

# Phytochemical Screening and Antibacterial Activity of *Syzygium cumini* (L.) (Myrtaceae) Leaves Extracts

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**ABSTRACT:** Phytochemical investigation was carried out on the crude methanol and aqueous extracts of the leaves of *Syzygium cumini* (L.) (MYRTACEAE). The antimicrobial activity of the extract was tested against standard strains and clinical isolates of some bacteria using the disc diffusion method. Preliminary phytochemical studies revealed the presence of flavonoids, alkaloids, glycosides, steroids, phenols, saponins, terpenoid, cardiac glycosides and tannins as the chemical class present in the extracts. The extracts showed inhibitory activity against clinical isolates of The gram negative bacteria such as *Salmonella enteritidis*, *Salmonella typhi*, *Salmonella typhi A*, *Salmonella paratyphi A*, *Salmonella paratyphi B*, *Pseudomonas aeruginosa* and *Escherichia coli*. gram positive bacteria are *Bacillus subtilis*, and *Staphylococcus aureus*. The results showed that the methanol extracts was more potent than the aqueous extracts.

**Key words:** *Syzygium cumini* (L.) (Myrtaceae) Leaves Extracts Phytochemical Screening ,Antibacterial Activity.

## INTRODUCTION

The medicinal properties of several herbal plants have been documented in ancient Indian literature and the preparations have been found to be effective in the treatment of diseases. Therefore to meet the increasing demand of manufacturing modern medicines and export, the need of the medicinal plants have enormously increased. This demand is generally met with by cultivating uprooted medicinal plants (1).

During the last ten years pace of development of new antimicrobial drugs has slowed down while the prevalence of resistance has increased astronomically (2). The problem of microbial resistance of growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce this problem, such as controlling the use of antibiotics and carrying out research for the better understanding of the genetic mechanism of resistance. This prompted us to evaluate plants as the source of potential chemotherapeutic agent, antimicrobial agent and their ethno medicinal use (3).

*Staphylococcus aureus* infections can spread through contact with pus from an infected wound, skin to skin contact with an infected person by producing hyaluronidase that destroy tissues. *Proteus vulgaris* can cause many different types of infection to humans such as urinary track infection, wound infections. *Proteus vulgaris* can be deadly when in the sinus or respiratory tissues, if left untreated or is treated with antibiotics that have only an intermediate effect on *Proteus vulgaris* (4). *Salmonella* are closely related to the *Escherichia* genus and are found worldwide in warm- and cold-blooded animals, in humans, and in nonliving habitats. They cause illnesses in humans and many animals, such as typhoid fever, paratyphoid fever and the food borne illness salmonellosis (5).

*Syzygium cumini* (L.) is belonging to the family Myrtaceae. Large trees cultivated throughout India for the edible fruits (Black Plum) and are reported to contain vitamin C, gallic acid, tannins, anthocyanins, includes cyanidin, petunidin, malvidin-glucoside and other components (6,7). The juice of unripe fruits is used for preparing vinegar that is

considered to be a stomachic, carminative and diuretic. The ripe fruits are used for making preserves, squashes and jellies. The fruits are astringent. A wine is prepared from the ripe fruits in Goa (7). Leaves have been used in traditional medicine as a remedy for diabetes mellitus in many countries (8, 9). The leaves are also used to strengthen the teeth and gums, to treat leucorrhoea, stomachalgia, fever, gastropathy, strangury, dermatopathy (10), constipation and to inhibit blood discharges in the faeces (11). Extract of seed, which is traditionally used in diabetes, has a hypoglycemic action and antioxidant property in alloxan diabetic rats (12) possibly due to tannins (13). The folkloric use of this species to treat infectious diseases stimulated the investigation of the antimicrobial activity of the methanol and aqueous extract from *Syzygium cumini* leaves against standard and multi drug resistant Gram positive and Gram negative bacteria.

## MATERIALS AND METHODS

### PLANT MATERIALS

The leaves of *Syzygium cumini* were collected from Coimbatore district and were shade dried, powdered and extracted in soxhlet apparatus successively with methanol and aqueous respectively due to their nature of polarity. After extraction, the hexane and aqueous extracts were filtered through Whatman No.1 filter paper and stored for further use.

### PHYTOCHEMICAL SCREENINGS

The leaf extracts of *Syzygium cumini* were analysed for the presence of flavonoids, alkaloids, glycosides, steroids, phenols, saponins, terpenoid, cardiac glycosides and tannins according to standard methods (14).

#### Alkaloids [Mayer's test]:

1.36gm of mercuric chloride dissolved in 60ml and 5gm of potassium iodide were dissolved in 10 ml of distilled water respectively. These two solvents were mixed and diluted to 100ml using distilled water. To 1ml of acidic aqueous solution of samples few drops of reagent was added. Formation of white or pale precipitate showed the presence of alkaloids.

#### Flavonoids:

In a test tube containing 0.5ml of alcoholic extract of the samples, 5 to 10 drops of diluted HCl and small amount of Zn or Mg were added and the solution was boiled for few minutes. Appearance of reddish pink or dirty brown colour indicated the presence of flavonoids.

#### Glycosides:

A small amount of alcoholic extract of samples was dissolved in 1ml water and then aqueous sodium hydroxide was added. Formation of a yellow colour indicated the presence of glycosides.

#### Steroids [Salkowski's test]:

About 100mg of dried extract was dissolved in 2ml of chloroform. Sulphuric acid was carefully added to form a lower layer. A reddish brown colour at the interface was an indicative of the presence of steroidal ring.

#### Cardiac glycosides [Keller killiani's test] :

About 100mg of extract was dissolved in 1ml of glacial acetic acid containing one drop of ferric chloride solution and 1ml of concentrated sulphuric acid was added. A brown ring obtained at the interface indicated the presence of a de oxy sugar characteristic of cardenolides.

#### Saponins:

A drop of sodium bicarbonate was added in a test tube containing about 50ml of an aqueous extract of sample. The mixture was shaken vigorously and kept for 3min. A honey comb like froth was formed and it showed the presence of saponins.

#### Resins:

To 2ml of chloroform or ethanolic extract 5 to 10ml of acetic anhydrite was added and dissolved by gentle heating. After cooling, 0.5ml of H<sub>2</sub>SO<sub>4</sub> was added. Bright purple colour was produced. It indicated the presence of resins.

#### Phenols [Ferric Chloride Test]:

To 1ml of alcoholic solution of sample, 2ml of distilled water followed by a few drops of 10% aqueous ferric chloride solution were added. Formation of blue or green colour indicated the presence of phenols.

#### Tannins [Lead acetate test]:

In a test tube containing about 5ml of an aqueous extract, a few drops of 1% solution of lead acetate was added. Formation of a yellow or red precipitate indicated the presence of tannins.

#### FeCl<sub>3</sub> test:

A 2ml filtrate [200mg of plant material in 10ml distilled water, filtered], and 2ml of FeCl<sub>3</sub> were mixed. A blue or black precipitate indicated the presence of tannins (15).

**Terpenoid:**

2ml of chloroform and 1ml of conc. H<sub>2</sub>SO<sub>4</sub> was added to 1mg of extract and observed for reddish brown colour that indicated the presence of terpenoid.

**MICROORGANISMS:**

The gram negative bacteria such as *Salmonella enteritidis*, *Salmonella typhi*, *Salmonella typhi A*, *Salmonella paratyphi A*, *Salmonella paratyphi B*, *Pseudomonas aeruginosa* and *Escherichia coli*. gram positive bacteria are *Bacillus subtilis*, and *Staphylococcus aureus*. were procured from Department of Microbiology, Kovai Medical Centre and Hospital, Coimbatore and used for the study.

**ANTIBACTERIAL TEST****PREPARATION OF CULTURE MEDIUM AND INOCULATION**

The petriplates and the nutrient agar medium were sterilized for 20 minutes at 120 °C. The rest of the procedure was carried out in laminar air flow. Approximately 20ml of the media was poured into the sterile petriplates and allowed to get solidified. After the media gets solidified the bacterial organisms were swabbed on the medium using cotton swabs.

**DISC DIFFUSION METHOD**

Antimicrobial activity of the leaf extracts was tested using the disc diffusion method (16). Sterile nutrient agar plates were prepared for bacterial strains and inoculated by a spread plate method under aseptic conditions. The filter paper disc of 5 mm diameter (Whatman's No. 1 filter paper) was prepared and sterilized. The leaf extracts to be tested were prepared various concentrations of 25 µl, 50 µl, 75 µl, and 100µl and were added to each disc of holding capacity 10 microlitre. The sterile impregnated disc with plant extracts were placed on the agar surface with framed forceps and gently pressed down to ensure complete contact of the disc with the agar surface. Filter paper discs soaked in solvent were used for negative controls. All the plates were incubated at 37 °C for 24 hours. After incubation, the size (diameter) of the inhibition zones was measured.

**RESULTS AND DISCUSSION**

In the present study, the phytochemical screening and antibacterial activities were performed with methanol and aqueous extracts of the leaf of *S. cumini*. The study was made against six gram positive pathogenic bacteria and two gram negative bacteria using the standard disc diffusion method. The leaves of *S. cumini* were rich in flavonoids, alkaloids, glycosides, steroids, phenols, tannins and saponins. These phytochemicals confer antimicrobial activity on the leaf extracts (Table-1).

**Table 1: Phytochemical screenings of *Syzygium cumini* leaf extracts.**

Phytochemical constituents		Methanol	Aqueous
Flavonoids		+++	+++
Alkaloids		++	+++
Glycosides		++	++
Steroids		+++	+
Phenols		++	+++
Terpenoid		+	++
Saponins		+	+
Resins		+	+
Tannins	1.FeCl <sub>3</sub> test	+	+
	2.Lead acetate test	+	+
Cardiac glycosides		+	++

+ = present, ++ = moderately present, +++ = Appreciable amount

The various phytochemical compounds detected are known to have beneficial importance in medicinal sciences. For instance, flavonoids have been referred to as nature's biological response modifiers, because of their inherent ability to modify the body's reaction to allergies and virus and they showed their anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities (17). Plant steroids are known to be important for their cardiogenic activities and also possess insecticidal and antimicrobial properties. They are also used in nutrition, herbal medicine and cosmetics (18).

Tannins were reported to exhibit antiviral, antibacterial and anti-tumour activities. It was also reported that certain tannins were able to inhibit HIV replication selectively and was also used as diuretic (18). Saponin is used as mild detergents and in intracellular histochemical staining. It is also used to allow antibody access in intracellular proteins. In medicine, it is used in hypercholesterolaemia, hyperglycaemia, antioxidant, anticancer, anti-inflammatory, weight loss, etc. It is also known to have antifungal properties (19).

The results of the methanol and aqueous extract of leaves exhibited antibacterial activity against all the tested strain viz. *Bacillus subtilis*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Proteus vulgaris* as shown in Table 2.

The zones of inhibitions were produced by both the aqueous and methanol extracts against all the test organisms. Methanol extracts were more active than the aqueous extract against all the microorganisms. The zones of inhibition were ranging from 6-22mm in diameter. The highest zone of inhibitions (22mm) noted in methanol extract against

some of the selected typhoid causing *micro organism* such as *Salmonella typhi* in 100 $\mu$ l concentration. The extracts of higher plants can be very good source of antibiotics (21) against various bacterial pathogens. Plant having antimicrobial compounds have enormous therapeutic potential as they can act without any side effect as often found with synthetic antimicrobial products.

Most antimicrobial medicinal plant are more effective against gram-positive than gram-negative bacteria (22, 23). However, our current findings show a remarkable activity against gram-negative bacteria including multi-resistant gram-negative strains. The antimicrobial activity of the *S.cumini* leaves methanol and aqueous extract may be due to tannins and other phenolic constituents. *S.cumini* is known to be very rich in gallic and ellagic acid polyphenol derivatives (25, 26). Also, acylated flavonol glycosides, kaempferol, myricetin and other polyphenols were isolated from *S.cumini* leaves (27,28).Tannins are

considered nutritionally undesirable because they precipitate proteins, inhibit digestive enzyme and affect the absorption of vitamins and minerals. However, some kinds of tannins can reduce the mutagenicity of a number of mutagens and display anticarcinogenic, antimicrobial and antioxidant activities (26).

The result obtained in this study suggests a potential application of *S. cumini leaves* for treatment of skin wounds, typhoid and further investigations should be conducted in order to explore there applications. Other medicinal plants containing phenolic compounds, including tannins, as major constituents are used topically for care and repair of skin wounds (27). The advantage of the use of topical antimicrobials is their ability to deliver high local concentrations of antibiotic irrespective of vascular supply. Further benefits include the absence of adverse systemic effects, and a low incidence of resistance (28).

**Table 2. Antibacterial activity of *Syzygium cumini* leaves using disc diffusion method**

EXTRACTS	STUDY OF INDICATOR TEST BACTERIA	ANTIBACTERIAL ACTIVITY OF PLANT EXTRACTS ( $\mu$ l)*				
		Control	25	50	75	100
METHANOL EXTRACTS	<i>Salmonella enteritidis</i>	5	7	12	15	17
	<i>Salmonella typhi</i>	5	8	15	19	22
	<i>Salmonella typhi A</i>	5	9	12	16	20
	<i>Salmonella paratyphi A</i>	5	6	7	9	11
	<i>Salmonella paratyphi B</i>	5	8	12	14	16
	<i>Pseudomonas aeruginosa</i>	5	7	9	14	17
	<i>Escherichia coli</i>	5	6	9	12	15
	<i>Bacillus subtilis</i>	5	7	12	15	18
	<i>Staphylococcus aureus</i>	5	8	10	14	16
AQUEOUS EXTRACTS	<i>Salmonella enteritidis</i>	5	9	12	14	16
	<i>Salmonella typhi</i>	5	7	11	14	18
	<i>Salmonella typhi A</i>	5	6	12	15	17
	<i>Salmonella paratyphi A</i>	5	7	13	15	18
	<i>Salmonella paratyphi B</i>	5	9	14	17	20
	<i>Pseudomonas aeruginosa</i>	5	8	12	14	17
	<i>Escherichia coli</i>	5	9	13	15	18
	<i>Bacillus subtilis</i>	5	6	8	8	13
	<i>Staphylococcus aureus</i>	8	10	14	16	18

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