Research Article

Phytochemical Investigation and Pharmacological Evaluation of *Syzygium cumini* Stem and Bark Extracts for Anti-Ulcer Activity

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ABSTRACT

Numerous plants have been investigated for their anti-ulcer properties, driven by the need for accessible and effective alternatives to synthetic medications, which can be costly and detrimental. *Syzygium cumini* L., a prominent member of the Myrtaceae family, is highlighted for its medicinal applications, particularly in treating diabetes mellitus. It contains several bioactive compounds, including alkaloids, carbohydrates, flavonoids, glycosides, tannins, and phenols, which contribute to diverse pharmacological effects, including antioxidant, antidiabetic, antimicrobial, gastroprotective, anti-inflammatory, antidiarrheal, and hepatoprotective activities. It aimed to assess the antiulcer potential of acetone and methanol extracts of *Syzygium cumini* stem in rat models. Various extracts were prepared, and preliminary phytochemical analysis, including TLC fingerprinting, was conducted to identify phytoconstituents. The acetone extract exhibited higher total phenolic and flavonoid content compared to the methanol and petroleum ether extracts, potentially correlating with its bioactivity. In vitro antioxidant activity assessed through the DPPH assay indicated superior performance of the methanol extract compared to the other extracts, benchmarking against the standard quercetin. For the in vivo studies, ulcers were induced using ethanol, and rats were treated with either the acetone or methanol extract at doses of 100 and 200 mg/kg, alongside the standard drug ranitidine. Results demonstrated significant protective effects of both extracts, as evidenced by increased gastric pH, decreased gastric volume, and reduced ulcer index compared with the control and standard groups. The study concluded that the extracts did not adversely affect the general health or weight of the animals, indicating their potential for clinical application in treating ulcers.

Keywords: *Syzygium cumini* stem, phytoconstituents, phytochemical screening, PUD, antioxidant, antiulcer activity.

INTRODUCTION

The main aim of the study is to do a pharmacological Assessment and Phytochemical Study of *Syzygium cumini* Stem and Bark Extracts for Anti-ulcer Activity."

Peptic ulcer disease is a chronic disease characterized by defects in the inner linings of the gastrointestinal (GI) tract. The ulcer penetrates through the layers of the mucosa, extending into the submucosa or muscularis propria.[1] It usually occurs in the stomach and proximal duodenum. It may involve the lower esophagus, distal duodenum, or jejunum. Gastric ulcers and duodenal ulcers are the two most prevalent kinds. Peptic ulcers are caused by an imbalance between aggressive factors like hydrochloric acid (HCL), pepsin, refluxed bile, leukotrienes (LTs), reactive oxygen species (ROS), and defensive factors like the mucus-bicarbonate barrier, prostaglandins (PGs), mucosal blood flow, cell renewal and migration, and nonenzymatic and enzymatic antioxidants. The goal of treating ulcers is to reduce the quantity of acid produced by your stomach, neutralize the acid produced, and protect the wounded area so it can recover.[2] *Syzygium cumini* L., a large evergreen tree from the Myrtaceae family, is native to the Indian subcontinent but has spread to regions including Eastern Africa, South America, and parts of the United States. [3] Known by various names such as jambolan and black plum, it has a long history of cultivation in South Asia. The tree is venerated in Buddhist culture and is often planted near Hindu temples. Additionally, it serves multiple purposes such as fruit production, ornamental use, and timber. In India, it grows across a wide range from the Himalayas to the southern regions.[4]

Essential oils from Jamun leaves exhibit significant antibacterial activity against various strains, including Bacillus sphaericus and Staphylococcus aureus. A 70% ethanol extract shows potent effects on both Gram-positive and Gram-negative bacteria.[5,6] The aqueous extract from Jamun stem and leaf is effective against several bacterial strains, while the fruit extract targets Pseudomonas aeruginosa and demonstrates antifungal activity against Penicillium chrysogenum and Candida albicans. Additionally, Jamun extracts show anti-inflammatory effects in rat models, gastroprotective properties against gastric ulceration, and hepatoprotective effects against drug-induced liver toxicity. [7-11]

MATERIALS AND METHODS

Fresh stems of *Syzygium cumini* L. were collected from the local region of Pune city and authenticated by Dr. S. M. Satpute, Associate Professor & Head of Botany & Horticulture, Shankarrao Mohite Mahavidyalaya, Akluj. This has been submitted to Vidya Niketan College of Pharmacy, Lakhewdi, and authenticated as *Syzygium cumini* L. (Family – Myrtaceae).

a. **Processing of crude drug**: The stem of the plant *Syzygium cumini* was subjected to shade drying and further crushed to coarse powder, and then the powder was passed through a mesh no. 14 and stored in an air-tight container for further use.

The dried stems of the plant were used for the determination of physicochemical parameters such as total ash value, acid-insoluble ash value, water-soluble ash value, and moisture content (LOD).

b. **Preparation of Extracts**:

Three extracts of powder of *Syzygium cumini* L. stem was prepared Petroleum ether, Acetone extract, and Methanol extract

1. **Preparation of Petroleum ether extract**: The coarse powder material was extracted with petroleum ether by continuous hot extraction method in Soxhlet apparatus for defatting. Petroleum ether extract of powdered stem of *Syzygium cumini* L. was prepared in Soxhlet extractor according to the standard method till colorless solution was observed in siphon tube. 210 gm of the powdered and 1500 ml petroleum ether was used for extraction. After completion of extraction, extract was cooled and dried. The extract as stored in air tight container till use. Percentage yield of extract was calculated.
2. **Preparation of Acetone extract:** Acetone extract of powdered *Syzygium cumini* L. stem was prepared by Soxhlet extraction according to the standard method till colorless solution was observed in siphon tube. 208 gm of the powdered husk and 1500 ml Acetone was used for extraction. After completion of extraction solvent was cooled and dried. The extract was stored in air tight container till use. Percentage yield of extract was calculated.
3. **Preparation of Methanol extract:** Methanolic extract of powdered stem was prepared in Soxhlet extractor according to the standard method till colorless solution was observed in siphon tube. 205 gm of the powdered and 1500ml Methanol was used for extraction. After completion of extraction extract was cooled and dried. The extract was stored in air tight container till use. Percentage yield of extract was calculated.

$$\%\ \text{yield} = \frac{\text{Wt. of extract}}{\text{Wt. of powdered drug}} \times 100$$

c. **In-vivo Anti-Ulcer activity**:

Animals used: For the study, Wistar rats of either sex, of weight 150-200 g, were selected.

Test group: For the study, seven groups of animals were made. Each group has six rats.

Route of administration:

Standard: IP

Test: Oral

Animals were housed in six groups in separate cages under controlled conditions of temperature (22±2 Celsius). All animals were given a standard diet (golden feed, New Delhi) and water regularly. Animals were divided randomly into six treatment groups. Each group consists of three rats.

IAEC Approval: Wistar rats of either sex weighing 150 to 200 g were used in the present study. The experimental animals were maintained under standard laboratory conditions in an animal house of Vidya Niketan college of pharmacy lakhewadi, which is approved by the committee for the purpose of control and supervision on experiments on animals (CPCSEA) Protocol. Animals were kept under 12 h light/dark cycles and controlled temperature (24 ± 2°C) and fed with commercial pellet diet and water ad libitum. All animals were acclimatized to the laboratory environment for at least one week before the commencement of experiment. The experimental protocol for the study was followed according to the norms of Institutional Animal Ethics Committee.

In-vivo studies: Model: Ethanol-induced gastric ulcer:

Ethanol induced gastric ulceration model is one of the widely used experimental model. Ethanol digests the mucosal layer and exposes the mucosa to the proteolytic and hydrolytic action of hydrochloric acid and pepsin. Ethanol induced microvascular injuries by reducing blood flow, increasing the production of reactive oxygen species (ROS)and proinflammatory cytokines, hence, reduced the cellular antioxidant levels.[12]

Grouping of Animals: Animals were divided into seven groups, each group consisting of six rats.

Grouping of animals

Group	Dose
Group I	Animals received 0.5%(1ml) DMSO/ Saline water orally. (Negative control)
Group II	Animals received 90%(1ml) Ethanol. (Positive control)
Group III	Animals received standard drug (Ranitidine 20mg/kg)
Group IV	Animals received 100mg/kg dose of Acetone extract of *Syzygium cumini L.* stem bark (SCAE100mg/kg oral).
Group V	Animal received 200 mg/kg dose of Acetone extract of *Syzygium cumini L.* stem bark (SCAE200 mg/kg oral)
Group VI	Animal received 100 mg/kg dose of Methanolic extract of *Syzygium cumini L.* stem bark (SCME100 mg/kg oral)
Group VII	Animal received 200 mg/kg dose of Methanolic extract of *Syzygium cumini L.* stem bark (SCME200 mg/kg oral)

All animals were weighed and housed individually to avoid coprophagy. They underwent a 24-hour fast (water allowed) before being divided into three groups: control, standard, and extract. The control group received only a vehicle, the standard group was administered Ranitidine (20

mg/kg), and the extract group received plant extract for seven days. Afterward, 90% ethanol was given orally to induce ulcers, except for the control group. Two hours post-injection, the animals were euthanized via cervical dislocation, and their stomachs were removed, washed, and assessed for gastric damage. Observations were made microscopically, and the ulcer index was calculated using a specific formula. After the animals are sacrificed pH, total acidity, and ulcer scoring, ulcer index and % protection were measured.[13]

Statistical Analysis:

Presentation of results was done in tabular form. All results are expressed as Mean ± Standard Error. The results were expressed as mean ± S.E.M. Data was analyzed by one way ANOVA test. (Followed by Tukey's test).

RESULTS AND DISCUSSION

1. Determination of Total Ash Content

Determination of Total ash content of *Syzygium cumini* L. stem powder.

Weight of empty crucible (g)	14.87
Weight of crucible + air-dried material (g)	16.87
Weight of crucible + ash (g)	15.03
Weight of total ash (g)	0.16
Total ash value (%w/w)	**8%**

The total ash content of *Syzygium cumini* L. stem powder was 8%.

2. Phytochemical evaluation of *Syzygium cumini* L. Stem extracts:

Table 1: Physical parameters and yield of extracts.

Sr. No.	Drug taken (gm)	Solvent used (ml)	Consistency	Color of extract	Yield (gm)	% Yield (w/w)
1	210	Petroleum ether	Sticky	Light Brown	**3**	**1.43**
2	208	Acetone	Sticky	Dark Brown	**5.2**	**2.5**
3	205	Methanol	Sticky	Dark Brown	**4.8**	**2.34**

Above observations table reveals that Petroleum ether, acetone and methanolic extracts have percentage yield respectively. Highest yield was obtained for acetone extract.

3. Phytochemical tests of *Syzygium cumini* L. stem extracts

Table 2: Observations for Phytochemical qualitative analysis

Sr. No.	Chemical test	Petroleum ether	Acetone	Methanol
1.	Test for Alkaloids			
	1. Dragendroff's test	-	-	-
	2. Mayer's test	-	+	+
	3. Wagner's test	-	+	-
	4. Hager's test	+	+	-
2.	Test for Carbohydrates			
	1. Molish's test	-	-	-
	2. Benedict's test	-	+	+
	3. Barfoed's test	-	-	-
	4. Fehling's test	-	+	+
3.	Test for Protein			
	1. Millions test	+	+	+
	2. Biuret test	-	-	-
4.	Test for Amino acid			
	1. Ninhydrin test	-	-	-
	2. Tyrosine test	-	-	-
5.	Test for Glycoside			
	1. Keller-Killani test	-	-	-
	2. Borntrager's test	+	+	-
	3. Legal test	+	+	+
6.	Test for Tannins and Phenols			
	1. Lead acetate test	+	+	+
	2. Dil. HNO3	-	+	-
7.	Test for Flavonoids			
	1. Lead acetate test	+	+	+
	2. Shinoda test	+	+	+
	3. Zinc dust & HCL test	-	+	+
8.	Test for Steroid			
	Salkowski's test	-	+	+

Above observation table shows the presence of phytoconstituents in the extracts. It reveals all three (i.e., Pet ether, Acetone and Methanolic) extracts contains Carbohydrates, Glycosides, Tannins, Alkaloids, Amino acid, Protein, Steroid and Flavonoids were presence or absence.

1. **Thin Layer Chromatography:**

Table 3: Results of TLC Profile of extracts

Sr. No.	Extracts	Solvent system	Proportions	Spraying agent	Rf value	Colour	Chemical constituent
1	Petroleum ether	Chloroform: Acetone	7.5: 2.5	10% H_2SO_4	0.15	Blue	Glycoside
					0.32	Pinkish red	Flavonoid
					0.43	Yellow	Flavonoid
2	Acetone	Toluene: Methanol	8: 2		0.28 0.36 0.42	Pink Green Blue Green Bluish	Glycoside Phenols Flavonoid Phenol Tannins
					0.58 0.82	white	
3	Methanol	Benzene Ethyl acetate Methanol	6: 3: 1		0.12 0.45 0.75	Pinkish Red	Anthocyanins Flavonoids Tannins

Pet ether Acetone Methanol

Image 1: TLC of Petroleum ether, Acetone and Methanol extract.

Petroleum ether extract of *Syzygium cumini* L. Stem when subjected to TLC showed Rf value at 0.15, 0.32, 0.43 in the solvent system of Chloroform : Acetone (7.5 : 2.5), Acetone extract of *Syzygium cumini* L. Stem when subjected to TLC showed Rf value at 0.28, 0.36, 0.42, 0.58,0.78 in the solvent system of Toluene: Methanol (8: 2). Methanolic extract of *Syzygium cumini* L. Stem when subjected to TLC showed Rf value at 0.12, 0.45, 0.75 in the solvent system of Benzene: Ethyl acetate: Methanol (6: 3: 1).

Table 4: Total Flavonoid Content of *Syzygium cumini* Stem Extracts

Sr. No.	Extracts	Concentration (µg//ml)	Absorbance	TFC (mg/QE/g)
1	Acetone	100	0.32±0.001453	65.07
2	Methanol	100	0.28±0.00176	48.38

Value represents mean ± SEM (n=3)

1. **Results of Total Phenolic Content:**

Acetone extract: Based on data, an acetone extract concentration of 100(µg/ml) yielded an absorbance of 0.232±0.001155, which corresponds to a Total Phenolic Content (TPC) value of 61.03±0.21 (milligrams of gallic acid equivalents per gram of extract).

Methanol extract: Based on data, a methanol extract concentration of 100(µg/ml) yielded an absorbance of 0.165±001528, which corresponds to a Total Phenolic Content (TPC) value of 42.5±0.28(milligrams of gallic acid equivalents per gram of extract).

This observation reveals that Acetone and Methanol

extracts of *Syzygium cumini* have found phenolic content as 61.03 mg GAE/g DW and 42.5mg GAE/g DW, respectively. Acetone extract shows more phenolic content than Methanol as per the comparative evaluation of phenolic content of extracts.

The Observation table reveals that Acetone and Methanol extract of *Syzygium cumini* have Flavonoid content as 65.07 and 48.38 mg/QE/g DW respectively. Acetone extract shows more flavonoid content than Methanol as per comparative evaluation of flavonoid content of extracts.

7. In-vivo Anti-Ulcer activity.: The in-vivo antiulcer study of *Syzygium cumini* stem extract was carried out by using most commonly used ulcer study model that is ethanol induced gastric ulcer model.

The study evaluated the antiulcer activity of acetone and methanol extracts of S. Cumini in rats. Rats were pre-treated with extracts at doses of 100 and 200 mg/kg for seven days, followed by ethanol-induced ulceration. Results indicated that the pH of gastric juice increased post-treatment, suggesting decreased acidity and reduced gastric content volume and ulcer index. The SCAC and SCME extracts displayed better ulcer protection at the 200 mg/kg dose compared to the control group, with the acetone extract providing superior protection over the methanol extract.

Table 5: Evaluation of Antiulcer activity of *Syzygium cumini* stem

Groups	Ulcer Index	Ulcer Protection	pH	Total acidity
Positive control (Group I)	83.52±0.2969	0	2.335±0.07	98.60±0.5465
Negative control (Group-II)	48.45 ±1.057	0	3.083±0.06	73.43±0.7706
Standard (Group-III)	50.40±0.6808**	38.36±0.00**	3.166±0.06**	22.33±0.9984 **
SCAE-100 (Group-IV)	61.76±0.292**	25.63±0.3332**	4.186±0.0021**	53.33±1.524**
SCAE-200 (Group-V)	52.71±0.1318**#	37.48±0.7364**#	5.211±0.0047**#	22.355±1.092**#
SCME-100 (Group-VI)	63.11±0.8806**	24.1516±0.5545*	3.964±0.03*	43.40±0.6047**
SCME-200 (Group-VII)	60.78±0.9310**	36.89±0.2087**	4.916±0.021**	25.55±0.5444**

n=6, * Significant difference when standard and test compared with positive control (P < 0.05); ** Highly significant difference when test compared with positive control (p< 0.001); # No significant difference when test compared with standard; Δ Significant difference when test compared with standard but more effect than standard. Data obtained was presented as means ± standard error of mean (S.E.M) for the number of animals in each group (n=6).The groups were compared using one-way analysis of variance (ANOVA), P< 0.05 was considered significant difference when standard and test compared with positive control in excision wound model and P < 0.001 was considered highly significant difference when test compared with positive control in ethanol induced ulceration model.

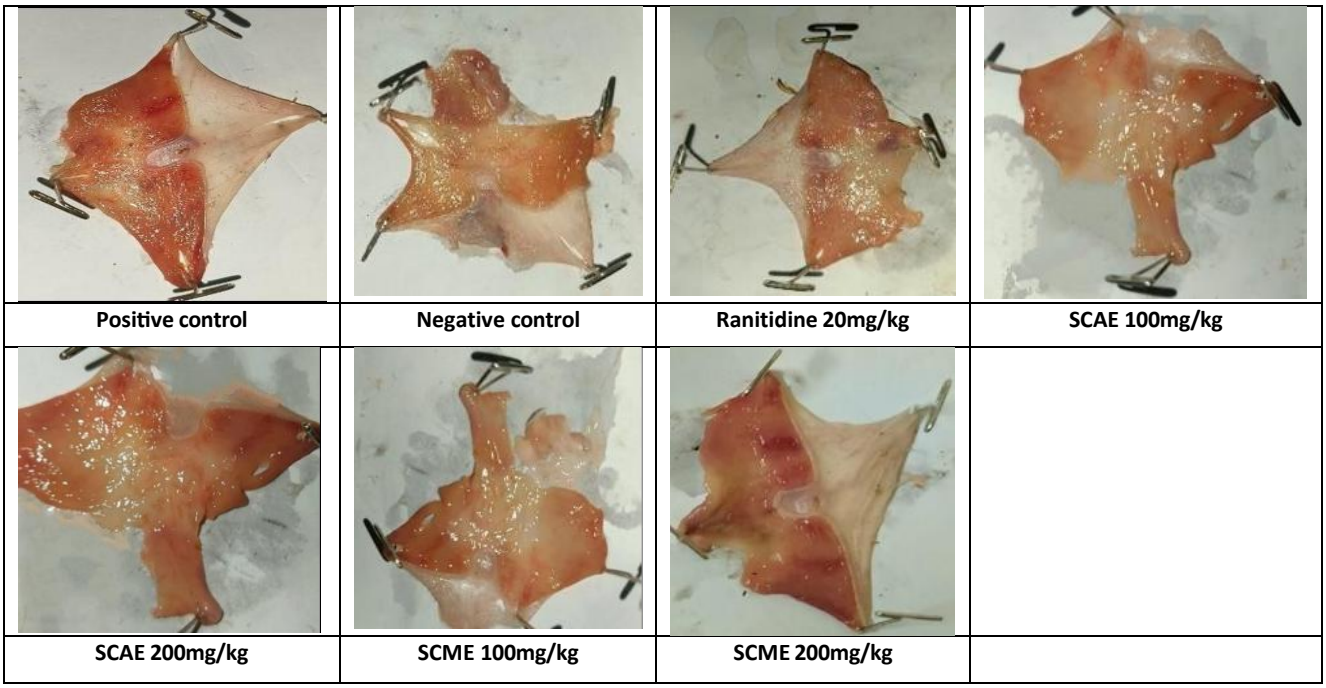
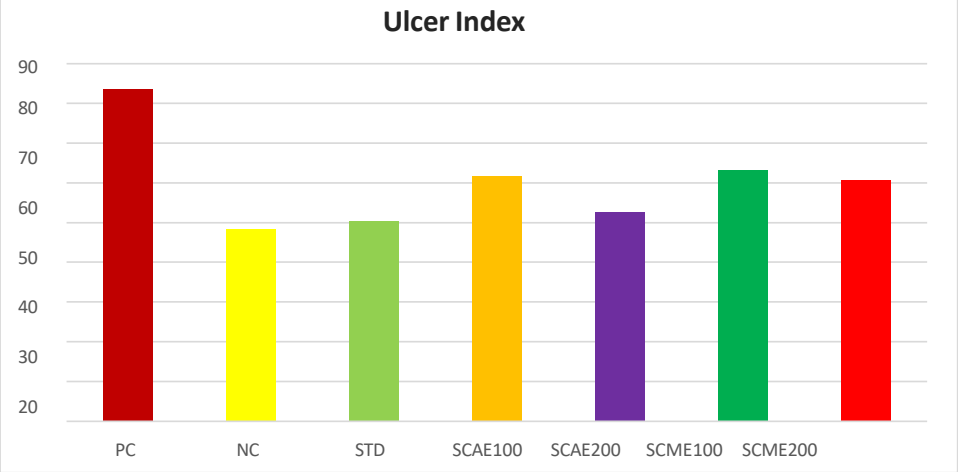


Image 2: Representation of ethanol-induced ulcer in Wistar rats



Graph 1: Effect of different extracts of *Syzygium cumini* L. on ethanol-induced gross morphological study of the effect of the stem of *Syzygium cumini* extracts in ethanol-induced gastric ulceration model in Wistar rats

DISCUSSION

A peptic ulcer is a lesion in the stomach or duodenum, often linked to impaired gastric mucosal defenses, Helicobacter pylori infection, anti-inflammatory medications, and alcohol use. Symptoms may not correlate with the ulcer's severity, and complications include bleeding, perforation, and gastric blockage. There is a growing interest in herbal treatments due to limitations of synthetic medications. This investigation explores the anti-ulcer potential of *Syzygium cumini* L. (Jamun) through morphological and phytochemical analysis. The study used acetone and methanol for extraction, yielding 2.5% and 2.34% respectively. The acetone extract revealed glycosides, carbohydrates, alkaloids, flavonoids, tannins, and proteins, while the methanol extract contained carbohydrates, tannins, and flavonoids.TLC for Acetone extract & Methanol extract of *Syzygium cumini* was carried out by using solvent system Toluene: Methanol & Benzene: Ethyl acetate: Methanol. respectively, spots were observed under UV, which helps in identification of specific chemical constituents present in the extracts of *Syzygium cumini*.In-vivo anti-ulcer studies on an ethanol-induced ulcer model indicate that both Acetone and Methanol extracts of *Syzygium cumini* L. stem exhibit significant anti-ulcer activity. The Acetone extract at a dose of 200 mg/kg provides 37.48% protection, while the Methanol extract offers 36.89%, compared to 38.36% protection from the standard Ranitidine at 20 mg/kg. The Acetone extract demonstrates slightly superior efficacy over Methanol. Additionally, gross morphological data were provided through photographs. Further research is required to explore the hypothetical mechanisms and the specific phytoconstituents responsible for the protective effects against ulcers.

CONCLUSION

The study examines the pharmacognostic, phytochemical, and pharmacological evaluations of *Syzygium cumini* L. (Jamun) stem. This large evergreen tree contains various phytoconstituents, including alkaloids, flavonoids, and tannins, linked to its medicinal properties such as antibacterial, anti-inflammatory, gastroprotective, hepatoprotective, and antidiabetic activities. The stem, collected from Nanded and authenticated by Dr. S. M. Satpute, was dried, powdered, and extracted using acetone and methanol via a Soxhlet apparatus. Preliminary investigations confirmed the presence of carbohydrates, glycosides, tannins, alkaloids, and flavonoids in both extracts. Notably, the acetone extract demonstrated a DPPH radical scavenging activity of 79.63%, while methanol showed 75.02%, compared to ascorbic acid's 88.92%. Acute oral toxicity studies indicated the extracts were safe at doses ≤200 mg/kg. The anti-ulcer activity was tested in ethanol-induced gastric ulcer models in Wistar rats, showing results similar to the reference drug Ranitidine. The acetone extract provided a percent ulcer protection of 37.48%, and the methanolic extract showed 36.89%, compared to Ranitidine's 38.36%.

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