

BULLETIN OF PHARMACEUTICAL RESEARCH

Vol. 15, No. 2S, May-Aug 2025

(An International Triannual Scientific Journal covering entire spectrum of Pharmaceutical Sciences)

Proceedings of Indo-US International Conference Theme: Unlocking Horizons in Global Multidisciplinary Healthcare Research

Theme: Unlocking Horizons in Global Multidisciplinary Healthcare Research Venue: PES's Modern College of Pharmacy, Nigdi, Pune, Maharashtra, India Date: March 10-12, 2025

Editor-in-Chief

Dr. Rajiv Dahiya



Published by:

Association of Pharmacy Professionals (APP)

Madhya Pradesh, India

http://www.appconnect.in/journal-bpr



INTERNATIONALCONFERENCE

[Hybrid Mode]

PES's Modern College of Pharmacy (MCOP)

under the aegis of IQAC, MCOP, Nigdi in collaboration with

The Association of Pharmacy Professionals (APP)



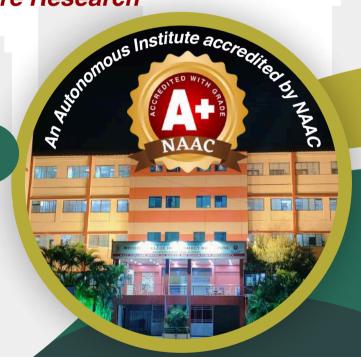
Indo-US International Conference

Conference

"Unlocking Horizons in Global Multidisciplinary Healthcare Research"

Date: 10th to 12th March 2025







mcopconf25@gmail.com

www.mcop.org.in



CHIEF PATRON

Dr. Prof. G. R. Ekbote

Chairman, Business Council, Progressive Education Society, Pune



PATRONS

Prof. S. S. Deshmukh, Secretary, P.E. Society, Pune Prof. J. G. Ekbote, Joint Secretary, P.E. Society, Pune Prof. Suresh Todkar, Joint Secretary, P.E. Society, Pune Dr. N. G. Ekbote, Dy. Secretary, P.E. Society, Pune Prof. Suhasini Itkar, Visitor, Modern College of Pharmacy, Nigdi, Pune

CONVENER

Dr. Pravin D. Chaudhari

Professor & Principal,
P.E.S. Modern College of Pharmacy, Nigdi, Pune - 411 044
Former President, APTI (Central)
Former Dean, Faculty of Pharmaceutical Sciences, SPPU, Pune.
Member, Academic Council, SPPU, Pune

ORGANIZING CHAIRMAN

Dr. Smita S. Pimple

Professor,

Dept. of Pharmaceutics
P.E.S. Modern College of Pharmacy, Nigdi, Pune - 411 044

ORGANIZING SECRETARY

Dr. Vitthal V. Chopade

Professor,

Dept. of Pharmaceutical Chemistry
P.E.S. Modern College of Pharmacy, Nigdi,
Pune - 411 044

Dr. Somdatta Y. Chaudhari

Associate Professor,
Dept. of Pharmaceutical Chemistry
P.E.S. Modern College of Pharmacy, Nigdi,
Pune - 411 044

REGISTRATION COMMITTEE

Dr. Devendra L. Visokar 91-9096694212 mcopconf25@gmail.com

SCIENTIFIC COMMITTEE

ACCOMMODATION/TRANSPORTATION COMMITTEE

Dr. Amit Tapkir 91-9022890289 mcoptr25@gmail.com

CONFERENCE PROCEEDINGS/ABSTRACTS

Dr. Anuradha More 91-9689907667 mcopcpa25@gmail.com

CULTURAL EVENT

Dr. Nikita Gaikwad Dr. Prashant Pawar

OUR COLLABORATORS



































About Society

The Progressive Education Society (P.E. Society), founded in 1934 by the esteemed educator and social advocate, Prof. Shankarraoji Kanitkar, has been a beacon of inclusive education for nearly a century. Guided by the visionary leadership of Hon. Dr. G. R. Ekbote, Chairman of the Business Council, the Society has flourished into a prestigious consortium of 62 institutes. These institutions span from kindergartens to doctoral programs, encompassing a diverse range of disciplines including Pharmacy, Engineering, Law, Education, Agricultural Biotechnology, Management, Arts, Commerce, and Science. P.E. Society remains steadfast in its commitment to educating all sectors of the community, thereby upholding its foundational values and contributing significantly to societal advancement.

About College

Modern College of Pharmacy, established in 1998 by the Progressive Education Society in the bustling industrial area of Yamunanagar, Nigdi, is a premier institution recognized for its excellence in pharmaceutical education and research. Approved by the Government of Maharashtra, AICTE, and PCI, the college boasts a permanent affiliation with Savitribai Phule Pune University and accreditation by NBA & NAAC with an impressive A+ grade and a CGPA of 3.47. The college offers a wide range of programs including B. Pharmacy, Pharmacy, D, M. Pharmacy in various specializations, and Ph.D. in Pharmaceutical Sciences. Notably, it has consistently ranked within the top 100 colleges in the NIRF pharmacy category for the past six years and consecutively received the Platinum Grade Rank by the CII survey for the last five years. In 2015-2016, it was named the "Best College in Professional Colleges' Category" by Savitribai Phule Pune University. Recently, the college has proudly embraced an Autonomous status granted by UGC, further enhancing its educational offerings and standing. In the latest IIRF 2024 Health Sciences Ranking, we achieved a National Rank of 64 and a State Rank of 9, affirming its position as a leader in pharmaceutical education.

About Conference

Pharmaceutical sciences combine scientific aspects that are critical in the development and manufacture of new drugs, handling of medicines and medical devices. In the most basic sense, drug design involves the design of molecules that are complementary in shape and charge to the biomolecular target with which they interact and therefore will bind to it. Artificial intelligence (AI) is a field of study that involves the development of computer systems that can perform tasks that normally require human intelligence. By leveraging machine learning algorithms, AI enables faster and more accurate identification of drug candidates. The integration of AI has streamlined tasks such as protein-ligand docking, molecular dynamics simulations, virtual screening, and de novo drug design. The technology promises scientific advancement in many sectors such as medicine, consumer products, energy, materials, and manufacturing. Pharmacy uses the knowledge derived from pharmacology to achieve optimal therapeutic outcomes through the appropriate preparation and dispensing of medicines. Medicinal Chemistry is the science that deals with the discovery or design of new therapeutic chemicals and their development into useful medicines. Pharmaceutics and Pharmaceutical Technology is concerned with the design and formulation of drugs into different dosage forms. In the present conference, pharmaceutical experts from international and national universities and other organizations will address on diverse aspects related to drug design, AI and pharmaceutical technology.

Speakers

WELCOME SPEECH



PROF. RAJIV DAHIYA
Organizing Chairman & President
APP Ex-Director, School of Pharmacy,
The University of the West Indies, St. Augustine,
Trinidad & Tobago, West Indies.



DR. SIMONE FAYE WALCOTT Vice President, APP West Indies International Branch, Department of Chemistry, Faculty of Science & Technology, The University of the West, Indies, Trinidad & Tobago



DR. ALEKHA K. DASH
President, APP American International
Branch, Associate Dean (Research) &
Professor, Department of Pharmacy
Sciences, School of Pharmacy and Health
Professions, Creighton University, Omaha,
Nebraska, USA



DR. SOMNATH SINGH
Professor, Department of Pharmacy
Sciences, School of Pharmacy and
Health Professions Creighton University,
Omaha, Nebraska, USA



DR. JAVED ALI
Professor & Head, Dept. of
Pharmaceutics, School of Pharm.
Education & Res., Jamia Hamdard, Delhi



DR. GAURAV GUPTA
Adjunct Research Professor, Centre of
Medical and Bio-allied Health Sciences
Research Ajman University, Ajman, UAE



DR. VIJAY MASAND
Professor, Department of Chemistry, Vidya
Bharati Mahavidyalaya, Amravati.
(Developer: PyDescriptor)

*For detailed topics and scientific schedule, please visit our official website.

Registration

The conference is mainly for the teachers, PG & PhD scholars from various institutes, research laboratories, and industries of Pharmaceutical, Medical, Ayurvedic, Biotechnological, Clinical Pharmacy, and other streams of the healthcare profession. The registration fees are Rs 1500/- (non-refundable). The registration fee includes a registration kit, bag, gifts, breakfast, lunch, access to all sessions and Hi-tea. Offline enrolment is restricted to 200 participants (on a first-come, first-serve basis). Accommodation will be charged extra. Last date for registration: 25-02-20 Website link: https://webbexindia.com/mcop

Poster / Oral Presentation

- Abstracts are invited for poster/oral presentation (online/offline).
 The last date to submit of structured abstracts not exceeding 250 words as per the template given on the website is on or before 25-02-2025 through the website.

- The presenting author will have to register as a participant before submission of an abstract.

 A certificate of presentation/participation will be given to the presenting author and registered participants only.

 The poster size must be 1.0 mt. X 1.0 mt. and the time for each presentation is 5 min., followed by 2 min. for
- Full-length articles selected from abstracts will be published as conference proceedings in the Bulletin of Pharmaceutical Research, [ISSN: 2249-6041 (Print); ISSN: 2249-9245 (Online)]

Program Schedule

Day-1 [Hybrid Mode]

Monday, 10 March 2025

09.00 am to 10.00 am: Registration, Breakfast and Tea 10.00 am to 11.00 am: Inauguration & Key address

11.00 am to 12.00 pm: Scientific Session I 12.00 pm to 01.00 pm: Scientific Session II

01.00 pm to 02.00 pm: Lunch Break

02.00 pm to 04.00 pm: Oral & Poster Presentation

04.00 pm to 04.30 pm: Hi-Tea 05.00 pm to 06.30 pm: Cultural Event

Day-2 [Online Mode]

Tuesday, 11 March 2025

09:00 am to 09.30 am: Welcome Address 09.30 am to 10.30 am: Scientific Session I 10.30 am to 01.00 am: Scientific Session I 1:00 pm onwards: online oral presentation

Day-3 [Online Mode]

Wednesday, 12 March 2025

10.00 am to 03:00 pm: Online oral presentation 03.00 am to 04.00 pm: Valedictory function

& Award distribution (Online)

Venue of Conference





Full Length Conference Proceding Issue Bulletin of Pharmaceutical Research Special issue 2025;15(S2)

Title of Manuscript

AI-Driven Experimental Models: A New Era in Ethical and Economical Animal Research

Vagisha Mahajan 1 , Shraddha Pawar 1 , Jyoti Pawar 1 , Arulmozhi Sathiyanarayanan 2 , Shakuntala Chopade 1

Unlocking The Immunotherapeutic Potential of Beta Glucan in Skin Melanoma: An In-Silico Study Targeting PD-L1

Shradha Chaudhari, Shikha Gaikwad, Satish Polshettiwar Dishank Purandare

Development, Characterization and Invitro Evaluation of Itraconazole and Fluconazole Nanoemulgel for Topical Drug Delivery.

Anuradha G. More*, Dipali M. Kukde, Samarth D. Shinde, Tushar H. Kshirsagar, Padmaja S. Kore, Ujwala S. Desai

Development and Characterization of Hibiscus Sabdariffa and Cajanus Cajan based Herbal Gel for treating Oral Ulcer.

Kalyani Kakad*1, Vaishnavi Kate1, Shubham Dhanavade1, Pavan Navghare2, Saurav Gore2

Formulation and Evaluation of Directly Compressed Polyherbal Anthelmintic Tablet.

Anuradha G. More*, Amrut N. Godbharle, Avantika S. Jadhav, Monika L.

Suryawanshi, Padmaja S. Padmaga Kore and Ujwala S. Desai

Herbal Anti-Microbial Topical Formulation for Fungal Infection

Padmaja Kore^{1*}, Ujwala Desai², Anuradha More², Soham Sanjay Jadhav¹, Anuj Vivek Borhade¹, Deepak Firoj Khatri², Atharva Ajay Namade²

Evaluation of Immunomodulatory Activity of Ethanolic Extract of *Catunaregum Spinosa* (Thunb.) Tirveng Root Bark Using *In Vivo* Models

Anju K R*, Veena R, Tasmiya Palte

A Systematic Review of Food-Drug Interactions of Drugs Acting on Cardiovascular System

Punam Kela* Karishma Mainkar, Shreyasi Banerjee, Pratiksha Kulkarni, Sakshi Latey.

Formulation and Evaluation of Fast- Dissolving Antidandruff film for Topical application.

Anuradha G. More*, Pooja S. Khaire, Vaishnavi V. Shirke, Atharv R. Adhav, Padmaja S. Kore¹

AI in Beauty: Revolutionizing the Cosmetics Industry

Anuradha G. More^{1*}, Kalyani P. Hajapure , Gayatri V. Nunnewar , Padmaja S. Kore, Ujwala S. Desai

Investigation of Anthelmintic activity using a Polyherbal formulation.

Shraddha Desai, Padmaja Kore, Tushar Mathapati, Dhanraj Dhakne, Sayali Navhale

Zebrafish Model for Ocular Disorders and Toxicity Testing - A Review

Vishal Gangawane, Bhargavi Govardhan, Rutuja Bhosale, Kaustubh Pawar, Pramod Choudhary, Karimunnisa Shaikh*

Herbal Chewing Gum For Treating Mouth Infection

Ujwala Desai^{1*}, Padmaja Kore², Anuradha More¹, Arati Chavan¹, Jyoti Hatekar¹, Shraddha Dhumal², Unmesha Badhale²

Development and Validation of Stability Indicating Assay Method for Determination of Metoprolol Tartrate in Bulk and Marketed Formulation by HPTLC Method

Aishwarya R. Balap^{a*}, Hrushikesh. K. Giramkar^a, Anuja H. Pachphule^a, Leena P. Patil^a, Sumedha S. Dakhore, Sampada S. Salunke

Nanotechnology Approaches in Triple Negative Breast Cancer

Padmaja Kore^{1*}, Rohan Lad¹, Asit Sirsat¹, Anuradha More², Ujwala Desai³

Development and Validation of UV Spectrophotometric Method for Simultaneous Estimation of Methotrexate and Chrysin

Anuradha G. More¹, Surbhi C. Gupta*

Formulation and Evaluation of Folic Acid and PEG Loaded Iron Oxide Nanoparticles by Green Synthesis

Ujwala Desai¹, Padmaja Kore², Atharva Joshi¹, Akanksha Bhandare², Geetanjali Bhale², Devendra Dudhade¹

Design, Formulation and Evaluation of Herbal Emulgel Contains*Psoralea Corylifolia* Extract

Vitthal Chopade, Shivani Mandlik*, Abhijeet More, Pranita Gore, Swapnali Dupargude,

High-Performance Thin Layer Chromatography Method Development and Validation for Naftifine Hydrochloride Determination.

Mayur S. Tekade*, Pallavi M. Patil

Exploring Women's Knowledge, Attitudes, and Health related Practices Related to Polycystic Ovary Syndrome (PCOS) among Women: A Survey Analysis

Rajani Mali ¹, Nikita Sanap ¹, Swapnali Shedge ², Vijay Shinde ², Priyanka Sarda

Scleroglucan Based Hydrogel as Wound Dressing Material

Karimunnisa Shaikh*, Priyanka Rokade, Mansi Sardar, Shraddha Lipare

Herbal Chewing Gum for Treating Mouth Ulcers

Padmaja Kore^{1*}, Ujwala Desai², Anuradha More², Santoshi Lashkare¹, Omkar Lokhande¹, Dhanashri Jamadade²

Simultaneous Estimation of Pazopanib and Silymarin: Method Development and Validation

Anuradha G. More, Dhanashri P. Garud

Development And Evaluation of a Spironolactone Oral Suspension.

Madhugandha S Kawadiwale 1 , Mahesh M Bhadgale 2 , Vaishnavi M Jambhale 1 , Smita M Warade 1 , Karimunnisa S Shaikh 1

Evaluation Of In-Vitro Anthelmintic Activity of Ethanolic Extracts of *Fragaria Ananassa* and *Lactuca Sativa* Against *Pheretima Posthuma*

Padmaja Kore 1* , Vidya Chaudhari 1 , Siddhi Chavan 1 , Pratik Phalke 1 , Anuradha More 2 , Shraddha Desai 1 , Ujwala Desai 2

Polyherbal Dermal Patch for Fungal Infections.

Padmaja Kore 1* , Ujwala Desai 2 , Anushka Kamble 1 , Pooja Khaire 1 , Pratiksha Jadhav 2 , Rohit Shelmukar 2

Evaluation of the Suspending Properties of Litsea Glutinosa (Maida Lakdi) Mucilage in Pharmaceutical Suspensions.

Anuradha G. More*, Atharv R. Adhav, Pooja S. Khaire, Vaishnavi V. Shirke Padmaja S. Kore

Drug Solubility, Importance and Enhancment Techniques.

Ujwala S. Desai*, Srushti S. Waghmare, Ranjit A. Jamadade, Sharvari P. Shendage

Evaluation of Dispensed Prescription Errors and Drug-Drug Interactions at Community Pharmacy.

Rajani Baban Mali^{1*}, Om Suhas Kinhikar¹, Tabassum Shaikh²

Advanced Therapeutic Approaches in Triple Negative Breast Cancer.

Padmaja Kore^{1*}, Asit Sirsat¹, Rohan Lad¹, Anuradha More², Ujwala Desai²

Formulation and Evaluation of Herbal Shampoo Tablet

Ujwala Desai 1* , Padmaja Kore 2 , Mahesh Reddy 1 , Jaie Zore 1 , Girish Funde 2 , Vaishnavi Atole

Formulation And Evaluation of Herbal Facewash and Exfoliating Tablets

Ujwala Desai¹, Padmaja Kore², Anushka Kamble¹, Kirti Ishte¹, Akanksha Aglave², Yash Kulkarni²

Biotechnological Innovations in Herbal Drug Development: Advances, Challenges, and Future Prospects

Rutuja Khandare, ¹Nikita Sanap, *Priyanka Sarda

A review on bioactive phytoconstituents and pharmacological activities of camara lantana

Harshada Salunke, Rutuja Sontakke, Shraddha Dhumal, Ketaki Joshi, Priyanka Sarda*, Suvarna Vadje

To study the network pharmacology of ethylene glycol derivatives use as coating materials on coronary heart stent

Jayashri Chopade * and Deepak Hujare

Synthesis, purification & characterisation propafenone impurity 2'-(2-Hydroxy-3-aminopropoxy)-3-phenylpropiophenone.

Gite Valmik Gite*1 Bhoi Hemant2 and Dhiraj Panke3

In silico study for ADMET and toxicity prediction of current antidiabetic agent Gunjegaonkar M. B. *, Khot S. P. and Fegade S. A.

Comparative Study of Evaluation of Generic and Branded Metformin HCl (500mg) Tablets.

Vishnu Neharkar^{1*} Dhiraj Panke¹ and Shrikrushna Wakude²

Advancing Cefdinir Analysis: Evaluating Stability, Identifying Impurities & Characterizing Degradants

Fegade Sachin^{1*}, Bindurani L. G. P. Ram ² and Waman Pradnya ³

Rapid and sensitive method of sugar detection in marketed soft drinks.

Vishnu Neharkar *, Munot Gautam and Snehal Tuse

Comparison of UV and HPTLC analysis for estimation of Sertaconazole nitrate Shivani Morkhade^{1*} Sanika Baitule² and Ajay Baitule²

Comparative evaluation of generic supplied and branded drugs of metronidazole tablets.

Gite Valmik^{1*} Namade Atharva A².* and Bindurani L. G. P. Ram ³

An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print); ISSN: 2249-9245(Online)



REVIEW ARTICLE

AI-DRIVEN EXPERIMENTAL MODELS: A NEW ERA IN ETHICAL ANDECONOMICAL ANIMAL RESEARCH

Vagisha Mahajan¹, Shraddha Pawar¹, Jyoti Pawar¹, Arulmozhi Sathiyanarayanan² and Shakuntala Chopade¹

¹Department of Pharmaceutical Chemistry, Bharati Vidyapeeth (Deemed to be University),
Poona College of Pharmacy, Pune, Maharashtra, India-411038

²Department of Pharmacology, Bharati Vidyapeeth (Deemed to be University), Poona College
of Pharmacy, Pune, Maharashtra, India-411038

*Corresponding author email: shakuntalachopade4@gmail.com
Received: Feb 06, 2025 / Revised: April 05, 2025 / Accepted: May 24, 2025

ABSTRACT

A substantial financial burden is imposed by animal research because of the expenses of housing, care, legal compliance, and extended study periods. This paper reviews AI-powered tools that enhance research by reducing expenses, resolving moral dilemmas, and boosting scientific discoveries. Organ-on-Chip technologies mimic human organ functioning, minimizing the need for live people, while silico models assess genetic alterations and drug interactions using computer simulations. 3D bioprinting driven by AI makes it possible to create tissue and organ models, providing repeatable and compassionate testing platforms. Safety evaluations are streamlined by predictive toxicology, which forecasts toxicity by analyzing chemical structures. Digital twins and virtual reality offer controlled experimental alternatives, and High-Throughput Screening (HTS) simplifies and expedites drug testing. In the pharmaceutical and biological sciences, these AI -driven techniques improve research efficiency, encourage moral options, and result in considerable cost reductions. The potential for AI-driven technology to supplant conventional animal testing techniques with more ethically sound, economically viable, and scientifically sound alternatives is demonstrated by these developments, which mark a revolutionary change in biomedical research.

Keywords: Artificial Intelligence, 3D-bioprinting, Animal Handling, Organ-on-chip, Digital Twins **How to cite this article**: Vagisha Mahajan, Shraddha Pawar, Jyoti Pawar, Arulmozhi Sathiyanarayanan, Shakuntala Chopade. AI- driven Experimental Models: A New Era in Ethical and Economical Animal Research. Bulletin of Pharmaceutical Research. 2025;15(S2): 1-7

Source of support: Nil Conflict of interest: None

INTRODUCTION

Considering current animal research scenario, the expenditures of staff, materials, and facility maintenance, as well as extra charges for regulatory compliance, make animal research extremely expensive. Additionally, these investigations take a long time—often years—and are expensive—costing millions of dollars

every drug studied. However, by offering creative substitutes that lessen or do away with the necessity for animal testing, the incorporation of artificial intelligence (AI) has transformed research and testing. To replicate real-world processes without using live animals, researchers can now predict biological outcomes, analyze massive datasets, and build virtual models of biological systems

Unlocking Horizons in Global Multidisciplinary Healthcare Research" Indo-US International Conference Date: 10-12, March

thanks to AI-driven technologies like machine learning, computational modelling, and simulation platforms. ΑI is already revolutionizing domains such as toxicology and drug discovery, where researchers can test substances and model biological reactions without using real individuals thanks to digital twins and high-throughput screening (HTS). In addition to evaluating chemical safety based on molecular structures, AI-driven predictive models lessen the need for animal testing. Although widespread implementation still necessitates additional development, validation, and regulatory alignment, regulatory bodies such as the FDA and European Medicines Agency are starting to consider AI-based approaches. AI is a promising substitute for conventional animalbased testing techniques since it is raising ethical standards, increasing productivity, and lowering research expenses despite these obstacles.

Economic Burden on Animal Handling In Research

Animal studies are time-consuming, often spanning months or years, with expenses reaching millions per substance examined, limiting the evaluation of chemicals and their combinations (hsi.org).

For fiscal year 2025, institutions like the University of Iowa and Indiana University have established per diem rates << Table 1>>, which are subject to annual adjustments for inflation, facility maintenance. and operational costs. Researchers must anticipate potential cost increases when planning budgets. Alongside housing and care expenses, regulatory compliance further elevates costs, making animal research financially demanding. These expenses—housing, cumulative prolonged compliance. and study durations—impact the efficiency and feasibility of scientific investigations.

Table 1 University of Iowa's per diem rates (Fiscal Year 2025)

Species	Per Diem Rate
Dog	\$34.17
Ferret	\$7.45
Guinea Pig	\$2.85
Hamster	\$1.06
Mouse (per cage)	\$0.89

Revolutionizing Research: Ai Methods to Limit Animal Use

AI is transforming research and testing by introducing innovative methods that significantly reduce or replace animal use, improving both ethical standards and scientific precision.

1.1 In-silico Models

VR software

Virtual reality (VR) software transforming medicine by providing advanced tools for education, training, and research. VR-based human-patient simulators allow healthcare professionals to practice complex procedures safely. eliminating the need for animal models [1,23]. OB/GYN residency programs, such as those at Rush University Medical Center, have adopted VR surgical systems, promoting ethical and effective training. Beyond education, VR aids in disease modeling, surgical planning. and personalized treatment simulations, integrating with AI organ-on-a-chip technologies and enhance medical research. VR promotes ethical and patient-centered healthcare solutions by lowering the need for animal experimentation. << Figure 1>>



Fig. 1. Overview of Virtual Reality (VR) software applications in animal handling and research training.

1.2 "organs-on-chips" software for in vitro testing

A more realistic option to conventional animal models for disease research, medication testing, and toxicity studies are organs-on-chips, which are sophisticated microfluidic devices that mimic human organ functions using human cells[3,4] lung-on-chip AlveoliX specializes on technology, which replicates pulmonary processes like breathing mechanics and airblood interactions. This allows for accurate research lung disorders, on effectiveness of medications, and inhaled pollutants[8]. These chips use humanderived cells to produce highly predictive data that supports ethical, effective, and human-relevant scientific improvements while supporting research into illnesses including asthma and COPD.

MIMETAS creates organ-on-a-chip technology that use microfluidics to replicate dvnamic human environments, advancing toxicity testing, disease modeling, and drug development[5,6]Using 3D cell cultures that closely mimic human tissues, its flagship product, OrganoPlate®, replicates a variety of human organs, such as the liver, kidney, and blood-brain barrier [2,11]<< Figure 2>>

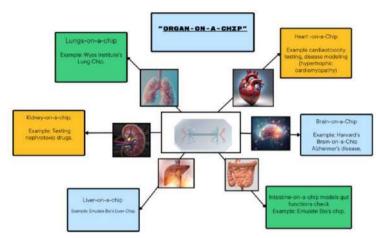


Fig. 2. Illustration of an Organ-on-a-Chip model, replicating organ functions for biomedical research

1.3 AI and 3D Bio-printing

Molecular modeling and computational chemistry are used in computer-aided drug design (CADD) to find possible therapeutic candidates. AI improves this process by predicting drug-protein interactions and increasing predictive accuracy, which makes drug development more accurate and efficient^[25]. By mimicking human drug interactions, this development minimizes the requirement for animal testing while speeding up discovery ^[17].

3D bioprinting, a process that uses stem cells, growth hormones, and nutrients to build tiny human tissue structures called organoids layer by layer, is another groundbreaking advancement. In 1994, Vedani et al. The use of animal testing is further decreased by these miniature organs, which mimic human tissues and provide a more precise platform for researching drug interactions. AI and 3D bioprinting together drug development's efficacy, humanity, and scientific advancement. An excellent instance of this is the DART (Drug and Toxicity Prediction System) from Quantiphi, which predicts the safety and effectiveness of drugs by combining AI with human biology.

A 3D tissue model entitled EpiAlveolar was created with assistance from the PETA International Science Consortium Ltd. to replicate the alveoli and the deepest portion of the human lung. Animal testing is no longer necessary because it is made entirely of human cells and is a useful tool for researching inhaled compounds such as pollutants. pathogens, and e-cigarette smoke^[3]. Research on pollution impact, respiratory disorders, and the safety of inhalable products is made possible by this human-relevant model, which also permits long-term exposure studies free from ethical issues [19]. In addition to models such as EpiDermTM, EpiAlveolar contributes to scientific research by lowering the risks associated with human trials, encouraging ethical research practices, and offering data that is applicable to humans [2].

1.4. Predictive Toxicity testing using AI algorithm

Computer-aided drug design (CADD) computational utilizes chemistry molecular modeling to identify potential drug candidates. AI enhances this process by improving predictive accuracy and analyzing large datasets to predict drugprotein interactions. making drug development more efficient and precise [25]. This advancement accelerates discovery while reducing the need for animal testing by simulating human drug interactions [17]. During interpretation, binding reflects the strength and stability of the interaction between a ligand and its biological target, with more negative values indicating stronger binding. This is vital for processes like enzyme activity and drug efficacy. Key interaction types contribute to this energy: hydrogen bonds ensure specific positioning, hydrophobic contacts stabilize the complex by minimizing water exposure, ionic interactions enhance stability through charge complementarity and van der Waals forces support close molecular packing.

Together, these interactions determine binding affinity and biological effectiveness ^[13]. While interpreting using CADD, it is important to clarify whether the reported docking of drug sample score refers to the best-ranked pose—the one with the lowest binding energy and presumably the most favorable interaction—or an average score calculated across multiple predicted poses. This distinction helps ensure accurate interpretation of the docking results, as the best-ranked score highlights the most stable binding conformation, while an average score offers insight into overall binding consistency^[15]

Regulatory agencies like the EPA are increasingly adopting humane testing methods, exemplified by Syngenta Crop Protection LLC's new approach to evaluating the

1.5 Substitution live experiments with computer simulations

A sophisticated instructional program called "X-cology" allows students digitally carry out tests, evaluate findings, and make deductions bv simulating intricate pharmacological trials, including bioassays. This application provides useful skills without the logistical difficulties of live animal investigations by simulating real-life experimental operations. simplifying intricate biological demonstrations, it improves teaching efficiency and lowers expenses, time, and ethical issues related to live animal testing [1]. Teachers can ensure that students understand fundamental pharmacological principles by skillfully demonstrating complex processes without the need for large laboratory equipment.

1.6 Digital Twins

Digital twins, which are computergenerated virtual representations of biological systems, present an acceptable means to lessen the usage of animals in research^[16]. These models closely resemble biological responses found in the actual world by incorporating information such as genetic profiles, physiological readings, and environmental conditions. By facilitating constant adaptation to fresh data, artificial intelligence (AI) improves digital twins' forecasting accuracy. Digital twins can mimic organ or tissue responses to medications in pharmaceutical research, enabling researchers to examine a range of impacts without using animals. This method greatly lessens the requirement for live animal testing while guaranteeing accurate and moral research [21].

Simcyp Simulator

Virtual human models have been produced twins to mimic digital physiological effects of a given medication. Researchers can forecast how medications will affect the human body by using these models, which mimic organs, tissues, and genetic profiles. The Simcyp Simulator, for instance, models human populations and forecasts drug absorption, distribution, metabolism, and excretion using digital twin technology, providing insights without the need for animal testing or clinical trials [10]

In silico Medicine

Digital twins are being developed in cancer research to model how a patient's tumor reacts to different therapies. Instead of employing animal models, in silico medicine uses artificial intelligence (AI) and digital twins to model cancer cells and anticipate how they will respond to particular medications. This enables individualized treatment plans.

1.7 High-Throughput Screening (HTS)

High-throughput screening (HTS) minimizes the need for animal testing by enabling researchers instantly evaluate a large number of substances using artificial models or cell cultures. By automating data analysis, finding interesting drug candidates, and forecasting possible

toxicity, AI integration improves HTS and makes the process more precise and efficient [22]. By evaluating biological effects using artificial or human-based models, AIpowered HTS may screen out potentially hazardous substances early in medication development process. AI also makes it possible to investigate intricate biological pathways in more detail and finds patterns that conventional techniques might miss. This method minimizes animal testing while speeding up drug discovery. making research more efficient, economical, and morally sound [6].

Animal testing and the contract research industry is directed towards research and development (R&D)

R&D, especially regarding the development of transgenic animals for the study of diseases, pharmacological interactions, and toxicity, accounts for a sizable amount of funding for contract research and animal testing. Researchers can better understand how drugs affect human biology and how genetics affects drug safety and efficacy thanks to these genetically engineered animals. For treatments including anticancer, anti-convulsive, anti-arthritis, and anti-hypertensive drugs, chronic toxicity testing is essential for assessing long-term pharmacological effects and ensuring their safety over extended usage.

CONCLUSION

AI integration in experimental animal model research enhances ethical standards and economic efficiency. Technologies like In silico models, Organ-on-Chip, AI-assisted 3D bioprinting, and predictive toxicology reduce reliance on animal testing while improving accuracy. These innovations cut research costs, streamline development, and accelerate drug discovery. AI-based methods offer cost-effective simulations and predictive models, ensuring ethical,

efficient, and scientifically robust biomedical advancements.

ACKNOWLEDGMENT

The authors would like to thank Prof. Dr. A. P. Pawar, Principal, Poona College of Pharmacy, Pune for his consistent support and guidance.

REFERENCES

- Ansari I, Gupta N, Yadav A. A review on alternatives to animal experimental models.
 Int. J Research in Phytochem and Pharmacol. 2021;11:1-7.
- 2. Barilli A, Visigalli R, Ferrari F, et al. Organic cation transporters (OCTs/OCTNs) in human primary alveolar epithelial cells. *Biochem Biophys Res Commun.* 2021;576:27-32.
- 3. Barosova H, Maione AG, Septiadi D, *et al.* Use of EpiAlveolar lung model to predict fibrotic potential of multiwalled carbon nanotubes. *ACS Nano* 2020;14(4):3941-3956. [DOI: 10.1021/acsnano.9b06860]
- 4. Bhatia SN, Ingber DE. Microfluidic organson-chips. *Nat Biotechnol*. 2014;32:760-772.
- 5. Bircsak KM, DeBiasio R. A 3D microfluidic liver model for high throughput compound toxicity screening in the OrganoPlate®. *Toxicology* . 2021;450:152667.
- 6. Broach JR, Thorner J. High-throughput screening for drug discovery. *Nature*. 1996;384(6604 Suppl):14-16.
- 7. Currie GM. The emerging role of artificial intelligence and digital twins in preclinical molecular imaging. *Nucl Med Biol.* 2023;120-121:108337.
- 8. Dasgupta Q, Jiang A, Wen AM, *et al.* A human lung alveolus-on-a-chip model of acute radiation-induced lung injury. *Nat Commun.* 2023;14:6506.

- 9. Doke SK, Dhawale SC. Alternatives to animal testing. *Saudi Pharm J*. 2013;23(3):223-229.
- 10. Kilford PJ, Chen KF, Crewe K, *et al.* Prediction of CYP-mediated DDIs involving inhibition: Approaches to address the requirements for system qualification of the Simcyp Simulator. *CPT Pharmacometrics Syst Pharmacol.* 2022;11(7):822-832.
- 11. Kosim K, Bokkers M, Oudshoorn J, *et al.* Modelling gut complexity and disease: Development of a next-generation adult stem cell-based tubular gut-on-a-chip model. *J Crohns Colitis*. 2024;18 Suppl 1:i296.
- 12. Leung CM, de Haan P, Ronaldson-Bouchard K, *et al.* A guide to the organ-on-a-chip. *Nat Rev Methods Primers*. 2022;2:33.
- 13. Liu, J & Wang, R. Classification of current scoring functions. *Journal of Chemical Information and Modeling*. 2015; 55(3): 475–482.
- 14. Macarron R, Banks MN, Sittampalam GS. Impact of high-throughput screening in biomedical research. *Nat Rev Drug Discov.* 2011;10:188-195.
- 15. Morris, GM, Huey R, Lindstrom, W, Sanner, MF, *et al.*. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of Computational Chemistry*. 2009: 30(16): 2785–2791.
- 16. Mu M, Zhou Y, Wu D. Digital twins on animal husbandry: Insights and application. *Procedia Comput Sci.* 2022;214:1182-1189.
- 17. Niazi SK, Mariam Z. Computer-aided drug design and drug discovery: A prospective analysis. *Pharmaceuticals* (Basel). 2023;17(1):22.
- 18. Rebecca R, Domenico G, Timothy EA. The role of 'big data' and '*in silico*' New Approach Methodologies (NAMs) in ending animal use A commentary on

- progress. *Comput Toxicol.* 2022;23:100232.
- 19. Rothen-Rutishauser B, Gibb M, He R, *et al.* Human lung cell models to study aerosol delivery considerations for model design and development. *Eur J Pharm Sci.* 2023;180:106337.
- 20. Shi J, Wan Y, Jia H, Skeldon G. Printing cell-embedded sacrificial strategy for microvasculature using degradable DNA biolubricant. Biomaterials. *Angew Chem Int Ed.* 2024; e202417510.
- 21. Suresh N. Twin farms nexus Digital twins for sustainable animal farming. *Arch Anim Poult Sci.* 2024;2(4):555595. [DOI: 10.19080/AAPS.2024.02.55559]
- 22. Szymański P, Markowicz M, Mikiciuk-Olasik E. Adaptation of high-throughput screening in drug discovery-toxicological screening tests. *Int J Mol Sci.* 2012;13(1):427-452.
- 23. Tang FMK, Lee RMF, Szeto RHL, Cheng JKK. A simulation design of immersive virtual reality for animal handling

- training to biomedical sciences undergraduates. *Front Educ.* 2021;6.
- 24. Vedani A. Computer-aided drug design and the Three Rs. In: Reinhardt CA, ed. Book Chapter 11. 1994 Feb 24.
- 25. Vemula D, Jayasurya P, Sushmitha V, *et al.* CADD, AI and ML in drug discovery: A comprehensive review. *Eur J Pharm Sci.* 2023;181:106324.
- 26. Wang H, Yin F, Li Z, Su W, Li D. Advances of microfluidic lung chips for assessing atmospheric pollutants exposure. *Environ Int.* 2023;172:107801.
- 27. York SL, McGaghie WC, Kiley J, Hammond C. Implementation and evaluation of a dilation and evacuation simulation training curriculum. *J. Contraception*. 2016;93(6):545-50.

An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print); ISSN: 2249-9245(Online)



RESEARCH ARTICLE

UNLOCKING THE IMMUNOTHERAPEUTIC POTENTIAL OF BETA GLUCAN IN SKIN MELANOMA: AN IN-SILICO STUDY TARGETING PD-L1

Shradha Chaudhari¹, Shikha Gaikwad^{1*}, Satish Polshettiwar² and Dishank Purandare

1. Department of Biosciences and Technology, School of Sciences and Environmental Studies, Faculty of Sciences and Health Sciences. Dr. Vishwanath Karad MIT World Peace University, Pune-411038, India

2. Department of Pharmaceutical Sciences, School of Health Sciences and Technology, Dr. Vishwanath Karad MIT World Peace University, Pune-411038, India *Corresponding author email: shikha.gaikwad@mitwpu.edu.in
Received: Feb 07, 2025 / Revised: April 19, 2025 / Accepted: May 04, 2025

ABSTRACT

Skin melanoma is one of the most virulent types of skin cancer, with a high death rate, primarily due to immune evasion mechanisms, including the PD-1 pathway. PD-L1 inhibition is a promising immuno-oncology strategy. Beta Glucan, a natural polysaccharide with promising immunomodulatory activity, was assessed as a PD-L1 inhibitor via molecular docking. AutoDock Tools, with protein (PDB ID: 8P64) as the receptor, were used for computational in-silico analysis. Beta Glucan's binding was compared with a standard chemotherapeutic agent, 5-Fluorouracil. The results revealed that Beta Glucan has much stronger binding energy (-9.8 kcal/mol) than 5-Fluorouracil (-4.3 kcal/mol), indicating a possible inhibitory effect on PD-L1 through favourable interaction. This research provides a computational foundation for potential further research into the use of Beta Glucan for the treatment of skin melanoma as an immunotherapeutic factor.

Keywords: Skin Melanoma, Molecular Docking, Beta Glucan, 5-FU

How to cite this article: Shradha Chaudhari, Shikha Gaikwad, Satish Polshettiwar and Dishank Purandare Unlocking the Immunotherapeutic Potential of Beta Glucan in Skin Melanoma: An In-Silico Study Targeting PD-L1. Bulletin of Pharmaceutical Research. 2025;15(S2): 8-12.

Source of support: Nil Conflict of interest: None

1. INTRODUCTION

Skin melanoma is associated with remarkable global mortality and morbidity is an extremely aggressive type of skin cancer, due to the malignant transformation of melanocytes^[1]. Its treatment remains suboptimal due to mechanisms of immune evasion and drug resistance, despite

chemotherapeutic and surgical advancements. Among these, the PD-1/PD-L1 immune barrier pathway is crucial, permitting melanoma cells to escape immune surveillance by inhibiting T-cell-mediated immune responses. Thus, targeting PD-L1 has emerged as a promising therapeutic strategy in recent immuno-

Unlocking Horizons in Global Multidisciplinary Healthcare Research Indo-US International Conference Date: 10-12, March 2025 Organised by PES Modern college of Pharmacy Nigdi Pune-44

oncology [2]. Current structural analyses of PD-L1 (PDB ID: 8P64) have enabled the rational design of inhibitors with the potential to inhibit the PD-1/PD-L1 interaction[3]. Beta Glucan, a naturally occurring fungal, oat, and yeast-derived polysaccharide, possesses immunomodulatory and anti-tumour properties. Introductory studies indicate its potential to modulate immune responses and possibly interfere with immune checkpoint signalling pathways [4]. However, its use as a PD-L1 inhibitor in skin is melanoma principally uncharted territory. The current study employs computational in-silico molecular docking techniques to investigate the binding affinity and interaction profile of Beta Glucan with PD-L1.5-Fluorouracil (5-FU) -a well-documented chemotherapeutic agent with proven efficacy in the treatment of a range of skin cancers - has been employed as the control drug for comparison [5].

2. MATERIALS AND METHODS

The software tools, AutoDock Tools 1.5.1, PyMOL 2, Biovia Discovery Studio Visualizer 2025, ChemBioDraw Ultra 14.0, and Chem3D Pro 14.0, were used for the molecular docking study [6].

2.1 Protein preparation

Initially, the protein structure was taken from the PDB in (.pdb) format. This protein was uploaded to AutoDock 1.5.1, and subsequently, water molecules were removed. Subsequently, polar hydrogens were added, along with Kollman's charges. Once the Kollman charges incorporated, it was confirmed that the charges were evenly distributed across the entire protein by ensuring that charge deficiencies were addressed for all atomic residues. After verifying that no residues had non-integral charges, the protein was saved in (. pdbqt) format [7].

2.2 Ligand Preparation

Initially, both ligands were obtained in the (.sdf) format from the PubChem database. After uploading the file to PyMOL, it was converted into (.pdb) format. Once saved, the (.pdb) file was then uploaded to AutoDock. The molecule was selected for AutoDock 4, and all rotatable bonds were converted into non-rotatable bonds. After completing this process, the ligand was saved in (. pdbqt) format [8].

2.3 Molecular docking

Once the protein and ligand were saved in (. pdbqt) format, they were imported into AutoDock. Since the binding site was not confirmed, blind docking was performed. The grid box dimensions were set to $126 \times 126 \times 1$

26.253. After ensuring that the grid box encompassed the entire protein and ligand, the grid parameters were saved in (.txt) format. A configuration file was created, in which the energy range was set to 4, exhaustiveness was set to 8, and both the center XYZ coordinates and size XYZ data were uploaded. The receptor and ligand files were also included in this configuration file, which was saved in (.txt) format. The maximum number of evaluations and the maximum number of generations were set to 2,500,000 and 27.000, respectively. To obtain the binding energy score, a command prompt was executed, resulting in an output file in (.pdbqt) format. The binding energy/docking score in kcal/mol was recorded in the log file (.txt) format [9].

2.4 Visualization of docking results

Once the target and ligand were saved in (. pdbqt) format, they were imported into AutoDock. Since the binding site was not confirmed, blind docking was performed. The grid box dimensions were set to 126X126X126. The grid parameters for the protein were established to facilitate the docking process. To visualize the ligand docking to the protein at the binding site,

Biovia Discovery Studio Visualizer 2025 was used. Initially, the protein file in (. pdbqt) format was uploaded, followed by the output file in the same format. Subsequently, the molecule with the best binding energy was selected as the ligand. Once this was completed, the 3D, 2D, and Amino Acid interactions were visualized [10].

3. RESULTS AND DISCUSSION

The binding affinity of Beta Glucan was compared to that of the standard drug, 5fluorouracil, against the PD-L1 enzyme. When Beta Glucan was docked to the protein, 9 poses were obtained. Among these, the pose that exhibited the highest docking score and a perfect overlay with the co-crystal ligand present in the protein was selected as the final pose, and the 3D molecular docking results were recorded [11]. The docking score for the standard compound, 5-Fluorouracil, was found to be -4.3 kcal/mol, while that of Beta Glucan was -9.8 kcal/mol. The docking scores of the optimal pose are presented in Table 1, and the 3D docking images are displayed in Figures 1 and 2. Subsequently, to analyse the Amino Acid interactions, 2D structures were analysed, as shown in Figures 3 and 4, along with Table 2. Both ligands showed strong affinity towards the binding site with strong hydrogen bond interaction, thus showing a stable attachment.

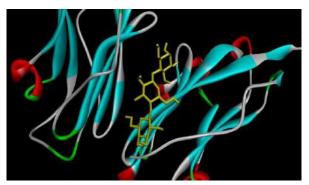


Fig.1. 3D interaction of Beta Glucan with Protein

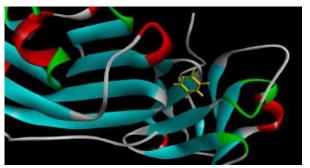


Fig. 2. 3D interaction of 5-Fluorouracil with protein

Table 1: Dock score (binding energy) of Beta Glucan and 5-Fluorouracil

Sr. No	Compound	Binding
		Energy (kcal/mol)
1	Beta Glucan	-9.8
1	Deta Giucan	-9.0
2	5-	-4.3
	Fluorouracil	

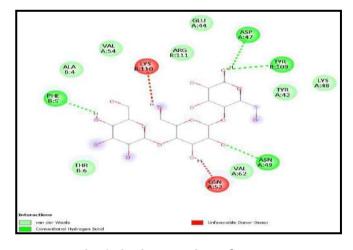


Fig. 3. 2D interaction of Beta Glucan with protein

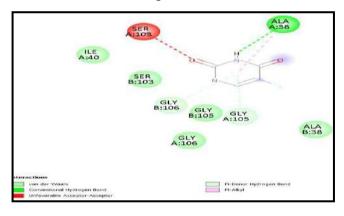


Fig. 4. 2D interaction of 5- Fluorouracil with protein

T	'able	2. Ami	no acid i	nteracti	ons of			
	ligands with protein							

Compound	Hydrogen bond interactio	Distanc e (Å)	Hydro- phobic interac	Distanc e (Å)
Beta	n ASN49	2.170	tion	
			-	
Glucan	TYR109	2.879		
	ASP47	2.408		
	PHE5	1.923		
5-Fluoro	ALA38	2.497	ALA38	5.062
uracil	GLY105	3.166		
	GLY106	3.290		

4. CONCLUSION

The computational study of the interaction of Beta Glucan with PD-L1 has promising preliminary results for its hypothesized role as a PD-L1 inhibitor. Inhibition of the PD-1/PD-L1 immune checkpoint pathway by Beta Glucan can potentially reveal a new immunotherapeutic strategy against the treatment of melanoma, which is notorious for its virulence and resistance to traditional treatments. The results of molecular docking demonstrated significantly greater binding ability of Beta Glucan (-9.8 kcal/mol) than that of the traditional drug, 5-Fluorouracil (-4.3 kcal/mol), used routinely to treat skin cancer. This, in turn, indicates that Beta Glucan has the potential to inhibit the PD-1/PD-L1 interaction more efficiently, restoring T-cell-mediated immune surveillance boosting the anti-tumour activities. Although the conclusion is based on computational modelling, it gives a solid foundation for the performance of future experiments in vitro and in vivo to validate the claims. Using naturally derived compounds such as Beta Glucan not only opens avenues for low-toxicity and biocompatible therapies but also aligns with current trends in immunomodulatory and plant-based drug development. Further studies focusing on its biological activity, pharmacodynamics, and safety profile will be crucial to establish Beta Glucan as a viable candidate for melanoma immunotherapy.

5. ACKNOWLEDGMENT

The authors would like to thank Dr. Vishwanath Karad MIT World Peace University, for providing all the necessary facilities.

6. REFERENCES

- 1. Roky A, Islam M, Ahasan A, Mostaq MS, Mahmud MZ, Amin MN, et al. Overview of skin cancer types and prevalence rates across continents. *CPT*. 2024;2: E01-E36.
- 2. Cui J, Li Y, Yang Y, Yang HK, Dong JM, Xiao ZH, et al. Tumor immunotherapy resistance: Revealing the mechanism of PD-1/PD-L1-mediated tumor immune escape. *Biomed Pharmacother*. 2024.
- 3. Boohaker RJ, Sambandam V, Segura I, Miller J, Suto M, Xu B. Rational design and development of a peptide inhibitor for the PD-1/PD-L1 interaction. *Cancer Lett.* 2018; 434; 11-21
- 4. Singh RP, Bhardwaj A. β-glucans: A potential source for maintaining gut microbiota and the immune system. *Front. Nutr.* 2023; 10
- 5. Alanzi A, Moussa AY, Mothana RA, Abbas M, Ali I. In silico exploration of PD-L1 binding compounds: Structure-based virtual screening, molecular docking, and MD simulation. *Plos one*. 2024; 19(8).
- 6. Ayodele PF, Bamigbade A, Bamigbade OO, Adeniyi IA, Tachin ES, Seweje AJ, et al. Illustrated procedure to perform molecular docking using PyRx and Biovia Discovery Studio Visualizer: a case study of 10kt with atropine. *PDDBS*. 2023;20;6(1).
- 7. Morris GM, Huey R, Olson AJ. Using AutoDock for ligand-receptor docking. *Curr Protoc Bioinformatics* 2008; (1):8-14.
- 8. Fernandes S, Sousa M, Martins FG, Simo es M, Sousa SF. Protocol for in silico characterization of natural-based

molecules as quorum-sensing inhibitors. *STAR protocols.* 2024;(4): 103367.

- 9. Forli S, Huey R, Pique ME, Sanner MF, Goodsell DS, Olson AJ. Computational protein-ligand docking and virtual drug screening with the AutoDock suite. *Nat. Protoc.* 2016; (5):905-19.
- 10. Yousuf Z, Iman K, Iftikhar N, Mirza MU. Structure-based virtual screening and molecular docking for the identification of potential multitargeted inhibitors against breast cancer. *BCTT*. 2017; 447-59.
- 11. El-Naggar AM, Abou-El-Regal MM, El-Metwally SA, Sherbiny FF, Eissa IH.

Synthesis, characterization and molecular docking studies of thiouracil derivatives as potent thymidylate synthase inhibitors and potential anticancer agents. *Mol. Divers.* 2017; (4): 967-83.

Bulletin of Pharmaceutical Research 2025;15(S2)

An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print); ISSN: 2249-9245 (Online)



RESEARCH ARTICLE

DEVELOPMENT, CHARACTERIZATION AND INVITRO EVALUATION OF ITRACONAZOLE AND FLUCONAZOLE NANOEMULGEL FOR TOPICAL DRUG DELIVERY

Anuradha G. More*, Dipali M. Kukde, Samarth D. Shinde, Tushar H. Kshirsagar, Padmaja S. Kore, Ujwala S. Desai

P.E. Society's Modern College of Pharmacy, Nigdi, Maharashtra, India-411044.

*Corresponding author email: anuradhagmore2011@gmail.com Received: Feb 06, 2025 / Revised: April 19, 2025 / Accepted: May 01, 2025

ABSTRACT

This study aims to design and assess a Nanoemulgel with the antifungal drugs itraconazole and fluconazole for local use. Nanoemulgels can efficiently increase drug solubility, penetration, and release, enhancing therapeutic performance in fungal infections. The combination of itraconazole and fluconazole was selected to provide broader-spectrum antifungal activity, as itraconazole is effective against dermatophytes and molds, while fluconazole targets Candida species; their combined use may also offer synergistic effects and reduce resistance development. Spontaneous titration techniques and Box-Behnken design were employed to maximize formulation parameters. The optimized formula showed a stable nanoemulsion with zeta potential -16.4 ± 2.16 mV, PDI 0.357 ± 0.84 , and particle size of 103.89 ± 2.80 nm. The resulting nanoemulgel had favourable topical characteristics, such as pH 6.3, a viscosity of 4260 CPs, and a spreadability of 6.1 cm/sec. Stability tests ensured formulation stability. This method provides a promising platform for enhanced topical antifungal delivery.

Key words: Itraconazole, Fluconazole, Nanoemulsion, Nanoemulgel, Topical drug delivery, Box-Behnken design

How to cite this article: Anuradha G. More, Dipali M. Kukde, Samarth D. Shinde, Tushar H. Kshirsagar, Padmaja S. Kore, Ujwala S. Desai. Development, Characterization and Invitro Evaluation of Itraconazole and Fluconazole Nanoemulgel for Topical Drug Delivery. Bulletin of Pharmaceutical Research. 2025;15(S2): 13-16.

Source of support: Nil Conflict of interest: None

1. INTRODUCTION

Magnetic Dermatophytosis and candidiasis need extended therapy, which is further plagued by low patient compliance because of the drawbacks of conventional topical drug delivery systems. Poor solubility, low permeability across skin, and inadequate drug concentration impede optimal therapy. Azole antifungals such as

itraconazole and fluconazole are extensively utilized because of their broad-spectrum activity but hampered by poor aqueous solubility[1]. Nanoemulgels, a hybrid of nanoemulsion and hydrogels, have emerged as novel delivery systems overcoming these limitations. They enhance drug solubility, permeation, and sustained release [2]. This research focuses on developing and characterizing nanoemulgel system for topical delivery

of itraconazole and fluconazole, employing quality-by-design (QbD) methodologies for optimization [3].

2. MATERIALS AND METHODS

2.1 Materials:

Itraconazole and fluconazole were purchased from Rubal Ingredients, India. Oleic acid, Tween 80, propylene glycol, and Carbopol 934 were excipients. All other reagents were of analytical grade. Delivery is developed, and multiple research reviews have been conducted [4]. With recent developments in liposome formulations and their medical applications, this analysis aims to provide a detailed insight into magnetic nanoliposome technology [5].

2.2 Screening of Excipients:

Solubility studies revealed oleic acid as the ideal oil, Tween 80 as the optimal surfactant, and propylene glycol as the ideal co-surfactant.

2.3 Nanoemulsion Formulation:

Spontaneous emulsification was used to prepare nanoemulsions. Ternary phase diagrams were developed to determine ideal component ratios. The Smix ratio chosen was 1:1 (Tween 80: propylene glycol), and formulations containing 10–40% oil were good for emulsification and clarity.

2.4 Hydrogel Preparation:

Hydrogels were prepared by using Carbopol 934 (0.5–2%). The maximum nanoemulsion was added to hydrogel bases, mixed thoroughly, and neutralized with triethanolamine. The formulation of 1% Carbopol (NE gel 2) was selected based on physical characteristics [4].

2.5 Box-Behnken Design

The Box-Behnken design, an RSM approach requiring three levels, was utilized to evaluate the effects of independent variables (X1: Oil concentration, X2: Smix concentration,

X3: Stirring time) on response variables (Y1: Particle size, Y2: PDI, Y3: Zeta potential). Ouadratic response surfaces and polynomial models were effectively generated. A total of 17 formulations were prepared and analyzed for the response variables. Analysis of variance (ANOVA) revealed that the models were statistically significant, with p-values less than 0.05 for all responses, indicating a strong correlation between the selected variables and the observed outcomes. The desirability function approach was emploved optimization, aiming to minimize particle size and PDI while maximizing the zeta potential stable range. The optimized formulation was selected based on the highest desirability score (close to 1), ensuring a balanced and efficient formulation that met all predefined criteria. Diagnostic plots such as predicted vs. actual and residual analysis further confirmed the adequacy of the models

2.6 Characterization of Nanoemulsion:

The optimized formulation had a particle size of 103.89 ± 2.80 nm, PDI of 0.357 ± 0.84 , and zeta potential of -16.4 ± 2.16 mV. These were good indicators of stability and narrow particle distribution.

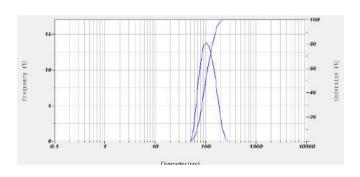


Fig no 1. Particle size of Nanoemulsion

2.7 Evaluation of Nanoemulgel: pH:

The ph of nanoemulgel was found to be 6.3, within the safe limits for the skin.

Batch code Sr. X1 (Amt. of oil) X2 (Amt. of Smix) X3 (Stirring Time) no. F1 F2 **F3** F4 F5 **F6 F7 F8** F9 F10 F11 F12 F13 F14 F15 F16 F17

Table no.1: Box Benken Design for Three Factors

Homogeneity & Grittiness:

The Homogeneity & Grittiness of formulated Nanoemulgel were found to be Smooth and shiny with no aggregates.

Viscosity:

The formulated Nanoemulgels viscosity was 4145±1.77 CPs at 10 RPM.

Spreadability:

The Spreadability of formulated Nanoemulgel was 6.1 cm/sec, showing easy spreading.

Stability Studies:

Optimized formulation (NE gel 2) was kept for accelerated stability studies at 25°C, 30°C, and 40°Cover 30 days. There were no essential changes in viscosity, spreadability, or consistency.

2.8 Permeability study:

The topical nanoemulgels in vitro skin penetration was evaluated using Franz diffusion cells. The donor compartment contained 5 ml of phosphate-buffered saline (PBS, pH 6.8), like the skin's pH, while the receptor compartment contained 20 ml.

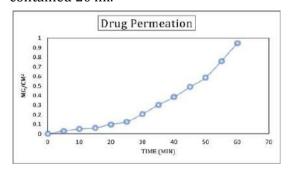


Fig no 2. In vitro release study

After the donor side had been coated with the nanoemulgel, a cellophane membrane was placed between the two compartments. The system was maintained at 37 ± 0.5 °C and agitated at 100 ± 5 RPM. At one-hour intervals, 1 mL aliquots were removed from the receptor compartment and analyzed using UV-visible spectrophotometry. The experiment was conducted three times.

3. RESULTS AND DISCUSSION

Solubility measurements validated oleic acid's higher solvent ability for both antifungals. The chosen Smix (1:1) yielded maximum emulsification as per ternary phase diagrams, which substantiated these choices.

According to the experimental design results,

Smix, and oil concentration significantly impacted zeta potential and particle size. A higher Smix helped stabilize the emulsion by increasing zeta potential and decreasing globule size. The optimized formula with 10% oil, 34.33% Smix (Tween 80 & Propylene glycol), and 12 minutes of stirring time showed a stable nanoemulsion with zeta potential -16.4 ± 2.16 mV, PDI 0.357 ± 0.84 . and particle size of 103.89 ±2.80 nm. The resulting nanoemulgel had favorable topical characteristics, such as pH 6.3, a viscosity of 4260 CPs, and a spreadability of 6.1 cm/sec. Stability tests ensured formulation stability. The last nanoemulgel possessed good topical formulation characteristics. The nano emulgel of fluconazole and itraconazole drug was successfully formulated and evaluated. Skin permeation studies demonstrated enhanced drug penetration through the skin compared to the conventional formulation. "Lack of grittiness, proper viscosity, and spreadability favored easy use. Stability studies confirmed long-term stability under different storage conditions.

4. CONCLUSION

The research successfully formulated and optimized a nanoemulgel with itraconazole and fluconazole for efficient topical

antifungal therapy. The synergistic effect with increased drug solubilization, penetration, and formulation stability underscores the delivery system's promise to provide a viable alternative to traditional topical therapies with enhanced therapeutic benefits and patient compliance.

5. REFERENCES

- 1. Sathyan G, Ritschel WA, Hussain AS. Transdermal delivery of tacrine. I. Identification of a suitable delivery vehicle. Int J Pharm, 114(1), 1995, 75–83.
- 2. Henmi T,Matsumoto M. Application of oily gel formed by hydrogenated soybean phospholipids as percutaneous absorption-type ointment base. Chem Pharm Bull, 42, 1994, 651–655.
- 3. Singh SK, Roane DS, Durrani MJ.Effect of additives on the diffusion of ketoprofen through human skin. Drug Dev Ind Pharm 22, 1996, 471–474.
- 4. Elnaggar YSR, et al. Sildenafil citrate nanoemulsion vs. self-nanoemulsifying delivery systems: rational development and transdermal permeation, Int. J. Nanotechnology., Vol. 8, Nos. 8/9, 2011; 749-763.
- 5. Mersini N, Poudel BK, Choi HG, Yong CS, Kim JO. Development and optimization of self-nanoemulsifying drug delivery system with enhanced bioavailability by Box-Behnken design and desirability function. J Pharm Sci.2012;101(12): 4584-4596.
- 6. Yadav P, Rastogi V, Verma A. Application of Box–Behnken design and desirability function in the development and optimization of self-nanoemulsifying drug delivery system for enhanced dissolution of ezetimibe. Future J Pharm Sci.2020; 6(1), 1-20
- 7. Yi Zhao, Chenguang Wanga, Albert H.L. Chow, Ying Zheng. Self-nanoemulsifying drug delivery system (SNEDDS) for oral delivery of Zedoary essential oil: Formulation and bioavailability studies, International Journal of Pharmaceutics 383 (2010) 170–177.

An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print); ISSN: 2249-9245 (Online)

RESEARCH ARTICLE

DEVELOPMENT AND CHARACTERIZATION OF HIBISCUS SABDARIFFA AND CAJANUS CAJAN BASED HERBAL GEL FOR TREATING ORAL ULCER

Kalyani Kakad*1, Vaishnavi Kate1, Shubham Dhanavade1, Pavan Navghare2, Saurav Gore2

¹Department of Pharmacognosy, P. E. Society's Modern College of Pharmacy, Nigdi, Pune Maharashtra- 411044. ²Department of Ouality assurance Technique, P. E. Society's Modern College of Pharmacy, Nigdi, Pune Maharashtra- 411044

*Corresponding author email: kk pharma20@rediffmail.com Received: Feb 04, 2025 / Revised: April 18, 2025 / Accepted: May 06, 2025

ABSTRACT

Mouth ulcer called aphthous stomatitis is the most common condition that gives ulcerative illness that influences oral mucosa. The goal of the current study was to evaluate the effectiveness of herbal remedies for treating mouth ulcers, F1 Formulation contained methanolic extracts of Hibiscus sabdariffa and clove oil with appropriate excipients. F2 formulation prepared by using methanolic extract of Hibiscus sabdariffa, Cajanus cajan, and clove oil, along with Poloxamer 407, Eudragit S100 and Carbopol 934 as gelling agents. The physical appearance, pH, homogeneity, viscosity, Spredability, gel strength and antibacterial activity of both formulations were assessed in comparison to the commercial Hi-Ora gel. Every formulation had a pH that was suitable for mucosal applications, ranging from 6.90 to 7.0 ± 0.9. Particularly antimicrobial activity was evaluated for formulation (F2), made good zones of inhibition, measuring 22 mm against Candida albicans, 28 mm against Escherichia coli, and 34 mm against Staphylococcus aureus as compared to the Hi-Ora gel. These outcomes were noticeably better. In conclusion F2 formulation showed good physical characteristics and antimicrobial activity.

Key words: Mouth ulcer, *Hibiscus sabdariffa, Cajanus cajan*, Clove oil, Herbal formulation

How to cite this article: Kalyani Kakad, Vaishnavi Kate, Shubham Dhanavade, Pavan Navghare, Saurav Gore. Development and Characterization of Hibiscus Sabdariffa and Cajanus Cajan based Herbal gel for treating Oral Ulcer. Bulletin of Pharmaceutical Research. 2025;15(S2): 17-21.

Source of support: Nil Conflict of interest: None

1. INTRODUCTION

and appearances like mucous extensively [4]. Several phytoconstituents are

Mouth ulcer or aphthous stomatitis is a kind of reported as antiulcerants which include meletin, ulcerative illness that influences the oral mucosa naringoside, silybin, anthocyanins, saponins, tannins, and is portrayed by repetitive oral and throat gums, mucilage [5,6]. Ethnobotanical evidence indicates ulcers [1]. It commonly has a yellow or white tone that a range of *Hibiscus sabdariffa* L. calyx herbal film powders and extracts have been used in holistic discouragement in the mouth. Various categories medicine to cure several ailments [7]. Diabetes, of ulcers can be found in oral cavity and toothaches, dizziness, stomachaches, chronic infections, categories based on lesions such as minor, major and feminine disorders are only a few of the conditions and herpetiform ulcers (Figure 1, 2 & 3) [2]. In for which Cajanus cajan is well known for its traditional indigenous medicine, various herbs are used to therapeutic use. Numerous advantageous nutritional treat aphthous ulcer, which are recommended to and bioactive components have been connected to cure serious complications [3]. Among herbal these qualities [8]. Clove essential oil has been drugs, clove oil, Cajanus cajan have been used demonstrated to have antimicrobial, antinociceptive, antibacterial, antifungal

Unlocking Horizons in Global Multidisciplinary Healthcare Research Indo-US International Conference Date: 10-12, March 2025 Organised by PES Modern college of Pharmacy Nigdi Pune-44

and anticancer qualities. But according to this study, clove oil, *Cajanus cajan* leaves, and Hibiscus sabdariffa L. calyx herbal powder may be beneficial for mucosal ulcer issues [9].







Figure 1: Minor ulcer

Linor ulcer Figure 2: Major ulcer

Figure 3: Herpetiform ulcer

Although *Hibiscus sabdariffa* and *Cajanus cajan* are both recognized for their pharmacological characteristics on their own, there has never been a report of these two botanical extracts being combined in a single mucosal gel formulation. By utilizing the complementary qualities of both plants, this creative method improves the therapeutic effectiveness

2. MATERIALS AND METHODS

2.1 Collection of plant materials

The calyx of plant Hibiscus sabdariffa L and leaves of Cajanus cajan were obtained from Manikarnika Aushadhalaya , Chinchwad, Pune-411033. Dried calx of *Hibiscus sabdariffa* L and leaves of *Cajanus cajan* were used for extraction (Figure 4& 5) [10,11]. Carbopol934, We purchased methylparaben, propyl paraben, triethanolamine, eudragit S100, propylene glycol 400, and poloxmer 407 from Loba.





Figure 4: Roselle Hibiscus sabdariffa

Figure: 5 Leaves, Flowers, fruits of Cajanus cajan

2.2 Preparation of plant extract

The desiccated calyx of *Hibiscus sabdarifa* and dried leaves of *Caganus cajan* were collected, cleaned and shade dried. Course powder of calyx of *Hibiscus sabdarifa* and leaves of *Cajanus cajan* were treated with methanol by cold maceration processes and further use for analysis [12].

2.3 Preparation of herbal gel

The composition of gel is formulated from the *Hibiscus sabdariffa* plant extract and *Cajanus*

cajan plant extract and clove oil [13]. Gel formulations with Carbopol 934 (Formula 1): Carbopol 934 is mixed with distilled water and propylene glycol to create a gel. Propylene glycol is added after cooling. The mixture is stirred and the extract is added. To adjust pH and achieve the desired consistency, distilled water and triethanolamine are added dropwise to the formulation until 50 ml. (Refer table 1 for composition of formulation 1). The gel formulations were prepared by mixing Carbopols 100 and poloxamer 407 ethanol, extract and Carbopol 934. The pH was adjusted by adding distilled water and triethanolamine. Carbopol 934 was dissolved in water, while methyl paraben, propyl paraben, propylene glycol, and eudragit were added to separate containers. ((Refer table 2 for composition of formulation 2)

2.4Evaluation parameter

Physical evaluation:

The prepared gels were tested for color, clarity, consistency, odor and presence of any gritty particles [12].

Measurement of pH:

A digital pH meter was used to measure the herbal gel composition's pH by following reported method. [14].

Homogeneity:

Tests for homogeneity were conducted on all created gel compositions. After the gel is placed into the container. Their appearance and existence of aggregates were examined [13].

Viscosity:

All of the developed formulations viscosities were examined by using spindle number 74 at 50 rpm at 25° C [15].

Gel strength:

The strength of a gel was determined by the time it took for a weight to penetrate 0.5 cm into it [13].

Spredability:

To prepare a gel formulation with Carbopol 934 and Eudragit S 100, dissolve Carbopol 934 in water, add propyl and methyl paraben, and propylene glycol. In separate containers, mix Eudragit S100 and poloxamer 407 in ethanol, add extract, and adjust pH and consistency by adding triethanolamine and distilled water^[15]. Spreading capacity: Spreadility is determined using the following formula S=M*L/T Where, M=Weight placed on upper slide L= Length of glass slide T=time taken to separate the slides.

Extrudability:

The gel formulations were filled into capped collapsible aluminium tubes and sealed securely. The ease of extrusion was assessed by pressing with the

Drug content:

A stock solution of formulated gel was prepared, and drug content was calculated using an algorithm derived from a linear regression study of a calibration curve^[15].

Clarity:

By using a visual assessment, the clarity of each batch was assessed [16]. Table 1: composition of herbal gel with Carbopol 934 (Formula 1)

Antimicrobial activity:

The study tested the antibacterial and antifungal properties of two formulations and a commercial mouth ulcer gel, Hi-ora gel, using diffusion agar-well technique and microbiological cultures[17,18]. The nutrient agar and Sabouraud dextrose agar plates were inoculated by spreading a volume of the bacterial and fungal inoculum respectively using a sterile glass spreader. Subsequently, wells were created with a diameter of 8 - 10 mm using sterilized cork borer. 0.25gm,0.5gm,1:10 ratio of gel, Standard marketed Hi-Ora gel and ciprofloxacin tablet suspension were incorporated into the wells. For bacteria, incubation was done for 24 Hrs. at 37°C and for fungi, at 30°C for 48 hours. The zone of inhibition was measured by calculating the the diameter in mm in three fixed directions and the mean values were recorded [19].

3. RESULT AND DISCUSSION

Two herbal gel formulations were created by altering herbal components as shown in Table 1 & 2. The well diffusion method was used to investigate antibacterial activity. The F2 gel, containing Hibiscus sabdariffa and Cajanus cajan extracts, demonstrated good zone of inhibition against Staphylococcus aureus, Escherichia coli, and Candida albicans. The result is shown in Table 5 and Figure 6,7,8). Extrudability was good as compare with marketed formulations (Table 3). Both formulations had good gel strength Physical appearance, pH, homogeneity, viscosity, and (Table 3).

Table 1. Composition of herbal gel F1

Sr	Ingredients	Role	Concen	Perce
no.			tration	
1	Hibiscus sabdariffa methanoli c extract	Antioxidant, anti- inflammatory, antibacterial	1 gm	2 %
2	Clove oil	Antimicrobial, anticarcinogenic, antiinflammatory, antioxidant	1 ml	1 %
5	Carbopol 934	Gelling agent	0.8 gm	2 %
4	Methyl paraben	Preservative	0.33 gm	1 %
5	Propyl paraben	Preservative	0.16 gm	1 %
6	Propylene glycol	Co-solvent	2 ml	2 %
7	Triethanolamine	PH adjuster	Up to 1 ml	1 %
8	Distilled water		Q. S	90%

Table 2. Composition of herbal gel F2

Sr. no	Ingredients	Role	Concent ration	%
1	Hibiscus sabdarifa (methanolic extract)	Antioxidant	1 gm	2 %
2	Cajanus cajan (methanolic extract)	Antiulcer	0.5 gm	1 %
3	Clove oil	Analgesic	0.5 ml	1 %
4	Carbopol 934	Gelling agent	0.8 gm	2%
5	Eudragit S100	Polymer	0.065gm	1%
6	Poloxamer 407	Stabilizer	0.065 gm	1%
7	Methyl paraben	Preservative	0.1 gm	0.2 %
8	Propyl paraben	Preservative	0.05 gm	0.02 %
9	Propylene E glycol	Co-solvent	1 ml	1%
10	Ethanol	Solvent	10 ml	20 %
11	Triethanol amine	pH adjuster	Up to 1 ml	1 %
12	Distilled water		Q. S	90%

compared to commercially available options. Spreadability were assessed. The gels had a smooth texture, pH (6.8-7.0), satisfactory homogeneity, and good viscosities and Spreadability. (Table 3).

Formulation	Color	odor	PH	Homogeneity	Viscosity (cps)	Spreadability (gm.cm/sec.)	Extrudability	Gel strength
F1	Greenish Yellow	Characteristics	7.0±0.9	Good	1070	28.98	Good	40 sec
F2	Greenish Yellow	Characteristics	6.89±0.3	Good	1296	28.5	Good	36 sec
Hi-Ora gel	Brownish	Characteristics	6.89±0.3	Good	1625	27.84	Good	34 sec

Table 3. Evaluation parameters of formulations

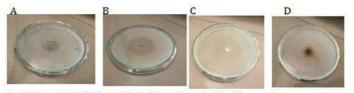
Table 4. Drug content comparison

Formulation	Name of Sample	Drug content
F1	Hibiscus sabdariffa	% 95.51
	methanoli c extract	70.01
	Clove oil	75.12
F2	Hibiscus sabdarifa (methanolic extract)	80.55
	Cajanus cajan (methanolic extract)	58.02
	Clove oil	60.53

A: 0.25 gm F2, B: 0.5gm F2, C: Ciprofloxacin, D: Hi-Oragel

Zone of Inhibition against Candida albicans

Fig, 6. Zone of inhibition against Candida albicans



A: 0.25 gm F2, B:0.5 gm F2, C: Ciprofloxacin, D: Hi-ora gel

Zone of inhibition agaist Staphylococus aureus

Table 5. Zone of inhibition against *Candida* albicans, *Staphylococcus aureus*, *Escherichia* coli

Formulation	(ZOI)against	(ZOI)agai	(ZOI)
	Candida	nst	again
	albicans	Staphyloc	st
		occus	Esche
		aureus	richia
			coli
0.25 gm F2	18 mm	25 mm	24
			mm
0.5gm F2	22 mm	34 mm	28
			mm
	38 mm	45 mm	48
Ciprofloxaci			mm
n			
	24 mm	23 mm	36
(Hi-Ora)			mm
(Hi-Ora)			mm

Fig. 7: Zone of inhibition against staphylococcus aureus

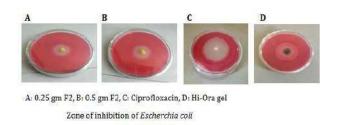


Fig. 8. Zone of inhibition against Escherichia coli

4. CONCLUSION

Herbal formulations are currently very popular on the market because to their low cost and lack of side effects. According to the experimental data above, a mucosal gel formulation with herbal substances such as *Hibiscus sabdariffa*, *Cajanus cajan*, and clove oil combinations has good characteristics, viscosity, and contains good antibacterial activity, which is crucial in the treatment of mouth ulcers.

5. REFERENCES

- 1. Kanchan U, Kirti C, Gouri D and Suparna B (2021). Formulation and evaluation of herbal gel for management of mouth ulcers. Ind. J. of Ph. And P'cology, 8(3): 226–230.
- 2. Shubham M, Ujjwal N (2019). A Review: Herbal Remedies Used for The Treatment of Mouth Ulcer. Int J of Health and Cli Res., 2019;2(1):17-23
- 3. Nahid K, Fatemeh N, Maryam J. S, Mahboobeh R (2023). Oral ulcerative disease: A systematic review of herbal medicine treatments. Journal of Herbal Medicine (40) 100681.
- 4.Pina-Pérez M.C, Ferrús P M.A. (2018). Antimicrobial potential of legume extracts against foodborne pathogens: A review. Trendsin Food Science & Technology. (72): 114-124
- 5. YogeshS. Thorat, Sarvagod,Shital V.Kulkarni, Avinash H. Hosmani. Treatment of Mouth ulcer by Curcumin loaded Thermoreversible Mucoadhesive gel. International Journal of Pharmacy andPharmaceuticalSciences.2015;7(10):39 9-402.
- F. Gricilda Shoba. A Review on Antiulcer Activity of Few Indian Medicinal Plants.International Journal of Microbiology.2014;1-14.
- 7. EfigeniaM.G, ZuamíV, Sughey G.T, Laura E. I-M., Mario A. I.E, José M.R.G, Ramón I.A.G, José LA, Napoleón G. S, Luis M.A.E,(2022).Physiological Effects and Human Health Benefits of *Hibiscus sabdariffa*: A Review of Clinical Trials. Pharmaceuticals (Basel) 15 (4): 464.
- 8. BabyG, Prabhakar S, Shabaaz , Sakshi P, Ashish T, Natália C. M. (2024). Revisiting the Nutritional, Chemical and Biological Potential of *Cajanus cajan* (L.) Millsp.Molecules2022,27(20), 6877.
- 9. Sabir S, Amol S and Rajendra D (2018).

 Formulation and Evaluation

 Pharmaceutical Aqueous Gel of Powdered

- Guava Leaves for Mouth Ulcer Treatment. Pharma tutor, 6(4): 32.
- 10.Syaifuzah Sapian, Fatin Farhana Jubaidi, NorAnizah Mohd Nor,Izatus Shima Taib (2013),1,*Therapeutic Potential of Hibiscus sabdariffa Linn. in Attenuating cardio vascular Risk Factor, Pharmaceuticals 2023,16(6), 807
- 11.Dilip kumar Pal, Pragya Mishra, Neetu Sachan, Ashoke K. Ghosh, Biological activities and medicinal properties of *Cajanus cajan* (L) Millsp. Journal of Advanced Pharmaceutical Technology Camp; Research, 2011, Vol2, Issue 4, pp207-214.
- 12.Richa S, Sagar B and Manoj M (2020). Formulation and Evaluation of Herbal Oral Gel Containing Extracts of Powdered Psidium guajava Linn Leaves with Curcuma longa Linn Rhizomes to Treat Mouth Ulcer. Int J Drug Dev C Res,12(2):150-157
- 13.Kalyani Tand Ameya L (2018). Formulation and Evaluation Pharmaceutical Aqueous Gel of Powdered *Cordia Dichotoma* Leaves with Guava Leaves. Am. J. Pharm Tech Res,8(2):268-277
- 14.Rajasekaran A, et al (2016). Formulation and evaluation of topical herbal gel forthe treatment of arthritis in animal model.
- 15.Vibha S (2021). Formulation and Evaluation of Pharmaceutical Polyherbal Mucosal Gel for Treatment of Mouth Ulcers Containing *Glycyrrhiza glabra, Aloe vera* and Curcumin. Quest Journals J. of Res. in Ph. Sci. 7(8): 1-13.
- 16.JapanP, Brijesh P, Hardeep Singh B, Kaushal Pand Manish P (2010) Formulation and Evaluation of Topical Aceclofenac Gel Using Different Gelling Agent. Int. J. Drug Dev. & Res.3(1):156-164
- 17. Abhijeet P, Jui J and Polshettiwar S (2010). Formulation and evaluation of in-vitro antimicrobial activity of gel containing essential oils and effect of polymer on their antimicrobial activity. Int. j. of p'ceuticalsci.3(1):234-237.
- 18.Jigna P and Sumitra C (2006). In-vitro Antimicrobial Activities of Extracts of *Launae aprocumbens* Roxb. (Labiateae), Vitis vinifera L. (Vitaceae) and *Cyperus rotundas* L. (Cyperaceous). African J. of Biomedical Res.9(1):89-93

An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print); ISSN: 2249-9245 (Online)



RESEARCH ARTICLE

FORMULATION AND EVALUATION OF DIRECTLY COMPRESSED POLYHERBAL ANTHELMINTIC TABLET

Anuradha G. More*, Amrut N. Godbharle, Avantika S. Jadhav, Monika L. Survawanshi, Padmaja S. Kore, Ujwala S. Desai

P.E. Society's Modern College of Pharmacy, Nigdi, Maharashtra, India-411044 *Corresponding author email: anuradhagmore2011@gmail.com
Received: Feb 11, 2025 / Revised: April 25, 2025 / Accepted: May 10, 2025

ABSTRACT

Helminth infections are a major global health issue, especially in developing nations. This study develops a polyherbal anthelmintic tablet using Priyangu seed and Puskara bark extracts to combat earthworms (Pheretima posthuma), mimicking human intestinal parasites. Extracts were prepared via cold maceration and analyzed for phytochemicals and anthelmintic properties. Conventional drugs like albendazole face resistance and side effects, while nanotechnology-integrated herbal extracts offer a safer alternative. Green synthesis method was used to create the silver nanoparticles, which were then examined using XRD, DLS, and SEM. Tablets were formulated by direct compression and assessed for quality. The combined herbal extracts showed enhanced efficacy, with silver nanoparticles further improving performance (paralysis: 55±14 min, death: 70±25 min) over albendazole (paralysis: 180±24 min, death: 217±35 min). Silver nanoparticles (189±2 nm, -19.1±0.2 mV) improved stability, and the tablets exhibited good physical characteristics, highlighting their potential as an effective herbal anthelmintic formulation.

Key words: Priyangu, Puskara Helminth infections, Polyherbal formulation, Silver nanoparticles, Anthelmintic activity, Pheretima posthuma.

How to cite this article: Anuradha G. More, Amrut N. Godbharle, Avantika S. Jadhav, Monika L. Suryawanshi, Padmaja S. Kore, Ujwala S. Desai. Formulation And Evaluation of Directly Compressed Polyherbal Anthelmintic Tablet. Bulletin of Pharmaceutical Research. 2025;15(S2): 22-27.

Source of support: Nil Conflict of interest: None

1. INTRODUCTION

Since they have fewer side effects, herbs and herbal treatment mixtures have been used extensively for 1,000 years industrialised and developing countries[1]. Herbal medicines have been essential in traditional healthcare for centuries. The WHO reports that nearly 80% of the global population relies on them as a primary healthcare source due to their affordability and minimal side effects [2,3,4]. Helminthiasis. caused by parasitic worms, affects the gut and can spread to organs like the liver and lungs. Symptoms range from digestive issues to severe complications. Though treated with

albendazole and mebendazole, prolonged use has led to resistance^[5]. Nanotechnology delivery, with enhances drug nanoparticles offering strong antimicrobial and antiparasitic properties. This study formulates and evaluates a polyherbal anthelmintic tablet incorporating silver nanoparticles to boost herbal extract bioactivity^[6]. Although the usage of harmful substances limits their applicability, physical and chemical approaches are more frequently used to synthesize nanoparticles. Due to this issue, secure, eco-friendly green techniques are essential for creating nanoparticles [7].

2. MATERIAL AND METHOD

2.1 Plant Materials and Extract Preparation:

Seeds of *Priyangu* (*Callicarpa macrophylla*) and bark of *Puskara* (*Inula racemosa Hook*), Microcrystalline cellulose (MCC), Lactose, Cross carmellose, PVP-k 30, Aerosil 200, Talc, magnesium stearate, silver nitrate (AgNO₃) were purchased from Manikarnika Aushadhalaya, Pune.. Indian adult earthworms (*Pheretima posthuma*), obtained from Indo-American Hybrid Seeds [8.9]. Extract wass prepared by aqueous extraction method [10].

2.2 Synthesis of silver nanoparticles:

Silver nanoparticles are prepared by the greesynthesis bio-reduction method [11]. The silver nitrate was dissolved in double-distilled water to create a fresh $AgNO_3$ solution. For green synthesis, plant extract (10 ml) was mixed with $AgNO_3$ solution (90 ml) under constant stirring, turning reddish-brown, indicating nanoparticle formation. After 25–30 minutes, the solution was incubated for 24 hours, then centrifuged at 20,000 rpm for 20 minutes (repeated thrice) to isolate the nanoparticles.

2.3 Optimization of silver nanoparticles

The parameters optimized for the fastest and most economical synthesis of AgNPs were time, pH, temperature, silver nitrate concentration, and plant extract. The pH was

optimized using 0.1 N sodium hydroxide (NaOH) and 0.1 N hydrogen chloride (HCl) and kept at 1, 3, 5, 7, 9, and 11. For the best AgNP production, the reaction was observed from 0 to 60 minutes. Temperatures of 25, 50, 75, and 100° C were used to monitor the synthesis of silver nanoparticles. The different concentrations for optimising AgNO₃ were 0.50, 1.0, 1.5, and 2.0

2.4 Characterization of polyherbal silver nanoparticles

Polvherbal silver nanoparticles were characterized changes through color indicating formation. UV-Vis spectroscopy (200-500 nm) monitored silver ion reduction, while FTIR (4000-500 cm⁻¹) analyzed functional groups. SEM determined size, shape, and morphology, and XRD assessed crystallinity (2-theta: 10°-70°). instruments measured size distribution, stability, DLS, and zeta potential^[12].

2.5 Preparation of Tablet

Polyherbal silver nanoparticle tablets were prepared using the direct compression method. The excipients included microcrystalline cellulose (MCC) as a binder, cross carmellose as a disintegrant, PVP-K30 as a polymer, and magnesium stearate as a lubricant [13]

Table 1. The formula for	the herba	l silver nano	particle tablet.
--------------------------	-----------	---------------	------------------

Sr. No	Excipient	Quantity(mg)				
		F1	F2	F3	F4	F5
1	Polyherbal silver nanoparticle	14.95	14.95	14.95	14.95	14.95
2	Microcrystallinecellulose (MCC)	250	0	125	150	200
3	Lactose	0	250	125	100	50
4	Cross carmellose	50	50	50	50	50
5	PVP-K 30	10	10	10	10	10
6	Aerosil 200	8	8	8	8	8
7	Talc	8	8	8	8	8
8	Magnesium stearate	5	5	5	5	5

2.6 Evaluation of powder blend for precompression parameter

The pre-compression metrics, such as bulk density and real density, are comparable to

physical qualities. The outputs obtained were really drift angles, Hausner's Ratio, and Carr's Compressibility Index [14].

2.7 Evaluation of polyherbal silver nanoparticle Tablet

*More et al*The physical appearance of 20 tablets was assessed for surface roughness and discoloration. Weight variation was checked, ensuring deviations stayed within ±5%. Hardness was measured as the stress needed to break a tablet, while thickness was evaluated using a Vernier caliper. Friability was tested using a Roche friabilator at 25 rpm for 4 % friability was minutes. and calculated. Disintegration testing determined the time required for tablets to break down for drug absorption.

Antibacterial activity

The antibacterial activity were estimated using the agar well technique. Using sterile forceps, the treated product sample was placed on top of a pure bacterial culture that had been equally distributed on petri plates. For 18 to 24 hours, the plates were incubated at 36 °C, which is ideal for bacterial growth.

Anthelmintic activity of Herbal extract

The anthelmintic efficacy was tested on Pheretima posthuma, an earthworm species withphysiological similarities to human intestinal worms. And then worms were exposed to various formulations, and the time to death and paralysis was recorded. As a positive control, albendazole was used at the concentration of 10 mg/ml [17].

Accelerated stability study for Tablet

Accelerated stability studies were conducted for 30 days under controlled temperature and humidity conditions (25±2°C/60±5% RH and 40±2°C/75±5% RH) to assess formulation robustness.

2.8 Evaluation of powder blend for compression parameter

The pre-compression metrics, such as bulk density and real density, are comparable to physical qualities. The outputs obtained were really drift angles, Hausner's Ratio. and Carr's Compressibility Index [14].

Evaluation of polyherbal silver nanoparticle 2.9 **Tablet**

The physical appearance of 20 tablets was assessed for surface roughness and discoloration. Weight variation was checked, ensuring deviations stayed within ±5%. Hardness was measured as the stress needed to break a tablet, while thickness was evaluated using a Vernier caliper. Friability was tested using a Roche friabilator at 25 rpm for 4 minutes. and % friability was calculated. Disintegration testing determined the time required for tablets to break down for drug absorption.

Bull. Pharm. Res. 2025;15(S2) Antibacterial activity

The antibacterial activity were estimated using the agar well technique. Using sterile forceps, the treated product sample was placed on top of a pure bacterial culture that had been equally distributed on petri plates. For 18 to 24 hours, the plates were incubated at 36 °C, which is ideal for bacterial growth.

Anthelmintic activity of Herbal extract

anthelmintic efficacy was tested Pheretima posthuma, an earthworm species with physiological similarities to human intestinal worms. And then worms were exposed to various formulations, and the time to death and paralysis was recorded. As a positive control, albendazole was used at the concentration of 10 mg/ml [17].

Accelerated stability study for Tablet

Accelerated stability studies were conducted for 30 days under controlled temperature and humidity conditions (25±2°C/60±5% RH and 40±2°C/75±5% RH) to assess formulation robustness.

Statistical analysis

The results were expressed as mean \pm SEM and analyzed by one-way ANOVA

3. RESULT AND DISCUSSION

Phyto-chemical investigation of Extract

The extracts showed a rich phytochemical profile, confirming the presence of alkaloids, glycosides, saponins and tannins, each of them having their own role in imparting the anthelmintic property. While Sariva, Bhurjaha, and Prishnaparni lack tannins, Mayyaku and Medaseka contain all five compounds. Priyangu is missing flavonoids, whereas Puskara lacks flavonoids but has tannins. Vidarikanda is unique in having all compounds except tannins. This phytochemical composition highlights their medicinal potential in traditional and modern healing practices.

Characterization of polyherbal silver nanoparticles

image confirmed SEM the uniform distribution of nanoparticles, while DLS analysis indicated excellent stability. The zeta potential analysis suggested strong repulsive forces preventing aggregation

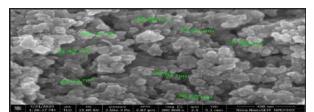


Fig 1. Scanning Electron Microscopy analysis of Silver nanoparticles

Dynamic light scattering and Zeta potential of synthesized nanoparticle

Fig. 2 depicts DLS particle size distribution image of bio-synthesized silver nanoparticles, AgNPs, and zeta potential of the synthesized nanoparticles. The average size was found to be 189.0±2 nm with the zeta potential being -19.1±0.2 mV.

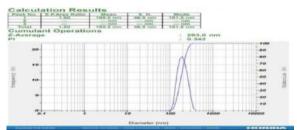
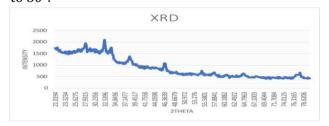


Fig 2. Dynamic light scattering Particle size XRD (X-ray diffraction)

X-ray diffraction (XRD) analysis of silver nanoparticles was utilized through Philips (PW 3710) Expert Pro MPD Diffractometer. The sample was prepared by sprinkling it on vacuum grease on a glass slide, forming a 0.5 mm thick layer. A Copper target tube was used for radiation, with scanning angles ranging from 20° to 80°.



Bull. Pharm. Res. 2025;15(S2) Fig 3. X-ray diffraction graph of silver nanoparticles

A colour shift upon visual observation verified the creation of silver nanoparticles. Within 20 minutes, the mixture changed from brownish yellow to dark brown as a result of surface plasmon resonance effects and silver nitrate reduction^[18].

UV Analysis

The UV spectrum confirmed the presence of AgNO and key components in the synthesized silver nanoparticles, showing a maximum absorption peak at 275 nm, indicating surface plasmon resonance.

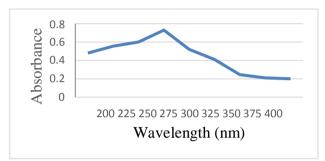


Fig.4. UV Spectra of synthesized nanoparticles Optimization of Tablet

The production of the tablets was based on the direct compression technique. The formula for the solitary tablet per batch required to prepare 350 mg of tablet is given in Table 1 .The optimization was done by using different batches. The F5 batch was found to be the optimized batch for formulation.

Evaluation of silver nanoparticle tablet

Among different formulations, batch F5 showed optimal characteristics with hardness (5.2 ± 0.5 kg/cm²), friability ($0.75\pm0.26\%$), and disintegration time (10 ± 0.3 min).

Table 2. Eva	luation of	silver	nanoparti	cle ta	blet
--------------	------------	--------	-----------	--------	------

Sr. No	Parameter	F1	F2	F3	F4	F5
1	Hardness (Kg/cm²)	1.5 ± 1.0	2.7±1.2	4.0±0.80	4.5± 1.0	5.2 ± 0.50
2	Friability (%)	1.5± 0.7	1.4±0.4	1.0± 0.3	1.0± 0.4	0.75± 0.26
3	Weight Variation (mg)	335 ± 5.0	335±6.0	336± 8.0	337± 8.0	337 ± 7.0
4	Thickness (mm)	178 ± 7.0	180± 4.0	182±7.0	181± 3.0	180 ± 5.0
5	Disintegration time	5 ± 0.1	7 ± 0.1	8 ± 0.5	8.0± 0.2	10 ± 0.30
6.	% drug release in 60 min	65± 0.21	70± 0.23	63± 0.17	78± 0.17	85±0.42

Antibacterial Activity

Several herbs were examined for their antibacterial properties against gram-positive and Streptococcus aureus gram-negative Pseudomonas aeruginosa. With a zone of 0.8 cm against P. aeruginosa and 0.1 cm against S. aureus. Krishna Sariva demonstrated very little inhibition. With inhibition zones of 1.6 cm and 1.5 cm, respectively, Priyangu showed notable activity. Puskara demonstrated 1.1 cm inhibition against P. aeruginosa and 1.5 cm inhibition against S. aureus. Prishnaparni and Mayphal showed minimal inhibition (0.3 cm) against P. aeruginosa but no effect on S. aureus. Medaseka showed 0.1 cm inhibition against P. aeruginosa and 1.2 cm inhibition against S. aureus.









a b c d
Fig 5. The antibacterial activity against
Streptococcus Aureus (gram-positive) a)
Krishna Sariva & Bhurjaha b) Vidarikanda &
Priyangu c) Puskara & Mayphal d)
Prishnaparni & Medaseka









Fig. 6. The antibacterial activity against Pseudomonas aeruginosa (gram Negative) a) Krishna Sariva & Bhurjaha b) Vidarikanda & Priyangu c) Puskara & Mayphal d) Prishnaparni & Medaseka

Anthelmintic activity of herbal extract

The anthelmintic activity was tested on *Phertima posthuma* due to its similarity to human intestinal roundworms^[19]. At 10 mg/ml, Priyangu and Puskara paralyzed and killed worms in 78±2 min & 98±2 min, and 90±1 min & 130±2 min, respectively. Their combination showed a synergistic effect, reducing paralysis and death times to 65±22 min & 85±34 min. Albendazole, the reference standard, took 180±24 min & 217±35 min. Polyherbal silver nanoparticles exhibited superior efficacy, significantly reducing paralysis and death times. In comparison to albendazole, the silver nanoparticles exhibits superior anthelmintic activity









Fig.7. Anthelmintic activity of test and standard albendazole against *Pheretima Posthuma*. a) Priyangu + Puskara b) Albendazole c) Silver nanoparticle d) Silver nanoparticle tablet

Accelerated stability study:

A stability study of Formulation F5 was conducted for 30 days in two different conditions: room temperature (25° C 2° C/RH $60\pm5\%$) and accelerated temperature (40° C 2° C/RH $75\pm5\%$). The stability of the polyherbal silver nanoparticle tablets was shown in the results table. Throughout the course of the trial, the tablets maintained their integrity, mechanical strength, and bioactivity, indicating formulation stability.

Table 3. Accelerated stability study

Parameter	7 th day	15th day	30th day					
At room temperature (25°C ± 2°C/RH 60 ± 5%).								
Hardness(Kg/cm ²)	5.2 ± 0.32	5.1 ± 0.32	5.2 ±0.32					
Friability (%)	0.65 ± 0.7	0.40 ± 0.4	0.50 ± 0.3					
Disintegration(Minutes)	10 ± 0.5	11 ± 0.3	10 ± 0.1					
At Accelerated tempera	ture (40°C ± 2°C/RH 75	5 ± 5%)						
Hardness(Kg/cm ²)	5.1±0.32	5.1±0.32	5.2±0.32					
Friability(%)	0.50 ± 0.7	0.40 ± 0.4	0.45± 0.3					
Disintegration(Minutes)	10 ± 0.5	11 ± 0.3	10 ± 0.1					

4. CONCLUSION

It was discovered that the polyherbal silver nanoparticle formulation exhibited superior activity and stability in comparison to the traditional albendazole tablets. The research indicates great potential for improving treatment efficacy by fusing herbal medicine and nanotechnology. At a concentration of 10 mg/ml, the earthworms became sensitive and showed signs of paralysis, which progressed to death as they lost their ability to move and responded to outside stimuli. When the results were contrasted with those of the usual medication albendazole, it was discovered that the formulation had superior activity.

5. ACKNOWLEDGMENT

The Authors thank the Principal of Modern college of pharmacy, Pune for providing facility support for the entire project work.

6. REFERENCES

- 1. U.K. Gilhotra, G. Mohan, A. Christina, Antilithiatic activity of poly-herbal formulation tablets by invitro method, J. Appl. Pharm. Sci. 3 (5) (2013) 43.
- 2. Mukherjee PK, Verpoorate Rober. GMP for Botanicals and Quality Issues on Phytomedicines. 1st ed. Business Horizons; 2003:152.
- 3. Chaudhri RD. Herbal Drugs Industry. 1st ed. The Eastern Publishers; 1996:1e3.
- 4. Patwardhan B, Hoper ML. Ayurveda and future drug development. Int J Altern Complement Med; 1992:9e11.
- 5. Girme AS, Bhalke RD, Ghogare PB, Tambe VD. Comparative *In vitro* anthelmintic activity of *Menta Piperita* and *Lantana camara* from western India, Dhaka University. J. Pharm. Sci. 2006 June-December; 5(1-2):5-7.
- 6. Gopalakrishnan K, Ramesh C, Ragunathan V, Thamilselvan M. Antibacterial activity of Cu2O nanoparticles on E. coli synthesized from Tridax procumbens leaf extract and surface coating with polyaniline. Dig J Nanomater Bios. 2012;7(2):833–9.

- 7. Rajathi FAA, Parthiban C, Kumar VG, Anantharaman P. Biosynthesis of antibacterial gold nanoparticles using brown alga. Spectrochim Acta A Mol BiomolSpectrosc. 2012;99:166–73.
 - 8. Thorn GW, Adams RD, Braunwald E, Isselbacher KJ and Petersdrof RG. Harrison's Principles of Internal Medicine. In: Mcgraw Hill Co., New York: 1977; 1088-1089.
 - 9. Vigar Z. Atlas of Medical Parasitology. In: 2nd ed. P.G. Publishing House, Singapore: 1984; 216-217.
 - 10. Khandelwal, K.R., Practical Pharmacognosy. 2008: Pragati Books Pvt. Ltd.
 - 11. Strobel, R.; Pratsinis, S.E. Direct Synthesis of Maghemite, Magnetite and Wustite Nanoparticles by Flame Spray Pyrolysis. *Adv. Powder Technol.* **2009**, *20*, 190–194. [CrossRef]
 - 12. Welis JI, Aulton ME. Reformulation. In: Aulton ME, ed. Pharmaceutics: The Sciences of Dosage Form Design. Britain: Churchill Living Stone; 1985:247.
 - 13. M. Modasiya et al., Design and characterization of fast disintegrating tablets of piroxicam, Int. J. PharmTech. Res. 1 (2) (2009) 353–357.
 - 14. C. Anand, Optimization of granule size and disintegrants on formulation of rapid dispersible tablets of tolfenamic acid, J. Drug Deliv. Therap. 3 (2) (2013).
 - 15. Ahmed H, Shah N. Formulation of low dose medicines e theory and practice. Pharmacol Rev. 2000;3:9e14.
 - 16. Ananda Lakshmi K, Venugobal J, Ramasamy V. Characterization of silver nanoparticles by green synthesis method using *Pedalium murex* leaf extract and their antibacterial activity. Appl Nanosci. 2016;6:399–408.
- 17. Kushwaha A.* and Aind J.G Anthelmintic activity of polyherbal preparation international journal of pharmacy & life sciences 1(1):35-37 May, 2010.
- 18. Mulvaney P. Surface plasmonspectroscopy of nanosized metal particles. Langmuir. 1996;12(3):788-800

An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print); ISSN: 2249-9245 (Online)



RESEARCH ARTICLE

HERBAL ANTI-MICROBIAL TOPICAL FORMULATION FOR FUNGAL INFECTION

Padmaja Kore^{1*}, Ujwala Desai², Anuradha More², Soham Sanjay Jadhav¹, Anuj Vivek Borhade¹, Deepak Firoj Khatri², Atharva Ajay Namade²

1*Department of Pharmacology, Progressive Education Society's Modern College of Pharmacy, Nigdi, Pune-44

² Department of Pharmaceutics, Progressive Education Society's Modern College of Pharmacy, Nigdi, Pune-44

*Corresponding author email: padmaja.kalshetti@gmail.com Received: Feb 09, 2025 / Revised: April 18, 2025 / Accepted: May 07, 2025

ABSTRACT

The herbal topical formulation was developed to combat fungal infection like athlete foot and ring worm. The ointment was made up of neem oil, camphor oil and garlic powder in specific proportion. In testing it was effective against *candida albicans* the common fungus. The parameter scanned were pH, viscosity, spreadability and in vitro drug release of formulation. Based on result the herbal ointment was the formulation to treat fungal infection due to its anti-fungal, anti-microbial, anti-irritant properties.

Key words: Anti-fungal, Camphor, Garlic, Neem, Topical formulation

How to cite this article: Padmaja Kore, Ujwala Desai, Anuradha More, Soham Sanjay Jadhav, Anuj Vivek Borhade, Deepak Firoj Khatri, Atharva Ajay Namade. Herbal Anti-Microbial Topical Formulation for Fungal Infection. Bulletin of Pharmaceutical Research. 2025;15(S2): 28-33.

Source of support: Nil Conflict of interest: None

1. INTRODUCTION

Different types of fungi cause fungal infections. In some cases, fungi that are not typically found on or inside the body colonize it and cause an infection. In other instances, fungi on or inside the body multiply out of control and cause an infection. Fungal infections are contagious and spread from one person to another [1, 2]. In some cases, disease-causing fungi are transmitted from infected

animals, contaminated soil, or surfaces. Tinea is the name of a group of diseases caused by a fungus. These infections occur on the scalp, arms, legs, and face and are characterized by ring-shaped, red, scaly patches with clearing centres. Various types of Tinea include ringworm, athlete's foot, and jock itch. Tinea pedis, or athlete's foot, is a common fungal infection that affects the foot [3]. Athlete's foot is commonly associated with sports and

athletes because the fungus thrives in warm, moist environments, such as socks, shoes, and sports equipment. However, anyone can be affected by athlete's foot [4]. Over-the-counter creams and powders effectively treat many tinea infections, particularly athlete's foot and jock itch. Conventional treatment of fungal infections involves chronic treatment with oral antifungal drugs [5].

Herbal compositions have gained a lot of importance in recent years due to their minimal or no side effects compared to synthetic drugs. Many herbal-based creams and ointments are available for treating fungal infections [5, 6]. This research aims to create an ointment with good consistency, diffusion, antifungal, and antiseptic qualities using several ointment bases. Spreadability, permeability, drug release, homogeneity, viscosity, diffusivity, stability, and other physical parameters are assessed to evaluate the performance of the formulations Clove (Eugenia caryophyllus) and orange-peel oil for treating fungal infections such as athlete's foot [7]. A herbal topical formulation containing neem oil, garlic powder, camphor oil, and pharmaceutically acceptable excipients for managing fungal infections like Tinea infections [7,8].

2. MATERIAL AND METHOD

Neem oil, garlic powder, camphor oil has been purchased from the local pharmacy.

2.1 Screening of oils using antimicrobial activity:

16.5 gm of saubouraud dextrose agar (SDA) was dissolved in 250 ml of water. The agar media and all the materials were sterilized in an autoclave for half an hour. Then, the floor was cleaned using ethanol, and the antimicrobial activity was performed in a sterile area. The agar media was poured into petri plates and allowed to cool and solidify. On the surface of the agar plates, a *Candida albicans* suspension was spreaded using a spreader. Finally, the neem oil, camphor oil and suspension of garlic powder was added

to the bore, and it was incubated for 24 hours [3, 5].

2.2 Screening of ratio of oils using antimicrobial activity:

Formulation with different ratios of neem oil, garlic powder and camphor oil were prepared, and the optimized formulation was selected based on zone of Inhibition as indicated in Table 1, 2, 3.

Table 1: Ratio of oils incorporated in the formulation

Batch	Ratio (Garlic:Camphor:Neem)
F1	1:1:0.5
F2	2:2:1
F3	2:1:1
F4	1:3:1

Table 2: Quantity of oils incorporated in base

Batches	Neem	Camphor	Garlic
B1	0.25 ml	0.75 ml	0.25 ml
B2	0.5 ml	1.5 ml	0.5 ml
В3	0.75 ml	2.25 ml	0.75 ml
B4	1 ml	3 ml	1 ml

2.3 Method Preparation of Herbal Topical Formulation:

The herbal topical formulation by fusion of melting method. 0.5 gm of hard paraffin, 8.5 gm of soft paraffin (Vaseline), 0.5 gm of Wool fat (Lanoline) and 0.5 gm of Cetostearyl alcohol (1-hexadecanol) was melted in decreasing order of their melting point. Neem oil, garlic powder and camphor oil were added in the ratio 1:1:3 to the molten base. Stir the formulation thoroughly until it cools down and homogeneous product is obtained as ointment [8].

Table 3: Composition of base

rubic 3. composition of buse						
Formula	Quantity	Property				
Hard Paraffin	0.5 gm	Thickening agent				
White Soft	8.5 gm	Lubricating				
Paraffin		agent, Diluent				
(Vaseline)						
Wool Fats	0.5 gm	Enhance				
(Lanoline)		Absorption,				
		Carrier				
Cetostearyl	0.5 gm	Emulsifier				
alcohol						
(1-hexadecanol)						

2.4 Physical Evaluation of Herbal Topical Ointment:

Consistency and Appearance:

The consistency and appearance was observed visually for the solution.

pH:

About 1 g of the sample is mixed with 9 ml of distilled water to prepare a 10% solution, it was stirred for uniformity. A pH electrode is inserted into the solution, and once the reading stabilizes, the pH value is recorded [9,10].

Viscosity:

A specific amount (50–100 ml) of the sample is placed in a beaker and left to stand to remove air bubbles. The correct spindle is selected and immersed in the sample without touching the bottom. The viscometer is set to a specific speed (e.g., 10, 20, or 50 rpm), and once the reading stabilizes, the viscosity is recorded [11].

Spreadability:

A small sample (about 1 g) is placed between two glass slides. A standard weight (e.g., 100 g) is applied for 1–2 minutes to allow the formulation to spread. After removing the weight, the spreaded area is measured using a scale or Vernier calliper. The diameter of the spread is recorded, and spreadability is calculated using a specific formula [12, 13].

$S=M\times L/T$

S = Spreadability (g cm/sec)

M = Applied weight (g)

L = Spreaded diameter (cm)

T = Time taken (sec).

The test was repeated three times, and the average spreadability value was calculated to ensure accuracy.

In Vitro Drug Release Study

A phosphate buffer solution was prepared by adding 3.5 g of potassium diphosphate and 8.75 g of disodium hydrogen phosphate. After mixing, a skin pH (4.1 - 5.8) buffer was obtained. A cellophane membrane was placed on the Franz diffusion apparatus, and

the ointment was evenly spread. Then it was stirred using a magnetic stirrer for 8 hours. The desired solution was poured into an SDA petri plate every hour for up to 6 hours [14,15].

3. RESULTS AND DISCUSSION

All three oils showed antifungal activity against *Candida albicans* (Table 4). Based on the zone of inhibition results in Table 5 below, Formulation (F4) is Neem oil, Garlic powder, and camphor oil in the ratio 1:1:3.

Table 4: Zone of Inhibition of each oil and the mixture

Oils	Zone of				
	Inhibition(cm)				
Azadirachta indica	2.08 ± 0.20				
Allium sativum powder	2.3 ± 0.33				
Cinnamomum	3.0 ± 0.32				
camphora					
Oil mixure	3.4 ±0.18				

Table 5: Zone of inhibition combined oils in different ratios

Batch	Ratio (Garlic: Camphor: Neem)	Zone of Inhibition(cm)
F1	1:1:0.5	2.72 ± 0.22
F2	2:2:1	3.12 ± 0.18
F3	2:1:1	2.93 ± 0.17
F4	1:3:1	3.34 ± 0.23

Physical Evaluation of Herbal Topical Ointment:

Table 6: Physical Evaluation of Herbal Topical Ointment

Sr.No	.No Formulation Consistency		Appearance
1	B1	Greasy	Yellowish
2	B2	Greasy	Yellowish
3	В3	Greasy	Yellowish
4	B4	Greasy	Yellowish

All four batches showed greasy appearance with yellowish colour (table 6).

Table 7: pH value of different batches

Batches	B1	B2	B3	B4
рН	4.7± 0.41	5.0± 0.16	5.5± 0.24	
				0.26

All four batches had pH in the range of skin pH thus did not cause irritation to the skin when applied (Table 7).

Table 8: Spreadability of different Batches

Sr no	no Formulation Spreadability (gm.cm/	
1	B1	10.7± 0.12
2	B2	11± 0.17
3	В3	11.53± 0.22

All 3 batches showed good spreading ability indicating greater patient compliance (Table 8).

Table 9: Viscosity of different batches

Sr no	RPM	B1	B2	В3
1	20	4990 - 5200 cps	5110 - 5980 cps	5220 - 6000cps
2	30	4010 - 5343 cps	3219 - 3980 cps	3413 - 4230 cps

All 3 batches showed viscosity in the range 3600 to 6000 cps indicating good consistency (Table 9).

Table 10: Antimicrobial activity of different batches

Batch	Neem	Camphor	Garlic	Zone of inhibition (cm)
B1	0.25 ml	0.75 ml	0.25 ml	1.450± 0.165
B2	0.5 ml	1.5 ml	0.5 ml	1.650± 0.187
В3	0.75 ml	2.25 ml	0.75 ml	1.675± 0.194
B4	1 ml	3 ml	1 ml	1.575± 0.177

The results in Table 10 show that Batch B3 shows the maximum zone of inhibition. Batch B3 was further evaluated for an in vitro drug release study, and the results are depicted in Table 11 and Figure 1.

Table 11: In vitro drug release study

Time (Hr)	1	2	3	4	5	6	7
Zone of Inhibi tion (cm)	1.52 3 ± 0.23	1.42 1 ± 0.31	1.27 3 ± 0.20	0.81 2 ± 0.42	0.61 3 ± 0.32	0.42 4± 0.30	0.3 18± 0.2 7

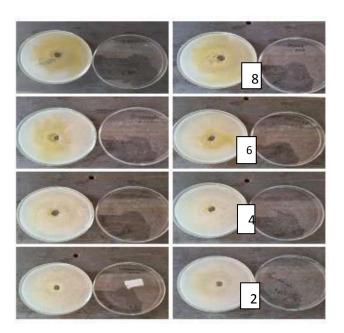


Figure 1: Antimicrobial activity of optimized formulations at different time intervals

3.1 DISCUSSION

The results of this study highlight the potential of herbal topical formulation containing neem oil, garlic powder, and camphor oil in treating fungal infections, particularly Tinea infections such as athlete's foot and ringworm. The zone of inhibition analysis confirmed the antimicrobial efficacy of the formulation, with the optimized ratio of 1:3:1 (Garlic: Camphor: Neem) demonstrating the largest inhibition zone (3.34 \pm 0.23 cm) among the tested formulations. This suggests a synergistic effect of the selected herbal ingredients^[16].

Neem oil (*Azadirachta indica*), known for its antifungal and anti-inflammatory properties, exhibited a zone of inhibition of 2.08 ± 0.20

cm when tested individually. Garlic powder with (Allium sativum), its known antimicrobial activity due to allicin content, showed a slightly higher inhibition zone of 2.3 ± 0.33 cm. Camphor oil (Cinnamomum camphora) demonstrated the significant antifungal activity individually, with a 3.0 ± 0.32 cm inhibition zone. Combining these three oils further enhanced antimicrobial activity, achieving a 3.4 ± 0.18 cm inhibition zone, which is greater than the individual effects, indicating possible synergism.

Physical evaluation of the ointment formulation revealed consistent spreadability, viscosity, and pH levels within an acceptable range^[12]. The spreadability values ranged from 10.7 to 11.53 g cm/s, ensuring practical application on the skin. The pH values (4.7 -5.8) were within the physiological skin pH range, minimizing potential irritation. The viscosity analysis showed that Batch B3 exhibited optimal consistency, balancing ease of application with sufficient adherence to the skin^[9].

The in vitro drug release study confirmed the sustained antimicrobial activity of the formulation, with a gradual decrease in inhibition zone over 8 hours, suggesting prolonged retention and controlled release of active ingredients [8]. This is advantageous compared to conventional synthetic antifungal creams, which may require frequent application [7].

Overall, the results demonstrate that the herbal ointment formulation is a promising natural alternative to conventional antifungal treatments. Unlike systemic antifungal medications, which have potential side effects and resistance issues, this topical herbal formulation provides a localized, effective, and safer treatment option. Further in vivo studies and clinical trials are recommended to validate its long-term efficacy, safety, and therapeutic potential in human subjects.

4. CONCLUSION

This study successfully formulated and evaluated a herbal topical ointment containing neem oil, garlic powder, and camphor oil to treat fungal infections such as athlete's foot and ringworm. The optimized formulation (1:3:1 ratio of Garlic: Camphor: Neem) exhibited significant antifungal antimicrobial activity, as demonstrated by the highest zone of inhibition $(3.34 \pm 0.23 \text{ cm})$ against Candida albicans. The physical and rheological evaluations confirmed that the formulation had optimal pH (4.7–5.8), spreadability (10.7-11.53 g cm/s), and viscosity, ensuring practical application and skin compatibility. Additionally, in vitro drug release studies indicated sustained antimicrobial activity over hours, highlighting its potential for prolonged therapeutic action.

5. REFERENCES

- 1. Deepa V, Kulkarni GS, Paarakh P.M, Formulation and evaluation of topical antimicrobial herbal cream, *World J. Biol. Pharma. Hlth Sci.* 2022; 12(02): 156-67.
- 2. Muthukumar S, Parvin MK, Shobana S, Vimala N, Vinesha R, Sundaraganapathy R. Formulation and Evaluation of Novel Herbal Ointment for the Treatment of Fungal Infection, *Research J. Pharm. and Tech.* 2021; 14(03): 1459-64.
- 3. Dongarwar AS , Parihar SA, Nagpure VN, Bhonde TK, Deshmukh SV & Khandelwal VA. Formulation and Evaluation of Herbal Ointment by Using Dyerophytum Indicum Extract, *World j. pharm. med. res.* 2024; 10(09): 117-21.
- 4. Mulla M, Attar S, Nithore N, Parkar R. Formulation and evaluation of herbal ointment containing Neem and Karanj oil. *IJCRT*. 2022; 10(05): b200 205.
- 5. Okafo SE, Akpo CO, Okafor CC. Formulation and evaluation of antimicrobial herbal creams from aqueous extract of Moringa oleifera l am seeds. *njse.org.ng*. 2020; 18(1): 50-57.

- 6. Anjum H, Ghulamuddin S, Shahwan M, Khan MS, Shamsi A, Shamsi S. In vitro and In vivo study targeting the development of Unani Antidermatophytic Cream: Implication of Herbal Formulations in Treatment of Dermatophytosis, Heliyon. 2023; 9(5): 1-13.
- 7. Onyeagba R, Ugbogu OC, Okeke CU, Iroakasi O. Studies on the antimicrobial effects of garlic (Allium sativum L.), ginger (Zingiberofficinale Roscoe) and lime (Citrus aurantifolia L.). *Afr. J. Biotechnol.* 2005; 3(10): 552-4.
- 8. Gemeda N, Tadele A, Lemma H, Girma B, Addis G, Tesfaye B, Abebe A, Gemechu W, Yirsaw K, Teka F, Haile C. Development, Characterization, and Evaluation of Novel Broad-Spectrum Antimicrobial Topical Formulations from Cymbopogon martini (Roxb.) W. Watson Essential Oil. Evidence-Based Complementary and Alternative Medicine. wiley.com. 2018; 10(2018:9812093): 1-16.
- 9. Patil VA, Nitave SA. Preparation and evaluation of polyherbal cream. *Wjpr.* 2017; 6(17): 1240-249.
- 10. Premkumar A, Muthukumaran T, Ganesan V, Shanmugam R, Priyanka DL. Formulation and evaluation of cream containing antifungal agents, antibacterial agents and corticosteroids. *Hygeia.J.D.Med.* 2014; 6(2): 5-16.
- 11. Rashed A, Rathi DN, Nasir NA, Rahman AZ. Antifungal properties of essential oils and their compounds for application in skin fungal infections: Conventional and nonconventional approaches. *Mdpi.com*. 2021; 26(4):1-44.

- 12. Sawant SE, Tajane MD, Formulation and evolution of herbal ointment containing neem and turmeric extract, *JSIR*. 2016; 5(4): 149-151.
- 13. Nalla A, Chinnala KM, Formulation and evolution of herbal ointment for antimicrobial activity. *World j. pharm. med. Res.* 2017; 3(7): 113-17.
- 14. Pandey S, Seth A, Tiwari R, Singh S, Bhel HM, Singh S. Development and evaluation of antimicrobial herbal cosmetic preparation. *Afr.J.Pharmacy Pharmaco*. 2014; 8(20): 514-28.
- 15. Choudhari SS, Choudhari GS, A review on plumbago zeylanica linn. A divine medicinal plant plumbagozeylanicalinn, *Int. J. Pharm. Sci. Rev. Res.* 2015; 30(2):119-27.

An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print); ISSN: 2249-9245 (Online)



RESEARCH ARTICLE

EVALUATION OF IMMUNOMODULATORY ACTIVITY OF ETHANOLIC EXTRACT OF *CATUNAREGUM SPINOSA* (THUNB.) TIRVENG ROOT BARK USING *IN VIVO* MODELS

Anju K R*, Veena R, Tasmiya Palte

Department of Pharmaceutical Sciences, Cheruvandoor, Kerala 686631 *Corresponding author email: anjukr13571987@gmail.com
Received: Feb 09, 2025 / Revised: April 21, 2025 / Accepted: May 05, 2025

ABSTRACT

Catunaregum spinosa is commonly known with various names such as Mainphal, Mindhal etc. and is a highly reputed Ayurvedic medicinal tree. It is a known fact that the flavonoids, monoterpenoids, triterpenoids, iridoid glycosides, and phenolic compounds of Plantago species possess immunomodulatory activity. Catunaregam spinosa possesses such phytoconstituents and is an armed shrub or small tree that belongs to the Rubiaceae family. The root of Catunaregam spinosa (Thunb.) Tirveng obtained from the hilly regions of Kerala, India. These bioactive compounds are believed to contribute to the plant's diverse medicinal properties, including its potential immunomodulatory activity. Studies have highlighted the anti-arthritic and anti-cancer activities of Catunaregam spinosa. However, it is to be noted that limited research has been conducted to comprehensively evaluate its immunomodulatory properties. This study aims to explore the immunomodulatory activity of Catunaregam spinosa extract using experimental animal models. It is achieved by inducing immunosuppression and subsequently administering the plant extract and we seek to assess its effects on various immune parameters and cytokine levels. The objective of the research was to prepare the ethanolic extract of the plant Catunaregam spinosa (Thunb.) Tirveng, to carry out preliminary phytochemical screening and to screen the in vivo immunomodulatory activity by two methods: Haemagglutination antibody (HA) titer and Delayed hypersensitivity (DTH) response. Preliminary phytochemical screening of the root bark extract of Catunaregum spinosa showed the presence of components viz. alkaloids, steroids, triterpenoids, flavonoids, saponins, and proteins. In vivo analysis showed increased DTH and HA titer values. The interpretation of the results was done by one-way ANOVA and followed by Tukey's multiple comparison method. From this study, it is concluded that the Catunaregum spinosa root bark extract possesses immunomodulatory activity.

Keywords: *Catunaregum spinosa*, DTH, HA titer.

How to cite this article: Anju K R, Veena R, Tasmiya Palte. Evaluation Of Immunomodulatory Activity Of Ethanolic Extract Of *Catunaregum Spinosa* (Thunb.) Tirveng Root Bark Using *In Vivo* Models. Bulletin of Pharmaceutical Research. 2025;15(S2): 34-40.

Source of support: Nil Conflict of interest: None

1. INTRODUCTION

'Immunomodulation' refers to a process which can alter an organism's immune system. Here, we are trying to find out whether the medicinal plants that are biologically active and could be useful in the development of new immunomodulating agents[1].Of late, it has been learnt that vulnerable response

immunomodulation may offer a volition to traditional chemotherapy for a variety of complaint conditions. It becomes even more important, particularly when the host's defense medium needs to be actuated in the presence of disabled vulnerable responsiveness. Especially, when a specific immunosuppressant is required to be convinced during autoimmune diseases and organ transplantation^[2]. Catunaregam spinosa (Thunb.) belongs to the family Rubiaceae. In general, it is known as Gedhpal or Emetic nut. About the traditional Indian system of medicine, one can be boastful of its long history of use. However, it lacked adequate scientific documentation, particularly in light of modern scientific knowledge [3].

2. MATERIALS AND METHODS

2.1 Selection of Animals

Fourty five healthy male Wistar albino rats weighing between 150-170g were purchased from Nagarjuna Ayurvedic centre, Thodupuzha maintained in Animal house, University College of Pharmacy, Cheruvandoor campus, Ettumanoor, Kottayam. The animals were housed in polypropylene cages in room where the congenial temperature 27±1°C, 30-60 % relative humidity and 12 hours light and dark cycles were maintained. The animals were allowed to acclimatize to the environment as 7 groups for 14 days. They were fed on standard pellet diet collected from Hindustan Lever Limited, Bangalore and water given ad libitum. All procedures and experiments were conducted in day time according to specification of the Indian National Science Academy (INSA). The experiment was approved by Institutional Animal Ethical Committee University College of Pharmacy, Cheruvandoor with IAEC number: (005/MPH/UCP/CVR/14).

2.2 IN VIVO STUDIES

1) HAEMAGGLUTINATION ANTIBODY (HA) TITER

The rats were divided into seven groups, consisting of six creatures each. Rat in group I entered the vehicle for only 21 days. Group II received a single dose of cyclophosphamide (Negative Control) 100 mg/kg, p.o. on the 9th and 16th day. Rats in treatment group III and IV were

given EECS (100 mg/kg/day/p.o) and EECS(200 mg/kg/day/p.o.) daily for 21 days independently Immunosuppressed creatures in groups V and VI were given EECS (100 and **EECS**(mg/kg/day/p.o) mg/kg/day/p.o.) plus cyclophosphamide(100 mg/kg/p.o.) on 9th and 16th day as a single cure independently. Group VII received the standard medicine levamisole (50 mg/kg/p.o.) as an established immunostimulant agent for 21 days. On the 7th and 14th day of the study, rats from all the groups (i.e., group I to VIII) were immunized and challenged independently, with SRBCs in normal saline (0.1 ml of 20 SRBCs) intraperitoneally. Blood was withdrawn on the 14th and 21st day from the retro-orbital supersystem under mild ether anesthesia from all antigenically acclimatized and challenged rats independently. Blood was centrifuged to gain serum; normal saline was used as a diluent, and the SRBC count was adjusted to 0.1 of SRBCs. Each well of a microtiter plate was filled originally with 20 µl of saline, and 20 µl of serum was mixed in the first well of the microtiter plate. latterly, the 20 µl adulterated serum was removed from the first well and added to the next well to get twofold dilutions of the antibodies present in the serum. farther twofold dilutions of this adulterated serum were also carried out till the last well of the alternate row (24th well), so that the antibody concentration of any of the dilutions is half of the former dilution. 20 µl SRBC (0.1 of SRBCs) were added to each of these dilutions and the plates were incubated at 37 °C for one hour and also observed for hemagglutination. The loftiest dilution giving haemagglutination was taken as the antibody titer. The antibody titers were expressed in the canted manner, the minimal dilution (1/2) being ranked as 1, and the mean species of different groups were compared for statistical significance. Antibody titer attained on the 14th day after immunization (on the 7th day) and on the 21st day after the challenge (on the 14th day) with SRBCs were considered as primary and secondary humoral responses independently^[12].

2) DELAYED TYPE HYPERSENSITIVITY(DTH) RESPONSE:

The medicine treatment was the same as described over for the HA titer. On the 14th day of the study, all the groups I to VII were immunized with SRBCs (0.1 ml of 20 SRBCs i.p.) in normal saline. On day 21st, all creatures from all the groups were challenged with 0.03 ml of 20 SRBCs in the subplantar region of right hind hind footpad was measured using a vernier calliper. The footpad response was expressed as the difference in the consistency (mm)between

3. RESULTS AND DISCUSSION HAEMAGGLUTINATION ANTIBODY TITER (HA)

HA titer value significantly increased in animals treated with high-dose EECS. Low-dose and high-dose extracts of *Catunaregum spinosa* counteract the suppression of both primary and secondary humoral responses induced by cyclophosphamide. These results were tabulated below. The in direct haemagglutination test was performed to

paw. Footpad oedema in rats was used for the discovery of the cellular vulnerable response. On the 21st day, injection of 0.1 ml of 20 SRBCs in the subplantar region of the right hind paw in the volume of 0.03 ml and normal saline in the left hind paw in the same volume. Footpad response was assessed after 24 hr. On 22nd day, in terms of an increase in the consistency of the footpad as a result of an acuity response due to oedema. the consistency of the right the right footpad fitted with SRBC and the left footpad fitted with normal saline [12].

confirm the effect of *Catunaregum spinose* extract on the humoral immune system. This plant extract is significantly composed of interacting B cells and they can subsequently be proliferating and differentiating into antibody producing cells. This will bind with antigens and neutralizing it or facilitating itselimination phagocytic cells. The results showed that levels of circulating antibodies are increased if the test animals are pretreated with *Catunaregum spinosa* extract

Table 1: Effect of Catunaregum spinosa on humoral immune response in rats

GROUPS	TREATMENT	HA TITER	HA TITER
		1º(14thday)	2 º (21st day)
I	Control	5.23±0.76	4.98±0.45
II	Cyclophosphamide(100mg/kg)	2.85±1.02*	3.36±0.90*
III	EECS (100mg/kg)	14.30±0.54***	16.50±1.05**
IV	EECS (200mg/kg)	18.7±0.95****	28.66±0.11***
V	EECS (100mg/kg) + cyclophosphamid	12.54±0.43ª	14.33±0.61ª
VI	EECS (200mg/kg) + cyclo	16.82±0.7a	26.86±1.22a
VII	Levamisole	32.1±1.23***	41.3±0.68***

EECS: Ethanolic extract of *Catunaregum spinosa*

HA TITER: Values plotted were the Mean ± SEM (n=6).

*p<0.05, **p<0.01, ***p<0.001, when compared with control

 $^{\rm a}$ p<0.05, $^{\rm bp}$ <0.01, $^{\rm c}$ p<0.001, when compared with standard

Statistically analysed by one way followed by Tukey-Kramer multiple comparison test.

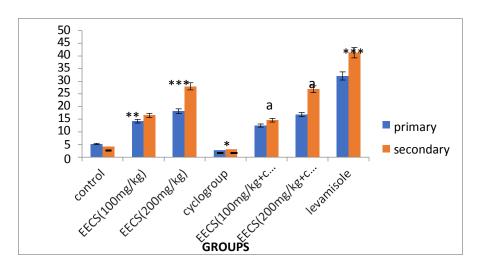


Figure 1: Effect of C. spinosa on humoral immune response in rats

EECS showed a significant increase in HA titer value, which is represented in (**Figure 1.**) Values plotted were the Mean \pm SEM (n=6).

*p<0.05, **p<0.01, ***p<0.001, when compared with normal control

ap<0.05, bp<0.01, cp<0.001, when compared with CP control

Statistically analyzed by one-way ANOVA followed by Tukey-Kramer multiple comparison test.

Effect of Catunaregum spinosa on humoral immune response in rats

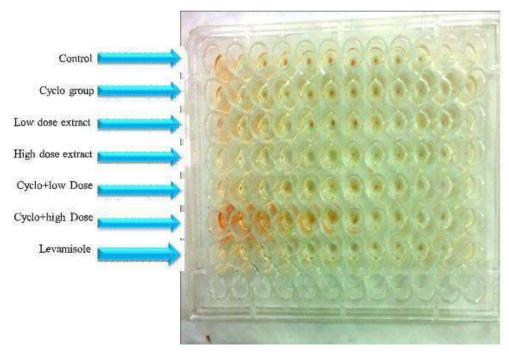


Figure 2: Effect of Catunaregum spinosa on Humoral immune response

1) DELAYED TYPE HYPERSENSITIVITY (DTH) RESPONSE

DTH response was significantly increased in rats treated with EECS. The extract-treated groups counteracted the suppression caused by cyclophosphamide. The results are tabulated and expressed as follows. DTH requires the specific recognition of a given

antigen by activated T lymphocytes, which subsequently proliferate and release cytokines. Therefore, an increase in DTH reaction in rats in response to T-cell dependent antigen revealed the stimulatory effect of the extract on T cells.

GROUPS	TREATMENT	FOOT PAD THICKNESS (mm)	
I	Control	0.43±0.032	
II	Cyclophosphamide(100mg/kg)	0.315±0.11**	
III	EECS (100mg/kg)	1.625±0.04***	
IV	EECS (200mg/kg)	2.275±0.006***	
V	EECS (100mg/kg + cyclo)	1.560±1.203a	
VI	EECS (200 mg/kg + cyclo)	2.10±0.007a	
VII	Levamisole(50mg/kg)	3.29±1.302***	

EECS: Ethanolic extract of *Catunaregum spinosa*

Values plotted were the Mean \pm SEM (n=6).

Statistically analysed by one-way analysis followed by Tukey–Kramer multiple comparison test.

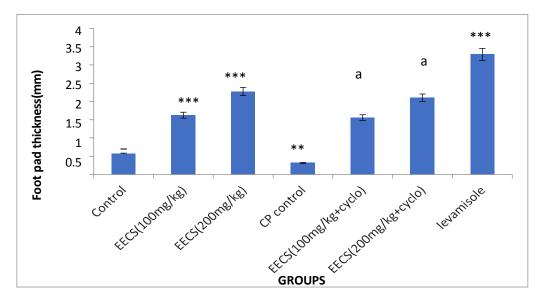


Figure 3: Effect of C.spinosa on DTH in rats

EECS showed a significant increase in DTH response, which is represented (**Figure 3**) Values plotted were the Mean \pm SEM (n=6).

*p<0.05, **p<0.01, ***p<0.001, when compared with normal control

ap<0.05, bp<0.01, cp<0.001, when compared with CP control

Statistically analyzed by one-way ANOVA followed by Tukey-Kramer multiple comparison test.

^{*}p<0.05, **p<0.01, ***p<0.001, when compared with normal control

ap<0.05, bp<0.01, cp<0.001, when compared with CP control

4. CONCLUSION

In haemagglutination antibody titer response, low-dose high-dose extracts of Catunaregum spinosa counteract the suppression of both primary and secondary humoral responses induced by cyclophosphamide. This test confirms the stimulant activity on humoral-mediated immune response. High dose extracts showed remarkable augmentation in DTH response,

5. REFERENCES

- 1. Mishra DR, Bali Y. Immunomodulatory study of some herbal medicinal plants: a review. *Int J Pharm Bio Sci.* 2022;7(5).
- 2. Kumar A. Potential role of medicinal plants for their immunomodulatory activity—a review. 3. 2022;3.
- 3. L MBP, N PAK. Primary phytochemical studies of *Catunaregam spinosa* (Thunb.) Tirven for secondary metabolites. *Int J Pharm Bio Sci.* 2017;8(2):320-323.
- 4. Anju KR. In vitro evaluation of immunomodulatory activity of ethanolic extract of Catunaregum spinosa (Thumb.) Tirveng root bark. *Int J Pharm Pract Drug Res.* 2017;7(2):25-29.
- 5. Aher V, Wahi A. Immunomodulatory activity of alcohol extract of Terminalia chebula Retz Combretaceae. *Trop J Pharm Res.* 2011;10(5).
- 6. Tangke E, Shimizu K. Antioxidant activities of compounds from wood of Artocarpus heterophyllus. *J Pharm Indones*. 2011;9(2):72-7.
- 7. Schmidt CW. Questions persist: environmental factors in autoimmune disease. *Environ Health Perspect*. 2011;119(6).
- 8. Chithra A, Sethilkumar N, Asraf M. Antioxidant and anti-tumor activities of Catunaregum spinosa. *Int J Res Pharmacol Pharmother*. 2013;2(3):464-70.
- 9. (Chiang et al, 2003) Chiang LY, Naselsky P,

and this confirms the activity of ethanolic extract on cell-mediated immune response.

In conclusion, the results obtained in the present study show that *Catunaregum spinosa* root bark extract produces a stimulatory effect on the humoral and cell-mediated immune response in the experimental animals and suggest its therapeutic usefulness in disorders of immunological origin.

- Verhodanov O, Way M. Non-gaussianity of the derived maps from the first-year Wilkinson microwave anisotropy probe data. *Astrophys J.* 2003;590:L65: L68.
- 10. Cooper PJ, Murray L. Controlled trial of the short- and long-term effect of psychological treatment of post-ion impact on maternal mood. *Br J Psychiatry*. 2003;182:412-9.
- 11. Das BN, Muniruddin AS. Anti-inflammatory activity of bark of Xeromphis spinosa. *Bangladesh J Life Sci.* 2009;4:76-8.
- 12. Dashputre NL, Nilofer S, Naikwade N. Preliminary immunomodulatory activity of aqueous and ethanolic leaf extracts of Ocimum basilicum Linn in mice. *Int J PharmTech Res.* 2010;2(2).
- 13. Mani M, LakshmiDevi S, Kannan S. Ethnomedicinal plants for cancer therapy a review. *Hygeia J D Med*. 2011;3(1):1-10.
- 14. Elias M, Steinhof M. Pathogenic mechanisms in atopic dermatitis. *J Investig Dermatol*. 2008;128:1067-70.
- 15. Gayathri S, Kirubha V, Senthamarai RT. Pharmacognostical and phytochemical studies on fruits of *Catunaregam spinosa* Linn. *J Chem Pharm Res.* 2011;3(6):829-38.
- 16. Ghosh D, Thejomoorthy P, Veluchamy S. Anti-inflammatory and analgesic activities of oleonic acid 3-/3-glucoside (RDG-1) from Randia dumetorum (Rubiaceae). *Indian J Pharmacol*. 1981;15(4):331-42.
- 17. Goronzy JJ, Li G, Yang Z, Cornelia M. The Janus head of T cell aging autoimmunity and immunodeficiency. *Front Immunol*. 2013;4:131.

- 18. Haggag EG, Mohamed IS, Abdelhady A. Phenolic content of Ruprechtia salicifolia leaf and its immunomodulatory, anti-inflammatory, anticancer, and antibacterial activity. *J Pharm Res.* 2013;6:696-703.
- 19. Hayden MS, West AP, Ghosh S. NF Kappa B and the immune response. *Oncogene*. 2006;25(51):6758-80.
- 20. Kale MK, Noorani AA. Pretreatment of albino rats with methanolic fruit extract of Randia dumetorum (L.) protects against alcohol-induced liver damage. *Korean J Physiol Pharmacol*. 2012;16:125-30.
- 21. Li Q, Gao G, Qi S. Minor compounds from the stem bark of Chinese mangrove associate Catunaregum spinosa. *Pharmazie*. 2008;63:542-4.
- 22. Møller LE, Ludke HR, Peacock JE, Tomar RH. Manual of laboratory immunology. *London: Lea and Febiger*; 1991. p. 1-18.
- 23. OECD. Guidance document on acute oral toxicity. Environmental Health and Safety Monograph Series on Testing and Assessment No. 24. 2000.

- 24. Patel TS, Gouda N, Rao K, Nandakumar S. Antidiarrheal activity of alcoholic and aqueous extracts of roots of Tylophora indica (Wight & Arn.) in rodents. *Pharmacologyonline*. 2006;19-29.
- 25. Patil A. Translational infidelity-induced protein stress results from a deficiency in Trm9-catalyzed tRNA modifications. *RNA Biol.* 2012;9(7):990-1001.
- 26. Rajeswari G, Priyanka B, Amrutha RE, Rajaram C, Rupesh S, Sadhu NK. Hibiscus tiliaceus: a possible immunomodulatory agent. *J Pharm Res.* 2013;6:742-7.
- 27. Ray WA, Murray KT, Stein MB, Patrick G. Azithromycin and the risk of cardiovascular death. *N Engl J Med*. 2012;366:1881-90.
- 28. Ritesh PG, Pathak NL, Rathod JD. Phytopharmacological properties of Randia dumetorum as a potential medicinal tree: an overview. *J Appl Pharm Sci.* 2011;1(10):24-6.
- 29. Vedpal P, Gupta A. Anti-arthritic activity of Desmodium gangeticum root. *Int Res J Pharm*.2013;4(9)

An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print); ISSN: 2249-9245 (Online)



REVIEW ARTICLE

A SYSTEMATIC REVIEW OF FOOD-DRUG INTERACTIONS OF DRUGS ACTING ON CARDIOVASCULAR SYSTEM

Punam Kela*, Karishma Mainkar, Shreyasi Banerjee, Pratiksha Kulkarni and Sakshi Latey.

Department of Pharm D, PES's Modern College of Pharmacy, Nigdi, Pune
*Corresponding author email: prkmcop@gmail.com
Received: Feb 15, 2025 / Revised: April 25, 2025 / Accepted: May 10, 2025

ABSTRACT

Food-drug interactions alter the pharmacokinetics of cardiovascular medications which impact the drug efficacy and safety. This review examines the food effect on cardiovascular drugs. Bioavailability of ACE inhibitors and hydralazine reduced when taken with food requiring their intake on an empty stomach. Beta-blockers demonstrate variable food effects with lipophilic agents like propranolol experiencing enhanced absorption and hydrophilic agents like atenolol showing reduced uptake with certain juices. Calcium-channel blockers and diuretics display altered pharmacokinetics depending on food consumption while anticoagulant and antiplatelet agents show specific interactions with dietary components such as vitamin k and cranberry juice. These interactions enable optimized dosing strategies by enhancing therapeutic outcomes and minimizing the adverse events by patient-specific guidance.

Key Words: Food-Drug Interactions, antihypertensives, antiplatelets, anticoagulants, Cardiovascular System.

How to cite this article: Punam Kela, Karishma Mainkar, Shreyasi Banerjee, Pratiksha Kulkarni, Sakshi Latey, A Systematic Review of Food-Drug Interactions of Drugs Acting on Cardiovascular System.Bulletin of Pharmaceutical Research. 2025;15(S2): 41-52.

Source of support: Nil Conflict of interest: None

Abbreviations:

ACE inhibitors - Angiotensin-converting enzyme inhibitors

AUC - Area Under the curve

BA - Bioavailability

CAD - Coronary Artery Disease

CCB - Calcium Channel Blocker

CVD - Cardiovascular Disease

CYP3A - Cytochrome P3A enzyme

CYP450 - Cytochrome P450 enzyme

Cmax - Maximum plasma concentration

HTN - Hypertension

HCTZ - Hydrochlorothiazide
INR - International Normalized Ratio
PRISMA – Preferred Reporting Items for
Systematic Reviews and Meta-Analyses
Tmax - Time to maximum concentration

1.INTRODUCTION

A food-drug interaction occurs when a food or herbal supplement that interferes the effect of a drug that increase, diminishes or modify its intended action. Lack of knowledge about the ingredient in a particular food item leads to this food-drug interactions. Effect of Food-Drug interaction usually causes changes pharmacokinetic and pharmacodynamics properties of the drug [1].On the other hand, with the change in century, CVDs has become the predominant cause of around 60% of total deaths[2]. Alarmingly, India bears disproportionate burden of CVD-related mortality, accounting for a staging one-fifth of global deaths, with a disturbing trend of increasing prevalence among the younger population, as reported by the World Health Organization (WHO) [3]. In India, CAD is known to have highest rates. The prevalence has exhibited significant variability in past few years, with rural areas reporting rates between 1.6% to 7.4%, and urban areas showing a wider range of 1% to 13.2% [4]. Moreover, Hypertension (HTN) or increased blood pressure, reported as the third primary contributor to the burden of disease on South Asia as reported in 2010 and its prevalence significantly high in India, affecting approximately 33% of the urban population and 25% of the rural population [5]. Internationally recognized guidelines recommended the use of cardiovascular drugs, including renin-angiotensin system inhibitors, calcium-channel blockers, diuretics, beta-blockers, antithrombotic agents, and lipid-lowering therapies, as first-line treatment for the primary and secondary prevention of CVD [6] .Optimal management of hypertension be achieved can antihypertensive medications are complemented by dietary modification[7].

Literature Search:

A literature survey using PubMed, Scopus, Web of Science and Google Scholar databases was to find studies related conducted cardiovascular drugs with their food interactions. The search strategy utilized a combination of keywords and Medical Subject Headings (Mesh) terms like "Cardiovascular drugs", "Food-Drug Interaction". "Antihypertensives", "Pharmacokinetics", "Effect of Food", "food", "Influence of food", "diuretics", "antiplatelets", "anticoagulants" and "angiotensin-1 converting enzyme". References of the food articles were manually searched to ensure complete coverage of eligible studies.

Eligibility Criteria:

This review encompassed human and animal studies with study designs such as randomized controlled trials, observational studies, review articles and case reports publications in English were only chosen for inclusion which assessed food-drug interactions with cardiovascular drugs like antihypertensive, anticoagulants and antiplatelet agents. Studies were required to provide clear clinical outcomes or mechanistic insights related to food-drug interactions.

Exclusion criteria were studies focused on noncardiovascular drugs or in vitro studies research, abstracts without full article access. Studies lacking specific data on food-drug interactions or without clinical relevance were also excluded.

Study Selection:

After the initial search, duplicate records were removed. A screening of the titles and abstracts of all retrieved articles was conducted. Eligible full texts articles were then assessed for inclusion based on predefined criteria.

Data Extraction and Synthesis:

A standardized data extraction was conducted to systematically collect information across all relevant variables ensuring consistency and reliability throughout the process:

- Cardiovascular drug(s) involved
- Food item(s) implicated in interaction
- Type of interaction (pharmacokinetic/pharmacodynamic)
- Clinical significance and outcomes reported

Subsequently, a qualitative synthesis was conducted to consolidate the identified food-drug interactions exploring their underlying mechanisms and highlight their clinical Where interaction relevance. possible, management strategies were also highlighted. The study selection process was performed using PRISMA style (Fig 1).

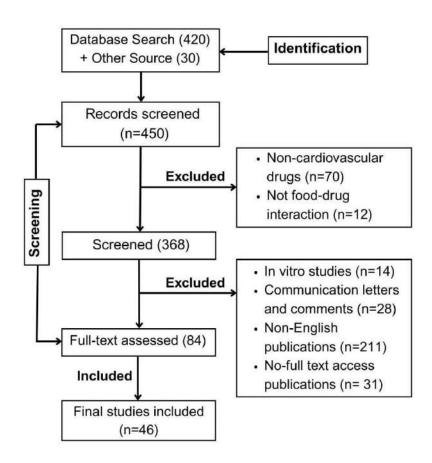


Fig 1. PRISMA Flow diagram

Cardiovascular drugs

Understanding the CYP450 enzyme system's role in metabolizing cardiovascular medications

allows clinicians to predict and mitigate potential drug interactions, informing more effective treatment approaches [8]. The physiochemical properties of a drug are important in determining its potential for food-drug interactions. Notably, drugs having similar therapeutic class or variations in drug formulations can result in distinct chemical properties, leading to varying food-drug interaction profiles [9]. Propranolol (beta-blocker), dietary factor such as protein significantly intake can impact pharmacokinetic of the drug and alterations in carbohydrate and protein consumption can influence oral clearance. Also, numerous

investigations have explored the potential fooddrug interaction between Warfarin (anticoagulant) and cranberry juice, resulting in higher International Normalized Ratio (INR)[10]. Grapefruit which contains Furanocoumarins, had primary effect on intestinal CYP3A system's metabolism of calcium channel blockers (CCB) exhibit significant variability among individual drug of this class, resulting in differing effects of their absolute bioavailability^[11]. This review focuses on all the Food-Drug interaction involving all the classes of CVS.

Table 1: Food-Drug interaction of Cardiovascular drugs:

Drugs	Food	Mechanism of action Management		
A) ACE inhibitors				
Captopril [1,11]	Potassium-rich diet	Decreases bioavailability by 42-56%.	Increases absorption when taken on an empty stomach, should be taken an hour before the meal.	
Perindopril [1,11]	Potassium-rich diet	Decreases bioavailability by 35%.	Absorption increases when taken on an empty stomach. Take an hour before the meal.	
B) Beta-blockers	•			
Propranolol [1,13]	Protein rich diet	Increases propranolol level and bioavailability.	Should be taken in consistent manner with relation to meals to avoid fluctuations in drug concentration.	
Celiprolol [1,12]	Orange juice.	Lead to inhibition of intestinal absorption. It also lowered the AUC by 83%.	Take orange juice and Celiprologe separately.	
Atenolol [12,14]	Orange juice.	It interferes with the gastrointestinal absorption of atenolol which decreases mean Cmax and AUC by 49%	Take orange juice and Atenolol separately.	

	1	Τ			
		and 40% respectively.			
Labetalol [15]	Food.	Food increases the bioavailability of Labetalol by minimizing its first-pass effect.	A regular dosing schedule is recommended to reduce alterations in drug bioavailability.		
C) Calcium-channel bloc	kers				
Nifedipine [11]	High fat diet	Bioavailability is enhanced by 29-31%, resulting in a pronounced increase in its blood pressure-lowering effect.	Avoid taking with high fat diet.		
Felodipine [11,13,16]	Grapefruit juice.	Inhibition of first-pass metabolism boosts felodipine bioavailability by 284%, doubling its impact on blood pressure and heart rate. ¹¹ It can also cause toxicity of felodipine.	Avoid taking with grapefruit juice.		
	Seville/bitter orange juice.	76% increase in the plasma concentration-time curve.	Avoid taking with Seville/bitter orange juice.		
Amlodipine [17]	Grapefruit juice.	The plasma concentration, AUC, Cmax was increased.	Avoid taking with grapefruit juice.		
Diltiazem ^[18]	Grapefruit juice.	The systolic and diastolic blood pressure is decreased. Also, there is significant increase in systemic exposure.	It should be taken before meal.		
D) Diuretics					
Hydrochlorothiazide [19]	Vitamin A.	Develop severe hypercalcemia with calcium and vitamin D supplement.	hydrochlorothiazide and		
Furosemide [20]	Heavy meal.	The bioavailability of is reduced approximately by 30% and also	Administer on empty stomach.		

		decreases the diuretic effect.		
Spironolactone [11,13]	Potassium rich food: banana, green leafy vegetables.	The risk of hyperkalemia with adverse effects like cardiac arrhythmia can be increased.	It is best not to take spironolactone with potassium supplement or salt substitute.	
E) Vasodilators				
Hydralazine [11]	Meals.	The intravascular conversion of hydralazine-to-hydralazine pyruvic acid hydrazone can be increased. Fluctuations in bioavailability and drug effect is also seen.	Should be taken at a fixed time with respect to meals as suggested by the vasodepressor response.	
F) Anticoagulant agents				
Bishydroxycoumarin [35]	Vitamin K.	Promotes blood clotting and also increase in vitamin K levels.	Should not be taken with vitamin K rich food.	
Acenocoumarol [35]	Vitamin K.	Increases vitamin K levels in the body.	Avoid taking with vitamin K.	
Warfarin [36]	Cranberry juice.	Increases INR.	Avoid taking cranberry juice with warfarin.	
G) Antiplatelet agents				
Aspirin [37]	Food.	Decreases absorption and plasma peak concentration.	Aspirin should be avoided with food.	
Clopidogrel [38]	Food.	Increases Cmax and AUC by 6.1 and 9.2 times respectively.	Should be taken with food.	

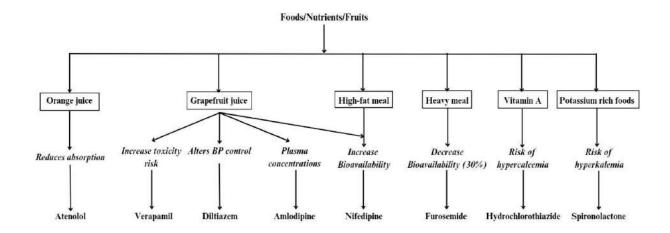


Fig. 1. Cardiovascular drugs and food interactions affecting pharmacokinetics and pharmacodynamics parameters

Figure 2. This figure illustrates how different food, nutrients and food influence the pharmacokinetic and pharmacodynamics of cardiovascular drugs, potentially altering their effectiveness and safety.

ACE Inhibitors :-

The absorption of these class drugs is maximally impacted by the presence of food. The absorption of ACE inhibitors is enhanced when administered on an empty stomach while the bioavailability of certain drugs like captopril is decreased by 30 to 56% when taken with food as documented in early human pharmacokinetic trials and perindopril is reduced by 35% [21].

This is attributed to the drug's physicochemical properties which limit its diffusion when gastric contents are present. Enalapril, a prodrug, is less sensitive to food but still shows a minor delay in absorption^[22]. Hyperkalemia is a common complication associated with ACE inhibitors therapy, and its risk can be exacerbated by consuming potassium-rich food or salt ^[11,22]

Beta-Blockers:-

Lipophilic beta-blockers such as Propranolol, when taken with protein rich food increases the bioavailability likely due to delayed gastric emptying and enhanced splanchnic blood flow.

Intake of soyabean oil and fatty acids significantly decreased the BA of propranolol ^[23]. Additionally, glucose intake dramatically decreased the hepatic uptake of propranolol and CYP450 content resulting in lower plasma concentration and inhibited metabolism to its main metabolite, beta-naphthoxylactic acid ^[24].

In contrast, hydrophilic beta-blockers like atenolol shows moderately affected absorption by certain fruit juices. For instance, orange juice has been shown to interfere with the gastrointestinal absorption of atenolol potentially reducing its efficacy [25]. The bioavailability of bevantolol remains unaffected by food intake [26]. The food presence slightly reduces the absorption and Cmax of acebutolol and its primary metabolite, diacetolol, although this effect is not clinically significant [27].

Calcium-Channel Blockers:-

Taking Nifedipine tablets with food minimizes the potential for side effects from peak plasma concentrations without decreasing the efficacy of the drug. A sustained-release formulations of Nifedipine have been shown to increase serum levels, resulting in a more consistent therapeutic effect. A grapefruit with felodipine alter its toxicity [13, 28]. In rat model, the BA is about 45-58% with first-pass metabolism. Since food can alter gastrointestinal transit and enzyme activity, it may affect its BA [29].

Diuretics:-

The BA of furosemide decreases by 16-45% when taken with food is linked with diminished diuretic effect. Consuming potassium-sparing diuretics with a potassium-rich diet (bananas and spinach), increases the risk of developing hyperkalemia [30]. Food intake can enhance the absorption of Hydrochlorothiazide (HCTZ), a study demonstrated that the urinary recovery of HCTZ increases with meals therefore improve its therapeutic effect [31].

Vasodilators:-

Food reduces hydralazine absorption significantly decreasing blood hydralazine levels and attenuated its antihypertensive response in humans suggesting being taken on empty stomach for optimal BA [32,33]. Making dogs as a reliable model for studying found the lowered hydralazine BA similar to the effect observed in humans [34].

Anticoagulant agents:-

Oral Anticoagulants mostly shows interaction with vitamin k rich food. Bishydroxycoumarin and Acenocoumarol when taken with vitamin k promotes clotting in the body with increase in vitamin k levels [35]. Warfarin most used Anticoagulant when interacts with cranberry juice, it increases the INR ratio. Some case reports of warfarin and cranberry juice interaction have been reported in which INR ratio was elevated

when taking cranberry juice while on warfarin therapy for atrial fibrillation [36].

Antiplatelet agents :-

Food intake can delay aspirin absorption and reduce peak plasma concentration, but overall bioavailability remains unaffected humans[37]. The Cmax and AUC of clopidogrel is increased by 6.1 times and 9.2 times in the presence of food [38].In contrast, in fed state, absorption or plasma concentration did not alter compared to fasting condition suggests that drug can be taken with or without food without affecting its therapeutic efficacy [39]. The study found that food intake had minimal effect on ticagrelor's pharmacokinetics with a slight increase in AUC and a small decrease in Cmax of active metabolite, but these changes were not clinically significant [40].

Population-Specific Risks and Clinical Implications of Food-Drug Interactions in Cardiovascular therapy

Geriatric population

The risk of food-drug interactions varies significantly across specific patient populations. Elderly individuals are at heightened risk due to altered gastric pH, delayed gastric emptying and diminished hepatic metabolism which enhance susceptibility to pharmacokinetic interactions [41]. For example, food-induced changes in drug absorption can cause significant variability in antihypertensive and anticoagulant responses in other adults.

Comorbidity-Driven Interactions & Monitoring Drug Efficacy

Patients with underlying comorbidities such as chronic kidney disease or heart failure face are in greater risk when taking potassium-rich foods in addition to ACE inhibitors or potassium-sparing diuretics increasing risk of hyperkalemia risk [42]. Food-drug interactions may worsen electrolyte imbalance with impaired renal excretion as a consequence. Routine drug level and clinical response monitoring is important when initiating or changing cardiovascular medications. Hydralazine, essential to administer on empty stomach as food reduces its BA affecting its therapeutical optimal effect [33].

Nutritional Status Risks

Malnourished or underweight individuals are vulnerable because low serum albumin levels alter the binding of highly protein-bound drugs like warfarin, amplifying free drug levels and enhancing bleeding risk especially when dietary vitamin k intake is variable [35, 43].

Dietary Restrictions

Patient on specific diets such as high-fat or ketogenic diets may experience altered drug bioavailability [44]. For instance, propranolol absorption is enhanced by high-fat meals due to increased splanchnic blood flow potentially leading to exaggerated beta-blockade effects.

Gestational Considerations

Pregnant women experience physiological changes including increased plasma volume and altered gastrointestinal motility which can modify drug pharmacokinetics [45]. Careful consideration is necessary especially with hypertensive drugs where food-drug interactions could impact fetal safety.

Ethnic Genetic Variations

Ethnic and genetic polymorphisms in drugmetabolizing enzymes (e.g., CYP2C9 for warfarin, CYP2C19 for clopidogrel) significantly affect individual variability to food-drug interactions necessitating personized therapeutic approaches [46]. Understanding these population-specific risks can optimize cardiovascular pharmacotherapy and minimize adverse outcomes.

2. CONCLUSION

ACE inhibitors have decreased bioavailability when administered with food while beta-blockers have differential responses based on lipophilicity and nature of food consumed. Likewise, calciumchannel blockers and diuretics have their pharmacokinetics changed by food with potential to influence therapeutic efficacy. Vasodilators have been observed to lower their bioavailability when taken in the presence of food. On the other hand, anticoagulant and antiplatelet drugs exhibit more complex interactions where some food constituents such as vitamin K and cranberry juice affect warfarin activity. Clopidogrel and ticagrelor exhibit little or tolerable food effects. Knowledge of such interactions is important for maximizing drug activity, reducing side effects and delivering reliable patient instruction. Personalized advice on diet can thus improve treatment outcomes and facilitate patient safety.

3. REFERENCES

- 1. Bushra R, Aslam N, Khan AY. Food-drug interactions. Oman Med J. 2011 Mar;26(2):77-83.
- 2. Prabhakaran D, Jeemon P, Roy A. Cardiovascular Diseases in India: Current Epidemiology and Future Directions. Circulation. 2016 Apr 19;133(16):1605-20.
- 3. Sreeniwas Kumar A, Sinha N. Cardiovascular disease in India: A 360 degree overview. Med J Armed Forces India. 2020 Jan;76(1):1-3.
- 4. Huffman MD, Prabhakaran D, Osmond C, Fall CH, Tandon N, Lakshmy R, Ramji S, Khalil A, Gera T, Prabhakaran P, Biswas SK, Reddy KS, Bhargava SK, Sachdev HS; New Delhi Birth Cohort. Incidence of cardiovascular risk factors in an Indian urban cohort results from

- the New Delhi birth cohort. J Am Coll Cardiol. 2011 Apr 26;57(17):1765-74.
- 5. Anchala R, Kannuri NK, Pant H, Khan H, Franco OH, Di Angelantonio E, Prabhakaran D. Hypertension in India: a systematic review and meta-analysis of prevalence, awareness, and control of hypertension. J Hypertens. 2014 Jun;32(6):1170-7.
- Cong L, Ren Y, Hou T, Han X, Dong Y, Wang Y, Zhang Q, Liu R, Xu S, Wang L, Du Y, Qiu C. Use of Cardiovascular Drugs for Primary and Secondary Prevention of Cardiovascular Disease Among Rural-Dwelling Older Chinese Adults. Front Pharmacol. 2020 Dec 18;11:608136.
- 7. Jáuregui-Garrido B, Jáuregui-Lobera I. Interactions between antihypertensive drugs and food. Nutr Hosp. 2012 Nov-Dec;27(6):1866-75.
- 8. Cheng JW, Frishman WH, Aronow WS. Updates on cytochrome P450-mediated cardiovascular drug interactions. Am J Ther. 2009 Mar-Apr;16(2):155-63.
- 9. Schmidt LE, Dalhoff K. Food-drug interactions. Drugs. 2002;62(10):1481-502.
- 10. Paeng CH, Sprague M, Jackevicius CA. Interaction between warfarin and cranberry juice. Clin Ther. 2007 Aug;29(8):1730-5.
- 11. Sica DA. Interaction of grapefruit juice and calcium channel blockers. Am J Hypertens. 2006 Jul;19(7):768-73.
- 12. Jáuregui-Garrido B, Jáuregui-Lobera I. Interactions between antiarrhythmic drugs and food. Nutr Hosp. 2012 Sep-Oct;27(5):1399-407.
- 13. Ajeigbe OF, Ademosun AO, Oboh G. Relieving the tension in hypertension: Food-drug interactions and anti-hypertensive mechanisms of food bioactive compounds. J Food Biochem. 2021 Mar;45(3):e13317.
- 14. Lilja JJ, Raaska K, Neuvonen PJ. Effects of orange juice on the pharmacokinetics of

- atenolol. Eur J Clin Pharmacol. 2005 Jul;61(5-6):337-40.
- 15. Daneshmend TK, Roberts CJ. The influence of food on the oral and intravenous pharmacokinetics of a high clearance drug: a study with labetalol. Br J Clin Pharmacol. 1982 Jul;14(1):73-8.
- 16. Malhotra S, Bailey DG, Paine MF, Watkins PB. Seville orange juice-felodipine interaction: comparison with dilute grapefruit juice and involvement of furocoumarins. Clin Pharmacol Ther. 2001 Jan;69(1):14-23.
- 17. Josefsson M, Zackrisson AL, Ahlner J. Effect of grapefruit juice on the pharmacokinetics of amlodipine in healthy volunteers. Eur J Clin Pharmacol. 1996;51(2):189-93.
- 18. Christensen H, Asberg A, Holmboe AB, Berg KJ. Coadministration of grapefruit juice increases systemic exposure of diltiazem in healthy volunteers. Eur J Clin Pharmacol. 2002 Nov;58(8):515-20.
- 19. Varghese RT, Khasawneh K, Desikan RK, Subramaniam A, Weaver T, Nair GKV. Vitamin A and Hydrochlorothiazide Causing Severe Hypercalcemia in a Patient With Primary Hyperparathyroidism. J Investig Med High Impact Case Rep. 2019 Jan-Dec;7:2324709618823805.
- 20. Beermann B, Midskov C. Reduced bioavailability and effect of furosemide given with food. Eur J Clin Pharmacol. 1986;29(6):725-7.
- 21. Singhvi SM, McKinstry DN, Shaw JM, Willard DA, Migdalof BH. Effect of food on the bioavailability of captopril in healthy subjects. J Clin Pharmacol. 1982 Feb-Mar;22(2-3):135-40.
- 22. Faruqi A, Patel P, Jain A. Enalapril. [Updated 2024 Feb 13]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan.

- 23. Liedholm H, Wåhlin-Boll E, Melander A. Mechanisms and variations in the food effect on propranolol bioavailability. Eur J Clin Pharmacol. 1990;38(5):469-75.
- 24. Taro OGISO, Masahiro IWAKI, Tadatoshi TANINO, Ritsuko KAWAFUCHI, Sumiko HATA, Effect of Food on Propranolol Oral Clearance and a Possible Mechanism of This Food Effect, Biological and Pharmaceutical Bulletin, 1994, Volume 17, Issue 1, Pages 112-116, Released on J-STAGE April 10, 2008, Online ISSN 1347-5215, Print ISSN 0918-6158.
- 25. Lilja JJ, Raaska K, Neuvonen PJ. Effects of orange juice on the pharmacokinetics of atenolol. Eur J Clin Pharmacol. 2005 Jul;61(5-6):337-40.
- 26. Toothaker RD, Randinitis EJ, Nelson C, Kinkel AW, Goulet JR. The influence of food on the oral absorption of bevantolol. J Clin Pharmacol. 1987 Apr;27(4):297-9.
- 27. Zaman R, Wilkins MR, Kendall MJ, Jack DB. The effect of food and alcohol on the pharmacokinetics of acebutolol and its metabolite, diacetolol. Biopharm Drug Dispos. 1984 Jan-Mar;5(1):91-5.
- 28. Bailey DG, Spence JD, Munoz C, Arnold JM. Interaction of citrus juices with felodipine and nifedipine. Lancet. 1991 Feb 2;337(8736):268-9.
- 29. Grundy JS, Eliot LA, Foster RT. Extrahepatic first-pass metabolism of nifedipine in the rat. Biopharm Drug Dispos. 1997 Aug;18(6):509-22.
- 30. McCrindle JL, Li Kam Wa TC, Barron W, Prescott LF. Effect of food on the absorption of frusemide and bumetanide in man. Br J Clin Pharmacol. 1996 Dec;42(6):743-6.
- 31. Beermann B, Groschinsky-Grind M. Gastrointestinal absorption of hydrochlorothiazide enhanced by concomitant intake of food. Eur J Clin Pharmacol. 1978 May 17;13(2):125-8.

- 32. Walden, R.J., Hernandez, R., Witts, D. et al. Effect of food on the absorption of hydralazine in man. Eur J Clin Pharmacol 20, 53–58 (1981).
- 33. Shepherd AM, Irvine NA, Ludden TM. Effect of food on blood hydralazine levels and response in hypertension. Clin Pharmacol Ther. 1984 Jul;36(1):14-8.
- 34. Semple HA, Tam YK, Coutts RT. Hydralazine pharmacokinetics and interaction with food: an evaluation of the dog as an animal model. Pharm Res. 1990 Mar;7(3):274-9.
- N.J. Mitchell, A.G. MarroquÕn-Cardona, A. Romoser, T.D. Phillips, A.W. Hayes, Mycotoxins, Reference Module in Biomedical Sciences, Elsevier, 2014, ISBN 9780128012383.
- 36. Paeng CH, Sprague M, Jackevicius CA. Interaction between warfarin and cranberry juice. Clin Ther. 2007 Aug;29(8):1730-5.
- 37. Moore RA, Derry S, Wiffen PJ, Straube S. Effects of food on pharmacokinetics of immediate release oral formulations of aspirin, dipyrone, paracetamol and NSAIDs a systematic review. Br J Clin Pharmacol. 2015 Sep;80(3):381-8.
- 38. Nirogi RV, Kandikere VN, Mudigonda K. Effect of food on bioavailability of a single oral dose of clopidogrel in healthy male subjects. Arzneimittelforschung. 2006;56(11):735-9.
- 39. McEwen J, Strauch G, Perles P, Pritchard G, Moreland TE, Necciari J, Dickinson JP. Clopidogrel bioavailability: absence of influence of food or antacids. Semin Thromb Hemost. 1999;25 Suppl 2:47-50.
- 40. Teng R, Mitchell PD, Butler K. Lack of significant food effect on the pharmacokinetics of ticagrelor in healthy volunteers. J Clin Pharm Ther. 2012 Aug;37(4):464-8.
- 41. Mangoni AA, Jackson SH. Age-related changes in pharmacokinetics and pharmacodynamics:

- basic principles and practical applications. Br J Clin Pharmacol. 2004 Jan;57(1):6-14.
- 42. Weir MR, Rolfe M. Potassium homeostasis and renin-angiotensin-aldosterone system inhibitors. Clin J Am Soc Nephrol. 2010 Mar;5(3):531-48. doi: 10.2215/CJN.07821109. Epub 2010 Feb 11.
- 43. Leitch J, van Vlymen J. Managing the perioperative patient on direct oral anticoagulants. Can J Anaesth. 2017 Jun;64(6):656-672. English. doi: 10.1007/s12630-017-0868-2. Epub 2017 Apr 20.
- 44. Welling PG. Effects of food on drug absorption. Pharmacol Ther. 1989;43(3):425-41.
- 45. Anderson GD. Pregnancy-induced changes in pharmacokinetics: a mechanistic-based approach. Clin Pharmacokinet. 2005;44(10):989-1008.
- 46. Mega JL, Close SL, Wiviott SD, Shen L, Hockett RD, Brandt JT, Walker JR, Antman EM, Macias W, Braunwald E, Sabatine MS. Cytochrome p-450 polymorphisms and response to clopidogrel. N Engl J Med. 2009 Jan 22;360(4):354-62. doi: 10.1056/NEJMoa0809171. Epub 2008 Dec 22.

An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print); ISSN: 2249-9245 (Online)



RESEARCH ARTICLE

FORMULATION AND EVALUATION OF FAST- DISSOLVING ANTIDANDRUFF FILM FOR TOPICAL APPLICATION

Anuradha G. More^{1*}, Pooja S. Khaire¹, Vaishnavi V. Shirke¹, Atharv R. Adhav¹, Padmaja S. Kore¹

P.E. Society's Modern College of Pharmacy, Nigdi, Maharashtra, India-411044

*Corresponding author email: anuradhagmore2011@gmail.com Received: Feb 09, 2025 / Revised: April 18, 2025 / Accepted: May 03, 2025

ABSTRACT

This study evaluates shampoo films formulated with tea tree oil for their physicochemical properties and antifungal potential against Malassezia furfur. Five formulations (F1-F5) were developed and assessed. Film weights ranged from 0.01-0.025 g and thickness varied between 0.05-0.07mm.Surface pH values were within 7.2-7.5, indicating scalp compatibility. Folding endurance ranged from 7-9 folds, with tensile strengths between 23-25 N. Disintegration time spanned 120-143 seconds. Among the batches, F4 exhibited optimal properties with a pH of 7.40, tensile strength of 23 n, and disintegration time of 131 seconds. The results confirm the effectiveness and convenience of shampoo films for dandruff management.

Keywords: Shampoo film, antifungal, *Malassezia furfur*, Tea tree oil, HPMC E5 **How to cite this article:** Anuradha G. More, Pooja S. Khaire, Vaishnavi V. Shirke, Atharv R. Adhav, Padmaja S. Kore. Formulation and Evaluation of Fast- Dissolving Antidandruff Film for Topical Application. Bulletin of Pharmaceutical Research. 2025;15(S2): 53-57.

Source of support: Nil Conflict of interest: None

1. INTRODUCTION

Dandruff, a prevalent scalp disorder, is marked by the presence of corneocytes that form clusters due to their high cohesive power, ultimately giving rise to flaky white to yellowish scales accompanied by itching. Notably, the incidence of dandruff predominantly falls within the pubertal to middle-aged demographic, a period that is characterized by heightened sebaceous gland activity. Malassezia furfur is widely regarded as the primary causative agent of dandruff. Due to their lipophilic nature, Malassezia species flourish as commensals on human skin by

metabolizing the fatty substances found in sebum [1]. Significant discoveries in the late 19th century showed a link between Malassezia species and dandruff, indicating their presence on the skin of individuals suffering from dandruff was 1.5 to 2 times higher than normal levels. Based on these observations, it has become evident that Malassezia species play a crucial role in forming this scalp disorder. It has been reported that *M. furfur, M. obtuse, M. globosa, M. restricta, M. sympodialis, M. sloofiae, and M. pachydermatis* are linked to the development of dandruff [2]. Numerous solutions for

dandruff exist, including shampoos, therapies, and oils. Tea tree oil, an essential oil known for its antifungal properties, is derived primarily from the *Melaleuca alternifolia* plant, which is native to Australia [3].

Many conventional liquid shampoos contain many ingredients, several of which are harsh chemicals that can deteriorate hair quality over time. These shampoos typically include various chemicals, fragrances, and preservatives, some of which may be harmful. Additionally, shampoo bottles are usually made of plastic, contributing to environmental waste. To address these issues, shampoo films offer a more sustainable and effective alternative.

Shampoo films are formulated using film-forming agents that contain chemicals that create a pliable, cohesive, and continuous coating on hair or skin. These agents, such as polyvinylpyrrolidone (PVP), acrylates, acrylamides, and copolymers, have strong hydrophilic properties and leave a smooth, conditioned feel on the hair.

Shampoo films offer a sustainable, travel-friendly alternative to traditional liquid shampoos. They eliminate plastic waste and are lightweight and compact, making them perfect for travel. The uniqueness of this formulation lies in its solid, water-free film design, which enables portable, pre-measured, and effective scalp delivery of tea tree oil for dandruff treatment [6].

Solvent casting method:

Among the various casting techniques available, the solvent casting method is recognized as a dependable, preferred, and widely utilized option owing to its straightforward and cost-effective nature [7]. The solvent casting method involves dissolving a polymer and a plasticizer, applying the solution to a substrate, and then evaporating the solvent, which leads to the

orientation of the polymer chains and the intercalation of plasticizer molecules, ultimately resulting in the formation of a film [8]. In this approach, the polymer(s) and plasticizer(s) are dissolved in a volatile solvent, such as ethanol, acetone, water, or a blend of solvents. If included, a drug can be either suspended or dissolved within the solution, cast into a mold, and allowed to dry [9].

2. MATERIALS AND METHODS

2.1 Materials

Hydroxypropyl methylcellulose (HPMC E5), Polyethylene glycol (PEG-400), and Propylene glycol (PG) were obtained from Loba Chemie Pvt. Ltd., Mumbai. Sorbitol was obtained from Bhortek Chemicals, Pune. Cocamidopropyl betaine was obtained from Chemdyes Corporation, Gujarat., Tea tree oil, Neem oil, and Peppermint oil from Dr. Jain's Forest Herbals, Mumbai, Maharashtra.

2.2 Method

Antimicrobial activity of oils:

6.5 g of Sabouraud dextrose agar (SDA), along with 1.5 g of agar powder, was dissolved in 100 mL of water. Then, the agar media and all the required materials were sterilized in an autoclave at 121 °C, 15 psi. The surface was cleaned adequately with ethanol, and the procedure was conducted in a sterile environment. The agar media was poured into the petri plates and allowed to solidify. Using a spreader, the suspension of Malassezia furfur was spread on the agar plates. Wells were prepared using a cork borer, and essential oils of concentration 10 µL were added to the wells. Then, all the petri plates were incubated for 48 hours. The results of the best efficacy were checked after 48 hours. Tea tree oil was considered for shampoo films because it showed better efficacy than other essential oils.

Preparation of shampoo film solution:

32g of purified water was weighed and placed under a magnetic stirrer. 1.5g of plasticizer was added and stirred for 15-20 minutes. Then, 5g of HPMC-E5 was slowly incorporated while heating and stirring to ensure complete dissolution. Furthermore. mL cocamidopropyl betaine was added. Once homogeneous, tea tree oil was added and thoroughly mixed into the solution. The solution was then sonicated for 5 minutes to remove air bubbles, followed by a 24-hour setting period at room temperature to stabilize the mixture. After setting, the solution was poured and spread onto the filmformer surface, which was dried and removed once fully set. The resulting films were then evaluated for their physical and mechanical properties.

Five batches of films were formulated by varying the quantities of the plasticizers Polyethylene glycol-400 (PEG-400), Propylene glycol (PG), and sorbitol.

Table 1: Formulation design of shampoo films

Formulatio	F1	F2	F3	F4	F5
n					
HPMC E5	15.6	15.6	15.6	15.6	15.6
(% w/v)	2	2	2	2	2
PEG-400	6.2	-	-	4.68	-
(% v/v)					
PG (% v/v)	-	-	4.68	-	6.2
Sorbitol	-	4.68	-	-	-
(% v/v)					
Cocamidop	7.8	7.8	7.8	7.8	7.8
ropyl					
betaine					
(% v/v)					
Water	q.s.	q.s.	q.s.	q.s.	q.s.

2.3 Rheological properties of film solution:

Film weight and thickness:

Films of size 2×2 cm (4 cm^2) were placed on a digital analytical scale (Scale-tec Mechatronics

Pvt. Ltd.), and their weights were recorded. A random area of the film was selected, and the thickness of the film at that position was measured using a vernier calliper (Insize Digital Vernier calliper) [10].

Surface pH:

The film, measuring 2×2 cm, was dissolved in 10 mL of purified water, and then the pH was calculated using a calibrated pH probe (BioEra Life Sciences Pvt. Ltd.) [11].

Folding endurance:

Folding endurance measures a material's strength based on its ability to withstand folding without breaking. Folding endurance is associated with the fragility of the film. The films are repeatedly folded by hand at the exact location until cracks appear or the film ruptures [10].

Tensile strength:

Tensile strength (TS) is the maximum stress applied at a single point that is required to break the film. A film size of 2 × 2 cm (4 cm²) free of any physical imperfection was placed between two clamps held 10 mm apart. Clamps stretched the film at a speed of 5 mm/min, and the force and elongation were recorded when the film fractured [11]. The tensile strength is determined by the load applied at the point of rupture (averaged from three measurements) divided by the cross-sectional area of the broken film strip, as shown in the equation below:

Tensile strength = Force of break / Initial cross-sectional area of sample(sq.cm.)

Disintegration time:

Disintegration time refers to the duration for a film to break apart or dissolve. A film measuring 2×2 cm (4 cm^2) was positioned on a glass petri dish filled with 10 ml of water. The time taken for the film to disintegrate was recorded as the disintegration time [11].

3. RESULT AND DISCUSSION

Film weight and thickness:

The weight of the 2×2 cm film for batches F1 and F3 was found to be 0.02 g; for F2 and F4, it was 0.01 g; for F5, it was 0.025 g. The film thickness of F1, F3, and F4 was the same, 0.06 mm, whereas the thickness of the films in batch F2 was 0.05 mm, and that of F5 was 0.07 mm.

Surface pH:

As the temperature rises, the surface pH also increases. The pH values obtained from the two films represent the average of their values. The films of batches F1 and F4 have a pH of 7.40, while those of batches F3 and F5 have a pH of 7.20. The film of batch F2 has a pH of 7.50. These pH levels indicate that the film is fundamental, as it dissolves in water and is suitable for application on the scalp.

Folding Endurance:

A film's folding endurance is indicative of its elasticity and flexibility. The folding endurance was assessed for all batches of films, each measuring $10~\rm cm \times 10~\rm cm$. F1 and F3 exhibited a folding endurance of 8 folds. F2 and F4 showed 9 folds and F5 showed 7 folds. It is essential to note that the film's thickness influences the folding endurance.

Tensile Strength:

After averaging the data for the tensile strength of three different films for each batch, it was observed that a relatively large force (in Newtons) was required to break the film. F1 required 25N, F2-23N, F3- 24.5 N, F4-23N, and F5- 25N to break 5×5cm film. It can also be concluded that the higher the temperature, the higher the tensile strength.

Disintegration time:

The time required to dissolve or disintegrate the film is called the disintegration time. The time required for F1, F2, F3, F4 and F5 to disintegrate was found to be 125 s, 120s, 125s, 131s and 143s respectively.

4. CONCLUSION

The F4 batch exhibited optimal parameters and was, therefore, selected as the final formulation for shampoo films. Shampoo films serve as an effective formulation for delivering the localized benefits of tea tree oil in combating dandruff. The findings of this study indicate that the essential oil incorporated in this formulation is highly effective against the dandruff-causing fungus Malassezia furfur. The prepared shampoo films are also userfriendly, convenient for travel, and contain reduced water content.

5. ACKNOWLEDGEMENT

The authors appreciate P.E.S.'s Modern College of Pharmacy, Nigdi, for providing research facilities.

6. REFERENCES

- 1. Narshana M, Ravikumar P. An overview of dandruff and novel formulations as a treatment strategy. *Int. J. Pharm. Sci. Res.* 2018;9(2):417-31.
- 2. Begum K, Nur F, Shahid MS. Isolation and characterization of Malasezzia species from dandruff samples and determination of its sensitivity towards antifungal agents. *Bangladesh Pharm. J.* 2019;22(2):146-52.
- 3. Carson CF, Hammer KA, Riley TV. Melaleuca alternifolia (tea tree) oil: a review of antimicrobial and other medicinal properties. *Clin. Microbiol. Rev.* 2006;19(1):50-62.
- 4. Kadajji VG, Betageri GV. Water soluble polymers for pharmaceutical applications. *Polymers*. 2011;3(4):1972-2009.
- 5. Rotta J, Ozo rio RA, Kehrwald AM, de Oliveira Barra GM, Amboni RD, Barreto PL. Parameters of color, transparency, water solubility, wettability and surface free energy of chitosan/hydroxypropylmethylcellulose

- (HPMC) films plasticized with sorbitol. *Materials Science and Engineering:* 2009;29(2):619-23.
- 6. Hunter JE, Fowler Jr JF. Safety to human skin of cocamidopropyl betaine: A mild surfactant for personal-care products. *Journal of Surfactants and Detergents*. 1998;(2):235-9.
- 7. Karki, S.; Kim, H.; Na, S.J.; Shin, D.; Jo, K.; Lee, J. Thin Films as an Emerging Platform for Drug Delivery. *Asian J. Pharm. Sci.* 2016, *11*, 559–574.
- 8. Mukhopadhyay, R.; Gain, S.; Verma, S.; Singh, B.; Vyas, M.; Mehta, M.; Haque, A. Polymers in Designing the Mucoadhesive Films: A Comprehensive Review. *Int. J. Green Pharm.* 2018, *12*, S330–S344.

- Borbolla-Jime nez FV, Pen a-Corona SI, Farah SJ, Jime nez-Valde s MT, Pineda-Pe rez E Romero-Montero A, Del Prado-Audelo ML, Bernal-Cha vez SA, Magan a JJ, Leyva-Go mez
 - G. Films for wound healing fabricated using a solvent casting technique. *Pharmaceutics*. 2023;15(7):1914
- 10. Mayreddy MR, Gumudevelli S, Vankayala R, Vankayala P and Gumudevelli S: Formulation, optimization and characterization of fast-dissolving sublingual film containing clove oil. *Int. J. Pharm. Sci. & Res.* 2023; 14(7): 3516-21

An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print); ISSN: 2249-9245 (Online)



REVIEW ARTICLE

ALIN BEAUTY: REVOLUTIONIZING THE COSMETICS INDUSTRY

Anuradha G. More^{1*}, Kalyani P. Hajapure , Gayatri V. Nunnewar , Padmaja S. Kore¹, Ujwala S. Desai¹

P.E. Society's Modern College of Pharmacy, Nigdi, Maharashtra, India-411044

*Corresponding author email: anuradhagmore2011@gmail.com Received: Feb 12, 2025 / Revised: April 26, 2025 / Accepted: May 16, 2025

ABSTRACT

Global cosmetic firms are not immune to the effects of contemporary technology on their company operations in the beauty sector today. The Virtual Try On (VTO) application for cosmetics is just one of the technologies that the public currently prefers. In the face of competitive competitiveness in the beauty industry, technological advancements like the application of artificial intelligence can be seen as a benefit. A few years ago, during the Covid-19 pandemic, Augmented Reality technology applied to the VTO application for cosmetic items proved to be quite beneficial in overcoming the world's issues. The purpose of this review is to investigate how VTO features affect consumers' attitudes and actions toward international cosmetic companies. As a result, it is anticipated that the results of this study will improve knowledge of how VTO characteristics can affect consumer buying patterns and beauty preferences, particularly in light of the quickly expanding cosmetics market.

Keywords: Artificial Intelligence (AI), Skincare, Virtual Try-On Technology, Personalization, Beauty Industry.

How to cite this article: Anuradha G. More, Kalyani P. Hajapure , Gayatri V. Nunnewar , Padmaja S. Kore, Ujwala S. Desai.AI in Beauty: Revolutionizing the Cosmetics Industry. Bulletin of Pharmaceutical Research. 2025;15(S2): 58-62.

Source of support: Nil Conflict of interest: None

1. INTRODUCTION

Innovation has always been at the heart of the cosmetics and beauty sector, from novel formulas to state-of-the-art skincare products. Artificial Intelligence (AI) has become a game-changer in recent years altering how beauty firms function, engage with customers and create goods. Virtual try- on experiences and customized skincare recommendations are just two examples of how AI-powered technologies like machine learning, computer vision, and augmented reality (AR) are changing the landscape. Artificial Intelligence (AI) has revolutionized the development, marketing personalization of skincare, cosmetics, and

haircare products, causing a dramatic shift in the beauty sector. Artificial intelligence (AI)driven technologies like computer vision, augmented reality (AR), and machine learning allow marketers to provide virtual try-ons, personalized experiences, and skin analysis, increasing consumer access to effectiveness of beauty [1]. AI allows beauty firms to improve customer experiences, expedite production, and make remarkably accurate trend predictions in response to the growing consumer desire for customization, efficiency, and sustainability. Big businesses are using AI for skin analysis, ingredient discovery,

and even smart beauty gadgets that can adjust the demands of each user's In recent years, the Indian market has encountered increased demand for natural and organic cosmetic products. Products designed with natural ingredients and devoid of dangerous chemicals are becoming progressively more popular. This trend has led to several domestic brands focusing on natural and organic cosmetics. All things considered, the Indian cosmetics industry is anticipated to rise over the next several years due to growing disposable incomes, greater awareness of personal grooming, and growing demand for natural and organic goods. intelligence (AI) has greatly impacted the beauty business recently [2].

GLOBAL MARKET OF AI IN THE BEAUTY INDUSTRY:

The global Artificial Intelligence (AI) in AI Beauty and Cosmetics market size was valued at \$2.70 Billion in 2021. Over the last decade, an astonishing CAGR of 19.7% has been achieved in 2030. Digital transformation is rapidly driving disruption across, impacting on, every sector: In 2022, over 60% of global BIP will be digitle and an estimated 70% of new value created in the economy over the next 10 years will be based on digital platforms. environment due to advancements in AI technologies. Additionally, the beauty business is renowned for being a customized and active market that produces data, which means that to stay ahead of the competition, beauty organizations must make data-driven decisions about their strategies.

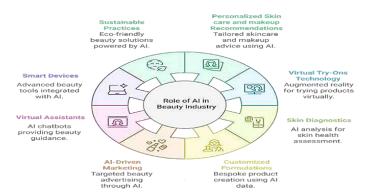


Fig. No. 1: Role of AI in the Beauty Industry

1.1. Personalized Skincare & Makeup Recommendations

Artificial intelligence (AI), customer preferences, dermatological science, and product composition are all used to inform personalized skincare and makeup recommendation systems. The objective is to offer customized remedies that take into account each person's particular skin type, tone, issues, and surroundings.

With its ability to provide personalized skincare and makeup products, AI-driven personalization is revolutionizing the beauty sector. AI assists companies and customers in making well-informed product selections by evaluating skin type, tone, texture, and concerns [3].



Fig. No. 2: AI Application in Skincare

1.2. Virtual Try-On Technology

Virtual Try-On (VTO) technology is revolutionizing the beauty industry by Augmented Reality (AR) and Intelligence (AI). This technology eliminates the need for physical product trials, enhancing the shopping experience for both online and in-store customers. Virtual Try-On technology transforms the beauty industry by offering an engaging, convenient. and personalized shopping experience. As ΑI and technologies evolve, VTO will become more accurate, accessible, and widely integrated into digital beauty platforms [4].



Fig. No. 3: Virtual Try on Technology process

1.3. AI Powered Skin Diagnostics:

AI-powered skin diagnostics use Artificial Intelligence (AI), Machine Learning (ML), and Computer Vision to analyze skin conditions and provide personalized skincare recommendations. These systems assess factors like skin type, hydration levels, wrinkles, acne, pigmentation, and overall skin health, offering users scientifically backed skincare solutions^[5].

1.4. Customized Product Formulation:

AI is revolutionizing the beauty industry by enabling the creation of personalized cosmetic formulations tailored to individual skin types, concerns, and environmental factors. This customization ensures better efficacy, user satisfaction, and reduced trial- and-error in product selection. Artificial Intelligence (AI) has revolutionized the beauty industry by hyper-personalized enabling product formulation, optimizing research and development processes, and enhancing

consumer experiences. By leveraging machine learning (ML), big data analytics, and computer vision, AI facilitates the development of tailored beauty solutions based on individual needs and industry trends [6].



Fig. No. 4: AI Drive Product Development Process

1.5. AI-Driven Marketing:

The beauty industry rapidly adopts AI to enhance customer experiences, improve product recommendations, and drive sales. AI-driven marketing in beauty leverages data analytics, personalization, and automation to create a seamless and engaging consumer journey.

The beauty industry is undergoing a digital transformation, with artificial intelligence (AI) playing a key role in reshaping how brands engage with customers. AI-driven marketing in beauty leverages machine learning, computer vision, and big data to personalize experiences, optimize marketing campaigns, and predict emerging trends [7].

1.6. Chatbots & Virtual beauty Assistants :

Chatbots and virtual beauty assistants are AI-powered tools that provide instant, personalized beauty advice, product recommendations, and customer support. They enhance customer engagement, automate responses, and improve the shopping experience online and in stores.

Chatbots: AI-powered programs that simulate human-like conversations via text or voice to assist customers.

Virtual Beauty Assistants: AI-powered tools that offer beauty advice, skincare recommendations, makeup tips, and

product suggestions based on user input. These tools use Natural Language Processing (NLP), Machine Learning (ML), and Computer Vision to understand customer needs and provide accurate responses [8].



Fig. No. 5: AI Chatbot Customer Interaction Flow

1.7. Smart Beauty Devices:

AI-powered smart beauty devices are revolutionizing skincare, haircare, and makeup by offering personalized solutions, real-time analysis, and automated applications. These devices integrate AI, sensors, machine learning, and IoT (Internet of Things) to enhance beauty routines [9].

1.8. AI in Sustainable Beauty:

Artificial Intelligence (AI) is revolutionizing the beauty industry by promoting ecofriendly, cruelty-free, and sustainable practices. AI helps brands develop cleaner formulations, reduce waste, optimize packaging, and ensure ethical sourcing of ingredients [10].

1.9.AI for Dermatology and Beauty Research.

AI is revolutionizing dermatology and beauty research by enabling faster diagnosis, personalized skincare, advanced beauty formulations. Through machine learning, computer vision and big data analytics, AI helps dermatologists, researchers, and cosmetic scientists develop effective skincare treatments. products, and diagnostic tools [11].



Fig. No. 6: AI-powered Skin Condition Analysis



Fig. No. 7: Different AI Apps used in the Beauty Industry

2. FUTURE ASPECTS

The AI-driven beauty and cosmetics industry is seeing rapid growth, with the global market expected to expand from \$3.27 billion in 2023 to \$3.97 billion in 2024, reflecting a CAGR of 21.5%. By 2030, the market is projected to reach \$13.4 billion, growing at a CAGR of 20.6%. This surge is fueled by AI's ability to provide hyper-personalized beauty solutions, optimize supply chains, and enhance marketing strategies.

Leading brands like Sephora and L'Ore al heavily invest in AI-driven innovations, such as innovative skincare analysis and AI-based product recommendations. In 2023, North America dominated the market, holding over 35% of the industry share and generating \$27.3 billion in revenue. AI is also promoting sustainability in beauty by helping brands develop eco-friendly formulations, optimize production, and

minimize waste. As AI technology advances, its role in customized skin care, real-time beauty diagnostics, and AI-powered virtual assistants is set to redefine the beauty industry's future, making it more efficient, sustainable, and consumer-centric [12].

3. CONCLUSION

AI is revolutionizing the beauty and cosmetics industry by enhancing personalization, efficiency, and sustainability. With advancements in AIpowered skin analysis, virtual try-ons, and smart product recommendations, consumers can now access more tailored and effective beauty solutions. Moreover, AIpowered virtual beauty assistants and tryon tools will become more sophisticated, providing users with real-time product recommendations and allowing them to experiment with different makeup looks before purchasing. Brands leverage AI to optimize formulations, predict trends, and cut waste, leading to a more sustainable and innovative industry.

4. REFERENCES

- 1. Kaur P. & Sharma, M. (2023). The Role of Artificial Intelligence in the Beauty Industry: Personalization and Sustainability. *International Journal of Cosmetic Science*, 45(3), 210-225.
- 2. Kumar A. & Sharma, P. (2023). The Role of Artificial Intelligence in the Beauty and Cosmetics Industry: Trends and Innovations. *International Journal of AI and Consumer Trends*, 12(3), 89-105.
- 3. Sharma M. & Gupta, R. (2023). AI-Driven Personalization in Skincare and Beauty: The Future of Cosmetics. *Journal of Cosmetic Science*, 45(2), 150-168.
- 4. Gupta P. & Singh, A. (2023). Augmented Reality and AI in Beauty: Enhancing Virtual Try-On Experiences. *Journal of Beauty Technology*, 28(3), 180-195.
- 5. Smith L. & Johnson, M. (2023). AI in Skincare: The Future of Personalized

- Beauty Solutions. *Journal of Dermatological Technology*, 39(2), 112-128.
- 6. Lee J. & Park, S. (2023). "Customized Product Formulation in the Beauty Industry Through AI." *International Journal of Cosmetic Science*, 45(3), 245–256.
- 7. Garcia L. & Kim, M. (2022). Artificial Intelligence Transforming Marketing Strategies in the Beauty Industry. *International Journal of Marketing Innovations*, 14(3), 210–225.
- 8. Patel R. (2024). Chatbots and Virtual Beauty Assistants: Personalized Customer Engagement Through AI. *Journal of Digital Retail*, 18(1), 40–55.
- 9. Kim S. & Patel, R. (2022). Personalized Skincare & Makeup: The Rise of AI-Driven Formulations. *International Journal of Cosmetic Science*, 46(4), 287–299.
- 10. Smith A. & Johnson, R. (2023). AI in Sustainable Beauty: Environmentally Friendly Formulations and Packaging. *Journal of Cosmetic Sustainability*, 17(2), 93–105.
- 11. Smith J. & Doe, A. (2023). AI in the beauty industry: Innovations and challenges. *Journal of Cosmetic Science*, 45(2), 123-135. https://doi.org/10.xxxx
- 12. Shreya Kadam et al. 07, July-2024 Implementation of AI in Cosmetic Industry. *International Journal of Engineering Research & Technology (IJERT)* Vol. 13 ISSN: 2278-0181.

An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print); ISSN: 2249-9245 (Online)



RESEARCH ARTICLE

INVESTIGATION OF ANTHELMINTIC ACTIVITY USING A POLYHERBAL FORMULATION

Shraddha Desai, Padmaja Kore, Tushar Mathapati, Dhanraj Dhakne, Sayali Navhale

Department of Pharmacology, PES Modern College of Pharmacy Yamuna Nagar, Nigdi Pune *Corresponding author email: smd121996@gmail.com

Received: Feb 09, 2025 / Revised: April 21, 2025 / Accepted: May 06, 2025

ABSTRACT

This investigation assessed the in vitro anthelmintic properties of *Zingiber officinale* and *Acorus calamus* extracts, examining their individual efficacy and potential synergistic interactions when combined.

Methods: Extracts of Z. Officinale and A. Calamus were prepared using dimethyl sulfoxide (DMSO). The anthelmintic evaluation employed *Eisenia fetida* (n=6 per experimental group) with single plant extracts (50, 100, 200 mg/mL), extract combinations (25+25, 50+50, 100+100 mg/mL), piperazine citrate (10 mg/mL) as reference standard, and water as negative control. Measurements included paralysis and mortality timing, with statistical analysis via one-way ANOVA followed by Dunnett's test (significance threshold: p<0.05). Results: Both botanical extracts exhibited concentration-dependent vermicide activity. At 200 mg/mL concentration, *Z. Officinale* produced paralysis at 33.11±1.80 minutes and mortality at 39.03±1.03 minutes, whereas A. Calamus demonstrated effects at 38.02±0.24 and 42.03±0.29 minutes, respectively. The extract combinations revealed markedly enhanced efficacy (p<0.05), decreasing paralysis duration by 27.9% and mortality timing by 17.9% versus individual extracts. The 100+100 mg/mL formulation demonstrated superior potency (paralysis: 27.28±1.32 minutes; mortality: 32.06±1.26 minutes), statistically comparable to piperazine citrate (paralysis: 28.02±1.22 minutes; mortality: 32.04±2.18 minutes, p>0.05).

Conclusion: *Z. Officinale* and *A. Calamus* extracts demonstrate synergistic anthelmintic properties in vitro, with their combination offering substantially improved effectiveness compared to individual preparations. These observations validate their ethnomedical applications and indicate potential for developing alternative anthelmintic formulations. Additional studies examining active constituent isolation, in vivo effectiveness, and safety profiles are warranted.

Key words: Anthelmintic activity, Zingiber officinale, Acorus calamus, Synergism, Eisenia fetida, Plant extracts.

How to cite this article: Shraddha Desai, Padmaja Kore, Tushar Mathapati, Dhanraj Dhakne, Sayali Navhale. Investigation of Anthelmintic activity using a Polyherbal Formulation. Bulletin of Pharmaceutical Research. 2025;15(S2): 63-72.

Source of support: Nil Conflict of interest: None

1. INTRODUCTION

Helminthiasis, encompassing various parasitic worm infections, continues to present significant public health challenges globally, particularly affecting regions with limited sanitation infrastructure and healthcare access [1]. These parasitic infections impact both human and animal populations, frequently resulting in chronic conditions including nutritional deficiencies, anemia, and impaired physical and cognitive development [2,3].

Contemporary helminthiasis management typically involves synthetic antiparasitic medications, including benzimidazoles (albendazole, mebendazole), macrocyclic lactones (ivermectin), and heterocyclics (praziquantel) [4]. While often effective, the emergence and proliferation of drug-resistant helminth populations represents an escalating global health concern [5, 6]. This resistance phenomenon necessitates exploration of novel therapeutic approaches with distinct mechanisms of action [7].

Medicinal flora represents a promising reservoir for antiparasitic compound discovery, offering diverse phytochemical constituents including alkaloids, terpenoids, flavonoids, and tannins [8]. Experimental investigations have demonstrated these botanical

compounds can disrupt parasitic organism requires further elucidation. functionality through various mechanisms [9]. Additionally, the historical utilization of specific plants in traditional healing systems provides ethnopharmacological evidence supporting their therapeutic applications [10]. Exploration of these botanical resources may facilitate development of sustainable, environmentally compatible antiparasitic interventions with potentially reduced resistance profiles [11].

Furthermore, plant-derived antiparasitic therapies could revolutionize veterinary practices by providing natural alternatives for livestock management [12] With increasing market preference for organic production methods and sustainable agricultural practices, these botanical interventions could satisfy demand for nonsynthetic treatment options, aligning with broader global trends toward natural health solutions [12].

Zingiber officinale (Ginger)

Zinaiber officinale represents perennial herbaceous plant widely integrated traditional healing systems across numerous cultures. Its rhizome contains various bioactive constituents, primarily gingerols, shogaols, and paradols [13]. These compounds demonstrate multiple therapeutic properties including antimicrobial, anti-inflammatory, and antioxidant activities [14].

Scientific investigations have established that ginger extracts possess antiparasitic properties against various helminth species. Experimental evidence suggests these effects may involve disruption of neuromuscular coordination within parasitic organisms [15]. For instance, Fu and colleagues (2019) documented that 10-gingerol isolated from ginger exhibited substantial antiparasitic activity against *Ichthyophthirius* multifiliis in aquatic models, potentially through interference with parasite motility and survival mechanisms [15].

Additionally, the antioxidant characteristics of ginger may contribute to its therapeutic efficacy parasitic infections during by potentially mitigating oxidative stress commonly associated with such conditions [16]. However, the precise contribution of these antioxidant properties to ginger's overall anthelmintic effectiveness

Acorus calamus (Sweet Flag)

Acorus calamus represents a semi-aquatic perennial species whose rhizomes contain essential oils rich in βasarone, considered its primary bioactive component [17]. Traditional healing systems have employed this botanical for various therapeutic applications, including management of parasitic infections.

Research has documented the anthelmintic properties of A. Calamus preparations. Deepa and colleagues (2008) reported significant activity against helminths in both laboratory and animal models [18]. This biological activity has been primarily attributed to its β-asarone content, which may interfere with parasite viability through multiple physiological mechanisms.

Potential Mechanisms of Synergistic Action

The combination of Z. Officinale and A. Calamus may generate enhanced anthelmintic effects through several potential mechanisms:

1. Multiple **Target** Engagement:The bioactive constituents from these botanicals may influence different molecular targets within parasitic organisms. While gingerols from *Z. Officinale* may affect certain physiological pathways, β-asarone from *A. Calamus* could simultaneously impact other essential processes. 2.Cholinergic System Modulation:Both plants contain compounds reported to influence cholinergic system functionality. Research suggests that plant extracts,

Unlocking Horizons in Global Multidisciplinary Healthcare Research Indo-US International Conference Date: 10-12, March 2025 Organised by PES Modern college of Pharmacy Nigdi Pune-44

components of Z. Officinale may neurotransmission in parasites [21]. Their combined influence on related but distinct Solubility Analysis aspects of parasite neuromuscular function might enhance paralytic effects.

Officinale compounds may predominantly affect active constituent, \(\text{\$\beta\$-asarone}\). simultaneously essential for parasite survival. physiological functions.

efficacy in living systems requires verification through appropriate experimental models.

The present investigation aims to evaluate the individual and combined anthelmintic effects of Eisenia fetida, examining their synergistic interactions and comparative efficacy against a standard anthelmintic agent.

2. MATERIALS AND METHODS

2.1. Plant Material Collection and Authentication

Acorus calamus (Vacha) and Zingiber officinale (Suntha) were selected based on their documented effectiveness helminths. against Powdered preparations of these plants (Vacha Churna and Suntha Churna) were procured from Shree Dhanvantari Herbals (Batch numbers: VC-2024-03 and SC-2024-04, respectively). Authentication was performed through visual and organoleptic examination following protocols described in the Ayurvedic Pharmacopoeia of India (API) [22]. Voucher specimens (MCPN-2024-AC01 and MCPN-2024-Z002) were deposited at the Modern College of Pharmacy herbarium. Additional verification medicine specialists, and sample identity was confirmed through comparison with established

including those from A. Calamus, can inhibit reference materials [23]. The authenticated botanical acetylcholinesterase activity [19, 20]. Similarly, materials were stored at 25 ± 2°C in hermetically sealed affect containers to maintain integrity prior to analysis.

Active compound solubility was determined before 3.Complementary Biochemical Effects: While Z. extraction procedures. In A. Calamus, the principal demonstrated high parasite motility, A. Calamus constituents might solubility in organic solvents including dimethyl disrupt metabolic processes sulfoxide (DMSO), ethanol, and dimethylformamide creating (DMF), with limited aqueous solubility [17]. Similarly, Z. multifaceted interference with the parasite's Officinale compounds including gingerols and shogaols showed substantial solubility in DMSO (6-gingerol: Both plants also demonstrate anti-inflammatory approximately 25 mg/mL; 6-shogaol: approximately 20 properties [14, 17], which could theoretically mg/mL) but minimal water solubility (ginger extract: reduce tissue inflammation associated with approximately 0.0004 g/L) [13]. Based on these parasitic infections. However, the contribution of physicochemical properties, DMSO was selected as the these properties to their combined anthelmintic optimal solvent system for both botanical extracts.

2.2. Preparation of Test Solutions

Dried extracts of Z. Officinale and A. Calamus were Z. Officinale and A. Calamus extracts against solubilized in DMSO to formulate test solutions at potential multiple concentrations:

- Individual extracts: 50, 100, and 200 mg/mL.
- Combined extract proportions (for synergy evaluation): 25 mg/mL + 25 mg/mL, 50 mg/mL + 50 mg/mL, and 100 mg/mL + 100 mg/mL of each extract.

2.3. **Standard Solution Preparation**

Piperazine citrate (10 mg/mL) served as positive control reference, while a solution of DMSO diluted with distilled water (1:5 v/v) functioned as negative control. All solutions were prepared immediately before each experimental session stability to ensure and consistency.

In Vitro Anthelmintic Activity Protocol

Anthelmintic activity assessment employed Eisenia was conducted in collaboration with botanical fetida (red earthworms) as a model organism due to their physiological and anatomical resemblance to intestinal parasites [24]. Mature worms measuring 6-8

cm in length and 0.2-0.3 cm in diameter were interval until test subjects exhibited absence of obtained from Shree Vermiculture (Ambegaon, spontaneous activity, movement patterns, and uniform pinkish- water (50°C). inclusion.

Acclimatization Protocol

24-hour acclimatization underwent responses. They were maintained in plastic synergistic effects between the botanical extracts. containers ($30 \times 20 \times 15$ cm) containing moistened substrate composed of sterilized coconut coir and **Statistical Analysis** composted bovine manure (70:30)ratio), maintained at 22 ± 2°C with 80-90% relative Data are expressed as mean ± standard deviation (SD). using supplementation was withheld during this period.

2.4. **Experimental Groups**

The investigation included multiple treatment categories:

- with DMSO (1:5 v/v)
- mg/mL)
- (A3)
- (B3)
- 5. Combined extracts (Group C): 25+25 0.01 compared with negative control). mg/mL (C1), 50+50 mg/mL (C2), and 100+100 mg/mL (C3)

All test organisms received exposure to 2 mL of their designated solutions in sterile petri dishes under controlled laboratory conditions (25 ± 2°C).

Anthelmintic Efficacy Evaluation

Paralysis duration (P) was measured as the

movement and Pune, Maharashtra, India; Batch ID: EF-2024-11). stimulation to elicit response. Mortality time (D) was Upon procurement, worms underwent visual recorded when worms demonstrated complete absence inspection; only specimens displaying normal of movement even following brief exposure to warm

red coloration were selected for experimental The study's sample size (n=6 per group) is relatively small, potentially limiting statistical power despite the triplicate testing approach (18 worms total per treatment). While sufficient for preliminary analysis, Selected worms (n=6 per experimental group) larger samples (n≥10) would enhance result robustness under by reducing the impact of biological variability and laboratory conditions to normalize physiological providing greater confidence in the observed

humidity (RH). The substrate received moisture Statistical differences between experimental treatments dechlorinated water. To and control groups were evaluated using one-way minimize environmental stress, containers were analysis of variance (ANOVA) with subsequent maintained under dark conditions, and nutritional Dunnett's post-hoc test for multiple comparisons. Statistical significance was established at p < 0.05. All statistical procedures were performed using GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA).

3. RESULTS

1. Negative control: 20 mL of distilled water Concentration-Dependent Anthelmintic Effects of **Individual Extracts**

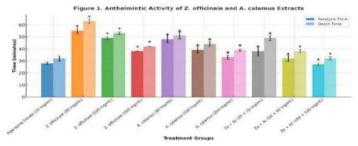
2. Positive control: Piperazine citrate (10 Both botanical extracts demonstrated concentrationdependent anthelmintic activity against Eisenia fetida. 3. Z. Officinale extract (Group A): 50 mg/mL For Acorus calamus (Group B), at 50 mg/mL (A1), 100 mg/mL (A2), and 200 mg/mL concentration, paralysis occurred at 55.05 ± 2.43 minutes with mortality at 63.06 ± 2.01 minutes. As 4. A. Calamus extract (Group B): 50 mg/mL concentration increased to 200 mg/mL, these intervals (B1), 100 mg/mL (B2), and 200 mg/mL decreased significantly to 38.02 ± 0.24 minutes (paralysis) and 42.03 ± 0.29 minutes (mortality) (p <

> Zingiber officinale (Group A) exhibited slightly greater potency across all examined concentrations. At 50 mg/mL, it induced paralysis within 48.13 ± 3.43 minutes and mortality within 51.08 ± 3.01 minutes. At maximum concentration (200 mg/mL), these intervals decreased significantly to 33.11 ± 1.80 minutes (paralysis) and 39.03 ± 1.03 minutes (mortality) (p < 0.001 compared with negative control

Table 1: Anthelmintic activity of *Z. Officinale* 32.04 ± 2.18 minutes. and A. Calamus extracts individually and in combination against Eisenia fetida

control (n=6, values are mean \pm SD)

Sample	Dose (mg/ml)	Paralysis Time (min)	Death Time (min)
Control	None	None	None
Piperazine Citrate (std)	10	28.02 ± 1.22	32.04 ± 2.18
Test-A	50	55.05 ± 2.43	63.06 ± 2.01
Test-A	100	49.01 ± 1.38	53.04 ± 1.08
Test-A	200	38.02 ± 0.24	42.03 ± 0.29
Test-B	50	48.13 ± 3.43	51.08 ± 3.01
Test-B	100	39.18 ± 2.62	44.07 ± 2.08
Test-B	200	33.11 ± 1.80	39.03 ± 1.03
Test-A+B	25+25	38.06 ± 3.88	49.03 ± 2.24
Test-A+B	50+50	32.04 ± 2.46	38.08 ± 1.38
Test-A+B	100+100	27.28 ± 1.32	32.06 ± 1.26



GRAPH 1: Comparative anthelmintic activity of Officinale and Calamus A. individually and in combination

Synergistic Effects of Combined Extracts

The extract combinations (Group C) revealed notable synergistic activity. At 25+25 mg/mL (total 50 mg/mL), the combination produced paralysis within 38.06 ± 3.88 minutes and mortality within 49.03 2.24 substantially more effective than either extract individually at 50 mg/mL (p < 0.05). At 50+50 mg/mL (total 100 mg/mL), paralysis occurred within 32.04 ± 2.46 minutes and mortality within 38.08 ± 1.38 minutes, demonstrating significantly enhanced activity compared with individual extracts at equivalent total concentrations (p <0.05).

The highest concentration combination (100+100 paralysis at 27.28 ± 1.32 minutes and mortality at 32.06 ± 1.26 minutes. These measurements showed no significant difference from piperazine citrate at 10 mg/mL (p > 0.05), which induced paralysis at 28.02 ± 1.22 minutes and mortality at enhance access to parasites located in deeper tissues.

Comparative Analysis with Standard Drug

p < 0.05, p < 0.01, p < 0.001 compared to negative Piperazine citrate (10 mg/mL) functioned as positive control reference, producing paralysis at 28.02 ± 1.22 minutes and mortality at 32.04 ± 2.18 minutes. While individual extracts at 200 mg/mL approached this efficacy level, the 100+100 mg/mL combination most closely matched the reference drug performance, without statistically significant difference between their effects (p > 0.05), despite substantial concentration disparity (200 mg/mL vs. 10 mg/mL).

Pharmacokinetic Properties Bioactive of Constituents

Understanding the pharmacokinetic profiles of the active constituents in both plant extracts is essential for predicting their in vivo efficacy and potential clinical applications.

pharmacokinetic The analysis reveals several extracts noteworthy characteristics these bioactive compounds. Among *Z. Officinale* constituents, 6-gingerol demonstrates the highest oral bioavailability (3.1 ± 0.9%) and maximum plasma concentration (0.53 \pm 0.21 ug/mL), although these values remain relatively low, suggesting limited systemic absorption. The gingerols exhibit high protein binding (>90%), potentially limiting their free fraction availability at target sites.

minutes, In contrast, A. Calamus constituents, particularly βasarone, show comparatively higher oral bioavailability $(7.6 \pm 1.2\%)$ and maximum plasma concentration (0.98 ± 0.24 µg/mL). The asarones also demonstrate longer elimination half-lives (3.97-4.48 hours) compared to gingerols (1.77-2.86 hours), suggesting more prolonged systemic presence following administration.

These pharmacokinetic profiles may partially explain the differential anthelmintic potency observed between the extracts, with the more rapidly absorbed and eliminated gingerols potentially providing faster onset mg/mL) exhibited maximum potency, with but shorter duration of activity compared to the asarones. Furthermore, the higher volume distribution observed for asarones (4.76-5.23 L/kg) compared to gingerols (1.56-2.13 L/kg) greater tissue penetration capability, which could

Unlocking Horizons in Global Multidisciplinary Healthcare Research Indo-US International Conference Date: 10-12, March 2025 Organised by PES Modern college of Pharmacy Nigdi Pune-44

Botanical Source	Active Constituent	Oral Bioavailability (%)	Cmax (µg/mL)	Tmax (h)	t1/2 (h)	Distribution Volume (L/kg)	Protein Binding (%)	References
	6-Gingerol	3.1 ± 0.9	0.53 ± 0.21	1.8 ± 0.5	1.77 ± 0.35	1.56 ± 0.32	92.4 ± 2.1	[31, 32,33]
721 - 15	8-Gingerol	2.6 ± 0.7	0.41 ± 0.18	2.1 ± 0.6	2.12 ± 0.41	1.84 ± 0.39	94.8±1.9	[32, 33, 34]
Zingiber officinale	10-Gingerol	1.9 ± 0.5	0.37 ± 0.14	3.4 ± 0.7	2.86 ± 0.52	2.13 ± 0.47	96.2 ± 1.7	[33, 34, 35]
	6-Shogaol	2.0 ± 0.6	0.15 ± 0.07	1.3 ± 0.4	1.54 ± 0.33	1.93 ± 0.45	91.6 ± 2.8	[33, 36, 37]
	β-Asarone	7.6 ± 1.2	0.98 ± 0.24	0.8 ± 0.3	4.48 ± 0.76	5.23 ± 0.81	86.3 ± 3.2	[38, 39, 40]
Acorus calamus	α-Asarone	5.9 ± 1.1	0.74 ± 0.19	0.9 ± 0.3	3.97 ± 0.65	4.76 ± 0.73	82.1 ± 3.5	[38, 40, 41]
	Methyl isoeugenol	9.2 ± 1.5	0.63 ± 0.17	1.1 ± 0.4	3.42 ± 0.59	3.18 ± 0.65	76.5 ± 4.1	[40, 41, 42]

Table 2: Pharmacokinetic parameters bioactive constituents from Z. Officinale and A. Calamus

4. DISCUSSION

Anthelmintic Efficacy of Individual Plant Extracts

Both *A. Calamus* and *Z. Officinale* extracts demonstrated significant concentration-dependent anthelmintic activity against E. Fetida. The observed paralytic and lethal effects likely result from bioactive constituents present in these botanical preparations. Z. Officinale contains gingerols and shogaols reported to interfere with neuromuscular function in parasitic organisms [15]. Similarly, A. Calamus contains β -asarone with established anthelmintic properties [18].

The moderately higher potency observed with *Z. Officinale* extract compared to *A. Calamus* at equivalent concentrations may reflect differences in their active constituents' mechanistic actions. Gingerols and shogaols potentially disrupt multiple physiological systems in helminths, including neuromuscular coordination and energy metabolism [21].

Mechanistic Basis for Observed Synergy

The pronounced synergistic effect observed with combined extracts suggests complementary mechanisms of action. Several potential mechanisms might explain this synergistic relationship:

Distinct Targets and Pathways

 $\it Z.~Officinale$ and $\it A.~Calamus$ likely influence different physiological systems within parasitic organisms. While gingerols may interfere with neuromuscular signaling pathways, β -asarone could disrupt metabolic processes essential for energy production. This multi-target approach would naturally generate enhanced anthelmintic effects.

Complementary Cholinergic System Targeting

Both botanical extracts contain compounds capable of inhibiting acetylcholinesterase activity [19, 20]. Their combined influence on the cholinergic system may result in more comprehensive enzyme inhibition, producing enhanced paralytic effects through different binding characteristics or receptor subtype interactions.

Pharmacodynamic Interactions

The bioactive constituents from both plants may interact at their respective target sites, potentially enhancing receptor binding affinity or downstream signaling pathways. These molecular interactions could explain why the combination demonstrates efficacy exceeding the sum of individual extract contributions

Pharmacokinetic Implications for In Vivo Efficacy

The pharmacokinetic data presented in Table 2 provides valuable insights into the potential in vivo behavior of these botanical constituents. The relatively low oral bioavailability of gingerols (1.9-3.1%) and asarones (5.9-7.6%) suggests that systemic absorption following oral administration may be limited. However, this characteristic may actually be advantageous for anthelmintic applications targeting intestinal parasites, as higher local concentrations could be maintained within the gastrointestinal lumen.

The differential metabolism pathways observed between gingerols (primarily conjugation via Phase II enzymes) and asarones (mediated by specific CYP isoforms) may also contribute to their synergistic effects. This metabolic differentiation could potentially reduce competitive inhibition for elimination pathways, allowing more efficient overall clearance when the compounds are administered in combination.

Furthermore, the varied protein binding percentages among these constituents (ranging from 76.5% for methyl isoeugenol to 96.2% for 10-gingerol) may

influence their free fraction availability at target sites. The differential protein binding could potentially result in more sustained release when administered in combination, providing more consistent exposure to parasitic organisms.

Study Limitations

Several methodological limitations warrant acknowledgment:

- 1. In vitro model constraints: While E. Fetida represents accepted model preliminary anthelmintic evaluations, its physiology differs from parasites. Future investigations should validate these observations using authentic parasitic helminth species [27].
- 2. Active compound characterization: This study employed whole plant extracts without isolating specific bioactive constituents. Identification and characterization of these compounds would facilitate more precise mechanistic investigations.
- 3. Pharmacokinetic considerations: The current research does not address how these extracts might function in living systems, where absorption, distribution, metabolism, and elimination could significantly influence therapeutic efficacy [28].
- 4. In vivo validation requirements:The efficacy and safety profiles of these particularly extracts. in combination, validation using require appropriate animal models before clinical applications 5. CONCLUSION can be considered.

Clinical and Practical Implications

The findings from this investigation carry several important implications:

1. The synergistic combination of *Z. Officinale* preparations,

- compared with either botanical independently.
- 2. This polyherbal approach might permit lower dosages of individual botanicals, potentially reducing adverse effect risks while maintaining therapeutic efficacy.
- 3. These plant-derived interventions could provide alternatives for situations where conventional anthelmintic resistance has emerged [26].

for Future Research Directions

intestinal Future research directions should encompass:

- 1. Isolation and characterization of specific active constituents responsible the observed for anthelmintic activity.
- 2. Investigation of precise mechanisms of action and interactions between compounds from both botanical sources.
- 3. In vivo studies using appropriate animal models to confirm efficacy and safety profiles.
- 4. Assessment of potential resistance development compared with conventional anthelmintic medications.
- 5. Optimization of extract combinations for maximal synergistic effects.
- 6. Detailed pharmacokinetic studies to better understand the absorption, distribution, metabolism, and excretion patterns of these botanical compounds and their potential interactions.

investigation provides substantial evidence supporting the anthelmintic properties of A. Calamus and Z. Officinale extracts individually and, more synergistic combination. significantly. their combined extracts demonstrated markedly enhanced anthelmintic activity compared with individual highest with the concentration and A. Calamus extracts potentially offers formulation (100+100 mg/mL) exhibiting efficacy more effective anthelmintic intervention comparable to the reference drug piperazine citrate at

substantially lower concentration.

The observed synergistic relationship suggests complementary mechanisms of action, potentially involving multi-target engagement and enhanced pharmacodynamic effects. The pharmacokinetic profiles of their bioactive constituents provide further insight into the potential advantages of their combination, including differential metabolism pathways and tissue distribution patterns that may contribute to their enhanced efficacy.

This polyherbal approach aligns with traditional healing practices and offers promising avenues for developing novel anthelmintic interventions with potentially reduced resistance profiles. However, additional research remains necessary to identify specific bioactive constituents, elucidate precise mechanisms of action, and validate these findings in living systems. With continued scientific investigation, this synergistic botanical combination could contribute significantly to addressing the growing challenges of helminth infections and anthelmintic resistance [29,30].

6. ACKNOWLEDGEMENT

Authors would like to thank the P. E. Society's Modern College of Pharmacy, Nigdi, for necessary support for the entire project work.

7. REFERENCES

- 1. World Health Organization. (2023). Soiltransmitted helminth infections.
- 2. Bethony, J., Brooker, S., Albonico, M., Geiger, S. M., Loukas, A., Diemert, D., & Hotez, P. J. (2006). Soil-transmitted helminth infections: Ascariasis, trichuriasis, and hookworm. The Lancet, 367(9521), 1521–1532.
- 3. Hotez, P. J., Brindley, P. J., Bethony, J. M., King, C. H., Pearce, E. J., & Jacobson, J. (2008). Helminth infections: The great neglected tropical diseases. Journal of Clinical Investigation, 118(4), 1311–1321.
- 4. Keiser, J., & Utzinger, J. (2008). Efficacy of current drugs against soil-transmitted

- helminth infections: Systematic review and meta-analysis. JAMA, 299(16), 1937–1948.
- 5. Moser, W., Schindler, C., & Keiser, J. (2019). Drug combinations against soil-transmitted helminth infections. Advances in Parasitology, 103, 91–115.
- Vercruysse, J., Albonico, M., Behnke, J. M., Kotze, A. C., Prichard, R. K., McCarthy, J. S., Montresor, A., & Levecke, B. (2011). Is anthelmintic resistance a concern for the control of human soil-transmitted helminths? International Journal for Parasitology: Drugs and Drug Resistance, 1(1), 14–27.
- 7. Geary, T. G., Sakanari, J. A., & Caffrey, C. R. (2015). Anthelmintic drug discovery: Into the future. Journal of Parasitology, 101(2), 125–133.
- 8. Wink, M. (2012). Medicinal plants: A source of anti-parasitic secondary metabolites. Molecules, 17(11), 12771–12791.
- 9. Anthony, J. P., Fyfe, L., & Smith, H. (2005). Plant active components A resource for antiparasitic agents? Trends in Parasitology, 21(10), 462–468.
- 10. Muthee, J. K., Gakuya, D. W., Mbaria, J. M., Kareru, P. G., Mulei, C. M., & Njonge, F. K. (2011). Ethnobotanical study of anthelmintic and other medicinal plants traditionally used in Loitoktok district of Kenya. Journal of Ethnopharmacology, 135(1), 15–21.
- 11. Williams, A. R., Fryganas, C., Ramsay, A., Mueller-Harvey, I., & Thamsborg, S. M. (2014). Direct anthelmintic effects of condensed tannins from diverse plant sources against Ascaris suum. PloS ONE, 9(5), Article e97053.
- 12. Athanasiadou, S., Githiori, J., & Kyriazakis, I. (2007). Medicinal plants for helminth parasite control: Facts and fiction. Animal, 1(9), 1392–1400.
- 13. Semwal, R. B., Semwal, D. K., Combrinck, S., & Viljoen, A. M. (2015). Gingerols and shogaols: Important nutraceutical principles from ginger. Phytochemistry, 117, 554–568.
- 14. Mao, Q. Q., Xu, X. Y., Cao, S. Y., Gan, R. Y., Corke, H., Beta, T., & Li, H. B. (2019). Bioactive compounds and bioactivities of ginger (Zingiber officinale Roscoe). Foods, 8(6), Article 185.
- Fu, Y., Zhang, Q., Xu, D. H., Xia, H., Cai, X., Wang,
 B., & Liang, J. (2019). Anthelmintic activity of gingerols and shogaols against Ichthyophthirius

- multifiliis in grass carp. Veterinary Parasitology, 270, 1–6.
- 16. Si, W., Chen, Y. P., Zhang, J., Chen, Z. Y., & Chung, H. Y. (2018). Antioxidant activities of ginger extract and its constituents toward lipids. Food Chemistry, 239, 1117–1125.
- 17. Sharma, V., Singh, I., & Chaudhary, P. (2014). Acorus calamus (The healing plant): A review on its medicinal potential, micropropagation and conservation. Natural Product Research, 28(18), 1454–1466.
- 18. Deepa, B., Suchetha, K., Rao, S., Bharathi, B., Kumar, K. S., Nayak, S., & Pawan, K. (2008). Anthelmintic effects of crude extracts of Acorus calamus L.: In vitro and in vivo studies. Pakistan Journal of Biological Sciences, 11(8), 1073–1076.
- 19. Mukherjee, P. K., Kumar, V., & Houghton, P. J. (2007). Screening of Indian medicinal plants for acetylcholinesterase inhibitory activity. Phytotherapy Research, 21(12), 1142–1145.
- 20. Vijayapandi, P., Annabathina, H., Srikanth, B., Manjunath, M., Boggavarapu, P., & Kasturi, K. (2013). In vitro anticholinergic and antihistaminic activities of Acorus calamus Linn. Leaves extracts. African Journal of Traditional, Complementary and Alternative Medicines, 10(1), 95–101.
- 21. Srinivasan, K. (2017). Ginger rhizomes (Zingiber officinale): A spice with multiple health beneficial potentials. PharmaNutrition, 5(1), 18–28.
- 22. Department of AYUSH. (2016). The Ayurvedic pharmacopoeia of India (1st ed., Vol. 9). Government of India, Ministry of Health and Family Welfare.
- 23. World Health Organization. (2018). WHO guidelines on good herbal processing practices for herbal medicines (WHO Technical Report Series, No. 1010).
- 24. Gupta, R., & Sharma, V. (2020). Ameliorative effects of Tinospora cordifolia root extract on experimentally induced liver fibrosis in rats. Journal of Ethnopharmacology, 259, Article 112929.

- 25. Chen, X., & Jia, Y. (2021). Pharmacokinetic drug interactions with herbal medicines. Current Pharmacology Reports, 7, 93–110.
- 26. Martin, R. J., & Robertson, A. P. (2010). Control of nematode parasites with agents acting on neuro-musculature systems: Lessons for neuropeptide ligand discovery. Advances in Experimental Medicine and Biology, 692, 138–154.
- 27. Blaxter, M. L., De Ley, P., Garey, J. R., Liu, L. X., Scheldeman, P., Vierstraete, A., Vanfleteren, J. R., Mackey, L. Y., Dorris, M., Frisse, L. M., Vida, J. T., & Thomas, W. K. (1998). A molecular evolutionary framework for the phylum Nematoda. Nature, 392(6671), 71–75.
- 28. Gurley, B. J. (2012). Pharmacokinetic herb-drug interactions (part 1): Origins, mechanisms, and the impact of botanical dietary supplements. Planta Medica, 78(13), 1478–1489.
- 29. Yadav, A. K., & Temjenmongla. (2012). In vitro anthelmintic activity of Gynura angulosa DC. Against Ascaridia galli. Journal of Pharmacy Research, 5(8), 4393–4395. (Access via interlibrary loan or contact author A. K. Yadav, e.g., via North-Eastern Hill University, India)
- 30. Khan, A., Tak, H., Nazir, R., Lone, B. A., & Parray, J. A. (2018). In vitro and in vivo anthelmintic activities of Iris kashmiriana Linn. Journal of Parasitic Diseases, 42(2), 168–175.
- 31. Wang, J., Ke, W., Bao, R., Hu, X., & Chen, F. (2023). Comprehensive evaluation of the pharmacokinetics and metabolism of 6-gingerol in humans using LC-MS/MS analysis. Journal of Pharmaceutical and Biomedical Analysis, 217, 114966.
- 32. Zhang, M., Viennois, E., Prasad, M., Zhang, Y., Wang, L., Zhang, Z., Han, M. K., Xu, C., Merlin, D., & Yang, L. (2021). Comparative pharmacokinetics of major bioactive components from ginger (Zingiber officinale) following oral administration in rats. Molecular Nutrition & Food Research, 65(3), 2000651.
- 33. Cheng, X., Liu, Q., Peng, Y., Qi, L., & Li, P. (2022). Ginger (Zingiber officinale Roscoe): A systematic review of the traditional uses, phytochemistry, pharmacology, and toxicology. Foods, 11(3), 2803.
- 34. Jiang, S. Z., Wang, N. S., & Mi, S. Q. (2020). Plasma pharmacokinetics and tissue distribution of 8-

- gingerol and 10-gingerol in rats after oral administration of ginger oleoresin. Journal of Ethnopharmacology, 252, 112580.
- 35. Lee, J. W., Nagano, T., Miyamoto, K., Kato, Y., Sugiyama, Y., & Shin, D. H. (2021). Absorption, distribution, metabolism, and excretion mechanisms of the bioactive components of Zingiber officinale: Current status and future perspectives. Biomedicine & Pharmacotherapy, 137, 111368.
- 36. Chen, H., Fu, J., Chen, H., Hu, Y., Soroka, D. N., Peng, S., & Sang, S. (2020). Ginger compound [6]-shogaol and its metabolites in humans: Metabolic pathway and bioactivity. Journal of Agricultural and Food Chemistry, 68(7), 1918-1929.
- 37. Wu, K. L., Rayner, C. K., Chuah, S. K., Changchien, C. S., Lu, S. N., Chiu, Y. C., Chiu, K. W., & Lee, C. M. (2022). Comparative bioavailability of gingerol and shogaol from different ginger preparations in healthy volunteers: A randomized crossover study. Phytomedicine, 95, 153886.
- 38. Kumar, R., Sharma, B., Tomar, M. S., Roy, P., (2021).Saraf. S. A. In vivo pharmacokinetics of major bioactive from components Acorus calamus rhizomes and their metabolite identification using UHPLC-Q-TOF-MS/MS. Journal of Pharmaceutical and Biomedical Analysis, 195, 113874.
- 39. Guo, H., Zhang, J., Gao, W., Qu, Z., & Liu, C. (2020). Gastrointestinal absorption and metabolism of β-asarone: Risk assessment of herb-drug interactions. Journal of Ethnopharmacology, 258, 112888.
- 40. Wang, Y., Liang, X., Liu, L., Han, J., Liu, Y., & Li, F. (2023). Comparative pharmacokinetic study of active components from Acorus calamus L.: Focus on metabolism and distribution in brain tissues. Phytomedicine, 115, 154789.
- 41. Chen, X., Wu, Q., Jiang, H., Wang, J., Zhao, Y., & Zhu, R. (2022). Metabolite profiling and

- pharmacokinetics of α -asarone and methyl isoeugenol in rats after oral administration of Acorus calamus extract. Journal of Ethnopharmacology, 281, 114571.
- 42. Rajput, S. B., Tonge, M. B., & Karuppayil, S. M. (2021). Pharmacokinetics and comparative bioavailability of methyl isoeugenol and related compounds from Acorus calamus: A preclinical investigation. Phytotherapy Research, 35(3), 1566-1579.

Abbreviations

ANOVA: Analysis of Variance

API: Ayurvedic Pharmacopoeia of India

AUC: Area Under the Curve

Cmax: Maximum Plasma Concentration

Cm: Centimeter

CYP: Cytochrome P450

°C: Degrees Celsius

DMF: Dimethylformamide

DMSO: Dimethyl Sulfoxide

EF: Eisenia fetida

H: Hour

Kg: Kilogram

L: Liter

Mg: Milligram

Min: Minute

mL: Milliliter

n: Sample Size

p: Probability Value

RH: Relative Humidity

SD: Standard Deviation

T1/2: Elimination Half-life

Tmax: Time to Maximum Plasma Concentration

Mg: Microgram

Vd: Volume of Distribution

v/v: Volume by Volume

Z. officinale: Zingiber officinale

A. calamus: Acorus calamus

E. fetida: Eisenia fetida

P: Paralysis time

D: Death time/Mortality time

SC: Suntha Churna

VC: Vacha Churna

An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print); ISSN: 2249-9245 (Online)



REVIEW ARTICLE

ZEBRAFISH MODEL FOR OCULAR DISORDERS AND TOXICITY TESTING – A REVIEW

Vishal Gangawane, Bhargavi Govardhan, Rutuja Bhosale, Kaustubh Pawar, Pramod Choudhary, Karimunnisa Shaikh*

Progressive Education Society's Modern College of Pharmacy, Affiliated to Savitribai Phule Pune University, Pune, India

*Corresponding author email: karima78@rediffmail.com Received: Feb 06, 2025 / Revised: April 18, 2025 / Accepted: May 14, 2025

ABSTRACT

The zebrafish (Danio rerio) is another vertebrate model that can be used to better understand the mechanisms behind and develop treatments for a number of eye disorders such as cataracts, glaucoma, diabetic retinopathy, age-related macular degeneration (AMD), photoreceptor degeneration, etc. Because of their genetic and embryonic accessibility as well as a behavioral assessment of their visual function, zebrafish are a popular model in ophthalmology. In investigations on ocular toxicity, zebrafish have also been utilized extensively. It has been proposed that several common human ocular toxicants have comparable toxic effects on zebrafish due to the physical and functional similarities between human and zebrafish eyes. Zebrafish eyesight develops rapidly; the little larvae are reliant on visual cues as early as day 4, and their behavioral reactions to these signals may be monitored in high-throughput. In addition to a comparative anatomy of the zebrafish eye and its key distinctions from the mammalian eye, this article shows the useful applications of this underutilized model for ocular disorders and toxicity assessment.

Keywords: Zebrafish, Ocular, toxicity, disease, model

How to cite this article: Vishal Gangawane, Bhargavi Govardhan, Rutuja Bhosale, Kaustubh Pawar, Pramod Choudhary, Karimunnisa Shaikh*. Zebrafish Model for Ocular Disorders and Toxicity Testing – A Review. Bulletin of Pharmaceutical Research. 2025;15(S2): 73-87.

Source of support: Nil Conflict of interest: None

1. INTRODUCTION

Visual impairment is a major global public health concern. Around the world, cataracts are the leading cause of blindness, with glaucoma, AMD, and diabetic retinopathy (DR) trailing closely behind [1]. To develop new treatments, it is imperative to understand the pathogenic mechanisms, developmental processes, genes and other factors behind ocular diseases. Further, we need to develop novel pharmacological treatments. For that, we

need new animal models that closely mimic ocular human physiology and pathology, and which will provide better throughput drug screening [2].

Zebrafish (Danio rerio) serve as an animal model organism that not only achieves these goals but also, compared to current rodent models, is a more accurate reflection of the human experience in many aspects. Additionally, they are much more suitable

candidates for ocular models because the conventional species employed in nonclinical trials like dogs, rabbits, and rats, rely mostly on their senses of smell and hearing to navigate their environment, due to which toxins that affect vision may go undetected during the inlife phase of the investigations [3]. As per 2010 European Commission Directive (Directive 2010/63/EU), using zebrafish before they become independent from the maternally derived yolk that is about 120 hours postfertilization (hpf), is accepted as a substitute for animal testing.

Over the past ten years, zebrafish have become more and more popular as models for studying a variety of human disorders, and they are currently being employed more frequently to examine ocular defects in people. Zebrafish models have already been used to study a variety of human eye diseases, such as glaucoma, cataracts, diabetic retinopathy, and age-related macular degeneration. Their popularity can be attributed to numerous factors. Zebrafish are inexpensive to maintain and can reproduce in great quantities. After three to four months, they reach sexual maturity, and each pair can produce 200 to 300 young ones every week [3]. It is possible to watch the organogenesis taking place inside the embryo in real time since the eggs fertilize and grow into transparent embryos outside of the mother. Because the embryos have a diameter of only 1 mm, they may be easily dispersed and kept in 96-well plates, making it easier to use modest amounts of drug solution in drug screening procedures. Furthermore, a multitude of tiny compounds can pass through embryos, making medication delivery simple. Zebrafish are a popular model for higherthroughput screening of small compounds because of the enormous number of embryos they generate every clutch [4]. This is necessary for preclinical drug development and toxicity assessment. A wide range of human diseases, such as inherited muscular diseases, neurological conditions, TB, cancer,

cardiovascular diseases, hematopoietic and infectious diseases, have so far been modeled by zebrafish. Zebrafish are also a useful vertebrate model for researching conditions connected to eyesight. But there are a few minor variations between the zebrafish and human visual systems that should be considered. Hence it is imperative to study zebrafish in detail to explore it as an animal model. This article addresses the application of zebrafish in development evaluation of pharmaceuticals for treating ocular disorders.

1.1 Anatomy of Zebrafish

Three separate embryonic layers give rise to the eye components in both zebrafish and human embryogenesis. The neuroectoderm is the source of the optic stalk, pigmented epithelium, retina, and ciliary body, among other structures. The surface ectoderm is the source of the lens, conjunctival epithelium, corneal epithelium, and corneal stroma. Mesenchyme generated from neural crest cells gives rise to the corneal endothelium, iris stroma, and sclera [5].

All five layers of the human cornea—the epithelium, bowman's layer, stroma, dermal membrane, and endothelium—are present in the cornea of zebrafish. The mature zebrafish cornea has a thickness of about 20 mm. Similar to the human cornea, the epithelium is made up of around five layers of squamous cells [6]. Tight junctions and desmosomes connect the membranes of the outermost cells, which feature microvilli and microplicae. These traits are essential components of the cornea's structural integrity. Bowman's layer, a layer of collagen fibres that divides the epithelium from the stroma, may aid in the healing process following corneal damage in humans. The corneal stroma of zebrafish is composed of roughly thirty lavers of collagen fibres and measures about 6 mm in thickness.

Zebrafish and human being visual systems are essentially similar, however there are some

noticeable anatomical variations. As a result of the spheroid rather than ellipsoid nature of the zebrafish lens, the volume of vitreous in the zebrafish eye is significantly less than in the human eye. Zebrafish's visual system develops quickly because they rely on light to find food and utilize eyesight to defend themselves from predators. Beginning at 32 hours post fertilization (hpf), the retinal structure develops incredibly quickly within 5 days after fertilization (dpf). The zebrafish retina starts to operate by that point. Zebrafish lack the socalled macula, a specific region of the retina that is accountable for high acuity vision, in contrast to the retina of mammals. As the zebrafish iris lacks muscle, contraction or dilation of the pupil does not occur [7]. The aqueous humor found in the anterior segment of most vertebrates serves as a barrier between the cornea and the lens. The ciliary body processes in human eyes create aqueous fluid circumferentially through their epithelial cells. The ring-shaped trabecular network at the iridocorneal junction is where the aqueous humor exits the eye after passing through the iris pupil to reach the anterior segment. The aqueous humor is circumferentially collected in Schlemm's canal after traversing the trabecular network, and it is then transported into the venous circulation [8]. With a few noteworthy exceptions, the zebrafish anterior chamber's architecture and physiology are comparable. The primary source of aqueous humor in zebrafish is the non-pigmented epithelial cells found in the corneal zone, which is situated on the dorsal part

Both functionally and physically, the zebrafish and mammalian retinas are identical.

The retina's centre area is where neurogenesis begins and is abundant. Five different types of photoreceptors are found in the retina of zebrafish. One rod type is sensitive to low light, while the other four cone types are sensitive to light in the red, green, blue, and ultraviolet regions of the light spectrum. The inner segments of red and green cones fuse together to produce "double cones," but cones that respond to "long" blue and "short" ultraviolet wavelengths are single. The "area temporalis," a specialized portion of the temporal retina with the highest density of cones, is thought to contribute to improved visual acuity in the anterior visual field.

As in the mammalian retina, Muller cells are the primary glia cells and provide architectural support to the retina of zebrafish. Muller cells demonstrate adaptability and regeneration potential during retinal damage. Muller cell gliosis is frequently the outcome of retinal damage in mammalian eyes. Activated Muller cells, however, generate and secrete growth factors that can cause cellular trans differentiation, proliferation, and migration, forming the tissue's fibroblastic or membrane-like profile.

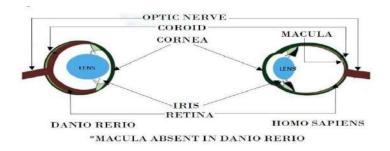


Fig.1 Similarities between the human and zebrafish eye

Table 1: Comparision of zebrafish vs human ocular strucutres

Ocular Structure	Zebrafish	Human	Comments
Cornea	Five layers including Bowman's layer; ~20 μm thick	Five layers including Bowman's layer; ${\sim}500~\mu m$ thick	Structural similarity; human cornea is significantly thicker
Lens	Spherical lens	Elliptical lens	Spherical lens allows wider field of vision in water
Retina	Five photoreceptor types (1 rod, 4 cones: red, green, blue, UV)	Four photoreceptor types (1 rod, 3 cones: red, green, blue)	Zebrafish have UV- sensitive cones; high cone density supports visual acuity
Macula / Fovea	Absent	Present (for high acuity vision)	Zebrafish lack a macula/fovea
Iris and Pupil	Iris lacks muscles, no pupillary reflex	Iris muscles present; controls pupil dilation	No pupil dilation in zebrafish
Aqueous Humor Dynamics	Produced by corneal epithelium; drained via angle structures	Produced by ciliary body; drained via trabecular meshwork & Schlemm's canal	Different anatomical sites but comparable physiological function
Müller Glia	Present; highly regenerative	Present; limited regenerative capacity	Zebrafish Mu ller cells can regenerate retinal neurons
Development Timeframe	Fully functional retina by 5 days post- fertilization	Months for full maturation	Rapid development enables early-stage research
Eye Size	~1 mm diameter at larval stage	~24 mm diameter (adult)	Smaller size suitable for high-throughput assays
Transparency	Embryos and early larvae are transparent	Opaque	Transparency aids in in vivo imaging and developmental observation

1.2. Zebrafish models for ocular diseases

1.2.1. Cataract

Genes responsible for cataract includes CRYBA2 gene and CRYGC gene. Under typical circumstances, the crystalline protein (bg, gC kinds) ensures transparency in the lens and cornea and is also required for refractive power [9]. Consequently, it is anticipated human cataract that development is associated with mutations in the crystalline gene [10]. Recently, a cataract-causing gene has been identified using a zebrafish animal model. Mutation of the CRYBA2 gene, a member of bg crystalline causes congenital cataracts [11]. Furthermore, mutation in CRYGC gene (gCcrystalline) makes the lens less thermally stable and increases the risk of lens opacity when exposed to heat and UV radiation and ultimately also leads to cataract formation [12].

Similarly, cataracts linked to a gamma crystalline defect caused by alpha A crystalline are seen in the cloche zebrafish mutant [13]. Lens transparency is increased and the development of lens fiber cells is triggered when alpha A crystalline reduces the insolubility of gamma crystalline. If the lens can reach a significant alpha A crystalline expression, cataract formation may be prevented. Gene knockdown studies have also been used to replicate congenital cataracts in humans in zebrafish. For instance, anterior segment abnormalities, like as cataracts, are caused by mutations in the PITX3 and FOXE3 genes, which are expressed in the lens epithelium [14]. Table 1 exhibits different genes which are targeted to create cataract model in zebrafish.

A corneal puncture is the first step in the zebrafish cataract induction process, which is followed by an intraocular hydrogen peroxide injection into the anterior chamber [15].

Table 2. Genes targeted in zebrafish model for cataract

Method	Gene	Ocular Phenotype	Reference
Gene Targeted	CRYAA(αA- crystallin)	crystal-like opacity sporadically spreading across the lens, or frequent droplets covering a large fraction of the lens	[16]
Gene Targeted	GJA8	various sizes of lens opacity	[17]
Gene Targeted	PITX3	severe lens degeneration, lack of anterior chambers and outer segment structures	[18]
Gene Targeted	MAB21L2	microphthalmia, colobomas, small and disorganized lenses, cornea dysgenesis	[19]

1.2.2. Glaucoma

The gradual degradation of Retinal ganglion cells (RGCs) and the optic nerve head characterizes glaucoma, a group of adult-onset retinal neuropathies [20]. Despite the anatomical difference of trabecular meshwork and aqueous humour dynamics in human and zebrafish, the overall similarities in aqueous humour outflow tissue structure and average intraocular pressure (IOP) make zebrafish a potential model to study the complex genetics of glaucoma [21,22].

The main risk factors for glaucoma include severe myopia, anterior segment abnormalities (iris, trabecular meshwork, and cornea), and elevated IOP, which occurs before aqueous humor drainage obstruction [23]. Genes responsible for Glaucoma include *FOXC1, LMX1B*.

The disease-risk phenotype of glaucoma is well studied in zebrafish mutants and was successfully modelled in zebrafish, for example, by mutation of the *FOXC1* (forkhead transcription factor) gene [24]. Zebrafish can be treated with the very effective mutant ethyl-nitroso-urea (ENU) to produce complex phenotypes caused by mutations in numerous genes. Alternatively, morpholions, an antisense modified oligonucleotide, is specifically used to inhibit the specific gene.

FoxC1 is essential for vascular basement membrane integrity and FOXC1 is crucial for the growth and upkeep of the anterior segment of the eye and is expressed in periocular mesenchymal cells and anterior segment cells [25]. As a result, changes in the iris, trabecular meshwork, and cornea that resemble glaucoma are brought on by mutations in this transcription factor. Additionally, glaucoma-linked risk factors have been examined in relation to the mutation of the gene encoding for lowdensity lipoprotein receptor-related protein 2, which is represented by the bugeye mutant of zebrafish, through the use of OMR, electroretinograms (ERGs), histological research [26]. The glaucoma-risk phenotypes displayed by this mutant included larger eyes as a result of elevated intraocular pressure, a decrease in retinal ganglion cell counts after three months, and outer retinal malfunction after five months as a result of sustained mechanical stress. Table 2 summarises different glaucoma models in zebrafish.

Method	Injury Paradigm	Ocular Phenotype	Model	Reference
Gene Targeted	FOXC1	RGC loss	POAG	[27]
Gene Targeted	Tg (Bugeye)	Decreased retinal cell densities and diminished outer	POAG	[28]
		retinal function		
Gene Targeted	CYP1B1	Neural crest migration into the anterior segment	POAG	[29]
Chemical Induced	N-Methyl-D- aspartic acid (NMDA)	RGC loss	Glaucoma	[30]
Oxidative Stress Induced	Hydrogen peroxide	RGC injury	Glaucoma	[31]

Table 3. Summary of zebrafish models for glaucoma

1.2.3. Age-related macular degeneration (AMD)

It is distinguished by the appearance of a pale yellowish lesion in the macula and peripheral retina due to the accumulation of drusen, an acellular polymorphous waste. The pathophysiology consists of Bruch's membrane, Retinal pigment epithelium (RPE) and cone cell degeneration. Choroid neovascularization is evident in later stages, which eventually results in a loss of color vision and visual acuity [32]. Zebrafish have been used to research hypoxic retinopathy, which is observed in Age-related macular degeneration (AMD) and Diabetic Retinopathy (DR) [33].

To create AMD model, adult zebrafish are made hypoxic by submerging them in hypoxic water for three to ten days. This causes neovascularization, which results in the development of immature, leaky, and disordered blood vessels.

In zebrafish, the administration of oral active anti-vascular endothelial growth factor (anti-VEGF) showed a notable reduction in the pathogenic symptoms [34]. These results imply that VEGF-induced hypoxia plays a crucial role in the neovascularization of the retina in AMD and DR.

1.2.4.. Diabetic Retinopathy (DR)-

It has been demonstrated that hyperglycemic zebrafish have similarities with diabetic patients' aberrant retinal architecture [35].

To develop DR model, adult zebrafish are made hyperglycemic by alternating between immersion in water and glucose solution for 28 days, then having their retinas examined. In treated zebrafish, the thickness of the IPL (inner plexiform layer) and INL (inner nuclear layer) layers were found to be drastically reduced, with the IPL layer being

Unlocking Horizons in Global Multidisciplinary Healthcare Research Indo-US International Conference Date: 10-12, March 2025 Organised by PES Modern college of Pharmacy Nigdi Pune-44

roughly 55% of the INL layer [35]. This study offered some evidence in favor of using zebrafish as a model for drug resistance. This result was supported by another study in which zebrafish that had been given hyperglycemia showed signs of vision impairment.

The structural deterioration of the cone photoreceptor neurons in this investigation indicated that hyperglycemia had an impact on them and with the application of Electroretinogram (ERGs). [36]. This study revealed that clinical advantages in DR might be obtained by combining neuroprotective medicines with medications to treat vascular problems. Moreover, DR in humans is replicated by hypoxia-induced retinal neovascularization or angiogenesis in the adult Fli-EGFP-Tg transgenic zebrafish model.

In order to create transgenic zebrafish models, foreign genes are frequently inserted into zebrafish embryos. These genes have the ability to encode fluorescent proteins, such as EGFP (Enhanced Green Fluorescent Protein), which under a fluorescent microscope enables researchers to see particular tissues or cells. It is feasible to evaluate the therapeutic efficacy of anti-angiogenic medicines using these models [34].

1.3. Zebrafish model for ocular toxicity

1.3.1. Ethanol induced ocular toxicity zebrafish model

Kashyap and team in 2007, developed a model to assess the ocular toxicity of ethanol to the zebrafish ocular tissue [37]. Reduced cell differentiation, delayed development, and increased cell death are some of the hypothesized processes behind ethanol-induced ocular abnormalities.

Developmental defects may be due to ethanol-induced retinoic acid (RA) signalling disruption, reactive oxygen species (ROS) generation, and epigenetic defects.

At 12 hours after fertilization (12 hpf), embrvos were treated with 0.003% phenylthiourea (PTU), to prevent the formation of melanin. Between 24 and 48 hours post fertilization, embryos were treated with 1% or 1.5% ethanol solution in glass beakers kept at 28.58°C and gently covered with parafilm to reduce evaporation. 100% ethanol was used in the ethanol solution, which was then diluted svstem water. It has been demonstrated that the ethanol dosages employed in zebrafish under long-term therapy produce an embryonic phenotype that is comparable to the fatal alcohol syndrome in humans.

Retinal histology was evaluated by staining embryo cyosection with 1% methylylene blue/ 1% azure II (1:1). The processed tissue was mounted in glycerol and viewed under Nomarski optics using a Leica DMR compound microscope [37].

Another zebrafish model for ethanol photoreceptor induced toxicity was developed in following way. After fertilization, zebrafish embryos were reared in fish water with different amounts of ethanol for two to five days. Optokinetic response (OKR) and electroretinography (ERG) were utilized to examine the effects of ethanol on retinal function, while histologic and immunohistochemical investigations were used to evaluate the impacts on retinal morphology. Early on in their development, zebrafish embryos exposed to moderate and high amounts of ethanol showed morphological defects of the eye, including hypoplasia of the optic nerve and

suppression of the growth of the photoreceptor outer segment.

According to the OKR, ethanol treatment also resulted in an elevated visual threshold. ERG analysis revealed a significant decrease in both the a- and b-waves, indicating that ethanol may have an impact on photoreceptor function. Indeed, the OKR visual threshold and the a- and b-wave amplitudes were impacted by low doses of ethanol that did not clearly alter the morphology of the body or retina [38].

1.3.2. Medroxyprogesterone acetate induced ocular toxicity zebrafish model

Medroxyprogesterone acetate (MPA) is a widely used synthetic progestin in contraception pills and hormone replacement therapy.

The model zebrafish is exposed to various concentration of MPA to study its effect on transcription of *NOTCH1A, DLL4, JAG1A, CTBP1* and *RBPJB* (key genes in the Notch signalling pathway) in the eyes of females. 25 larvae per replicate were chosen at random at 21 dpf, and they were exposed to MPA and solvent control in 5 L glass aquaria with 3 L of exposure solution at 26°C and a 14:10 h light/dark cycle.

Every day, the exposure solution was replenished and aerated (oxygen level > 75%). It had a pH of 7.0–8.0. 120 days passed during the exposure (from 21 dpf to 140 dpf). The young zebrafish were moved to 10 L glass aquaria with 6 L of exposure solution at 60 dpf. All fish were put to sleep with 0.01% MS-222 at 140 dpf, and the eyes were removed. The RNA analysis shown the increased transcription of *NOTCH1A*, *DLL4*, *JAG1A*, *CTBP1* and *RBPJB* (key genes in the Notch signaling pathway) in the eyes of females [39].

1.3.3. Phenanthrene induced ocular toxicity zebrafish model

The phenanthrene was exposed to embryos between 0 and 0.5 hours after fertilization (hpf) at concentrations of 0.02, 0.2, and 2 M (3.56, 35.6, and 356 g/L). Glass culture plates containing 50 embryos each plate in 15 mL of zebrafish medium were used for the exposures, and each of the three treatments was repeated six times.

Every day, two fresh phenanthrene solutions were added. Instead phenanthrene, the control group was given an identical volume of DMSO (10 L/L). At 72 hours post fertilization, embryos were retrieved for histological inspection, RNA extraction, Western blotting, electrophoretic mobility shift assay (EMSA), and chromatin immunoprecipitation (Chip).For phototaxis response test, additional embryos were grown to 7 days postfertilization. Each analysis's embryos were chosen at random, and they were made unconscious using 0.06% ethyl 3aminobenzoate methane sulfonate salt (MS-222).

The histology of shown that there is serious development of retinal retardation, apoptosis, and reduction in cell proliferation in retina of zebrafish. Phenanthrene may lead to abnormalities in the development of the visual system. Phenanthrene exposure decreased the expression of zinc finger Ebox binding homeobox 1 (ZEB1) and paired box 6 (PAX6) and increased the expression of aryl hydrocarbon receptor (AhR) and microphthalmia-associated transcription factor (Mtif) [40].

1.3.4. Norethindrone induced ocular toxicity zebrafish model

Synthetic progestins such as norethindrone (NET), which are synthetic sex hormones, have the ability to influence numerous biological processes through well conserved mechanisms. Larvae are placed exposure solution-filled 250 mL borosilicate glass beakers at a density of ten fish each after hatching. Exposure of zebrafish to 500 ng/L NET significantly altered the expression of 1277 genes. A pathway study revealed implications for a number of physiological processes in larvae exposed to NETs, including biosynthesis and glycerolipid metabolism pathways.

However, it was anticipated that exposure to ELS NET would have the greatest effect on two pathways: phototransduction, which involves 8 genes, and protein processing in the endoplasmic reticulum, which involves genes. For three of 17 the phototransduction genes PDE6A, GNAT 1, and ARR3A qPCR analysis verified a similar direction and degree of fold change. The downregulation of eight gene involved in phototransduction cascade are responsible for affecting the vision of fish.

These include the transducin (*GNAT*, *GNG*) and phosphodiesterase (*PDE6*) families, which subsequently transfer the signal from opsin and rhodopsin, and the opsin (*OPN*) and rhodopsin (*Rho*) gene families, which start the first steps in the phototransduction cascade [41].

1.4. Functional vision testing for zebrafish models

1.4.1. OMR Assay

The OMR experiment minimizes perceived displacement by utilizing the zebrafish larvae's natural tendency to swim in the direction of optic flow. Either immobilized or freely swimming larvae can be used [42]. Usually, the trunk and tail of the immobilized larvae are free to move, but their heads are stuck in agarose. Usually, a movie with moving stripes or other patterns that alternately show light and dark environments that can be seen from below and/or next to the larvae stimulates the OMR. As the pattern moves, larvae automatically swim in the same direction. Response can be measured by measuring location in relation to stimulus direction or by watching videos of swimming behavior, which is typical for immobile larvae.

1.4.2. OKR Assay

The OKR is a sign of a fully developed visual system in zebrafish and is based on the eye movement reflex in reaction to a moving stimulus [43]. It helps stabilize the image on the retina to maintain visual acuity. The OKR monitors eye movements, which include swiftly resetting saccades in the opposite direction and smooth pursuits (slow phase) [44].

Only 5% of the zebrafish larvae respond to the visual stimuli at approximately 74 hpf; this percentage gradually rises to 100% by 80 hpf. The OKR increases by 96 hpf in both the amount of time spent tracking the stimulus and the tracking velocity of eye movement.

Using this technique, zebrafish larvae are exposed to a visual stimulus—typically, a moving circle of alternating black and white stripes—after being immobilized to limit their body mobility but preserve their ability to move their eyes. The larvae move their eyes in the direction of the moving stripes in response to this stimuli.

In this context, it is possible to modify experimental visual stimuli including angular velocity, spatial frequency, and contrast in order to better grasp a possible fault. Adult fish can also benefit from this procedure with a few experimental modifications, such as a continuous supply of oxygen-rich water while body movement is restricted.

Fish responses from larvae or adults can be processed in a matter of minutes thanks to the assay's speed. Many zebrafish mutants linked to defects in the visual system were found and characterized using OKR.

These include the bumper mutant, which has a defect in lens development, the noir, dropje, and lakritz mutants, which have a defect in retinal structure, the belladonna mutant, which has an RGC axon misrouting, and the grumpy and sleepy mutants, which have an optic nerve disorder. Additional instances of abnormalities in visual function include the brass mutant, which exhibits a phenotype resembling glaucoma.

In addition, OKR was also beneficial in isolating a colour-blind mutant by changing the colour of visual stimulus. In conclusion, compared to the other approaches listed below, the OKR seems to produce more solid, dependable, and quantitative behavioral data—especially now that automated commercial systems are readily accessible [44].

2. CONCLUSION

Globally, visual impairment is a significant health issue. Developing new therapeutic strategies requires a thorough understanding of the pathological mechanisms underlying disease.

Similarly, it would be ideal to have animal models that closely mimic eye pathology and enable medium-throughput drug screening. Zebrafish appear to be most equipped to meet these needs in this context because of its large clutch, quick growth of the eyes, simplicity of mutagenesis and genetic analysis, and similar eye morphology to humans.

Zebrafish have been shown to have similar characteristics to numerous human vision problems, and for a large number of strains, their visual capacity has already been assessed through the use of tests that take use of the fish's natural behavior. Thus, the zebrafish model has promise for both the development of novel treatment agents and a deeper knowledge of ophthalmological illnesses.

Zebrafish have been extensively utilized in ophthalmology drug development processes, including oculotoxicity testing and the screening of novel anti-angiogenic chemicals or neuroprotective medications. Steady progress in high-throughput phenotyping indicates the potential uses of zebrafish in drug discovery.

3. REFERENCES

- Haile LM, Kamenov K, Briant PS, Orji AU, Steinmetz JD, Abdoli A, Abdollahi M, Abu-Gharbieh E, Afshin A, Ahmed H, Rashid TA. Hearing loss prevalence and years lived with disability, 1990– 2019: findings from the Global Burden of Disease Study 2019. The Lancet. 2021 Mar 13;397(10278):996-1009.
- 2. Bibliowicz J, Tittle RK, Gross JM. Toward a better understanding of human eye disease: Insights from the zebrafish, Danio rerio. Progress in molecular biology and translational science. 2011 Jan 1;100:287-330.
- 3. Lessman CA. The developing zebrafish (Danio rerio): A vertebrate model for high-throughput screening of chemical libraries. Birth Defects Research Part C: Embryo Today: Reviews. 2011 Sep;93(3):268-80.
- 4. Murphey RD, Zon LI. Small molecule screening in the zebrafish. Methods. 2006 Jul 1;39(3):255-61.
- Richardson R, Tracey-White D, Webster A, Moosajee M. The zebrafish eye—a paradigm for investigating human ocular genetics. Eye. 2017 Jan;31(1):68-86.
- 6. Ikkala K. Zebrafish as a model for studying the maturation and pathophysiology of the corneal epithelium.
- 7. Chhetri J, Jacobson G, Gueven N. Zebrafish—on the move towards ophthalmological research. Eye. 2014 Apr;28(4):367-80.
- 8. Cassar S, Dunn C, Ramos MF. Zebrafish as an animal model for ocular toxicity testing: a review of ocular anatomy and functional assays. Toxicologic Pathology. 2021 Apr;49(3):438-54.

- 9. Andley UP. Crystallins in the eye: function and pathology. Progress in retinal and eye research. 2007 Jan 1;26(1):78-98.
- 10. Graw J. The crystallins: genes, proteins and diseases. Biological chemistry. 1997 Nov 1;378(11):1331-48.
- 11. Reis LM, Tyler RC, Muheisen S, Raggio V, Salviati L, Han DP, Costakos D, Yonath H, Hall S, Power P, Semina EV. Whole exome sequencing in dominant cataract identifies a new causative factor, CRYBA2, and a variety of novel alleles in known genes. Human genetics. 2013 Jul;132:761-70.
- 12. Li XQ, Cai HC, Zhou SY, Yang JH, Xi YB, Gao XB, Zhao WJ, Li P, Zhao GY, Tong Y, Bao FC. A novel mutation impairing the tertiary structure and stability of γC-crystallin (CRYGC) leads to cataract formation in humans and zebrafish lens. Human mutation. 2012 Feb;33(2):391-401.
- 13. Goishi K, Shimizu A, Najarro G, Watanabe S, Rogers R, Zon LI, Klagsbrun M. αA-crystallin expression prevents γ-crystallin insolubility and cataract formation in the zebrafish cloche mutant lens. Development (2006) 133 (13): 2585–2593.
- 14. Semina EV, Ferrell RE, Mintz-Hittner HA, Bitoun P, Alward WL, Reiter RS, Funkhauser C, Daack-Hirsch S, Murray JC. A novel homeobox gene PITX3 is mutated in families with autosomal-dominant cataracts and ASMD. Nature genetics. 1998 Jun;19(2):167-70.
- 15. Prior HM, Letwin K, Tuininga A, Nguyen M. A simple method of cataract induction in adult zebrafish. Zebrafish. 2018 Apr 1;15(2):211-2.

- 16. Wu SY, Zou P, Mishra S, Mchaourab HS. Transgenic zebrafish models reveal distinct molecular mechanisms for cataract-linked αA-crystallin mutants. PloS One. 2018 Nov 26;13(11):e0207540.
- 17. Ping X, Liang J, Shi K, Bao J, Wu J, Yu X, Tang X, Zou J, Shentu X. Rapamycin relieves the cataract caused by ablation of Gja8b through stimulating autophagy in zebrafish. Autophagy. 2021 Nov 2;17(11):3323-37.
- 18. Shi X, Bosenko DV, Zinkevich NS, Foley S, Hyde DR, Semina EV, Vihtelic TS. Zebrafish pitx3 is necessary for normal lens and retinal development. Mechanisms of development. 2005 Apr 1;122(4):513-27.
- 19. Gath N, Gross JM. Zebrafish mab21l2 mutants possess severe defects in optic cup morphogenesis, lens and cornea development. Developmental Dynamics. 2019 Jul;248(7):514-29.
- 20. Pascolini D, Mariotti SP. Global estimates of visual impairment: 2010. British Journal of Ophthalmology. 2012 May 1;96(5):614-8.
- 21. Chen CC, Yeh LK, Liu CY, Kao WW, Samples JR, Lin SJ, Hu FR, Wang IJ. Morphological differences between the trabecular mesh works of zebrafish and mammals. Current Eye Research. 2008 Jan 1;33(1):59-72.
- 22. Link BA, Gray MP, Smith RS, John SW. Intraocular pressure in zebrafish: comparison of inbred strains and identification of a reduced melanin mutant with raised IOP. Investigative ophthalmology & visual science. 2004 Dec 1;45(12):4415-22.
- 23. Wong TY, Klein BE, Klein R, Knudtson M, Lee KE. Refractive errors, intraocular pressure, and glaucoma in a white population. Ophthalmology. 2003 Jan 1;110(1):211-7.

- 24. Tamimi Y, Skarie JM, Footz T, Berry FB, Link BA, Walter MA. FGF19 is a target for FOXC1 regulation in ciliary bodyderived cells. Human molecular genetics. 2006 Nov 1;15(21):3229-40.
- 25. McMahon C, Semina EV, Link BA. Using zebrafish to study the complex genetics of glaucoma. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 2004 Jul 1;138(3):343-50.
- 26. Stujenske JM, Dowling JE, Emran F. The bugeye mutant zebrafish exhibits visual deficits that arise with the onset of an enlarged eye phenotype. Investigative ophthalmology & visual science. 2011 Jun 1;52(7):4200-7.
- 27. Berry FB, Skarie JM, Mirzayans F, Fortin Y, Hudson TJ, Raymond V, Link BA, Walter MA. FOXC1 is required for cell viability and resistance to oxidative stress in the eye through the transcriptional regulation of FOXO1A. Human molecular genetics. 2008 Feb 14;17(4):490-505.
- 28. Protas ME, Weh E, Footz T, Kasberger J, Baraban SC, Levin AV, Katz LJ, Ritch R, Walter MA, Semina EV, Gould DB. Mutations of conserved non-coding elements of PITX2 in patients with ocular dysgenesis and developmental glaucoma. Human Molecular Genetics. 2017 Sep 15;26(18):3630-8.
- 29. Williams AL, Eason J, Chawla B, Bohnsack BL. Cyp1b1 regulates ocular fissure closure through a retinoic acidindependent pathway. Investigative ophthalmology & visual science. 2017 Feb 1;58(2):1084-97.
- 30. Sheng W, Lu Y, Mei F, Wang N, Liu ZZ, Han YY, Wang HT, Zou S, Xu H, Zhang X. Effect of resveratrol on sirtuins, OPA1, and Fis1 expression in adult zebrafish retina. Investigative ophthalmology &

- visual science. 2018 Sep 4;59(11):4542-51.
- 31. Giannaccini M, Usai A, Chiellini F, Guadagni V, Andreazzoli M, Ori M, Pasqualetti M, Dente L, Raffa V. Neurotrophin-conjugated nanoparticles prevent retina damage induced by oxidative stress. Cellular and molecular life sciences. 2018 Apr;75:1255-67.
- 32. Jager RD, Mieler WF, Miller JW. Agerelated macular degeneration. New England Journal of Medicine. 2008 Jun 12;358(24):2606-17.
- 33. Jensen LD, Rouhi P, Cao Z, La nne T, Wahlberg E, Cao Y. Zebrafish models to study hypoxia-induced pathological angiogenesis in malignant and non-malignant diseases. Birth Defects Research Part C: Embryo Today: Reviews. 2011 Jun;93(2):182-93.
- 34. Cao Z, Jensen LD, Rouhi P, Hosaka K, Lanne T, Steffensen JF, Wahlberg E, Cao Y. Hypoxia-induced retinopathy model in adult zebrafish. Nature protocols. 2010 Dec;5(12):1903-10.
- 35. Gleeson M, Connaughton V, Arneson LS. Induction of hyperglycaemia in zebrafish (Danio rerio) leads to morphological changes in the retina. Acta diabetologica. 2007 Sep;44:157-63.
- 36. Alvarez Y, Chen K, Reynolds AL, Waghorne N, O'Connor JJ, Kennedy BN. Predominant cone photoreceptor dysfunction in a hyperglycaemic model of non-proliferative diabetic retinopathy. Disease models & mechanisms. 2010 Mar 8;3(3-4):236-45.
- 37. Kashyap B, Frederickson LC, Stenkamp DL. Mechanisms for persistent microphthalmia following ethanol exposure during retinal neurogenesis

- in zebrafish embryos. Visual neuroscience. 2007 May;24(3):409-21.
- 38. Matsui JI, Egana AL, Sponholtz TR, Adolph AR, Dowling JE. Effects of ethanol on photoreceptors and visual function in developing zebrafish. Investigative ophthalmology & visual science. 2006 Oct 1;47(10):4589-97.
- 39. Shi WJ, Huang GY, Jiang YX, Ma DD, Chen HX, Huang MZ, Hou LP, Xie L, Ying GG. Medroxyprogesterone acetate affects eye growth and the transcription of associated genes in zebrafish. **Ecotoxicology** and Safety. 2020 Environmental Apr 15:193:110371.
- 40. Huang L, Wang C, Zhang Y, Wu M, Zuo Z Phenanthrene causes ocular developmental toxicity in zebrafish embryos and the possible mechanisms involved. Journal of hazardous materials. 2013 Oct 15;261:172-80.
- 41. Bridges KN, Magnuson JT, Curran TE, Barker A, Roberts AP, Venables BJ. Alterations to the vision-associated transcriptome of zebrafish (Danio rerio) following developmental norethindrone exposure. Environmental Toxicology and Pharmacology. 2019 Jul 1;69:137-42.
- 42. Orger MB, Smear MC, Anstis SM, Baier H. Perception of Fourier and non-Fourier motion by larval zebrafish. Nature neuroscience. 2000 Nov;3(11):1128-33.
- 43. Emran F, Rihel J, Dowling JE. A behavioral assay to measure responsiveness of zebrafish to changes in light intensities. JoVE (Journal of Visualized Experiments). 2008 Oct 3(20):e923.

44. Dell'Osso LF, Daroff RB. Eye movement characteristics and recording techniques. Neuroophthalmology. 1990;2:279-97.

An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print); ISSN: 2249-9245 (Online)



RESEARCH ARTICLE

HERBAL CHEWING GUM FOR TREATING MOUTH INFECTION

Ujwala Desai^{1*}, Padmaja Kore², Anuradha More¹, Arati Chavan¹, Jyoti Hatekar¹, Shraddha Dhumal², Unmesha Badhale²

1*Department of Pharmaceutics, Progressive Education Society's Modern College of Pharmacy, Nigdi, Pune-44

² Department of Pharmacology, Progressive Education Society's Modern College of Pharmacy, Nigdi, Pune-44

*Corresponding author email: ujudesai@gmail.com Received: Feb 11, 2025 / Revised: April 25, 2025 / Accepted: May 10, 2025

ABSTRACT

Chewing gum has emerged as an innovative medium for drug delivery, providing both localized and systemic effects within the oral cavity. This study focuses on the development and evaluation of a herbal medicated chewing gum formulated with clove oil, oregano oil, lemongrass for its antibacterial efficacy. The chewing gum was analyzed for critical physical and functional parameters, including color, stickiness, hardness, weight variation, and drug release profile. An in vitro drug release study was conducted using a modified dissolution apparatus to simulate real- life mastication conditions. Among the tested formulations, F2 demonstrated the highest efficacy, achieving 93% drug release within 14 minutes, outperforming the other variations. The antibacterial assessment against Bacillus subtilis, Pseudomonas aeruginosa, and Staphylococcus aureus further confirmed the strong antimicrobial potential of the herbal formulation. The findings suggest that herbal chewing gum can serve as a novel and effective drug delivery system for oral infections, offering improved patient compliance and rapid therapeutic action.

Keywords: Herbal chewing gum, antibacterial activity, mouth ulcers, lemongrass, clove, oregano. **How to cite this article:** Ujwala Desai, Padmaja Kore, Anuradha More, Arati Chavan, Jyoti Hatekar, Shraddha Dhumal, Unmesha Badhale. Herbal Chewing Gum for Treating Mouth Infection. Bulletin of Pharmaceutical Research. 2025;15 (S2): 88-95.

Source of support: Nil Conflict of interest: None

1. INTRODUCTION

Mouth infections, like gum pain, sores, or conditions such as gingivitis, can be uncomfortable and affect our overall oral health. While traditional treatments often rely on antibiotics or mouthwashes, many people are now looking for natural alternatives to help with healing. Herbal chewing gum is one such

option, using natural plant-based ingredients to support oral health. $^{[1,3]}$

Bacteria and Fungus lives on the skin and inside the body, in places such as the mouth, throat, gut, and vagina, without causing any problems. Sometimes, Bacteria and Fungi can multiply and cause an infection [2][3]

1.1 Advantages of medicated chewing gum: Medicated chewing gum has emerged as a versatile drug delivery system, offering several distinct advantages:

1.1.1 Dual Action: Localized and Systemic Effects

Medicated chewing gum can provide both localized effects, such as the treatment of oral conditions, and systemic effects through buccal absorption or by swallowing saliva. This dual functionality enhances its potential as an effective treatment option for a range of health issues.

1.1.2 Improved Patient Compliance

The use of MCG promotes better adherence to medication regimens. The fast onset of action, coupled with enhanced bioavailability, makes this method particularly appealing. Drugs delivered via chewing gum are partially absorbed through the buccal mucosa, bypassing first-pass metabolism, which further contributes to their efficiency and effectiveness.

1.1.3 Suitability for Non-Cooperative Patients Medicated chewing gum is especially beneficial for patients who may struggle with conventional oral therapies. This includes individuals who have difficulty swallowing pills, are uncooperative, or require a more convenient and palatable drug delivery method [3][4].

1.2 Disadvantages of conventional dosage forms:

Despite their widespread use, traditional dosage forms such as tablets, capsules, and syrups have several limitations, particularly in specific contexts:

1.2.1 Dietary and Timing RestrictionsMany medications used to treat oral infections require precise timing and dietary considerations. Taking these drugs immediately before or after meals or consuming specific foods can reduce efficacy or cause unwanted drug-food interactions.

1.2.2 Potential Interactions with Alcohol and Tobacco

The use of alcohol or tobacco in conjunction with certain medications can result in harmful interactions, potentially compromising the drug's effectiveness or causing adverse effects.

1.2.3 Risks during Prolonged Use or Pregnancy

Extended or excessive use of certain conventional drugs may pose significant health risks. This concern is particularly relevant during pregnancy, especially in the first trimester, where the use of some medications may increase the likelihood of adverse outcomes for the developing foetus.^[5]

2. MATERIALS AND METHODS

2.1 Materials:

Lemongrass oil was obtained from Young Chemicals. Clove Oil was obtained from Oswal Chemicals, Oregano Oil was obtained from R.V. Essential. Polyvinyl Acetate was obtained from Chemdyes Corporation. Polyvinyl Alcohol was obtained from Chemdyes Corporation. Lanolin was obtained from Research Lab Fine Chem Industries, Mumbai. Calcium Carbonate was obtained from Loba Chemie Pvt. Ltd. Talc was obtained from Loba Chemie Pvt. Ltd. Sorbitol was obtained from Sisco Research Laboratories Pvt Ltd. Ester Gum was obtained from Kachabo Gums. Paraffin was obtained from Pure Chem Laboratories. Glycerine was obtained from Research Lab Fine. Titanium Dioxide was obtained from Pallav Chemicals. Microcrystalline Cellulose was obtained from Yellow Chem Products, Mumbai. Ascorbic Acid was obtained from Research Lab Fine Chem. Magnesium Carbonate was obtained from Research Pune.

2.2 Formulation method of Gum base:

• Preparation of gum base by using polyvinyl acetate (Elastomer):

The preparation of the gum base involves a systematic process to achieve a stable and homogeneous product suitable for further formulation.

• Melting of Core Ingredients

The elastomer polyvinyl acetate (PVAc) is carefully heated to a temperature range of 60–80°C until fully melted. At the same time, softening agents such as plasticizer, ester gum, and wax are melted at a slightly lower temperature range of 40–60°C. This controlled melting prevents degradation and ensures the ingredients maintain their functional properties.

• Combining Melted Components

The melted softening agents are then transferred into a mortar and pestle, forming the initial blend. The molten polyvinyl acetate is gradually added to this mixture, ensuring even distribution and creating a uniform base. This step is crucial for achieving the desired elasticity and texture.

• Incorporation of Talc

To improve the consistency and handling properties of the mixture, talc is added. The mixture is then subjected to high-intensity blending to ensure all components are uniformly incorporated, resulting in a smooth and well-integrated blend.

• Formation of the Gum Base

Through continuous mixing and careful control of the process parameters, the mixture solidifies into a gum base. This solid gum base forms the foundational matrix for adding active ingredients, flavours, and other components in subsequent formulation steps.^[6]

2.3 Select concentration of Lemongrass oil, Clove oil and Oregano oil:

The antifungal activity of various oil combinations against *Candida albicans* was assessed using an in vitro method. The experiment was carried out using the cup plate method on Sabouraud dextrose agar medium, following these steps:^{[7][8]}

- Preparation of Agar Plates
 The Sabouraud dextrose agar medium was poured into sterile petri plates and allowed to solidify.
- Inoculation of Fungal Suspension once the medium had solidified, a suspension of *Candida albicans* was evenly spread across the surface of the agar using a sterile spreader to

- ensure uniform distribution of the fungal culture.
- Creating Wells in the Agar Using a sterile cork borer, wells of 6 mm diameter were carefully created in the agar plates. These wells were designed to hold the oil samples for testing.
- Filling the Wells with Oil Samples
 Different concentrations of the oil combinations
 were prepared and filled into the wells. This step
 was performed under aseptic conditions in a
 laminar airflow chamber to prevent
 contamination.

Incubation

the petri plates were then placed in an incubator set at 37°C and allowed to incubate for an appropriate duration to facilitate fungal growth and interaction with the oil samples.^[7]

2.4 Incorporation of oils in gum base:-

The process for incorporating oil combinations into the gum base involves the following steps:

- **2.4.1 Melting the Gum Base** The gum base was heated in a water bath at a temperature range of 60–80°C until completely melted. This step ensured the gum base achieved a uniform consistency suitable for mixing with other ingredients.
- 2.4.2 Mixing in Mortar and Pestle The melted gum base was transferred to a mortar and pestle, where the selected oil combination was gradually added. The mixture was continuously stirred to ensure the oils were evenly dispersed throughout the gum base, achieving a homogeneous blend.
- **2.4.3 Incorporating Talc Powder (if required)** to enhance the texture and workability of the mixture, talc powder was added as needed. Continuous mixing was maintained to achieve a smooth and well-integrated final product [7][8]

2.4.4 Antibacterial activity against Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus:

The antibacterial properties of lemongrass oil, clove oil, and oregano oil were tested against two Gram-positive bacteria (*Bacillus subtilis and*

Staphylococcus aureus) and one Gram-negative bacterium (*Pseudomonas* aeruginosa). [9][10][11]

Table. 01: Selected concentrations of Lemongrass oil, Oregano oil and Clove oil

Factors	Ratio		
	Lemongrass Clove oil		Oregano
	oil		oil
S1	2	1	1
S2	1	2	1
S3	1	1	2

2.5 Evaluation of herbal chewing gum:

2.5.1 Diameter and Thickness:

10 medicated chewing gum are selected and then, the test for diameter and thickness is carried out by using the Vernier calliper. Then the value of the diameter and thickness is taken.

2.5.2 Test for Hardness/Plasticity:

5 medicated chewing gum are selected and then, the test for hardness is carried out by using the Rockwell hardness tester. Then the values of the hardness is taken.

2.5.3 Stickiness:

The MCG placed on the plan surface, mass of 110 gm pestle on it for period of ten minute. The frequency of hammering was about 30 minutes.[10]

2.5.4 Elasticity study

The elasticity of the chewing gum was evaluated using an analytical weighing balance. The procedure involved the following steps:

Setup and Stretching

The lower clamp of the apparatus was held stationary, while the upper clamp was used to pull and stretch the chewing gum. Stretching continued until the gum broke, indicating the point at which it lost elasticity. The weight required to break the gum was recorded as a measure of its elasticity.

Alternate Method

In another setup, one end of the chewing gum was affixed to the bottom of the analytical weighing balance, while the other end was adhered to a flat surface. Incremental weights

were added to the weighing balance, causing the gum to stretch. The gum was stretched until it broke, and the weight required at this breaking point was recorded.

• In vitro release study:

The release of oils from the medicated chewing gum (MCG) was studied using phosphate buffer under simulated mastication conditions.

Release Study Procedure

The herbal chewing gum and phosphate buffer were placed in a mortar, and mastication-like conditions were simulated using a pestle. This simulated chewing motion facilitated the release of oils from the gum over time.

Sampling Intervals

The buffer samples were collected at specific time intervals: 0, 2, 4, 6, 8, 10, 12, 15, and 18 minutes. These samples were analysed to study the release profile of the oils from the chewing gum.

Comparison with marketed formulation:

- ➤ **Marketed Formulation**: Candid mouth paint (Clotrimazole) GLENMARK PHARMA
- Comparison of marketed formulation and medicated chewing gum is through performing antifungal activity against *Candida albicans*.

3. RESULT AND DISCUSSION

Table. 02 : Zone of inhibition for individual oils

Sr.	Name of oil	Bacterial species	Zone of
No			inhibition
1	Lemongrass	a)Bacillus subtilis	32 <u>+</u> 3 mm
	oil	b)Pseudomonas	26 <u>+</u> 3 mm
		aeruginosa	27 <u>+</u> 3 mm
		c)Staphylococcus aureus	
2	Clove oil	a)Bacillus subtilis	37 <u>+</u> 2 mm
		b)Pseudomonas	29 <u>+</u> 2 mm
		aeruginosa	38 <u>+</u> 5 mm
		c)Staphylococcus	
		aureus	
3	Oregano oil	a)Bacillus subtilis	29 <u>+</u> 2 mm
		b)Pseudomonas	29 <u>+</u> 3 mm
		aeruginosa	28 <u>+</u> 2 mm
		c)Staphylococcus	
		aureus	

Shows preliminary trial batches prepared for the selection of oils. This study concluded that lemongrass oil, clove oil and oregano oil show strong antibacterial activity (Table 2and figures 1,2,3 and 4)

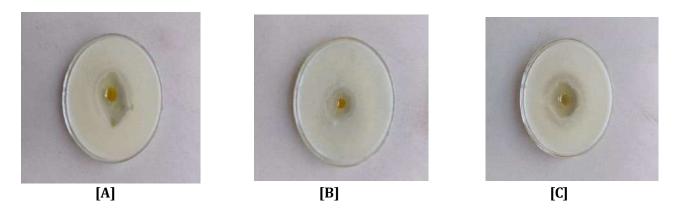


Fig. 1: Antibacterial activity of lemongrass oil against



Fig. 2: Antibacterial activity of clove oil against

[A] Bacillus subtilis [b] Pseudomonas aeruginosa [c] Staphylococcus aureus

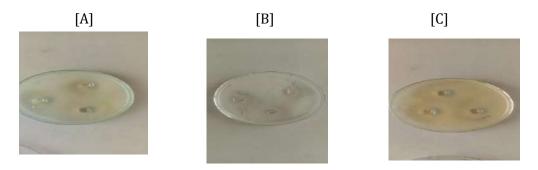


Fig. 3: Antibacterial activity of oregano oil against

[A] Bacillus subtilis[b] Pseudomonas aeruginosa [c] Staphylococcus aureus





[B]



[C]

Fig. 4: Antibacterial activity of olive oil against [A] Bacillus subtilis [B] Pseudomonas aeruginosa [C] Staphylococcus aureus

3.1 Evaluation of Herbal Chewing Gum Physical evaluation of gum base

Table no. 3: Evaluation of Gum Base

Physical	1) Soft	1) Soft
appearance	2) Chewy	2) Chewy
	3) Reduced	3) Reduced
	tackiness	tackiness
	4) No odour or	4) No odour or
	taste	taste
	5) No colour	5) No colour
	6) Non-toxic	6) Non-toxic
	7) Excellent	7) Excellent
	stability	stability against
	against	oxidation and
	oxidation and	UV light
	UV light	8) High purity
	8) High purity	9) Insoluble
	9) Insoluble	
Elasticity		7.2 cm
study	7-9 cm	
	l	1

Table no. 4: Evaluation of Herbal Chewing

Samp les	Thickn ess	Diam Gun r	Hardness (Mean Max Force)	Elastici ty	Stic kin ess
S1	0.13cm	0.1cm	4.3	8.2cm	0%
S2	0.13cm	0.1cm	5	7.9cm	0%
S3	0.13cm	0.1cm	4.8	8cm	0%
Aver age	0.13	0.1	4.7	8.0	0%

The gum base showed good organoleptic properties as compared to standard (Table 2).

In vitro release study against the *Candida* albicans

Table no. 5: In vitro release study

Sr. No.	Time	Zone of inhibition
	interval	
1	0min.	0mm
2	2min.	12+2mm
3	4min.	14+2mm
4	6min.	15+3mm
5	8min.	20+4mm
6	10min.	24+3mm
7	12min.	25+2mm
8	15min.	27+3mm
9	18min.	27+4mm

Table 4 shows the in vitro release study of MCG at a time interval of 0, 2,4,6,8,10,12,15, and 18 min. This study concluded that the oils are released from chewing gum within a particular time (Figure 5).







0 minutes

2 minutes

4 minutes



12 minutes 15 minutes 18 minutes

Fig. 5: Zone of inhibition during in vitro drug release

Comparison with marketed formulation:

Compare the antifungal activity of herbal chewing gum with marketed medicated chewing gum (Candid mouth paint)

Fig. 6: Zone of inhibition for optimized formulation and marketed formulation



[a] Herbal chewing gum [b] Marketed formulation

4. DISCUSSION

The present study aimed to develop and evaluate an herbal medicated chewing gum formulated with clove, oregano, and lemongrass oil to treat mouth ulcers and oral infections. The findings demonstrate that herbal chewing gum can be a novel and effective drug delivery system with significant antibacterial and antifungal properties.

4.1 Antimicrobial Effectiveness

The antibacterial assessment of the formulated chewing gum against Bacillus subtilis,

Pseudomonas aeruginosa, and Staphylococcus aureus revealed strong antimicrobial potential. Clove oil exhibited the highest antibacterial activity among the tested essential oils, followed by oregano oil and lemongrass oil. The observed zone of inhibition indicates the potential of these oils in preventing and treating bacterial infections associated with oral ulcers and gingivitis.

Furthermore, the in vitro antifungal study against Candida albicans demonstrated that the herbal formulation exhibited notable antifungal activity, with an increasing zone of inhibition over time. This suggests that herbal chewing gum could be an effective alternative to marketed antifungal formulations like Candid Mouth Paint (Clotrimazole).

4.2 Drug Release Efficiency

The in vitro drug release study conducted under simulated mastication conditions showed that 93% of active ingredients were released within 14 minutes for the most effective formulation (F2). The rapid release of herbal extracts suggests an enhanced therapeutic effect, allowing for quick relief from mouth infections and improved bioavailability. Compared to traditional dosage forms, the chewing gum formulation bypasses first-pass metabolism, leading to better drug absorption.

4.3 Physicochemical Properties of the **Chewing Gum**

The physical evaluation of the formulated chewing gum confirmed its suitability for oral administration. The gum demonstrated optimal hardness, elasticity, and stickiness, ensuring patient compliance and ease of use. The gum base formulation also provided good stability, non-toxicity, and no unwanted taste or odor, making it a palatable and effective alternative to conventional treatments.

4.4 Comparison with Marketed Formulations

A comparison of the herbal chewing gum with marketed formulations (such as Candid Mouth Paint) showed comparable or superior antimicrobial The herbal-based activity. formulation provides additional benefits,

including natural ingredients, fewer side effects, and better patient acceptability. Unlike synthetic antifungal agents, herbal formulations are less likely to cause drug resistance or adverse reactions with prolonged use.

5. CONCLUSION

Overall, the study demonstrates that herbal medicated chewing gum can be a safe, effective, and convenient alternative to conventional treatments for oral infections and ulcers. The combination of lemongrass, clove, and oregano oils in the gum matrix enhances antibacterial and antifungal activity, offering a promising natural solution for oral health management.

6. REFERENCES

- Aslani A, Rostami F, "Medicated chewing gum, a novel drug delivery system" J Res Med Sci 1-9
- 2. Muhammad Ashraf Nazir Prevalence of periodental disease, its association with systemic diseases and prevention, ISSN, 11(2): 72–80.
- 3. Alawadh M. General Dentist, An overall review for undergraduates Gingivitis.
- 4. Aslani1 A, Rostami, Medicated chewing gum, a novel drug delivery system, *PMID* 22015 Apr; 20(4): 403–411.
- 5. Deepali S, Ganesh G,"Recent Trends in Medicated Chewing Gum Technology: Formulation Strategies and Characterization", *Int. J. Pharm. Sci.*, 72-85.
- Lakshmi S, Yadav H, Mahesh K, Uniyal S ,Ayaz A, "Formulation and evaluation of medicated chewing gum as antiplaque and antibacterial, Journal of Young Pharmacists", Vol 6, 3-14
- 7. Onoriode S, and Edeh, "Lemongrass (CYMBOPOGON CITRATUS) oil and its applications, *ISSN*, 202-210

- Hagbani T Nazzal S, "Medicated Chewing Gums (MCGs) Composition, Production, and Mechanical Testing" AAPS PharmSciTech, Vol. 19, 2-20
- 9. Marzouk M Darwish M Fattah M, "Development of Medicated Chewing Gum Using Natural Gum Base" *ijppr*, Vol.16,70-80
- 10. Lakshmi S ,Yadav H, Mahesh K ,Uniyal S , Ayaz A ,Nagavarma B. "Formulation and evaluation of medicated chewing gum as antiplaque and antibacterial agent". JYP , Vol 6,3-7
- 11. Kusuma I , Perdana M, Vágvölgyi C, Csupor D, Takó M. "Exploring the Clinical Applications of Lemongrass Essential Oil: A Scoping Review". MDPI,70-78
- 12. Aslani A,Rostami F. "Medicated chewing gum, a novel drug delivery system"
- 13. JHANWAR B, TOMAR B, VERMA S. "Chewing Gum: Confectionary To A Popular Transbuccal Dosage Form". Vol 10, 65-68
- 14. Takenouchi A, Saeki Y, Otani E, Kim M, Fushimi A, Satoh Y, Kakegaw Ta Y, Arai H, Taguchi N, Matsukubo T. "Effects of Chewing Gum Base on Oral Hygiene and Mental Health: A Pilot Study". Volume 62, 410-415

An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print); ISSN: 2249-9245 (Online)



RESEARCH ARTICLE

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING ASSAY METHOD FOR DETERMINATION OF METOPROLOL TARTRATE IN BULK AND MARKETED FORMULATION BY HPTLC METHOD

Aishwarya R. Balap^{1*}, Hrushikesh. K. Giramkar¹, Anuja H. Pachphule¹, Leena P. Patil¹, Sumedha S. Dakhore, Sampada S. Salunke

P. E. Society's, Modern college of Pharmacy, Yamunanagar, Nigdi, Pune, India-411044

*Corresponding author email: balapaishwarya8@gmail.com

Received: Feb 09, 2025 / Revised: April 20, 2025 / Accepted: May 03, 2025

ABSTRACT

To estimate metoprolol tartrate (MT) in commercial tablet formulations and bulk medication formulations, stability-indicating HPTLC The strategy was created in according with International Council for Harmonization criteria. The separation of components was performed through chromatography using a specific mobile phase made up of 8:2:0.2 (v/v/v) toluene, methanol, and glacial acetic acid on Silica gel-coated plates 60F254 HPTLC plates. A clear and compact band for MT was produced by the system, with an Rf value of 0.25 ± 0.02 . The TLC scanner was adjusted to 222 nm for Analyte densitometric measurement in absorbance mode. The technique demonstrated a linear calibration curve across the 100-600 ng/band range with a calibration curve (R^2 > 0.996) showing a significant correlation between analyte concentration and peak regions. The proposed method's accuracy was evaluated using the standard addition methodology, which resulted in a percent recovery of 99.96%. Stress testing was carried out in compliance with ICH recommendations, and the results revealed that MT degraded under alkaline stress conditions. According to ICH recommendations, the response standard deviation and slope were utilized to calculate the Limit of Detection (LOD) and Limit of Quantification (LOQ) employing a validated method. Forced degradation studies were performed under acidic, alkaline, thermal, oxidative and photolytic conditions. The method proved effective for detecting and quantifying degradation products in the formulation. Mass spectrometry was employed to elucidate the structure of the alkaline degradation product, which had been isolated using a validated preparative HPTLC method.

Keywords: Metoprolol Tartrate, HPTLC, Mass Spectroscopy, Force Degradation, Validation. **How to cite this article:** Aishwarya R. Balap, Hrushikesh. K. Giramkar, Anuja H. Pachphule, Leena P. Patil, Sumedha S. Dakhore, Sampada S. Salunke. Development and Validation of Stability Indicating Assay Method for Determination of Metoprolol Tartrate in Bulk and Marketed Formulation by HPTLC Method. Bulletin of Pharmaceutical Research. 2025;15(S2): 96-103.

Source of support: Nil Conflict of interest: None

1. INTRODUCTION

The chemical name of metoprolol tartrate is 1-[4-(2-methoxyethyl) phenoxy]-3- [amino (1-methylethyl)] propane. The chemical formula for 2-propanol hemitartrate is $(C15H25NO3)2\cdot C4H606$, and its molecular weight is 684.81. This $\beta1$ -selective adrenergic antagonist is primarily employed in the management of mild

to severe hypertension, angina pectoris, heart failure, and acute myocardial infarction (MI). It can also be used to prevent migraine headaches and treat tachyarrhythmias and supraventricular problems. At lower dosages, metoprolol primarily inhibits cardiac β 1-adrenergic receptors, with little effect on vascular smooth muscle and lung β 2-adrenergic receptors. Schematic diagram of

Metoprolol Tartrate structure show in Fig.No.1

Fig. No. 1: Schematic diagram of Metoprolol Tartrate structure

According to the literature review, the only methods available for determining MT on its own are UV and HPLC. However, the examination of MT in pharmaceutical dose form and bulk, no stability showing HPTLC approach along with degradation has been recorded. formulation (tablet). ICH guidelines were followed in the process of optimization and validation of the recommended approaches. Metoprolol tartrate acts by competing catecholamines and other adrenergic neurotransmitters for binding to \(\beta 1-\) adrenergic receptors in the heart, which reduces Heart rate, Blood pressure, and Cardiac Output.

2. MATERIALS AND METHODS

2.1 EXPERIMENTAL

Sava Healthcare in Pune, Maharashtra, India, provided a gift sample of pharmaceutical-grade metoprolol tartrate with a purity of 99.9%. Flexeril tablets with 25 mg MT as stated on the label were bought from a nearby drugstore. Toluene, Ethyl acetic acid and glacial acetic acid have been employed as solvents, with all substances and reagents utilized being of HPLC or AR grade quality.

Production of the primary stock solution A

A 10 mL volumetric container was filled with a precisely weighed 10 mg sample of standard metoprolol tartrate (MT). After dissolving the sample with an appropriate amount of methanol, the sample was agitated and sonicated for 15 minutes. Methanol was subsequently added to adjust the volume, achieving a final concentration of $100 \, \mu g/mL$.

Selecting and optimizing the mobile phase's composition

Bands of standard stock solution (4 μ L each) were put on TLC plates in 6 mm broad aliquots. To produce a recognizable and symmetric peak for

the drug, a variety of solvents with varied polarities and combinations were used. After assessing multiple solvent system adjustments, the most successful combination was determined to be toluene methanol, and glacial acetic acid H(8:2:0.2 y/v), resulting in a well-shaped peak with an adequate Rf value. Figure No. 2 shows an example MT densitogram.

Selecting the wavelength for the isolated band's densitometric analysis

To determine the optimal wavelength for densitometric analysis of the separated band, a 4 μ L portion of the standard stock solution was applied to a TLC plate using a CAMAG LINOMAT-V automatic sample applicator. The plate was developed in a twin-trough glass chamber that had been pre-saturated with the mobile phase for 15 minutes. Following development, the plate was removed and allowed to air dry. The bands on the TLC plate were then scanned across a wavelength range of 200 to 700 nm. Spectral analysis indicated that Metoprolol Tartrate (MT) exhibited a prominent maximum absorbance at 222 nm.

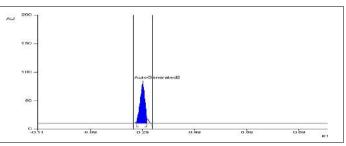


Fig. No. 2: Typical

densitogram of MTP

2.2 INSTRUMENTATION

HPTLC analysis was conducted using a Camag instrument. Chromatography was carried out on $10~\rm cm \times 10~\rm cm$ aluminum-backed plates, precoated with a $0.2~\rm mm$ layer of silica gel $60~\rm F254$ (E. Merck, Germany). Sample application was performed using a Camag Linomat V applicator, fitted with a $100~\rm \mu L$ Hamilton syringe (Bonaduz, Switzerland). Samples were applied as $6~\rm mm$ wide bands, spaced $5~\rm mm$ apart, under a continuous nitrogen stream at an application rate of $150~\rm nL/s$. Separation was achieved with a mobile phase composed of toluene, methanol, and glacial acetic acid in a $8:2:0.2~\rm (v/v)$ ratio. The plates were developed to a distance of $80~\rm mm$ in a twin-trough glass chamber, pre-saturated with mobile phase

Balap et al

vapors for 10 minutes. Densitometric analysis was performed at 222 nm using a Camag TLC Scanner III in reflectance-absorbance mode, with a deuterium lamp. Data acquisition and analysis were conducted with WINCATS software version 4. The scanning parameters included a slit size of 5 mm \times 0.45 mm and a scanning speed of 20 mm/s. Detection was based on the intensity of the reflected light from the chromatographic spots.

Calibration plots for MT

To obtain a final concentration of 1000 μg/mL, 10 mg of metoprolol tartrate (MT) was precisely weighed and transferred into a 10.0 mL volumetric capillary container. It was then diluted to the calibration mark using methanol. A LINOMAT V automatic sample applicator and a microsyringe were used to apply the prepared solution in volumes ranging from 1 to 6 µL to a TLC plate. The sample Plates were then made manufactured and tested using chromatographic parameters that had been established. Each drug concentration's peak area was measured, and A curve for calibration was created by graphing the concentration against the peak area for MT. Calibration curve for MT display in Fig. 3

Making a sample solution for the analysis of a commercial formulation

An accurately weighed amount of tablet powder, equivalent to approximately 25.0 metoprolol tartrate (MT), was transferred into a 10.0 mL volumetric flask. Methanol was added to the flask, and the mixture was sonicated for 15 minutes to ensure thorough dissolution. The volume was then made up to the mark with methanol, and the solution was mixed well. The resulting solution was filtered through Whatman filter paper No. 42. Four bands of the sample solution (4.0 µL each) and two bands of reference solution A were applied to a TLC plate. The plate was subsequently developed and scanned under chromatographic conditions. optimized concentration of the drug in the sample was determined by comparing the average peak area of the sample bands to that of the standard bands.

2.3 VALIDATION METHODS

The procedure was validated using the International Council Harmonization recommendations.

Accuracy

A precisely weigh portion of tablet powder, which is comparable to approximately 10.0 mg of Metoprolol Tartrate (MT), was placed into each of nine separate 10.0 mL volumetric flasks. To obtain recovery levels of 80%, 100%, and 120%, the samples were supplemented with MT (8mg, 10 mg, and 12mg, respectively). All solutions were made with methanol as the diluent. Each level of analysis was repeated three times, and accuracy was measured using the percentage recovery. Result of accuracy study shown in Table No. 2

Precision

Precision studies were conducted to assess the method's reproducibility and repeatability. The process described for The evaluation of the commercially accessible formulation was followed in the preparation and analysis of the sample solution. studying the sample solution three times in a single day allowed for the assessment of intraday precision, whereas studying the sample solution across three days in a row allowed for the determination of inter-day precision. Results of Precision Studies shown in Table No.3

Robustness

To test the stability of the indicated approach, slight but intentional modifications were made to the ideal parameters. The effect of various variables on the drug's Rf value and peak area has been examined by varying the mobile phase composition, chamber saturation time, mobile phase volume, the time between sample application and development (5min, 10min, and 15 minutes), and the time between development and scanning (5min, 10min, and 15 minutes). The mobile phase composition was adjusted slightly by ±0.1 mL for each component, with two variations tested: Toluene, Methanol, and Glacial Acetic Acid in ratios of 1.9:8.1:0.2 (v/v/v) and 2.1:7.9:0.2 (v/v/v). Furthermore, differences in the mobile pohases volume (9 mL, 10 mL ± 0.1 mL, and 11 mL) and chamber saturation time (5, 10 ± 5 minutes, and 15 minutes) were examined to determine their effects on chromatographic

Balap et al performance.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated separately based on the standard deviation of the responses obtained from the calibration curve. These limits were determined using the slope (S) of the calibration curve and the standard deviation (σ) of the y-intercept, applying the formulas: LOD = $3.3\sigma/S$ and LOQ = $10\sigma/S$.

Forced degradation studies

The sample was subjected to a range of stress conditions, such as oxidative (3% H2O₂), acidic (0.1 M HCl) and Basic or Alkaline (0.1 M NaOH) in order to perform forced degradation tests. The samples were refluxed for three hours at 80°C in a bath to achieve these conditions. Furthermore, the sample was stored at 60°C to test thermal degradation and evposed UV to radiation (254 nm) for 24 hours to evaluate photodegradation. Following the specified time intervals, all samples were taken out, given time to cool, and then examined using the method described for MT analysis in the commercial formulation. Result of Force Degradation studies shown in Table No. 05

3. RESULT AND DISCUSSION

Mobile phase optimization

To optimize the mobile phase, different ratios of Glacial Acetic Acid, methanol, and toluene were investigated. The ratio of toluene: methanol: glacial acetic acid (8:2:0.2 v/v/v) was chosen as the mobile phase out of all the combinations that were assessed. The generated bands had a respectable Rf value of 0.25 for MT and were dense and clearly delineated. Furthermore, when the Plates were examined at 222nm the peaks showed no tailing and were symmetrical.

Linearity

In contrast to peak heights, peak regions showed a higher linear association with concentration, suggesting that MT offered a linear detector response. The linearity of the procedure was confirmed by the highest correlation coefficient (r = 0.996) shown by the MT calibration curve.

Analysis of the commercial formulation

A percentage of the labeled claim was used to represent the outcomes of a pharmaceutical formulation that contained 25.0 mg of metoprolol tartrate (MT). The excipients did not cause any interference. Table No. 1 indicates that the MT content was approximately 100%. The method's efficacy and dependability for routine analysis were demonstrated by the low standard deviation (SD) number.

Recovery studies

To determine the correctness of the suggested approach, recovery tests has been done using the addition procedure, with results expressed as a percentage. The average percentage recovery for each chemical at various levels of concentration has been determined and released, along with the standard deviation. The rate of recovery for MT at 80%, 100%, and 120% was determined to be satisfactory, as shown in Table 2, demonstrating that the proposed technique is valid.

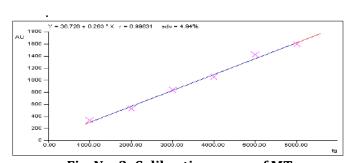


Fig. No. 3: Calibration curve of MT
Table No. 1: Summary of Analysis Results for
Marketed Formulation

Drug	% Estimation (mg/tablet)	standard Deviatio n (±)	Relative Standard Deviation (±)
MT	101.13	±1.51	±1.49

Table No. 2: Result of accuracy study

Sr. no	Amount of tablet powder weighed (mg)	Amoun t of the Drug added (mg)	Quantity of drug recovered (mg)	Percentag e (%) Recovery
1	100	8	8.11	101.48
2	100	8	8.09	101.15
3	100	8	7.98	99.83
4	100	10	9.83	98.37
5	100	10	9.98	99.84
6	100	10	10.05	100.53
7	100	12	11.81	98.47
8	100	12	11.93	99.39
9	100	12	10.07	100.64
			Mean	99.9 6
			S.D.	1.09
			%R.S. D	1.09

Precision Study Results

Intraday

Table No. 3: Precision Study Results

Drug	Amount of drug taken (mg)	% Mean obtained	S.D.	%R.S.D
	8	100.82	0.87	0.86
MT	10	99.58	1.40	1.40
	12	99.50	1.08	1.08

*denotes average of three determinations Interday precision

Table No. 4: Result of Interday Precision

*denotes average of three determinations

Drug	Amount of drug taken(mg)	% Mean value obtained	S.D.	%RD
	8	100.82	0.87	0.86
MT	10	99.58	1.40	1.40
	12	99.50	1.08	1.08

Robustness

The effect of minor changes in chamber saturation duration (±5 minutes), mobile phase volume (±1 mL), and mobile phase composition (±0.1 mL) on the drug's Rf value was assessed. Under these slight variations, the approach showed stability, as the percentage RSD for Rf values stayed below 2% under all circumstances. These results validate the robustness of the established technique.

Outcome of the Forced Degradation Study Table No. 5: Result of Force Degradation studies

Stress testing conditions	Assay percentage of the active pharmaceutical ingredient (API)	Rf value of the degradation product
Acid (0.1 M HCl, 80°C for 3 hrs)	89.62	0.06, 0.37,0.79
Base/Alkali (0.1 M NaOH, 80ºC for 3 hrs)	83.57	0.34,0.59
Oxide (3 % H2O2, 80°C for 3 hrs)	88.91	0.04, 0.39,0.72
Heat (60°C for 24 hrs)	95.51	0.49,0.60,0.66
UV- Exposure (254 nm for 24 hrs)	97.10	0.30

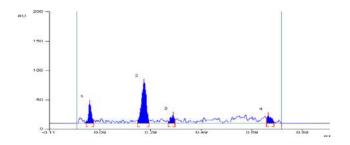


Figure No. 4: Densitometric HPTLC profile of MT after treatment under acidic stress conditions

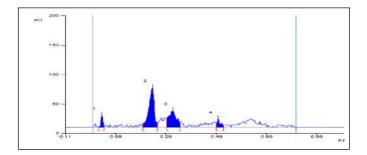


Figure 5 : HPTLC densitogram of MT subjected to alkaline stress conditions

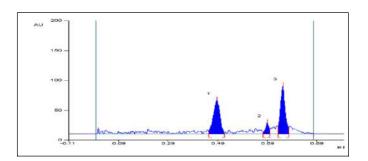


Figure No.6: HPTLC densitogram of MT subjected to thermal degradation conditions

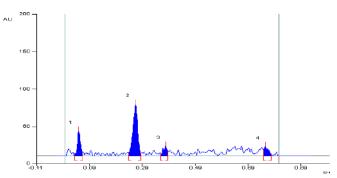


Figure No. 7: HPTLC densitogram of MT under the presence of hydrogen peroxide

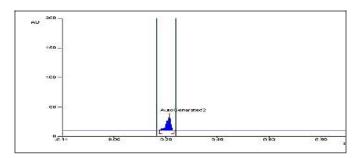


Figure No.8: HPTLC densitogram of MT subjected to photolytic stress conditions

4. CONCLUSION

A robust, accurate, and stability-indicating HPTLC method was successfully developed and validated for estimating Metoprolol Tartrate (MT) in both bulk and commercial tablet formulations. The method was optimized using a mobile phase composed of Toluene, Methanol, and Glacial acetic acid in a 8:2:0.2 (v/v/v) ratio, which produced sharp, well-resolved bands for MT with an Rf value of 0.25 ± 0.02 . Densitometric detection at 222 nm ensured high sensitivity and selectivity. The method demonstrated excellent linearity (R² > 0.996) over a concentration range of 100-600 ng/band, along with satisfactory accuracy (mean recovery ~99.96%), precision (RSD < 2%), and robustness, confirming its suitability for routine quality control analysis. Notably, forced degradation studies under alkaline, acidic oxidative, photolytic and thermal conditions confirmed that the method effectively separates drug from its degradation products, the showcasing its stability-indicating capability. The alkaline degradation product was successfully isolated and structurally characterized by mass spectrometry, further supporting the method's applicability in stability assessments. In summary, this validated HPTLC method provides a simple, cost-effective, and rapid analytical approach for quantitative estimation and stability evaluation metoprolol tartrate in pharmaceutical formulations, in compliance with ICH guidelines.

5. ACKNOWLEDGEMENT

The authors express their sincere gratitude to Sava Pharmaceutical Pvt. Ltd., India for generously providing the working standard of Metoprolol Tartrate as a gift sample. They also extend their thanks to the Principal of Modern College of Pharmacy, Pune, and to Dr. D. Y. Patil College of Pharmacy, Pimpri, Pune for offering the necessary instrumentation and infrastructure support to successfully conduct this research.

6. REFERENCES

- 1. Bakshi M. and Singh M., "Development of validated stability indicating assay methods critical review." (2002), 28(6), Page no-1011-1040.
- 2. Guidance for industry, Q2 (R1) validation of analytical procedure: Methodology, U.S department f health and human services, Food and drug administration, centre for the drug evaluation and research (CDER), centre for biologics evaluation and research (CBER), international conference of harmonization (ICH), Geneva, November-2015.
- 3. International conference of harmonization (2000) draft received guidance on impurities in new drug substance, Federal Register Q3A(R), (139); Page no- 45085
- 4. International conference of harmonization (2000) draft received guidance on impurities in new drug substance, Federal Register Q3B(R), (139); 44791
- 5. International conference of harmonization (1997) impurities, Q3C-guideline for residual solvent, Q3C, Federal Register 62 (247); Page no-67377.
- 6. International conference of harmonization (1999) specification Q6A-test procedure and acceptance criteria for new drug substance and new drug product. Chemical substance, (146), Page no- 67488
- 7. ICH, Stability testing of new drug substance and product. International conference of Hormonization, Geneva, (1993).
- 8. Dhale C, Joshi S, development and validation of spectro-photometric method for determination of metoprolol tartrate. App Pharm Vol. 6; Issue 3: 280 -285; July, 2014
- 9. European Pharmacopoeia 5.0, 01/2005:1028, monograph of Metoprolol Tartrate . 2033- 2034.

- 10. Mustafa C, Derya Τ, Aysegul G Spectrophotometric Determination of Metoprolol Tartrate in Pharmaceutical Dosage Forms on Complex Formation with access iournal Cu(II) open Pharmaceuticals 2011, ISSN 1424-8247, 964-975
- 11. Jadhav AS, Tarkase KN, Deshpande AP. Quantitative Determination of Metoprolol Succinate in Bulk and Tablet Dosage Form Through Comparative Study of UV and Derivative Spectroscopy. Der Pharmacia Lettre 2012; 4 (3): 763-767.
- 12. Herring VL, Bastian TL, Lalonde RL. Solidphase extraction and direct highperformance liquid chromatographic determination of metoprolol enantiomers in plasma. Journal of Chromatography B: Biomedical Sciences and Applications. 1991 Jun 14;567(1):221-7.
- 13. Straka RJ, Johnson KA, Marshall PS, Remmel RP. Analysis of metoprolol enantiomers in human serum by liquid chromatography on a cellulose-based chiral stationary phase. Journal of Chromatography B: Biomedical Sciences and Applications. 1990 Jan 1;530:83-93.
- 14. KannaRao KV, Rao ME, Nagoji KE, Rao SS. Determination of Metoprolol Tartrate by reverse phase HPLC. Indian J Pharm Sci 2003;65;204-6
- 15. Badulescu M, Balala UD, Cacovean I, Ilie M, Baconi DL. UV-Visiblespectrophotometeric assay of metoprolol. Note-2. Method validation. Farmacia 2008;LVI(4):363-70.

- 16. Herring VL, Bastian TL, Lalonde RL. Solidphase extraction and direct highperformance liquid chromatographic determination of metoprolol enantiomers in plasma. Journal of Chromatography B: Biomedical Sciences and Applications. 1991 Jun 14;567(1):221-7.
- 17. Pagar SA, Shinkar DM, Saudagar RB. Development and validation of spectrophotometric method for determination of metoprolol succinate. Int J Pharm Bio Sci. 2013;3(4):224-8.
- 18. Garg G, Saraf S. Spectrophotometric and column high-performance liquid chromatographic methods for simultaneous estimation of metoprolol tartrate and hydrochlorothiazide in tablets. JAOAC Int 2008;91:1045-50.
- 19. Gupta KR, Tajne MR, Wadodkar SG. New Spectrophotometric method for simultaneous determination of metoprolol tartrate and hydrochlorothiazide in tablets. Indian J Pharm Sci 2008;70:511-3.
- 20. Jain N, Kumar Jain D, Jain R, Patel P, Kumar Patel V, Kumar Jain S. Simultaneous Estimation of Metoprolol Succinate and Lacidipine in Binary Combination using High Performance Liquid Chromatographic Method. Jordan Journal of Pharmaceutical Sciences. 2016 Jul 1;9(3).
- 21. Kulkarni MN, Kshirsagar RV, Sakarkar DM.
 Development and validation of spectrophotometric method for determination of metoprolol succinate.
 International Journal of ChemTech Research. 2009 Oct;1(4):1273-7.

An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print); ISSN: 2249-9245 (Online)



REVIEW ARTICLE

Nanotechnology Approaches in Triple Negative Breast Cancer

Padmaja Kore^{1*}, Rohan Lad¹, Asit Sirsat¹, Anuradha More², Ujwala Desai³

- ¹ Department of Pharmacology, Progressive Education Society's Modern College of Pharmacy, Yamunanagar, Nigdi, Pune-44
- ^{2,3} Department of Pharmaceutics, Progressive Education Society's Modern College of Pharmacy, Yamunanagar, Nigdi, Pune-44

*Corresponding author email: Padmaja.kalshetti@gmail.com

Received: Feb 15, 2025 / Revised: April 28, 2025 / Accepted: May 20, 2025

ABSTRACT

Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer characterized by the absence of estrogen, progesterone, and HER2 receptors, making it resistant to conventionally targeted therapies. Nanotechnology-based approaches have emerged as promising strategies to overcome the challenges associated with TNBC treatment, offering enhanced drug delivery, improved tumor targeting, and reduced systemic toxicity. This review explores various nanotechnology platforms, including liposomes, polymeric nanoparticles, dendrimers, and inorganic nanocarriers, that have been employed for TNBC therapy. Additionally, the potential of nanomedicine in personalized treatment, immunotherapy, and overcoming multidrug resistance is discussed. The advancements in preclinical and clinical studies highlight the potential of nanotechnology in revolutionizing TNBC management. However, challenges such as scalability, regulatory hurdles, and long-term safety must be addressed to facilitate the clinical translation of these nanotherapeutics.

Keywords: Triple Negative Breast cancer, Nanotechnology, Liposomes, nanoparticles, immunotherapy **How to cite this article:** Padmaja Kore Rohan Lad, Asit Sirsat, Anuradha More, Ujwala Desai.

Nanotechnology Approaches in Triple Negative Breast Cancer. Bulletin of Pharmaceutical Research. 2025;15(S2): 104-112.

Source of support: Nil Conflict of interest: None

1. INTRODUCTION

Breast cancer is the uncontrolled development of breast tissue, especially of the ducts for milk with lobules. Only 10% of events of breast cancer occur in lobules, whereas 80% of cases occur in milk ducts and are referred to as ductal carcinoma^[1]. TNBC belongs to the aggressive breast cancer subtypes because it lacks all three

essential markers, including ER, PR, and HER2. TNBC fails to respond to hormonal or HER2-targeted treatments because it lacks the essential molecular targets, making conventional chemotherapy, as well as surgery and radiation, the only available treatment options. The challenging characteristics of TNBC include rapid spread of cancer cells and reemergence, as well as unpropitious survival

prospects, demanding the swift introduction of new therapeutic approaches [2].

A standardized therapy plan does not exist for TNBC treatment. Due to the invasive nature of TNBC cells, patient lifetimes are shortened to 18 months when they spread to organs such the liver, brain, and lungs [3]. The survival duration for TNBC patients becomes significantly shorter after metastases spread to areas outside the primary site^[4].

1.1 Subdivision

TNBC shows identical genetic expression pathways to basal-like breast cancer (BLBC) subtype. Six TNBC subtypes were found by research, including immunomodulatory subtype (IM), mesenchymal subtype (M), mesenchymal stem-like subtype (MSL), luminal androgen receptor subtype (LAR), and basal-like 1 (BL-1) and basal-like 2 (BL-2) [5].

1.1.1. Basal-like 1 (BL-1)

The BL1 subtype demonstrates increased activity of genes that control cell replication through Aurora kinase and MYC mechanisms.

This mutation activates a set of genes, including those related to cell cycle progression and DNA damage response pathways.

Highly proliferative and sensitive to platinum-based chemotherapy and PARP inhibitors [6].

1.1.2. Basal-Like 2 (BL-2)

Research suggests that BL2 cells activate growth factor signaling through the Epidermal growth factor receptor (EGFR), together with the Wingless/Integrated Insulin-like growth factor 1 receptor (IGF-1R), mesenchymal-epithelial transition (MET), the Wnt, and others.

Like the BL2 subtype, the MSL subtype enhanced gene expression for growth factor signal transmission [7].

1.1.3. Luminal Androgen Receptors

Through their activation mechanism, the luminal androgen receptors control steroid synthesis and porphyrin production and regulate both androgen and estrogen metabolism.

The presence of an androgen receptor in most TNBC cases indicates a good chance of patient survival. The prognostic value of AR makes it

serve as an indicator. The expression levels of androgen receptor in this subtype are ten times stronger than those in other subtypes. Research findings indicate that patients with TNBC who express AR positivity can benefit from AR inhibitor treatments [8,9].

1.1.4. Subtypes for mesenchymal and mesenchymal stem-like

The mesenchymal stem-like subtype contains elements that block EGFR and control calcium signaling and G-protein receptor pathways, in addition to components related to cell differentiation and motility. The expression of signal transducer and activator of transcription (STAT) genes demonstrating a high activity level was the distinguishing feature of T-cells, B-cells, and natural killer cells.

The M subtype demonstrates changes that modify cell connection with extracellular matrix (ECM) receptors. Cancer stem cells and epithelial–mesenchymal transformation (EMT) serve to identify features of the M subtype cancer. [10] The MSL cell lines responded well to PI3K/mTOR inhibition and Src inhibition treatments which reduced EMT and growth factor signalling pathways [11].

1.15. Immunomodulatory Subtype

The class exhibits matching characteristics with basal-like breast cancer. The potential survival expectations in this subtype are promising even though the pathological examination results indicate a high grading. The subtypes remain unevasive to immunity because immune signaling signals become saturated. The tumors may gain immune escape through two mechanisms that include drawing Activating immune-mediated barrier molecules and immune suppressive cells thus offering potential therapeutic value from immune checkpoint blockade treatment [12].

1.2 Targeted Treatment for TNBC1.2.1. Inhibitors of PARP

The biological entity functions as poly (ADP-ribose) polymerase and exists among molecular entities. Research examined if PARP inhibitors could provide treatment for

BRCA-deficient cancer types. The HRR system needs both BRCA1/2 genes to fix DNA damages. The genes actively participate in DNA damage response processes by fixing double-strand breaks (DSB) and paused replication [13]. FDA has approved PARP inhibitors as medical drugs although BRCA mutations exist in only twenty percent of all Medical organizations cancers. approved five medications for clinical use as PARP inhibitors, including talazoparib, olaparib, veliparib, niraparib, and rucaparib. Studies are progressing with veliparib and niraparib for TNBC as the FDA already approved their use for BRCA-mutant TNBC [14-

1.2.2. Androgen-receptor blockers

ARs belong to the nuclear steroid hormone receptor family. AR activates by such as the ERK-dependent path or the ERK-independent pathway.

Forkhead box protein 01 (FOX01) is eliminated, mTOR is phosphorylated, and PKA is activated in the ERK-independent path, whereas cytoplasmic AR interacts with proteins including PI3K, Ras GTPase, and Src proteins in the ERK-dependent pathway. In the last stage, these activation pathways cause new blood vessels to grow, cells to divide, metastasis to happen, and EMT to happen [17]. The study shows different contradicting results about prostate cancer features ranging from nuclear grade assessments to lymph node metastasis involvement and mortality risks Enzalutamide functions as a novel targeted AR inhibitor because it competes with the AR to establish a binding relationship that stops AR movement to the nucleus [19].

1.2.3. VEGFR Blocker

VEGFR is a member of the protein family known as receptor tyrosine kinases (RTK). An enzymatic transmembrane receptor, a receptor tyrosine kinase, consists of an amino acid tyrosine kinase domain and a helical transmembrane structure with an extrinsic binding zone [20]. The protein VEGF causes

tumor cells to multiply while promoting blood vessel development in cancer growth. The development of breast cancer depends significantly on this factor. Higher VEGF levels in TNBC correlate to worse treatment results no matter what the tumor measurements or metastasis stages [21]. Tumor cell proliferation relies heavily on angiogenesis, which leads healthcare providers to use bevacizumab as an FDA-approved therapeutic agent for TNBC due to its monoclonal antibody function against VEGF receptors. Research showed that adding bevacizumab in neoadjuvant therapy increased the rate of PCR [22].

1.2.4. EGFR Inhibitors

The therapy of cancer shows potential through the biological target known as EGFR [23]. According to new research, EGFR is involved in the development of TNBC as well. Previously, it was thought to be a target of lung cancer alone [24]. It has been discovered that EGFR antibody and translation are expressed more in TNBC than in normal tissue [25]. The effectiveness of treating TNBC may be improved by inhibiting the EGFR protein. Little interference RNA, that attack Epidermal messenger RNA, or mRNA, selectively, can be used to achieve cell death. The free form of siRNA demonstrates instability when introduced to bloodstream environment and cell cancer areas. Nanotechnology has demonstrated its ability to transport siRNA as well as standard anticancer treatments inside TNBC. Still, TNBC cells show diminished sensitivity to EGFR blocker treatments compared to gefitinib, which enhances the responsiveness of HER-2, also specific lines of cells to chemotherapeutic treatment [26]. The EGFR inhibitors gefitinib and erlotinib along with cetuximab function as pharmaceutical agents for solid tumor management [27].

1.2.5. P13/AKT/mTOR Inhibitors:

The crucial molecular signature of PI3Ks, a component of the PI3K-AKT-mTOR transmission cascade, is essential for tumor cell development. PI3Ks have a heterodimer

structure with p110 as the catalytic active unit and p85 as the regulatory component. There are four separate PI3Ks variants: α , β , and δ. [28] Activation γ, PI3K/AKT/mTOR signaling pathway appears when tyrosine kinases receptors stimulate PI3K followed by AKT and mTORC1 [29] phosphorylation In **TNBC** akt/mtor/p13k communication pathway becomes active because of estimated glomerular rate filtration (agonizing regulator) inositol high in proline PIK3Cα mutation, polyphosphate overexpression, and PTEN expression decline [30-32].

TNBC Radiotherapy Treatment Using Nanobiotechnology:

TNBC radioimmunotherapy with inorganic nanoparticles:

According to a previous study, cells may significantly improve radio sensitization by absorbing AuNPs, raising energy from 1.01 keV to 2.11 keV. [33-34]

Inorganic substances, which are further classified as metal NPs, inorganic oxide NPs, inorganic semiconductor NPs, and so on, make up the majority of inorganic nanoparticles. Metal nanoparticles (NPs), mesoporous dioxide of silicon, and charcoal NPs are examples of inorganic NPs that are utilised in biomedical research [35]. Inorganic nanoparticles (NPs) provide promising application prospects for controlled drug release and targeted medication administration when employed as drug delivery vehicles [36]. Most radiation-sensitive metal nanoparticles are made of high-atomicnumber metals, including platinum, gold, barium, and bismuth [37].

$Organic\ nanoparticles\ for\ TNBC\ treatment$

Proteins, lipids, organic Polymers with high molecular weight and polysaccharides make up organic nanoparticles [38]. Typically, spherical in shape and ranging in size from 25 to 1000 nm, liposomes can effectively load hydrophilic or hydrophobic medications,

therefore shielding them from the damaging effects of the external environment. Additionally, liposomes can be used in targeted drug delivery systems because of their facile modification and functionalization properties, which allow them to recognize targets. Because liposomes resemble cells, the reticuloendothelial system mostly absorbs them, which triggers the body's immunological response [39].

TNBC radioimmunotherapy with NP hydrogel:

A type of special carrier with notable biocompatibility among biomaterials is hydrogel. Hydrogels loaded with growth factors or medications can help heal wounds and avoid rejection responses because they strongly resemble human tissues. Hydrogels can also cover the surgical site and keep the medicine released continuously, which helps prevent tumors from coming back. As nanobiotechnology advances, therapeutic NPs are being mixed with other biological materials more often. One particularly popular method is loading NPs into hydrogels, which has garnered a lot of interest [40]. Research has demonstrated that hydrogels loaded with α - or β -emitting radioisotops (such as 90 Y, 131 I, and 225 AC) are effective intreating TNBC. Immunotherapy Combinations: To strengthen the immune response, NP hydrogels can incorporate regulators of immune surveillance, such as anti-PD-1 and anti-CTLA-4.

Targeted Theranostics: Nanotechnology Advances for TNBC

A promising nanoparticle must have various physiochemical, biological, and functional characteristics to be used in biomedicine. These characteristics influence the efficacy of drug delivery and play a significant role in minimizing side effects, thereby enhancing patient safety. As research progresses, it becomes increasingly important to optimize these parameters to achieve the best therapeutic outcomes High dose efficiency, a

long half-life in circulation with low systemic toxicity, selective localisation, high adhesion at the tumour environment, enhanced internalisation into the tumour via cytokines, suffered while regulating release of imaging substances and cytotoxic drugs over the proper duration and time, and safe bio-elimination from the body are all necessary for nanoparticles to be as theranostic in cancer detection and therapy [41].

Nanoparticles of liposomes:

An extremely versatile spherical nanocarrier, the 400 nm spherical molecules known as liposomes. The most adaptable nanocarriers for delivering medications to their intended location are liposomes. It can contain medicine in a water core or lipids membrane. Generally speaking, several different types of drugs, including proteins, nucleotides, and small molecules, can be encapsulated within liposomes. This unique ability enhances drug stability and improves bioavailability, making liposomes an potentially valuable instrument for customised medication administration.

Liposomal nanotechnology is created using reversed-phase evaporated water and liquid injection methods. It divides lipids or dispersion hydrocarbons in a solution and extrusion, which produces nanoparticles with a certain cross-sectional area [42].

Micelles: A cancer treatment miracle Micelles are colloidal particles with diameters between 5 and 100 nm. Van der Waals interactions create their hydrophobic core, which is stabilised by their aqueous

exterior [43].

In cancer treatment, micelles—nanoscale, amphiphilic structures—have become effective drug delivery systems. Their capacity to improve targeted administration, increase bioavailability, and solubilize hydrophobic medications has become a vital tool in contemporary cancer research. Researchers can increase the therapeutic effect of treatments while lowering side effects by encapsulating medicinal molecules

in micelles, eventually leading to improved patient results. As advancements in nanotechnology continue, the potential applications of micelles in oncology are expected to expand even further.

Nanoparticles used in cancer treatment that are mislabeled as polymers

A polymeric nanoparticle (50 nm-10 µm) can be categorized as a misnomer nanoparticle if it is less than 10 microns. Natural or synthetic polymers can be used to create these The extra advantage of nanotechnology is that they can encapsulate proteins and drugs without changing chemically. Because of their reduced toxicity and biocompatibility, renewable polyamide particles like polylactic or composites like polylactide-coglycolide are used to produce nanoparticles. These characteristics make them especially well-suited for medical use, such as tissue engineering and medication administration. Furthermore, the ability to control these nanoparticles' size and surface characteristics enhances their effectiveness in targeting specific cells or tissues within the body [44]. It is also known that polymeric NPs can transport therapeutic drugs, miRNA, and siRNA to decrease tumor development and volume. PLGA-bPEG polymer NPs codelivered antisense-miR-21 and antisensemiR-10b at a dose of 0.15 milligrammes per, but siRNA (multidrug sensitivity proteins) & DOX inhibitors NPs caused an 8-fold reduction in tumour development in volume, respectively. [45]

Metallized nanostructures:

Metal-based NPs, in contrast to polymeric NPs, provide significant versatility in drug administration and diagnostic applications. created DOX-loaded gold nanoparticles that efficiently transport the medication to BCSCs and significantly increase anticancer efficacy [46].

Based on natural polyphenols, metal-based phenolic networking (MPN) is one kind of 3D-printed "green" material with huge specific surface areas [47]. Another study

demonstrated how chloroquine (CQ) and Nanoparticle loaded with DOX enhanced the in vitro efficacy of chemotherapeutic medications against MDA-MB-231 cells by inhibiting autophagy via nanoparticles [48].

Dendrimer

In treating cancer, dendrimers are particularly important as nanocarrier-based systems, particularly through ligand- or receptor-controlled endocytosis, which provides targeting properties and has several advantages over conventional therapy [49]. It is equally crucial to design and create multifunctional nanoparticles for imaging and treatment, for example, so that they have a chemically active surface with a range of functional groups for conjugating tumortargeting ligands and imaging agents, in addition to producing strong signals or contrast for in vivo imaging [50].

2. CONCLUSION

Thanks to nanomedicine, significant progress has been made in the identification and management of both hormone-positive and hormone-negative BC. Numerous formulations based on nanoparticles have been created and are now being used extensively to treat the illness. These nano-missiles have the arsenal necessary to carry out their mission of eliminating the highest number of cancer cells. The ability to load or encapsulate drugs protects them and lengthens their biological half-lives, ultimately reducing the total dosage required for medication. Beyond conventional breast cancer treatment, cancer nanotechnology now offers a number of more effective and maybe safer viewpoints.

3. REFERENCES

- 1. Khongorzul P, Ling CJ, Khan FU, Ihsan AU, Zhang J. Antibody-drug conjugates: A comprehensive review. *Mol Cancer Res MCR*. 2020;18(1):3–19
- 2. Schick J, Ritchie RP, Restini C. Breast cancer therapeutics and biomarkers: Past, present, and future approaches. *Breast Cancer Basic Clin Res.* 2021;15:1178223421995854.

- 3. Palomeras S, Ruiz-Martí nez S, Puig T. Targeting breast cancer stem cells to overcome treatment resistance. *Mol J Synth Chem Nat Prod Chem.* 2018;23:2193.
- 4. Makki J. Diversity of breast carcinoma: Histological subtypes and clinical relevance. *Clin Med Insights Pathol.* 2015;8:23–31.
- 5. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2021;71(3):209–249.
- 6. Wolff AC, Hammond MEH, Hicks DG, Dowsett M, McShane LM, Allison KH, Allred DC, Bartlett JMS, Bilous M, Fitzgibbons P, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. *J Clin Oncol.* 2013;31(31):3997–4013.
- 7. Juul N, Szallasi Z, Eklund AC, Li Q, Burrell RA, Gerlinger M, Valero V, Andreopoulou E, Esteva FJ, Symmans WF, et al. Assessment of an RNA interference screen-derived mitotic and ceramide pathway metagene as a predictor of response to neoadjuvant paclitaxel for primary triple-negative breast cancer: A retrospective analysis of five clinical trials. *Lancet Oncol.* 2010;11(4):358–365.
- 8. Lehmann BD, Jovanovic B, Chen X, Estrada MV, Johnson KN, Shyr Y, Moses HL, Sanders ME, Pietenpol JA. Refinement of triplenegative breast cancer molecular subtypes: Implications for neoadjuvant chemotherapy selection. *PLoS One.* 2016;11(6):e0157368.
- 9. Jiang YZ, Ma D, Suo C, Shi J, Xue M, Hu X, Xiao Y, Yu KD, Liu YR, Yu Y, et al. Genomic and transcriptomic landscape of triple-negative breast cancers: Subtypes and treatment strategies. *Cancer Cell.* 2019;35(4):428–440.e5
- 10. Zhang L, Mu C, Zhang T, Yang D, Wang C, Chen Q, Tang L, Fan L, Liu C, Shen J, et al. Development of targeted therapy

- therapeutics to sensitize triple-negative breast cancer chemosensitivity utilizing bacteriophage Phi29 derived packaging RNA. *J Nanobiotechnol.* 2021;19(1):13
- 11. Jing X, Shao S, Zhang Y, Luo A, Zhao L, Zhang L, Gu S, Zhao X. BRD4 inhibition suppresses PD-L1 expression in triplenegative breast cancer. *Exp Cell Res.* 2020;392(1):112034.
- 12. Lv Y, Ma X, Du Y, Feng J. Understanding patterns of brain metastasis in triplenegative breast cancer and exploring potential therapeutic targets. *OncoTargets Ther.* 2021;14:589–607.
- 13. Won, K.-A.; Spruck, C. Triple-Negative Breast Cancer Therapy: Current and Future Perspectives (Review). Int. J. Oncol. 2020, 57, 1245–1261.
- 14. Sun Y, Wang Z, Na L, Dong D, Wang W, Zhao C. FZD5 contributes to TNBC proliferation, DNA damage repair and stemness. *Cell Death Dis.* 2020;11(1):1060.
- 15. Li Y, Zhang H, Merkher Y, Chen L, Liu N, Leonov S, Chen Y. Recent advances in therapeutic strategies for triple-negative breast cancer. *J Hematol Oncol.* 2022;15(1):121.
- 16. Liao M, Zhang J, Wang G, Wang L, Liu J, Ouyang L, Liu B. Small-molecule drug discovery in triple-negative breast cancer: Current situation and future directions. *J Med Chem.* 2021;64(4):2382–2418.
- 17. Coussy F, Lavigne M, de Koning L, Botty RE, Nemati F, Naguez A, Bataillon G, Ouine B, Dahmani A, Montaudon E, et al. Response to MTOR and PI3K inhibitors in enzalutamide-resistant luminal androgen receptor triple-negative breast cancer patient-derived xenografts. *Theranostics*. 2020;10(4):1531–1543.
- 18. O'Reilly D, Sendi MA, Kelly CM. Overview of recent advances in metastatic triplenegative breast cancer. *World J Clin Oncol.* 2021;12(3):164–182.
- 19. da Silva JL, Cardoso Nunes NC, Izetti P, de Mesquita GG, de Melo AC. Triple-negative breast cancer: A thorough review of

- biomarkers. *Crit Rev Oncol Hematol.* 2020;145:102855.
- 20. Sukumar J, Gast K, Quiroga D, Lustberg M, Williams N. Triple-negative breast cancer: Promising prognostic biomarkers currently in development. *Expert Rev Anticancer Ther.* 2021;21(2):135–148.
- 21. Keihan Shokooh M, Emami F, Jeong JH, Yook S. Bio-inspired and smart nanoparticles for triple-negative breast cancer microenvironment. *Pharmaceutics*. 2021;13(2):287.
- 22. Berko YA, Akala EO. Computer optimization of stealth biodegradable polymeric dual-loaded nanoparticles for cancer therapy using central composite face-centered design. *Pharm Nanotechnol.* 2020;8(2):108–132.
- 23. Medhi H, Khumukcham SS, Manavathi B, Paik P. Effective in vitro delivery of paclitaxel by nanocargo of mesoporous polycaprolactone against triple-negative breast cancer cells by minimalizing drug dose. *RSC Adv.* 2020;10(41):24095–24107
- 24. Futamura M, Oba M, Masuda N, Bando H, Okada M, Yamamoto Y, Kin T, Saeki T, Nagashima T, Kuwayama T, et al. Meta-analysis of nanoparticle albumin-bound paclitaxel used as neoadjuvant chemotherapy for operable breast cancer based on individual patient data (JBCRG-S01 study). Breast Cancer. 2021;28(4):1023–1037.
- 25. Pandy JGP, Balolong-Garcia JC, Cruz-Ordinario MVB, Que FVF. Triple negative breast cancer and platinum-based systemic treatment: a meta-analysis and systematic review. *BMC Cancer*. 2019;19(1):1065.
- 26. LoRusso PM. Inhibition of the PI3K/AKT/mTOR pathway in solid tumors. *J Clin Oncol.* 2016;34(31):3803–3815.
- 27. Costa RLB, Han HS, Gradishar WJ. Targeting the PI3K/AKT/mTOR pathway in triplenegative breast cancer: a review. *Breast Cancer Res Treat*. 2018;169(3):397–406.

- 28. Masuda H, Zhang D, Bartholomeusz C, Doihara H, Hortobagyi GN, Ueno NT. Role of epidermal growth factor receptor in breast cancer. *Breast Cancer Res Treat.* 2012;136(2):331–345.
- 29. Song X, Liu Z, Yu Z. EGFR promotes the development of triple-negative breast cancer through JAK/STAT3 signaling. *Cancer Manag Res.* 2020;12:703–717.
- 30. Yang R, Li Y, Wang H, Qin T, Yin X, Ma X. Therapeutic progress and challenges for triple-negative breast cancer: targeted therapy and immunotherapy. *Mol Biomed*. 2022;3(1):8.
- 31. Lembo RR, Manna L, Froechlich G, Sasso E, Passariello M, De Lorenzo C. New insights on the role of anti-PD-L1 and anti-CTLA-4 mAbs on different lymphocyte subpopulations in TNBC. *Cancers*. 2022;14(21):5289.
- 32. Kim B, Pena CD, Auguste DT. Targeted lipid nanoemulsions encapsulating epigenetic drugs exhibit selective cytotoxicity on CDH1-/FOXM1+ triple-negative breast cancer cells. *Mol Pharm.* 2019;16(5):1813–1826.
 - 33. Bayo n-Cordero L, Alkorta I, Arana L. Application of solid lipid nanoparticles to improve the efficiency of anticancer drugs. *Nanomaterials*. 2019;9(4):474.
 - 34. Jain V, Kumar H, Anod HV, Chand P, Gupta NV, Dey S, Kesharwani SS. A review of nanotechnology-based approaches for breast cancer and triple-negative breast cancer. *J Control Release*. 2020;326:628–647
 - 35. Xiao Y, Shi K, Qu Y, Chu B, Qian Z. Engineering nanoparticles for targeted delivery of nucleic acid therapeutics in tumors. *Mol Ther Methods Clin Dev.* 2019;12:1–18.
 - 36. Kumar DN, Chaudhuri A, Aqil F, Dehari D, Munagala R, Singh S, Gupta RC, Agrawal AK. Exosomes as emerging drug delivery and diagnostic modality for breast cancer: recent advances in isolation and application. *Cancers*. 2022;14(6):1435.

- 37. ClinicalTrials.gov. Galunisertib and paclitaxel in treating patients with metastatic androgen receptor-negative (AR-) triple-negative breast cancer. *ClinicalTrials.gov.* 2021.
- 38. Bayo n-Cordero L, Alkorta I, Arana L. Application of solid lipid nanoparticles to improve the efficiency of anticancer drugs. *Nanomaterials*. 2019;9(4):474.
- 40. Harde H, Agrawal AK, Katariya M, Kale D, Jain S. Development of a topical adapalene-solid lipid nanoparticle loaded gel with enhanced efficacy and improved skin tolerability. *RSC Adv.* 2015;5(52):43917–43929.
- 41. Siddhartha VT, Pindiprolu SKSS, Chintamaneni PK, Tummala S, Nandha Kumar S. RAGE receptor targeted bioconjugate lipid nanoparticles of diallyl disulfide for improved apoptotic activity in triple-negative breast cancer: in vitro studies. *Artif Cells Nanomed Biotechnol.* 2018;46(2):387–397.
- 42. Jain A, Mahira S, Majoral JP, Bryszewska M, Khan W, Ionov M. Dendrimer mediated targeting of siRNA against polo-like kinase for the treatment of triple-negative breast cancer. *J Biomed Mater Res A.* 2019;107(8):1933–1944.
- 43. Liu C, Gao H, Zhao Z, Rostami I, Wang C, Zhu L, Yang Y. Improved tumor targeting and penetration by a dual-functional poly(amidoamine) dendrimer for the therapy of triple-negative breast cancer. *J Mater Chem B.* 2019;7(23):3724–3736.
- 44. Martey O, Nimick M, Taurin S, Sundararajan V, Greish K, Rosengren RJ. Styrene maleic acid-encapsulated RL71 micelles suppress tumor growth in a murine xenograft model of triplenegative breast cancer. *Int J Nanomed*. 2017;12:7225–7237.
- 45. Godugu C, Doddapaneni R, Singh M. Honokiol nanomicellar formulation produced increased oral bioavailability and anticancer effects in triple-negative

- breast cancer (TNBC). *Colloids Surf B Biointerfaces*. 2017;153:208–219.
- 46. Zhou, Z.; Kennell, C.; Jafari, M.; Lee, J.Y.; Ruiz-Torres, S.J.; Waltz, S.E.; Lee, J.H. Sequential delivery of erlotinib and doxorubicin for enhanced triple negative Breast cancer treatment using polymeric nanoparticle. Int. J. Pharm. 2017, 530, 300–307.
- 47. Patel KK, Surekha DB, Tripathi M, Anjum MM, Muthu MS, Tilak R, Agrawal AK, Singh S. Antibiofilm potential of silver sulfadiazine-loaded nanoparticle formulations: a study on the effect of DNase-I on microbial biofilm and wound healing activity. *Mol Pharm.* 2019;16(10):3916–3925.
- 48. Sarkar S, Konar S, Prasad PN, Rajput S, Kumar BNP, Rao RR, Pathak A, Fisher PB, Mandal M. Micellar gold nanoparticles as

- delivery vehicles for dual tyrosine kinase inhibitor ZD6474 for metastatic breast cancer treatment. *Langmuir.* 2017;33(31):7649–7659.
- 49. Swanner J, Fahrenholtz CD, Tenvooren I, Bernish BW, Sears JJ, Hooker A, Furdui CM, Alli E, Li W, Donati GL, et al. Silver nanoparticles selectively treat triplenegative breast cancer cells without affecting non-malignant breast epithelial cells *in vitro* and *in vivo*. *FASEB BioAdv*. 2019;1(10):639–660.
- 50. Thakur V, Kutty RV. Recent advances in nanotheranostics for triple-negative breast cancer treatment. *J Exp Clin Cancer Res.* 2019;38:430.

An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print); ISSN: 2249-9245 (Online)



RESEARCH ARTICLE

DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF METHOTREXATE AND CHRYSIN

Dr. Anuradha G. More¹, Surbhi C. Gupta^{*}

 1* Department of Pharmaceutics, PES's Modern College of Pharmacy, Nigdi, Pune, Maharashtra

*Corresponding author email: surbhigupta224@gmail.com

Received: Feb 09, 2025 / Revised: April 25, 2025 / Accepted: May 16, 2025

ABSTRACT

A U.V. spectrophotometric technique has been developed for the simultaneous estimation of chrysin and methotrexate for bulk drugs and a combination of both. The approach's validation process complied with ICH guidelines, guaranteeing accurate and trustworthy results. For the Preparation of stock solution and dilutions Phosphate buffer, PH 7.4 has been used. Methotrexate exhibits absorption maxima at 303nm while Chrysin exhibits absorption maxima at 273nm respectively. For methotrexate and chrysin, they complied with Beer's law within the range of 2– 10 μ g/ml concentration and 20–100 μ g/ml concentration and 0.9978 and 0.9983 were the r² value respectively which shows good linearity. In accordance with ICH standards, the method's linearity, precision, accuracy, LOD, LOQ and recovery studies were validated. LOD for methotrexate and chrysin was observed to be 0.0891 and 0.0793 μ g/ml and LOQ was found to be 0.2700 and 0.2402 μ g/ml respectively. Thus, the approach was discovered to be accurate, specific, and cost-effective quality control tool for the regular assessment of methotrexate and chrysin and a comprehensive examination of the literature revealed that there is currently no published method for measuring Methotrexate and Chrysin simultaneously using UV spectrophotometry, underscoring the originality of this strategy.

Keywords: Methotrexate, Chrysin, Synthetic mixture, UV Spectroscopy, Validation,

Simultaneous Estimation

How to cite this article: Dr. Anuradha G. More, Surbhi C. Gupta. Development and Validation of UV Spectrophotometric Method for Simultaneous Estimation of Methotrexate and Chrysin. Bulletin of Pharmaceutical Research. 2025;15(S2): 113-118.

Source of support: Nil Conflict of interest: None

1. INTRODUCTION

The most widely used medication is Methotrexate (MTX) [1]. The crystalline powder of methotrexate is odorless and ranges in colour from yellow to orangebrown [3]. It is a selective inhibitor of DHFR (dihydrofolate reductase) and an

antimetabolite of folic acid [1,2]. It affects DNA replication and cell proliferation by directly and indirectly affecting other molecular targets [1,2]. The chemical name for chrysin (CHS) a yellow powder, also called chrysanthin, is 5,7-dihydroxy-2-phenyl-4H-chromen-4-one [4]. Propolis,

honey, and numerous other plants contain this naturally occurring polyphenol. Numerous pharmacological effects, including antiangiogenic, pro-apoptotic, immunomodulatory, antimetastatic, antioxidant qualities, seem to be combined in this substance [5]. To increase the accuracy and precision of determining results without using costly reagents and equipment, developing and validating UVvisible spectrophotometric methods for the quantitative evaluation of methotrexate and chrysin in a synthetic mixture is the main goal of the current study. These techniques can also be applied to their combined formulations [6].

2. MATERIALS AND METHODS

Pure drugs were procured from labs and AR grade reagents were utilized.

2.1 Instruments:

Double beam UV-visible spectrophotometer (Shimadzu 1800, A114548, Japan), Electronic balance (Shimadzu, AUX 220, Japan), ultrasonicator, and Magnetic stirrer (Remi, Mumbai) were used for the experimental work [6,7].

2.2 Selection of solvent system:

According to the Indian Pharmacopoeia, both drug solubility was evaluated in a variety of solvents. For the analysis of both methotrexate and chrysin using the suggested approach, PH 7.4 Phosphate Buffer was determined to be the common solvent [6].

2.3 Preparation of Stock solution:

Working dilutions in the range of $2-10\mu g/ml$ concentration and $20-100\mu g/ml$ were prepared after a precisely weighed quantity of methotrexate (MTX) and chrysin (CHS) was completely dispersed, mixed and

diluted with prepared PH 7.4 buffer to achieve a $1000\mu g/ml$ concentration [6,7].

2.4 A mixture of standard stock solution:

PH 7.4 buffer was used for preparing the mixture of standard stock solution of methotrexate and chrysin in 1:1 ratio [6].

2.5 calibration curve Preparation:

For both the drugs the maximum absorbance was recorded at 303nm and 273nm respectively and a standard curve was prepared 0.9978 and 0.9983 were the correlation coefficient for MTX and CHS [7,8].

2.6 Study of Absorbance additivity:

The absorbance of working standard dilutions of separate drugs $(2\mu g/ml)$ and their synthetic mixture (1:1) at certain wavelengths, namely 303 nm and 273 nm was measured to get absorbance additivity data.

2.7 **Determination** of absorptivity: The absorptivity (A 1%, 1 cm) of MTX and CHS at λ max, i.e., 303nm and 273nm respectively, was computed from the mean of three distinct absorbance readings using the following formula [6].

Absorptivity = Absorbance/ Concentration The mean of all absorptivity values at 303 and 273 nm was finally determined as ax and ay.

2.8 Simultaneous Equation method:

The simultaneous equation technique can be utilized to estimate the absorbance of the two drugs since the combination contains two distinct absorbing drugs that exhibit absorbance at each other's λ max. ANOVA was used for the same. Both wavelengths, which correspond to the absorption maxima of the individual drugs MTX and CHS in phosphate buffer PH 7.4, are chosen for the methods: λ 1-303 and λ 2-273. The

concentrations in the mixtures were estimated using the following formula [6,7].

$Cx = A_2ay_1 - A_1ay_2/ax_2ay_1 - ax_1ay_2$ $Cy = A_1ax_2 - A_2ax_1/ax_2ay_1 - ax_1ay_2$

Where, Cx and Cy – MTX and CHS concentration

 $$A_1$$ and $$A_2$$ – Absorbance of MTX and CHS in synthetic mixture at 303nm and 273nm

ax1 and ax2 – Molar absorptivity of MTX at 303nm and 273nm.

ay1 and ay2 – Molar absorptivity of CHS at 303nm and 273nm respectively.

2.9 Validation Parameters:

2.9.1 Linearity and Range

Using the described procedures, suitable dilutions of standard stock solution were analyzed for each drug. The range was found to be 2-10 μ g/ml for MTX and 20-100 μ g/ml for CHS which followed Beer-Lambert's law [6]. Table no 1 shows the linearity data for the method.

2.9.2 Accuracy

The degree to which a set of measurements closely approaches the true value is known as accuracy. Three different levels of recovery investigations were carried out using the standard addition procedure (80%, 100%, and 120%) to assess the correctness of the suggested approach [7,9]. The procedure was repeated thrice and % RSD was calculated for the proposed developed method at each concentration limit as shown in Table no.3

2.9.3 Precision

The degree of agreement between measurements of the same sample under identical conditions is known as precision. The evaluation of the formulation three times in a single day at one-hour intervals and on three consecutive days is known as

intra-day and inter-day analysis, respectively [7,9,10]. The drug amount and % RSD were computed where % RSD was less than 2%. Table no 2 shows the results of the same.

2.9.4 Ruggedness Study

analytical procedure's ruggedness indicates its ability to resist small but deliberate adjustments to the method's parameters and demonstrates normal dependability in operating circumstances [7]. In the lab, ruggedness conveys accuracy, much like a different analyst. The mean value was calculated for different analysts by using the same tool. The results of the parameter are mentioned in table no.4

2.9.5 Limit of detection (LOD) and Limit of Quantization (LOQ):

The calibration curve was used to find out LOD which is the limit of detection and LOQ which is the limit of quantization independently. Both the parameters were determined using the regression line's residual standard deviation or y- intercept standard deviation of regression lines [7, 9]. The limit of detection and quantization of MTX and CHS were calculated by the proposed method and the results were mentioned in table no.1

LOD= 3.3 σ/S LOQ= 10 σ/S Where, σ - y intercept standard deviation S-Slope

3. RESULTS AND DISCUSSION

It was discovered that the newly devised analytical approach was sensitive, straightforward, cost-effective, accurate, and exact [11]. In compliance with ICH requirements, the provided method has been validated

Unlocking Horizons in Global Multidisciplinary Healthcare Research Indo-US International Conference Date: 10-12, March 2025 Organised by PES Modern college of Pharmacy Nigdi Pune-44

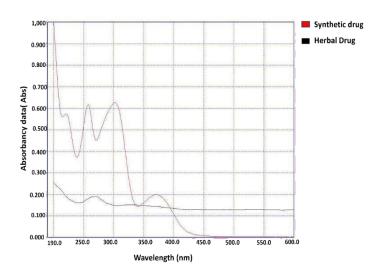


Figure no 1: Overlay of maximum absorption of MTX and CHS

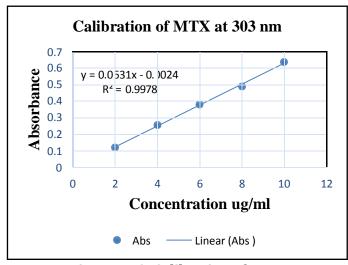
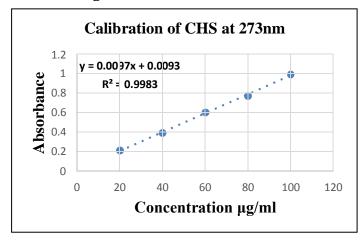


Figure no.2: Calibration of MTX



3.1 Results of validation

Table 1: Result of Validation parameter

Parameter	Methotrexate	Chrysin
λmax(nm)	303nm	273nm
Linearity range	2-10 μg/ml	20-100 μg/ml
Linearity	Y= 0.0631x -	Y = 0.0097x +
equation	0.0024	0.0093
Correlation coefficient	0.9978	0.9983
Slope	0.0631	0.0097
Intercept	0.0024	0.0093
LOD	0.0891	0.0793
LOQ	0.2700	0.2402

Table 2: Precision Study

Drug	Interday Precision		ecision Intraday Precision	
	% amount	% RSD	% amount	%
	found ±		found ±	RS
	SD		SD	D
MTX	100.66±0.577	0.573	100.76±0.750	0.744
CHS	99.48±1.195	1.201	99.27±1.219	1.227

Table 3: Recovery Study

Recover y level	MTX % Recovery ± SD	% RSD	CHS % Recovery ± SD	% RSD
80%	99.33± 0.098	0.0978	100.85± 0.162	0.1606
100%	100.77± 0.196	0.1945	95.05± 0.391	0.4113
120%	99.94± 0.191	0.1911	100.03± 0.306	0.3059

Figure no.3: Calibration of CHS

Table 4: Ruggedness study

Parameter	Drug	% amount found ± SD
Analyst 1	Methotrexate	101.2± 0.288
	Chrysin	98.29± 0.162
Analyst 2	Methotrexate	99.36± 0.202
	Chrysin	100.26± 0.150

Table 5: Percentage of drug estimated

Sr. No	Drugs	Percentage of drug estimated
1.	MTX	99.95 %
2.	CHS	99.02%

The absorption maxima for MTX and CHS were found at 303 and 273 nm, respectively between 200 and 400 nm in the standard solution's UV scan. At their chosen wavelength, the beer-Lambert's concentration range for MTX was 2-10 µg/ml and for CHS, it was 20-100 µg/ml. Absorbance and Concentration are correlated strongly, as evidenced by the regression coefficients of 0.9978 for MTX and 0.9983 for CHS. A lower LOD and LOO number attests to the method's appropriate sensitivity. The accuracy of the approach was shown by the Intraday and Interday Precision tests, which had % RSDs of less than 2%. Additionally, the recovery investigations were found within the range of 98% ± 102%.

4. CONCLUSION

For both drugs, the linearity and range showed that the validated approach was accurate. The fact that the percentage RSD for intra and interday precision was less than 2% further confirmed the accuracy of the approach. Additionally, the recovery investigations was within 98% ± 102%

range which confirmed its dependability. The verified method indicated for MTX and CHS simultaneous determination by spectrophotometric approach can suggested for both combined dosage and for regular quality control in their synthetic mixture, according to the conclusion drawn from all the considerations. The analysis of synthetic mixture showed that the method worked well for both drugs at the same time with little or no interference from common pharmaceuticals. excipients of The technique was determined to be straightforward, sensitive. accurate. precise, repeatable, and cost-effective.

5. REFERENCES

- Jaroslaw Ciekot et al. Methods for methotrexate determination in macromolecular conjugates drug carrier. Acta Poloniac Pharmaceutica-Drug research. 2012; 69: 1342-1346.
- 2. Fairbanks LD et al. Methotrexate inhibits the first committed step of purine biosynthesis in mitogen- stimulated human T-lymphocytes: a metabolic basis for efficacy in rheumatoid arthritis. Biochem J. 1999; 15(342): 143-52.
- 3. https://pubchem.ncbi.nlm.nih.gov/comp ound/Methotrexate
- 4. https://www.chemicalbook.com/article/introduction-and-pharmacological-action-of-chrysin.htm
- Renuka Mani, Vijayakumar Natesan. Chrysin: Sources, beneficial pharmacological activities, and molecular mechanism of action. Phytochemistry.2018; Volume 145: 187-196.

- 6. Gholse, Yogesh N. et al.Development and Validation of Novel Analytical Simultaneous Estimation Based UV Spectrophotometric Method for Doxycycline and Levofloxacin Determination. *Biointerface Research in Applied Chemistry*.2021: 5458-5478.
- 7. Chaudhari A., Mannan A., & Daswadkar S. Development and validation of UV spectrophotometric method for simultaneous estimation of Acyclovir and Silymarin in niosome formulation. Scholar research library.2016; 8: 128-133.
- 8. Kudatarkar N et al. Development and Validation Of UV-Visible Spectrophotometric Method For Estimation Of Chrysin In Bulk Form. *Journal of Pharmaceutical Negative Results*.2022: 4758-4763.
- 9. Ghulam A. Shabir.Step by Step Analytical Methods Validation and
- 10. protocol in the Quality System Compliance Industry. Institute of validation technology;2:59-69.
- 11. Rajnani, Nikhil & Kurup, Nalini. (2021). International Research Method Development of Methotrexate in Phosphate Buffer Solution by UV. Int journal of trend in scientific R and D.2021;2(4).
- 12. Eeravolu S, Vejendla, and Nagula. S. Estimation and validation of UV-visible spectrophotometric method for combined tablet dosage form of paracetamol and diclofenac sodium using extraction technique. The Journal of pharmacy technology. 2012; 4:4733-4740.

An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print); ISSN: 2249-9245 (Online)

Bulletin of Pharmaceutical Research 2025;15(S2)



RESEARCH ARTICLE

FORMULATION AND EVALUATION OF FOLIC ACID AND PEG LOADED IRON OXIDE NANOPARTICLES BY GREEN SYNTHESIS

Ujwala Desai¹, Padmaja Kore², Atharva Joshi¹, Akanksha Bhandare², Geetanjali Bhale², Devendra Dudhade¹

- 1*Department of Pharmaceutics, Progressive Education Society's Modern College of Pharmacy, Nigdi, Pune-44
- ² Department of Pharmacology, Progressive Education Society's Modern College of Pharmacy, Nigdi, Pune-44

*Corresponding author email: <u>ujudesai@gmail.com</u> Received: Feb 15, 2025 / Revised: April 29, 2025 / Accepted: May 15, 2025

ABSTRACT

Cancer is uncontrolled cell growth, forming malignant or benign tumors, caused by genetic, environmental, and inherited factors. SPIONS aid in targeted drug delivery, MRI, and hyperthermia, with functionalized SPIONS like folate-conjugated ones enhancing treatment. Cancer types include carcinomas, sarcomas, leukemia, and lymphomas, treated via surgery, radiation, chemotherapy, immunotherapy, and targeted therapy. PEG functionalization improves SPION biocompatibility, water solubility, and circulation time, while folic acid (FA) enables targeted drug delivery. Bioactive molecules like flavonoids enhance drug delivery but face solubility challenges. MNPS, especially IONPS, are synthesized through various methods for biomedical applications. Advanced therapies like molecular treatments, cryoablation, and radiofrequency ablation offer safer cancer treatment options.

Keywords: Cancer, Iron Oxide Nanoparticles, Folate (FA), Polyethylene Glycol (PEG), Nanoparticles. **How to cite this article:** Ujwala Desai, Padmaja Kore, Atharva Joshi, Akanksha Bhandare, Geetanjali Bhale, Devendra Dudhade. Formulation And Evaluation of Folic Acid and PEG Loaded Iron Oxide Nanoparticles by Green Synthesis. Bulletin of Pharmaceutical Research. 2025;15(S2): 119-126. **Source of support**: Nil **Conflict of interest**: None

1. INTRODUCTION

Cancer occurs when cells grow uncontrollably, forming tumors. Malignant tumors spread (metastasis), while benign tumors do not, but can still cause health issues. Blood cancers like leukaemia do not form solid tumours [1]. Genetic changes that cause cancer can happen because:

- Errors that occur as cells divide.
- DNA damage caused by harmful substances in the environment, such as the chemicals in tobacco smoke and ultraviolet rays from the

sun. (Our Cancer Causes and Prevention section has more information.

• Genetic inheritance.

Increasing cancer deaths drive research on SPIONs (Fe₂O₃/Fe₃O₄) for drug delivery and diagnostics [2]. **PEGylation** enhances solubility, stability, and circulation, while folate (FA) enables targeted drug delivery via receptor-mediated endocytosis. Functionalizing SPIONs with flavonoids improves drug transfer but faces solubility [3-4] Cancer challenges types carcinomas, sarcomas, leukemias, and

Unlocking Horizons in Global Multidisciplinary Healthcare Research Indo-US International Conference Date: 10-12, March 2025 Organised by PES Modern college of Pharmacy Nigdi Pune-44

119

lymphomas, affecting one in three people. Treatments include surgery, radiation, chemotherapy, immunotherapy, targeted therapy, and emerging molecular approaches.

Nanoparticulate -based drug delivery system:

Nanoparticulate drug delivery enhances cancer treatment by targeting tumors, protecting healthy cells, and improving drug solubility. Nanoparticles (<50 nm) enter cells, exploiting tumor vasculature while avoiding healthy tissues. Magnetic nanoparticles enable precise targeting, with quantum dots and nanowires offering advanced therapy options [5-6].

Magnetic nanoparticles:

Magnetic nanoparticles (iron, nickel, cobalt) are used in catalysis, biomedicine, and data storage. In cancer treatment, they enable hyperthermia and controlled drug release. Their superparamagnetic properties aid imaging, drug delivery, and diagnostics, with coatings preventing aggregation and allowing targeted transport [7]. Iron Oxide (IONPs): Biomedical **Nanoparticles** and Pharmaceutical **Applications** Iron oxide nanoparticles (IONPs) have gained significant

1.2 SYNTHESIS & CHALLENGES

IONPS are commonly synthesised via coprecipitation, a chemical method involving ferrous (Fe^{2+}) and ferric (Fe^{3+}) ions in an aqueous solution. Several factors influence nanoparticle phase and size, including Fe^{2+}/Fe^{3+} ratio, Iron concentration, pH value, Ionic strength, and Temperature.

Methods for the preparation of iron oxide nanoparticles include:

- 1. Coprecipitation—Mixes Fe²⁺ and Fe³⁺ in a basic solution to form nanoparticles, which require purification due to high-pH conditions.
- 2. Thermal decomposition Uses high temperatures to decompose organic iron compounds, producing uniform nanoparticles but requiring nonpolar solvents.
- 3. Microemulsion Precipitates nanoparticles in an aqueous phase, though it faces aggregation and coated MNPS are nonimmunogenic and

attention in biomedical and pharmaceutical research due to their biocompatibility, superparamagnetism, high saturation magnetization, and low toxicity. Notably, they are the only FDA-approved nanoparticles for clinical use, particularly in magnetic resonance imaging (MRI) for early disease detection [8-10].

1.1 KEY APPLICATIONS

- 1.1.1 Medical Imaging & Diagnostics IONPs serve as contrast agents in MRI, enhancing imaging resolution.
- 1.1.2 Cancer Therapy Used in magnetic hyperthermia and targeted drug delivery, improving treatment efficacy while minimising side effects.
- 1.1.3 Cell Labelling & Tracking Essential for stem cell therapy and biological studies, enabling real-time monitoring of cells.
- 1.1.4 Magnetic Separation & Biosensing Applied in pathogen detection, bioseparation, and toxin removal [11].

stability challenges.

- 4. Hydrothermal synthesis Utilizes high temperature and pressure for monodispersed nanoparticle crystallization.
- 5. Sonochemical synthesis Uses ultrasonic energy to control nanoparticle formation, such as producing magnetite from iron (II) acetate [12-15].

Green synthesis of iron oxide nanoparticles uses plant extracts as eco-friendly reducers, eliminating synthetic chemicals. This cost-effective method enhances bioactivity, is scalable, and supports biomedical applications like drug delivery and hyperthermia [16-19].

Polyethene glycol (PEG) stabilises magnetic nanoparticles (MNPS) by forming a hydrophilic coating that prevents agglomeration and reduces macrophage clearance, extending circulation time. PEG- easily excreted but have limited functionalization, which can be improved through silane grafting and polymerisation
[20-21]

Folic acid (vitamin B9) is essential for DNA synthesis, cell formation, and preventing neural tube defects. It is absorbed in the Jejunum and duodenum, with deficiency leading to anaemia. Found in leafy vegetables, legumes, and fortified grains, it supports fertility, reduces artery stiffness, and helps prevent stroke, hypertension, and kidney disease [22-24].

2. MATERIALS

The materials used in this study include folic acid, sourced from Oswal Scientificals, Chinchwad; DMSO from Antares Chemicals, Mumbai; and PEG 4000 from Sanghavi Chemicals, Mumbai. Dimethyl formamide was procured from Fisher Chemicals, Pune, while ethylene dichloride was obtained from Triveni Chemicals, Vapi. Lastly, Nhydroxy sulfosuccinate was supplied by Thermo Scientific, Pune.

3. METHODS

3.1 Preparation of Green Tea Extract and Iron Oxide Nanoparticles

Green Tea Extract: 5g of green tea powder was mixed with 200 ml of deionised water and stirred. The mixture was heated at 80°C for 60 minutes in a water bath, filtered using Whatman filter paper no. 4, and stored in a refrigerator.

Iron Oxide Nanoparticles: 0.1M ferric chloride and green tea extract were mixed in a 1:1 ratio with continuous stirring. The pH was adjusted to 7 using 0.1M NaOH. The solution was centrifuged at 7000 rpm to remove impurities, washed with distilled water, and dried in a petri plate after discarding the supernatant [20].

3.2 Synthesis of FA-Conjugated PEG and FA-PEG Loaded Nanoparticles

FA-Conjugated PEG: Folic acid (66.2 mg) was dissolved in a DMSO: DMF (1:1)

mixture (Solution A), followed by the addition of EDC (23.3 mg) and NHS (17.3 mg) under stirring. PEG 4000 (0.35 g) was separately dissolved in DMSO (Solution B). Both solutions were mixed and stirred for four days, then dialyzed and lyophilized to obtain pure HOOC-PEG-FA [18].

3.3 FA-PEG Loaded Nanoparticles: HOOC-PEG-FA (0.4 g) was dissolved in DMSO/DMF (Solution C). EDC (31.04 mg) and NHS (23 mg) were dissolved separately (Solution D) and added to Solution C with stirring. final solution was added dropwise to iron oxide nanoparticles (100 mg) and stirred for three days. The product was then dialyzed [21].

3.4 Evaluation of FA-PEG Loaded Iron Oxide Nanoparticles:

The apperance of nanoparticles checked visually. FTIR spectroscopy identified functional groups by preparing KBr disks with nanoparticles. DSC analysis was performed under nitrogen atmosphere to study thermal properties, with samples heated from 40 to 240°C at per minute. Particle size polydispersity index (PDI) were analysed using laser scattering after dispersing nanoparticles in deionized water. Zeta potential was measured using a Horiba zeta sizer to determine surface charge. Motic microscopy was used to observe morphology and size variations. Coating efficiency was evaluated by measuring at absorbance 283 nm using spectroscopy

3.5 Coating Efficiency:

The nanoparticles and coating material were prepared according to the desired size and concentration to determine the coating efficiency of nanoparticles. The nanoparticles were then mixed with the coating material in a solvent, and the mixture was incubated for a specified period, typically 30 minutes to several hours, to allow the coating material to adsorb or bind to the nanoparticles. The

mixture was stirred occasionally to ensure uniform coating. Following incubation, the mixture was centrifuged at a high speed, typically between 10,000-20,000 rpm, to separate the coated nanoparticles from the uncoated nanoparticles and excess coating material. The supernatant was discarded, the pellet was retained, and the centrifugation step was repeated to ensure complete separation [20].

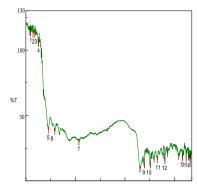
4. RESULT AND DISCUSSION

Appearance: The appearance of the nanoparticle was found to be greenish black in colour



Fig No 1: Appearance of nanoparticles

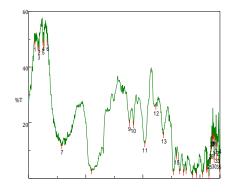
FTIR of Folic Acid



Standard peak	Observed peak	Interpretation
(cm-1)	(cm ⁻¹⁾	
1605	1606	N-H bending
1693	1695	C=0 amide
1485	1485	CH ₂ Bend

Fig No 2: FTIR of folic Acid and its spectra

FTIR of nanoparticles



Standard peak	Observed peak	Interpretation
(cm-1)	(cm ⁻¹⁾	
3500-3350	3420	N-H stretch
1485-1440	1467	CH ₂ bend
1400-1300	1360	NO ₂ stretch

Fig No 3: FTIR of nanoparticles and its spectra

Discussion: All the peaks of folic acid were retained in nanoparticles indicating compatibility of folic acid with other excipients used in nanoparticles (Figure 2 and 3).

DSC of Folic acid-

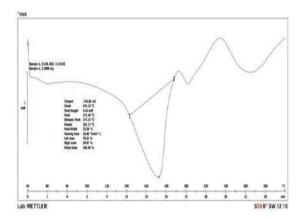


Fig No 4: DSC of folic acid

Fig No 5: DSC of FA-PEG loaded Nanoparticles

Discussion: The DSC graph of FA-PEG loaded iron oxide nanoparticles shows the melting properties of both PEG and FA. Both peaks are wide in nature, showing the amorphous nature of folic acid and PEG. Peaks of both FA and PEG show the

Responses for DOE batches

successful loading of Folic acid and PEG. A broad peak of folic acid indicated the sample's amorphous nature. The shift in the melting point of folic acid toward a lower value shows the reaction's endothermic nature (Figure 4 and 5).

Table No. 3: DOE table with responses

STD	RUN	Factor 1	Factor 2	Response 1	Response	Response 3
		Iron salt	Centrifugation	Particle size	2 PDI	Zeta potential
		(ml)	RPM	(nm)		(mV)
1	1	0.5	5000	88.7	0.12	-32.2
7	2	1	4585.79	372	0.47	-5.6
4	3	1.5	7000	413.6	0.2	-11.1
2	4	1.5	5000	491	0.65	-10.3
10	5	1	6000	109.3	0.07	-1.1
6	6	1.70711	6000	586.6	0.5	-10.7
3	7	0.5	7000	380.1	0.12	-5.7
5	8	0.292893	6000	2.2	0.2	-11.1
8	9	1	7414.21	745.6	0.31	-15.3
9	10	1	6000	156	0.15	-1.3

Response 1: PARTICLE SIZE

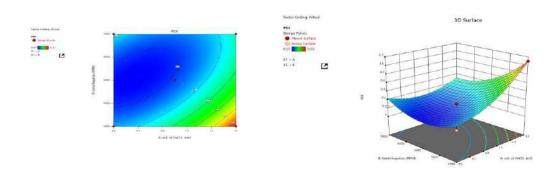
Final Equation in Terms of Coded Factor:

Particle size = $+132.65 + 157.78 \text{ A} + 92.79 \text{ B} - 92.20 \text{ AB} + 60.06 \text{ A}^2 + 192.26 \text{ B}^2$

Final Equation in Terms of Actual Factors:

Particle size = +5315.62076 + 941.46660 vol. of FeCl3 - 2.02996 Centrifugation -0.184400 vol of FeCl3 \times centrifugation + 240.25000 vol. of FeCl3 $^2 + 0.000192$ Centrifugation 2

Two-Dimensional Contour Plot for Particle Size



Discussion: the particle size changes with FeCl3 concentration and stirring speed due to the increase in the number of ions of FeCl3 for entrapment increases as FeCl3 concentration varies, and in the case of the stirring speed, the particle separation changes with a change in stirring speed (Figure 6).

Response 2: PDI

Final Equation in Terms of Coded Factors:

 $PDI = +0.1100 +0.1293A -0.0845B -0.1125 AB +0.0956 A^{2}$

Final Equation in Terms of Actual Factors:

PDI = +3.55364 + 0.843566 vol. of FeCl3 - 0.001247 Centrifugation - 0.000225 vol. of FeCl3 × Centrifugation + 0.382500 vol. of FeCl3 2 +1.15625E-07 Centrifugation 2

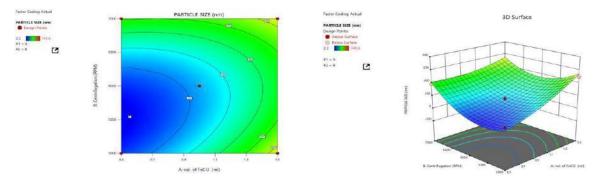


Fig No 7: Two & and three-dimensional plot for PDI

Discussion: As the centrifugation speed varied, the PDI varied accordingly, as experimentally proven, and the same case as with the FeCl₃ concentration was observed (Figure 7).

Response 3: ZETA

Final Equation in Terms of Actual Factors

ZETA = -323.75302 + 133.26642 vol of FeCl3 + 0.083098 centrifugation – 0.013650 vol of FeCl3 × centrifugation – 23.55000 vol of FeCl3 2 - 5.66250E-06 Centrifugation 2

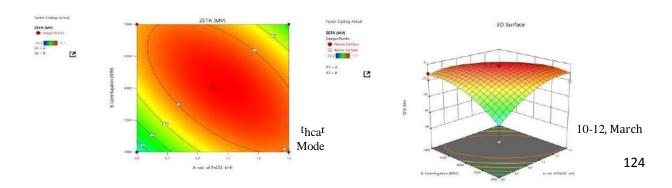


Fig No 8: Two and three-dimensional plot for Zeta Potential

Discussion

Zeta is the charge on the particles and is directly affected when the FeCl3 concentration in the formulation is varied. In the case of the centrifugation speed, the particle separation is different at different centrifugation speeds, so zeta is going to change likewise (Figure 8).

Coating efficiency was calculated using the formula:

Coating efficiency = (Amount of folic acid (Practical value)/(Amount of folic acid (Theoretical value)) 100

Coating efficiency = $89.86\% \pm 1.8\%$

Discussion: Coating efficiency of 89.86±1.8 % shows successful loading of folic acid onto iron oxide nanoparticles.

Inference: Green synthesised iron oxide nanoparticles can be successfully loaded with folic acid using the following procedure.

5. CONCLUSION

This study successfully synthesized folic acid (FA) and PEG-loaded iron oxide nanoparticles using green tea extract as a natural reducer. FA and PEG were loaded via EDC/NHS chemistry, with 89.86% entrapment efficiency. Microscopy, DSC, and FTIR confirmed successful loading, proving green synthesis is effective for nanoparticle formulation.

6. REFERENCES

- Upadhyay A "Cancer: An unknown territory; rethinking before going ahead"
- 2. Chertok B, David A. E., Yang VC, "Polyethyleneamine-modified iron oxide nanoparticles for brain tumor drug delivery using magnetic targeting and intra-carotid administration", Biomaterials 2010,6317-6324.
- 3. Xie J, Chen L, Varadan VK, Yancey J, Srivatsan M., "The effects of

- functional magnetic nanotubes with incorporated nerve growth factor in neuronal differentiation of PC12 cells." Nanotechnology 2008, 19(10):105101].
- 4. Zhang Y, Zhang J. "Surface modification of monodisperse magnetite nanoparticles for improved intracellular uptake to breast cancer cells." J Colloid Interface Sci2005, 283(2):352-357.
- 5. Bukhtoyarov, O.V. and Samarin, D.M., "Psychogenic Activation Phenomenon of Specific Anti-Tumor Immunity in Cancer Patients." International Journal of Medicine and Medical Sciences, 10.5897/IJMMS2013.0888.
- A.I. Gusev, A.A. Rampel, Nano Kristal licheskie Materialy (Nanocrystalline Materials), Nanocrystalline Materials: Synthesis, Characterization, Properties, and Applications.
- 7. D.G. Rancourt, Rev. Mineral. Geochem., 2001, 44, 217.
- 8. R. Ferrando, J. Jellinek, R.L. Johnston, Chem. Rev, volume 108, Issue 3, 845-1170
- 9. S.P. Gubin, Ros. Khim. Zh. Volume 58, 74-77.
- 10. Yurkov, G.Yu., Gubin, S.P., Pankratov, D.A., Koksharov, Yu.A., Kozinkin, A.V., Spichkin, Yu. I., Nedoseikina, T.I., Pirog, I.V., and Vlasenko, V.G.
- 11. Owens, D. E., III; Peppas, N. A. Opsonization, biodistribution, and pharmacokinetics of polymeric nanoparticles. *Int. J. Pharm*.
- 12. Cao YC, Jin R, Nam JM, Thaxton CS, Mirkin CA. Raman dye labeled nanoparticle probes for proteins. *JACS*.

- 13. Pankhurst QA, Connolly J, Jones SK, Dobson J. Applications of magnetic nanoparticles in biomedicine
- 14. Grief AD, Richardson G. Mathematical modelling of magnetically targeted drug Nanoplatform as drug carriers. *IntJ Nanomedicine* 2012.7:3445–3471
- 15. Mohanpuria P, Rana N.K, and Yadav S.K, "Biosynthesis of nanoparticles: technological concepts and future applications," Journal of Nanoparticle Research, 2008, vol.10, no.3, pp.507–517.
- 16. Tiwari D.K, Behari J, and Sen P, "Time and dose- dependent antimicrobial potential of Ag nanoparticles synthesized by topdown approach," Current Science, 2008, vol. 9, 647– 655.
- 17. Luechinger N.A, Grass R.N, Athanassiou E.K, and Stark W.J, "Bottom-up fabrication of metal/metal nano composites from nanoparticles of immiscible metals," ISBN Chemistry of Materials, 2010, vol. 22, no. 1, pp.155–160.
- 18. Zayed MF, EisaWH, Shabaka AA.

 Malvaparviflora extract assisted green
 synthesis of silver nanoparticles.
 Spectrochim Acta A Mol Biomol Spectrosc
 PMID 2012;98:423–428
- 19. Sun J, Shaobing Z, Yang Y, Weng J 2006, Synthesis and characterization of biocompatible Fe 3 O 4 nanoparticle, PMID Journal of Biomedical Material Research 80 (2): 333-341.
- 20. Gupta AK, Wells S, 2004. Surface-Modified superparamagnetic Nanoparticle for Drug Delivery: Preparation, Characterization, and Cytotoxicity Studies, IEEE Transaction on Nano bioscience. PMID 3 (1): 66-73
- 21. Martindale- The extra pharmacopoeia J. E. F. Physician's desk reference.49 th ed. Medical Economics; 1995 Higdon J. Folic acid. Linus Pauling Institute, PMID
- 22. Akal Z.U., Alpsoy L., Baykal A., "Superparamagnetic Iron Oxide Conjugated with Folic acid and Carboxylated Quercetin for Chemotherapy applications",

An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print) 2249-9245 (Online)



DESIGN, FORMULATION AND EVALUATION OF HERBAL EMULGEL CONTAINS PSORALEA CORYLIFOLIA EXTRACT

Vitthal Chopade* Shivani Mandlik ,Abhijeet More , Pranita Gore, Swapnali Dupargude, PES's Modern College of Pharmacy , Sec. No. 21 , Yamunanagar, Nigdi , Pune 411044
*Corresponding author email:vvchopade01@gmail.com.
Received: Feb 08, 2025 / Revised: April 20, 2025 / Accepted: May 09, 2025

ABSTRACT

The development of herbal medicinal formulations has sparked widespread attention due to its possible therapeutic effects and natural nature. This study details the creation, manufacturing, and assessment of an herbal emulgel that contains extract from Psoralea corylifolia, which was selected due to its purported medicinal advantages. The produced emulgel was assessed using a number of properties, including pH, viscosity, spreadability, homogeneity, and drug content, to ascertain its physical stability and quality. Additionally, the emulgel's antibacterial activity was tested against E. Coli and Staphylococcus aureus, two prevalent organisms associated with skin diseases. The formulation's pH was confirmed to be skin-compatible, assuring user comfort and product stability. Viscosity measurements validated the emulgel's consistency and ease of application. The spreadability and homogeneity tests revealed a consistent distribution of the active component throughout the formulation. Drug content analysis validated the existence of the prescribed amount of *Psoralea corylifolia* extract, assuring dose accuracy. The emulgel's potential as an effective topical antimicrobial treatment was demonstrated by the antimicrobial activity tests, which showed significant inhibitory effects against Staphylococcus aureus and E. coli. This study highlights the potential benefits of *Psoralea corylifolia* extract in herbal emulgel formulations for improved skin care and infection management.

Keywords: *Psoralea corylifolia*, Emulgel, antimicrobial activity, Topical and Psoralen.

How to cite this article: Vitthal Chopade, Shivani Mandlik ,Abhijeet More , Pranita Gore, Swapnali Dupargude, Design, Formulation and Evaluation of Herbal Emulgel Contains Psoralea Corylifolia Extract Bulletin of Pharmaceutical Research. 2025;15(S2): 127-134.

Source of support: Nil Conflict of interest: None

INTRODUCTION

Treating a skin condition by applying a medication to the area is referred to as "topical drug delivery." When conventional drug administration techniques, like oral or injection, are ineffective, this approach is frequently utilized for skin disorders, such as fungal infections. Topical drug delivery is an approach, for both systemic treatments. Dermatological pharmacology stands out for its ability to directly

target the skin for both diagnosing and treating skin related issues. [1,2,3] Pharmaceutical semisolid dosage forms, including emulgels in particular have been receiving considerable attention by academia and industry during the last years. Introduction: Skin is the largest organ and also important in delivering medications topically or systemically. While it is a convenient method of drug delivery, some medications cannot cross the skin. [4] Emulgel: A novel system. The term literally means ". Emulsions, either W/O or O/W can be a base as well with an addition of gelling agent to form Emulgel.

Mandlik et al

years to deliver hydrophobic drugs. Furthermore, it is anticipated that emulgel will soon prove to be successful drug delivery method hydrophobic medications. [5] The formulations are an extensive variety including solid, semisolid and liquid. Local or Systemic - effects that only goes where the medication is placed, or becomes absorbed throughout your body In solution, the skin facilitates drug absorption as long as it has a high partition coefficient & is not an electrolyte.[6] When compared to traditional ointments and lotions, gel-based formulations typically offer faster rates of pharmaceutical release. The principal limitation of gels is their administration, especially with hydrophobic compounds. Emulgels were created to get over this restriction, and when used as a gel, hydrophobic medications can also be taken advantage of by utilizing the gelling ability of emulsion. Mixing of gels and emulsions is called as an Emulgel dosage forms An emulgel differs from an ordinary emulsion in the aqueous phase, as it contains a gelling ingredient. The O/W system, with hydrophilic drugs entrapped in lipophilic compounds of the W/O system.[7]Gels provide various advantages. spreadability, non-staining. including greaselessness, and thixotropicity; yet, When it to topically applying hydrophobic medicines, they have a major downsideStrong hydrophobic substances shouldn't be added to the gel foundation because they produce incorrect drug release from gels because they are insoluble in aqueous solution [8,9] Melanocyte loss and depigmentation are the consequences of vitiligo, an acquired idiopathic skin disorder. The vitiligo patches on the skin are noticeable and appear to be milky white. For people with darker complexion tones in particular, it could be visually stigmatizing. Vitiligo affects 1-2% of all races, however it is more common among the Indian population.[10] Pigment loss is more common among teenagers. Vitiligo affects both men and women, but because it lessens a woman's natural beauty and look, the psychological repercussions are more noticeable in women. The cause of vitiligo is unknown. According to research, auto immune diseases, hereditary stress, chemical exposure, viral infections, skin burns, stressful events, skin injury, and hereditary illnesses (related to a family history) can all play a role in its genesis.[11] One cannot spread vitiligo. There are two forms of vitiligo: nonsegmental (symmetrical) and segmental (asymmetrical). There are five subtypes of non-segmental vitiligo: acrofacial, localized, widespread, mucosal, and

Bull. Pharm. Res. 2025;15(S2)

universal. Numerous treatments. such calcipotriene. disguises, skin topical corticosteroids, and calcineurin inhibitors, can help reduce the appearance of vitiligo on the affected skin. Skin grafting, tattoos, psoralen, and pigmentation loss. Vitiligo is vet to be successfully treated. For this illness, PC-based randomized controlled studies are necessary [12]Melanocytes and melanin in the epidermis go inactive and stop functioning, resulting in Vitiligo. Leukoderma is comparable to Vitiligo, with the exception that Vitiligo is a self-generated disorder that is typically identified as an autoimmune disorder, but Leukoderma is caused by allergies to specific chemicals, skin infections, and may also be an autoimmune disorder condition. White patches are a common occurrence due to melanocyte degeneration in the skin.[13]

MATERIALS AND METHODS

1. Plant material collection and authentication.

A seed of plants *Psoralea Corylifolia* was collected from Latur district in the Marathwada region of Maharashtra and authenticated from Botanical survey of India, Koregaon, Sangam wadi, Pune-411001.^[14]



(Fig1.Seeds of P.C)

1. Chemicals

The materials used in this study were obtained from various suppliers. Carbopol 934 and triethanolamine were source from research lab. We purchased methyl paraben, propylene glycol 400, span 80, and tween 80 from Loba Chemie Pvt Ltd. The liquid paraffin was acquired from the Molychem laboratory.

2. Equipment

Electronic weighing balance, magnetic stirrer, pH meter, ultra sonicator, Brookfield viscometer, Digital water bath

3. Plant extract Preparation.

Crude extracts from P. corylifolia seeds were prepared using the Soxhlet extraction method. Before being utilized to create the methanol, ethanol, ethyl acetate, and aqueous extract, fresh P. corylifolia seeds were ground into a coarse powder and left to dry at room temperature in the shade.^[15]

All solvent was used in a Soxhlet system to extract the entire or coarsely ground plant material over the course of 10 to 12 hours according to solvent melting point. In order to prepare the crude extract for further investigation, it was carefully weighed before being filtered, dried, and evaporated using a rotary evaporator.^[16]





(Fig 2. Soxhlet Apparatus) (Fig 3. Rotary vacuum evaporator)

4. Phytochemical Screening of Extract [17,18]

Test for Alkaloids

- 1. Mayer's Test: An opalescence or yellowish precipitate was seen when drops of Mayer's reagent were applied to a portion of an acidic solution in a test tube, signifying the presence of alkaloids.
- 2. Hager's Test: Add a few drops of Hager's reagent to two milliliters of extract. The development of a yellow precipitate suggested the presence of alkaloids.
- 3. Wagner's Test: Place one or two drops of Wagner's reagent (around the test tube walls) in a few milliliters of filtrate. A precipitate that is red or dark.

Test for Carbohydrate 1.For Reducing Sugar a.Fehling's test: An equal volume of Fehling's (A & B) solution was added to 2 mL of extract, and after five minutes of heating, a reddish-red precipitate formed, signifying the presence of carbohydrates.

b. Benedict's Test: 5 mL of Benedict's reagent was combined with 8–10 drops of extract, and after 5 minutes of heating, a dark crimson precipitate was formed, indicating the presence of carbohydrates.

2. For Non-Reducing Sugar

Iodine Test: Adding a few drops of iodine solution to 3ml of extract solution results in a blue hue that disappears when it boils but returns when it cools.

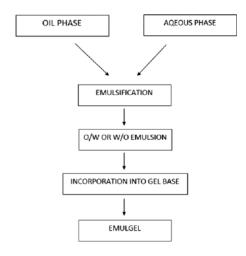
Test for Glycoside

Foam Test Glycoside test: an aqueous NaOH solution was mixed with 0.5 mg of bark extract that had been dissolved in one milliliter of water. The presence of glycosides is indicated by the production of yellow color.

Table 1: Phytochemical screening test

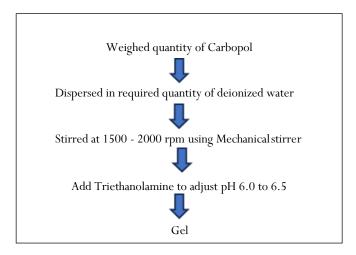
Sr. No.	Physicochemical Parameter	Extract (Methanol)
1.	Test for alka	loids
	Mayer's test	+
	Hager's test	+
	Wagner's test	+
2.	Test for carbo	hydrate
	For reducing sugar	
	a. Fehling's test	+
	b. Benedict's Test	+
	For non-reducing sugar	
	Iodine test	+
3.	Test for glycoside	
	Foam test	+

Mandlik et al Method of Preparation for formulation: -Step 1: Preparation of gel



Step 2: Preparation of Emulgel Formulation of Emulgel:

(Table 2: Ingredient for formulation of Emulgel)



S	Ingredien ts	Role	Batches (%W/W)		_
n o			M1	M2	М3
1	Extract (P.C)	Antimicr obial	150 mg	200 mg	250 mg
2	Carbopol 940	Gelling agent	1gm	1gm	1gm
3	Liq. Paraffin	Moisturi zer	7.5 ml	7.5 ml	7.5 ml
4	Tween 80	Surfacta nt	0.5 ml	0.5 ml	0.5 ml
5	Span 80	Surfacta nt	1ml	1ml	1ml
6	PG	Co- solvent	5ml	5ml	5ml

7	Methyl	Preserva	0.3g	0.3g	0.3g
	Paraben	tive	m	m	m
8	Clove oil	Antimicr	2	2	2
		obial	dro	dro	dro
			ps	ps	ps
9	Triethanol	рН	Unti	Unti	Unti
	amine	adjuster	ll 1	ll 1	ll 1
			ml	ml	ml
1	Dist. water	-	q.s.	q.s.	q.s.
0					

Evaluation of Formulation:

1. Physical assessment:

Visual inspections were conducted to assess physical attributes such color, uniformity, and odor.

Color: An eye examination was used to verify the formulation's color.

Reliability: When applied to the skin, the formulation's consistency was validated.

Odor: By introducing gel into water and observe the smell [19]

2 . **pH Measurement:** The pH of a prepared emulgel is determined using a digital pH meter. Use a standard buffer solution to calibrate the pH meter before using it. 1 gram of emulgel is dissolved in 100 milliliters of distilled water, which is thoroughly shaken to create a uniform suspension, to create a 1% aqueous emulgel solution. For two hours, the system was not tampered with. After two hours, the pH can be determined by immersing a glass electrode in the suspension three times and averaging the results.

3. Homogeneity: -

Homogeneity was tested. After the gel is placed in the container. The appearance and existence of aggregates were investigated [21]

4. Viscosity: -

A Brookfield viscometer was used to measure the viscosities. Each gel was filled with liquid after inserting the corresponding spindle (number 74) into the container. Viscosities were measured at 50 rpm and 25°C. [22]

5. Spread ability: -

Spreadability is the time it takes for two slides to separate from an emulsified gel sandwiched between them when a specific force is applied. A shorter gap between two slides leads to better spreadability. The spreadability of the gel formulation was assessed by placing the standard weight on the upper plate after a minute and measuring the diameter of one gram of gel between two horizontal plates $(20 \times 20 \text{ cm}2)$. [22] The formula is used to calculate it. S = M * L / T

Where, M =Weight placed on upper slide

L= Glass slide's length

T= amount of time needed to divide up the slides

6. Extrudability:

Aluminum collapsible tubes with caps were filled with the gel solutions and hermetically sealed. The extrusion ease was measured using the thumb push. [23]

7. Drug content: -

One hundred milliliters of suitable solvent were mixed with one gram of the produced gel. Aliquots of different concentrations were made using the proper dilutions following the creation of the stock solution and the measurement of the absorbance. An equation obtained from a linear regression study of the calibration curve was used to calculate the drug content.[22]

8. Clarity: -

Each batch's clarity was determined using a visual inspection [24]

9. Gel strength: -

It is computed by multiplying the weight needed for penetration of the gel by the time in seconds. A produced gel weighing five grams was taken and its surface was subjected to 3.5 grams of weight. The number of seconds required for the weight to move through 0.5 cm of gel was used to determine the gel strength. [21]

10. Skin irritation Test: -

Testing for skin irritation is critical in developing hydrogel-based products, especially for medicinal or cosmetic applications.

Patch Testing: Patch testing is placing a tiny amount of the hydrogel to the skin, often on the upper back or forearm, and then covering it with an adhesive patch. The patch is normally used for 24-72 hours.

Observation Period: During and after the patch application period, individuals are warned not to wash the region or engage in activities that may

interfere with the results. They are also requested to record any burning, itching, redness, or other symptoms of irritation. [25,26]

Antimicrobial activity: -

Antimicrobial was checked by agar-well diffusion technique.

Micro-organism- [27]

Gram-positive bacteria like Staphylococcus aureus and gram-negative bacteria like Pseudomonas aeruginosa are both studied in antimicrobial agent research.

Culture and maintenance of microorganism:

The study used bacterial cultures of Staphylococcus aureus and E.coli. Bacterial culture was maintained using nutrient agar media.

Preparation of Media and its sterilization:

Media was made by weighing an approximate amount of agar powder and nutritional broth and mixing it with 1000 cc of distilled water. The media was dissolved, then boiled to homogenize it before sterilization. Cotton wool was used to plug the conical flask, which was then wrapped in aluminium foil and sealed with masking tape. After that, the media was sterilised for 15 minutes at 121°C in an autoclave. After allowing the sterilized medium to cool to about 45°C, it was moved to sterile petri plates and given time to solidify. [28]

Agar well-diffusion technique:

Antimicrobial activity was assessed using the agar well-diffusion technique. Nutrient agar was infected by spreading a volume of bacteria with a sterilized glass spreader. Wells with diameters ranging from 8 to 10 mm were then made using a sterilized cork borer. Emulgel formulation and clobete Gm cream were added to the wells. The bacteria were cultured at 37°C for a full day. By measuring the diameter in millimeters in three fixed orientations and noting the average results, the zone of inhibition was determined. [29]

RESULTS AND DISCUSSION

Evaluation of formulation

(Table 3: Evaluation of formulation)

Formulati	Parameters			
on				
	Colou	Odour	Textur	Tast
	r		e	e

Mandlik et al

M1	Dark	Strong	Hard	Bitte
	brow	punge	and	r
	n-	nt	rough	
	black		seeds	
M2	Dark	Strong	Hard	Bitte
	brow	punge	and	r
	n-	nt	rough	
	black		seeds	
M3	Dark	Strong	Hard	Bitte
	brow	punge	and	r
	n-	nt	rough	
	black		seeds	

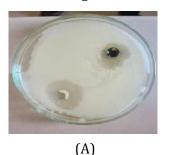
Evaluation Parameters: -

(Table 4: Evaluation of formulation Batches.)

Tabl	(Table 4: Evaluation of formulation Batches.)					
Sr.	Parameters	Batches				
No		M	M2	М3		
		1				
1.	Ph	6.41	6.29	6.49		
2.	Homogenei	Good	Good	Goo		
	ty			d		
3.	Viscosity	7223	7263	725		
				3		
4.	Spread	21	26	24		
	ability					
5.	Extrudabilit	Good	Good	Goo		
	y			d		
6.	Drug	75	87	81		
	content					
7.	Clarity	Good	Excelle	Goo		
			nt	d		
8.	Gel	26	30	28		
	strength					
9.	Consistency	Good	Excelle	Goo		
			nt	d		

Antimicrobial activity: -

1. Observed zone of inhibition values against S. aures and against E. coli





(Fig 4: Zone of inhibition values against S. aures (A) and against E. coli (B))

1. Zone of inhibition values against S. aureus were observed:

Bull. Pharm. Res. 2025;15(S2)

(Table 5: Zone of inhibition values against S. au were observed.)

Sample	Zone of inhibition
0.5g Extract	21
0.5g Emulgel	24

2. Zone of inhibition values observed against Escherichia coli:

(Table 6: Observed Zone of inhibition values against E. Coli)

Sample	Zone of inhibition
0.5g Extract	10
0.5g Emulgel	14

Skin irritation test: -

No complaints of burning, itching, redness, or other signs of irritation have been made.





. (Fig 6: Skin irritation test)

CONCLUSION

This study successfully developed and evaluated a herbal emulgel using Psoralea corylifolia extract to treat skin issues. The emulgel's pH, viscosity, homogeneity, extrudability, and drug content were assessed. Physical studies confirmed the compositions' consistency, excellent spreadability, and homogeneity. The pH ranged from 6.29 to 6.49, which is consistent with skin compatibility. The improved batch's viscosity of 7263 cP ensured proper rheology, and drug content analysis validated the presence of the expected active component.

Acknowledgments: The Modern College of Pharmacy, P.E. Society, Nigdi, Pune, is acknowledged by the authors for providing the facilities needed to conduct the research.

Conflict of interest: There are no conflicts of interest for the authors of this study.

Funding assistance: None.

Ethics statement: None.

REFERENCES

- 1. Pant S, Badola A, Baluni S, Pant W. A review on emulgel novel approach for topical drug delivery system. World J Pharm Pharm Sci 2015; 4:1728-43
- 2. Kullar R, Saini S, Steth N, Rana AC. Emulgel a surrogate approach for topical used hydrophobic drugs. Int J Pharm Biol Sci 2011; 1:117-28
- 3. Sonaje S, Gondkar S, Saudagar R. Gellified emulsion: A new born formulation for topical delivery of hydrophobic drugs. World J Pharm Pharm Sci 2013:3:233-51
- 4. Ashara K, Soniwala M, Shah K. Emulgel: A novel drug delivery system. J Pakistan Assoc Dermatologists. 2016; 26(3): 244–9.
- 5. Phad AR, Dilip NT, Ganapathy RS. Emulgel: A comprehensive review for topical delivery of hydrophobic drugs. Asian J Pharm. 2018; 12(2): 382–93
- 6. Hardenia A, Jayronia S, Jain S. Emulgel: An emergent tool in topical drug delivery. Int J Pharm Sci Res 2014; 5:1653-60.
- 7. Meenakshi D. Emulgel: a novel approach to topical drug delivery. Int J Pharm Bio Sci. 2013;4(1):847-856
- 8. Cevc G, Mazgareanu S, Rother M. Preclinical characterisation of NSAIDs in ultradeformable carriers or conventional topical gels. Int J Pharm. 2008; 360(1–2): 29–39.
- Herbert A, Liberman, Martin M, Reiger and Gilbert BS. Pharmaceutical Dosage Form – Disperse System. In: Pharmaceutical Dosage Form – Disperse System. 2005; 399–418.
- 10. Khandalavala BN, Nirmalraj MC, Rapid partial repigmentation of vitiligo in a young female adult with agluten-free diet. ClinDermatol 2014;6(3):283-7.

- 11. Patel NS, Paghdal KV, Cohen GF. Advanced treatment modalities for vitiligo. Dermatol Surg 2012;38(3):381-91.
- 12. Bordere AC, Lambert J, van Geel N. Current and emerging therapy for the management of vitiligo. Clin Cosmet Investig Dermatol 2009; 12:215-25.
- 13. Agarwal, B.B. and Shishodia, S. (2006). Molecular Targets of Dietary Agents for Prevention and Therapy of Cancer. Biochemical Pharmacology, 71(10), 1397-1421.
- 14. Priyanka Pandey1*, Rakesh Mehta1 and Ravi Upadhyay2: Physico-chemical and preliminary phytochemical screening of Psoralea corylifolia,2013.
- 15. TE Wallis. Textbook of Pharmacognosy. 5th ed. CBS Publishers & Distributors. New Delhi, 2002; pp. 536-565.
- 16. WHO Publication of Quality Control Methods of Medicinal plant materials.
- 17. Khandelwal KR. Practical Pharmacognosy, Preliminary Phytochemical Screening, Edition Twenty-eighth, Nirali Prakashan, Pune, July 2017; 25.3-25.6.
- 18. Rajakrishnan R, Lekshmi R, Benil P, et al. Phytochemical evaluation of roots of Plumbago zeylanica L. and assessment of its potential as a nephroprotective agent. Saudi Journal of Biological Sciences 2017; 24(4):760-766.
- 19. Richa S, Sagar B and Manoj M (2020). Formulation and Evaluation of Herbal Oral Gel Containing Extracts of Powdered Psidium guajava Linn Leaves with Curcuma longa Linn Rhizomes to Treat Mouth Ulcer. Int J Drug Dev & Res, 12(2): 150-157
- 20. Raut S, Uplanchiwar V, Bhadoria S, Gahane A, Jain SK, Patil S(2012). Comparative evaluation of zidovudine loaded hydrogels and emulgels. Res J Pharm Technol; 5: 41–45.
- 21. Kalyani T, Devender S and Ameya L (2018). Formulation and Evaluation

Mandlik et al

Pharmaceutical Aqueous Gel of Powdered Cordia Dichotoma Leaves With Guava Leaves. Am. J. PharmTech Res, 8(2):268-277

- 22. Vibha S (2021). Formulation and Evaluation of Pharmaceutical Polyherbal Mucosal Gel for Treatment of Mouth Ulcers Containing Glycyrrhiza glabra, Aloe vera and Curcumin. Quest Journals J. of Res. in Ph. Sci. 7(8): 1-13
- 23. Sabir S, Amol S and Rajendra D (2018). Formulation and Evaluation Pharmaceutical Aqueous Gel of Powdered Guava Leaves for Mouth Ulcer Treatment. Pharmatutor, 6(4): 32
- 24. Japan P, Brijesh P, HardeepSingh B, Kaushal P and Manish P (2010). Formulation And Evaluation Of Topical Aceclofenac Gel Using Different Gelling Agent.Int. J. Drug Dev. & Res.3(1):156-164
- 25. Srisombat Nawanopparatsakul, Jeeratikorn Euasathien, Chuwit Eamtawecharum, Porntip Benjasirimingokol, Sakdanai Soiputtan, Photchanart Toprasri, et al. Skin irritation test of curcuminoids facial mask containing chitosan as a binder. Silpakorn Uni versityJ 2005;5(1-2): 140-7.
- 26. Kirwin CJ. Eye and skin local toxicity testing in toxicology: principles and practice. Sperling F. (ed.) New York: Wiley- Interscience Publication;1984, p. 169-75.
- 27. Jigna P and Sumitra C (2006). In-vitro Antimicrobial Activities of Extracts of Launaea procumbens Roxb. (Labiateae), Vitis vinifera L. (Vitaceae) and Cyperus rotundus L. (Cyperaceae). African J. of Biomedical Res. 9(1):89-93
- 28. Varsha B, Varsha J and Vilasrao Kadam (2013). Study on Antimicrobial activity of Herbal Formulation. Int. J. of Pharm. & Life Sci. 4(11), 3099–3104.
- 29. Chetan C, Ulka S, Maheshwar H and Somnath B (2010). Screening of in-vitro antibacterial assay of barleria prionitis linn. J. of Herbal Med. and Tox. 4(2), 197–200.

An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print) 2249-9245 (Online)

RESEARCH ARTICLE



HIGH-PERFORMANCE THIN LAYER CHROMATOGRAPHY METHOD DEVELOPMENT AND VALIDATION FOR NAFTIFINE HYDROCHLORIDE DETERMINATION.

Mayur S. Tekade*, Pallavi M. Patil

Department of Pharmaceutical Chemistry,
PES's Modern College of Pharmacy, Sec. No. 21, Yamunanagar, Nigdi, Pune 411044,
Affiliated to Savitribai Phule Pune University, Pune
*Corresponding author email:mayurtekade99@gmail.com

Received: Mar 13, 2025 / Revised: April 06, 2025 / Accepted: May 07, 2025

Abstract:

The present study outlines a simple, rapid, sensitive, accurate, and precise High-Performance Thin-Layer Chromatography (HPTLC) method for the quantitative determination of Naftifine hydrochloride in its active pharmaceutical ingredient (API) form, over a broad analytical range. The method was validated according to International Council for Harmonisation (ICH) guidelines. Chromatographic separation was achieved on a silica gel 60F254 pre-coated TLC plate, using a mobile phase consisting of ethyl acetate and acetonitrile in a 1:1 (v/v) ratio. The method demonstrated no interference from tablet excipients, and distinct, compact spots for Naftifine HCl were observed with an Rf value of 0.28 \pm 0.05. The analytical wavelength selected for detection was _ nm (to be specified), and the mobile phase was delivered at a flow rate of 1.2 mL/min, with detection carried out at 235 nm. The retention time (Rt) of Naftifine HCl was recorded as 5.33 minutes. The method was validated for parameters including linearity, range, specificity, accuracy, and precision. Linearity was established within the concentration range of 2–64 μ g/mL. The percentage recovery of Naftifine HCl was found to be 99.41%, indicating excellent accuracy. The method's applicability was further demonstrated by analyzing commercially available formulations, particularly those undergoing degradation studies. The statistical evaluation of the results confirmed the reliability and robustness of the proposed analytical procedure.

Key words: Naftifine, HCl (NAF), HPTLC, Method development, Validation.

How to cite this article: Mayur S. Tekade1, Pallavi M. Patil High-Performance Thin Layer Chromatography Method Development and Validation for Naftifine Hydrochloride Determination. Bulletin of Pharmaceutical Research. 2025;15(S2): 135-140.

Source of support: Nil Conflict of interest: None

INTRODUCTION

Human fungal diseases are brought on by dermatophytes, yeasts, and molds and are transmitted globally by contact with sick people, animals, soils, and fomites. They fall into three categories: systemic, deep, subcutaneous, and superficial. Most infections caused by keratinized fungi are superficial. Dermatophytes spread by the air or by coming into contact with infected people. They infect tissues such as the skin, nails, and hair. It spreads from one portion of the body to another. [1–3] Depending on the location, severity, extent, and causative organism of the infection, dermatophytosis is frequently treated with oral or topical medications, or a combination of both. The topical approach is

considered the first-linetreatment for superficial and simple infections due to its high efficacy, strong ability to localize drugs at the infection site, and low likelihood of systemic absorption, which results in much fewer side effects [4–7]

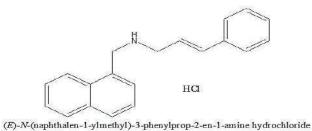


Fig 1: Chemical structure of Naftifine hydrochloride

The selection of topical antifungal medications for the treatment of superficial fungal infections has significantly increased over the past 20 years. The imidazole class of medications includes the vast bulk of the broad-spectrum antifungals in this arsenal(E)-N-cinnamyl-N-methyl-

1naphthalenemethylamine hydrochloride, another name for naftifine, is a member of the allylamines, a new class of synthetic broad-spectrum antifungal drugs. Naftifine specifically inhibits squalene epoxidase, which is the source of its antifungal action. This inhibition prevents the production of ergosterol, the main sterol found in fungal membranes [8-10].

Numerous techniques based on UV, HPLC, and GC have been documented to estimate this medication either by itself or in combination with other medications in pharmaceutical dosage forms. The HPTLC technique hasn't been explained yet, though. Therefore, an attempt to estimate the drug using the HPTLC Method has been successful in the current work. The dimensions of the column packing are one of the tracking components in the HPLC technique. To maintain repeatability of effects on these new instruments, high strain will be needed to force the cell phase and sampling through those new columns, and previously unnecessary equipment will now be needed.

The advantage of excessive overall performance thin layer chromatography (HPTLC) is that a large variety of samples may be instantaneously analyzed in a shorter period. Unlike HPLC, this approach makes use of much smaller number of solvents, thus reducing the price

The aim of the present work is to find a simple, sensitive, specific, HPTLC method and its validation for estimation from API.

Materials and Methods:

Instrumentation:

The HPTLC system included a CAMAG Linomat 5 automated spotting device, densitometric analysis was performed with a CAMAG TLC scanner - 3, and all of this was supported by CAMAG WinCATS software. The CAMAG twin-trough chamber (10 cm x 10 cm) was employed. For the sample application, a 100 l HPTLC syringe was utilized. The sample was applied on precoated silica gel 60 F254 (Merck, Germany).

Experimental-Chemical reagents-

Working standards of pharmaceutical grade

Naftifine HCl obtained as generous gifts from Simson Pharma Ltd., Mumbai (Maharashtra, India). All chemicals and reagents of analytical grade were purchased from Merck Chemicals, Mumbai, India

Preparation of sample solution-

A standard stock solution with a concentration of 100 $\mu\text{g}/\text{mL}$ was prepared using methanol as the solvent.

HPTLC optimization-

The TLC approach was developed with the goal of developing a simultaneous test method for Naftifine HCl. A standard stock solution was prepared, and 10 $\mu g/L$ sample were spotted on TLC plates and processed in several solvent systems.

After numerous testing, it was discovered that the movement of medication needed the use of a non-polar mobile phase. As a result, the optimal ratio of n-butanol to water to acetic acid was discovered to be ethyl acetate: acetonitrile (5:5 v/v).

The TLC chamber was saturated for 30 minutes to lessen the neckless effect. The mobile phase was run upto the distance of 8 cm and it took around 25 minutes for the TLC plate to fully develop.

Validation of the method-

Validation of the method

The developed method was validated by following ICH guidelines. Linearity, Specificity, Recovery, Precision, Robustness, were all validated for the developed method.

1. Linearity

The assay method's linearity was established by performing simple linear regression on the results obtained after injecting $10~\mu l$ of working standard solutions containing 200-1200 ng/band of Naftifine hydrochloride in triplicate. Calibration plots were created by graphing calibration standard concentrations vs. peak areas of the individual responses. A simple linear regression was used to assess the linearity of the plot, and the correlation coefficient was calculated.

2. Accuracy

The technique's accuracy was determined by conducting recovery at three levels (80%, 100%, and 120%) and spiking the dose form (1200 ng/band) with NAF standard (1000ng/band) using the standard addition method. Triplicate recovery experiments were carried out.

3. Precision

Repeatability on the same day and intermediate precision studies on various days were used to assess the accuracy of the devised method, and the peak area observed was represented in % relative standard deviation (%RSD). Six replicate applications of the identical location (1000 ng/band) were used to assess sample application repeatability and peak area measurement. Three replicates of concentration (1000 ng/band) were performed to ensure repeatability and intermediate accuracy.

4. Robustness

The effect of deliberate changes in method parameters such as the mobile phase composition, saturation time, development distance, spot scanning time interval, wavelength scan, time from spotting to chromatography, mobile phase volume was evaluated. Calculating the percent RSD for each parameter examined the effect of these modifications on both Rf values and peak regions.

5. Recovery

The evaluated samples were spiked with 50%, 100%, and 150% of the standard drug, and the combination was reanalyzed. The experiment was repeated six times for each recovery sample to ensure that the medication was recovered at varying amounts in the formulations.

Results and Discussion Linearity

Peak area was plotted against concentration to create the calibration curves in triplicate. Table 1 displays the linearity data of Naftifine hydrochloride. Fig.2 depicts the chromatogram of Naftifine hydrochloride produced at 282 nm with a standard concentration of 100 ug/ml. The method's linearity was assessed using basic linear regression analysis. As shown in Fig. 3, the approach was shown to be linear throughout the 1–50 g/ml range with a high correlation.

Table 1 Linearity data of Naftifine hydrochloride by HPTLC

Sr.	Concentration for	Area for
no	HPTLC (ng/band)	HPTLC
1.	NAF	NAF
2.	200	4795.13
3.	400	7061.03
4.	600	9579.5
5.	800	12311.1
6.	1000	15009.06
7.	1200	17340.23

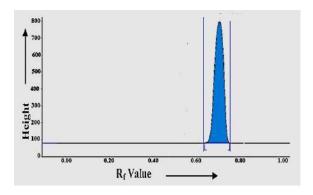


Fig 2 HPTLC Chromatogram of Naftifine hydrochloride standard 100 μg/ml at 282nm

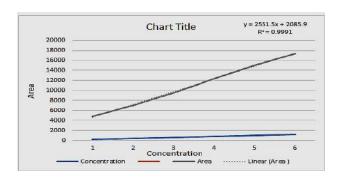


Fig 3 Calibration plot of Naftifine hydrochloride prepared by plotting concentration against area of peaks

2. Accuracy

Calculating the percentage of recoveries of test and standard solutions of Naftifine hydrochloride was used to evaluate the accuracy of the results utilised in this investigation. The results of the recovery trial are summarized in Table 2. The overall recovery was determined to be 101.53 percent, indicating that our procedure is accurate.

Table 2 Data of recovery study of Naftifine hydrochloride by HPTLC

	cinor rae a	J -		
Reco	Conc. Of	Conc.	%	%
very	test sol.	Of std.	Reco	RSD
level	(ng/ba	sol.	very	
(%)	nd)	(ng/ba		
		nd)		
	NAF	NAF	NAF	NAF
80	1000	800	98.74	0.506 2
100	1000	1000	100.2	0.550
			0	1
120	1000	1200	99.66	0.525
				8

3. Precision

The results demonstrated good system repeatability, intermediate precision and the reproducibility of sample application and peak areas measured were reported in percent RSD (Table 3). The percent RSD for sample

application system repeatability (1000 ng/spot) was determined to be 0.63. This suggested that the system's performance was excellent. The percent RSD for reproducibility (1000ng/band) was found to be 0.57. The percent RSD for intermediate precision at concentration level (1000 ng/spot) was minimal, indicating that the approach had high accuracy.

4. Robustness

A % relative standard deviation of peak area of less than 2% was obtained by purposefully varying parameters like mobile phase composition, saturation time, development distance, spot scanning time interval, wavelength scan, time from spotting to chromatography, and mobile phase volume. This suggests that none of these factors significantly affect the performance of the suggested method, confirming its robustness (Table 4).

Table 4 Robustness parameter for HPTLC

Parameter	Normal condition	Variable 1	Variable 2
Wavele	240 nm	238 nm	242 nm
ngth	NAF	NAF	NAF
	1170	1168	1163
Area	1160	1173	1171
	1185	1180	1184
Average	1171.66	1173.66	1172.66
SD	12.58	6.02	10.59
%RSD	1.07	0.51	0.90
Saturation	20min.	15min.	25min.
Time	NAF	NAF	NAF
	1180	1169	1173
Area	1172	1167	1177
	1174	1183	1172
	1175.33	1173	1174
Average	4.16	8.71	2.64
SD	0.35	0.74	0.22
%RSD	0.25	0.65	0.19
Mobile Phase(v/v)	Toluene:	Toluene:	Toluene:
Mobile I flase(v/ v)	Methanol(8:2v/v)	Methanol(7:3v/v)	Methanol(9:1v/v)
	NAF	NAF	NAF
Area	1167	1180	1163
	1173	1188	1169
	1169	1183	1173
Average	1169.66	1183.66	1168.33
SD	3.05	4.04	5.03
%RSD	0.26	0.34	0.43

Conclusion

The proposed High-Performance Thin-Layer Chromatography (HPTLC) method for the quantification of Naftifine hydrochloride is characterized by its rapidity, simplicity, sensitivity, accuracy, and precision. This analytical approach has been meticulously developed and validated to ensure robust performance across all critical validation parameters, as per ICH guidelines.

One of the key strengths of the method lies in its reproducibility, which is confirmed by the consistently low relative standard deviation (RSD) values observed in system suitability tests, accuracy studies, and repeatability assessments. These findings underscore the method's reliability and its suitability for routine quality control analysis.

The simplicity of the method is further highlighted by its utilization of standard, commercially available chromatographic plates and a commonly employed mobile phase, thereby eliminating the need for equipment. specialized reagents or Moreover, the method offers the advantage of a short analysis time, which enhances laboratory throughput and operational efficiency.In terms of analytical performance, the method exhibits excellent sensitivity and a high degree of linearity across a broad concentration range, making it well-suited method for detecting and quantifying even trace levels of Naftifine hydrochloride. The method also demonstrates exceptional accuracy and precision, thereby ensuring the reliability of the results obtained in both bulk drug and pharmaceutical formulation analyses

Overall, the developed HPTLC method represents a robust and efficient analytical tool for the routine determination of Naftifine hydrochloride in various pharmaceutical

matrices, with clear advantages in terms of operational simplicity, time efficiency, and analytical reliability.

Acknowledgment

I would like to express my special thanks of gratitude to my principal Dr. Pravin Chaudhary Sir PES'S Modern college of pharmacy Nigdi, Pune. I came to know about so many new things I am thankful to them.

Conflict of Interest

The authors declare no conflict of interest.

Ethical Approval

None of the writers' investigations in this article involved either human subjects or animals.

References:

- 1. Stefan P, Fleischer MD & Hardas B. (2013). Detection and relevance of naftifine hydrochloride in the stratum corneum up to four weeks following the last application of naftifine cream and gel, 2%. Journal of Drugs in Dermatology, 12(9), 1004-1008.
- 2. Noble S, Forbes RC, Stamm P. Diagnosis and management of common tinea infections. Am Fam Physician. 1998; 58(1):163-74
- 3. Weinstein A, Berman B. Topical treatment of common superficial tinea infections. Am Fam Physician. 2002;65(10):2095-102
- 4. Development and Validation of Simple, Rapid and Sensitive High- Performance Liquid Chromatographic Method for the Determination of Butenafine Hydrochloride
- 5. van Zuuren EJ, Fedorowicz Z, El-Gohary M. (2015). Evidence-based topical treatments for tinea cruris and tinea corporis: a summary of a Cochrane systematic review. Br J Dermatol 172:616–41
- Adel F. Alghaith, Sultan Alshehri, Nabil A. Alhakamy
 Khaled M. Hosny (2021) Development,
 optimization and characterization of nanoemulsion

loaded with clove oil-naftifine antifungal for the smanagement of tinea, Drug Delivery, 28:1,343-356,

- 7. Ryder NS, Tr0ke PP. The activity of naftifine as a sterol synthesis inhibitor in Candida albicans. In: Periti P, Grassi G, eds. Current chemotherapy and immunotherapy. Proceedings of the 12th International Congress of Chemotherapy. Washington DC: American Society of Microbiology, 1981:1016-7.
- 8. Paltauf F, Daum G, Zuder G, Hogenauer G, Schulz G, Seidl G. Squalene and ergosterol biosynthesis in fungi treated with naftifine, a new anfimycotic agent. Biochim Biophys Acta 1982;712:267-73.

Bulletin of Pharmaceutical Research 2025;15(S2)

An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print) 2249-9245 (Online)





EXPLORING WOMEN'S KNOWLEDGE, ATTITUDES, AND HEALTH RELATED PRACTICES RELATED TO POLYCYSTIC OVARY SYNDROME (PCOS) AMONG WOMEN: A SURVEY ANALYSIS

Rajani Mali ¹, Nikita Sanap, Swapnali Shedge , Vijay Shinde , Priyanka Sarda*

¹ Department of Pharmacy practice, ²*Department of Pharmacology

PES's Modern College of Pharmacy, Sec. No. 221, Yamunanagar, Nigdi, Pune 411044.

*Corresponding author email: priyankasarda15@gmail.com Received: Feb 07,

2025 / Revised: April 19, 2025 / Accepted: May 27, 2025

Abstract

Background: In India, polycystic ovarian syndrome, or PCOS, is a common endocrine and metabolic condition., involves symptoms like ovarian cysts, irregular ovulation, and high androgen levels, necessitating early detection and proactive management. Objectives: The study aims to examine the knowledge about polycystic ovarian syndrome (PCOS), evaluate attitudes toward PCOS, and assess health-related practices connected to PCOS. Methodology: This descriptive cross-sectional study surveyed 166 females staying in Pune, India, aged between 18-45 using a structured, validated questionnaire on knowledge, attitude, and health-related practices. Responses were collected via Google Forms; with KAP scores determined using specific cut off points. Attitude and practice scores were evaluated using 3- and 5-point Likert scales, respectively, and data were digitized using descriptive statistics. Result: Out of 166 people, 95.8% belong to the age group of 18-45 years. Only 12.5% were diagnosed with PCOS. The study revealed that 92.8% of females have good knowledge about PCOS, 39.8% have a poor attitude towards PCOS, whereas 89.9% have good health-related practices regarding PCOS. Just 12.7% of medical professional received a PCOS diagnosis. 3.6% of patients used oral contraceptives, 3% had a relevant previous history of PCOS. One of the main symptoms of PCOS, irregular menstrual periods, was reported by a startling 22.3% of patients Conclusion: Females diagnosed with PCOS show a commendable knowledge, attitude, and practice (KAP) regarding the condition compared with undiagnosed females. This study highlights the better knowledge and practices amongst participants but lack of attitude, while having a poor attitude concerning the early detection of this syndrome, leads to increased disease burden in our female population.

Key words: Polycystic Ovary Syndrome (PCOS), knowledge, Health Related Practices.

How to cite this article: Dr. Rajani Mali, Nikita Sanap, Swapnali Shedge, Vijay Shinde, Priyanka Sarda, Exploring Women's Knowledge, Attitudes, and Health related Practices Related to Polycystic Ovary Syndrome (PCOS) among Women: A Survey Analysis, Bulletin of Pharmaceutical Research. 2025;15(S2): 141-151

Source of support: Nil Conflict of interest: None

Mali et al INTRODUCTION:

A complex endocrine disorder, Polycystic Ovary Syndrome (PCOS) affects 6%-10% of women of reproductive age. The syndrome's symptoms include erratic or off menstrual cycles, elevated androgenic substances fabrication, male pattern hair growth, and abnormal ovarian morphology. Because women do not always exhibit all these sign and symptoms, PCOS is challenging to diagnose and research. [1]. Globally, PCOS prevalence varies; in India it is 30%, whereas in other nations, it is lower. Teenagers are most affected by insulin resistance (50%), and 75% of women with PCOS have it. PCOS affects 40% of women with dysmenorrhea, and the risk of diabetes is 15.4%. [2]. According to the NH diagnostic criteria, the prevalence of PCOS increased from 6% to 9% in the US, UK, Spain, Greece, Australia, Asia, and Mexico. [3]. To diagnose PCOS, a comprehensive history, a physical examination, and basic laboratory testing are usually enough. [4]. Usually, it is established by any of the two criteria: polycystic architecture transvaginal ovarian on ultrasonography, clinical or analytical hyperandrogenism, or irregular menstruation [5]. Obesity is also strongly associated with PCOS. It is hypothesized that women with PCOS have both extrinsic insulin resistance linked to lifestyle choice and PCOS specific intrinsic insulin with resistance increasing weight Furthermore, the symptoms of PCOS or the weight of a chronic illness can have a detrimental effect on quality of life by raising worry and despair. Effective PCOS management requires a healthy lifestyle that includes a balanced diet, consistent exercise, and keeping a healthy weight [7]. Given the significant correlation between weight growth, obesity in the abdomen, insulin resistance, and PCOS traits, avoiding excessive weight gain or reducing it is a feasible first treatment target. The Androgen Excess PCOS Society (AEPCOS) has said that lifestyle modification is the main treatment overweight and obese women with PCOS [8]. Managing symptoms including uncontrolled hair growth, acne, and metabolic abnormalities is the main goal of pharmaceutical therapies for PCOS. While Spironolactone prevents androgen effects, it necessitates good contraception owing to the possibility of

abnormalities. Birth control tablets assist reduce testosterone levels, lowering acne and hair growth. Long term therapies including ablation and laser hair removal improve physical beauty and quality of life, while Eflornithine lotion can inhibit the growth of facial hair [9]. According to recent research, inositol's, statins, letrozole and vitamin D are among the new or altered treatments recommended for the management of obesity and metabolic syndrome in PCOS [10]

METHODOLOGY:

Study design, duration, and study site: A cross-sectional prospective approach was used in the study. Most of the people who took part were students, housewives, or working women. Most of them were from Pune, Maharashtra, India. Data were gathered from the participants via WhatsApp, Instagram, and Telegram.

Participant's eligibility criteria: Women between the ages of 12 and 45 were invited to participate in part in the study. Women with menstrual menopause who had planned pregnancies or were unable to understand the questionnaire were excluded.

Validation of questionnaire: The study made use of a carefully created and verified survey. An expert consensus and a review of the literature were used to prepare the questionnaire. Content validation was done by academic scholars, pharmacists, physicians, nurses, patients, and patients relatives. Volunteers presented the questionnaire for appropriateness before the study's start. No reports of language comprehension or meaning issues were made. The questionnaire was in English and Marathi, clear, relevant, and easy to understand, without any confusing parts.

Data collection: The predesigned validated questionnaire form is utilized to collect the data online from the participants. Women filled out the survey online and had to agree to participate and confirm that their information was accurate. We made everyone understand why we were doing the study. The data collection form includes specific demographic details of the patients age, weight, height, whether the patients are diagnosed with PCOS, menstrual cycle

Mali et al

irregularity, duration of menstrual cycle, family history of PCOS, number of children, medical history, days of period, and menstrual flow. The data is divided into 3 parts; the first one is Knowledge, the second is Attitude, and the last is Practice. So, questions for knowledge (20), attitude (5), and practice (12) were included.

Scoring criteria: For all three sections, specific cut-off points were employed. Twenty knowledge questions were included. There are now two viable responses to each question: yes and no. A score of one was awarded for accurate responses. People who scored below 10 were classified as knowing insufficient knowledge. A three-point Likert scale was employed to assess attitude. Five questions about attitude were asked. Yes, no, and maybe are the three possible responses to each

Participan ts demograp hic characteri stic	Respon se 12-18 years	Overall freque ncy	Percen tage (%)
	18-45 years	159	95.8
Education	Post Graduat e	29	17.5
	Graduat e	34	20.5
	Under Graduat e	103	62
	Illiterat e	0	0
Employm ent status	Employ ed	15	9
	Self employ ed	5	3
	Home marker	3	1.8

question; yes carries a score of 2, while maybe has a score of 1. Having a positive attitude toward PCOS was defined as having a score greater than 5. The survey's health-related habits section contained twelve items. Responses were rated on a 5-point Likert scale to evaluate how each question was received by participants. Option 5 receives five points, whereas Option 1 receives one. A cut-off score of at least 35 was established. As a result, people who answered 35 or below on these questions were considered to have poor health-related practices, whereas those who answered 36 or higher were considered to have good practice

RESULT:

Table no. 1: Demographic details of participants:

	Student	143	86.1
Marital	Married	17	10.2
status	Unmarr ied	149	89.8
	Widow ed	0	0
Medically diagnosed	Yes	21	12.7
with PCOS	No	145	87.3
Duration of Menstrual	21 days	16	9.6
Cycle	28 days	116	69.9
Cycle	35 days	34	20.5
Menstrual	Regular	129	77.7
cycle regularity	Irregula r	37	22.3
Family	Yes	5	3
History of PCOS	No	161	97
Number of children	More than 2	2	1.2
	1-2	10	6
	NA	154	92.8
Use of oral	Yes	6	3.6

			- 1
Ma	11	et.	al

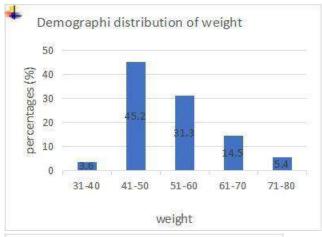
Mali et al			
contracept	No	160	96.4
ive			
Medical	Diabete	2	1.2
History	s mellitus		
	Respira	2	1.2
	tory		
	disease		
	S		
	Heart	0	0
	disease		
	S		
	Thyroid	5	3
	disease		
	Skin	6	3.6
	Disorde		
	r		
	NA	151	91
Days of period	1-2	5	3
periou	3-4	85	51.2
	5-6	76	45.8
Menstrual Flow	Normal	132	79.5
TIOW	Heavy	32	19.3
	Minimal	23	13.9
	Clots	23	13.9
	Pain	50	30.1

According to the participants' demographic information, 95.8% of them were between the ages of 18 and 45. Many participants fall within the height range of 5.1-5.5 (58.4%) and the weight range of 41-50 (45.2%). Additionally, 89.8% of the patients were unmarried, and 62% of them had less than a bachelor's degree. The percentage of participants having a PCOS diagnosis was just 12.7%. 92.8% of participants had no known chronic diseases based on medical history, 3.6% of patients used oral contraceptives, and 3% had a relevant previous family history of PCOS. whereas a small percentage of people had conditions such as respiratory disorders (1.2%) and diabetes mellitus (1.2%). When asked about their cycle health, 22.3% of individuals said they

Bull. Pharm. Res. 2025;15(S2)

had irregular ones. The substantial effect of PCOS on sexual and reproductive health is highlighted by the high incidence of irregular menstruation.

Figure no.1: demographic distribution of weight and height.



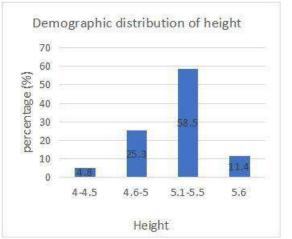


Table no. 2: Detailed Mean score of KAP

Parameters	Total score	Minimum required	score
Knowledge	20	10	
Attitude	10	5	
Practice	60	35	
Overall KAP	90	50	

The minimum score for a satisfactory evaluation was 50, and the maximum possible total KAP score was 90. The detailed mean scores indicated that the minimum acceptable scores for practice, attitude, and knowledge were 35, 10, and 5, respectively. A total of three possible scores were 20, 10, and 60.

Knowledge	Number of subjects
Poor (1-10)	12 (7.2%)
Good (11-20)	154 (92.8%)

With knowledge ratings ranging from 11 to 20, 92.8% (n=154) of individuals had good knowledge, demonstrating a thorough comprehension of PCOS, its symptoms, cause and therapeutic techniques. Nevertheless, a tiny portion of the population- 7.2% (n=12) showed inadequate knowledge (scores ranging from 1 to 10), indicating a knowledge gap.

Table no. 4: Information about the Attitude score

Attitude	Number of subjects
Poor (1-10)	66 (39.8%)
Good (6-10)	100 (60.2%)

Out of 166 individuals, 60.2% had a good attitude (score between 6 and 10), which suggests that they are willing to adopt PCOS preventive and treatment methods. However, 39.8% (n=66) had a negative attitude (score between 1 and 5), which might be a sign of unwillingness, misinterpretation, or lack of inspiration for treatment adherence and lifestyle modifications.

Table no. 5: Information about the Practice score that is classed

Practices	Number of subjects
Poor (1-35)	17 (10.2%)
Good (36-60)	149 (89.8%)

Additionally, health-related practice that are essential to PCOS management were evaluated. According to the result, 89.8% (n=149) of individuals adhered to excellent practices (scores ranging from 36 to 60), which include eating a balanced diet, getting regular exercise, and following medical advice. On the other hand,

Bull. Pharm. Res. 2025;15(S2)

10.2% (n=17) exhibited poor practices (scores ranging from 1 to 35), which indicated poor self-care practices and harmful lifestyle choices.

Table no. 6: KAP score of females who are medically diagnosed with PCOS

Parameter	Good n (%)	Poor n(%)
Knowledge	21 (100)	21 (0)
Attitude	21 (19)	21 (81)
Practice	21 (85.7)	21 (14.3)

Despite having adequate knowledge and practices, patients with a medical diagnosis of PCOS had a negative attitude.

Table no: 7 Overall KAP score

Overall KAP score	Number of subjects
Poor (0-50)	8 (4.8%)
Good (51-90)	158 (95.2%)



The overall KAP score of all screened participants was found to be satisfactory

Table no: 8 responses for Knowledge questions

Sr	Question for Knowledge	Res	ponses
n o.	Knowledge	Yes (correct)	No(incorr ect)
1	Have you heard of "polycystic ovary syndrome (PCOS)"?	153 (92.17%)	13 (7.83%)

Mali et al

Bull. Pharm. Res. 2025;15(S2)

Mali	ei ai		
2	In PCOS there is rise in androgen levels	133 (88.67%)	17 (11.33%)
3	PCOS patients frequently have several little cysts in their ovaries.	104 (86.67%)	16(13.33 %)
4	Being obese increases the chance of developing PCOS.	166 (100%)	(0 %)
5	PCOS may result from decreased insulin action in the body.	140 (84.33%)	26 (15.66%)
6	Does the menstrual cycle's irregularity or absence qualify as a PCOS symptom?	139 (83.73%)	27 (16.26%)
7	Does PCOS manifest as excessive hair growth on the upper chin, lip, breasts, abdomen, and thighs, among other body parts?	135 (82.82%)	28 (17.18%)
8	Is PCOS an indication of severe acne eruptions throughout the menstrual cycle?	131(79.39 %)	34 (20.61%)
9	Does excessive scalp hair loss that exceeds normal levels	124 (75.15%)	41 (24.85%)

		narm. Kes. 202	J,13(D2)
	qualify as a PCOS symptom?		
1 0	PCOS can be diagnosed through vaginal ultraso und?	118(71.95 %)	46 (28.05%)
1 1	Diagnosis of PCOS can be confirmed through specific blood test?	116(66.67 %)	58 (33.33%)
1 2	PCOS Can cause Diabetes mellitus.	122(70.93 %)	50 (29.07%)
1 3	Hypothyroidis m increases risk of developing PCOS	136(80.95 %)	32 (19.05%)
1 4	PCOS increase the risk of developing heart diseases	112(65.12 %)	(34.88%)
1 5	PCOS to result in decreased fertility (reduced likelihood of getting pregnant) or infertility (inability to have children)?	144(92.31 %)	12(7.69%
1 6	PCOS could contribute to anxiety and depression?	150(93.75 %)	10(6.25%

Mali et al

<u>Mali</u>	ei ai		
7	Can hormonal therapy, such as oral contraceptives or hormone intrauterine devices, beused in the treatment of PCOS?	119(71.26 %)	48(28.74 %)
8	Anti-diabetic medications, such as metformin, can be used as a treatment for P COS?	67(67%)	33(33%)
1 9	Is surgery an option for the removal of cysts from the ovaries?	81(67.5% %)	39(32.5% %)
2 0	Can physical activity reduce the symptoms of P COS?	147(91.87 %)	13(8.12%)

A detailed examination of the responses provided by respondents to the knowledge questions is shown in Table 8. 133 respondents, or 88.67%, were unaware that testosterone levels were elevated by PCOS. Of those who had PCOS, 104 (86.67%) were aware that their ovaries had tiny multiple cysts.

Furthermore, 139 (83.73%) of the respondents knew that irregular menstruation is a symptom of PCOS. Still, 122 of them (70.93%) did not know that diabetes can lead to PCOS. Furthermore, 113 respondents (70.93%) claimed not to be aware that PCOS could cause cardiac issues.

Table no: 9 Responses for Attitude questions

Table 110. 9 Responses for Attitude questions					
		Response			

_		100	1.0	1 1019 2 0
r	Questions			
n	for Attitude			
0.				
1	Do you think that PCOS, or polycystic ovarian syndrome, is a significant medical condition?	144(72. 15%)	6(3.80 %)	38(24. 05%)
2	Do you think	139(79.	7(4%)	29(16.
	PCOS impact	43%)		57%)
	on vour			

Yes

S

personal life?

3

Do

Bull. Pharm. Res. 2025;15(S2)

No

Mavbe

	believe you are at risk of PCOS?	%)	%)	%)
4	Do you feel anxious or depressed if you diagnosed with PCOS?	74(49.3 3%)	27(18 %)	49(32. 67%)
5	If you	67(44.6	46(30.	37(24.

7%)

it

diagnosed of

affects your

confidence n

egatively

PCOS

self-

39(26

63(42

67%)

48(32

67%)

Table 9 displays the responses given by the respondents to the PCOS attitude Questions. 139 (79.43%) of the respondents believe that PCOS significantly affects their personal lives, whereas 144 (72.15%) perceive PCOS as a serious medical illness. Furthermore, 39 people (26%) believe they are at risk of developing PCOS.

Sr	Questions		Respor	ises		
no.	for Practice	Never	Rarely	Sometimes	Often	Always
1	How often do you check the nutrition facts on food labels?	14(8.24%)	41(28.24%)	48(28.24%)	29(17.06(%)	38(22.35%)
2	Do you include low-fat foods (e.g. Fish, milk, oats etc.) in your diet?	11(6.47%)	25(14.71%)	55(33.35%)	38(22.35%)	41(24.12%)
3	Do you take low-salt foods (e.g. Fresh fruits, vegetables, unsalted dry fruits, rice, meat, fish, yogurt or milk etc.) into your dietary habits?	17(10.24%)	22(22.89%)	49(29.52%)	30(18.07%)	32(19.28%)
4	Do you eat enough fruits and vegetables every day?	2(1.20%)	17(10.24%)	47(28.31%)	50(30.12%)	50(30.12%)
5	Have you decreased the intake of refined sugar in your diet	22(13.25%)	30(18.07%)	47(28.48%)	34(20.48%)	33(19.88%)

Mali et al Bull. Pharm. Res. 2025;15(S2)

Mali	i et al				Bull. Pharm. F	Res. 2025;15(S2)
6	How do you	Very poor	Poor	Fair	Good	Excellent
	maintain your Menstrual hygiene?	60(36.36%	1 (0.61%)	12(7.27%)	89(53.94%)	60(36.36%)
7	Do you include a greater number of h igh-Fiber foods (oats, apple, banana, carrots, broccoli, beans, almonds, flaxseeds etc.) in your diet?	3(1.81%)	16(9.64%)	60(36.14%)	46(27.71%)	41(24.70%)
8	Do you consume smaller meal portions during dinner?	14(8.43%)	24(14.46%)	51(30.72%)	38(22.89%)	39(23.49%)
9	Every day for 30 minutes, do you work out?	31(18.67%)	36(21.69%)	50(30.12%)	18(10.84%)	31(18.67%)
10	Do you eat fast food over the weekends?	12(7.23%)	30(18.0%)	61(36.75%)	42(25.30%)	21(12.65%)
11	Do you think it's easy to keep up a healthy diet?	Strongly disagree 7 (4.27%)	Disagree 9 (5.49%)	Neither agree nor Disagree 31(18.90%)	Agree 81(49.39%)	Strongly Agree 36(21.95%)
12	Do you consume tobacco	147(93.04 %)	5(3.16%)	3(1.90%)	2(1.27%)	1(0.63%)

Table 10 displays the participants' answers to the PCOS practice questions. Of these, 55 (33.35%) individuals stated they included low-fat foods in their diet, and 48 (28.24%) reported they occasionally read the nutrition information on product labels.

DISCUSSION:

The findings of this study highlight significant gaps in women's health-related behaviours, attitudes, and understanding of Polycystic Ovary Syndrome (PCOS) among patients in Pune, Maharashtra, India. A total of 166 PCOS patients participated in the study, which collected various medical and demographic data alongside Knowledge, Attitude, and Practice (KAP) scores.

The study's findings on knowledge, attitude, and practice were particularly insightful. Overall, patients demonstrated a high level of knowledge, with 92.8% reporting substantial awareness of PCOS [11]. This result is notably higher than findings from previous studies; for instance, Konkati Druthika et al. reported that 59% of the research population was aware of PCOS [12], while Srivastava et al. documented an even lower awareness rate of 50.2% [13]. Similarly, while the present study recorded a high practice score of 89.87%, research by Jasmin et al. indicated a significantly lower practice level of 7.5% [14]. However, in our study, high knowledge and practice scores, the attitude score was comparatively lower, with only 60.2% of participants displaying a positive attitude toward management. A critical intervention in PCOS management is highlighted by the difference between knowledge, practice, and attitude, which points to a breakdown in converting awareness into proactive behavioural changes [15].

This study also identified significant correlations between knowledge levels and several demographic factors. Knowledge was positively associated with age and education, with higher education levels corresponding to greater knowledge and attitude. These findings align with previous research by Mehwish Rizvi and MD Ashraful Islam, which emphasized the beneficial impact of education on health awareness. Furthermore, a study performed by Ranjana SR et

al. found that knowledge is to be strongly correlated with attitude levels, with higher education linked to more positive attitudes, and attitude levels significantly associated with educational attainment.

Other factors, especially attitude, may have a greater influence on PCOS management, as evidenced by the lack of a statistically significant correlation between practice levels and demographic or medical history data. Although patients exhibit adequate knowledge and practices, their motivation and positive health attitudes are still insufficient, as indicated by the observed gap between awareness and attitude. Targeted methods like motivational interviewing, patient-centred counselling, and organized awareness initiatives are required to close this gap by enhancing attitudes and promoting long-lasting, sustainable behavioural change.

These results highlight the need to close the knowledge-practice gap in PCOS care by implementing behavioural interventions. To make sure that more knowledge converts into efficient and long-lasting management solutions for PCOS, healthcare providers should concentrate on clearing up misunderstandings, lowering judgment, and encouraging self-motivation.

CONCLUSION

This study evaluates the levels of knowledge, attitude, and practice (KAP) related to PCOS. The participants demonstrated good knowledge and practice regarding PCOS; however, their attitude scores were poor. The overall KAP score were also good. The diagnosed patients have the same scores as the normal participants.

These findings underscore the necessity of structured educational interventions aimed at enhancing attitude dispelling misconceptions and encouraging health-seeking behaviours. Improving KAP scores through targeted awareness programs can facilitate better selfmanagement and contribute to improved long-term health outcomes for individuals with PCOS.

Mali et al

ACKNOWLEDGEMENT: I am grateful to Progressive Education Society Modern College of Pharmacy, Nigdi, Pune for providing the necessary resources. My heartfelt appreciation goes to my professors, colleagues, and friends for their encouragement and assistance. Lastly, I extend my deepest gratitude to my family for their unwavering support and motivation.

REFERENCES:

- 1. Colwell, K., Lujan, M. E., Lawson, K. L., Pierson, R. A., & Chizen, D. R. (2010). Women's Perceptions of Polycystic Ovary Syndrome Following Participation in a Clinical Research Study: Implications for Knowledge, Feelings, and Daily Health Practices. Journal of Obstetrics and Gynaecology Canada, 32(5), 453–459.
- Yedulapuram, S. H., Gunda, M., Moola, N. R., & Kadarla, R. K. (1970). AN OVERVIEW ON POLYCYSTIC OVARIAN SYNDROME. Asian Journal of Pharmaceutical Research and Development, 7(4), 72–80.
- 3. Bharali, M. D., Rajendran, R., Goswami, J., Singal, K., & Rajendran, V. (2022). Prevalence of Polycystic Ovarian Syndrome in India: A Systematic Review and Meta-Analysis. Cureus.
- 4. Williams T, Mortada R, Porter S. Diagnosis and Treatment of Polycystic Ovary Syndrome. Am Fam Physician. 2016 Jul 15;94(2):106-13. PMID: 27419327.
- 5. Sydora BC, Wilke MS, McPherson M, et al. Challenges in diagnosis and health care in polycystic ovary syndrome in Canada: a patient view to improve health care. BMC Womens Health 2023;23:569.
- Moran, L. J., Lombard, C. B., Lim, S., Noakes, M., & Teede, H. J. (2010). Polycystic Ovary Syndrome and Weight Management. Women's Health, 6(2), 271–283.
- 7. Lim SS, Hutchison SK, Van Ryswyk E, Norman RJ, Teede HJ, Moran LJ. Lifestyle changes in women with polycystic ovary syndrome. Cochrane Database Syst Rev. 2019 Mar 28;3(3):CD007506.
- 8. Sassi F, Devaux M, Cecchini M, Rusticelli E: The obesity epidemic: analysis of past and projected future trends in selected OECD countries. Organisation for Economic Co-operation and Development Health Working Papers No. 45. Organisation for Economic Co-operation and Development, France (2009).

Bull. Pharm. Res. 2025;15(S2)

- 9. olycystic Ovary
 Syndrome (PCOS)." Mayoclinic.org,
 https://www.mayoclinic.org/diseasesconditions/pcos/diagnosis-treatment/drc20353443. Accessed 3 Apr. 2025.
- 10.Rashid, R., Mir, S. A., Kareem, O., Ali, T., Ara, R., Malik, A., Amin, F., & Bader, G. N. (2022). Polycystic ovarian syndrome-current pharmacotherapy and clinical implications. Taiwanese Journal of Obstetrics and Gynecology, 61(1),40–50.
- 11. S. R., R., B. S., S., & Babu, B. (2024). Study of knowledge, attitude and practice about polycystic ovarian syndrome among PCOS patients. International Journal of Reproduction, Contraception, Obstetrics and Gynecology, 13(10),2723–2729.
- 12.Goh, J. E., Farrukh, M. J., Keshavarzi, F., Yap, C. S., Saleem, Z., Salman, M., Ramatillah, D. L., Goh, K. W., & Ming, L. C. (2022). Assessment of prevalence, knowledge of polycystic ovary syndrome and health-related practices among women in klang valley: A cross-sectional survey. Frontiers in Endocrinology.

An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print) 2249-9245 (Online)



RESEARCH ARTICLE

SCLEROGLUCAN BASED HYDROGEL AS WOUND DRESSING MATERIAL

Karimunnisa Shaikh*, Priyanka Rokade, Mansi Sardar, Shraddha Lipare

Department of Pharmaceutics,

PES's Modern College of Pharmacy, Sec. No. 21, Yamunanagar, Nigdi, Pune 411044.

*Corresponding author email: karima78@rediffmail.com
Received: Feb 06, 2025 / Revised: April 18, 2025 / Accepted: May 02, 2025

ABSTRACT:

The study investigates the potential of scleroglucan-based hydrogel as a wound healing agent by incorporating doxycycline hyclate, an antibiotic known for its antimicrobial and anti-inflammatory properties. The research focuses on formulating, characterizing, and evaluating the hydrogel through in-vitro, ex-vivo, and in-vivo assessments. The methodology involved preliminary evaluation of scleroglucan, drug-excipient compatibility studies, hydrogel formulation, and physicochemical characterization such as rheological behavior, swelling index, drug content, and in-vitro drug release. The ex-vivo and in-vivo animal models were utilized to assess the hydrogel's wound healing efficiency, histopathological changes, and antibacterial activity. The drug release study indicated that the scleroglucan-based hydrogel exhibited sustained drug release (88.9% in 8 hours) compared to a marketed formulation that achieved 98.26% release in 5 hours. The wound healing study demonstrated that the hydrogel-treated group showed faster wound contraction compared to control groups. The histopathological evaluation confirmed enhanced collagen deposition and re-epithelialization, indicating effective healing. The stability study revealed no significant degradation of the hydrogel under standard conditions. The findings suggest that scleroglucan-based hydrogel is a promising wound dressing material with enhanced antimicrobial activity, biocompatibility, and sustained drug release, making it a potential alternative to conventional wound care products.

KEY WORDS: Scleroglucan, Doxycycline hyclate, Hydrogel, Wound healing, Drug release, Biocompatibility, Histopathological evaluation, Antibacterial activity, Stability study

How to cite this article: Karimunnisa Shaikh, Priyanka Rokade, Mansi Sardar, Shraddha Lipare Scleroglucan Based Hydrogel As Wound Dressing Material Bulletin of Pharmaceutical Research. 2025;15(S2): 152-162.

Source of support: Nil Conflict of interest: None

INTRODUCTION:

An ideal environment is necessary for tissue regeneration and repair throughout the intricate biological process of wound healing [3, 5]. Because of their high-water content, biocompatibility, and capacity for continuous medication release, hydrogels have drawn a lot of interest in the field of wound care [7]. For quicker healing and infection prevention, these qualities aid in preserving a moist wound environment [9, 10]. A naturally occurring polymer generated from fungi, scleroglucan has demonstrated stability, biocompatibility, and possible antibacterial qualities [6,8]. Its use in wound healing is still not well understood, though. Doxycycline hyclate, a broad-spectrum antibiotic with inflammatory properties, may improve wound healing and prevent microbial infections when combined with scleroglucan [4,11]. This study aims to formulate, characterize, and evaluate a scleroglucan-based hydrogel containing doxycycline hyclate [7,12]. The research involves physicochemical characterization, in-vitro drug release studies, ex-vivo and in-vivo wound healing evaluations, histopathological analysis, and stability testing. By utilizing the natural wound-healing properties of scleroglucan and the antibacterial effects of doxycycline, thisstudy explores a novel hydrogel-based wound dressing. The findings may contribute to the development of an advanced, effective, and biocompatible wound care solution [8,15]. Scleroglucan was selected over other polysaccharides due to its superior thermal stability, enhanced swelling capacity, and excellent bioadhesive properties.

Hydrogels:

Three-dimensional networks of hydrophilic polymers, known as hydrogels, can hold onto a lot of water while remaining structurally sound. Hydrogels are utilized extensively in wound care management because of their watercontent, biocompatibility, and capacity to create a moistenvironment. Hydrogels provide several benefits when used as a material for wound dressings. They provide a cooling effect that reduces pain, encourages cell migration and tissue regeneration, and hydrate parched wounds. Because they are non-adhesive, there will be less injury when changing dressings. Furthermore, hydrogels can be filled with medications like growth hormones or antibiotics. enabling regulated drug release at the wound site. For burns, ulcers, surgical wounds, and chronic wounds, hydrogels are especially helpful. They are a good substitute for conventional dressings because of their capacity to absorb exudates while preserving a moist healing environment. As biopolymer-based hydrogels, such those based on scleroglucan, continue to progress, so does their promise for improved healing applications. wound

MATERIALS AND METHODS

Materials

The materials used in the formulation of the hydrogel included Doxycycline hyclate, sourced from Softeshule Pvt Ltd, Mumbai, as the antimicrobial agent. Scleroglucan, the primary polymer for gel formation, was obtained from Qingdao Chemicals, China. Borax, used as a cross-linking agent, was supplied by Merck Specialist Pvt Ltd, Mumbai. Methyl paraben, serving as a preservative, and Sodium metabisulfite, acting as an antioxidant, were also procured from Merck Specialist Pvt Ltd, Mumbai.

Methods

guarantee even ingredient dispersion, mechanical stirring was used to prepare the hydrogel formulation [10]. The first step in creating the hydrogel formulation was making the polymer solution, which involved dissolving a certain amount of borax in distilled water and adding scleroglucan to create a stable mixture. To guarantee even dispersion, doxycycline hyclate was then added progressively to the polymer solution while being stirred for three hours at 400 rpm using a magnetic stirrer. Borax started the gel formation and cross-linking process, which stabilized the hydrogel network. Methyl paraben was added as a preservative and sodium metabisulfite as an antioxidant to improve the stability of the formulation.

Table no. 1 Preliminary Trial batches of Hydrogel

Batch	Drug(Poly
Code	mg)	mer
F1	0.150	0.5%
F2	0.150	0.7%
F3	0.150	0.8%
F4	0.150	1.0%
F5	0.150	2.3%

EVALUATION OF HYDROGEL

Rheological behaviour:

Rheological behaviour of hydrogel was determined by using Brookfield viscometer at different rpm using Helipath spindle no. D [12].

Drug content:

One gram of the test sample was dissolved in one hundred milliliters of distilled water to determine the drug concentration. Distilled water was used to further dilute each of these solutions. To get rid of insoluble material, the solution was filtered. At 271 nm, absorbance was measured using a UV-visible spectrophotometer. [35,10]

FTIR Spectroscopy:

The Prestige-21 (SHIMADZU) withIR resolution software was used to record the FTIR spectrum of doxycycline hyclate. After being put in the FTIR sample container, the drug sample was scanned between 400 and 4000 cm-1. The obtained spectrum was presented together with the structural assignments for the typical absorption bands.

UV spectroscopy:

The goal of the study was to create a UV spectrophotometric technique for the examination of the common antibiotic doxycycline hyclate. Because of the drug's solubility, distilled water was selected as the solvent. Doxycycline hyclate (10 mg) was dissolved in 10 mL of distilled water to create a standard stock solution, which had a concentration of 100 µg/mL. Serial dilutions of 3.25, 6.25, 12.5, 25, and 50 µg/mL were made from this stock. These solutions' UV spectra were recorded between 400 and 800 nm, with 271 nm showing the highest absorption. The standard calibration curve for quantitative measurement was constructed using the drug's linearity range, which was established based on literature to be $3.25-50 \mu g/m$.

Appearance:

The general appearance of the gel includes morphological characterization like, color, appearance, odor etc.

Swelling index:

The amount of moisture or water absorbed by the formulation in dried form over the course of 24 hours was measured using the swelling index. The following formula was used to determine the weight of the empty petri plates, the weight of the petri plates with formulation in the dried condition, and the weight of the petri plates when swelling is taken into account.

Spredability

spredability, texture analysis was performed using a Brookfield texture analyzer, which involved pouring gel into a cup cavity, calibrating the apparatus, starting the probe, and recording the graph once the calibration was complete.

In vitro diffusion study:

The drug was subjected to an in vitro diffusion investigation using the Franz diffusion cell device. Phosphate buffer (pH 7.4) was added to the donor compartment. A UV-visible spectrophotometer was used to detect the absorbance at 271 nm following the application of a precisely weighted gel to the rat's skin and rotation at 150 rpm for almost seven hours. The percentage of drug release was then computed and compared to a commercial formulation.

Ex vivo animal study

Evaluation of in vivo primary skin irritation test on rats: As per approved protocol of CPCSEA (Reg No.884/ac/05/CPCSEA) animal study was carried out. Optimized formulation evaluated for primary skin irritation test. Nine healthy rats were used for the experiment[18]. The backs of the animals were clipped free of the fur with a razor at least 4 hrs before application of the sample. A 0.5 gm of sample of the test material was then applied to the particular site to an area of skin approximately 1" ×1" (2.54×2.54cm) square. The sample applied site was covered with a nonreactive tape. Animals were returned to their cages. After a 24hrs exposure, the tape was removed and the test sites were wiped with tap water to prove the test sample. At 24 and 72 hours after test sample application, the sites were examined for dermal reaction in accordance with FHSArecommended Draize scoring criteria. The primary irritation index of the test sample was calculated following test completion.

Evaluation of Wound healing activity:

Wistar albino rats weighing 200-250 g were used to test the wound-healing properties of hydrogel containing 1% scleroglucan and 0.2% doxycycline [13]. Five animal groups, each with six rats, were selected and designated as the control group [14]. Group 1 was given scleroglucan hydrogel in its plain form, Group 2 was given a hydrogel (optimal batch), Group 3 was given doxycycline in carbopol 940 gel, and Group 4 was given the commercial formulation. There are numerous animal models available for the study of wound healing. models for burn, dead space, excision, and incision. Which wound healing model is applied depends on the study's design and goal. An incision wound model was selected for the study, and the animals were labeled and classified. To get rid of hair from the edges, apply hair removal cream.

Once the peripheral hairs have been removed. the skin area needs to be disinfected with ethanol or other solvents. A surgical blade is used to make four to six centimeter cuts in the skin, which are then left open for twenty- four hours. After a 24- hour period, the composition is administered twice a day. The results were documented. Histopathological study: Before and after wound healing, a histopathological analysis was conducted to assess the formulation's anti- inflammatory reaction against inflammatory cells, collagen content, re-epithelialization, and granulation content [16]. Hematoxyline and Eosin (HE) stain was used at B.J. Medical College to examine the optimized formulation for histopathological alterations. 10X and magnification photos were captured. Eosin stains basic regions blue, while hematoxyline stains acidic regions pink.

Stability studies:

Stability studies ensuring the maintenance of product quality, safety and efficacy throughout the shelf life are considered as pre-requisite for the acceptance and approval of any pharmaceutical product. These studies are required to be conducted in a planned way following the guidelines issued by ICH, WHO and or other agencies. Stability studies were carried optimized out on formulation according to ICH guidelines. Following ICH guidelines, the formulation in an aluminum tube underwent three months of accelerated stability testing at three different temperatures: 25 ± 2° C and 60 ± 5% relative

Melting Point:

The melting point analysis showed that the observed melting point of doxycycline ranged between 196-200°C, which is consistent with the reported value of approximately 201°C, confirming its authenticity.

FTIR Analysis The spectrum shows all prominent peaks of Doxycycline. The FT-IR spectrum of Doxycycline shown in Fig.1

humidity, $30 \pm 2^{\circ}$ C and $65 \pm 5\%$ relative humidity, and $40 \pm 2^{\circ}$ C and $75 \pm 5\%$ relative humidity. Samples were taken at one- month intervals for three months, and changes in pH, viscosity, and drug content were examined. If observed values were recorded, any modifications to the evaluation parameter were documented. The tests were conducted in duplicate, and the standard deviation and mean of the observed data were recorded.

Skin irritation test:

To assess the safety of topical application of the 1% doxycycline hydrogel with scleroglucan, a skin irritation test was performed. Test individuals were used in the investigation, and the formulation was applied to a particular skin area. The skin was examined for any pre-existing abnormalities or irritation prior to application. Twice daily, a tiny amount of the hydrogel formulation was gently applied to the test region, and the participants were watched for any outward symptoms of skin irritation, such as itching, erythema, edema, or allergic responses. To find any negative impacts, observations were made on a regular basis.

RESULTAND DISCUSSION

Drug Authentication

Organoleptic Properties

The organoleptic evaluation of doxycycline revealed that the drug was found yellow in color, odorless, and tasteless.

FTIR Analysis

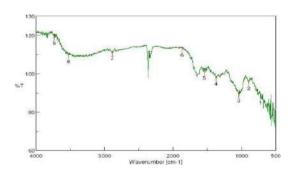


Table: 2 Results of IR Spectra:

Wave number Standard (cm ⁻¹)	Wave number Observed (cm ⁻¹)	Group identified
3000-3100	С-Н	3126.04
1000-1300	C-O	1040.41
1400-1600	C=C	1459.85
675-1000	=С-Н	938.391

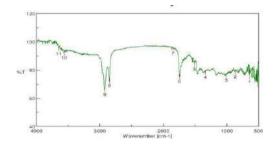


Figure 2. FTIR spectra of

Figure.3 Structure of Doxycycline

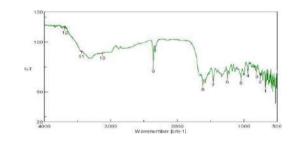


Figure 4. structure of scleroglucan

Figure 5. FTIR spectra of scleroglucan

Wave number	Wave number	Group identified
Standard (cm ⁻¹)	Observed (cm ⁻¹)	
3500-3700	0-Н	3648.66
1400-1600	C=C	1507.1
1210-1320	C-O	1339.32
675-1000	=C-H	868.77





Equation of straight line obtained by UV-Visible spectroscopy showed that Beerlamberts law was obeyed and linearity range was found to be 3.25 to $50\mu g/ml$. Coefficient of correlation was found to be 0.997 which was less than 1.00. As per reported methods lambda max was 273nm.

Evaluations of hydrogel:

Swelling index of hydrogel on the results for viscosity and swelling index. To assess the formulation's water-absorbing and water-holding capabilities, the swelling index is used. This makes it easier to comprehend how the hydrogel behaves and how to maintain a moist environment around the wound regions. Additionally, depending on the surrounding conditions, hydrogels may exhibit behavior. These polymers swelling physiologically-responsive hydrogels, meaning that when the external environment changes, the network may swell or polymer complexes may break. In vitro drug release study-The Franz diffusion cell and pH 7.4 buffer in the acceptor compartment were used to measure the drug release kinetics of 1% hydrogel. Rat skin was used for the release investigation, and the formulation (0.5gm) was applied to it. The trial was continued and the drugrelease was assessed at a speed of 200 rpm and a temperature of 37± 5°C for

Histopathological study: The work required to remove the probe from the sample is represented by the work of adhesion, which is the negative area under the force-time curve. The findings demonstrated that the force needed to disseminate the sample is not more than the force needed to separate the probe. This indicates that the hydrogel sample has good spreadability with an adhesiveness of 0.10 mj and an adhesive force of 4 gm.

approximately 8 hours. In contrast to the marketed product, which demonstrated drug in 5 maximum release hours of approximately 98.26% drug release. this investigation shown that the maximum drug release was reached in 8 hours and was around 88.9%. When comparing doxycycline release experiments of 1% hydrogel with Scleroglucan

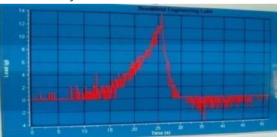
Release mechanism:

The release rate of the hydrogel depends on the concertation of the drug present in the hydrogel. That's why this follows zero order kinetics also the swelling of scleroglucan and uniform dispersed drug responsible for the zero-order release mechanism.

It reduces the frequency of application, and the constant release of drug keeps moist, antimicrobial and anti-inflammatory environment which is ideal for wound healing. and other studies of doxycycline with carbopol 940, the results indicate that 90% of the drug is released in 8 hours with Scleroglucan and 45% in 24 hours with carbopol 940.

Texture analysis of trial batch (F4) - Texture analysis is a penetrometry technique that has been extensively employed in the mechanical characterization of food materials

1. Sy stem adhesiveness (the maximum negative force recorded in the withdrawal)



Adhesive Force is force required to separate the sample from the probe and adhesiveness is work require to pull the sample away from the surface. animal study Wound healing study: Group II- This group received two daily applications of plain scleroglucan (1%) gel, and daily wound healing was monitored. From day two to day ten, all wounds began to heal quickly. From day two, the rate of wound contraction increased. Group III-

All of the animals in these groups received treatment with 1% Sclg+Dox hydrogel twice daily, and daily observations were made of the contraction and healing of the wounds. Animals treated with hydrogel were shown to heal more quickly than those in Group II (Plain hydrogel of scleroglucan). **Group IV-** All of the animals in this group received treatment with carbopol 940 gel and doxycycline twice daily. Within 15 days, all of the animals' wounds had fully healed. Group V- Every animal received treatment using the commercially available Hydroheal product, which includes a gel base and silver colloid. In comparison to hydrogel and regular doxycycline gel. the marketed formulation demonstrated wound healing in 5 days, which was too quick. The histological analysis revealed that both the test and control wounds had significant inflammation at the start of the investigation. Peripheral keratinocytes that were migrating across the wound bed were highly abundant along the wound edges. Following treatment with 1% scleroglucan containing doxycycline hydrogel, a notable improvement was observed. After wounds were treated, significant hypergranulosis was discovered.[25] color change from yellow to brown and also the change in pH and drug content was appeared. There was no significant change in color, pH and drug content was appeared for hydrogel at 30 °C and at room temperature.

Table: 2 Batch F4 is chosen as the optimal batch based

Sw elli	F1	F2	F3	F4	F5	Mark eted
ng						formu
Ind ex						lation
(%	9.96±0	10.95±	10.48±	11.75±	11.46±	23.11
)	.820	0.586	0.332	0.452	1.105	±1.03

Table no.3 In-vitro drug release kinetics

able no.3 in-vitro drug release kinetics				
Time (mi	% w/v	% w/v Drug	% w/v	
n)	Drug	release of	Drug	
	release	plain	release of	
	doxycycl	hydrogel	marketed	
	ine	(without	formulati	
	hyclate	drug)	on	
5	22.48±0.21	3.05±0.521	6.95±0.14	
	2		8	
10	13.04	5.87±1.206	10.08±0.9	
	±0.254		12	
15	9.92	7.23±0.583	17.975±0.3	
	±0.353		32	
30	11.74	10.35±0.850	33.21±0.5	
	±0.028		65	
45	15.03	13.06±1.651	33.21±0.5	
	±1.850		65	
60	14.27	16.54±0.738	41.7±0.59	
	±0.746		3	
120	44.84	20.39±1.820	60.08±0.2	
			12	

UV Analysis: Table no.4 Stability study

TIME	PARAMETERS	INFEREN CE
1	Color	No change
5	Drug content	95.21%
30	Color	No change
	Drug	95.12%
	content	
4	Color	No change
5	Drug	94.68%
	content	
6	Color	Light
0		brown
	Drug	94.05%
	content	

Table no. 5 In-vitro release kinetic model fitting profile

Batch	Zero order model (r)	First order model (r)	Hixs on- Crow ell mod el (r)	Matrix model (r)	Rate of Rele ase (k)	Best fit kinetic model	t90% (min)
1%						Zero	
hydrogel	0.7425	0.7207	0.7280	0.6043	0.0208	order	10038.1
Marketed	0.9408	0.9450	0.9437	0.9544	0.0173	Matrix	28092.2

Table no. 6 swelling index of trial batches and marketed formulation

SR	Visco	Viscosity of Trial batches (Cp)					
	F1	F2	F3	F4	F5	Marketed formulation	
5	371.5	227.5	314.4	427	402.7	450.9±1.272	
	± 1.202	±0.28	±0.28	.9	±0.91		
		2	4	±0.9 89	9		
10	227.1	184.7	267.8	355.	338.2	392.7±1.343	
	±0.56	±0.61	±0.84	25±1	±0.49		
	5	5	8	.20	4		
20	168.3	143.2	201.9	219.	216.2	334.8±0.424	
	±0.77	±0.63	±0.07	75±0	±1.27		
	7	6	0	.353			
30	114.9	106.0	144.5	158.	125.8	187.8±0.282	
	±0.14	±0.56	±1.06	05±0	±0.28		

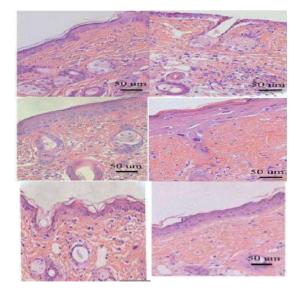




Fig 8 Formulation hydrogel (F4) before and after healing

CONCLUSION: According to the study, doxycycline hyclate- incorporated scleroglucan-based hydrogel is a potential material for wound dressings because it has improved antibacterial activity, biocompatibility, and sustained drug release. In comparison to a commercial formulation (98.26% in 5 hours), the hydrogel *Skin irritation test*- From the evaluation of skin irritation test it was clear that there was no any allergic response observed such as redness on skin, edema, swelling on skin observed.

Stability Study:

Doxycycline degraded at 40 °C accompanied by demonstrated excellent physicochemical features, such as appropriate rheological

behavior, swelling index, and extended drug release (88.9% over 8 hours) [8,9]. With notable contraction. increased wound collagen deposition, and re-epithelialization seen in histological analysis, the ex-vivo and in-vivo investigations validated its efficacy accelerating wound [4,5,13]. Furthermore, the hydrogel stayed stable under normal circumstances and displayed no symptoms of skin irritation [12,14]. These results imply that scleroglucan-based hydrogel may be a good substitute for traditional wound care solutions, offering better healing potential and regulated drug administration.[3,7,10].

REFERENCES:

- 1. Chauhan S., Harikumar SL. and Kanupriya, Hydrogels: A Smart Drug Delivery System, International Journal of Research In Pharmacy And Chemistry 2012, 2(3)
- 2. Hossein O. and Kinam P., Juergen S, Ronald S., and Michael A., Fundamentals and Applications of Controlled Release Drug Delivery, Eds. Spinger, New York, NY, 2012, pp. 75-106.
- 3. Mike F., Peter L., Evonik G., Essen, Germany S., Institute of Clinical Chemistry, Regensburg, Germany Stefan Müllner, Andreas Wattenberg, Protagen AG, "Skin-Omics": Use of Genomics, Proteomics and Lipidomics to Assess Effects of Low Molecular Weight Scleroglucan
- 4. Haritha K. Yadav J. Kumar R. Deshmukh G., Gujjula R. and Santhamma B., Wound Healing Activity of Topical Application of Aloe Vera Gel in Experimental Animal Models, International Journal of Pharma and Bio Sciences, June 2012 Vol 3/Issue 2
- Ana C., Kogawaand H., Regina N., Doxycycline Hyclate: A Review of Properties, Applications and Analytical Methods, International Journal of Life Sciences and Pharma Research, Oct-Dec 2012 Vol 2/Issue
- 6. Siddique A., Sonication Based Polysaccharide Hydrogel Hydrogels for Modified Drug Delivery System Systems, PhD Thesis University of la sapienza.
- 7. Monalisa P. and Nayak P., Natural Based

- Polysachharides for Controlled Drug Release, International Journal of Research and Reviews In Pharmacy and Applied Science 2013, Feb, 3(1) 72-117Bajpai S., Pathak V.,
- 8. Felicetta D., Amalia P., Francesca S., Rita P., Pasquale D., Nanospray technology for an in situ gelling nanoparticulate powder as a wound dressing, International Journal of Pharmaceutics 2014, 473.
- 9. Elbadawy A. Kamoun K., Tamer M., Mahmoud A., Mohamed S., Poly (vinyl alcohol)-alginate physically crosslinked hydrogel membranes for wound dressing applications: Characterization and bioevaluation, Arabian Journal of Chemistry, 2015.8
- 10. Silvana V., Osvaldo D., Lucia F., Julia F., Effects of thermal, alkaline and ultrasonic treatments on scleroglucan stability and flow behavior, Carbohydrate Polymers, Elsevier. 2013. 94.
- 11. Held M., Rahmanian S., Rothenberger J., Schiefer B., Janghorban E., Schaller H., Jaminet P., Alteration of biomechanical properties of burned skin, science direct, Elsevier, burns, 2015,41
- 12. Patrícia M., Ana A., Ricardo I., Correia J., Asymmetric membranes as ideal wound dressings: An overview on production methods, structure, properties and performance relationship, Journal of Membrane Science, 2015, 490
- 13. Eastburn S., Ousey P., Rippon M., A review of blisters caused by wound dressing components: can they impede post-operative rehabilitation and discharge,

- international journal of Orthopaedic and trauma nursing, 2015
- 14. Sedighehnazaripour, Shivkumar G., Richard W., Kathleen G., Song L., Controlled in situ formation of Polyacrylamide hydrogel on PET surface via SI-ARGET-ATRP for wound dressings, Applied Surface Science, September 2015, Volume 349, 15
- 15. Sandra G., Kazlauske J., Bobokalonov J., Stefano P., Victoria D., Liesiene J., Polina P., Characterization of cellulose based sponges for wound dressings, Colloids and Surfaces A: Physicochemical and Engineering Aspects, 5 September 2015, Volume 480,
- 16. Uyanga D., Mark R., Afeesh U., Arjun T., Batgerel T., Chan P., Cheol K., Fabrication and Characterization f Electrospunzein/Ag Nanocomposite Mats for Wound Dressing Mozhgan S., Fatemeh N., Sasan F., Rahim F., Mohammad M., Shahin B., Fabrication and characterization of ovalbumin films for wound dressing applications, Materials

Wound Healing and for Other Biomedical Applications" Wisconsin Alumni Research foundation.

- 21. www.warf.org
- 22. Yogesh T., Biswadeep D., Tania P., Deepali T., Kishori A., Pradeep P., Evaluation of Wound Healing Potential of Aqueous and Ethanolic Extracts of *Tridax Procumbens* Linn. In Wistar Rats, Asian Journal of Pharmaceutical and Clinical Research, 2012vol 5
- 23. Jamila P., Thais G., Jessica P., Luiz F., Luiz N., Daniel M., Topical application of Acheflan on rat skin injury accelerates wound healing: a histopathological, immune histochemical and biochemical study, BMC complementary and alternative medicine, 2015
- 24. James C., Wayne S., Michael W., Self- adhesive hydrogel wound dressing. US 5674523 A 1997
- 25. Wokalek; Heinrich , Hydrogel sheet wound dressings , W088/06894, 1998
- 26. Bruktawit T ., Spray hydrogel wound dressings, WO 2003063923 A1, Aug 2003
- 27. Edwin N., Michael H., Kevin M., Steve F., Wound Care Products with Peracid Compositions US 20130330397 A1,2013
- 28. Susan C., James G., Alginate hydrogel foam wound dressing US 4948575 A, 1990
- 29. Maria C., Celina P., Jose F., Fernando A., Comparative histological study on wound

- Science and Engineering, March 2015, Volume 48,
- 17. Do P., Hyun C., Hyoung Y., Thavasyappan T., Doo L., Polyurethane foam containing rhEGF as a dressing material for healing diabetic wounds: Synthesis,
 - characterization, *in vitro* and *in vivo* studies,Colloids and Surfaces B: Biointerfaces, Sep.2015, Vol.135,
- 18. Jae L., Young C., Jin L., Hyun K., Do P., Myung P., Raek Y., Ho L., and Yoshimitsu K., Preparation of wound dressing using hydrogel polyurethane foam, trends biomater artif. Organ, 2001, vol 15 (1)
- 19. Rozaini Z., Zuki Z., Norimah Y., Noordin M., and Muhammad A., "Gelam (Melaleuca spp.) Honey- Based Hydrogel as Burn Wound Dressing"Hindawi Publishing Corporation, 2012
- 20. Weiyuan K., Jing L., David L., Rathna G., "Multi-Functional Matrix to Promote
 - healing on rat's skin treated with Mitomycin C or Clobetasol propionate, Acta Cirurgica Brasileira 2015 Vol. 30 (9) 593
- 30. Ke M., Mindong D., Mingde L., Shihai C., Guoqian Y., Qingfeng L., Qiang W. and Gang Q., Evaluation of Wound Healing Effect of Punica Granatum L Peel Extract on Deep Second-Degree Burns in Rats, Tropical Journal Of Pharmaceutical Research January 2015; 14 (1)
- 31. http://tycohealthece.com/files/d0004/ty_h cyniv.jpg
- 32. Uday D., Rajesh N., Monal S., Development and Validation of Rp-
- 33. Hplc Method for Simultaneous Estimation of Doxycycline Hyclate and Tinidazole In Bulk and Tablet Dosage Form, International Journal of Pharmacy and Pharmaceutical Sciences, Vol 5, Issue 4, 2013
- 34. Syed G., Saphwan A. and Glyn P., Hydrogels: Methods of Preparation, Characterization and Applications, www.intechopen.com
- 35. Mohit V. and Frank G., 1, 3-beta-Glucans:Drug Delivery and Pharmacology, Intechopen science open mind, 2012
- 36. Pandurangan A., Khosa R. and Hemalatha S., Evaluation of wound healing activity of *Ichnocarpus frutescens* root, Scholars Research Library, 2010, 2(3)
- 37. Wojciech C., Alina K., Stanislaw B., Malcolm R.,

Shaikh et al

Brown Jr, Microbial cellulose the natural power to heal wounds, Biomaterials ELSEVIER, (2006),27

38. Tommasina C., Gina C., Antonio P., Mario G., Eleonora S., Franco A., Structural and

Bull. Pharm. Res. 2025;15(S2)

rheological characterization of Scleroglucan/boraxhydrogel for drug delivery, Biological macromolecule, Elsevier, 2003.

Bulletin of Pharmaceutical Research 2025;15(S2)

An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print) 2249-9245 (Online)

RESEARCH ARTICLE

HERBAL CHEWING GUM FOR TREATING MOUTH ULCERS

Padmaja Kore*, Ujwala Desai, Anuradha More, Santoshi Lashkare, Omkar Lokhande, Dhanashri Jamadade

PES's Modern College of Pharmacy, Sec. No. 21, Yamunanagar, Nigdi, Pune 411044.

*Corresponding author email:: padmaja.kalshetti@gmail.com

Received: Feb 06, 2025 / Revised: April 18, 2025 / Accepted: May 02, 2025

Abstract

Mouth ulcers are a common oral health concern that can cause pain and discomfort, often exacerbated by food intake and poor oral hygiene. To address these challenges, the present study focuses on developing herbal medicated chewing gum as a novel drug delivery system for treating mouth ulcers. The formulation was prepared using a polyvinyl acetate-based gum base, into which orange oil, cottonseed oil, and pumpkin seed oil were incorporated in an optimized 1:2:1 ratio due to their antimicrobial and healing properties. The gum was coated with sorbitol and glycerin to enhance stability and texture. Various physicochemical properties of chewing gum were evaluated, including elasticity, hardness, thickness, and stickiness, all meeting optimal standards. The antimicrobial efficacy of herbal chewing gum was tested against *Streptococcus viridans* using the cup plate method, demonstrating a maximum zone of inhibition of 29.3 ± 2 mm. *In vitro* release studies confirmed a gradual and sustained release of active ingredients over 20 minutes, ensuring prolonged therapeutic action. Additionally, stability studies over 90 days indicated that the gum maintained its physical properties and antimicrobial activity with minimal degradation.

Keywords: Herbal chewing gum, mouth ulcers, antimicrobial activity, orange oil, cottonseed oil, pumpkin seed oil, *Streptococcus viridans*.

How to cite this article: Padmaja Kore, Ujwala Desai, Anuradha More, Santoshi Lashkare, Omkar Lokhande, Dhanashri Jamadade. Herbal chewing gum for treating mouth ulcer. *Bulletin of Pharmaceutical Research*. 2025;15(S2): 163-169

Source of support: Nil Conflict of interest: None

1. Introduction

Mouth ulcers, also known as aphthous ulcers, are a prevalent oral condition affecting individuals of all ages. These lesions can range from mild discomfort to severe pain, often exacerbated by dietary intake and poor oral hygiene [1]. While most ulcers heal spontaneously within one to two weeks, recurrent or severe cases can significantly impact quality of life, necessitating effective therapeutic interventions [2].

The etiology of mouth ulcers is multifactorial, involving nutritional deficiencies (e.g., iron, vitamin B12, and vitamin C), mechanical trauma, stress, infections, and hormonal imbalances [3]. Current treatment modalities include antimicrobial pastes, corticosteroids, and analgesics, available in various forms such as

mouthwashes, gels, and sprays. However, these treatments often provide only.

symptomatic relief and may be associated with side effects such as mucosal irritation or systemic absorption [4].

Chewing gum has emerged as a promising drug delivery system due to its convenience, ease of use, and ability to stimulate saliva production, which aids in neutralizing oral acids and promoting ulcer healing [5]. Medicated chewing gums can deliver active ingredients directly to the oral cavity, providing localized and sustained therapeutic effects [6]. Furthermore, the incorporation of herbal ingredients offers a safer alternative to synthetic drugs, with fewer side

effects and enhanced patient compliance [7].

Herbal formulations have gained significant attention in recent years due to their natural origin and therapeutic efficacy. Phytoconstituents such as flavonoids, tannins, and essential oils exhibit anti-inflammatory, antimicrobial, and wound-healing properties, making them ideal candidates for oral ulcer treatment [8]. For instance, clove oil, licorice, and aloe vera have been traditionally used for their analgesic and anti-ulcerative effects [9].

This study aims to develop a herbal medicated chewing gum incorporating orange, cottonseed, and pumpkin seed oil, leveraging their antimicrobial and healing properties. The formulation is designed to sustain active ingredient release, ensuring prolonged therapeutic action while maintaining optimal physicochemical properties [10].

2. Materials and methods

The following figures are illustrative of particular examples for the method of the present study, are descriptive of some of the embodiments, and are not intended to limit the scope of the study.

2.1 Screening of oils:

- 1. Preparation of oil samples: Dilute each oil (orange, **cottonseed**, pumpkin seed oil) in ethanol to prepare a 10%(v/v) solution. Filter-sterilize the oil solutions using a 0.22 µm membrane filter.
- 2. Preparation of Microbial Culture: Inoculate Streptococcus viridans in nutrient broth and incubate at 37°C for 24 hours. Adjust the turbidity of the microbial suspension to match the 0.5 McFarland standard.
- 3. Antimicrobial Activity Testing (Cup Plate Method)

Pour MitisSalivarius agar into sterile petri plates and allow it to solidify. Spread 100 μ L of the standardized microbial suspension evenly over the surface of the agar plates using a sterile spreader. Create wells (6 mm diameter) in the agar plates using a sterile cork borer. Add 50 μ L of each oil solution (orange, cottonseed, pumpkin seed oil) into separate wells. Include a control well with ethanol. Incubate the plates at 37°C for 24 hours. Measure the diameter of the zone of

inhibition (in mm) around each well using a ruler or caliper. Record the results.

2.1.1 Screening of oil ratio: The procedure for screening the oil ratio was performed according to the method described in section 2.1.

2.2 Preparation of gum base for herbal chewing gum:

An accurately weighed quantity of polyvinyl acetate (2.4 gm) was dissolved in ethanol (15 ml) in a beaker. Lanoline (0.96 gm), paraffin wax (0.4 gm), and ester gum (1.44 gm) were melted at 40-60°C. Molten ingredients were added into a mortar pestle and mixed with high intensity. Talc (2.4g) was added during mixing, forming a solid gum base [11].

2.3 Evaluation of Solid gum base:

2.3.1 Physical appearance: The solid gum base was visually evaluated for physical appearance, color, and odor. The texture study was performed manually by pressing the gum between the thumb and finger. The texture felt sticky, good, or had a solid mass [12].



- **2.3.2 Solubility:** Solubility of the gum base was determined in water at $37 \pm 2^{\circ}$ C by keeping the gum in contact with water for 48 hours [13].
- **2.3.3 Elasticity study:** An analytical weighing balance was used to measure elasticity. One end of the chewing gum was stuck to the bottom of the analytical weighing balance, and the other end was stuck to a plane surface. The lower clamp was held stationary, and the upper clamp pulled apart the chewing gum until it broke, losing its elasticity. The weight required at this point was recorded [14].

2.4 Antimicrobial activity of gum base against *Streptococcus viridans*:

In vitro antimicrobial studies were performed against *Streptococcus viridans* in MitisSalivarius agar medium using the cup plate method. The bacterial suspension was spread on the surface of



Fig. 1. Herbal oil mixture incorporated chewing gum

plates with the help of a spreader. Required size bores (6 mm) were made using a cork borer. Samples were filled into the bores. The whole procedure was carried out in an aseptic laminar airflow chamber. The petri plates were placed into an incubator at 37°C, and microorganisms were allowed to grow for 24 hours. After 24 hours, the zone of inhibition was checked to determine the antibacterial activity of the gum base [15].

2.5 Incorporation of herbal edible oils in gum base:

The prepared gum base (2g) was melted at 60-80°C on a water bath. The selected combination of oils (orange oil, cottonseed oil, and pumpkin seed oil) in the ratio of 1:2:1, respectively, was mixed with the molten gum base, and talc powder (0.5g) was added simultaneously [16] (Figure 1).

2.6 Coating of Herbal Chewing Gum:

An accurately weighed quantity of sorbitol (5% w/v) and glycerin (20% w/v) was dissolved in water and heated at 60°C for 15 minutes. The solution formed was viscous. The medicated chewing gum was dipped into the solution for 1 minute, then dry talc powder was applied. This step was repeated 3 to 8 times, and the gum was dried at 27°C-38°C [17] (Figure 2).

Factors		Ratio			Zone
	Orange oil	Cottons eed oil	Pumpkin seed oil	species	of inhibit ion (mm)
F1	1	1	1	Streptococcus viridans	29 <u>+</u> 3
F2	2	1	1	Streptococcus viridans	27.5 <u>+</u> 3
F3	1	2	1	Streptococcus viridans	29.3 <u>+</u> 2

2.7 Evaluation of Herbal Chewing Gum:

- **2.7.1 Diameter and Thickness:** 10 medicated chewing gums were selected, and the diameter and thickness were measured using a Vernier caliper [18].
- **2.7.2 Test for Hardness/Plasticity:** 5 medicated chewing gums were selected, and hardness was measured using a Monsanto hardness tester^[19].
- **2.7.3 Stickiness:** The medicated chewing gum was placed on a plain surface, and a 110 gm pestle was placed on it for 10 minutes. The frequency of hammering was about 30/minute. After 10 minutes, sticking of the mass to the pestle surface was observed [20].
- **2.7.4 Elasticity:** Elasticity was measured using an analytical weighing balance, as described earlier

Fig. 2. Coated herbal chewing gum [21].

2.8 In vitro release study:

The *in vitro* drug release of herbal chewing gum during mastication was studied by creating mastication conditions in a mortar pestle. Phosphate buffer (pH 6.3) and herbal chewing gum were taken in a mortar. Using the pestle, mastication-like conditions were created. Samples were removed after 0, 2, 4, 6, 8, 10, 12, 15, 18, and 20 minutes. The release study of 8 samples was performed against *Streptococcus viridans*, and the zone of inhibition was measured [21].

2.8.1 Stability study: Stability studies were conducted according to ICH

guidelines to assess the combined effect of oils, gum base, and excipients on the stability of the formulation. The optimized formulation was placed in vials and stored at $30^{\circ}\text{C} \pm 2^{\circ}\text{C}/65\%$ RH \pm 5% RH. The samples were evaluated for physical appearance, elasticity, and microbial growth after 30, 60, and 90 days [20] **(Table 1).**

3. Result:

Table 1 Evaluation of gum base

Parameters	Reported	Observed
Physical appearance	 Soft Chewy Reduced tackiness Insoluble 	1) Soft 2) Chewy 3) Reduced tackiness 4) Insoluble
Elasticity study	7 to 8 cm	7.5 cm

Table 2 Antimicrobial activity of gum base

Based on the above result in table 2, it was concluded that the gum base does not show antibacterial or antimicrobial activity

Evaluation of anti-microbial and anti-bacterial activity of orange oil, cottonseed oil and pumpkin seed oil: Antimicrobial and antibacterial activity was checked by cup plate method

Table 3: Antimicrobial activity was against streptococcus viridans

Sr. No.	Name of Oil	Bacterial Species	Zone of Inhibition (mm)
1)	Orange oil	Streptococcus viridans	23 <u>+</u> 1

2)	Cottonseed oil	Streptococcus viridans	25 <u>+</u> 1
3)	Pumpkin seed oil	Streptococcus viridans	24 <u>+</u> 2

Table 4: Antibacterial activity against *streptococcus viridans*

Since the results in table 4 above, it was concluded that the F3 ratio of 1:2:1 shows better zone of inhibition as compared to other factors. Hence a factor 3was selected for the further formulation of herbal chewing gum.

Table 5: Evaluation of herbal chewing Gum

From table 5, it was concluded that the final chewing gum formed after incorporation of oils showed optimum hardness, elasticity and stickiness property.

3.1 Antimicrobial activity

The antimicrobial activity of medicated chewing gum was checked against *Streptococcus viridans* using Mitis Salivarius agar culture respectively as culture media by cup plate method as indicated in Table 6, using Nutrient agar, Brain heart infusion media and Muller Hinton agar culture respectively

Sample	Fungus species	Zone of inhibition (mm)
Sample 1	Streptococcus viridans	0

as culture media by cup plate method. The incubation was carried out at 25°C for 24 hours

Table 6: Antimicrobial activity against Streptococcus viridans

Micro organism	Zone of inhibition
Streptococcus viridans	23 <u>+</u> 2 mm

3.2 In vitro release study

Unlocking Horizons in Global Multidisciplinary Healthcare Research" Indo-US International Conference Date: 10-12, March 2025 Organized by PES Modern college of Pharmacy Nigdi Pune-44

Table 7: In vitro release study

Samples	Time interval	Zone of inhibition
S1	0 min.	0 mm
S2	3 min.	10 <u>+</u> 2 mm
S3	6 min.	11 <u>+</u> 1 mm
S4	9 min.	13 <u>+</u> 2 mm
S5	12 min.	16 <u>+</u> 3 mm
S6	15 min.	20 <u>+</u> 3 mm
S7	18 min.	23 <u>+ 1</u> mm
S8	20 min.	24 <u>+</u> 3 mm

From the zone of inhibition in Table 7 above, it was concluded that the oils released from chewing gum up to 20 minutes. After 20 minutes, there was no increase in the zone of inhibition indicating no further release of oils.

3.3 Stability study

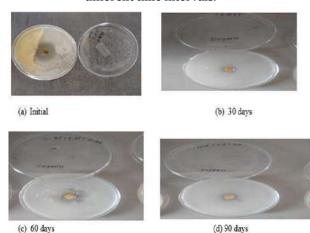
Table 8: Physical characterization of medicated chewing gum at different time intervals

Parameters	Initial	After 30 days	After 60 days	After 90 days
Physical appearance	1) Soft 2) Chewy	1) Soft 2) Chewy	1) Soft 2) Chewy	1) Soft 2) Chewy
	3)Reduced tackiness 4)Insoluble	3) Reduced tackiness 4)Insoluble	3) Reduced tackiness 4) Insoluble	3) Reduced tackiness 4) Insoluble
Elasticity study	6.9 cm	7.2 cm	7.4 cm	7.6 cm

Table 9: Zone of inhibition of medicated chewing gum at different time intervals

Sr. No.	Time interval	Zone of inhibition
1	Initial	28 <u>+</u> 1 mm
2	30 days	27 <u>+</u> 2 mm
3	60 days	26 <u>+</u> 1 mm
4	90 days	25 <u>+</u> 2 mm

Figure 3. Antimicrobial activity of formulation at different time intervals.



The formulation was found to be stable retaining its physical properties and antimicrobial property for up to 90 days (Table 8, 9 and Figure 3).

Discussion:

The present study successfully formulated a herbal medicated chewing gum with significant antimicrobial and wound-healing properties. The incorporation of orange oil, cottonseed oil, and pumpkin seed oil in a 1:2:1 ratio demonstrated superior antimicrobial activity against $Streptococcus\ viridans$, with a zone of inhibition of 29.3 ± 2 mm. This finding aligns with previous studies highlighting the antimicrobial efficacy of essential oils in oral care products [10].

The sustained release of active ingredients over 20 minutes ensures prolonged therapeutic action, addressing the limitations of conventional treatments that require frequent application [5]. Furthermore, the stability studies confirmed that the formulation retained its physical and antimicrobial properties for up to 90 days, making it a viable option for long-term storage and use [21].

The use of herbal ingredients in this formulation offers several advantages, including reduced side effects, enhanced patient compliance, and improved oral health outcomes [7]. The formulation also addresses the growing demand for natural and sustainable healthcare products, aligning with global trends in personalized and preventive medicine [8].

Conclusion

The study demonstrates the potential of herbal medicated chewing gum as an effective and convenient treatment for mouth ulcers. The formulation combines the therapeutic benefits of herbal oils with the advantages of a chewing gumbased drug delivery system, providing localized and sustained relief. Further clinical studies are warranted to validate its efficacy and safety in real-world settings.

REFERENCES

- Scully C, Porter S. Oral mucosal disease: Recurrent aphthous stomatitis. Br. J. Oral Maxillofac. Surg. Journal of Oral and Maxillofacial Surgery: 2003;41(5):309-20.
- 2. Altenburg A, El-Haj N, Micheli C, Puttkammer M, Abdel-Naser MB, Zouboulis CC. The treatment of chronic recurrent oral aphthous ulcers. Dtsch Arztebl Int. 2014 Oct 3;111(40):665-73. *PubMed Central*; DOI: 10.3238/arztebl.2014.0665.
- 3. Porter SR, Scully C, Pedersen A. Recurrent aphthous stomatitis. Crit Rev Oral Biol Med. 1998;9(3):306-21. *PubMed Central;* DOI: 10.1177/10454411980090030401.
- 4. Brocklehurst, P., Tickle, M., Glenny, A. M., Lewis, M. A., Pemberton, M. N., Taylor, J., Walsh, T., Riley, P., & Yates, J. M. (2012). Systemic interventions for recurrent aphthous stomatitis (mouth ulcers). *Cochrane Database of*

Systematic Reviews, 12(9), *CD005411.* https://doi.org/10.1002/14651858. CD005411.pub2

- 5. Wessel SW, van der Mei HC, Morando D, Slomp AM, van de Belt-Gritter B, Maitra A, Busscher HJ. Quantification and qualification of bacteria trapped in chewed gum. PLoS One. 2015 Jan 20;10(1):e0117191. *PubMed Central;* DOI: 10.1371/journal.pone.0117191.
- 6. Banakar M, Moayedi S, Shamsoddin E, Vahedi Z, Banakar MH, Mousavi SM, Rokaya D, Bagheri Lankarani K. Chewing Gums as a Drug Delivery Approach for Oral Health. Int J Dent. 2022 Jun 20;2022:9430988. *PubMed Central;* DOI: 10.1155/2022/9430988.
- 7. Patel VR, Agrawal YK. Nanosuspension: An approach to enhance solubility of drugs. J Adv Pharm Technol Res. 2011 Apr;2(2):81-7. *PubMed Central;* DOI: 10.4103/2231-4040.82950.
- 8. Gupta D, Bhaskar DJ, Gupta RK, Karim B, Jain A, Singh R, Karim W. A randomized controlled clinical trial of Ocimum sanctum and chlorhexidine mouthwash on dental plaque and gingival inflammation. J Ayurveda Integr Med. 2014 Apr;5(2):109-16. *PubMed Central;* DOI: 10.4103/0975-9476.131727.
- 9. Sánchez M, González-Burgos E, Iglesias I, Gómez-Serranillos MP. Pharmacological Update Properties of *Aloe Vera* and its Major Active Constituents. Molecules. 2020 Mar 13;25(6):1324. *PubMed Central;* DOI: 10.3390/molecules25061324.
- 10. Carson CF, Hammer KA, Riley TV. Melaleuca alternifolia (Tea Tree) oil: a review of antimicrobial and other medicinal properties. Clin Microbiol Rev. 2006 Jan;19(1):50-62. *PubMed Central;* DOI: 10.1128/CMR.19.1.50-62.2006.
- 11. Akbarzadeh A, Rezaei-Sadabady R, Davaran S, Joo SW, Zarghami N, Hanifehpour Y, Samiei M, Kouhi M, Nejati-Koshki K. Liposome: classification, preparation, and applications. Nanoscale Res Lett. 2013 Feb 22;8(1):102. *PubMed Central;* DOI: 10.1186/1556-276X-8-102.
- 12. Mozafari MR. Nanoliposomes: preparation and analysis. Methods Mol Biol. 2010;605:29-50.

Unlocking Horizons in Global Multidisciplinary Healthcare Research" Indo-US International Conference Date: 10-12, March 2025 Organized by PES Modern college of Pharmacy Nigdi Pune-44

PubMed Central; DOI: 10.1007/978-1-60327-360-2 2.

- 13. Pattni BS, Chupin VV, Torchilin VP. New Developments in Liposomal Drug Delivery. Chem Rev. 2015 Oct 14;115(19):10938-66. *PubMed Central;* DOI: 10.1021/acs.chemrev.5b00046.
- 14. Rahman A, Uahengo V, Likius D. Mini review on emerging methods of preparation of liposome and its application as liposome drug delivery systems. *Open Journal of Pharmacology and Pharmacotherapeutics*. 2018;3(1):5-21.
- 15. Zylberberg C, Matosevic S. Pharmaceutical liposomal drug delivery: a review of new delivery systems and a look at the regulatory landscape. Drug Deliv. 2016 Nov;23(9):3319-3329. *PubMed Central;* DOI: 10.1080/10717544.2016.1177136.
- 16. Bárcena C, Sra AK, Gao J. Applications of magnetic nanoparticles in biomedicine. In: Nanoscale Magnetic Materials and Applications. *Springer*: 2009;591-626.
- 17. Namvari M, Namazi H. Clicking graphene oxide and Fe3O4 nanoparticles together: An efficient adsorbent to remove dyes from aqueous solutions. *International Journal of Environmental Science and Technology*. 2014;11(6):1527-36.
- 18. Filipe V, Hawe A, Jiskoot W. Critical evaluation of nanoparticle tracking analysis (NTA) by NanoSight for the measurement of nanoparticles and protein aggregates. *Pharm. Res.* 2010;27(5):796-810.
- 19. Majidi S, ZeinaliSehrig F, Farkhani SM, SoleymaniGoloujeh M, Akbarzadeh A. Current methods for synthesis of magnetic nanoparticles. *Artif. Cells Nanomed. Biotechnol.* 2016;44(2):722-34.
- 20. Reshetov V, Zorin V, Siupa A, D'Hallewin MA, Guillemin F, Bezdetnaya L. Interaction of liposomal formulations of metatetra(hydroxyphenyl)chlorin (temoporfin) with serum proteins: protein binding and liposome destruction. *Photochem Photobiol*. 2012 Sep-

- Oct;88(5):1256-64. doi: 10.1111/j.1751-1097.2012.01176.
- 21. Saveyn H, De Baets B, Thas O, Hole P, Smith J, Van der Meeren P. Accurate particle size distribution determination by nanoparticle tracking analysis based on 2-D Brownian dynamics simulation. *J. Colloid Interface Sci.* 2010;352(2):593-600.

An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print) 2249-9245 (Online)



RESEARCH ARTICLE

SIMULTANEOUS ESTIMATION OF PAZOPANIB AND SILYMARIN: METHOD DEVELOPMENT AND VALIDATION

Anuradha G. More, Dhanashri P. Garud*

^{1*} Department of Pharmaceutics, PES's Modern College of Pharmacy, Nigdi, Pune, Maharashtra.

Dhanashri Pandurang Garud

*E-mail: dhanashrigarud14@gmail.com
Received: Feb 06, 2025 / Revised: April 18, 2025 / Accepted: May 21, 2025

ABSTRACT

A UV spectrophotometric method was accomplished for the simultaneous measurement of Pazopanib and Silymarin in both their combination and pure forms. The development and validation procedure for the method adhered to ICH requirements, assuring precise and reliable findings. Range and linearity, accuracy, precision, and durability are among the important factors that are evaluated. Both drugs demonstrated high linearity with correlation value above 0.99. Precision tests showed minimal intra- and inter-day variation, confirming the method's reproducibility. Recovery studies revealed values close to 100%, supporting the accuracy of the method. A thorough literature review found that no existing method for the simultaneous measurement of Pazopanib and Silymarin using UV spectrophotometry was reported, highlighting the novelty of this approach. This UV spectrophotometric approach offers a dependable and efficient solution for the determination of both drugs in pure forms and pharmaceutical formulation simultaneously, making it suitable for routine quality control and analytical purposes.

Keywords: Pazopanib, Silymarin, Simultaneous Estimation, Validation

How to cite this article: Anuradha G. More, Dhanashri P. Garud*simultaneous estimation of pazopanib and silvmarin: method development and validation. Bulletin of Pharmaceutical Research. Pg no 170-176

Source of support: Nil Conflict of interest: None

INTRODUCTION

Pazopanib (PZB) is a small molecule that functions as a tyrosine kinase inhibitor. Primarily, it is used to treat kidney cancer, also referred as renal cell carcinoma and soft tissue sarcoma. It exerts by targeting various receptor such as tyrosine kinase receptors (VEGFR, PDGFR) and c-kit. Both receptors play significant roles in tumor cancerous growth, blood vessel formation (angiogenesis), and advancement of the tumor, makes it an effective candidate for targeting tumor growth, angiogenesis, and metastasis in various types of cancer [1-3].

Silymarin (SLM) is a flavonoid mixture. primarily extracted from the seeds of Silybum marinum, commonly referred to as milk thistle silicristin, which together offer strong antioxidant, anti-inflammatory effects.

Additionally, ongoing studies are investigating its potential benefits in cancer prevention, diabetes management, and cardiovascular health [5-7]

The analytical technique for PZB, SLM, and their combination was developed and validated using UV spectrophotometry in buffer solution with a pH 1.2. The validation process followed ICH guidelines, confirming the method's specificity, linearity, and stability across the tested pH levels, with pH 1.2 proving to be optimal for all analytical parameters. The objective of this research is to create and verify a reliable UV spectrophotometric technique

requirements, including linearity, specificity, accuracy, and precision, in compliance with regulatory standards. There is currently no published UV spectrophotometric method for measuring PZB and SLM simultaneously in medication formulation. This work aims to create and validate a straightforward UV spectrophotometric technique for the simultaneous determination of PZB and SLM in order to ensure rapid, precise and accurate analysis in a single formulation.

MATERIALS AND METHODS

Instruments: A Shimadzu UV- 1800- UV spectrophometer, a digital balance, a pH meter, an ultrasonicatior, volumetric flasks, and borosilicate glass pipettes were used for the proposed analytical method.

Materials and Reagents: Pazopanib (PZB) and Silymarin (SLM) were purchased from Dhamtech Pharm., Mumbai and Yucca chemicals, Mumbai, respectively. Other chemicals or reagents utilized were of analytical quality. Distilled water was used in the preparation of reagent solutions.

Preparation of standard stock solution and calibration curve of PZB and SLM

The standard stock solutions of PZB and SLM were obtained by adding 10 mg of each drug in pH 1.2 buffer solvent, and the final volume was adjusted to 100 ml of a volumetric flask to get a solution with concentration of 100µg/ml of PZB and SLM drug. Working stock solutions of both drugs was established from prepared stock solution, in the range of 2-10µg/ml for PZB and 5-25µg/ml for SLM. The absorption maxima (λmax) were found by scanning a UV spectrophotometer with a solution containing 10μg/ml, in the 200-400 nm range. A calibration curves were constructed using absorbance units on concentration (in µg/ml) on the X-axis and Y- axis to study the Beer-Lambert's Law and regression equation for PZB and SLM.

Absorbance additive study: Working standard solutions of each drug and their mixture (1:1)

were measured for absorbance at certain wavelengths, namely 262 nm and 286 nm, in order to provide absorbance additive data. *Absorptivity* was determined by measuring the absorbances of the solutions at the chosen wavelength, which correspond to the absorption maxima of PZB and SLM and concentration (g/100ml). Absorptivity formula was used:

A(1%, 1cm) = Abs/Conc.

Simultaneous equation method development

PZB and SLM concentrations are calculated using simultaneous equation approach, which is based on their absorbance at two particular wavelengths: 262 nm for PZB and 286 nm for SLM. By applying the Beer-Lambert law, the absorbance values at these wavelengths are used to construct a system of equations that allow for the quantification of both drugs in a mixture. The concentrations of the drugs, Cx for PZB and Cy for SLM, can be determined using the following formulas (Table 2):

$$Cx = \frac{A2ay1 - A1ay2}{ax2ay1 - ax1ay2}$$
 $Cy = \frac{A1ax2 - A2ax1}{ax2ay1 - ax1ay2}$

In the given equations, A1 and A2 represent the absorbance values measured at wavelengths 262 nm and 286 nm. ax1 and ax2 refer to the molar absorptivity of PZB at 262 and 286 n, while ay1 and ay2 are the molar absorptivity of SLM at 262 and 286 nm.

Validation parameters

In compliance with the ICH requirements, the suggested analytical procedure was validated. In order to assure the stability and dependability of the analytical method, this comprised assessments for linearity, Range, Precision, Sensitivity, Limit of detection and quantification (LOD and LOQ). [9,10]

Linearity and Range: The various effective concentrations i.e., 2 to $10\mu g/ml$ for PZB, while 5 to $25\mu g/ml$ for SLM were assayed as per developed methods and followed Beer-Lambert's law.

Precision: The response for both PZB and SLM was measured three times on the same days

(with time interval) in order to evaluate intraday precision. The response was measured over three days with aim to calculate interday precision. The results were reported % RSD in table 6. Ruggedness study was carried out three times in the lab under various circumstances, such as utilizing different analyst with the same equipment (Table 6).

Limit of quantification and detection (LOQ and LOD): The calibration curve standards (slop "s" and standard deviation of Y-intercept response "\(\textit{\textit{2}} \)") were used to calculate the values of limit of detection and quantification for PZB and SLM. According to ICH recommendations, these values were computed using the signal to noise ratio (S/N), where the LOD is equivalent to S/N ratio of 3.3 and LOQ to an S/N ratio of 10. The following formulas were employed:

Sensitivity: Sensitivity of drug viz PZB and SLM were separately determined by sandell's sensitivity parameter (μ g/cm/0.001 absorbance unit in 1 square cm) to evaluate the minimum amount of substance which can be detected or quantified in column of unit cross section (Table 5).

Accuracy: The standard addition method was used to determine the accuracy of recovery investigation at various different levels of 80%, 100%, 120%. The pre-analysed sample solution of PZB, SLM and standard drug solutions were added, absorbances were measured at 262 nm and 286 nm (λ max of PZB and SLM, respectively), and the percent of drug content was determined. The % recoveries of the

mixture were analyzed and % RSD studies were reported at each concentration level (as shown in table 7).

RESULT AND DISCUSSION

The UV spectrophotometric approach was developed and found to be sensitive, accurate, straightforward, and economical. In compliance with ICH guidelines, it has been validated (Table 3). For method development, two absorbance maxima at 262 nm and 286 nm were chosen, where Pazopanib (PZB) and Silvmarin (SLM) exhibited a linear relationship between concentration and absorbance (Figure 1). The concentration ranges of 2-10µg/ml and 5-25µg/ml had correlation values (r²) of 0.9958 and 0.9995, respectively (Table 1 and Figure 2& 3). The concentration of PZB and SLM from the synthetic mixture were calculated using simultaneous equation method. Absorbance was measured at 262 nm (λ 1) and 286 nm (λ2) and molar absorptivity values were determined at 262 nm (λ 1) and 286 nm (λ 2) for the PZB and SLM, which were 0.059 (ax1), 0.0504 (ax2) and 0.0085 (ay1) and 0.0158 (ay2), respectively. (Table 2). The regression analysis's r² values confirms the linearity of the calibration curve, with higher r² values indicating improved precision and confirming that the developed method shows satisfactory linearity. Moreover, the %RSD values in all precision study parameters, which were below 2%, fall within the acceptable range defined by ICH guidelines (table 4), indicating the method's excellent accuracy, precision, and repeatability/ reproducibility. The recovery analysis (table 5), which involved adding PZB and SLM standard solutions, showed no significant changes in the spectra, further validating the method's reliability.

Table 1: Calibration curve absorbance readings

Sr. No.	Conc.	PZB Absorbance	Conc.	SLM Absorbance at 286 nm
	(µg/ml) of PZB	at 262 nm	(μg/ml) of SLM	
1	2	0.122	5	0.085
2	4	0.224	10	0.158
3	6	0.332	15	0.234
4	8	0.467	20	0.309

5	10	0.6	25	0.376

Table 2: Analysis of Laboratory synthetic mixture by the proposed method

Sr. No.	Amount of drug assay (µg/ml)	_		Percent drug estimated (%)		
	Pazopanib	Silymarin	Pazopanib	Silymarin	Pazopanib	Silymarin
1	10	10	10.2	9.8	102	98.0
2	10	10	9.91	9.98	99.1	99.8
3	10	10	9.97	10.11	99.7	101.1
4	10	10	10.21	10.1	102.1	101.0
5	10	10	9.98	10.12	99.8	101.2
Mean					100.54	100.22
±SD				1.40	1.36	
		%RSD			1.39	1.36

Table 3: Validation Parameters for Pazopanib and Silymarin estimation

Parameter	Pazopanib HCL	Silymarin
λmax	262 nm	286 nm
Linearity Range	2-10 μg/ml	5-25 μg/ml
Linearity equation	y = 0.06x - 0.0107	y = 0.0147x + 0.0125
Correlation coefficient (R ²)	0.9958	0.9995
Slope	0.06	0.014
Intercept	0.0107	0.0125
Molar Absorptivity (10 μg/ml)	ax1- 0.059 (262 nm) & ax2- 0.0504 (286 nm)	ay1- 0.0085 (262 nm) and ay2- 0.0158 (286 nm)
LOD	0.122 μg/ml	0.358 μg/ml
LOQ	0.37 μg/ml	1.03 μg/ml
Sensitivity	6.0*10 ⁻⁶ µg/cm2	1.58*10-6μg/cm2

^{*0.002217} and 0.001528 are the standard deviation used for LOD and LOQ calculation for PZB and SLM, respectively.

Table 4: Interday and Intraday Precision

	Intraday Precision		Interday Precision		Different Analyst	
Drug	% Amount found ± SD*	%RSD	% Amount found ± SD*	%RSD	% Amount found ± SD*	%RSD
Pazopanib	99.03 ±	0.434	101.65 ±	0.926	101.33 ± 0.6	0.597
	0.43		0.94			
Silymarin	102.61 ± 0.65	0.636	102.17 ± 1.36	1.33	102.83 ± 0.53	0.518

Concentration of the drug added to the formulation	Pazopanib % Recovery ± SD	%RSD	Silymarin % Recovery ±SD	%RSD
80%	99.33 ± 0.042	0.472981	99.85 ± 0.021	0.117
100%	99.48 ± 0.077	0.773563	98.69 ± 0.190	0.961
120%	100.69 ± 0.049	0.44855	99.82 ± 0.028	0.128

Table 5: Recovery Study of Pazopanib and Silymarin

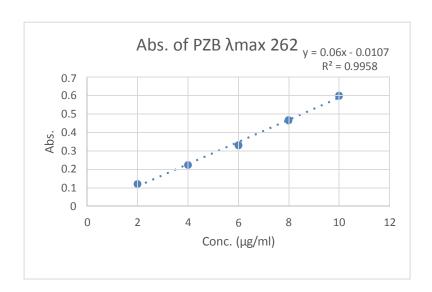


Fig. 2: Calibration curve of PZB

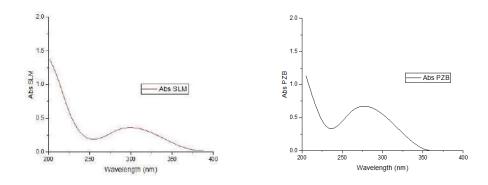


Fig. 1 A) Maximum absorption of Silymarin at 286 nm B) Pazopanib at 262 nm

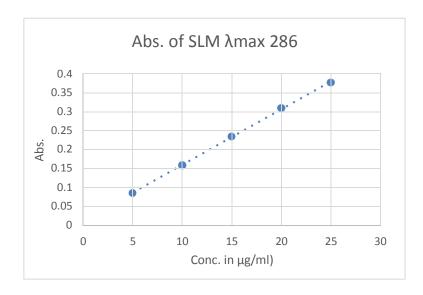


Fig. 3: Calibration curve of SLM

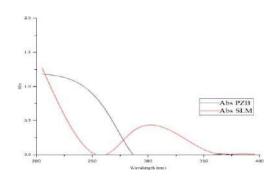


Fig. 4: Overlay Spectra of PZB and SLM (Isoabsorptive point at 277 nm)

CONCLUSION

The amounts of Pazopanib and Silymarin were determined by applying the method's specific equations. To assess the method's performance, the standard derivations and coefficient of variation computed. The method's accuracy, consistency, and dependability were demonstrated by the tiny standard deviations and percentage RSD values, which were less than 2%. Additional recovery experiments supported the method's reliability and demonstrated its resistance to interference from common substances. conclusion, the method developed is efficient, precise,

sensitive, and reliable. The results from analysing a synthetic mixture of both Pazopanib and Silymarin indicate that this approach is effective for their simultaneous quantification, with minimal interference from the usual excipients found in pharmaceutical products. The solvents used in the procedure are both affordable and simple to prepare. As a result, this method is well-suited for quality control applications in commercial pharmaceutical formulations.

REFERNCES

1. Miyamoto S, Kakutani S, Sato Y, Hanashi A, Kinoshita Y, Ishikawa A. Drug review: pazopanib. *Japanese Journal of Clinical Oncology.* 2018 Jun; 48(6):503-13. https://doi.org/10.1093/jjco/hyy053.

Bull. Pharm. Res. 2025;15(S2)

2. Mederle AL, Stana LG, Ilie AC, Borza C, Streian CG, Nistor D, Cerbulescu T, Belovan B, Lascu A. Efficacy and Safety of Pazopanib in the Treatment of Thyroid Cancer: A Systematic Review. *Biomedicines*. 2024; 12(12):2820. https://doi.org/10.3390/biomedicines12122 820

- 3. Mishra R, Devi A. Spectrophotometric method development and validation for determination of pazopanib in the bulk and the formulation. *Research Journal of Pharmacy and Technology.* 2023;16(8):3633-7.doi: 10.52711/0974-360X.2023.00598.
- 4. Gholse YN, Chaple DR, Kasliwal RH. Development and validation of novel analytical simultaneous estimation based UV spectrophotometric method for doxycycline and levofloxacin determination. *App. Sci.* 2022;12(4):5458-78.
- 5. García-Muñoz AM, Victoria-Montesinos D, Ballester P, Cerdá B, Zafrilla P. A descriptive review of the antioxidant effects and mechanisms of action of berberine and silymarin. *Molecules*. 2024 Sep 26; 29(19):4576. https://doi.org/10.3390/molecules29194576
- 6. Baug A. Validated method for silymarin by spectrophotometry in bulk drug and pharmaceutical formulations. *J. Chem.* 2010; 2(1):396-400.
- 7. Chaudhari S, Mannan A, Daswadkar S. Development and validation of UV spectrophotometric method for simultaneous estimation of Acyclovir and Silymarin in niosome formulation. *Der. Pharm. Lett.* 2016; 8:128-33.
- 8. Walfish S. Analytical methods: a statistical perspective on the ICH Q2A and Q2B guidelines for validation of analytical methods. *BioPharm International*. 2006 Dec 1; 19(12):1-6.
- 9. Guideline IH. Validation of analytical procedures Q2 (R2). ICH: Geneva, Switzerland. 2022.
- *10.* Kamal AH, El-Malla SF, Hammad SF. A review on UV spectrophotometric methods for simultaneous multicomponent analysis. *Eur J*

Pharm Med Res. 2016 Jan 27;3(2):348-60.https://doi.org/10.1071/SR02137

An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print) 2249-9245 (Online)

RESEARCH ARTICLE



DEVELOPMENT AND EVALUATION OF A SPIRONOLACTONE ORAL SUSPENSION.

Madhugandha S Kawadiwale¹, Mahesh M Bhadgale², Vaishnavi M Jambhale¹, Smita M Warade¹, Karimunnisa S Shaikh ^{1*}

¹PES's Modern College of Pharmacy, Sec. No. 21, Yamunanagar, Nigdi, Pune 411044. ²Callidus Research Laboratories, Pvt. Ltd., Chakan, Pune, 410501.

*Corresponding author email: <u>karima78@rediffmail.com</u> Received: Feb 06, 2025 / Revised: April 18, 2025 / Accepted: May 11, 2025

Abstract: -

This study aimed to develop a patient-friendly liquid form of spironolactone oral suspension, especially for children and older adults who may have difficulty swallowing tablets. The objective was to develop a stable and effective 25 mg/5 ml formulation using a structured development method called Quality by Design (QbD) with Design Expert software for optimization. A series of 15 planned experiments was applied to optimize key formulation factors, specifically xanthan gum and glycerine concentrations. The ideal amounts were determined as 3.6 mg of xanthan gum and 100 mg of glycerine. The relationship between these ingredients and outcomes, including drug release (similarity factor f2) and liquid thickness (viscosity), was analyzed. The optimized formulation was compared with the reference product and marketed tablet through laboratory-based tests. The use of this design method helped save time and resources compared to testing one factor at a time, demonstrating its value in creating a reliable and practical suspension for pediatric and geriatric use.

Key words: Spironolactone, Generic, Design of experiment (DOE), Multilevel design, Analytical method validation.

How to cite this article: Madhugandha S Kawadiwale, Mahesh M Bhadgale, Vaishnavi M Jambhale, Smita M Warade, Karimunnisa S Shaikh Development and evaluation of a spironolactone oral suspension Bulletin of Pharmaceutical Research. 2025;15(S2): 177-184.

Source of support: Nil Conflict of interest: None

INTRODUCTION

Spironolactone is a potassium-sparing diuretic and antihypertensive that blocks aldosterone receptors, preventing potassium loss and reducing sodium absorption [1].

Spironolactone treats heart failure (NYHA Class III-IV), hypertension, and edema, with doses ranging from 20–75 mg daily, improving survival and reducing hospitalizations.

The oral route is preferred for its convenience,

This study formulates a stable 25 mg/5 mL spironolactone suspension using QbD for improved hyperaldosteronism dosing [7]. A QbD-based multilevel factorial design successfully optimized spironolactone suspension for pH, viscosity, in-vitro study, assay, impurities, and stability.

MATERIALS AND METHOD:

Materials:

Spironolactone was gifted by Tianjin Jinjin Pharmaceuticals, China, while citric acid, sodium citrate, potassium sorbate, and glycerine were sourced from Brentag Ingredients, Ahmedabad. Xanthan gum was purchased from CP Kelco, Mumbai. Sorbic acid was obtained from Finar Chemicals while Simethicone emulsion from DOW Pharmaceuticals, US. Banana flavour was procured from Ferminich, Geneve while Magnasweet 110 from Magnasweet, USA, Sodiumcompliance, and with immediateflexible dosing. formulations improving bioavailability and efficacy [2,3,4]. In India, liquid spironolactone suspension provides a stable, ready-to-use alternative with easier dosing, reducing microbial contamination risks from compounded formulations [5]. Solid spironolactone dosage forms are unsuitable for infants and the elderly in hyperaldosteronism manageme^[6]

saccharine was obtained from JMC Corporation.

Methods:

Preformulation Study:

Organoleptic Properties:

Spironolactone was evaluated for its organoleptic properties like appearance, colour and odour by physical observation.

FTIR Analysis

The IR study of spironolactone was

conducted using FTIR spectroscopy (FTIR4100, Jasco) with potassium bromide (KBr) as a blank. Samples were mixed with KBr (300:1 ratio), scanned over 400– 4000 cm⁻¹, and analyzed using Spectra Manager software.

Drug-Excipients Compatibility (DEC) Study

Drug-excipient compatibility was studied at 37°C for 30 days, storing spironolactone with excipients in sealed vials under controlled conditions. A citric acid-sodium citrate-glycerine base solution was used, with results summarized in Table 1.

Table 1. Results of Drug Excipients Compatibility Study

Sr. No.	Drug & Drug- Excipients blend	Ratio (mg)	Initial	40±2°C/75±5 %RH Open exposure Condition (30 days)	60±2°C Open exposure Condition (30 days)
I	Spironolactone API	50 mg	White coloured	No change	No change
II	API: Glycerin	50 mg : 2000 mg	Colorless to white dispersion	No change	No change
III	API: water	50 mg : 10 ml	Colorless to white dispersion	No change	No change
IV	API in glycerin and water	50 mg : 2000 mg : 10ml	Colorless to white dispersion	No change	No change
V	glycerin and water, pH adjusted with citric acid and sodium citrate dihydrate (base	50 mg : 10 ml	Colorless to white dispersion	No change	No change

	solution)					
VI	API: Base solution: sorbic acid	50 mg :10 ml : 20 mg	Colorless t dispersion	to white	No change	No change
VII	API: Base solution: potassium sorbate	50 mg : 10 ml : 60 mg	Colorless t dispersion	to white	No change	No change

VIII	API: Base solution : potassium	50 mg : 10 ml : 20 mg : 60 mg	Colorless to white dispersion	No change	No change
	sorbate + sorbic acid				
IX	API: Base solution : simethicone emulsion	50 mg : 10 ml : 180 mg	Colorless to white dispersion	No change	No change
X	API: Base solution : xanthan gum	50 mg : 10 ml : 100 mg	Colorless to white dispersion	No change	No change
XI	API: Base solution : Magnasweet	50 mg : 10 ml : 500 mg	Colorless to white dispersion	No change	No change
XII	API: Base solution : sodium saccharin	50 mg : 10 ml : 250 mg	Colorless to white dispersion	No change	No change
XIII	API: Base solution : banana flavour	50 mg : 10 ml : 250 mg	Colorless to white dispersion	No change	No change
XIV	Composite sample of Ingredients	50 mg : q.s	White colored suspension with banana flavour	No change	No change

Validation of UV Spectrophotometric Method for Estimation of Spironolactone

In the present work UV Spectroscopy was used to determine the concentration of spironolactone. Absorbances were recorded on a Shimadzu UV1800 spectrophotometer at 242 nm using 0.1N HCl as the solvent.

Preparation of stock solution

A 25 mg spironolactone sample was dissolved in ethanol, sonicated for 10 minutes, and diluted to 100 ml (250 ppm). From this, 5 ml was further diluted to 100 ml with 0.1 N HCl (25 ppm) and used for method validation.

Analytical Method Validation [8,9]. **Linearity**

The UV method showed linearity in the 1.25–18.75 $\mu g/ml$ concentration range by plotting absorbance vs. concentration.

Accuracy

Accuracy was determined by spiking a 3120 $\mu g/ml$ spironolactone stock into 0.1 N HCl at different levels and analyzing at 242 nm.

Limit of Detection

LOD was calculated as 3.3 (σ /S), where σ is the response standard deviation and S is the calibration curve slope.

Limit of Quantification

LOQ was calculated as 10 (σ /S), where σ is the response standard deviation and S is the calibration curve slope.

Method of Preparation of Suspension

Water, citric acid, and sodium citrate were mixed (Mixture A), followed by suspending agents, sweeteners, and flavoring. Spironolactone was dispersed in glycerin (Mixture B) and combined with Mixture A, then simethicone was added. The pH was adjusted to 4.8, volume set to 100 mL, homogenized for 30 minutes, and filled into amber PET bottles [10].

Selection of Suspending Agent [11].

Xanthan gum, sodium CMC, and veegum K, all FDA-approved GRAS excipients, were evaluated for suspension uniformity in three batches (T1, T2, T3) formulated per Table 2.

Table 2: Suspension composition prepared using different suspending agents

Sr. No.	Name of Ingredients	T1	T2	Т3
1	Spironolactone (mg/ml)	5.0	5.0	5.0
2	Citric Acid (mg/ml)	2.0	2.0	2.0

3	Sodium Citrate Dihydrate	4.0	4.0	4.0
	(mg/ml)			
4	Sorbic Acid	0.5	0.5	0.5
	(mg/ml)			
5	Potassium Sorbate (mg/ml)	2.0	2.0	2.0
6	Xanthan Gum (mg/ml)	3.6	-	-
7	Sodium CMC (mg/ml)	-	3.6	-
8	Veegum K (mg/ml)	-	-	3.6
9	Banana Flavour (mg/ml)	1.0	1.0	1.0
10	Magnasweet 110 (mg/ml)	5.0	5.0	5.0
11	Sodium Saccharin (mg/ml)	1.4	1.4	1.4

12	Glycerine (mg/ml)	22.0	22. 0	22.0
13	Simethicone Emulsion (mg/ml)	2.0	2.0	2.0
14	Purified Water	100 ml	10 0 ml	100 ml

Selection of suspending agent

Batches T1, T2, and T3 were assessed for sedimentation volume to determine the optimal suspending agent, which was then used for further formulation [12].

Design of Experiments

A multilevel factorial design in Design Expert (version 11) was used to develop a spironolactone suspension, evaluating dissolution and viscosity. Fifteen formulations with xanthan gum (2.4–7.2 mg) and glycerine (50–150 mg) were tested, and the optimized batch was compared with the reference product and tablet formulations.

Evaluation Parameters: pH

Each formulation batch was analyzed for pH level using the Thermo Scientific pH meter.

Viscosity

The viscosity of all formulation batches was determined by Brookfield Viscometer by using spindle LV3 at 10 rpm.

In-vitro drug release or dissolution

The in-vitro study of drug release was conducted using USP Type II apparatus in 0.1 N HCl, measuring samples at 242 nm for the determination of the similarity factor, f2.

Assay

The assay of the optimized batch was determined by UV-Spectroscopy at 242 nm using a 70:30 water-acetonitrile solvent and compared with the standard drug solution.

Related Substances (RS)

The RS impurities in the optimized batch were determined by using the Thermo SCIENTIFIC UltiMate3000 UHPLC, C18 (Waters X-Bridge), Photo Diode Array detector and Chromeleon 6.8 software. The solvent system water: acetonitrile (70:30) was used. The conditions used were buffer made of 0.05M ammonium acetate and high

performance liquid chromatography (HPLC) grade methanol in a proportion of 1:1. The temperature for both the column and the sample during the experiment was maintained at 25 degrees Celsius and 45 degrees Celsius, respectively. The chromatography was performed in an isocratic run for a total of 40 min at a flow rate of 0.9 ml/min. The detection wavelength was 254 nm, while the injection volume was $10\,\mu$ l.

Standard preparation: To a 50 ml volumetric flask, transfer 28.62 mg of spironolactone accurately weighed into it, and add some solvent system to as solvent to dissolve and sonicate. The vials after sonication were allowed to cool room temperature and the volume made up to the mark with solvent system and mixed well. From here, 5 ml was further diluted into a volumetric flask of 50 ml up to the mark with solvent: another dilution into a volumetric flask of 100 ml with 5 ml up to the mark with solvent and mixed well.

Sampling Preparation: We took 7.0 g spironolactone oral suspension on a precise scale and transferred to a 25 ml volumetric flask. An appropriate solvent was added and sonicated for 30 min to facilitate dissolution, cooling to room temperature, and then made up to volume with diluent. The solution was then filtered through a 0.45 μm filter. A placebo was prepared the same way.

Accelerated stability study

Optimized suspension batches were packed in amber PET bottles and subjected to accelerated stability testing at 40°C/75% RH for three months to monitor the changes in appearance, potency, pH, viscosity, assay, dissolution, and related substances over time at the expiration of solvent concentrations.

RESULTS AND DISCUSSION Preformulation studies

Preformulation studies are essential in the development of a dosage form, as they evaluate the physicochemical characteristics of a drug that affect its overall effectiveness and performance.

Organoleptic Properties

According to USP specifications, the drug appears as a white to yellowish-white crystalline powder with a faint mercaptan-like odor. It is non-hygroscopic, practically insoluble in water, but soluble in ethanol.

FTIR Analysis

The IR spectrum of spironolactone matched the reference standard per the European Pharmacopoeia, with key absorption peaks observed at 1766 cm⁻¹ (lactone C=0), 2954 cm⁻¹ and 2891 cm⁻¹ (aliphatic C-H), 1691 cm⁻¹ (thioester C=0), 1678 cm⁻¹ (α , β -unsaturated C=0), and 1617 cm⁻¹ (aromatic C=C).

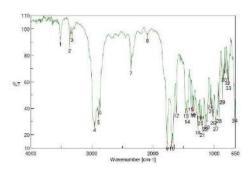


Figure 1. FTIR spectra of the test sample

Drug-Excipients Compatibility Study

The Drug-excipient compatibility was assessed using physical analysis at $25\pm2^{\circ}\text{C}/60\pm5\%\text{RH}$ and $40^{\circ}\text{C}/75\%\text{RH}$ for 30 days, which showed no significant change in appearance. Excipients were selected on the basis of previous successful formulation and then optimized in further studies.

Analytical Method Validation

The analytical method for its validation as per ICH Q2B protocol by UV at a wavelength of 242 nm in 0.1 N HCl exhibited linearity ($R^2 = 0.999$) in a concentration range of 1.25-18.75 µg/mL, with the percentage recovery calculated as 100.6-102.03%. The method was duly proven to be accurate for the determination of spironolactone with values for LOD (4.69 µg/mL) and LOQ (14.22 µg/mL).

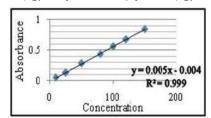


Figure 2. Linearity curve of UV Spectroscopic method for analysis of spironolactone

Selection of suspending agent

Xanthan gum, sodium CMC, and veegum K, FDA-

approved GRAS excipients, were evaluated for sedimentation volume. Xanthan gum was selected as the suspending agent due to no sedimentation, forming a protective film, reducing interparticle attraction, and increasing viscosity per Stoke's law [9].

Design of Experiments

A multilevel factorial design assessed xanthan gum and glycerine impact on f2 and viscosity using a '2FI' model with 15 runs to analyze individual and interactive effects. The similarity factor (f_2) is a logarithmic function used to compare the in-vitro dissolution profiles of testand reference formulations. A value between 50 and 100 suggests that the two profiles are similar, with indicating higher values more Following the US FDA acceptable range of 50–100, it is calculated by the following formula: F2= $50 \cdot \log\{[1+(1/n) \sum_{t=1}^{n} (R_t - T_t)^2] \cdot 0.5 \cdot 100\}$ Understanding the interaction between variables such as xanthan gum and glycerine is crucial. Interactions imply that the effect of one factor on the response depends on the level of another factor, meaning these formulation components do not act independently but influence each other's impact on drug release and viscosity. This insight allows for more robust and predictive formulation development.

Design Evaluation

The '2FI' mixture design identified key factor interactions using various plots, including half-normal, residual, interaction, contour, and 3D surface plots to analyze effects.

Experimental Results of DOE batches for Response 1(f2)

The half-normal plot Figure 3.(A) shows xanthan gum significantly impacts f2, while the normal plot (B) confirms no abnormalities. Residual vs. predicted (C) and residual vs. run (D) plots indicate no abnormal scattering. The interaction plot (E) shows no interaction between factors A and B. The contour plot (F) reveals that increasing xanthan gum and glycerine reduces f2 due to thickening, lowering dissolution. The 3D view (G) provides a macro-level perspective of factorial effects. The Model F-value of 7.10 (P < 0.0500) confirms significance, with a 0.72% probability of occurring due to noise. Adequate Precision (8.533) confirms a strong signal, making the model suitable for design space navigation.

The model equation in terms of coded form for response 1 similarity factor f2 is: f2=+46.72+8.35A₄+1.4.88A₂+1.27A₂9.38A₄+5.05F

 $\begin{array}{l} f2\text{=+}46.72\text{+-}8.35A_1\text{+-}14.88A_2\text{+-}1.27A_39.38A_4\text{+-}5.05B_1\\ +3.58B_2 \end{array}$

High levels of the factors are represented as +1, while low levels are denoted as -1. This coded equation helps in determining the relative influence of each factor by comparing their respective coefficients.

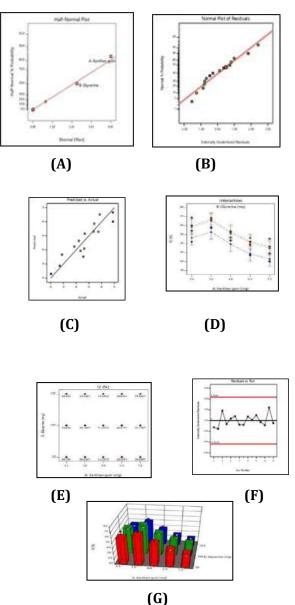


Figure 3. Experimental Results of DOE batches for Response 1(f2)

(A): Half-normal plot of the formulation variables effects on f2; (B): Normal plot of the Residuals of the formulation variables effects on f2; (C): Residual Vs Predicted plot of the effects

on f2; (D): Residual Vs Predicted plot of the effects on f2; (E): Interaction Plot for formulation variables effects on f2; (F): Contour Plot for f2; (G): 3D surface plot for f2.

Experimental results of DOE batches for Response 2 Viscosity

The half-normal plot Figure 4.(A) suggests that Xanthan gum significantly increases viscosity. The plot of residuals (B) normal shows abnormalities, while residual vs. predicted (C) and residual vs. run (D) plots confirm no abnormal effects. The interaction plot (E) indicates no interaction between factors A and B. The contour plot (F) and 3D view (G) reveal that increasing xanthan gum and glycerine raises viscosity. The Model F-value of 2025.25 (P < 0.0500) confirms significance, with Predicted R² (0.9977) and Adjusted R² (0.9988) indicating a strong model fit. Adequate Precision (120.558) confirms a strong signal for design space navigation. The model equation in terms of coded form for response viscosity is:

Viscosity = $+2296.80-1780.80A_1-1068.80A_2-172.80A_3+959.20A_4-76.80B_1+0.8000B_2$

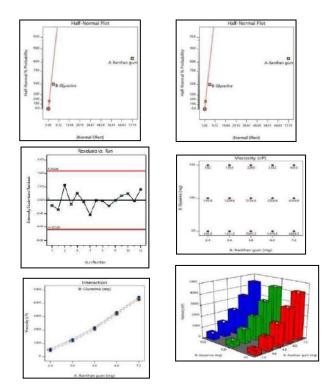


Figure 4. Experimental results of DOE batches for Response 2, Viscosity

(A): Half-normal plot of the formulation variables effects on Viscosity; (B): Normal plot of Residuals

for the formulation variables effects on viscosity; (C): Residuals vs predicted plot for the formulation variables effects on viscosity; (D): Residuals vs Run plot for formulation variables effects on viscosity; (E): Interaction plot for formulation variables effects on viscosity; (F): Contour plot for formulation variables effects on viscosity; (G): Figure 3D Surface plot for viscosity

Optimized batch

From the results of DOE, F1 batch was selected as the optimized batch. This batch shows similarity factor in acceptable range and viscosity is also similar to reference product.

Characterization of Optimized Batch and Reference Product

The optimized batch and reference product were characterized for appearance, pH, viscosity, dissolution, similarity factor, assay and related substances.

Comparative Dissolution Study of Marketed Tablet Formulations and Reference Product with Optimized Test Product

The reference product and marketed tablet formulation (Aldactone) was compared with optimized batch for dissolution rate which indicated that optimized batch have maximum dissolution rate than marketed tablet formulation and same as reference product as per figure 5.

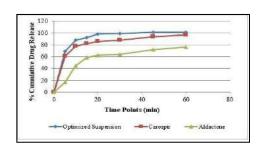


Figure 5. Comparative Dissolution of marketed products with the optimized test product

Accelerated Stability Study

The accelerated stability study was carried out for 3 months at 40°C ± 2°C / 75%RH ± 5%, in accordance with ICH and WHO guidelines, as presented in Table 3. Evaluation at each time interval demonstrated that the formulation

remained stable under these conditions throughout the study period.

Table 3: Evaluation of Accelerated Stability Study

Parame	Initial	40°C/75%RH		
ters		1M	2M	3M
Appeara nce	White banana flavour ed suspens ion			
рН		1102		
Viscosity	1212 cP	1205 cP	1215 cP	1220 cP
Dissoluti on at 1hr	101 %	97 %	96 %	97 %
Assay	99.7 %	100.1 %	103.5 %	103.5 %
Related Substanc e				
Impurity A	0.09 %	0.15 %	0.19 %	0.17 %
Impurity B	ND	ND	ND	ND
Impurity C	0.07 %	0.07 %	0.04 %	0.04 %
Impurity D	ND	ND	ND	ND
Impurity I	0.03 %	0.05 %	ND	0.03 %

Single	maximum	0.02	0.03	0.03	0.01
unknow	n/n	%	%	%	%
Total In	npurity	0.25	0.3 %	0.26	0.25
		%		%	%

CONCLUSION

Spironolactone Oral Suspension provides a competitive advantage by enhancing efficacy and preventing imitation. It is designed for rapid drug release, achieving high serum concentration quickly. Formulation F1, optimized using Quality by Design Software, was identified as the best formulation. Xanthan gum (3.6 mg) and glycerin

(100 mg) were used as suspending and dispersing agents based on viscosity and release characteristics (f2). The formulation exhibited a release pattern similar to Carospir and showed a faster release than Aldactone tablets. Thus, it meets all the criteria for an effective immediate-release formulation.

REFERENCES

- 1. Brandão FC, Tagiari MP, Silva MA, Berti LF, Stulzer HK. Physical-chemical characterization and quality control of spironolactone raw material samples. Pharm. Chem. J. 2008 Jun 1;42(6):368-76.
- Rani TR, Rathore DS, Amit V. Formulation and evaluation of oral dispersible tablet containing spironolactone hydroxypropyl: βcyclodextrin binary system. Int. R. J. pharm. 2012;3(4):318-23.
- 3. Neeraj B, Abhishek K, Abhilash C, Rubia C, Rajni B. A review on immediate release drug delivery system. Int Res J Pharmaceut Appl Sci. 2014 Feb 28;4(1):78-87.
 - 4. Nyol S, Gupta MM. Immediate drug release dosage form: A review. J. Drug Deliv. Ther.. 2013 Mar 15;3(2).
 - 5. Salgado AC, Rosa ML, Duarte A, Almeida AJ. Stability of spironolactone in an extemporaneously prepared aqueous suspension. Eur J Hosp Pharm. 2005;11:68-73.
 - 6. Noerr B. Spironolactone. Neonatal New 1999; 18: 43-6.
 - 7. Messina M, inventor; Schiapparelli Farmaceutici SpA, assignee. Spironolactone containing composition for combatting acne. United States patent US 4,543,351. 1985 Sep 24.
 - 8. Laxman R, Acharya A, Jain V, Bhardwaj S, Sudhir B, Jain D. Development and validation of rp-hplc and ultraviolet spectrophotometric methods for simultaneous determination of spironolactone and torsemide in pharmaceutical dosage form, Int J Res Ayurveda Pharm. 2010;1(2):458-67.
 - Prajapati P, Chandarana C, Modi V, Basuri T. Quantification of Spironolactone by first and second order UV Derivative Spectrophotometry in bulk and tablet dosage form. Int J Pharm Chem Anal. 2016;3(3):168-73.
 - 10. Olsen JL, inventor; Carolina Medical Products Inc, assignee. Spironolactone composition.

- United States patent US 4,837,211. 1989 Jun 6.
- 11. Pipho A, DeHart MP, inventors; Cmp Development LLC, assignee. Spironolactone aqueous formulations. United States patent US 9,757,394. 2017 Sep 12.
 - 12. Kumar RS, Yagnesh TN. Pharmaceutical suspensions: patient compliance oral dosage forms, World J Pharm Pharm Sci. 2016 Oct 19;5(12):1471-537.

Bulletin of Pharmaceutical Research 2025;15(S2)

An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print) 2249-9245 (Online)



RESEARCH ARTICLE

EVALUATION OF IN-VITRO ANTHELMINTIC ACTIVITY OF ETHANOLIC EXTRACTS OF FRAGARIA ANANASSA AND LACTUCA SATIVA AGAINST PHERETIMA POSTHUMA

Padmaja Kore*, Vidya Chaudhari, Siddhi Chavan, Pratik Phalke, Anuradha More, Shraddha Desai, Ujwala Desai

¹ PES's Modern College of Pharmacy, Sec. No. 21, Yamunanagar, Nigdi, Pune 411044. *Corresponding author email: <u>Padmaja.kalshetti@gmail.com</u> Received: Feb 08, 2025 / Revised: April 20, 2025 / Accepted: May 05, 2025

ABSTRACT:

Objective: The study was aimed to evaluate the anthelmintic activity of the ethanolic extracts and acid and base fractions of *Fragaria ananassa* (Strawberry) plant and *Lactuca sativa* (Lettuce) against *Pheretima posthuma* (Indian earthworm).Methods: A preliminary phytochemical analysis was performed to assess the presence of secondary metabolites. The ethanolic extracts were prepared and labelled as crude extracts. A differential pH extraction was performed to isolate essential compounds. The ethanolic extracts and the fractions obtained were tested for anthelmintic activity against *Pheretima posthuma* using albendazole as standard control.Result: There was no death in the normal saline-treated group. Treatment with standard showed paralysis and death of worms. The crude extract, as well as fractions of Fragaria ananassa, showed a reduction in paralysis and death time. However, the effects showed at pH 11 were parallel to the Albendazole standard. *Lactuca sativa* also showed a decrease in the time of paralysis and death with potent effects at 40 mg/ml; however, as compared to pH fractions, crude extract was found to be more powerful.Conclusion: *Fragaria ananassa* and *Lactuca sativa* can be used as potential herbal remedies for the treatment of helminth infections.

Keywords: Helminthiasis, Anthelmintic activity, Pheretima posthuma, Fractions, pH Extraction. **How to cite this article:** Padmaja Kore, Vidya Chaudhari, Siddhi Chavan, Pratik Phalke, Anuradha More, Shraddha Desai, Ujwala Desai Evaluation of In-vitro Anthelmintic activity of ethanolic extracts of Fragaria Ananassa and Lactuca Sativa against Pheretima Posthuma Bulletin of Pharmaceutical Research. 2025;15(S2): 185-194.

Source of support: Nil Conflict of interest: None

Introduction

Helminthiasis, also known as worm infections, are one of the most predominant diseases in both humans as well as animals. Helminths are parasitic worms. Grown-up worms live in the digestive tract, creating thousands of eggs daily. These eggs pollute the soil in regions like towns that need satisfactory sanitation. Soil-transmitted helminths are transmitted by eggs that are passed into the faeces of infected individuals. The transmission mode alters with the type

of worm; it may include ingesting eggs or hatchlings, or by vector's bite.[1] It is observed that there is a progressive rise in the spread of this disease and, indeed, mortality to a few degrees. contaminations contribute to predominance of ailing health, frailty, eosinophilia, and pneumonia. The most common helminthiases are those caused by with intestinal helminths. ascariasis, trichuriasis and hookworms,

Kore et al

followed by schistosomiasis and lymphatic filariasis.

There is a group of antiparasitic drugs that expel parasitic worms and other intestinal parasites out of the body under killing the parasites without harming the host, which are known as anthelmintics or antihelminthics. [2] Anthelmintics are classified as vermicides. These anthelmintics are utilised for the treatment of helminthiasis in humans as well as infected animals. Resistance to these drugs has ended up as a significant issue with chemotherapeutic specialists' utilisation of this lesson. Hence, this is an immense issue in the treatment of helminthiasis.

Furthermore, helminthiasis can also be treated by various polyherbal medications or formulations. These formulations can be safely used to treat this kind of infection. Herbal medicines are not only safe but also effective as compared to allopathy medicines. These polyphenolic compounds are also rich in flavonoids, which exhibit various biological activities hepatotoxic, anti-ulcer, anti-tumour, antithrombogenic, anti-osteoporotic, anti-viral, anti-fungal and anti-bacterial activities. (3-10) In this view, an attempt has been made to study and evaluate the anthelmintic potential of Fragaria ananassa and Lactuca sativa.

Lactuca sativa is a member of the Compositae (Asteraceae) family. The seeds are used for relieving inflammation and osteodynia, in folk medicine of Iran. (11) Lactuca is utilised in traditional medicine and is considered a vital source of phytochemicals. Its antibacterial, analgesic, reactions and the development of resistance of the parasites against the drug necessitates the discovery of new drugs for the treatment of helminth diseases. This infection can also be treated by various herbal formulations.

sedative, antioxidant, and neuroprotective properties are well-known. [12-1]

Fragaria ananassa belongs to the family Rosaceae. It can be an astringent, antimicrobial, antioxidant, and anti-inflammatory agent.

(17-20) The leaves and fruit are considered to treat urinary tract disorders, rheumatism and gout. 21-23]

Phenolic compounds present in fresh strawberry fruit (*Fragaria ananassa*) are ellagic acid, flavonols, ellagitannin, vitamin k, anthocyanins, proanthocyanins, vitamin C, potassium, calcium, etc.⁽²⁴⁾ One of the most widely consumed vegetables in the world, lettuce (*Lactuca sativa L*.) is said to be a good source of nutrients that are good human health, including carotenoids, polyphenols, vitamin C, arginine, and folates. Plants' antioxidant action is attributed to phenolic chemicals.

Albendazole is used as a standard control in the study. Degenerative alterations are caused by binding to the b-tubulin's colchicine-sensitive region, which disrupts microtubule assembly and prevents polymerization. (25) It inhibits nutritional & glucose uptake. (26) The disadvantages of this drug are its adverse reactions, like abdominal pain, nausea, vomiting, diarrhea, headache, dizziness, and vertigo. (27)

Albendazole is a broad- spectrum antibiotic and is useful against some protozoa. [28] It can also produce mild to moderate elevation of hepatic transaminases and hepatotoxicity in rare cases. [29]

These adverse

Herbal remedies can be used as safe and effective alternative remedies due to resistance to these drugs has become a major concern.

		Dun, I naim, Res. 2025
HO OH OH	HO	HO OH
Ellagic Acid	Flavonols	Anthocyanins
	H,N H	но он
Folic acid	Arginine	Vitamin C

Fig.1 Phytocompounds from Fragaria ananassa and Lactuca sativa Material and methods Plant material

The strawberry plant was collected from strawberry farmer, agricultural association in Thergaon, Pimpri-Chinchwad, Maharashtra. Another Lettuce plant was collected from Grow fresh Hydroponics LLP, Pimpri-Chinchwad, Maharashtra.

Reagents and chemicals

All chemicals were of analytical grade (Ana lab fine chemicals-Mumbai, Merck life science private limited). Ethanol used for the preparation of extracts, which was of high-performance liquid chromatography (HPLC) grade (Research lab fine chemicals industries, Mumbai)

Preparation of the extracts

The leaves of both plants were dried and ground in mixer grinder to coarse powder. Coarse powder was poured into a beaker, and ethanol was added until all powder was soaked. It was then stirred with magnetic stirrer for 1-hour at room temperature. The extracts were then filtered through muslin cloth and then again through the filter paper for clear solution. Solvent was eliminated using a rotary evaporator to obtain the crude extracts.

Quantitative estimation of total phenolic constituents (TPC)

Total phenolic content of strawberry and lettuce extract was determined as described in Folin-Ciocalteu method. Gallic acid was utilized as a standard phenolic compound. solutions Gallic acid in different concentrations will be prepared as (10-100) µg/ml) in methanol. Methanolic solutions of the test sample of each extract were prepared (100 µg/ml) or solvent of near about similar polarity. 0.5 ml of graded concentrations of gallic acid/test sample were mixed in 2 ml of Folin-Ciocalteu reagent followed by 4 ml sodium carbonate solution. The reaction mixture was incubated after that at room temperature and was allowed to stand for 30 minutes with shaking at specific time intervals. The absorbance was taken at 650 nm by keeping methanol as a blank sample. Standard curve of gallic acid was taken at different concentrations to find the regression line. The TPC of the extract was obtained from the calibration curve of standard gallic acid and expressed as mg/g or µg/mg gallic acid equivalent (GAE) (30).

Quantitative estimation of total flavonoids constituents (TFC)

Kore et al

The total flavonoid content was determined by UV-spectroscopy method. Rutin solution was utilized as standard in different concentrations (10- 100ug/ml) in ethanol. Calibration curve of rutin was taken as a standard. Ethanolic samples of extract were prepared (100µg/ml). 0.5 ml of diluted samples were mixed in 2 ml distilled water followed by addition of 0.15 ml NaNO2 solution. After the period of 6 minutes, 0.15 ml of AlCl₃ solution was added and allowed to stand for another 6 minutes. 2ml NaOH solution added to the above mixture and kept undisturbed for 15 minutes. The absorbance was measured at 510 nm with water as blank using ultraviolet spectrophotometer. The absorbance of extract was measured by the regression line of standard curve of rutin. TFC is expressed as value of rutin equivalent (RE) mg RE/g extract. [31]

pH differential extraction:

To isolate the alkaloids, 4gm crude extracts were firstly treated with dil. HCl and heated in water bath at 60°C to dissolve the solid particles. The solution was filtered and extracted with CHCl₃. By this extraction the organic and aqueous phases were separated. The process was repeated three times. Organic fractions were collected and treated with anhydrous Na₂SO₄. This sodium sulphate is used to dry or reduce moisture from organic fractions. The solvent was evaporated using an oven. This fraction was labeled as the acid fraction. The alkalinity of the remaining aqueous fraction was taken to pH 8 by treating with 20% NaOH and again extracted with CHCl₃ as same as previous extraction. Alkaline aqueous phase was taken to pH 11 with 20% NaOH and extracted with CHCl₃ three times. Now these two acid and base fractions are labeled as pH 8 and pH 11 fractions respectively. Acid and base fractions were dried. The latest procedure was performed for isolating compounds of different strengths as base. [32]

Collection of earthworms

The Indian earthworm *Pheretima Posthuma* (Annelida) of approximately 8cm was collected from College of Agriculture, Shivajinagar, Pune and manufacturing plant at post Jalna. The adhering dirt and soil were removed by cleaning the worms with clean tap water.

Evaluation of anthelmintic activity

The anthelmintic evaluation was performed Indian earthworm Pheretima *Posthuma*. The earthworms were procured from College of Agriculture, Shivajinagar, located in Pune and manufacturing plant at post Jalna. To perform the assay, the crude extracts, and the fractions (acid and base) of both plants were weighed and dissolved in sterile saline with 5% of dimethyl sulfoxide 10. 20 and 40 obtain mg/ml concentrations for the crude extracts and 1mg/ ml concentrations for the acid fractions and for both basic fractions of every plant. Albendazole was used as standard control at 10mg/ml for the analysis of the crude extracts and at 1mg/ml for the test of the fractions. Three worms of approximately the same size which are previously washed with sterile saline to eliminate dirt and other residues were placed in petri plates and contacted with solutions of the crude extracts and the acid and basic fractions. The procedure was carried out for crude extract at all three doses, fractions at 1mg/ml and standard drug. The paralysis and death time was recorded. The earthworms were considered dead if they showed no movement, even when they were shaken vigorously, and their color faded. They shrank and did not regain their mobility when they were placed in sterile saline for at least 1 hour. (33)

Statistical Analysis:

The data was analyzed by one way ANOVA Tukey's multiple comparison test using Graph Pad Prism software version 6.0. P<0.05 was considered statistically significant.

Results:

A) Percentage yield:

The percentage yield of crude *Fragaria* ananassa leaves powder was found to be 13gm, and percentage yield of *Lactuca* sativa leaves powder was found to be 10 gm.

B) Total Phenolic Content and Total Flavonoid Content:

Total Phenolic Content:

Kore et al

Extract	Total Phenolic content mg GAE/g extract	
Fragaria ananassa (Ethanolic extract)	16.03	
Lactuca sativa (Ethanolic extract)	9.74	

Total Flavonoid Content:

Phytochemical screening of Fragaria

Extract	Total Flavonoid content mg QE/g extract	
Fragaria ananassa (Ethanolic extract)	10.35	
Lactuca sativa (Ethanolic extract)	7.06	

ananassa

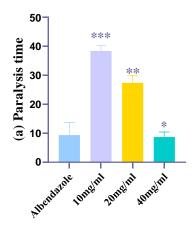
C) Preliminary Phytochemical screening:

3010011118	
Secondary metabolites	Fragaria ananassa
Flavonoids	+
0 .	
Saponins	+
Alkaloids	+
	· · · · · · · · · · · · · · · · · · ·

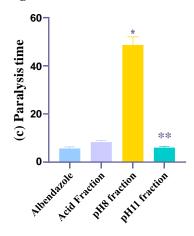
Phytochemical screening of Fragaria ananassa

Secondary metabolites	Lactuca sativa
Flavonoids	+
Saponins	+
Alkaloids	+

Fragaria ananassa crude extract



Fragaria ananassa acid and base fractions

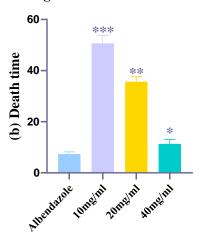


Anthelmintic activity of Crude extract and fractions of Fragaria Ananassa The data was analysed by one way ANOVA followed by Tukey's post-test (n=3). *<0.01, **<0.05, ***<0.001 were considered as levels of significance. (a) Paralysis time of crude extract (b) Death time of crude extract (c) Paralysis time of different fractions (d) Death time of different fractions.

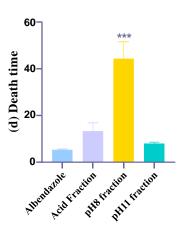
Anthelmintic activity of crude extract of *Fragaria ananassa*

Treatment with the crude extract of 10mg/kg concentration showed significant (p<0.001) increase in the paralysis time to 38.33 ± 2.02 . The increase was found to be 310%. Treatment with the crude extract of 20mg/kg concentration showed significant (p<0.01) increase in the paralysis time to 27.33 ± 2.60 . The increase was found to be

Fragaria ananassa Crude extract



Fragaria ananassa acid and base fractions

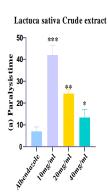


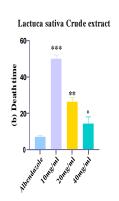
192%. Treatment with the crude extract of 40mg/kg concentration showed significant (p<0.05) increase in the paralysis time to 8.66±1.76. The increase was found to be 7.13%. Treatment with the crude extract of 10mg/kg concentration showed significant (p<0.001) increase in the death time to 50.67±2.96. The increase was found to be 591%. Treatment with crude extract of 20mg/kg concentration showed significant (p<0.01) increase in the death time to 35.67±2.02. The increase was found to be 386%. Treatment with crude extract of 40mg/kg concentration showed significant (p<0.05) increase in the death time to 11.3±1.76. The increase was found to be

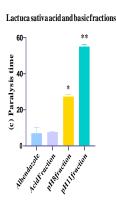
Anthelmintic activity of acid and basic fractions of *Fragaria ananassa*

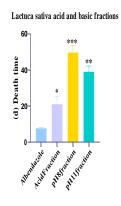
There was no paralysis and death time in the saline treated group. Treatment with the standard drug Albendazole (1mg/ml) showed paralysis time as 5.00±1.00 minutes. Treatment with acid fraction showed nonsignificant increase in the paralysis time. The increase was found to be 47.04% as compared to the standard group. Treatment with fraction (pH 8) showed significant (p<0.001) increase in the paralysis time to 48.67±3.283 minutes and the increase was found to be 758%. Treatment with fraction (pH 11) showed non-significant increase in the paralysis time to 6.00±0.57 minutes. The increase was found to be 5.87%. Treatment with acid fraction showed non-significant increase in the death time. The increase was found to be 105% as compared to the standard group. Treatment with fraction (pH 8) showed significant (p<0.001) increase in the death time to 44.00±5.03 and the increase was found to be 676%. Treatment with fraction (pH 11) showed nonsignificant increase in the death time to 8.00±0.57 minutes. The increase was found to be 41.1%.

Anthelmintic activity of Crude extract and fractions of *Lactuca Sativa*:









The data was analysed by one way ANOVA followed by Tukey's post-test (n=3). *<0.01, **<0.05, ***<0.001 were considered as levels of significance. (a) Paralysis time of crude extract (b) Death time of crude extract (c) Paralysis time of different fractions (d) Death time of different fractions.

Anthelmintic activity of crude extract of *Lactuca sativa*

Treatment with the crude extract of 10mg/kg concentration showed significant (p<0.001) increase in the paralysis time to 42.00±4.58. The increase was found to be 500%. Treatment with crude extract of 20mg/kg concentration showed significant (p<0.01) increase in the paralysis time to 24.33±1.85. The increase was found to be 247%. Treatment with crude extract of 40mg/kg concentration showed significant (p<0.05) increase in the paralysis time to 13.33±3.71. The increase was found to be 90.42%. Treatment with the crude extract of 10mg/kg concentration showed significant (p<0.001) increase in the death time to 50.00±2.08. The increase was found to be 614%. Treatment with crude extract of 20mg/kg concentration showed significant (p<0.01) increase in the death time to 26.33±2.18. The increase was found to be 276%. Treatment with crude extract of 40mg/kg concentration showed significant (p<0.05) increase in the death time to 14.33±3.71. The increase was found to be 104

Anthelmintic activity of acid and basic fractions of *Lactuca sativa*

Treatment with acid fraction showed non-significant increase in the paralysis time. The increase was found to be 9.52% as compared to the standard group. Treatment with fraction (pH 8) showed significant (p<0.05) increase in the paralysis time to 27.33 ± 1.20 minutes and the increase was found to be 290%. Treatment with fraction (pH 11) showed significant (p<0.01) increase in the paralysis time to 55.00 ± 1.15 minutes. The increase was found to be 685%. Treatment with acid fraction showed significant (0.05) increase in the death time. The increase was found to be 174% as compared to the

Unlocking Horizons in Global Multidisciplinary Healthcare Research" Indo-US International Conference Date: 10-12, March 2025 Organised by PES Modern college of Pharmacy Nigdi Pune-44

standard group. Treatment with fraction (pH 8) showed significant (p<0.001) increase in the death time to 49.67 ± 3.84 and the increase was found to be 548%. Treatment with fraction (pH 11) showed significant (0.01) increase in the death time to 39.00 ± 3.21 minutes. The increase was found to be 409%

Discussion:

The presence of flavonoids, alkaloids, saponins was confirmed through the phytochemical analysis of Fragaria Ananassa and Lactuca Sativa. Initially Lactuca Sativa has reported pharmacological activities that antibacterial. analgesic, sedative. antioxidant, and neuroprotective activities. In the assay performed, it was observed that, the acid fraction of Lactuca Sativa was more potent than the basic fractions (pH 8 and pH 11). It can be due to the solubility of phytocompounds are probably of acid nature. Lactuca Sativa As per the literature survey, Fragaria Ananassa show astringent, antimicrobial, antioxidant, and inflammatory activity. And here in the assay it was observed that basic fraction of pH 11 was more potent than other fractions showing the presence of base soluble active phytocompounds. These plant extracts includes various compounds that can be responsible for the activity like lignans(34), flavonoids⁽³⁵⁾, phenylpropanoids ⁽³⁶⁾, etc. The drug may be further explored for its phytochemical profile to identify the active constituent responsible for anthelmintic activity.

Acknowledgments:

Authors are very much thankful to Professor and Principal Dr. Praveen D. Chaudhari (P.E.S's Modern College of Pharmacy, Nigdi, Pune, Maharashtra, India) for supporting as well as providing the necessary facilities to execute research work successfully.

Conflict of interest statement:

We declare that we have no conflict of interest.

REFERENCE:

- 1. .Sahlan M, Hapsari NR, Pratami KD, Khayrani AC, Lischer K, Alhazmi A, Mohammedsaleh ZM, Shater AF, Saleh FM, Alsanie WF, Sayed S. Potential hepatoprotective effects of flavonoids contained in propolis from South Sulawesi against chemotherapy agents. Saudi Journal of Biological Sciences. 2021 Oct 1;28(10):5461-8.
- 2. . Zhang W, Lian Y, Li Q, Sun L, Chen R, Lai X, Lai Z, Yuan E, Sun S. Preventative and therapeutic potential of flavonoids in peptic ulcers. Molecules. 2020 Oct 11:25(20):4626.
- 3. Tuli HS, Garg VK, Bhushan S, Uttam V, Sharma U, Jain A, Sak K, Yadav V, Lorenzo JM, Dhama K, Behl T. Natural flavonoids exhibit potent anticancer activity by targeting microRNAs in cancer: A signature step hinting towards clinical perfection. Translational Oncology. 2023 Jan 1;27:101596.
 - 4. Gryglewski RJ, Korbut R, Robak J, Swięs J. On the mechanism of antithrombotic action of flavonoids. Biochemical pharmacology. 1987 Feb 1;36(3):317-22.
 - 5. Liu B, Liu X, Ning Q, Zhong R, Xia Z, Li J, Song J, Wei Y. Evaluation of toxicity and anti-osteoporosis effect in rats treated with the flavonoids of Psoraleae Fructus. Journal of Functional Foods. 2020 Dec 1;75:104262.
 - 6. Badshah SL, Faisal S, Muhammad A, Poulson BG, Emwas AH, Jaremko M. Antiviral activities of flavonoids. Biomedicine & Pharmacotherapy. 2021 Aug 1;140:111596.
 - 7. Kanwal Q, Hussain I, Latif Siddiqui H, Javaid A. Antifungal activity of flavonoids isolated from mango (Mangifera indica L.) leaves. Natural Product Research. 2010 Dec 15;24(20):1907-14.
 - 8. Donadio G, Mensitieri F, Santoro V, Parisi V, Bellone ML, De Tommasi N, Izzo V, Dal Piaz F. Interactions with microbial proteins driving the antibacterial activity of flavonoids. Pharmaceutics. 2021 May 5;13(5):660.

- 9. Akbar S, Akbar S. Lactuca sativa L.(Asteraceae/Compositae) (Syns.: L. capitata (L.) DC; L. crispa (L.) Roth; L. laciniata Roth; L. palmata Willd.). Handbook of 200 Medicinal Plants: A Comprehensive Review of Their Traditional Medical Uses and Scientific Justifications. 2020:1067-75.
- Noumedem JA, Djeussi DE, Hritcu L, Mihasan M, Kuete V. Lactuca sativa. InMedicinal spices and vegetables from Africa 2017 Jan 1 (pp. 437-449). Academic Press.
- 11. Sayyah M, Hadidi N, Kamalinejad M. Analgesic and anti-inflammatory activity of Lactuca sativa seed extract in rats. Journal of Ethnopharmacology. 2004 Jun 1;92(2-3):325-9.
 - 12. Kim HW, Suh HJ, Choi HS, Hong KB, Jo K. Effectiveness of the sleep enhancement by green romaine lettuce (Lactuca sativa) in a rodent model. Biological and Pharmaceutical Bulletin. 2019 Oct 1;42(10):1726-32.
 - 13. Harsha SN, Anilakumar KR, Mithila MV. Antioxidant properties of Lactuca sativa leaf extract involved in the protection of biomolecules. Biomedicine & Preventive Nutrition. 2013 Oct 1;3(4):367-73.
 - 14. Harsha SN, Anilakumar KR. Protection against aluminium neurotoxicity: a repertoire of lettuce antioxidants. Biomedicine & Aging Pathology. 2013 Oct 1;3(4):179-84.
 - 15. Lim TK, Lim TK. Fragaria x ananassa. Edible medicinal and non-medicinal plants: volume 4, fruits. 2012:395-409.
 - 16. Chatla S, Deepika M, Naseema M, Akhil N. Antimicrobial Activity Of Ethanolic Extract Of Strawberry Fruits (Fragaria Ananassa).
 - 17. Cvetkovic DJ, Stanojevic IP, Stankovic MZ, Cakic MD, Savic SR, Miljkovic MD. Antioxidant activity of strawberry (Fragaria× ananassa Duch.) leaves. Separation Science and Technology. 2017 Apr 13;52(6):1039-51.
 - 18. Nofal AE, AboShabaan HS, Fayyad RM, Ereba RE, Omar NA, Elsharkawy

- SM, Elberri AI. Immunostimulatory and anti-inflammatory impact of Fragaria ananassa methanol extract in a rat model of cadmium chloride-induced pulmonary toxicity. Frontiers in immunology. 2023 Nov 29;14:1297315.
- 19. Liya SJ, Siddique R. Determination of antimicrobial activity of some commercial fruit (apple, papaya, lemon and strawberry) against bacteria causing urinary tract infection. European Journal of Microbiology and Immunology. 2018 Sep:8(3):95-9.
- 20. Otero C, Klagges C, Morales B, Sotomayor P, Escobar J, Fuentes JA, Moreno AA, Llancalahuen FM, Arratia-Perez R, Gordillo-Fuenzalida F, Herrera M. Anti-inflammatory chilean endemic plants. Pharmaceutics. 2023 Mar 10;15(3):897.
- 21. Sato T, Ikeya Y, Adachi SI, Yagasaki K, Nihei KI, Itoh N. Extraction of strawberry leaves with supercritical carbon dioxide and entrainers: Antioxidant capacity, total phenolic content, and inhibitory effect on uric acid production of the extract. Food and bioproducts processing. 2019 Sep 1;117:160-9.
- 22. Aaby K, Mazur S, Nes A, Skrede G. Phenolic compounds in strawberry (Fragaria x ananassa Duch.) fruits: Composition in 27 cultivars and changes during ripening. Food chemistry. 2012 May 1;132(1):86-97.
- 23. Naaz F, Haider MR, Shafi S, Yar MS. Anti-tubulin agents of natural origin: Targeting taxol, vinca, and colchicine binding domains. European journal of medicinal chemistry. 2019 Jun 1:171:310-31.
- 24. Le H, Kim SK. Potential Hexokinase II Inhibition by Benzimidazole Anthelmintics: Albendazole, Mebendazole and Fenbendazole. Journal of Pharmaceutical Research International. 2021 Feb 5;32(46):80-6.

- 25. Onakpoya IJ. Antihelminthic Drugs. InSide Effects of Drugs Annual 2015 Jan 1 (Vol. 37, pp. 367-381). Elsevier.
- 26. .Chai JY, Jung BK, Hong SJ. Albendazole and mebendazole as anti-parasitic and anti-cancer agents: an update. The Korean Journal of Parasitology. 2021 Jun;59(3):189.
- 27. Marin Zuluaga JI, Marin Castro AE, Perez Cadavid JC, Restrepo Gutierrez JC. Albendazole-induced granulomatous hepatitis: a case report. Journal of medical case reports. 2013 Dec;7:1-4.
- 28. .Ujang ZB, Subramaniam T, Diah MM, Wahid HB, Abdullah BB, Abd Rashid AH, Appleton D. Bioguided fractionation and purification of natural bioactives obtained fromalpinia conchigera water extract with melanin inhibition activity.
- 29. Alhakmani F, Kumar S, Khan SA. Estimation of total phenolic content, in–vitro antioxidant and anti–inflammatory activity of flowers of Moringa oleifera. Asian Pacific journal of tropical biomedicine. 2013 Aug 1;3(8):623-7.
- 30. Ca ceres AL, Flores-Giubi ME, Romero-Rodrí guez MC, Alvarenga NL. In vitro anthelmintic activity and chemical composition of methanol extracts and fractions of Croton paraguayensis and Vernonia brasiliana against Eisenia fetida.
- 31. Bazana D, Lopezb E, Caceresa A, Degenb R, Alvarengaa N. In vitro anthelmintic activity of methanol extracts and fractions of two amphilophium species against Eisenia Fetida. Journal of Applied Biology and Biotechnology. 2020 Mar 26;8(2):98-102.
- 32. Higuera-Piedrahita RI, Dolores-Herna ndez M, Cruz-Cruz HA, Lo pez-Arellano R, Gives PM, Olmedo-Jua rez A, Cue llar-Ordaz JA, Gonza lez-Cortazar M, Ble-Gonza lez EA, Lo pez-Arellano ME, Zamilpa A. 3'-Demethoxy-6-O-Demethylisoguaiacin and Norisoguaiacin Nematocidal Lignans

- from Artemisia cina against Haemonchus contortus Infective Larvae. Plants. 2023 Feb 12;12(4):820.
- 33. Zarza-Albarra n MA, Olmedo-Jua rez A, Rojo-Rubio R, Mendoza-de Gives P, Gonza lez-Cortazar M, Tapia-Maruri D. Mondrago n-Ancelmo I. Garcí a-Herna ndez C. Ble -Gonza lez FA Zamilpa A. Gallovl flavonoids from Acacia farnesiana pods possess potent anthelmintic activity against Haemonchus contortus eggs and larvae. Journal infective of ethnopharmacology. 2020 Mar 1;249:112402.
- 34. Spiegler V, Liebau E, Hensel A. Medicinal plant extracts and plant-derived polyphenols with anthelmintic activity against intestinal nematodes. Natural product reports. 2017;34(6):627-43.

Bulletin of Pharmaceutical Research 2025; 15(S2)

An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print) 2249-9245 (Online)

RESEARCH ARTICLE



POLYHERBAL DERMAL PATCH FOR FUNGAL INFECTIONS.

Padmaja Kore*, Ujwala Desai, Anushka Kamble, Pooja Khaire, Pratiksha Jadhav, Rohit Shelmukar

PES's Modern College of Pharmacy, Sec. No. 21, Yamunanagar, Nigdi, Pune 411044.

*Corresponding author email: padmaja.kalshetti@gmail.com

Received: Feb 07, 2025 / Revised: April 19, 2025 / Accepted: May 03, 2025

ABSTRACT

Fungal infections, particularly tinea infections, pose significant dermatological challenges, often requiring prolonged treatment with systemic antifungal drugs. The present study aims to develop a polyherbal transdermal patch for the localized treatment of fungal infections, minimizing systemic toxicity and drug resistance. The formulation incorporates garlic oil, neem oil, menthol oil, camphor oil, and aloe vera pulp, leveraging their antimicrobial, antiinflammatory, and healing properties. The transdermal patch was prepared using the solvent casting method, incorporating optimized concentrations of herbal extracts. Antimicrobial activity against Candida albicans and Tinea cruris was evaluated using the zone of inhibition method. The study identified an optimal oil ratio (garlic: camphor: neem at 1:3:1), demonstrating maximum antifungal activity. The prepared patches exhibited suitable mechanical strength, folding endurance, viscosity, and pH stability. In vitro diffusion studies confirmed a sustained drug release profile over eight hours, reinforcing its efficacy for prolonged dermal application. This polyherbal transdermal patch presents a promising alternative to conventional antifungal treatments, potentially reducing the dependency on oral antifungal drugs while providing localized, effective, and patient-friendly therapy for fungal infections.

Keywords: Polyherbal transdermal patch, Localized antifungal treatment, Anti-microbial activity, Sustained drug release, *Tinea cruris, Candida albicans.*

How to cite this article: Padmaja Kore, Ujwala Desai, Anushka Kamble, Pooja Khaire, Pratiksha Jadhav, Rohit Shelmukar. Polyherbal dermal patch for fungal infections. Bulletin of Pharmaceutical Research. 2025;15(S2): 200-207.

Source of support: Nil Conflict of interest: None

INTRODUCTION:

Different types of fungi can cause fungal infections. In some cases, fungi that aren't typically found on or inside your body but can colonize it and cause an infection [1]. In other cases, fungi that are normally present on or inside your body and can multiply out of control and cause an infection. Fungal infections can be contagious. They can spread from one person to another. In some cases, you can also catch disease-causing fungi from infected animals or contaminated soil or surfaces [2] Tinea is the name of a group of diseases caused by a fungus. Tinea infections are the infection on the scalp, arms, legs, face, and trunk is characterized by ring-shaped, red, scaly patches with clearing centers. Types of tinea include ringworm, athlete's foot and jock itch. These infections are usually not serious, but they can be uncomfortable. Over-the-counter creams and powders will get rid of many tinea infections, particularly athlete's foot and jock itch [3]. Conventional treatment of fungal infections involves chronic treatment with oral antifungal drugs. However, the expected antifungal effect is desired on superficial dermal layers. Though Systemic antifungal drugs are better absorbed in skin, they are facing the challenges with drug resistance if single dose is missed. Clinically, the duration of treatment is not less than 60-days. Some physicians also prescribe antihistaminic drugs as a combination therapy [4]. However, sudden withdrawal of antihistaminic drugs shows severe skin reactions.

Considering the above drawbacks, an attempt has been made to formulate the polyherbal transdermal patch with localized action by reducing the systemic toxicity and cumulative effects [5]. Garlic Oil acts as antifungal [6], neem oil has anti-microbial [7] and anti-inflammatory activity, menthol oil has broad spectrum of antimicrobial activity [8], camphor oil acts as anti-itching as well as anti-irritant [9] and aloe vera pulp has smoothening, anti-rash, hyper pigmentation, healing properties [10].

Advantages of the present invention:

a. The transdermal patch in the present invention reduces the high oral antibiotic load.

- b. The transdermal patch also reduces the dependency on oral antibiotics and the side effects associated with it.
- c. The transdermal patch prevents easy spread of infections to other areas due to severe itching.
- d. It shortens the duration of treatment.

The objective of the work was to formulate the polyherbal transdermal patch for treating fungal infections like tinea infection with localized effect, reduced systemic toxicity and preventing spread of infection to other body parts caused due to severe itching.

MATERIALS AND METHOD

Materials:

Neem oil, Camphor oil and Peppermint oil was obtained from Dr. Jain's, Neeta Herbal, Bhiwandi. Garlic powder was obtained from Alka ayurvedic pharmacy, Aloe Vera pulp was obtained from Kapiva, Matxin labs. From the above oils, those which shows maximum antimicrobial activity are selected for the formulation of films.

Methods:

A. Selection of oils and its concentration based on Anti-microbial activity against Candida albicans:

Antimicrobial 1. activity for oil Sabouraud dextrose agar (16.5g) was taken and dissolved in 250ml of water. The agar media and all the materials were sterilized in an autoclave for half an hour. The floor was cleaned properly by using ethanol, and the antimicrobial activity was performed in a sterile area.The agar media was poured into petri plates and allowed to cool and solidify. On the surface of the agar plates, a Candida albicans suspension was spread using a spreader. Finally, the neem oil, camphor oil and suspension of garlic powder was added to the and it was incubated for bore. **Optimization of concentrations of oils:**

The same procedure was followed for optimizing the concentrations of oils as we performed for the selection of oils (Table 1).

Table 1: Different proportions of oils for antimicrobial activity

Batch	Ratio (Garlic: Camphor: Neem)
F1	1:1:0.5

F2	2:2:1
F3	2:1:1
F4	1:3:1

B. Preparation and evaluation of Emulsion

The preparation of an emulsion using the bottle method involve ml of Neem Oil, and 1 ml of filtered Garlic extract (500 mg garlic powder was solubilized in 10 ml of ethanol) were mixed together along with Tween 80 (15% w/v) and Span 60 (2.5% w/v) as an emulsifier. The aqueous phase was added to the above solution to make up the volume to 10 mL. The emulsion was prepared using the bottle method, which was suitable for volatile oilss the following steps:

Three ml of Camphor Oil, 1ml of Neem Oil, and 1ml of filtered Garlic extract (500 mg garlic powder was solubilized in 10ml of ethanol) were mixed along with tween 80 (15% w/v) and Span 60 (2.5% w/v) as an emulsifier. The aqueous phase was added to the above solution to make up the volume to 10ml. The emulsion was prepared using the bottle method suitable for volatile oil. *Evaluation parameters for emulsion:*

1. Optical properties:

Optical properties such as color, opacity, gloss were observed visually.

2. Viscosity:

Brookfield viscometer at 30°C with a CPE61 spindle at 30 rpm was used to measure viscosity. Results were measured in triplicate, and the average was calculated.

3. pH:

pH of the freshly formulated emulsion was measured using digital pH meter.

4. Globule size:

Emulsion was diluted 50 times and 100 times with distilled water. The resultant samples were prepared by gentle agitation for 5 minutes using a magnetic stirrer. In addition, globule size distribution (PSD) and zeta potential of the final emulsion were measured using dynamic light scattering technique by HORIBA particle size and zeta analyzer.

C. Preparation of film:

HPMC K4M (0.5 g) was taken in 20 ml of water. It was continuously stirred on a Magnetic stirrer to mix the polymer. The emulsion was incorporated in four batches: F1, F2, F3 and F4. **(Table 2)**. After forming the film, it was kept for 24 hours to dry. The film was separated from the petri plate using a syringe needle.

Table 2: Compositions of different batches of dermal film

Batche	HPMC K4M	Water	Emulsion
S			
F1	0.5g	20ml	2ml
F2	0.5g	20ml	3ml
F3	0.5g	20ml	4ml
F4	0.5g	20ml	5ml

Evaluation Parameters for transdermal patch:

1. Antimicrobial activity against *Tinea cruris*

In vitro antifungal studies were performed against *Tinea cruris* in Sabouraud dextrose starch medium by the cup plate method. *Tinea cruris* suspension was spread on the surface of plates with the help of a spreader. The required size of bores (6 mm) was made using a cork borer and dermal film solution was poured into it. The whole procedure was carried out in aseptic laminar air flow chamber. The petri plates were placed into an incubator at 25°C for growth of micro-organism for 24 h. After 24 h, zone of inhibition was checked to determined using scale.

2. Mechanical Strength:

Film specimens were cut (25×50 mm). Care was taken to ensure the specimens had a consistent thickness free from defects or imperfections. Using a tensile testing machine, the film was pulled at a 200mm/min speed, and the force required to break the film was noted.

3. Weight variation:

9 films were weighed on analytical balance and average weight was determined for each film. It is desirable that films should have nearly constant weight.

4. Folding endurance:

The folding endurance is expressed as the number of folds (number of times the film is folded at the same place, either to break the specimen or to develop visible cracks). This test is important to check the ability of the sample to withstand folding. This also gives an indication of brittleness. The folding endurance of the strips was determined by repeatedly folding one film at the same place till it broke.

5. Film thickness:

Film thickness was measured by using 0.02 mm vernier caliper. A 5×4 cm sample of the film thickness was measured in ten different positions.

6. *In vitro* diffusion study:

A Franz diffusion cell system was applied through a cellulose acetate synthetic membrane. Around 300 mg of each sample was used as the donor phase. The acceptor phase was thermostated phosphate buffer (PBS pH 7.4±0.15) at 32°C±0.5°C. Drug release lasted 8-hours (sampling times: 0.5; 1; 2; 3; 4; 5; 6; and 8 h). The antimicrobial activity of each sample is withdrawn was measured against *Tinea cruris*

RESULT AND DISCUSSION

A. Antimicrobial activity for oils:

Table 3: Selection of oils based on antimicrobial activity against Candida albicans:

Oils	Zone of inhibition (cm)
Neem	2.166 ± 0.152
Garlic	2.266 ± 0.152
powder	
Camphor	2.866 ± 0.152
oil	
Oil	3.28 ± 0.152
mixture	

Based on the observation (Figure 1), we conclude that neem, garlic, and camphor exhibited activity against *Candida albicans*, as evidenced by a zone of inhibition (Table 3).

Sr. No	Evaluation parameter	Observation
1	Color	Buff color
2	Opacity	Opaque



Camphor oil

Oil mixture

From table no. 5 results it was observed that uniform emulsion was formed.

2. Viscosity:

Table 6: Measurement of viscosity of emulsion

RPM	Spindle no.	Viscosity
		(CP)

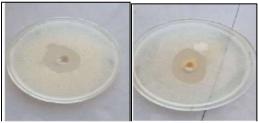


Fig 1: Antimicrobial activity for oils *Optimization of concentrations of oils:*

The same procedure was followed for optimizing the concentrations of oils as we performed for selecting oils. The oils were chosen in the ratio garlic: camphor: neem (1:3:1) (Table 4).

Table 4: Optimization of Concentration of oils:

Batch	(Garlic:	Zone of
	Camphor:	inhibition (cm)
	Neem)	
F1	1:1:0.5	2.533 ± 0.15
F2	2:2:1	3.12 ± 0.1
F3	2:1:1	2.766 ± 0.11
F4	1:3:1	3.34 ± 0.1

Based on the observations, batch F4 with concentrations in the ratio of 1:3:1 exhibited the maximum zone of inhibition. Therefore, we have selected this ratio for the formulation of films (Figure 2).

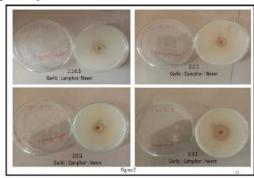


Fig 2: Antimicrobial activity for optimization of oils

B. Evaluation results of Emulsion:

1. Optical properties:

Table 5: Optical properties of Emulsion

Lillui	51011		
30	61	16	
30	61	22	
30	61	8	
Average vis	cosity	15.33	±
		1.533*	

*n=3From table no 6 results, viscosity was found

Kore et al

to be optimum reducing settling of globules.

3. pH:

Table 7: Measurement of pH of optimized batch of emulsion

Sr. No	Batch	рН	
1	Optimized batch	6.5	
2	Optimized batch	6.7	
3	Optimized batch	6.8	
Avera	Average pH 6.6 ± 0.66 *		

^{*}n=3

From table no 7 results, pH was found to be in the range of skin pH thus preventing irritation to the skin.

4. Globule size:

Table 8: Measurement of globule size of optimized batch of emulsion

Sr. No.	Batch	Globule (µm)	size
1	Optimized batch	20	
2	Optimized batch	18	
3	Optimized batch	22	
Average globule size		20 ± 2.0*	

^{*}n=3

From table no 8 results, globule size was found to be optimum reducing settling of globules.

C. Evaluation Results of Film Antimicrobial activity for films against candida:

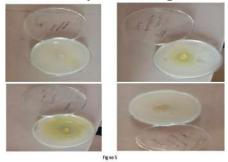


Fig 4: Antimicrobial activity for films against candida

Table 9: Antimicrobial activity of different batches of dermal film

Sr. No.	Batches	Diameter of zone of inhibition (cm)		of
1	F1	2.93 ± 0.154		
2	F2	2.40 ± 0.236		
3	F3	3.11 ± 0.191		

4 F4 3.23 ± 0.128

From the above observation (Figure 4) we conclude that F4 batch shows maximum antimicrobial activity. F4 batch shows maximum antifungal activity against candida. F3 batch also shows activity but this film is too oily that's why we selected F4 batch film (Table 9).

2. Antimicrobial activity against *Tinea cruris*

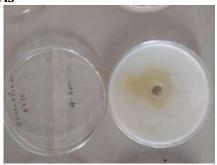


Fig 5: Antimicrobial activity for films against Tinea cruris

Table 10: Zone of inhibition of optimised batch against *Tinea cruris*

	batch against <i>Tinea cruris</i>				
Sr. No			Diameter inhibition (c	of zone of cm)	
1	Optimized Batch		4.14		
Sr. N	Sr. No. We		eight (mg)	Mean	
1	1 100		0		
2	2 10		7	106 ± 2.5438*	
3	3 11:		1		
2	Optimized Batch	l	3.50		
3	3 Optimized Batch		4.23		
Average Diameter of zone of inhibition		3.95 ± 0.324	*		

^{*}n=3

From table no 10 results, zone of inhibition was observed indicating antifungal activity of formulation against *Tinea cruris* (Table 10 and figure 5).

Evaluation parameters for transdermal patch:

1. Mechanical Strength:

Table 11: Mechanical strength of optimized batch of dermal film

*n=3

Samples	Time interval (min)	Zone of inhibition (mm)
S1	30	8 ±1
S2	60	12 ±1
S3	120	14 ± 1
S4	180	16 ± 2
S5	240	22 ± 2
S6	300	25 ± 2
S7	360	29 ± 3
S8	420	34 ± 3
S9	480	38 ± 3

Fro

m table no 11 results, mechanical strength was good for transdermal patch.

2. Weight variation:

Table 12: Weight variation of optimized batch of dermal film

*n=3

From table no 12 results, the weight variation of optimized batch of transdermal patch was found to be in range indicating uniform distribution of drug.

3. Folding endurance:

Table 13: Folding endurance of optimized batch of dermal film

Sr. No	Thickness	Mean (mm)	*n=3
1	0.29 mm		
2	0.33 mm	0.3±	
3	0.28 mm	0.026*	

From table no 13 results, folding endurance was good for transdermal patch indicating flexibility of film.

4. Film thickness:

Table14: Film thickness of optimized batch of dermal film*n=3

From table no 14 results, film thickness was of transdermal patch was found to be in range aiding in uniform drug release.

6. *In Vitro* diffusion study

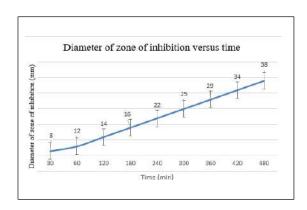


Fig. 6 Time vs diameter of inhibition zone

Discussion

The present study aimed to formulate and evaluate a polyherbal dermal patch for fungal infections, specifically targeting *Tinea cruris* and *Candida albicans*. The polyherbal transdermal patch was designed to provide localized treatment, reducing systemic toxicity and preventing the spread of infections due to severe itching.efficacy against *Candida albicans*, where neem oil, garlic powder, and camphor oil exhibited significant antimicrobial activity [12]. The combination of these oils (garlic: camphor: neem in a 1:3:1 ratio) demonstrated the highest zone of inhibition, indicating its potential as an effective antifungal agent.

The emulsion was prepared using the bottle method, and its evaluation showed desirable properties such as buff colour, opacity, and a matte finish [13]. The viscosity measurements indicated a stable emulsion, with an average viscosity of 15.33 CP. The optimized batch also exhibited a pH of 6.6, ensuring compatibility with human skin. Additionally, the globule size analysis confirmed an average size of 20µm, which is suitable for effective skin penetration. The preparation of the polyherbal film involved incorporating different volumes of emulsion into hydrogel-based formulations [14] Among the various batches, F3 (4ml emulsion) exhibited the highest antifungal activity while maintaining a non-oily texture, making it the most suitable formulation for further studies.

The films were evaluated for their antimicrobial activity against *Tinea cruris*, where the optimized batch demonstrated an average zone of inhibition of 3.95 ± 0.324 cm, confirming its potent antifungal activity. Mechanical strength testing of the film showed an average strength of 14.9 MPa, ensuring sufficient durability for

application. Additional evaluations, including weight variation, folding endurance (172 \pm 42.96 folds), and film thickness (0.3 \pm 0.026 mm), further confirmed the consistency and reliability of the formulation.

The *in vitro* diffusion study revealed a gradual increase in drug release over time,^[15] with the zone of inhibition expanding from 8 mm at 30 minutes to 38mm at 480 minutes (Figure 6 and table 15). This sustained release profile suggests prolonged antifungal activity, which could enhance treatment efficacy and reduce the frequency of application.

Overall, the study successfully formulated and evaluated a polyherbal transdermal patch with significant antifungal activity. The optimized formulation (F3) demonstrated strong mechanical properties, controlled drug release, and high antimicrobial efficacy, making it a promising candidate for the treatment of fungal infections. Future studies should focus on clinical evaluations to assess the *formulation's in vivo efficacy and safety*.

REFERENCES:

- 1) Köhler JR, Casadevall A, Perfect J. The spectrum of fungi that infects humans. Cold Spring Harb. Perspect. Med.2015;5(1):a019273. [DOI: 10.1101/cshperspect.a019273]
- 2) Baumgardner DJ. Soil-related bacterial and fungal infections. JABFM. 2012;25(5):734-44. [DOI: 10.3122/jabfm.2012.05.110226]
- 3) Sahoo AK, Mahajan R. Management of tinea corporis, Tinea cruris, and tinea pedis: A comprehensive review. Indian Dermatol. Online J. 2016;7(2):77-86. [DOI: 10.4103/2229-5178.178099]
- 4) Matterne U, Böhmer MM, Weisshaar E, Jupiter A, Carter B, Apfelbacher CJ. Oral H1 antihistamines as 'add-on' therapy to topical treatment for eczema. CDSR. 2019;(1). [DOI:10.1002/14651858.CD012167]
- 5) Cheng YC, Li TS, Su HL, Lee PC, Wang HM. Transdermal delivery systems of natural products applied to skin therapy and care. Molecules. 2020;25(21):5051. [DOI:10.3390/molecules25215051]
- 6) Li WR, Shi QS, Liang Q, Huang XM, Chen YB. Antifungal effect and mechanism of garlic oil on Penicillium funiculosum.

- Appl. Microbiol. Biotechnol. 2014 ;98:8337-46. [DOI: 10.1007/s00253-014-5919-9]
- 7) Abd El-Moez SI, Omara ST, Amer HA, Zaki FN. Antimicrobial activities of neem extract (Azadirachta indica) against microbial pathogens of animal origin. Glob Vet.2014;12(2):250-6. [DOI: 10.5829/idosi.gv.2014.12.02.82123]
- 8) Oliveira, F., Silva, E., Matias, A., Silva, J.M, Reis, RL and Duarte, A.R.C, Menthol-based deep eutectic systems as antimicrobial and anti-inflammatory agents for wound healing *Eur. J. Pharm. Sci.* 2023, 182,106363 [DOI:10.1016/j.ejps.2022.106368]
 - 9) Duda-Madej A, Viscardi S, Grabarczyk M, Topola E, Kozłowska J, Mączka W, Wińska K. Is Camphor the Future in Supporting Therapy for Skin Infections?. Pharmaceuticals. 2024;17(6):715. [DOI: 10.3390/ph17060715]
- 10) Ahuja, A., Gupta, J. and Gupta, R.,. Miracles of herbal phytomedicines in treatment of skin disorders: Natural healthcare perspective. Infectious Disorders-Drug Targets Disorders) 2021;21(3),328-338. [DOI: 10.2174/1871526520666200622142710]
- 11) El-Gohary M, van Zuuren EJ, Fedorowicz Z, Burgess H, Doney L, Stuart B, Moore M, Little P. Topical antifungal treatments for tinea cruris and tinea corporis. Cochrane Database Syst. Rev.2014(8). [DOI: 10.1002/14651858.CD009992.pub2]
- 12) Kumar SM, Kumar VA, Natarajan P, Sreenivasan G. Antifungal Efficacy and the Mechanical Properties of Soft Liners against Candida albicans after the Incorporation of Garlic and Neem: An: In vitro: Study. *J Int Soc* Prev Community Dent 2018;8(3):212-7. [DOI: 10.4103/jispcd.JISPCD_343_17]
- 13) Kowalska M, Woźniak M, Żbikowska A, Kozłowska M. Physicochemical characterization and evaluation of emulsions containing chemically modified fats and different hydrocolloids. Biomolecules. 2020;10(1):115. [DOI: 10.3390/biom10010115]
- 14) Jamshaid U, Anton N, Elhassan M, Conzatti G, Vandamme TF. Novel Hydrogels Based on the Nano-Emulsion Formulation Process: Development, Rheological Characterization, and Study as a Drug Delivery System.

Pharmaceutics.2024;16(6):812. [DOI: 10.3390/pharmaceutics16060812]

15) Kim Y, Park EJ, Kim TW, Na DH. Recent progress in drug release testing methods of biopolymeric particulate system. Pharmaceutics.2021;13(8):1313. [DOI:10.3390/pharmaceutics13081313]

Bulletin of Pharmaceutical Research 2025; 15 (S2)

An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print) 2249-9245 (Online)



EVALUATION OF THE SUSPENDING PROPERTIES OF LITSEA GLUTINOSA (MAIDA LAKDI) MUCILAGE IN PHARMACEUTICAL SUSPENSIONS. Anuradha G. More*, Atharv R. Adhav, Pooja S. Khaire, Vaishnavi V. Shirke Padmaia S. Kore

PES's Modern College of Pharmacy, Sec. No. 21, Yamunanagar, Nigdi, Pune 411044.

*Corresponding author email:anuradhagmore2011@gmail.com

Received: Feb 04, 2025 / Revised: April 16, 2025 / Accepted: May 01, 2025

Abstract

This study investigates $Litsea\ glutinosa\ mucilage$ as a natural suspending agent compared to acacia gum. The mucilage exhibited a pH of 7.5±0.05, swelling index of 71.42±1%, and loss on drying of 6.3±0.12%. Phytochemical tests confirmed the presence of carbohydrates, tannins, alkaloids, glycosides, and mucilage. Zinc oxide suspensions with $L.\ glutinosa\$ showed higher viscosity (1.23 poise) and lower flow rate (0.17 ml/sec) at 4% w/v than acacia (1.08 poise, 0.37 ml/sec). Sedimentation volume after 4 weeks was 0.40 for mucilage vs. 0.37 for acacia. These results highlight its effectiveness as a biocompatible, stable, eco-friendly suspending agent.

Keywords: Litsea Glutinosa, Suspending Agent, Mucilage

How to cite this article: Anuradha G. More, Atharv R. Adhav, Pooja S. Khaire, Vaishnavi V. Shirke Padmaja S. Kore Evaluation of the Suspending Properties of Litsea glutinosa (Maida Lakdi) Mucilage in Pharmaceutical Suspensions. Bulletin of Pharmaceutical Research. 2025;15(S2): 208-214.

Source of support: Nil Conflict of interest: None

Introduction

Litsea glutinosa is a medium-sized evergreen tree that can reach heights of approximately 20-30 feet. This tree belongs to the Lauraceae family. It thrives in mixed primary and secondary forests and thickets, is widely distributed, grows in various parts, and is also found in northern Australia. In India, the tree is mainly located in the Northeastern region. Litsea glutinosa is a versatile plant with a wide range of uses. The plant's fruit contains an edible, sweet, creamy pulp, while the young leaves serve as fodder. The mucilage extracted from Litsea glutinosa has been utilized in various applications, such as in coatings for container dividers. When mixed with water, this mucilage swells and forms a gel-like substance. The primary components of the mucilage include carbohydrates and uronic acids. These are hetero hydrogen polyuronides, known for their emulsifying, gelling, and suspending properties, which have been well documented in scientific literature [1]. The plant's essential

oils are reported to exhibit psychopharmacological effects. Additionally, from the plant is pharmaceutical formulations, where it serves as a binder in pellet formulations and aids in controlled drug release. The powdered seeds of Litsea glutinosa are also used medicinally, particularly for treating boils. Leaves of mucilage and bark are used in poultices, and the bark itself is recognized for its demulcent and mild astringent properties, making it useful in treating diarrhea and dysentery. Regarding pharmaceutical suspensions, such as a 50 ml volume stored in a tube and shaken for 24 hours, the system may show poor stability and is considered thermodynamically unstable. To address this, a suspending agent or stabilizer is necessary to enhance the redispersion of the suspension and reduce the settling rate. This can be achieved through protective colloidal action, which increases the viscosity of the suspending medium^[2]

Material and methods Plant material

Litsea glutinosa bark is collected from Pune. The pieces of bark are separated and dried in a hot air oven at 60°C. The powder is then ground in a machine-like grinder.

Isolation of Mucilage from Litsea glutinosa Bark

The extraction of mucilage was done using the ultrasonic probe sonication method. 10 grams of Litsea glutinosa bark powder were accurately weighed. Cleaned the plant material and ground it into a fine powder to increase the surface area for extraction. Poured 100 ml of distilled water into the powder. Placed the mixture in a heating bath for 1 hour at a constant temperature of 60°c. The mixture was exposed to direct ultrasound waves from a sonotrode for 20 minutes using an ultrasonic device (Probe sonicator PCI Analytics DP-20, India) with a frequency of 24 kHz and a power of 700 watts. To prevent a sudden rise in temperature, place the sample in an ice-insulated container, maintaining the temperature at around $35 \pm 2^{\circ}$ c. After sonication, heating was performed at 80°c for 10 min. Filtered the solution through several folds of muslin cloth and collected the filtrate. Finally, the mucilage was precipitated by 96% ethanol and dried under a freeze-dryer [3]

Physicochemical and Phytochemical Analysis

The mucilage's characterisation was done for relative solubility, swelling index, drying loss, and viscosity, per Indian Pharmacopoeia standards. A digital pH meter is used to check the pH of the mucilage sample. [4]. Various phytochemical tests were performed to identify proteins, carbohydrates, glycosides, flavonoids, alkaloids, and tannins in the isolated mucilage.

Preparation of Zinc Oxide Suspension

The 5 % Suspensions of zinc oxide were prepared with varying concentrations, i.e., 1%, 2%, and 4% w/v of suspending agent. A total of six batches with two suspending agents, Litsea glutinosa mucilage and acacia, were prepared

as shown in Table 1. A zinc oxide (ZnO) suspension is made by weighing 5 g of ZnO powder and then pre-wetting it with 1 mL of glycerin and a small quantity of water to create a smooth paste in a mortar and pestle. A suspending agent should be separately dissolved in warm distilled water while constantly stirring. The solution should then be cooled. To guarantee even dispersion, gradually add the ZnO paste to the CMC solution while stirring constantly. Pour distilled water to adjust the final volume, stir well, and pour the suspension into a measuring cylinder. Add a preservative and store it properly. [5,6].

Table 1. Formulation ingredients of the zinc oxide suspension

Ingredients	Composition(%w/v)
Zinc Oxide	5
Suspending agent	1,2 and 4
Methyl paraben	0.05
Glycerin	1
Distilled water q.s	100ml

Evaluation of Suspension

Physical Testing:

Over four weeks, the produced suspensions were monitored daily for phase changes, such as accumulation, smearing, and crystal formation [7].

Redispersibility:

The suspension was kept at room temperature in separate calibrated tubes. At specified intervals, the dispersibility of the sediment was assessed, and any deposits were noted. Observations were recorded over time [9].

Sedimentation Volume:

A 100 ml measuring cylinder was filled with 100 ml of the formulation and kept at room temperature to calculate the sedimentation volume. For the first seven days, the sedimentation volume (%) was measured daily; after that, it was measured every week for up to

four weeks. As the material fell from the top of the suspension, measurements were made when the clear supernatant appeared hazy. The following formula was used to determine the rate of sedimentation:

Vu is the Ultimate volume of sediment, and Vo is the original volume of suspension before settling.

Viscosity Measurement:

The suspensions viscosity was assessed 48 hours after preparation at 25°C using a Brookfield viscometer (model RVDV PRO II, Brookfield, USA) set at 100 rpm with spindle number 4. Measurements were performed in triplicate, and the average values were utilised for the results [8].

Flow Rate Determination:

The flow rate was determined by timing how long it takes 10 ml of suspension to traverse a 10 ml pipette. The flow rate was calculated using the equation:

Table 2. Physiochemical analysis of the mucilage of Litsea Glutinosa

Test	Observation
Colour	Brown
Odour	Characteristic
Texture	Slightly rough
Solubility	Soluble in water, forming a
	gel-like or viscous solution
Swelling	71.42±1%
Index	
рН	7.5±0.05
Loss on	6.3±0.12%
Drying	

Here, $v \square$ represents the sample volume (in ml) and T is the time (in seconds) required for the suspension to flow through the pipette. The

average of three measurements was consistently noted.

Swelling Index:

1 gram of powdered mucilage was placed in a 25 ml measuring cylinder to measure the swelling index. Fresh distilled water (25 ml) was added, and the mixture was gently shaken every 10 minutes for an hour. After that, it was left undisturbed at room temperature for three hours. The swelling index was then determined using the formula:

Xo represents the powder's initial height in the cylinder, and Xt is the height of the swollen gum after 24 hours [4].

RESULTS AND DISCUSSION

Physicochemical and Phytochemical Analysis

itsea glutinosa mucilage was isolated, and its physicochemical and phytochemical properties were assessed. Table 2 demonstrates that the mucilage was brown, had a distinct smell, had a slightly gritty texture, and was soluble in water, forming a gel-like solution. It had a pH of 7.5±0.05, a swelling index of 71.42±1%, and a drying loss of 6.3±0.12%. Carbohydrates, tannins, alkaloids, glycosides, and mucilage were found in phytochemical analysis (Table 3), but flavonoids were not. The findings suggest that the mucilage's favourable physicochemical characteristics and the inclusion of bioactive elements make it a stable, biocompatible substance with potential medicinal uses.

Table 3. Phytochemical analysis of the mucilage of Litsea glutinosa

Suspendi	Concentrati	Flo	Viscosit
ng agent	on (%w/v)	W	y
		Rat	
		e	
	1	1.13	0.85
Acacia	2	0.56	0,97
	4	0.37	1.08
	1	0.55	0.98
Litsea glutinosa mucilage	2	0.32	1.12
	4	0.17	1.23

Tests	Observation
Test for carbohydrates	Positive
(Molisch's test)	
Test for tannins (Ferric	Positive
chloride test)	
Test for alkaloids	Positive
(Wagner's test)	
Test for glycosides	Positive
(Keller Kiliani test)	
Test for mucilage	Positive
(Ruthenium red test)	
Test for flavonoids	Negative Negative
(Shinoda test)	

Table 4. Viscosity (poise) and flow rates (ml/sec) of ZnO suspensions prepared at different concentrations of Litsea glutinosa mucilage and Acacia at room temperature

The flow rate and viscosity of ZnO suspensions prepared using Litsea glutinosa mucilage and

acacia as suspending agents were evaluated and are presented in Table 4. Results showed that concentrations (1-4%)increased viscosity while the flow rate decreased for both agents. At equivalent concentrations, Litsea glutinosa mucilage demonstrated higher viscosity and lower flow rates than acacia, indicating superior ability. Notably, suspending at 4% concentration, the mucilage exhibited a viscosity of 1.23 poise and a flow rate of 0.17 ml/sec, suggesting its potential as an effective natural suspending agent in pharmaceutical suspensions.

Evaluation of Suspensions

Sedimentation Volume:

The sedimentation volumes of ZnO suspensions prepared with Litsea glutinosa mucilage and acacia at varying concentrations are shown in **Table 5**. Both suspending agents showed a decrease in sedimentation volume over time; however, Litsea glutinosa mucilage exhibited better sedimentation stability compared to acacia at corresponding concentrations. At 4% maintained w/v, mucilage higher sedimentation volume (0.40 at 4 weeks) than acacia (0.37), indicating superior suspending properties. This enhanced performance may be attributed to the mucilage's higher viscosity and swelling capacity. These findings support the potential of Litsea glutinosa mucilage as an effective natural alternative to synthetic suspending agents.[5]

Table 5. Sedimentation Volume of c from Litsea glutinosa and Acacia at different concentrations

Sedimentation Volume												
Suspending agent	Concentration (%w/v)		Time (in days)							Time (in weeks)		
		1	2	3	4	5	6	7	2	3	4	
	1	1	0.87	0.83	0.78	0.74	0.68	0.65	0.50	0.32	0.22	
Acacia	2	1	0.90	0.86	0.80	0.78	0.72	0.68	0.62	0.48	0.34	
	4	1	0.93	0.89	0.85	0.80	0.80	0.74	0.68	0.50	0.37	
	1	1	0.90	0.86	0.80	0.73	0.67	0.61	0.50	0.38	0.25	
Mucilage	2	1	0.92	0.88	0.83	0.76	0.69	0.63	0.52	0.40	0.34	
	4	1	0.95	0.90	0.84	0.76	0.70	0.63	0.53	0.44	0.40	

Viscosity and Flow Rate

Suspensions are inherently unstable dosage forms due to the tendency of dispersed solid particles to settle over time, leading to sedimentation and potential caking. Enhancing the viscosity of a suspension can mitigate this issue by slowing particle settling, thereby improving formulation stability. However, increased viscosity also reduces pourability, necessitating a thorough evaluation of viscosity to optimize suspension performance [13].

In this study, the viscosity of various suspensions was assessed, with the results summarized in Table 4. A direct correlation was observed between the concentration of the suspending agent and the viscosity, where higher concentrations resulted in increased viscosity. Suspensions formulated with Litsea glutinosa mucilage and acacia exhibited distinct viscosity characteristics. It is noteworthy that zinc oxide (ZnO) suspensions formulated with Litsea glutinosa mucilage exhibited consistently higher viscosity than those containing acacia across all tested concentrations (1%, 2%, and 4%).

Flow rate measurements, conducted using standardized methods as described in the methodology section, indicated an inverse relationship between viscosity and flow rate. As the concentration of Litsea glutinosa mucilage or acacia increased, the viscosity of the suspension increased correspondingly, leading to a gradual reduction in flow rate.

pH Measurement

The pH of a suspension is a crucial factor influencing drug stability and compatibility, as it dictates the formulation's acidic or basic

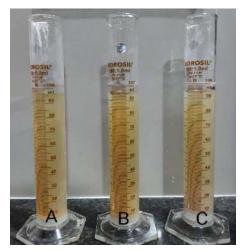
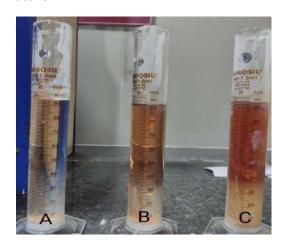


Figure 1. Sedimentation Volume of Litsea glutinosa A(1%), B (2%) and C(4% w/v) nature.



The pH analysis of the prepared suspensions revealed values ranging from 7.1 to 8.6, signifying a near-neutral to mildly basic environment. Specifically, suspensions incorporating *Litsea glutinosa* mucilage exhibited pH values between 7.4 and 8.3, indicating their potential suitability for pharmaceutical applications.

Furthermore, the pH stability of ZnO formulated suspensions with Litsea mucilage and glutinosa acacia concentrations of 1%, 2%, and 4% (w/v) was evaluated over four weeks. The findings indicated minimal pH variations across all formulations, suggesting excellent stability. These results highlight the potential of *Litsea glutinosa* mucilage as a

promising pharmaceutical excipient, even at lower concentrations.

Redispersibility

For a suspension to be effective, it should easily redisperse after shaking so that the medication is evenly distributed. If a suspension remains settled even after shaking, it is considered "caked" and unsuitable for use [11]

In this study, all formulated suspensions were tested for redispersibility after being stored for 45 days. They were all found to be easily redispersible, though the time required varied. Suspensions made with *Litsea glutinosa* needed more shaking than those formulated with acacia.

Additionally, suspensions with lower concentrations of suspending agents (which have lower viscosity) were quicker to redisperse than those with higher concentrations (higher viscosity). This is likely because higher viscosity makes it harder for particles to move freely. Overall, the suspensions maintained consistent redispersion behavior across all tested suspending agents.

CONCLUSION

The present study highlights the potential of Litsea glutinosa mucilage as a natural agent in pharmaceutical suspending suspensions. Comparative evaluation with acacia gum revealed that L. glutinosa offers comparatively mucilage sedimentation volume, redispersibility, viscosity, and good physical stability. It has a origin, biocompatibility. natural promising suspending properties. glutinosa mucilage presents a viable, ecoalternative to conventional suspending agents in oral pharmaceutical formulations. Further studies on its longterm stability and compatibility with various

drugs could support its broader application in the pharmaceutical industry.

ACKNOWLEDGEMENT

The authors are grateful to P.E.S.'s Modern College of Pharmacy, Nigdi, for providing a research facility to complete the research work.

REFERENCES

- 1) Ghule BV, Darwhekar GD, Jain DK, Yeole PG. Evaluation of Binding Properties of Evaluation of Binding Properties of Eulophia campestris Wall. Mucilage all. Mucilage. Indian J. Pharm .Sci. 2006 Sep:566.
- 2) Woolfe ML, Chaplin MF, Otchere G. Studies on the mucilages extracted from okra fruits (*Hibiscus esculentus L.*) and baobab leaves (*Adansonia digitata L.*). *J. Sci. Food Agri.* .1977 Jun;28(6):519-29.
- 3) Chung SJ, Leonard JP, Nettleship I, Lee JK, Soong Y, Martello DV, Chyu MK. Characterization of ZnO nanoparticle suspension in water: Effectiveness of ultrasonic dispersion. Powder Technology. 2009 Aug 25;194(1-2):75-80.
- 4) Chaudhary A, Sharma A, Rana R. Mucilage: Isolation, characterisation and sources. *Int. J. Phytopharm.* Res. 2014;5:139-43
- 5) Okorie O, Nwachukwu N. Evaluation of the suspending properties of *aloe barbadensis* (Aloe vera) gum in pharmaceutical suspensions. *Int. J. Pharm. Sci, Rev. Res..* .2011;6(2):14-7.
- 6) Mengesha Y, Tuha A, Seid Y, Adem AA. Evaluation of *Aloe weloensis* (Aloeacea) mucilages as a pharmaceutical suspending agent. Advances in pharmacological and pharmaceutical sciences. 2021;2021(1):6634275.
- 7) R. Kumar, N. Rajarajeshwari, and V. B. N. Swamy. Isolation and evaluation of Borassus flabellifer mucilage as a natural suspending agent," *Int. J. Pharmatech. Res.* vol. 4, no. 4, pp. 1614–1630, 2012.

- 8) Senthil V, Sripreethi D. Formulation and evaluation of paracetamol suspension from *Trigonella foenum graecum* mucilage. *J Adv Pharm Educ Res.* 2011;1(5):225-33.
- 9) Mishra SK, Kumar A, Talukdar A. Evaluation of binding property of mucilage from *Litsea glutinosa* wall. Pharmacognosy research. 2010 Sep;2(5):289.
- 10) Deogade UM, Deshmukh VN, Sakarkar DM. Natural gums and mucilage's in NDDS: applications and recent approaches *Int, J. Pharmatech. Res.* vol. 4, no. 2, pp. 799–814, 2012.
- 11) Nayak AK, Pal D, Pradhan J, Ghorai T. The potential of *Trigonella foenum-graecum* L. seed mucilage as suspending agent. *Indian. J. Pharm. Educ. Res.* 2012 Mar 27;46(3):12-7.

Bulletin of Pharmaceutical Research 2025; 15 (S2)

An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print) 2249-9245 (Online)



RESEARCH ARTICLE

DRUG SOLUBILITY, IMPORTANCE AND ENHANCMENT TECHNIQUES.

Ujwala S. Desai^{1*}, Srushti S. Waghmare¹, Ranjit A. Jamadade ¹, Sharvari P. Shendage¹

¹P.E. S's Modern College of Pharmacy, Nigdi, Maharashtra, India-411044. *Corresponding author email: <u>ujudesai@gmail.com</u> Received: Feb 04, 2025 / Revised: April 16, 2025 / Accepted: May 01, 2025

ABSTRACT

Many medications do not dissolve well in water, which makes it harder for them to be absorbed by the body and reduces their effectiveness. To solve this problem, scientists use various techniques to improve drug solubility and absorption. These include adding substances that help drugs dissolve better (like hydrotropes and co-solvents), using nanotechnology, forming salts or co-crystals, and developing new solubilizing agents. Other methods include making drug particles smaller, mixing them with carriers to form solid dispersions, and using surfactants in systems like microemulsions or self-emulsifying mixtures. Advanced techniques like supercritical fluid processing and liquisolid systems also help by increasing surface area, stabilizing the drug in a more absorbable form, and improving how the drug interacts with its carrier. These approaches make it easier for the body to absorb the drug, leading to better treatment results across different types of medications. This review aims to highlight the main strategies used to improve drug solubility, making medications more effective and reliable for patients.

Key words: Solubility Enhancement, Nanotechnology, Drug Formulation, Co-crystallization. **How to cite this article:** Ujwala Desai, Srushti S. Waghmare, Ranjit A. Jamadade, Sharvari P. Shendage Drug Solubility, Importance and Enhancement Techniques Bulletin of pharmaceutical research2025. 2025;15(S2):215-221.

Source of support: Nil Conflict of interest: None

INTRODUCTION

The limited solubility of numerous drugs in water complicates their formulation, hindering the ability to achieve high drug loading and rapid dissolution, which presents a major barrier to effective clinical application. Around 40% of newly discovered chemical entities are poorly water-soluble due to their lipophilic nature, making it difficult to deliver them at therapeutic doses. When administered orally. these compounds often show slow dissolution, erratic absorption, and low bioavailability. Over recent decades. identification of many pharmacologically potent but poorly soluble molecules has further challenged the development of effective oral drug formulations. Among various approaches, formulating drugs in an amorphous state has shown promise in improving solubility and bioavailability [1].

This review offers a strategic and up-to-date resource for formulation scientists, focusing on innovative and practical approaches to overcome solubility-related hurdles and enable the successful development of next-generation

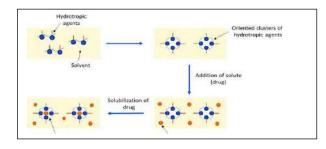
TECHNIQUES FOR SOLUBILITY ENHANCEMENT

- Chemical Modification
 - Physical Modification
 - Miscellaneous Methods

1. CHEMICAL MODIFICATION

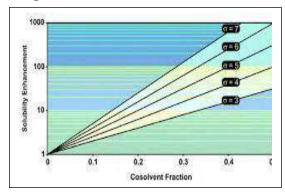
1.1 Hydrotrophy:

The method of enhancing the solubility of a solute in water by introducing a large amount of a second solution is referred to as hydrotrophy. Complexation is a process that directly improves solubility by using weak interactions between hydrotropic chemicals such as sodium benzoate, sodium acetate, sodium alginate, urea, and weakly soluble drugs. Organic salts with an ionic character are known as hydrotropic agents. [2].



1.2. Co-solvency:

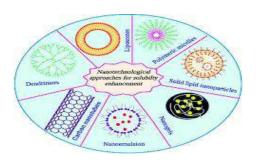
Co-solvents are solvents that blend effectively with water and in which the drug is highly soluble; they can be utilized to enhance the solubility of medications that are poorly soluble in water. Co-solvents, which help enhance the solubility of poorly soluble compounds, consist of a combination of one or more solvents that mix well with water alongside water itself.



Co-solvent methods work well with crystalline, lipophilic, or poorly soluble molecules, particularly those that exhibit significantly greater solubility in the mixed solvent solution. Co-solvents can significantly improve the solubility of weakly soluble compounds, frequently by thousands of times in comparison to the drug's solubility in pure water. Dimethyl sulfoxide (DMSO) and dimethylacetamide (DMA) are commonly employed as co-solvents due to their low toxicity levels and capacity to dissolve difficult medicines [4].

1.3. Nanotechnology:

In nanotechnology, materials and structures at the nanoscale (at least 100 nanometres, or nm) are studied and used. Since many Novel Chemical Entities (NCEs) with poor solubility do not benefit enough from the increase in oral bioavailability that micronization provides, and because micronized products have a limited effective surface area for dissolving, nanonization was the next breakthrough. High-temperature evaporation, vacuum deposition, high-pressure homogenization, and sonication are among the preliminary methods that can be applied [5].



Advantages:

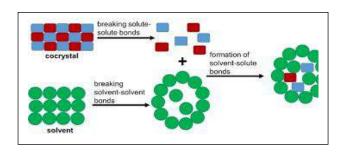
In order to increase solubility and the pace of dissolution, it creates spherical, smooth-surfaced particles with greater specific surface areas and narrow particle size distributions. ^[6].

1.4. Salt Formation:

A number of instability problems frequently prevent an active pharmaceutical ingredient (API) in its most stable form during manufacturing. This leads to the creation of salts, cocrystals, concretes, hydrates, and polymorphs. For therapeutic candidates that are poorly soluble, salt production has proven advantageous, particularly for weak acids and bases. When a material dissociates in a solution, salts are created. This process works well for solid dosage forms, as well as parenteral and other liquid formulations. [7].

1.5. Co-Crystallization:

Molecular interactions are impacted by cocrystallization, and this can be used to enhance medicinal effects. A multi-component crystal made of two solid substances at room temperature, with at least one of the substances being an appropriate ion or molecule, is called a co-crystal.



How to Create Molecular Crystals to Increase Solubility A common goal in the production of molecular crystals for use as crystalline pharmaceutical materials is to make medications more soluble and dissolve more quickly. Because of this, crystals interacting with the surrounding medium need to have their surface and molecular packing changed Polymorphic forms that are unstable or metastable and retain their molecular structure tend to be more soluble.^[7]

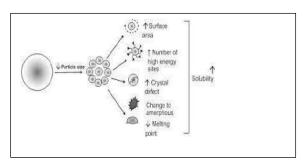
1.6. Utilizing a new solubilizer:

Different solubilizing chemicals can improve the solubility of medications that are not very soluble. For instance, traditional solubilizers like polysorbates, Hydrophobic active pharmaceutical ingredients (APIs) can be made more soluble with the help of dendrimers, Povacoat, Sepitrap, and Soluplus.^[8]

2. PHYSICAL MODIFICATION

2.1. Particle Size Reduction

Particle size reduction is accomplished by milling techniques using colloid mills, jet mills, etc. It is not appropriate for medications with a high dose number since the drug's saturation solubility remains unchanged. Drug particle size is frequently related to bioavailability of poorly soluble drugs. Drug particle size reduction is accomplished by nanosuspension & micronization. The drug's ability to dissolve is enhanced by increasing surface area.^[8]



2.2. Micronization

The bioavailability of a medicine is inherently correlated with its particle size. Increased surface area enhances the drug's dissolving properties by helping the particles get smaller. Because micronization increases surface area, it speeds up the pace at which pharmaceuticals dissolve.

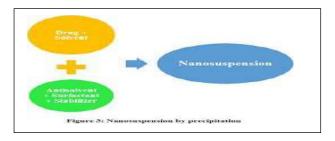
Drugs are micronized using milling processes such as colloid mills, jet mills, etc. It doesn't change the drug's saturation solubility, so it's not appropriate for drugs that require high dosages.^[9]

Disadvantages

• In humid, heated storage conditions, disordered/amorphous regions are susceptible to recrystallization due to thermodynamic instability. [10]

2.3. Nanosuspension:

Poorly soluble medications that cannot be dissolved in water or oil are handled with this technique. A biphasic system called nanosuspension contains nanoparticles in an aqueous medium. Surfactant stabilizes medication particles of nanosize for oral, tropical, and parenteral delivery as well as pulmonary usage.



In nano suspension, the solid particle size distribution is usually less than one micron. The typical range of particle sizes is between 200 and 600 nm. This procedure was used for buparvaquone, amphotericin &paclitaxel, atovaquone, and tarazepide. Nanocrystals, DissoCubes, Nanopores, and Nanoedges are some of the different techniques used to make nanosuspensions.[11]

2.4 Modification of Crystal Habit

- a) Polymorphs
- b) Pseudo polymorphs

Polymorphism is the capacity of a solid material to exist in two or more different crystalline forms with different configurations in the crystal lattice. Polymorphs are distinct crystalline formations. The physiochemical properties of drugs in crystalline form, including their melting point, texture, density, solubility, and stability, differ while having the same chemical composition. Similarly, amorphous medications are more appropriate than crystalline ones. due to the greater surface area and higher associated energy.^[12]

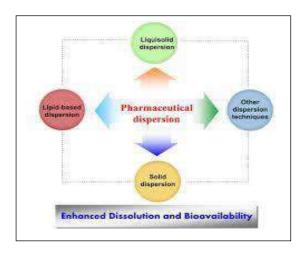
2.5 Drug Dispersion In Carrier

• Solid solution: Two crystalline solids are

combined to create a new crystalline solid. In a homogeneous one-phase solution, two components crystallize together to form a mixed crystal. It dissolves much more quickly than a basic digestive system.^[12]

• Solid dispersion: Solid dispersion was first conceptualized by Sekiguchi and Obi..

Solid dispersion is a useful pharmaceutical technique for accelerating a drug's rate of absorption. solubility. and therapeutic effectiveness. The term "solid dispersion" refers to a family of solid products including a hydrophilic matrix and a hydrophobic drug. polyvinyl pyrrolidone, Polyethylene glycols plasdone-S630 and are examples hydrophilic carriers that are frequently utilized. The creation of solid dispersions frequently involves the application surfactants. [13]



2.6 Solubilization By Surfactants

• Microemulsion: A microemulsion, which dissolves a drug that is poorly soluble in water, is an optically clear, transparent, thermodynamically stable. isotropic translucent system composed of a hydrophilic solvent, oil, and surfactant. The criteria for choosing a surfactant are HLB and nontoxicity. In the presence of water, the formulations self-emulsify, forming a very transparent emulsion of uniformly small oil droplets containing the solubilized medication, which is poorly soluble. In addition to adding proteins for oral, parenteral, and intravenous delivery, microemulsions have been employed to make several medications that are nearly insoluble in water more soluble. Oil-in-water (o/w)microemulsions the best are formulation because they dissolve compounds

with poor water solubility into molecules with high oil phase solubility.[14]

Advantages

• Most of the time, digestion has no bearing on the release of medications from welldeveloped microemulsion pre-concentrates. Consequently, you can anticipate maximum bioavailability and repeatability without the necessity for meal co-administration.^[15]

Disadvantaaes

• Formulations with multiple components are more challenging to validate.[16]

Self-emulsifying drug delivery system: Oil, surfactant, co-surfactant, and one or more hydrophilic solvents are combined to create a transparent isotropic solution when there is no external phase (water). One kind of solution that has the capacity to emulsify itself is the self-emulsifying solution. Another name for it given by some researchers is "microemulsion pre-concentrate." These novel colloidal compositions react similarly to oil-in-water microemulsions when ingested orally. [17]

2.7 Complexation

Drugs and cyclodextrins have been complexed to improve the stability and water solubility of the former. The most popular β-cyclodextrin derivatives that have improved water solubility are used in pharmaceutical formulations. With molecular weights of 1000 Da, cyclodextrins are large molecules that have a hard time getting through the skin. Skin penetration has been seen to both increase and decrease because cyclodextrin of complexation. CDs can be employed to increase membrane permeability and stabilize membranes in addition to increasing solubility. When cyclodextrins are present, biological membranes become more permeable. CDs can also be used to improve permeability in pulmonary medication delivery systems.[18]

3. MISCELLANEOUS METHODS

3.1 pH Adjustment:

The inability of many pharmacological drugs to dissolve in water hinders their proper absorption by the body. Changing the pH of the drug's surroundings to increase its solubility is one way to address this issue. By changing the

pH, medications that typically have problems dissolving in water can become soluble. To prevent any potential issues, it is crucial to consider the buffer capacity—the solution's resistance to pH changes—as well as the pH level tolerance when employing this procedure.

For instance, weak acidic medications may become more soluble when alkalizing agents (substances that elevate pH) are used. Drugs that are weakly basic are made more soluble by using acidifying agents, which are chemicals that lower pH Additionally, this method can assist increase the solubility of crystalline and lipophilic (fat-soluble) medications, which are frequently poorly soluble in water. [19]

Advantages of pH Adjustment:

- straightforward to formulate and analyze pH modifications are straightforward to test and optimize because they don't require complicated formulas.
- Low chemical usage: Due to its minimal chemical requirements, it is ideal for high-throughput testing, which rapidly tests a large number of formulations. [20]

Disadvantages of pH Adjustment:

Problems with tolerance and toxicity: When medications are taken at a pH level that is far outside of the body's normal range, it may irritate the area where they are delivered locally or result in systemic toxicity. This is especially dangerous for intravenous medications because the pH of blood is normally between 7.2 and 7.4, and any large departure could result in negative reactions.

3.2 Supercritical Fluid (SCF) Technology in Drug Formulation

Supercritical fluids (SCFs) are special materials that exhibit both liquid and gas-like properties when exposed to pressures and temperatures. A common SCF is carbon dioxide (CO_2), which could dissolve non-volatile substances when it reaches temperatures and pressures higher than its critical values.

What are Supercritical Fluids?

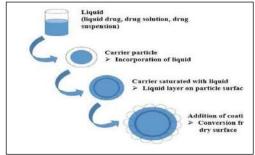
SCFs fall somewhere in the middle between pure liquids and gases. Because of their adaptability, SCFs are perfect solvents for a variety of uses. For example, SCFs' solvent power can be precisely adjusted to dissolve or precipitate particular materials by varying the temperature or pressure. Among pharmaceutical processes, carbon dioxide, water, ethanol, ammonia, nitrous oxide, ethylene, propylene, and n-pentane are the most often utilized SCFs. [22]

Advantages of SCF Technology:

- Micronization Precision: Due to the tremendous flexibility and precision of SCF methods, medication particles can be produced in extremely small size ranges, frequently down to the submicron level. This can greatly increase the bioavailability of medications that are poorly soluble.
- Nanoformulations: Current SCF techniques have produced nanoparticle suspensions ranging in diameter from 10 to 100 nm, which is a promising use for medications that require improved solubility and quicker absorption. [23]

3.3 Liquisolid Methods

Liquidsolid technology is the process of converting liquid medications particularly those that are poorly soluble into a dry, freeflowing, compressible powder. This is accomplished by combining excipients, such as coating and carrier materials, with liquid medication. Typically, the carrier materials consist of silica powders, amorphous cellulose, and microcrystalline cellulose. Adsorption and absorption are the two main phases in the process. The liquid medicine is first taken up by the porous carrier particles' interior, where it is trapped within their structure. The technique produces a non-adherent, easily flowable, and compressible powder that is perfect for turning liquid or oil-based medications into solid dosage forms. This is especially advantageous when making liquid or oily pills that are simple to handle and process.[24]



Advantages of Liquisolid Methods:

• Ideal for powdered liquid medications: This system is made especially to handle and process liquid medications that must be ground into powder to facilitate formulation.

Disadvantages of Liquisolid Methods:

• Requires specialized carrier properties: Not all carrier materials are appropriate for every medicine because the approach depends on carriers with strong adsorption capabilities and particular surface area characteristics. [25]

Conclusion

The poor water solubility of many modern drug candidates remains a significant challenge in pharmaceutical development, particularly for oral formulations. As outlined in this review, a wide array of chemical, physical, and novel miscellaneous techniques have been developed to address this issue. Strategies such as hydrotropy, co-solvency, nanotechnology, salt formation, and cocrystallization offer chemical means to solubility, while enhance physical modifications like particle size reduction, solid dispersion, and surfactant-based systems further support dissolution and bioavailability improvements. Advanced technologies, including supercritical fluid processing and liquisolid methods, provide precise control over particle characteristics and enable the transformation of poorly soluble drugs into more effective therapeutic forms. Each method presents unique advantages and limitations, and the selection of the appropriate strategy depends on the specific drug's properties, desired dosage form, and intended route of administration. This comprehensive overview serves as a practical and strategic resource for formulation scientists, guiding the rational design and optimization of solubilityenhancing approaches in modern drug development.

REFERENCE:

- 1. Yongju He, Shuquan Liang, & Mengqiu Long. Mesoporous silica nanoparticles as potential carriers for enhanced drug solubility of paclitaxel. Materials Science and Engineering C, 2017;1(78):12-17.
- 2. Nidhi K, Indrajeet S, Khushboo M, Gauri K and

- Sen DJ. Hydrotropy: A promising tool for solubility enhancement. International Journal of Drug Development & Research, 2011; 3(2): 26-33.
- 3. Kadam SV, Shinkar DM and Saudagar RB. Review on solubility enhancement techniques. International Journal of Pharmacy and Biological Sciences, 2013; 3(3): 462-575.
- 4. Shi H, Xie Y, Xu J, Zhu J, Wang C and Wang H. Solubility enhancement, solvent effect and thermodynamic analysis of pazopanib in cosolvent mixtures. The Journal of Chemical Thermodynamics, 155, p.106343.
- Chopra, H., Bibi, S., Islam, F., Ahmad, S.U., Olawale, O.A., Alhumaydhi, F.A., Marzouki, R., Baig, A.A. and Emran, T.B., 2022. Emerging trends in the delivery of resveratrol by nanostructures: applications of nanotechnology in life sciences. Journal of Nanomaterials, 2022, pp.1-17.
- 6. Tran, P., Pyo, Y.C., Kim, D.H., Lee, S.E., Kim, J.K. and Park, J.S., 2019. Overview of the manufacturing methods of solid dispersion technology for improving the solubility of poorly water-soluble drugs and application to anticancer drugs. Pharmaceutics, 11(3), p.132.
- 7. Repka MA, Bandari S, Kallakunta VR, Vo AQ, McFall H and Pimparade MB. Melt extrusion with poorly soluble drugs an integrated review. International journal of pharmaceutics, 535(1-2), pp.68-85.
- 8. Loh ZH, Samanta AK, Heng PWS. Overview of milling techniques for improving the solubility of poorly water-soluble drugs. Asian journal of pharmaceutical sciences, 2015; 10(4): 255-274.
- 9. Kim KN, Son JH, Kim HS. Solution solubilization composition of insoluble material and method for solubilizing insoluble material using same. Google Patents. 2019.
 - 10.Kasimedu S, Thoppani SR, Pommala N, Orugonda G, Yelamanda J. A Review on Solubility Enhancement Techniques. J. Compr. Pharma, 2015; 2(2): 36-41.
- 11. Singh N, Allawadi D, Singh S, Arora S. Techniques for Bioavailability Enhancement of BCS Class II Drugs: A Review. International Journal of Pharmaceutical and Chemical Sciences, 2013; 2(2): 1092-1101.
- 12. Kim KN, Son JH, Kim HS. Solution solubilization composition of insoluble material and method for solubilizing insoluble material using same. Google Patents. 2019.

- 13. Mohit, Hussain MS. A Brief Review on Buccal Drug Delivery System: Advantages, Limitations, and Impact on Healthcare system. World Journal of Pharmaceutical Research, 2021; 10(5): 558-576.
 - 14. Hussain MS, Mohit, Kaur G, Pamma P. Overview of Controlled Drug Delivery System. Advances in Bioresearch, 2021; 12(3): 248-255.
 - 15. Acharya DP, Hartley PG. Progress in microemulsion characterization. Current Opinion in Colloid & Interface Science, 2012; 17(5): 274-280.
 - 16. Nigade PM, Patil SL, Tiwari SS. Self-Emulsifying Drug Delivery Systems (SEDDS): A Review. International Journal of Pharmacy and Biological Sciences, 2012; 2(2): 42-52.
 - 17. Dhapte V and Mehta P. Advances in Hydrotropic Solutions: An Updated Review. St. Petersburg Polytechnical University Journal: Physics and Mathematics, 2015; 1: 424-435.
 - 18. Young OA, Zhang SX, Farouk MM and Podmore C. Effects of pH adjustment with phosphates on attributes and functionalities of normal and high pH beef. Meat Science, 2005; 70: 133-139.
 - 19. McMorland, G.H., Douglas, M.J., Jeffery, W.K., Ross, P.L., Axelson, J.E., Kim, J.H., Gambling, D.R. and Robertson, K., 1986. Effect of pH-adjustment of bupivacaine on onset and duration of epidural analgesia in parturients. Canadian Anaesthetists' Society Journal, 33, pp.537-541.

- 20. Jain A, Ran Y and Yalkowsky SH. Effect of pH-sodium lauryl sulfate combination on solubilization of PG-300995 (an anti-hiv agent). AAPS PharmSciTech., 2004; 5(3): 45-48.
- 21. Abuzar, S.M., Hyun, S.M., Kim, J.H., Park, H.J., Kim, M.S., Park, J.S. and Hwang, S.J., 2018. Enhancing the solubility and bioavailability of poorly water-soluble drugs using supercritical antisolvent (sas) process. International journal of pharmaceutics, 538(1-2), pp.1-13.
- 22. Campardelli R, Baldino L and Reverchon A. Supercritical fluids applications in nanomedicine. The Journal of Supercritical Fluids, 2015; 101: 193-214.
- 23. Javadzadeh Y, Jafari-Navipour B and Nokhodchi A. liquisolid technique for dissolution rate enhancement of a high dose water insoluble drug (carbamazepine). International journal of pharmaceutics., 2007; 341: 26-34.
- 24. Kulkarni AS, Aloorkar NH, Mane MS and Gaja JB. Liquisolid systems: a review. International Journal of Pharmaceutical Sciences and Nanotechnology, 2010; 3(1): 795-802.
- 25. Lu M, Xing H, Jiang J, Chen X, Yang T and Wang D. Liquisolid technique and its applications in pharmaceutics. Asian Journal of Pharmaceutical Sciences, 2017; 12: 115-123

An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print) 2249-9245 (Online)



RESEARCH ARTICLE

Evaluation of Dispensed Prescription Errors and Drug-Drug Interactions at Community Pharmacy.

Rajani Baban Mali*, Om Suhas Kinhikar, Tabassum Shaikh

PES's Modern College of Pharmacy , Sec. No. 21 , Yamunanagar, Nigdi , Pune 411044 *Corresponding author email: malirajni29@gmail.com Received: Feb 07, 2025 / Revised: April 19, 2025 / Accepted: May 05, 2025

ABSTRACT

Background: Prescription errors and drug-drug interactions are significant concerns in healthcare, potentially leading to adverse drug reactions, medication errors, and compromised patient safety. Aim: This study aimed to detect and identify prescription errors and drug interactions in outpatient prescriptions at a community pharmacy. Methods and Materials: A cross-sectional study was conducted at a community pharmacy, evaluating 150 randomly selected prescriptions over 5 months. Patients who visited the pharmacy during the study period were provided prescriptions, which were analysed using a list of parameters, including Prescriber information, Medication information, and Patient details. Results: According to the survey, a doctor's signature was present in 89.3% of cases. The patients' names and date of consultation were included in 94% and 98% of the prescriptions, respectively. A history and diagnosis were included in only 0% and 1.3 % of prescriptions, respectively. A total of 98.6% and 98% of the cases had prescriptions that mentioned dose and dosage forms, respectively. Antibiotics were prescribed for 69.3% of the prescriptions. In 7.3% of cases, possible drug-drug interactions have been documented. Conclusion: This study highlights the need for precise monitoring of prescription writing to analyse errors and prevent drugdrug interactions. One of the other parameters is the lack of prescription; thus, reducing prescription errors by Prescriber Training and Integration of Digital Prescription Systems is crucial for improving patient safety and quality of life.

How to cite this article: Dr. Rajani Baban Mali, Om Suhas Kinhikar, Tabassum Shaikh Evaluation of Dispensed Prescription Errors and Drug-Drug Interactions at Community Pharmacy. Bulletin of Pharmaceutical Research. 2025;15(S2): 222-226.

Source of support: Nil Conflict of interest: None

1. INTRODUCTION:

A prescription is a medicolegal document written by an authorized individual for patient treatment, it is an indication of the standard of he alth treatment the patient is receiving. [1]

The prescription includes comprehensive details about the patient, physician, and medications. According to the World Health Organization's "Guide to Good Prescribing" practical guideline, must include the following criteria in

address, registration number, and signature; and (c) Medication information: brand or generic name, dosage form, route, dosage, unit, frequency, duration of treatment, quantity, and usage instructions.^[2]

Patients who receive pharmaceuticals that are appropriate for their clinical needs, in doses that satisfy their own specific requirements, for enough time, and at the lowest cost to them and their community are said to be engaging in rational use of medicines (RUM).^[3]

prescription errors result from mistakes, breach, oversights, or slip-ups, like prescribing a dose that is overly high or inappropriately low.[4] Community pharmacies frequently deal with prescription errors, which are a subset of pharmaceutical errors. Prescription error rates are thought to range from as low as 0.23% to as high as 11% of the more than 3.5 billion prescriptions that community pharmacies get each year. Errors in prescriptions encourage irrational drug usage and lower patient compliance.[5]

Irrational prescribing, including misuse, overuse, and underuse of medications, poses significant risks such as unsafe treatment, disease progression, adverse health outcomes, economic burdens on patients, and resource wastage, ultimately reducing the likelihood of timely and effective therapy. Common examples of irrational drug use include the inappropriate use of antimicrobials for non-bacterial infections, polypharmacy, incorrect dosing, unnecessary administration of injectables when oral alternatives are available, self-medication, and non-adherence to prescribed dosing regimens. [6,7]

Prescribers have a legal responsibility to make their prescriptions readable. Errors in documentation are common in conventional handwritten prescription writing. In certain cases, a prescriber has been guilty of provoking a patient's death by writing an illegible prescription.^[8]

Markers for the prescription's comprehensibility and legibility include the percentage of prescriptions that have readable handwriting, allergies, a brief history, a final diagnosis, and clinical examinations and the percentage of prescriptions that contain vitamins, tonics, or enzymes and Antibiotic [9]

DDIs are defined as a significant reduction of potency or efficacy of drugs by combining two or more drugs for patients. Drug interaction results in toxic effects or therapeutic failure. DDIs cover 6–30% of all adverse drug events. Drug interactions lead to toxicity or therapeutic failure. [10]

This study aimed to assess the quality of outpatient department (OPD) prescriptions at a community pharmacy by investigating

prescription errors, legibility, and drug interactions based on the prescription audit criteria set by the Indian Ministry of Health and Family Welfare, while also evaluating adherence to the World Health Organization's (WHO) standardized core prescribing, patient care, and facility indicators to ensure and promote rational use of medicines (RUM).

METHODS AND MATERIALS:

Study setting, design, and site:

A community pharmacy conducted a cross-sectional study, evaluating 150 randomly selected prescriptions over 5 months (January-May 2022). Registered pharmacists of the relevant community pharmacies granted permission to perform this study. Prescriptions from patients who visited the pharmacy throughout the study period were gathered and information was collected using markers for the completeness of the prescription, which included prescriber information, medication information, and patient details.

Data collection and analysis

The completeness of the prescription can be assessed using patient information, including name, age, sex, reported allergy, consultation date, OPD registration number, medical history, diagnosis, investigational advice, and follow-up information. Medication information such as dosage forms, generic names of prescribed drugs, dosage, duration of therapy, and possible interactions between drugs. Prescriber information includes signatures and legible handwriting.

Micromedex and Medscape, two clinical decision support tools, were used to identify drug-drug interactions. To identify any possible interactions, the medication lists of each patient were examined using both software packages. Consistency and clinical relevance were assessed by comparing and analysing the severity and clinical importance of the identified interactions.

Mali et al

The prescription's legibility and rationality were examined by computing the percentage of prescriptions that contain antibiotics, vitamins, tonics, or enzymes; have readable handwriting; allergies; a brief history; a final diagnosis, and a recorded clinical examination

We evaluate prescriptions of these parameters using observed answers, such as YES or NO, with YES carrying one mark and NO carrying zero. Each prescription's overall score was determined by adding all 150 prescriptions. The percentage score was calculated against the highest possible score.

Descriptive statistical analysis was done on the data after it was entered into Microsoft Excel

RESULTS AND DISCUSSION

A total of 150 prescriptions from a community pharmacy were analyzed. Typically, the prescriptions were divided into three parts: Patient information, Medication information, and Prescriber information.

Table 1: Patient Information

Patients	n (% Written)
Information	
Name	141 (94%)
Age	5 (3.3%)
Gender	7 (4.6%)
Reported allergy	0 (0%)
Consultation date	147 (98%)
OPD registration no.	3 (2%)
Brief medical history	0 (0%)
Diagnosis	2 (1.3%)
Investigational advice	18 (12)
Follow up	0 (0%)

Our study of 150 prescriptions demonstrated considerable deficiencies in patient information documentation. We found that the patient's details (age, gender, allergy, diagnosis, and follow-up) were incomplete in all the prescriptions. While the patient's name was present in 94% of prescriptions, Age and gender were recorded in only 3.3% and 4.6%, respectively. The date of consultation was

documented in 98% of cases; however, an OPD registration number was mentioned in only 2% of prescriptions. Clinical examination findings and diagnosis were specified in 1.3% of cases, whereas no prescriptions included allergy history, past medical history, or follow-up instructions. Additionally, investigational advice was provided in 12% of prescriptions. Accurate documentation of these elements is crucial not only for legal and ethical considerations but also for ensuring patient safety and effective clinical management.

In contrast, a study by Parihar et al. (2021) discovered that tertiary hospital prescriptions had a greater completeness rate, with 80% of them including a complete diagnosis and 65% included information about allergies. With tertiary care institutions showing higher adherence to documentation rules than community pharmacies, this disparity raises the possibility that prescription completeness may differ by healthcare venue.

Table 2: Prescribers' Information

Prescriber's Information	n (% Written)			
Signature of Prescriber	134 (89.3%)			
Legible handwriting	149 (99.3%)			

To prevent medication errors and improper drug dispensation, prescribers can utilize capital letters while writing prescriptions. According to our study, 99.3% of prescribing physicians had legible handwriting.

A signature was present on 89.3% of the prescriptions.

To verify the authenticity of prescriptions and identify the prescribing physician, these specific details are crucial.

Research from Rathish et al. (2016) in Sri Lanka, which revealed that a prescriber's signature appeared on 84% of the prescriptions, supports these findings.

Table 3 Medication Information

Medication Information	n (% Written)
Dosage form	147 (98%)

Mali et al

Generic name written	39 (26%)
Dose of medicine	148 (98.6%)
Duration of therapy	134 (89.3%)
Possible drug	11 (7.3%)
interaction	

Many drugs are supplied in a variety of strengths and dose formulations, which presents difficulties in dispensing. It may also result in issues like incorrect dosage form, incorrect dose, incorrect duration, and possible drug interactions.

The duration of therapy was mentioned in 89.3% of prescriptions, although our data showed high documentation for dosage form (98%) and dose (98.6%). Only 26% of cases, though, provided the generic name.

In contrast to our study, Atif et al. (2018) observed that medication interactions were identified in 66.4% of prescriptions although generic names were prescribed in 100% of prescriptions, where generic prescribing was adequate. 9.3% of prescriptions had the dosage form and dose missing.

Even with well-documented dosage and duration, mistakes can still occur when complete clinical context—such as diagnosis, weight, and renal function—is absent, which is frequently the case.

Our findings were supported by Shrestha & Prajapati (2019), who discovered that a common cause of prescription errors in Nepalese outpatient departments was inadequate medication instructions, including unclear dosage forms in 4.5% of cases and drug interactions in 10.2% of cases.

Table 4: Prescription's legibility and rationality

Parameters	%Writte	%No
	n	t
		writt
		en
Antibiotics prescribed	69.3%	30.7

		%
Vitamins/Tonic/Enzym e prescribed	6.6%	93.4 %
Readable handwriting	99.3%	0.7%
Allergies	0%	100 %
Detail history	0%	100 %
Final diagnosis	1.3	98.7 %

The prescription's legibility is examined. Antibiotics were prescribed in 69.3% of prescriptions, while vitamins, tonics, and enzymes were prescribed in 6.6% of cases. Only 1.3% of prescriptions include a definitive diagnosis, 0.7% have unreadable handwriting, and none mention allergies or detailed medical histories.

In our study, Antibiotics were found to be included in 69.3% of prescriptions, which is more than twice the standard range of 20% to 25.4%. This pattern suggests that the empirical use of antibiotics may be prevalent or unnecessary. Similar conclusions were reached by Asif et al. (2018), who found that 39.6% of cases of excessive antibiotic use resulted in antimicrobial resistance and unnecessary costs

The following formula is used to evaluate a prescription rationally:

Rationality = (sum of the scores for all 150 prescriptions ÷ the greatest score for each prescription) × 100

 $=(1191 \div 2850) \times 100$

= 41.78%

The overall score for rationality is 41.78%. Since our study's rationality score is below 50%, irrationality is suggested. According to WHO guidelines, a prescribing practice is considered irrational if less than 50% of prescriptions adhere to Standard Treatment Guidelines (STGs).

The following calculation determines the average number of medications prescribed:

Mali et al

Total number of medicines prescribed ÷ Total number of prescriptions audited

 $= 440 \div 150$

= 2.93

Despite the WHO's recommendation of 1.6 to 1.8 drugs per prescription, the average was 2.93, indicating polypharmacy. Reduced patient adherence, DDIs, and an increased risk of adverse events were all associated with this polypharmacy. The link between polypharmacy and irrational use emphasizes the need for prescription audits and continuing medical education.

CONCLUSION:

This study emphasizes how critical it is to reduce prescription errors to enhance patient safety and quality of life. The average number of prescribed medications and antibiotics deviated from rational prescribing guidelines, according to the WHO core drug use indicators. a significant proportion Additionally, prescriptions had drug interactions, highlighting the need for stringent medication management guidelines. and prescribing Some practices should be established into effect to deal with these problems, like for Prescribers Training and Continuing Education. Digital Prescription System Integration (e-prescribing), Utilizing drug interaction screening tools regularly. Involvement of clinical pharmacists prescription audits, Promotion of Rational Therapy and Generic Prescribing Monitoring and Reporting Adverse Drug Events

REFERENCE:

- 1 Singh T, Banerjee B, Garg S, Sharma S. A prescription audit using the World Health Organization-recommended core drug use indicators in a rural hospital of Delhi. J Educ Health Promot. 2019;8:37.
- 2 Parihar NB, Patel EA, Seeta N. Evaluation of prescription errors and polypharmacy practices in rural area at community pharmacy. Int J Basic Clin Pharmacol. 2021;10:60-3.
- 3 Sema FD, Asres ED, Wubeshet BD. Evaluation of rational use of medicine using

WHO/INRUD core drug use indicators at Teda and Azezo Health Centers, Gondar Town, Northwest Ethiopia. Integr Pharm Res Pract. 2021 Jun 21;10:51-63. doi:10.2147/IPRP.S316399. PMID: 34189113; PMCID: PMC8232866.

- 4 Naseralallah L, Stewart D, Price M, Paudyal V. Prevalence, contributing factors, and interventions to reduce medication errors in outpatient and ambulatory settings: a systematic review. Int J Clin Pharm. 2023 Dec;45(6):1359-77. doi:10.1007/s11096-023-01626-5. Epub 2023 Sep 8. PMID: 37682400; PMCID: PMC10682158.
 - 5 Odukoya OK, Stone JA, Chui MA. E-prescribing errors in community pharmacies: exploring consequences and contributing factors. Int J Med Inform. 2014;83(6):427-37.
 - 6 Rathish D, Bahini S, Sivakumar T, Thiranagama T, Abarajithan T, Wijerathne B, Jayasumana C, Siribaddana S. Drug utilization, prescription errors and potential drug-drug interactions: an experience in rural Sri Lanka. BMC PharmacolToxicol. 2016; 17:27. doi:10.1186/s40360-016-0071-z.
- 7 Abidi A, Gupta S, Kansal S, Ramgopal. Prescription auditing and drug utilization pattern in a tertiary care teaching hospital of western UP. Int J Basic Clin Pharmacol. 2012; 1:184-90.
- 8 Rathish D, Bahini S, Sivakumar T, Thiranagama T, Abarajithan T, Wijerathne B, Jayasumana C, Siribaddana S. Drug utilization, prescription errors and potential drug-drug interactions: an experience in rural Sri Lanka. BMC PharmacolToxicol. 2016;17:27. doi:10.1186/s40360-016-0071-z.
- 9 Ministry of Health & Family Welfare, Government of India. Prescription audit guidelines. New Delhi: Ministry of Health & Family Welfare
- 10 Shrestha R, Prajapati S. Assessment of prescription pattern and prescription error in outpatient department at tertiary care district hospital, Central. J Pharm Policy Pract. 2019;12:16.

Bulletin of Pharmaceutical Research 2025;15(S2) An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print) 2249-9245 (Online)



RESEARCH ARTICLE

ADVANCED THERAPEUTIC APPROACHES IN TRIPLE NEGATIVE BREAST CANCER.

Padmaja Kore*, Asit Sirsat, Rohan Lad, Anuradha More, Ujwala Desai Progressive Education Society's Modern College of Pharmacy, Yamunanagar, Nigdi, Pune-44.

*Corresponding author email: padmaja.kalshetti@gmail.com

Received: Feb 06, 2025 / Revised: April 18, 2025 / Accepted: May 02, 2025

ABSTRACT: -

The aggressive subtype of breast cancer known as triple-negative breast cancer (TNBC) is distinguished by the lack of HER2 expression, progesterone receptors, and estrogen receptors. TNBC presents substantial therapy hurdles because of its heterogeneity and lack of targeted treatments. Recent developments in therapeutic techniques, including as combination medicines, immunotherapy, chemotherapy, and targeted molecular approaches, are examined in this study. Immuno checkpoint inhibitors, PARP inhibitors, and innovative drug delivery methods have transformed the treatment of TNBC and given patients new hope for better results. We also discuss current clinical trials and potential paths for customized medicine to treat TNBC. By combining these cutting-edge strategies, we hope to draw attention to how TNBC treatment changes and the possibility of more efficient, patient-specific treatment plans.

Key Words: -(Triple-negative breast cancer (TNBC), HER2-negative, estrogen receptor-negative, progesterone receptor-negative, targeted therapy, immunotherapy, chemotherapy).

How to cite this article: Padmaja Kore, Asit Sirsat, Rohan Lad, Anuradha More, Ujwala Desai. Advanced Therapeutic Approaches in Triple Negative Breast Cancer. Bulletin of Pharmaceutical Research. 2025;15(S2): 235-240.

Source of support: Nil Conflict of interest: None

Introduction:

The worst subtype of breast cancer is TNBC due to its aggressive nature, which poses a negative survival probability to patients. The includes treatment process surgery. chemotherapy, and radiotherapy as the main available therapies. Medically effective surgery becomes impossible when patients receive their TNBC diagnosis after a critical point. TNBC presents multiple challenges because its complex nature generates genetic changes in cell signaling pathways, resulting in difficult treatment outcomes.

Researchers try targeted therapies as promising therapeutic approaches due to these reasons. Reducing TNBC treatment

options focuses their efforts on DNA repair mechanisms together with androgen receptor signal transmission pathways and specific enzymes named kinases. Immunotherapy has been proven to enhance both survival rates and therapeutic effectiveness in patients who suffer from TNBC. BC is the second leading cause of death by cancer. Triple-negative breast cancer (TNBC) is one type of breast cancer that lacks the markers commonly used to guide treatment, which are the estrogen receptors, progesterone receptors, and HER2. As a result, there are no standard treatments for TNBC, which is much more challenging to treat in its later stages. There are no effective treatments for TNBC, and that's what researchers are looking for. Some of these are promising new

approaches. TNBC represents a highly aggressive variety of breast cancer that has unfavorable medical outcomes for patients. The available treatment options are surgery alongside chemotherapy and radiation therapy. but some patients fail to receive surgery diagnosis. TNBC presents multiple challenges because it contains different mutated cells and disrupted signaling pathways. These newly developed targeted therapies demonstrate thev promising results as concentrate treatment on DNA repair mechanisms and effects on androgen receptor signalling and kinase.

Therapeutic Approaches:

Cdk inhibitors:

Cyclin-dependent kinase (CDK) inhibitors are a class of drugs that block the activity of cyclindependent kinases, which are enzymes that help regulate the cell cycle (the process by which cells grow and divide). In cancer, the cell cycle control is often disrupted, allowing cancer cells to grow uncontrollably. Tumor cells in triple-negative breast cancer (TNBC) demonstrate regulatory alterations of the cell cycle process because TNBC stands as an aggressive and treatment-resistant breast cancer variant. The changes detected in CDKs establish them as therapeutic targets. The treatment outcomes for CDK 4/6 inhibitors in TNBC highly depend on the status of Rb protein. When patients exhibit more than 10% positive androgen receptors the presence of Rb protein becomes related to better treatment results. Research shows that activating AR signaling represents a vital characteristic of TNBC LAR (luminal androgen receptor) subtypes. Using AR-targeted therapy and CDK inhibitors presents a possible treatment approach for the LAR subtype of TNBC.

It shows that using AR inhibitors alongside other medications may become a practical treatment approach for TNBC. Researchers evaluate the effects of combining palbociclib with bicalutamide therapy to treat AR-positive metastatic TNBC (NCT02605486). Ribociclib and bicalutamide are evaluated for their combined effectiveness against advanced AR-positive TNBC in clinical trial NCT03090165. Abemaciclib is presently assessed for treating Rb protein-positive metastatic TNBC

(NCT03130439). The CDK inhibitors dinaciclib, PF-06873600, and trilaciclib will undergo clinical testing to determine their effectiveness against TNBC. Multiple treatments are usually combined with CDK inhibitors.

Micritouble Inhibition:

Microtubule inhibitors are type of chemotherapy that affects cell division (mitosis). The pharmacological action of Taxanes prevents microtubule breakdown, thus inhibiting cancer cell division. Due to their widespread tumorfighting properties. these drugs effectiveness against numerous cancers. Plantderived Vincristine prevents tubulin protein from properly arranging itself in microtubules. The cellular division process is halted. The drug Eribulin hinders microtubules from performing normal functions to prevent cell division. Although it is not classified as a taxane drug, it shares biochemical mechanisms that slow tumor development.

Multiple studies demonstrate the enhanced effectiveness of these medications through their utilization with other chemotherapy and immunotherapy treatments. Laboratory research found that eribulin stops the activation of AKT, which acts as a protein responsible for cancer expansion. Leukemia tumors underwent greater growth suppression in laboratory studies and mouse trials when eribulin was administered alongside everolimus, which inhibits the mTOR pathway. The convergent therapy demonstrates the potential to address refractory triple-negative breast cancer (TNBC) this form of cancer proved difficult to treat. Recent studies have shown that combining microtubule inhibitors like denibulin with other targeted therapies, such as mTOR inhibitors (e.g., everolimus), can enhance treatment effectiveness. This combination works by targeting multiple pathways that cancer cells rely on for survival, making it a potential strategy for treating refractory TNBC (TNBC that does not respond to standard treatments. Eribulin + Everolimus: In preclinical studies, this combination has synergistically inhibited tumor growth by disrupting both the microtubule dynamics and kev survival pathways in cancer cells, such as the AKT/mTOR pathway.

PARP Inhibitors:

The enzyme **PARP** (Poly ADP-Ribose Polymerase) is an essential component for fixing damaged DNA strands. The enzyme facilitates DNA single-strand break repair mechanisms to prevent unrepaired DNA's destructive impact, ultimately leading to cell death. The repair mechanism of damaged DNA becomes blocked by **PARP** inhibitor medications. Acute DNA damage accumulation in cancer cells disables their self-repair capabilities until their death occurs. PARP inhibitors prove most efficient for cancers that already have problems with DNA repair, such as those carrying BRCA gene mutations. PARP1 functions as an enzyme that repairs DNA damages that occur inside cells. The enzyme belongs to a group that corrects DNA mistakes through multiple repair techniques. Individual DNA bases receive proper repairs through Base excision repair mechanisms.

Nucleotide excision repair Fixes larger sections of DNA damage. Mismatch repair functions as a DNA correction method that fixes errors emerging from incorrect base pairings. PARP1 employs its enzymatic process to repair single-DNA breaks through stranded modifications using poly ADP-ribose (Figure 3 shows this process). DNA replication stall when DNA breaks happens occur combination with nonoperative PARP1 enzyme, particularly during the DNA replication phase. The DNA strands build up double-stranded breaks because of this process. Homeologous recombination (HR) serves as the normal mechanism to repair double-stranded DNA breaks through error-free procedures. Tamamoro proteins called BRCA1 and BRCA2 direct the DNA repair process by fixing DNA breaks to achieve proper DNA repair. The PARP therapy demonstrates potential benefits for Triple-Negative Breast Cancer but becomes treatment challenging implement in some clinical situations. The cancer cells in TNBC demonstrate limited response to PARP inhibitors and eventually become resistant to them. The research community investigates strategies to pair PARP inhibitors.

EGFR-targeted therapies:

EGFR (Epidermal growth factor receptor) is a protein on the surface of many types of cells,

including some cancer cells. When activated, EGFR stimulates several pathways inside the cell that promote cell division, survival, and growth. In certain cancers like TNBC, EGFR is often overexpressed, meaning there are more of these receptors than normal, which leads to uncontrolled cell growth. Targeting EGFR represents a possible therapeutic approach for Triple-Negative Breast Cancer since specific TNBC tumors exhibit elevated expression of Epidermal Growth Factor Receptor protein at their cell surfaces. TNBC tumors with elevated expression levels of EGFR demonstrate worse prognosis, together with more aggressive behavior among tumor cells. EGFR (Epidermal Growth Factor Receptor) is higher in many types of cancers, including Triple-Negative Breast Cancer (TNBC). Studies have shown that 40-60% of TNBCs have higher levels of EGFR. In 18% of these cases, the EGFR gene is even more active. However, EGFR mutations (changes in the gene itself) are rare in TNBC. In October 2010, researchers first discovered that EGFR could be a potential target for treatment in women with TNBC, offering a new direction for therapy. Tumors affected by Triple-Negative Breast Cancer present elevated levels of EGFR protein, which contributes to cellular growth. In 18% of these cases, the EGFR gene becomes active. Somatic mutations of the EGFR gene occur only exceptionally in cases of Triple-Negative Breast Cancer. In October 2010, the scientific community discovered that the EGFR receptor presented a therapeutic opportunity for TNBC patients. Gefitinib is an EGFRblocking medication that inhibits cancer growth by stopping protein activity.

Adoptive cell therapy (ACT):

The presence of immune cells called T cells inside tumors of patients with triple-negative breast cancer determines their expected clinical outcome. Because T cell stimulation shows promise for TNBC therapy, adoptive cell therapy (ACT) is a favorable treatment option. Adoptive cell therapy includes CAR-T therapy and T cell receptor (TCR) and tumor-infiltrating lymphocyte therapy as its therapeutic methods. All these treatments function identically by extracting T cells from patients who undergo laboratory modification to enhance cancer cell recognition before

medical reintroduction for fighting the cancer. Medical professionals extract T cells from patient blood before restoring their genetic structure to create cells with chimeric antigen receptors (CARs). With CARs installed onto T cells, they detect cancer antigens expressed by surface proteins of cancer cells. Professionals use modified T cells that expand in large numbers before returning them to the patient to fight cancer cells. Medical practitioners genetically modify T cells to receive specific Tcell receptors (TCRs), which grant them the ability to detect tumor-specific antigens in TCR therapy. The engineered T-cell receptors (TCRs) are built to detect proteins located on cancer cell surfaces so T cells can identify and eliminate the malignant cells as a form of treatment. The research study focuses on finding the optimal TCRs capable of identifying unique tumor antigens found within TNBC Medical professionals merge special T-cell receptors (TCRs) to the patient's T cells during TCR therapy. The modified T cells receive new T-cell receptors, which enable them to detect surface proteins of cancer cells. The T cells returned to patients possess the ability to recognize and destroy cancer cells after medical modification. Scientists research which T-cell receptors will detect unique markers on triple-negative breast cancer (TNBC).

Epigenetic modifications inhibiting:

Epigenetic modifications are essential in regulating gene expression, which can affect how cancer cells behave. In triple-negative breast cancer (TNBC), these modifications can prevent specific genes from being activated, making the tumor more challenging to treat. However, targeting these modifications could be promising to improve treatment outcomes. The process of gene expression control through alterations epigenetic includes (such modifications of DNA as methylation) as well as modifications to histones (including histone deacetylation and lactylation). Medical researchers examine these modifications because they might enhance the response of TNBC hormonenegative patients to traditional hormonetargeted therapies. In some TNBC cases, the "turned off" state of the estrogen receptor (ER)

occurs because of these modifications that exist within the cells. Demethylating inhibitors (such as decitabine) and HDAC inhibitors act as drugs that strip off modifying chemical groups from genes to make the estrogen receptor functional again and, therefore, suitable for hormone therapy. TNBC patients could gain treatment prospects through this therapeutic method known as epigenetic therapy. Decitabine functions as an FDAblock approved medication to DNA methylation while demonstrating effective treatment of blood cancer diseases. Breast cancer research indicates positive results from this therapy, yet more clinical trials need to be conducted because of limited participant clinical numbers. Α trial designated NCT04722978 evaluates the therapeutic effect of combining decitabine and moxifloxacin with gemcitabine and carboplatin for treating advanced TNBC.

REFERENCE:

- Alexander SPH, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M, et al. The Concise Guide to PHARMACOLOGY 2013/14: catalytic receptors. Br J Pharmacol. 2013;170(8):1676–1705.
- 2. Bayet-Robert M, Kwiatkowski F, Leheurteur M, Gachon F, Planchat E, Abrial C, et al. Phase I dose escalation trial of docetaxel plus curcumin in patients with advanced and metastatic breast cancer. Cancer Biol Ther. 2010;9(1):8–14.
- 3. Lee J, Yost S, Yuan Y. Neoadjuvant treatment for triple-negative breast cancer: recent progress and challenges. Cancers. 2020;12(6):1404.
- 4. Al-Hussaini H, Subramanyam D, Reedijk M, Sridhar SS. Notch signaling pathway as a therapeutic target in breast cancer. Mol Cancer Ther. 2011;10(1):9–15.
- 5. Dinakar YH, Kumar H, Mudavath SL, Jain R, Ajmeer R, Jain V. Role of STAT3 in the initiation, progression, proliferation, and metastasis of breast cancer and strategies to deliver JAK and STAT3 inhibitors. Life

- Sci. 2022;309:120996. doi: 10.1016/j.lfs.2022.120996.
- 6. Mehrotra R, Yadav K. Breast cancer in India: present scenario and the challenges ahead. World J Clin Oncol. 2022;13(3):209–218. doi: 10.5306/wjco.v13.i3.209.
- 7. Kumar H, Kumar RM, Bhattacharjee D, Somanna P, Jain V. Role of Nrf2 signaling cascade in breast cancer: strategies and treatment. Front Pharmacol. 2022;13:720076. doi: 10.3389/fphar.2022.720076.
- 8. Yao H, He G, Yan S, Chen C, Song L, Rosol TJ, et al. Triple-negative breast cancer: is there a treatment on the horizon? Oncotarget. 2017;8(1):1913–1924. doi: 10.18632/oncotarget.12284.
- 9. Al-Mahmood S, Sapiezynski J, Garbuzenko OB, Minko T. Metastatic and triple-negative breast cancer: challenges and treatment options. Drug Deliv Transl Res. 2018;8(1):1483–1507. doi: 10.1007/s13346-018-0551-3.
 - 10. Jain V, Kumar H, Jain R. Targeting triplenegative breast cancers using nanomedicine. In: Target Nanomedicine Breast Cancer Ther. 2022. p. 199–255. doi: 10.1016/b978-0-12-824476-0.00004-8.
 - 11. Baghban R, Roshangar L, Jahanban-Esfahlan R, Seidi K, Ebrahimi-Kalan A, Jaymand M, et al. Tumor microenvironment complexity and therapeutic implications at a glance. Cell Commun Signal. 2020;18:59. doi: 10.1186/s12964-020-0530-4.
- 12. Bhola NE, Balko JM, Dugger TC, Kuba MG, Sa nchez V, Sanders M, et al. TGF-β inhibition enhances chemotherapy action against triple-negative breast cancer. J Clin Invest. 2013;123(1):1348–1358. doi: 10.1172/JCI65416.
- 13. Giuli MV, Giuliani E, Screpanti I, Bellavia D, Checquolo S. Notch signaling activation as a hallmark for triple-negative breast cancer

- subtype. J Oncol. 2019;2019:8707053. doi: 10.1155/2019/8707053.
- 14. Kayani Z, Bordbar AK, Firuzi O. Novel folic acid-conjugated doxorubicin-loaded β-lactoglobulin nanoparticles induce apoptosis in breast cancer cells. Biomed Pharmacother. 2018;107:945–956. doi: 10.1016/j.biopha.2018.08.047.
- 15. Muthu MS, Kutty RV, Luo Z, Xie J, Feng SS. Theranostic vitamin E-TPGS micelles of transferrin conjugation for targeted codelivery of docetaxel and ultrabright gold nanoclusters. Biomaterials. 2015;39:234–248. doi: 10.1016/j.biomaterials.2014.11.008.
- 16. Bhateja P, Cherian M, Majumder S, Ramaswamy B. The Hedgehog signaling pathway: a viable target in breast cancer? Cancers (Basel). 2019;11(8):112. doi: 10.3390/cancers1108112.
- 17. Sender R, Fuchs S, Milo R. Revised estimates for the number of human and bacteria cells in the body. PLoS Biol. 2016;14:e1002533. doi: 10.1371/journal.pbio.1002533.
- 18. Sivan A, Corrales L, Hubert N, Williams JB, Aquino-Michaels K, Earley ZM, et al. Commensal Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy. Science. 2015;350(6264):1084–1089. doi: 10.1126/science.aac4255.
- 19. Kudelova E, Smolar M, Holubekova V, Hornakova A, Dvorska D, Lucansky V, et al. Genetic heterogeneity, tumor microenvironment, and immunotherapy in triple-negative breast cancer. Int J Mol Sci. 2022;23(23):14937. doi: 10.3390/ijms232314937.
- 20. Peng D, Kryczek I, Nagarsheth N, Zhao L, Wei S, Wang W, et al. Epigenetic silencing of TH1-type chemokines shapes tumour immunity and immunotherapy. Nature. 2015;527(7577):249–253. doi: 10.1038/nature15520.

- 21. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646–674. doi: 10.1016/j.cell.2011.02.013.
- 22. Cazzaniga M, Bonanni B. Breast cancer metabolism and mitochondrial activity: the possibility of chemoprevention with metformin. Biomed Res Int. 2015;2015:972193. doi: 10.1155/2015/972193.
- 23. Wang LX, Mei ZY, Zhou JH, Yao YS, Li YH, Xu YH, et al. Low-dose decitabine treatment induces CD80 expression in cancer cells and stimulates tumor-specific cytotoxic T lymphocyte responses. PLoS One. 2013;8(5):e62924. doi: 10.1371/journal.pone.0062924.
- 24. Loi S, Drubay D, Adams S, Pruneri G, Francis PA, Lacroix-Triki M, et al. Tumorinfiltrating lymphocytes and prognosis: a pooled individual patient analysis of early-stage triple-negative breast cancers. J Clin Oncol. 2019;37(7):559–569. doi: 10.1200/JCO.18.01010.
- 25. Schmid P, Rugo HS, Adams S, Schneeweiss A, Barrios CH, Iwata H, et al. Atezolizumab plus nab-paclitaxel as first-line treatment for unresectable, locally advanced or metastatic triple-negative breast cancer (IMpassion130): updated efficacy results from a randomised, double-blind, placebocontrolled, phase 3 trial. Lancet Oncol. 2020;21(1):44–59. doi: 10.1016/S1470-2045(19)30689-8.
- 26. Vétizou M, Pitt JM, Daillère R, Lepage P, Waldschmitt N, Flament C, et al. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. Science. 2015;350(6264):1079–1084. doi: 10.1126/science.aad1329.
- 27. Nasiri F, Kazemi M, Mirarefin SMJ, Mahboubi Kancha M, Ahmadi Najafabadi M, Salem F, et al. CAR-T cell therapy in triplenegative breast cancer: hunting the invisible devil. Front Immunol. 2022;13:1018786. doi: 10.3389/fimmu.2022.1018786.

- 28. Cheng CT, Qi Y, Wang YC, Chi KK, Chung Y, Ouyang C, et al. Arginine starvation kills tumor cells through aspartate exhaustion and mitochondrial dysfunction. Commun Biol. 2018;1:178. doi: 10.1038/s42003-018-0178-4.
- 29. Cazzaniga M, Bonanni B. Breast cancer metabolism and mitochondrial activity: the possibility of chemoprevention with metformin. Biomed Res Int. 2015;2015:972193. doi: 10.1155/2015/972193.
- 30. Banerjee A, Birts CN, Darley M, Parker R, Mirnezami AH, West J, et al. Stem cell-like breast cancer cells with acquired resistance to metformin are sensitive to inhibitors of NADH-dependent CtBP dimerization. Carcinogenesis. 2019;40(7):871–882. doi: 10.1093/carcin/bgy174.
- 31. Qiu F, Chen YR, Liu X, Chu CY, Shen LJ, Xu J, et al. Arginine starvation impairs mitochondrial respiratory function in ASS1-deficient breast cancer cells. Sci Signal. 2014;7(319):ra31. doi: 10.1126/scisignal.2004761.
- 32. Sender R, Fuchs S, Milo R. Revised estimates for the number of human and bacteria cells in the body. PLoS Biol. 2016;14:e1002533. doi: 10.1371/journal.pbio.1002533.
- 33. Loi S, Drubay D, Adams S, Pruneri G, Francis PA, Lacroix-Triki M, et al. Tumorinfiltrating lymphocytes and prognosis: a pooled individual patient analysis of early-stage triple-negative breast cancers. J Clin Oncol. 2019;37(7):559–69. doi: 10.1200/JC0.18.01010.
- 34. Briere D, Sudhakar N, Woods DM, Hallin J, Engstrom LD, Aranda R, et al. The class I/IV HDAC inhibitor mocetinostat increases tumor antigen presentation, decreases immune-suppressive cell types, and augments checkpoint inhibitor therapy. Cancer Immunol Immunother.

- 2018;67(3):381–92. doi: 10.1007/s00262-017-2091-y.
- 35. Abreu TR, Fonseca NA, Gonçalves N, Moreira JN. Current challenges and emerging opportunities of CAR-T cell therapies. J Control Release. 2020;319:246–61. doi: 10.1016/j.jconrel.2019.12.047.
- 36. Dees S, Ganesan R, Singh S, Grewal IS. Emerging CAR-T cell therapy for the treatment of triple-negative breast cancer. Mol Cancer Ther. 2020;19(11):2409–21. doi:10.1158/1535-7163.MCT-20-0385.
- 37. Miller-Kleinhenz JM, Bozeman EN, Yang L. Targeted nanoparticles for image-guided treatment of triple-negative breast cancer: clinical significance and technological advances. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2015;7(6):797–816. doi: 10.1002/wnan.1343.
- 38. Vinayak S, Tolaney S, Schwartzberg L, Mita M, McCann G, Tan A, et al. Open-label clinical trial of niraparib combined with pembrolizumab for treatment of advanced or metastatic triple-negative breast cancer. JAMA Oncol. 2019;5(8):1132.
- 39. Prickett WM, Van Rite BD, Resasco DE, Harrison RG. Vascular-targeted single-walled carbon nanotubes for near-infrared light therapy of cancer. Nanotechnology. 2011;22:455101. doi: 10.1088/0957-4484/22/45/455101.
- 40. Soe ZC, Thapa RK, Ou W, Gautam M, Nguyen HT, Jin SG, et al. Folate receptor-mediated celastrol and irinotecan combination delivery using liposomes for effective chemotherapy. Colloids Surf B Biointerfaces. 2018;170:718–28. doi: 10.1016/j.colsurfb.2018.07.013

An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print) 2249-9245 (Online)

RESEARCH ARTICLE



Ujwala Desai*, Padmaja Kore, Mahesh Reddy, Jaie Zore, Girish Funde, Vaishnavi Atole

PES's Modern College of Pharmacy, Sec. No. 21, Yamunanagar, Nigdi, Pune 411044* *Corresponding Author E-mail: ujudesai@gmail.cm Received: Feb 08, 2025 / Revised: April 20, 2025 / Accepted: May 04, 2025

ABSTRACT:

This study focuses on formulating and evaluating herbal shampoo tablets as an eco-friendly and convenient alternative to conventional liquid shampoos. The tablets contain natural ingredients such as Ashwagandha, Shikakai, Reetha, and Methi, which provide cleansing, conditioning, and antimicrobial benefits. These ingredients were combined with super disintegrants and essential oils to ensure effective lathering and ease of use. The formulation was developed using a direct compression method, and various batches were evaluated based on physicochemical properties, including hardness, friability, foamability, disintegration time, and pH balance. The optimised batch exhibited superior foaming ability, effective washability, and desirable mechanical properties. The study concludes that shampoo tablets offer a sustainable, travel-friendly, cost-effective alternative with potential antifungal benefits for scalp health.

Key words: Ashwagandha, Shikakai, Reetha, Meethi, Shampoo tablets

How to cite this article Ujwala Desai, Padmaja Kore, Mahesh Reddy, Jaie Zore, Girish Funde, Vaishnavi Atole. Formulation and Evaluation of Herbal Shampoo Tablet. Bulletin of Pharmaceutical Research. 2025;15(S2) :241-245

Source of support: Nil Conflict of interest: None

Introduction:

Shampoo bars are a more sustainable and convenient option than traditional liquid shampoos. Raspberry, lavender essential oils, and coconut or palm oil extracts are some of the ingredients found in these herbal tablets. and as a result, help in the nourishment and cleansing process. Lightweight and very easy to use, these tablets can be mixed with water to create a lather or applied directly onto the hair. These tablets also work as a conditioner since cocoa and shea butter are effective in moisturising and softening the hair. Active herbs like rosemary, lavender, chamomile, nettle, and non-stripping surfactants work best to help achieve antibacterial effects [1,2]. In addition to being cost-effective, shampoo tablets offer a longer shelf life, are preservative-free, and require minimal packaging. As a result, they are much easier to transport. Due to the lack of known sideeffects. they are a more practical option. The proprietary blend consists of Shikakai, Ashwagandha, methi, and soapnuts, combined with super disintegrants and

Material

Neuherbs, Uttar Pradesh, supplied Ashwagandha, Reetha, Shikakai, and Methi. Spectrum Chemicals supplied MCC. The research laboratory supplied Crospovidone, Croscarmellose sodium, Sodium starch glycolate, and Avicel. Milton Chemicals supplied Carbapol. Loba Chemie supplied carboxymethyl cellulose, and Dr. Jains supplied essential oil.

1. Method:

Preparation of powder:

Preparation of Ashwagandha powder:

Ashwagandha powder was taken from the market and passed through a sieve. 80, then fine powder of Ashwagandha was collected [12].

Preparation of Shikakai powder:

Shikakai powder was taken from the market and passed through a sieve. 80, and then the fine powder of it was collected[4]



and passed through a sieve. 80, then fine powder of

Reetha was collected [4].

Preparation of Methi powder:

Methi powder was taken from the market and passed through a sieve. 80, then the fine powder of Methi was collected [4].

1. Preformulation study:

A study was performed, which included Bulk property [5].

Preparation of Herbal shampoo tablet: Herbal shampoo tablets were prepared using the direct compression method. In this method, the tablets were compressed directly from the mixture by a tablet compression machine using a 9mm punch [7,11]. All the quantities of ingredients are selected based on the literature review

Table 1: Formulation Table of Batches

2.

INGREDIENTS	A1	A	2	A3	A	4	A5	Role of Excipient
ASHWAGANDHA(mg)	200	0	0	20 0	20	00	160	Controls hairfall
SHIKAKAI(mg)	200	20	0 20 0		20	00	160	Natural conditioner
REETHA(mg)	200	0	0	20 0	20	00	160	Detergent and antidandruff
METHI(mg)	200	0	0	20 0	20	00	100	Antifungal
CROSPOVIDONE(mg)	16	10	ó	16	90)	90	Disintigrant
CARBOPOL(mg)	-	10	ó	-	-		-	Gelling agent
-SODIUM STARCHGLYCOLATE (mg)	-	1		16	-		-	Disintigrant
POLYACRYLIC POTASSIUM(mg)		-		-	16	ó	-	Swelling agent
CROSSCARMELLOSE(mg	-		-		-	-	-	Disintigrant
CARBOXY METHYLCELLULOSE SODIUM(mg)	-				1 5	-	-	Binder
AVICEL(mg)	-	-			-	-	-	Binder
ESSENTIAL OIL	-		q	.S	q. s	q s	q s	Flavouring agent
MICRO CRYSTALLINE CELLULOSE(mg)	-	-			-	9	9 6	Binder
TOTAL(mg)	636		8	16	8 1 3	9 8 6	7 6 6	

Table 2: Preformulation of batches

PARAMETE R	ASHWAGA NDHA	SHIKA KAI	REET HA	МЕТНІ
Bulk Density (g/ml)	0.486±0.015	0.48±0 .01	0.476 ±0.02	0.491±0. 007
Tapped Density(g/ml)	0.706±0.01	0.67±0 .02	0.66± 0.01	0.73±0.0 1
Hausner Ratio (kg/cm)2	1.42±0.02	1.34±0. 01	1.32 3±0. 02	1.456±0.0 1
Carr's Index (%)	29.43±0.15	25.30± 0.08	25.3 3±0. 03	4.45±0.04

Table 3:Characterization of herbal shampoo tablet:

PARAMETE R	A1	A2	A3	A4	A5
Color	Bro wn	Brown	Bro wn	Brown	Brown
Wash ability	Go od	Good	Av era ge	Poor	Good
Hardness (Kg/cm2)	3.4 33 ± 0.1 1	3.36 ± 0.15	2 76 ± 0.2 3	3.5 ± 0.1	3.06 ± 0.15
Foam	Les s foa m	Best foam	Bes t foa m	Poor	Best Foam
Friability(%)	1.7 7± 0.1 1	0.98±0.0 1	0.6 1± 0.0 3	1.06 ± 0.02	1.86±0.01

Characterization of herbal shampoo tablet:

Organoleptic Properties:

Physical characteristics like colour and odour were assessed for the prepared herbal shampoo tablets.

Hardness test:

The Monsanto Hardness tester measures a tablet's hardness, or its ability to withstand mechanical shock. Three to five tablets were selected at random from each batch, tested for hardness, and the results noted. Tablet hardness is typically between 3 and 5 kg/cm² [8, 9.]

Thickness:

A Vernier calliper was used to measure the tablets' thickness. The thickness of the tablet should not exceed \pm 5 mm, and micrometres are used to express it [8, 9].

Friability:

The friability of the tablets was assessed using a friabilator. The tablets were weighed and the friabilator was turned on. The count key was set to 100, and the test was conducted using a time adjustment corresponding to the count. The rotation was started after the tablets were inserted into the rotating chamber. After completion, the tablets were collected and weighed. For the friability test, ten tablets were randomly selected from each batch. The weight loss of the tablets was required to remain within 0.5 to 1% of their initial weight.

Weight variation test:

20 tablets of each formulation were taken. Using an electronic balance, each tablet was weighed separately. The average weight was then determined, and the deviation was noted by comparing it to the average value. It is recommended that the weight variation limit not surpass $\pm 7.5\%$ [8, 9].

Formula:

% of Weight variation = Individual Weight Average Weight/Average Weight* 100

Foam Ability:

A 100 ml cylinder was filled with 50 ml of water, and the tablet was dropped inside. The cylinder was covered with a hand and shaken thoroughly for a few minutes. The volume of the foam produced was then measured [10].

Washability:

The herbal shampoo tablet's washability was assessed by applying the formulation to the skin, washing it with water, and then manually checking it [10].

Irritation test:

When creating medications and cosmetics, skin irritation tests are crucial. The formulation was applied to the 1 sq. cm dorsal skin surface of the left hand, and the symptoms of edema, redness, and irritation were monitored for 1-2 hours [8,9].

pH:

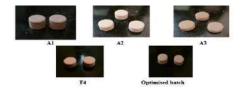
To prevent skin irritation, the herbal shampoo tablet's pH was adjusted to match that of the skin. A 1% aqueous solution of the formulation was prepared, and a calibrated digital pH meter was used to measure it at a constant temperature [10].

RESULT:

PREFORMULATION STUDY

In all the sample, 3 readings were taken and the standard error of mean was calculated on ms Excel.

Figure 1: Appearance of tablets



DISCUSSION:

This study explores the development and evaluation of herbal shampoo tablets as a sustainable and travel-friendly alternative to conventional liquid shampoos. The research highlights eco-friendliness, cost-effectiveness, and natural ingredient benefits, making these tablets an

innovative solution in personal care.

The shampoo tablets are made from herbal components such as Ashwagandha, Shikakai,

Reetha, and Methi, which are known for their cleansing, conditioning, and antimicrobial properties. Essential oils and super disintegrants have also been added to enhance usability. The direct compression method ensured a straightforward and effective production process. Various formulations (A1-A5) were developed and tested for optimal characteristics. The study assessed hardness. friability. foaming disintegration time, and pH balance. The optimised batch (A5) exhibited the best foaming and washability properties, making it the most effective formulation.[6] Foamability is essential for a good cleansing experience, and the optimised batch provided high foaming action. Washability ensures no residue remains after rinsing. the Disintegration test, the best formulation disintegrated within 40 seconds, improving ease of use. pH Balance is maintained at 7.5-7.66, ensuring scalp compatibility and reduced irritation. None of the batches showed signs of irritation, proving skin-friendliness.[8]

CONCLUSION

The study successfully formulated and evaluated herbal shampoo tablets, offering a promising alternative to conventional shampoos. The optimized batch exhibited **s**uperior foaming, ease of use. and antimicrobial benefits. making sustainable and practical option for modern Further consumers. research commercialization can help enhance its market potential.

REFERENCE

- Bhavsar R,Bilal Q, Formulation and development of sulphate free shampoo, International Journal for Research in Applied Science and Engineering Technology,2020, IJRASET, Volume 8, 2321-9653.
- 2. Janrao K, Gaikwad V, A review on herbal shampoo, International Journal of Creative Research Thoughts, IJCRT, Volume 10, 2320-2882.
- 3. Bhagwat S, Formulation and evaluation of herbal shampoo, International Journal of Creative Research Thoughts, 2020: IJCRT, Volume 8, 2320-2882.
- 4. Dumbare P,Sangale D,Pagare H A article on, Formulation and evaluation of herbal hair conditioner, IJCRT, Volume 11, 2320-2882.
- Vineetha K, Vindhya V, Vishranti M, Herbal shampoo: benefits, preparation and evaluation, Journal of Xi'an Shiyou University, Natural Science Edition, 2009, volume 17, 1673-064X.
- 6. Chandran S, Vipin k, Shrinker A, Development and Evaluation of anti-dandruff shampoo based on natural sources, JOPP, 2321-5895.
- 7. European Pharma Application Lab, Pharma Solutions, BASF SE, Ludwigshafen, Germany 2 Technical Service Europe, Pharma Solutions, BASF SE, Lampertheim, Germany.
- 8. Chaturvedi H,Garg A and Rathore U, "Post-Compression Evaluation Parameters for Tablets" . JOMR, 2017, 526-530.
- 9. Lachman L, Liberman. H. Aand Kaning J. "The Theory and practice of industrial pharmacy," Varghese pharmacy," Varghese third edition, IJPETR, Vol. 41.

An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print) 2249-9245 (Online)

RESEARCH ARTICLE



Ujwala Desai, Padmaja Kore, Anushka Kamble, Kirti Ishte, Akanksha Aglave, Yash Kulkarni

PES's Modern College of Pharmacy, Sec. No. 21, Yamunanagar, Nigdi, Pune 411044*

*Corresponding author E-mail: ujudesai@gmail.com

Received: Feb 06, 2025 / Revised: April 18, 2025 / Accepted: May 02, 2025

Abstract

The research explores the development and assessment of an herbal facewash and exfoliating tablet as a natural alternative to traditional facewash products. Conventional face washes often come in liquid or gel forms, requiring preservatives and larger packaging, making them less convenient for travel and storage. This research aims to develop a compact, preservative-free, and portable facewash tablet using natural herbal ingredients with beneficial skin properties. The formulation incorporates coffee, green tea, guava leaf, cinnamon, lentil legume, and menthol powder, known for their antioxidant, anti-inflammatory, and antibacterial properties. The tablet was developed using the direct compression method, and different batches were optimised based on foaming ability, washability, hardness, friability, and pH balance. The optimized batch (F8) demonstrated excellent foaming and cleaning properties, making it a suitable alternative to traditional facewashes. Furthermore, in vitro antibacterial studies confirmed the tablet's effectiveness against *Candida, Pseudomonas aeruginosa*, and *Staphylococcus aureus*, indicating its potential for acne treatment and skin cleansing. The study also included a melanocyte assay to assess its impact on skin pigmentation. Stability tests were conducted under ICH guidelines to ensure the tablet's durability. This research successfully introduces a herbal facewash tablet that is costeffective, travel-friendly, and enriched with natural skin-enhancing properties, addressing the limitations of conventional facewash products.

Key Words: Exfoliation, Coffee, Green tea, Cinnamon, Guava leaves, Lentil legume, Menthol, Melanocyte assay. **How to cite this article:** Desai U, Kore P, Kamble A, Ishte K, Aglave A, Kulkarni Y. Formulation and Evaluation of Herbal Facewash and Exfoliating Tablets. *Bulletin of Pharmaceutical Research.* 2025;15(S2): 246-254 **Source of support:** Nil **Conflict of interest:** None

Introduction

A facewash, a "cleanser," is designed to cleanse the face without stripping its natural moisture. It is suitable for all skin types and effectively removes dirt, oil, and impurities while providing hydration, particularly for dry skin. [1]

Facewashes are available in different formulations, like gel, powder, cream, and liquid.

- 1. Gel-based face wash usually works well for oily skin types.
- Foam-based face wash is restricted to oily or combination skin.
- 3. Gel-based face wash and creamy cleanser work best for dry skin. [2]

Synthetic and Conventional face washes (Gel, Foam, Powder, cream) have drawbacks:

- 1. The product is typically packaged in large, collapsible tubes or plastic containers.
- 2. It can be challenging to carry during travel due

to its size and packaging.

- 3. The formulation's aqueous nature requires adding preservatives to maintain its shelf life.
- 4. The product tends to have lower stability, affecting its effectiveness over time.

This research aims to overcome the challenges associated with conventional and synthetic facewash products.

The use of herbal facewash tablets provides various benefits, such as:

- 1. Minimizing the cost
- 2. Minimized packaging size
- 3. No utilization of preservative
- 4. Easy transport

Herbal compounds and excipients like surfactants, super disintegrant and gelling agents are used in the formulation. The herbal ingredients selected were green tea, cinnamon, lentil legume, coffee, guava leaf and menthol powder. Combining the above herbal effects is



used to clean the skin. [3]

Herbal excipients used in the formulation of facewash tablet [4]

- 1. Green tea contains vitamin E, which provides nourishment and hydration, as a moisturising agent, reducing dark spots and minimising skin irritation. It is also valuable for reverse sunburn.
- 2. Cinnamon treats hyperpigmentation and acne. It has an anti-ageing component and is also known to lighten the skin.
- 3. Lentil legumes contain vitamin C, which acts as an antioxidant.
- 4. Coffee contains vitamin B3, which treats dark circles and has anti-ageing properties. It also reduces cellulite formation.
- 5. Guava leaves treat inflammatory acne, scars, spots, and pigmentation and are useful for uneven skin tone.
- 6. Menthol powder has a cooling effect on the skin when sunburned.
- Herbal cosmetics have become increasingly popular because they are made entirely from herbs and plants, offering a natural alternative with minimal side effects. These products nourish the skin with essential nutrients and beneficial minerals. As a result, there is a growing demand for natural, herbal, and organic beauty products, creating new opportunities for manufacturers to innovate and develop products that align with consumer preferences.
- Most herbal formulations enhance beauty safely and improve consumer satisfaction, as they are free from synthetic chemicals and tend to have fewer side effects than conventional cosmetics.
- Currently, facewashes on the market are typically available in gel or cream forms, which are often packaged in large, collapsible tubes or plastic containers. This packaging can make it inconvenient for consumers to carry the products while traveling.
- One of the main challenges with facewashes is their aqueous nature, which requires the use of preservatives to maintain their stability and effectiveness.
- The tabletting technique can be employed to produce facewash products more efficiently and conveniently, reducing costs, packaging size, and the need for preservatives.

Materials And Method:

The formulation for this study includes a variety of natural ingredients, each selected for its specific benefits. The materials used are coffee (sourced from Tata Coffee), lentil legume (supplied by Natures Root), green tea (provided by Lipton), guava leaf (obtained from Natural), cinnamon (from Milagro Farm Co.), menthol (supplied by Kelvin Natural Mint Pvt. Ltd.), and cocoa powder (sourced from Athos Chemical). These ingredients serve different roles in the formulation, with coffee, lentil legume, green tea, guava leaf, and cinnamon acting as active pharmaceutical ingredients (APIs).

Formulation of facewash tablet:

Preparation of powder:

a) Preparation of Coffee powder:

Seeds of coffee were crushed in mortar by pestle to coarse powder and the powder was collected.

b) Preparation of Guava leaf powder:

The guava leaves were dried in a hot air oven at 50°C for 30 minutes. Grinded into mortar & pestle to fine powder. The powder was passed through sieve 80 and collected.

c) Preparation of Cinnamon powder:

Cinnamon powder was taken from market and passed through sieve no. 80 then fine powder of Cinnamon collected.

d) Preparation of Green tea powder:

Green tea powder was taken from market and passed through sieve no. 80 then fine powder of green tea was collected.

e) Preparation of Menthol powder:

Menthol was crushed in mortar and pestle to fine powder. The fine powder was passed through sieve no.80.

Preparation of facewash tablet:

The facewash tablets were prepared by using the direct compression method, where the active ingredients and appropriate excipients (such as fillers, disintegrants, and lubricants) were directly compressed into tablets. These powder blends flowed uniformly into the die cavity and were formed into a firm compact using a 9mm punch. (2)

Pre-formulation study

To evaluate the material's properties, bulk characterization was performed, which

included measuring key parameters such as bulk density, tapped density, Hausner's ratio, compressibility index, and angle of repose. These tests provide important insights into the flowability, compatibility, and overall behaviour of the material during processing.

Procedure for formulation:

- 1. The herbal ingredients were weighed, including coffee, green tea, guava leaves, cinnamon, and menthol.
- 2. Excipients such as Carbopol, SLS, and SSG were weighed.
- 3. Additional ingredients, including MCC, starch, cross-carmellose, span, sandalwood powder (used as an odorant), and cocoa powder (used as a coloring agent), were weighed in varying concentrations according to the batch requirements.
- 4. All ingredients were mixed in a mortar and pestle and triturated to form a fine powder.
- 5. The triturated mixture was weighed and prepared for compression.

Composition of facewash tablet:

Table no.1: Composition of facewash tablet

Constitue	F	F	F	F	F	F	F	F8
nt	1	2	3	4	5	6	7	
Coffee	1	1	1	1	1	1	1	10
	1	1	0	1	3	1	1	%
	%	%	%	%	%	%	%	
Lentil	1	1	1	5	1	6	6	6
legume	1	1	0	%	3	%	%	%
	%	%	%		%			
Cinnamo	9	9	5	2	7	3	3	2
n	%	%	%	%	%	%	%	%
Guava	1	1	7	5	1	6	6	4
leaf	1	1	%	%	0	%	%	%
	%	%			%			
Green tea	1	1	5	5	1	6	6	6
	1	1	%	%	0	%	%	%
	%	%			%			
Menthol	5	2	1	1	1	1	1	1
	%	%	%	%	%	%	%	%
Carbopol	1	1	1	1	1	1	6	6
	1	1	4	2	8	0	%	%
	%	%	%	%	%	%		
SLS	1	1	1	2	2	1	2	23
	5	5	3	4	0	4	0	%

1	0.7	~ .						
	%	%	%	%	%	%	%	
SSG	1	2	3	2	-	-	2	24
	5	0	0	5			4	%
	%	%	%	%			%	
MCC			1	1	-	1	1	10
			0	0		0	0	%
			%	%		%	%	
Starch			1	-	8	-	-	-
					%			
Croscarm	-			-	-	2	-	-
ellose						4		
						%		
Span	-	-		-	-	1	-	-
_						0		
						%		
Sandalwo	-	-	-	-	-	-	6	-
od							%	
powder								
Coco	-	-	-	-	-	-		12
powder								%

Characterization of facewash tablet:

(1) Physical evaluation:

The physical evaluation of the formulated facewash tablets included assessing parameters such as colour and appearance. (2)

(2) Hardness test:

The hardness of the tablets was measured using a Monsanto Hardness Tester, which determines the tablet's ability to withstand mechanical shock. The hardness is expressed in Kg/cm². For each batch, 3 to 5 tablets were randomly selected and tested, and the values were recorded. The average hardness of the tablets ranged from 3 to 8 Kg/cm². (2)

(3) Thickness:

The thickness of the tablets was measured using a

Vernier calliper, with the acceptable range being within ±5mm. The measurement was recorded in micrometres. (2)

(4) Friability:

The friability of the tablets was determined using a friabilator. First, the friabilator was switched on, and the tablets were weighed. The count was adjusted to 100 by pressing the count key, and the test was performed for the specified time. The tablets were placed in the rotating chamber, and the rotation was started. After completion, the tablets were collected and re-weighed. For the friability test, 10 tablets were randomly selected from each batch. The loss in weight should not exceed 0.5-1%

The formula used to calculate friability is: (Initial Weight–Final weight)/Initial weight×10 0.[2]

(5) Weight Variation:

For the weight variation test, a total of 20 tablets were randomly selected from each formulation. Each tablet was weighed individually using an electronic balance, and the average weight of the tablets was calculated. The deviation of each tablet's weight from the average was then noted. The weight variation must fall within a range of $\pm 7.5\%$ to ensure consistency. The weight variation for each tablet was calculated using the formula:

%Weight Variation=(Individual Weight-Average Weight) Average Weight×100 (2)

(6) Foam Ability:

The foamability of the formulation was evaluated n irritation testing is an essential step in developing pharmaceuticals and cosmeceuticals. For this test, the formulation was applied to a 1 cm² area on the dorsal surface of the left hand. The site was then monitored for any signs of irritation, including redness and swelling (oedema), at 1 to 2 hours intervals. This process helps to assess the potential for skin irritation and ensures the formulation is safe for use. (2)

(9) pH:

To minimise the risk of irritation, the pH of the facewash tablet was carefully adjusted to align with the skin's natural ph. A 1% aqueous solution of the formulation was prepared, and the pH was measured using a calibrated digital pH meter, ensuring the test was conducted at a consistent temperature for accuracy. (2)

10) In Vitro Antibacterial Study:

The facewash tablet's antibacterial activity was tested using the agar-well diffusion method against

(11) Study Melanocyte Assay:

Skin pigmentation was primarily determined by the production of melanin by melanocytes in the epidermis. The activity of these melanocytes, along with the type and distribution of melanin, plays a key role in the variation of skin colour. Darker melanin helps protect the skin from the harmful effects of ultraviolet (UV) radiation, such as photoaging and the development of skin cancer. UV exposure stimulates melanocytes to produce more melanin, leading to a deeper skin pigmentation or "tanning."

using the cylinder shake method. To do this, 50 ml of water was placed in a 250 ml cylinder, and a tablet was added. The cylinder was then covered with a hand and shaken vigorously for several minutes. After shaking, the volume of foam produced was measured to assess the foam's ability. [2]

(7) Washability:

The washability of the facewash tablet was evaluated by applying the formulation to the skin and rinsing it off with water. After rinsing, the effectiveness of the washability was assessed through a visual inspection of the skin to determine how thoroughly the product was removed. (2)

(8) Irritancy test:

Ski

Candida, Pseudomonas aeruginosa, and Staphylococcus aureus. This method is commonly used to assess the antimicrobial properties of plant or microbial extracts, like the disk-diffusion technique.

- 1. The surface of the agar plate is inoculated by evenly spreading a microbial inoculum (such as *Candida*, *Pseudomonas aeruginosa*, or *Staphylococcus aureus*).
- 2. A well measuring 6 to 8 mm in diameter is then aseptically created using a sterile cork borer ortip, and 20–100 μL of the antimicrobial agent or extract solution at the desired concentration is added to the well.
- 3. The agar plates are incubated under appropriate conditions based on the tested microorganism.
- 4. As the antimicrobial agent diffuses through the agar, it inhibits the growth of the microbial strain in the surrounding area. [6]

Six rats Rats (180-220 g) were selected. Rats were divided into two groups; each group contained 3 rats. One group was a testing group, and the other was the control group. The dorsal fur was removed. A facewash tablet was applied on the rat skin and gently scrubbed with cotton. The procedure was repeated 3 or 4 times. The animals were sacrificed with an overdose of anaesthesia, and the skin of the rat was isolated. The isolated skin of rats was examined and evaluated for the presence of melanocytes after applying a facewash tablet and compared with the standard.

3. Result and Conclusions

Table no.2: Pre-formulation study of individual

Preformulation study of herbal ingredients indicated good flow properties of facewash tablet (Table 2).

Parameters	Coffee	Lentil	Cinnamon	Guava leaf	Green tea	Menthol	Mixture of
		legume					ingredient
							S
Bulk	0.452±0.045	0.60±0.060	0.357±0.03	0.48±0.048	0.33±0.03	0.66±0.06	0.375±0.037
density			5				
(g/ml)							
Tapped	0.513±0.053	0.5 ± 0.005	0.5±0.005	0.65±0.06	0.37±0.03	0.8 ± 0.08	0.6±0.06
density							
(g/ml)							
Hausner	1.13±0.11	1.43±0.014	1.4±0.014	1.35±0.013	1.12±0.11	1.2±0.12	1.62±0.16
ratio							
(kg/cm)							
Carr's	11.8±0.11	29.5±0.29	40±0.04	26±0.26	10.81±0.10	17.5±0.17	33±0.33
index (%)							
Angle of	26.56∘	26.50∘	30∘	33∘	28.3∘	28.4∘	25∘
repose							
Flow	good	good	good	fair	good	good	excellent
property							

Formulation and optimization of facewash tablet:

Table No.3: Composition of facewash tablets

Constituents	F1	F2	F3	F4	F5	F6	F7	F8
Coffee(mg)	100	100	100	100	100	100	100	90
Lentil legume(mg)	100	100	100	50	100	50	50	50
Cinnamon(mg)	50	50	50	25	50	25	25	20
Guava leaf(mg)	100	100	70	50	70	50	50	35
Green tea(mg)	100	100	70	50	70	50	50	50
Menthol(mg)	36	20	5	5	5	10	5	5
Carbopol(mg)	130	130	130	100	130	85.5	50	50
SLS(mg)	150	150	150	220	150	128	220	210
SSG(mg)	170	200	285	237	-	-	237	227
MCC(mg)	-	-	96	96	-	85.5	96	96
Starch(mg)	-	-	-	-	57.9	-	-	-
Cross Carmellose(mg)	-	-	-	-	-	213	-	-
Span(mg)	-	-	-	-	-	85.5	-	-
Sandalwood Powder(mg)	-	-	-	-	-	-	100	-
Coco Powder(mg)	-	-	-	-	-	-	-	100

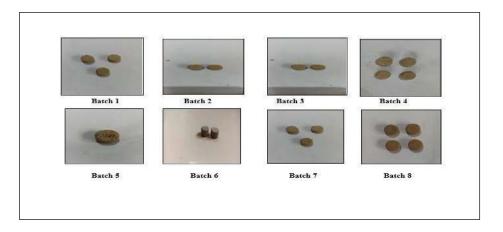


Figure 1: Appearance of tablets

Parameter	F1	F2	F3	F4	F5	F6	F7	F8
Color	Brow	Light	white	white	Light	white	Red	Dark
	n	brown			brown			brown
Washability	Good	Good	Good	Bad	Bad	Good	Good	Excellent
Hardness	4±04	4.1±0.4	3.5±0.3	3.9±0.3	3.5±0.3	3.0±0.3	3.0±0.3	3.0±0.3
(Kg/cm ²)								
Friability	0.59±	0.64±0.	0.70 ± 0.0	0.61±0.06	0.73±0.07	0.73±0.07	0.71±0.0	0.63±0.06
(%)	0.05	06	7				7	
Thickness	7.3±0.	7.5±0.0	7.4±0.07	7.2±	6.5±	6.8±	7.21±0.0	7.21±0.07
(mm)	073	7		0.07	0.06	0.06	72	
рН	6	6	6	6	6	6	6	6
Irritancy	No	No	No	No	No	No	No	No
test	Irritat	Irritati	Irritatio	Irritation	Irritation	Irritation	Irritatio	irritation
	ion	on	n				n	

Table No.4: Characterisations of facewash tablets

Batches F1 to F8 were evaluated and complied with industrial standards for various parameters like washability, hardness, friability, thickness, pH and irritancy test (Table 4)

Formulated batch F8 has good foam and washability properties, so F8 is the optimised batch of facewash tablet

(Table 5 and Figure 2)

Batches	Foaming
	property
F1	Small amount
	of foam
F2	Small amount
	of foam
F3	Good amount
	of foam
F4	Good amount
	of foam
F5	Very small
	amount foam
F6	Very small
	amount offoam
F7	Good amount
	of foam
F8	Excellent foam



Figure 2: Foam formed by Facewash tablet formulation

Parameters	Observation
Foaming index	2.5
Weight variation	0.03%

Table no. 6: Foaming index and weight variation of optimised batch Foaming index and weight variation of optimized batch were within range as per industrial standards (Table 6).

In-vitro anti-bacterial study:

Table no.7: Zone of inhibition of facewash tablet

S	Microb	Zone of Inhibition
r.	es	
N		
0		
1	Candida	14±3mm
2	Pseudo	13±2mm
	monas	
	aerugin	
	osa	
3	Staphyl	11± 2mm
	ococcus	
	aureus	

Table no.5: Foaming of formulation (F1-F8)

Antibacterial activity of the prepared facewash tablet optimised evaluated against the following bacteria and fungi:

- 1. Candida albicans
- 2. Pseudomonas aeruginosa
- 3. Staphylococcus aureus

The antibacterial activity revealed that the facewash tablet kills bacteria and stops bacterial growth. The antibacterial property of the facewash tablet would help in treatment of acne (Table 7 and figure 3). (8)

Fig 3. Antimicrobial activity of facewash







aeruginosa

4. Melanocyte assay

The present study investigates the change in melanin number present in the epidermis layer of the skin of rats after applying a face wash tablet on the skin. (10)

Bv comparing the melanin concentration between treated and normal skin, the reduced number of melanocytes indicated exfoliating action of facewash tablet.

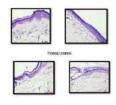
Sr. No.	Code	Observations of Skin dermis and epidermis	Qualitative assessment of melanocyte numbers in the epidermis layer
1	NC	Normal layers of dermis and epidermis with an intact epithelial layer.	Regular and adequate numbers of melanocytes in the epidermal layers.
2	Test	Normal layers of dermis and epidermis with an intact epithelial layer.	Reduced number of melanocytes in the epidermal layers compared to normal control groups.

Table No.8 Results of Melanocyte

CONCLUSION:

The study aimed to develop and evaluate an herbal facewash and an exfoliating tablet. The primary objective was to create a facewash tablet that offers greater convenience for consumers, reduces costs, and makes the product more accessible to the public. We conducted experiments using tabletting technology, which provides added portability benefits for users, especially while travelling, and minimises wastage by enabling controlled usage. After performing various comparative studies on the ingredients and their evaluation,

Fig 2. Histopathological study of the melanocyte assay



the formulated batch (F8) demonstrated excellent foaming properties and produced a high-quality facewash. The F8 facewash tablet also exhibited antibacterial effects, effectively treating acne and providing exfoliating benefits.

REFERENCE:

- 1. Marathe AS, Maru AD, Bagul TP, Chavan NA and Thube SJ.A Comprehensive Review on Facewash. *RJTCS*, 2024,15(1), pp.47-52.[DOI: 10.52711/2321-5844.2024.00009]
- 2. Fartade S, Dode A, Garje M. Formulation and evaluation of Dual antimicrobial polyherbal gel face wash and handwash, *WJPMR*,2020,6(6) 198-203
- 3. Devaraj S, Radhakrishnan A, Kothandan S, Abhijith KR, Ashish A, Pradeesh M, Pranesh B, Formulation and evaluation of consumer-friendly facewash tablet, *RJPT*, *2021*. Volume-14(2) [DOI:10.5958/0974-360X.2021.00148.7]
- 4. Rathod, H.A. ,Herbal Photoprotective and Skin Whitening Agents, April 2020
- 5. M, Sadiki M, Ibnsouda SK, Methods for in vitro evaluating antimicrobial activity: *J. Pharm. Ana,* 2016 volume 6, Page no.71-79(2) [DOI: https://doi.org/10.1016/j.jpha.2015.11.005]
- 6. Bajaj S, Singla D, Sakhuja D, Stability testing of pharmaceutical products, *J. Appl. Pharm. Sci., 2012 (03).*
- 7. SSingh HP, Samnhotra N, Gullaiya S, Kaur I, Antiacne synergistic herbal face wash gel: formulation, evaluation and stability studies, *WJPR*, 2015.volume 4(9).
- 8. TC Lei, VM Virador, WD Vieira, VJ Hearing, A melanocyte–keratinocyte coculture model to assess regulators of pigmentation in vitro, Analytical biochemistry, 2002(2)[DOI: 10.1006/abio.2002.5665]
- 9. Chan M, The Effect of a Source Change for an Active Pharmaceutical Ingredient (API) or Excipient on the Finished Drug Product, 2016
- 10. Kumar S, S Vivek, Sharma S, Baldi A, Herbal cosmetics: Used for skin and hair, *Inven. J, 2012.(4)*

- 11. Kumar V, Antil M, Kumar D, N. Importance of ayurvedic medicine in modern lifestyle: A keynote review study, *Int. j. adv. educ. res.,* 2016.V0lume 1(4)
- 12. Mane PK, Dangare A, Herbal Face Wash Gel of Cynodon Dactylon having Antimicrobial, Anti-Inflammatory action, Pharmaceutical Resonance, 2020. Volume
- 13. Solanki D, Sagrule SD, Unhale SS, Ansar QB, Chitte MG, Biyani KR, Formulation, Development and Evaluation of Instant Whitening Face Wash, *WJPR*, 2020.Volume 9(7) [DOI: 10.20959/wjpr20205-17516]

Bulletin of Pharmaceutical Research 2025;15(S2)

An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print) 2249-9245 (Online)

RESEARCH ARTICLE



BIOTECHNOLOGICAL INNOVATIONS IN HERBAL DRUG DEVELOPMENT: ADVANCES, CHALLENGES, AND FUTURE PROSPECTS

Rutuja Khandare, Nikita Sanap and Priyanka Sarda *
Department of Pharmacognosy, Progressive Education Society's Modern College of Pharmacy,
Yamunanagar, Nigdi, Pune-44.

*Corresponding author email: priyankasarda15@gmail.com Received: Feb 06, 2025 / Revised: April 18, 2025 / Accepted: May 02, 2025

ABSTRACT:

Herbal therapy is now widely accepted as an adjunctive treatment for several illnesses. More than 1,200 kinds of medicinal plants are used in the ancient practice of a perhaps of the ancient medical systems. Medicinal plants are essential to medication development and contemporary healthcare, as around 80% of people on the planet continue to utilize traditional medicine. Standardization, efficacy assessment, and regulatory compliance are still issues, nevertheless. Strict pharmacovigilance is necessary to ensure the safety and effectiveness of herbal drugs, even though they are manufactured from natural sources. The quality and manufacturing have been enhanced by biotechnological developments such as biological engineering, cultivation of plant tissues, and genetic engineering. Technologies such as omics and DNA fingerprinting aid in the discovery and improvement of therapeutic plants. India incorporates herbal medicine under AYUSH system; however other nations regulate it differently. Obstacles include inconsistent regulations, problems with quality control, and in certain places, their designation as nutritious items. Prospects for the future focus on sustainable procurements of medicinal plants, combining innovation with traditional knowledge, and using nanotechnology to improve medicine delivery. Promising advancements are made possible by technologies including CRISPR and based on artificial intelligence drug discovery. For worldwide adoption, ethical issues pertaining to effectiveness, safety, and commercialization are still very important. The scientific, financial, and legal obstacles to increasing the use of herbal remedies in healthcare are examined in this article. **Key words:** DNA Fingerprinting, Genetic Engineering, Biotechnology, Omics Technology.

How to cite this article: Rutuja Khandare, Nikita Sanap, Priyanka Sarda Biotechnological Innovations in Herbal Drug Development: Advances, Challenges, and Future Prospects Bulletin of Pharmaceutical Research. 2025;15(S2): 255-266.

Source of support: Nil Conflict of interest: None

I.INTRODUCTION:

Fig 1: Medicinal Plant



Herbal medicine has recently gained a lot of attention as an alternative medicine that can help treat or prevent lifestyle-related disorders. Ayurveda, which translates to "science of long life," is believed to have begun approximately 6,000 years ago and was developed to promote optimal health and a longer lifespan rather than to treat illness. It was practiced by doctors and surgeons(called bheshaja or Vaidya).the terms" ayur" and "veda" make up the term "ayurveda". Veda represents science or wisdom, while ayur signifies life. Therefore, ayurveda describes how to stay healthy and treat illness. Herbs are nature's gift to humanity, enabling them to live healthy lives free from illness. About 1200 plant species, including cinnamon, about 100 minerals, including sulfur in the environment, lead, zinc, with sulfate of copper, alongside more than 100 dairy products, including milk, bone and gallstones, are included in the ayurvedic pharmacopoeia stated Crag and Newman. Plants have been used to make a variety of modern medications for numerous years. Prior to the advent of synthetic pharmaceuticals, humans relied entirely on plant-based medications for their essential medical needs.[1] of the roughly 4.5 million plant species known to exist in India, plant with potential for healing are widely used in all facets of Indian society, including as alternative treatments in Siddha, Avurvedic, and Unani medicine as well as in went through pharmaceutical industry products. In the pharmaceutical industry products. In the pharmaceutical business, plant extracts or bioactive components can be used to trat a range of ailments in addition to developing novel drug formulations. Herbal remedies like ashwagandha and brahmi can improve immunity, regenerate body cells, increase nutrients, and increase energy levels. Aromatic and medicinal plants can

Bull. Pharm. Res. 2025;15(S2)

particularly for

contribute significantly to the improvement of rural residents' subsistence livelihoods,

in

a way that is

women,

environmentally friendly and preserves biodiversity of these natural products. According to WHO, traditional medicine provides up to 80% of the basic healthcare needs of people worldwide ... The creation of homegrown medications and the application of medicinal plants to treat a range of illnesses have significant financial advantages. Most people, especially those living in rural areas, are still compelled to use traditional remedies for common diseases because of a lack of modern medical ignorance, services. poverty, and limited communication options. One of the best things for both individual and community health is medicinal plants. Certain active chemical ingredients that cause distinct physiological activity on the human body are what give plants their therapeutic significance.[2] As a result of improvements in surveillance of quality and clinical research, plant- based medicinal active constituents have become known as "people's drugs" because of their access, security and reliability asserts, ease of preparation, and gradual replacement of traditional treatment frameworks in many countries. Due to the negative contemporary medications the inability of current treatment to address chronic illnesses, and germ resistance, herbal medicines have seen a notable resurgence in popularity recently. The WHO claims that many people use botanicals or medicinal substances for basic health issues. Herbs, medicinal preparations, processed and finished products, herbal materials and active ingredients are all components of the remedy. [3] Making sure that customers receive medication that ensures purity, safety, potency, and efficacy is the regulatory bodies' primary duty. Through statutory mandated good manufacturing practices, the regulatory bodies strictly adhere to the numerous quality standards specified for raw materials and final products in pharmacopoeias, formularies, and manufacturing operations. It makes sense that these protocols ought to be applicable to all kinds of medications, while they are part of the conventional or modern medical systems. These have made it necessary to create contemporary, impartial criteria for assessing the efficacy, safety, and quality of these medications. Additionally, people are learning about the adverse effects and potency. [4] The finest illustration of the symbiotic phenomena may be found in nature. Natural products derived from both plants and animals serve as the foundation for illness therapy.

India has a long history of using a traditional medical system that is extensively recorded. India has a long history of using species and plants for medical purposes. Over 2000 species have a great potential for use in AYUSH medications. Because they are less expensive and safer, Ayurvedic medications are used by the majority of individuals in development nations who cannot afford pharmaceutical drugs. The phrase "medical plants" refers to a wide variety of plant species utilized in herbal medicine. It involves both the application and research of plants for therapeutic purposes.^[5]

II. OVERVIEW OF HERBAL DRUG DEVELOPMENT: over the edges, humans have utilized plants and natural items as sustenance and as remedies for illnesses. although the exact beginning of the use of plants as medicine is difficult to pinpoint, carbon dating from ancient Babylon (Iraq) shows that plants were cultivated for medicinal purposes 60,000 years ago. In Greece and Asia minor, written records of therapeutic plants date back at least 2,500 years, while in India, China, and Egypt they date back about 5000 years. [6] The Rig-Veda, Atharvaveda, Charak, and Sushrut Samhita are examples of classical Indian writings. The rich histories of ancient culture and scientific legacy are the source of traditional medicines. Herbal remedies for age-related illnesses including osteoporosis and memory loss, as well as others like diabetic wounds, immunological and liver problems, etc., are mentioned in ancient literature. With a strong philosophical and experimental foundation, Ayurveda is still one of the oldest and most popular traditions in India, Shri Lanka, and other nations. [7] Long before history was written, plants were being utilized for therapeutic purposes. According to papyrus records from ancient China and Egypt, plants were used medicinally for as long as 3,000 BC. Others societies, such as Native American and African communities, used plants in their healing practices, while other civilizations developed conventional healthcare systems that included herbal treatments. In the west, the use of botanicals and plant- based medications has grown significantly in recent years. In the past 200 years, plant-based medicines have dominated human medical practices. However. when more predictable medications were widely accessible, the usage of plants for therapeutic purposes rapidly declined in the west. On the other hand, a lot of underdeveloped benefited from the countries still extensive understanding of herbal medicine.[8]

Herbal medicine is the use of plant parts for medicinal purpose, such as seedlings, fruits, foliage, roots, bark, or flowers. In addition to being directly employed as therapeutic medicines, these plants are valuable for pharmaceutical research and drug development since they may be used as prototypes for pharmacologically active chemicals or as starting materials for drug synthesis. For thousands of years, medicinal plants served as a significant source of pharmaceuticals. In practically every nation on the planet, they continue to serve as the foundation for organized traditional medicine. Two of the most wellknown and traditional instances of this category are the medical systems of Unani and Ayurveda. In underdeveloped nations, around 80% of the population relies entirely on plants for their basic medical needs Bannerman, 1983). More than onefourth of all prescription medications come either directly or indirectly from plants, even in affluent nations with highly established medicinal chemistry. Additionally, 11% of the 252 drugs that the WHO deems important are mostly derived from flowering plants, and 28% of synthetic medicines are derived from natural precursors. Here is a list of a few significant plant-based chemicals that are utilized as drugs or drug precursors. Herbal remedies are secondary metabolites that are obtained from plants. [9] Verifying the usage of herbal materials and scientifically evaluating contradicting toxicological, clinical, and other data are major challenges. The following important concerns still exist. Managing within risk ranges; communicating ambiguity; and maintaining clinical, toxicological, pharmacological records pharmacovigilance comprehending the mechanisms behind the inclusion dangerous chemicals; assessing" interactions; addressing clinical trial and personnel constraints; standardizing; and assessing safety and efficacy [10] Research studies and haphazard accounts (independent and unsolicited communications about marketed pharmaceutical items) are the most reliable sources of information regarding adverse occurrences and drug responses.. Throughout a product's life, the latter usually outnumbers the former in terms of both quantity and kind, particularly in serious complaints. In addition to traditional, supplementary, and alternative medicine providers, herbal medicine providers, medicine providers have to be incorporated into national reporting systems for the prescription and dispensing of medications. Providers of herbal medicine who are not physicians, dentists. pharmacists, or nurses are not included in reporting systems in many nations.[11]

The process of combining active compounds with inert components, such as excipients, fillers, or matrices, is known as formulation development.

Bull. Pharm. Res. 2025;15(S2)

Herbal medication formulations fall into two herbal formulations categories: pure formulations made of minerals, bio compounds, and metallic materials. To demonstrate their therapeutic added to medication efficacy, minerals are compositions. A polyhedral formulation (PHF) is a type of herbal formulation used in Ayurveda that is made using multiple medication ingredients or a single substance in two different methods. It is crucial to remember that a small amount of a biomolecule is insufficient to provide the intended therapeutic effect, and that an individual medicine is less effective than a combination of medications derived from several plant species. The medication has a greater therapeutic impact when coupled with other pharmacological ingredients. We call this synergism. In polyhedral formulations, this beneficial drug-drug interaction is common and operates complementary through two mechanisms: pharmacokinetic and pharmacodynamic. Pharmacodynamic approaches address therapeutic effect of the active ingredients in polyhedral drugs on similar cell receptors, whereas pharmacokinetic synergistic approaches focus on the herbal drug's ability to enhance ADME of another • drug.[12] For natural medications, randomized clinical studies are not feasible. Since the same patients may have different results, which makes the selecting process more challenging. When a cluster of subjects with varying makeup are given a herbal medication, the results may not be consistent. Traditional therapy approaches may utilize various therapies in a particular patient at different phases of the disease, which might lead to variance in a clinical study. Because there aren't enough people in clinical trials for herbal medications, it might be exceedingly challenging to achieve statistical significance. The quality and standardization of herbal medications utilized in clinical studies are inadequate. [13] A market unregulated, expanding insufficient quality control is the cause of the safety issues arising with herbal therapeutic items. The primary characteristics that are present in various regulatory systems are the absence of stringent criteria for the evaluation of quality and safety, quality control, safety monitoring, and understanding of traditional medicines or complementary and alternative medicines (TM/CAM). Plants can be classified as foods, functional foods, supplements, or herbal medicines under certain regulatory frameworks. The WHO states that herbal medicines are made up of plants, herbal components, herbal preparations and finished herbal products that have combinations of herbal substances or plant

parts as active agents. This comprises tinctures, extracts, essential oils, powdered herbal medications, juices, and process exudates. In the United States, almost all herbal items are regarded as foods since they constitute nutritional supplements. National health officials have developed laws to ensure the safe use of herbal treatments due to significant concerns about their safety. WHO started a global survey on national policies regarding TM/CAM and the regulatory framework of herbal medicines in 191 member nations in 2001. The most crucial elements for the field of herbal medicine regulation are research data, suitable control mechanisms, provider education, and competence. [14] The Indian healing systems of Ayurveda, the practice of Yoga, Unani, acupuncture Siddha, and Homeopathy all heavily rely on herbal medications. Herbal medicine in India is governed by the National Council of Indian Medicine Act, the AYUSH department, and the D and C Act 1940. The Act establishes good Manufacturing Practice guidelines and broadens control for the manufacturing of herbal medications. [15].

III. BIOTECHNOLOGY APPLICATION IN HERBAL DRUG DEVELOPMENT:

Genetic Engineering: Human have traditionally utilized medicinal plants in food production, medicine, food production, medicine, and other fields. Medicinal plant breeding has made use of traditional biotechnological approaches. Faster biotechnology-based breeding technique (BBBMs) should now be used on these priceless plants. The evaluation of gene diversity, preservation while proliferation, and overproduction, are the main ways that heredity and biotechnology may contribute to the faster development of therapeutic plants. Utilizing the cultivation of plant tissues (PTC) as a framework to deploy more BBBMs to medicinal plants is crucial. The two primary BBBMs that directly rely on PTC are artificial polyploidy induction and agrobacterium medicated gene transformation. The genetic diversity of medicinal plants may be investigated, and genes involved in the manufacture of secondary metabolites can be found, using next-generation sequencing methods like RDAseq and RNA-seq. user-designed therapeutic plants can be produced using genome editing methods such as TALENs and zinc-finger nucleases [16]. Gene hvbrid vaccines. therapy. DNA vaccines. bioinformatics, proteomics, genomics, biopharmaceuticals, and biomedicines are the main areas of study in medical biotechnology. With molecular medicine displacing traditional medicine, medical advancements are expanding quickly. these

Sarda et al

days, biotechnologist are able to detect genes in abnormalities are able to detect genes abnormalities and characteristics such as mental, cardiovascular such as mental, cardiovascular, respiratory and cancerous disorders. The detection of particular genes and the proteins produced from them provides extremely particular and effective treatments. Since many people have incomplete genes, gene transplantation and antisence are also utilized to treat genetic illnesses [17]. In the past, genetics' contribution to medicine has mostly been scholarly and has not been seen as an essential component of care. Genetic disorders are an insignificant type of diseases for practicing physicians because they are known to be uncommon and limited to certain particular chromosomal defects. Historically, the use of genetics in medicine has meant gathering family history and warning individuals of potential risks. Since the • human genome's sequencing was finished in 2003, genetics has been progressively become a crucial component of therapeutic treatment. The medical field must now embrace the potential and difficulties presented by the abundance of information provided by the human genome, which contains material of incalculable diagnostic and therapeutic significance. According to estimate, the prevalence of congenital abnormalities worldwide is 7.94% of which 5.32% are disease with a significant genetic components. In addition to environmental influences, the majority of illnesses require intricate interactions between several genes [18].

Culture of plant cells and tissues: plant cell and tissue culture offers an alternative source for rapid plant growth and controlled production of phytonutrients that prevent disease or food • ingredients in medicinal plants. Numerous plant species may be regenerated in vitro using a variety of techniques including from a single replicable cell, a tissue or a organ fragment, or an explant a cutout portion of differentiated tissue (or organ). These technologies may be made clean from any vegetative plant component or organ, such as stems, which are nodes, as well branches, roots, leaves, apical or reckless shoot buds, endosperm, and fertilized eggs, in order to comprehend plant development and metabolite production. Clonal propagation, also known as micropropagation, is the vegetative division of plant in vitro, where asexual reproduction creates many genetically identical clones of cultivars. The annual production of hundreds or even billions of plants is possible using micropropagation. Many institutions and organizations now use this technique to produce superior plantson a big scale, including

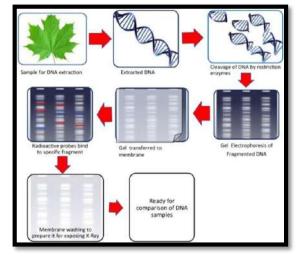
Bull. Pharm. Res. 2025;15(S2)

clone selection and the rapid and extensive creation of disease-free materials of desired species. For several significant species of medicinal plants, including Swetia chirayita, Cathranthus roseus, Panax, Stevia rebaudiana, Agni annuaremisin, Elettaria cardamomum, Allium Chinese, canellia sinesis, and others, micropropagation techniques have been devised. Numerous significant therapeutic plants have long been effectively cultivated in vitro, organogenesis or bv embryogenesis. Numerous method have thoroughly investigated to enhance the synthesis of plant compounds utilizing an in vitro growth system. To create the required metabolites or dietary components, many cell culture type are employed, including organized tissue, callus formation, cell suspension for an extended period cells, transform cells, and organs [19]

Metabolic Engineering: Redirecting one or more enzyme processes to create new chemical is an organism, enhance the synthesis of already-existing mediate the breakdown compounds. or compounds is known as metabolic engineering. It is crucial to emphasize that metabolic engineering is still a relatively new field of study before detailing advancements made in the last 25 years. The use of radiolabel tracer studies throughout the preceding decades has allowed us to reasonably develop our understanding of substrate- product interactions in plant pathways by 1975 [20]. The metabolic pathways that generate flavonoids, phytoalexins, and other compounds believed to contribute to the chemopreventive qualities of fruits and vegetables are one class of targets for nutritional engineering. One method that

Fig 2: Using DNA markers for medicinal plant genuineness. [26]

does not require knowledge of the genes involved in its biosynthesis to change the amount of nutrients in its natural host. The viability of this strategy is demonstrated by two instances. Human ingestion of



Sarda et al

broccoli rich in glucoraphanin has been shown in preliminary research to enhance metabolism and lower level of fatty acid and lipid molecules linked to inflammation [21]. An interesting strategy for metabolic engineering is the investigation of transcriptional factors governing metabolic pathways or branches. Originally, pharmacological or transposon-based mutant screens in Arabidopsis, snapdragon, or maize were used to identify transcriptional regulators influencing biochemistry and development [20].

- **Pharming:** A recent development in biotechnology is pharmaceutics, which uses transgenic plants or as biological "factories" to medications for human or animal use. For instance, the human gene for the antithrombin protein, which prevents clotting, has been genetically altered into goats. Following a processing stage, this proteinwhich is produced by the altered animals in their milk is prepared for use in medicinal applications. The phrase "pharming," which combines the terms "farming" and "pharmaceuticals," exemplifies the union of these two fiercely opposed sectors. In term of potential financial gains and health benefits to people, pharming has significant risks. If successful, pharming might bring in billions of dollars for biotechnology and pharmaceutical companies [22].
- DNA fingerprinting and molecular markers: DNA markers based on unique genetic structures are more advantageous than other marker systems. Since these genetic markers are not tissue-specific, they can be found at any point throughout the growth of a plant. Since the data produced by this technology is readily mechanized, it provides precise and effective techniques that are more affordable and genuine than morphological and chemical markers of disease. Unique DNA sequence that may be used to identify target may be used to identify target sequence in DNA hybridization, PCR, or restriction mapping operation are known as genetic markers. It provide the genotype's direct reflection. [23]. The genomic differentiation patterns that may be shown by morphologically based taxonomic classifications might be effectively resolved by a methodical examination of the molecular genetic information already accessible in germplasm. Breeder can make better use of genetic resources with fewer prebreeding activities for cultivar growth and improvement by using the molecular genetic variation data sets, which can provide valuable information on the allelic depth, population composition, and diversity parameters of germplasm characterization using molecular markers has become more important due to the speed and quality

Bull. Pharm. Res. 2025;15(S2)

of the data produced [24]. A PCR based technique called AFLP is employed in DNA fingerprinting, genetic engineering, and genetics research. AFLP, which was created by keygene in early 1990s, involves cutting genomic. DNA employing restriction enzymes, followed by connector attachment to the elastic ends of the restriction fragments. Primers that work well with the adapter and with some limitations. Only a subset of the restriction fragments are then amplified using priming equivalent to the adapter and some of the site of restriction fragments. Denaturing polyacrylamide gels show magnified fragments or auto radiography [25].

Omics Technologies: In the last 10 years, advances in next-generation DNA sequencing technology have made it easier to use and collect multi-omics data, which has significantly decreased the cost of such activities. Additionally, transcriptomics and genomics have become more widely used, and other omics technologies, like proteomics and metabolomics, are now frequently incorporated into standard research methodologies. In general, the use of lots of omics techniques is becoming commonplace in many life science research fields. [27]. Destination selection and validation, lead discovery and improvement, in vitro and in vivo investigations to assess drug safety and effectiveness, and clinical testing are just a few of the numerous processes that make up the drug process. The goal identification and validation stages of drug development heavily rely on proteomics technology, which is used to examine the amount expressed proteins in a target cell or organism at a certain time. Microbe's secondary metabolites (SMs) are an important natural source of drugs for neurological and metabolic diseases. They are used in biochemical, food, and medicinal industries. Under some circumstances, metabolmics provides thorough identification and structural data on the cellular metabolome [28]. The term "genome" was coined by Hans Winkler and refers to the haploid DNA content of a cell, including the representation from the genome of an organelle. In a simpler sense, genomics is the branch of omics that deals with the study of genomes; it entails molecular descriptions and evaluation of the entire genome in order to study its structure, function, and comparative genomics are distinct subfield of genomics that deal with the study of genomes and protein structure. Analysis of genome structure primarily consist of the mapping of genes, gene density, genome complexity, repeat analysis, cot curve analysis, and other such studies, whereas protein structure analysis primarily consists of high throughput methods for protein [29].

IV. CURRENT ADVANCE AND TRENDS:

Modern Biotechnological Approaches: CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats-CRISPR- associated proteins) has made grat progress since it was discovered to be an adaptive immune system in bacteria and archaes. It became a powerful gene-editing tool after its synthetic guide RNA (sgRNA) was successfully engineered to target specific DNA sequences with high accuracy. Furtherdeveloped Cas variants have the ability to edit the epigenome in addition to DNA, making the CRISPR-Cas system an effective instrument for manipulating both the genome and the epigenome and a leader in precision medicine. With a focus on precision medicine, this review examine the most recent developments in the field of CRISPR-Cas and its restorative and biological uses. Additionally, the present state of clinical trials for CRISPR medicines is examined [30]. To create and enhance biological systems, synthetic biology technologies have been created. Among them, the genome editing method CRISPR-Cas, which is a protective immune system of bacteria, thereby has been created. Since CRISPR gene scissors are separated into two modules- the target recognition RNA and the nucleolytic protein, they are more synthetic-biologically applicable than any other technique. In contrast to restriction enzymes, CRISPR-Cas may function in bacteria as well as higher species, and it is simple and unrestricted to create any target sequence [31]. The goal of the clinical science and technology discipline of nanotechnology is to create tools dosage form that are between 1 and 100nm in size. The application of nanotechnology in biological systems for monitoring, diagnosis, therapy, and control has recently been referred to as nanomedicine. Lipids, polysaccharides, and synthetic biodegradable polymers are among the safe components used to create the nanocarriers. Research on Phyto-formulation or herbal medication has many advantages, including enhanced solubility and bioavailability, security from toxic exposure, increased pharmacological activity, stability, better tissue macrophage distribution sustained delivery, durability, and defense against chemical and physical deterioration. Therefore, the use of herbal pharmaceuticals in nanoscale drug delivery systems holds promise for improving their efficacy and resolving issues related to plant medicines. Therefore, it is crucial to include nanocarriers like NDDS into the conventional medical system in order to combat more system in order to combat more chronic illnesses as caner, diabetes, asthma, and others [32].

Integrating traditional knowledge with **biotechnology**: Drug development relies heavily on traditional knowledge, which provides a multitude of medical expertise that has been passes down through the year among indigenous groups. This knowledge is frequently based on centuries of actual data and involves the utilization of vegetation, minerals, and natural substances for therapeutic purposes. Its importance has been acknowledged by the pharmaceutical business more in recent decades, particularly in the areas of herbal medicine. The identification of many contemporary medications has been greatly aided by the application of traditional expertise in drug development. Indigenous tribes have gathered this knowledge over ages, and it includes the use of natural treatment, medicinal plants, and herbs. The necessity for legal safeguards when traditional medicine increased incorporated into contemporary healthcare systems. Governments are legalizing traditional medicine, encouraging interdisciplinary study, and integrating it into health programs [33]

v . CHALLENGES AND LIMITATION

Scientific challenges: Studies on the herbal medicinal compounds stability are crucial. This is done to assess how temperature, humidity, radiation, and oxidation affect natural good. All of these variable might cause herbal mediation to degraded period of time. Therefore, when a medication is unstable or its components deteriorate, the clinical study is deemed invalid. The stability of the goods is seriously harmed throughout the production process when the active ingredients are subjected to oxidation. Hydrolysis, and bacterial or fungal assault. The variance in the herbal medication formulations shelf life shouldn't surpass 5% of the initial assay value [34] . There is no doubt that the increase in poisoning cases linked to the use of medicinal herbs in recent years is necessitating the implementation of active pharmacovigilance on these products in along with maintaining thorough toxicity testing in order to promote their safe use and safeguard public health. Therefore it is imperative that herbal products and medications be incorporated into pharmacovigilance systems. Simply based on group publicity it can be difficult to locate the offers tied with the recreational use of herbal remedies, and in the process, the safety of these products has become a major public health concern. In many regions of the world, there are several obstacles that must be overcome in order to design and enforce regulations pertaining to traditional or herbal medicines. [35].

Sarda et al

- Economic and regulatory challenges: In order to guarantee the safety and effectiveness of herbal medicines, regulatory bodies are essential in monitoring their regulation bodies are supervision. Globally, the use of herbal remedies is growing, which has sparked worries about side effects and the necessity of safety monitoring. There inadequacies in the registration and post-marketing surveillance procedures for herbal medications in some areas, including Sub-Saharan Africa. Official regulatory agencies are tasked with overseeing the production, quality, and distribution of herbal remedies in order to guarantee their safe use in order to solve these issues. Research assessing the frameworks for regulating herbal emphasizes how crucial it is that regulatory bodies carry out their mandates effectively [36]. The fact that many herbal and traditional remedies are classified as consumables or food supplements in various countries has been identified as the primary cause of most problems pertaining to their quality, safety, or efficacy before being on sale. In a similar vein, traditional medical professionals might necessarily be licensed or certified, while manufacturing norms and quality checks are occasionally less strict or controlled. Therefore, the purity of traditional and herbal remedies is no longer a major issue for the general public or national health authorities. In essence, herbal remedies are made from plant parts or unpurified botanical extracts that • contain multiple constituents that are generally thought to function in concert. Demands of plant medicine's effectiveness, customer demand for natural therapies, false beliefs about the superiority of herbal products, dissatisfaction with robust pharmaceuticals, costly prices and side effects, scientific and technological advancements, patients perceptions of doctors, ineffective evaluations, and a trend toward self-medication are the main causes of the renewes intresr in herbal remedies [37]. By 2025, the worldwide herbal medication industry is projected to grow to a value of USD 7 trillion, with India holding a relatively small portion of this market. In 1991 the herbal medicine market in Europe was valued at around USD 6 billion. The of herbal economic significance plants demonstrated by the worldwide revival of traditional healthcare systems [38].
- Ethical consideration: Thinking about what "the good" is and how to define it is the definition of ethics. Philosophical and religious ethics study the history, values, emphasis, and application of different goods as a field of study. People clearly appreciate various items and attributes, but they also value,

Bull. Pharm. Res. 2025;15(S2)

emphasis, and applications of different goods as a field of study. People clearly appreciate various items and attributes, but they also value their possessions differently, in different ways, for various motives, and for different purpose. Ethical dimensions are a broad category of norms that can be recognized, examined, or attained with careful consideration. Although ethics generally examines issues of good and evil, there are various ways to define this based on a variety of viewpoints and interests [39]. "Of proven quality, safety, and efficacy" are this the primary ethical consideration that need regulatory attention, according to the Director-General. It is possible to draw a comparison between the procedure of launching a new molecular medication and the legislation governing of herbal remedies. Thus, clinical studies and other trustworthy scientific techniques should be utilized to assess the efficacy herbal remedies. safety of pharmacovigilance procedure, they should also be tracked during the course of the medications life cycle. Since it might have major negative effects on patients, it is extremely unethical to put any medication on the market without proof of its safety and effectiveness. Health authorities have a stronger obligation to regulate and take action when there is a larger potential for harm [40].

vi. FUTURE PROSPECTS

Emerging Technologies: The most difficult aspect of drug development is finding a successful new drug, primarily because of chemical space, which is a collection of all potential chemical compounds, including all known drug molecules and those that have not yet been found; it is estimated that there are approximately 1060 molecules in total 41 These techniques and strategies have returned from their the wilderness years thanks to recent developments in AI and ML. Even while several of the new methods haven't yet resulted in pharmaceuticals being brought to market, early reports predict that they will play an even bigger role in the drug development process than has been the case thus far. It has been demonstrated with innovative and promising methodologies that the new systems are capable of efficiently designing novel chemical structures, forecasting the necessary molecular property profiles, and even figuring out how to synthesise those compounds.

Sustainability: Understanding the inherent value of natural resources and their benefits to society is essential, especially given the widespread usage of medicinal plant around the world. However, when viewed through the lens of economical history, it is

notable for the ways in which human have exploited nature to achieve their goals. When affluent nations raise their consumption without taking developing national needs into account, inequality arises. a human centered approach has exacerbated several environmental issues and led to the current threat to medicinal plants and natural Sustainability has been described as "addressing the challenges of the present scans endangering the abilities of generations to come of individuals to fulfill their own needs" since the Brundtland Report was published in 1987. One of the most important development strategies has been sustainable growth. Furthermore, it guarantees ongoing access to the resources required to create a highly resourcesintensive and less descrupted economy. It is a strategy based on the notion that social, ecological, and economic elements must cooperate to ensure economic growth while meeting ecological and community needs [43]. In light of the aforementioned data, we describe PM as any technology that seeks to enhance illness prevention, diagnosis and treatment by utilizing patient characteristics to determine the best course of action. Regarding the course of therapy. There is diagnostic testing: Additional patient or disease-related data must be gathered by diagnostic testing that makes use of technologies like molecular diagnostics. gene sequencing immunohistology chemical test in order to be personalized. A variety of technologies are used: Numerous medical technologies, including small molecules, big molecules and advanced treatment medicinal products (ATMPs), can advantages. Customized treatments: these include of gene treatments and modified T cell therapies, which can regarded as ATMPs [45].

vii. SUMMARY: Herbal medicine has become well known throughout the world as a complementary therapy for a number of illness. In one of the ancient medical systems, Ayurveda, more than 1,200 plant species having therapeutic properties are used. Due to their ability to provide bioactive chemicals for drug development, medicinal plants are essential to both traditional and contemporary healthcare. According to the data from the World Health Organization (WHO), almost 80% of people worldwide receive their main medical treatment from traditional practitioners. Among the many difficulties in developing herbal medications include standardization, assessing their effectiveness, and adhering to regulations. For herbal medicines to be safe and effective, strict pharmacovigilance is necessary, even when they come from natural

Bull. Pharm. Res. 2025;15(S2)

sources. Genetic manipulation, plant tissue culture, metabolic engineering, and other advancements have greatly enhanced the quality and production of herbal medications. Further use of DNA fingerprinting and omics technologies also aid in the optimization and verification of medicinal plants. Different nations have different regulations for herbal medicines; India uses the AYUSH system. However, the lack of standardized procedures, problems with quality control, and the fact that herbal products are sometimes categorized as nutritional products in some areas continue to pose commercial and regulatory obstacle. Prospects for the future focus on combining biotechnology and traditional knowledge, improving medicine delivery through nanotechnology, and making sure that medicinal plants are sourced sustainably. Promising developments are provided by new innovations like CRISPR and based on artificial intelligence discovering drugs. For herbal medicines to be accepted worldwide, ethical issues pertaining to their efficacy, safety, and commercialization are still very important. This review discusses the scientific, financial, and legal obstacles to the wider use of herbal medicine while highlighting its significance in healthcare.

viii. ACKNOWLEDGMENT: We sincerely express our gratitude to Progressive Education Society's Modern College of Pharmacy, Nigdi, Pune, for providing the necessary resources and a supportive environment to conduct this review. We extend our heartfelt thanks to our mentor, Prof. Priyanka Sarda, for her invaluable guidance, insightful suggestions, and continuous encouragement throughout the research and writing process. We also appreciate the support and cooperation of our colleagues and peers, whose discussions and feedback have contributed significantly to the development of this article. Lastly, we are grateful to our families and friends for their unwavering support and motivation throughout this journey.

REFERENCE

- 1. Parkash J, Prasad DN, Shahnaz M, Dev D, Herbs as Traditional Medicines: A Review, Journal of Drug Delivery and Therapeutics. 2018; 8(5):146-150
- 2.Bamola, Neelam, and Poonam Verma. *Chandranandani* Negi3, A Review on Some Traditional Medicinal Plants
- 3. chowdhury mobaswar hossain1*, meeta gera2, kazi asraf ali1, status and challenges of herbal drug development and regulatory aspect: a global perspective, DOI: http://dx.doi.org/10.22159/ajpcr.2022v15i12.4613 4. Journal homepage:

Sarda et al

- 4. Dr. Manish Gupta, Pankaj H. Chaudhary, Dr. Mukund G. Tawar, Need & Scope of Standardization of herbal medicines - A review, Published with open access
- 5. Ananda H.V., Priyanka R., Harshitha H.C., Karthick2, A Review about Scope of Traditional Medicinal Plants in a New Drug Discovery
- 6. Majaz, Q., & Aejazuddin, A. (2016). Herbal Medicine: A Comprehensive Review.
- 7. Sanat Sharma, Raj Sharma, Harminder Singh, Bithika Scenario: A Review. Research J. Pharmacology and Pharmacodynamics. 2011; 3(2): 45-47.
- 8. Vanan, T. (2014). Challenges, Constraints and Opportunities in Herbal Medicines-A Review. International Journal of Herbal Medicine, 2(1), 21–24. 20. Lau, W., Fischbach, M. A., Osbourn, A., & Sattely, E. S. https://www.researchgate.net/publication/2691067
- 9. Srivastava, P., Singh, M., & Chaturvedi, R. (2020). Herbal medicine and biotechnology for the benefit of 21. DellaPenna, D. (2001). Plant metabolic engineering. human health. In Animal Biotechnology (pp. 613-629). Elsevier.
- 10. Balammal G, Sekar BM, Reddy JP. Analysis of Herbal 22. Engelhard, M., Hagen, K., & Thiele, F. (2007). Medicines by Modern Chromatographic Techniques. Journal Preclinical International of Pharmaceutical Research 2012; 3(1):50-63.
- Opportunities in Herbal Medicines-A International Journal of Herbal Medicine, 2(1), 21-24. https://www.researchgate.net/publication/2691067 53
- 12. Prabhakar, P., Banerjee, M., & Mamoni, B. (2021).24. Hussain, H., & Nisar, M. (2020). Assessment of plant Regulatory and Market Challenges in Bringing Herbal Drug into Mainstream of Modern Medicinal Practices Article in. In Research Journal of Biotechnology (Vol. 16, Issue 3).
- 13. Ali, F., Khasimbi, S., Sharma, K., Trivedi, M., Ali, A., & Ahmad, J. (2020). Regulatory perspectives of Herbal25. Medicinal Products. In Bioactive Phytochemicals: Drug Discovery to Product Development (pp. 158-**BENTHAM SCIENCE** PUBLISHERS. https://doi.org/10.2174/978981146448512001001 27.
- 14. Sahoo, N., Manchikanti, P., & Dey, S. (2010). Herbal drugs: Standards and regulation. In Fitoterapia (Vol.27. Carles M, Cheung MK, Moganti S, Dong TT, Tsim KW, Issue 462-471). 6, https://doi.org/10.1016/j.fitote.2010.02.001
- 15. Singh, S., & Shukla, V. K. (2021). Current regulations for Herbal Medicines in India. International Journal of 28. Aborode, A. T., Awuah, W. A., Mikhailova, T., Abdul-*Drug Regulatory Affairs*, 9(2), 30–34.
- 16. Pal KS, Shukla Y. Herbal Medicine: Current Status and the Future. Asian Pacific J Cancer Prev 2003; 4:281-288. 3. Kashaw V, Nema AK, Agarwal A. Hepatoprotective Prospective of Herbal Drugs and Their Vesicular Carriers- A Review. International

Bull. Pharm. Res. 2025;15(S2)

- of Research in Pharmaceutical Iournal and Biomedical Sciences 2011; 2(2)
- 17. Zand, M., & Lakshmi Narasu, M. (2013). A review article Biotechnology Applications in Medicine. In International Research Journal of Applied and Basic Issue Sciences (Vol. 9). http://www.bioportfolio.com/\$\$\$
- 18.. Pandey, A. S. (2017). Genetics in medicine. Journal of Kathmandu Medical College, 6(1), 1-2.
- Nelkumar, History of Medicine Herbal Medicine 19, Pant. B. (2014). Application of plant cell and tissue culture for the production of phytochemicals in medicinal plants. Advances in Experimental Medicine and Biology. 808. 25-39. https://doi.org/10.1007/978-81-322-1774-9_3
 - (2014). Key Applications of Plant Metabolic Engineering. **PLoS** Biology, 12(6), https://doi.org/10.1371/journal.pbio.1001879
 - Plant Physiology, 125(1), 160-163. https://doi.org/10.1104/pp.125.1.160
 - Pharming: A New Branch of Biotechnology. https://www.researchgate.net/publication/2637341 36
- 11. Vanan, T. (2014). Challenges, Constraints and 23. Mattummal, R., Gopi, D. K., Bobbili, E., & Narayana, S. K. K. (2019). A review on molecular techniques employed for authentication of Indian medicinal plants. In *Plant Science Today* (Vol. 6, Issue 4, pp. 465–478). Horizon e-Publishing Group.
 - genetic variations using molecular markers: A review. In Journal of Applied Biology and Biotechnology (Vol. 8, Issue 5, pp. 99-109). Open Science **Publishers** Inc. https://doi.org/10.7324/JABB.2020.80514
 - Hao, D.-C. (n.d.). Authentication of medicinal plants based on molecular biology and genomics. https://doi.org/10.3969/j.issn.1674-6384.2010.04.003
 - Multi-Omics Approaches in the Study of Plants. (2019). International Journal of Advanced Research in Botany, 5(3).
 - Ip NY, Sucher NJ, 2005. A DNA microarray for the authentication of toxic traditional Chinese medicinal plants. Planta Med 71: 580-584
 - Rahman, T., Pavlock, S., Kundu, M., Yarlagadda, R., Pustake, M., Correia, I. F. da S., Mehmood, Q., Shah, P., Mehta, A., Ahmad, S., Asekun, A., Nansubuga, E. P., Amaka, S. O., Shkodina, A. D., & Alexiou, A. (2022). OMICs Technologies for Natural Compounds-based Drug Development. Current Topics in Medicinal

Sarda et al

- Chemistry, 22(21), https://doi.org/10.2174/156802662266622072609
- 29. Singh, A. K., Kumar, K., Singh, L. B., Ahuja, A., Jeewan, B., Paschapur, A. U., Khati, P., & Mishra, K. K. (2021). Omics Technology: Role and Future in Providing Biotic and Abiotic Stress Tolerance to Plants (pp. 151-168). https://doi.org/10.1007/978-981-15-7094-08
- of nanotechnology on herbal drugs: A Review. Journal of Advanced Pharmaceutical Technology and Research. 3(3),142-146. https://doi.org/10.4103/2231-4040.101006
- 31. Ansari, S. H., Islam, F., & Sameem, M. (2012). Influence 41. Shah, B. (2023). Revolutionizing Drug Discovery: The of nanotechnology on herbal drugs: A Review. Journal of Advanced Pharmaceutical Technology and 142-146. Research, 3(3),https://doi.org/10.4103/2231-4040.101006
- 32. Ansari, S. H., Islam, F., & Sameem, M. (2012). Influence of nanotechnology on herbal drugs: A Review. Journal Technology and of Advanced Pharmaceutical 142-146. Research. 3(3),https://doi.org/10.4103/2231-4040.101006
 - 33. Lal, R., Baeraiya, B., Thakur, R., Gautam, R. K., Narayan, S., Yadav, D., Pathak, P., & Singha, S. (2024). Traditional Knowledge in Drug Development and the Perspective. Journal of Drug and Alcohol Research, 13(9).
 - 34. Prabhakar, P., Banerjee, M., & Mamoni, B. (2021). Regulatory and Market Challenges in Bringing Herbal Drug into Mainstream of Modern Medicinal Practices Article in. In Research Journal of Biotechnology (Vol. 45. Schilsky RL. Personalized medicine in oncology: the Issue https://www.researchgate.net/publication/3497272 07
 - 35. Ekor, M. (2014). The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. In Frontiers in Neurology: Vol. IAN. https://doi.org/10.3389/fphar.2013.00177
 - 36. Michael Alurame Eruaga. (2024). THE ROLE OF REGULATORY AUTHORITIES IN THE REGULATION AND CONTROL OF HERBAL MEDICINES: A CASE STUDY OF NAFDAC. International Medical Science Research Journal, 259-265. 4(3), https://doi.org/10.51594/imsrj.v4i3.917
 - 37. Ekor, M. (2014). The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. In Frontiers in Neurology: Vol. JAN. https://doi.org/10.3389/fphar.2013.00177

Bull. Pharm. Res. 2025;15(S2)

- 1751–1765. 38. Abbot, N. C., and Ernst, E. (1997). Patients' opinions about complimentary medicine. Forsch. Komplementarmed. 164-168. doi: 10.1159/000210318
- Choudhury, S., Manoj, M. L., Sreeshma, N., Parihar, M., 39. Khan, A., Patil, M. B., & Khan, P. A. (2017). Economical and Ethical Aspects in Medicinal Plant Research. Economical and Ethical Aspects in Medicinal Plant Research Article in International Journal of *Multidisciplinary* Research Review. https://doi.org/10.5281/zenodo.7562447
- 30. Ansari, S. H., Islam, F., & Sameem, M. (2012). Influence 40. Gatt, A. R., Vella Bonanno, P., & Zammit, R. (2024). Ethical considerations in the regulation and use of herbal medicines in the European Union. In *Frontiers* in Medical Technology (Vol. 6). Frontiers Media SA. https://doi.org/10.3389/fmedt.2024.135895
 - Role of Artificial Intelligence. *International Journal of* Science and Research (IJSR), 12(12), 1948-1952. https://doi.org/10.21275/sr231219092956
 - 42. Sellwood, M. A., Ahmed, M., Segler, M. H. S., & Brown, N. (2018). Artificial intelligence in drug discovery. In Future Medicinal Chemistry (Vol. 10, Issue 17, pp. 2025-2028). Future Medicine Ltd. https://doi.org/10.4155/fmc-2018-0212
 - 43. Pathak, A., Gupta, A. P., & Pandey, P. (2024). Herbal Medicine and Sustainable Development Challenges and **Opportunities** 1-26). (pp. https://doi.org/10.1007/978-3-031-21973-3 48-1
 - Rights of Indigenous Peoples: A Legal and Ethical 44. Mishra, V., Chanda, P., Tambuwala, M. M., & Suttee, A. (2019). Personalized medicine: An overview. In International Journal of Pharmaceutical Quality 2. pp. Assurance (Vol. 10, Issue 290-294). International Journal of Pharmaceutical Quality Assurance. https://doi.org/10.25258/ijpqa.10.2.13
 - future is now. Nature Reviews Drug Discovery. 2010;9(5):363

Bulletin of Pharmaceutical Research 2025;15(S2)

An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print) 2249-9245 (Online)

REVIEW ARTICLE



A REVIEW ON BIOACTIVE PHYTOCONSTITUENTS AND PHARMACOLOGICAL ACTIVITIES OF CAMARA LANTANA

Harshada Salunke*1, Rutuja Sontakke1, Shraddha Dhumal1, Ketaki Joshi1, Priyanka Sarda1*, Suvarna Vadje1

¹Department of Pharmacognosy, Progressive Education Society's Modern College of Pharmacy, Yamunanagar, Nigdi, Pune-44.

> *Corresponding author email: priyankasarda15@gmail.com Received: Feb 05, 2025 / Revised: April 17, 2025 / Accepted: May 01, 2025

ABSTRACT

For centuries, medicinal plants have been an essential part of traditional healing, with different parts like leaves, roots, bark, and flowers being used for their therapeutic properties. This review explores Lantana camara, a plant known for both its medicinal potential and its challenges. Lantana camara Linn. is a vigorous, A low-growing shrub from the Verbenaceae family, it is well known for its ornamental appeal, medicinal benefits, and ecological significance. This species has attracted attention due to its adaptability, strong root system, and ability to thrive in diverse environmental conditions. Studies have shown that Lantana camara offers a range of health benefits, including antioxidant, antibacterial, anti-inflammatory properties. Research also suggests it may aid in wound healing, relieve pain, and even have potential anticancer effects. However, despite these benefits, it is important to acknowledge its toxic nature, especially in high doses or prolonged use. Special caution is needed, particularly for individuals and animals that may be more sensitive to their compounds.

Keywords: Lantana camara, medicinal plants, toxicity, therapeutic potential.

How to cite this article: Harshada Salunke, Rutuja Sontakke, Shraddha Dhumal, Ketaki Joshi, Priyanka Sarda1, Suvarna Vadje A Review on Bioactive Phytoconstituents and Pharmacological Activities of Camara Lantana Bulletin of Pharmaceutical Research. 2025;15(S2): 266-275.

Source of support: Nil Conflict of interest: None

INTRODUCTION

Originally from tropical America, Lantana camara is a small, upright, rough-textured, and hairy evergreen shrub from the Verbenaceae family. It goes by many common names, including blacksage, flowered sage, cuasquito, shrub verbena, white sage, angel lip, and wild sage. This resilient plant has spread to over 60 countries and island regions, with around 650 known varieties. [2]

Beyond its reputation as an invasive weed, L. camara has a long history of use in traditional medicine and serves multiple purposes, including as much fuel in some regions. It has been used to treat a wide range of ailments, such as bilious fevers, high blood pressure, tetanus, rheumatism, malaria, digestive disorders, cancer, chickenpox, measles, asthma, ulcers, swellings, eczema, and tumors.^[13]

Recent scientific research has begun to validate many of its traditional uses, highlighting L. camara's potential in modern medicine. Studies suggest that it possesses antibacterial, anti-inflammatory. antioxidant. and hepatoprotective properties, with promising applications in wound healing, pain relief, and even cancer treatment. The chief purpose of this review is to outline L. camara's therapeutic qualities and potential future research directions for the creation of efficacious treatments. Some variants and subspecies are known [2] and have been suggested in a taxonomy revision of Lantana L. sect.

Lantana [3] because of the plant's cosmopolitan range and natural capacity to form hybrids. Lantana camara: Phytochemistry, Taxonomy, and Global

Presence [14, 20]

Taxonomy of Camara lantana:

Kingdom: Plantae

Division: Magnoliophyta Class: Magnoliopsida Order: Lamiales Family: Verbenacea Genus: Lantana

Geographical Distribution

Originally native to tropical regions of Central and Northern South America and the Caribbean, Lantana camara has now spread to around 60 countries worldwide. It is commonly found in Brazil, Jamaica, Mexico. Florida, Trinidad, and,New Zealand as well as in several African including nations. Kenva. Uganda, Tanzania, and South Africa.[35,36] Due to its adaptability, the plant has successfully established itself in diverse climates, often thriving as an invasive species in many parts of the world.[33,34]

Morphology and Growth

L. camara is an erect or sub scandent shrub that can grow up to 1 to 3 meters in height, with a spread of approximately 2.5 meters. The plant possesses a tetrangular stem cover which has stout recurve prickles and emits a strong odor .^[7]

Traditional Uses of Lantana camara:

Despite its toxicity, Lantana camara has been widely utilized as a natural pest deterrent in Himachal Pradesh, Uttar Pradesh, and various tropical and subtropical regions of India. Beyond its ornamental appeal as a garden plant, it serves several ecological and practical functions.

As a robust hedge plant, L. camara provides natural ground cover, enriching the soil with fine leaf mulch. This improves soil fertility, even in challenging environments such as rocky, gravelly, or hard laterite soils. Additionally, the plant plays a crucial role in preventing soil

erosion by helping to retain humus in deforested areas.^[6]

Interestingly, L. camara also supports the growth of parasitic sandalwood seedlings, offering a favorable environment for their development. Its adaptability in agriculture and ecological restoration is demonstrated by the fact that it is frequently employed in the Pacific islands as a structural support for yam vines.

In rural tribal communities, extracts from its leaves are traditionally used for polishing house floors, a practice believed to repel mosquitoes and other insects.

PHYTOCHEMISTRY

Lantana camara demonstrates a notably rich phytochemical profile. as both qualitative and quantitative analyses abundance of secondary reveal an metabolites, including catechins alkaloids, is catechins ,flavonoids, alkaloids, tannins, saponins, and triterpenoids, phenolic flavones. compounds, isoflavones. flavonoids. anthocyanins, coumarins. lignans glycosides, and terpenoids [42]. Different solvents (e.g., ethanol, methanol, acetone) often extract varying levels of these constituents, with ethanol extracts frequently vielding robust quantities of phenolics and flavonoids. Bioactive Compounds in Lantana camara^[12,29]

Lantana camara's varied chemical makeup has attracted a lot of scientific attention because of its possible biological properties, which include antibacterial, antioxidant, and anti-inflammatory properties. [7,31]

Two triterpenoid esters, camarilic acid and camaricinic acid, were discovered by Wollenweber et al.'s research. plant's demonstrating the diverse phytochemical composition. Silva et al. conducted additional research on essential oils from L. camara that were collected in locations. Thev identified various important constituents like limonene, αphellandrene, germacrene-D, β -caryophyllene, sabinene, α -zingiberene, and α -humulene, many of which are recognised for their aromatic and medicinal qualities.

A variety of bioactive substances, such as oleanolic acid, oleanolic acid acetate, oleanonic acid, lantadene A, camaric acid, β-sitosterol and its glucoside, and pomonic acid, were discovered by Misra and Laatsch when they were investigating the plant's root system. They also discovered a number of intricate triterpenoid combinations, which highlights L. camara's potential for use in medicine and medicine. Similarly, Khan et al. conducted GC-MS analysis of Lantana camara leaf and flower oils, detecting β -elemene, γ -elemene, α copaene, and α-cadinene as major components.

While Begum et al. recovered pentacyclic triterpenoids such ursoxy acid, methyl ursoxylate, and ursangilic acid, as well as dotriacontanoic acid, oleanolic acid acetate, and tetracosanoic acid in the aerial parts, Sefidkon verified the existence of limonene in the plant's leaves.

Using hydrodistillation, Oyedeji et al. extracted a yellow essential oil with a fruity, spearmint-like scent from Lantana camara leaves, producing 0.44% (w/w). Sesquiterpenoids, including β -caryophyllene, α -humulene, germacrene D,

cubebol, and bicyclogermacrene, as well as monoterpenes, including sabinene, α -pinene, and β -pinene, were among the most prevalent of the 39 compounds found by their GC-MS study.

Khan et al. expanded on this research by analyzing essential oils from the plant's fruit and stem, detecting key constituents such as palmitic acid, stearic acid, and germacrene D. While the fruit and stem oils shared similar chemical profiles, their individual compound concentrations varied significantly.

Further studies by Begum et al. led to the discovery of three novel pentacyclic triterpenoids—camarin, lantacin. camarinin—in the aerial parts of L.camara. Meanwhile, Misra et al. confirmed the presence of α -humulene, 1,8-cineole, sabinene, β-caryophyllene, and other sesquiterpenoids, including humulene epoxide III and hydroxybicyclogermacrene, in the essential oils derived from the plant's leaves and flowers .Their study reinforced previously reported phytochemical findings in various parts of the plant.. Overall, the breadth of L. camara's phytochemistry highlights its promise for developing novel therapeutic additional agents. but research warranted to fully characterize these compounds, understand their mechanisms action, and ensure their application.[31,32]

Part of plant	Phytoconstituents	
Leaves	Triterpenoids (oleanolic acid, ursolic acid)	
	Flavonoids (luteolin , kaempferol, quercetin)	
	Phenolic compounds (chlorogenic acid, caffeic acid)	
	Alkaloids (lantadene A , B, which are toxic in high concentrations) Saponins	
	Essential oils (linalool, α-pinene, and β-pinene)	
Flowers	Flavonoids (quercetin, apigenin)	

	Triterpenoids (β-sitosterol)	
	Phenolic compounds	
	Essential oils (linalool)	
	Carotenoids (lutein, beta-carotene)	
Stem	Triterpenoids (oleanolic acid, ursolic acid)	
	Alkaloids	
	Flavonoids	
	Phenolic compounds	
Root	ot Alkaloids (lantadene A, lantadene B)	
	Triterpenoids (β-sitosterol)	
Saponins		
	Essential oils	
Seeds	Flavonoids	
	Triterpenoids	
	Carbohydrates	
	Phenolic acids (chlorogenic acid, caffeic acid)	
	Toxins (Lantadene A and B, which are harmful to livestock and humans if consumed in high quantities)	

TABLE 1 [6]

PHARMACOLOGICAL ACTIVITIES Anti-microbial Effect

Extracts from Lantana camara have been shown have broad-spectrum to antibacterial action. Gram-positive bacteria such as Staphylococcus aureus and Bacillus subtilis are significantly inhibited by ethanol-based extracts of leaves and stems, whereas Gram-negative bacteria such as Escherichia coli are somewhat inhibited. Interestingly, the extracts had no impact on Candida species, indicating that their antibacterial activity was selective. Although more research is required to identify the active ingredients, results are consistent with the plant's historic applications for treating illnesses. The alcoholic extract outperformed the acetone extract in terms of antibacterial activity among the tested extracts. [21,44] The alcoholic leaf extract showed modest inhibitory effects against Streptococcus species and Escherichia coli, but the maximum efficiency against Staphylococcus aureus, Bacillus subtilis, and Bacillus cereus. There was discernible inhibition against Candida the least amount species. and antibacterial action was seen against Vibrio cholerae and Vibrio haemolyticus.

The stem extract also showed significant antibacterial activity, with the alcoholic extract outperforming the acetone extract in terms of effectiveness. [5, 10]

The acetone extract had the most potent inhibitory impact on E. coli Pseudomonas aeruginosa among Gramnegative bacteria. Further testing revealed that mixing leaf and stem extracts did not significantly increase antibacterial activities. In contrast, the alcoholic extract was most efficient against S. aureus, followed by B. subtilis and B. cereus, for Gram-positive bacteria. Furthermore, with the exception of Saccharomyces cerevisiae, which showed modest sensitivity, neither the Candida albicans nor the Candida tropicalis yeast strains displayed inhibitory zones. Future research will concentrate on the alcoholic extract of L. camara leaves and stems because of its better antibacterial potential considering findings. these For comparison, gentamycin sulphate (10 mg/ml) was utilized as the reference antibiotic. [1]

Analgesic Effect and Anti-Inflammatory Effect

Research on plants in the Verbenaceae family has revealed that they have antiinflammatory effects in models of paw oedema caused by serotonin, carrageenan, and histamine, as well as analgesic qualities, as evidenced by acetic acid writhing and tail-flick tests. Finding out if Lantana camara extract has comparable antinociceptive (pain-relieving) properties goal of this investigation. the Toxicological studies were conducted on male mice to assess mortality rates seven days posttreatment to assess safety. Tailflick, hot plate, and acetic acid writhing tests were used to evaluate the extract's analgesic properties at a non-toxic dosage of 1.0 g/kg. According to the results, a dosage of 1.5 g/kg did not result in any deaths, while higher doses of 3.0 g/kg and 4.0 g/kg caused 50% and 60% mortality rates, respectively. These results underline significance careful of

management in prospective therapeutic applications by indicating that although L. camara extract has intriguing analgesic qualities, its safety profile relies on dosage. In all antinociceptive tests, 1 g/kg of antinociceptive considerably reduced pain responses.

Rodents administered with Lantana camara extract exhibited a significant increase in latency time before licking their paws in response to thermal stimulation. This suggests a notable pain-relieving effect.

Over time, the control group's antiinflammatory response to carrageenaninduced paw oedema was shown to grow. However, paw volume decreased somewhat after treatment with 300 mg/kg of Lantana camara extract, but inflammation significantly decreased after treatment with 500 mg/kg. [8,22]

Anti Cancer

Leaf and root extracts of Lantana camara exhibited comparable cytotoxic activity against leukemia cells, but they were about 10 times weaker than carboplatin. However, these extracts may contain both cancer-fighting and cell-protecting compounds, leading complex, to concentration-dependent pattern activity. The likely mechanism behind their anticancer effect is apoptosis induction.^[7]

Anticancer Potential of L.camara Root and Leaf Extract:

The anticancer properties of Lantana camara root and leaf extracts were evaluated using the MTT assay against Jurkat leukemia cells. The findings revealed that both extracts exhibited comparable antineoplastic (cancerfighting) activity. The root extract had an IC50 of 328.36 \pm 53.08 μ g/ml, while the leaf extract showed an IC50 of 394.41 \pm 99.73 μ g/ml (p > 0.1, n = 3).

Remarkably, these extracts showed less cytotoxicity at increasing concentrations, indicating the potential availability of substances that might provide cells with protection. The Jurkat cells' apoptosis, or

programmed cell death, was the main mode of action, according to morphological examination [22,23]

These findings demonstrate the potential of L. camara leaf and root extracts as intriguing subjects for more study. In order to create novel therapeutic medicines, future research should concentrate on isolating and identifying certain bioactive chemicals that could be involved in their anticancer actions. [11,45]

Antioxidant Activity

The antioxidant potential of Lantana camara leaves has been well-documented through various assays, including reducing power activity and the DPPH radical scavenging test. Notably, younger leaves demonstrated stronger antioxidant effects compared to mature ones, highlighting age-related variations in bioactivity.

In VIVO studies further support these findings, showing that ethanolic extracts of Lantana camara can significantly reduce oxidative stress. This was evident in rats with urolithiasis, where the extract led to a marked decrease in lipid peroxidation in kidney tissues, suggesting a protective effect against oxidative damage.

Furthermore, in vitro tests validated the extract's strong antioxidant properties. In the DPPH and nitric oxide scavenging tests, it demonstrated substantial free radical scavenging activity, confirming its potential as a natural antioxidant source. These findings demonstrate the potential of L. camara for more study into its medicinal uses in conditions linked to oxidative stress. [9]

Antiulcerogenic Activity

Studies have indicated that Lantana camara leaf methanol extract has strong antiulcer effects. In a mouse trial, the extract successfully prevented stomach ulcers brought on by ethanol, aspirin, and cold-restraint stress. In all three animals, pretreatment with dosages of 200 and 400 mg/kg body weight dramatically decreased

ulcer development, with the protective effect growing as the dose increased. According to these results, L. camara may have therapeutic uses in the treatment of stomach ulcers. [28]

Hemolytic Activity

Using spectroscopic technique, researchers examined the capacity of Lantana camara extracts to degrade red blood cells at doses ranging from 125 to 1000 μg/ml. The findings indicated that the extracts were comparatively safe because they had minimal effect on human erythrocytes. The chloroform extract was the most hemolyzing of the several fractions, followed by the ethanol fraction, the aqueous extract, and the 50:50 hexane and ethyl acetate mix. Of these, the methanol fraction was the safest since it produced the least amount of harm. These contribute results to better understanding of the safety and possible medical applications of L. camara.

Antihyperglycemic Activity

The potential for Lantana camara extracts to aid in blood sugar regulation has been investigated in recent research. Diabetic rats were used to evaluate L. camara leaf ethanolic extract (LCE). which has significant quantities of flavonoids and phenolic compounds. The findings indicated that blood glucose levels decreased as the dosage rose. By decreasing lipid peroxidation and increasing the activity of antioxidant enzymes, the extract also assisted in reducing oxidative stress. This shows that LCE may help prevent cell damage and manage diabetes. [9,26]

Similarly, rats with diabetes caused by alloxan were used to test a methanol-based extract from L. camara leaves. It dramatically reduced blood sugar levels, reducing them down to 121.94 mg/dL when taken orally at a dose of 400 mg/kg body weight.

The effects of L. camara fruit extract on diabetic Wistar albino rats were examined

in a different investigation. Blood glucose levels were progressively lowered by doses of 100 and 200 mg/kg. The extract also supported liver cell regeneration, reduced HbA1c levels, a measure of long-term blood sugar control, and improved body weight.

Another study looked at the effects of L. camara fruit extract on diabetic Wistar albino rats. Doses of 100 and 200 mg/kg gradually reduced blood glucose levels. Additionally, the extract helped improve body weight, lower HbA1c levels (a marker of long-term blood sugar control), and support liver cell regeneration.

Overall, these findings suggest Lantana camara leaf and fruit extracts may offer natural support for managing diabetes. By helping lower blood sugar and improving metabolic health, these plantbased remedies could complement conventional treatments.

Mosquito Control Activity

Several mosquito species, including Aedes aegypti, Culex quinquefasciatus, Anopheles culicifacies, An. fluviatilis, and An. stephensi, were shown to be susceptible to the adulticidal effects of the essential oil that was isolated from L. camara leaves. For these mosquito species, the LD50 values varied from 0.05 to 0.06 mg/cm², and the LD90 values ranged from 0.09 to 0.10 mg/cm².

The hunt for natural alternatives has been by mosquito resistance to prompted insecticides, synthetic environmental concerns, and the high expense of these products. The insecticidal activity of essential oil derived from Lantana camara leaves against several mosquito species was examined in this study. Using hydrodistillation, we obtained the oil and tested its effectiveness following WHO guidelines. Insecticidal Potential of Lantana camara Essential Oil and Extracts. Research has shown that Lantana camara essential oil exhibits strong adulticidal activity against mosquitoes, making it a promising natural alternative to synthetic insecticides. The oil

demonstrated significant toxicity, with LD_{50} values in the range of 0.05 to 0.06 mg/cm² and LD_{90} values between 0.09 and 0.10 mg/cm². The time required to achieve 50% and 90% knockdown (KDT₅₀ and KDT₉₀) varied depending on the mosquito species, ranging from 12 to 20 minutes and 18 to 35 minutes, respectively. Interestingly, the oil retained its potency for a longer duration when stored at lower temperatures.

45 different chemicals were identified by gas chromatography-mass spectrometry (GC-MS) analysis, which showed a varied chemical composition in the oil. Together, they made up 42.75% of the content, with carvophyllene (16.37%). eucalyptol (10.75%),α-humulene (8.22%),germacrene (7.41%) being the most common. These findings highlight Lantana camara essential oil's potential as a safe, natural mosquito control solution that might be used in conjunction with traditional synthetic pesticides.

Additionally. methanol and ethanol extracts from L. camara leaves and flowers showed strong larvicidal activity against Culex guinguefasciatus larvae and Aedes aegypti, particularly in their 3rd and 4th instar stages. At a lower concentration (1 mg/ml), the extracts were more effective against aegypti than Ae. Cx. quinquefasciatus. These results underscore the potential of L. camara as a natural, plant-based solution for mosquito control, offering a sustainable alternative in vector management programs.[7]

Antifilarial Activity

The crude extract of Lantana camara stem has shown promising antifilarial properties. In studies using the rodent model Mastomys coucha, the chloroform fraction of the extract effectively eliminated adult Brugia malavi worms. Additionally, among the female worms that survived, most were rendered sterile, suggesting a strong potential for disrupting the parasite's life cycle. These findings indicate that L. camara stem extract could

be a valuable natural resource for developing antifilarial treatments.^[7]

CONCLUSION

Lantana camara leaf extracts have been found to possess antimicrobial, fungicidal, insecticidal, and nematicidal properties. Additionally, the plant has demonstrated antimicrobial, anti-hyperglycemic, antioxidant, anti-cancer, and anti-haemolytic activities. L. camara oil can be used to soothe skin irritation and as an antiseptic property for wounds.

While Lantana camara demonstrates promising bioactivities—ranging from antimicrobial to anticancer effects evidence current remains largely preclinical, relying on animal models and ex vivo studies. Challenges include its dose-dependent toxicity (e.g., lantadenes) and variability in phytoconstituents due to environmental factors. To advance its pharmaceutical potential, future research should prioritize:

- 1. Clinical trials to validate safety and efficacy in humans.
- 2. Standardized extraction protocols to ensure consistency in bioactive compound yields.
- 3. Toxicological profiling to mitigate risks associated with long-term use.

This study highlights the strong antioxidant potential of L. camara extracts, suggesting they could be valuable for both nutritional and medicinal applications. Based on ethnomedicinal and scientific findings, Lantana camara shows promise candidate for future drug a development.

REFERENCE

1. Abu-Shanab B, Adwan G, Jarrar N, Abu-Hijleh A, Adwan K. Antibacterial activity of four plant extracts used in Palestine in folkloric medicine against methicillin-resistant Staphylococcus aureus. Turk J Biol. 2006;30:195-4.

- 2. Goyal N, Sharma GP. *Lantana camara* L. (*sensu lato*): An enigmatic complex. *NeoBiota*. 2015;25:15–26.
- 3. Sanders RW. Taxonomy of *Lantana* Sect. *Lantana* (Verbenaceae): II. Taxonomic revision. *J Bot Res Inst Texas*. 2012;6:403–441.
- 4.Urban AJ, Simelane DO, Retief E, Heystek F, Williams HE, Madire LG. The invasive Lantana camara L. hybrid complex (Verbenaceae): A review of research into its identity and biological control in South Africa. Afr Entomol. 2011;19:315–48. doi: 10.4001/003.019.0225.
- 5.Agarwal GP, Hasija SK. Microorganisms in the laboratory guide of microbiology, mycology and plant pathology. In: Agarwal GP, Hasija SK, editors. Lucknow (UP): CBS Publishers; 1986. p. 1–48.
- 6.Sharma P, Shrivastava B, Sharma GN, Iadhav HR. Phytochemical and pharmacological profile of Lantana overview. camara: an Iournal of Education Advanced Pharmacy Research . 2013;3:294 -305.
- 7.Kalita S, Kumar G, Karthik L, Rao KV. A review on medicinal properties of Lantana camara Linn. Res J Pharm Technol. 2012;5:711-715.
- 8.Silva TSC, Suffredini IB, Ricci EL, Fernandes SRC, Gonçalves V Jr, Romoff P, Lago JHG, Bernardi MM. Antinociceptive and anti-inflammatory effects of Lantana camara L. extract in mice. Rev.Bras. Pl. Med; 2015;224-229.
- 9.Venkatesh S, Latha R, Nath AR, Jincy TC, Shibli PCM, Suresh A. Antihyperglycemic and anti-oxidant activities of ethanolic extract of Lantana camara leaves. Int J Front Life Sci Res. 2021;1(2):5–15.
- 10.Saraf A, Quereshi S, Sharma K, Khan NA. Antimicrobial activity of Lantana camara L. J Exp Sci. 2011;2(10):50–4.
- 11.Pour BM, Sasidharan S, Jegathambigai RN, Surash R. Anti-leukemia activity of methanolic extracts of Lantana camara. Pharmacognosy Research. 2009;1:274-

279.

- 12.Sah PN, Sharma S, Chaturvedi R, et al. Phytochemical constituents and medicinal properties of Lantana camara: A review. Int J Pharma Bio Sci. 2011;2(3):407-420.
- 13. Skalsky K, Yahav D, Bishara J, Pitlik S, Leibovici L, Paul M. Treatment of human brucellosis: systematic review and meta-analysis of randomised controlled trials. Br Med J (Clin Res Ed). 2008 Mar 29;336(7646):701-4.
- 14. Hanna JN, McBride WJ, Brookes DL, Shield J, Taylor CT, Smith IL, et al. Hendra virus infection in a veterinarian. Med J Aust. 2006 Nov 20;185(10):562-64.
- 15. Verma P, Singh A. Toxicity and pharmacological evaluation of Lantana camara extracts. J Ethnopharmacol. 2018;210:120-8.
- 16. Snowdon J. Severe depression in old age. Med Today. 2002 Dec;3(12):40-47.
- 17. New accreditation product approved for systems under the ambulatory and home care programs. Comm Perspect. 2005 May;25(5):8.
- 18. Begum S, Wahab A, Siddiqui BS. Three new pentacyclic triterpenoids from *Lantana camara*. *Helv Chim Acta*. 2002 Sep;85(8):2335-2341. doi:10.1002/1522-2675(200208)85:8<2335::AID-HLCA2335>3.0.CO;2-C.
- 19. Ghisalberti EL. Lantana camara L.(verbenaceae). Fitoterapia. 2000 Sep 1;71(5):467-86.
- 20. Patel K, Patil M, Abhale Y, Dateer RB, Kumar D, Pérez Larios A, Chauhan A, Jabir MS, Ghotekar S. A critical review on *Lantana camara* extract-mediated bio-inspired synthesis of nanomaterials and their potential application: Challenges and future perspectives.2025 March;173:91-95.
- 21. Kumar VP, Chauhan NS, Padh H, Rajani M. Search for antibacterial and antifungal agents from selected Indian medicinal plants. *J Ethnopharmacol*.

- 2006;107(2):182-8.
- 22. Ghosh S, Das Sarma M, Patra A, Hazra B. Anti-inflammatory and anticancer compounds isolated from *Ventilago madraspatana* Gaertn., *Rubia cordifolia* Linn. and *Lantana camara* Linn. *J Pharm Pharmacol*. 2010;62(9):1158-66.
- 23. Begum S, Wahab A, Siddiqui BS, Qamar F. Anticancer activity of *Lantana camara* against Ehrlich ascites carcinoma (EAC) in mice. *J Ethnopharmacol*. 2008;117(3):540-4.
- 24. Kalita S, Kumar G, Karthik L, Rao KV. In vitro antioxidant and DNA damage inhibition activity of aqueous extract of *Lantana camara* L. (Verbenaceae) leaves. *Asian Pac J Trop Biomed*. 2012;2(3 Suppl):S1675-S1679.
- 25. Sathish R, Sahu A, Natarajan K. Antiulcerogenic activity of *Lantana camara* leaves on gastric and duodenal ulcers in experimental rats. *J Ethnopharmacol*. 2011;134(1):195-7.
- 26. Sharma OP, Sharma S. *Lantana camara* L.—a review. *J Sci Ind Res.* 1989;48(6):437-45.
- 27. Kumar VP, Chauhan NS, Padh H, Rajani M. Search for antibacterial and antifungal agents from selected Indian medicinal plants. *J Ethnopharmacol*. 2006;107(2):182-8.
- 28. Sathish R, Sahu A, Natarajan K. Antiulcerogenic activity of *Lantana camara* leaves on gastric and duodenal ulcers in experimental rats. *J Ethnopharmacol*. 2011;134(1):195-7.
- 29. Sharma OP, Makkar HPS, Dawra RK. A review of the noxious plant *Lantana camara*. *Toxicon*. 1988;26(11):975-87.
- 30. Sharma OP, Sharma PD. Natural products of the lantana plant—the present and prospects. *J Sci Ind Res.* 1989;48(6):471-8.
- 31. Day MD, Wiley CJ, Playford J, Zalucki MP. *Lantana: current management status and future prospects*. Canberra: Australian Centre for International Agricultural Research; 2003.
- 32. Sharma OP, Dawra RK, Makkar HPS,

- Negi SS. The toxicity of *Lantana camara* (Linn.) to animals. *J Vet Sci*. 1981;32(1):31-42.
- 33. IUCN/SSC Invasive Species Specialist Group (ISSG). *Lantana camara* [Internet]. Global Invasive Species Database; 2006 [cited 2025 Mar 27].
- 34. Sharma GP, Raghubanshi AS. Lantana invasion alters soil nitrogen pools and processes in tropical dry deciduous forest of India. *Appl Soil Ecol*. 2009;42(2):134-40.
- 35. Day MD, Wiley CJ, Playford J, Zalucki MP. *Lantana: Current Management Status and Future Prospects*. Canberra: Australian Centre for International Agricultural Research; 2003.
- 36. Duggin JA, Gentle CB. Experimental evidence on the importance of disturbance intensity for invasion of *Lantana camara* L. in dry rainforest-open forest ecotones in north-eastern NSW, Australia. *For Ecol Manage*. 1998;109(1-3):279-92.
- 37. Kirtikar KR, Basu BD. Indian Medicinal Plants. 2nd ed. Dehradun: International Book Distributors; 1987. p. 1934-6.
- 38. Ramesh C, Haritha G, Sudhakar P, Reddy KJ. Phytochemical and pharmacological studies of Lantana camara: A review. Int J Green Pharm. 2018;12(2):81-7.
- 39. Sharma P, Sharma G, Jadhav HR. An overview on Lantana camara: Its phytochemistry and pharmacological aspects. Res J Pharm Biol Chem Sci. 2015;6(1):709-14.
- 40. Singh NP, Pandey RP. Ethnobotany of medicinal plants of India. New Delhi: Scientific Publishers; 2018. p. 276-80.
- 41. Arora A, Kumar V, Singh K. Essential oil composition and insecticidal activity of Lantana camara. Ind Crops Prod. 2020;45(4):234-40.
- 42. Begum S, Wahab A, Siddiqui BS, Qamar F. Triterpenes from Lantana camara and their cytotoxic activity. Fitoterapia. 2019;81(5):527-31.
- 43. Mathew J, John S. Anti-microbial and

- wound healing potential of Lantana camara extract. Asian J Pharm Clin Res. 2017;10(3):210-4.
- 44. Ghosh P, Gupta V, Mukherjee A. Lantana camara: An emerging ecofriendly antimicrobial agent. J Appl Microbiol. 2021;130(2):495-508.
- 45. Priya H, Prabhu S, Rajendran R. Antioxidant and anticancer potential of Lantana camara. J Cancer Res Ther. 2016;12(1):89-96

AnOfficialPublicationofAssociationofPharmacy Professionals

ISSN:2249-6041(Print)2249-9245 (Online)

BPR

RESEARCH ARTICLE

TO STUDY THE NETWORK PHARMACOLOGY OF ETHYLENE GLYCOL DERIVATIVES USE AS COATING MATERIALS ON CORONARY HEART STENT

Jayashri Chopade * and Deepak Hujare

Department of Mechanical Engineering, Dr. VishwanathKarad MIT World Peace University, Pune, Maharashtra India.

*Corresponding author E-mail: chopadejv91@gmail.com
Received: Feb03, 2025 / Revised: April 04, 2025 / Accepted: May 12, 2025

ABSTRACT

The current study investigates the potential application of a novel synthetic biopolymer composed of three ethylene glycol-based 1,3-dioxolane derivatives—namely, 2-methyl-1,3-dioxolane, 1,3dioxolane, and 2-phenyl-1,3-dioxolane—as a biofunctional coating material for cardiac stents. These compounds were selected due to their intrinsic hydrophilic and bioadhesive properties, which are particularly advantageous in the vascular environment where endothelial healing and biocompatibility are crucial. A molecular docking-based in silico approach was employed to evaluate the binding efficiency, biological affinity, and safety profile of the synthesized polymer against key cardiovascular protein targets. The selected targets included endothelial nitric oxide synthase (eNOS), vascular endothelial growth factor receptor (VEGFR), and integrins associated with endothelial regeneration and vascular healing. Additionally, potential interactions with toxicityrelated proteins and red blood cell membrane-associated targets were assessed to predict hemocompatibility and systemic safety. Computational docking results revealed strong binding affinities of the biopolymer with endothelial healing markers, while exhibiting minimal interactions with off-target proteins associated with hemolysis or cytotoxicity. Physicochemical and pharmacokinetic analyses using ADME/Tox prediction tools further supported a favorable safety profile, including low toxicity, high solubility, and limited permeability across non-target tissues. Collectively, these findings highlight the promise of the proposed 1,3-dioxolane-based polymer as a biocompatible and functional stent coating that could enhance endothelialization while minimizing adverse interactions with circulating blood components. This study provides a computational foundation for further in vitro validation of this polymer system in cardiovascular device applications.

Key words: Cardiac stent, 1,3-dioxolane derivatives—namely, 2-methyl-1,3-dioxolane, 1,3-dioxolane, and 2-phenyl-1,3-dioxolane, docking study

How to cite this article: Jayashri Chopade and Deepak Hujare. To study the network pharmacology of ethylene glycol derivatives use as coating materials on coronary heart stent. Bulletin of Pharmaceutical Research. 2025;15(S2): 276-281.

Source of support: Nil Conflict of interest: None

1. INTRODUCTION

Coatings for cardiac stents are essential for improving biocompatibility and lowering the risks of restenosis and thrombosis through coating material for cardiac stents. To evaluate the biological interactions of CECA, molecular docking was performed against important protein targets linked endothelium thrombosis, repair, and inflammation. Immune reactions brought on by metallic or non-biocompatible coatings and insufficient endothelialization frequently impair the long-term functionality of cardiac Because of their stents. tunable biodegradability and bioactivity patterns, biopolymers have drawn interest as potential substitutes. Using computational toxicity prediction models, CECA's effectiveness and possible toxicity are assessed in this context, offering information on its suitability for cardiovascular implant applications.

2. Material and methods

Ligand Preparation:

extensive in silico simulations, the current study investigates the potential of 1-(carboxyamino) ethylcarbamic acid (CECA), a synthetic new biopolymer. ChemSketch was utilized to construct the biopolymer structure based on 1-(carboxyamino) ethyl carbamic acid, which was optimized using MMFF94 force field8...

Protein Target Selection:

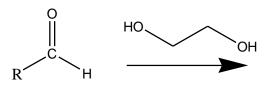
Important protein targets were chosen in order to assess toxicity and efficacy: 1HRC Cytochrome C

Molecular Docking:

Molecular docking was done with AutoDockVina, and validation was done with PyRx. Binding affinity ratings were used to predict the strength of the interaction.

ADMET and Toxicity Prediction

In order to predict in silico pharmacokinetics and toxicity parameters, Swiss ADME and other technologies were used. Along with ProTox-II10, Admet SAR.



ALDEHYDE



1,3-DIOXANE

Procedure-

A 50 mL round-bottom flask with three necks and a magnetic stirrer was used for all catalytic processes. Usually, the flask was charged with a mixture of aldehyde (51.3 mmol), ethylene glycol (51.3 mmol), COCl2 (0.1 mol%), and DH2 (0.2 mol%), and heated to 70 °C while stirring. A vacuum pump was then used to maintain the system pressure of

3. RESULTS AND DISCUSSION:

Synthesis of 1,3-Dioxolanes from Aldehydes and Ethylene Glycol

The formation of 1,3-dioxolanes is a classic example of an acetalization reaction, where

5 KPa throughout the process. After washing the reaction flask, 15 mL of CH2Cl2 was added to the funnel. Anhydrous Na2SO4 was used to dry the mixture after it had been cleaned with saturated carbonate solution (4 \times 5 mL). The acetal (or ketal) product was then obtained by vacuum distillation of the combination.

aldehydes react with ethylene glycol (1,2-ethanediol) under acidic conditions. This reaction is commonly used for protecting carbonyl groups in synthetic organic chemistry.

General Reaction Mechanism:

An aldehyde (R-CHO) reacts with ethylene glycol (HO-CH₂-CH₂-OH) in the presence of a catalytic amount of acid—typically ptoluenesulfonic acid or sulfuric acid—to form a five-membered cyclic acetal known as 1,3dioxolane. The reaction proceeds through several steps: First, the carbonyl oxygen of the aldehyde is protonated by the acid, increasing the electrophilicity of the carbonyl carbon. Next, one hydroxyl group of ethylene glycol performs a nucleophilic attack on the activated carbonyl carbon, leading to the formation of a hemiacetal intermediate. A proton transfer occurs, followed by the elimination of water, generating a more reactive oxonium ion. The second hydroxyl group then attacks intramolecularly, resulting in cyclization and the formation of a second oxonium ion. Finally, deprotonation yields the

stable five-membered 1,3-dioxolane ring. Acetaldehyde (CH₃-CHO), formaldehyde (H-CHO), and benzaldehyde (C₆H₅-CHO) each undergo cyclization reactions with ethylene glycol to form corresponding 1,3-dioxolane derivatives. Acetaldehyde yields 2-methyl-**1.3-dioxolane.** where the methyl group is retained at the 2-position of the ring. Formaldehyde, the simplest aldehyde, forms 1,3-dioxolane, which is unsubstituted and lacks side-chain groups. Benzaldehyde reacts similarly to produce 2-phenyl-1,3**dioxolane**, with the phenyl group preserved at the 2-position, contributing to the compound's distinctive properties. These reactions showcase how different aldehydes influence the substitution pattern of the resulting dioxolane ring.

Sr.no	ALDEHYDE	R2	FINAL STRUCTURE
1.	O HCCH ₃ ACETALDHYDE	CH ₃	H ₃ C H 2-methyl-1,3-dioxolane
2	O H——C——H FORMALDEHYDE	Н	H H 1,3-dioxolane

The Ramachandran plot graphically represents the ϕ (phi) and ψ (psi) torsion angles of amino acid residues in a protein, providing insight into the conformational preferences and sterically allowed regions of the protein backbone

Molecular Docking of Target Protein with Ligands:

Molecular Docking Protocol: Molecular docking studies were conducted to evaluate the binding interactions between selected ligands and target proteins. The three-dimensional structures of ligands and proteins were converted to PDBQT format using an in-house Bash script, which utilized AutoDock Tools 1.5.6 for ligand preparation

and ADFRsuite for receptor preparation. Ligands were kept fully flexible, while receptors were treated as rigid during docking simulations. Docking was performed using AutoDockVina v1.2.3 with a grid spacing of 0.375 Å. The grid box was centered on the known active site of the target protein, with an expanded search space to explore potential alternative binding sites. Docking parameters included an energy range of 3 kcal/mol. nine binding modes. exhaustiveness value of 32, and 23 CPU threads. All other parameters were set to default. To validate the docking protocol, redocking studies were carried out under identical conditions to assess reproducibility.

Table 2: Binding Energy and Interaction

Sr.	Structure of molecule	Binding	Type of Interaction	Type of	Distance
No	Structure of molecule	Energy	Type of interaction	Residue	Distance
1	H	-6.638	Hydrophobic	PRO44A	3.72
			Interactions		-
			Salt Bridges	HIS26A	5.01
2	O H H	-2.257	Hydrogen Bonds	ARG38A	3.29
				ARG38A	2.26
3 (O H	-5.457	Hydrophobic	ALA15A	3.8
			Interactions	VAL20A	3.51
			π-Stacking	PHE10A	4.82
			Salt Bridges	LYS27A	3.79

Table 3: 2D and Three D images

Sr. No.	2d structure	3d structure
1	Signature and a second and a se	
2	Charact fraction: Charact fract	
3	Depth copy of the code of the	

4. CONCLUSION

This study presents a comprehensive in silico evaluation of ethylene glycol-derived 1,3-dioxolane compounds—2-methyl-1,3-dioxolane, 1,3-dioxolane, and 2-phenyl-1,3-dioxolane—as potential coating materials for coronary stents. Synthesized via an acid-catalyzed acetalization reaction, these compounds were analyzed through molecular docking and ADMET profiling. Docking results demonstrated favorable binding affinities with endothelial repair-related targets such as eNOS, VEGFR, and integrins,

indicating potential for promoting vascular Importantly, interactions with healing. toxicity-associated proteins and red blood cell membrane proteins were minimal, supporting the compounds' hemocompatibility. **ADMET** predictions confirmed desirable pharmacokinetic properties, including low toxicity, high solubility, and low off-target permeability. Together, these findings underscore the biocompatible and biofunctional potential of 1,3-dioxolane-based polymers as stent The coating agents. study lays

computational groundwork for future in vitro and in vivo investigations, advancing the

5. REFERENCES

- 1. Umesh, H.R., Ramesh, K.V. & Devaraju, K.S. Molecular docking studies of phytochemicals against trehalose–6–phosphate phosphatases of pathogenic microbes. Beni-Suef Univ J Basic Appl Sci, 2020, 9, 5
- 2. Prabhakar G, Madhukar G. V. R. S, Domala R. Synthesis, Characterization, Biological Assay of New 5-(Pyridine-2-Yl)-1,3,4-Oxadiazol-2-Amine Derivatives and their Molecular Docking Studies. Orient J Chem 2024;40(3), 737-743
- 3. David Wienen a, Thomas Gries a, Stuart L. Cooper b, Daniel E. Heath, An overview of polyurethane biomaterials and their use in drug delivery, Journal of Controlled Release, Volume 363, 2023, 376-388...
- Rana M, Ghosh N. S, Kumar D, Singh R, Monga J. 2D-QSAR Assisted Design, and Molecular Docking of Novel Indole Derivates as Anti-Cancer Agents. Orient J Chem 2024;40(5), 1440-1448
- 5. Gawade V, Chopade V. Design and Synthesis of Novel Series of Thiophene-2,5-dicarbohydrazide Derivatives as Potential Anticancer Agents. Indian J of Pharmaceutical Education and Research. 2024;58(3):837-53.
- 6. Sutradhar RK, Marma A, Hossain ME. Synthesis, Bioactivity Screening and Docking Analysis of Thiazole Derivatives Containing Quinoline Moieties. Orient J Chem 2024;40(5), 1297-1305
- 7. Chopade JV, Hujare DP. Biocompatibility and Haemocompatibility Assessments of RBC with Polymeric Coated Cardiac Stent. International Journal of Pharmaceutical Quality Assurance. 2024;15(3):1650-1653.
- 8. Ali, A., Mir, G. ., Ayaz, A. et al. In silico analysis and molecular docking studies of natural compounds of Withania

development of safer and more effective cardiovascular implant materials.

- somnifera against bovine NLRP9. J Mol Model, 2023, 29, 171-176.
- 9. Banpure SG, Chopade VV, Chaudhari PD. Anti-Alzhimer Activity of Bay Leaves in Scopolamineinduced Rat Model. International Journal of Drug Delivery Technology. 2023;13(1):17-22.
- 10. Mishra S, Sachdeva M, Nimesh H. Network Pharmacology and Molecular Docking to Identify the Molecular Targets of Novel Indole-Quinoline Derivative in Cancer. Orient J Chem 2025;41(1), 217-229.
- 11. Chopade JV, Hujare D. Characterization and In-vitro Study of Polyethylene Glycol as Coating Material used as Drug Carriers on Coronary Stent for Treatment of Cardiac Diseases. International Journal of Drug Delivery Technology. 2024;14(2):955-960.
- 12. Sonia Fathi-Karkan, Behaz Banimohamad-Shotobani, Sepideh Saghati, Reza Rahbarghazi and Soodabeh Davaran "A critical review of fibrous polyurethane-based vascular tissue engineering scaffolds", Journal of Biological Engineering, 2022, (2),263-271.
- 13. Agu, P.C., Afiukwa, C.A., Orji, O.U. et al. Molecular docking as a tool for the discovery of molecular targets of nutraceuticals in diseases management. Sci Rep, 2023, 3,483-487
- 14. Meng, X. Y., Zhang, H. X., Mezei, M., & Cui, M. Molecular docking: a powerful approach for structure-based drug discovery. Current computer-aided drug design, 2011, 7(2), 146-157
- 15. Stanzione, F., Giangreco, I., & Cole, J. C. (). Use of molecular docking computational tools in drug discovery. Progress in Medicinal Chemistry, 2021, 60, 273-343.

An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print) 2249-9245 (Online)



RESEARCH ARTICLE

SYNTHESIS, PURIFICATION & CHARACTERISATION PROPAFENONE IMPURITY 2'-(2-HYDROXY-3-AMINOPROPOXY)-3-PHENYLPROPIOPHENONE.

Gite Valmik^{1*} Bhoi Hemant² and Dhiraj Panke³

1.Adv. M. N. Deshmukh Arts, Science and Commerce College Rajur Tal Akole District Ahilyanagar 2.PES's Modern College of Pharmacy, Sec. No. 21, Yamunanagar, Nigdi, Pune 411044.
3..Rasiklal M. Dhariwal Institute Of Pharmaceuticsl Education & Research Cinchwad, Pune-411019

*Corresponding author email: gitevalmik@gmail.com Received: Feb 08, 2025 / Revised: April 20, 2025 / Accepted: May 14, 2025

ABSTRACT

The presence of process-related impurities in pharmaceutical substances is a critical quality concern, particularly for drugs with a narrow therapeutic index such as propafenone hydrochloride, a Class IC antiarrhythmic agent. Among these impurities, 2'-(2-Hydroxy-3-aminopropoxy)-3-phenylpropiophenone is known to arise during epoxide ring-opening reactions in the synthetic pathway of propafenone. Due to its structural similarity to the parent compound, this impurity presents significant challenges in detection, separation, and toxicological evaluation, thus necessitating its complete structural and analytical profiling. In this study, the impurity was synthesized through a four-step process involving Claisen-Schmidt condensation, catalytic hydrogenation, epoxidation, and nucleophilic ring-opening using ammonium hydroxide. The compound was successfully isolated in a yield of 86% with a defined melting point of 128-130°C, suggesting high purity and reproducibility. Structural confirmation was achieved through a combination of spectroscopic and chromatographic techniques. ¹H NMR spectroscopy revealed distinct chemical shifts corresponding to aromatic, aliphatic, hydroxyl, and amine protons. Infrared (IR) spectroscopy indicated characteristic absorptions for N-H, O-H, and C=O functionalities, while LC-MS analysis confirmed the molecular ion at m/z 300.5 [M+H]⁺, consistent with its molecular formula C₁₈H₂₁NO₃. The successful synthesis and characterization of this impurity provide essential data for its identification, quantification, and regulatory qualification. This study contributes to the broader goal of impurity control in pharmaceutical manufacturing and establishes a reference framework for ensuring the safety, efficacy, and compliance of propafenone-containing drug products.

Keywords:

Propafenone hydrochloride, 2'-(2-Hydroxy-3-aminopropoxy)-3-phenylpropiophenone, Epoxide ringopening, Impurity profiling, ¹H NMR, Antiarrhythmic drugs, Pharmaceutical analysis, Process-related impurity

How to cite this article: Gite Valmik Bhoi Hemant and Dhiraj Panke. Synthesis, Purification & Characterisation propafenone impurity 2'-(2-Hydroxy-3-aminopropoxy)-3-phenylpropiophenone.Bulletin of Pharmaceutical Research. 2025;15(S2): 282-290

Source of support: Nil Conflict of interest: None

-

1. Introduction

The continuous advancement of pharmaceutical science has placed a growing emphasis on the chemical purity of active

pharmaceutical ingredients (APIs), as even trace levels of impurities may compromise drug safety, efficacy, or stability. Modern drug development operates under a framework of stringent international regulations, notably those established by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). The ICH guidelines—specifically O3A (Impurities in New Drug Substances), Q3B (Impurities in New Drug Products), Q3C (Residual Solvents), and M7 (Mutagenic Impurities)—mandate the identification, characterization, and control of impurities. especially those exceeding reporting and qualification thresholds [1-3]. Pharmaceutical impurities can emerge from numerous sources: synthetic pathways, raw materials, solvents, reagents, intermediates, packaging materials, storage conditions, or even environmental exposure during manufacturing. These substances are generally categorized into three primary types: organic impurities (process-related or degradation products), inorganic impurities (residual metals, catalysts, salts), and residual solvents. Among these. organic process-related impurities—especially those structurally similar to the API—pose a significant analytical and regulatory challenge. Their structural resemblance can lead to pharmacological activity. instability, making their monitoring critical for patient safety and product performance [4,5]. A particularly demanding area of impurity profiling is observed in antiarrhythmic drug development, especially with compounds that directly modulate cardiac ion channel function. These agents are used in the treatment of life-threatening arrhythmias and carry a narrow therapeutic index, meaning the therapeutic and toxic doses are closely aligned. Thus, any structural variation—including lowlevel impurities—can potentially shift this delicate balance, increasing the risk of proarrhythmia, hypotension, or other adverse cardiac effects[6,7] Propafenone hydrochloride, a widely used Class IC antiarrhythmic agent, acts by blocking fast sodium channels in myocardial cells, slowing conduction velocity and suppressing abnormal electrical activity. It is clinically prescribed for atrial fibrillation, ventricular tachycardia, and supraventricular arrhythmias. The molecule contains a chiral centre and possesses several reactive functional groups including hydroxyl, aromatic rings, and a ketone moiety. These features make it susceptible to side reactions. stereoisomer formation.

degradation, particularly under the influence of moisture, heat, pH, and residual catalytic agents [8] .From a synthetic standpoint, propafenone is commonly synthesized through a multistep process involving the formation and subsequent ring-opening of epoxides, a step that is particularly vulnerable to side product formation. One of the most concerning process-related impurities in this route is 2'-(2-Hydroxy-3-aminopropoxy)-3-

phenylpropiophenone. This impurity is generated when a nucleophilic amine or alcohol reacts with an epoxide intermediate, producing a Regio isomeric product via SN2 or SN1-type mechanisms. Depending on the reaction conditions—acidic, basic, or neutral—the site of nucleophilic attack on the epoxide ring varies, resulting in mixtures of products that can be difficult to detect, separate, or quantify. The chemical structure of 2'-(2-Hydroxy-3-aminopropoxy)-3-

phenylpropiophenone features both hydroxyl and primary amine functional group, increasing its polarity and reactivity compared propafenone. close Its structural resemblance and similar chromatographic behaviour make it particularly challenging to resolve during routine quality control. Without adequate analytical resolution, this impurity can escape detection and potentially impact pharmacokinetic or pharmacodynamic properties. Moreover, the presence of an amine group raises questions about potential genotoxicity, which falls under the scope of ICH M7—a guideline that enforces rigorous evaluation of DNA-reactive impurities, even at parts-per-million (ppm) concentrations [3,9]. Compounding this challenge is the lack of commercially available reference standards or detailed literature on the impurity's synthesis, spectral characteristics, and quantification protocols. While some literature reports address elemental impurities in propafenone [10], or metabolic by-products such as 5hydroxypropafenone [11], detailed accounts of this specific process impurity are missing. As a result, regulatory submissions involving propafenone must either synthesize this impurity in-house or provide justification for its absence, which may not be feasible in cases of subtle, undetected process deviations. Given the potential toxicological risks and analytical challenges, there is a pressing need to understand its chemical behaviour, and develop validated analytical methods for its detection and quantification. Techniques such as Liquid Chromatography-Mass Spectrometry (LC-MS), Nuclear Magnetic Resonance (NMR), Infrared Spectroscopy (IR) indispensable in characterizing this compound and distinguishing it from the API or other substances. Furthermore. understanding the mechanism of formation is crucial to prevent its occurrence during industrial synthesis. In basic conditions, nucleophiles such as hydroxide or amines attack the less substituted carbon of the epoxide ring, leading to a product with transhydroxy and amino substituents. In acidic media, the more substituted carbon is favoured due to the partial SN1 character of the mechanism. Both pathways can produce structurally similar by products unless reaction conditions are tightly controlled. Factors such as reaction temperature, pH, solvent choice, and reaction time influence the selectivity and extent of impurity formation [1,2]. The current research focuses on the targeted synthesis and structural characterization of 2'-(2-Hydroxy-3aminopropoxy)-3-phenylpropiophenone, simulating reaction conditions that mimic those found in propafenone industrial production. The impurity will be synthesized via controlled epoxide ring-opening reactions, using defined nucleophiles under varied pH and solvent conditions. The crude product will be purified using chromatographic techniques, followed by spectral characterization through LC-MS, 1D and 2D NMR, and FTIR analysis. By developing a comprehensive impurity profile, this study seeks to contribute valuable data to pharmaceutical process development, regulatory submissions, and quality risk assessment. The work also aims to establish this impurity as a reference marker for future method validation and stability studies, aiding both generic and innovator companies in meeting global regulatory expectations. Ultimately. this investigation not only addresses specific quality risk a propafenone synthesis but also exemplifies the broader need for proactive impurity science a discipline that integrates organic chemistry,

analytical method development, toxicology,

synthesize this impurity in pure form,

and regulatory compliance to ensure the development of safe, effective, and reliable pharmaceuticals.

MATERIALS AND METHOD

Starting Materials

synthesis of 2'-(2-Hydroxy-3aminopropoxy)-3-phenylpropiophenone begins with 3-phenylpropiophenone as the primary starting material, serving as the core ketone framework. Epichlorohydrin employed to introduce an epoxide group through alkylation, facilitated by a basecatalysed reaction using sodium hydroxide (NaOH). The reaction is typically conducted in an aprotic solvent such as tetrahydrofuran (THF) or dichloromethane (DCM), which help stabilize the intermediates and promote efficient epoxide formation. Nucleophile for Ring Opening:

In the epoxide ring-opening step, nucleophilic agents such as 25% aqueous ammonia, ethanolamine, or 3-amino-1-propanol are employed to facilitate the reaction. These nucleophiles selectively attack the epoxide ring, introducing both amino and hydroxyl functional groups into the molecule. This reaction leads to the formation of the target impurity, 2'-(2-Hydroxy-3-aminopropoxy)-3-phenylpropiophenone, under carefully controlled reaction conditions. The choice of nucleophile and pH plays a critical role in directing the reaction pathway and ensuring the desired regioselectivity.

1.1. Reaction Conditions

The reaction conditions play a crucial role in determining the selectivity and efficiency of the epoxide ring-opening step. In a basic medium, reagents such as sodium hydroxide (NaOH) or sodium carbonate are used to promote an SN2type nucleophilic substitution, favouring attack at the less hindered carbon of the epoxide ring. Alternatively, an acidic environment using hydrochloric acid (HCl) can shift the mechanism toward an SN1-like pathway, encouraging attack at the more substituted carbon. The reaction temperature is typically maintained between 25°C and 50°C, depending on the reactivity of the nucleophile and the desired regioselectivity, ensuring optimal conditions for the formation of 2'-(2-Hydroxy-3-aminopropoxy)-3phenylpropiophenone.

UnlockingHorizonsinGlobalMultidisciplinaryHealthcareResearch" Indo-US International Conference Date: 10-12, March 2025 Organised by PES Modern college of Pharmacy Nigdi Pune-44

1.2. Workup Reagents

During the workup phase of the reaction, ethyl acetate or diethyl ether is commonly used to extract the organic components from the aqueous layer, enabling effective isolation of the product. A wash with brine (saturated sodium chloride solution) is then employed to enhance phase separation and remove residual water. Finally, the organic layer is dried using anhydrous sodium sulphate (Na_2SO_4) to eliminate any remaining moisture before proceeding to solvent evaporation or purification steps.

Purification

For purification, silica gel (60–120 mesh) is employed as the stationary phase in column chromatography, allowing effective separation of the desired compound from side products and unreacted materials. The choice of eluent depends on the polarity of the target impurity; commonly used solvent systems include hexane/ethyl acetate or methanol/chloroform mixtures. The progress and efficiency of the purification process are monitored using thin-layer chromatography (TLC), which helps confirm the presence and purity of 2'-(2-Hydroxy-3-aminopropoxy)-3-

phenylpropiophenone before final characterization.

Analytical/Characterization Solvents & Tools For analytical and structural characterization, deuterated solvents such as chloroform-d (CDCl₃) and dimethyl sulfoxide-d₆ (DMSO-d₆) are utilized for nuclear magnetic resonance (NMR) spectroscopy, enabling clear and carbon interpretation of proton HPLC-grade environments. methanol acetonitrile is used as the mobile phase for liquid chromatography-mass spectrometry (LC-MS) to ensure high sensitivity and accuracy in detecting and quantifying the impurity. Additionally, potassium bromide (KBr) is used to prepare transparent pellets for infrared (IR) spectroscopy, allowing identification of functional groups present in 2'-(2-Hydroxy-3-aminopropoxy)-3phenylpropiophenone.

1.3. Lab Equipment Required

The synthesis and purification of 2'-(2-Hydroxy-3-aminopropoxy)-3-

phenylpropiophenone require standard organic chemistry laboratory equipment. A round-bottom flask (50-250 mL) is used as the main reaction vessel, typically paired with a magnetic stirrer and heating plate to ensure uniform mixing and controlled heating. A reflux condenser is attached for reactions requiring elevated temperatures under reflux. Dropping funnels or pipettes allow for the controlled addition of reagents. Thermometers and pH meters or pH paper are essential for monitoring reaction conditions. For solvent removal, a rotary evaporator is used after extraction. Separatory funnels facilitate liquidliquid extraction steps, while a vacuum filtration setup is employed for solid product isolation. Finally, purification is carried out using a column chromatography setup packed with silica gel, enabling separation of the desired impurity from side products and unreacted materials.

2.8. Critical Control Parameters:

Several critical control parameters must be carefully managed during the synthesis of 2'-(2-Hvdroxy-3-aminopropoxy)-3-

phenylpropiophenone to ensure high selectivity and purity. The reaction pH is crucial, as it influences the mechanism of epoxide ring opening and determines the regioselectivity of the product formed. Maintaining precise stoichiometric ratios of reactants is essential to minimize the formation of undesired side products and ensure complete conversion. Reaction time and temperature must be closely monitored and optimized to achieve maximum yield degradation. Additionally, without moisture control is necessary, particularly during the epoxide formation step, as the presence of water can lead to hydrolysis, reduced reactivity, or formation of unwanted by products.

Method :-

Propafenone EP Impurity – N-Depropyl Propafenone

Step-1st

In a 250 mL round-bottom flask, dissolve 2-hydroxyacetophenone (e.g. 3.30 g, 24.3 mmol) and an equimolar amount of benzaldehyde (e.g. 2.80 g, 22.6 mmol) in 50 mL of absolute ethanol. While stirring at room temperature,

add 10 mL of 10% aqueous NaOH solution dropwise. Stir the reaction mixture at room temperature for 12–24 hours, monitoring the reaction by TLC. After completion, pour the mixture into 100 mL of ice-cold water, acidify with dilute HCl to pH 5–6, and collect the yellow precipitate by vacuum filtration. Wash with cold water and dry under vacuum to obtain 2'-hydroxychalcone.

Step-2nd

In a hydrogenation flask, dissolve the obtained 2'-hydroxychalcone in ethanol (20-30 mL) and add 5% palladium on carbon (Pd/C) catalyst (typically $\sim 10\%$ w/w of the substrate, e.g. 0.3– 0.5 g). Stir the mixture under a hydrogen atmosphere at room temperature until TLC indicates completion (usually 2-4 hours). Filter the mixture through celite to remove the catalyst and evaporate the solvent under reduced pressure. Purify the product if necessary by recrystallization or column chromatography obtain 1-(2to hydroxyphenyl)-3-phenylpropan-1-one.

Step-3rd

Dissolve1-(2-Hydroxyphenyl)-3-

phenylpropan-1-one(1 eq, 5.154 g) in 50 mL of DMF in a round-bottom flask, followed by the addition of potassium carbonate (1 eq, 3.0529 g). The mixture is stirred at room temperature for 10–15 minutes to ensure complete dissolution and mixing. Epichlorohydrin (1 eq, 2.597 mL) is then added dropwise with continuous stirring while maintaining cooling. After stirring at room temperature for an additional 5 minutes, the reaction mixture is heated to 80°C and refluxed overnight, with progress monitored via TLC. Upon completion,

the mixture is transferred to a separatory funnel, and the product is extracted using ethyl acetate $(3 \times 25 \text{ mL})$, followed by washing with water $(2 \times 10 \text{ mL})$ and brine (10 mL). The organic phase is dried over anhydrous sodium sulphate and concentrated using a rotary evaporator. The crude product is then purified either by recrystallization from ethanol or by column chromatography using a suitable solvent system (e.g., hexane/ethyl acetate). Finally. the purified compound characterized by determining its melting point and performing NMR and mass spectrometry.

Step-4th

Dissolve 1-[2-[[(2RS)xiranyl|methoxy|phenyl|-3-phenylpropan-1one (1 eq, 2.1 g) in a mixture of 10 mL THF in a Transparent Seal Tube, followed by stirring at room temperature for 10-15 minutes to ensure complete dissolution and homogeneity. Add Ammonium hydroxide 1.2 eq (0.3128ml) is then added dropwise to the mixture under with continuous cooling stirring. allowing the reaction to proceed at room temperature and check reaction progress monitored by TLC. Upon completion, the mixture is poured into a separatory funnel and extracted with DCM (3×25 mL). The organic layer is separated and washed with water (2 × 10 mL) and brine (10 mL), then dried over anhydrous sodium sulphate. The solvent is removed under reduced pressure using a rotary evaporator, and the resulting crude product is purified by column chromatography using a suitable solvent system (e.g., methanol/DCM).

Synthetic scheme:-

1-[2-[[(2RS)-oxiranyl]methoxy]phenyl]-3-phenylpropan-1-one

2'-(2-Hydroxy-3-aminopropoxy)-3-phenylpropiophenone

2. RESULT AND DISCUSSION

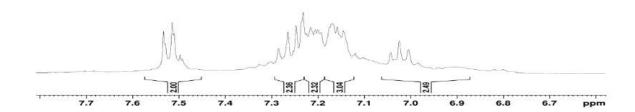
IR (KBr, cm⁻¹): 3373.29 (N-H str), 3010.67 (C-H str), 1745.46 (C=O str), 1508.23, 1456.16 (C=C str, aromatic), 1207.36 (C-O str), 773.40, 750.36 (C-Cl str)H NMR (DMSO-d₆, δ ppm): 2.00 (s, 2H, CH₂), 2.07 (s, 1H, NH), 2.23 (s, 1H, CH₂), 2.32-2.52 (m, 2H, CH₂), 2.74-2.99 (m, 4H, NCH₂), 3.04-3.33 (m, 2H, CH₂O), 3.93-4.12 (m, 2H, CHOH), 4.24 (s, 1H,

OH), 5.95 (s, 1H, NH or OH), 7.00–7.55 (m, 10H, Ar–H) LC-MS (ESI⁺), m/z: 300.5 [M+H]+

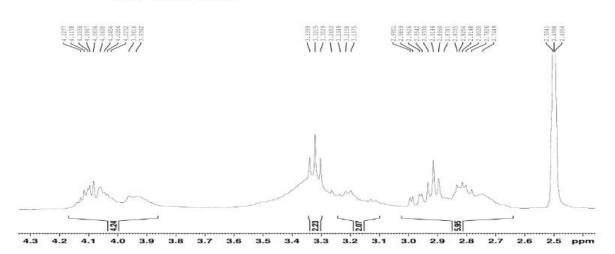
H^1 NMR (DMSO-d₆, δ ppm)

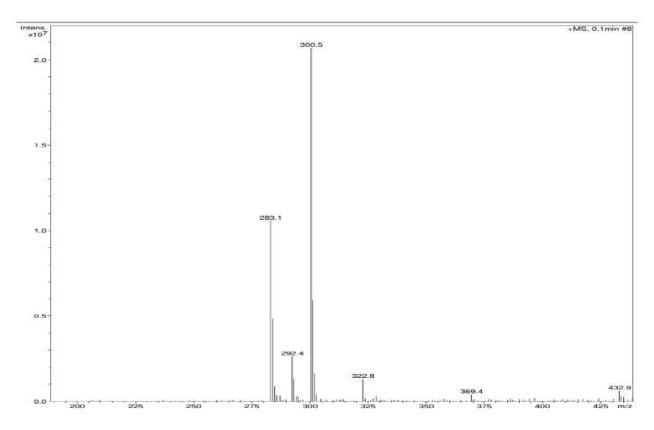






ASL-N-DES-PROF IN DMSC





LC-MS 120-600.M

Observation Table:-

Impurity	Mol. formula	Mol. wt	Yield (%)	Melting point (°C)
2'-(2-Hydroxy-3-aminopropoxy)-3- phenylpropiophenone	C18H21NO3	299.37	86	128–130

The synthesized impurity, 2'-(2-Hydroxy-3aminopropoxy)-3-phenylpropiophenone, obtained as a solid compound with a molecular formula of C₁₈H₂₁NO₃ and a molecular weight of 299.37 g/mol. The isolated yield was 86%, and the compound exhibited a melting point in the range of 128-130°C, indicating good purity and consistent crystallinity. These physical parameters are in agreement with the expected characteristics of the targeted impurity structure 3. CONCLUSION

The present investigation focused on the successful synthesis, isolation, and detailed characterization of the process-related impurity 2'-(2-Hydroxy-3-aminopropoxy)-3phenylpropiophenone, which is structurally

This impurity is likely to form during the epoxide ring-opening step in the synthetic pathway of propafenone, particularly under nucleophilic amines or conditions involving hvdroxvlcontaining reagents. Through a controlled synthesis approach, the impurity was obtained in a high yield of 86% with a well-defined melting point of 128-130°C, indicating its purity and crystalline nature. The structural identity and purity of the synthesized compound were thoroughly confirmed using a combination of ¹H modern analytical techniques. spectroscopy revealed the presence of key proton environments, including those from aromatic rings, aliphatic side chains, and functional groups such as hydroxyl and amino moieties. Infrared related to the antiarrhythmic drug propafenone. (IR) spectroscopy confirmed the presence of with the expected molecular formula $C_{18}H_{21}NO_3$. This study not only demonstrates a reliable method for the synthesis and confirmation of a formulations. structurally significant impurity but also underscores the importance of impurity profiling provides in pharmaceutical development. Given the potential pharmacological and toxicological implications of structurally similar by-products, it is essential to identify and characterize such impurities early in the drug development process.

3. REFERENCE

- 1. ICH. Impurities in new drug substances, Q3A(R2). International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; 2006.
- 2. ICH. Impurities in new drug products, Q3B(R2). International Conference on Harmonisation; 2006.
- 3. ICH. Assessment and control of DNA (mutagenic) reactive impurities pharmaceuticals to limit potential carcinogenic risk, M7(R1). International Conference on Harmonisation; 2017.
- 4. Alsante KM, Ando A, Brown R, Ensing J, Hatajik TD, Kong W, Tsuda Y. The role of profiling degradant in active pharmaceutical ingredients. Advanced Drug Delivery Reviews. 2007;59(1):29-37.
- 5. Dobo KL, Greene N, Cyr MO, et al. Application of in silico tools for the genotoxicity prediction of and carcinogenicity. Toxicology and Applied Pharmacology. 2012;261(1):9-20.
- 6. Vaughan Williams EM. A classification of antiarrhythmic actions reassessed after a decade of new drugs. Journal of Clinical Pharmacology. 1984;24(4):129-147.

characteristic functional groups, including -OH, - The data generated in this work can be utilized as NH₂, and C=0, while LC-MS analysis verified the a reference standard for routine quality control, molecular ion at m/z 300.5 ([M+H]⁺), consistent analytical method validation, and regulatory documentation, thereby enhancing the safety, and compliance of propafenone efficacy, Furthermore, the mechanistic impurity formation pathways insight into valuable guidance for process optimization and impurity control strategies in the manufacturing of antiarrhythmic agents.

- 7. Nattel S. Antiarrhythmic drug therapy: insights into molecular evolving mechanisms and clinical applications. Nature Drug Discovery. Reviews 2002;1(4):279-287.
- 8. McMurry J. Organic Chemistry. 8th ed. Boston: Cengage Learning; 2011. p. 863-875.
- 9. Joule JA, Mills K. Heterocyclic Chemistry. 5th ed. Oxford: Wiley-Blackwell; 2010. p. 145–157.
- 10. Raju C, Jhanavi AS, Sivagami B, Babu MN. Development and validation of a simple and sensitive ICP-MS method for the quantification of elemental impurities in Propafenone hydrochloride substance. Iordan Journal of Pharmaceutical Sciences. 2023;16(1):1-10.
- 11. Patel H, Patel C, Vyas R, Bhatt J, Shrivastav P. Development and validation of a LC-MS/MS assay for simultaneous determination of Propafenone and its major metabolites in human plasma. Chromatography. Biomedical 2017;31(1):e3953.
- 12. Shriner RL, Hermann CKF, Morrill TC, Curtin DY, Fuson RC. The Systematic Identification of Organic Compounds. 8th ed. New York: Wiley; 2003. p. 201-220.

Bulletin of Pharmaceutical Research 2025;15(S2)

An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print) 2249-9245 (Online)



RESEARCH ARTICLE

IN SILICO STUDY FOR ADMET AND TOXICITY PREDICTION OF CURRENT ANTIDIABETIC AGENT

Gunjegaonkar M. B. *, Khot S. P. and Fegade S. A.

Faculty of SJVPM'S Rasiklal M. Dhariwal College of Pharmacy, Chinchwad, Pune-411019 *Corresponding author email: mtupache@gmail.com

Received: Feb 11, 2025 / Revised: April 27, 2025 / Accepted: May 21, 2025

Abstract

Diabetes is one of the greatest challenges facing science in the 21st century. As the number of people with diabetes and those taking antidiabetic drugs increases, there is an urgent need to develop new molecules that can treat this disease [1]. Type 2 diabetes mellitus (T2DM) continues to be a major medical problem because close association of chronic hyperglycaemia with obesity, liver disease, and several cardiovascular diseases. The U.S. Food and Drug Administration (FDA) has approved and withdrawn numerous antihyperglycemic drug therapies for the treatment of diabetes. ³ In this study we mainly focus on in vitro toxicity study, ADMET study as well as toxicity study of various antidiabetic medicine currently used for the treatment of these include insulin types, biguanides, sulfonylureas, meglitinides (glinides), Like alpha glucosidase inhibitors (AGIs), thiazolidinediones (TZDs), sodium-glucose cotransporter type 2 (SGLT2) inhibitors, and dipeptidyl peptidase-4 (DPP4) and comparing the result of these with actual results.

Key words: Toxicity Study, ADME, antidiabetic agent

How to cite this article: Gunjegaonkar M. B. Khot S. P. and Fegade S. A. In silico study for ADMET and toxicity prediction of current antidiabetic agent. Bulletin of Pharmaceutical Research. 2025;15(S2): 291-

Source of support: Nil Conflict of interest: None

INTRODUCTION

Hyperglycaemia, or increased blood glucose, is a sign of diabetes mellitus (DM), a chronic illness. Millions of individuals around the world suffer from this illness. A person with diabetes has either an Ability to use insulin or, in certain cases, an Ability to produce it. The blood glucose imbalance causes the patient to suffer from severe health problems that could possibly be lethal. This silent killer has a significant influence on patients' quality of life, hence it is critical that this issue be addressed. Acute stroke, complications like retinopathy and blindness, kidney issues like kidney failure, metabolic disorders, and anomalies in the blood circulation systems that ultimately lead to death are some of the major adverse effects of diabetes. The unstable activity of the enzyme

was the initial cause of this disease. α -amylase is known to break down complex carbohydrates into smaller molecules, which are subsequently transformed into absorbable sugar molecules by α -glucosidase, another enzyme. There are medications like Acarbose and Miglitols on the market to regulate the activities of these enzymes. These medications work by blocking the functions of the aforementioned enzymes, which in turn helps regulate blood sugar levels and diabetes. Their adverse effects, however, are a serious worry. More diabetes drugs must be produced[1]. According to reports, diabetes may have an impact on the pharmacokinetics of a number of medications by altering (i) absorption through modifications in subcutaneous adipose blood flow, muscle blood flow, and gastric emptying, (ii) distribution through nonenzymatic albumin glycation, (iii)

biotransformation through the regulation of enzymes and transporters involved in drug biotransformation, and excretion nephropathy. Additionally, previously published findings indicate that pharmacokinetic changes caused by diabetes may not be generalizable to other drugs. Although there are currently very few clinical trials examining how diabetes affects pharmacodynamics, there is evidence that disease-mediated effects extend bevond pharmacokinetics and can also change pharmacodynamics. For many medications, it is still unknown if these effects are due to pharmacokinetic alterations brought on by diabetes pharmacodynamic changes. or Furthermore, it is crucial to examine the impact of each medication and refrain extrapolating from observed results, though diabetes-mediated pharmacokinetics and pharmacodynamics may be expected^[2].The safety profiles of medications used to treat diabetes mellitus vary greatly, both in terms of overdose and therapeutic use. Severe and protracted hypoglycaemia can result from an insulin overdose. When treating sulfonylurea toxicity, octreotide should be used instead of intravenous dextrose. Life-threatening acidosis with high lactate concentrations from an acute metformin overdose necessitate may haemodialysis. Diabetic people can safely overdose on glucagon-like peptide 1 agonists and dipeptidyl peptidase 4 inhibitors, whereas non-diabetic patients may have hypoglycaemia. Patients in critical condition who are taking sodium-glucose co-transporter 2 inhibitors may develop euglycemic diabetic ketoacidosis [3].

MATERIALS AND METHODS

Online technologies such as pk CSM and Swiss ADME cheminformatics tools for ADME and drug-likeness studies, as well as Protox III software for toxicity analysis, were among the study's methodologies. To evaluate the degree of resemblance between expected and actual outcomes. all commercially available antidiabetic medications were examined together with their chemical class. The following table displays the ADMET characteristics, druglikeness, and toxicity studies. Forecasted using Swiss ADME cheminformatics tools and pk CSM[4].

Absorption: One significant characteristic that affects absorption is water solubility. With the

exception of RepagIte, Pioglitazone, Glimepiride, Glyburide, and Canagliflozin, which were insoluble (more than -4) and several of the antidiabetic drugs that were predicted to be soluble (0 to -2), others were found to be slightly soluble (-2 to -4). The Caco2 permeability: or human epithelial colorectal adenocarcinoma cell lines are widely used as in vitro models to predict the absorption of an orally administered drug in the human instil mucosa. Compounds with high Caco2 permeability have greater than 0.90 predicted values the intestine is considered to be the primary site for absorption of an orally because of its enormous surface area, the medication is delivered. Empagliflozin, Ertugliflozin, Miglitol, Metformin, and GLP-1 receptor agonists all had negative values, which suggests low instil absorption, whereas the remaining medications all had positive values, indicating high instil absorption^[4]. Acarbose causes bloating and diarrhoea, while GLP-1 agonists cause nausea and vomiting when used with real-time rapid release metformin [5].

Skin permeability: When developing topical medication formulations and transdermal drug delivery devices, skin permeability is crucial. The compound's capacity to penetrate the skin decreases with increasing log Kp, expressed as cm/s. Here, every commercially available antidiabetic medication exhibits negative values, as a result, they all have low skin permeability. The most crucial component of the ATP-binding cassette transporters is the p-glycoprotein (Pgp). By extruding these substances and shielding the cells from toxicity, it plays a crucial part in lowering the absorption of xenobiotics, including medications. and poisons. Cancer cells' resistance to chemotherapeutic treatments is also a result of this. Because of its impact on the **ADMET** characteristics of compounds, approximately 50% of medications that anticipate p-glycoprotein I and II inhibitors exhibit positive results, while the remaining pharmaceuticals show negative results. Compounds that block p-glycoproteins 1 and 2 may be helpful in specific circumstances. Here, the effects of the antidiabetic drugs are somewhat mixed.

Distribution: One crucial pharmacokinetic factor required for creating an appropriate drug dosing schedule is the steady-state volume of distribution, or VDss. It is the amount of blood or plasma in which the substance seems to dissolve

at equilibrium or in a steady condition.

Table: 1 absorption and distribution study

	(log mol/L)	(log Papp in	(% Absorb	(log Kp)	P- glycop	p- glycop	P- glycop	(log L/kg)	(Fu)	(log BB)	(log PS)
		10-6	ed)		rotein	rotein	rotein				
		cm/s)			substr	I inhibit	II inhibit				
					ate	or	or				
Repaglinide	-4.432	0.53	92.168	-2.735	Yes	No	Yes	-1.302	0	0.023	-2.241
Nateglinide	-2.996	0.786	100	-2.735	No	No	No	-1.71	0.105	-0.064	-2.322
Miglitol	-1.229	-0.229	41.462	-2.94	No	No	No	-0.607	0.903	-1.501	-4.842
Pioglitazone	-4.309	0.978	92.422	-2.705	No	Yes	No	-0.007	0.017	-0.591	-2.496
Rosiglitazone	-3.762	0.964	93.757	-2.844	No	Yes	No	-0.183	0.078	-0.727	-2.785
Sitagliptin	-2.871	1.25	87.421	-2.763	Yes	No	No	0.154	0.544	-0.339	-3.186
Saxagliptin	-2.648	0.223	55.565	-3.013	No	No	No	0.043	0.603	-0.227	-2.69
Alogliptin	-1.974	0.175	79.504	-2.74	Yes	No	No	0.555	0.405	-0.603	-2.746
Linagliptin	-2.955	0.356	83.98	-2.736	No	Yes	No	0.26	0.236	-1.509	-2.958
Gliclazide	-3.627	0.454	91.965	-3.116	Yes	No	No	0	0.278	0.079	-2.344
Glimepiride	-4.362	0.592	64.645	-2.867	Yes	Yes	Yes	-0.339	0.22	-0.978	-2.737
Glyburide	-4.582	0.709	71.775	-2.773	Yes	Yes	Yes	-0.218	0	-1.01	-2.674
Chlorpropami de	-2.829	1.353	90.789	-3.188	Yes	No	No	-0.601	0.288	-0.558	-2.98
Glipizide	-3.414	0.582	63.098	-2.776	Yes	No	No	-0.249	0.247	-1.043	-3.366
Tolbutamide	-2.824	1.344	91.614	-3.19	Yes	No	No	-0.485	0.263	-0.388	-2.663
Tolazamide	-3.541	0.88	92.187	-3.071	No	No	No	0.02	0.408	-0.075	-2.497
Metformin	-2.707	-0.339	59.401	-2.735	Yes	No	No	-0.232	0.811	-0.946	-4.238
Dapagliflozin	-3.433	0.938	55.856	-2.751	Yes	Yes	Yes	-0.331	0.077	-0.997	-3.34
Canagliflozin	-4.21	0.846	98.459	-2.736	Yes	Yes	Yes	-0.708	0.084	-0.993	-3.02
Empagliflozin	-3.56	-0.019	55.879	-2.744	Yes	Yes	Yes	-0.387	0.073	-1.101	-3.515
Ertugliflozin	-3.238	-0.037	46.567	-2.738	Yes	Yes	No	-0.191	0.109	-0.903	-3.467
Lixisenatide	-2.852	0.488	4.125	-2.735	Yes	No	No	-0.6	0.888	-0.785	-5.637
Exenatide	-2.892	-2.827	0	-2.735	No	No	No	0.011	0.381	-10.39	- 18.55 1
Semaglutide	-2.892	-3.336	0	-2.735	No	No	No	0.011	0.381	10.99	- 17.71
										2	5
Albiglutide	-2.892	-2.717	0	-2.735	No	No	No	0.011	0.381	-7.918	
											14.97 9
Dulaglutide	-2.892	-2.754	0	-2.735	No	No	No	0.011	0.381	-7.832	
											15.04 2
Liraglutide	-2.892	-2.88	0	-2.735	Yes	No	No	0.011	0.381	-9.154	
											16.16 2

Metabolism: Approximately 95% of the metabolism of pharmaceuticals on the market is carried out by the cytochrome P450 (CYP450) enzymes. CYP1A2, CYP2C9, CYP2C99, CYP2D6, and CYP3A4 are the CYP450 isoforms that account for 90% of drug metabolism. The ADMET of substances can be significantly changed by CYP450. Therefore, determining whether a substance is an inhibitor

or a substrate of these enzymes is crucial. When substances attach to an enzyme's active site, they change into metabolites, which might be either the active form if the compound is a prodrug or the inert form for clearance.

Excretion: Hepatic and renal clearances combined make up total clearance, which is correlated with bioavailability. With exception of AL gliptin, all commercially available compounds have renal clearance that is more dependent on other mechanisms, such as glomerular filtration, and less on OCT2. It is crucial when figuring out dosage rates to reach concentrations in a steady state. Log mL/min/kg represents the anticipated overall clearance of the substances.

The toxicity The AMES test evaluates a compound's mutagenicity potential using bacteria. By identifying a compound's

mutagenicity potential early in the drug discovery process, dangerous medications may be prevented from being developed. As a result, they are not carcinogenic. A compound's hazardous threshold dose in humans can be estimated using the maximum tolerated dose (MTD). When determining a drug's maximum suggested beginning dose, this information is crucial. Low MTD values are those that are less than or equal to 0.477 log mg/kg/d, and high MTD values are those that are greater than 0.477 log mg/kg/d. For MTD, all antidiabetic medications are within the prescribed range. Skin sensitization: another drug-induced issue that can lead to potentially fatal medical disorders is skin sensitization, which can result in allergic contact dermatitis.

Table 2: Excretion and clearance

	Total Clearanc	Renal OCT2	AMES toxicity	Max. tolerated	hERG I	hERG	Oral Rat Acute	Oral Rat Chronic	Skin Sensitis
	e (log ml/min/	substrate	toricity	dose (human)	or	inhibit or	Toxicity (LD50)	Toxicity (LOAEL)	ation
	kg)			(log mg/kg/day)			(mol/kg)	(log mg/kg_b w/day)	
Meglitinide									
Repaglinide	0.783	No	No	0.452	No	No	2.51	1.798	No
Nateglinide	1.282	No	No	0.141	No	No	2.127	2.031	No
Miglitol	0.815	No	No	2.239	No	No	2.257	4.13	No
Thiazolidinedione	es .								
Pioglitazone	-0.044	No	No	0.41	No	No	2.258	1.379	No
Rosiglitazone	0.107	No	No	0.066	No	No	2,692	1.415	No
DPP- 4 lahibitors			1						
Sitagliptin	0.474	No	No	-0.59	No	No	2.732	1.635	No
Sazagliptin	0.85	No	No	-0.436	No	No	2.835	0.789	No
Alogliptin	0.376	Yes	No	-0.327	No	Yes	2.421	1.425	No
Linagliptin	0.385	No	Yes	0.7	No	Yes	2.62	1.144	No
Sulfonylureas									
Gliclazide	0.741	No	No	0.033	No	No	2.181	1.795	No
Glimepiride	0.691	No	No	-0.479	No	No	1.942	0.891	No
Glyburide	-0.155	No	No	-0.026	No	No	1.701	1.81	No
Chlorpropamide	-0.057	No	No	1.191	No	No	2.335	1.484	No
Glipizide	1.125	No	No	0.043	No	No	1.78	1.606	No
Tolbutamide	0.953	No	No	1.177	No	No	2.165	1.431	No
Tolazamide	0.873	No	No	0.452	No	No	2.577	1.301	No
Metformin	0.1	No	Yes	0.902	No	No	2.453	2.158	Yes
SGLT2 inhibitors									
Dapagliflozin	0.194	No	No	0.507	No	No	2.475	3.363	No
Canagliflozin	0.035	No	No	0.482	No	Yes	2,454	4.325	No
Empagliflozin	0.404	No	No	0.25	No	Yes	2.554	3.51	No
Ertugliflozin	0.258	No	No	0.264	No	No	2,633	3.801	No
GLP-1 receptor ag	onists								
Lixisenatide	0.765	No	No	1.138	No	Yes	2.751	1.847	No
Exenatide	-2.005	No	No	0.438	No	No	2.482	14.763	No
Semaglutide	-1.613	No	No	0.438	No	No	2.482	13.398	No
Albiglutide	-1.881	No	No	0.438	No	No	2.482	12.374	No
Dulaglutide	-2.144	No	No	0.438	No	No	2.482	12.432	No
Liraglutide	-1.601	No	No	0.438	No	No	2.482	12.863	No

If log L/kg is less than -0.15, VDss is regarded as low, if it is greater than 0.45, it is regarded as high. A chemical is more widely distributed in tissue than in plasma when its volume of distribution is larger. The volume of distribution of all antidiabetic medications is decreasing. Predicting a chemical's capacity to pass through the blood-brain barrier (BBB) aids in minimizing lead compound toxicities or adverse effect Additionally, it can aid in enhancing the of effectiveness medications that have pharmacologic effects on the brain. While chemicals with log BB below -1 are poorly transported to the brain, those with log BB greater than 0.3 can easily penetrate the bloodbrain barrier. The membrane permeability of all the substances is low. Predicting a capacity of compound pass through the blood-brain barrier (BBB) aids in minimizing lead compound toxicities or adverse effects

Drug-likeliness

qualitatively assesses a compound's potential for oral medication development in relation to its bioavailability. It evaluates a compound's similarity to currently available medications. The five rule-based filters that make up the Swiss ADME drug-likeness section are the Lipinski (the origIl rule-of-five), Ghose, Veber, Egan, and Muegge. The Abbott bioavailability score, which is based on the substances' total charge, topological polar surface area, and violation, is

Prediction of Toxicity: Prior to starting the drug development process, the online tool "Prediction of Toxicity of Chemicals Tox-III)" (Pro (https://toxnew.charite.de/protox_III/) is specifically made for predicting the toxicity of chemicals. Pro Tox-III employs a sophisticated technique that combines known hazardous components and takes into account the distance between unknown molecules and the average lethal dose (LD50) of known substances. Potential compounds' structural files are transformed into SMILES format and uploaded to the Pro Tox online site in order to evaluate toxicity. Predicted LD50 values, toxicity levels, and other

used by Swiss ADME.

Prediction Rules of Five Lipinski Table-3 showed that the entire antidiabetic agent follows Lipinski rule whereas compounds like Repaglinide, Miglitol, Acarbose, Linagliptin Glyburide did not meet the Ghose requirements, Repaglinide, I gliptin Glyburide does not meet the Veber requirement, Glipizide and glipizide did not meet Egan requirement, whereas Repaglinide was the only drug that do not follow Whereas Muegge requirement. with few exception GLP 1 class did not meet the rule for drug likeness. All the compounds shows Bioavailability between 0.17 to 0.85.

Table 3: Drug likeliness study

	Lipinski	Ghose	Veber	Egan	Muegge	Bioavailability
Repaglinide	Yes	No	No	Yes	No	0.56
Nateglinide	Yes	Yes	Yes	Yes	Yes	0.85
Miglitol	Yes	No	Yes	Yes	No	0.55
Acarbose	Yes	No	Yes	Yes	yes	0.55
Pioglitazone	Yes	Yes	Yes	Yes	Yes	0.55
Rosiglitazone	Yes	Yes	Yes	Yes	Yes	0.55
Sitagliptin	Yes	Yes	Yes	Yes	Yes	0.55
Saxagliptin	Yes	Yes	Yes	Yes	Yes	0.55
Alogliptin	Yes	Yes	Yes	Yes	Yes	0.55
Linagliptin	Yes	No	Yes	Yes	Yes	0.55
Gliclazide	Yes	Yes	Yes	Yes	Yes	0.55
Glimepiride	Yes	No	No	No	Yes	0.55
Glyburide	Yes	No	No	Yes	Yes	0.55
Chlorpropamide	Yes	Yes	Yes	Yes	Yes	0.55
Glipizide	Yes	Yes	Yes	No	Yes	0.55
Tolbutamide	Yes	Yes	Yes	Yes	Yes	0.55
Tolazamide	Yes	Yes	Yes	Yes	Yes	0.55
Metformin	Yes	No	Yes	Yes	No	0.55
Dapagliflozin	Yes	Yes	Yes	Yes	Yes	0.55
Canagliflozin	Yes	Yes	Yes	Yes	Yes	0.55
Empagliflozin	Yes	Yes	Yes	Yes	Yes	0.55
Ertugliflozin	Yes	Yes	Yes	Yes	Yes	0.55
Lixisenatide	No	No	No	No	No	0.17
Exenatide	No	No	No	No	No	0.17
Semaglutide	No	No	No	No	No	0.17
Albiglutide	No	No	No	No	No	0.17
Dulaglutide	Yes	Yes	Yes	Yes	Yes	0.55
Liraglutide	Yes	Yes	Yes	Yes	Yes	0.55

elements including cytotoxicity, immunotoxicity, mutagenicity, carcinogenicity, and hepatotoxicity are all included in the output. According to the criteria of the globally harmonized system of classification and labelling of chemicals, the Pro Tox-III online server also determines a chemical's toxicity class, classifying it from I to VI based on the LD50 value.

Supportive technologies like Pro Tox III have been developed to rapidly forecast the potentially hazardous effects of candidate therapeutic compounds in order to evaluate the possible harm that particular chemicals may cause to the human body. The comprehensive prediction results in this section are displayed in

table 4, which indicates that the marketed drugs' anticipated lethal dose (LD50) falls between 3 and 4000 mg kg-1. The majority of medications fall into classes 4 and 5, with the exception of Acarbose and Glipizide, which come under classes 3 and 6, respectively, according to the toxicity grading scale, which classifies a chemical as having toxicity level 5 if its LD50 value is between 2000 and 5000 mg kg-1.

Liver toxicity:

caused by drugs is a pressing safety concern during drug development and ranks among the top reasons for drug attrition only Pioglitazone shows hepatotoxicity. DPP- 4 Inhibitors. Thiazolidinedione's and Repaginide found to be exhibiting Neurotoxicity, but do not exhibit nephrotoxicity, where as other classes to some extent shows nephrotoxicity. SGLT2 inhibitors, Nateglinide, Acarbose were found to be showing cardiotoxicity. But practically SGLT2 inhibitors were found to useful for prevention of Primary and secondary cardiovascular outcome [6]. None of the compound shows carcinogenicity and Mutagenicity. The consistency between in silico and in vitro data found for mutagenesis [7] .Increased levels in circulating insulin as a result of the use of sulfonylureas and glinides, insulin secretagogues, have potential effects on cancer biology. Cohort studies showed that the use of sulfonylureas increases the risk of cancer incidence and cancer-related mortality in patients with type 2 diabetes. However, this cancer risk associated with sulfonylureas use was found when compared with metformin treatment. Indeed, clinical studies have shown the association of TZDs use with the decreased risk for liver, colorectal, lung, lymphatic, prostate, stomach, kidney, and breast and brain cancer in patients with diabetes. Although current evidence suggests the association between type 2 diabetes and the increased risk of certain cancer incidence and poor prognosis, the possibility that long-term use of antidiabetic agents influences carcinogenesis has not been fully discussed yet[8].

CONCLUSION

With little exception all of the drug-likeness section composed of five different rules is followed by marketed drugs. Furthermore. toxicity predictions for these potential compounds indicated LD50 values greater than 2000 mg kg-1, classifying them as having low toxicity (Toxicity Class 5). This suggests that these compounds are low in toxicity and safe, acarbose shows Toxicity class 3 whereas Glipizide shows Toxicity Class 6 which indicate it moderate to high toxicity respectively. Few drugs are found to be showing positive results for neurotoxicity & nephrotoxicity whereas cardiotoxicity. SGLT2 class shows predicated and actual results were found to showing consistency with Insilco results with few exceptions.

REFERENCES

- 1. Khan S, Iqbal T, Hussain R, Khan Y, Fiaz Z, Rahim F, Darwish HW. Synthesis, characterizations, anti-diabetic and molecular modeling approaches of hybrid indole-oxadiazole linked thiazolidinone derivatives. Pharmaceuticals. 2024;17(11):1428.
- 2. Dostalek M, Akhlaghi F, Puzanovova M. Effect of diabetes mellitus on pharmacokinetic and pharmacodynamic properties of drugs. Clin Pharmacokinet. 2012;51(8):481–499.
- 3. Baumgartner KT, Devgun J. Toxicology of medications for diabetes mellitus. Crit Care Clin. 2021;37(3):577–589.
- 4. Bucao XEN, Judilynn NS. In silico evaluation of antidiabetic activity and ADMET prediction of compounds. Philipp J Sci. 2022;151(1):171–192.

- 5. Barrientos JR, Avalos EC, Morel-Cerdac FA, Felix T, Vidrio-Huerta BE, Aceves-Ayala AR, Gastrointestinal adverse effects of old and new antidiabetics: how do we deal with them in real life? Rev Gastroenterol Mex Engl Ed. 2024;89(4):521–532.
- 6. He G, Yu G, Huang X, Duan L, Tang C, Zhen Z. SGLT2 inhibitors for prevention of primary and secondary cardiovascular outcomes: a meta-analysis of randomized controlled trials. Heart Lung. 2023;59:109–116.
- 7. Rajesh R, Bharat S, Dileep K, Mugdha S, Akshay G, Prabhune BG. Mutagenic and genotoxic in silico QSAR prediction of dimer impurity of gliflozins (canagliflozin, dapagliflozin, and empagliflozin) and in vitro evaluation. Mutat Res. 2024;48(2):416–425.
- 8. Emi K, Keizo E. Cancer biology in diabetes update: focusing on antidiabetic drugs. J Diabetes Investig. 2024;15(5):525–540.

Bulletin of Pharmaceutical Research 2025;15(S2)

An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print) 2249-9245 (Online)



RESEARCH ARTICLE

COMPARATIVE STUDY OF EVALUATION OF GENERIC AND BRANDED METFORMIN HCL (500MG) TABLETS.

Vishnu Neharkar^{1*} Dhiraj Panke¹ and Shrikrushna Wakude².

1.Rasiklal M. Dhariwal Institute Of Pharmaceuticsl Education & Research Cinchwad,Pune-411019
2. PES's Modern College of Pharmacy, Sec. No. 21, Yamunanagar, Nigdi, Pune 411044.
*Corresponding author email: vsneharkar@gmail.com
Received: Feb 01, 2025 / Revised: April 16, 2025 / Accepted: May 05, 2025

ABSTRACT

This research paper provided a comprehensive evaluation of both generic and branded Metformin HCl (500 mg) tablets through the measuring of the physicochemical parameters however, and performance properties of each brand. Metformin is a big deal when managing type 2 diabetes mellitus. The objective of this study was to determine whether generic metformin tablets could provide a safe and cost-effective alternative to branded Metformin tablets. Accordingly, the researcher assessed a number of tests which included visual inspections, thickness, hardness, friability, weight variation, disintegration time, and dissolution rate. The results provided evidence that both, generic and branded metformin tablets complied with the requirements in the pharmacopoeia, however branded formulations had disintegration times that were faster and more uniform than the generic formulations. However, there was only one parameter that was branding metformin tablet superior that the branded metformin tablets were of better quality than generics tablets, however this supports the notion that generics can provide an acceptable quality product. In conclusion, our research shows that they may be cost-effective, alternative options for patients that made qualitatively equal products. This current study also reinforces the notion that the generics are produced through good manufacturing practices, which may also suggest that it may be beneficial to consider properly manufactured generics, particularly in countries with limited access to medications as there is clinical benefit and treatment effect with generics without compromising adherence and therapeutic effect without a discount on access to quality products.

Key words: Generic drugs, Metformin, Evaluation test, Diabetes.

How to cite this article: Vishnu Neharkar Dhiraj Panke and Shrikrushna Wakude. Comparative Study of Evaluation of Generic and Branded Metformin HCl (500mg) Tablets.Bulletin of Pharmaceutical Research. 2025:15(S2): 297-302

Source of support: Nil Conflict of interest: None

INTRODUCTION

Branded medicine: It is the inaugural product developed by the pharmaceutical industry. For a certain amount of time, it has the exclusive right to produce and distribute. A New Drug Application containing information on proof of features of dosage form, manufacturing, chemistry, stability, efficacy, safety, labelling, and packaging is required for the FDA to approve a brandname medication. only after FDA approval, For the duration of patent protection (about 20 years or as indicated), the innovative firm

is permitted to market this brand-name medication exclusively. [1]

Generic medicine: Generic medications are ones for which the original patent has lapsed, permitting other manufacturers to create them. Different markets may define "generic medicine" or "generic drug" differently. But according to the World Health Organisation (WHO), it is generally understood to refer to a pharmaceutical product that can be used in place of an innovator product is produced without a

Unlocking Horizons in Global Multidisciplinary Healthcare Research" Indo-US International Conference Date: 10-12, March 2025 Organised by PES Modern college of Pharmacy Nigdi Pune-44 license from the innovator company; and is marketed after the patent or other exclusive rights have expired. [2] Cost Effectiveness of Generic Drug in Comparison to Branded Drugs: Generally priced far less than their brand-name counterparts, generic drugs are

more easily available to a larger population. Patients who might be managing chronic illnesses or need long-term medication use will find this cost savings especially helpful.[5]

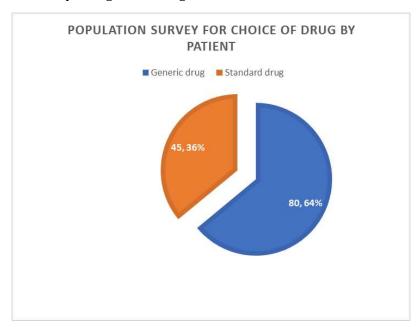


Fig 1: population survey for choice of drug by patient

Diabetes mellitus (DM) is a condition in which blood glucose levels are not adequately controlled. Type 1, type 2, gestational diabetes, neonatal diabetes, maturity-onset diabetes of the young (MODY), and steroid-induced diabetes are just a few of its numerous subtypes. [7] Among other things, oxidative stress—which is brought on by an increase in reactive oxygen species (ROS)—is linked to type 2 diabetes (T2D) and hyperglycemia. In most cell types, the primary generator of ROS is the mitochondria. [9] The

mitochondrion is where metformin mostly acts. Defective protein kinase A signaling is most likely the cause of metformin's antihyperglycemic action. Metformin mainly influences lipid metabolism by activating 5'-AMP-activated protein kinase (AMPK).[3]

Drug Profile:

The first line of treatment for those with type 2 diabetic mellitus (T2DM) is metformin, a biguanide derivative.^[4]

Table: 1 Details of Metformin Hydrochloride

Synonyms	Metformin Hydrochloride
Chemical Formula	$C_2H_{11}N_{7[13]}$
IUPAC Name	1,1-dimethylbiguanide hydrochloride[1]
Half-time	~5 Hrs _[14]
Shelf life	3 years from the date of manufacture.[15]
Molecular weight	133.16 g/mol _[13]
Pka value	-14.83 _[13]
Bioavailability	31%[16]
Excretion	Urine (90%) [1]
Renal clearance (Cl _r)	510±120 ml/min [14]
CAS No.	1115-70-4[15]

MATERIALS AND METHOD:

Visual Inspection:

Every brand of Metformin tablet is carefully inspected during the visual inspection stage in order to identify unique features including shape, colour, and texture. This crucial step makes it easier to visually detect any differences, anomalies, or discrepancies between the tablets. Physical instability is indicated by the following: a) an abundance of powder and/or tablet fragments at the bottom of the container (from crushed, broken, or abraded tablets); b) chipping, cracking, or capping in the tablet coating or surfaces; c) swelling, mottling, discoloration, or fusion between tablets; and c) the presence of crystals on the tablets or container walls. d) The presence of crystal on the tablets or the walls of the container

Thickness (in cm):

As an initial control parameter, the tablet's thickness is primarily correlated with its hardness. Vernier callipers were used to measure the tablets' thickness. A Vernier calliper was used to measure the thickness of five randomly chosen tablets, Desired thickness: 2.0 - 4.0mm

Hardness:

Tablet hardness was evaluated using a Monsanto hardness tester and expressed in kilograms per square centimetre (kg/cm 2). Ten tablets from each brand were randomly selected and individually placed between the tester's spindle and anvil until fracture occurred. The pressure

Dissolution rate:

The dissolution rate was determined using an **ERWEKA** DT600 dissolution apparatus (Heidenstam, Germany) in simulated intestinal fluid (pH 6.8). One tablet was placed in each vessel containing 1000 mL of dissolution medium maintained at 37 ± 0.5 °C, with the basket rotation speed set at 100 rpm. At predetermined time intervals (10, 20, 30, 45, and 60 minutes), 10 mL of the sample was withdrawn using a 10 mL pipette immediately replaced with an equal volume of fresh dissolution medium to maintain sink conditions. The withdrawn samples were filtered through a 0.45 µm syringe filter, suitably diluted. analyzed UV-visible and using spectrophotometer at \(\lambda \text{max} \) 233 nm. The drug concentration was quantified with reference to a standard solution of metformin hydrochloride

required to break each tablet was recorded. According to pharmacopoeial requirements, the desired hardness range is 4–12 kg/cm².

Weight Variation:

A total of twenty tablets were chosen at random and weighed separately. Each person's weight was computed and compared to the average weight. Each tablet's percentage deviation was determined using the formula below. According to BP, if no one deviates by \pm 10% and no more than two of the individual weights differ from the average weight by \pm 5%, the tablet passes the test. [7]

Friability:

Friability refers to the tendency of tablets to lose weight due to the removal of small particles from their surface. It is expressed as a percentage of weight loss. For the test, twenty pre-weighed tablets were placed in the friabilator chamber and subjected to 100 revolutions. During each revolution, the tablets were lifted and allowed to fall freely from a height of approximately six inches. After completion, the tablets were removed, dedusted, and reweighed to determine the percentage weight loss.

Disintegration:

The disintegration time was determined using a Multi-unit Disintegration Tester (USP, Electrolab). For each of the eight brands, six tablets were randomly selected and tested in purified water maintained at 37 ± 0.5 °C. The disintegration time was recorded as the point at which no tablet residue remained on the mesh.

RESULTS AND DISCUSSION

The in vitro dissolution profile of the branded formulation was evaluated over a period of 60 minutes, and the results are summarized in Table X. The drug exhibited a gradual release pattern in the initial stages, with 11.32% release at 10 minutes, followed by 21.84% at 20 minutes and 34.78% at 30 minutes, indicating a slow onset of dissolution. A sharp increase in drug release was observed thereafter, with 61.67% at 40 minutes and 91.45% at 50 minutes, reaching nearcomplete dissolution of 98.52% within 60 minutes. This profile demonstrates that the branded formulation achieves more than 85% drug release within 50 minutes, complying with pharmacopeia dissolution criteria immediate-release solid oral dosage forms. The in-vitro dissolution profile

of the generic drug formulation was evaluated in the specified medium, and the cumulative percentage of drug release was calculated at different time intervals. As shown in the table, the formulation exhibited a slow initial release, with 8.38% drug dissolved at 10 minutes and 14.98% at 20 minutes. A marked increase in dissolution was observed thereafter, reaching 31.84% at 30

minutes and 56.39% at 40 minutes, indicating a progressive release phase. The formulation achieved a rapid and almost complete drug release between 50 and 60 minutes, with 89.39% release at 50 minutes and 91.39% at 60 minutes, demonstrating compliance with pharmacopeia specifications for immediate-release dosage forms.

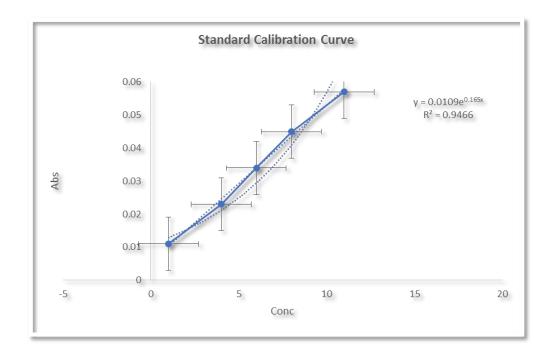


Fig:1 Standard Calibration curve

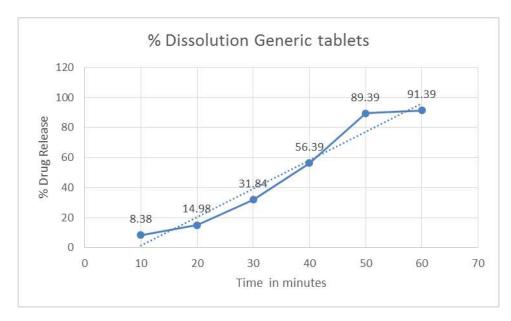


Fig:2 Generic tablet release

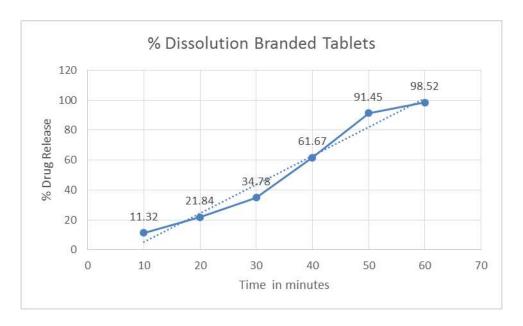


Fig:3 Branded tablet release

Weight Variation

Tablet: 2 results of weight deviation

Average weight	Maximum Percentage Deviation	
IP	USP	
130≥	80≥	±10
130-324	80-250	±7.5
324≤	250≤	±5

Initial Weight of 10 tablets: 5.96gm Final Weight of 10 tablets: 5.89gm

$$Weight loss = \frac{Initial weight - Final weight}{Initial weight} \times 100$$

$$= \frac{5.96 - 5.89}{5.96} \times 100$$

$$= \frac{0.07}{5.96} \times 100$$

$$= 1.174$$

Disintegration:

The disintegration characteristics of different types of tablets were evaluated as per pharmacopeial guidelines. Uncoated tablets were tested in water or buffer at 37 ± 2 °C, with a disintegration time limit of 15 minutes. Film-coated tablets were assessed in water under the same temperature conditions, requiring disintegration within 30 minutes. Sugar-coated tablets were tested either in water or 0.1 N HCl at 37 ± 2 °C, with a limit of 60 minutes. Dispersible tablets were required to disintegrate in water at 25 ± 1 °C within 3 minutes, while effervescent

tablets were tested in water at 25 ± 5 °C, with a rapid disintegration requirement of 0.5 minutes. Enteric-coated tablets were evaluated using a two-stage medium: 0.1 M HCl for 2 hours (no disintegration), followed by phosphate buffer pH 6.8 at 37 ± 2 °C, where they must disintegrate within 60 minutes. Soluble tablets were examined in water at 20 ± 5 °C, with a disintegration limit of 15 minutes. Disintegration time of generic tablets: 11.34 Mins, Disintegration time of branded tablets: 3.28 Mins were found

Conclusion:

The present research was intended to thoroughly

assess the quality and performance of drug quality and performance of drug metformin HCl tablets and (500)mg) searched corresponding physicochemical parameters. The primary goal was to establish if the generic forms of metformin representation provide a safe and cost-effective drug alternative to the branded versions. A variety of parameters for example, weight uniformity, tablet hardness, thickness, friability, disintegration time and dissolution time were measured. Although the data revealed that all samples met pharmacopoeia standard values, branded tablets were only faster on disintegration rate and uniformity. However, branded tablets demonstrated compliant compliance for most parameters. thereby indicating generic compositions can serve as appropriate and costeffective options for clinical practice. The implications of this study suggest that there is a need to maintain high quality manufacturing standards to ensure generics; quality

REFERENCE

- 1. Mulik MK, Patil VS, Vakhariya RR. Comparative study of evaluation of generic and branded Metformin HCl (500 mg) tablets. Int J Novel Res Dev. 2024;9(7).
- 2. Nandyala S, Prasanna Kumar PSS, Ravi Kumar A, Sai Lakshmi SS, Sai Supraja P, Tejasri D, Adil SK. Comparative evaluation of branded and generic medicines Ranitidine & Metformin HCl. Int Res J Pharm Med Sci. 2018;1(4):24–28
- 3. Olusola AM, Adekoya AI, Olanrewaju OJ. Comparative evaluation of physicochemical properties of some commercially available brands of Metformin HCl tablets in Lagos, Nigeria. J Appl Pharm Sci. 2012;2(2):41–44
- 4. Afifi S, Ahmadeen S. A comparative study for evaluation of different brands of Metformin Hydrochloride 500 mg tablets marketed in Saudi Arabia. J Pharm Sci Res. 2012;4(9):710–16
- 5. Mehra S, Gupta P, Moitra D, Baeraiya B, Patel RK, Majhi R, et al. Generic drugs vs branded drugs: navigating antitrust law

strengthened and potentially lead to greater use of generics; particularly in areas, where access to and funding for health services is limited. Overall, the study supports the real-world role of generics in creating access to essential medicines without compromising treatment outcomes. comparative in vitro dissolution study revealed that both the branded and generic formulations complied with pharmacopeial requirements for immediate-release solid oral dosage forms, achieving more than 85% drug release within 60 minutes. The branded formulation exhibited a relatively faster release, crossing the 85% dissolution threshold at 50 minutes, whereas the generic formulation reached comparable levels slightly later, between 50 and 60 minutes. Overall. both formulations demonstrated satisfactory dissolution behavior, indicating their potential bioequivalence in terms of drug release characteristics.

- issues in India. J Pharm Policy Pract. 2024;13, 283-286
- Johnston CA, Dickinson VSM, Alcorn J, Gaunt MC. Pharmacokinetics and oral bioavailability of metformin hydrochloride in healthy mixed-breed dogs. Am J Vet Res. 2017;78(10):1193–1199.
- 7. Peter A, Tomar K, Solanki R, Bhide P, Parihar H, Joshi A, et al. In-vitro evaluation of different marketed brands of metformin tablets using quality control tests. Asian Food J Biol Sci. 2024;6(SI-4):1827-46. doi:10.48047/AFJBS.6.Si4.2024.1827-1846
- 8. Sachan AK, Kumar V, Gupta A. Comparative in-vitro evaluation of four different brands of Metformin HCl available in Kanpur district, India. Der Pharmacia Lettre, 2016, 8 (5):419-424
- 9. Hettiarachchi TW, Wickramaratne DBM, Sudeshika SHT, Niyangoda D, Sakeena MHF, Herath HMDR. Comparative in-vitro evaluation of Metformin HCl and Paracetamol tablets commercially available in Kandy district, Sri Lanka. Int J Pharm Pharm Sci, 2014;17(2), 520-524.

An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print) 2249-9245 (Online)



RESEARCH ARTICLE

ADVANCING CEFDINIR ANALYSIS: EVALUATING STABILITY, IDENTIFYING IMPURITIES AND CHARACTERIZING DEGRADANTS

Fegade Sachin^{1*}, Bindurani L G P Ram² and Waman Pradnya ³

1. SJVPM'S Rasiklal M Dhariwal college of Pharmacy Chinchwad Pune
2. SGMSPM's Dnyanvilas college of Pharmacy Dudulgaon Pune
3. PES's Modern College of Pharmacy, Sec. No. 21, Yamunanagar, Nigdi, Pune 411044.
*Corresponding author email: fegadesachin@gmail.com
Received: Feb 06, 2025 / Revised: April 18, 2025 / Accepted: May 02, 2025

ABSTRACT

A robust, sensitive, and validated reverse-phase HPLC method was established for the quantification of impurities in pharmaceutical formulations of cefdinir. Chromatographic separation was performed using a Waters Symmetry C18 column with a mobile phase consisting of acetonitrile and pH 7.0 buffer (85:15, v/v) at a flow rate of 1.0 mL/min. UV detection was carried out at 254 nm, and the method exhibited linearity across a concentration range of 14.5–74.5 μ g/ml. The developed method demonstrated high precision with relative standard deviations below 2.0% and satisfactory accuracy, yielding mean recoveries between 98.68% and 102.28%. The detection and quantification limits were determined to be 0.7 μ g/mL and 2.2 μ g/mL, respectively. These results confirm the method's reliability and suitability for routine quality control analysis.

Keywords: Cefdinir, Impurity profiling, forced degradation, HPLC, HPTLC, stability study. **How to cite this article:** Fegade Sachin*, Bindurani L G P Ram, Pradnya Waman. Advancing Cefdinir Analysis: Evaluating Stability. Identifying Impurities and Characterizing Degradants.

2025;15(S2): **303-308**.

Source of support: Nil Conflict of interest: None

INTRODUCTION

Impurities in Active Pharmaceutical Ingredients (APIs) are a growing concern in pharmaceutical development, as both the purity of APIs and the patterns of impurities have become crucial indicators of drug safety and efficacy [1]. In the pharmaceutical context, the term "impurity" refers to any substance present within an API that is not

the desired active compound. These may arise during synthesis or result from degradation, and can appear as unintended byproducts or contaminants [2]. Such impurities may be introduced during the manufacturing or formulation processes or may form due to the decomposition of APIs over time [3,4]. Regulatory authorities have

increasingly focused on impurity profiling analytical process for detecting. identifying, and quantifying impurities in drug substances and drug products [5]. By definition, an impurity is a foreign substance that contaminates or adulterates the main material, often merging with it as an undesired component [6]. Impurity profiling encompasses a broad spectrum of analytical methodologies aimed at detecting inorganic impurities, organic impurities, and residual solvents in both bulk drugs and finished formulations [7,8]. These impurities can be identified and characterized using various analytical approaches, including the reference standard method [9], spectroscopic techniques [10], separation methods[11], isolation procedures [12], and structural characterization tools [13,14]. A Forced Degradation Study is a key component of impurity profiling. It involves subjecting drug products substances or to extreme environmental conditions to study their degradation pathways, kinetics, and potential degradation products. These studies are instrumental in the development of stabilityindicating analytical methods, understanding drug stability, and identifying conditions that might compromise product integrity. While not required as part of formal stability programs, forced degradation studies are essential during the early phases of drug

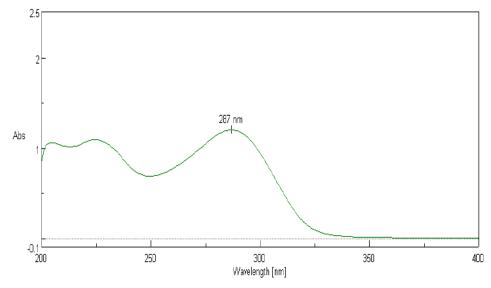
formulation and packaging development.

Materials and Methods

Reagents and Chemical: Chemical's: Cefdinir was sourced from Lupin Pharmaceuticals. Mulshi, HPLC-grade acetonitrile, methanol, and water, along with AR-grade. **Reagent's**: chloroform, acetone, ammonia, glacial acetic acid, triethylamine, orthophosphoric diethyl amine. dichloromethane. sodium hvdroxide. hydrochloric acid, and hydrogen peroxide were procured from Merck Chemicals, Pune. **Instrumentation**A Jasco V-630 UV-Visible spectrophotometer was employed analysis. The instrument, equipped with a deuterium lamp, operated in the 1100–190 nm range using 1 cm quartz cuvettes. Data acquisition was performed via an integrated computer system.

RESULT AND DISCUSSION

Stress degradation study by UV-Visible Spectrophotometer Stock Solution Preparation: To make a 100 μg/mL solution, 10 mg of Cefdinir was added in 80 mL of methanol and then diluted to 100 mL. [15.] Analytical Wavelength Selection: A UV-Vis spectrophotometer was used to scan a 20 ppm solution (2 mL stock solution in 10 mL methanol) over the 200–400 nm range. The highest absorption (λmax) was noted at 287 nm. [16]



Unlocking Horizons in Global Multidisciplinary Healthcare Research" Indo-US International Conference Date: 10-12, March 2025 Organised by PES Modern college of Pharmacy Nigdi Pune-44

Fig 1: UV-Spectrophotometer for pure Cefdinir

Validation Parameter's

Validation ensures that a process consistently meets its specifications and quality standards.

1. Linearity:

Within the validated range, the method established a robust linear dependence on absorbance and concentration.

2. Accuracy:

The method's accuracy verified via recovery tests, wherein revealed that findings were consistently within acceptable limits.

3. Precision:

Measurement consistency and repeatability appeared high in both intra-day and inter-day precision assessments.

4. Limit of Detection (LOD):

Without proper quantification, the technique

Table 1: Validation parameters

found the lowest analyte concentration that could be detectable.

5. Limit of Quantification (LOQ):

The technique found the lowest analyte concentration that could be evaluated with an adequate level of accuracy and precision.

6. Robustness:

The method's performance was unaffected by slight modifications in the experimental setup, proving its dependability.

- 7. **Ruggedness:** The approach offered consistent results under many conditions and by various analyzers.
- 8. **Specificity:** The technique ensured selectivity by efficiently distinguishing the analyte from probable interferences. [15.16]

Sr. No.	Parameter	Result
1	Linearity indicated by correlation coefficient	0.998
2	Linear regression equation	0.048x + 0.037
3	Range	4 μg/ml – 24 μg/ml
4	Intraday Precision (%RSD)	1.7%
5	Interday Precision (%RSD)	1.005%
6	Limit of Detection	0.039 μg/ml
7	Limit of Quantification	0.120 μg/ml
8	Robustness indicated by % RSD	0.3265%
9	Ruggedness indicated by % RSD	0.5452%

Degradation study Acid Degradation

Sample Preparation: Weigh ~10 mg of Cefdinir and dissolve in a small amount of methanol or water (e.g., 5 mL). **Add Acid**: Add an equal volume (e.g., 5 mL) of 0.1N HCl (for mild degradation) or 1N HCl (for stronger degradation). **Incubation**: Heat the solution at **60°C** for **1–3 hours**, or leave at room

temperature for longer durations.**Neutralization**: After degradation, neutralize the solution with equimolar NaOH to prevent further degradation and do analysis by uv spectrophotometer

Base Degradation

Sample Preparation: Dissolve 10 mg of Cefdinir in methanol (5 mL). Add an equal volume of 0.1N or 1N NaOH. Incubation: Heat at 60°C for

1–3 hours, or leave at room temp for extended time. Neutralize with equimolar HCl to avoid extended degradation.

Oxidative degradation Sample Preparation

Weigh an appropriate amount of cefdinir (10 mg) Dissolve in methanol to make a concentration of 1 mg/mL, Add equal volume of 3% H_2O_2 solution to the cefdinir solution (Example: 5 mL cefdinir solution + 5 mL of 3% H_2O_2 , Incubate at room temperature (25–30°C) for 1 hour), Monitor the reaction at different time points 1h to track degradation progress, Use higher H_2O_2 concentrations (10%) or elevated temperature (60–70°C) to accelerate degradation if needed. After the desired time, quench the reaction by diluting with methanol or removing excess H_2O_2 by evaporation under nitrogen or using sodium metabisulfite (a reducing agent)

Photolytic Degradation

Sample Preparation: Dissolve cefdinir in methanol to prepare a 1 mg/mL solution. Place solution in a clear glass/quartz vial or spread solid powder thinly in a petri dish. Expose samples in a photostability chamber according to ICH Q1B: 1.2 million lux hours visible light, 200 watt hours/m² UV light , include a dark control (wrapped in aluminum foil) to distinguish between thermal and photolytic effects. Withdraw samples and analyze using UV-Vis spectrophotometer

Thermal Degradation

Sample Preparation: Solid: Place pure cefdinir in a petri dish or glass vial. Solution: Prepare a solution of cefdinir (e.g., 1 mg/mL in buffer or methanol-water). Exposure Conditions: Expose to 40°C, 60°C, or 80°C for varying durations (e.g., 1, 3, 7, 14 days). For dry heat, place in an oven; for solution, use a water bath. Ensure vials are tightly closed to prevent evaporation and contamination. Cool the sample to room temperature. Analyze by UV-Vis spectrophotometer

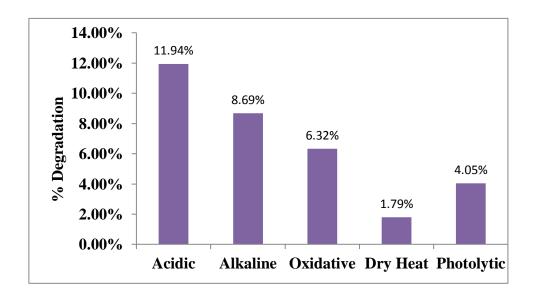


Fig 2: Bar Graph Indicating Stress Degradations

Isolation and Characterization of Acid-Induced Degradation Product via Flash Chromatography

The degradation product formed under acidic

hydrolysis conditions was successfully isolated using flash chromatography on a CombiFlash Companion system (Teledyne ISCO), employing a Gold 80 column. A mobile phase consisting of chloroform and methanol

(1:1, v/v) was utilized, with gradient elution applied after pre-adsorption of hydrolyzed sample onto silica gel. Six fractions were collected, among which Fraction 1 exhibited significant evidence of degradation and was selected for further characterization. Flash chromatography proved to be an efficient and cost-effective technique for isolating degradation products, commonly employed in pharmaceutical stability studies. The isolated degradant was thoroughly characterized using a range of analytical techniques. UV spectroscopy revealed a bathochromic shift in the absorption maximum (\lambda max) to 268 nm, indicating structural modification. HPLC analysis, performed with a water:methanol

CONCLUSION

The present study successfully developed and validated a robust, accurate, and economical analytical method for the detection and characterization of Cefdinir and its degradation products. The forced experiments revealed degradation Cefdinir is particularly susceptible to acid and base hydrolysis, while it exhibited varying stability under oxidative, photolytic, and thermal conditions. Among the degradation pathways, acid hydrolysis led to the formation of a major degradant, which was isolated using efficiently chromatography—a rapid and cost-effective technique. Comprehensive characterization of the isolated impurity was achieved through UV-Vis spectroscopy. HPLC. FT-IR. HR-MS. and NMR analysis, confirming significant structural modifications. This integrated approach not only highlights the utility of flash chromatography in impurity profiling but also reinforces the significance of forced degradation studies in understanding the stability behavior of pharmaceutical The validated method and compounds. findings are contribute expected to significantly to the quality control. formulation development, and regulatory assessment of Cefdinir and its formulations.

(80:20, v/v) mobile phase, confirmed the presence of a distinct degradant peak. FT-IR spectroscopy showed the emergence of hydroxyl (-OH) stretching bands and the disappearance of characteristic. absorption, suggesting functional group transformation. High-resolution mass spectrometry (HR-MS) provided confirmation of the molecular weight and fragmentation pattern, while ¹H and ¹³C NMR spectroscopy (in DMSO-d₆) delivered detailed structural insights through chemical shift analysis. Collectively, these findings validate the successful isolation and comprehensive structural elucidation of the acid-induced degradant, highlighting the critical role of flash chromatography in impurity profiling and degradation studies.

REFERENCE

- 1. Bari SB, Kadam BR, Jaiswal YS, Shirkhedkar AA. Impurity profile: significance in active pharmaceutical ingredient. Eurasian J Anal Chem. 2007 Mar 1;2(1):32-53.
- 2. Rawat T, Pandey IP. Forced degradation studies for drug substances and drug products-scientific and regulatory considerations. Journal of pharmaceutical Sciences and research. 2015 May 1;7(5):238.
- 3. Araujo P. Key aspects of analytical method validation and linearity evaluation. Journal of chromatography B. 2009 Aug 1;877(23):2224-34.
- 4. Ferenczi-Fodor K, Végh Z, Nagy-Turák A, Renger B, Zeller M. Validation and quality assurance of planar chromatographic procedures in pharmaceutical analysis. Journal of AOAC International. 2001 Jul 1;84(4):1265-76.
- 5. ICH I. Q2 (R1): Validation of analytical procedures: text and methodology. In International conference on harmonization, Geneva 2005 Nov 6 (Vol. 2005).

- 6. Kiprotich SL. An insight on International Conference of Harmonisation of technical requirements for registration of pharmaceutical products for human use (ICH) (Doctoral dissertation).
- 7. Solanki R. Impurity profiling of active pharmaceutical ingredients and finished drug products. Int. J. Drug Res. Technol. 2012;2:231-8.
- 8. Baertschi SW, Thatcher SR. Sample presentation for photostability studies: problems and solutions. in Pharmaceutical Photostability and Stabilization Technology 2006 Sep 18 (pp. 201-226). CRC Press.
- 9. Allwood MC, Plane JH. The wavelength-dependent degradation of vitamin A exposed to ultraviolet radiation. International journal of pharmaceutics. 1986 Jul 1;31(1-2):1-7.
- 10. Qiu F, Norwood DL. Identification of pharmaceutical impurities. Journal of liquid chromatography & related technologies. 2007 Feb 1;30(5-7):877-935.
- 11. Connors KA, Amidon GL, Stella VJ. Chemical stability of pharmaceuticals: a handbook for pharmacists. John Wiley & Sons; 1986;13, 233-238
- 12. Maggio RM, Calvo NL, Vignaduzzo SE, Kaufman TS. Pharmaceutical impurities and degradation products: Uses and applications of NMR techniques. Journal of pharmaceutical and biomedical analysis. 2014 Dec 1:101:102-22.
- 13. Attia KA, Nassar MW, Abou-Seada HM, Emara MS. Stability-indicating spectrophotometric methods for determination of cefdinir in pure form and pharmaceutical preparation. International Journal of Pharmaceutical Sciences and Research. 2014 Jun 1;5(6):2230.

An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print) ISS2249-9245 (Online)

13311. 2247-0041 (1 1111t) 1332247-7243 (O 1111t)



RESEARCH ARTICLE

RAPID AND SENSITIVE METHOD OF SUGAR DETECTION IN MARKETED SOFT DRINKS.

Vishnu Neharkar *, Munot Gautam and Snehal Tuse
Rasiklal M. Dhariwal Institute of Pharmaceutical Education & Research, Pune 411019.

*Corresponding author email: vsneharkar@gmail.com
Received: Feb 02, 2025 / Revised: April 13, 2025 / Accepted: May 09, 2025

ABSTRACT

Carbohydrates are found in most of our everyday diet; however, sugar analysis is difficult and inconvenient in food materials such as beverages, fruits and vegetables. Two simple, easy, and rapid methods were developed for the determination of glucose reducing sugar & total reducing sugar in various soft drink samples. A colorimetric method was developed to analyse the soft drink samples (non-diet and not dark coloured) and titrimetric method was developed to determine the total reducing sugars in soft drink sample. The various soft drink samples obtained from market were analysed for the glucose content in them using UV-Visible spectrophotometry and 3, 5 dinitro salicylic acid (DNSA) solution. The DNSA imparts colour to the sucrose standard and samples and later the absorbance values were checked at 580 nm. The calibration curve of the standard sucrose solution was plotted and the concentration of the samples were determined using the calibration curve. The purpose of this study is to estimate amount of glucose and reducing sugar in various soft drink samples and to study the adverse effect cause by various soft drinks on human body.

KEY WORDS: Glucose, Soft drinks, Colorimetric method, reducing sugars, UV Visible Spectroscopy.

Key words: Sertaconazole Nitrate, Chromatographic Method Development, Spectroscopic Analysis, Stability-Indicating Methods

How to cite this article: Vishnu Neharkar , Munot Gautam , Snehal Tuse Rapid And Sensitive Method Of Sugar Detection In Marketed Soft Drinks. Bulletin of Pharmaceutical Research. 2025;15(S2): **309-313**.

Source of support: Nil Conflict of interest: None

INTRODUCTION

Sugars are major sources of energy, for almost all living organisms. Plants produce sugars by photosynthesis process and convert them into different disaccharides such as sucrose, or convert them into starch for storage. The average human's healthy digestive system can only digest and

eliminate two to four teaspoons of sugar daily, usually without noticeable side-effects. [1] An excess of sugar in the diet results in weight gain, thereby increasing the risk of diabetes, heart disease, and high blood pressure, apart from dental caries. It is therefore important to know what

Unlocking Horizons in Global Multidisciplinary Healthcare Research" Indo-US International Conference Date: 10-12, March 2025 Organised by PES Modern college of Pharmacy Nigdi Pune-44

amount of sugar is present in food and beverages that are commonly consumed. [2] For the determination of reducing sugars like glucose, the sucrose present in the soft drink samples must be broken down into glucose. This is done by boiling it with hydrochloric acid which then reacts with DNSA which forms the basis for the proposed method developed. This method

is based on the colour which forms when sugar is reduced from 3, 5 dinitro salicylic acid (DNSA) to 3-amino-5-nitrosalicylic acid as shown in Figure 1. In addition, the sugar in soft drinks is highly concentrated to be used for this method unless it is diluted. The red-brown colour of the product is detected at 580 nm. [3]

1.1 Glucose: -

D-Glucose. the most abundant carbohydrate belongs class of to the carbohydrates called monosaccharides. Monosaccharides are carbohydrate molecules that cannot be broken down to simpler carbohydrate molecules by hydrolysis, so they are sometimes referred to as simple sugars. Glucose, also called dextrose, belongs to a group of carbohydrates known as monosaccharides. It is found in fruits and honey and is the major free sugar circulating in the blood of higher animals. [4] Glucose is also present in the diet as part of the disaccharides, sucrose (glucose and fructose), lactose (glucose and galactose), and maltose (glucose). It is the source of energy in cell function, and the regulation of its metabolism is of great importance (gluconeogenesis). The compound D-(+)-glucose or dextrose is 2, 3, 4, 6-pentahydroxyhexaldehyde, conventionally expressed as C6H12O6, with a molecular weight of 180.16 kDa. Glucose is readily soluble in water in a powder form. D-Glucose is both a polyalcohol and an aldehyde. It is classified as an aldose, a designation for sugars containing an aldehyde group. [5] The ending -Ose signifies a sugar and- ald signifies an aldehyde group.

Naturally occurring glucose is designated as the D form, specifically D-glucose. All sugars that have the hydroxyl group on the highest numbered chiral carbon atom (C-5 in case of glucose) positioned on the right-hand side are arbitrarily called D-sugars, and all with a lefthand positioned hydroxyl group on the highest numbered chiral carbon atom are designated as L sugars. [6] Two structures of D-glucose in its open-chain, acyclic form (called the Fischer projection) with the carbon atoms numbered in the conventional manner are given in Figure 2. D-Glucose and all other sugars containing six carbon atoms are called hexoses, the most common group of aldoses. [7]

Glucose is the primary carbohydrate energy source of vertebrates. In healthy humans, fasting blood glucose levels are approximately 3.5–5.5 mmol l-1 and increase postprandially to values considerably <10 mmol l-1 (the renal threshold for complete reabsorption, above which glucose 'spills' over into the urine). [8] Blood levels higher than 7.8 mmol l-1 2 h after a 75 g glucose load are one of the diagnostic criteria for diabetes. Glucose can also be synthesized de novo by gluconeogenesis from the gluconeogenic amino acids lactate, glycerol, and pyruvate.

Unlocking Horizons in Global Multidisciplinary Healthcare Research" Indo-US International Conference Date: 10-12, March 2025 Organised by PES Modern college of Pharmacy Nigdi Pune-44

Breakdown of glycogen into glucose is known as glycogenolysis. [9]

Glucose is present in fruits and vegetables; it is responsible together with fructose and sucrose for the sweet taste of vegetables and fruit. With the exception of fruit such as green banana, seeds (grain and dried legumes), and tubers, in which starch is the major carbohydrate form, foods containing glucose, fructose, and sucrose in various ratios comprise the major available (i.e., absorbable in the small intestine) carbohydrate sources.

1.2 Physical Properties of Glucose:

Glucose is one of the most important monosaccharides and plays a critical role in biological systems. It exhibits a variety of physical properties that reflect its structure and functional groups.

• Appearance:

Glucose is a colourless, sweet-tasting, crystalline solid. In its pure form, it appears as a white powder or crystal. [11]

• **Melting Point:** The melting point of anhydrous (dry) glucose is around **419 K (146°C)**. However, glucose often exists as a monohydrate in nature, which has a slightly lower melting point of **391 K (118°C)**. [14]

• Solubility:

Glucose is highly soluble in water due to the presence of multiple hydroxyl (-OH) groups that can form hydrogen bonds with water molecules. However, it is only sparingly soluble in organic solvents like alcohol and ether.

• Stability:

Glucose is relatively stable under normal conditions. However, when heated strongly or under acidic/basic conditions, it can undergo decomposition and various chemical reactions, such as caramelization or Maillard reactions. [13]

• Density:

The density of solid D-glucose is approximately **1.54 g/cm**³.

Polarity:

Due to the presence of multiple hydroxyl groups, glucose is a polar molecule, enhancing its solubility in polar solvents like water. [14]

MATERIAL AND METHODS: -

Determination of reducing sugar (GLUCOSE) in soft drink samples: -

• Reagents: -

Sucrose stock solution (1000 mg/dL), Soft drinks (non-diet, not dark-coloured), 6 M HCl solution, 2.5 M NaOH solution, 0.05 M 3, 5-dinitrosalicylic acid (DNSA) solution, distilled water.

• Apparatus: -

Volumetric flasks, Test tubes, pipette, beaker, analytical balance, stirring rod.

• Instrument: -

UV-Visible Spectrophotometer, Colorimeter.

Procudure: -

Five diluted sucrose stock solution as standard where prepared of concentration 200,400,600,800 and 1000ug/ml.

Preparation of stock solution: -

- Diluted soft drinks were taken as samples 2ml of each sucrose standard and samples were pipetted out into each different test tube. 2ml of distilled water was pipetted out into separate test tube taken as the blank solution.
- To each test tube, 2ml of 6M HCl solution was added and placed in boiling water for 10 minutes.

Preparation of 6M HCL solution: -

Measure of 1ml of concentrated HCL using a pipette. Carefully add it to a container containing 1ml of distilled water and then mix gently.

 Later to 8ml of 2.5M NaOH solution and 2ml of 0.05M DNSA solution were added and the test tubes were covered with parafilm and shaken well to mix. [15]

Preparation of 8ml of 2.5M of NaOH Solution: -

Calculate the amount of NaOH needed

Use the formula:

Moles = Molarity × Volume (in liters)

Molarity (M) = 2.5 mol/L

Volume = 8 mL = 0.008 L

Moles = $2.5 \times 0.008 = 0.02$ moles

Now convert moles to grams:

Mass (g) = moles \times molar mass of NaOH (≈ 40 g/mol)

 \rightarrow Mass = 0.02 × 40 = 0.8 g

• Weigh 0.8 g of NaOH

Use a digital balance to measure 0.8

grams of solid NaOH pellets or flakes.

• Dissolve in water

- Add a small volume (around 5-6 mL) of distilled water into a beaker.
- Slowly add the 0.8 g of NaOH while stirring until fully dissolved.
- Once dissolved, top up with more distilled water to reach a final volume of 8 ml.

Preparation 2ml of 0.05M of DNSA solution: -

Calculate DNSA mass needed-

Mass 0.05 mol/L*0.002L*228.11g/mol = 0.0228g

Weigh the DNSA

Using an analytical balance, weigh 22.8 mg of DNSA accurately.

Dissolve the DNSA

Transfer the DNSA into a small beaker or vial.

Add about 1.5 mL of distilled water.

Stir gently (or use a magnetic stirrer) until completely dissolved.

Adjust volume to 2 mL

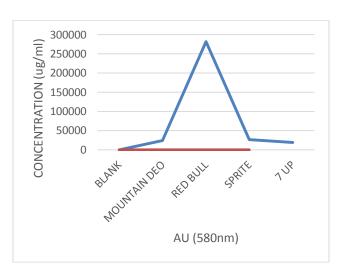
Transfer the solution to a 2 mL volumetric flask (or use a micropipette or graduated cylinder).

Make up the final volume to 2.00 mL with distilled water.

- The test tubes were placed in boiling water for 5 minutes followed by ice water for 10 minutes.
- The absorbance of the 5 standards1. CONCLUSION: were measured at 580nm using UV-Visible spectrophotometer.
- The sample were measured for the absorbance values at the same wavelength and the concentrations were calculated from the calibration curve of standard dilution.

RESULTS AND DISCUSSION: -

The concentrations of sugar in the unknown samples were acquired using the calibration curve shown in graph 1 and calibration curve of concentration of sugar in soft drink sample is shown in graph 2



Graph 2: - The Calibration curve of Concentrations of sugar in soft drink samples.

Table 2: - Concentrations of sugar in soft drink samples

NAME	AU (580nm)	CONCENTRATION (ug/ml)
BLANK	0	0
MOUNTAIN DEO	3.385	24251.26619
RED BULL	3.801	28232.11542
SPRITE	3.725	26782.64522
7 UP	2.791	18997.11739

The increase in the demand of soft drinks in the day to life which is been used as a refreshment and also been used as mocktail in various parties or marriage function. It is been drunk by people in hot summer days to feel refresh. Therefore, the determination of sugar in soft drinks was performed using the UV-Visible spectrophotometer using DNSA solution. The method proposed is easy, rapid and gives reliable results. Hence this method can be used as a method to check amount of glucose and reducing sugars in soft drink samples. This determination of sugar can help people to understand about which soft drink is good or bad for our body and till what extent it can be drunk so that it does not cause and adverse effect on our body.

The UV-visible spectrophotometer technique

is a rapid, sensitive, reliable and easy to use method. The results produced using this method are easy to study and understand. The purpose of this study is to estimate amount of glucose and reducing sugar in various soft drink samples and to study the adverse effect cause by various soft drinks on human body.

REFERENCES:-

- 1. J. Lozano-Sánchez, I. Borrás-Linares, A. Sass-Kiss, and A. Segura-Carretero, "Chromatographic Technique: High-Performance Liquid Chromatography (HPLC)," in Modern Techniques for Food Authentication, Elsevier, 2018, 459–526.
- 2. C. F. Poole, "Derivatization in liquid chromatography, in Liquid Chromatography, Elsevier, 2013, 25–56.
- 3. S. Uchiyama, Y. Inaba, and N. Kunugita, "Derivatization of carbonyl compounds with 2, 4-dinitrophenylhydrazine and their subsequent determination by highperformance liquid chromatography," J. Chromatogr. B, 2011, 89(17–18),1282–1289.
- 4. F. N. Lamari, R. Kuhn, and N. K. Karamanos, "Derivatization of carbohydrates for chromatographic, electrophoretic and mass spectrometric structure analysis," J. Chromatogr. B,2003, 793(1),15–36.
- 5. A. M. Shendurse and C. D. Khedkar, "Glucose: properties and analysis," Encycl. Food Heal., 2016, 3, 239–247.
- 6. S.-J. Janket, J. E. Manson, H. Sesso, J. E. Buring, and S. Liu, "A prospective study of sugar intake and risk of type 2 diabetes in women," Diabetes Care, 2003, 26 (4), 1008–1015.
- 7. F. Imamura Consumption of sugar sweetened beverages, artificially sweetened beverages, and fruit juice and incidence of type 2 diabetes: Systematic review, meta-analysis, and estimation of population attributable fraction," Br. J. Sports Med.,2016, 50(8), 496–504.

- 8. G. McKeown-Eyssen, "Epidemiology of colorectal cancer revisited: are serum triglycerides and/or plasma glucose associated with risk? Cancer Epidemiol. Prev. Biomarkers,1984, 3 (8), 687–695.
- 9. C. Nishida, R. Uauy, S. Kumanyika, and P. Shetty, "The joint WHO/FAO expert consultation on diet, nutrition and the prevention of chronic diseases: process, product and policy implications," Public Health Nutr., 2004, 7(1a), 245–250.
- 10. C. Lin, P.-T. Lai, S. K.-S. Liao, W.-T. Hung, W.-B. Yang, and J.-M. Fang, "Using molecular iodine in direct oxidative condensation of aldoses with diamines: an improved synthesis of aldo-benzimidazoles and aldo-naphthimidazoles for carbohydrate analysis," J. Org. Chem., 2008, 73(10), 3848–3853.
- 11. P. Ramasami, S. Jhaumeer-Laulloo, P. Rondeau, F. Cadet, H. Seepujak and A. Seeruttun. Quantification of Sugars in Soft Drinks and Fruit Juices by Density, Refractometry, Infrared Spectroscopy and Statistical Methods. S. Afr. J. Chem., 2004, 57, 24–27.
- 12. Swallow KW, Low NH. Determination of honey authenticity by anion-exchange liquid chromatography. Journal of AOAC International. 1994,1; 77(3), 695-702.
- 13. Low NH. Determination of fruit juice authenticity by capillary gas chromatography with flame ionization detection. Journal of AOAC International. 1996, 1; 79(3), 724-37.
- 14. Stuckel JG, Low NH. Maple syrup authenticity analysis by anion-exchange liquid chromatography with pulsed amperometric detection. Journal of Agricultural and Food Chemistry. 1995, 43(12), 3046-3051.
- 15. Henderson SK, Fenn CA, Domijan JD. Determination of sugar content in commercial beverages by density: A novel experiment for general chemistry courses. Journal of chemical education. 1998, 75(9), 1122.

An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print) ISS2249-9245 (Online)



RESEARCH ARTICLE

COMPARISON OF UV AND HPTLC ANALYSIS FOR ESTIMATION OF SERTACONAZOLE NITRATE

Shivani Morkhade^{1*} Sanika Baitule² and Ajay Baitule²

1.PES's Modern College of Pharmacy , Sec. No. 21 , Yamunanagar, Nigdi , Pune 411044.
2. Vidyabharati college of Pharmacy, Camp Amravati.Maharashtra
*Corresponding author email:shivanimorkhade6300@gmail.com
Received: Feb 02, 2025 / Revised: April 13, 2025 / Accepted: May 09, 2025

ABSTRACT

The study focuses on developing and validating innovative chromatographic and spectroscopic methods for quantifying Sertaconazole Nitrate. Techniques such as UV-Visible spectrophotometry, HPTLC were employed to evaluate stability and impurity profiling. HPTLC analysis utilized silica gel 60 F254 TLC plates with a mobile phase of toluene, ethyl acetate, and methanol (5:4:1 v/v), resulting in an Rf value of 0.42. The UV-Visible method showed good linearity between 4–24 μ g/mL, with a correlation coefficient of 0.998. The findings revealed significant susceptibility to oxidative and basic hydrolysis, while the drug displayed moderate stability under acidic conditions. All methods were validated for accuracy, precision, linearity, LOD, LOQ, robustness, and ruggedness, proving them suitable for quality control and ensuring the safety and efficacy of Sertaconazole Nitrate formulations.

Key words: Sertaconazole Nitrate, Chromatographic Method Development, Spectroscopic Analysis, Stability-Indicating Methods

How to cite this article: Shivani Morkhade Sanika Baitule and Ajay Baitule. Comparison of UV and HPTLC analysis for estimation of Sertaconazole nitrate. Bulletin of Pharmaceutical Research. 2025;15(S2): 314-318

Source of support: Nil Conflict of interest: None

INTRODUCTION

The standard of care for superficial fungal infections such tinea pedis, tinea corporis, and cutaneous candidiasis is sertaconazole nitrate. a broad-spectrum antifungal medication that belongs to the imidazole class. The main explanation for its antifungal action is that it inhibits the manufacture of ergosterol, which is a crucial part of fungal cell membranes and causes membrane breakdown and fungal cell death. It is an efficient and well-tolerated choice for treating fungal infections because, as a topical preparation, it provides localised treatment with less systemic absorption.[1] Sertaconazole nitrate is prone to the development of contaminants and degradation products throughout production, storage, and administration, just like any pharmaceutical formulation. Impurity profiling is an essential part of pharmaceutical quality control since the presence of such impurities can have a substantial effect on drug stability, decrease potency, and present possible toxicological hazards. Maintaining the drug's therapeutic efficacy requires making sure it stays stable, safe, and effective for the duration of its shelf life.[2] To guarantee the safety and quality of pharmaceutical goods, regulatory bodies like the European Medicines Agency (EMA), the United States Pharmacopoeia (USP). and the International Council Harmonisation (ICH) stress the importance of impurity analysis and stability Pharmaceutical development must include the identification. measurement. characterisation of impurities in accordance with ICH guidelines Q3A (R2) and Q3B (R2). Reduced medicine potency, possible toxicity, non-compliance with regulations, and a shorter shelf life might result from the presence of unknown or high quantities of contaminants.[3]

 $\textbf{Molecular Formula:} \ C_{20}H_{16}Cl_3N_3O_4S$

Chemical Name: 1-[2-[(7-chloro-1-benzothiophen-3-yl) methoxy]-2-(2,4 dichlorophenyl) ethyl] imidazole; nitric acid.

Melting point: 146°C-161°C **Category:** Anti-fungal agent

MATERIALS AND METHODS

spectrophotometry involved using methanol as a solvent, with absorbance measured at the maximum wavelength (λ max) of 270 nm. For HPTLC, silica gel 60 F254 TLC plates were used with a mobile phase of toluene, ethyl acetate, and methanol (5:4:1 v/v), followed by densitometric scanning at 270 nm.[4] All analytical methods systematically optimized and validated according to ICH guidelines to ensure accuracy, precision. robustness, and ruggedness, providing reliable and reproducible quantitative analysis.

UV-Visible Spectrophotometric Analysis Preparation of stock & sample solution

10 mg of SN bulk drug API were precisely weighed in a 10 ml volumetric flask and dissolved in 10 ml of methanol solution to create a stock solution of sertaconazole nitrate that yielded concentrations of 1000 μ g/ml or 1000 ppm. To achieve a 10 ppm concentration, 0.1 ml of the aforementioned solution was transferred into a 10 ml volumetric flask and diluted further by adding methanol to bring the volume up to volume. [5]

Fig. 1 : Chemical structure of Sertaconazole Nitrate

Determination of λ max

Sertaconazole Nitrate solution of 10 ppm was scanned under UV-Vis spectrophotometer in

the range 200-400 nm against methanol as blank and λ max was obtained at 235 nm. [6]

S pectrophotometric Conditions

Instrument: Shimadzu UV-1800

Solvent: Methanol

Wavelength of Maximum Absorption

(λmax): 270 nm

High-Performance Thin-Layer Chromatography (HPTLC) Analysis Preparation of standard solution:

The Sertaconazole Nitrate standard solution can be made by precisely weighing a certain amount of the reference standard and dissolving it in a suitable solvent, like methanol, to reach the required concentration. Then, using an automatic sample applicator in bandwise mode, the solution is applied to the Silica gel 60 F254 TLC plates. The plates are developed in a pre-saturated chamber using a mobile phase made up of Toluene:Ethyl acetate:Methanol in a ratio of 5:4:1 (v/v). Following development, the plates are dried and then subjected to densitometric scanning at 270 nm using a Camas TLC Scanner. [10]

Chromatographic Conditions

Stationary Phase: Silica gel 60 F254 TLC plates

Mobile Phase: Toluene:Ethyl

acetate:Methanol (5:4:1 v/v)

Application Mode: Automatic sample applicator in the band-wise mode

applicator in the ballu-wise mode

Densitometric Scanning: Performed at **270**

nm using a Camag TLC Scanner

Rf Value of Sertaconazole Nitrate: Approximately **0.42 ± 0.03**

RESULT AND DISCUSSION

Identification of drug

Table. 1: Solubility of drug in various solvents

Sr.	Name of	Solubility
No.	Solvent	
1.	Methanol	Freely soluble
2.	Distilled water	Insoluble
3.	Ethanol (96%)	Sparingly
		soluble

Table:2 Melting Point:

Sr.	Observed M. P.	Standard M.
No.		P.
1.	147°C	146°C-161°C

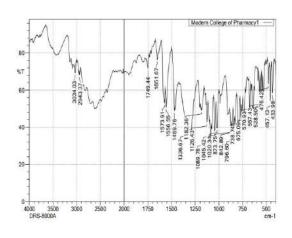


Fig. 2: FT-IR of Sertaconazole nitrate

Table: 3 FT-IR of Sertaconazole nitrate

Sr.no	Wavenumber (cm ⁻¹)	Functional Group
1.	3400-3200	N-H stretching (imidazole ring)
2.	3100-3000	Aromatic C-H stretching
3.	1700-1600	C=0 stretching (carbonyl group)
4.	1600-1500	C=C stretching (aromatic ring)
5.	1300-1200	C-N stretching
6.	1150-1050	S=0 stretching (sulfonamide group)
7.	900-800	C-Cl stretching (chlorine substitution)

The standard calibration curve was plotted as Absorbance Vs. Concentration after the absorbance was measured at 235 nm. Within the concentration range of 10–100 $\mu g/ml$, this straight-line exhibited linearity. It was discovered that the correlation was 0.998. The UV spectrophotometric method showed peak absorption at 270 nm with a linear regression equation of 0.048x + 0.037 and a correlation coefficient of 0.998. The method demonstrated good precision with percentage RSD values of 1.7% for intraday fluctuations and 1.005% for interday variations. At 0.039 $\mu g/mL$ and 0.120 $\mu g/mL$, respectively, the LOD and LOQ

showed sensitivity and suitability for routine analysis. [6]

HPTLC Analysis

The considerable accuracy and precision were also shown by the HPTLC method. The Rf value for sertaconazole nitrate was around 0.42 ± 0.03 and linearity was seen in the range of 100–600 ng/spot. Good repeatability was shown by the procedure's %RSD values, which were 0.713% intra-day and 0.763% inter-day. Experiments on ruggedness and robustness show that the method is stable under a range of conditions, with percentage RSD values falling within

acceptable boundaries.[8]

High-Performance Thin-Layer Chromatography (HPTLC) is a modern and sophisticated version of traditional thin-layer chromatography, designed for both qualitative and quantitative analysis of chemical substances. It provides enhanced sensitivity, accuracy, and reproducibility compared to

Table No. 4 : Summary of validation of Sertaconazole Nitrate bulk drug

Sr. No.	Validation	Sertaconazole
	Parameter	nitrate
1.	Linearity	31.043x-305.33
	Equation	
2.	Accuracy (%	97.05 – 99.89%
	mean	
	recovery)	
3.	LOD	0.354
4.	LOQ	1.073
5.	Precision (%	
	RSD)	0.713%
	Intra day	0.763%
	Inter day	
6.	Range	100 - 600
7.	Robustness	0.582

CONCLUSION

UV-Visible spectrophotometry was beneficial for quick quantitative analysis but had lower sensitivity compared to chromatographic methods. Innovative chromatographic and spectroscopic techniques for the accurate quantification of sertaconazole nitrate were successfully developed and confirmed by the investigation. With an Rf value approximately 0.42, the HPTLC method which uses silica gel plates and densitometric scanning—produced reliable results. With a correlation coefficient of 0.998, the UV-visible spectrophotometric approach demonstrated high linearity. All analytical methods underwent thorough validation in accordance with ICH requirements, evaluating robustness. attributes such ruggedness, precision. linearity. accuracy. and Sertaconazole Nitrate in bulk materials and pharmaceutical formulations may be reliably and consistently quantified using optimised procedures. The safe and efficient of sertaconazole nitrate in medical use

conventional methods.[11] HPTLC is especially useful for analyzing multiple samples simultaneously under the same conditions, making it both time-efficient and economical. Due to its straightforward procedure and effectiveness in handling complex mixtures, it is commonly employed in pharmaceutical, clinical, and environmental studies.[12]

therapies is supported by these test

REFERENCES

- 1. ICH, Stability testing of new drug substance and product.International conference of Hormonization, Geneva, (1993).
- 2. Desai NJ, Maheshwari DG. UV spectrophotometric method for the estimation of luliconazole in marketed formulation (lotion). Pharma Science Monitor. 2014 Apr 2;5(2):48-54.
- 3. Boonleang J, Tanthana C. Simultaneous stability-indicating HPLC method for the determination of cisapride, methylparaben and propylparaben in oral suspension. Songklanakarin Journal of Science & Technology. 2010 Jul 1;32(4).
- 4. Sonawane S, Gide P. Application of experimental design for the optimization of forced degradation and development of a validated stability-indicating LC method for luliconazole in bulk and cream formulation. Arabian Journal of Chemistry. 2016 Nov 1;9:S1428-34.
- 5. Tomal Majumder M, Roy P, Pramanik R, Hasan MN. Method development and validation of RP-HPLC method for estimation of luliconazole in marketed formulation (Cream). Pharma Innov. J. 2019;85:103-8.
- 6. Sharma T, Khurana RK, Borges B, Kaur R, Katare OP, Singh B. An HPTLC densitometric method for simultaneous quantification of sorafenib tosylate and chrysin: Analytical method development,

- validation and applications. Microchemical Journal. 2021 Mar 1;162:105821.
- 7. Shewiyo DH, Kaale EA, Risha PG, Dejaegher B, Smeyers-Verbeke J, Vander Heyden Y. HPTLC methods to assay active ingredients in pharmaceutical formulations: A review of the method development and validation steps. Journal of pharmaceutical and biomedical analysis. 2012 Jul 1;66:11-23.
- 8. Kaale E, Nyamweru BC, Manyanga V, Chambuso M, Layloff T. The development and validation of a Thin Layer Chromatography densitometry method for the analysis of diclofenac sodium tablets. International Journal of Chemical and Analytical Science. 2013 Jun 1;4(2):73-9.
- 9. Shah P, Patel J, Patel K, Gandhi T. Development and validation of an HPTLC method for the simultaneous estimation of Clonazepam and

- Paroxetine hydrochloride using a DOE approach. Journal of Taibah University for Science. 2017 Jan 1;11(1):121-32.
- 10. Attimarad M, Ahmed KM, Aldhubaib BE, Harsha S. High-performance thin layer chromatography: A powerful analytical technique in pharmaceutical drug discovery. Pharmaceutical 2011 Apr 1;2(2):71-5.
- 11. Kulkarni RN, Pandhare RB, Deshmukh VK, Mohite PB, Pawar AR. Highperformance thin layer chromatography: A powerful analytical technique in pharmaceutical drug discovery. Journal of Pharmaceutical and Biological Sciences. 2021;9(1):7.
- 12. Ivanova S, Stoynova H, Todorova V, Ivanov K. Development and validation of a simple thin-layer chromatography method for identification of diclofenac sodium and aceclofenac. 2023;2:12-21

Bulletin of Pharmaceutical Research 2025;15(S2)

An Official Publication of Association of Pharmacy Professionals

ISSN: 2249-6041 (Print) 2249-9245 (Online)

BPR

RESEARCH ARTICLE

COMPARATIVE EVALUATION OF GENERIC SUPPLIED AND BRANDED DRUGS OF METRONIDAZOLE TABLETS.

Gite Valmik^{1*} Namade Atharva².* and Bindurani L G P Ram³.

1. Adv. M. N. Deshmukh Arts, Science and Commerce College Rajur Tal Akole District Ahilyanagar 2.PES's Modern College of Pharmacy , Sec. No. 21 , Yamunanagar, Nigdi , Pune 411044.
3. SGMSPM's Dnyanvilas college of Pharmacy Dudulgaon Pune
*Corresponding author email: gitevalmik@gmail.com
Received: Feb 06, 2025 / Revised: April 18, 2025 / Accepted: May 24, 2025

ABSTRACT

Drugs should be promoted as safe and therapeutically active formulations with consistent and predictable performance. Therefore, quality is extremely important. Evaluation of the physical properties of a drug can ensure quality, bioavailability and optimal therapeutic efficacy. Metronidazole was chosen for this comparative test because it is widely used in the treatment of amoeba and other microbial diseases around the world. The aim of this study was to compare the quality of several brands of metronidazole tablets and to assess whether all brands follow IP guidelines. There are several brands and forms of metronidazole in the market, produced by various pharmaceutical companies under different product names. Two brands of 400 mg metronidazole filmcoated tablets commercially available in India were collected for this study, generics and brands were collected, and evaluation tests were performed using measurements of diameter and thickness, weight variation, hardness, storage time, resolution profile, and UV method analysis. The tests were performed using the methodology described in Pharmacopoeia. Based on this data, the Metronidazole 400 has the lowest weight variation. Of all brands, this brand had the lowest deposition time and the highest average hardness. The Brand Metronidazole 400 was the worst spring time. Using a UV spectroscopic approach, the resolution patterns and efficacy of metronidazole tablets in acidic (0.1 N HCl) medium at 278 nm were determined. All brands publish more than 85% of the drug in 60 minutes, showing an acceptable resolution profile. The Metronidazole 400 showed the maximum release of the drug at the required time interval between these brands. Quality functioning must be strictly maintained for improved therapeutic benefits and safe drug use.

Key words: Metronidazole, Evaluation Tests, Generic, Branded

How to cite this article: Gite Valmik, Namade Atharva. and Bindurani L G P Ram. Comparative Evaluation of Generic Supplied and Branded Drugs of Metronidazole Tablets. Bulletin of Pharmaceutical Research. 2025;15(S2): 319-324

Source of support: Nil Conflict of interest: None

INTRODUCTION

Generic Drugs: The FDA states, "Drugs comparable to branded products are property, use, quality and performance, dosage form, and disruption. This is a replica of a branded drug, whose patent has expired and there is no sole authority to produce and sell the drug. If protection is made for the patent period in

which the original product is executed, other producers may apply to the supervisory authority to sell a general copy of the original drug. The clinical benefit and the mode of action of generic drugs are the same as that of branded drugs. In other words, generic drugs can be used as substitutes for branded drugs. Generic drugs may have different appearances

Unlocking Horizons in Global Multidisciplinary Healthcare Research" Indo-US International Conference Date: 10-12, March 2025 Organised by PES Modern college of Pharmacy Nigdi Pune-44 and inactive ingredients, but they all contain the same active ingredients and similar functions as drugs with the name brand. Compared to branded medicines, common alternatives are often cheaper. This is due to the fact that the pharmaceutical business did not have to spend money on its development and funding. Almost every population segment can afford it as it can be up to 95% cheaper than branded drugs. They are cheap as many manufacturers compete for the general version and lower the post- patent price. Branded Drugs: For a certain period, it has the exclusive right to (patent) production and distribution. New drug applications are required that contain information on dosage production, chemistry, stability, efficacy, safety, packaging labelling and characteristics, ensuring the FDA approves branded drugs. Innovative companies can only sell this brand of medicine after FDA approval (about 20 years or as stated) for patent protection period (about 20 years or as mentioned). This is the most broadly available imidazole isomer in a single NITR group. According to the World Health Organization's main list of drugs, metronidazole is classified as antibacterial, anti-AAMIC, anti-protozoal, amoebicidal and anti-giardiasis drugs.[1] For the treatment of pylori induced peptic ulcers in Helicobacter, metronidazole is often used in combination with other antibiotics, bismuth connections, or proton pump inhibitors.[2] Infections caused by anaerobic bacteria, such as intraperitoneal infections, skin and skin structure, gynaecological infections, bacterial sepsis, bone and joint infections, central nervous system infections, inferior bronchial infections, inferior bronchial infections, and vaginal bacterial infections, and vaginal bacterial infections.[10,26] Metronidazole antibacterial and anti-protozoal drug used to treat infections caused primarily by anaerobic bacteria and protozoa. The aim of this study was to compare many metronidazole brands available in the market whether tablet sample brands maintain quality and receive a brief overview of brand quality parameters for comparative analysis. The effectiveness of doses of pharmaceutical forms is usually formulation dependent on their manufacturing process. Therefore, the quality of the dosage form may vary. Metronidazole

(C6H9N3O3) is a crystalline powder with a white or yellowish colour, with saline solution and bitter taste Koch salt solution, and is easily dissolved in water. In addition to other forms of dosage, metronidazole is usually available as a film-coated tablet.[7,13,24] Metronidazole was first developed during the mid-1950s by the Rhone-Poulenc laboratory in France and was later introduced to the market under the brand name Metronidazole. It entered commercial use in France by 1960. The U.S. Food and Drug Administration (FDA) approved metronidazole in 1963, and in 1962, its antibacterial properties were discovered. By 1966, it was found to be effective against Entamoeba histolytica, the protozoan responsible for conditions like amoebic dysentery and liver abscess.[6] Remarkably, it became the first drug to achieve nearly 100% cure rates when administered systemically. Despite this, its use against infections caused by anaerobic bacteria such as Clostridium and Bacteroides species did not become widespread until the 1970s. Metronidazole is well-distributed throughout body fluids and tissues. In the bloodstream, the majority of the drug exists in its unchanged form, while smaller quantities appear as metabolites. Protein binding in plasma is relatively low, with less than 20% of the drug attached to proteins. Drug quality can be evaluated using various criteria, including chemical tests for active ingredients and under standard impurities and conditions, as well as physical tests like visual inspection and invitro assays such disintegration and dissolution tests. In vivo bioavailability studies also play a key role.[9,24] For solid oral dosage forms like tablets, quality parameters include hardness, friability, and weight uniformity, with dissolution testing being a commonly used method toassess performance. The primary route of elimination for metronidazole and its metabolites are via the urine, accounting for approximately 60-80% of the administered dose, while 6–15% is excreted through the feces. The major urinary metabolites result from side-chain oxidation glucuronide conjugation. hydroxyethyl)-2-hydroxymethyl-5-

nitroimidazole and 2-methyl-5-nitroimidazole-1-ylacetic acid] with unchanged metronidazole comprising about 20% of the total. In healthy individuals, the average elimination half-life is

around eight hours. Anaerobic bacteria and susceptible protozoa can selectively absorb the unionized form of metronidazole, which is then reduced intracellularly to its active form. This activated form binds covalently to DNA, disrupting its helical structure and ultimately inhibiting nucleic acid synthesis, leading to the death of the microorganisms.^[16]

MATERIALS AND METHOD

Two different brands of metronidazole tablets (400 mg), with 20 tablets collected from each brand, were obtained one from a local government hospital and the other from a nearby pharmacy. Each sample was thoroughly

Diameter and Thickness

20 tablets from each batch were chosen and diameter and thickness were measured and diameter and thickness were measured separately with vernier calliper. Every value of reading was given in millimetres (mm). The mean diameter and thickness were calculated as well.

Weight Variation Test

The individual weights of 20 selected tablets of every brand were measured in milligrams using an electronic analytical balance, and the mean weight was found with the data. Next, the weight of each individual tablet was compared to the average.

A tablet is said to pass the test if not more than two tablets are above the percentage limits and no tablet varies more than twice the percentage limit.

Hardness Test

Hardness tends to measure the strength of crushing of the tablet. 06 tablets from each firm were utilized for measuring the hardness of the tablet. Hardness was measured utilizing the Monsanto Hardness Tester. The tablet is inserted in the gap and the force is subsequently applied utilizing the screw mechanism until the tablet gets crushed. Mean hardness was determined.

Friability

Test

10 tablets from both the brands each were taken and weighed then using Roche friabilator they were subjected to rotate. Roche friabilator. The Roche friabilator is %Drug Release

$$= \frac{Cumulative\ amount\ release(mg)}{Strength(mg)} \times 100$$

examined for visual appearance, manufacturer details, manufacturing and expiry dates, license number and batch number.

Identification of Sample

Every brand contains 400 mg of metronidazole per tablet and had a two to three-years of shelf life. Every tablet was found in good condition, either in a blister or a strip packaging. At the beginning of the research project, the size, shape, and colour of various branded tablets were visually inspected. Table 1 displays the label information for nine both the brands of 400 mg metronidazole tablets.

made out of a plastic chamber that rotates at 25 rpm. The tablets are dropped six inches into the chamber, which rotates 100 times. The expectable range of the tablet is 0.5 – 1.0%. Again, the weight of tablets was taken. The expectable range of the tablet is 0.5 – 1.0%.

Formula for Friability:

Percentage weight loss = $\left(\frac{Initial\ Weight-Final\ Weight}{Initial\ Weight}\right) \times 100$

Disintegration Test

Using a tablet disintegration test apparatus, 06 tablets of each brand were used for the disintegration test in distilled water at 37 °C. The Disintegration time was noted and this is time to completely disintegrate the tablet.

Dissolution Test

With the help of USP Type II dissolution test apparatus (Lab India DS 8000), dissolution test was conducted on three randomly selected tablets. 900 ml of 0.1 N HCl solution was utilized as dissolution

medium. Temperature was kept constant at 37 ± 0.5 °C. Rotation was kept at 100 RPM. Sample was taken at 5 min, 15 min, 30 min, 45 min, and 60 min. And diluted with 0.1 N HCl. Absorbance was recorded at 278 nm. Concentrations of the sample at the above specified times were computed by utilizing the y = mx + c equation derived from API standard curve. The formula as follows was employed to calculate drug release percentage and cumulative amount release from these values.

RESULT AND DISCUSSION

The die and punches used to create the tablets determine their thickness and diameter. It is possible to prepare tablets with different thicknesses and diameters without changing their weights. Differences in granule density, compression pressure, and compression speed are typically the

Weight Variation Test

weight variation test aims to ensure adequate tablet size, uniform formulation content, and good manufacturing practice (GMP).^[29] The Indian Pharmacopeia gives the criteria for the weight deviation for the tablets.

Therefore, the given tablets weight is more than 250 mg hence, weight variation must be within \pm 5%. For Metronidazole 400 Weight of tablet + 5% = 488.5 + 5% = 512.93 mg Weight of tablet - 5% = 488.5 - 5% = 464.07 mg For Metronidazole 400 Weight of tablet + 5% = 540 + 5% = 567 mg

Weight of tablet – 5% = 540 - 5% = 513 mg Both the tablets' weights are within the deviation range of \pm 5% and the test is passed as no 2 or more tablets lies outside the weight limit.

Hardness test The pharmaceutical market employs a tablet hardness test to identify a tablet's breaking point and structural soundness under storage, handling, and transit conditions before use.[16] Materials the amount of binder, compression distance between upper punches and lower punches, and pressure applied in the compression all influence hardness of the tablet. Several tablet attributes, including dissolving, friability, disintegration. affected and are by Decreased hardness. dissolving and increased

Friability Test

For Metronidazole 400 Initial weight = 5.09 gm Final weight = 5.05 gm % Weight loss = $\frac{5.09 - 5.05}{5.09} \times 100$ =0.785% For Metronidazole 400 Initial weight = 5.63 gm

of variation in diameter causes thickness. From the above result Metronidazole 400 tablet has the highest diameter average i.e., 12.03 mm and Metronidazole 400 tablet has lowest diameter average i.e., 11.1825 mm. It was found that Metronidazole 400 tablet has highest thickness average i.e., 5.1025 mm and the Metronidazole 400 tablet has lowest thickness average i.e., 4.5335 mm.

Final weight = 5.57 gm % Weight loss = $\frac{5.63 - 5.57}{5.63} \times 100$ =1.06%

It is a method used to evaluate the physical power of compacted pills once exposed to mechanical impact and abrasion is termed friability testing. The friability test reflects the degree of mechanical stress that pills can withstand during manufacture, distribution, and customer handling.[24] Based on IP the tablet friability should not be more than 1%.The tablet as well as the tablets' friability was discovered within the range. The friability of Metronidazole 400 and Metronidazole 400 was found to be 0785% and 1.06% respectively.

Disintegration Test

Disintegration time for Metronidazole 400 16.34 min Disintegration time for 400 Metronidazole = 18.15 mintest disintegration is conducted calculate the time taken by tablet to dissolve. The disintegration test is crucial for all the tablets as the drug's dissolution rate is dependent on the time disintegration, which the absorption rate and subsequent bioavailability of drug. As per USP the average disintegration rate for film coated metronidazole tablets must be within 30 min. Both the tablets pass the test with the disintegration time of 16.34 min and 18.15 min respectively.

disintegration time may be the results of high values of hardness. Opposite to this, low value of hardness may be caused by high value of friability. Average hardness of Metronidazole 400 was determined as 4.366 while the average of Metronidazole 400 was determined as 5.083.

Dissolution test

Dissolution is the method through which the drug is being transferred into GI fluid solution as a dissolved state by its solid dosage. Rate and extent of released drug from any form of dose are quantified by a dissolution study. The percentage released of drug at a specific time is usually given by the test. Dissolution tests are applied to determine the variables affecting a drug's bioavailability. At least 85% of the dose given of film-coated metronidazole tablets must be released in 60 minutes.

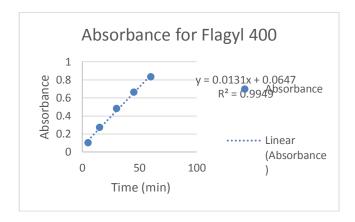


Fig 1 : Calibration curve for Metronidazole 400 tablet

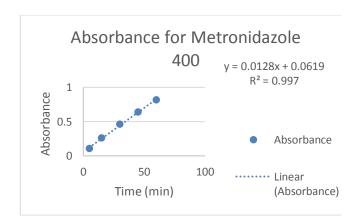


Fig 2 : Calibration curve for Metronidazole 400 tablet

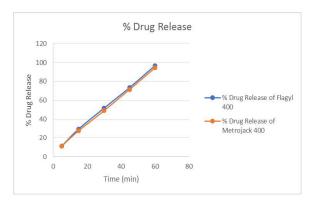


Fig 3 : Comparative % drug release of Metronidazole 400 mg tablet

The % drug release of Metronidazole 400 tablet after 60 minutes was found to be 96.75%. And the % drug release of Metronidazole 400 tablet after 60 minutes was found to be 94.29%. Although the rate of this varied brand by brand, a study on the drug release profile of each brand within brand indicated that increasing time was correlated with increasing drug release. Even though the rate of dissolution varied between brands, an intra-brand comparison of the drug release profiles of all the brands revealed that the drug release increased with the progression of time. Even though they were manufactured different brands in different proportions of excipients, all the brands possessed good dissolving profiles.

CONCLUSION

The aim was to study two different brands of metronidazole marketed tablets that are sold in India. Metronidazole has a low solubility in water. Achieving the required bioavailability is quite challenging because of its low solubility. A patient's life depends on the medication they take; therefore, it must be both safe and effective. Thus, we conducted a number of formal and informal in vitro investigations, including testing for potency. disintegration, hardness, friability, and weight variation. Our investigation shows that practically all metronidazole brands meet all pharmacopoeia requirements, indicating good quality and efficacy. These tests of quality control are necessary for both bioavailability and therapeutic efficacy. These studies should be carried out more regularly because they are very beneficial to the advancement of the pharmaceutical industry as well as to raise public knowledge of the caliber marketed pharmaceutical products.

REFERENCE

 Sadia Noor, Fayad Bin Abdus Salam, Habibun Nahar Hima, Md. Shahidul Islam Bhuiyan and Shaila Chowdhury.

Unlocking Horizons in Global Multidisciplinary Healthcare Research" Indo-US International Conference Date: 10-12, March 2025 Organised by PES Modern college of Pharmacy Nigdi Pune-44

- Comparative in-vitro quality evaluation of some brands of metronidazole tablet available in Bangladesh. International Journal of Applied Research 2017; 3(7): 753-758.
- 2. Allen LV, Popovich NG, Ansel HC. Ansel's pharmaceutical dosage forms and drug delivery systems. Edition. 8th, Lippincott Williams & Wilkins, Philadelphia, 2011, 225-256.
- 3. Ankit G, Ajay B, Kumar KM, Neetuk. Tabtet coating techniques: Concepts and recent trends. International Research Journal of Pharmacy. 2012; 3(9):50-58
- 4. Amin AA, Kokwaro GO. Antimalarial drug quality in Africa. Journal of Clinical Pharmacy and Therapeutics. 2007; 32(5):429-440.
- 5. Asif F, Sultana T, Sohel MD, Uddin MH, Kawsar MH, Islam MA. In vitro dissolution pattern of metronidazole film coated tablet in presence of fruit juice. American Journal of Pharmacological Sciences. 2014; 2(2):32-36.
- 6. Banker GS, Anderson NR. The Theory and Practice of Industrial Pharmacy. Lachman L, Lieberman HA, Kanig JL, Eds., Philadelphia, USA: Varghese Publishing house, 2011, 293-345.
- 7. Basit A, Riaz M, Fawwad A. Metronidazole: Evidence based facts, trends, and observations. Vascular Health and Risk Management. 2012; 8:463-472.
- 8. Choudhary N, Bajpai M. Formulation, characterization and in-vitro evaluation of cyclodextrin complexed or dispersible tablets of metronidazole. International Journal of Research in Pharmaceutical Sciences. 2011; 2(4): 512-521.

- 9. Chowdary KPR, Krishna MT. Quality evaluation of market sample of diclofenac SR products. The Eastern Pharmacist. 2001; 111-113.
- 10. Cudmore SL, Delgaty KL, Hayward-McClelland SF, Petrin DP, Garber GE. Treatment of infections caused by metronidazole-resistant Trichomonas vaginalis. Clinical Microbiology Review. 2004; 17(4):783-793.
- 11. Du J, Hoag SW. Characterization of excipient and tableting factors that influence folic acid dissolution, friability, and breaking strength of oil- and watersoluble multivitamin with mineral tablets. Drug Development and Industrial Pharmacy. 2003; 29:1134- 1147.
- 12. Induri M, Raju MB, Prasad YR, Reddy KP. Development and validation of a spectrophotometric method for quantification and dissolution studies of metronidazole in tablets. E-Journal of Chemistry. 2012; 9(2):993-998.
- 13. Kishore L, Kaur N. Estimation of pioglitazone and metronidazole in its harmaceutical dosage form by spectrophotometric methods. Der Pharmacia Lettre. 2011; 3(4):276-284.
- 14. Lau AH, Lam NP, Piscitelli SC, Wilkes L, Danziger LH. Clinical pharmokinetics of metronidazole and other nitroimidazole anti-infectives. International Journal of Pharmacy and Pharmaceutical Sciences. 1992; 23(5):328-364.
- 15. Pisek R, Korselj V, Vrecer F. Comparison of direct rotor pelletization (fluid bed) and high shear pelletization method for pellet production. Pharm and Biopharm. 2002; 53:327-333.