

PHYTOCHEMICAL SCREENING AND IN VIVO ANTIULCER ACTIVITY OF ETHANOLIC EXTRACT OF *SPILANTHES OLERACEA***Shaikh Faizur Rahman*, Khushboo Chauhan, Satkar Prasad****RKDF School of Pharmaceutical Sciences, Bhabha University, Bhopal***Corresponding Author's E mail: rahmansf001@gmail.com

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ABSTRACT

The present study aimed to evaluate the phytochemical composition and anti-ulcerogenic potential of the hydroalcoholic extract of *Spilanthus oleracea* roots. The extract showed a yield of 11.25% w/w and was found to contain flavonoids, phenols, proteins, carbohydrates, and diterpenes. Quantitative estimation revealed total flavonoid and phenolic contents of 0.854 mg/100 mg and 0.625 mg/100 mg, respectively. The gastroprotective activity was assessed in indomethacin-induced ulcer models in rats by measuring the number of ulcers, ulcer index, gastric pH, and gastric juice volume. Indomethacin caused severe gastric lesions, acidification, and increased gastric volume, while treatment with the extract showed a dose-dependent reduction in ulcer number and index, restoration of gastric pH, and normalization of gastric volume. The extract at 200 mg/kg exhibited significant protection, comparable to standard cimetidine therapy. These findings suggest that the hydroalcoholic extract of *Spilanthus oleracea* roots possesses notable anti-ulcerogenic activity, likely due to its flavonoid and phenolic constituents, and may serve as a potential natural remedy for gastric ulcers.

Keywords: *Spilanthus oleracea*, Hydroalcoholic extract, Anti-ulcerogenic, Flavonoids, Phenolic compounds, Gastric ulcer, Indomethacin-induced ulcer, Gastroprotection.

INTRODUCTION

Gastric ulcer is a common gastrointestinal disorder characterized by the disruption of the gastric mucosal lining due to an imbalance between aggressive factors, such as gastric acid, pepsin, and reactive oxygen species, and defensive mechanisms, including mucus secretion, bicarbonate production, and prostaglandin synthesis¹. Non-steroidal anti-inflammatory drugs (NSAIDs), particularly indomethacin, are well-known ulcerogenic agents that cause gastric mucosal injury by inhibiting prostaglandin synthesis and increasing oxidative stress². Conventional anti-ulcer drugs, such as H₂ receptor antagonists and proton pump inhibitors, though effective, are associated with several adverse effects, including headache, diarrhea, and long-term risk of gastric infections³.

Medicinal plants are gaining attention as alternative therapies due to their bioactive phytoconstituents, which can provide cytoprotection, antioxidant activity, and acid modulation with minimal side effects ⁴. *Spilanthes oleracea*, commonly known as the toothache plant, belongs to the family Asteraceae and is traditionally used for treating pain, inflammation, and gastrointestinal disorders. The plant is rich in bioactive compounds such as flavonoids, phenolics, and diterpenes, which are reported to exhibit anti-inflammatory, antioxidant, and cytoprotective properties ⁵.

Phytochemical screening of plant extracts provides essential information about the presence of secondary metabolites responsible for pharmacological activities. Flavonoids and phenolic compounds, in particular, are known to protect gastric mucosa by scavenging free radicals, enhancing mucus secretion, and inhibiting gastric acid hypersecretion. High-performance thin-layer chromatography (HPTLC) is a reliable, rapid, and sensitive method for the qualitative and quantitative analysis of bioactive markers in plant extracts, enabling standardization and quality control ⁶.

The present study focuses on the phytochemical evaluation and in vivo anti-ulcerogenic potential of ethanolic extract of *Spilanthes oleracea* roots against indomethacin-induced gastric ulcers in rats. The study aims to explore the protective effect of the extract on ulcer parameters, including number of ulcers, ulcer index, gastric pH, and gastric juice volume, along with estimation of bioactive constituents, providing a scientific basis for its traditional use in gastrointestinal disorders.

MATERIAL AND METHODS

Material

The study was carried out using the roots of *Spilanthes oleracea*, which were extracted with a hydroalcoholic solvent (ethanol and water). Phytochemical analysis and quantification of bioactive compounds were performed using standard reagents, including quercetin, gallic acid, ferric chloride, lead acetate, Benedict's and Fehling's solutions, Molisch's reagent, copper acetate, and xanthoproteic reagent. Indomethacin was used to induce gastric ulcers in Wistar rats, while cimetidine served as the standard anti-ulcer drug. Analytical and experimental procedures were supported by instruments such as an HPTLC system, UV-Vis spectrophotometer, pH meter, micropipettes, and standard laboratory glassware.

Methods

Extraction by maceration process

The cool, stable samples obtained from the plants were cut into small pieces and dried under shade for 3 to 4 weeks. 50 gram shade dried roots of *Spilanthes oleracea* were coarsely powdered. Powdered of

Spilanthes oleracea has been extracted with hydroalcoholic solvent (ethanol: water; 70:30v/v) solvent using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40°C⁷.

Determination of percentage yield

The yield of the collected plant extract was measured in grams after extraction, and then converted into percentage. 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 examinations were carried out for all the extracts as per the standard methods⁸.

Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method⁹. 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% AlCl₃ 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.

6.7 Estimation of total phenol content

The total phenol content of the extract was determined by the modified folin-ciocalteu method¹⁰. 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 folin-ciocalteu 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 a spectrophotometer.

6.8 *In vivo* antiulcer activity of *Spilanthes oleracea* extract

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

Toxicity study

Healthy adult male Wistar rats were fasted overnight before the experiment. Different doses (50-2000 mg/kg, P.O) of *Spilanthes oleracea* extract were administered to each group of six rats. The rats were observed continuously for 1 hour, then at half-hourly intervals for 4 hours, and further monitored up to 72 hours for any gross behavioral changes. Additionally, observations continued for 14 days to check for mortality, in accordance with OECD Guideline 425 ¹¹. The *Spilanthes oleracea* 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.

Indomethacin-induced gastric ulcer

The rats were divided into five groups (n=6) randomly, and six animals were placed in each group ¹².

Group I: The normal group was treated with distilled water

Group II: The control group was induced with indomethacin (40 mg/kg) administered to rats after fasting for 36 h

Group III: The standard drug group was treated with Cimetidine 100 mg/kg/p.o

Group IV: Ulcerated rats pretreated with *Spilanthes oleracea* extract 100 mg/kg

Group V: Ulcerated rats pretreated with *Spilanthes oleracea* extract 200 mg/kg

Animals were treated with vehicle, extracts or Cimetidine orally 30 min prior to induction of gastric lesions. The animals were sacrificed 5 h after treatment with the ulcerogenic agent to assess the ulcer score and mucous-producing activity. The stomach was cut open along the greater curvature, and ulcer scoring was done by using a magnifying lens, and the ulcer was scored according to its severity in comparison with that of the standard. The ulcer index was determined using the formula:

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

Where $X = \text{Total mucosal area} / \text{Total ulcerated area}$.

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.

Determination of volume and pH

The volume of gastric juice of each rat was measured after centrifugation at 1000 rpm for 10 min and analysed. An aliquot of 1 ml of gastric juice was diluted with 1 ml of distilled water, and the pH of the solution was measured using a pH meter ¹².

Statistical analysis

The experimental data are presented as the mean $\pm$ 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 evaluated the phytochemical composition and in vivo anti-ulcerogenic activity of the hydroalcoholic extract of *Spilanthes oleracea* roots. The extractive yield was 11.25% w/w (Table 1), indicating efficient extraction of bioactive compounds. Phytochemical screening (Table 2) revealed the presence of flavonoids, phenols, proteins, carbohydrates, and diterpenes, while alkaloids, glycosides, saponins, and tannins were absent. This suggests that the gastroprotective activity of the extract may be mainly due to flavonoids and phenolic compounds, which are known for their antioxidant, cytoprotective, and anti-secretory properties.

Quantitative estimation showed that the total flavonoid content was 0.854 mg/100 mg, and total phenolic content was 0.625 mg/100 mg (Table 3). These bioactive compounds can enhance gastric mucosal defense, scavenge free radicals, and inhibit acid hypersecretion, contributing to protection against ulcerogenic agents.

Indomethacin administration (Group-II) caused severe ulceration with a high number of ulcers (15.2) and ulcer index (18.1), accompanied by a significant decrease in gastric pH (3.15) and an increase in gastric volume (4.89 ml) (Tables 4–7). This is consistent with the ulcerogenic effect of NSAIDs, which involves inhibition of protective prostaglandins, increased acid secretion, and reduced mucus production.

Treatment with cimetidine (Group-III) significantly reduced the number of ulcers (4.5), ulcer index (5.1), restored gastric pH (6.02), and normalized gastric volume (2.48 ml), demonstrating its protective effect.

The hydroalcoholic extract of *Spilanthes oleracea* showed a dose-dependent gastroprotective effect. At 100 mg/kg (Group-IV), the number of ulcers and ulcer index were reduced to 8.3 and 9.4, respectively, with moderate restoration of gastric pH (5.34) and a decrease in gastric volume (2.96 ml). At 200 mg/kg (Group-V), ulcer number and ulcer index further decreased to 5.4 and 5.7, while gastric pH increased to 5.96 and gastric volume decreased to 2.41 ml, indicating enhanced protection at higher doses.

The gastroprotective effect of the extract can be attributed to its bioactive constituents, which strengthen the gastric mucosal barrier, reduce oxidative damage, enhance mucus secretion, and modulate gastric acid secretion. The hydroalcoholic extract of *Spilanthes oleracea* roots demonstrated significant anti-ulcerogenic activity, comparable to standard therapy, supporting its potential use in the management of gastric ulcers.

Table 1: % Yield of roots extract of *Spilanthes oleracea*

S. No.	Extract	% Yield (w/w)
1.	Hydroalcoholic	11.25

Table 2: Phytochemical screening of extract of *Spilanthes oleracea*

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

[+ve=Positive, -ve=Negative]

Table 3: Estimation of total flavonoids and phenol content of extract of *Spilanthes oleracea*

S. No.	Extract	Total flavonoids content	Total phenol content
		(mg/ 100 mg of dried extract)	
1.	Hydroalcoholic	0.854	0.625

**Table 4: Anti-ulcerogenic effect of *Spilanthes oleracea* extract against ulcerogenic agents in rats
(Number of Ulcers)**

Group	Treatment	Number of Ulcers
Group-I	Distilled water	0.00 ± 0.00
Group-II	Indomethacin (40 mg/kg)	15.2 ± 0.41#
Group-III	Cimetidine (100 mg/kg) + Indomethacin (40 mg/kg)	4.5 ± 0.26***
Group-IV	<i>Spilanthes oleracea</i> extract (100 mg/kg) + Indomethacin (40 mg/kg)	8.3 ± 0.35*
Group-V	<i>Spilanthes oleracea</i> extract (200 mg/kg) + Indomethacin (40 mg/kg)	5.4 ± 0.28***

**Table 5: Anti-ulcerogenic effect of *Spilanthes oleracea* extract against ulcerogenic agents in rats
(Ulcer index)**

Group	Treatment	Ulcer Index
Group-I	Distilled water	0.00 ± 0.00
Group-II	Indomethacin (40 mg/kg)	18.1 ± 0.45#
Group-III	Cimetidine (100 mg/kg) + Indomethacin (40 mg/kg)	5.1 ± 0.24***
Group-IV	<i>Spilanthes oleracea</i> extract (100 mg/kg) + Indomethacin (40 mg/kg)	9.4 ± 0.36*
Group-V	<i>Spilanthes oleracea</i> extract (200 mg/kg) + Indomethacin (40 mg/kg)	5.7 ± 0.30***

Table 6: Anti-ulcerogenic effect of *Spilanthes oleracea* extract on gastric pH in rats

Group	Treatment	pH
Group-I	Distilled water	6.52 ± 0.14
Group-II	Indomethacin (40 mg/kg)	3.15 ± 0.12
Group-III	Cimetidine (100 mg/kg) + Indomethacin (40 mg/kg)	6.02 ± 0.16
Group-IV	<i>Spilanthes oleracea</i> extract (100 mg/kg) + Indomethacin (40 mg/kg)	5.34 ± 0.13
Group-V	<i>Spilanthes oleracea</i> extract (200 mg/kg) + Indomethacin (40 mg/kg)	5.96 ± 0.15

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

Table 7: Anti-ulcerogenic effect of *Spilanthes oleracea* extract on volume in rats

Group	Treatment	Volume (ml)
Group-I	Distilled water	2.18 ± 0.11
Group-II	Indomethacin (40 mg/kg)	4.89 ± 0.15
Group-III	Cimetidine (100 mg/kg) + Indomethacin (40 mg/kg)	2.48 ± 0.09
Group-IV	<i>Spilanthes oleracea</i> extract (100 mg/kg) + Indomethacin (40 mg/kg)	2.96 ± 0.12
Group-V	<i>Spilanthes oleracea</i> extract (200 mg/kg) + Indomethacin (40 mg/kg)	2.41 ± 0.08

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 hydroalcoholic extract of *Spilanthes oleracea* roots demonstrated significant anti-ulcerogenic activity in indomethacin-induced gastric ulcer models in rats. The extract exhibited dose-dependent protection by reducing the number of ulcers and ulcer index, restoring gastric pH, and decreasing gastric juice volume. Phytochemical analysis revealed the presence of flavonoids, phenols, proteins, carbohydrates, and diterpenes, which likely contributed to its gastroprotective effects. These findings suggest that *Spilanthes oleracea* has potential as a natural remedy for the prevention and management of gastric ulcers.

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