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Research Article

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

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ABSTRACT

The phytochemical analysis of the medicinal plants are important and have commercial interest in both research institutes and pharmaceutical companies for the manufacturing of the new drugs for treatment of various diseases. The present study was aimed to investigate the preliminary phytochemical screening of the seeds of *Syzygium cumini* belonging to family Myrtaceae. The results revealed the presence of medicinally important phytochemical constituents in the ethyl acetate and methanol extracts of *Syzygium cumini* seeds and it justifies their use in the traditional medicines for the treatment of different diseases.

Phytochemical investigation was carried out on the crude methanol and aqueous extracts of the leaves of *Syzygium cumini* (L.) 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, Antiviral, antibacterial and anti-tumor activities.

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INTRODUCTION

Medicinal plants are the local heritage with global importance and world is endowed with a rich wealth of medicinal plants^[1]. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs^[2]. Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases^[3]. *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,

malvidinglucoside and other components^[4]. (Wealth of India Raw materials 1976). *Syzygium cumini* is a medicinal plant, whose parts were pharmacologically proved to possess hypoglycaemic, antibacterial, anti-HIV activity and anti-diarrhea effects. Traditionally, the leaves of this plant were used as astringent, treat fever and halt diarrhea^[6]. As a potential plant, many publications had reported that the leaves extract of this plant had pharmacological activity like antibacterial, Antidiabetic, immunomodulatory, anticancer^[5, 7, 8, 9]. The previous phytochemical study of the leaves of *S. samarangense* showed the presence of triterpenoids and flavonoids^[7, 10, 11]. For antibacterial properties, the in vitro study of the leaves extract of *S. samarangense* from India showed strong antibacterial

activity against both Gram (+) and Gram (-) bacteria using diffusion agar method with inhibition zone value are 20-25 mm/50µL^[9].

MATERIALS AND METHODS

Collection of Plant Materials : The leaves of *Syzygium cumini* were collected from Nashik (Farm) 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.



Figure 1: Syzygium cumini Leaves



Figure 2: Syzygium cumini Leaves Powder

PHYTOCHEMICAL SCREENINGS

The leaf extracts of *Syzygium cumini* were analyzed for the presence of flavonoids, alkaloids, glycosides, steroids, phenols, saponins, terpenoid, cardiac glycosides and tannins according to standard methods^[12, 14]

Table 1: Phytochemical Screenings

Sr. No	Test	Observation	Inference
1	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	Presence of alkaloids
2	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 color.	Presence of flavonoids.
3	Glycosides: A small amount of alcoholic extract of A sample was dissolved in 1ml water and then aqueous sodium hydroxide was added.	Formation of a yellow color	Presence of glycosides.
4	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 color at the interface	Presence of steroidal ring
5	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	Presence of a de oxy sugar characteristic of cardenolides.
6	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	Presence of saponins.
7	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 H2SO4 was added.	Bright purple color was produced	Presence of resins.
8	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 was added.	Formation of blue or green color	Presence of phenols.

9	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	Presence of tannins.
	FeCl3 test: A 2ml filtrate 200mg of plant material in 10ml distilled water, filtered, and 2ml of FeCl3 were mixed.	A blue or black precipitate	Presence of tannins
10	Terpenoid: 2ml of chloroform and 1ml of conc. H ₂ SO ₄ was added to 1mg of extract and observed.	reddish brown color	Presence of terpenoid.



Methanolic leaves Extract



Aqueous Leaves Extract

Figure 3: Syzygium cumini Leaves Extraction

Table 2: Phytochemical screenings of Syzygium cumini leaf extracts:

Phytochemical constituents	Methanol	Aqueous
Alkaloids	++	+++
Flavonoids	+++	+++
Glycosides	++	++
Steroids	+++	+
Cardiac glycosides	+	++
Saponins	+	+
Resins	+	+
Phenols	++	+++
Tannins	+	+
Terpenoid	+	++

+ = Present , ++ = Moderately present, +++ = Appreciable amount.

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 was performed with methanol and aqueous extracts of the leaf of *S. cumini*. The leaves of *S. cumini* were rich in flavonoids, alkaloids, glycosides, steroids, phenols, tannins and saponins. It revealed the presence of alkaloids, flavonoids, glycosides, steroids, Cardiac glycosides, saponins, resin, phenol, tannin and terpenoid in the extracts of *Syzygium cumini* seed.

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, antimicrobial and anti-cancer activities [15]. Plant steroids are known to be important for their cardiotoxic activities and also possess insecticidal and antimicrobial properties. They are also used in nutrition, herbal medicine and cosmetics [16].

Tannins were reported to exhibit antiviral, antibacterial and anti-tumor activities. It was also reported that certain tannins were able to inhibit HIV replication selectively and was also used as diuretic [16]. 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 hypercholesterolemia,

hyperglycemia, antioxidant, anticancer, anti-inflammatory, weight loss, etc. It is also known to have antifungal properties [17].

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 3. 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 100µl concentration. The extracts of higher plants can be very good source of antibiotics [18]. 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.

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 their applications. Other medicinal plants containing phenolic compounds, including tannins, as major constituents are used topically for care and repair of skin wounds [19]. 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 [20].

Table 3: Antibacterial activity of *Syzygium cumini* leaves using disc diffusion method

EXTRACTS	STUDY OF INDICATOR TEST BACTERIA	ANTIBACTERIAL ACTIVITY OF PLANT EXTRACTS (µl)*				
		Control	25	50	75	100
METHANOL EXTRACTS	<i>Salmonella enteritidis</i>	5	8	13	15	18
	<i>Salmonella typhi</i>	5	9	13	16	20
	<i>Salmonella typhi A</i>	5	7	12	19	22
	<i>Salmonella paratyphi A</i>	5	8	15	08	21
	<i>Salmonella paratyphi B</i>	5	9	14	15	22
	<i>Pseudomonas aeruginosa</i>	5	8	14	15	20
	<i>Escherichia coli</i>	5	10	07	16	18
	<i>Bacillus subtilis</i>	5	8	12	17	18
	<i>Staphylococcus aureus</i>	7	7	12	13	19
AQUEOUS EXTRACTS	<i>Salmonella enteritidis</i>	5	7	14	12	20
	<i>Salmonella typhi</i>	5	9	15	12	20
	<i>Salmonella typhi A</i>	5	9	13	13	22
	<i>Salmonella paratyphi A</i>	5	8	15	17	25
	<i>Salmonella paratyphi B</i>	5	9	13	15	23
	<i>Pseudomonas aeruginosa</i>	5	8	13	19	22
	<i>Escherichia coli</i>	5	7	13	15	18
	<i>Bacillus subtilis</i>	5	10	08	09	17
	<i>Staphylococcus aureus</i>	8	8	15	16	18

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