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**Research Article**

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# Antimicrobial Potency of Isolated Compounds from *Syzygium cumini* (L.) (Myrtaceae) root

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**Abstract** *Objective*: To observe antibacterial, antifungal, MIC of two compounds from methanol root extract.
Methodology: The powdered root extracted n-Hexane, acetone, chloroform and methanol using separating funnel. The antibacterial activity of *S. cumini* were tested against thirteen bacteria following by disc diffusion assay method and Ciprofloxacin was used as a control. Antifungal activity were tested against five pathogenic fungi and Nystatin used as control. Serial dilution technique was used for Minimum Inhibitory Concentrations (MIC).
*Results*: Ciprofloxacin responsive with highest inhibition of zones at 30 µg/disc concentration. Both the compounds were highly responsive of tested bacteria. Nystatin showed the larger zone of inhibition for all the tested fungi. *C. albicans* possessed higher responsive zone of inhibition than *A. niger* but there was no zone of inhibition recorded on other tested fungi. The MIC of the purified compound $S_2$ were 128 µg/ml against *S. -β-haemolyticus*, *S. dysenteriae* and *B. cereus*.
*Conclusion*: The results summarized that antibacterial and antifungal activities of both the compounds *S. cumini* against life threatening pathogens which appears to be an effective material for development of antimicrobial drugs and ecofriendly biopesticides.

**Keywords** Antimicrobial potency, disk diffusion method, compounds, *Syzygium cumini*

## 1. Introduction

In treatment and prevention of number of ailments, man is using various parts of a number of plants [1]. The active compounds of plants have medicinal values, and the first such compound is the morphine isolated from the opium in the early 19$^{th}$ century [2, 3].

Out of 2000 species of plants 170 odd families which are said to have some insecticidal and anti-pathogenic values [4]. Constituents of many aromatic plants are used for flavoring or medicinal purpose possesses insecticidal properties. The interest in plant derived drugs has been mainly has been increasing because they are safer, less costly and without side effects in comparison with synthetic drugs. Moreover, development of resistance of the pests to these synthetic drugs occur which is absent in botanicals.

An indigenous and widely distributed plant tropics is *Syzygium cumini* (L.) Skeels. This evergreen tree is a member of the family Myrtaceae and widely distributed throughout India, Bangladesh, Ceylon, Pakistan and Indonesia.

It is important both in Ayurveda and Unani system of medication because of its therapeutic properties. *S. cumini* has anti-inflammatory [5], antipyretic [6] and antioxidant [7, 8] activities. It has also medicinal value for the treatment of fever, gastropathy, strangury, dermopathy [9], diarrhea, digestive problems, cough and diabetes [10].

In the present study the antimicrobial potency of the methanolic root extract of *S. cumini* is evaluated for its antimicrobial potency.

## 2. Materials and Methods

*Plant*: The roots of *S. cumini* were collected from Rajshahi University Campus and authenticated by the authority of the Department of Botany, University of Rajshahi where a voucher specimen (# 735) has been deposited.

*Preparation of extracts and isolation of compound:*

*S. cumini* (500g) roots were dried and powdered followed by extraction with methanol (MeOH) (BDH, England) using separating funnel. The crude extract was stored in a refrigerator at $-20^0$C. Subsequently the crude extracts was subjected to isolation, identification and finally structural elucidations. Two compounds namely Quercetin 3-β-D-glucoside ($S_1$) and Isorhamnetin 3-O-rutinoside ($S_2$) were identified.

*Antibacterial assay:*

The antibacterial activity of the extracts was performed by the agar well diffusion method [11]. Fourteen pathogenic bacteria (six gram-positive and eight gram-negative) were selected for the antibacterial test and were cultured at the Molecular Laboratory, Institute of Biological Sciences, Rajshahi University.

Six gram positive bacterial strains, viz., *Staphylococcus aureus* (ATCC-259233), *Bacillus cereus* (ATCC-14603), *Bacillus megaterium* (QL-38), *Bacillus subtilis* (QL-40), *Sarcina lutea* (QL-166), *Streptococcus β-haemolyticus* (CRL) and seven gram-negative bacterial strains, viz., *Salmonella typhi* (ATCC-14028), *Shigella dysenteriae* (AL-35587), *Shigella shiga* (ATCC-26107), *Shigella boydii* (AL-17313), *Escherichia coli* (FPFC-1407), *Pseudomonas aeruginosa* (ATCC-27853), *Pseudomonus aeruginosa* (ATCC-27853) were obtained from the rearing cultures maintained in the Molecular Biology Laboratory.

For determining the optimal concentration of extracts, sterile 7.5 mm filter paper discs were treated with 50 and 200 µl of methanol extracts. The bacteria were inoculated on full–strength nutrient agar (Qualigens Fine Chemicals, Prod # 58673) by suspending loops in sterile de-ionized water. The bacterial suspension was then smeared on agar plates with a sterile glass-rod to ensure the entire surface of the agar had an even coating of the bacterial suspension. The test plates were divided into several areas and one filter paper disk was placed on each of the areas. The plates are then kept in an incubator (37 ºC) for 12-18 h to allow the growth of the organisms. Biological activity of the *S. cumini* components on bacterial growth was quantified by measuring the diameter of zones of inhibition (mm) deducing the size of the treated filter paper discs. Ciprofloxacin 30 µg/disc was used as control.

*Determination of minimum inhibitory concentration*: The minimum inhibitory concentration (MIC) of pure compounds was determined by serial dilution technique. The test organisms were *S. β haemolyticus*, *S. dysenteriae* and *B. cereus*. Compounds $S_1$ and $S_2$ in different concentrations (2-512 µg/ml) and 10 µl of bacterial culture ($10^7$ cells/ml) were added in culture tubes containing 1 ml sterile broth medium separately. The cultures were mixed and incubated at 37 ºC for 24 h and bacterial growth was observed.

*Antifungal activity*: The same procedure was followed as that for antibacterial activity. Nystatin (50 µg/disc) was used as reference and from the stock culture solution (5 µg/µl). The incubation period was 48-72 h. The fungi (*F. vasinfectum*, *A fumigatus*, *A. niger*, *A. flavus* and *C. albicans*) were collected from Department of Pharmacy, University of Rajshahi.

## 3. Results

Two purified compounds of *S. cumini* isolated from the root were active against Gram positive and Gram negative bacteria and against the selected fungi (Tables 1-4).

### *3.1 Antibacterial activity of the purified compounds*

Among the test bacteria all were responsive to the $S_1$ and $S_2$ compounds with the zones of inhibition given in the (Table 1) below in comparison to the inhibition by the standard Ciprofloxacin. Both the compounds showed prominent clear zone of inhibitions at 200 µg/disc. Higher zone of inhibition was recorded in *S. lutea* and *S. dysenteriae*.

**Table 1:** Antibacterial activity of pure compounds $S_1$ and $S_2$ of S. *cumini* and the standard ciprofloxacin

| Test organisms | Diameter of zone of inhibition (in mm) | | | | |
|---|---|---|---|---|---|
| | $S_1$ µg/disc | | $S_2$ µg/disc | | Ciprofloxacin |
| | 50 | 200 | 50 | 200 | 50 µg/disc |
| | Gram positive bacteria | | | | |
| *S. aureus* | 6 | 10 | 8 | 12 | 30 |
| *B. cereus* | 6 | 12 | 9 | 15 | 28 |
| *B. megaterium* | 7 | 11 | 8 | 13 | 31 |
| *B. subtilis* | 9 | 13 | 7 | 12 | 29 |
| *S. lutea* | 8 | 16 | 7 | 14 | 30 |
| *S.- β –haemolyticus* | 8 | 14 | 0 | 15 | 28 |
| | Gram negative bacteria | | | | |
| *S. typhi* | 8 | 12 | 6 | 13 | 30 |
| *S. dysenteriae* | 0 | 16 | 8 | 12 | 28 |
| *S. shiga* | 8 | 12 | 8 | 12 | 31 |
| *S. boydii* | 10 | 14 | 8 | 12 | 29 |
| *E. coli* | 10 | 14 | 7 | 15 | 30 |
| *K. pneumoni.* | 8 | 12 | 6 | 10 | 28 |
| *P. aeruginosa* | 8 | 14 | 7 | 13 | 30 |

*3.2. Minimum inhibitory concentration (MIC)*:

The tested bacterial species did not show any growth when culture medium was supplemented with 128, 256 and 512 µg/ml of compound $S_1$ and compound $S_2$. *B. cereus* was responsive to 64 µg/ml whereas *S. β. haemolyticus* and *B. cereus* were responsive to 32 µ g/ml in both the compounds. There were no inhibition zone was observed in test tubes containing compound $S_1$ and compound $S_2$ at a concentration less than 64 µg/ml. Among three control tests, only $C_i$ showed bacterial growth. It is evident from the results that both the compounds have property to inhibit bacterial growth even at low concentration (64 µg/ml) (Tables 2 and 3).

**Table 2:** Minimum inhibitory concentrations (MIC) of the purified compound $S_1$ against test pathogenic bacteria

| Test tube No. | Nutrient broth medium added (ml) | Root extract (µg/ml) | Inoculum added (µl) | *S. -ß-haemolyticus* | *S. dysenteriae* | *B. cereus* |
|---|---|---|---|---|---|---|
| 1 | 1 | 512 | 10 | - | - | - |
| 2 | 1 | 256 | 10 | - | - | - |
| 3 | 1 | 128 | 10 | - | - | - |
| 4 | 1 | 64 | 10 | - | - | + |
| 5 | 1 | 32 | 10 | + | - | + |
| 6 | 1 | 16 | 10 | + | + | + |
| 7 | 1 | 8 | 10 | + | + | + |
| 8 | 1 | 4 | 10 | + | + | + |
| 9 | 1 | 2 | 10 | + | + | + |
| 10 | 1 | 1 | 10 | + | + | + |
| Cm | 1 | 0 | 0 | - | - | - |
| Cs | 1 | 512 | 0 | - | - | - |
| Ci | 1 | 0 | 10 | + | + | + |
| Results of MIC values in (µg/ml) | | | | 16 | 64 | 32 |

"+" = Growth "-" = No growth

**Table 3:** Minimum inhibitory concentrations (MIC) of the purified compound $S_2$ against test pathogenic bacteria

| Test tube No. | Nutrient broth medium added (ml) | Root extract (µg/ml) | Inoculum added (µl) | *S. -ß-haemolyticus* | *S. dysenteriae* | *B. cereus* |
|---|---|---|---|---|---|---|
| 1 | 1 | 512 | 10 | - | - | - |
| 2 | 1 | 256 | 10 | - | - | - |
| 3 | 1 | 128 | 10 | - | - | - |
| 4 | 1 | 64 | 10 | - | - | + |
| 5 | 1 | 32 | 10 | + | - | + |
| 6 | 1 | 16 | 10 | + | + | + |
| 7 | 1 | 8 | 10 | + | + | + |
| 8 | 1 | 4 | 10 | + | + | + |
| 9 | 1 | 2 | 10 | + | + | + |
| 10 | 1 | 1 | 10 | + | + | + |
| Cm | 1 | 0 | 0 | - | - | - |
| Cs | 1 | 512 | 0 | - | - | - |
| Ci | 1 | 0 | 10 | + | + | + |
| Results of MIC values in (µg/ml) | | | | 64 | 32 | 64 |

"+" = Growth "-" = No growth

*3.4. Antifungal activity*: Among the five tested fungi *A. niger* and *C. albians* were responsive to the $S_1$ and $S_2$ compounds with the zones of inhibition given (Table 4) below in comparison to the inhibition by the standard Nystatin. There was no activity in case of *F. vasinfectum*, *A. fumigatus* and *A. flavus* in both the tested compounds. However, Nystatin showed the prominent zone of inhibition at 50 µg/disc in all tested fungi.

**Table 4:** *In vitro* antifungal activity of compounds $S_1$ and $S_2$ of *S. cumini* and the standard Nystatin.

| **Test Fungus** | **Diameter of zone of inhibition (in mm)** | | | | |
|---|---|---|---|---|---|
| | **$S_1$ µg/disc** | | **$S_2$ µg/disc** | | **Nystatin 50 µg/disc** |
| | **50** | **200** | **50** | **200** | |
| *F. vasinfectum* | - | - | - | - | 20 |
| *A. fumigatus* | - | - | - | - | 21 |
| *A. niger* | 6 | 12 | 6 | 14 | 18 |
| *A. flavus* | - | - | - | - | 20 |
| *C. albicans* | 8 | 14 | 8 | 16 | 18 |

## 4. Discussion

Findings on antimicrobial activity reported by previous workers support the recent outcome of this investigation. Imran *et al*. [12] determined the antibacterial activity of *S. cumini* leaf extracts against in solvents and found higher efficacy in petroleum ether and ethanolic solvents with the inhibition of zone 8-24 mm than other solvent extracts tested. In another study Khan *et al*. [13] also reported *Candida* spp. resistant to antifungal drugs against *S. cumini* ethanol extracts and was effective against MDR with inhibition of zone ranging from 10-18mm in diameter.

Antimicrobial activity of *S. calophyllifolium* Walp methanol leaf extract was evaluated [14] in different gram-positive and gram-negative bacteria using agar diffusion method, fungi and MIC by macro-broth dilution methods. They reported largest zone of inhibition against *Escherichia coli*, (25 mm in diameter) and fungal *Saecharomyces cerevisiae* ( 25 ± mm in diameter) at the highest concentration (15 l) and according to them *Bacillus cerus*, *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *E. coli* showed the highest MIC (10 ± mg/ml). Finally they concluded that methanol extract of *S. calophyllifolium* leaf has a broad spectrum antimicrobial activity.

Antimicrobial activity of the leaf oil of *S. caryophyllatum* (L.) Alston was studied using diffusion method *in vitro* [15]. They found strong inhibition against *Cardida albicons* compared to Fluconazole. They also found pronounced activity against *C. albicans* with a minimum-inhibitory concentration (MIC) of 44.0 g/ml.

Antimicrobial activities of *S. cumini* leave extracts against selected microorganisms were studied [16]. The collected leaves were extracted by petrolium ether, methanol and water by using different concentrations (15%, 10% and 5%). Bioassay *in vitro* for its bioactivity to inhabit the growth of four types of bacteria (*S. aureus*, *E. coli*, *P. aeroginosa* and *B. subtilis*) and two types of fungi (*A. niger* and *Candida albicans*). The extract showed different activities against the selected bacteria at all concentration levels. By using cup plate diffusion method, petroleum ether extract of *S. cumini* leaves showed activity against *E. coli* only. Methanol extract showed variable activities against bacteria in different concentrations especially 15% concentration showed high activity against most of the tested bacteria. *E. coli* was found to be sensitive to all concentrations.

The present result also support the antibacterial screening of the stem and leaf of *S. cumini* as demonstrated [17]. All extracts (stem, leaf) showed antibacterial activity against all bacteria's tested. Maximum zone of inhibition was observed against *Roultella plantikola* (25 mm). Minimum zone of inhibition was observed against *Pseudomonas aeruginosa* by using fruit extract (14 mm). Inhibition zones of 25 mm and 14 mm were observed by using leaf and fruit extract against *R. plantikola.*

Chanudom *et al*. [18] studied antioxidant and antimicrobial activities of aqueous and ethanol crude extracts of 13 Thai traditional plants. Out of thirteen plants, *S. cumini* showed the highest inhibition zone against *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus* (MRSA) at the concentration of 100mg/ml. Minimum inhibitory concentration (MIC) and minimal bacteriocidal concentration (MBC) of *S. cumini* aqueous crude extracts against *S. aureus* and MRSA were 6.25 and 12.5 mg/ml, respectively. They recorded that *S. cumini* possessed antimicrobial activities.

*In vitro* antimicrobial potential of endophytic fungi isolated from *Eugenia jambolana* Lam. was studied [19] who reported gram negative bacteria (*E. coli*, *P. mirabilis*, *P. aeruginosa*, *K. pneumonia*, *S. typhi*, *S. flexneri*, *Serrata marcescens*) and gram positive bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*) and 68% fungi having significant antibacterial and antifungal activities. MIC range of endophytic fungal extract varied from 1. 87 mg/ml to 7.50 mg/ml.

Chikowe *et al*. [20] evaluated antibacterial activity of leaf extracts of some selected australian *Syzygium* species at a concentration of 10 mg/ml in the disc diffusion assay against 14 bacteria. *S. fortes*, *S. francissi*, *S. moorei*, *S. puberulum* and *S. S. wilsonii* leaf methanolic extracts inhibited the growth of 5 (36%), 3 (21%), 3 (21%), 5 (36%) and 2 (14%) of the bacteria tested respectively. Both gram-positive and gram-negative bacterial growth was inhibited by the *Syzygium* extracts, although gram-positive bacteria found to be slightly more susceptible.

Priya *et al*. [21] studied methanol, ethyl acetate and dichloromethane extracts of shade dried *S. cumini* fruit peel was screened for antimicrobial effects using disc diffusion and minimum inhibitory concentration (MIC) methods. Methanol extract showed maximum antimicrobial potency against all the test microorganisms with inhibition zone diameter (IZD) ranging from 9.35±0.49 to 19.25±0.35 mm and the lowest MIC of 0.18 mg/ml. Ethyl acetate and dichloromethane extracts moderately inhibited the growth of microorganisms [21].

Devakumar *et al*. [23] studied phytoconstituents and in vitro antifungal activity of *S. cumini* and *S. jambos* through acetone and aqueous extracts. The most significant 100% inhibition was observed on SCACE with MIC at 500 µg/ml with their $IC_{50}$ values from 291 µg/ml they recorded.

The present work revealed that the plant extract inhibited fungal growth but their effectiveness varied. The antifungal activity has been attributed to the presence of some active constituents in the extracts. These results also support the results of Madhu *et al*. [24]. They evaluated the antifungal potential of aqueous extracts of leaves, bark, seeds and fruits of *S. cumini* against two important fungal plant pathogens, *A. alternata* and *F. oxysporum.* Results revealed that among various plant parts, aqueous extract of fruit was most effective against the growth of *F. oxysporum* as compared to other extracts whereas, the aqueous extract of bark showed potential to inhibit the growth of *A. alternata*. Leave extracts were not much effective against the both test organisms.

These findings support the traditional knowledge of antifungal activity of acetone and aqueous leaf extract of *S. cumini* and *S. jambos* against *A. niger, C. albicans, C. neofromans* and *T. rubrum*. Complete growth inhibition was noticed in positive control (Fluconazole 10µg/ml) and full growth was observed in negative control (DMSO). The leaf extracts of two plants showed varying degree of antifungal activity against fungal strains. The most significant 100% inhibition was observed on SCACE against *C. neoformans* and *T. rubrum* and SJACE against *C. neoformans* with MIC at 500µg/ml and 250µg/mL with $IC_{50}$ values 291 µg/ml, 52.67 µg/ml and 34.36 µg/ml respectively. The inhibitory level 80-100 µg/ml at MIC >1000 µg/mL was observed on SJACE against *A. niger, C. albicans, C. neoformans, P. chrysogenum* and *T. rubrum* with their $IC_{50}$ 42.39 µg/ml, 9.59 µg/ml, 34.36 µg/ml and 98.12 µg/ml respectively. The average fungicidal action was observed for SCACE and SCAQE against *A. niger, C. albicans* and *C. neoformans* with their $IC_{50}$ values of 246.66µg/mL, 67.23µg/ml and 637.94µg/ml individually [25].

Bangladesh being the homeland of this famous plant might have a bright future of earning foreign currency by exporting different products or preparations of this plant. The root offered 100% highest activity to the multicellular test organisms; followed by the leaf extracts. So, seed could be an export item, as well as the leaf products (for what there is an international market), and easy formulation of the products is necessary while the folk use of the *S. cumini* products given hints in this regard. To achieve the goal with much success in proper utilization of this promising plant and for the marketing of its products it needs further investigation.

**Conflict of Interest Statement**

We declare that we have no conflict of interest.

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