Full Length

ANTIMICROBIAL, ANTIFUNGAL AND ANTIOXIDANT ACTIVITY OF AQUEOUS EXTRACT OF *SYZGIUM CUMINI* LEAF

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ABSTRACT

In the present study, the phytochemical constituents of Syzygium cumini (Naval) leaf extract were analyzed, and its antibacterial, antifungal, and antioxidant activities were evaluated. The phytochemical screening shown the presence of alkaloids, phenols, flavonoids, tannins, glycosides, and saponins in the extract. Antibacterial and antifungal activities were evaluated against Escherichia coli, Staphylococcus aureus, and Aspergillus flavus using the agar well diffusion method, which showed clear dose-dependent inhibition of the selected organisms.The zone diameters indicating antibacterial activity ranged from 1.3 to 1.8cm against Escherichia coli and from 1.0 to 1.6 cm against Staphylococcus aureus. In addition, the zone diameters for antifungal activity against Aspergillus flavus were observed to range between 1.2 and 1.6 cm. The antioxidant activity of the leaf extract demonstrated substantial free radical scavenging potential, reaching its maximum effect at 250 µg/ml is 61.32±0.44%. The aqueous leaf extract of Syzygium cumini exhibits strong antioxidant and antimicrobial properties.

Key words: *Syzygium cumini,*antimicrobial activity,antifungal activity, antioxidant activity

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INTRODUCTION

Various parts of the *Syzygium cumini* (jambolan) plant exhibit significant biological activities, making it valuable for developing products with potential applications. They are rich in phenolic compounds and possess strong anti-inflammatory properties. In India, *S. cumini* is popularly known by several names,

including Indian black plum, Java plum, Indian blackberry, and jamun (Lima *et al*., 2007).

Jamun, also referred to as *Syzygium cumini*, is a significant and widely cultivated member of this family (Fiqri *et al*., 2020). *Syzygium cumini* fruits, stems, and leaves have been studied for their antibacterial properties.Tannins and other phenolic components may be responsible for the *S. cumini* leaf hydroalcoholic extract's antibacterial action. Gallic and ellagic acid polyphenol derivatives are reported to be abundant in *Syzygium cumini.*There have been reports of *Syzygium* species having antimicrobial properties (Abdulrahman *et al*., 2018).

Water and methanol extracts from *Syzygium* spp. have demonstrated the ability to suppress the propogation of some certain fungal microorganisms included with skin diseases, including *Candida albicans, Trichophyton rubrum*, and *Staphylococcus aureus*.

Bacteria are simple microorganisms that vary in shape and size. They are single-celled and are considered prokaryotes. Bacteria are found in different environments, such as air, water, and soil, andshows a vital role in life cycles on the surface of Earth. The virulence characteristics that pathogenic bacteria possess, their capacity to evade the host's defenses, and their resistance to antibiotics all influence and it shows much virulent of pathogenic bacteria. Among the most important species of pathogenic bacteria that are resistant to many antibiotics are *E. coli, S. aureus, P. aeruginosa*, and others(Chua *et al*., 2019).

The current work aimed to define the phytochemical profile of aqueous *S. cumini* leaf extract and examine its antibacterial, antifungal, and antioxidant activities in order to determine its potential as a natural bioactive agent.

MATERIALS AND METHODS

Materials

Fresh naval (*Syzygium cumini*) leaves were collected from the College of Food and Dairy Technology, Koduveli, Chennai – 52. Analytical reagent (AR) grade zinc acetate dihydrate (LOBA CHEMIE Pvt. Ltd.,), DPPH2, 2-diphenyl-1-picrylhydrazyl (Sisco Research Laboratories Pvt. Ltd.,) was used for the naval leaf extract. The antibiotic kanamycin monosulfate powder(Sisco Research Laboratories Pvt. Ltd.) was used for antibacterial assays, and potato Dextrose Agar (PDA) medium (HiMedia Laboratories Pvt. Ltd.) and standard antifungal agent fluconazole (Sisco Research Laboratories Pvt. Ltd.) were used for antimicrobial assays.

Preparation of aqueous leaf extract of naval leaf (*Syzgium cumini*)

The naval leaves were plucked and thoroughly cleaned with running tap water and carried outby distilled water. The cleaned leaves were desiccated in a hot air oven at 60°C. The desiccated leaves were finely powdered and stored in an airtight

container.To prepare the extract, weigh 15 g of leaf powder and transfer it to a 200 mL beaker containing 100 mL of distilled water. To ensure appropriate mixing, the mixture was agitated at 600 rpm using a magnetic stirrer for 10 minutes. The solution was then cooked in a water bath at 70°C for an hour. After heating, the mixture was filtered using Whatman No. 1 filter paper. The filtrate (leaf extract) was collected and stored in screw-capped borosilicate bottles at 4°C for later use.

Phytochemical test of naval leaf extract
Test for Alkaloids

Add 200 mg of plant extract with 2% H_2SO_4 and heat for two minutes. After boiling, the liquid was completely filtered and a few drops of Dragendorff's reagent were included. The production of an orange-red precipitate revealed the presence of alkaloids in the extract (Adil *et al.*, 2024).

Test for Flavonoids test

The plant extract (200 mg) was first dissolved in NaOH, followed by the addition of HCl. The solution colorchanged from yellow to colorless, confirmed the presence of flavonoids (Adil *et al*., 2024).

Test for Tannins test

Aliquots of sample was mixed with distilled water and boiled. The resulting solution was filtered, and the filtrate was mixed with a few drops of ferric chloride. The appearance of a blackish-green color showed the presence of tannins in the leaf extract. (Adil *et al.*, 2024).

Test for phenolic groups

The plant extract of 1 ml was mixed with two millilitres of distilled water, added by a few drops of 10% ferric chloride solution. The formation of a blue or black suggested the existence of phenolic groups (Roy *et al*., 2020).

Test for saponins

The extract of 2 mL was added with 5 mL of distilled water and briskly shaken. The results formation of steady and persistent foam suggests the presence of saponins (Roy *et al.*, 2020).

Test for glycosides

The plant extract was then mixed with 2 ml of 50% H_2SO_4 in a boiling tube. The mixture was boiled in a ring water bath for 5 minutes. Next, 10 ml of Fehling's solution was included in the boiling tube. Boiling produces a precipitation of brick red, which confirmed the presence of glycosides (Roy *et al.*, 2020).

Antibacterial activity of naval leaf extract

The agar well diffusion assay was utilized to determine the antibacterial activity of the naval leaf extract against *E. coli* and *S. aureus*. Mueller-Hinton Agar (MHA) served as the growth medium for the assay. Overnight cultures of *E. coli* and *S. aureus* were evenly swabbed onto Petri plates and allowed to stand for 10 minutes. A sterile well borer was used to bore 6-mm-diameter wells in the petri plates. Various

concentrations of naval leaf extract were added to the wells, with kanamycin (100 µg/mL) serving as the standard antibacterial agent (Oliveira *et al.,* 2007).

Antifungal activity of naval leaf extract

The antifungal activity of naval leaf extract against *Aspergillus flavus* was analyzed using the agar well diffusion technique. Potato Dextrose Agar (PDA) was used as the growth medium for the assay. An overnight culture of *A. flavus* was evenly swabbed onto the Petri plates and allowed to solidify for 10 min. Wells with a diameter of 6 mm were created in the petri plates using a sterile well borer. Different concentrations of naval leaf extract were added to the bore wells in the plate, and Fluconazole (100 µg/mL) was used as the standard antifungal agent (Jabeen and Javaid, 2010).

Antioxidant activityof naval leaf extract

The antioxidant activity of the *Syzygium cumini* leaf extract was evaluated using the DPPH assay. Briefly, 100 µL aliquots of different concentrations of the naval leaf extract were added to 100 µL of 0.1 mM DPPH (2,2-Diphenyl-1-picrylhydrazyl) solution in ethanol, contained in flat-bottom 96-well plates (Ruan *et al*., 2008). The mixtures were allowed to stand in the dark at ambient temperature for 30 minutes. Ethanolic DPPH was employed as the blank control, and aqueous ascorbic acid (1 mg/mL) was used as the reference standard to compare the antioxidant activity of the anthocyanin extract. After incubation, the absorbance was measured at 517 nm using an Epoch microplate spectrophotometer

%Radical scavenging activity = (AC–AS) / AC ×100
Where,

AC – absorbance of the control;

AS– absorbance of the sample or standard sample.

RESULTS AND DISCUSSION

Phytochemical test of naval leaf extract

The result phytochemical analysis of naval leaf extract shown in Table.1 revealed that the confirmation of alkaloids, phenols,flavonoids, tannins,glycosides,saponin in the aqueous extract of leaves. Kadlag, (2023) reported the presence of alkaloids, glycosidess, phenolicss, flavonoidss, tanninss, saponins, steroids and triterpenoids inaqueous and alcoholic leaf extract and Hingmire *et al*., (2024) reportedthe presence ofalkaloidss, tannins, flavonoids, steroids, saponins, glycosides and anthraquinones in the aqueous plant extract of different plant parts.

Antibacterial activity of naval leaf extract

The antimicrobial potential of *S. cumini* leaf extract was evaluated against two bacterial strains, *E. coli* and *S. aureus.* Figure 1 depicts the inhibition zones produced by the standard antibiotics and *S. cumini* (naval) leaf extract within a concentration range of 1 mg/mL to 125 µg/mL (Table 2). The experiments were done in triplicate, the measurements and mean values were recorded. Compared to the standard antibiotics, the leaf extract demonstrated a

noteworthy antibacterial effect. For *E. coli,* the inhibition zone produced by the standard drug was 2.5 cm, whereas the naval leaf extract exhibited zones ranging from 1.3 cm to 1.8 cm. In the case of *S. aureus*, the leaf extract's inhibition zones varied from 1.0 cm to 1.6 cm, demonstrating antibacterial activity. Imran *et al.* (2017) reported inhibition zones for leaf extracts using various solvents against clinical strains of *E. coli* and *S. aureus* in the range of 0.8–2.4 cm. Additionally, (Shidiki and Vyas, 2022) reported inhibition zones of 2.2 cm and 2.6 cm for *S. aureus* at 100 µg/mL leaf extract concentration.

Antifungal activity of naval leaf extract

The antifungal activity of *Syzygium cumini* leaf extract was assessed against *Aspergillus flavus*. Figure 2 illustrates the inhibition zones produced by both the standard antifungal agents and the *S. cumini* (naval) leaf extract at concentrations varying from 1 mg/mL to 125 µg/mL (Table 3). The mean inhibition zone diameters (cm) were obtained from three replicates to ensure accuracy and reproducibility. Compared to standard antifungal drugs, the leaf extract exhibited a considerable inhibitory effect. For *A. flavus*, the inhibition zone of the standard drug measured 2.5 cm, while the zones produced by the leaf extract ranged between 1.2 and 1.6 cm, indicating moderate antifungal activity. Chandrasekaran and Venkatesalu (2004) reported that *S. cumini* aqueous leaf extract produced inhibition zones ranging from 1.2 cm –1.2 cm against *A. flavu*s, and Devi *et al.* (2025) reported inhibition zones of 1.22 cm against *A. flavus* for *S. cumini* leaf extract.

Antioxidant activity of naval leaf extract

The antioxidant activity of the sample was evaluated by determining the absorbance at 517 nm through the DPPH antioxidant assay (Table.4). At the highest tested concentration (250 µg/mL), ascorbic acid exhibited a maximum inhibition of 75.22 ± 0.35%, whereas the *S. cumini* leaf extract exhibited 61.32 ± 0.44%. A gradual reduction in activity was observed with decreasing extract concentrations. At 125 µg/mL, the scavenging activity of the leaf extract was 58.52 ± 0.50%, whereas the standard retained 73.31 ± 0.38% activity. At the lowest concentrations (15.63 µg/mL and 7.81 µg/mL) is (8.12 ± 0.71% and 4.71 ± 0.73% compared to the standard 65.89 ± 0.69% and 31.63 ± 0.38%. (Ruan *et al*., 2008) reported around 60–65 % inhibition at similar concentrations and (da Rosa *et al*., 2024) showed around 60–63% DPPH inhibition at higher concentrations (200–250 µg/mL), while ascorbic acid demonstrated about 75–78% inhibition at the same concentration.

Table.1. Phytochemical screening of aqueous naval leaf extract

Phytoconstituents	Aqueous leaf extract
Alkaloids	+
Phenols	+
Flavonoids	+
Tannins	+
Glycosides	+
Saponin	+

+ present; - absent

Table.2. Antimicrobial activity of naval leaf extract

Concentration	*E. coli* (Zone of inhibition in cm)	*S. aureus* (Zone of inhibition in cm)
1mg/ml	1.83^{c}±0.133	1.83^{b}±0.324
500µg/ml	1.63^{bc}±0.084	1.60^{b}±0.113
250µg/ml	1.36^{ab}±0.038	1.00^{a}±0.104
125µg/ml	1.16^{a}±0.121	0.96^{a}±0.078
Kanamycin (1 mg/mL)	2.66^{d}±0.069	2.66^{c}±0.252
F value	36.683**	12.285**

Table.3. Antifungal activity for naval leaf extract

Concentration	Aspergillus flavus (Zone of inhibition in cm)
1mg/ml	1.66^{a} ±0.261
500µg/ml	1.46^{a} ±0.098
250µg/ml	1.23^{a} ±0.080
125µg/ml	1.16^{a} ±0.095
Fluconazole (1 mg/mL)	2.56^{b} ±0.276
F value	9.377**

Values are expressed as the Mean±SDE according to Dunkan test. [a]Significance difference($p<0.05$), [b] Significance difference($p<0.05$), [c] Significance difference($p<0.05$),
[d] Significance difference($p<0.05$),**Highly significant($p<0.01$)difference

Table.4. Antioxidant activity for naval leaf extract

Concentration (µg/ml)	Radical scavenging activity of ascorbic acid (%)	Radical scavenging activity of naval leaf extract (%)
250 µg/ml	75.22±0.35	61.32±0.44
125 µg/ml	73.31±0.38	58.52±0.50
62.5 µg/ml	71.12±0.11	32.89±0.63
31.25 µg/ml	71.01±0.43	16.69±0.69
15.63 µg/ml	65.89±0.69	8.12±0.71
7.81 µg/ml	31.63±0.38	4.71±0.73

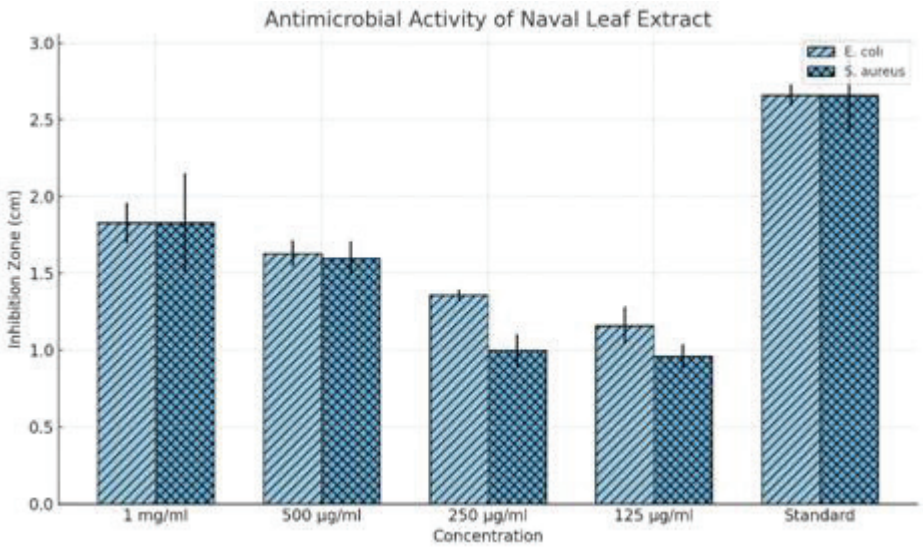

Fig.1. Antimicrobial activity of naval leaf extract(Mean±SE)

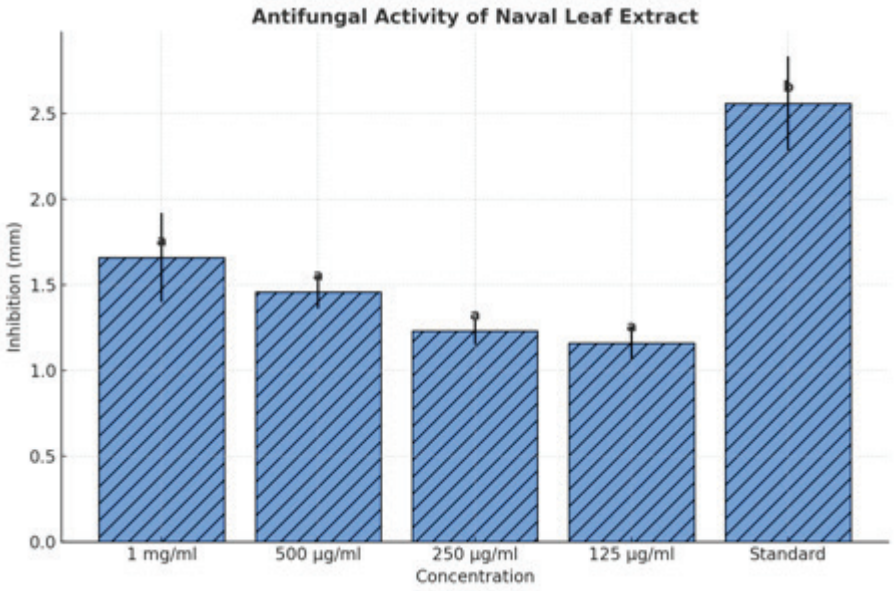

Fig.2. Antifungal activity of naval leaf extract(Mean±SE)

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