



Antimicrobial Activity of *Syzygium Cumini*

KEYWORDS

Syzygium cumini, Antibacterial activity, *Pseudomonas aeruginosa*, *Rouletella planticola*, Antifungal activity, *Penicillium chrysogenum*, *Candida albicans*.

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ABSTRACT *Syzygium cumini*, jambul, jambolan, jamblang, or jamun, is an evergreen tropical tree in the flowering plant family Myrtaceae. *Syzygium cumini* is native to Bangladesh, India, Pakistan, Sri Lanka, Malaysia, the Philippines, and Indonesia. The name of the fruit is sometimes mistranslated as blackberry, which is a different fruit in an unrelated family. All two plants extracts (Stem, Leaf) showed antibacterial activity against all used bacteria. Maximum zone of inhibition was observed against *Rouletella planticola* (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 *Rouletella planticola*. The plant extract showed maximum zone of inhibition (18 mm) activity against fungal strains viz. *Penicillium chrysogenum* and minimum (7 mm) against *Candida albicans*.

INTRODUCTION

Syzygium cumini (L.) Skeels is one of the best known species and it is very often cultivated. The synonyms of *S. cumini* are *Eugenia jambolana* Lam., *Myrtus cumini* Linn., *Syzygium jambolana* DC., *Syzygium jambolanum* (Lam.) DC., *Eugenia djouant* Perr., *Calyptanthus jambolana* Willd., *Eugenia cumini* (Linn.) Druc. and *Eugenia caryophyllifolia* Lam. It is commonly known as jambolan, black plum, jamun, java plum, Indian blackberry, Portuguese plum, Malabar plum, purple plum, Jamaica and damson plum. For long in the period of recorded history, the tree is known to have grown in the Indian sub-continent, and many others adjoining regions of South Asia such as India, Bangladesh, Burma, Nepal, Pakistan, Sri Lanka and Indonesia. It was long ago introduced into naturalized in Malaysia. In southern Asia, the tree is venerated by Buddhists, and it is commonly planted near Hindu temples because it is considered sacred to Lord Krishna. The plant has also been introduced to many different places where it has been utilized as a fruit producer, as an ornamental and also for its timber.

METHODOLOGY

Plants are the oldest source of pharmacologically active compounds and have provided mankind with many useful medicines for years. Biologically active plant extracts, pure compounds and their semi-synthetic derivatives serve as a promising therapeutic alternative to the currently available cost intensive options for the treatment of infection due to superbugs. Numerous plants have been screened for anti-infective properties as the probability of finding diverse chemistries have been implicated to serve as leads for the new anti-infective drugs. Thus, antimicrobial research is geared toward the discovery and development of novel antibacterial and antifungal agents

The systematic screening of plant species with the purpose of discovery of new bioactive compounds is a routine activity in many laboratories. In particular, the search for components with antimicrobial activity is gaining increasing importance in recent times, due to growing world wide

concern about the alarming increase in the rate of infection by antibiotic-resistant microorganisms. Hence, there is a constant need for new and effective therapeutic agents. Many plant species have been utilized as traditional medicines but it is necessary to establish the scientific basis for the therapeutic actions of traditional plant medicines as these may serve as the source for the development of more effective drugs.

Plant collection

Fresh plants or plant parts were collected from Botany Department, University of Rajasthan Jaipur. Fresh plant material was washed under running tap water, air dried, homogenized to fine powder, and stored in tightened light protected containers.

Preparation of Extract

Plant parts (leaf, fruit) were washed, air dried and grinded into powder form for preparation of extract. Aqueous plant extract was prepared by macerating powdered plant sample with 50 ml sterile distilled water. The macerate was filtered and filtrate was centrifuged at 8000 rpm for 15 minutes. Supernatant obtained after centrifugation was heat sterilized at 1200 C for 30 minutes. Extract obtained was preserved aseptically. Solvent extracts of plant parts were prepared in 70% methanol using Soxhlet extraction for 72 hours and extract was preserved at 40 C in air tight bottles. They were air dried and dissolved in Dimethyl sulfoxide (DMSO) in 1mg/1ml concentration and stored in refrigerator.

The reference antibiotic discs and antifungal:-

Antibacterial activity of the test samples was compared with antibiotics known to be effective against the test bacteria in their established doses. Amoxycilin was used for bacteria and Glucanazole for fungi as reference for comparison.

Fungal Media (Potato dextrose sugar)

200 gm of potato slices were boiled with distilled water.

The potato infusion was used as water source of media preparation. 20 gm of dextrose was mixed with potato infusion. 20 gm of agarose was added as a solidifying agent. These constituents were mixed and autoclaved. The solidified plates were bored with 6mm diameter cork borer. The plates with wells were used for antifungal studies.

Test Fungal Strains:

The test fungal strains namely *Aspergillus niger* MTCC 282, *Penicillium chrysogenum* MTCC 161, *Candida albicans* MTCC 183, *Fusarium solani* MTCC 9667 were used to study antifungal potential. They were collected from Botany Department, University of Rajasthan, Jaipur, India.

Antifungal activity assessment:

In vitro antimicrobial activity was screened by using Potato Dextrose agar (PDA) using agar well diffusion method. Fungal strains were activated in Potato Dextrose broth (PDB) and incubated for 24 hours. 0.05ml of inoculum was uniformly spread on agar plates. Ethanolic, methanolic and aqueous extracts were introduced in agar wells in concentration of 20 mg/10 ml. Control experiment was carried out with **Glucanazole**. Antifungal potential was then determined on the basis of diameter of zone of inhibition.

Media preparation for nutrient agar media:

The bacterial cultures of gram positive and gram negative bacteria were maintained on nutrient agar medium (agar 15 gm, beef extract 3 gm, sodium chloride 5 gm and peptone 5 gm, in one litre distilled water). These micro-organisms were allowed to grow at 35°C-37°C temperature. A fresh inoculum of test microorganism in saline solution was prepared from a freshly grown agar slant before every antibacterial assay by adjusting the concentration of micro-organism in the medium using spectronic-20 colorimeter (Bausch and Lomb) set at 630 nm, transmittance used bacteria was 40%.

Preparation of Inoculum

The antibacterial activity was tested by Whatman filter paper disc method. Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring loopful bacterial cells from the stock cultures to Erlenmeyer flask of nutrient broth that were incubated with agitation for 24 hrs at 37°C. The bacterial cultures of gram positive and gram negative bacteria were maintained on nutrient agar medium (agar 15 gm, beef extract 3 gm, sodium chloride 5 gm and peptone 5 gm, in one liter distilled water). These micro-organisms were allowed to grow at 35°C-37°C temperature. A fresh inoculum of test microorganism in saline solution was prepared from a freshly grown agar slant before every antibacterial assay by adjusting the concentration of micro-organism in the medium using spectronic-20 colorimeter (Bausch and Lomb) set at 630 nm, transmittance used bacteria was 40%.

Test microorganisms

The bacterial strains studied are *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*. Microorganisms were maintained at 4 °C on nutrient agar slants. These test organisms were clinical isolates obtained from patients diagnosed for having bacterial infections and procured from the Durlabhji Hospital Jaipur.

Antibacterial screening

The filter paper disc method was used for screening the extract for antibacterial activity. Standard size Whatman fil-

ter paper disc (6.0 mm diameter) were sterilized in an oven at 140°C for one hour, saturated with three plant extracts such as root, stem, leaf different streptomycin and air dried at room temperature to remove any residual solvent that might interfere with the determination of activity. The discs were then placed on the surface of sterilized nutrient agar medium that had been inoculated with test bacteria (using saline solution) and air dried to remove the surface moisture. The thickness of the agar medium was kept equal in all the petriplates and the standard disc (streptomycin) was used as a control. Before incubation, the petriplates were placed for one hour in a cold room (5°C) to allow the diffusion of the compounds from the disc into the medium. Plates were incubated at 37°C for 20-24 hours after which the zone of inhibition or depressed growth could be easily measured. All the experiments were done in five replicates and the activity index was calculated for each of these.

$$\text{Activity index (A.I.)} = \frac{\text{Inhibition zone of the sample}}{\text{Inhibition zone of the standard}}$$

RESULTS AND DISCUSSION

According to the antibacterial assay done for screening purpose, all these selected gram positive and gram negative microorganisms showed zone of inhibition against test plant extract. Among these test micro organisms *Rouletella plantikola* are the most susceptible to methanol extract of *Syzygium cumini*. An important characteristic of plant extracts and their components is their hydrophobicity, which enable them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable. Extensive leakage from bacterial cells or the exit of critical molecules and ions will lead to death. This antibacterial study of the plant extract demonstrated that folk medicine could be as effective as modern medicine to combat pathogenic microorganisms. The millenarian use this plant in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases. The results of the antibacterial activity are presented in Table-1 and of antifungal are presented in Table 2. The two Plants extracts (Stem, Leaf) showed antibacterial activity against all used bacteria. Maximum zone of inhibition was observed against *Rouletella 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 *Rouletella plantikola*. The plant extract showed maximum zone of inhibition (18mm) activity against fungal strains viz. *Penicillium chrysogenum* and minimum (7mm) against *Candida albicans*.

The antimicrobial principles and their distribution have been extensively reviewed by (1) followed by (2) who surveyed 174 plants belonging to 157 families of vascular plants. Antimicrobial activity of various plant parts has also been observed by several workers viz; *Begonia malabarica* (3) *Azadirachta indica*, (4) The vital role of flavonoids in defence against microorganism, due to antimicrobial activity has already been discussed by (5). Flavonoids such as quercetin, isorhamnetin and kaempferol have antimicrobial activity. (6) have carried out antimicrobial studies in *Syzygium cumini* leaf extracts and have proved that the methanolic extracts were more potent than the aqueous extracts. (7) have successfully carried out the phytochemical evaluation and antimicrobial activity for determination of bioactive compounds from the leaves of *Aegle marmelos*. Thus, it can be concluded that antimicrobial activity in plants is

neither a generic character nor a family one but it is the feature of active principles present in the plant.

This research work states the presence of phytoconstituents in the chloroform extract of *Syzygium cumini* were responsible for its antimicrobial activity. This study concludes that the crude extract of chloroform has potent antibacterial and antifungal activity against clinical isolates of bacteria and plant pathogens of fungi. Traditional herbal medicines must be granted the benefits of modern science and technology to serve further global needs. The drugs derived from herbs may have the possibility of use in medicine because of their antimicrobial activity. It is therefore; from above findings recommended for further investigation on isolation and purification of bioactive compounds responsible for the antimicrobial activity. These findings support the traditional knowledge of local users and it is a preliminary, scientific, validation for the use of these plants for antibacterial activity to promote proper conservation and sustainable use of such plant resources. Awareness of local community should be enhanced incorporating the traditional knowledge with scientific findings. In conclusion, the results of the present study support the folkloric usage of the *Syzygium cumini*

Antimicrobial activity in Methanol extract of *Syzygium cumini*

Table 1: Antibacterial Activity of *Syzygium cumini*

Bacterial Strain	Standard	Zone of inhibition					
		Methanol		Petroleum ether		Chloroform	
	Amoxycilin	Fruit	Leaf	Fruit	Leaf	Fruit	Leaf
<i>Rouletella planticola</i>	55 mm	21mm	25mm	23 mm	24 mm	22 mm	27 mm
<i>Pseudomonas aeruginosa</i>	20 mm	13 mm	14 mm	15 mm	17 mm	16mm	17 mm
<i>Bacillus subtilis</i>	48 mm	18 mm	19 mm	19 mm	23 mm	20mm	21 mm
<i>Agrobacterium tumifacian</i>	35 mm	10mm	12mm	21 mm	22 mm	20 mm	22 mm

Table 2: Zone of Inhibition of leaf and fruit of Methanol, Ethanol and Aqueous Extract with test fungal cultures and control drug

Fungal strain	Zone of inhibition					
	Methanolic Aqueous			Ethanolic		
	Leaf	Fruit	Control	Leaf	Fruit	Glucanazole
<i>Penicillium chrysogenum</i>	9	15	12	18	9	9
<i>Fusarium solani</i>	11	12	12	-	13	10
<i>Aspergillus niger</i>	9	13	13	15	12	17
<i>Candida albiconis</i>	10	17	7	13	14	12

Conclusion

The plant *Syzygium cumini* showed good results in both antibacterial and antifungal activity of various extracts. Traditional herbal medicines must be placed in a position so that they support the benefits of modern science technology as global needs. The plant also contain phytomedicine, antioxidant and anti-depressive agents, Thus *Syzygium cumini* drugs may have the possibility of use in modern medicine.

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