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Research Article

EVALUATION OF ANTIBACTERIAL AND DPPH RADICAL SCAVENGING ACTIVITIES OF THE LEAF EXTRACTS AND LEAF ESSENTIAL OIL OF SYZYGIUM CUMINI LINN. FROM SOUTH INDIA

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ABSTRACT

In the present study, the antibacterial and DPPH radical scavenging activities of the leaf extracts and leaf essential oil of *Syzygium cumini* Linn were investigated. The antibacterial potential of the leaf essential oil, petroleum ether, chloroform, ethyl acetate and methanol extracts of the leaves of *Syzygium cumini* Linn were studied against human pathogenic bacteria viz. *Bacillus cereus, Enterobacter faecalis, Salmonella paratyphi, Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Serratia marcescens* by 'agar well diffusion' method and DPPH activity by spectrophotometer assay using ascorbic acid as standard. Leaf essential oil as well as leaf ethyl acetate, chloroform and methanol extracts of *Syzygium cumini* Linn exhibited pronounced activity against Gram-positive and Gram-negative bacteria and their activity is quite comparable with the standard antibiotics such as tobramycin, gentamicin sulphate, ofloxacin and ciprofloxacin screened under similar conditions. Among the leaf essential oil and leaf extracts of *S. cumini* studied, methanol extract and leaf essential oil showed potent scavenging activity on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical. The remarkable antibacterial and antioxidant activity exhibited by the plant extracts and essential oil can be attributed to the synergic effect of the active compounds present in it. The results obtained showed that the leaf methanol extract and leaf essential oil of *S. cumini* can be considered as good sources of natural antioxidants and antimicrobial compounds and can be incorporated into the drug formulations.

Keywords: *Syzygium cumini*, antibacterial activity, agar well diffusion method, DPPH radical scavenging activity, drug formulations

INTRODUCTION

Syzygium cumini Linn (family Myrtaceae), commonly known as Jamun (Hindi), is a medicinal plant and utilizable species. Common names are Java plum, Black plum, Jambul and Indian Blackberry. The original home of jamun is India, distributed throughout India, in forest up to 1800m usually along the bank and moist localities. The sprouts are refrigerant, carminative & astringent to bowels. *Syzygium cumini* is one of the most commonly medicinal plants used to treat diabetes mellitus in Brazil [1]. Different parts of this plant such as seeds, bark, fruit and leaves have been used in traditional medicine as a remedy for diabetes mellitus in many countries [2, 3]. Oliveira and co-workers [4] evaluated the antimicrobial activity of the crude hydro alcoholic leaf extract of *S. cumini*. The leaves of *Syzygium cumini* are used to strengthen the teeth and gum, to treat leucorrhoea, fever, gastropathy, strangury, dermopathy [5], constipation and to inhibit blood discharges in faeces [6].

Based on review of literature no reports are available regarding antibacterial and DPPH radical scavenging activities of *Syzygium cumini* leaf essential oil and leaf extracts. In this work, the antibacterial property of the *Syzygium cumini* leaf oil and leaf extracts were checked against various multi-drug resistant Gram positive and Gram negative bacterial strains by 'agar well diffusion method'. The antioxidant activity of the leaf essential oil and extracts were studied by DPPH radical scavenging assay. The results showed that the leaf essential oil and methanolic extracts of the leaves of *Syzygium cumini* is a good source of active compounds and antioxidants.

MATERIALS AND METHODS

Plant Material

The leaves of *Syzygium cumini* were collected from Thrissur district of Kerala, South India and authenticated by Dr. Kochuthressia M.V., HOD, Department of Botany, Vimala College, Thrissur. Voucher specimen is deposited in the specially maintained herbarium, Department of Botany, Vimala College, Thrissur.

Essential oil extraction

Fresh leaves of *Syzygium cumini* (250g) were ground to a paste using an electric mixer grinder and subjected to steam distillation for three hours. About 2 liters of the distillate were collected and extracted with diethyl ether (3X100 mL) and dried using anhydrous sodium sulphate. The dry ether extract on evaporation yielded 0.50g (0.20% of fresh weight of the sample) of pale yellow leaf oil.

Preparation of Plant Extracts

Fifty grams of the powered plant material were extracted successively with 150mL of petroleum ether, chloroform, ethyl acetate and methanol as solvents for 24hours by Soxhlet equipment.

Test microorganisms

The microorganisms used for antibacterial activity evaluation were obtained from Microbial Type Culture Collection and gene bank (IMTECH, Chandigarh, India), which were maintained on Nutrient broth media. They were Gram-positive bacteria such as *Bacillus cereus* (MTCC-1305), *Staphylococcus aureus* (MTCC-96) and *Enterobacter faecalis* (MTCC-5112) and Gram-negative bacteria such as *Salmonella paratyphi* (MTCC-735), *Escherichia coli* (MTCC-729), *Klebsiella pneumoniae* (MTCC-109), *Pseudomonas aeruginosa* (MTCC-647), *Proteus vulgaris* (MTCC-426) and *Serratia marcescens* (MTCC-86).

Culture medium and inoculums

The stock cultures of microorganisms used in this study were maintained on Plate Count Agar slants at 4^0C. Inoculum was prepared by suspending a loop full of bacterial cultures into 10mL of nutrient broth and was incubated at 37^0C for 24hours. On the next day Muller-Hinton agar (MHA) (Merck) sterilized in a flask and cooled to 45-50^0C was distributed by pipette (20mL) into each sterile Petri dish and swirled to distribute the medium homogeneously. About 0.1mL of bacterial suspension was taken and poured into Petri plates containing 20mL nutrient agar medium. Using the L-shaped sterile glass spreader bacterial suspensions were spread to get a uniform lawn culture.

Antibacterial activity assay

The agar well diffusion method is used for the antimicrobial evaluations. Wells of 8mm (0.8cm) diameter were dug on the inoculated nutrient agar medium with sterile cork borer and 50µL of the petroleum ether, chloroform, ethyl acetate and methanol extracts of the leaves of *Syzygium cumini* were added in each well. Wells introduced with 50µL of pure petroleum ether, chloroform, ethyl acetate and methanol served as negative controls. The plates

were incubated at 37^{0}C over night and examined for the zone of inhibition. The diameter of the inhibition zone was measured in mm. The standard antibiotic drugs such as tobramycin, gentamicin sulphate, ofloxacin and ciprofloxacin were also screened under similar conditions for comparison. An extract was classified as active when the diameter of the inhibition was equal to or larger than 8mm [7]. All the assays were performed in triplicate and expressed as average values.

Preliminary Phytochemical analysis

The sample extracts were analysed for the presence of various phytoconstituents like flavonoids, alkaloids, glycosides, steroids, phenols, saponins and tannins according to standard methods [8].

DPPH free radical scavenging assay

The DPPH free radical is a stable free radical, which has been widely accepted as a tool for estimating free radical-scavenging activities of antioxidants [9]. Hydrogen or electron donation abilities of the compounds were measured from the bleaching of the purple-colored methanol solution of 1, 1-diphenyl-2-picrylhydrazyl (DPPH). This spectrophotometer assay uses the stable radical DPPH as a reagent. The sample solution of material (50 µL) at four concentrations (1.0, 0.5, 0.25 and 0.125 mg/mL) was mixed with freshly prepared methanolic solution of DPPH (634 μM) and allowed to stand for 30 min at room temperature. The absorbance was then measured at 515nm using a spectrophotometer and the inhibition of free radical DPPH in percent (%) was calculated using the formula below:

The percent of inhibition of DPPH reduction (decolourization)

$$\% \text{ of inhibition} = \frac{A_0 - A_{sample}}{A_0} \times 100$$

Where, (A_0) is the absorbance of the control (blank) and (A_{sample}) is the absorbance of the test compound. The compound concentration demonstrating 50% inhibition (IC_{50}) was calculated from the plot of inhibition percentage against sample concentration. Tests were carried out in triplicate. Samples and DPPH were dissolved in methanol. L-ascorbic acid was used as positive control.

RESULTS

Antibacterial screening

The leaf extracts and leaf essential oil of *Syzygium cumini* showing the zone of inhibition in millimeters, for Gram positive and Gram negative bacteria are summarized in Table1. In addition, the inhibition zones formed by standard antibiotics and those of negative controls are listed in Table 2.

Table 1: Inhibition zones formed by *Syzygium cumini* leaf essential oil and leaf extracts

Microorganisms	Diameter of inhibition zones (mm/50µL)								
	S.cumini Leaf oil					Leaf extracts			
	50%	25%	10%	5%	1%	A	B	C	D
1. Bacillus cereus	32	26	22	20	18	18	14	12	10
2. Enterobacter faecalis	32	30	26	22	20	24	14	11	10
3. Salmonella paratyphi	28	24	22	20	18	22	16	11	10
4. Staphylococcus aureus	44	40	32	26	24	26	15	11	10
5. Escherichia coli	28	26	20	14	10	25	16	14	12
6. Proteus vulgaris	48	30	28	24	20	24	18	12	11
7.Klebsiella pneumonia	30	28	24	20	18	20	14	12	11
8.Pseudomonas aeruginosa	32	30	28	24	22	22	20	16	14
9.Serratia marcescens	34	28	25	20	16	24	20	14	12

A: methanol; B: ethyl acetate; C: chloroform; D: petroleum ether

Used concentrations: 50µL of 50%,25%,10%, 5% and 1% essential oil samples in DMSO

and 50µL of 10mg/mL of plant extracts.

Table 2: Inhibition zones formed by the standard antibiotics and negative controls

Microorganisms	Diameter of inhibition zones (mm/50µL)				
	Tob 10µg	Gen 10µg	Oflo 10µg	Cip 10µg	Control A, B, C, D
1. *Bacillus cereus*	28	32	34	30	--
2. *Enterobacter faecalis*	26	32	32	26	--
3. *Salmonella paratyphi*	25	30	28	30	--
4. *Staphylococcus aureus*	26	28	24	24	--
5. *Escherichia coli*	30	36	32	34	--
6. *Proteus vulgaris*	26	30	24	32	--
7. *Klebsiella pneumoniae*	26	32	32	36	--
8. *Pseudomonas aeruginosa*	26	24	32	28	--
9.*Serratia marcescens*	24	32	30	30	--

Controls- A: methanol; B: ethyl acetate; C: chloroform; D: petroleum ether; Tob: tobramycin, Gen: gentamicin sulphate, Oflo: ofloxacin, Cip:ciprofloxacin

Phytochemical screening

Phytochemical evaluation was performed with methanol, ethyl acetate, chloroform and petroleum ether extracts of the leaves of *Syzygium cumini* (Table 3).

Antioxidant activity

The antioxidant activity of *Syzygium cumini* leaf essential oil and leaf extracts in solvents of varying polarity were measured in terms of hydrogen donating or radical scavenging ability, using the stable radical, DPPH. The method is based on the reduction of alcoholic DPPH· solutions in the presence of a hydrogen donating antioxidant. DPPH· solutions show a strong absorption band at 515 nm appearing as a deep violet color. The absorption vanishes and the resulting decolourization is stoichiometric with respect to degree of reduction. The remaining DPPH·, measured after a certain time, corresponds inversely to the radical scavenging activity of the antioxidant. The results of the free radical scavenging activity of the leaf extracts and leaf essential oil of *S. cumini* assessed by DPPH assay and amount of the sample needed for 50% inhibition of free radical activity, IC_{50} values were summarized in table 4.

Table 3: Phytochemical screening of *Syzygium cumini* leaf extracts

Phytoconstituents	MeOH extr.	EA extr.	$CHCl_3$extr.	PE extr.
Flavonoids	+ + +	-	-	-
Alkaloids	+ +	-	-	-
Glycosides	+ +	-	-	-
Steroids	+ + +	-	-	-
Phenols	+ +	-	-	-
Terpenoid	+	-	-	-
Saponins	+	-	-	-
Resins	+	-	-	-
Tannins	+	-	-	-

+ Present + + Moderately present + + + Appreciable amount

Table 4: DPPH free radical scavenging activity of the leaf essential oil and leaf extracts of *Syzygium cumini*

Samples	Concentration (mg/ml)				IC_{50} (µg/ml)
	1.0	0.5	0.25	0.125	
	Radical scavenging effect (%)				
*S.cumini*eaf methanol extract	93.39	87.22	84.14	81.05	83.02
S.cumini leaf ethyl acetate extract	89.86	83.7	81.93	77.97	88.04
S.cumini leaf chloroform extract	86.34	81.93	78.49	69.6	95.06
S.cumini leaf pet.ether extract	84.14	80.61	77.53	67.84	97.08
S.*cumini* leaf oil	90.0	87.56	80.98	72.68	76.40
L-ascorbic acid	96.03	93.83	91.18	86.34	70.40

DISCUSSION

Antibacterial screening of leaf essential oil

As can be seen from table 1, the leaf essential oil and leaf extracts of *Syzygium cumini* showed pronounced antibacterial activity against all the microorganisms tested. *S.cumini* leaf oil at various concentrations was evaluated for antimicrobial activity against Gram-positive and Gram-negative bacteria strains and the oils exhibited marked activity against all tested microorganisms. The leaf oil (50%) showed pronounced activity against *Bacillus cereus, Enterobacter faecalis, Salmonella paratyphi, Escherichia coli, Proteus vulgaris*, *Pseudomonas aeruginosa, Serratia marcescens, Staphylococcus aureus* and *Klebsiella 360ethanol360* (28-48mm/50µl inhibition zone).

The inhibitory effect of 50% leaf oil of *S.cumini* on *Escherichia coli* and *Klebsiella 360ethanol360* was comparatively less than that of standard antibiotics whereas the activity of the leaf oil (50%) against other tested bacteria like *Enterobacter faecalis, Salmonella paratyphi*, *Bacillus cereus* and *Pseudomonas aeruginosa* was comparable with that of all standard antibiotics; *Serratia marcescens*, *Staphylococcus aureus* and *Proteus vulgaris* was found to be higher than the all standard antibiotics(10µg each)screened under similar conditions.

The activities of 20% (24-40mm/50µl inhibition zone), 10% (22-32mm/50µl inhibition zone), 5% (14-26mm/50µl inhibition zone) and 1% (10-24mm/50µl inhibition zone) of the leaf oil samples were also studied against various pathogenic bacteria and were found to be active on all microorganisms tested.

As the leaf oil exhibited pronounced antibacterial activity comparable with standard antibiotics, it can be used as an external antiseptic in prevention and treatment of bacterial infections.

The remarkable antibacterial activity exhibited by the *S. cumini* leaf oil can be attributed to the synergic effect of the antimicrobial agents present in the oil. The leaf oil contains pinocarveol, α-terpeneol, myrtenol, eucarvone, muurolol, myrtenal, geranyl acetone, α-cardinol and pinocarvone as the major constituents [10] and reported to have antimicrobial activities [11].

Antibacterial screening of leaf extracts

Among the leaf extracts, methanol extract exhibited higher activity than the other extracts and petroleum ether extract showed least activity. Methanol (18-26mm/50µl inhibition zone), ethyl acetate (14-20mm/50µl inhibition zone), chloroform (11-16mm/50µl inhibition zone) and petroleum ether (10-14mm/50µl inhibition zone) extracts of the leaf exhibited marked activity against all the tested organisms such as *Bacillus cereus, Enterobacter faecalis, Salmonella paratyphi, Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Klebsiella 360ethanol360, Pseudomonas aeruginosa* and *Serratia marcescens.*

The leaf methanol extract exhibited significant activity against *Pseudomonas aeruginosa* (22mm/50µl inhibition zone) and *Serratia marcescens* (24mm/50µl inhibition zone) which is comparable with the standard antibiotic tobramycin (10µg). The activity of leaf methanol extract against *Proteus vulgaris* (24mm/50µl inhibition zone) which is comparable with the standard antibiotic ofloxacin (10µg) screened under similar conditions.

Phytochemical analysis

Phytochemical studies revealed the presence of various secondary metabolites in the leaf extracts of *S. cumini*. Various phytochemical compounds detected are known to have beneficial importance in medicinal sciences. The leaf methanol extract of *S.cumini* was rich in phenols, saponins, glycosides, flavinoids, alkaloids, steroids, terpenoids, resins and tannins. Antibacterial and antioxidant potential of leaf extracts can be attributed to the presence of these phytochemicals [12, 13]. The results of this study support the use of this plant for human diseases and reinforce the ethnobotanical importance of plant as a potential source of bioactive substances.

DPPH free radical scavenging activity assay

The DPPH free radical scavenging activity of the leaf extracts and leaf oil of *S.cumini* are sorted in descending order: leaf oil > Leaf methanol extract > Leaf ethyl acetate extract > Leaf chloroform extract > Leaf petroleum ether extract.

Out of the five samples tested, *S. cumini* leaf oil showed the highest scavenging activity (% inhibition 90.0, 87.56, 80.98 and 72.68 at 1.0, 0.5, 0.25 and 0.125mg/ml respectively), followed by *S. cumini* leaf methanol extract. Leaf petroleum ether extract exhibited least DPPH radical scavenging ability with % inhibition 84.14, 80.61, 77.53and 67.84 at 1.0, 0.5, 0.25 and 0.125mg/ml respectively.

S. cumini leaf oil possesses potent free radical-scavenging activity. The amount of the sample needed for 50% inhibition of free radical activity is expressed by IC_{50}. Lower IC_{50} value indicates higher antioxidant activity. By comparing the IC_{50} value of the leaf extracts of *S. cumini* with that of the authentic antioxidant L-ascorbic acid, it was found that the antioxidant activity of *S. cumini* leaf essential oil (76.4µg/ml) was quite comparable with that of L-ascorbic acid (IC_{50}: 70.40µg/ml). IC_{50} value of *S. cumini* leaf methanol extract (IC_{50}: 83.02 µg/ml) is not significantly different from that of L-ascorbic acid (IC_{50}: 70.40/ml).

CONCLUSIONS

The essential oil from the leaves of *S. cumini* showed varying degrees of antibacterial activity on the microorganisms tested. It is interesting to note that even crude extract of this plant showed prominent activity against various pathogenic bacteria where modern therapy has failed. Due to the emergence of the antibiotic resistant pathogens, plants are being looked upon as an excellent alternate to combat the spread of multi drug resistant microorganisms.

From the above experiment it can be inferred that leaf essential oil of *Syzygium cumini* as well as leaf methanol extract showed significant activity against Gram-positive and Gram-negative bacteria. The activity of leaf oil and leaf methanol extract was found to be quite comparable with the standard antibiotics screened under similar conditions. So they can be used as an external antiseptic in the prevention and treatment of bacterial infections caused by various pathogenic bacteria such as *Bacillus cereus, Enterobacter faecalis, Salmonella paratyphi, Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Klebsiella 361 ethanol 361 , Pseudomonas aeruginosa* and *Serratia marcescens*, which have developed resistance to antibiotics. The incorporation of these samples into the drug formulations is also recommended. This study demonstrated that the essential oil and methanolic leaf extracts of *Syzygium cumini* is as effective as modern medicine to combat pathogenic microorganisms.

Among the leaf essential oil and leaf extracts of *Syzygium cumini* studied, *Syzygium cumini* leaf oil extract and leaf [361] ethanol showed potent scavenging activity on DPPH free radical comparable with the standard antioxidant L-ascorbic acid. Antioxidant activities of the extracts and essential oils from medicinal plants are mainly attributed to the active compounds present in them. This can be due to the high percentage of main constituents, but also to the presence of other constituents in small quantities or to synergy among them. The methanolic leaf extract of *S. cumini* was rich in phenolic compounds, saponins, flavonoids and tannins. The antioxidant activity of *S. cumini* leaf extracts may be related to their phenolic substrates [14, 15]. The results obtained showed that the leaf methanol extract and leaf essential oil of *S. cumini* can be considered good sources of natural antioxidants.

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