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Regular Article

Fractionation of antibacterial extracts of *Syzygium cumini* (Myrtaceae) seeds

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Antibacterial activity of *Syzygium cumini* seed extracts prepared in methanol and ethanol was evaluated by disc diffusion and broth dilution assays. Both extracts exerted a broad spectrum of bacteriostatic action against different gram-positive and gram-negative bacteria. Their minimum inhibitory concentration (MIC) against susceptible organisms ranged from 154-656 µg/mL. Highest total activity was registered by the ethanol extract against *Staphylococcus epidermidis*. Extracts were separated on TLC (thin-layer chromatography) plates, and separated components were individually tested for their antibacterial activity. HPLC (high performance liquid chromatography) analysis indicated presence of gallic acid and quercetin in the methanolic extract.

Keywords: Bacteriostatic; Microwave assisted extraction (MAE); MIC; TLC; HPLC

Abbreviations: DDA- Disc Diffusion Assay; NCCLS- National Committee for Clinical Laboratory Standards; MeOH- Methanol

Natural products have been a significant source of commercial medicines and drug leads. Screening of crude plant extracts paves the way for discovery of novel bioactive compounds, and elucidation of their structures can open the door for new synthetic preparations (Colegate and Molyneux, 2008). Scope of phytochemicals in dealing with drug-resistant bacteria has been well recognized (Gibbons, 2008). Pathogen resistance to synthetic drugs and antibiotics already in use makes search for plants with antimicrobial activity more important, as they can substitute for synthetic antibiotics and drugs (Kothari *et al*., 2010). Seed extracts have been reported by several researchers for their antimicrobial activity (Nascimento *et al*., 2000; Kothari and Seshadri, 2010; Kothari, 2011).

Present study was aimed at investigation on antibacterial activity of *Syzygium cumini* seed extracts. *Syzygium cumini* (Linn.) skeels (Myrtaceae) is popularly known as Jaamun or black plum. It bears other names viz. *Syzygium jambolanum* and *Eugenia jambolana*. Its leaves are reported for antibacterial and antidysenteric action. Its seeds are believed to possess hypoglycemic action. Water extract of the seed exhibited antibacterial property against *S. boydi* and *S. dysenteriae*. Ethyl acetate extract of the seeds was reported to exert antibacterial action against gram-positive and gram-negative

bacteria (Sagrawat *et al.*, 2006; Khare, 2007). Vibrioicidal activity of *S. cumini* bark was reported by Sharma et al. (Sharma *et al.*, 2009). Satish *et al.* (2007) reported antifungal activity from aqueous extract of this plant.

Materials and Methods

Plant material: Seeds of *S. cumini* were procured from its fruit available in local market of Ahmedabad city. They were authenticated for their unambiguous identity by Prof. Y. T. Jasrai, Head of Botany Department, Gujarat University, Ahmedabad.

Extraction: Seeds were extracted in two different solvents (Merck, Mumbai, India)- Methanol, and Ethanol (50%) by microwave assisted extraction (MAE) method (Kothari *et al.*, 2009). One gram of dry seed powder was soaked into 50 mL of solvent, and subjected to microwave heating (Electrolux EM30EC90SS) at 720 W. Total heating time was kept 90 and 70 second for methanol and ethanol, respectively, with intermittent cooling. This was followed by centrifugation (at 10,000 rpm for 15 min.), and filtration with Whatman paper # 1 (Whatman International Ltd., Maidstone, England). Solvent was evaporated from the filtered extract and then the dried extracts were reconstituted in: (i) their respective solvents for disc diffusion assay, and (ii) dimethyl sulfoxide (DMSO) for broth dilution assay. Reconstituted extracts were stored under refrigeration for further use. Extraction efficiency was calculated as percentage weight of the starting dried plant material. Extraction efficiency was 16.4% and 29% for methanol and ethanol extracts, respectively.

Bacterial strains: Test organisms used were- *Escherichia coli* (MTCC 723), *Salmonella typhi* (MTCC 734), *Staphylococcus aureus* (MTCC 737), *Streptococcus pyogenes* (MTCC 442), *Staphylococcus epidermidis* (MTCC 435), *Aeromonas hydrophila* (MTCC 1739), *Shigella flexneri* (MTCC 1457), *Vibrio cholerae* (MTCC 3906), *Salmonella paratyphi* A (MTCC 735), and *Pseudomonas oleovorans* (MTCC 617). One additional strain of *S. paratyphi A* (designated as R3 owing to its resistance to three antibiotics- nalidixic acid, tetracycline, and cotrimexaxole) was taken from our institute's culture collection, while the rest were obtained from Microbial Type Culture Culture (MTCC), Chandigarh.

Antibacterial activity assay: Disc diffusion assay (DDA) was performed by Kirby-Bauer method as per NCCLS guidelines (Jorgensen and Turnidge, 2003). 500 µL of inoculum (adjusted to 0.5 McFarland standard) was spread on surface of Muller-Hinton agar medium (HiMedia, Mumbai, India). Sterile discs (6 mm diameter) made of Whatman paper # 1 were dipped into the test extract and were put onto the agar surface after complete drying. Discs dipped into respective solvent (separate disc for each solvent after drying) was applied as negative control. Commercially available discs of either streptomycin or ofloxacin (HiMedia) served as positive control. Plates were then incubated at 35ºC for 24 h. After incubation plates were observed for zones of inhibition (ZOI), and their diameter were measured. Studies were performed in triplicates.

MIC (minimum inhibitory concentration) determination was carried out using microbroth dilution method as per NCCLS guidelines (Jorgensen and Turnidge, 2003). Assay was performed in 96-well microtitre plates. Total volume of the assay system in each well was kept 200 µL. Cation-adjusted Muller-Hinton broth (HiMedia) was used as growth medium. Inoculum density of the test organisms was adjusted to that of 0.5 McFarland standard. Broth was dispensed into wells of microtitre plate followed by addition of test extract and inoculum. Extracts (reconstituted in DMSO) were serially diluted into each of the wells. A DMSO control was included in all assays (Wadhwani *et al.*, 2009). Gentamicin (HiMedia) served as positive control. Plates

were incubated at 35ºC for 16-20 h, before being read at 655 nm in a plate reader (BIORAD 680). MIC was recorded as the lowest concentration at which no growth was observed. All MICs were determined on three independent occasions. Concentration at which growth was inhibited by 50% was recorded as IC_{50} value.

After reading the plates for MIC, subculturing was made on nutrient agar from the wells showing no growth, so as to determine whether the extract is bactericidal or bacteriostatic. Total activity (mL/g) was calculated as (Eloff, 2004): Amount extracted from 1 g (mg) / MIC (mg/mL).

Phytochemical tests: Extracts were subjected to qualitative tests for presence of alkaloids, phenolics, and flavonoids (Wagner *et al*., 1983; Borgio *et al*., 2008). Total phenol content was quantified by Folin-Ciocalteau method as described by Singleton and Rossi (Singleton and Rossi, 1965). Total flavonoid content was quantified by aluminum chloride colorimetric method (Chang *et al*., 2002).

TLC: TLC separation was carried out on silica gel 60 F_{254} plates (Merck) of 0.25 mm thickness, and of 20X20 cm size. Solvent system applied was- n-butanol:water (1:1). Separated components were identified by observing the plate under UV light (254 and 365 nm; Uvitec-LF-206.LS, UK). Separated components were recovered (Stahl, 1969) by scrapping from the adsorbent, reconstituted in methanol followed by centrifugation at 14,000 rpm for 30 min so as to remove any silica from it. Supernatant was then stored in sterile glass vials under refrigeration. Quercetin (SD fine chemicals, New Delhi) was run as a standard along with the test extracts.

HPLC: HPLC was performed in gradient HPLC PU-2080 plus system (Jasco, Japan) incorporated with a Jasco PU 2080 Plus pump and a Jasco MD-2015 Plus UV detector. The column used was HiQ Sil C18, 4.6 × 250 mm, 5 micron. All the solvents (CDH, New Delhi) used were of HPLC grade. Injection volume was 20 µL in all cases with run time of 20 min and flow rate either 0.7 mL/min or 0.5mL/min, as required. Detection was made at wavelength 270 nm. Quercetin and gallic acid (SRL, Mumbai) were run as standards.

Results and Discussion

***Antibacterial activity of crude extracts*:** Results of DDA of *S. cumini* extracts are presented in table-1. ZOI obtained in case of methanol extract of *S. cumini* seeds at 110 mg/mL and its ethanol extract at 250 mg/mL against *E. coli* and *V. cholerae* were of identical diameter. This means that methanol extract is 2.27 times more potent than the ethanol extract against these organisms. *S. paratyphi* A proved to be most susceptible among all test organisms, against both the extracts. *A. hydrophila* and *S. pyogenes* were not inhibited by either of the extracts.

Results of broth dilution assay which led to the determination of IC_{50}, MIC, and total activity are put in table-2. MIC values of extracts against susceptible organisms ranged from 154-656 µg/mL. Both the extracts being active against gram-positive and gram-negative bacteria can be labeled as having a broad spectrum of antibacterial activity (Romich, 2004; Talaro, 2008). Gram- negative bacteria are generally much harder to find "hits" against, presumably as a result of their outer membrane in cell wall which greatly decreases permeability and because they are intrinsically resistant through the expression of membrane bound efflux pumps (Gibbons, 2008). Interestingly both the test extracts are effective against multiple gram-negative bacteria. Both extracts exerted a bacteriostatic action against susceptible organisms. However none of them was effective against *S. typhi* and drug-resistant strain of *S. paratyphi* A (R3).

Table 1. Disc diffusion assay of *S. cumini* seed extracts

Organism	**Diameter of zone of inhibition** (Mean±SD) (mm)			
	Metahnolic extract[a]	**Ethanolic extract**[a]	**Ofloxacin** (5 µg/disc)	**Streptomycin** (10 µg/disc)
A. hydrophila	0	0	-	18±2.0
E. coli	13±1.0	13±1.8	28±1.8	-
S. aureus	FI[b]	FI[b]	17±2.0	28±2.5
S. epidermidis	13±2.2	15±2.4	-	19±1.8
S. paratyphi A	20±2.6	28±2.2	-	22±1.0
S. flexineri	8±1.2	10±1.0	31±2.4	-
V. cholerae	12±1.8	12±1.0	27±1.4	20±2.2
P. oleoverans	12±1.8	14±1.0	26±1.4	-
S. pyogenes	0	0	15	20±2.2

[a]Methanolic and ethanolic extracts were tested at concentration of 110 and 250 mg/mL respectively;
[b]FI: Faint inhibition without clear zone; Negative controls did not cause any inhibition of growth.

Table 2 Broth dilution assay of *S. cumini* seed extracts

Extract	**Organism**	**IC_{50}**	**MIC**	**Total activity**	**Average total activity**
		(µg/mL)		(mL/g)	
Methanol extract of *S. cumini* seeds	*S. paratyphi* A	200	271	605.16	404.67
	P. oleoverans	400	656	250	
	S. epidermidis	<387	494	331.98	
	E. coli	~724	-	-	
	V. cholerae	<225	380	431.57	
Ethanol extract of *S. cumini* seeds	*S. paratyphi* A	<124	159	1823.89	1276.66
	P. oleoverans	<303	399	726.81	
	S. epidermidis	<140	154	1883.11	
	V. cholerae	<425	431	672.85	

Extract of *S. cumini* seeds prepared in ethyl acetate was earlier shown to be effective against both gram-positive and gram-negative bacteria (Sagrawat *et al.*, 2006). Extracts prepared from leaves of *S. cumini* also have previously been reported for their antibacterial action (Kaneria *et al.*, 2009). Ethanol extract of *S. cumini* bark was reported to inhibit various isolates of *V. cholerae* (Sharma *et al.*, 2009) with MIC values much higher (2.5-20 mg/mL) than those found in present work. This indicates seeds to have better anti-vibrio activity than bark of same plant.

Ethanol extract registered an average total activity 3.15 times higher than that of methanol extract. Highest total activity (1883.11 mL/g) was exerted by ethanolic extract of *S. cumini* seeds against *S. epidermidis.* It means that 1 g of it can be diluted to 1883 mL and still be able to inhibit *S. epidermidis*. Total activity is a measure of the amount of material extracted from a plant in relation to the MIC of the extract, fraction or isolated compound. Total activity is expressed in mL/g which is an indication of the degree to which active extracts of one gram can be diluted and still inhibit the

growth of the test organisms (Katerere and Eloff, 2005; Eloff, 2004). It is a way of measuring the potency of antibacterial constituents.

Phytochemical tests: Phytochemical tests of methanol and ethanol extracts of *S. cumini* seeds revealed the presence of phenols, alkaloids, and flavonoids. Kumar *et al*. (2009) had also shown methanol extract of *S. cumini* seeds to contain alkaloids and flavonoids. They additionally reported presence of saponins, tannins, and triterpenoids too. Presence of phenols in ethanol extract of *S. cumini* seeds was also reported by Sagrawat *et al* (2006). Antibacterial action of phenols, flavonoids, and alkaloids is well documented (Cowan, 1999). Alkaloids are able to intercalate with DNA, whereas phenols inhibit bacterial growth by substrate deprivation and membrane disruption. Total flavonoid content in methanol and ethanol extract was found to be 27.38 and 2.20 µg quercetin equivalent per mg of dry extract, respectively. Whereas total phenol content was determined to be 76 and 360 mg gallic acid equivalent per mg of dry extract, in methanol and ethanol extract respectively. High flavonoid content of the methanol extract may be responsible for its antibacterial activity. Flavonoids have been reported as effective antimicrobials against a wide array of microbes (Dixon *et al*., 1983). Their antibacterial activity may probably be due to their ability to complex with extracellular and soluble proteins, and the cell wall.

TLC (thin-layer chromatography) separation: Results of TLC fractionation of ethanol and methanol extracts (Figure 1) of *S. cumini* seeds are recorded in table-3. They yielded 3 and 7 fractions, respectively. One fraction (R_f 0.72) was common to both of them. Fraction-7 of methanol extract had R_f value near identical to that of quercetin.

Figure 1. Appearance of TLC plate under 365 nm after separation of methanolic extract of *S. cumini* seeds on it. Fraction 4 and 5 are visible as red spots.

Antibacterial activity of TLC fractions: All the fractions obtained through TLC of *S. cumini* methanol extract were tested against two bacteria by broth dilution assay (Table-4). Both fraction 1 and 7, were capable of inhibiting growth of gram-positive as well as gram-negative bacteria, indicating their activity spectrum to be broad. Both fractions recorded MIC values lesser than those of parent extract against same test organisms. With respect to MIC fraction 1 proved 3.12 and 2.11 times more potent than the crude preparation against *P. oleovorans* and *S. epiermidis*, respectively. Similarly fraction 7 was found to be 3.91 and 1.23 times more potent than the crude preparation against *P. oleovorans* and *S. epiermidis*, respectively. As compared to the crude extract, fraction 2 proved 3.45 times more potent against *P. oleovorans.* As only limited amount of

separated components could be recovered after running many TLC plates, MIC endpoints were not attained in all cases. Ofloxacin (HiComb™ strips) registered MIC of 0.15 µg/mL against both organisms.

HPLC (high performance liquid chromatography) analysis: Fractions separated through TLC were injected into HPLC system to achieve further separation/identification. Summary of HPLC experiments is presented in table-5. All chromatograms are not shown here. When fraction 7 was run in the mobile phase methanol:water (90:10), it recorded a retention time of 6.6 min, which is identical to that of quercetin run in same system. When fraction 7 was spiked with quercetin, it recorded an increased peak height at almost same retention time (6.8 min), indicating presence of quercetin in fraction 7 (Figure 2). To confirm the presence of quercetin further, fraction 7 was run in another solvent system of different composition- methanol:water (70:30), retention time found was 5.4 min (with an additional peak at 6.1 min), which was comparable to that of quercetin (5.6 min) in same solvent system. Further when the fraction was spiked with quercetin and gallic acid, an increase in height of the peak for quercetin was obsereved, indicating presence of quercetin in fraction 7. A separate peak of gallic acid at 3.17 min was observed.

Fraction 7 and quercetin were also run in acetonitile:water (90:10) + acetic acid, where they recorded peak at nearly identical retention time of 4.2 and 4.3 min, respectively. In addition to that, fraction 7 also showed six other peaks at different retention times. This demonstrated presence of few other components too, other than quercetin in fraction 7. Thus after running fraction 7 in three different mobile phases along with quercetin as a marker, similarity in their retention time values confirmed presence of quercetin in fraction 7. Results from TLC (Table-3) and HPLC, put together establish quercetin's presence in fraction 7.

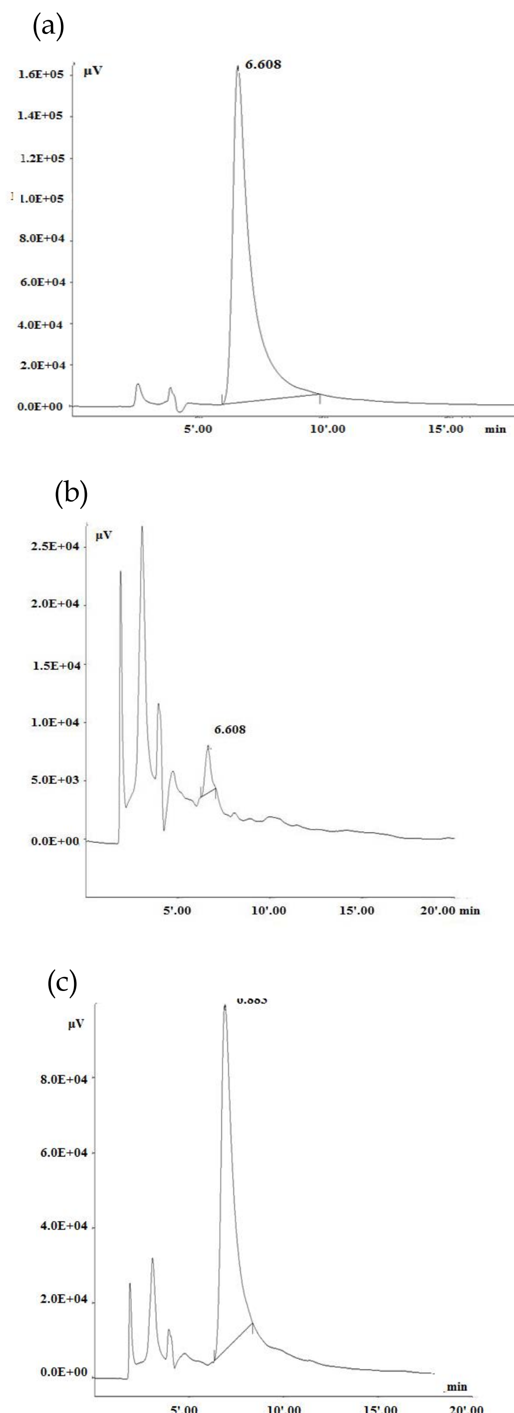

Figure 2. Indication of quercetin's presence in Fraction 7: (a) Quercetin; (b) Fraction 7; (c) Fraction 7 spiked with quercetin; mobile phase methanol:water (90:10)

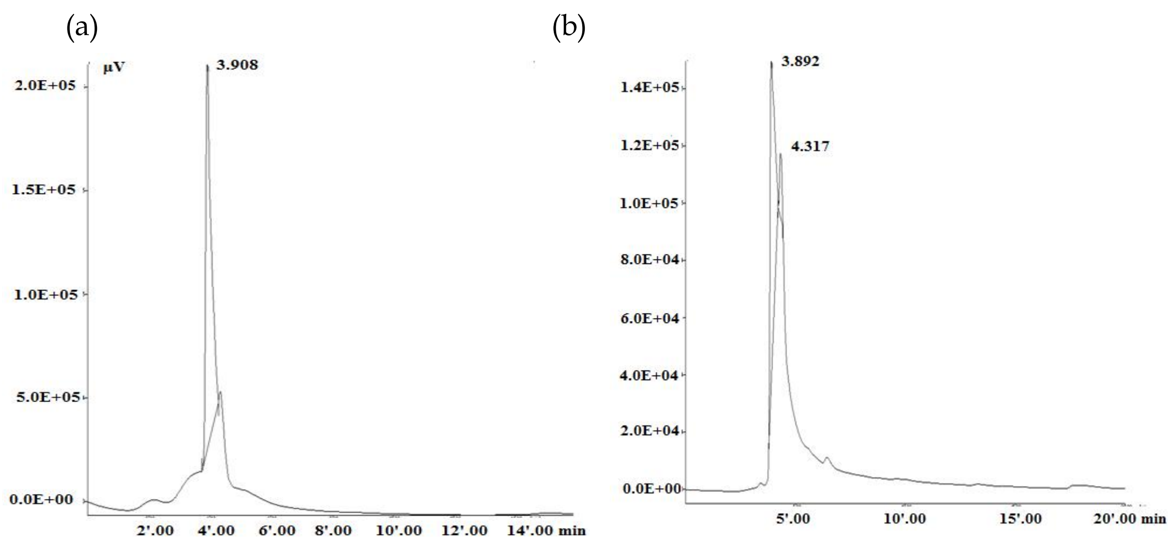

Figure 3. Indication of gallic acid's presence in Fraction 1: (a) Gallic acid; (b)Fraction 1; mobile phase acetonitrile:water (90:10) + acetic acid at flow rate 0.7 mL/min

Table 3. Results of TLC separation of *S. cumini* seed extracts

Extract	Fraction no.	Appearance of separated spot		R_f	hR_f
		365 nm	254 nm		
Methanol extract	1	Translucent	Not visible	0.12	12
	2	Translucent	Not visible	0.27	27
	3	Translucent	Not visible	0.38	38
	4	Red	Not visible	0.62	62
	5	Red	Not visible	0.72	72
	6	Not visible	Brown	0.78	78
	7[a]	Faint red	Brown	0.87	87
Ethanol extract	1	Transleucent	Not visible	0.65	65
	2	Red	Not visible	0.72	72
	3	Translucent	Not visible	0.91	91
Quercetin		Faint red	Brown	0.88	88

[a]In day light only fraction 7 of methanol extract was visible as a brown coloured component.

When fraction 1 and 2 were run in mobile phase- acetonitrile:water (90:10) + acetic acid + phosphoric acid, retention times of 3.91 and 3.94 respectively was recorded, which were nearly identical to that of gallic acid (3.89 min). When fraction 1 and gallic acid were run in acetonitrile:water (90:10) + acetic acid, they registered a retention time of 3.89 and 3.90 min, respectively (Figure 3). An additional peak was observed in fraction 1 with retention time of 4.31 min. The same experiment was repeated at a reduced flow rate (0.5 mL/min), there was an increase in retention times both for gallic acid and fraction 1. Thus, results of HPLC analysis indicated the possibility of presence of gallic acid in fraction 1 and 2.

Separation of fraction 6 in solvent system- acetonitrile : water (90:10) + acetic acid + phosphoric acid- generated 4 peaks with different retention times. Satisfactory separation for fractions other than those described above was not achieved in any of the solvent systems tried.

HPLC and TLC analysis confirmed presence of quercetin (3,3',4,5,7-pentahydroxy flavone) and gallic acid (3,4,5 trihydroxy benzoic acid) in different fractions of methanol extract of *S. cumini.* Both of these phytochemicals are widely distributed in the plant kingdom. Brito et al. (2007) revealed the presence of tannins (ellagitannin and gallotannin) and flavonoids in aqueous extract of *S. cumini* leaves through HPLC fingerprinting. Various bioactive derivatives of flavonoids (e.g., rutin and quercetin) and their glycosides were detected in ethanol extract of *S. cumini* seeds using HPLC (Sharma *et al.*, 2009). Earlier quercetin has been reported as a constituent of *S. cumini* seeds (Karthic *et al*., 2008; Bhatia and Bajaj, 1972). Quercetin is the most abundant of the flavonoids. It is also a building block for other flavonoids (http://www.phytochemicals.info /phytochemicals/quercetin.php). Earlier flavanones isloted from *S. cumini* seeds have been shown to act as α-amylase inhibitors (Karthic *et al.,* 2008). Mingyu and Zhuting (2008) reported quercetin in lotus leaves as a component that may be a potential antibacterial agent.

Gallic acid has earlier been known to be a constituent of *S. cumini* seeds (Khare, 2007; http://www.eudra.org/emea.html). It shows absorption peaks at 220 and 271 nm (in ethanol). Fraction 1 and 2 from the methanolic extract also registered absorption maxima at 271 nm (spectrum not shown). Gallic acid occur as a free molecule or as part of a tannin molecule. The mode of antimicrobial action of tannins seems to be based on their ability to inactivate microbial adhesins, enzymes and cell envelope transport proteins (Samy and Gopapa-krishnakone, 2008). Gallic acid is reported to have antifungal, antibacterial and antiviral properties. Gallic acid and tannin in extracts of *S. cumini* bark has been suggested to be responsible for its antibacterial activity (Sharma *et al.*, 2009). Their antimicrobial properties seemed to be associated with the hydrolysis of an ester linkage between gallic acid and polyols hydrolyzed after the ripening of many edible fruits.

Table 4. Summary of broth dilution assay of TLC fractions of *S. cumini* methanol extract

Sample	*P. oleovorans*		*S. epidermidis*	
	MIC (µg/ mL)	**IC $_{50}$** (µg/mL)	**MIC** (µg/mL)	**IC $_{50}$** (µg/ mL)
Crude MeOH extract of *S. cumini*	656	400	494	< 387
Fraction 1	210	> 186	234	-
Fraction 2	190	-	-	>327
Fraction 3	-	175	-	~175
Fraction 4	-	150	-	>150
Fraction 5	-	< 176	-	>176
Fraction 6	-	108	-	>160
Fraction 7	167.5	-	400	-

Table 5. Summary of HPLC results for methanol extract of *S. cumini* seeds

Expt. number	Sample	Mobile phase	Retention time (min)	Flow rate (mL/min)
1	Quercetin	Methanol:Water (90:10)	6.6	0.7
2	Fraction 7		6.6	
3	Fraction 7 spiked with quercetin		6.8	
4	Quercetin	Methanol:water (70:30)	5.6	
5	Fraction 7		Peak I-5.4 Peak II- 6.1	
6	Fraction7 + quercetin + gallic acid		Peak I- 3.17 Peak II- 5.7	
7	Quercetin (100 ppm)	AcN:water (90:10) + acetic acid (Run time: 25 min)	4.2	
8	Fraction 7		Peak I- 4.3 Peak II- 7.4 Peak III- 8.9 Peak IV-13.6 Peak V-14.7 Peak VI-18.5 Peak VII-21.2	
9	Gallic acid (50 ppm)	AcN:water (90:10) + acetic acid +OPA (pH 2)	3.9	
10	Fraction 1		3.9	
11	Fraction 2		3.9	
12	Gallic acid	AcN:water(90:10)+ acetic acid	3.9	
13	Fraction 1		Peak I- 3.9 Peak II- 4.3	
14	Gallic acid (100 ppm)	AcN:water(90:10)+ acetic acid	5.6	0.5
15	Fraction 1		6.0	
16	Fraction 6	AcN:water(90:10)+ acetic acid+ OPA (pH 2)	Peak I- 6.5 Peak II- 8.5 Peak III- 12.7 Peak IV- 15.5	0.7

This study showed the *S. cumini* seed extracts to possess broad spectrum antibacterial activity. Such findings provide preliminary scientific validation towards therapeutic applications of plant extracts. Further investigation on fractions separated but not identified is warranted using compatible techniques such as mass spectrometry, IR and NMR spectroscopy for structural studies.

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References

Bhatia, I., Bajaj, K. 1972. Tannins in black-plum (*Syzygium cumini* L.) seeds. *Biochem. J.*, **128(1)**:56.

Borgio, J.F., Thorat, P.K. and Lonkar, A.D. 2008. Antimycotic and antibacterial activities of *Gynandropis pentaphylla* DC

extracts and its phytochemical studies. *The Internet J. Microbiol*., **5(2).**

Brito, F., Lima, L., Ramos, M., Nakamura, M., Siani, A., Henriques, M. and Sampaio, A. 2007. Pharmacological study of anti-allergic activity of *Syzygium cumini* (L.) Skeels. *Braz. J. Med. Biol. Res*., **40**:105-115.

Chang, C., Yang, M., Wen, H. and Chern, J. 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food Drug Anal*., **10**:178-182.

Colegate, S., Molyneux, R. 2008. An introduction and overview. In *Bioactive Natural Products-Detection, Isolation and Structural Determination* (2nd edition), Eds. S Colegate and R Molyneux, Boca Raton: CRC Press, pp. 1-9.

Cowan, M. 1999. Plant products as antimicrobial agents. *Clin. Microbiol. Rev*., **12(4):**564-582.

Dixon, R.A., Dey, P.M. and Lamb, C.J. 1983. Phytoalexins: enzymology and molecular biology. *Adv. Enzymol*., **55**: 1-69.

Eloff, J.N. 2004. Quantifying the bioactivity of plant extracts during screening and bioassay-guided fractionation. *Phytomed.,* **11**: 370-371.

Gibbons, S. 2008. Phytochemicals for bacterial resistance – Strengths, weaknesses and opportunities. *Planta Medica*, **74**:594-602.

Jorgensen, J.H., Turnidge, J.D. 2003. Susceptibility test methods: Dilution and disk diffusion methods. In *Manual of Clinical Microbiology* (8th edition), Eds. PR Murray, New York, USA: ASM International, pp. 1108-1127.

Kañería, M., Baravalia, Y., Vaghasiya, Y. and Chanda, S. 2009. Determination of antibacterial and antioxidant potential of some medicinal plants from Saurashtra region, India. *Ind. J. Pharma. Sci*., **71(4):**406-412.

Karthic, K., Kirthiram, K., Sadasivam, S. and Thayumanavan, B. 2008. Identification of α amylase inhibitors from *Syzygium cumini* Linn seeds. *Ind. J. Exp. Biol*., **46**:677-680.

Katerere, D., Eloff, J. 2005. Antibacterial and antioxidant activity of *Sutherlandia frutescens* (Fabaceae), a reputed anti-HIV/AIDS phytomedicine. *Phytotherapy Res*., **19**:779-781. doi: 10.1002/ptr.1719.

Khare, C. 2007. *Indian Medicinal Plants: An Illustrated Dictionary*, Germany: Springer, pp.53-812.

Kothari V. 2011. *In vitro* antibacterial activity in seed extracts of *Phoenix sylvestris* Roxb (Palmae), and *Tricosanthes dioica* L (Cucurbitaceae). *Curr. Trends Biotechnol. Pharm*., **4(5):**993-997.

Kothari, V., Seshadri, S. 2010. In vitro antibacterial activity in seed extracts of *Manilkara zapota, Anona squamosa*, and *Tamarindus indica*. *Biol. Res*., **43**:165-168.

Kothari, V., Shah, A., Gupta, S., Punjabi, A. and Ranka, A. 2010. Revealing the antimicrobial potential of plants. *Int. J. Biosci. Technol.,* **3(1):**1-20.

Kothari, V.O., Punjabi A.B. and Gupta S.R. 2009. Optimization of microwave assisted extraction of *Anona squamosa* seeds. *The Icfai Univ. J. Life Sci*., **3(1)**: 55-60.

Kumar, A., Ilavarasan, R., Jayachandran, T., Decaraman, M. and Aravindhan, P. 2009. Phytochemical investigation on a tropical plant, *Syzygium cumini* from Kattup-palayam, Erode district, Tamilnadu, South India. *Pak. J. Nutri*., **8(1):**83-85.

Mingyu, L., Zhuting, X. 2008. Quercetin in a lotus leaves extract may be responsible for antibacterial activity. *Arch. Pharm. Res*., **31(5)**:640-644. doi: 10.1007/s12272-001-1206-5.

Nascimento, G., Locatelli, P., Freitas, C. and Silva, G. 2000. Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria. *Braz. J. Microbiol.,* **31**:247-256.

Romich, J. 2004. *Antimicrobial Terminology: Fundamentals of Pharmacology for Veterinary Technicians*, Delmar Cengage Learning, pp. 210-220.

Sagrawat, H., Mann, A. and Kharya, M. 2006. Pharmacological potential of *Eugenia jambolana*: A review. *Pharmacog. Mag.*, **2(6)**:96-105.

Samy, R., Gopalakrishnakone, P. 2008. Therapeutic potential of plants as antimicrobials for drug discovery**.** *Compl. Alt. Med*., **7(3)**: 283-294.

Satish, S., Mohana, D., Ranhavendra, M. and Raveesha, K. 2007. Antifungal activity of some plant extracts against important seed borne pathogens of *Aspergillus* sp. *J. Agri. Technol.*, **3(1):** 109-119.

Sharma, A., Patel, V. and Ramteke, P. 2009. Identification of vibrioicidal compounds from medicinal plants using chromate-graphic fingerprinting. *World J Microbiol. Biotechnol.*, **25:** 19-25. doi: 10.1007/s11274-008-9855-7.

Singleton, V, Rossi, J. 1965. Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *Am. J. Enology and Viticulture*, **16**: 144-58.

Stahl, E. 1969. *Thin-Layer Chromatography: A laboratory Handbook* (2nd ed.), Springer.

Talaro, K.P. 2008. *Foundations in Microbiology: Basic Principles* (6th edition), New York, USA: McGraw Hill, pp. 350.

Wadhwani, T., Desai, K., Patel, D., Lawani, D., Bahaley, P., Joshi, P. and Kothari, V. 2009. Effect of various solvents on bacterial growth in context of determining MIC of various anti-microbials. *The Internet J. Microbiol.*, **7(1).**

Wagner, H., Bladt, S. and Zgainski, E. 1983. *Plant Drug Analysis* (2nd edition), Berlin, Germany: Springer.