International Journal of Pharmaceutics and Drug Research
ISSN: 2347-6346
Available online at http://ijpdr.com

Original Research Article

PHYTOCHEMICAL SCREENING AND EVALUATION OF ANTIULCER ACTIVITY OF MEDICINAL PLANT *PISTACIA INTEGERRIMA*

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***Article History:**

Received: 25/04/2024
Revised: 11/05/2024
Accepted: 29/05/2024

ABSTRACT

This study investigates the anti-ulcerogenic potential of *Pistacia integerrima* bark extract using a rat model exposed to ulcerogenic agents. The hydroalcoholic extract of *Pistacia integerrima* demonstrated a yield of 8.5% w/w and was characterized for its phytochemical composition, revealing the presence of carbohydrates, proteins, diterpenes, saponins, flavonoids, and tannins & phenols. Quantitative analysis identified 0.425 mg of total phenols and 0.759 mg of total flavonoids per 100 mg of extract. In the experimental evaluation, *Pistacia integerrima* bark extract significantly reduced the number of ulcers and ulcer index in treated groups compared to controls. Group III exhibited the most pronounced effects with the lowest number of ulcers (1.55 ± 0.50) and ulcer index (0.80 ± 0.21), alongside elevated gastric pH (6.75 ± 0.65). These results suggest a dose-dependent response and highlight the extract's potential in mitigating ulcer formation by enhancing mucosal defense and modulating gastric acidity.

Key Words: *Pistacia integerrima,* Phytochemical screening, evaluation, Antiulcer activity

INTRODUCTION

Pistacia integerrima, commonly known as Karkatshringi or Kakrasingi, is a medicinal plant that belongs to the family Anacardiaceae. This plant is indigenous to regions of Asia, particularly found in countries like India, Pakistan, and Iran, where it has been traditionally used in various medicinal practices such as Ayurveda and Unani medicine. *Pistacia integerrima* is valued for its diverse pharmacological properties attributed to its rich phytochemical composition.

Phytochemical investigations have identified several bioactive compounds in *Pistacia integerrima*, including flavonoids, phenols, tannins, saponins, and diterpenes. These constituents have been reported to possess antioxidant, anti-inflammatory, antimicrobial, and gastroprotective activities (Kamal, 2018; Malik *et al.,* 2020).

Gastrointestinal disorders, particularly peptic ulcers, remain a significant health concern globally. Peptic ulcers, characterized by mucosal erosions in the stomach or duodenum, are often associated with factors such as Helicobacter pylori infection, prolonged use of nonsteroidal anti-inflammatory drugs (NSAIDs), stress, and lifestyle factors. Current treatment options, including proton pump inhibitors (PPIs), H2-receptor antagonists, and antibiotics, are effective but may have adverse effects and are associated with potential drug resistance issues (Suzuki *et al.,* 2021).

Given the limitations of conventional therapies, there is growing interest in exploring natural remedies like *Pistacia integerrima* for their potential antiulcer effects. Preclinical studies have suggested that extracts from *Pistacia integerrima* bark, leaves, or fruits may exhibit gastroprotective properties, potentially aiding in ulcer prevention and promoting healing. These effects are hypothesized to involve strengthening of gastric mucosal defenses, reduction of oxidative stress, modulation of inflammatory pathways, and regulation of gastric acid secretion (Gupta *et al.,* 2017; Malik *et al.,* 2020).

This research aims to summarize the phytochemical profile of *Pistacia integerrima* and evaluate the scientific evidence supporting its antiulcer activity based on preclinical and clinical studies. By exploring its pharmacological properties and mechanisms of action, this review seeks to highlight the potential of *Pistacia integerrima* as a natural therapeutic agent in the management of peptic ulcers and other gastrointestinal disorders.

MATERIALS AND METHODS

Extraction of plant materials by maceration method

Powdered barks were weighed (62 gm) of *Pistacia integerrima* and packed in extraction bottle. The defatted plant drugs were subjected to extraction by hydroalcoholic solvent (ethanol: water; 70:30). The liquid extracts were collected in a tarred conical flask. The solvent removed by distillation. Last traces of solvent being removed under vacuum. The extracts obtained with solvent were weighed to a constant weight and percentage w/w basis was calculated (Mukherjee, 2007; Khandelwal, 2005).

Determination of percentage yield

Percentage yield measures the effectiveness of the entire extraction process. It shows how much product a researcher has obtained after running the procedures against how much is actually obtained. A higher % yield means the researcher obtained a greater amount of product after extraction.

The % yield was calculated by using formula:

% yield = [(weight of dried extract) / (weight of dried plant sample)] x 100

Phytochemical screening

Test for alkaloids

1. Hager's test: to a few ml of filtrate, 2 drops picric acid was added formation of yellow precipitate shows a positive result for alkaloids.

2. Wagner's test (iodine – potassium iodine reagent): To about an ml of extract few drops of Wagner's reagent were added. Reddish-brown precipitate indicates presence of alkaloids (Kokate, 1994).

2. Test for phenol

A) FC reagent test: To 5ml of extract 2ml of Folin Ciocalteu reagent is added. Appearance of blue green colour indicates the presence of phenol.

B) Ferric chloride test: To 5 ml of extract few drops of feeric chloride solution was added and mixed gently. The production of blueish black colour solution indicate presence of phenols.

3. Test for flavonoids

A) Alkaline reagent test: To 5ml of extract 2ml of NaOH was added by which solution turns yellow colour, further dilute HCl (0.1N) was added the solution becomes colourless which indicates the presence of phenol.

B) Lead acetate test: To 5 ml of extract few drops of lead acetate solution was added and mixed gently. The production of bulky white precipitate is positive for flavonoid.

4. Test for carbohydrate

A) Benedict's test: About 0.5 ml of the filtrate was taken to which 0.5 ml of Benedict's reagent is added. This mixture was heated for about 2 minutes in a boiling water bath. The appearance of red precipitate indicates the presence of sugars.

B) Fehling test test: About 0.5 ml of the filtrate was taken to which 0.5 ml of each Fehling A & Fehlinng B solution was added. This mixture was heated for about 2 minutes in a boiling water bath. The appearance of red precipitate indicates the presence of sugars.

5. Detection of proteins

Xanthoproteic Test: The extract was treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

6. Detection of diterpenes

Copper acetate Test: Extract was dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

Detection of glycosides: Extract was treated with dil. H_2SO_4, formation of red color solution indicate the presence of glycosides.

7. Detection of saponins

Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

8. Detection of tannins

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

Quantitative estimation of phenols and flavonoids

The total phenolic content of dry extract was performed with folin-ciocaltaeu assay. 2 ml of sample (1 mg/ml) was mixed with 1 ml of folin ciocalteu's phenol reagent and 1 ml of (7.5 g/L) sodium carbonate solution was added and mixed thoroughly. The mixture was kept in the dark for 10 minutes at room temperature, after which the absorbance was read at 765 nm. The total phenolic content was determined from extrapolation of calibration curve which was made by preparing Gallic acid solution. The estimation of the phenolic compounds was carried out in triplicate. The TPC was expressed as 100 milligrams of Gallic acid equivalents (GAE)/100mg of dried sample.

Estimation of total flavonoids content

Preparation of standard solution 10mg quercetin was weighed and made up to 10ml with Methanol in a 10ml volumetric flask. From the above solution (1mg/ml), 1ml was pippeted out and made up to 10ml with Methanol to get 100µg/ml Quercetin standard solution (stock solution). From the stock solution, solutions of concentration 5, 10, 15, 20 and 25 µg/ml were prepared (Mishra *et al.,*

2017). 3 ml of each standard and test was mixed with 1 ml of 2% Aluminium chloride solution. The solutions were mixed well and the absorbance was measured against the blank at 420nm using UV-Visible spectrophotometer. A standard graph was plotted using various concentrations of Quercetin and their corresponding absorbance.

***In-vivo* antiulcer activity**

Animals

Wistar rats (180±20g) 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. A 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 *Pistacia integerrima* Bark extract 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 72hours, followed 14 days for any mortality as per the OECD (Organization for Economic Co-operation and Development) Guideline 425 (OECD; 2001). The *Pistacia integerrima* Bark extract was found to be non-toxic up to the maximum dose of 2000 mg/kg body weight. The dose selected for antiulcer evaluation was 100 and 200 mg/kg respectively.

Experimental designs

Indomethacin-induced gastric ulcer

The rats were divided to 5 groups (n=6) randomly and 6 animals were placed in each group (Eraslan *et al.,* 2020).

Group I: The normal group was applied diluted DMSO (750 µl/250 g bw) only

Group II: indomethacin (100 mg/kg) was dissolved in DMSO and was orally administered to rats to induce a maximum level of acute ulcer

Group III: omeprazole (30 mg/kg) was dissolved in DMSO and was orally administered

to the animals

Group IV: Ulcerated rats pretreated with *Pistacia integerrima* Bark extract 100 mg/kg,

Group V: Ulcerated rats pretreated with *Pistacia integerrima* Bark extract 200 mg/kg,

After 90 min, animals were sacrificed by an overdose of general anesthetic and stomach tissues were collected. Stomach was cut open in the greater curvature and ulcer scoring was done by using magnifying lens and the ulcer scored according to its severity in comparison with that of standard. 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.

Statistical analysis

The results are expressed as the mean± SD for each group. Statistical differences were evaluated using a One-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test. Results were statistically significant at $P<0.05$.

RESULTS AND DISCUSSION

Pistacia integerrima, a medicinal plant known for its diverse phytochemical composition, has been evaluated in this study for its potential anti-ulcerogenic properties using a rat model exposed to ulcerogenic agents. The hydroalcoholic extract of *Pistacia integerrima* bark demonstrated a yield of 8.5% w/w (Table 1), indicating efficient extraction of bioactive compounds from the plant material. Preliminary qualitative phytochemical tests (Table 2) revealed the presence of carbohydrates, proteins, diterpenes, saponins, flavonoids, and tannins & phenols, while amino acids, fats and oils, steroids, triterpenoids, glycosides, and alkaloids were absent. These findings suggest that the extract contains a variety of bioactive constituents that could contribute to its pharmacological activities.

Quantitative analysis (Table 3) further characterized the extract, showing it contains 0.425 mg of total phenols and 0.759 mg of total flavonoids per 100 mg of extract. Phenols and flavonoids are known for their antioxidant and anti-inflammatory properties, which are relevant in the context of ulcer prevention and healing.

In the evaluation of anti-ulcerogenic effects (Table 4), the efficacy of *Pistacia integerrima* bark extract was assessed based on the number of ulcers, ulcer index, and gastric pH levels in different experimental groups compared to controls. Group II, the untreated control, exhibited a significantly higher number of ulcers (11.35 ± 0.60) and ulcer index (5.21 ± 0.71), indicating severe ulceration induced by the ulcerogenic agents. The gastric pH in Group II was markedly low (2.45 ± 0.50), indicative of increased gastric acidity, which is detrimental to ulcer healing.

In contrast, groups treated with *Pistacia integerrima* bark extract (Groups III-V) demonstrated significant reductions in the number of ulcers and ulcer index compared to the control. Group III showed the most pronounced effect, with the lowest number of ulcers (1.55 ± 0.50) and ulcer index (0.80 ± 0.21), alongside a higher gastric pH (6.75 ± 0.65). Groups IV and V also exhibited substantial reductions in ulcer parameters, albeit to a lesser extent than Group III, suggesting a dose-dependent response or varying efficacy among different concentrations of the extract.

The observed anti-ulcerogenic effects of *Pistacia integerrima* bark extract can be attributed to its bioactive compounds, particularly phenols, flavonoids, and other phytochemicals identified in the extract. These compounds are known to exert gastroprotective effects by enhancing mucosal

defense mechanisms, reducing oxidative stress, and modulating inflammatory responses implicated in ulcer formation.

In conclusion, the findings of this study support the potential of *Pistacia integerrima* bark extract as a natural therapeutic agent for preventing and treating peptic ulcers. Further research is warranted to elucidate the specific mechanisms of action, optimize dosage regimens, and evaluate long-term safety and efficacy in clinical settings. If validated, *Pistacia integerrima* bark extract could offer a promising alternative or adjunctive treatment for gastrointestinal disorders, benefiting individuals at risk of or affected by peptic ulcers.

Table 1: % Yield of crude extract of *Pistacia integerrima*

Extract	Colour	Consistency	Yield (% w/w)
Hydroalcoholic	Brown	Solid	8.5%

Table 2: Preliminary qualitative phytochemical tests for *Pistacia integerrima* extract

Phytoconstituents	*Pistacia integerrima* extract
Primary Metabolites	
Carbohydrates	(+)
Amino acids	(-)
Proteins	(+)
Fats and oils	(-)
Secondary metabolites	
Steroids	(-)
Triterpenoids	(-)
Volatile oils	(-)
Diterpenes	(+)
Glycosides	(-)
Saponins	(+)
Flavonoids	(+)
Tannins & Phenol	(+)
Alkaloids	(-)
HE = Hydroalcoholic extract; '+' = Present; '-' = Absent	

Table 3: Total bioactive constituents content of *Pistacia integerrima*

S. No.	Extract	Total phenol content	Total flavonoid content
		mg/ 100mg	
1.	Hydroalcoholic extract	0.425	0.759

Table 4: Anti-ulcerogenic effect of *Pistacia integerrima* bark extract against ulcerogenic agents in rats

Group	Number of Ulcers	Ulcer Index	pH
Group-I	-	-	7.00 ± 0.91
Group-II	11.35 ± 0.60#	5.21±0.71#	2.45 ± 0.50#
Group-III	1.55 ± 0.50***	0.80±0.21***	6.75 ± 0.65***
Group-IV	4.85 ± 0.75*	2.75±0.61**	4.45 ± 0.50*
Group-V	2.70 ± 8.21**	1.50±0.40***	5.70 ± 0.25**

Values are expressed as mean±S.E.M. (n = 6).

#P<0.001 vs Group I; ***$P < 0.001$, ** $P < 0.01$, * $P < 0.05$ vs Group II (One-way ANOVA followed by Tukey's post hoc test).

CONCLUSION

In conclusion, *Pistacia integerrima* bark extract demonstrates promising anti-ulcerogenic properties in this study. The extract's efficacy in reducing ulcer severity and increasing gastric pH levels supports its potential therapeutic use in managing peptic ulcers. These findings underscore the importance of exploring natural remedies like *Pistacia integerrima* as alternatives to conventional treatments, offering potential benefits in terms of efficacy, safety, and affordability. Further research is warranted to elucidate its underlying mechanisms of action, optimize dosage regimens, and validate its efficacy in clinical settings. If validated, *Pistacia integerrima* bark extract could represent a valuable addition to the armamentarium against gastrointestinal disorders, benefiting individuals at risk of or affected by peptic ulcers.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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