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Research Article

BACTERICIDAL ACTIVITY OF WILD AND HYBRID VARIETIES OF *SYZIGIUM CUMINI* L. AGAINST A FEW PLANT PATHOGENS

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ABSTRACT

Objective: The objective of the present study was to analyze the antibacterial activity of seeds of wild and hybrid varieties of *Syzygium cumini* against plant pathogens such as *Erwinia herbicola*, *Pseudomonas syringae*, and *Xanthomonas campestris.*

Methods: The phytochemicals from the wild and hybrid varieties of *S. cumini* seeds collected from the Tirunelveli region (Tamil Nadu) were extracted by cold methanol method. Antibacterial activity of the methanol extract of seeds has been tested against *E. herbicola*, *P. syringae*, and *X. campestris* by disc diffusion assay.

Results: The seed extract of wild variety revealed more effective antimicrobial activity than a hybrid variety with minimum inhibitory concentration ranging from 8 µg/ml to 128 µg/ml. The highest inhibition zone was exhibited by wild seed extract against *X. campestris* (16.3 mm).

Conclusions: This result showed that the wild variety of *S. cumini* seeds may be the best antibacterial agent against plant pathogens.

Keywords: Myrtaceae, Medicine, Phytochemicals, Antimicrobial activity, Plant pathogens.

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INTRODUCTION

Medicinal and aromatic plants are a potential source of raw materials used for the manufacture of drugs and perfumery products. More than a quarter of all the medicines used in the world today contain natural compounds derived from plants that often serve lead molecules whose activities can be enhanced by manipulation through combinations with chemicals and by synthetic chemistry that can be exploited in the field of new drugs research and development [1]. The primary benefits of using plants derived medicines are that they are relatively safer than synthetic alternatives offering profound therapeutic benefits and more affordable treatment.

Plants and herbal extracts have formed an important position in modern medicine due to their chemical and medicinal contents found in natural form. Their secondary metabolites represent a large reservoir of structural moieties which work together, exhibiting a wide range of biological activities. Microorganisms have the genetic ability to transmit and acquire resistance to antibiotics and have become a major global health problem. This compelled the scientists to search out new drugs from plant origin. Plant-derived antimicrobial compounds might inhibit bacteria through different mechanisms and provide clinical values for the treatment of infections caused by resistant microbes [2].

The fruit of *Syzygium cumini* (Naval fruit) is one of the important edible fruits in India. The fruit is available in a particular season only. *S. cumini* is also known as Jamun or black plum or jambolan. *S. cumini* showed various phytoconstituents such as tannins, alkaloids, steroids, flavonoids, terpenoids, fatty acids, phenols, minerals, carbohydrates, and vitamins [3]. All parts of the plant such as seeds, fruits, leaves, flowers, bark are used in folk medicine [4].

Each part of the plant *S. cumini* is used as medicine for various diseases. Many review works have been made about the phytochemical properties of this plant. Many phytochemical tests have been conducted in various parts like leaf, bark, stem, and fruit of the selected plants [5,6]. However, the research work is less on the seed analysis of wild and hybrid varieties. Hence, the present study was focused to analyze the antibacterial activity of seeds of wild and hybrid variety of *S. cumini.*

METHODS

Collection of plant materials

The stem leaves and fresh seeds of *S. cumini* were collected randomly from the region of Tirunelveli, South India and identified. A voucher specimen was deposited in the Herbarium of Sarah Tucker College. Fresh seeds were washed and then shade dried. Dried seeds were powdered using the blender and stored in airtight bottles.

Methanol extraction

Ten gram of seed powder was added to 100 ml of methanol in a conical flask and plugged with cotton wool. After 24 h, the supernatant was collected and the solvent was evaporated to make the crude extract and stored at 4°C [7].

Bacterial strain

The microorganisms used to examine the antibacterial activity were *Xanthomonas campestris* (MTCC 2286), *Erwinia herbicola* (MTCC 3609), and *Pseudomonas syringae* (MTCC 1604). The microorganisms were procured from IMTECH, Chandigarh and were maintained at 4°C on nutrient agar slants.

Antibacterial activity

The antibacterial activity of the methanol extracts was tested by disc diffusion method [8]. Mueller–Hinton agar medium was seeded with 100 µl of each inoculum (1×10^8 CFU/ml). The impregnated discs containing the extracts (100 µg/disc) were placed on the agar medium seeded with tested microorganisms. Standard antibiotic discs (Chloramphenicol 5 µg/disc) and blank discs (impregnated with solvent) were used as a positive and negative control. The plates were then incubated at 37°C for 24 h to allow maximum growth of the microorganisms. The antibacterial activity of the test samples was determined by measuring the diameter of the zone of inhibition

expressed in millimeter. Experiments were done in triplicate and mean of the three experiments was recorded.

Determination of minimum inhibitory concentration (MIC)

The MIC of the methanol extracts of wild and hybrid varieties of *S. cumini* was determined using serial dilution technique. 1 mg/ml of the sample solutions of the extracts were prepared using dimethyl sulfoxide. In this technique, a large number of test tubes were used and each of the test tubes was filled with 1 ml of sterile nutrient broth media and graded doses of sample solution were added. Then, these test tubes were inoculated with the selected organisms (inoculum contains 1×10^6 cells/ml) followed by incubation at 37°C for 24 h to allow the growth of the bacteria. The test tubes which showed minimum concentration as well as clear content were selected. This lowest or minimum concentration was considered as MIC. Another three test tubes containing medium, medium and sample, medium, and inoculum were used as control. Bacterial growth observed was only in test tubes (solution content was cloudy) containing medium and inoculum and the other two were clear showing no growth [9]. Experiments were done in triplicate and repeated twice.

RESULTS

The methanol extracts of wild seed of *S. cumini* showed the highest inhibition against all the selected plant pathogens. The inhibition zones were 14 mm, 16 mm, and 15 mm against *P. syringae*, *X. campestris*, and *E. herbicola*, respectively. Among these, the highest inhibition was 16 mm against *X. campestris* and the lowest inhibition zone was 14 mm against *P. syringae.* However, the inhibition rate was high compared with positive control and hybrid seed extract (Table 1).

The methanol extract of hybrid seed *of S. cumini* exhibited the inhibition zones, 10 mm against *P. syringae* and 12 mm against *X. campestris* and 14 mm against *E. herbicola.* The highest inhibition was observed against *E. herbicola* and the lowest inhibition against *P. syringae*. However, the inhibition rate was low compared with positive control and wild seed extract.

MIC

The MIC value of methanol extracts of wild seeds was 8 µg/ml against *P. syringae* and 64 µg/ml against *X. campestris* and *E. herbicola*, respectively. Similarly, the MIC value of methanol extracts of hybrid seeds was 16 µg/ml against *P. syringae* and 128 µg/ml against *X. campestris* and *E. herbicola,* respectively. The highest MIC value was found in the methanol extracts of hybrid seeds, i.e., 128 µg/ml. The lowest MIC value was found in the methanol extract of wild seeds, i.e., 8 µg/ml. Hence, it is concluded that the methanol extracts of wild variety of *S. cumini* showed inhibition of bacterial growth even at low concentrations (Table 2).

DISCUSSION

Essential oil present in the leaves of *S. cumini* showed good antibacterial activity [10]. Leaf extract showed activity against *Escherichia coli* and *Staphylococcus aureus* [11]. Shaikh *et al*. [12] reported the antibacterial activity of ethanolic extracts of *Eugenia jambolana* against Gram-positive and Gram-negative bacteria. Bhuiyan *et al*. [13] reported the antibacterial activity of methanol and ethyl acetate extracts of the seeds of *E. jambolana* at a concentration of 200 µg/disc against *Bacillus cereus*, *Bacillus subtilis*, *Bacillus megaterium*, *Streptococcus ß–haemolyticus*, *S. aureus*, *Shigella dysenteriae*, *Shigella shiga*, *Shigella boydii*, *Shigella flexneri*, *Shigella sonnei*, *E. coli*, *Salmonella typhi B*, *S. typhi*, and *Klebsiella* species. Pragada *et al*. [14] carried out preliminary phytochemical analysis and quantification of total phenols, *in-vitro* antioxidant, and antibacterial activities of the hydroalcoholic (70% ethanol) extract of *Acalypha indica*. Ramamoorthy *et al*. [15] screened the methanol extract of roots of *Gentiana kurroo Royle* (Gentianaceae), an important and endemic medicinal plant of Kashmir Himalaya for the presence of various bioactive plant metabolites and analgesic activity. The phytochemical analysis revealed the presence of tannins, alkaloids, saponins, cardiac glycosides, terpenes, flavonoids, phenolics, and carbohydrates. Daisy [16] assayed the antibacterial activity of the extract of *S. cumini*. Methanol, acetone, and hexane extract of *S. cumini* seeds were examined for antibacterial activity against *Aeromonas hydrophila*, *Acinetobacter baumannii*, *Citrobacter freundii*, *E. coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Proteus mirabilis*.

Table 1: Antibacterial activity of the selected seed extracts

S. No.	Name of the sample	Pathogens (zone of inhibition in mm)		
		P. syringae	*X. campestris*	*E. herbicola*
1.	Wild seeds	14.0±0.5	16.3±0.2	15.1±0.4
2.	Hybrid seeds	10.3±0.2	12.2±0.3	14.0±0.5
3.	Chloramphenicol	10.1±0.4	07.2±0.3	06.2±0.3

P. syringae: Pseudomonas syringae, X. campestris: Xanthomonas campestris, E. herbicola: Erwinia herbicola

Table 2: MIC of the selected seed extract

Name of the pathogens	Wild seeds (µg/ml)	Hybrid seeds (µg/ml)
P. syringae	8.00±0.00	16.00±0.00
X. campestris	64.00±0.00	128.00±0.00
E. herbicola	64.00±0.00	128.00±0.00

P. syringae: Pseudomonas syringae, X. campestris: Xanthomonas campestris, E. herbicola: Erwinia herbicola, MIC: Minimum inhibitory concentration

But here, we have analyzed the bactericidal activity of methanol extract of wild and hybrid variety of *S. cumini* seeds against the harmful plant pathogens *P. syringae*, *X. campestris*, and *E. herbicola.* The wild seeds extract exhibited the highest antibacterial activity when compared to hybrid seeds extract against tested bacteria.

CONCLUSIONS

In the antibacterial assay, the extracts of wild variety showed significant bactericidal activity against all the three pathogens, namely, *P. syringae*, *X. campestris,* and *E. herbicola.* The methanol extracts of hybrid variety showed moderate bactericidal activity against all the three pathogens, namely, *P. syringae*, *X. campestris,* and *E. herbicola.* However, both seeds extracts exhibited the highest bactericidal activity when compared to positive control, i.e., standard antibiotics (chloramphenicol). In the MIC test, the lowest MIC value was found in the methanol extract of wild seeds against the tested pathogens. Hence, it is concluded that the wild variety of *S. cumini* seed is the best antibacterial agent against plant pathogens compared to hybrid seeds.

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AUTHORS' CONTRIBUTIONS

The author declares that this work was done by the author named in this article.

CONFLICTS OF INTEREST

We declare that we have no conflicts of interest.

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