Analgesic and Anti-inflammatory activity of *Syzygium Cumini* leaves

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Abstract

Extensive investigations into the curative attributes of various plant species are prompting a reassessment of traditional medical approaches on a global scale. The vast range of plant diversity offers exciting prospects as an untapped reservoir of novel chemicals that could be employed for medicinal purposes. Herbal medicines are particularly advantageous due to their perceived effectiveness and cost-effectiveness.

The fruit commonly known as BlackBerry is scientifically referred to as Syzygium cumini (L.) Skeels or Eugenia jambolana Lam., belong to the species of Black Plum. Historically, people have utilized the bark, stem, and leaves of this plant due to their diverse properties. The fruits and seeds of the Black Plum are utilized for managing diverse conditions such as pharyngitis, diabetes etc

The study, therefore, presents preliminary results on the analgesic and anti-inflammatory effects of *S. cumini* leaves, aiming to validate traditional and folkloric beliefs.

This study aimed to evaluate the assessment of ethanol extract from cumin leaves to prevent edema in Wistar rats. Oral doses during the study were 200 and 400 mg/kg. Two doses of the extract were found to inhibit the effects, confirming the use of the plant in traditional Medicine. Our research focused specifically on investigating the anti-inflammatory properties of cumin leaves.

Additionally, the analgesic effects of *S. cumini* leaves were studied using the acid-induced writhing method in mice. The ethanolic extract obtained from *S. cumini* leaves was compared to a 300 mg dose of acetylsalicylic acid (Aspirin). The study explored two different doses of the ethanolic extract, 300 mg/kg and 500 mg/kg. The results indicated that *S. cumini* leaves have significant analgesic effects. Both the 300 mg/kg and 500 mg/kg doses demonstrated considerable pain-relieving activity, comparable to the reference medication, aspirin.

Keywords: Anti-inflammatory; S.Cumini; Traditional medicine

INTRODUCTION:

Jamun, a plant with a long tradition of ethno medical usage, has been utilized in diverse treatments to address a broad spectrum of human ailments. Traditional practices involving Jamun have focused on ailments such as diabetes, dental issues, intestinal problems, liver disorders, and skin conditions. In the practice of Ayurvedic medicine, it is customary to give individuals 1-3 grams of dried seed powder as a remedy for diabetes. Numerous components of the plant have been utilized to treat conditions such as colic, mouth blisters, diarrhea, dysentery, diabetes, acne, piles, stomachaches, digestive disorders, and cancer [1]

The black plum has significant historical use in complementary and alternative medicine, with all its different parts traditionally employed for various purposes. People across the world have employed its fruits to treat various conditions such as inflammation, cough, ringworm, diarrhea, and diabetes. Jamun is a highly regarded medicinal plant that holds significant prominence in India. Within the practice of Ayurvedic medicine, it is specifically recommended for the management of diabetes mellitus.To treat diabetes in humans, the ripe fruit juice of jamun is commonly consumed three times a day, with doses typically ranging from 0.5 to 2 teaspoons. In addition to its use for diabetes, jamun is also recognized as a general health stimulant and blood purifier, providing overall health benefits [1]. The bark of the jamun stem has a wide range of uses and can be employed for various purposes. It is utilized as a natural sweetener, possesses astringent properties, acts as an anthelmintic to combat intestinal worms, exhibits antibacterial effects, serves as a carminative to alleviate gas and flatulence, acts as a constipating remedy, functions as a diuretic to promote urine production, aids in digestion, acts as a febrifuge to reduce fever, serves as a refrigerant to cool the body, and has stomachic properties to support a healthy stomac. The fruits and seeds of the jamun plant are used to cure asthma, bronchitis, diabetes, and spleenopathy. Additionally, the fresh pulp of jamun fruit blended with honey helps maintain a healthy body, while the powder from its seeds aids in treating skin blemishes and enlarged spleen. Jamun seed powder mixed with jaggery is typically given to patients suffering from diarrhea and dysentery. The leaves, when extracted as juice or used as a poultice, are beneficial in treating skin diseases and diarrhea [1].

Similar to Ayurvedic medicine, Unani medicine also utilizes jamun for strengthening gums and teeth and deworming against ringworm infection. The pulp of jamun fruit has potential in treating

gingivitis, and regular consumption of jamun for two to four months can aid in the healing of hemorrhoids. As per Jagetia [1], the consumption of an infusion made from jamun seeds may assist in relieving feelings of exhaustion and fatigue. Numerous studies have investigated the traditional applications of different components of the Jamun plant using diverse research approaches.

Inflammation serves as the body's protective response against foreign substances, including pathogens or irritants. It can be categorized into two types: acute inflammation, which is a short-term response typically occurring after an injury, and chronic inflammation, a long-term condition associated with serious diseases like COPD and rheumatoid arthritis. During inflammation, the immune system sends white blood cells to surround and safeguard the affected area, resulting in visible signs such as swelling, redness, heat, and pain. In some cases, the body mistakenly identifies its own cells or tissues as harmful and triggers an autoimmune response, leading to autoimmune diseases like type 1 diabetes [2].

The extensive study of the therapeutic properties of plants is prompting a worldwide reconsideration of herbal medicine. The wide variety of these plants offers a valuable reservoir of new compounds that hold the potential for therapeutic effects. Herbal medicine is often praised for its perceived effectiveness, fewer side effects, and affordability.

Syzygium cumini (L.), also called as Eugenia jambolana Lam., is commonly referred to as BlackBerry. Its stem and bark have traditionally been used for their astringent, stomachic, and antibacterial properties. The fruits and seeds are employed in treating several ailments such as spleenopathy, diabetes etc.

Syzygium cumini leaves are known for their antibacterial properties and their ability to enhance dental health by strengthening teeth and gums. Additionally, they have been broadly utilized in the healing of various diseases. While there have been several studies on the phytochemical and pharmacological aspects of the plant, most of them have focused on its stem bark, fruits, and seeds, with limited research conducted on the leaves. Therefore, this study aims to present initial findings of the analgesic and anti-inflammatory effects of *S. cumini* leaves in experimentally induced inflammations in rats.

Approximately 82% of the oil content in freshly harvested jamun leaves comprises essential oils [3]. These oils extracted contain a diverse range of compounds, such as α-Pinene, camphene, β-Pinene, myrcene, limonene, cis-Ocimene, trans-Ocimene, γ-Terpinene, terpinolene, bornyl acetate, α-

Copaene, β-Caryophyllene, α-Humulene, γ-Cadinene, and δ-Cadinene [4]. Additionally, other constituents identified in the essential oils include trans-ocimene, cis-ocimene, β-myrcene, α-terpineol, dihydrocarvyl acetate, geranyl butyrate, and terpinyl valerate [5].
The researchers also investigated the chemical composition of unsaponifiable substances in the seed oil [6,7].

MATERIALS AND METHODS

Plant materials

The *S. cumini* leaves were collected in June 2022 from the Department of Agriculture, University of Karachi, Pakistan.

Ethanolic extract

Fresh leaves of the plant were collected and sent to the Department of Botany, University of Karachi, for identification. After collection, the leaves were dried for 2 weeks, and once completely dried, they were ground into a fine powder. This powder was then subjected to maceration with ethanol for a duration of 14 days. After filtration, the resulting mixture was further concentration using a rotary evaporator.

Experimental Animals

This study utilized Wistar rats of both sexes, with a weight of 160-180 g. The rats were kept in appropriate plastic cages under controlled conditions, maintaining a temperature of 25 ± 2 °C. They were provided with unrestricted access to a standard diet. The ethical treatment and care of these animals adhered to the guidelines presented by the National Institute of Health (NIH).

Drug treatment

The animals were divided into four groups, each consisting of 7 rats. The first group received a vehicle and served as the control group. Two groups were administered the ethanolic extract *of S. cumini* at doses of 200 mg/kg and 400 mg/kg, respectively. The remaining group received Diclofenac sodium at a dose of 5 mg/kg. All substances, including the vehicle, *S. cumini* ethanolic extract, and the standard drug (Diclofenac sodium), were administered orally for a duration of 15 days.

Evaluation of anti-inflammatory activity

Carrageenan-induced rat paw oedema

The animals, irrespective of their sex, were divided into four groups, each consisting of seven animals:

Group I: Control animals received 10 ml/kg of 1% Sodium Carboxymethyl Cellulose (SCMC) orally (p.o.).

Group II: Animals received the ethanolic extract at a dose of 200 mg/kg orally (p.o.).

Group III: Animals received the ethanolic extract at a dose of 400 mg/kg orally (p.o.).

Group IV: Animals were administered the standard drug Diclofenac sodium at a dose of 5 mg/kg orally (p.o.).

$$\text{Percentage inhibition of edema} = \frac{(Vc - Vt)}{(Vc)} \times 100$$

In this experiment, Vc represents the volume of the inflamed paw in the control group of animals, while Vt represents the inflamed paw volume in the drug-treated animals.
To trigger paw edema, a solution containing 0.1 ml of 1% carrageenan in physiological saline was injected into the sub-plantar tissue of the left hind paw of each rat, using the approach outlined by Winter et al. in 1962 [8]. Prior to the administration of carrageenan, the animals were given the ethanol extract orally, 30 minutes beforehand. Paw volume measurements were then taken at specific time intervals (60, 120, 180, and 240 minutes) using the mercury displacement method with a plethysmograph.

The percentage of paw volume inhibition in the drug-treated group was then compared to the control group (I-group) injected with carrageenan only. Additionally, a reference agent, Diclofenac sodium (at a dose of 5 mg/kg orally), was used for comparison purposes.

ASSESSMENT OF ANALGESIC ACTIVITY

A modified method was utilized for the experiment, adapted from the procedure introduced by Koster et al. in 1959 [9]. Mice were used as the test subjects for this particular experiment. To induce writhing, the mice were given an intraperitoneal injection of a 10ml/kg acetic acid solution. Prior to the acetic acid administration, the test material was orally administered to the mice, and a waiting period of thirty minutes was observed.

The number of writhes was then recorded over the next 30 minutes immediately after the administration of acetic acid. The degree of writhing inhibition was expressed as a percentage, providing evidence of the presence of analgesia. We juxtaposed this evidence with the frequency of writhing incidents observed in the control group of animals.

The mice were divided into four groups: Group-A served as the control, while Group B and Group C received oral doses of 300mg/kg and 500mg/kg of ethanol extract, respectively. Group D was given a standard reference compound. Each group consisted of 5 animals, with weights ranging from 25-34 grams. The reference compound used was Acetylsalicylic acid (Aspirin) at a dose of 300mg/kg, administered orally. Both the test drug and Acetylsalicylic acid were diluted in distilled water and orally administered. The control animals were treated orally with the same volume of saline as the test drug.

Statistical analysis of the data obtained from the pharmacological experiments was expressed as mean ± SEM. The difference between the control and treatment groups was tested for significance using ANOVA followed by Dunnet's t-test.

RESULTS

ANTI-INFLAMMATORY ACTIVITY

The research aimed to explore the anti-inflammatory properties of S. cumini ethanolic leaf extract in rats with carrageenan-induced acute paw edema, with the results presented in Table 1. Animals treated with the extract exhibited significantly smaller hind paw volumes compared to the control group, indicating the potential anti-inflammatory effects of Syzygium cumini crude extract. Two different doses of the ethanolic extract, 200 mg/kg and 400 mg/kg were administered to the test animals. The higher dose of 400 mg/kg showed more notable anti-inflammatory activity than the lower dose of 200 mg/kg. However, when

compared to the standard dose of diclofenac sodium (5 mg/kg), the extract's anti-inflammatory effectiveness was found to be less potent than that of diclofenac sodium. The anti-inflammatory activity of *S. cumini* ethanolic leaf extract was evaluated against carrageenan-induced acute paw edema in rats and the results are summarized in Table 1

ANALGESIC ACTIVITY

Aspirin commonly known as Acetyl salicylic acid, was given orally at a dosage of 300 mg/kg and used as a standard chemical in the writhing test induced by acetic acid. When the ethanolic extract of Syzygium cumini was administered, there was a significant decrease in the number of writhes observed, which was dose-dependent. According to Table-2, the average number of writhes in animals treated with a control substance (vehicle) and injected intra-peritoneal with acetic acid was 126. However, this number decreased to 47.4 and 41 in animals treated with oral doses of 300mg/kg and 500mg/kg of the test material, respectively. The results from the writhing test, as shown in Table-2, were highly significant and comparable to the writhes induced by aspirin, which resulted in 36.4 writhes. In terms of writhing inhibition, aspirin exhibited a percentage inhibition of 71.11%, while the ethanolic extract at doses of 300mg/kg and 500mg/kg showed percentages of inhibition of 62.8% and 67.47%, respectively.

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DISCUSSION

The broad study conducted on various genera of plants and their beneficial properties is prompting a reconsideration of traditional medicine practices worldwide. The wide variety of plants offers significant possibilities for uncovering novel medicinal compounds. Herbal medicines are valued for their perceived effectiveness, minimal occurrence of notable side effects, and affordability, which are regarded as their main benefits.

Syzygium cumini (L.), generally known as BlackBerry or Black plum, is associated with the Myrtaceae family. In conventional medicine, the stem and bark of this plant have traditionally been used for their properties that include antibacterial, and diuretic effects. The plant *S.cumini* have been used to treat ailments such as splenomegaly, urethrorrhea, and parasite infection. Syzygium cumini leaves have antibacterial properties and are used to strengthen oral diseases. They also have been extensively used in therapeutic treatment such as fever, gastric pain, skin constipation, and

leucorrhoea, and to inhibit excretion in the feces.

While numerous phytochemical and pharmacological studies have been conducted on *S. cumini*, most have focused on the seed, fruits, and bark. However, there are limited reports of experimental biological research conducted specifically on its leaves. Therefore, this study aims to provide preliminary data on the anti-inflammatory properties of *S. cumini* leaves in experimentally induced inflammation in mice, confirming traditional and folklore beliefs associated with this plant. While there have been numerous phytochemical and pharmacological studies conducted on Syzygium cumini, most of them have focused on its stem bark, fruits, and seeds.

However, reports of experimental biological studies performed specifically on leaves are limited. Hence, the present study aims to present preliminary findings of the anti-inflammatory effect of Syzygium cumini leaves in experimentally induced inflammations in mice, supporting the traditional and folkloric beliefs surrounding the plant [10].

The findings of the study indicate that the ethanolic extract of Syzygium cumini leaves revealed note-worthyt anti-inflammatory activity at doses of 200mg/kg and 400mg/kg, comparable to the reference drug Diclofenac sodium. The extract effectively inhibited carrageenan-induced inflammation in rat paws. Moreover, the extract demonstrated analgesic activity at doses of 300mg/kg and 500mg/kg, comparable to Aspirin as the reference medication. The methanolic extract of Syzygium cumini exhibited the highest analgesic efficacy among various fractions tested. The anti-inflammatory and analgesic effects of Syzygium cumini support its potential in treating inflammatory and painful conditions. Furthermore, these findings provide valuable leads for researchers in the search for new compounds from Syzygium cumini with biological potential for treating various ailments [11].

Indeed, Syzygium cumini has shown the potential in possessing both analgesic and anti-inflammatory effects. Nevertheless, it is essential to highlight that further research is needed to comprehensively comprehend the mechanisms behind these effects and to ascertain their practical applications in clinical settings. Additional studies and investigations will help to unlock the full therapeutic potential of Syzygium cumini and its potential benefits for human health.

Conclusion

In conclusion, the findings of the current research endorse the regular utilization of S. cumini for treating pain and inflammation. The extract obtained from *S. cumini* leaves has demonstrated anti-inflammatory properties, whichc can be linked to the existence of

triterpenoids, saponins, and tannins. However, more investigation is necessary to develop deeper into their therapeutic potential and gain a comprehensive understanding of the specific mechanisms by which they exert their anti-inflammatory effects. Continued research in this area will help to elucidate the precise benefits of these compounds and their potential applications in medical treatments.

Conflicts of Interest:

The authors declare no conflicts of interest related to the research, content, or publication of this article. The authors solely hold responsibility for the content and writing presented in this article.

Table 1. Anti-inflammatory evaluation of *Syzygium cumini* extracts against carrageenan-induced paw edema in rats.

Group	**Paw edema (ml)**			
	1 hr	2 hr	3 hr	4 hr
control	0.38±.0053	0.4487±.00262	0.5±4.00437	0.6242±.01042
SC200	0.3615±.00858* (5.26%)	0.4085±.0044** (8.88%)	0.3972±00643*** (27.27%)	0.3462±.005*** (43.54%)
SC400	0.3172±.00521** (15.8%)	0.3783±.00397** (18.42%)	0.3887±.00596*** (29.09%)	0.31±.00489*** (48.4%)
Diclofenac	0.2487±.00262*** (34.21%)	0.3272±.00422*** (26.66%)	0.2787±.0068*** (49.09%)	0.1987±.0127*** (67.74%)

Values are mean ± SEM of 7 animals in each group.

P- values: *p<0.05, **p<0.01, ***p<0.001

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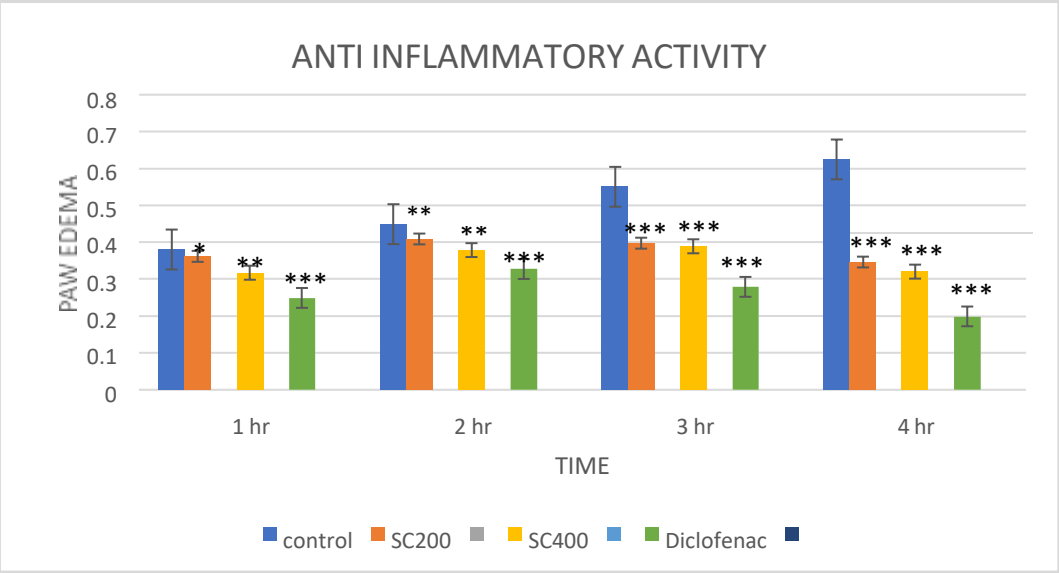


Figure 1

{Anti-inflammatory effect of *Syzygium cumini* extracts on carrageenan induced paw edema in rats. Values are mean ± SEM of 7 animals in each group (P- values: *p<0.05, **p<0.01, ***p<0.001)}

TABLE 2
ASSESSMENT OF ANALGESIC ACTIVITY

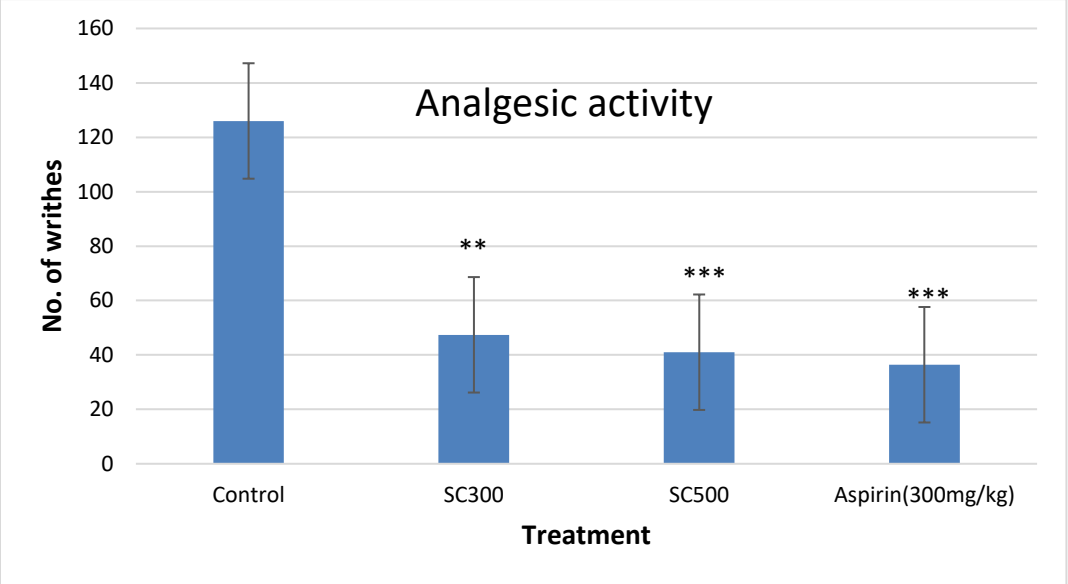
Effects of ethanolic extract of *Syzygium cumini* and Aspirin on acetic acid induced writhing in mice.

Treatment	Dose mg/kg Orally	Mean No. of writhes±S.E.M	Inhibition(%)
Control	0.5ml saline orally	126.1±5.7098	-
Ethanolic extract of *Syzygium cumini*	300mg/kg Orally	47.5±2.1588**	62.9%
	500mg/kg Orally	41.2±1.9494***	67.47%
Aspirin	300mg/kg	36.5±2.2718***	71.13%

{All values are presented as mean ± SEM; n = 5, *p < 0.05, **p < 0.01, ***p < 0.001 indicates significance compared to control}.

FIGURE 2

ASSESSMENT OF ANALGESIC ACTIVITY
(Writhing Test)



{Effects of ethanolic extract of *Syzygium cumini* and Aspirin on acetic acid induced writhing in mice.
All values are expressed as mean ± SEM; n = 5, *p < 0.05, **p < 0.01, ***p < 0.001 indicates significant compared to control}

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