



## Antimicrobial Potential of Leaf, Seed Coat, and Bark Extracts of Sri Lankan *Syzygium cumini* (L.) Skeels: A Comparative Study

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### Abstract

**Background and Objectives:** Emergence of antimicrobial resistance emphasizes the requirement for new plant-derived bioactive compounds. *Syzygium cumini* (L.) Skeels (*S. cumini*) has been reported to possess various pharmacological properties; however, systematic comparison of different parts of the plant and extraction solvents under similar conditions is lacking. This study aimed to evaluate and compare the antifungal and antibacterial activities of extracts obtained from leaves, seed coats, and bark of the plant using ethanol, methanol, and water.

**Methods:** The extracts were evaluated for antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas* sp. Antifungal activity was evaluated using the agar well diffusion method against *Mucor* sp., *Aspergillus niger*, and *Fusarium* sp. through an *in vitro* bioassay. Zones of inhibition were measured and statistically analyzed.

**Results:** Antimicrobial activities varied with plant parts and solvent. The ethanolic seed coat extract showed the maximum antibacterial activity against *Staphylococcus aureus* ( $27.56 \pm 0.46$  mm), while the aqueous bark extract showed the minimum antibacterial activity against *Pseudomonas* sp. ( $11.50 \pm 0.66$  mm). The leaf methanolic extract exhibited the highest antifungal activity against *Fusarium* sp. ( $15.87 \pm 0.73$  mm), whereas the bark aqueous extract exhibited the lowest antifungal activity against *Aspergillus* sp. ( $37.93 \pm 0.80$  mm). Seed coat extracts were more efficacious against bacteria, and leaf extracts were relatively more potent against fungi.

**Conclusion:** This comparative study identified the most effective plant part–solvent combinations of *S. cumini*, supporting its potential as a naturally occurring source of antimicrobial agents for pharmaceutical and nutraceutical applications.

**Keywords:** Antimicrobial activity, Inhibition zone, Plant extracts, *Syzygium cumini*, Sri Lanka

### Introduction

Medicinal plants are an important source of bioactive compounds and continue to contribute to the discovery of novel therapeutic agents (1-7).

Plant-derived constituents promote human health by reducing the risk of cardiovascular diseases, inflammation, cancer, and diabetes (8). Despite their natural origin, these plants contain powerful



bioactive compounds and should be used with the same caution as conventional therapeutic medications. Certainly, many modern medications are synthetic derivatives of naturally occurring plant compounds (9, 10). Global use of herbal remedies, phytonutrients, and nutraceuticals is increasing rapidly, reflecting growing interest in natural therapeutic alternatives (11).

Numerous studies have confirmed the antibacterial properties of therapeutic plants, representing their capacity to prevent the growth and virulence of pathogens (12–16). However, despite the vast diversity of bioactive natural products, relatively few compounds have successfully advanced to approved antibacterial therapeutics due to challenges related to efficacy, toxicity, standardization, and clinical development (17, 18). Increase of antibiotic-resistant bacteria, frequently driven by overuse of existing antibiotics, has further limited the efficiency of conventional therapies, highlighting the urgent requirement for new antimicrobial agents (19).

*Syzygium cumini* (L.) Skeels (Myrtaceae), commonly known as Jamun, Black Plum, or Jambul, is an evergreen tree rich in tannins, alkaloids, flavonoids, terpenoids, fatty acids, phenols, minerals, and other phytochemicals that have been related to various pharmacological activities, including antimicrobial effects (20–26). Conventionally, it has been used for managing diabetes, cancer, asthma, arthritis, and cardiovascular conditions and is commonly found in regions such as Jaffna District, Sri Lanka (27, 28).

Universal rise of *Escherichia coli* presents a foremost treatment challenge, as multidrug-resistant strains are progressively reported in both hospital- and community-acquired infections (29, 30). Multidrug resistance in clinical bacteria, such as *Staphylococcus aureus*, further contributes to hospital-acquired infections (31). *Pseudomonas aeruginosa* is normally used to

assess the antimicrobial potential of therapeutic plants due to its occurrence and broad antibiotic resistance. Fungal pathogens, as well as *Mucor* sp., *Aspergillus niger*, and *Fusarium* sp., can cause invasive infections in several human organs (32). Their inadequate treatment possibilities and resistance mechanisms, such as biofilm formation, make them suitable models for evaluating antifungal activity (33). These strains were selected to evaluate the antimicrobial potential of plant extracts.

Earlier studies of *S. cumini* have reported antimicrobial activity in various parts of the plant; however, direct comparisons amongst leaf, seed coat, and bark extracts using different extraction solvents remain limited. Consequently, this study aimed to compare the antibacterial and antifungal activities of ethanol, methanol, and aqueous extracts prepared from the leaf, seed coat, and bark of *S. cumini* to identify the most effective plant part–solvent combinations.

## Materials and Methods

### Plant Materials

In November, *S. cumini* leaves, seed coat, and bark were collected from Jaffna District, Sri Lanka. The whole specimen was confirmed botanically by the Department of Botany, University of Jaffna, Sri Lanka.

### Preparation of Plant Materials

Selected plant parts were washed thoroughly with running tap water, air-dried at room temperature for two weeks to avoid the direct loss of phytoconstituents, and pulverized into a fine powder. Each powdered sample was labeled and kept in sealed plastic jars until extraction.

### Preparation of Extracts

At this stage, 30 g of each powdered sample was extracted separately with 150 mL of 100% ethanol, methanol, or water (1:5 w/v) in 250 mL culture bottles. The mixtures were agitated using an orbital shaker at 100 rpm for 2 h daily over 5

days at room temperature. After maceration, extracts were filtered through Whatman No. 1 filter paper, concentrated by a rotary evaporator, and kept at 4 °C. Ethanol, methanol, and water were selected to evaluate the influence of solvent polarity on the extraction of bioactive phytoconstituents.

### Evaluation of Antimicrobial Activities Microorganisms

Three bacterial strains and three fungal strains (Tables 1 and 2) were obtained from the Department of Botany, University of Jaffna. Bacterial cultures were sub-cultured in nutrient broth for 24 h and kept on nutrient agar slants at 4 °C. Fungal cultures were kept on potato dextrose agar (PDA).

**Table 1.** Selected bacterial strains for the study

| Name                          | Type          | ATCC No |
|-------------------------------|---------------|---------|
| <i>Escherichia coli</i>       | Gram negative | 25922   |
| <i>Staphylococcus aureus</i>  | Gram positive | 29213   |
| <i>Pseudomonas aeruginosa</i> | Gram negative | 27853   |

ATCC: American Type Culture Collection

**Table 2.** Selected fungal strains for the study

| Name                     | Division   |
|--------------------------|------------|
| <i>Mucor</i> species     | Zygomycota |
| <i>Aspergillus niger</i> | Ascomycota |
| <i>Fusarium</i> species  | Ascomycota |

### Preparation of bacterial suspensions

Aseptic techniques were followed using a laminar airflow chamber and 70% ethanol for surface sterilization. Single colonies from nutrient agar plates were shifted into 2 mL sterile distilled water to prepare bacterial suspensions.

### Antibacterial Activity: Agar Well Diffusion Method

Nutrient agar (22.68 g in 810 mL distilled water) was autoclaved at 121 °C for 20–30 min, transferred into sterile Petri dishes, and allowed to solidify. Bacterial suspensions (0.1 mL in 10 mL sterile saline) were spread evenly on agar surfaces. Wells (0.5 cm diameter) were filled with 15 µL of each plant extract. Plates were incubated at 37 °C for 24 h. Streptomycin (100 µg/µL) and distilled water were used as positive and negative controls, respectively (34, 35). Inhibition zones were measured in four orientations, and assays were executed in triplicate.

### Antifungal Activity: In Vitro Bioassay

Sterile filter paper discs (14 mm) were soaked with 20 µL of each crude extract and placed on PDA plates. A 9 mm mycelial plug of each fungus was placed on the discs. Plates were incubated at room temperature for six days. Homai (20 µL) and sterile distilled water (20 µL) were used as positive and negative controls. Each assay was performed in three replicates. After six days on PDA supplemented with 100 µg/mL streptomycin, the maximum fungal growth was regarded as 0% growth suppression (34, 35). Fungal growth inhibition (%) was calculated based on the following formula:

$$\text{Percentage of growth inhibition (\%)} = \frac{(D_c - D_s)}{D_c} \times 100$$

where  $D_c$  = diameter of fungal growth in the control, and  $D_s$  = diameter of fungal growth in extract-treated plates.

### Crude Extract Concentration

All the extracts were used at a standardized concentration of 100 mg/mL for antibacterial and antifungal assays, ensuring consistent comparison across plant parts and solvents.

### Statistical Analysis

All antibacterial and antifungal tests were performed in triplicate, and the results were

presented as Mean  $\pm$  Standard Deviation. Minitab 17 was used to conduct statistical analysis. Extracts, plant parts, and solvent types were compared using one-way analysis of variance (ANOVA). To find specific pairwise differences, Tukey's multiple comparison test was used in cases where the ANOVA indicated significant differences ( $p < 0.05$ ).

## Results And Discussion

### Antibacterial Activity

Antibacterial activity of ethanolic, methanolic, and aqueous extracts of *S. cumini* leaves, seed coat, and bark against specific bacterial strains is compiled in Table 3.

**Table 3.** Inhibition of bacterial growth of various *Syzygium cumini* extracts

| Parts                              | Extracts | Inhibition zone diameter of bacterial strains (mm) |                               |                               |
|------------------------------------|----------|----------------------------------------------------|-------------------------------|-------------------------------|
|                                    |          | <i>Staphylococcus aureus</i>                       | <i>Escherichia coli</i>       | <i>Pseudomonas aeruginosa</i> |
| Leaf                               | Ethanol  | 26.25 $\pm$ 0.96 <sup>a</sup>                      | 24.62 $\pm$ 0.69 <sup>a</sup> | 23.75 $\pm$ 0.66 <sup>a</sup> |
|                                    | Methanol | 22.50 $\pm$ 0.70 <sup>b</sup>                      | 20.75 $\pm$ 0.35 <sup>b</sup> | 20.00 $\pm$ 0.70 <sup>b</sup> |
|                                    | Aqueous  | 19.00 $\pm$ 0.70 <sup>c</sup>                      | 18.31 $\pm$ 0.96 <sup>c</sup> | 15.00 $\pm$ 0.70 <sup>c</sup> |
| Seed coat                          | Ethanol  | 27.56 $\pm$ 0.46 <sup>a</sup>                      | 25.00 $\pm$ 0.70 <sup>a</sup> | 24.50 $\pm$ 0.70 <sup>a</sup> |
|                                    | Methanol | 24.37 $\pm$ 0.69 <sup>b</sup>                      | 22.37 $\pm$ 0.69 <sup>b</sup> | 20.50 $\pm$ 0.50 <sup>b</sup> |
|                                    | Aqueous  | 17.31 $\pm$ 0.78 <sup>d</sup>                      | 16.37 $\pm$ 0.54 <sup>d</sup> | 13.06 $\pm$ 0.72 <sup>d</sup> |
| Bark                               | Ethanol  | 19.81 $\pm$ 0.99 <sup>c</sup>                      | 18.62 $\pm$ 0.73 <sup>c</sup> | 16.93 $\pm$ 0.72 <sup>c</sup> |
|                                    | Methanol | 18.25 $\pm$ 0.61 <sup>d</sup>                      | 16.81 $\pm$ 0.70 <sup>d</sup> | 14.25 $\pm$ 0.79 <sup>d</sup> |
|                                    | Aqueous  | 14.43 $\pm$ 0.76 <sup>e</sup>                      | 13.81 $\pm$ 0.70 <sup>e</sup> | 11.50 $\pm$ 0.66 <sup>e</sup> |
| Positive control (Streptomycin)    |          | 33.31 $\pm$ 0.42 <sup>f</sup>                      | 30.25 $\pm$ 0.35 <sup>f</sup> | 29.31 $\pm$ 0.42 <sup>f</sup> |
| Negative control (distilled water) |          | 0 $\pm$ 0 <sup>g</sup>                             | 0 $\pm$ 0 <sup>g</sup>        | 0 $\pm$ 0 <sup>g</sup>        |

Mean  $\pm$  SD, n = 3; Different superscript letters (a-g) within a column indicate significant differences ( $p \leq 0.05$ ).

Diameter of the inhibitory zone reflected the antibacterial effectiveness of the extracts. The ethanolic seed coat extract revealed the highest inhibition against *Staphylococcus aureus* (27.56  $\pm$  0.46 mm), while the aqueous bark extract displayed the lowest effect against *Pseudomonas aeruginosa* (11.50  $\pm$  0.66 mm). Among the tested bacteria, *S. aureus* and *P. aeruginosa* were the most and least susceptible, respectively. Streptomycin, used as a positive control, produced the largest inhibitory zone against *S. aureus*, while the negative control revealed no activity. Overall, ethanolic extracts consistently verified superior antibacterial activity compared to methanolic and aqueous extracts.

### Antifungal Activity

In comparison to specific fungal strains, Table 4 presented the fungal growth diameter of

ethanolic, methanolic, and aqueous extracts of the leaf, seed coat, and bark sections of *S. cumini*.

Antifungal activity was inversely associated with fungal growth diameter, with smaller diameters indicating stronger inhibition. Amongst the tested extracts, the methanolic leaf extract exhibited the highest antifungal efficacy, producing the minimum growth diameter (15.87  $\pm$  0.73 mm) against *Fusarium* sp. In contrast, the aqueous bark extract displayed the largest growth diameter (37.93  $\pm$  0.80 mm) and the lowest activity against *Aspergillus niger*. Across all plant parts and extracts of *S. cumini*, *Fusarium* sp. was commonly the most susceptible fungus, while *Aspergillus* sp. was the least inhibited. Positive controls with fungicide entirely suppressed fungal growth, whereas negative controls (distilled water) displayed maximum growth.

**Table 4.** Fungal growth in various *Syzygium cumini* sections against certain fungal strains

| Parts                              | Extracts | Fungal growth diameter (mm) for fungal strains |                           |                           |
|------------------------------------|----------|------------------------------------------------|---------------------------|---------------------------|
|                                    |          | <i>Mucor</i> sp.                               | <i>Aspergillus niger</i>  | <i>Fusarium</i> sp.       |
| Leaf                               | Ethanol  | 22.87 ± 0.81 <sup>a</sup>                      | 28.12 ± 0.59 <sup>b</sup> | 17.06 ± 0.63 <sup>b</sup> |
|                                    | Methanol | 20.62 ± 0.41 <sup>b</sup>                      | 26.62 ± 0.64 <sup>c</sup> | 15.87 ± 0.73 <sup>c</sup> |
|                                    | Aqueous  | 24.87 ± 0.59 <sup>a</sup>                      | 29.87 ± 0.59 <sup>a</sup> | 19.50 ± 0.70 <sup>a</sup> |
| Seed coat                          | Ethanol  | 25.75 ± 0.50 <sup>a</sup>                      | 31.50 ± 0.70 <sup>a</sup> | 21.87 ± 0.78 <sup>a</sup> |
|                                    | Methanol | 24.56 ± 0.58 <sup>a</sup>                      | 28.93 ± 0.63 <sup>b</sup> | 19.75 ± 0.66 <sup>b</sup> |
|                                    | Aqueous  | 27.50 ± 0.43 <sup>a</sup>                      | 33.75 ± 0.82 <sup>a</sup> | 23.62 ± 0.48 <sup>a</sup> |
| Bark                               | Ethanol  | 31.93 ± 0.88 <sup>b</sup>                      | 35.37 ± 0.85 <sup>b</sup> | 25.25 ± 0.66 <sup>b</sup> |
|                                    | Methanol | 29.62 ± 0.69 <sup>b</sup>                      | 31.87 ± 0.59 <sup>c</sup> | 23.62 ± 0.69 <sup>b</sup> |
|                                    | Aqueous  | 33.37 ± 0.48 <sup>c</sup>                      | 37.93 ± 0.80 <sup>c</sup> | 27.37 ± 0.85 <sup>c</sup> |
| Positive control (Fungicide)       |          | 0 ± 0 <sup>d</sup>                             | 0 ± 0 <sup>d</sup>        | 0 ± 0 <sup>d</sup>        |
| Negative control (Distilled water) |          | 39.25 ± 0.66 <sup>e</sup>                      | 40.20 ± 0.34 <sup>e</sup> | 38.45 ± 0.41 <sup>e</sup> |

Mean ± SD, n = 3; Different superscript letters (a-e) within a column indicate significant differences ( $p \leq 0.05$ ).

Results of this study confirmed that *S. cumini* exhibits significant antimicrobial potential, which is in line with reports from several countries (36–58). A closer analysis suggests that the differences in activity among plant parts and extraction solvents are connected to their phytochemical composition. For example, the seed coat and pulp are predominantly rich in phenolic compounds, flavonoids, and tannins, which are recognized to disrupt microbial cell walls, inhibit enzymes, and interfere with nucleic acid synthesis, explaining the heightened activity of seed extracts (55, 59).

Choice of extraction solvent significantly influences antimicrobial efficacy. Alcoholic solvents, such as methanol and ethanol, dissolve both polar and non-polar phytochemicals, creating extracts with a broader spectrum of bioactive compounds, which correlates with larger inhibition zones observed in methanolic or ethanolic extracts, compared to aqueous ones (37–53). Water, being highly polar, primarily extracts hydrophilic compounds, which may lack potent antimicrobial constituents, such as phenolics or alkaloids, reducing the efficacy of the extract.

Antibacterial activity of seed extracts on cotton fabric demonstrates their practical potential and is attributed to the high concentration of bioactive

compounds in the seeds (54). Hydroalcoholic seed extracts also demonstrated efficacy against multidrug-resistant pathogens, such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* (36, 56–58), highlighting their potential as a naturally occurring source of antimicrobial agents in medical and dental contexts.

In summary, the antimicrobial potential of *S. cumini* is strongly dependent on both plant part and extraction solvent, reflecting the distribution and solubility of bioactive phytochemicals. These findings underscore the potential of this plant as a source of antimicrobial agents and support its further development into nutraceutical and pharmaceutical products for the treatment of microbial infections (56, 59).

Although the agar well and disc diffusion methods provided clear evidence of the antimicrobial action of *S. cumini* extracts, these assays have inherent limitations. Inhibition zone diameters can be influenced by factors such as extract solubility, diffusion rate, and agar composition, and may not precisely reflect the potency of bioactive compounds. Subsequently, while the results indicate relative efficacy among plant parts and solvents, they do not provide exact concentrations required to inhibit microbial growth. Incorporating minimum inhibitory

concentration and minimum bactericidal/fungicidal concentration determinations in future studies would allow a more quantitative assessment of antimicrobial action and reinforce the assessment of *S. cumini* as a potential foundation of natural antibacterial and antifungal agents.

### Conclusion

*Syzygium cumini* demonstrated significant antimicrobial activity, with ethanolic seed coat extracts showing the strongest antibacterial activity and methanolic leaf extracts exhibiting the highest antifungal activity. These findings support its potential as a natural source of antimicrobial agents and provide scientific evidence for its traditional therapeutic use. Further studies are warranted to identify the active compounds and evaluate their safety and efficacy.

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### Author Contributions

**Gowri Rajkumar:** Conceptualization, methodology, investigation design, formal analysis, supervision, validation, manuscript review and editing.

**Kolamugi Vethanayahan:** Performance of the experiments, data collection and analysis, and preparation of the initial draft of the manuscript.

**Vinotha Sanmugarajah:** Contribution to the development of the research proposal and assistance in manuscript preparation and revision.

**All authors** read and approved the final version of the manuscript.

### Funding Statement

None.

### Conflict of Interest

The authors declare no conflicts of interest.

### Ethics Statement

Ethical approval was not required for this study because it involved only plant materials and *in vitro* antimicrobial assays and did not involve human participants or experimental animals.

### Data Availability Statement

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

### Artificial Intelligence Statement

The authors declare that no artificial intelligence tools were used in the preparation, analysis, or writing of this manuscript.

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