

#### International Journal of Pharmaceutics and Drug Research

ISSN: 2347-6346 Available online at <u>http://ijpdr.com</u>

#### **Original Research Article**

STUDY OF PLANTS SECONDARY METABOLITES AND ANTIULCER ACTIVITY OF ACACIA MODESTA LEAVES EXTRACT

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\*Article History:

Received: 19/02/2025 Revised: 05/03/2025 Accepted: 27/03/2025

#### ABSTRACT

The present study was undertaken to evaluate the phytochemical composition and antiulcer activity of the hydroalcoholic extract of Acacia modesta leaves. The extract was prepared using successive solvent extraction, with a maximum yield of 7.25% obtained from the hydroalcoholic solvent system. Preliminary phytochemical screening revealed the presence of flavonoids, phenols, proteins, carbohydrates, and tannins, while alkaloids and saponins were absent. Quantitative estimation confirmed notable amounts of flavonoids (0.952 mg/100 mg) and phenols (0.645 mg/100 mg). The antiulcer potential was assessed using the ethanol-induced ulcer model in rats. The extract demonstrated a significant, dose-dependent reduction in ulcer index, comparable to the standard antiulcer drug, Ranitidine. The treated groups showed a marked increase in gastric pH and a decrease in total acidity, free acidity, and pepsin activity. These findings suggest that the hydroalcoholic extract of Acacia modesta leaves possesses promising antiulcer activity, likely attributed to its phytoconstituents with antioxidant and gastroprotective properties.

**Keywords:** *Acacia modesta*, antiulcer activity, secondary metabolites, flavonoids, ethanol-induced ulcers, gastric protection

#### **INTRODUCTION**

Medicinal plants have long been recognized as a valuable source of bioactive compounds, particularly secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids, and saponins, which play vital roles in plant defense and therapeutic applications (Pandey & Rizvi, 2009). These natural compounds have demonstrated significant pharmacological effects. including antioxidant, anti-inflammatory, antimicrobial, and antiulcer activities (Balunas & Kinghorn, 2005). Among gastrointestinal disorders, peptic ulcers remain a major global health concern, often caused by factors such as Helicobacter pylori infection, stress, NSAID use, and alcohol consumption (Sung et al., 2009). Conventional antiulcer therapies are often associated with side effects, which necessitates the exploration of safer, plantbased alternatives.

Acacia modesta, a plant native to South Asia and traditionally used in folk medicine, is wide range known to contain а of pharmacologically active constituents including flavonoids, tannins, and saponins (Khan et al., 2016). Although its various parts have been explored for analgesic, antiinflammatory, and antimicrobial properties, limited scientific data exist regarding the antiulcer potential of its leaves extract. The current study is therefore aimed at evaluating the phytochemical profile and antiulcer activity of Acacia modesta leaves extract in experimental models. By identifying and quantifying secondary metabolites, and correlating their presence with biological activity, this research seeks to validate traditional claims and explore the plant's potential as a natural antiulcer agent.

#### MATERIALS AND METHODS Materials

Various analytical-grade chemicals and reagents were utilized for the phytochemical screening and antiulcer activity assessment of Acacia modesta leaves extract. These included Potassium Mercuric Iodide, Picric Acid, Ferric Chloride, and Lead Acetate (Thomas Baker, Mumbai); Iodine, Potassium Iodide, Sodium Nitroprusside, Sodium Hydroxide, and Folin-Ciocalteu reagent (Loba Chemie Pvt. Ltd., Mumbai); Potassium Bismuth Iodide, Pyridine, Gelatin, Nitric Acid, Copper Acetate, and Sodium Chloride (S.D. Fine Chem. Ltd., Mumbai); and Ethanol. and Methanol, Chloroform Fine Chemicals, (Qualigens Mumbai). Fehling's solution was procured from Central Drug House Ltd., New Delhi. All chemicals used were of analytical grade and used without further purification.

#### Methods

#### **Collection of plant material**

The plants have been selected on the basis of its availability and folk use of the plant. Leaves of *Acacia modesta* were collected from local area of Bhopal in the month of January, 2025. Drying of fresh plant parts was carried out in sun but under the shade. Dried leaves of *Acacia modesta* were preserved in plastic bags, closed tightly and powdered as per the requirements.

#### **Extraction procedure**

#### **Defatting of plant material**

50 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 *Acacia modesta* has been extracted with hydroalcoholic solvent (ethanol: water; 70:30) 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 For into percentage. calculating the percentage yield of selected plant products, formula following was introduced. By using the following formula the percentage yield of extract was calculated:

% Yield  $\frac{\text{Weight of Extract}}{\text{Weight of powdered drug}} X100$ 

#### **Phytochemical Screening**

Medicinal plants are traditional pharmaceutical commodities and many of the current medicinal drugs are derived indirectly from plants. Phytochemical materials consist two main bioactive of 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 (Mishra *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. 2 ml (1mg/ml) of this solution was used for the estimation of flavonoid. 2 ml of 2% AlCl<sub>3</sub> solution was added to 2 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

#### Estimation of total phenol content

The total phenol content of the extract was determined by the modified folin-ciocalteu method (Mishra et al., 2017). 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10-50µg/ml was prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this solution was used for the estimation of phenol. 2 ml of each extract or standard was mixed with 1 ml of folinciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/L) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15 min for colour development. The absorbance was measured at 765 nm using а spectrophotometer.

#### *In vivo* anti-ulcer activity of hydroalcoholic extract of *Acacia modesta* 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 ( $25\pm2$  °C, 55–65%). Rats received standard rodent chow and water *ad libitum*.

Rats were acclimatized laboratory to 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 Acacia modesta were administered to each group of rats (Each group carries 6 rats) and they were observed continuously for 1 hour and then at halfhourly 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 Acacia modesta 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 hydroalcoholic extract of *Acacia modesta* 100 mg/kg/p.o.

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

The animals were treated with ranitidine (100 mg/kg), dose of hydroalcoholic extract of *Acacia modesta* 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 current study explored the phytochemical profile and antiulcer potential of the hydroalcoholic extract of Acacia modesta leaves. The extraction process yielded 1.42% for the petroleum ether extract and 7.25% for the hydroalcoholic extract (Table 1). The higher vield and solubility of the hydroalcoholic extract justified its selection for pharmacological evaluation. Preliminary phytochemical screening indicated the presence of flavonoids, phenols, proteins, and tannins, while alkaloids, glycosides, saponins, and diterpenes were absent (Table 2). The presence of flavonoids (0.952 mg/100 mg) and phenols (0.645 mg/100 mg) was quantitatively confirmed (Table 3), both of are known to possess strong which antioxidant and mucosal protective properties. In the ethanol-induced gastric ulcer model, the hydroalcoholic extract demonstrated a dosedependent reduction in ulcer index, with the 200 mg/kg dose showing a significant effect  $(3.15 \pm 0.20)$  comparable to the standard drug Ranitidine  $(2.65 \pm 0.20)$ , as shown in Table 4. This suggests a protective effect of the extract against ethanol-induced mucosal damage, potentially through antioxidant mechanisms and acid suppression.

The gastric pH was significantly increased in treated groups, especially at the 200 mg/kg dose ( $4.10 \pm 0.10$ ), in contrast to the control ( $2.85 \pm 0.15$ ), supporting reduced gastric acidity (Table 5). Additionally, total acidity and free acidity were significantly reduced in extract-treated groups compared to the control (Tables 6 and 7), indicating a suppressive effect on acid secretion. This may be attributed to the phenolic constituents, which are known to reduce histamine- and stressinduced acid secretion.

Moreover, the pepsin activity was also significantly reduced at higher doses of the extract ( $2.86 \pm 0.10$ ), suggesting a role in reducing proteolytic degradation of gastric mucosa (Table 8). This combined reduction in acid and enzyme activity, along with mucosal protection, strongly supports the antiulcer efficacy of the extract.

S. No.	Extracts	% Yield (w/w)
1.	Pet. ether	1.42%
2.	Hydroalcoholic	7.25%

### Table 1: % Yield of extract of Acacia modesta

S. No.	Constituents	Hydroalcoholic 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

#### Table 2: Phytochemical screening of extract of Acacia modesta

Table 3: Estimation of total flavonoids and phenol content of extract of Acacia modesta

S. No.	Extract	Total flavonoids content (mg/ 100 mg of dried extract)	Total phenol content (mg/ 100 mg of dried extract)
1.	Hydroalcoholic	0.952	0.645

# Table 4: Effect of hydroalcoholic extract of Acacia modesta on ulcer index by ethanol induced ulcers in rats

Treatment and dose	Ulcer Index
Control	$6.8 \pm 0.15$
Ranitidine (50 mg/kg, p.o.)	2.65±0.20***
Hydroalcoholic extract of Acacia modesta (100 mg/kg, p.o.)	3.45±0.10 <sup>**</sup>
Hydroalcoholic extract of Acacia modesta (200 mg/kg, p.o.)	3.15±0.20***

Table 5: Effect of hydroalcoholic extract of Acacia modesta on gastric parameters i.e. pH by

#### ethanol-induced ulceration in rats

Treatment and dose	рН
Control	2.85±0.15
Ranitidine (50 mg/kg, p.o.)	4.65±0.25***
Hydroalcoholic extract of Acacia modesta (100 mg/kg, p.o.)	3.92±0.15**
Hydroalcoholic extract of Acacia modesta (200 mg/kg, p.o.)	4.10±0.10***

Table 6: Effect of hydroalcoholic extract of Acacia modesta on gastric parameters i.e. total

#### acidity ethanol- induced ulceration in rats

Treatment and dose	Total acidity (mEq/lt)
Control	76.15±0.10
Ranitidine (50 mg/kg, p.o.)	35.15±0.15***
Hydroalcoholic extract of Acacia modesta (100 mg/kg, p.o.)	54.45±0.25*
Hydroalcoholic extract of Acacia modesta (200 mg/kg, p.o.)	43.35±0.15***

Table 7: Effect of hydroalcoholic extract of Acacia modesta on gastric parameters i.e. free

#### acidity by ethanol-induced ulceration in rats

Treatment and dose	Free acidity (mEq/lt)
Control	55.25±0.15
Ranitidine (50 mg/kg, p.o.)	24.45±0.15 ***
Hydroalcoholic extract of Acacia modesta (100 mg/kg, p.o.)	40.36±0.10**
Hydroalcoholic extract of Acacia modesta (200 mg/kg, p.o.)	35.45±0.20 ***

Table 8: Effect of hydroalcoholic extract of Acacia modesta on gastric parameters i.e. pepsinactivity by ethanol-induced ulceration in rats

Treatment and dose	Pepsin activity (Per ml/h)
Control	3.36±0.20
Ranitidine (50 mg/kg, p.o.)	2.25±0.10 ***
Hydroalcoholic extract of Acacia modesta (100 mg/kg, p.o.)	3.25±0.15**
Hydroalcoholic extract of Acacia modesta (200 mg/kg, p.o.)	2.86±0.10***

#### CONCLUSION

In conclusion, the hydroalcoholic extract of *Acacia modesta* leaves demonstrates significant antiulcer activity, likely due to its flavonoid and phenolic content and its ability to modulate gastric acidity and enzyme activity. These findings validate its traditional medicinal use and provide a basis for future studies to isolate active constituents and explore clinical relevance.

#### **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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