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PHYTOCHEMICAL ANALYSIS AND EVALUATION OF ANTI -ULCER POTENTIAL OF EXTRACT OF *BARRINGTONIA ACUTANGULA*

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Abstract

One of the most common gastrointestinal conditions, gastric ulcers affect 5–10% of people at some point in their lives. Much research has been done on herbal medicine in recent years to determine whether or not it can be used to prevent or treat stomach ulcers. Here this study deals with assessing the anti -ulcer activity of *Barringtonia acutangula*. The plant material was gathered and subjected to extraction by methanol. Further qualitative, quantitative studies along with In vivo anti -ulcer activity was conducted. Results showed that the % yield in pet ether and methanolic extract was found to be 3.4 % & 8.2 % respectively. The phytochemical test confirmed the presence of alkaloid, flavonoid, protein and saponin. The total flavonoid and alkaloid content in extract was observed to be 0.721 and 0.645 mg/ 100 mg respectively. Further the effect of methanolic extract of *Barringtonia acutangula* on ulcer index was checked In case of *Barringtonia acutangula* 100 & 200 mg/kg treated rats the ulcer index was estimate to be 3.25±0.10 and 3.45±0.20 respectively. The gastric pH was noted to be 3.95±0.15 and 4.20±0.10 for 100 & 200 mg/kg respectively. For the rats treated with drug the pH was observed to be 4.38±0.20 as compared to control group which is 2.65±0.10. The acidity of stomach was seen to be reduced upto 54.63±0.15 mEq/lt and 42.41±0.15 mEq/lt in *Barringtonia acutangula* extracts 100 and 200 mg/kg treated rats respectively. The pepsin activity in Ranitidine treated rats was estimated to be 2.45±0.20 ml/h. In rats treated with *Barringtonia acutangula 100 and 200* mg/kg treated rats the pepsin activity was estimated to be 3.25±0.20 ml/h and 2.58±0.18 ml/h respectively. Thus, from results it can be assumed that *Barringtonia acutangula* has potent anti-ulcer pharmacologic action.

Keywords: Peptic ulcer, Medicinal plants, Phytochemicals, *Barringtonia acutangula*, Ulcer index, Pepsin activity, Rantidine

Introduction

A skin or mucous membrane ulcer is an open sore that is characterized by the sloughing off of inflamed, dead tissue. Lesions on the skin's surface or a mucous membrane that exhibit a superficial tissue loss are called ulcers. Although they can occur almost anywhere, ulcers are most frequently found on the skin of the lower extremities and in the gastrointestinal tract. There are numerous varieties of ulcers, including genital, peptic, esophageal, and mouth ulcers. Among these, peptic ulcers are common in many individuals (Ramakrishnan and Salinas, 2007).

The older population is now primarily affected by ulcer disease, with the peak incidence occurring between the ages of 55 and 65. Duodenal ulcers were found to be more common in men than gastric ulcers; the opposite was observed in women. Of patients diagnosed with gastric ulcers, 35% will experience severe complications. Despite having a low death rate, peptic ulcer disease is highly prevalent and causes significant financial, medical, and emotional hardship (Yeomans and Naesdal, 2008).

Traditionally, an acidic environment that is hypersecretory in addition to dietary factors or stress are thought to be the cause of mucosal disruption in patients with acid peptic disease. H. pylori infection, alcohol and tobacco use, use of non-steroidal anti-inflammatory drugs (NSAIDs), and Zollinger-Ellison syndrome are risk factors for developing peptic ulcer. The primary risk factors for H. pylori infection and NSAID use are gastric and duodenal ulcers. Nonetheless, the development of peptic ulcer disease is uncommon in individuals with H. pylori infection or NSAID use, indicating that individual susceptibility plays a significant role in the early stages of mucosal damage. Peptic ulcers and functional polymorphisms in various cytokine genes are linked. For instance, H. pylori-associated gastroduodenal disease is caused by polymorphisms in interleukin 1 beta (IL1B), which alter mucosal interleukin 1β production (Coleman *et al*., 2013; Sverdén *et al*., 2019).

Many chemical agents are available to treat peptic ulcers, but many of them have serious side effects. For example, H2 antagonists can cause impotence, headaches, skin rashes, and arrhythmias, while using proton pump inhibitors can unexpectedly lead to

hypergastrinemia and atrophic gastritis. Antacid use causes belching, constipation, and stomach distention. It also increases the risk of ulcer perforation. Other medications, such as anticholinergics, can cause constipation, dry mouth, urine retention, blurred vision, xerostomia, and the early onset of glaucoma. While prostaglandin analogs are likely to cause abdominal cramps, uterine bleeding, and abortion, ulcer protectives induce constipation, diarrhea, dizziness, edema, and hypophosphatemia. Because they are less harmful, more palatable to different cultures, more compatible with the human body, less likely to cause side effects, affordable, efficient, and readily available, herbal medications have thus maintained their significance. The characteristics of certain medicinal plants with antiulcer activity are described in this paper (Kuna *et al*., 2019; Mouly *et al*., 2013).

Since the chemical components of plants and herbal medicines are involved in the physiological processes of living flora, it is thought that these substances are more suited to the human body. Plant-based natural products have been utilized for centuries to treat a wide range of illnesses. Today, it is imperative that natural medicine be used to treat a variety of conditions, including peptic ulcers. Thus, an alternative strategy that has gained traction recently is the study of traditional medicine's medications. In addition to being clinically effective and comparatively less toxic than current medications, the use of phyto-constituents as drug therapy to treat major ailments also lowers the offensive factors, which can be used as a preventative measure against peptic ulcers (Gadeka *et al*., 2010; Saxena *et al*., 2014; Sen *et al*., 2009)

One of India's most significant medicinal plants is *Barringtonia acutangula* Linn. (Family: Lecythidaceae), commonly known as Samudraphal (or "Indian Oak" in English). This evergreen tree, which can reach a height of 9 to 12 meters, is widespread in the sub-Himalayan regions that stretch from the Ganges eastward to Assam, Madhya Pradesh, and the Indian peninsula. A plant called Barringtonia acutangula L. has long been used to treat and cure a wide range of illnesses. Its roots, leaves, and fruits have been used for many centuries in Ayurveda medicine to treat spleenic disorders, leprosy, stomach disorders, and jaundice. In folklore, it is used as an anthelmintic for vitiated conditions of kapha and pitta, leprosy, arthralgia, dysmenorrhea, plumbago, skin

disease, diarrhea, inflammation, and flatulence. Many medical conditions, including hemiplegia, joint pain, eye disorders, stomach disorders, cough, dyspnea, leprosy, intermittent fever, spleenic disorders, and poisoning, have been treated with various parts of Barringtonia acutangula. This study provides additional insight into the anti-ulcer potential of *Barringtonia acutangula* extract in relation to its wide range of medicinal uses (Padmavathi *et al*., 2011; Kaur *et al*., 2013).

Materials and Methods

Collection of plant material

The seeds of *Barringtonia acutangula* were collected from local area of Bhopal in the month of January, 2023. Drying of fresh plant parts was carried out in sun but under the shade. Dried seeds of *Barringtonia acutangula* were preserved in plastic bags, closed tightly and powdered as per the requirements.

Defatting of plant material

58 gram shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place.

Extraction by maceration process

Defatted powdered of *Barringtonia acutangula* has been extracted with methanol solvent using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40°C (Chen *et al*., 2016).

Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method (Shraim *et al.,* 2021). 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filtered. 3 ml (1mg/ml) of this solution was used for the estimation of flavonoid. 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

Estimation of total alkaloids content

The plant extract (1mg) was dissolved in methanol, added 1ml of 2 N HCl and filtered (John *et al.,* 2014). This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (40, 60, 80, 100 and 120 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/100mg of extract.

***In vivo* anti-ulcer activity**

Animals

Wistar rats (150–200 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Toxicity study

Healthy adult male albino rats were fasted overnight prior to the experiment. Different doses (50-2000 mg/kg, P.O) of the methanolic extract of *Barringtonia acutangula* were administered to each group of rats (Each group carries 6 rats) and they were observed continuously for 1 hour and then at half-hourly intervals for 4 hour, for any gross behavioural changes and further up to 72 hour, followed 14 days for any mortality as per the OECD (Organization for Economic Co-operation and Development) Guideline

425 (OECD, 2008). The methanolic extract of *Barringtonia acutangula* was found to be non-toxic up to the maximum dose of 2000 mg/kg body weight. Dose selected for antiulcer evaluation was 100 and 200 mg/kg respectively.

Ulcer induced by absolute ethanol

The rats were divided into four groups of six each.

Group I (Toxicant control) received absolute ethanol (1 ml/animal)

Group II was treated with ranitidine (50 mg/kg)

Groups III was treated with methanolic extract of *Barringtonia acutangula* 100 mg/kg/p.o.

Groups IV was treated with methanolic extract of *Barringtonia acutangula* 200 mg/kg/p.o.

The animals were treated with ranitidine (100 mg/kg), dose of methanolic extract of *Barringtonia acutangula* 100 and 200 mg/kg (once daily) for 5 days after the induction of ulcer, while the control group received only the vehicle. The rats were fasted for 24 h and they received 1 ml of absolute ethanol orally. The animals were sacrificed after 1 h of ulcerogen administration, and their stomachs were excised and the gastric contents were aspirated. The contents were subjected to centrifugation at 1000 rpm for 10 min and then analyzed for pH (digital pH meter), pepsin activity, total and free acidity (Mousa *et al.,* 2019).

Antiulcer screening

The ulcer index was determined using the formula

Ulcer index = 10/X

Where X = Total mucosal area/Total ulcerated area.

Based on their intensity, the ulcers were given scores as follows:

0 = no ulcer, 1 = superficial mucosal erosion, 2 = deep ulcer or transmural necrosis,

3 = perforated or penetrated ulcer.

Results and Discussion

The % yield in pet ether and methanolic extract was found to be 3.4 % & 8.2 % respectively. The phytochemical test confirmed the presence of alkaloid, flavonoid,

protein and saponin. The total flavonoid and alkaloid content in extract was observed to be 0.721 and 0.645 mg/ 100 mg respectively. Further the effect of methanolic extract of *Barringtonia acutangula* on ulcer index was checked. It was observed that control group had ulcer index of 6.8 ±0.20. In case of ranitidine 50 mg/kg treated rats the ulcer index was observed to be 2.45±0.15. In case of *Barringtonia acutangula* 100 & 200 mg/kg treated rats the ulcer index was estimate to be 3.25±0.10 and 3.45±0.20 respectively.

The extract greatly decreased ulcers caused by ethanol. This could be the result of the extract's cytoprotective action through antioxidant properties. The extract exhibits defense against the typical lesions brought on by ethanol ingestion. *B. acutangula* may have an antiulcer effect because it lowers both gastric cytoprotection and gastric acid secretion.

The gastric pH was noted to be 3.95±0.15 and 4.20±0.10 for 100 & 200 mg/kg respectively. For the rats treated with drug the pH was observed to be 4.38±0.20 as compared to control group which is 2.65±0.10.

The acidity of stomach was seen to be reduced upto 54.63±0.15 mEq/lt and 42.41±0.15 mEq/lt in *Barringtonia acutangula* extracts 100 and 200 mg/kg treated rats respectively. This is comparable to that of rats treated with rantidine with acidity of 37.85±0.30 mEq/lt. Further the free acidity was found to be 41.15±0.10 and 36.50±0.20 in group of rats treated with 100 and 200 mg/kg treated rats.

These findings indicate that the extracts blocked the formation of acid in parietal cells in response to histamine, inhibited H+/K + ATPase, the gastric proton pump, and interfered with the digestive effect of accumulated gastric juice. They also showed a reduction in both gastric acidity and gastric secretory volume, which may be partially attributed to their flavonoid component.

The pepsin activity in Ranitidine treated rats was estimated to be 2.45±0.20 ml/h. In rats treated with *Barringtonia acutangula 100 and 200* mg/kg treated rats the pepsin activity was estimated to be 3.25±0.20 ml/h and 2.58±0.18 ml/h respectively.

The antiulcer medication may selectively increase prostaglandins, shielding the mucosa from the effects of acid. Prostaglandins have a critical role in protection. The gastric mucosa's epithelial cells, which create a physical barrier by being impermeable to H+ ions, may be the cause of the mucosal defense mechanism.

Due to known adverse effects of current antiulcer medications. The focus has switched to natural goods as the new anti-ulcer agents suppliers. with the interest in alternative medicine expanding. Based on the conventional understanding of their pharmacological characteristics, a variety of plants have been investigated and found to be helpful in the treatment and management of ulcers. Moreover, studies on medicinal plants have demonstrated encouraging outcomes in the treatment of a number of illnesses, including duodenal and stomach ulcers.

Table 1: % Yield of methanolic extract of *Barringtonia acutangula*

S. No.	Extracts	% Yield (w/w)
1.	Pet. ether	3.4%
2.	Methanolic	8.2%

Table 2: Phytochemical screening of extract of *Barringtonia acutangula*

S. No.	Constituents	Methanolic extract
1.	**Alkaloids**	
	Mayer's Test	-ve
	Wagner's Test	-ve
	Dragendroff's Test	+ve
	Hager's Test	+ve
2.	**Glycosides**	
	Legal's Test	-ve
3.	**Flavonoids**	
	Lead acetate	+ve
	Alkaline test	+ve
4.	**Phenol**	
	Ferric chloride test	-ve
5.	**Proteins**	
	Xanthoproteic test	+ve
6.	**Carbohydrates**	
	Molisch's Test	-ve
	Benedict's Test	-ve
	Fehling's Test	-ve
7.	**Saponins**	
	Froth Test	+ve
8.	**Diterpenes**	
	Copper acetate test	-ve
9.	**Tannins**	
	Gelatin Test	-ve

Results of estimation of total flavonoids and alkaloid content

Total flavonoids content estimation (TFC)

Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: **$Y=0.030X - 0.008$, $R^2=0.998$**, where X is the quercetin equivalent (QE) and Y is the absorbance.

Total alkaloid content estimation (TAC)

Total alkaloid content was calculated as atropine equivalent mg/100mg using the equation based on the calibration curve: **$Y=0.008X- 0.016$, $R^2=0.997$**, where X is the Atropine equivalent (AE) and Y is the absorbance.

Table 3: Estimation of total flavonoids and alkaloid content of extract of *Barringtonia acutangula*

S. No.	Extract	Total flavonoids content (mg/ 100 mg of dried extract)	Total alkaloid content (mg/ 100 mg of dried extract)
1.	Hydroalcoholic	0.721	0.645

Table 4: Effect of methanolic extract of *Barringtonia acutangula* on ulcer index by ethanol induced ulcers in rats

Treatment and dose	Ulcer Index
Control	6.8 ±0.20
Ranitidine (50 mg/kg, p.o.)	2.45±0.15***
Methanolic extract of *Barringtonia acutangula* (100 mg/kg, p.o.)	3.25±0.10**
Methanolic extract of *Barringtonia acutangula* (200 mg/kg, p.o.)	3.45±0.20***

Values are expressed as mean ± S.E.M. (*n* = 6).Values are statistically significant at p<0.05 vs. control group respectively (One-way ANOVA followed by Dunnett's test).

Table 5: Effect of methanolic extract of *Barringtonia acutangula* on gastric parameters i.e. pH by ethanol-induced ulceration in rats

Treatment and dose	pH
Control	2.65±0.10
Ranitidine (50 mg/kg, p.o.)	4.38±0.20***
Methanolic extract of *Barringtonia acutangula* (100 mg/kg, p.o.)	3.95±0.15**
Methanolic extract of *Barringtonia acutangula* (200 mg/kg, p.o.)	4.20±0.10***

Table 6: Effect of methanolic extract of *Barringtonia acutangula* on gastric parameters i.e. total acidity ethanol- induced ulceration in rats

Treatment and dose	Total acidity (mEq/lt)
Control	76.45±0.20
Ranitidine (50 mg/kg, p.o.)	37.85±0.30 ***
Methanolic extract of *Barringtonia acutangula* (100 mg/kg, p.o.)	54.63±0.15*
Methanolic extract of *Barringtonia acutangula* (200 mg/kg, p.o.)	42.41±0.15***

Table 7: Effect of methanolic extract of *Barringtonia acutangula* on gastric parameters i.e. free acidity by ethanol-induced ulceration in rats

Treatment and dose	Free acidity (mEq/lt)
Control	56.32±0.40
Ranitidine (50 mg/kg, p.o.)	24.58±0.20 ***
Methanolic extract of *Barringtonia acutangula* (100 mg/kg, p.o.)	41.15±0.10**
Methanolic extract of *Barringtonia acutangula* (200 mg/kg, p.o.)	36.50±0.20 ***

Table 8: Effect of methanolic extract of *Barringtonia acutangula* on gastric parameters i.e. pepsin activity by ethanol-induced ulceration in rats

Treatment and dose	Pepsin activity (Per ml/h)
Control	3.34±0.10
Ranitidine (50 mg/kg, p.o.)	2.45±0.20 ***
Methanolic extract of *Barringtonia acutangula* (100 mg/kg, p.o.)	3.25±0.20**
Methanolic extract of *Barringtonia acutangula* (200 mg/kg, p.o.)	2.58±0.18***

Conclusion

The results of this investigation validate that *Barringtonia acutangula* has anti-ulcer pharmacologic action and does not exhibit oral acute toxicity at the levels used. Its effectiveness is on par with that of regular medications; multiple doses are more beneficial than one. One or more of the discovered phytochemicals may have cytoprotective and/or antisecretory properties that contribute to their anti-ulcer actions. Consequently, the current study supports the use of *Barringtonia acutangula* for gastrointestinal ulcers in traditional medicine, and future research will concentrate on isolating particular phytochemicals and clarifying their modes of action.

References

1. Ramakrishnan K, Salinas RC. Peptic ulcer disease. American family physician. 2007 Oct 1;76(7):1005-12.
2. Yeomans ND, Naesdal J. Systematic review: ulcer definition in NSAID ulcer prevention trials. Alimentary pharmacology & therapeutics. 2008 Mar;27(6):465-72.
3. Coleman S, Gorecki C, Nelson EA, Closs SJ, Defloor T, Halfens R, Farrin A, Brown J, Schoonhoven L, Nixon J. Patient risk factors for pressure ulcer development: systematic review. International journal of nursing studies. 2013 Jul 1;50(7):974-1003.
4. Sverdén E, Agréus L, Dunn JM, Lagergren J. Peptic ulcer disease. Bmj. 2019 Oct 2;367.
5. Kuna L, Jakab J, Smolic R, Raguz-Lucic N, Vcev A, Smolic M. Peptic ulcer disease: a brief review of conventional therapy and herbal treatment options. Journal of clinical medicine. 2019 Feb 3;8(2):179.

6. Mouly C, Chati R, Scotté M, Regimbeau JM. Therapeutic management of perforated gastro-duodenal ulcer: literature review. Journal of visceral surgery. 2013 Nov 1;150(5):333-40.
7. Gadekar R, Singour PK, Chaurasiya PK, Pawar RS, Patil UK. A potential of some medicinal plants as an antiulcer agents. Pharmacognosy reviews. 2010 Jul;4(8):136.
8. Saxena A, Kaur K, Hegde S, Kalekhan FM, Baliga MS, Fayad R. Dietary agents and phytochemicals in the prevention and treatment of experimental ulcerative colitis. Journal of traditional and complementary medicine. 2014 Oct 1;4(4):203-17.
9. Sen S, Chakraborty R, De B, Mazumder J. Plants and phytochemicals for peptic ulcer: An overview. Pharmacognosy Reviews. 2009 Jul 1;3(6):270.
10. Padmavathi D, Susheela L, Bharathi RV. Pharmacognostical evaluation of Barringtonia acutangula leaf. International Journal of Ayurveda Research. 2011 Jan;2(1):37.
11. Kaur M, Singh G, Mohan C. Barringtonia acutangula: A traditional medicinal plant. Int. J. Pharm. Sci. Rev. Res. 2013;23(1):168-71.
12. Chen Q, Fung KY, Lau YT, Ng KM, Lau DT. Relationship between maceration and extraction yield in the production of Chinese herbal medicine. Food and Bioproducts Processing. 2016 Apr 1;98:236-43.
13. Shraim AM, Ahmed TA, Rahman MM, Hijji YM. Determination of total flavonoid content by aluminum chloride assay: A critical evaluation. Lwt. 2021 Oct 1;150:111932.
14. John BI, Sulaiman CT, George S, Reddy VR. Spectrophotometric estimation of total alkaloids in selected Justicia species. International journal of pharmacy and pharmaceutical sciences. 2014;6(5):647-8.