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Research Article

## Phytochemical screening and evaluation of antiulcer activity of ethanolic extract of *Spathodea campanulata* leaves

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### ABSTRACT

Peptic disorders like Gastroesophageal reflux disease, gastritis, peptic ulcer, duodenal ulcer, etc., are the common in today's life style. This may be due to stressful life style or improper balance diet. The pathology behind these disorders may be discrepancy between offensive and defensive mechanisms either by excess secretion of acid and pepsin or diminished ability of the gastro-duodenal mucosal barrier to protect against stomach acid-pepsin secretion. Non-steroidal anti-inflammatory drugs (NSAIDs) are a class of the most commonly used medicines and proven to be effective for certain disorders. Some people use NSAIDs on daily basis for preventive purpose. But a variety of severe side effects can be induced by NSAIDs. Studies have shown that edible natural ingredients exhibit preventive benefit of gastric ulcer. Therefore present study was designed to evaluate antiulcer activity of ethanolic extract of *Spathodea campanulata* (*S. campanulata*) leaves in rats. Qualitative analysis of various phytochemical constituents was determined by the well-known test protocol available in the literature. The *in vivo* anti-ulcer activity of ethanolic extract was assessed against aspirin-induced gastric ulcer in rats. Depending on the model, outcome measures were volume and pH of gastric fluid, free acidity, total acidity and ulcer index as well as percent inhibition of ulcer index. Preliminary phytochemical screening revealed the presence of carbohydrates, glycosides, alkaloids, flavonoids, phenols & tannins, saponin. Further ethanolic extract of 200 and 400mg/ kg /p.o reduced the gastric volume, pH, ulcer number, ulcer index, free acidity and total acidity in aspirin induced ulcer models in rats. The findings of this study confirmed that *S. campanulata* extract has anti-ulcer pharmacologic activity due to one or more of the secondary metabolites present in it. Therefore, this study validates its anti-ulcer use in Indian folk medicine. Further investigations on isolation of specific phytochemicals and elucidating mechanisms of action are needed.

**Keywords:** *Spathodea campanulata*, Phytochemical constituents, Antiulcer, Non-steroidal anti-inflammatory drugs, Aspirin-induced gastric ulcer.

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### INTRODUCTION

Gastric ulcer is a common gastrointestinal tract (GIT) disorder that affects about 10% of the world population<sup>1</sup>. It's characterized by GIT bleeding, perforation and erosion of the mucosa wall due to imbalance between aggressive (acid, pepsin and *Helicobacter pylori*) and defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide, mucosal blood flow and growth factors)<sup>2,3</sup>. The incidence of this disease is multifactorial which include *H. pylori*, non-steroidal anti-inflammatory drugs (NSAIDs), smoking, stress, chronic alcohol, altered prostaglandin E series metabolism and bad dietary habits<sup>4,5</sup>. Aspirin is acetylsalicylic acid often used to treat pain, fever and inflammation<sup>6-8</sup>. Despite its therapeutics benefits, it's known to induce gastric ulcer in both human and animals<sup>9, 10</sup>. The pathogenesis of aspirin-

induced gastric ulceration includes the aspirin blocking the activities of the cyclooxygenase enzymes (COX-1 and COX-2) hence leading to reduced mucus and bicarbonate secretion, decreased mucosal blood flow, impaired platelet aggregation, alteration of microvascular structures leading to epithelia damage, increased leukocyte adherence and increased production of reactive oxygen species (ROS), increased lipid peroxidation and neutrophil infiltration as well as decreased antioxidant enzymes<sup>11-13</sup>. Treatment of gastric ulcer using a conventional therapy faces a major setback because most of the drugs currently available in the market show limited efficacy against gastric diseases and are often associated with severe side effects. However, several medicinal plants are used in folk medicine to treat gastrointestinal disorders and they have been shown to

produce promising results in the treatment of these pathologies<sup>14</sup>. *S. campanulata* P. Beauv species belonging to the Bignoniaceae family. Bark contains sterols, triterpenoids, tranorin, vanillic acid, ferulic acid, verminoside, pelargonidin diglycoside, maldivin, tannin. Leaves contain polyhydroxy sterol spathodol, quercetin, chlorogenic acid<sup>15</sup>. This species has many uses in folk medicine such as; the flowers are employed as diuretic and anti-inflammatory, while the leaves are used against kidney diseases, urethra inflammations and as an antidote against animal poisons. The stem bark preparations are employed against enemas, fungus skin diseases, herpes, stomachaches and diarrhea<sup>16</sup>. The leaves have furnished spathodol, caffeic acid, other phenolic acids and flavonoids<sup>17-19</sup>. Several medicinal property have been reported, including flavonoids of eight bignoniaceous plants<sup>20</sup>, Leaf has been reported to possess antiplasmodial activity<sup>21</sup>, analgesic and anti-inflammatory properties<sup>22</sup>, hypoglycemic, anti-HIV and antimalarial activity were also observed in stem bark extracts<sup>23,24</sup>. The literature survey revealed that so far no scientific studies carried out on its *in vivo* anti-ulcer activity of the ethanol extract of leaves of *S. campanulata*. Hence, in the present study, we focused to evaluate the *in vivo* anti-ulcer activity of the ethanol extract of *S. campanulata* leaves on aspirin-induced gastric ulcers in rats.

## MATERIALS AND METHODS

### Plant material

The leaves of *S. campanulata* were collected from local area of Bhopal (M.P.) in the month of July, 2019.

### Chemicals and reagents

All the drugs, solvents and chemicals used in the study were of analytical grade. Omeprazole was obtained as a gift sample from PBRI Lab, Bhopal, MP, India. All other chemicals e.g. methanol, ether, formalin, sodium hydroxide, citric acid monohydrate, trichloroacetic acid, sodium nitrate, sodium potassium tartrate, ethylene diamine tetra acetic acid disodium salt were purchased from S. D. Fine Chemicals, Mumbai, India. Tris buffer, Topfer's reagent, Folin's Reagent and Phenolphthalein were purchased from Hi-Media Pvt. Ltd., Mumbai, India.

### Extraction

Dried pulverized leaves of *S. campanulata* were placed in thimble of soxhlet apparatus. Soxhlation was performed at 40-60°C using petroleum ether as non-polar solvent at first. Exhausted plant material (marc) was dried and then extracted with ethanol. Soxhlation was continued till no colour was observed in siphon tube. For confirmation of exhausted plant marc (i.e. completion of extraction), colorless solvent was collected from siphon tube and completion of extraction was confirmed by absence of any residual solvent, The entire extract was concentrated to dryness using rotary flash evaporator under reduced pressure and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts<sup>25</sup>.

### Phytochemical screening

The crude ethanolic extract of *S. campanulata* was qualitatively tested for the detection of alkaloids, flavonoids, saponins, tannins, glycosides, carbohydrates, reducing sugars, proteins, glucosides, terpenoids, and steroids following standard procedures<sup>26</sup>.

### Animals

Wistar rats (150-200 gm) 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 of PBRI, Bhopal, constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

### Acute toxicity test

Preliminary experiments were carried out on rats (n=6). Ethanolic leaves extract of *S. campanulata* were administered orally in different doses to find out the range of doses which cause zero and 100 % mortality of animals. Acute oral toxicity was conducted according to the method of Organization for Economic Co-operation and Development (OECD) <sup>27</sup>. Animals were kept fasting providing only water, ethanolic leave extract of *S. campanulata* were given p.o. in doses of 5,50, 300 and 2000 mg/kg/p.o. administered orally for 14 days of six groups of rats (n=6) and the animals were kept under observation for mortality as well as any behavioral changes for evaluation of a possible anti-ulcer effect.

### Experimental designs

#### Aspirin-induced gastric ulcer

Group -1: Control

Group -2: Omeprazole (Standard)

Group -3: Ethanolic leave extract of *S. campanulata* (200mg/kg, p.o.)

Group -4: Ethanolic leave extract of *S. campanulata* (400mg/kg, p.o.)

The animals were fasted for 24 hr prior to the experiment. Under anesthesia, ulcers were induced by applying aspirin (200 mg/kg, p.o.) over the anterior serosal surface of the stomach for 60 seconds. The animals were treated with Omeprazole (20mg/kg, p.o.), low dose of ethanolic leave extract of *S. campanulata* (200 m/kg p.o.) or high dose of ethanolic leave extract of *S. campanulata* (400 m/kg p.o.) [Once daily, for 5 days after the induction of ulcer, while the control group received only the vehicle. The rats were sacrificed on the 5<sup>th</sup> day, the stomachs removed and cut open along the greater curvature<sup>28</sup>.

### Biochemical estimations

#### Determination of gastric volume

After sacrificing the rat, the stomach portion was removed. The gastric contents were transferred into the centrifuge tube, centrifuged and filtered. The supernatant liquid was then transferred to a measuring cylinder and the volume was measured.

#### Determination of pH of gastric content

One ml of the gastric juice was collected and the pH was directly measured by using Digital pH meter.

#### Determination of ulcer index

The stomachs were opened along the greater curvature; the number of ulcers was counted.

Ulcer scoring was done by the following scoring system:

- 0=no ulcer,
- 1=superficial ulcer,
- 2=deep ulcer,
- 3=perforation.

Ulcer index was calculated by using following formula

$$UI=UN+US+UP \times 10^{-1}$$

Where,

UI=ulcer index,

UN=mean of ulcer number,

US=mean of ulcer score,

UP=ulcer probability for each group.

**Determination of free acidity and total acidity**

The total volume of gastric content was measured. The gastric contents were centrifuged and filtered. One ml of the gastric juice was pipette out and the solution was titrated against 0.1N sodium hydroxide using 2 to 3 drops of topfer's reagent as indicator, to the end point when the solution turned to yellowish orange colour was observed. This indicated the volume of NaOH required neutralizing the free hydrochloric acid present in the gastric juice. Then 2 to 3 drops of phenolphthalein solution was added and titration was continued until a definite red colour appears. The difference between the two readings indicated the volume of NaOH required neutralizing the combined acid present in the gastric juice. The sum of the two titrations was the total acid present in the gastric juice.

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH}}{\text{Volume of gastric juice used}}$$

**RESULTS AND DISCUSSIONS**

The crude extracts so obtained after the soxhlet apparatus, extracts was further concentrated on water bath for evaporate the solvents completely to obtain the actual yield of extraction. The yield of *S. campanulata* extracts was 4.3 %w/w. The results of preliminary phytochemical screening of ethanolic extract of *S. campanulata* leaves are shown in Table 1. The extract showed the presence of carbohydrates, glycosides, alkaloids, flavonoids, phenols & tannins, saponin.

The acute oral toxicity study was done according to the OECD 425 guidelines. No adverse changes and mortality were observed in animals, which orally received ethanolic extract (2000 mg/kg) of *S. campanulata* leaves. This indicates that 2000 mg/kg is maximum safe dose. So 1/10<sup>th</sup> and 1/5<sup>th</sup> i.e. 200 and 400 mg/kg of body weight, of the maximum safe dose were selected for studying *in vivo* anti-ulcer effects. Aspirin induced ulcer was used to study the effect of ethanolic extract of *S. campanulata* leaves on gastric acid secretion and mucus secretion. Ethanolic leave extract of *S. campanulata* revealed that it has significant anti-ulcer activity. Usually, NSAIDs and corticosteroids are widely used in clinical practice as anti-inflammatory agents. With the exception of newer highly selective COX-2 inhibitors, NSAID's and corticosteroids produce significant gastric irritation resulting in gastritis and gastric ulceration, especially on long-term treatment. Present study revealed that ethanolic leave extract of *S. campanulata* has ulcer protective properties. Previous studies showed its potent anti-inflammatory activity. Therefore, it can be consider as an ideal substitute for conventional NSAIDs and glucocorticoid. Further studies have to be conducted to explain precisely the mechanism of action of this drug. Ethanolic leave extract of *S. campanulata* has an antiulcer effect. It increased healing of aspirin induced ulcer. The ethanolic leave extract of *S. campanulata* and Omeprazole significantly decreased the ulcer index and significantly enhance the pH; this suggests that it having an anti secretory effect. Aspirin induced ulcer control rats shown perforated ulcer. There is a dose-dependent increase in anti-ulcer effect of ethanolic leave extract of *S. campanulata* (Table 2).

**Table 1 Result of phytochemical screening of extracts of *S. campanulata***

S. No.	Constituents	Ethanolic extract
1.	Alkaloids	+
2.	Glycosides	+
3.	Flavonoids	+
4.	Saponins	+
5.	Phenolics	+
6.	Amino Acids	+
7.	Carbohydrate	+
8.	Proteins	+
9.	Diterpenes	-

**Table 2 Anti-ulcerogenic effect of ethanolic leave extract of *S. campanulata***

Groups	Ulcer Index	% Protection	pH of Gastric Juice	Vol of Gastric Juice (ml)	Total acidity
Group I- Control	9.833±1.343	--	1.166±0.372	4.00±0.816	28.16±2.266
Group II- Omeprazole	2.166± 1.067	77.90 %	5.00±1.247	1.333±0.471	11.166±1.067
Group III -SCEE400mg	4.5±0.957	54.20%	4.333±0.942	2.66±0.471	14.66±2.211
Group IV -SCEE200mg	7.5±1.258	18.60 %	3.333±1.374	3.500±0.500	19.00±2.160

**CONCLUSION**

The preliminary phytochemical investigation of ethanolic extract of *S. campanulata* leaves showed the presence of polyphenolic compounds, saponins, flavonoids and alkaloids. Ethanolic extract was screened for acute oral toxicity and was found to be non toxic. Ethanolic extract of *S. campanulata* leaves possesses significant anti-ulcer activity. In conclusion, our results showed that the anti-ulcer activity

of the extract was a result of the probable gastric ulcer healing mechanism (anti-secretory, cytoprotective and the antioxidant properties) of its active phytoconstituents. These findings suggest the potential for use of *S. campanulata* as an adjuvant in the treatment of gastric ulcer. Further, studies are needed for the isolation of active constituents responsible for the anti-ulcer activity and to elucidate the exact mechanism of action in gastric ulcer healing.

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