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Evaluation of the anti-ulcer activity of seeds of *Syzyzium cumini* on experimental animal models

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Syzyzium cumini (Myrtaceae) is an evergreen tropical tree mostly found in South East Asia. Extracts of fruits and seeds are extensively used in the treatment of diabetes. Other folkloric uses include treatment of cold, cough, fever, skin problem and genitourinary tract ulcer. The present study evaluated the anti-ulcer activity of the seeds of *S. cumini* against different ulcer models in rats. The anti-ulcer activity of the methanol extract and ethyl acetate fraction of methanol extract of *S. cumini* seed was evaluated in ethanol, pylorus ligation, Aspirin + pylorus ligation and stress-induced gastric ulcer model. The study biomarkers used were gastric volume, pH, ulcer index and free acidity. The extract and fraction at the dose level of 200 mg/kg caused a significant (P <0.05) reduction in the ulcer index, free acidity while there was an increase in the pH value of the test in comparison to the solvent control group. Experimentally, it was observed that animals who received ethyl acetate fraction had relatively improved protection of gastric ulcer as demonstrated by different biomarkers. The present study showed that the ethyl acetate fraction of methanol extract of *S. cumini* seed possesses potential anti-ulcer activity. This may be due to a linear relationship between the antioxidant value of the seeds as reported earlier by several authors due to rich in phenolic compounds, which in turn is responsible for mucosal cytoprotection of the stomach and hence, participate in the enhancement of mucosal defence mechanism.

Keywords: Anti-ulcer activity, Pyloric ligation, Ranitidine, Stress-induced, *Syzyzium cumini*, Ulcer index.

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Introduction

Syzygium cumini L., better known as *Jamun* in India; Jambolan in Spain and Black plum in Europe which is an important member of the Myrtaceae family of plants consisting of trees and shrubs[1]. Peptic ulcer and gastric ulcer are one of the leading cause of significant morbidity worldwide. Although, many anti-ulcer drugs are available in the market but they are producing several side effects. Gastric ulcer is a very familiar disorder of the gastrointestinal system, responsible for causing much discomfort to patients, disrupting their daily routines and causing mental discomfort. The desensitization of the internal surface of the stomach to external stimulus causes excessive gastric secretions leading to chronic gastritis and indigestion and prolonged gastric secretion[2]. *S. cumini* is commonly used in traditional medicine as a remedy for treating various diseases. It has been used therapeutically in Ayurveda and Unani systems of medicine. It is an evergreen tree with brown thick bark, fruits are like berries which can be edible and ripe fruits are dark violet coloured. The taste of the fruits is sweet and sour to the tongue[3]. The seeds and fruits of *S. cumini* have been used to treat diabetes mellitus in South Asia countries as traditional medicine for several centuries. The seeds are used as hypoglycemic; antibacterial; anticancer; antioxidant; anti-leishmania; immunomodulatory activity and also having many nutritive values[4-9]. Previous studies have demonstrated that *S. cumini* possess anti-diarrhoeal, anti-allergic, antihyperlipidemic, antiviral, antifungal and trypanocidal properties[10,11]. In addition, this species has also been used for the treatment of allergics[12], hyperlipidemic[13] and viral activities[14]. Seeds from this plant are reported to contain certain important phytochemicals like jamboline, alkaloid jambosine, gallic acid, oleanolic acid, ellagic acid, quercetin, tannins, etc.[15-20]. Plants derived drugs are found to be safe and less toxic as compared to synthetic ones. However, no study has been carried out to evaluate the antiulcer activity of *S. cumini* seeds. The present study was focused on the evaluation of the antiulcer activity

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of seeds of *S. cumini* in various experimental animal models which have not been explored earlier.

Materials and Methods

Plant materials

The fruits of *Jamun* (*S. cumini)* were collected from Khandagiri, Bhubaneswar during the month of July 2018 and authenticated by Dr P. C. Panda, Principal Scientist, Regional Plant Resource Center, Bhubaneswar. The collected fruits were washed and pulp was separated from the seeds. Seeds were shed dried at room temperature for 30 days and coarsely powdered before being used in the extraction process.

Extraction and fractionation of seeds

The powder plant material was defatted with n-hexane over a period of 72 h at room temperature to avoid the destruction of any thermolabile substances. The defatted powder sample was subjected to methanol extraction in a stoppered container over a period of 3 days with periodical agitation by cold maceration technique. The crude extract was collected, filtered, and dried in a rotary evaporator at 40 °C until complete dehydration.

The dry methanolic extract was dissolved in water and filtered through Whatman filter paper. The filtrate was fractioned with ethyl acetate solvent in 1:1 ratio with vigorous and uniform shaking in a separating funnel by solvent separation technique. The mixture of solvent and extract was kept over a period of 24 h for the separation of organic and aqueous layers respectively. The separated fractionate was collected in a beaker, dried, and stored in an airtight container for further study.

Animals

Animals were procured from M/s Saha Enterprise, Kolkata and stored in the animal house of the institute. Wistar albino rats of both sexes weighing around 120-150 g were used and kept in polycarbonate cages, in 12 h light followed by 12 h dark condition. Standard pellet diets were used and water *ad libitum* at room temperature i.e. 25–30 ºC, with 45-55% humidity. The healthy animals of either sex were acclimatized for 72 h before the commencement of the experiment. All the animal experiments were approved by the Institutional Animal Ethics Committee (IAEC), bearing approval number IAEC/SPS/SOA/08/2018 and registration number 1171/PO/RE/S/08/CPCSEA and executed as per the CPCSEA guidelines.

Acute oral toxicity study

Acute oral toxicity was performed according to OECD test guidelines 423-acute toxic class method[21]. Six rats were divided into two groups of three animals each. A single dose of 2000 mg/kg body weight of methanol extract (ME) and ethyl acetate fraction (EAF) was administered orally to the animals and observed for any signs of toxicity for a period of 24 h, followed by 13 days, for the recording of any mortality. Bodyweight was recorded before dosing and after that once a week till the completion of the experiments. Gross pathological changes were also observed at the end of the experiment. In the above study procedure, no marked toxic signs or death occurred till the end day of the experiment. Both the test drugs were found to be safe up to a dose level of 2000 mg/kg and a dose of 200 mg/kg was selected for both of the test drugs for further study[21,22].

Anti-ulcer activity

Aspirin-induced gastric ulceration in pylorus ligated rats

Adult albino rats were fasted for 24 h with water *ad libitum* before the experiment and were randomly divided into four groups of six animals each. The animals were treated with methanol extract-ME (200 mg/kg) and ethyl acetate fraction (200 mg/kg) of seeds of *S. cumini* or control vehicle (2 drops of tween 80+ water) or standard drug ranitidine (100 mg/kg) orally 1 h prior to ligation. The rats were anaesthetized with the combination of ketamine (90 mg/kg) and xylazine (10 mg/kg) by i.p. route and followed by the opening of the abdomen by a small midline incision below the xiphoid process. The pylorus was secured and ligated with silk sutures after which the abdomen wall was closed by sutures and animals were allowed to recover from anaesthesia. Aspirin (100 mg/kg) suspended in 2 drops of tween 80+ water was given orally to all the rats 15 min after pylorus ligation. The animals were sacrificed after 7 h, the stomach was removed, gastric juice was collected, then the stomach was cut open through its greater curvature and ulcer index was determined for every rat. The collected gastric juice was centrifuged and the supernatant was measured. Gastric contents were analyzed for total acidity by titrating against 0.01 N NaOH using phenolphthalein as an indicator. The pH of gastric juice was measured using pH meter[23] (Table 1).

Gastric secretion in pylorus ligated rats

Pylorus ligation was done by ligating the pyloric end of the stomach of rats under anaesthesia using the

combination of ketamine and xylazine as mentioned in the previous model and animals were divided into four groups of six animals each. The stomach was replaced carefully and the abdomen was closed with sutures. Animals were allowed to recover and stabilize in the individual cages and were deprived of water during the post-operative period. After 7 h of the surgery, rats were sacrificed and ulcer scoring was done. Gastric juice was collected for performing gastric secretion study[23] (Table 2).

Alcohol-induced gastric lesion

Four groups of six adult Wistar rats were made to fast for 24 h and water was given *ad libitum*. Extract and fraction of seeds of *S. cumini* at a dose level of 200 mg/kg or control vehicle (tween + water) and standard drug were administered by oral route. Gastric ulcers were induced by the administration of absolute alcohol at a dose of 1 mL/200 g of body weight, orally. All the drug treatments (test, standard and control) were given to all the respective groups of animals, after 45 min of administration of alcohol. One hour after the animals were sacrificed, stomachs were removed and hemorrhagic length was measured to calculate ulcer index[24] (Table 3).

Measurement of ulcer index

Ulcer scoring was done by viewing ulcers under a magnifying glass. The ulcer was scored with the help of a scoring technique as per the standard procedure. Erosions of gastric mucosal of length 1 mm or less, 1 mm to 2 mm, more than 2 mm registered as score values of 1, 2 and 3 respectively[25].

Statistical analysis

The data were analyzed using one-way ANOVA, followed by Dunnett's t test. All values were reported as mean±SEM. Statistical significance was set at $P<0.05$.

Results and Discussion

Anti-ulcer activity

Table 1 gives the effects of ethyl acetate fraction and methanol extract on *S. cumini* Seeds in pylorus ligation induced ulcers in rats. Treatments with test and standard drugs decreased the volume of gastric secretion but increased the pH as compared with the

Table 1 — The effect of methanol and extract ethyl acetate fraction of *S. cumini* in Aspirin+Pylorus ligation induced rat ulcer model

Group	Treatment	Dose mg/kg	Gastric volume/100 g	pH	Free acidity (mEq/L/100 g)	Ulcer index
I	Control	10 mL/kg	3.62±0.31	3.26±0.10	47.16±1.01	11.51±0.10
II	Ranitidine	100	1.98±0.04$^{\#}$	4.65±0.13$^{\#}$	9.31±0.2$^{\#}$	3.41±0.05$^{\#}$
III	ME	200	1.76±0.11$^{\#}$	4.63±0.06$^{\#}$	21.83±0.40$^{\#}$	4.31±0.01$^{\#}$
IV	EAF	200	1.77±0.38$^{\#}$	4.58±0.13$^{\#}$	19.67±0.33$^{\#}$	3.25±0.03$^{\#}$

Values expressed as Mean±SEM, n=6, $^{\#}P<0.05$ treated groups Vs solvent control; ME: Methanol Extract; EAF: Ethyl Acetate Fraction

Table 2 — The effect of methanol extract and ethyl acetate fraction of *S. cumini* seeds in pylorus ligation induced ulcer in rats

Group	Treatment	Dose mg/kg	Gastric volume/100 g	pH	Free acidity(mEq/L/100 g)	Ulcer index
I	Control	10 mL/kg	2.9±0.07	3.08±0.05	48.67±0.42	8.40±0.04
II	Ranitidine	100	1.30±0.03$^{\#}$	6.1±0.04$^{\#}$	9.16±0.27$^{\#}$	1.61±0.07$^{\#}$
III	ME	200	1.54±0.05$^{\#}$	6.24±0.06$^{\#}$	21.83±0.31$^{\#}$	3.2±0.03$^{\#}$
IV	EAF	200	1.7±0.11$^{\#}$	6.22±0.04$^{\#}$	11.67±0.56$^{\#}$	2.23±0.05$^{\#}$

Values expressed as Mean±SEM, n=6, $^{\#}P<0.05$ treated groups Vs solvent control; ME: Methanol Extract; EAF: Ethyl Acetate Fraction

Table 3 — The effect of methanol extract and ethyl acetate fraction of *S. cumini* in ethanol-induced ulcer model.

Group	Treatment	Dose mg/kg	Ethanol-induced ulcer		Stress-induced ulcer	
			pH	Ulcer index	pH	Ulcer index
I	Control	10 mL/kg	2.98±0.07	6.24±0.08	2.18±0.07	6.24±0.08
II	Ranitidine	100	4.57±0.14$^{\#}$	2.16±0.30$^{\#}$	4.57±0.14$^{\#}$	2.16±0.30$^{\#}$
III	ME	200	4.62±0.04$^{\#}$	3.33±0.33$^{\#}$	3.87±0.30$^{\#}$	3.82±0.49$^{\#}$
IV	EAF	200	4.61±0.09$^{\#}$	2.17±0.30$^{\#}$	4.50±0.19$^{\#}$	2.12±0.71$^{\#}$

*Values expressed as Mean±SEM, n=6, $^{\#}P<0.05$ treated groups Vs solvent control; ME: Methanol Extract; EAF: Ethyl Acetate Fraction

control group. There is a significant (P <0.05) reduction of ulcer index found in the case of standard ranitidine as well as test groups. However, ethyl acetate fraction showed better effects than that of methanol extract here.

Table 2 depicts the effects of both the test drugs of *S. cumini* in aspirin + pylorus ligation induced rat ulcer model. Both the ulcer index and gastric secretion were significantly (P <0.05) reduced by the standard drug ranitidine and test drugs as compared with the control group.

Table 3 represents the effects of ethyl acetate fraction and methanol extract of *S. cumini* in ethanol-induced ulcer model. The standard drug Ranitidine and ethyl acetate fractions showed a significant reduction in ulcer index than methanol fraction of *S. cumini* as compared with the control group. However, the ethyl acetate fraction showed better results than that of the methanol fraction.

The fundamental theory behind the development of an ulcer is the unevenness between aggressive factors and the maintenance of mucosal integrity through the endogenous defence mechanism[26]. The potential anti-ulcerogenic drugs are known to act on the defensive and offensive axis. *S. cumini* is a notable plant to possess a wide range of biological properties. In the present study, two test samples i.e., methanol extract (ME) and ethyl acetate fraction (EAF) from the seed part of the plant *S. cumini* were selected as per the literature survey and reported phytochemicals. The dose selected was 200 mg/kg for the test groups as per the results of the acute toxicity study. Both the test compounds significantly reduced the development of gastric ulcers in rats through different ulcerogenic models. The models used for the purpose are pylorus ligated, pylorus + aspirin, ethanol and stress-induced ulcerogenic models in rats. The test substances EAF and ME showed promising anti-ulcer activity and somehow, the data showed a similar kind of activity to that of standard drug Ranitidine in all tested models. In the acute pyloric model, the methanol extract and the ethyl acetate fraction reduce the ulcer index, free acidity significantly to that of solvent control. Moreover, the ethyl acetate fraction, a fractionated part of methanol extract that narrow down the phytoconstituents, showed better potency than that of the methanol extract-treated group in both pylorus and aspirin + pylorus ligated models. A similar observation was also seen in the stress-induced ulcer model. Pylorus ligated and stress-induced ulcers are results of auto degradation of the gastric mucosal barrier due to excess production and accumulation of hydrochloric acid in the stomach[27].

In the ethanol-induced model, the development of gastric lesion formation may be contributed due to a decrease in gastric blood flow. Similarly, it is reported that leukotriene antagonists and 5-lipoxygenase inhibitors are capable of inhibiting alcohol and NSAID-induced gastric ulceration in rats[28]. EAF fraction reduces the ulcer index as compared to control and showed a similar result to that of standard drug ranitidine treated group. Pylorus ligation induced ulcer is caused due to increase in the amount of acid and pepsin in the stomach thereby causing damage to the wall of the mucosa. EAF tends to decrease this secretion of acid and perhaps increase the defensive factor by increasing the mucous secretion. Aspirin induces the ulcer by damaging the mucosa layer, interfering with the prostaglandin (PG) synthesis and causing a back diffusion of H^+ ion thereby enhancing acid secretion. Ethanol-induced ulcers are caused by different factors including decreased mucosal blood flow, damage to capillary endothelium, and release of arachidonate metabolites specifically LTC4 /D4, PAF, and histamine. EAF probably increase the defensive factors including the synthesis of endogenous PGs respectively[29]. The plant is reported to be a rich source of polyphenols, tannins, and flavonoids in the seed. These polyphenols display many pharmacological properties in the Gastrointestinal tract area, acting as anti-secretory and gastro-protective activity. It is reported that polyphenolic constituents promote defensive factors and have potential antioxidant and radical scavenging properties. Flavonoid diglycosides (quercetin and myricetin), hydrolysable tannins (1-0-galloylcastalagin and casuarinin) and a triterpene, oleanolic acid have been isolated from seeds of *S. cumini*[30]. The gastric ulcer healing activity of ME and EAF in rats may be due to the presence of flavonoids as they have been reported to possess both hepatoprotective and anti-inflammatory activities[31]. Flavonoids have been reported to affect arachidonate metabolism where cyclo-oxygenase products like Prostaglandin E1 and Prostaglandin I2 protect gastric mucosa against damage. Therefore, it is suggested that there can be a linear relationship between anti-oxidant potency, free radical-scavenging ability, and the content of phenolic compounds of the seeds of *S. cumini.* Henceforth, the ethyl acetate fraction may

have the potential to prevent ulcerogenic effects as observed in different test models.

Conclusion

From the above findings, we have confirmed the presence of potential anti-ulcer activities by the plant *Syzygium cumini* and the absence of acute oral toxicities. Thus, the present work validates about the anti-ulcer effects of ethyl acetate fractions of methanol extract of this plant which is comparable with that of the standard drug in rat models. It may be due to the anti-secretory and mucosal protection effects of the identified phytochemicals. Thus, further investigations should be carried out to know the specific phytochemicals that are responsible for the anti-ulcer activity and mechanism pathway of this plant.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1 Chagas V T, França L M, Malik S and Paes A M A, *Syzygium cumini* (L.) skeels: A prominent source of bioactive molecules against cardiometabolic diseases, *Front Pharmacol*, 2015, **6**, 259.

2 Jamal A, Siddiqui A, Tajuddin and Jafari M A, A review on gastric ulcer remedies used in unani system of medicine, *Nat Prod Rad*, 2006, **5**(2), 153-159,

3 Singh N and Gupta M, Effect of ethanolic extract of *Syzygium cumini* (Linn) seed powder on pancreatic islets of alloxan diabetic rats, *Indian J Exp Biol*, 2007, **45**(10), 861-867.

4 Das S, Das A and Dharani N, Application of jamun (*Syzygium cumini* Linn) seed extract on cotton fabric for antibacterial activity, *Indian J Fibre Text Res*, 2019, **44**(3), 365-368.

5 Yadav S S, Meshram G A, Shinde D, Patil R C, Manohar S M, *et al.*, Antibacterial and anticancer activity of bioactive fraction of *Syzygium cumini* L. seeds, *Hayati J Biosci*, 2011, **18**(3), 118-122.

6 Sehwag S and Das M, Nutriative, therapeutic and processing aspects of Jamun, *Syzygium cumini* (L) skeels- An overview, *Indian J Nat Prod Resour*, 2014, **5**(4), 295-307.

7 Azima A M S, Noriham A and Manshoor N, Phenolic, antioxidants and color properties of aqueous pigmented plant extracts: *Ardisia colorata* var. elliptica, *Clitoria ternatea*, *Garcinia mangostana* and *Syzygium cumini*, *J Funct Foods*, 2017, **38**, 232-241.

8 Rodrigues K A D F, Amorim L V, Dias C N, Moraes D F C, Carneiro S M P, *et al.*, *Syzygium cumini* (L.) Skeels essential oil and its major constituent α-pinene exhibit anti-Leishmania activity through immunomodulation *in vitro*, *J Ethnopharmacol*, 2015, **160**, 32-40.

9 Veigas J, Narayan M S, Laxman P M and Neelwarne B, Chemical nature, stability and bio efficacies of anthocyanins from fruit peel of *Syzygium cumini* Skeels, *Food Chem*, 2007, **105**(2), 619–627.

10 Dos Santos A O, Costa M A, Ueda-Nakamura T, Dias-Filho B P, DaVeiga-Júnior V F, *et al.*, *Leishmania amazonensis*: Effects of oral treatment with copaibaoilin mice, *Exp Parasitol*, 2011, **129**(2),145–151.

11 Chandrasekaran M and Venkatesalu V, Antibacterial and antifungal activity of *Syzygium jambolanum* seeds, *J Ethnopharmaco*, 2004, **91**(1), 105–108.

12 Brito F A, Lima L A, Ramos M F S, Nakamura M J, Cavalher-Machado S C, *et al.*, Pharmacological study of anti-allergic activity of *Syzygium cumini* (L.) Skeels, *Braz J Med Biol Res*, 2007, **40**(1),105–115.

13 Ravi K, Rajasekaran S and Subramanian S, Antihyperlipidemic effect of *Eugenia jambolana* seed kernel on streptozotocin-induced diabetes in rats, *Food Chem Toxicol*, 2005, **43**(9), 1433–1439.

14 Sood R, Swarup D, Bhatia S, Kulkarni D D, Dey S, *et al*., Antiviral activity of crude extracts of *Eugenia jambolana* Lam against highly pathogenic avian influenza (H5N1) virus, *Indian J Exp Biol*, 2012, **50**(3), 179–186.

15 Mir Q Y, Ali M and Alam P, Lignan derivatives from the stem bark of *Syzygium cumini* (L.) Skeels, *Nat Prod Res*, 2009, **23**(5), 422–430.

16 Srivastava S and Chandra D, Pharmacological potentials of *Syzygium cumini*: A review, *J Sci Food Agric*, 2013, **93**(9), 2084–2093.

17 Nadkarni A, *Indian Materia Medica*, (Popular Book Depot, Bombay), 1976.

18 Rastogi R and Malhotra B, *Compandium of Indian Medicinal Plant*, (Publication and Information Director, New Delhi), 1970.

19 Kirtika K and Bas B, *Indian Medicinal Plant*, (Periodical Expert, New Delhi), 1975.

20 *Ayurvedic Pharmacopoeia*, Govt. of India, Ministry of Health and Family Welfare, Department of ISM and Health, 2nd ed, 1999.

21 OECD, guidelines for testing of chemicals/section: Health effects test No.423: acute oral toxicity-Acute toxicity class methods, (Organization for Economic Cooperation and Development, Paris), 2002.

22 Vimala G, Gricilda S F, Pandikumar P and Sukumar E, Pharmacological evaluation of ethanol extract of *Ficus benghalensis* seeds for antiulcer and antimicrobial efficacy, *Indian J Nat Prod Resour*, 2017, **4**, 329-334.

23 Dharmani P, Mishra P K, Maurya R, Chauhan V S and Palit G, *Desmodium gangeticum*: A potential anti-ulcer agent, *Indian J Exp Biol*, 2005, **43**, 517-521.

24 Suleyman H, Akcay F and Altinkaynak K, Effect of nimesulide on indomethacin and ethanol-induced gastric ulcer in rats, *Pharmacol Res*, 2002, **45**(2), 155-158.

25 Main I H M and Whittle B J R, Investigation of the vasodilator and antisecretory role of prostaglandins in the rat gastric mucosa by use of non-steroidal anti-inflammatory drugs, *Br J Pharmacol*, 1975, **53**(2), 217-224.

26 Piper D W and Stiel D D, Pathogenesis of chronic peptic ulcer, current thinking and clinical implications, *Med Prog*, 1986, **2**(1), 7-10.

27 Sairam K, Priyambda S, Aryya N C and Goel R K, Gastroduodenal ulcer protective activity of *Asparagus racemosus*; an experimental, biochemical and histological study, *J Ethnopharmacol*, 2003, **86**(1), 1-10.

28 Singh S, Evaluation of gastric anti-ulcer activity of fixed oil of *Ocimum basilicum* Linn. and its possible mechanism of action, *Indian J Exp Biol*, 1999, **37**(3), 253-257.

29 Vimla G, Shoba F G, Pandikumar P and Sukumar E, Pharmacological evaluation of ethanol extract of *Ficus benghalensis* seeds for antiulcer and antimicrobial efficacy, *Indian J Nat Prod Resour*, 2017, **8**(4), 329-334.

30 Ayyanar M and Subash-Babu P, *Syzygium cumini* (L.) Skeels: A review of its phytochemical constituents and traditional uses, *Asian Pac J Trop Biomed*, 2012, **2**(3), 240-246.

31 Bijauliya R K, Alok S, Sabharwal M and Chanchal D K, *Syzygium cumini* (linn.) - an overview on morphology, cultivation, traditional uses and pharmacology, *Int J Pharm Sci Res*, 2018, **9**, 3608-3620.