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EXTRACTION, PHYTOCHEMICAL ANALYSIS AND ANTI-ULCER ACTIVITY OF *ZIZIPHUS ROTUNDIFOLIA*

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ABSTRACT

The present study was carried out to investigate the extraction, phytochemical analysis, and anti-ulcer activity of the hydroalcoholic extract of *Ziziphus rotundifolia*. The extractive value of the hydroalcoholic extract was found to be 7.25% (w/w) with a dark brown appearance, indicating efficient extraction of phytoconstituents. Preliminary phytochemical screening revealed the presence of flavonoids, phenols, tannins, carbohydrates, and proteins, while alkaloids, glycosides, steroids, resins, and saponins were absent. Quantitative estimation showed that the extract contained 0.72 mg/100 mg of total phenolic content (GAE) and 0.91 mg/100 mg of total flavonoid content (QE), suggesting significant antioxidant potential. Acute oral toxicity studies confirmed that the extract was safe up to 2000 mg/kg, with no signs of toxicity or mortality. The anti-ulcer activity was evaluated using an ethanol-induced gastric ulcer model in rats. The hydroalcoholic extract of *Ziziphus rotundifolia* demonstrated a significant, dose-dependent reduction in ulcer index. It also increased gastric pH and decreased total acidity, free acidity, and pepsin activity. The higher dose (200 mg/kg) showed effects comparable to ranitidine, indicating strong gastroprotective activity. The anti-ulcer effect may be attributed to the presence of flavonoids, phenols, and tannins, which exhibit antioxidant, cytoprotective, and antisecretory properties. The findings suggest that *Ziziphus rotundifolia* has promising potential as a natural agent for the management of gastric ulcers.

Keywords: *Ziziphus rotundifolia*, Hydroalcoholic extract, Phytochemical screening, Anti-ulcer activity, Ethanol-induced ulcer, Gastric parameters, Phenolic content, Flavonoid content, Gastroprotective activity.

INTRODUCTION

Peptic ulcer disease is one of the most prevalent gastrointestinal disorders, affecting a significant proportion of the global population. It is primarily characterized by lesions in the gastric or duodenal mucosa due to an imbalance between aggressive factors such as gastric acid, pepsin, reactive oxygen species (ROS), and defensive mechanisms including mucus secretion, bicarbonate production, and mucosal integrity (Tanyeli *et al.*, 2023). Various etiological factors such as excessive alcohol consumption, stress, Helicobacter pylori infection, and prolonged use of non-steroidal anti-inflammatory drugs (NSAIDs) contribute to ulcer formation. Among these, ethanol is widely used in experimental models as it rapidly penetrates

the gastric mucosa, causing cellular damage, oxidative stress, and mucosal erosion (Beiranvand *et al.,* 2021).

Despite the availability of conventional anti-ulcer drugs such as H_2 receptor antagonists and proton pump inhibitors, their long-term use is often associated with adverse effects and recurrence of ulcers. This has led to increasing interest in the use of medicinal plants as alternative therapeutic agents due to their safety, efficacy, and affordability (Begg *et al.,* 2023).

Plant-derived compounds, particularly phenolics and flavonoids, have been extensively studied for their gastroprotective properties. These compounds exert their effects by scavenging free radicals, enhancing mucus production, and strengthening the gastric mucosal barrier (Kmail, 2024).

The genus *Ziziphus* (family Rhamnaceae) comprises numerous species that are widely distributed in tropical and subtropical regions and are traditionally used in various systems of medicine. These plants are known to contain a wide range of bioactive constituents, including alkaloids, flavonoids, tannins, saponins, and terpenoids, which contribute to their diverse pharmacological activities. Several species of *Ziziphus*, such as *Ziziphus jujuba* and *Ziziphus nummularia*, have been reported to possess significant anti-ulcer, antioxidant, anti-inflammatory, and gastroprotective properties. Studies have shown that extracts of these plants reduce gastric lesions, decrease acid secretion, and enhance mucosal defense in experimental models (Ara *et al.,* 2008).

Ziziphus rotundifolia, a medicinal plant belonging to the same genus, has been traditionally used in the treatment of various ailments, including gastrointestinal disorders. However, scientific validation of its anti-ulcer potential is still limited. Considering the rich phytochemical composition of *Ziziphus* species and their established therapeutic importance, it is hypothesized that *Ziziphus rotundifolia* may also exhibit significant gastroprotective activity (Gaur *et al.,* 2024).

Therefore, the present study was undertaken to evaluate the extraction, phytochemical analysis, and anti-ulcer activity of the hydroalcoholic extract of *Ziziphus rotundifolia*. The study aims to investigate its phytochemical constituents, quantify important bioactive compounds such as phenols and flavonoids, and assess its anti-ulcer potential using an ethanol-induced gastric ulcer model in rats. This investigation may provide scientific evidence supporting the traditional use of the plant and contribute to the development of novel plant-based anti-ulcer therapies.

MATERIALS AND METHODS

Materials

The materials used in the present study included fresh plant material of *Ziziphus rotundifolia*, which was collected, authenticated, and processed for extraction. Analytical grade solvents such as ethanol and distilled water were used for the preparation of the hydroalcoholic extract. Various chemical reagents including Mayer's reagent, Dragendorff's reagent, Fehling's solution, Benedict's solution, ferric chloride, lead acetate, and vanillin-HCl were employed for preliminary phytochemical screening. Standard compounds such as gallic acid and quercetin were used for the estimation of total phenolic and flavonoid content, respectively. Ranitidine was used as the standard anti-ulcer

drug. Wistar rats were utilized for in vivo anti-ulcer studies, and all other chemicals and reagents used were of analytical grade.

Methods

Extraction by maceration method

Powdered leaves were weighed (50 gm) and packed in (250 ml) air tight glass Bottle. The leaves of *Ziziphus rotundifolia* were subjected to extraction by Hydroalcoholic (Ethanol: water; 70:30v/v) as solvents for about 24 hrs (Kokate, 1994). The liquid extract was collected in a tarred conical flask. The solvent removed from the extract by evaporation method. The extracts obtained with different solvents were weighed to a constant weight and percentage w/w basis was calculated.

Determination of percentage yield

The percentage yields of extract were calculated by using following formula:

Percentage yield = Weight of extract/ Weight of powder drug Taken×100

Phytochemical analysis

Preliminary phytochemical screening means to investigate the plant material in terms of its active constituents. In order to detect the various constituents present in the different extract of *Ziziphus rotundifolia*, were subjected to the phytochemical tests as per standard methods. Phytochemical screening was revealed for the presence of alkaloids, glycosides, carbohydrates, tannins, resins, flavonoids, steroids, proteins and amino acids (Audu *et al.,* 2007).

Quantitative estimation of phenols and flavonoids

Estimation of total phenol content

Estimation of total phenol content Total phenol content of the extracts was evaluated with folin-ciocalteu method (Gaur Mishra *et al.,* 2017). Samples containing polyphenols are reduced by the folin-ciocalteu reagent there by producing blue colored complex. The phenolic concentration of extracts was evaluated from a gallic acid calibration curve. To prepare a calibration curve, 2 ml aliquots of 5, 10, 15, 20, and 25 µg/ml gallic acid solutions were mixed with 1ml folin ciocalteu reagent (diluted ten-fold) and 1 ml (7.5 g/L) sodium carbonate. After incubation at 25°C for 10 min, the quantative phenolic estimation was performed at 765 nm against reagent blank by UV Spectrophotometer.

The calibration curve was constructed by putting the value of absorbance vs. concentration. A similar procedure was adopted for the extract (1000µg/ml) as above described in the preparation of calibration curve. All determinations were performed in triplicate. Total phenolic content was expressed as milligrams of gallic acid equivalent (GAE) mg per 100mg of extract.

Estimation of total flavonoids content

The aluminum chloride colorimetric method was modified (Gaur Mishra *et al.,* 2017). Quercetin was used to make the calibration curve. Ten milligrams of quercetin was dissolved in 10 ml methanol and then diluted for 5-25 µg/ml. The diluted standard solutions (2 ml) were separately mixed with 1 ml of 2% aluminum chloride. After incubation at room temperature for 10 min, the absorbance of the reaction mixture was measured at 420 nm with a UV spectrophotometer. Similarly, extracts solutions (1000 µg/ml) were reacted with aluminum chloride for determination of Flavonoid content. Total flavonoid content was expressed as milligrams of Quercetin equivalent (QE) mg per 100mg of extract.

Ethanol induced *in vivo* antiulcer activity of extract of *Ziziphus rotundifolia*

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 rotundifolia* 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 (Narra *et al.*, 2015).

Hydroalcoholic extract of *Ziziphus rotundifolia* 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.

Experimental designs

The rats were divided into four groups of six each (Khandare *et al.*, 2009).

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

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

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

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

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

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.

Measurement of gastric acidity

The evaluation of gastric juice pH was conducted, as described by Beiranvand *et al.*,

(2021). In a concise manner, the gastric content from each rat was subjected to centrifugation at a speed of 5000 revolutions per minute for duration of 10 min. The resulting supernatant was carefully collected, and subsequently, 1 mL of the supernatant was mixed with an equal volume of distilled water. The pH of this mixture was then determined using a pH meter.

Statistical analysis

The experimental data are shown as the mean ± S.E.M for every experimental group (Kaur and Sen, 2017). A one-way analysis of variance (ANOVA) was used to evaluate statistical differences among groups, followed by Tukey's post hoc test for multiple comparisons. A significant effect was found ($p < 0.05$).

RESULTS AND DISCUSSION

The present study was undertaken to evaluate the phytochemical profile and anti-ulcer potential of the hydroalcoholic extract of *Ziziphus rotundifolia* using an ethanol-induced gastric ulcer model in rats. The results obtained from extractive value determination, phytochemical screening, quantitative estimation, and in vivo anti-ulcer studies provide substantial evidence supporting the therapeutic significance of the plant.

The extractive value study (Table 1) revealed that the hydroalcoholic extract yielded 7.25% (w/w) and exhibited a dark brown colour. This indicates the efficiency of the hydroalcoholic solvent system in extracting a broad range of phytoconstituents, particularly polyphenolic compounds. The yield suggests that the plant is a good source of bioactive constituents suitable for further pharmacological evaluation.

Preliminary phytochemical screening (Table 2) demonstrated the presence of important secondary metabolites such as flavonoids, phenols, tannins, carbohydrates, and proteins, while alkaloids, glycosides, steroids, resins, and saponins were absent. The presence of flavonoids and phenolic compounds is particularly significant, as these are well known for their antioxidant and gastroprotective properties. Tannins may contribute to ulcer protection by forming a protective layer over the gastric mucosa, thereby reducing irritation and preventing further damage.

Quantitative estimation further supported these findings, with total phenolic content reported as 0.72 mg/100 mg (Table 3) and total flavonoid content as 0.91 mg/100 mg (Table 4). These compounds play an important role in scavenging free radicals and reducing oxidative stress, which is a major contributing factor in ethanol-induced gastric mucosal injury.

The anti-ulcer activity of the extract was evaluated using an ethanol-induced ulcer model, which is widely accepted for assessing gastroprotective agents. The ulcer index (Table 5) was significantly reduced in extract-treated groups compared to the control group, indicating effective protection against gastric mucosal damage. The reduction was dose-dependent, with the higher dose (200 mg/kg) showing greater protection, comparable to the standard drug ranitidine.

Evaluation of gastric pH (Table 6) revealed that the extract significantly increased pH levels compared to the control group, indicating a reduction in gastric acidity. This suggests that the extract may possess antisecretory or acid-neutralizing properties,

which help in protecting the gastric mucosa from acid-induced damage.

Similarly, total acidity (Table 7) and free acidity (Table 8) were significantly reduced in the treated groups, further confirming the antisecretory activity of the extract. The decrease in acidity indicates that the extract may inhibit gastric acid secretion, possibly through modulation of proton pumps or histamine-mediated pathways.

Pepsin activity (Table 9), an important factor in ulcer formation, was also significantly reduced following treatment with the extract. Since pepsin contributes to proteolytic damage of the gastric mucosa, its inhibition plays an important role in ulcer healing. The reduction in pepsin activity may be secondary to increased pH or a direct inhibitory effect of phytoconstituents present in the extract.

The results indicate that the hydroalcoholic extract of *Ziziphus rotundifolia* exerts a multifaceted gastroprotective effect, involving antioxidant activity, inhibition of gastric acid secretion, reduction of pepsin activity, and enhancement of mucosal defense mechanisms. The observed effects are likely due to the presence of flavonoids, phenols, and tannins, which collectively contribute to its pharmacological activity.

Table 1: Extractive values obtained from *Ziziphus rotundifolia*

S. No.	Extract	Colour	% Yield (w/w)
1	Hydroalcoholic	Dark brown	7.25

Table 2: Preliminary phytochemical screening of *Ziziphus rotundifolia* extract

S. No.	Phytoconstituents	Test Name	Hydroalcoholic extract
1	Alkaloids	Mayer's Test	-ve
		Dragendorff's Test	-ve
		Wagner's Test	-ve
		Hager's Test	-ve
2	Glycosides	Raymond's Test	-ve
		Killer Killani Test	-ve
		Legal Test	-ve
3	Carbohydrates	Molisch's Test	-ve
		Fehling's Test	+ve
		Benedict's Test	+ve
4	Tannins	Vanillin- HCl Test	-ve
		Gelatin Test	+ve
5	Flavonoids	Lead acetate Test	+ve
		Alkaline Reagent Test	+ve
6	Resins	Turbidity Test	-ve
7	Steroids	Libermann- Bur chard Test	-ve
		Salkowski Reaction	-ve
8	Proteins & Amino acids	Biuret Test	+ve

		Precipitation test	-ve
9.	Phenols	Ferric chloride test	+ve
10.	Saponins	Froth Test	-ve

[+ve = Positive; -ve = Negative]

Table 3: Total phenolic content of hydroalcoholic extract of *Ziziphus rotundifolia*

S. No.	Extract	Total phenol content (mg/100mg)
1	Hydroalcoholic	0.72

Table 4: Total Flavonoid content of hydroalcoholic extract of *Ziziphus rotundifolia*

S. No.	Extract	Total Flavonoid content (mg/100mg)
1	Hydroalcoholic	0.91

Table 5: Effect of Hydroalcoholic extract of *Ziziphus rotundifolia* on ulcer index by ethanol induced ulcers in rats

Treatment and Dose	Ulcer Index (Mean ± SEM)
Control	7.65 ± 0.25
Ranitidine (50 mg/kg, p.o.)	2.98 ± 0.15***
Hydroalcoholic extract of *Ziziphus rotundifolia* (100 mg/kg, p.o.)	3.75 ± 0.25**
Hydroalcoholic extract of *Ziziphus rotundifolia* (200 mg/kg, p.o.)	3.28 ± 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 6: Effect of Hydroalcoholic extract of *Ziziphus rotundifolia* on gastric parameters i.e. pH by ethanol-induced ulceration in rats

Treatment and Dose	Gastric pH (Mean ± SEM)
Control	2.95 ± 0.25
Ranitidine (50 mg/kg, p.o.)	4.75 ± 0.25***
Hydroalcoholic extract of *Ziziphus rotundifolia* (100 mg/kg, p.o.)	3.82 ± 0.20**
Hydroalcoholic extract of *Ziziphus rotundifolia* (200 mg/kg, p.o.)	4.21 ± 0.15***

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 7: Effect of Hydroalcoholic extract of *Ziziphus rotundifolia* on gastric parameters i.e. total acidity ethanol- induced ulceration in rats

Treatment and Dose	Total Acidity (mEq/L, Mean ± SEM)
Control	75.25 ± 0.15
Ranitidine (50 mg/kg, p.o.)	42.25 ± 0.25***
Hydroalcoholic extract of *Ziziphus rotundifolia* (100 mg/kg, p.o.)	65.58 ± 0.20*
Hydroalcoholic extract of *Ziziphus rotundifolia* (200 mg/kg, p.o.)	45.65 ± 0.25***

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 8: Effect of Hydroalcoholic extract of *Ziziphus rotundifolia* on gastric parameters i.e. free acidity by ethanol-induced ulceration in rats

Treatment and Dose	Free Acidity (mEq/L)
Control	58.12 ± 0.15
Ranitidine (50 mg/kg, p.o.)	26.12 ± 0.25***
Hydroalcoholic extract of *Ziziphus rotundifolia* (100 mg/kg, p.o.)	37.15 ± 0.15**
Hydroalcoholic extract of *Ziziphus rotundifolia* (200 mg/kg, p.o.)	35.45 ± 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 9: Effect of Hydroalcoholic extract of *Ziziphus rotundifolia* on gastric parameters i.e. pepsin activity by ethanol-induced ulceration in rats

Treatment and dose	Pepsin activity (Per ml/h)
Control	3.85 ± 0.15
Ranitidine (50 mg/kg, p.o.)	2.75 ± 0.17 ***
Hydroalcoholic extract of *Ziziphus rotundifolia* (100 mg/kg, p.o.)	3.42 ± 0.20 **
Hydroalcoholic extract of *Ziziphus rotundifolia* (200 mg/kg, p.o.)	2.56 ± 0.25 ***

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).

CONCLUSION

The present study demonstrates that the hydroalcoholic extract of *Ziziphus rotundifolia* possesses significant anti-ulcer activity against ethanol-induced gastric ulcers in rats. The extract showed a dose-dependent reduction in ulcer index along with improvement in gastric parameters such as increased pH and decreased total acidity, free acidity, and pepsin activity. Phytochemical investigations confirmed the presence of bioactive constituents such as flavonoids, phenols, and tannins, which are known to exhibit antioxidant and gastroprotective properties. The quantitative estimation of phenolic and flavonoid content further supports the therapeutic potential of the extract. The anti-ulcer effect of the extract may be attributed to its ability to reduce gastric acid secretion, inhibit pepsin activity, and enhance mucosal defense mechanisms. Additionally, the absence of toxicity at higher doses indicates that the extract is relatively safe for use. The findings validate the traditional use of *Ziziphus rotundifolia* in the management of gastrointestinal disorders and suggest its potential as a natural and effective anti-ulcer agent. Further studies are recommended to isolate the active constituents and to explore the exact mechanism of action.

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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