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Research Article

# EFFICIENCY OF *IN-VITRO* ANTIBACTERIAL ACTIVITY OF *SYZYGIUM CUMINI* PHENOLIC EXTRACT FROM LEAVES

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**ABSTRACT**

**Objective:** The fundamental objective of this analysis is to assess the potential effect of the phenolic plant extract from *Syzygium cumini* against bacterial culture. This response indicates the antibacterial properties of the phenolic extract of the plant which can be exploited as a feasible antibacterial agent.

**Methods:** The antibacterial activity of the phenolic-rich extract was tested against human pathogenic bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa* using the disc diffusion method and minimum inhibitory concentration. Preliminary phytochemical studies revealed the presence of alkaloids, flavonoids, polyphenols, glycosides, terpenoid, and tannins.

**Results:** The phenolic extract showed potent inhibitory activity against Gram-positive bacterium *S. aureus* and Gram-negative bacterium *P. aeruginosa*. The zone of inhibition obtained in the antibacterial assay was scrutinized, and the results obtained were analyzed in terms of the antibacterial activity.

**Conclusion:** Hereby, inferring from the experimental outcomes, the phenolic plant extract of *S. cumini* can be used as an effective antibacterial agent.

**Keywords:** Polyphenol, Antibacterial activity, Disc diffusion, *Syzygium cumini.*

## INTRODUCTION

Antibacterials were considered miracle drugs when they were first introduced. Resistant strains of bacteria began to emerge. Today, roughly 50 years after antibiotics were introduced; antibiotic resistance is a serious problem, and antibiotics are losing their effectiveness. One response to this phenomenon is the greater public use of a variety of antibacterial agents designed to remove disease-causing organisms from external surfaces before they can enter the body. Hence, the need for effective and novel antibacterial agents is significantly increasing. The genus Syzygium is one of the genera of the myrtle family Myrtaceae, which is native to the tropics, particularly to tropical America and Australia. Plants of this family are known to be rich in volatile oils which are reported for their uses in medicine, and many fruits of the family have a rich history of uses both as edibles and as traditional medicines in divergent ethnobotanical practices throughout the tropical and subtropical world [1,2]. Some of the edible species of Syzygium are planted throughout the tropics worldwide.

All parts of the jambolan can be used medicinally, and it has a long tradition in alternative medicine. From all over the world, the fruits have been used for a wide variety of ailments, including a cough, diabetes, dysentery, inflammation, and ringworm [2]. It is widely distributed throughout India, and Ayurvedic medicine (Indian folk medicine) mentions its use for the treatment of diabetes mellitus. Various traditional practitioners in India use the different parts of the plant in the treatment of diabetes, blisters in mouth, cancer, colic, diarrhea, digestive complaints, dysentery, piles, pimples, and stomach ache [3]. During last four decades, numerous folk medicinal reports on the antidiabetic effects of this plant have been cited in the literature. In Unani medicine, various parts of jambolan act as a liver tonic, enrich blood, strengthen teeth and gums, and form good lotion for removing ringworm infection of the head [4].

The leaves are also used to strengthen the teeth and gums, to treat leucorrhea, stomachalgia, fever, gastropathy, strangury, dermopathy, constipation, and to inhibit blood discharges in the feces [5,6]. The folkloric use of this species to treat infectious diseases stimulated the investigation of the antimicrobial activity of the hydroalcoholic extract from *Syzygium cumini* leaves against standard and multi-drug resistant Gram-positive and Gram-negative human pathogenic bacteria.

The traditional medicines based mostly on medicinal plants have been used for the treatment of various diseases by mankind for centuries. Plants are also well-known to be the rich source of biologically active compounds. The use of natural products in disease prevention and control as well as in drug development has received increased attention in recent timing; about 25% of globally prescribed drugs are obtained from plants [7]. Therefore, one approach being used for the discovery of antibacterial agents from natural sources is based on the evaluation of traditional plant extracts. The aim of the study report is to examine the antibacterial activity of alkaloid extract from the leaves of *S. cumini*.

## METHODS

### Preliminary screening of plant phytoconstituents

The S. *cumini* leaves were collected from the Herbal Garden of Sri Sai Ram Siddha Medical College and Research Centre, Chennai. The leaves were further processed to obtain the plant extract. The leaves of *S. cumini* are dried with sunshade condition and made into fine powder. Before the extraction of the phytochemicals from the plant material, they are subjected to the screening process for the identification of the phytoconstituents, if present. The aqueous-methanol extract is freshly prepared and divided into different test tubes, and various chemical constituents were analyzed according to methods described by Trease and Evans [8] and Harborne [9]. The different chemical constituents tested for included tannins, polyphenols, saponin, glycosides, glucosides, alkaloids, triterpenes, and flavonoids.

### Partial characterization of phenolic fraction from the leaves of *S. cumini* by thin-layer chromatography (TLC)

The partial characterization of the alkaloid extract from the leaves of *S. cumini* was done on pre-coated silica gel TLC plates (60 $F_{254}$ Merck, USA). The efficient solvent system used for the different phytochemical extract was as follows: Crude alkaloid extract was run on pre-coated aluminum silica gel 60 $F_{254}$ plates for further confirmation. For the

mobile phase, hexane, chloroform, and methanol were used in the ratio of 1:0.5:0.1 which was efficient in the separation of alkaloid. The chromatogram was developed and viewed under ultraviolet (UV) 240 and 360 nm.

**Determination of antibacterial activity by the disc diffusion method**

Antibacterial activity was carried out using disc diffusion method [10]. Petri plates were prepared with 20 ml of sterile nutrient agar (HIMEDIA). The tested cultures were swabbed on top of the solidified media and allowed to dry for 10 minutes. The crude extract impregnated discs (Whatmann NO.1 Filter paper was used to prepare discs) were prepared and air dried well. The test was conducted at four different concentrations of the alkaloid extract with three replicates. The loaded discs were placed on the surface of the medium and incubated at room temperature for 24 hrs. The relative susceptibility of the organisms to crude extract indicated by the clear zone of inhibition around the discs were observed, measured, and recorded in millimeters.

**Minimum inhibitory concentration (MIC)**

The MIC was determined according to method Sathyabama *et al*., [11]. The different concentration of the flavonoid-rich fraction (5-20 µg/ml) was mixed with 0.5 ml bacterial cultures were incubated at 37°C for 18 hrs, and OD value was measured spectrophotometrically at 580 nm.

**Inhibition of biofilm formation**

After completion of MIC procedures, the solution in the test tubes is discarded for measuring the biofilm formed over on the test tubes. Add about 0.5 ml of phosphate buffer rinse in a rotatory motion and discard it. 2.5 ml of 0.25% crystal violet was added in the test tubes. It was incubated for about 30 minutes. To this about 0.5 ml of 90% ethanol was added and the OD value was measured at 595 nm.

**Contemplation of antibacterial compounds by TLC bioautography**

Agar overlay TLC bioautography method Sawaya *et al*., [12] was used to detect the effective antibacterial compounds from S. *cumini* leaves. The inoculum of bacteria containing $10^6$ CFU/ml in molten Mueller–Hinton agar was distributed over the already prepared TLC plate. After solidification, the TLC-bioautographic plate was incubated at 37°C for 24 hrs. The bioautogram developed was sprayed with 1% aqueous solution of methyltetrazolium and incubated at 37°C for 4 hrs. Inhibition zones indicated the presence of active compounds. Growth inhibition areas were identified by the retention factor (RF) of the related spots on the reference TLC plate. Preparative TLC plates with a thickness of 1 mm were prepared using same stationary and mobile phases as above with the objective of isolating the components of plant extracts that inhibited the growth of bacteria isolates.

## RESULTS

**Screening of plant phytoconstituents**

The leaves of *S. cumini* aqueous-methanol extract revealed the following phytochemicals, alkaloids, flavonoids, polyphenols, glycosides, tannins, and triterpenoids. Furthermore, the phytochemical extract was characterized and tested for its antibacterial activity.

**Partial characterization of phenolic fraction of *S. cumini* by TLC**

The phenolic fraction of *S. cumini* loaded on pre-coated TLC plates (60 $F_2$ 54 Merck) and developed with a solvent system of 9.5:2.5:0.4 (toluene, tetrahydrofuran, and acetic acid) was efficient to extract the antibacterial compound that was used for further studies. The developed plate was viewed under UV 240 and 360 nm (Fig. 1).

**Antibacterial assay by disc diffusion method**

The antibacterial activity of a phenolic fraction of *S. cumini* leaves at different concentration was screened by disc diffusion technique, and the zone of inhibition was measured in mm diameter (Table 1). The phenolic fraction was effective against *Pseudomonas aeruginosa* and *Staphylococcus aureus* with a zone of inhibition percentage of 16.5 and 17.8, respectively, at the concentration of 20 µg/ml (Fig. 2).

**Table 1: Antibacterial activity of *S. cumini* phenolic fraction**

| Phenolic fraction concentration µg/ml | Zone of inhibition (in mm diameter)* | |
|---|---|---|
| | *P. aeroginosa* | *S. aureus* |
| 5 | 7.4±0.78 | 8.7±0.69 |
| 10 | 10.6±0.33 | 10.8±0.62 |
| 15 | 12.7±0.56 | 13.4±0.44 |
| 20 | 14.9±0.22 | 16.1±0.71 |

*The antibacterial activity was determined by measuring the diameter of zone of inhibition that is the mean of triplicates±standard error of the mean of three replicates. *P. aeroginosa: Pseudomonas aeruginosa, S. aureus: Staphylococcus aureus, S. cumini: Syzygium cumini*

**MIC**

The phenolic fraction of *S. cumini* leaves showed the maximum inhibitory activity at the higher concentration (20 µg/ml) than, the lower concentration (5 µg/ml) against Gram-negative bacteria *P. aeruginosa* and Gram-positive bacteria *S. aureus* (Fig. 3).

**Effect of phenolic fraction from *S. cumini* leaves on inhibition of biofilm formation on pathogenic bacteria**

The phenolic fraction from the leaves of *S. cumini* exhibited more toxicity on the biofilm formation of *S. aureus* and *P. aeruginosa*. The potential of 20 µg/ml concentration of *S. cumini* phenolic fraction in inhibiting biofilm formation after 18 hrs incubation was maximum in *S. aureus* (81%) and *P. aeruginosa* (78%) than the corresponding control (Fig. 4).

**Antibacterial activity assay by TLC bioautography of phenolic fraction from the leaves of *S. cumini* against pathogenic bacteria**

TLC bioautography agar overlay method is considered as one of the most efficient methods for the detection of antibacterial compounds. It involves the transfer of the active compounds from the stationary phase into the agar layer through a diffusion process. Eventually, the bioautography of the TLC plates (Fig. 5) of phenolic fraction of *S. cumini* showed an area that inhibited the growth of pathogenic bacteria over the region containing the active compounds. Consequently, it was found that the active compound of phenolic fraction of *S. cumini* was at RF 0.67.

## DISCUSSION

In spite of the enormous developments in medicinal sciences, the microbial infections remain a scourge of humanity. This may be due to the lack of vaccines against some infections, emergence of drug resistant phenotypes, and the resurgence of infections among others [13]. Hence, there exists a constant quest for the development of novel therapeutic approaches. Indulging a wide range of medicinal plants in various therapeutic approaches has been scientifically proven worth, which had led to the development of potent plant-based drugs.

Affirming this theory, Kumar *et al*. [14] investigated the ethyl acetate and methanol extracts of the seeds of *S. cumini* that showed the presence of alkaloids, amino acids, flavonoids, glycosides, phytosterols, saponins, steroids, tannins, and triterpenoids relatively similar to that of the phenolic extract of the leaves of *S. cumini*. Furthermore, the ethyl acetate and methanol extracts of the seeds showed the absence of anthraquinones. Likewise, Gowri and Vasantha [15] studied aqueous and methanol of *S. cumini* leaves in which the zones of inhibitions were produced by both the extracts against all the test organisms. Methanol extracts were more active than the aqueous extract against all the human pathogenic organisms. However, the zones of inhibition produced were comparatively higher in the phenolic extract from leaves of *S. cumini*.

Priya *et al*., [16] showed the MICs obtained from the three SC leaf extracts with values ranging from 0.18 to 1.0 mg/ml that are comparatively higher than the MIC values obtained for the phenolic extract *viz*.,

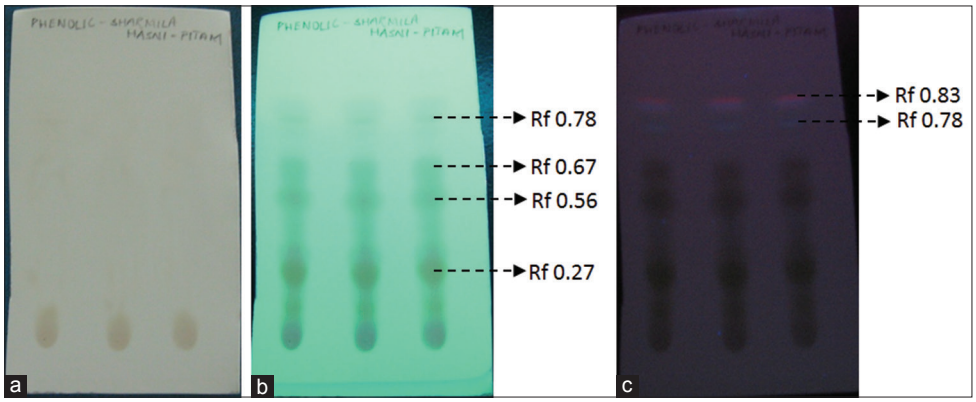


**Fig. 1: Depiction of thin-layer chromatography performed with phenolic extract of *Syzygium cumini,* (a) Normal light, (b) 240 nm ultraviolet (UV) light, (c) 360 nm UV light**

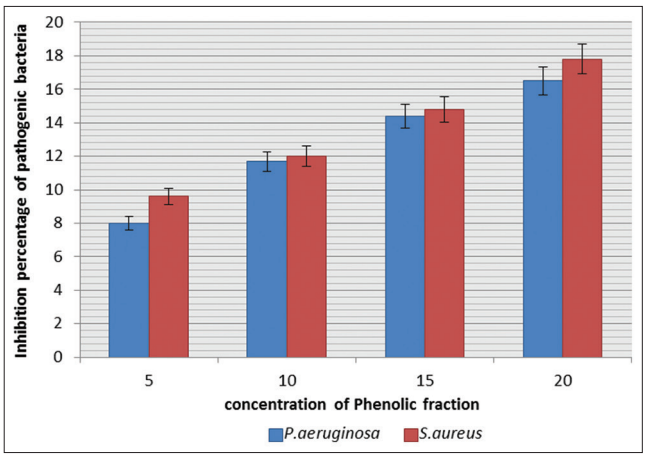


**Fig. 2: Inhibition percentage of phenolic fraction of *Syzygium cumini* leaves against pathogenic bacteria**

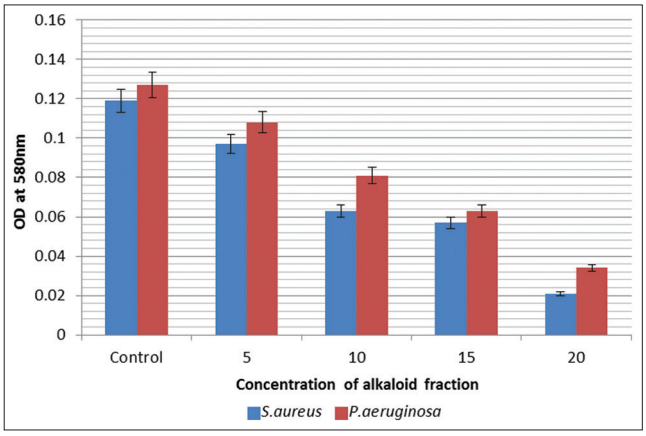


**Fig. 3: Minimum inhibitory concentration of phenolic fraction of *Syzygium cumini* leaves against pathogenic bacteria**

5-20 µl/ml, thereby indicating the phenolic extract to be more effective. The extracts strongly inhibited the growth of both Gram-positive and Gram-negative strains. Methanolic extract of *S. cumini* registered identical MIC values (500 µg/ml) against both planktonic and biofilm form of *S. mutans;* however, it's minimal bactericidal concentration against biofilm form was four times higher than that against planktonic form. Seed extracts reported in this study were able to kill ≥80% cells of *S. mutans* in biofilm, in the concentration range of 500-1000 µg/ml. These extracts were able to achieve ≥95% killing of *S. mutans* biofilm

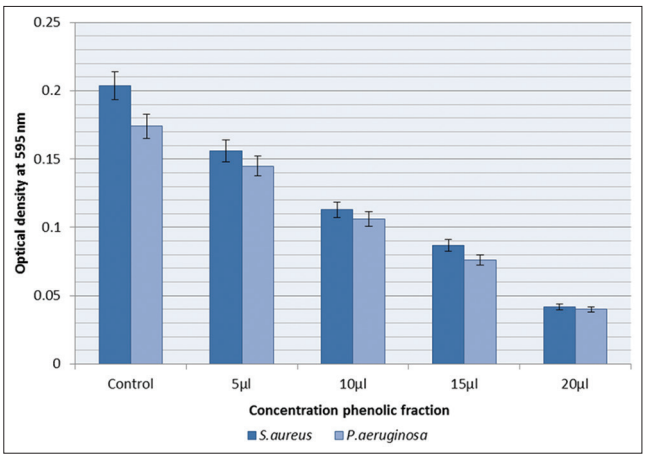


**Fig. 4: Inhibition activity of biofilm formation *Syzygium cumini* phenolic fraction against pathogens**

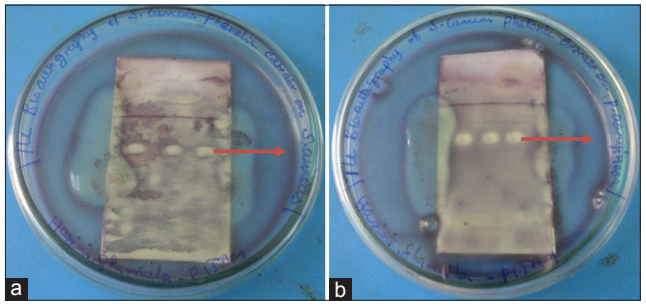


**Fig. 5: Thin-layer chromatography bioautography of phenolic fraction from the leaves of *Syzygium cumini* against pathogenic bacteria, (a) *Staphylococcus aureus* (b) *Pseudomonas aeruginosa***

at concentrations ranging from 600 to 2000 µg/ml [17]. However, the phenolic extract of the leaves were observed to produce inhibition of about 81% in *S. aureus* and 78% in *P. aeruginosa* at a very low concentration of 20 µg/ml.

## CONCLUSION

This analysis was carried out in pursuit of a proficient antibacterial agent with which human pathogenic microorganisms might be efficiently inhibited from their growth and deprived from causing any diseases. The antibacterial assay carried out for studying the effect of the phenolic extract of *S. cumini* revealed that the plant extract might serve as a potential agent for bacterial inhibition, hereby proving as a reliable source for antibacterial action.

**REFERENCES**

1. Mahmoud II, Marzouk MS, Moharram FA, El-Gindi MR, Hassan AM. Acylated flavonol glycosides from *Eugenia jambolana* leaves. Phytochemistry 2001;58(8):1239-44.
2. Reynertson KA, Basile MJ, Kennelly EJ. Antioxidant potential of seven myrtaceous fruits. Ethnobot Res Appl 2005;3:25-35.
3. Jain SK. Dictionary of Indian Folk Medicine and Ethnobotany. New Delhi: Deep Publications Paschim Vihar; 1991.
4. Sagrawat H, Mann AS, Kharya MD. Pharmacological potential of Eugenia jambolana: A review. Pharmacogn Mag. 2006;2:96-104.
5. Warrier PK, Nambiar VP, Ramankutty C. Indian Medicinal Plants. Hyderabad: Orient Longman Ltd.; 1996. p. 225-8.
6. Bhandary MJ, Chandrashekar KR, Kaveriappa KM. Medical ethnobotany of the Siddis of Uttara Kannada district, Karnataka, India. J Ethnopharmacol 1995;47(3):149-58.
7. Rates SM. Plants as source of drugs. Toxicon 2001;39(5):603-13.
8. Trease GE, Evans WC. Pharmacognsy. 11th ed. London: Brailliar Tiridel Can Macmillian Publishers; 1989.
9. Harborne JB. Phytochemical Methods: London. Chapman and Hall, Ltd.; 1973. p. 49-188.
10. Velickovic DT, Randjelovic NV, Ristic MS, Velickovic AS, Smelcerovic AA. Chemical constituents and antimicrobial activity of the ethanol extracts obtained from the flower, leaf and stem of *Salvia officinalis* L. J Serb Chem Soc 2003;68(1):17-24.
11. Sathyabama S, Kinsley SJ, Sankaranarayanan S, Bama P. Antibacterial activity of different phytochemical extracts from the leaves of *T. procumbens*: Identification and mode of action of terpenoid compound as antibacterial. Int J Pharm Pharm Sci 2011;4:557-64.
12. Sawaya AC, Kirllian SS, Maria CM. Analysis of the composition of Brazilian propolis extracts by chromatography and evaluation of their *in vitro* activity against gram-positive bacteria. J Microbiol 2004;35:104-9.
13. Njimoh DL, Assob JC, Mokake SE, Nyhalah DJ, Yinda CK, Sandjon B. Antimicrobial activities of a plethora of medicinal plant extracts and hydrolates against human pathogens and their potential to reverse antibiotic resistance. Int J Microbiol 2015;2015:547156.
14. Kumar A, Ilavarasan R, Jayachandran T, Decaraman M, Aravindhan P. Phytochemicals investigation on a tropical plant, *Syzygium cumini* from Kattuppalayam, Erode District, Tamil Nadu, South India. Pak J Nutr 2009;8(1):83-5.
15. Gowri SS, Vasantha K. Phytochemical screening and antibacterial activity of *Syzygium cumini* (L.) (Myrtaceae) leaves extracts. Int J Pharm Technol Res 2010;2(2):1569-73.
16. Priya SS, Devi PR, Eganathan P, Kingsley J. *In vitro* antimicrobial activity of *Syzygium cumini* fruit peel and identification of anthocyanins. Afr J Pharm Pharmacol 2013;7(25):1719-28.
17. Patel I, Patel V, Thakkar A, Kothari V. *Tamarindus indica* (Cesalpiniaceae), and *Syzygium cumini* (Myrtaceae) seed extracts can kill multidrug resistant *Streptococcus mutans* in biofilm. J Nat Remedies 2013;13(2):81-94.