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RESEARCH PAPER

FORMULATION AND EVALUATION OF ANTIFUNGAL CREAM USING NELUMBO NUCIFERA AND AZADIRACHTA INDICA LEAVES EXTRACTS

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The main aim of this investigation was to evaluate the antifungal activity of leaves of *Nelumbo nucifera* (Nelumbonacaea) and *Azadirachta indica* (Meliacaea). Study involved qualitative estimation of the methanolic and ethanolic extracts of *N. nucifera* and *A. indica*. The results showed good antifungal activity against *Candida albicans*. Further, it was found that F1 formulation has better antifungal action against *C. albicans* in comparison to F2 formulation. F1 Formulation has given equivalent antifungal effect in comparison to standard formulation. It was revealed that both plant leaves showed significant antifungal activity against *C. albicans* and may be used as an antifungal agent in the form of cream formulation.

Key words: *Nelumbo nucifera*, *Azadirachta indica*, *Candida albicans*, Antifungal activity, Cream.

INTRODUCTION

Fungal infections are common infection in the natural world. In humans, fungal infections occur when a fungus takes over an area of the body and it is difficult to handle by immune system. There are many fungi like helpful fungi and harmful fungi, harmful fungi invade the body, they can be difficult to kill and re-infect the person trying to get better and they can survive in the environment. Fungal infections are common in humans and usually not a serious issue if they are treated quickly and correctly. With a weakened immune system, anyone can be more likely to contact a fungal infection, as well as anyone who is taking antibiotics. Cancer treatment and diabetes may also make a person more suffer to fungal infections (Johnson, 2018). Literature is enriched with several reports which prove plants as a potential source of bioactive secondary metabolites (Shrestha *et al* 2016; Dahiya *et al* 2019; 2017; Dahiya and Singh, 2017a; 2017b; Viana *et al* 2017; Senthil Kumar *et al* 2017). *Azadirachta indica*, commonly known as neem and nimtree, belongs to the family Meliaceae (**Figure 1a**). In the genus Azadirachta, it is one of two species and easily available all over the world like India, Nepal, Maldives, Sri Lanka, Bangladesh etc. *A. indica* is easily grown in semi-tropical and tropical regions and shows many activities like antifungal, antiviral, antibacterial, contraceptive, antiproliferative, antioxidant etc. Its constituents are applied in alternative Ayurveda, Unani, Homeopathy and modern medicinal system. *e.g.* for the treatment of infections, cancer diseases etc. So, evergreen neem shows many activities and medicinal use (Kausik *et al* 2002). *Nelumbo nucifera*, also known as Indian lotus, sacred lotus and American lotus, is an aquatic plant belongs to family Nelumbonaceae (**Figure 1b**). Reomerine is a main chemical constituent present in the leaves of *N. nucifera* and

(a) (b)

Fig. 1. (a) *Azadirachta indica* (neem) (b) *Nelumbo nucifera* (lotus)

responsible for antifungal activity of leaf of this plant. More than 400 years before, it has been recorded in the most famous medicinal book in china. It shows many bioactivities including antioxidant, anticancer and antimicrobial. Leaves, seeds, flower, rhizome and other part of this plant are useful in traditional system of medicine (Shen-Miller *et al* 2002).

Fungal infections are common among all people of all age groups and development of new natural and safe therapeutic antifungal topical preparation is the plan of our study. The aim of the present study was to investigate the antifungal activity of methanolic and ethanolic extract of *Nelumbo nucifera* and *Azadirachta indica* and to formulate a natural, safe antifungal cream containing the combination of both extract and to evaluate its physicochemical properties (Agnihotri *et al* 2008).

MATERIALS AND METHODS

Materials

Excipients

Modern Institute of Pharmaceutical Sciences, Indore provided all excipients including Liquid paraffin, Stearic acid, Bees wax, Stearyl alcohol, Glycerin, Tween-80, Methyl parabens, Sorbitol solution, Potassium hydroxide. All reagents and chemicals that were used in the study were of analytical quality.

Fungal species

Candida albicans was obtained from the Department of Microbiology, Modern Laboratory Pvt. Ltd, Sanwer Road, Indore, Madhya Pradesh, India

Nutrient media

Nutrient agar (Hi-media) and Nutrient broth (Hi-Media) was obtained from the Department of Microbiology, Modern Laboratory Private Limited, Sanwer Road, Indore, Madhya Pradesh.

Collection of plant material

Leaves of *N. nucifera* and *A. indica* were collected from Modern Institute of Pharmaceutical Sciences, College Campus, Indore, Madhya Pradesh, India. Authentication of the plant was done and Voucher specimens were deposited at the Pharmacognosy Department, Modern Institute of Pharmaceutical Sciences, College Campus, Indore, Madhya Pradesh.

Extract preparation

Preparation of ethanolic extract of plant A. indica

Fresh *A. indica* A. Juss (neem) leaves were shade-dried for few days at room temperature and powdered with a grinder. Dried powder of *A. indica* leaves was mixed with 70% ethyl alcohol and kept at room temperature for 36 hr. The slurry was stirred intermittently for 2 hr and left overnight using mechanical stirrer. The mixture was then filtered and the filtrate was concentrated using water bath at 50°C and finally dried to form the extract which is kept for phytochemical screening (Chattopadhyay, 1998).

Preparation of methanolic extract of plant N. nucifera

The collected leaves were thoroughly washed and dried in hot air oven at 20° C after washing. 50 g of the powder was macerated in 150 ml of methanol for 20 mins. The prepared suspension was stirred using a magnetic stirrer for 3 hr. Then, the methanolic suspension of powder leaves of *N. nucifera* was kept in standing for 2 days and filtered. The filtrate was kept on water bath at 40° C for evaporation to form the extract which is kept for phytochemical screening (Lee *et al* 2015).

Methods

Formulation development

Formulation of the herbal antifungal cream

The formulation trails were done as per formula given in the **Table 1**. The formulation containing *N. nucifera* and *A. indica* was formulated by the following method: Different amount of ingredients were incorporated together in 2 phase *i.e.* oil phase and aqueous phase separately. The oil phase consists of liquid paraffin, bees wax, stearyl alcohol, tween-80 and stearic acid while the aqueous phase was composed of methyl paraben, sorbitol solution and potassium hydroxide. Both aqueous and oil phases were heated to 75 °C on a water bath separately. The aqueous phase was then added drop wise to the oil phase with continuous stirring and finally the herbal extracts of *N. nucifera* and *A. indica* were incorporated in the emulsion. Gradually temperature was decreased with continuous stirring and emulsion was formed which was then stored in the air tight wide-mouth container.

Table 1. Formula for preparation of antifungal cream

	Ingredients	F1 Formulation	F2 Formulation	Uses
1	*Nelumbo Nucifera*	2.5 gm	2 gm	A.P.I.
2	*Azadirachta Indica*	2.5 gm	2 gm	A.P.I.
3	Liquid paraffin	2.5 ml	2.5 ml	Softening agent
4	Stearic acid	1.5 gm	1.5 gm	Cleansing Property
5	Bees wax	2.5 gm	2.5 gm	Hydrating agent
6	Stearyl alcohol	5 gm	5 gm	Emollient
7	Glycerin	10 ml	10 ml	Humectant
8	Tween-80	4 ml	4 ml	Solubilizer
9	Methyl parabens	0.6 gm	0.6 gm	Preservative
10	Sorbitol solution	3 gm	3 gm	Moisturizing
11	Potassium hydroxide	2.5 gm	2.5 gm	pH Adjuster
12	Distilled water	16.5 ml	16.5 ml	Diluent

Evaluation (Chen *et al* 2016; Pal *et al* 2013)

Evaluation of extract (Phytochemical screening)

1) Test for alkaloids (Dragendroffs test)

0.1 ml of extract solution + 1 drop of Dragendroffs reagent.

Inference: Orange ppt. shows presence of alkaloid.

2) Test for glycosides (*Legal's test)*

Extract dissolved in pyridine **+** sodium nitroprusside solution and solution was made alkaline.

Inference: Red or pink colour shows presence of glycoside.

3) Test for flavonoids (Shinoda test)

Extract + pinch of magnesium + Conc. HCl.

Inference: pink color shows presence of flavonoids.

Evaluation of formulation

pH measurement

The determination of pH was done by using digital pH meter. Dissolved one gram cream in 100 ml of distilled water of each formulation (cream *i.e.* 1% of aqueous solution) and stored for two hrs. The pH measurement of each formulation was done three times and average was calculated.

Homogeneity

All formulation produces uniform distribution on skin and this was confirmed by visual appearance and by touch.

Consistency

Consistency was estimated by visual detection.

Washability

The ease and extent washing of formulation with water were checked manually after formulations were applied on the skin.

Spreadability

Two sets of the glass slides were taken. The cream was placed over one of the slides and other slide was placed on the top of the cream, such that the cream was sandwiched between the two slides in area occupied by a distance of 6.0 cm along the slide. 100 gm weight was placed upon the upper slide so that cream between the two slides was pressed uniformly to form thin layer. Position of two slides were fixed to a stand without slightest disturbance and in such a way that only the upper slide to slip off freely by the force of weight tied to it. Carefully, 20 gm of

weight was tied to the upper slide. The time taken of upper slide to travel the distance of 6.0 cm and separated away from the lower slide under the influence of the weight was noted. This experiment was repeated three times and the mean time taken was calculated. Spreadability was calculated by using the formula:

$S = M * L / T$

where, S = spreadability; M = weight tied to the upper slide, L = length moved on the glass slide, T = time (in sec) taken to separate the slide.

Antifungal testing

Fungal species

The test organisms (*Candida albicans)* were further subcultured at 37°C for 24 hrs. The fungus cultures were maintained in their appropriated agar slant at 4°C throughout the study and used as stock cultures.

Nutrient Media Used

Nutrient agar and Nutrient broth (Hi-Media)

Preparation of plates

Two plates were sterilized in hot air oven at 160°C for 2 hrs. Out of these, two Petridish were used for preparation of plates using nutrient agar as a media and other four being used for preparation of plate using nutrient agar as a media for well diffusion assay. The antibacterial activity was evaluated by the zone of inhibition (in mm).

RESULTS AND DISCUSSION

Evaluation of extract (Phytochemical screening)

Phytochemical screening of methanolic extract showed presence of alkaloid, glycoside and flavanoid.

Table 2. Phytochemical screening of *N. nucifera* and *A. indica* extract

S. No.	Name of test	Result of extract	
		Nelumbo nucifera	*Azadirachta indica*
1	Alkaloid	+++	+++
2	Flavonoid	+++	+++
3	Glycoside	+++	+++

Evaluation of Formulation

The formulated cream was evaluated using various physicochemical parameters. The pH of the formulation ranged from 6.40 to 6.56, which lie in the normal pH range of the human skin. Spreadability values showed that formulation spread with an ease and the type of smear is non greasy. In stability studies, creams showed no changes in viscosity, pH, spreadability and consistency.

Table 3. Physical evaluation of cream

S. No.	TEST	F1	F2
1	pH	6.57	6.49
2	Colour	Creamy Green	Creamy Green
3	Consistency	Semi-solid	Semi-solid
4	Homogeneity	Homogenous	Homogenous
5	Washability	Good	Good
6	Spreadability	20.16	19.06

Antifungal testing

The results of antifungal activity revealed that the formulation containing methanolic extract of *Nelumbo nucifera* and ethanolic extract of *Azadirachta indica* leaves exhibited significant antifungal activity.

Both the standard sample and test sample were compared on the antifungal testing. The result showed good antifungal activity of formulated cream (**Table 4**). It was found that F1 formulation (containing 2.5 gm extract of *Nelumbo nucifera* and *Azadirachta indica)* (**Figure 2a, 2b**) has better antifungal action against *C. albicans* in comparision with F2 formulation (containing 2.0 gm extract of *N. nucifera* and *A. indica)*.

Table 4. Antifungal testing

Zone of inhibition (mm)	Standard (Griseofulvin)	F1 Formulation	F2 Formulation
	9.6 ± 0.5	9.3 ± 0.3	8.6 ± 0.3

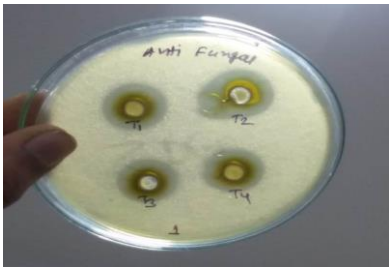

Fig. 2a. F1 Formulation

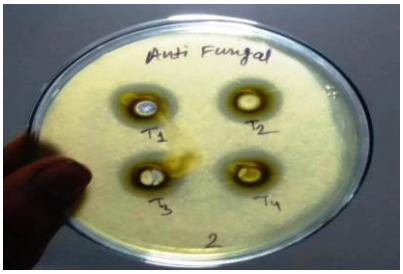

Fig. 2b. F2 Formulation

CONCLUSION

From the overall results, it was concluded that the topical formulation containing ethanolic extract of *Azadirachta indica* and methanolic extract *Nelumbo nucifera* possess significant antifungal activity and it can be used as an herbal product in the treatment of fungal infection. The preliminary phytochemical screening showed the presence of alkaloid, glycoside and flavonoid in the extract of *Azadirachta indica* and *Nelumbo nucifera* and it was present in its leaves and other chemical constituents like which might be in part responsible for antifungal effect against *Candida albicans*. It was found that F1 formulation has better antifungal action against *Candida albicans* in comparision with F2 formulation. Further, F1 formulation has showed equivalent antifungal action as standard formulation containing 2.5 gm of Griseofulvin.

REFERENCES

Agnihotri VK, ElSohly HN, Khan SI, Jacob MR, Joshi VC, Smillie T, Khan IA, Walker LA. Constituents of *Nelumbo nucifera* leaves and their antimalarial and antifungal activity. *Phytochem. Lett.* 2008;1(2):89-93.

Chattopadhyay RR. Possible biochemical mode of antiinflammatory action of *Azadirachta indica* A. Juss. in rats. *Indian J. Exp. Biol.* 1998;36(4):418-20.

Chen MX, Alexander KS, Baki G. Formulation and evaluation of antibacterial creams and gels containing metal ions for topical application. *J. Pharm. (Cairo)* 2016;2016:5754349.

Dahiya R, Singh S. Synthesis, characterization and biological screening of diandrine A. *Acta Pol. Pharm.* 2017a; 74(3):873-80.

Dahiya R, Singh S. Synthesis, characterization, and biological activity studies on fanlizhicyclopeptide A. *Iran. J. Pharm. Res.* 2017b;16(3):1176-84.

Dahiya R, Singh S, Kaur K, Kaur R. Total synthesis of a natural cyclooligopeptide from fruits of sugar-apples. *Bull. Pharm. Res.* 2017;7(3):151. [DOI: 10.21276/bpr.2017.7.3.4]

Dahiya R, Singh S, Varghese Gupta S, Sutariya VB, Bhatia D, Mourya R, Chennupati SV, Sharma A. First total synthesis and pharmacological potential of a plant based hexacyclopeptide. *Iran. J. Pharm. Res.* 2019;18(2):938-47.

Johnson J. What you need to know about fungal infections. *Medical News Today* 2018.

Kausik B, Chattopadhyay I, Banerjee RK, Bandyopadhyay U. Biological activities and medicinal properties of Neem (*Azadirachta indica*). *Curr. Sci.* 2002;82(11):1336-45.

Lee JS, Shukla S, Kim JA, Kim M. Anti-angiogenic effect of Nelumbo nucifera leaf extracts in human umbilical vein endothelial cells with antioxidant potential. *PLoS One* 2015;10(2):e0118552. [DOI: 10.1371/journal.pone.0118552]

Pal TK, Dutta D, Banerjee R, Maity S. Formulation and evaluation of antimicrobial topical semisolid dosage form containing whole plant extract of *Biophytum sensitivum*. *J. Pharm. Res.* 2013;1(7):641-6.

Senthil Kumar R, Vinoth Kumar S, Sudhakar P. Anticancer activity of methanolic leaf extract of Morinda tinctoria roxb. against ehrlich ascites carcinoma in mice. *Bull. Pharm. Res.* 2017;7(2):146. [DOI: 10.21276/bpr.2017.7.2.4]

Shen-Miller J, Schopf JW, Harbottle G, Cao RJ, Ouyang S, Zhou KS, Southon JR, Liu GH. Long-living lotus: Germination and soil-irradiation of centuries-old fruits, and cultivation, growth, and phenotypic abnormalities of offspring. *Am. J. Bot.* 2002;89(2):236-47. [DOI: 10.3732/ajb.89.2.236]

Shrestha DK, Sapkota H, Baidya P, Basnet S. Antioxidant and antibacterial activities of Allium sativum and Allium cepa. *Bull. Pharm. Res.* 2016;6(2):50-5. [DOI: 10.21276/bpr.2016.6.2.3]

Viana CB, Carbonezi LH, Martins RCC. Isolation of pentacyclic triterpenes from Simira sampaioana (standl.) steyerm (rubiaceae) as possible anticancer agents. *Bull. Pharm. Res.* 2017;7(1):142. [DOI: 10.21276/bpr.2017.7.1.5]

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