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In vitro antimicrobial activity of *Syzygium cumini* fruit peel and identification of anthocyanins

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The present investigation evaluated the antimicrobial potential of *Syzygium cumini* (SC) fruit peel against fourteen microorganisms and identified major anthocyanins. Methanol, ethyl acetate and dichloromethane extracts of shade dried SC fruit peel were screened for antimicrobial effects using disc diffusion and minimum inhibitory concentration (MIC) methods. Methanol extract showed maximum antimicrobial potency against all the test microorganisms with inhibition zone diameter (IZD) ranging from 9.35±0.49 to 19.25±0.35 mm and the lowest MIC of 0.18 mg/ml. Ethyl acetate and dichloromethane extracts moderately inhibited the growth of microorganisms. Separation of anthocyanins from crude methanol extract was carried out by thin layer chromatography (TLC) and the compounds were isolated with Liquid Chromatography-Electrospray-Ionization Tandem Mass Spectrometry (LC-ESI-MS/MS). The compounds identified were malvidin, petunidin and cyanidin. Our results indicate the antimicrobial efficacy of SC fruit peel and subsequent identification of anthocyanins. However, further studies are required to explore anthocyanins in order to develop novel drug candidates.

Key words: *Syzygium cumini,* antimicrobial, anthocyanins, Liquid Chromatography-Electrospray-Ionization Tandem Mass Spectrometry (LC-ESI-MS/MS), malvidin, petunidin, cyanidin.

INTRODUCTION

Plants have long been employed in treating and preventing various ailments. According to World Health Organization (WHO) reports, 80% of the world's population relies on traditional medicine (Anon, 2002). The adverse effects of synthetic antibiotics and multidrug resistant pathogens are the major cause of several diseases. The search for effective therapeutic alternatives led to the profound interest in medicinal plants and their pharmacological activities over the past two decades. Crude plant extracts or plant derived components are a reliable source to discover new antimicrobial agents (Hemaiswarya et al., 2008).

The tropical fruit, *Syzygium cumini* (L.), widely known as black plum belonging to the family of Myrtaceae is an indigenous plant grown in different parts of India (Kirtikar and Basu, 1975). The plant was demonstrated to contain substantial hypoglycemic, anti-inflammatory and anticancerous activities (Muruganandhan et al., 2001; Afify et al., 2011). Antibacterial effects of *S. cumini* leaves, fruits and seeds (Migliato et al., 2010; Kothari et al., 2011)

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have been investigated. Vibriocidal activity of SC bark has also been reported (Sharma et al., 2009).

Anthocyanins are water-soluble pigments, omnipresent in higher plants imparting colour to the plants (Harborne, 1988). Flowers and fruits are attractive owing to the presence of anthocyanin pigments. Berries, grapes, plums, black currants possess different groups of anthocyanins (Riihinen et al., 2008), and are commonly included in diet. The predominantly occurring anthocyanin compounds are cyanidin, petunidin, malvidin, delphinidin and peonidin, although pelargonidin (Zhang et al., 2011) occurred in some plants. Several workers proposed the positive therapeutic effects of anthocyanins in treating diabetic retinopathy, improving visual acuity (Boniface and Robert, 1996; Sole et al., 1984; Mazza and Miniati, 1993), lowering the risk of cardiovascular diseases (Wallace, 2011) and inhibiting the growth of cancer cells (Malik et al., 2003). Previous studies investigated antimicrobial properties of anthocyanins (Jimenez et al., 2011).

Few authors advocated that fruit peels possess remarkably higher amount of bioactive constituents than fruits or fruit pulp (Lim et al., 2006). Antioxidant activities of anthocyanins from SC fruit peel was evaluated (Veigas et al., 2007). To the best of our knowledge reports on antimicrobial activity of SC fruit peel is not reported. Therefore, the objective of our investigation was to determine the antimicrobial potential of SC fruit peel extracts against human pathogenic microorganisms. Furthermore, fruit peel was investigated for the presence of anthocyanins by using thin layer chromatography (TLC) (Stahl, 1958). We partially purified the methanolic TLC fraction, separated and identified major compounds using Liquid Chromatography-Electrospray Ionization-Tandem Mass Spectrometry (LC-ESI-MS/MS) method.

MATERIALS AND METHODS

Chemicals and reagents

Analytical grade chemicals and reagents purchased from Hi-media, Sigma and Merck were used throughout the study.

Plant source

Ripened fruits of *S. cumini* were collected from Sirumalai hills of Dindigul district of Tamil Nadu India, from a single tree during the months of August and September, 2011. The plant was authenticated at Department of Biotechnology, Anna University, Coimbatore and voucher specimen deposited (Voucher number: AUCBE-BT-0028-2011).

Preparation of SC fruit peel extracts

Decayed and damaged fruits were removed from the collection and fruits washed with sterile distilled water for removing dust. Subsequently, fruit skin was peeled, allowed to dry under room temperature at 31°C until complete disappearance of moisture. Dried fruit peel was ground to fine powder using Thomas Wiley Machine (Model 5 USA). A 100 g of fine powdered fruit peel was consecutively extracted in 1.5 L dichloromethane → ethyl acetate → methanol using Soxhlet apparatus at 39, 77, and 65°C each for 3 h under specific boiling temperature of the solvents (Rossenthaler, 1993). Solvents were evaporated using rotary evaporator at respective solvent boiling temperature and crude extract dried under laminar air flow and powders packed separately stored at room temperature for further investigations.

Determination of antimicrobial susceptibility

Microbial cultures used

SC fruit peel were screened for *in vitro* antimicrobial activities against fourteen test microorganisms, consisting of five Gram-positive, seven Gram-negative bacterial strains and two fungal strains. The freeze-dried microbial strains of *Staphylococcus aureus* (ATCC 29213), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus vulgaris* (ATCC 13315), *Serratia marcescens* (ATCC 13880), *Bacillus subtilis* (ATCC 6633), *Bacillus cereus* (ATCC 11778), *Salmonella typhimurium* (ATCC 23564), *Enterobacter aerogenes* (ATCC 13048), *Candida albicans* (ATCC 10231), and *Aspergillus niger* (ATCC 16404), were procured from National Chemical Laboratory, Pune, India. Methicillin resistant *Staphylococcus aureus* (MRSA) and *Salmonella paratyphi* B (SPB) were two clinical isolates obtained from Prof. A. Lebel, Department of Plant Biology and Biotechnology, Loyola College, Chennai (India). The microbial cultures were revived in Luria-Bertani (LB) broth, and incubated for 18 h at 37°C. The stock was maintained in Mueller-Hinton agar (MHA) slant at 4°C.

Preparation of inoculum

Microbial cells from the stock culture were transferred to Muller-Hinton broth and Sabouraud dextrose broth for bacteria and fungi, respectively. The turbidity of the suspension was adjusted to the McFarland 0.5 turbidity standard (approximately equal to 1×10^8 cells/ml). Following incubation of the cultures for 24 h at 37°C for bacteria and at 25°C for fungi active growth of the cells were accomplished.

Test concentration of SC fruit peel

The crude SC fruit peel extract from methanol, ethyl acetate, dichloromethane were dissolved in dimethyl sulphoxide (DMSO) and each disc was loaded with 25 µl of the extract at 5 and 10 mg concentrations, respectively. The diameter of the sterile disc was 6 mm (Hi-Media, Mumbai, India).

Disk diffusion method

By employing the Kirby-Bauer method, the antimicrobial activity was performed (Bauer et al., 1996). Sterile petriplates containing molten MHA were prepared before commencement of the assay and of 0.1 ml of the inoculum suspension was uniformly spread onto surface of MHA. Using DMSO the crude fruit peel extracts of methanol, ethyl acetate and dichloromethane were dissolved and placed on a 6 mm diameter sterile disc. Each plate contained seven paper discs wherein six discs contained the SC fruit peel extracts in 5 and 10

mg concentrations in each disc and one disc in the centre served as a positive reference standard with 10 µg/ml ampicillin for bacteria and nystatin for fungi. DMSO was used as negative control (Wadhwani et al., 2009). The test was carried out in triplicates and mean values calculated. The plates were incubated for 24 h at 37°C allowing maximum proliferation of the microorganisms. The zones of inhibitions were measured using a Hi-Media Zone scale. Difference between disc diameter (6 mm) and diameters of inhibition was taken to calculate the inhibition zones (Hewitt and Vincent, 1989).

Determination of minimum inhibitory concentration (MIC) of SC fruit peel extracts

MIC of SC fruit peel extracts against the microorganims was assessed using macro broth dilution method (Washington et al., 2006). The crude methanol extract was distributed in 8 test tubes at various concentrations ranging from 0.1 to 1.0 mg/ml. Overnight microbial cultures (100 µl, 10^5 colony forming units) were added to each test tube. Broth with bacterial suspension served as positive control (9th test tube), while blank Mueller Hinton broth solution with SC fruit peel extract (10th test tube) was the negative control. Ampicillin and nystatin were used as controls during the MIC determination. Finally, the tubes were made up to 2 ml of Mueller Hinton broth and incubated for 24 h at 37°C. The experiment was conducted in triplicates. Similar procedure was followed for determining MIC of ethyl acetate and dichloromethane extracts of the fruit peel. The lowest concentrations of the extract completely inhibiting the visual bacterial growth were recorded as MIC values.

Separation of anthocyanin pigments

Chromatographic fractionation of methanol extract of the fruit peel was carried out using precoated (20 × 20 cm) TLC plate made of silica gel 60 F254 (Merck, 0.25 mm thickness). Ten micrograms of the methanolic extract was spotted onto the silica gel plate. Development of chromatogram was accomplished with three types of solvents systems Butanol:Acetic acid:Water (BAW), Acetone:Formic acid:water (AFW), Methanol:Hydrochloric acid:Water (MHW) in ratios of 4:1:1, 4:1:2, and 4:1:3, respectively at room temperature. The chromatograms were allowed to dry after separation. Retardation factor (Rf value) of each spot was calculated based on ratio of distance traveled by the solute to the distance traveled by the solvent (Sherma, 1991). The separated spots on the TLC plate were observed under UV-Visible spectrophotometer between 450 and 650 nm ranges. Prominent bands formed were recovered by scrapping and eluting with 100% methanol and 0.1% HCl. Using standard reference table (Harborne, 1984), the values were compared.

LC-ESI-MS/MS anthocyanin analysis

TLC eluted fractions (R_f values- 0.67, 0.57, 0.52) were dissolved with methanol, filtered with 0.22 µm nylon membrane filter. Using LC-ESI-MS/MS method, individual anthocyanin compounds from these fractions were achieved. The chromatographic system comprised of an Agilent 1100 system coupled with a G1311A quaternary pump, G1367A autosampler, a G1316A thermostatic column control and equipped with an electrospray ionization source. The separation of compounds was performed on a 75 × 4.6 mm i.d. Symmetry C_{18} column, with a particle size of 5 micron (Waters, MA, USA). Mobile phase consisted of 5 mM ammonium acetate with 0.1% formic acid (Solvent A) and 0.1% formic acid in acetonitrile (Solvent B) by applying the following gradient: 0 to 2 min: 80% A, 20% B, 2.50 to 12 min: 100% in B, 12.50 to 15 min: 80% in A and 20% in B. The solvents were filtered prior to use. Temperature of the column was 55°C and flow rate was set to 400 µl. The sample injection volume was 25 µl. Anthocyanins were recorded between a wavelength range of 350 and 655 nm.

LC-ESI-MS/MS analyses were performed using 3200 Q TRAP AB (Sciex Instruments, Singapore) coupled with Linear Ion Trap Quadrupole Mass Spectrometer and an Electrospray Ionization Source (ESI). Chromatographic conditions were maintained as described earlier. The MS/MS parameters included a declustering potential, adjusted to obtain maximum sensitivity. Analyst 1.4.1 software generated the mass data. The linear ion trap MS was operated in a positive ionization mode with curtain gas (N_2) set to 15 psi, heater, nebulizer gas were set to 10 psi and ambient source temperature was maintained.

RESULTS

Antimicrobial activity

The crude methanol, ethyl acetate and dichloromethane extracts of SC fruit peel exhibited significant antimicrobial activity against the fourteen tested microorganisms assessed by agar well diffusion are depicted in Table 1. Methanolic extract presented strong antimicrobial effect with highest mean inhibition zone diameters (IZD) ranging from 9.35±0.49 to 19.20±0.35 mm. *E. faecalis* was the most sensitive bacterial strain against methanolic extract of the fruit peel having maximum IZD of 19.20±0.35 mm. Moderate antimicrobial effect was observed in *P. vulgaris* and *A. niger* (9.35±0.49 and 12.65±0.21 mm IZD). Ethyl acetate extract of SC fruit peel displayed considerable IZD of 12.45±0.35 mm against *E. faecalis*. Minimal IZD was found against *S. paratyphi* B and *E. coli* (6.17±0.14 and 6.80±0.14 mm). *C. albicans* was inhibited by ethyl acetate fraction forming an IZD of 8.65±0.21 mm. The extract was inactive against *P. aeruginosa, B. subtilis* and *A. niger*. Dichloromethane extract of the fruit peel showed good antimicrobial activity against Gram-positive *E. faecalis and S. aureus* with IZD of 11.60±0.21 and 10.35±0.49 mm, respectively whereas the Gram-negative bacteria displayed minimal susceptibility (6.40±0.28 to 10.35±0.21 mm IZD). The microorganisms resistant against the extract were *B. subtilis,* MRSA*, E. aerogenes* and *A. niger*. There was no activity observed in the negative control (DMSO) against the test microorganisms.

In general, the three SC fruit peel extracts showed substantial antimicrobial activity against both Gram-positive (*B. cereus, S. aureus*), Gram-negative (*E. coli, P. vulagris, S. typhimurium, S. marcescens, S. paratyphi* B) and the fungal strain *C. albicans.* Methanolic extract exhibited higher degree of antimicrobial activity when compared with ethyl acetate and dichloromethane extracts.

Table 2 presents the MICs obtained from the three SC fruit peel extracts with values ranging from 0.18 to 1.0 mg/ml. The methanolic extract strongly inhibited the

Table 1. Antimicrobial effects of SC fruit peel extracts on fourteen test microorganisms.

Tested microorganisms	Mean inhibition zone diameter (mm) with standard deviation						Ampicillin (10 µg/disc)	Nystatin (10 µg/disc)
Gram- positive bacteria	M		EA		DCM			
	5 mg	10 mg	5 mg	10 mg	5 mg	10 mg		
Enterococcus faecalis	17.20±0.28	19.25±0.35	11.2±0.42	12.45±0.35	10.35±0.35	11.6±0.21	40.20±0.28	-
Bacillus subtilis	12.35±0.21	15.20±0.28	NIZ	NIZ	NIZ	NIZ	40.25±0.21	-
Methycillin resistant *Staphylococcus aureus* (MRSA)	12.20±0.28	13.15±0.21	8.85±0.07	10.3±0.42	NIZ	NIZ	11.25±0.35	-
Bacillus cereus	11.20±0.28	13.25±0.21	7.80±0.14	9.15±0.07	9.75±0.21	10.15±0.21	40±0.0	-
Staphylococcus aureus	10.30±0.28	12.50±0.28	10±0.42	10.25±0.07	10.1±0.28	10.35±0.49	40.10±0.14	-
Gram -negative bacteria								
Salmonella typhimurium	13.50±0.28	14.35±0.49	7.70±0.28	8.10±0.14	6.00±0.14	6.40±0.28	27.15±0.21	-
Escherichia coli	11.15±0.21	13.20±0.28	6.25±0.21	6.80±0.14	6.90±0.28	7.20±0.21	40.50±0.07	-
Pseudomonas aeruginosa	10.15±0.21	12.30±0.42	NIZ	NIZ	9.20±0.28	10.35±0.21	-	-
Proteus vulgaris	8.05±0.07	9.35±0.49	9.1±0.14	11.30±0.14	6.35±0.49	7.20±0.14	40.9±0.56	-
Serratia marcescens	10.20±0.07	13.30±0.42	8.15±0.21	9.5±0.07	7.75±0.07	8.25±0.49	30.45±0.21	-
Enterobacter aerogenes	11.40±0.21	14.30±0.14	7.05±0.21	10.6±0.35	NIZ	NIZ	12.55±0.21	-
Salmonella paratyphi B (SPB)	10.20±0.28	13.05±0.07	6.17±0.14	6.20±0.21	6.40±0.07	6.85±0.07	21.30±0.42	-
Fungi								
Candida albicans	10.05±0.07	10.55±0.07	7.90±0.28	8.65±0.21	6.70±0.14	6.15±0.28	-	40.5±0.28
Aspergillus niger	10.55±0.35	12.65±0.21	NIZ	NIZ	NIZ	NIZ	-	-

M: Methanol extract; EA: Ethyl acetate extract; DCM: Dichloromethane extract; NIZ: No inhibition Zone; -: No activity.

growth of both Gram-positive and Gram-negative strains. *E. faecalis* (least MIC value of 0.18 mg/ml) was highly sensitive to the action of methanolic fruit peel extract followed by *B. subtilis*, *S. typhimurium, E. coli* and other bacteria. Among the two fungal species tested, *C. albicans* was susceptible to all the three extracts. Ethyl acetate and dichloromethane extracts remained inactive for *A. niger,* while methanol extract presented an MIC value of 0.50 mg/ml. The inhibitory effect of ethyl acetate extract against Gram-positive (0.48 to 0.75 mg/ml) and Gram-negative test micro-organisms (0.55 to 0.92 mg/ml) was moderate. Dichloromethane fraction of SC fruit peel indicated low inhibitory activity against the tested human pathogens with MIC values from 0.52 mg/ml (*E. faecalis*) to 1.0 mg/ml (*E. coli*). No activity was found against *E. aerogenes* and MRSA.

TLC analysis of anthocyanins

TLC fractionation of the methanol extract revealed the presence of three spots S1, S2 and S3. The rate of migration of anthocyanin pigments is estimated based on retardation factor (R_f). The R_f values of each spot were calculated (Table 3). In BAW solvent, the R_f values are 0.67 (S1), 0.57 (S2), and 0.52 (S3).

Anthocyanin identification using LC-ESI-MS/MS

Specific anthocyanins detected by LC-ESI-MS/MS analyses revealed three distinct peaks based on their retention times and mass to charge ratio (Table 4). Retention times of malvidin, cyanidin and petunidin were nearly similar to earlier reported

Table 2. MIC values of SC fruit peel extracts against fourteen test microorganisms.

Test microorganism	MIC (mg/ml)			Ampicillin (µg/ml)	Nystatin (µg/ml)
	M	EA	DCM		
Gram-positive bacteria					
Enterococcus faecalis	0.18	0.48	0.52	1.7	-
Bacillus subtilis	0.20	0.67	NMIC	3.7	-
Bacillus cereus	0.27	0.71	0.46	3.5	-
Staphylococcus aureus	0.45	0.64	0.61	1.4	-
Methycillin resistant *Staphylococcus aureus (MRSA)*	0.30	0.53	NMIC	12.7	-
Gram-negative bacteria					
Salmonella typhimurium	0.21	0.69	0.65	5.1	-
Escherichia coli	0.22	0.92	1.00	5.6	-
Pseudomonas aeruginosa	0.46	0.77	0.65	8	-
Proteus vulgaris	0.70	0.55	0.68	11.4	-
Serratia marcescens	0.24	NMIC	0.77	6.1	-
Enterobacter aerogenes	0.36	0.56	NMIC	10.1	-
Salmonella typhi B (SPB)	0.28	0.88	0.71	6	-
Fungi					
Candida albicans	0.50	0.83	0.66	-	1.5
Aspergillus niger	0.47	NMIC	NMIC	-	1.6

M: Methanol extract; EA: Ethyl acetate extract; DCM: Dichloromethane extract; NMIC: No minimum Inhibitory concentration; -: No activity.

Table 3. TLC profile of anthocyanins from SC fruit peel.

Spot	Colour of the identified pigments	R_f Value			Absorption spectra (nm)
		BAW	AWH	MFW	
S1	Dark purple	0.67	0.30	0.27	515
S2	Purple	0.56	0.41	0.34	524
S3	Purple	0.52	0.46	0.30	525

BAW: Butanol, acetic acid, water; AWH: actone, formic acid, water; MFW: methanol, formic acid, water.

data (Li et al., 2009). MS/MS analysis confirmed the structure of anthocyanins. The fragmentation of molecular ions at m/z=655 and its respective product ions are m/z=493/331 (Figure 1) assigned as aglycon malvidin 3,5 diglucoside. Similarly, the MS^2 spectra of ions at m/z=641 tentatively corresponds to petunidin 3,7 diglu-coside (Figure 2). The corresponding molecular product ions have an m/z of 479/317. The fragmentation pattern at m/z 611 resulting in product ions at m/z 449/287 may be attributed to cyanidin 3, 5 diglucoside (Figure 3).

DISCUSSION

The results obtained indicate the antimicrobial efficacy of SC fruit peel extracts. Previous studies have reported antimicrobial effects of various fruit peels (Al-Zoreky, 2009; Chanda et al., 2010). However, no reports on antimicrobial effect of SC fruit peel are available. Among the three different extracts that were investigated, methanol showed the highest activity against all the tested microorganisms. In general, Gram-positive bacteria were more susceptible than Gram-negative bacteria, due to the cell wall differences between these microorganisms (Suffredini et al., 2006). Contrarily, in our investigations, equal competence was observed against both Gram-negative and Gram-positive bacteria. According to a previous study (Loguercio et al., 2005), the hydro-alcoholic extracts of SC leaves inhibited growth of Gram-positive and Gram-negative bacteria. Phenolic compounds greatly influence the antimicrobial properties of plant extracts in negating the human pathogens (Cavanagh et al., 2003). Therefore, SC fruit peel may be a source of compounds possessing broad spectrum of

Table 4. Anthocyanins from SC fruit peel using LC-ESI-MS/MS.

Compound	Retention time (min)	ESI-[Glu-Glu]+		
		M+ (m/z)	[M+-Glu]+ (m/z)	[M+GluGlu]+ (m/z)
Malvidin 3,5 diglucoside	31.0	655	493	331
Petunidin 3,7 diglucoside	27.5	641	479	317
Cyanidin 3,5 diglucoside	25.2	611	449	287

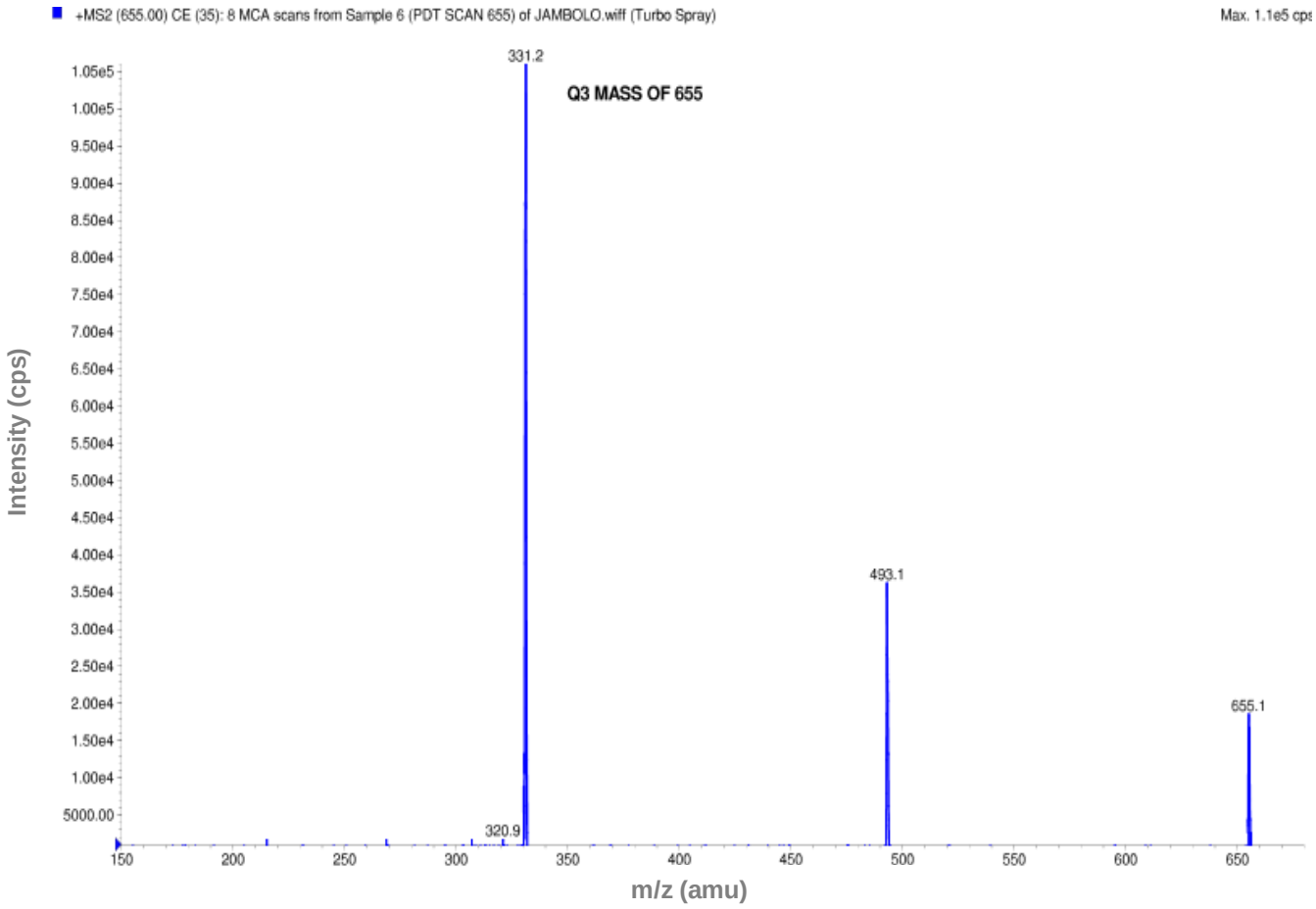

Figure 1. ESI-MS/MS spectrum of product ion at m/z 655 (Malvidin 3,5 digucoside).

antimicrobial activity.

Although, numerous reports demonstrated the inhibitory effect of SC leaves, seeds, bark extracts (Bag et al., 2012; Ugbabe et al., 2010), antimicrobial activity of SC fruits were limited. In this context, the SC fruit peel was found to have appreciable antimicrobial activity comparable to other parts of SC. Maximum IZD was found against *E. faecalis* in methanol extract of SC fruit peel and this is in complete agreement with a study on SC leaves (Mohamed et al., 2013). The seed extract of SC was inactive against *P. vulgaris*, (Duraipandiyan et al., 2006), however, moderate inhibition was found in the fruit peel. Consistent with methanolic SC seed extracts (Kothari.et al., 2011), SC fruit peel was observed to be active against *E. coli*. Bark extracts of SC was inactive against *B. subtilis*, while moderate inhibition (<10 mm IZD) was observed against *S. aureus, S. paratyphi and E. coli* (Iqbal and Arina, 2001). Interestingly, our results have shown considerable antimicrobial effect on *S. aureus*, *S. paratyphi*, and *E. coli* with IZD>10 mm. Ethanol extract of SC fruit was more active against Gram-positive *S. aureus* than Gram-negative *P. aeruginosa* (Migliato et al., 2010), which is implied in our MIC values of ethyl acetate extracts. Our data of methanol and ethyl

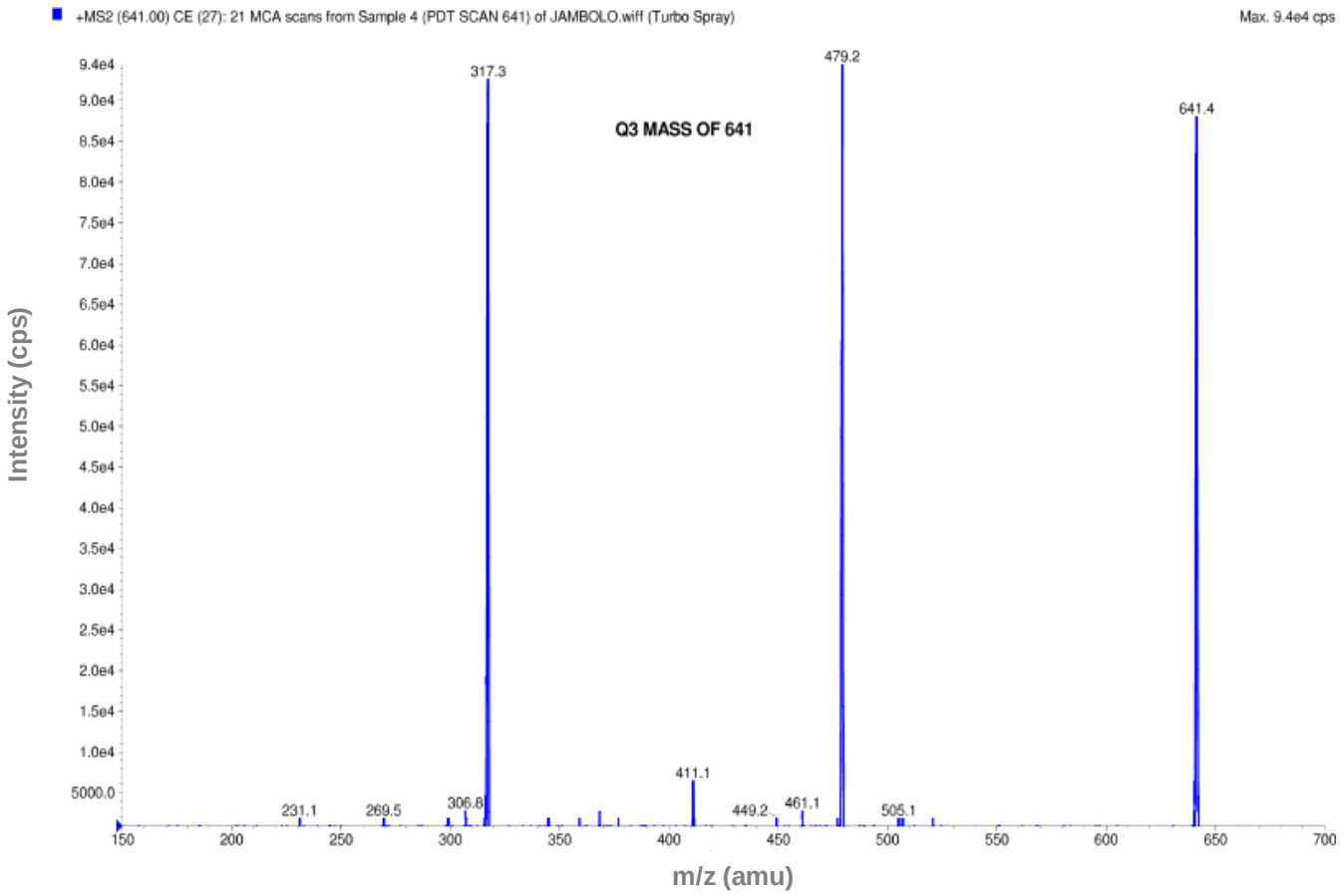

Figure 2. ESI-MS/MS spectrum of product ion at m/z 641 (Petunidin 3,7 digucoside).

acetate extracts show inhibitory activity against *S. typhimurium* and *E. aerogenes* corroborating the results of SC leaves (Kaneria et al., 2009). *C. albicans* is resistant to SC bark (Iqbal and Arina, 2001) while seeds of the fruit possess higher antifungal activity (Höfling et al., 2010). In the present study, *C. albicans* was found to be susceptible to the methanol, ethyl acetate and dicholoromethane extracts of SC fruit peel. Our findings showed antifungal effect of methanol extract against *A. niger* while previous work on SC seeds lacked activity (Duraipandiyan and Ignacimuthu, 2011). SC leaves, wood and bark assessed by disc diffusion and MIC were more active against *S. marcescens* (Aly et al., 2013), compared to our results of the SC fruit peel. In concurrence with disk diffusion and MIC results of antibacterial effect on SC leaves (Mohanty and Cock, 2009), *B. cereus* and *S.aureus* were moderately susceptible in our findings. Varied levels of antimicrobial activity of plant extracts may be attributed to the geographical location of the plant or the method of extraction (Viljakainen et al., 2002). Based on the presence of secondary metabolites in different parts of the plant, the degree of antimicrobial potency differs.

Plant extracts and their derived compounds have remarkable bioactivity contributing to the development of new drug leads (Carvalho and Ferrreira, 2001). The R_f values obtained in our study corroborated with the findings of various authors (Lestario et al., 2004). In a study conducted on a Brazilian variety of SC fruit peel (Santos et al., 2013), five different anthocyanins were identified, however in our TLC separation of South Indian SC fruit peel, three anthocyanins were present. Generally, intensity of anthocyanin pigments in all plants depends on the variety, regional source and climatic conditions during the growth period (Lago et al., 2004). The efficient combination of chromatography with LC-ESI-MS/MS enables fast and promising characterization of anthocyanins in several plants (Huang et al., 2012). The LC-ESI-MS/MS data is used to clearly identify the compounds present in the fruit peel. Major anthocyanins identified were cyanidin 3, 5 diglucoside, petunidin 3, 7 diglucoside and malvidin 3, 5 diglucoside. Our results are in agreement with previous reports indicating the presence of diglucosides of malvidin, petunidin and cyanidin (Benherlal and Arumughan, 2007; de Brito et al., 2007). Various workers studied the whole fruits of *S.*

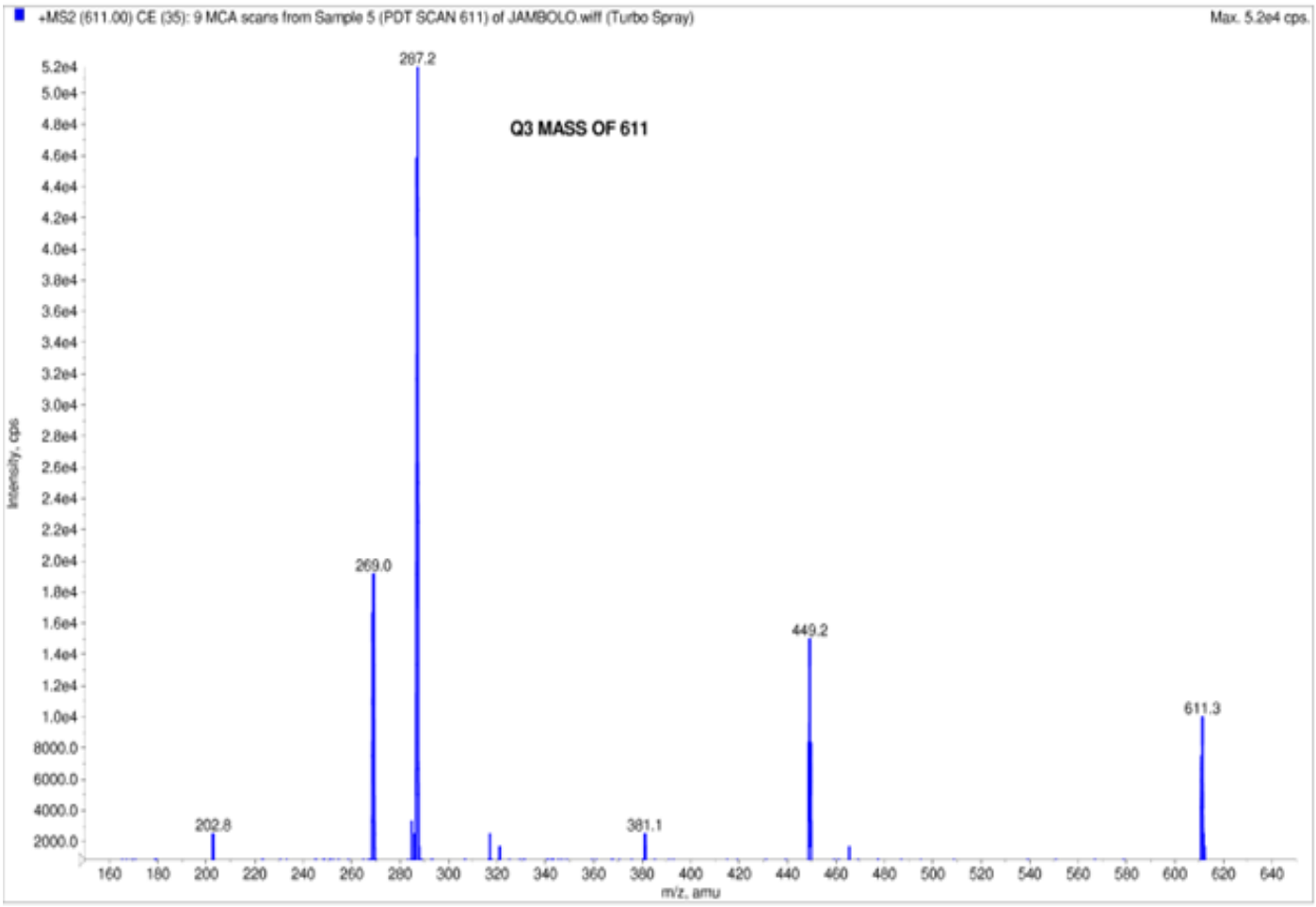

Figure 3. ESI-MS/MS spectrum of product ion at m/z 611 (Cyanidin 3,5 digucoside).

cumini for its potential bioactive anthocyanin compounds (Nuengchamnong and Ingkaninan, 2009; Arun et al., 2011). In the present study, the fruit peel was investigated for the detection and listing of anthocyanin compounds.

Conclusions

Conclusively, our study suggests SC fruit peel as a potential source of antimicrobial agents due to the presence of bioactive compounds. All the solvent extracts highlight the effectiveness of fruit peel, with methanol extract exhibiting the highest inhibitory activity as compared to ethyl acetate and dichloromethane. Fractionation performed by TLC and LC-ESI-MS/MS results confirmed the existence of major anthocyanins. However, further *in vivo* studies are required to develop potential drug candidates for the antimicrobial activity.

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