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**SCREENING OF SOLVENT EXTRACT OF SYZYGIUM
CUMINI LEAF FOR ITS ANTIBACTERIAL ACTIVITY**

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ABSTRACT

With the recent years, infections have increased to a great extent and resistant against antibiotics, become an ever increasing therapeutic problem. Therefore the present study was carried out to investigate the antibacterial activity of leaf extracts of Syzygium cumini against the selected gram positive and gram negative bacteria viz., S. aureus and S. typhimurium. Standard Ampicillin was used as a control. The antibacterial activity was done by using agar well diffusion method. Phytochemical investigation was carried out on the Acetone, Ethanol and Chloroform extracts of S.cumini leaves to evaluate the phytochemicals. The preliminary phytochemicals revealed the presence of many secondary metabolites. The result showed that maximum antibacterial activity was exhibited by chloroform extract of S. cumini against S. typhimurium (32.7 mm), which was followed by acetone extract which showed a zone of inhibition of 30 mm. Among the two test organisms S. typhimurium showed more resistance against the leaf extract of S. cumini than the bacterium S. aureus.

KEYWORDS: Plant extracts; Bacteria; Ampicillin; Antibacterial activity; Antibiotic; Phytochemicals; Solvent extracts.

1. INTRODUCTION

Antibiotics are undeniably one of the most important therapeutic discoveries of the 20th century that had effectiveness against serious bacterial infections. However, only one third of the infectious diseases are known to have been treated from the synthetic products [1]. With the advancement in science and technology, remarkable progress has been made in the field of medicine with the discoveries of many natural and synthetic drugs [2]. Several plants and herbs that are used traditionally have potential antimicrobial and antiviral properties and such reports have raised the optimism about phyto-antimicrobial agents [3]. During the last decade, reports of prevalence of resistance among microbes have increased but the development of antimicrobial drugs has not maintained the pace with the rate of development of drug resistance. A probable way to overcome this trend is the identification of more plant extracts with potential antimicrobial activities [4].

Some medicinal herbs for some reasons have not found wider application and sometimes are referred as 'Forgotten Plants'. Taking into account the increasing demand for natural ingredients for preventing plant diseases it is reasonable to revise the 'forgotten plants' by assessing their applicability and benefits using modern scientific analysis methods [5]. Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganism has increased. In general bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents [6]. In addition in developing countries, synthetic drugs are not only expensive but also inadequate for the infection-fighting strategies to control microbial infections [7].

For a long period of time plants has been a valuable source of natural products for maintaining human health, especially in the last decades, with more intensive studies for natural therapies. Now a days the use of phytochemicals for pharmaceutical purpose has gradually increased in many countries. According to World Health Organization (WHO) medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants [8]. Plant produces a wide variety of secondary metabolites which are used either directly as precursors or as lead compounds in the pharmaceutical industry. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant microbial pathogens. However very little information is available on such activity of medicinal plants and out of the 400,000 plant species on earth, only a small

number has been synthetically investigated for their antimicrobial activities [9].

Some organisms have developed resistance to the existing antibiotics; therefore the development of bacterial resistance to the currently available antibiotics has necessitated the research for new antibacterial agents [10]. As *Syzygium cumini* leaves have lot of medicinal significance, the present work was aimed to evaluate its antibacterial activity and phytochemical screening.

2. METHODOLOGY

2.1 Collections of test materials

Leaves of *Syzygium cumini* was collected from the College Campus and the specimens were identified; certified (BSI/SRC/5/23/2017/Tech) and the voucher specimen number were deposited at the Botanical Survey of India, Southern Circle, Coimbatore.

2.2 Preparation of leaf powder and extracts

Fresh leaves of *Syzygium cumini* were collected, and air dried under shade. Dried leaves were powdered using an electric pulverizer. Fine powder was obtained by sieving. The powder was subjected to extraction [11,12]. Acetone extraction was followed by chloroform extraction and ethanol extraction so that the powders were subjected to extraction with solvents of increasing polarity. The leaf extracts thus obtained were concentrated by distillation and dried by evaporation in a water bath at 40°C. The residue thus obtained was stored in tightly closed glass vials in the refrigerator for further use. Antibacterial activity of the leaves of *Syzygium cumini* was investigated.

2.3 Test microorganism

The bacterial strains used were the clinical isolates obtained from P.S.G. College, Coimbatore. The bacterial strains used were *Staphylococcus aureus* and *Salmonella typhimurium*.

2.4 Antibacterial assay

The activity of various solvent extracts of leaves of *S. cumini* on selected bacterial strains were assayed by agar well diffusion method.

2.5 Agar- Well Diffusion Method Media Preparation and Its Sterilization

For agar well diffusion, method of Murray *et al* [13] later modified by Olurinola [14] was used. Antibacterial susceptibility was tested on solid media in petriplates. For bacteria Nutrient agar was used for developing surface colony growth.

Reagents

Nutrient Agar

Nutrient agar medium was prepared and poured on to the petriplates and was left on sterile surface until the agar has solidified. The plates were swabbed (sterile cotton swabs) with 24 h old culture of bacterial strains. Wells were made in each of these plates using sterile cork borer. Stock solution of each solvent extract viz., Acetone, Chloroform and Ethanol was

prepared at a concentration of 1 mg/ml. About 50 μ l of different solvent extracts of the leaves of *S. cumini* was added using sterile syringe into the wells and allowed to diffuse at room temperature for 2 h. Ampicillin was used as positive antibacterial control and respective solvents were used as negative control.

The plates were incubated at 37°C for 18-24 h for bacterial pathogens. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around well [15]. Triplicates were maintained and the experiment was repeated thrice, for each replicates the readings were taken in three different fixed directions and the average values were recorded.

2.6 Statistical Analysis

The antimicrobial data was interpreted by calculating standard deviation and mean of three replicates.

2.7 Phytochemical screening

Preliminary phytochemical screening of leaf extract of selected plant was carried out using the standard procedures.

A. Test for Alkaloids

- **Mayer's test [16]:** A fraction of extract was treated with a drop or two of Mayer's test reagent along the sides of test tube and observed for the formation of white or cream coloured precipitate.
- **Wagner's test [17]:** A fraction of extract was treated with Wagner's reagent along the sides of the test tube and observed for the formation of reddish brown colour precipitate.
- **Hager's test [18]:** A few ml of extract was treated with 1 or 2 ml of Hager's reagent and observed for the formation of prominent yellow precipitate.

B. Test for Tannins

- **Ferric chloride test [19]:** About 0.5 g extract was stirred with about 10 ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2 ml of the filtrate, and observed for the blue-black, green or blue-green precipitate.

C. Test for Phenols

- **Ferric chloride test [20]:** The extract (50mg) was dissolved in 5 ml of distilled water and treated with few drops of 5% ferric chloride and observed for the formation of dark green colour
- **Lead acetate test [21,22]:** The extract (50 mg) was dissolved in 5 ml of distilled water and 3 ml of 10% lead acetate solution was added and observed for the formation of bulky white precipitate.

D. Test for Flavonoids

- **NaOH test [19]:** Few quantity of the extract was dissolved in water and filtered; to this 2 ml of the 10% aqueous sodium hydroxide was later added to produce a yellow colouration. A change in colour from yellow to colourless on addition of dilute hydrochloric acid was an indication for the presence of flavonoids.
- **Lead acetate test [21,22]:** Test extract (50 mg) was taken in a test tube and few drops of lead acetate solution was added to it and observed for yellow coloured precipitate.

F. Test for Sterols

- **Liebermann-Burchard test [23]:** The extract (50 mg) is dissolved in 2 ml of acetic anhydride. To this one or two drop of Conc. H₂SO₄ is added along the side of the test tube and observed for an array of colour changes.

G. Test for Terpenoids

- **Liebermann-Burchard test [24]:** A little of extract (50 mg) was dissolved in ethanol. To it 1 ml of acetic anhydride was added followed by the addition of Conc. H₂SO₄. A change in colour from pink to violet showed the presence of terpenoids.

H. Test for Saponins

- **Foam Test:** The extract (50 mg) or dry powder was diluted with distilled water and made up to 20 ml. The suspension is vigorously shaken in a graduated cylinder for 15 minutes and observed for the formation of 2 cm layer thick foam.

I. Test for Anthraquinones

- **Borntreger's test [24]:** About 0.2 g of extract to be tested was shaken with 10 ml of benzene and then filtered. 5 ml of the 10% ammonia solution was then added to the filtrate and thereafter shaken and observed for the appearance of a pink, red or violet colour in the ammoniacal (lower) phase.

J. Test for Proteins

- **Ninhydrin test [25]:** Two drops of ninhydrin solution (10 mg of ninhydrine in 200 ml of acetone) are added to 2 ml of aqueous filtrate and observed for the present of characteristic purple colour.
- **Biuret test [26]:** An aliquot of 2 ml of filtrate is treated with one drop of 2% copper sulphate solution. To this 1 ml of 95% ethanol was added followed by excess of potassium hydroxide pellets and observed for the formation of pink ethanolic layer.

K. Test for Quinones

- **H₂SO₄ test [22]:** To 1 ml of extract add 1 ml of Conc. H₂SO₄ and observed for the formation of red colour.
- **HCl test [27,28]:** To 1 ml of the extract 5 ml of HCl and observed for the presence of yellow colour precipitate.

3. RESULTS AND DISCUSSION

Plants have been a source of medicine from time immemorial. This is because it holds many antibacterial, antiseptic, antibiotic properties. The antibacterial activity of plant extracts is mainly due to the presence of chemical compounds obtained from the plants. They are named as phytochemicals. Result of the antibacterial activity of leaf extract of *S. cumini* is shown in Fig 1. Figure 2 exhibits the plate that shows the zone of inhibition produced against the selected two bacterial strains by the plant extracts and both positive and negative controls.

In this study against the bacterium *S. aureus* maximum antibacterial activity was exhibited by acetone extract (Fig 2) in which a zone of inhibition was reported as 24.7 mm (Fig 1). Whereas ethanol extract showed a sensitivity of 21.7 mm, thereby displaying a moderate antibacterial efficacy. The

positive control Ampicillin showed a zone of inhibition of only 6 mm compared to the leaf extract. Minimum antibacterial potential was found in chloroform extract which tabulated an inhibitory zone of 12 mm. which was followed by chloroform extract zone of inhibition of 12.

Among the three extracts, against the bacterium *S. typhimurium* maximum antibacterial potency was shown by chloroform extract (Fig 2) of *S. cumini* leaf giving a inhibitory zone of 32.7 mm. Positive control provided protection zone of 13.33 mm. Negative control treatment didn't provide any protection. Followed by the activity of chloroform was that of acetone extract which provided a zone of inhibition of 30 mm. Minimum antibacterial efficacy among the three extracts was that of ethanol extract, providing an inhibitory zone of 28 mm.

Among the two test organisms *S. typhimurium* showed more resistance against the leaf extract of *S. cumini* than the bacterium *S. aureus*. In this study maximum antibacterial activity was exhibited by chloroform extract of *S. cumini* against *S. typhimurium* (32.7 mm), which was followed by acetone extract which showed a zone of inhibition of 30.

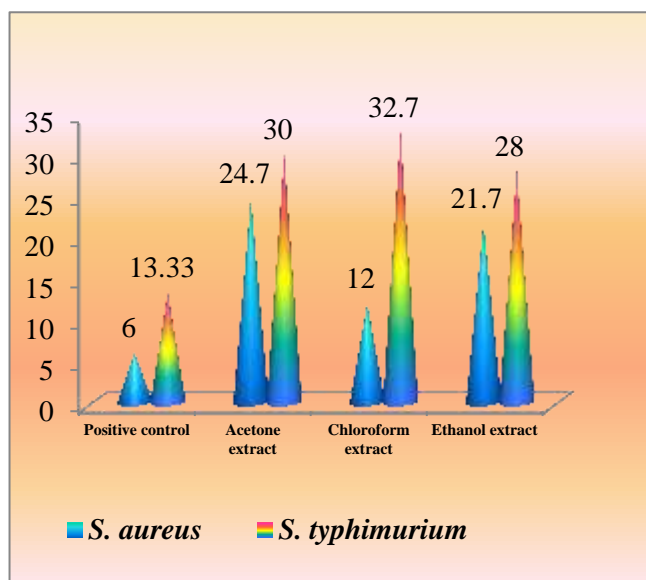


Fig 1: Antibacterial activity of *S. cumini* on selected bacterial strains

High antibacterial activity against the bacteria strain *S. aureus* and moderate antibacterial activity against *S. typhimurium* was exhibited by acetone extract of *Syzygium cumini*. Therefore acetone extract was tested for phytochemical which showed the presence of secondary metabolites like alkaloids, flavonoids, sterols, anthroquinones, saponins and proteins. Ethanol extract of *S. cumini* exerted moderate antibacterial activity against *S. aureus* and minimum

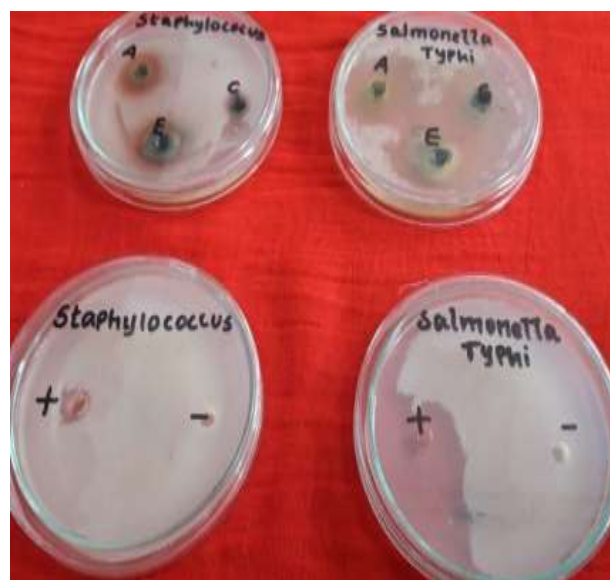


Fig 2: Plate showing antibacterial activity of *S. cumini* against selected bacterial strains

activity against *S. typhimurium*. The phytochemical analysis of ethanol extract of *S. cumini* showed the presence of phytochemical compounds such as alkaloids, flavonoids, sterols, terpenoids, tannins and protein.

Against the bacterium *S. typhimurium* and *S. aureus* maximum and minimum antibacterial activity respectively was exhibited by chloroform extract of *S. cumini*. The phytochemical analysis of chloroform

extract showed the presence of alkaloids, flavonoids, saponins, terpenoids, sterols, quinones, anthroquinones and proteins.

Table 1: Phytochemicals present in the extracts of *Syzygium cumini* leaves

Sl. No.	Constituents	<i>Syzygium cumini</i> leaf		
		Acetone extract	Chloroform extract	Ethanol extract
1	Alkaloids	+	+	+
2	Flavonoids	+	+	+
3	Sterols	+	+	+
4	Terpenoids	-	-	+
5	Anthroquinones	+	-	-
6	Phenols	-	+	-
7	Saponins	+	+	-
8	Tannins	-	+	+
9	Proteins	+	+	+
10	Quinones	+	+	-

‘+’ Detected ‘-’ Not Detected

An alarming increase in bacterial strains resistant to a number of antimicrobial agents demands that a renewed effort be made to seek antibacterial agents effective against pathogenic bacteria resistant to current antibiotics. Many of these plants have been investigated scientifically for antimicrobial activity and a large number of plant products have been shown to inhibit growth of pathogenic bacteria according to reports of Salman *et al* [29]. In the present study among the two test organisms *S. typhimurium* which is a gram negative strain showed more resistance against the leaf extract of *S. cumini*, than the bacterium *S. aureus* which is a gram positive strain. This may be because of the reason that Gram-positive bacterial strains were more susceptible to the extracts when compared to Gram negative bacteria.

Similar results in accordance with present study were reported by Zhao *et al* [30] in which it is stated that Gram negative bacteria are surrounded by the cell wall which restricts diffusion of hydrophobic compounds through its lipopolysaccharide covering. The absence of this barrier in Gram positive bacteria allows the direct contact of the essential oil's hydrophobic constituents with the phospholipids bilayer of the cell membrane, causing either an increase of ion permeability and leakage of vital intracellular constituents, or impairment of the bacterial enzyme systems. Venkataswamy *et al* [31] has reported that two groups of bacteria differ in their structure of cell wall. Ability of tannin to disintegrate bacterial colonies is hindered with bacterial cell wall. Medicinal plants which are rich in tannins are used to treat inflamed or ulcerated tissues [32].

Phytochemical analysis conducted on the plant extracts of *S. cumini* has revealed the presence of phytochemical constituents considered as active medicinal chemical constituents. Analysis of the plant extracts revealed the presence of phytochemicals such as alkaloids, flavonoids, sterols, saponins, tannins, terpenoids, quinones, anthraquinones, proteins and phenols. Similar results were reported in earlier study of Kathirvel and Sujatha [33]. Gowri and Vasantha [34] have reported antimicrobial activity of *S. cumini* leaves in methanol extract and has recorded the presence of tannins and other phenolic compounds.

Syzygium cumini is a good resource of bioactive compounds due to its content of various phytochemicals. However, most of the literature shows that the compounds of *S. cumini* are antibiotic in nature and the present study supports this statement. The present study also observed the sensitivity pattern of the selected pathogens towards the extracts of *S. cumini*, as well as standard antibiotics.

4. CONCLUSION

The present study the leaf extracts of *S. cumini* appears to be a rich source of different phytoconstituents, with antibacterial compounds and also supported the statement of applicability of plant in traditional system of treatment. The result suggested that both the plants could be used as a curative agent for different ailments. In addition, phytochemicals evaluation of *S. cumini* leaf provided information about a number of medicinally important secondary metabolites, which impart antibacterial characteristics. It may be concluded from this study that *S. cumini* leaf extracts has antimicrobial activity against *S. aureus* and

S. typhimurium. It is suggested that using natural products as therapeutic agents will probably not cause any health issues and instead of using synthetic drugs, botanical sources can be used as a valuable therapeutic index. In conclusion, the results of this study showed that *S. cumini* leaf extracts have antibacterial activity against the most common bacterial strains *S. aureus* and *S. typhimurium* involved in human infectious diseases.

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