Original Research Article

GC-MS analysis and antimicrobial assessment of *Syzygium cumini* (L.) Skeels seed ethanol extract

Abstract

People use plants to treat infections, and this has led to search of antimicrobials from medicinal plants. In this work, we evaluated the ethanol extract of *Syzygium cumini* seeds for their antibacterial and antifungal activities. Extraction was performed by cold maceration method using ethanol. The antimicrobial efficacy of the extract was assessed by agar well diffusion method against ten bacterial species: *Bacillus cereus*, *Bacillus subtilis*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Serratia marcescens*, *Staphylococcus aureus* and *Streptococcus mutans*, and five fungal species: *Candida albicans*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger* and *Mucor sp*. Minimum inhibitory concentrations (MICs) of the extract were determined by resazurin microtiter plate assay. Phytochemicals in the extract was identified by gas chromatography mass spectrometry (GC-MS) information. In agar well diffusion method, the Gram-negatives, *P. aeruginosa* and *S. marcescens*, Gram-positives, *B. subtilis* and *E. faecalis* and fungi *A. fumigatus* were more susceptible showing larger zones of inhibition. In resazurin method, low MICs were recorded for bacteria, *B. cereus* (<7.8 µg) and *P. aeruginosa* (15.6 µg) and fungi, *A. fumigatus* (31.2 µg). Fifteen compounds were identified by GC-MS profiling of the extract. The antimicrobial activity of the extract can be rightly related to the secondary metabolites in the ethanol extract of *Syzygium cumini* seeds.

Keywords: *Syzygium jambolanum*, Herbal drugs, Phytochemicals, Antibacterial, Minimum inhibitory concentration

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Introduction

Microbial infections cause millions of deaths annually worldwide. Every year globally, almost 700,000 lives are lost while presently 50,000 children die every year in India alone, from antibiotic-resistant infections, according to a release from Center for Disease Dynamics, Economics & Policy (CDDEP, 2015; Jain, 2015). Two million deaths per annum are expected to occur in India by the year 2050 (Subramaniam & Girish, 2020). According to Centers for Disease Control and Prevention's (CDC) Antibiotic Resistance Threats in the Unites States, 2019 report nearly 3 million antibiotic-resistant infections occur in U.S. every year where recording around 35,000 death (CDC, 2019). The greatest challenges and concern is the appearance of multi-drug resistant strains arising from indiscriminate use of broad-spectrum antibiotics. These synthetic drugs are expensive, ineffective, adulterated and not without severe side effects (Dabur et al., 2007). According to CDDEP, there is a need to reduce the use of antibiotics and to innovate to find new methods of disease treatment (Sriram et al., 2021). For this one can resort to search for new and effective antimicrobial drugs of natural origin.

Medicinal plants are used across the globe as remedies for the treatment of various diseases because they synthesize a diverse array of biologically active compounds (Egamberdieva et al., 2017). Despite many antimicrobial studies, the assessment of antimicrobial efficacy in medicinal plants still continue (Chassagne et al., 2021; Hlashwayo et al., 2020; Nigussie, Davey, Tufa, et al., 2021; Yan et al., 2021) to discover better novel drugs. In the present work, we have tested the antimicrobial potency of one such medicinal plant, *Syzygium cumini* L., its seeds extracted in ethanol. *S. cumini* is a medicinal

plant of the Myrtaceae family which is native to the tropics and commonly known as black plum or Indian blackberry. All parts of the plant are traditionally used in alternative medicine for the treatment of a wide variety of ailments, including cough, diabetes, dysentery, inflammation and ringworm. Traditional practitioners in India use the different parts of the plant in the treatment of diabetes, blisters in mouth, cancer, colic, diarrhoea, digestive complaints, dysentery, piles, pimples and stomach-ache. The fruit contains a single large seed, which is sweet, astringent to the bowels and the powder is widely used in India to control diabetes. The seed extract is also used to treat cold, cough, fever, and skin problems such as rashes and the mouth, throat, intestines and genitourinary tract ulcers. Seeds contain alkaloid, flavonoid and many essential oils (Ayyanar & Subash-Babu, 2012).

Having such miraculous power to cure many diseases, the current exploratory study was designed to evaluate the antimicrobial activity of *Szygygium cumini* seed ethanol extract and to identify the compounds in it by GC-MS analysis.

Materials and methods

Plant collection

Fruits of *S. cumini* were collected from Tirunelveli District, Tamil Nadu, India. The plant was authenticated by Prof. P. Jayaraman, Plant Anatomy Research Center, Chennai and a voucher specimen (PARC/2021/4520) was deposited there for future reference. Seeds were removed from the fruit, washed in distilled water, shade-dried for a week, powdered with a laboratory blender and preserved in sealed plastic bags.

Extraction

Extraction was performed by cold maceration technique in which 50 g of the powdered seeds were weighed in a digital balance and soaked in 200 ml ethanol (Handa, 2008). The conical flask with the solvent and the powdered seeds was covered with aluminium foil to prevent evaporation. The content was kept in constant shaking on a flask shaker for 72 h at room temperature to ensure thorough extraction. After 3 days, the mixture was filtered in Whatman filter paper No. 1 and concentrated in a hot water bath to obtain crude seed ethanol extract. The extract was stored in air-tight container to avoid contamination and used for further assays.

Microorganisms

Ten bacterial cultures (*Bacillus cereus*, *Bacillus subtilis*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Serratia marcescens*, *Staphylococcus aureus* and *Streptococcus mutans*) and five fungal cultures (*Candida albicans*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger* and *Mucor sp*.) were used in the study. All the cultures were obtained from Royal Bioresearch Centre, Chennai, Tamil Nadu, India. Stock cultures were maintained at 4 °C on slant of nutrient agar. Active cultures for experiments were prepared by transferring a loop of cells from the stock cultures to test tubes of nutrient broth that were incubated for 24 h at 37 ºC. The turbidity of the suspension was adjusted to obtain a final concentration to match that of a 0.5 McFarland standard (10^8 CFU/ml).

Antibacterial assay

Antibacterial activity was determined by well diffusion method (Valgas et al., 2007) with few modifications. 3.8 g Muller Hinton agar medium was weighed and dissolved in 100 ml distilled water. After sterilization the media was poured into sterile Petri plate. Once the medium had solidified, the agar was inoculated by spreading 1 ml of the bacterial inoculum over the entire agar surface. Six wells each with a diameter of 6 mm was cut aseptically with

a sterile cork borer. 20 µl of the extract dissolved in 1% DMSO at desired concentrations (200 µg, 400 µg, 600 µg and 800 µg) was poured into four wells. In the remaining two wells 20 µl of 1% DMSO and 20 µl of 10 µg streptomycin were loaded as negative and positive controls, respectively. Then, agar plates were incubated overnight at 37 °C. Bacterial growth was determined by measuring the diameter of zone of inhibition in mm.

Antifungal assay

Antifungal activity was determined by well diffusion method (Magaldi et al., 2004) with minor modifications. 4.4 g Potato Dextrose Agar medium was weighed and dissolved in 100 ml distilled water. After sterilization the media was poured into sterile Petri plate. Once the medium had solidified, the agar plate surface was inoculated by spreading 1 ml of the fungal inoculum over the entire agar surface. Six wells each with a diameter of 6 mm was cut aseptically with a sterile cork borer. 20 µl of the extract dissolved in 1% DMSO at desired concentrations (200 µg, 400 µg, 600 µg and 800 µg) was poured into four wells. In the remaining two wells 20 µl of 1% DMSO and 20 µl of 10 µg ketoconazole were loaded as negative and positive controls, respectively. Then, agar plates were incubated 48 h at 35 °C. Fungal growth was determined by measuring the diameter of zone of inhibition in mm.

Minimum inhibitory concentration determination

The minimum inhibitory concentration (MIC) was determined by the modified resazurin method (Sarker et al., 2007) in a 96 well plate under aseptic conditions. 100 µl of extract or control dissolved in 1% DMSO was pipetted into the first row. To all other wells 50 µl of nutrient broth was added and serially diluted to obtain eight concentrations (1000, 500, 250, 125, 62.5, 31.2, 15.6, 7.8 µg). To each well 10 µl resazurin indicator solution (270 mg dissolved in 40 ml distilled water) was added. 10 µl of bacterial suspension was added to each well to achieve a concentration of 5×10^5 cfu/ml (for bacterial isolates) and/or 10 µl of fungal suspension was added to each well to achieve a concentration of 10^5 spores/ml (for fungal isolates). 30 µl of 3.3X strength iso-sensitised broth was added to each well to ensure that the final volume was single strength of the nutrient broth. Each plate had a set of controls. The plates were prepared in triplicate, and placed in an incubator set at 37°C for 24 h. The colour change was then assessed visually. Any colour change from purple to pink was recorded as positive. The lowest concentration at which colour change occurred was taken as the MIC value.

Gas chromatograph – Mass Spectrum (GC-MS) analysis

For gas chromatograph analysis, a Shimadzu GC-2010 Plus gas chromatograph was equipped with a straight deactivated 2 mm direct injector liner and a 15 m Alltech EC-5 column (250µ I.D., 0.25 µ film thickness). A split injection was used for sample introduction and the split ratio was set to 10:1. The oven temperature program was programmed to start at 35 °C, held for 2 min, then ramped at 20 °C per min to 450 °C and held for 5 min. The helium carrier gas was set to 2 ml/min flow rate (constant flow mode). For mass spectrum analysis, a direct connection with capillary column metal quadrupole mass filter pre-rod mass spectrometer operating in electron ionization (EI) mode with software GCMS solution ver. 2.6 was used. Low-resolution mass spectra were acquired at a resolving power of 1000 (20% height definition) and scanning from m/z 25 to m/z 1000 at 0.3 sec per scan with a 0.2 sec inter-scan delay. High resolution mass spectra were acquired at a resolving power of 5000 (20% height definition) and scanning the magnet from m/z 65 to m/z 1000 at 1 sec per scan. Identification of the components of the compound was done by matching their recorded spectra with the data bank mass spectra of NIST library V 11 provided by the instruments software. GC/MS metabolomics database was used for the similarity search with retention index.

Results

Antibacterial activity

Antibacterial activity of ethanol extract of *S. cumini* seeds has been assessed by measuring the diameters of zones of growth inhibition on ten strains bacteria and the results are presented as shown in Table 1. Irrespective of being Gram-positive or Gram-negative, all the tested bacteria were susceptible to the ethanol extract of *S. cumini* seed as evidenced by formation of zones of inhibition ranging from 20 mm to 12 mm. With 200 µg of extract, largest zone was formed in *P. aeruginosa* culture (20 mm) and *S. marecescens* (20 mm) both being Gram-negative. Following which moderate zones of inhibition observed in cultures of Gram-positive bacteria like *B. subtilis* (19 mm), *B. cereus* (15 mm), *E. faecalis* (15 mm), *S. aureus* (14 mm) and *S. mutans* (14 mm). Relatively smaller inhibitory zones were formed in cultures of the remaining three Gram-negative strains namely, *K. pneumoniae* (13 mm), *S. typhi* (13 mm) and *E. coli* (12 mm) registering the least of all. Subsequent higher concentrations of the *S. cumini* seed extract resulted in wider zones of inhibition in all the bacterial cultures. However with 800 µg of the extract, a pattern similar to 200 µg was seen. Larger zones in Gram-negatives, *S. marcescens* (30 mm) and P. aeruginosa (26 mm) followed by moderate zones in cultures of both Gram-positives *B. subtilis* (23 mm), *E. faecalis* (22 mm), *B. cereus* (19 mm), *S. mutans* (19 mm) and Gram-negatives like *K. pneumoniae* (20 mm) and *S. typhi* (19 mm). Smallest zone was recorded in the Gram-negative *E. coli* (17 mm) culture. Wells with negative control 1% DMSO did not inhibit the growth of bacteria around them. But wells with the antibiotic streptomycin as one would expect, formed broader inhibition zones in all the cultures, from the largest being in *S. marcescens* (31 mm) to the smallest in *E. coli* (15 mm).

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Antifungal activity

Antifungal potential of *S. cumini* seed ethanol extract was also assessed in terms of zone of inhibition of fungal growth. The results of antifungal activity is presented in Table 2. Among the five fungal species, *A. fumigatus* was more susceptible to the extract in all the four concentrations tested showing increasing zones from 14 mm to 20 mm with increasing concentration. *Mucor sp.* formed relatively moderate zones in three concentrations: 400 µg (13 mm), 600 µg (13 mm) and 800 µg (14 mm). Clear zones were formed around the extract in fungal cultures *A. flavus* at 600 µg (12 mm) and 800 µg (18 mm) concentration. The extract was effective in preventing growth of *C. albicans* only with 800 µg of the extract (12 mm). Surprisingly, *A. niger* did not develop any zones. The standard antifungal drug used as a positive control in the study developed zones the largest being in *A. flavus* (25 mm), followed by *C. albicans* (24 mm), *A. fumigatus* (24 mm), *Mucor sp.* (16 mm) and *A. niger* (14 mm).

Minimum inhibitory concentrations

Table 3 lists the MIC values of *S. cumini* seed ethanol extract against bacteria and fungal species. In bacterial study, the MIC concentrations ranged from less than 7.8 to 125 µg. For fungi, the MIC ranged from 31.2 to 250 µg. Very high MIC values indicate a very limited antibacterial or antifungal efficacy. Gram positive, *B. cereus* and Gram-negative, *P. aeruginosa* showed low MIC value of less than 7.8 µg and 15.6 µg respectively followed by Gram-positive, *E. faecalis*, *B. subtilis* and Gram-negative, *S. marcescens* each having an MIC of 31.2 µg. Gram-positive, *S. mutans* recorded 62.5 µg as its MIC. Relatively high MIC 125 µg was against Gram positive, *S. aureus* and Gram-negative, *K. pneumoniae*, *S. typhi* and *E. coli*. In fungal study, MIC of the extract was lowest for *A. fumigatus* (31.2 µg), high (125

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µg) for three fungal species: *C. albicans, A. flavus* and *A. niger* and highest MIC 250 µg for *Mucor sp.*

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Phytochemicals by GC-MS analysis

The GC–MS chromatogram of ethanol seed extract of *S. cumini* recorded 15 peaks corresponding to the bioactive molecules that were identified by relating their peak retention time, peak area (%), height (%) and mass spectral fragmentation patterns to that of the known compounds described by the National Institute of Standards and Technology (NIST) library. Results revealed 15 compounds as shown in Table 4. The chemical formula, CAS number, molecular weight and the retention index for each compound identified in *S. cumini* seed ethanol extract are summarized in Table 5.

Discussion

The use of and search for antimicrobial drugs from medicinal plants have increased significantly in the recent few years. These plants are a repository of phytochemicals in the form of secondary metabolites, such as steroids, phenols, tannins, terpenoids, alkaloids, and flavonoids, which have established antimicrobial properties and could be developed for treatment of infectious diseases (Cowan, 1999). Innumerable studies worldwide have reported the antimicrobial potency of medicinal plants with a handful focussed on expounding the antimicrobial potency of isolated compounds (Behiry et al., 2019; Chassagne et al., 2021; Kebede et al., 2021; Naeim et al., 2020; Nigussie, Davey, Legesse, et al., 2021; Okla et al., 2021; Owusu et al., 2021).

This study is an attempt to screen the antibacterial and antifungal property of ethanol extract of *Syzygium cumini* seeds in vitro and to analyse the compounds in the extract. Jamun as the plant is called, has been used for the treatment of diabetes since ancient times in Ayurvedic system of medicine. Additionally, it is reported as an anti-allergic, anti-cancer, anti-diarrhoeal, anti-fertility, anti-hyperlipidemic, anti-hypertensive, anti-inflammatory, anti-lieshmanial, anti-nociceptive, anti-oxidant, anti-viral, cardioprotective, diuretic, gastroprotective, hepatoprotective, and radioprotective agent (Katiyar et al., 2016). Because of its numerous medicinal use, the plant was selected for the present study.

Extraction was performed by cold maceration method. It is a conventional method that uses simple equipment, widely in practice, does not require much skilled labour and energy consumption, cheap technique, allows sufficient time for the solvent to diffuse through the cell wall to solubilize the constituent present in plant and most suitable for extraction of heat labile compounds (Rasul, 2018). Ethanol was our choice of solvent since plant materials extracted with ethanol appears to have good extraction yield, high content of phytochemicals both polar and a great percentage of weak-polar compounds and thereby enhancing the pharmacological activity in many aspects (Sun et al., 2015).

Agar well diffusion method was adopted to test the antimicrobial efficacy that offers quite a lot of advantages over the other methods in use. The presence of suspended particulate matter hardly interferes with the diffusion of the drug into the agar. Cheap and simplicity are other reasons. Higher sensitivity is also detected than the disc method where disc might adsorb phytochemicals interfering with diffusion. Another benefit is the usage of carrier compounds such as DMSO that diffuse easily across the medium and yields relatively larger growth inhibition zones with natural products (Valgas et al., 2007). In our study 1% DMSO was used as the solvent to dissolve the ethanol extract of Syzygium cumini seeds which apparently do not affect the bacterial growth (Wadhwani et al., 2012).

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We selected five Gram-positive strains and five Gram-negative strains to find if the extract significantly controls the growth of both the bacterial groups. In our results we noticed that the ethanol extract of *S. cumini* seeds sizeably prevented the bacterial growth irrespective of the strains used. This is in accordance with a study on the methanol extract of *S. cumini* seeds against *B. subtilis*, *E. coli*, *S. aures* and *Klebsiella pneumonia* showing good antibacterial results (Amutha & Aishwarya, 2010). There is also a report on high antimicrobial property of the ethanolic extracts of leaves and aqueous extracts of seeds for 19 gram positive and gram negative bacterial strains (Prabhakaran et al., 2011). In another study, purified methanol fraction from ethanol extract of the seed of S. *cumini* L. exhibited significant antibacterial activity against *E. coli*, *S. aureus*, *B. subtilis* and *P. aeruginosa* suggesting its broad range of antibacterial activity (S. S. Yadav et al., 2011). Several other studies have been reported likewise with methanol (Anupam et al., 2017; Devi et al., 2020), petroleum ether (Aziz & Banerjee, 2018), aqueous (Sonali & Snehal, 2018) and acetone extracts able to achieve ≥95% killing of bacterial biofilm at concentrations ranging from 600-2000 µg/ml (Patel et al., 2013). With regard to the antifungal property of the ethanol extract of *S. cumini* seeds, we could trace only moderate activity. Though there are evidences demonstrating antifungal activity of *S. cumini* extracts obtained from different parts of the plant against fungi of the genus *Candida* (Júnior et al., 2021), here *Candida* was vulnerable only at 800 µg of the extract. In few other previous reports, methanolic extract of seed acted against *C. albicans* and *A. niger* (Saha et al., 2013). *S. cumini* seeds prepared with polymeric nanoparticles have proved its antifungal activity against *C. guilliermondii* and *C. haemulonii* (Bitencourt et al., 2016). A similar study with nanoparticles against pathogens of medical and dental interest, have also contributed to bacteriostatic and fungistatic effects, but at significantly lower concentrations than crude plant extracts (Bernardo et al., 2021)

The MIC was determined by resazurin method, an accurate, rapid and economical method in the determination of drug resistance and minimal inhibitory concentration of antimicrobial agents, tested successfully against a wide range of pathogens (Khalifa et al., 2013). Resazurin is pink with live cells and turns blue with dead cells. MIC in our study was in the range of 7.8 to 125 µg for bacteria and 31.2 to 250 µg for fungal species. This is in agreement with earlier investigations (Hanif et al., 2020; Saeloh et al., 2020).

The antimicrobial activity can be rightly attributed to the phytoconstituents in the extract that act synergistically to curb the growth of microbes. Hence analysing the extract for the constituents becomes mandate. In recent years, gas chromatography-mass spectrometry (GC–MS) has commonly been employed for detection of functional groups and identification of various bioactive therapeutic compounds that are present in medicinal plants. It is by far the best, fast and accurate technique to detect various compounds, including alcohols, alkaloids, nitro compounds, long chain hydrocarbons, organic acids, steroids, esters and amino and requires a small volume of plant extracts (Konappa et al., 2020). We have detected 15 compounds in the ethanol extract of *S. cumini* seeds. Previous studies have reported many such compounds from seed extract (Amutha & Aishwarya, 2010), compounds such as alkanes, sesquiterpenes, polyphenols (Banerjee & Narendhirakannan, 2011), gallic acid and quercetin in the methanolic extract (Kothari et al., 2011), compounds 4-(2-2-dimethyl-6-6-methylenecyclohexyl) butanol, decahydro-8a-ethyl-1,1,4a,6-tetramethylnaphalene, octadecane, 1-chlorooctadecane and tetratetracontane and many more in seed extract (Agarwal et al., 2019; Jagetia, 2017; Kumar et al., 2009; Ramya et al., 2012; Srivastava & Chandra, 2013).

Though we have not explored the mechanism by which these metabolites act on the microbes one can be sure that any antimicrobial agent act by either interfering chemically with the synthesis or function of vital components, and/or avoiding the conventional mechanisms of antibacterial resistance that comprise interrupting the bacterial protein biosynthesis or bacterial cell-wall biosynthesis, promoting bacterial cell membrane destruction, mistakes in bacterial DNA replication and repair, or inhibition of a metabolic pathway (Khameneh et al., 2019). One good evidence comes from a recent study of methanolic seed extract of *S. cumini* on *B. subtilis* revealing genomic DNA degradation, cell wall cracking and antimicrobial-induced permeabilization by acting against four enzymes which are crucial for plasma membrane synthesis in *B. subtilis* (A. K. Yadav et al., 2017).

Conclusion

The antimicrobial screening of ethanol extract of *Syzygium cumini* seeds by agar well diffusion method and resazurin-based broth microdilution assay proves it to be a potential source of antimicrobial agent as tested against the ten bacteria and five fungal species. From the GC-MS analysis individual components were identified from the complex mixture at a molecular level. We suggest further investigations with other parts of the plant, other extraction methods and in vivo assays to evaluate antimicrobial activity alongside antiviral, and antiparasitic activity that might exhibit better results.

Table 1: Zones of inhibition (mm) of test bacterial strains to *Syzygium cumini* seed ethanol extract

Bacteria	*S. cumini* seed extract				Streptomycin
	200 µg	400 µg	600 µg	800 µg	10 µg
Bacillus cereus	15	18	19	19	23
Bacillus subtilis	19	22	23	23	20
Enterococcus faecalis	15	18	20	22	22
Escherichia coli	12	14	15	17	15
Klebsiella pneumoniae	13	16	19	20	24
Pseudomonas aeruginosa	20	24	25	26	25
Salmonella typhi	13	14	17	19	18
Serratia marcescens	20	26	28	30	31
Staphylococcus aureus	14	15	17	17	17
Streptococcus mutans	14	17	18	19	20

Table 2: Zones of inhibition (mm) of test fungal strains by *Syzygium cumini* seed ethanol extract

Fungi	*S. cumini* seed extract				Ketoconazole
	200 µg	400 µg	600 µg	800 µg	10 µg
Candida albicans	-	-	-	12	24
Aspergillus flavus	-	-	12	18	25
Aspergillus fumigatus	14	15	17	20	24
Aspergillus niger	-	-	-	-	14
Mucor sp.	-	13	13	14	16

Table 3: Minimum Inhibitory Concentration (MIC) values of *Syzygium cumini* ethanol seed extract for the tested bacteria and fungi

Bacteria	MIC (µg)

Bacillus cereus	< 7.8
Bacillus subtilis	31.2
Enterococcus faecalis	31.2
Escherichia coli	125
Klebsiella pneumoniae	125
Pseudomonas aeruginosa	15.6
Salmonella typhi	125
Serratia marcescens	31.2
Staphylococcus aureus	125
Streptococcus mutans	62.5
Fungi	
Candida albicans	125
Aspergillus flavus	125
Aspergillus fumigatus	31.2
Aspergillus niger	125
Mucor sp.	250

Table 4: GC-MS chemical analysis of *Syzygium cumini* seed ethanol extract

Peak #	R. Time	Name	Area	Area%	Height	Height%
1	3.191	3-Furaldehyde	33638269	6.38	20704522	17.83
2	4.387	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	8758391	1.66	4625418	3.98
3	5.433	1,3,5-Triazine-2,4,6-triamine	26129860	4.96	6572085	5.66
4	6.090	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	31822265	6.04	6697607	5.77
5	7.163	5-Hydroxymethylfurfural	238305275	45.23	22657338	19.51
6	10.677	4-Chromanol	5449160	1.03	2316778	2.00
7	12.230	1,3-Cyclohexadiene, 1,3,5,5,6,6-hexamethyl-	3002076	0.57	1667352	1.44
8	12.711	1H-Cycloprop[e]azulen-7-ol	8330762	1.58	3802342	3.27
9	14.056	Tetradecanoic acid	9566946	1.82	4916051	4.23
10	16.426	Isopropyl palmitate	74995414	14.23	17138579	14.76
11	18.761	9,12-Octadecadienoic acid (Z,Z)-	18634169	3.54	4912346	4.23
12	18.829	Oleic Acid	9299571	1.77	4327870	3.73
13	19.085	l-(+)-Ascorbic acid 2,6-dihexadecanoate	12008047	2.28	3749416	3.23
14	24.758	Hexadecanoic acid	11770662	2.23	2636111	2.27
15	38.751	.gamma.-Sitosterol	35158406	6.67	9390057	8.09
			526869273	100.00	116113872	100.00

Table 5: Chemical formula, CAS number, molecular weight and retention index of compounds in *S. cumini* seed ethanol extract screened with GC-MS

Peak#	Formula	CAS number	Molecular weight	Retention index
1	$C_5H_4O_2$	498-60-2	96	831
2	$C_6H_80_4$	10230-62-3	144	1173

3	$C_3H_6N_6$	108-78-1	126	1597
4	$C_6H_80_4$	28564-83-2	144	1269
5	$C_6H_60_3$	67-47-0	126	1163
6	$C_9H_{10}O_2$	1481-93-2	150	1344
7	$C_{12}H_{20}$	0-00-0	164	1132
8	$C_{15}H_{24}O$	6750-60-3	220	1536
9	$C_{14}H_{28}O_2$	544-63-8	228	1769
10	$C_{19}H_{38}O_2$	142-91-6	298	2013
11	$C_{18}H_{32}O_2$	60-33-3	280	2183
12	$C_{18}H_{34}O_2$	112-80-1	282	2175
13	$C_{38}H_{68}O_8$	28474-90-0	652	4765
14	$C_{19}H_{38}O_4$	23470-00-0	330	2498
15	$C_{29}H_{50}O$	83-47-6	414	2731

Figure 1: Bacterial and fungal culture plates treated with *Syzygium cumini* seed ethanol extract in various concentrations and standards using agar well diffusion method (1: 200 µg extract, 2: 400 µg extract, 3: 600 µg extract, 4: 800 µg extract, 5: 10 µg standard drug)

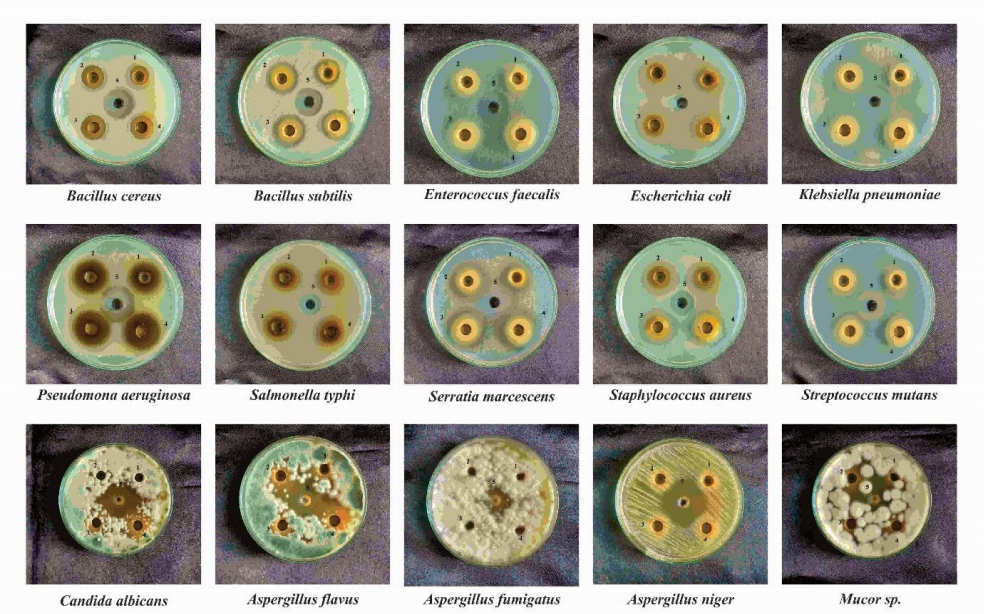


Figure 2: Micro-titre plates showing resarzurin method for determining Minimum Inhibitory Concentration of *Syzygium cumini* seed ethanol extract against the tested bacteria (a: *E. coli*, b: *S. aureus*, c: *S. mutans*, d: *S. typhi*, e: *S. marcescens*, f: *E. faecalis*, g: *K. pneumoniae*, h: *P. aeruginosa*, i: *B. subtilis*, j: *B. cereus*, 1– 8: 1000, 500, 250, 125, 62.5, 31.25, 15.6, 7.8 µg extract, 9: 10 µg standard drug, 10: control DMSO, 11: Culture alone)

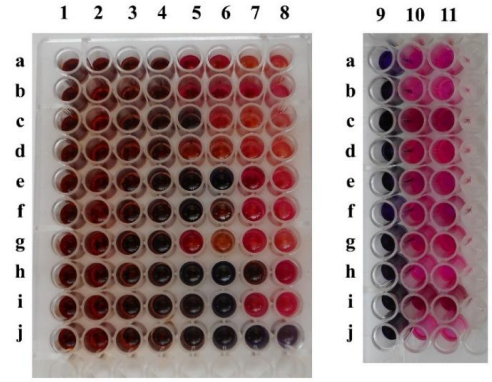


Figure 3: Micro-titre plates showing resarzurin method for determining Minimum Inhibitory Concentration of *Syzygium cumini* seed ethanol extract against the tested fungi (a: *A. niger*, b: *A. flavus*, c: *A. fumigatus*, d: *C. albicans*, e: *Mucor sp.*, 1– 8: 1000, 500, 250, 125, 62.5, 31.25, 15.6, 7.8 µg extract, 9: 10 µg standard drug, 10: control DMSO, 11: Culture alone)

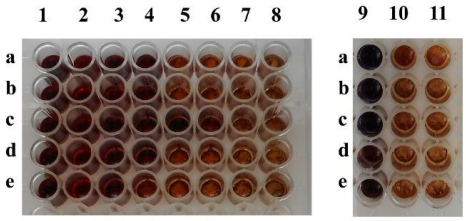
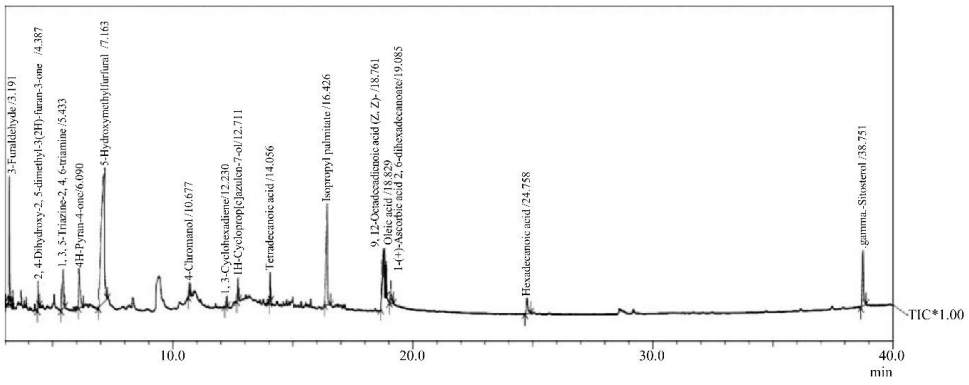


Figure 4: GC-MS chromatogram of compounds identified in *Syzygium cumini* seed ethanol extract



NOTE:

The study highlights the efficacy of "Ayurved" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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