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

Original Research Article

PHYTOCHEMICAL ANALYSIS AND INDOMETHACIN INDUCED ANTIULCER ACTIVITY OF *CATUNAREGAM SPINOSA*

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***Article History:**

Received: 11/02/2025
Revised: 25/02/2025
Accepted: 09/03/2025

ABSTRACT

The present study was undertaken to evaluate the anti-ulcer potential of ethanolic extract of *Catunaregam spinosa* using an indomethacin-induced gastric ulcer model in rats, alongside comprehensive phytochemical screening, extract standardization, and assessment of bioactive contents. Phytochemical screening of *Catunaregam spinosa* revealed the presence of significant secondary metabolites, including flavonoids, phenols, alkaloids, tannins, and saponins, which are known for their gastroprotective and antioxidant properties. The extract demonstrated total phenolic and flavonoid content of 0.958 mg/100 mg and 0.647 mg/100 mg, respectively. In the in vivo anti-ulcer model, *Catunaregam spinosa* at 200 mg/kg significantly reduced the ulcer index (1.90 ± 0.55) and number of ulcers (2.35 ± 0.50) while elevating the gastric pH (5.75 ± 0.85), compared to the ulcer control group. The results were comparable to those obtained with the standard drug omeprazole (30 mg/kg). *Catunaregam spinosa* demonstrated significant anti-ulcer activity supported by both phytochemical richness and *in vivo* pharmacological evidence, indicating its potential as a natural therapeutic agent.

Keywords: Gastric ulcer, Anti-ulcer potential, ulcer index, *Catunaregam spinosa*, Ethanolic extract, Phytochemical screening.

INTRODUCTION

Peptic ulcer disease (PUD) is a prevalent gastrointestinal disorder characterized by mucosal erosions in the stomach or duodenum, largely caused by an imbalance between aggressive factors such as acid, pepsin, Helicobacter pylori infection, and non-steroidal anti-inflammatory drugs (NSAIDs), and the defensive mechanisms of the gastrointestinal mucosa (Sung *et al.*, 2009). Among NSAIDs, indomethacin is widely recognized for its ulcerogenic potential due to its inhibition of prostaglandin synthesis, which compromises mucosal protection (Wallace, 2008). Despite the availability of synthetic antiulcer agents, their long-term use is associated with adverse effects, which has intensified interest in natural products and medicinal plants as alternative therapies.

Catunaregam spinosa (Thunb.) Tirveng., formerly known as *Randia dumetorum*, is a small thorny tree belonging to the Rubiaceae family and is traditionally used in various systems of medicine across Asia for its anti-inflammatory, antimicrobial, and wound healing properties (Kirtikar and Basu, 2006). Several parts of the plant, including its fruits and roots, are used in traditional formulations for treating gastric ailments, yet scientific validation of its antiulcer potential remains limited. Phytochemical investigations have revealed that *C. spinosa* contains a wide array of secondary metabolites, including alkaloids,

flavonoids, saponins, tannins, glycosides, and terpenoids, many of which possess known anti-inflammatory and gastroprotective effects (Chaudhary *et al.,* 2012; Rahmatullah *et al.*, 2010). These constituents are thought to contribute to the plant's medicinal properties by enhancing mucosal defense mechanisms, reducing oxidative stress, and modulating inflammatory pathways.

The present study aims to evaluate the phytochemical composition of *Catunaregam spinosa* extracts and investigate its antiulcer activity using an indomethacin-induced ulcer model in rats. This approach not only validates traditional knowledge but also provides a scientific basis for its potential application in the development of herbal antiulcer agents.

MATERIALS AND METHODS

Extraction of plant materials by maceration method

Powdered leaves were weighed (50 gm) of *Catunaregam spinosa* and packed in extraction bottle. The plant powders were subjected to extraction by ethanol solvent. 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

Plants generate compounds known as phytochemicals. These are created by the primary and secondary metabolisms of the plant. These phytochemicals are necessary for plants to survive or to fend off other plants, animals, insects, microbial pests, and pathogens. They also protect plants from illness and damage induced by environmental threats such as pollution, UV, stress, and drought. They have been employed as traditional medicine and as poisons since ancient times (Kokate, 1994).

Quantitative estimation of phenols and flavonoids

Estimation of total phenol content

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 phenol 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* anti-ulcer activity of *Catunaregam spinosa* extract using Indomethacin-induced gastric ulcer**

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 ℃, 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 to control and supervise experimental animals by the Ministry of Environment and Forests, Government of India, New Delhi, India.

Toxicity study

Healthy adult male albino rats were fasted overnight before the experiment. Different doses (50-2000 mg/kg, P.O) of the *Catunaregam spinosa* 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 hours, 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 (Schlede, 2002). The *Catunaregam spinosa* extract was found to be non-toxic up to the maximum dose of 2000 mg/kg body weight. The doses selected for antiulcer evaluation were 100 and 200 mg/kg, respectively.

Table 1: Toxicity study

Observations	Acute toxicity
Skin and Fur	Normal
Eyes	Normal
Respiration	Normal
Sleep	Normal
Coma	Not seen
Mortality	Not seen

Experimental designs

Indomethacin-induced gastric ulcer

The rats were divided to 5 groups (n=6) randomly and 6 animals were placed in each group:

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 *Catunaregam spinosa* extract 100 mg/kg,

Group V: Ulcerated rats pretreated with *Catunaregam spinosa* 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 experimental data are presented as the mean ± SD for each experimental group. One-way analysis of variance (ANOVA) was employed to assess statistical differences between groups, followed by Tukey's post hoc test for multiple comparisons. A statistically significant effect was observed ($p < 0.05$).

RESULTS AND DISCUSSION

The present study aimed to investigate the phytochemical profile and antiulcer activity of the ethanolic extract of *Catunaregam spinosa*. The findings provide promising evidence for the gastroprotective potential of the plant.

The percentage yield of the ethanolic extract of *Catunaregam spinosa* was 8.52% (Table 2), which is within the expected range for medicinal plant extractions. The extract displayed a green color and solid consistency, indicative of concentrated phytoconstituents.

Preliminary phytochemical screening revealed the presence of several primary and secondary metabolites (Table 3). The extract tested positive for carbohydrates, amino acids, and proteins, which are essential primary metabolites. Among the secondary metabolites, flavonoids, saponins, tannins, alkaloids, and phenols were present, while diterpenes and glycosides were absent.

The significant presence of phenolic compounds (0.958 mg/100 mg) and flavonoids (0.647 mg/100 mg) as reported in Table 4 further supports the potential of the extract in exerting antioxidant and antiulcer effects. Phenolic compounds are well known for their ability to scavenge free radicals and reduce oxidative damage in the gastric mucosa, while flavonoids contribute to enhancing mucosal protection by promoting mucus secretion and modulating inflammation.

The antiulcer activity of *C. spinosa* was evaluated using the indomethacin-induced gastric ulcer model in rats. Indomethacin is a well-established ulcerogenic agent that works primarily by inhibiting cyclooxygenase-mediated prostaglandin synthesis, thereby disrupting mucosal defense mechanisms (Wallace, 2008). As shown in Table 5, the ulcer number significantly increased in the indomethacin-treated group (Group II), confirming the successful induction of gastric ulcers.

Treatment with the ethanolic extract at different doses (Groups III–V) significantly reduced the number of ulcers compared to the ulcer control group. The lowest ulcer number (1.56 ± 0.25, ***P<0.001) was observed in Group III, indicating strong antiulcerogenic activity. Similarly, the ulcer index (Table 6) was markedly reduced in extract-treated groups, with Group III showing the most

significant reduction (0.75 ± 0.25, ***P<0.001).

The gastric pH data (Table 7) further corroborate the antiulcer effect. Indomethacin treatment drastically lowered the gastric pH (3.25 ± 0.45), suggesting increased acidity. In contrast, treatment with *C. spinosa* extract elevated the pH towards normal levels, indicating decreased acidity and enhanced mucosal protection. The highest pH value was observed in Group III (6.65 ± 0.25, ***P<0.001), which aligns with the lowest ulcer index and ulcer number in the same group.

The observed antiulcerogenic activity may be attributed to the synergistic action of flavonoids, tannins, alkaloids, saponins, and phenols present in the extract. Flavonoids and tannins are known to strengthen the gastric mucosa and inhibit acid secretion, while saponins can enhance mucus production and alkaloids exert anti-inflammatory effects.

Table 2: % Yield of crude extract of *Catunaregam spinosa*

Extract	Colour	Consistency	Yield (% w/w)
Catunaregam spinosa			
Ethanol	Green	Solid	8.52%

Table 3: Preliminary phytochemical tests of *Catunaregam spinosa* extract

Phytoconstituents	Ethanolic extract of *Catunaregam spinosa*
i) Primary Metabolites	
Carbohydrates	(+)
Amino acids	(+)
Proteins	(+)
ii) Secondary metabolites	
Diterpenes	(-)
Glycosides	(-)
Saponins	(+)
Flavonoids	(+)
Tannins	(+)
Alkaloids	(+)
Phenol	(+)
'+' = Present; '-' = Absent	

Table 4: Total phenol and flavonoid content of *Catunaregam spinosa*

S. No.	Extract	Total phenol content	Total flavonoid content
		mg/ 100mg	
1.	Ethanolic extract	0.958	0.647

Table 5: Anti-ulcerogenic effect of *Catunaregam spinosa* extract against ulcerogenic agents in rats (Number of Ulcers)

Group	Number of Ulcers
Group-I	-
Group-II	11.25 ± 0.70#
Group-III	1.56 ± 0.25***
Group-IV	3.85 ± 0.25*
Group-V	2.35 ± 0.50**

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).

Table 6: Anti-ulcerogenic effect of *Catunaregam spinosa* extract *against* ulcerogenic agents in rats (Ulcer index)

Group	Ulcer Index
Group-I	-
Group-II	5.50 ± 0.65#
Group-III	0.75 ± 0.25***
Group-IV	2.85 ± 0.45**
Group-V	1.90 ± 0.55***

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

Table 7: Anti-ulcerogenic effect of *Catunaregam spinosa* extract *against* ulcerogenic agents in rats (pH)

Group	pH
Group-I	7.02 ± 0.70
Group-II	3.25 ± 0.45#
Group-III	6.65 ± 0.25***
Group-IV	5.05 ± 0.65*
Group-V	5.75 ± 0.85**

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

The present study demonstrated that the ethanolic extract of *Catunaregam spinosa* possesses significant antiulcer activity in an indomethacin-induced gastric ulcer model in rats. Phytochemical screening confirmed the presence of bioactive constituents such as flavonoids, tannins, alkaloids, phenols, and saponins, which are known to contribute to gastroprotective effects. The extract not only reduced the number of ulcers and ulcer index but also normalized gastric pH, suggesting both antisecretory and mucosal protective mechanisms. These results provide scientific validation for the traditional use of *C. spinosa* in managing gastric disorders and highlight its potential as a natural therapeutic agent for peptic ulcer disease. Further research is warranted to isolate the active compounds, understand their mechanisms of action, and evaluate their clinical efficacy and safety in human studies.

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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