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RESEARCH PAPER

EVALUATION OF ANTI-ULCEROGENIC PROPERTIES OF ETHANOLIC EXTRACT OF *HIBISCUS SABDARIFFA* (EEHS) IN GASTRIC ULCER MODELS

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***Hibiscus sabdariffa* L. (Roselle) is widely recognized for its therapeutic benefits. This study assessed the anti-ulcerogenic properties of an ethanolic extract from its dried calyces (EEHS) in various gastric ulcer models in Wistar albino rats. Oral administration of EEHS at doses of 250 and 500 mg/kg body weight significantly mitigated gastric mucosal damage induced by cold restraint stress, pylorus ligation, necrotizing agents (80% ethanol, 0.2 M NaOH, and 25% NaCl), and indomethacin. EEHS notably reduced basal gastric acid secretion while enhancing gastric wall mucus (GWM) secretion and non-protein sulfhydryl (NP-SH) concentrations in gastric tissues. Furthermore, the extract decreased elevated malondialdehyde (MDA) levels caused by ethanol exposure, indicating strong antioxidant properties. These pharmacological and biochemical outcomes were corroborated by histological examinations, which showed preserved gastric mucosal integrity in treated animals. The anti-ulcer effects of *H. sabdariffa* calyces can be attributed to their phytochemical components, likely acting through mechanisms such as antioxidant activity and inhibition of gastric acid secretion. These findings suggest EEHS as a potential natural remedy for gastric ulcers, warranting further exploration for therapeutic applications.**

Key words: *Hibiscus sabdariffa*, Ethanolic extract, Gastric ulcer models, MDA, Roselle, Calyces.

INTRODUCTION

H. sabdariffa has drawn interest as a potential phyto-therapeutic agent for both the prevention and treatment of stomach ulcers. The therapeutic potential of this plant, often referred to as Roselle, is enhanced by a wide range of bioactive substances, including phenolic acids, flavonoids, anthocyanins, and organic acids [1].
Approximately, 80% of people in under-developed nations get their medical care from traditional medicines [2]. Just 1% of the 500,000 plant species found worldwide have undergone phytochemical analysis [3]. Around 6000 years ago, roselle was first planted in Sudan. The well-known versatile medicinal plant (*H. sabdariffa* Linn.) is found throughout the world's tropical and subtropical climates and grows as an annual tropical short shrub [4]. Warm nations including India, Indonesia, the Philippines, Malaysia, and tropical Africa, as well as Brazil, Australia, Hawaii, and Florida, are among those that cultivate it. Hs comes in two primary varieties: Hs var. altissima Wester, which is grown for its jute-like fibre, and Hs *var.* sabdariffa. The shorter bushy varieties of the second variety-bhagal prurience, intermedius, albus, and rubber have been referred to as races. While the second and third races have yellow-green edible calyces

(*var.* rubber) and also produce fibre, the first type has green, red-streaked, inedible calyces [5]. Depending on the sepal hue, Roselle comes in a variety of varieties. Numerous epi-demiological studies have demonstrated the anti-analgesic, hepato- protective, hair-growth, and coronary heart disease, cancer, and atherosclerosis-inhibiting effects of vitamins that contain antioxidants and carotenoids [6]. The most prevalent gastro-intestinal condition linked to substantial morbidity and mortality is gastric ulcer. Finding natural items with possible antiulcer effects is therefore crucial. The treatment for stomach ulcers involves the use of Hibiscus sabdariffa. Peptic ulcer disease (PUD) is distinguished by denuded mucosal with the fibrin-covered gap extending into the submucosa or muscularis propria. It is an acid-induced lesion of the gastrointestinal system that usually takes place in the stomach, proximal duodenum, or lower esophagus [7].

Fig. 1. Image of medicinal plant - *H. sabdariffa*

PUD, often known as a GI ulcer, is the most common gastrointestinal ailment and accounts for approximately 15 out of every 15,000 complex cases that occur globally each year. Under normal conditions, the stomach mucosa is shielded from ulceration by acidity from the stomach, pepsin, bile salts, H. pylori infection, and NSAIDs by substances such gastric mucus, prostaglandins, and bicarbonates. When aggressive stimuli override the defensive processes, gastric ulcers develop [8]. One plant product that has been utilized in traditional medicine to treat PUD is *Hibiscus sabdariffa*. Several pathways are involved in *H. sabdariffa's* mechanism of action in both the prevention and management of stomach ulcers, which is reinforced by its bioactive constituents found in the rosella.

Oxygen species that are reactive (ROS) harm stomach mucosal cells under oxidative stress, which frequently results in or exacerbates gastric ulcers. Antioxidants including poly-phenols, flavonoids, and anthocyanins are abundant in Hibiscus sabdariffa. By scavenging free radicals, these substances lessen oxidative stress. It keeps mucosal integrity intact by reducing oxidative damage and lipid per-oxidation to the stomach mucosa [9].

Pro-inflammatory mediators such as leuko-trienes and prostaglandins, as well as pro-inflammatory cytokines like TNF-α and IL-6, are overproduced in gastric ulcers. The bioactive substances in *H. sabdariffa* inhibit the synthesis of cytokines that promote inflammation. It reduces inflammation and harm to tissues in the stomach lining by blocking the enzymes, lipoxygenase and cyclooxygenase (COX) [10].

Cytoprotection entails fortifying the mucosal defenses against harm from irritants, pepsin, or stomach acid. *H. sabdariffa* promotes the production of bicarbonate ions and increases the synthesis of gastric mucus, both of which shield gastrointestinal lining from acidic degradation. *H. sabdariffa* encourages the production of stomach heat shock proteins (HSPs), which protect cells from harm [11].

Extracts of *H. sabdariffa* lower gastric acid secretion by blocking histamine (H_2) the receptors or proton pumps in cells in the parietal area. High secretion of stomach acid and pepsin leads to mucosal erosion and the development of ulcers. As a result, the acid load is decreased, and the pH of the stomach is balanced [12].

Because *Helicobacter pylori* infections damage the stomach mucous barrier and produce inflammation, they are a significant cause of gastric ulcers. The role of H. sabdariffa is to show antimicrobial activity towards H. pylori, mainly because of its organic acids (like hibiscus acid) and polyphenolic components. It stops the development of ulcers and gastritis brought on by bacteria by decreasing its colonization [13].

Maintaining oxygenation of tissues and supplying nutrients needed for repair depend heavily on gastric mucosal blood flow. The function of H. sabdariffa is to enhance blood supply to the stomach mucosa through its vasodilatory actions, which are mediated by anthocyanins. Improved blood flow encourages injured tissues to heal and regenerate [14].

Stress can cause ulcers by increasing the creation of stomach mucus, decreasing blood flow, and increasing the secretion of gastric acid.

Its antioxidant and adaptogenic qualities lessen the adverse impacts of stress on the physiology of the stomach. By strengthening antioxidant defenses and mucus secretion, it stops stress-induced erosion of the stomach mucosa [15]. The plant material utilized to make the ethanolic extract was dried the calyces of Hibiscus sabdariffa. For the experimental models, Wistar albino rats were chosen as the animals. In order to induce stomach ulcers and perform biochemical experiments, basic compounds and reagents were used.

H. sabdariffa solvent extraction method

In the solvent mixture (solvent-to-material ratio ~10:1), powdered calyx powder was added and the mixture was stirred or agitated for 24 hours at an ambient temperature or while it is mildly heated (30 to 50°C). A rotating evaporator or freezing and drying was used to filter the extracted material and to remove the solvent [16].

Analysis of H. sabdariffa phytochemistry

Hibiscus sabdariffa phytochemical analysis entails determining and measuring the different bioactive substances found in the plant, especially in its calyces. The analysis is separated into two parts *viz*. quantitative analysis, which determines the concentrations of the key phytochemical groups, and qualitative screening, which determines their presence.

Animals and dosing

In this investigation, albino Wistar rats of both sexes, roughly equal in age, weighing 150–200 g, and fed a regular chow diet were employed. They were split up into six-rat experimental groups at random. Prior to dosing, fresh aqueous solutions of EEHS and ulcerogenesis were made. EEHS was administered intraperitoneally for the assessment of gastric secretion as well as orally during anti-ulcer tests at dosages of 250 and 500 mg/kg [17]. The stomachs of the sacrificed rats were taken out and separated along the larger curvature. A person who was not informed of the treatments measured the number of stomach lesions following saline washing [18].

Ulcers caused by hypothermic restraint stress

For this purpose, Senay and Levine's approach was used with minor adjustments. The animals had unlimited access to water during their 36-hour fast. Rats were restrained in constraint cages and kept in a vented refrigerator at 3±1 °C for 3 hours after being given oral EEHS (250 and 500 mg/kg) thirty minutes earlier. The guts of the living things were then removed during the sacrifice process. The arbitrary scale, as described in literature, was used to assess them for ulceration and the degree of intraluminal bleeding [19].

Pylorus-ligated (Shay) rats (Anti-secretory research)

Prior to pylorus ligation, rats were given unlimited access to water and fasted for 36 hours. Care was required to avoid obstructing blood vessels or causing hemorrhage [19]. Immediately following pylorus ligation, EEHS was given intraperitoneally (Shay). Six hours after the pylorus ligation, the rats were sacrificed. Following the removal of the stomachs, the contents were gathered, their quantities measured, centrifuged, and their acidity titratable versus 0.01 mol/l NaOH at pH 6.5–7 was assessed [20].

Necrotizing agent-induced gastric lesions (Cytoprotection)

One milliliter of a necrotizing substance (either 80% ethanol, 0.2 mol/l NaOH, or 25% NaCl) was given to each rat. Thirty minutes before to the administration of necrosis of drugs, EEHS was administered. The rats were sacrificed and checked for stomach lesions one hour after the ethanol and alkalis were administered. The stomach lesions were scored as follows: patchy stomach lesions brought on by ethanol. utilizing the scale that follows: Normal mucosa is represented by 0; hyperemic mucosa or up to three tiny patches are represented by 1; four to ten small patches are represented by 2; more than ten little or up to 3 medium-sized patches are represented by 3; and four to six medium-sized patches are represented by 4, 5 indicates that there are more than six medium-sized or as many as three large patches; 6 indicates that there are 4-6 large patches; 7 indicates that there are 7-10 large patches; and 8 indicates that there are over 10 large patches or numerous necrotic zones. "Medium-sized" was defined as being from 2 to 4 mm across, "large" as being more than 4 mm across, and "small" as being up to 2 mm around (max. diameter) [1].

Measurement of gastric wall mucus (GWM)

Separation from the stomach's rumen, the glandular segment was weighed and promptly placed in 10 milliliters of 0.1% *w/v* Alcian blue

solutions (in 0.16 mmol/l solution of sucrose buffer with 0.05 ml of sodium acetate at pH 5). Alcian blue was used to stain the tissue for two hours. After 15 and 45 minutes, respectively, the tissue was rinsed twice using 10 ml of 0.25 mmol/l sucrose to remove any remaining color. Ten milliliters of 0.5 mmol/l magnesium chloride were used to extract the dye complexed with the GWM.

The mixture was agitated intermittently for one min at 30-min intervals of 2 hours. Next, an equivalent amount of diethyl ether was shaken vigorously with four ml of blue extract. After centrifuging the resultant emulsion for 10 min at 4,000 rpm, the absorbing capacity of the liquid layer was measured at 580 nm. Next, quantity of Alcian blue that was removed per gram of moist glandular tissue, was determined [21].

Gastric lesions caused by indomethacin

The rats were fasted for 36 hours and given 30 mg/kg body weight of Indomethacin, which was dissolved in 1.0% carboxy methylcellulose (CMC) in water (6 mg/ml). An identical volume of vehicle was administered to control rats in a similar manner. A single dose of 250 and 500 mg/kg of the sabdariffa plant extract was administered 30 minutes before indomethacin was administered. Six hours following treatment, the animals were sacrificed. After being removed, the stomachs were cleaned with regular saline and checked for ulcers [22].

Total protein (TP) determination

Crescent Testing and Diagnostics, Jeddah, Saudi Arabia, provided the kit method for estimating TP. For non-protein sulfhydryls (NP-SH) estimation, the Sedlak and Lindsay method was used to measure the NP-SH in the gastric mucosa. In ice-cold 0.02 mmol/l ethylene diaminetetraacetic acid (EDTA), the glandular portion of the stomach was homogenized [23].

Five milliliters of the homogenates were combined with one milliliter of 50% trichloroacetic acid (TCA) and four milliliters of distilled water in 15 milliliter test tubes. After ten minutes of sporadic shaking, the tubes were centrifuged at 3,000 rpm.

The sample was shaken after 0.1 ml of 5,5´-dithio bis (2-nitrobenzoic acid) (DTNB) was added to two milliliters of supernatant and 4 ml of 0.4 mol/l Tris buffer at pH 8.9. At 412 nm, the absorbance was determined five minutes after the addition of DTNB in comparison to a blank reagent [24].

Malondialdehyde (MDA) measurement

One hour after the animals were given ethanol, they were put down. After removing the stomachs, each was homogenized with 0.15 mol/l KCl (at 4°C) to produce a 10% *w/v* homogenate. A metabolic shaker was used to incubate 1 ml aliquots of homogenate for 3 hours at 37 °C. Next, a 10% aqueous TCA (1 ml) was added and combined. After that, the mixture was centrifuged at 800 g.

In 15 milliliter test tubes, five milliliters of the homogenates were mixed with 1 milliliter of 50% trichloroacetic acid (TCA) and four ml of distilled water. The tubes were shaken intermittently for ten minutes before they were centrifuged at 3,000 rpm. After adding 4 milliliters of 0.4 mol/l Tris buffer at pH 8.9 and 0.1 ml of 5,5´-dithio bis (2-nitrobenzoic acid) (DTNB) to two milliliters of supernatant, the sample was shaken.

Five minutes after, DTNB was added, the absorbance at 412 nm was measured and compared to a control reagent. After removing one milliliter of the supernatant, it was combined with one milliliter of 0.67% thiobarbituric acid in water and heated to a boiling point for 10 minutes. After cooling, 1 milliliter of distilled water was added to dilute the liquid. At 535 nm, the solution's absorbance was then measured. Next, the amount of MDA (nmol/g wet tissue) [an indicator of the degree of lipid per oxide (LPO)] was computed using a standard curve of MDA solution [25].

RESULTS AND DISCUSSION

The goal of *H. sabdariffa* phytochemical analysis is to find out which phytochemical classes are present in the extracts of the plant (**Table 1**).

Impact of EEHS on gastric mucosal lesions caused by hypothermic restraint stress

According to **Table 2**, EEHS significantly reduced intra luminal bleeding and lesion development brought on by hypothermic restraint stress at doses of 250 and 500 mg/kg body weight. Even though a dosage of 250 mg/kg body weight decreased ulcer index, this decrease was not deemed statistically significant.

Impact of EEHS on gastric secretions in rats with a 6-hour pylorus litigation

When compared to the control group, the treatment with EEHS at the two dosages (250 and 500 mg/kg) significantly decreased the

amount of basal gastric secretions, titratable acidity, and ulceration in the gastrointestinal secretion determination model, which used ligated pylorus for 6 hours (**Table 3**).

Table 1. Detection of the presence of various phytochemical classes in *H. sabdariffa* extracts

Phytochemical Class	Test Performed	Observation
Alkaloids	Dragendorff's test: Add the Dragendorff's reagent to the extract Mayer's test: Add Mayer's reagent.	Orange/brown or white precipitate
Flavonoids	Shinoda test: Add magnesium ribbon and HCl to the extract.	Pink or red coloration
Phenolics/Tannins	Ferric chloride test: Add 1% $FeCl_3$ to the extract.	Blue-green or black color
Saponins	Foam test: Shake extract with water vigorously.	Persistent froth/foam
Steroids	Liebermann-Burchard test: Add acetic anhydride and sulfuric acid.	Green or Bluish-green color
Terpenoids	Salkowski test: Add chloroform and concentrated H_2SO_4.	Red or reddish-brown color
Anthocyanins	pH test: Add acidic (HCl) and basic (NaOH) solutions.	Color change (red at acidic pH, green/blue in basic pH)
Carbohydrates	Molisch's test: Add α-naphthol and H_2SO_4.	Purple or violet ring
Proteins	Biuret test: Add NaOH and $CuSO_4$ to the extract.	Violet or purple color

Table 2. Impact of EEHS ethanolic extract on rats' intraluminal hemorrhage and stomach lesions caused by hypothermic restraint stress (mean ± SE)

Group serial	Treatment	Dose (mg/kg, i.g.)	Intraluminal bleeding	Gastric lesion
1	Control	-	2.51 ± 0.84	20.67 ± 5.26
2	EEHS	250	00 ± 00	10.32 ± 3.71
3	EEHS	500	00 ± 00	5.82 ± 2.03

*Each group consisted of six rats. $P < 0.05$ in comparison to the student's t-test control group (distilled water)

Table 3. Effect of ethanolic extract of EEHS on the gastric lesions index, acidity, and secretion in pylorus-ligated shay rats (mean ± SE)

Group serial	Treatment	Dose (mg/kg, i.g.)	Volume of gastric content (ml)	Titratable acidity (mEq/l)	Ulcer index
1	Control	-	8.01 ± 0.90	170.27 ± 8.59	0.84 ± 0.76
2	EEHS	250	2.67 ± 0.82	46.00 ± 9.53	00 ± 00
3	EEHS	500	2.17 ± 0.76	34.98 ± 10.06	00 ± 00

*Each group consisted of six rats, and the student's t-test showed that $P < 0.001$ compared to the control group (distilled water)

Table 4. Mean±SE effect of ethanolic extract of EEHS on stomach lesions caused by necrotizing agents

Group serial	Treatment	Dose (mg/kg, i.g.)	80% EtOH	0.2 mol/L NaOH	25% NaCl
1	Control#	-	5.6 ± 0.84	7.6 ± 0.84	7.34 ± 0.82
2	EEHS	250	5.2 ± 1.48	3.84 ± 1.33	4.84 ± 0.76
3	EEHS	500	2.01 ± 0.90	2.01 ± 1.10	3.34 ± 0.52

*Each group consisted of six rats; the lesion index was considerably lower in the student's t-test, #distilled water

Impact of EEHS on gastric lesions caused by necrotizing agents

All of the control rats developed large ulcers in the glandular mucosal of the stomach after being treated with 80% ethanol, 0.2 mol/l NaOH, and 25% NaCl pretreatment. EEHS at dosages of 250 and 500 mg/kg compared to the control (distilled water) group ($P < 0.05$, $P < 0.01$). **Table 4** indicates that while the animal groups that got a 250 mg/kg dose of extraction in

ethanol-induced mucosal injury showed a decrease in ulcer intensity, this decrease was statistically insignificant.

Impact of EEHS on ethanol-induced GWM alterations

Compared to control rats, rats given ethanol exhibited a marked reduction in GWM's Alcian blue binding ability. The Alcian blue binding capacity of the stomach mucosa was significantly increased in rats pretreated with EEHS at a dose of 500 mg/kg; however, this increase in binding capacity was not statistically significant at a dose of 250 mg/kg (**Figure 2**).

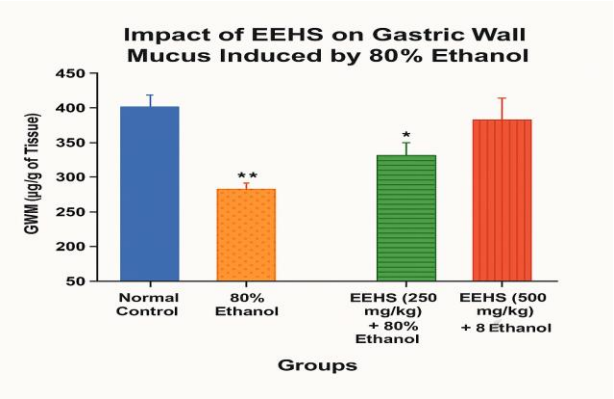

Fig. 2. Impact of EEHS on 80% ethanol-induced alterations in gastric wall mucus (GWM)

Table 5. Impact of EEHS ethanolic extract on gastric mucosal lesions caused by indomethacin (mean ± SE)

Group serial	Treatment	Dose (mg/kg, i.g.)	Ulcer Index
1.	Indomethacin	30	40.51 ± 4.64
2.	EEHS	250	30.17 ± 7.06
3.	EEHS	500	22.01 ± 3.90

*Each group consisted of six rats for the student's t-test $P < 0.05$ comparison to the control group (indomethacin alone)

Impact of EEHS on indomethacin-induced gastric lesions

The rat glandular stomach suffered significant damage as a result of oral indomethacin dosing. The development of lesions in the rat stomach was considerably inhibited by EEHS at a dose of 500 mg/kg ($P < 0.05$). However, in patients treated with indomethacin, EEHS at the 250 mg/kg dose had no discernible preventative effect (**Table 5**).

Impact of EEHS on ethanol-induced TP depletion

Figure 3 showed that the ethanol-only treated group's TP levels were considerably lower. The amount of protein in the stomach tissue was markedly and dose-dependently increased by EEHS at both dosages.

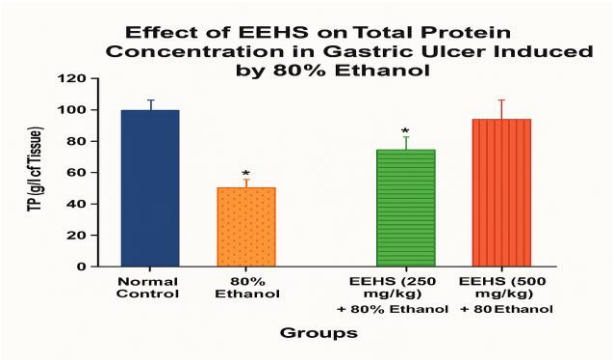

Fig. 3. Effect of EEHS on TP conc. in gastric ulcer induced by 80% ethanol

Effect of EEHS on ethanol-induced depletion of gastric mucosal NP-SH

The level of NP-SH in the gastric mucosa significantly decreased following the administration of 80% ethanol. Pre-treatment of rats with EEHS at 500 mg/kg significantly replenished the ethanol-induced depletion of NP-SH concentration in the stomach. However, this increase was not significant with the 250 mg/kg dose (**Figure 4**).

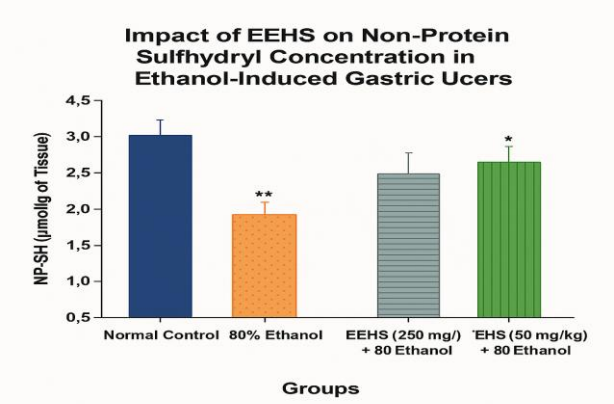

Fig. 4. Impact of EEHS on NP-SH conc. in 80% ethanol-induced gastric ulcers

Impact of EEHS on the rise in MDA caused by ethanol

MDA levels in the stomach mucosa were considerably greater in the ethanol-treated group compared to the group receiving no

treatment, as seen in **Figure 5**. However, the MDA content was considerably reduced by EEHS at a dose of 500 mg/kg. The MDA content was, however, marginally reduced by the lower dosage (250 mg/kg).

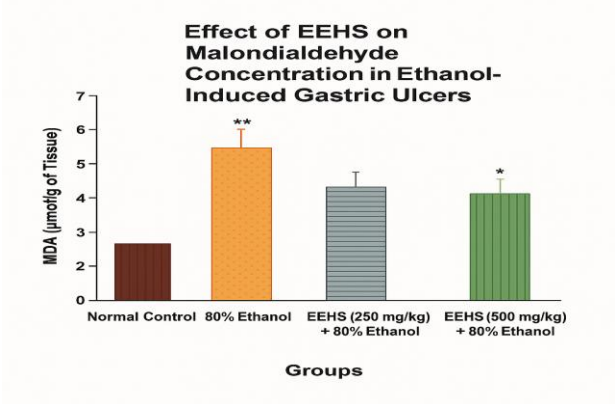

Fig. 5. Effect of EEHS on MDA conc in gastric induced by 80% ethanol

CONCLUSION

By reducing gastric mucosal lesions, inhibiting gastric acid secretion, and increasing gastric mucus production in a variety of experimental models, the current study showed that the ethanolic extract of *Hibiscus sabdariffa* (EEHS) has strong anti-ulcerogenic and gastroprotective properties. According to the results, *H. sabdariffa's* anti-ulcer efficacy is mediated by a number of mechanisms, including its cyto-protective, anti-inflammatory, antioxidant, and anti-secretory qualities. Additionally, it helps prevent and treat stomach ulcers by improving blood flow and having antibacterial properties against Helicobacter pylori. The existence of bioactive substances such flavonoids, anthocyanins, phenolics, and terpenoids was verified by the phytochemical study; these substances most likely contribute significantly to the pharmacological effects that were noted. The findings demonstrate *H. sabdariffa's* potential as a natural alternative for treating peptic ulcer disease (PUD) and support its traditional use as a medicinal agent for gastric ulcers. To prove its effectiveness and safety in people, more clinical research is required.

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