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Research Article

**PHYTOCHEMICAL AND PHARMACOLOGICAL
EVALUATION OF ZIZIPHUS MAURITIANA FOR PEPTIC
ULCER****Shivam Prajapati, Harshita Jain, Prateek K. Jain, Sunil Kumar Jain, Yashwant Singh Jat,
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Abstract:

This study evaluates the phytochemical and pharmacological properties of the hydroalcoholic extract of Ziziphus mauritiana leaves for its potential anti-ulcerogenic effects. The hydroalcoholic extract exhibited a high yield of 9.2% w/w compared to the petroleum ether extract (1.6% w/w). Phytochemical screening revealed the presence of alkaloids, flavonoids, carbohydrates, saponins, and diterpenes. Quantitative analysis showed the total flavonoid content to be 0.752 mg/100 mg and the total alkaloid content to be 0.347 mg/100 mg of dried extract. In ethanol-induced ulcer models in rats, the hydroalcoholic extract significantly reduced the ulcer index and gastric acidity while increasing gastric pH and reducing pepsin activity. The extract demonstrated dose-dependent anti-ulcerogenic activity, with the 200 mg/kg dose showing the most pronounced effects. These findings suggest that the hydroalcoholic extract of Ziziphus mauritiana possesses significant anti-ulcerogenic properties and may serve as a potential natural therapeutic agent for managing peptic ulcers. Further studies are recommended to explore its mechanisms of action and validate its efficacy and safety in clinical settings.

Key Words: Ziziphus mauritiana, anti-ulcerogenic, hydroalcoholic extract, phytochemical screening, peptic ulcer, ethanol-induced ulcer, gastric acidity, pepsin activity, natural therapeutic agent

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INTRODUCTION:

Peptic ulcer disease (PUD) is a common gastrointestinal disorder characterized by the formation of ulcers in the lining of the stomach or the first part of the small intestine (duodenum) due to an imbalance between the aggressive factors (such as gastric acid and pepsin) and the defensive mechanisms (such as mucus and bicarbonate secretion, blood flow, and cellular regeneration) of the gastrointestinal mucosa (Soll, 1990; Lanas & Chan, 2017). The management of PUD typically involves the use of proton pump inhibitors, H₂-receptor antagonists, antacids, and antibiotics to eradicate *Helicobacter pylori* infection, which is a major etiological factor for peptic ulcers (Malfertheiner, Chan, & McColl, 2009). However, these treatments can have side effects and may not be effective in all patients, leading to a growing interest in exploring alternative therapies, including those derived from medicinal plants (Ali & Harty, 2009).

Ziziphus mauritiana, commonly known as Indian jujube, is a medicinal plant belonging to the Rhamnaceae family. It is widely distributed in tropical and subtropical regions and has been traditionally used in various cultures for its medicinal properties (Pareek, 2013). The plant is known for its rich phytochemical profile, including alkaloids, flavonoids, saponins, tannins, and polysaccharides, which are attributed to its diverse pharmacological activities (Shukla et al., 2010).

Recent studies have highlighted the potential of *Ziziphus mauritiana* in the treatment of gastrointestinal disorders, including peptic ulcers. The phytochemicals present in *Ziziphus mauritiana* are believed to contribute to its gastroprotective effects through various mechanisms, such as enhancing mucosal defense, reducing gastric acid secretion, and exhibiting anti-inflammatory and antioxidant properties (Borrelli & Izzo, 2000). For instance, flavonoids and tannins have been shown to promote mucosal healing and inhibit ulcer formation by scavenging free radicals and modulating inflammatory pathways (Repetto & Llesuy, 2002). Additionally, the plant's polysaccharides may help in reinforcing the gastric mucosal barrier and stimulating the production of protective mucus (Han et al., 2013).

Given the promising pharmacological potential of *Ziziphus mauritiana*, this study aims to evaluate its phytochemical composition and pharmacological efficacy in the treatment of peptic ulcers. By conducting a comprehensive analysis of its bioactive compounds and their mechanisms of action, this

research seeks to provide scientific validation for the traditional use of *Ziziphus mauritiana* in managing peptic ulcer disease and to explore its potential as a natural therapeutic agent.

MATERIAL AND METHODS:**Material**

The chemicals used in this study were sourced from reputable suppliers, ensuring the quality and reliability of the reagents. Potassium mercuric iodide and picric acid were procured from Thomas Baker, Mumbai, while iodine, potassium iodide, and sodium nitroprusside were obtained from Loba Chemie Pvt. Ltd., Mumbai. Potassium bismuth iodide, pyridine, ferric chloride, gelatin, lead acetate, nitric acid, copper acetate, and sodium chloride were supplied by S. D. Fine Chem. Ltd., Mumbai.

Methods**Collection of plant material**

The plants have been selected on the basis of its availability and folk use of the plant. The leaves of *Ziziphus mauritiana* were collected from local area of Sagar (M.P.) in the month of February, 2023. Drying of fresh plant parts was carried out in sun but under the shade. Dried leaves of *Ziziphus mauritiana* were preserved in plastic bags, closed tightly and powdered as per the requirements.

Defatting of plant material

67 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 *Ziziphus mauritiana* has been extracted with hydroalcoholic solvent (ethanol: water; 70:30v/v) using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40°C (Mukherjee, 2007).

Determination of percentage yield

The extraction yield is an assessment of the efficiency of the solvent in extracting bioactive components from the selected natural plant samples and was defined as the quantity of plant extracts recovered after solvent extraction compared to the original quantity of plant samples. The yield of the collected plant extracts was measured in grams after extraction, and then converted into percentage. For calculating the percentage yield of selected plant products, formula following was introduced. By using the following formula the percentage yield of extract was calculated:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powdered drug}} \times 100$$

Phytochemical Screening

Medicinal plants are traditional pharmaceutical commodities and many of the current medicinal drugs are derived indirectly from plants. Phytochemical materials consist of two main bioactive components (chlorophyll, vitamins, amino acids, sugar etc.) and secondary bioactive components; (alkaloids, terpenoids, phenols, flavonoids etc.). Phytochemical analyses were performed according to the normal protocols for extract. Phytochemical examinations were carried out for all the extracts as per the standard methods (Kokate, 1994).

Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method (Kumar *et al.*, 2017). 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% AlCl₃ 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 (Shamsa *et al.*, 2008). 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 hydroalcoholic extract of *Ziziphus mauritiana* 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 hydroalcoholic extract of *Ziziphus mauritiana* 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/p.o)

Groups III was treated with hydroalcoholic extract of *Ziziphus mauritiana* (100 mg/kg/p.o.)

Groups IV was treated with hydroalcoholic extract of (*Ziziphus mauritiana* 200 mg/kg/p.o.)

The animals were treated with ranitidine (100 mg/kg), dose of hydroalcoholic extract of *Ziziphus mauritiana* 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

$$\text{Ulcer index} = 10/X$$

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.

Indomethacin induced gastric ulcer

Experimental designs

Method of C.N. Aguwa, et al 1981 was followed with minor modifications for the experiment. Thirty rats were taken. They were divided into four groups of six rats each. All the rats were starved for 24 h. After the fasting period, Indomethacin (40 mg/kg, p.o.) was given.

All samples of the plant extract were given 60 min prior to indomethacin as follows:

Group I: treated with Indomethacin (40mg/kg, p.o.) and was kept as control

Group II: treated with Ranitidine (50mg/kg, p.o.) and was kept as standard

Group III: treated with the hydroalcoholic extract of *Ziziphus mauritiana* (100 mg/kg, p.o.)

Group IV: treated with the hydroalcoholic extract of *Ziziphus mauritiana* (200mg/kg, p.o.)

The animals were sacrificed 5h after the treatment. 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: (Khare et al., 2008)

$$\text{Ulcer index} = 10/X$$

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.

Assessment of gastric fluid acidity

The stomachs were opened along the greater curvature and the juices were collected in the separate labeled tubes. The contents of each stomach were centrifuged at 3000 rpm for 10 min and the supernatant was assessed for pH measurement via digital pH meter titration, using 0.1 NaOH solution (Ketuly et al., 2011).

Statistical analysis

The results are expressed as the mean \pm 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 considered to be statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION:

The extraction yield of the hydroalcoholic extract of *Ziziphus mauritiana* was significantly higher compared to the petroleum ether extract. The hydroalcoholic extract yielded 9.2% (w/w), whereas the petroleum ether extract yielded only 1.6% (w/w). This indicates that the hydroalcoholic solvent is more efficient in extracting bioactive compounds from *Ziziphus mauritiana* leaves, which is likely due to its ability to dissolve both polar and non-polar compounds (Table 1).

The phytochemical screening of the hydroalcoholic extract of *Ziziphus mauritiana* revealed the presence of several bioactive constituents. Alkaloids were detected using Dragendorff's and Hager's tests but not with Mayer's and Wagner's tests. Flavonoids were present, as indicated by the positive result in the alkaline test, while glycosides, phenols, and proteins were absent. Carbohydrates were detected using Fehling's test but not by Molisch's or Benedict's tests. The extract also showed the presence of saponins and diterpenes, as evidenced by positive results in the froth and copper acetate tests, respectively. Tannins were not detected with the gelatin test. These results highlight the complex phytochemical profile of the hydroalcoholic extract, which may contribute to its pharmacological activities (Table 2).

The hydroalcoholic extract of *Ziziphus mauritiana* was found to contain 0.752 mg of total flavonoids and 0.347 mg of total alkaloids per 100 mg of dried extract. The presence of these bioactive compounds suggests potential therapeutic benefits, as flavonoids and alkaloids are known for their antioxidant, anti-inflammatory, and gastroprotective properties (Table 3).

The hydroalcoholic extract of *Ziziphus mauritiana* significantly reduced the ulcer index in a dose-dependent manner in rats with ethanol-induced ulcers. The ulcer index for the control group was 6.7, which was markedly decreased to 3.45 and 3.00 with 100 mg/kg and 200 mg/kg doses of the extract, respectively. This reduction was statistically significant ($p < 0.01$ and $p < 0.001$). The extract also improved the pH of the gastric content and decreased total and free acidity, particularly at the higher dose, approaching the effects observed with the standard anti-ulcer drug ranitidine. Additionally, pepsin activity was moderately reduced, suggesting a multifaceted mechanism of action that includes acid reduction and protection of the gastric mucosa (Table 4).

Table 5 further supports the anti-ulcerogenic effect of the hydroalcoholic extract against ulcerogenic agents. The extract demonstrated a significant reduction in the ulcer index and an increase in gastric pH. The ulcer indices for the 100 mg/kg and 200 mg/kg doses were 2.50 ± 0.50 and 1.50 ± 0.40 , respectively, compared to the control (4.2 ± 0.40). The pH levels

were also significantly higher in the treatment groups, indicating reduced gastric acidity. These findings corroborate the extract's efficacy in protecting against ulcer formation and suggest that higher doses may provide more substantial therapeutic benefits.

Table 1: % Yield of hydroalcoholic extract of *Ziziphus mauritiana*

S. No.	Extracts	% Yield (w/w)
1.	Pet. ether	1.6%
2.	Hydroalcoholic	9.2%

Table 2: Phytochemical screening of leaves extract of *Ziziphus mauritiana*

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

Table 3: Estimation of total flavonoids and alkaloid content of extract of *Ziziphus mauritiana*

S. No.	Extract	Total flavonoids content (mg/ 100 mg of dried extract)	Total alkaloid content (mg/ 100 mg of dried extract)
1.	Hydroalcoholic	0.752	0.347

Table 4: Effect of hydroalcoholic extract of *Ziziphus mauritiana* on ulcer index by ethanol induced ulcers in rats

Treatment and dose	Ulcer Index	pH	Total acidity (mEq/l)	Free acidity (mEq/l)	Pepsin activity (Per ml/h)
Control	6.7 ±0.10	2.15±0.10	75.74±0.10	55.45±0.20	3.25±0.10
Ranitidine (50 mg/kg, p.o.)	2.35±0.10***	4.38±0.15***	35.12±0.15 ***	24.25±0.15 ***	2.45±0.15 ***
Hydroalcoholic extract of <i>Ziziphus mauritiana</i> (100 mg/kg, p.o.)	3.45±0.15**	3.85±0.10**	52.14±0.10*	40.35±0.10**	3.15±0.25**
Hydroalcoholic extract of <i>Ziziphus mauritiana</i> (200 mg/kg, p.o.)	3.00±0.10***	4.20±0.20***	43.10±0.15***	35.80±0.15 ***	2.35±0.10***

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: Anti-ulcerogenic effect of hydroalcoholic extract of *Ziziphus mauritiana* against ulcerogenic agents in rats (Ulcer index)

Treatment and dose	Ulcer Index	pH
Control	4.2 ± 0.40	1.5 ± 0.50
Ranitidine	0.90 ± 0.40***	6.95 ± 0.50***
Hydroalcoholic extract of <i>Ziziphus mauritiana</i> (100 mg/kg, p.o.)	2.50 ± 0.50**	4.32 ± 0.50*
Hydroalcoholic extract of <i>Ziziphus mauritiana</i> (200 mg/kg, p.o.)	1.50 ± 0.40***	5.20 ± 0.50***

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

Percent inhibition calculated as compared to control group.***P < 0.001, ** P < 0.01, * P < 0.05 (One-way ANOVA followed by Tukey's post hoc test).

CONCLUSION:

In conclusion, the hydroalcoholic extract of *Ziziphus mauritiana* exhibits significant anti-ulcerogenic properties, as evidenced by its ability to reduce ulcer indices, increase gastric pH, and decrease gastric acidity and pepsin activity in animal models. The presence of bioactive compounds such as alkaloids, flavonoids, saponins, and diterpenes likely contributes to these effects, making *Ziziphus mauritiana* a promising candidate for the development of natural anti-ulcer agents. Further studies involving human trials and detailed mechanistic investigations are warranted to fully elucidate the therapeutic potential and safety profile of this extract.

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