



ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL CONSTITUENTS IN SEED, LEAF AND BARK EXTRACT OF *SYZYGIUM CUMINI* (L.)

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Abstract

Syzygium cumini is a well known traditional medicinal plant. The phytochemical constituents of the plant are responsible for its medicinal properties. Investigations were carried out on the crude methanol, ethanol and aqueous extracts of the seed, leaves and bark of *Syzygium cumini* (Magnoliopsida: Myrtaceae). The antimicrobial activity of the extract was tested against standard strains of bacteria using the agar well diffusion method. Phytochemical studies showed the presence of flavonoids, alkaloids, glycosides, steroids, phenols, saponins, terpenoid, cardiac glycosides and tannins as the class of chemicals present in the extracts of seed, leaves and bark. The extracts reflected inhibitory activity against clinical isolates. The gram negative bacteria such as *Salmonella typhi*, *Pseudomonas aeruginosa* and *Escherichia coli*; and the gram positive bacteria like *Bacillus subtilis* and *Staphylococcus aureus* were inhibited with the methanol and ethanol extract on contrary to aqueous extract. The methanolic extract of seed was found more potent antimicrobial agent than the leaves and bark extract. The present study provides a support for the use of different part of *Syzygium cumini* as potent antimicrobial agents for sustainable and ecofriendly management of various bacterial strains and further investigation are required for field application.

Key words: *Syzygium cumini* L., Leaves extracts, Bark extract, Seed extract, Phytochemical screening, Antibacterial activity.

Introduction

In most of the developed and developing countries people are expending traditional medicine to cure different types of diseases by using compounds derived from medicinal plants. Therefore, the plants were investigated to understand their properties, constituents, safety and efficiency against the disease and disease causing agents (Cowan, 1999; Mubassara *et al.*, 2015; Singh *et al.*, 2019). Medicinal plants in different areas were studied to produce new drug and pharmacological research development (Singh *et al.*, 2018; Singh *et al.*, 2020). The common wild medicinal plants were widely used and accepted as home remedies and applied as raw materials for the pharmaceutical industry (Rates, 2001; Nayami *et al.*, 2016). The *S. cumini* is widely used as antibacterial and reported to have a strong therapeutic value. All parts of the plant have different uses as well as medicinal properties and therapeutic values to make it a multipurpose

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plant (Singh *et al.*, 2019). *S. cumini* seeds used in diarrhoea and dysentery leaves used in treatment of bleeding gums and bark act as antimicrobial against different bacteria (Pushpahasni *et al.*, 2015). Therefore, one approach being used for the discovery of antibacterial agents from natural sources is based on the evaluation of traditional plant extracts. In present study, we report the antibacterial activity of seeds, leaves and bark extract of *S. cumini* L. against pathogenic bacteria.

Materials and Methods

The leaves, bark and seeds of *Syzygium cumini* L. were collected from the campus of Maharishi Markandeshwer (Deemed to be University), Mullana-Ambala (HR), India. The leaves, bark and seeds were washed and dried at room temperature. The dried seeds, leaves and bark were then crushed into fine powder and used for further study. Extracts were prepared by addition of 5g dried powder into the 50ml of solvent using hot water, cold water, methanol and ethanol separately. The

Table 1: Presence of different phyto-constituents in methanolic and ethanolic crude extract of seeds, leaves and bark of *Syzygium cumini*.

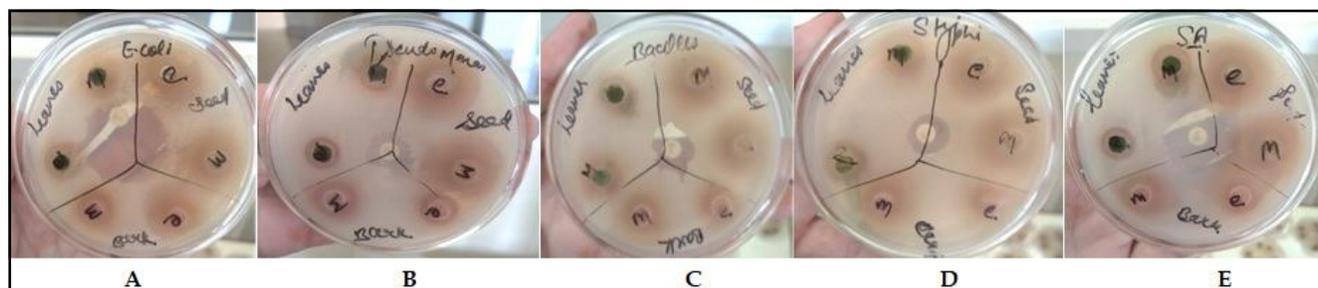
Test for phyto-constituents	Methanol Extract			Ethanol Extract		
	Seeds	Leaves	Bark	Seeds	Leaves	Bark
Mayer's test for alkaloids	+	+	+	+	+	+
Barfoed's test for carbohydrates	+	-	+	+	-	+
Foam test for saponins	+	+	+	+	+	+
Test for steroids	+	+	+	+	+	+
Test for tannins	+	+	-	+	+	-
Test for flavinoids	-	+	-	-	+	-
Test for amino acids	-	-	-	-	-	-
Test for terpenoids	+	-	+	+	-	+
Follin's test for proteins	+	-	-	+	-	-

Table 2: Antimicrobial assay (in mm) of methanolic and ethanolic crude extract of seeds leaves and bark vs chloramphenicol as control with test organisms.

Test organism	Methanol Extract			Ethanol Extract			Control Chloromphenicol
	Seed	Leaves	Bark	Seed	Leaves	Bark	
<i>E. coli</i>	17	13	10	12	12	10	24
<i>P. aeruginosa</i>	12	12	10	14	13	11	14
<i>B. subtilis</i>	13	11	11	13	10	12	16
<i>S. aureus</i>	12	14	12	13	13	10	18
<i>S. typhi</i>	12	12	12	12	11	13	14

solutions were kept in shaking incubator for 24hrs. The mixture was filtered using Whatman filter paper and filtrate was collected and stored at 4°C for further use. The crude plant extract prepared in various solvents were tested for the antibacterial activity against pathogenic bacteria through well diffusion method. The pure culture of five test organisms including *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Bacillus subtilis* and *Staphylococcus aureus* were obtained from Department of Biotechnology Maharishi Markandeshwar (Deemed to be University), Mullana, Ambala (HR). The cultures were maintained and preserved on nutrient agar slants. During experimental analysis the pure preserved culture were inoculated in the broth medium.

a. Phytochemical screening: Different Phyto-

**Fig. 1:** Antimicrobial activity of crude extract of *S. cumini* seeds, leaves and bark against different pathogenic bacteria: (A) *E. coli*, (B) *P. aeruginosa*, (C) *B. subtilis* (D) *S. typhi* and (E) *S. aureus*.

chemical test were performed by using standard method described by (Gowri *et al.*, 2010; Hasanuzzaman *et al.*, 2016).

b. Antimicrobial activity: Antibacterial activity was carried out by Agar well diffusion method described by (Perez *et al.*, 1990). In each well 100µl of crude plant extract was added and pure organic solvents were used as control. The antibacterial activity of each extract expressed in terms of diameter of zone of inhibition (in mm) produced by respective extract.

Minimum inhibitory concentration: Minimum inhibitory concentration of crude plant extracts against pathogenic strains was determined by dilution method (Sathyabama *et al.*, 2011). To test each plant crude extract Nutrient agar plates were prepared and chloremphenicol was used as control The plates of nutrient agar were lawn cultures with test organisms and plant extracts *viz.* seed, leaves and bark (methanolic and ethanolic extract, 100µl) was added to each well. The plates were incubated at 37°C for 24hrs. The inhibition of bacterial growth due to crude plant extract in the wells was detected.

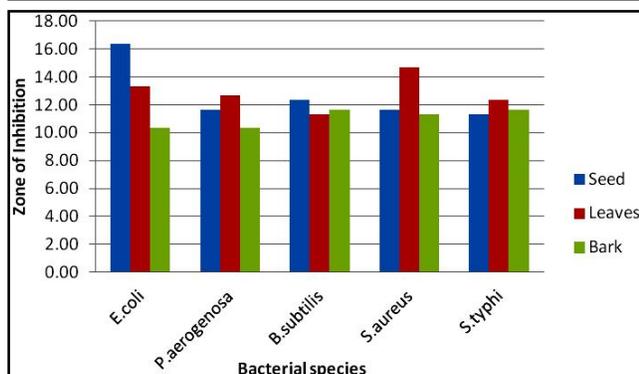
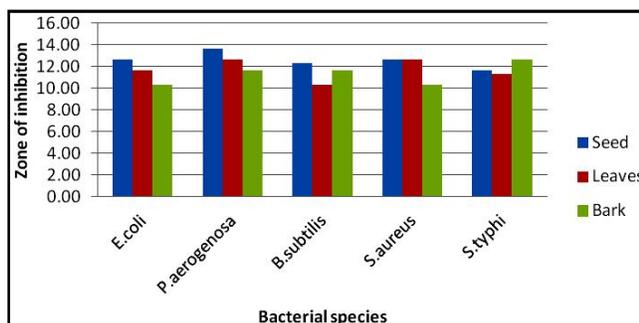
Results and Discussion

The methanolic and ethanolic crude extracts of *S. cumini* shows antibacterial property except the aqueous extract against test organisms. All the extracts showed presence of alkaloids, saponins, tannins and some shows presence of flavanoids.

Phytochemical analysis: All the plant extracts showed the presence of alkaloids, saponin and steroids. Only the leaves extract showed presence of flavinoids. Amino acid and proteins were absent in all the plant extracts except seed (Jagetia, 2017). The leaves of *S. cumini* were rich in alkaloids, flavonoids, tannins, saponins, steroids and terpenoids table 1. The methanolic leaf

Table 3: The minimum inhibitory concentration of different plant extract against selected pathogenic bacterial species.

Organisms	Seed		Leaves		Bark	
	Methanolic	Ethanollic	Methanolic	Ethanollic	Methanolic	Ethanollic
<i>E. coli</i>	83.25	41.62	333	41.625	83.25	41.625
<i>P. aeruginosa</i>	41.62	41.62	83.25	333	41.625	41.625
<i>B. subtilis</i>	83.25	83.25	83.25	41.625	83.25	83.25
<i>S. aureus</i>	333	333	333	83.25	333	333
<i>S. typhi</i>	83.25	41.62	83.25	83.25	83.25	41.625

**Fig. 2:** Inhibitory action of methanolic extracts of seeds, leaves and bark. (Data as mean \pm SE values of three replicates).**Fig. 3:** Inhibitory activity of ethanolic extract of seeds, leaves and bark. (Data as mean \pm SE values of three replicates).

extract of *S. cumini* was rich in phenols, saponins, glycosides, flavanoids, alkaloids, steroids, terpenoids, resins and tannins. Similar phytochemical constituents were reported by (Sharma *et al.*, 2012; Satyavathi and Bhawani, 2014).

Antimicrobial activity: Antimicrobial activity of seeds, leaves and bark was performed against five different pathogenic bacteria *viz.* *E. coli*, *P. aeruginosa*, *B. subtilis*, *S. aureus* and *S. typhi* Fig. 1; table 2. Methanol and ethanol extract of seeds showed maximum inhibitory activity on *E. coli* with a zone of 17mm and against *P. aeruginosa* with 14mm respectively. The growth of *S. aureus* was inhibited by methanolic and ethanolic extracts of leaves and showed a zone of 14

and 13mm respectively. The bark extract shows inhibitory action against *S. typhi* with a zone of 12-13mm (Bhatia and Bajaj 1975). From the above results, it was found that the seed extracts of *S. cumini* reflected efficient activity against pathogenic bacteria in various extracts and is comparative to control chloramphenicol (Gangadhar *et al.*, 2011; Sharma *et al.*, 2012). Similar activities have been reported by Gowri and Vasantha (2010) using *S. cumini* leaves in methanol extract. The studies by Yadav *et al.*, (2017) using methanolic and ethanolic extracts showed similar inhibitory activity against clinical isolates of gram negative bacteria such as *S. typhi*, *Shigella dysenteriae*, *Klebsiella pneumonia*, *P. aeruginosa*, *E. coli* and gram positive bacteria such as *B. subtilis* and *S. aureus*.

Determination of minimum inhibitory concentration (MIC) of plant extracts: The lowest concentration at highest dilution of the plant extract required to inhibit visible growth of the tested microorganism was designated as the MIC table 3; Fig. 2, 3.

Conclusions

The present study has helped in demonstrating the potential bioactive compound of natural plant extracts. The seeds, leaves and bark extract of *S. cumini* were evaluated for the presence or absence of diverse phytochemicals and antibacterial activity. The seed and leaves extract in methanol and ethanol has high antibacterial activity in comparison to bark extract. The activity can be used against multidrug resistant bacteria and as an herbal medicine alternative to the antibiotics. This study suggests that the petroleum ether extract of *S. cumini* seed has potent antibacterial activities. A result was found that the seed extracts of *S. cumini* was shown efficient activity against pathogenic bacteria in various extracts. The seed extract is shown the high therapeutic value against pathogenic bacteria and documented for futures.

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