Research Paper

Evaluation of *in vitro* Antibacterial and Antioxidant Activity of Aqueous Extracts of *Olax psittacorum*

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Present study aimed to evaluate the phytoconstituents of the aqueous extracts of stem and fruits of *Olax psittacorum* as well as the free radical scavenging and the antibacterial activity of the extracts. Free radical scavenging activities were evaluated through 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay, (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)di-ammonium salt radical scavenging assay, phosphomolybdate radical scavenging assay, hydrogen peroxide assay and reducing power assay using ascorbic acid as the standard. Well diffusion method was adopted for antibacterial activity evaluation of the aqueous extract of fruits and stem against *Staphylococcus aureus*, *Bacillus stereothermophillus*, *Pseudomonas aeruginosa*, *Vibrio cholera*, *Escherichia coli* and *Acinetobacter baumanii*. Phytochemical screening showed presence of tannins, saponins, steroids and terpenoids in the aqueous extracts of both stem and fruits, while glycosides, flavonoids, carbohydrates and reducing sugars were found in the aqueous extract of fruit only. Total phenolic, tannin and saponin contents were found to be higher in the aqueous extract of fruits. Total flavonoid content of aqueous extract of fruit was found to be 279.33 mg quercetin equivalent per gram of dry extract. Variations in phytoconstituents and *in vitro* experimental data obtained through antioxidant as well as antimicrobial assay methods indicated the existence of significant difference ($p<0.05$) between the antioxidant potency and zones of inhibition of *Staphylococcus aureus*, *Vibrio cholera*, *Acinetobacter baumanii*, which aqueous extract of stem failed to show at a dose of 100 mg/ml clearly demonstrated the superiority of the aqueous extract of fruits.

Key words: Antioxidant, antibacterial, flavonoid, fruit, stem

From ancient times in India many plants are being used for treating different sicknesses[1,2]. Still there remain gaps in terms of deep understanding and thus remains the extent of filling these gaps with the information about the medicinal properties of different species. It is a very interesting fact that even different parts of a plant show different level of activities due to different phytoingredients in different concentrations. Herbal remedies possess an edge over their synthetic counter parts in being cost-effectual and safe with minimal side effects. In the scenario of increasing resistance of bacteria towards existing antibacterial drugs, amelioration through treatment with herbal antimicrobial agents could become an important option[3]. Antioxidants have the property to stabilize free radicals leading to cytoprotection from the deleterious effects of free radicals. Antioxidant phytoingredients such as phenolics, flavonoids, tannins, saponins and proanthocyanidines are commonly present in numerous medicinal plants, vegetables and fruits indicating their antioxidant potential[4]. In the form of crude extract or as isolated compounds there from, these can be used to reduce oxidative stress[5]. *Olax psittacorum* (Willd.)Vahl. belonging to the family Olacaceae, order Scrophulariales, is a flowering plant found throughout the topical area of the world. All components of this plant after methanol extraction have shown the presence of a saponin (olaxoside), which when given orally to mice exhibited antiswelling properties and decreased oedema caused by carrageenan. Olaxoside additionally possessed laxative action[5]. Another report demonstrated the total absence of saponin

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and low contents of flavonoids, triterpines and proanthocianidines after phytochemical screening of methanol extract of stem and leaf, but had shown potential antiviral activity against polio virus[6]. The leaf of *O. psittacorum* also showed body-protecting chemical activity[7].

Aim of the present study was to evaluate and compare the phytoconstituents both qualitatively and quantitatively, the free radical scavenging activity and the antibacterial properties of the aqueous extract of stem (SAE) and fruit (FAE) of *O. psittacorum*.

MATERIALS AND METHODS

Fruits and stem of *O. psittacorum* was procured from Andharua, Bhubaneswar, Odisha during summer season, in the second week of May, 2015. The plant was identified and authenticated in the Regional Plant Resource Centre (RPRC), Bhubaneswar (Authentication Field Number, RMOP-1). All the chemicals and solvents utilized in the study were procured from Merck Specialties Private Limited.

Extraction:

Fresh stem and fruits of *O. psittacorum* were washed in tap water to remove any contaminants[8] and then dried to get rid of water from the surface of the stem and fruits. The stem was then cut into small pieces and dried under shade at room temperature for 30 d, crushed and ground to coarse powder[9]. Fruits on the other hand were crushed directly. Course powder of dried stem and crushed fruit of *O. psittacorum* were subjected to maceration with distilled water for 72 h each. The extract was then filtered and lyophilized to obtain SAE and FAE. Percent yield was calculated utilizing the following Eqn. 1, yield (%) = (weight of extract g)/(weight of plant material set for maceration g)×100.

Phytochemical screening:

SAE and FAE were subjected to various chemical procedures for the detection of alkaloids[10], glycosides[11], steroids and triterpenoids[10,11], flavonoids[12,13], proteins[11], carbohydrates and reducing sugars[11], tannins and phenolic compounds[13] and saponins[12] present in them, utilizing standard methods.

Quantitative estimation of total phenolic content (TPC):

The amount of TPC was determined using the Folin-Ciocalteu's reagent (FCR) taking gallic acid as a standard. In a test-tube, 0.5 ml of the dissolved extracts (1 mg/ml) and 0.5 ml of gallic acid solution were taken and 2.5 ml of FCR (10 fold diluted) was added followed by addition of 2 ml sodium carbonate (7.5 %). Due to the presence of phenolic compounds the yellow colour of FCR turns blue. The test-tube was allowed to stand for 30 min at room temperature and absorbance was measured at 760 nm by UV-spectrophotometer and the results expressed as mg gallic acid equivalents/g dry extract[14].

Quantitative estimation of total tannin content (TTC):

The amount of TTC was determined using Folin-Denis reagent (FDR) taking tannic acid as a standard. One millilitre of dissolved extracts (1 mg/ml) and 1 ml of tannic acid solution from its different concentrations were taken into a test-tube and 1 ml of FDR was added followed by addition of 2 ml of 7.5 % sodium carbonate. Absorbance was measured on a spectrophotometer at 700 nm. Results were expressed as mg tannic acid equivalent/g in dry extract[15].

Quantitative estimation of total flavonoid content (TFC):

The amount of TFC was determined by aluminium chloride ($AlCl_3$) method taking quercetin as a standard. In a test-tube 1 ml of plant extracts (1 mg/ml) and 1 ml of quercetin solution from its different concentrations were taken diluted with 4 ml of distilled water, followed by the addition of 0.3 ml sodium nitrite (10 %) and 0.3 ml of $AlCl_3$ (10 %). After 6 min incubation at room temperature, 2 ml of 1 % sodium hydroxide solution was added and absorbance was measured at 510 nm on a spectrophotometer. Results were expressed as mg quercetin equivalent/g dry extract[16].

Quantitative estimation of total saponin content (TSC):

The amount of TSC was determined taking diosgenin as a standard. Both the aqueous extracts were dissolved separately in 80 % methanol followed by addition of 2 ml of vanillin solution and 72 % sulphuric acid (H_2SO_4). Mixing was done properly and heated on water bath for 10 min at 60°. Against reagent blank, absorbance was measured at 544 nm and all the determinations were done in triplicate. The TSC was expressed as mg diosgenin equivalent/g dry extract[17].

In vitro antioxidant study:

The free radical scavenging activities of SAE and FAE and ascorbic acid (standard) were measured in

relation to the hydrogen donating or radical scavenging capacity using the stable DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals, ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)di-ammonium salt) radicals and phosphomolybdate radical. Hydrogen peroxide assay and reducing power properties were further performed to confirm the antioxidant property of SAE and FAE. The percent scavenging of free radical activity was calculated by means of the following Eqn. 2, percent scavenging = (A0–A1)/A0×100, where, A0 is the absorbance of the control and A1 is the absorbance of the sample. The concentration of extract at which 50 % inhibition is observed (IC_{50}) is calculated in µg/ml. DPPH radical scavenging assay is one of the prevalent methods of determining the antioxidant activity of plant extracts. Decrease in the absorbance was checked spectrophotometrically at 517 nm after 15 min and the percent scavenging (inhibition) was taken out using the Eqn. 2 along with their IC_{50}[4]. ABTS radical scavenging assay: decrease in the absorbance was checked spectrophotometrically at 745 nm and the percentage scavenging (inhibition) was taken out using the Eqn. 2 along with their IC_{50}[18]. In the hydrogen peroxide assay, the absorbance was quantified spectrophotometrically at 230 nm after 10 min of reaction time[19]. In the phosphomolybdate assay (TAC) the absorbance was quantified spectrophotometrically at 765 nm[14]. Absorbance was quantified at 700 nm for reducing power assay. Higher reducing power is denoted by high absorbance[20].

Antibacterial study:

The antibacterial activity of the plant extracts were tested against two Gram-positive bacteria, *Bacillus stereothermophillus* (ATCC 7953) and *Staphylococcus aureus* (ATCC 29737) and four Gram-negative bacteria, *Escherichia coli* (ATCC 11229), *Pseudomonas aeruginosa* (ATCC 9027), *Vibrio cholera* (ATCC 51395) and *Acinetobacter baumannii* (ATCC 17978). All the microorganisms with ATCC number were obtained from the Laboratory of Microbiology, NICU, SUM Hospital, Bhubaneswar.

The antibacterial study of SAE and FAE was executed using the well diffusion method[21]. 15 ml of nutrient clear jelly (HiMedia) medium was poured in clean sterilized petri-plates and allowed to cool (and turn into a concrete). One hundred microlitres of the broth of bacterial strain was pipette out and spread over the medium evenly by a spreading rod till it is dried properly. Wells of 6 mm in through its centre were bored using sterile cork borers. Solutions of both the extracts (100 mg/ml) in distilled water were prepared. One hundred microlitres of the plant extract solutions were added to the wells. The petri-plates were created and grown at 37° for 24 h. *Streptomycin* (1 mg/ml) was used as a positive control and distilled water was taken as the negative control. Antibacterial activity was estimated by measuring the diameters of zones of inhibition. All measurements were done in triplicate.

Gas chromatography-mass spectrometry (GC-MS) Analysis:

The GC-MS analysis of FAE and SAE was executed using Thermo Trace 1300 Gas chromatograph-Mass spectrophotometer amalgamated with TSQ 8000 Mass spectrophotometer, fitted with TG 5MS (30 m×0.25 mm, 0.25 µm) column. Helium was used as a carrier gas at a flow rate of 1 ml/min. Oven temperature was fixed from 60 to 280° at 10°/min. The injection temperature was set at 250° and the injection volume was taken as 1.0 µl with split ratio 1:10. MS transfer line temperature and ion source temperature was maintained at 280 and 230°, respectively and the mass spectra was taken at 70 eV (ionizing energy) for a total run time of 31 min.

Interpretation of the GC-MS spectrum was conducted by the help of data base obtained from the National Institute of Standard and Technology (NIST) library, which had been installed to the computer system attached with the instrument. Comparison of the mass spectrum of unknown components with the known one from NIST library on the basis of name, structure, molecular weight and fragmented ions achieved from the MS spectrum helps to identify the components present in the test substances were ascertained for GC-MS analysis.

Statistical analysis:

SPSS version 20 was used for statistical analysis. One-way ANOVA with $p \leq 0.05$ was used to spot the significant difference between SAE and FAE in *in vitro* antioxidant studies.

RESULT AND DISCUSSION

Regarding the selection of water as a solvent for extraction procedure attention has been given to the safety of the extract for consumption by human and cost effectiveness[22]. Literature revealed the fact that water can be used as a suitable solvent for extraction of polyphenols[22,23] and also support regarding the presence of flavonoids, steroids and terpenoids in water

extract that being evaluated through phytochemical screening[24,25]. Percent yield (%) was calculated using Eqn. 1, which illustrates that percent yield of FAE (7.89 %), is more than that of SAE (4.9 %).

Quantitative estimation of phytoconstituents was ended up using the standard methods. The list of phytoconstituents in SAE and FAE is illustrated in Table 1 indicating that both SAE and FAE are devoid of any alkaloid. Both the extracts have tannin, saponin, steroids and terpenoids in them. Only SAE contain proteins and amino acids, while, glycoside, flavonoids and carbohydrate and reducing sugar is present in FAE.

Results of quantitative estimation of TPC, TTC, TFC and TSC in SAE and FAE are depicted in fig. 1 indicating that TPC, TTC and TSC in FAE are remarkably more than SAE. TFC of FAE is found to be 279.33 mg quercetin equivalent per gram of dry extract. TPC of SAE and FAE were 22.28 mg GAE/g of dry extract and 95.06 mg GAE/g of dry extract, respectively. TTC of SAE and FAE were 74.00 and 207.18 mg TAC/g of dry extract, respectively and TSC of SAE and FAE were 104.71 and 318.62 mg diosgenin equivalent/g of dry extract, respectively. Moreover, it is apparent that TSC>TFC>TTC>TPC in FAE and TSC>TTC>TPC in SAE. Thus, in both the extracts TSC was found to be more than the other phytoconstituents.

Using all the methods stated above, for antioxidant study of SAE and FAE illustrated a clear picture of FAE being more potent than SAE (fig. 2A-D). In all the cases, percent inhibition increased with the increase in concentration. The IC_{50} values depicted in Table 2 supported the observation in fig. 2 where the IC_{50} values of FAE with four different methods of free radical scavenging assay was clearly lower than that of SAE[26]. Reducing power of any compound is the reflection of its antioxidant potency[27]. The yellow colour of Fe^{3+}/ferricyanide complex on reaction with the sample changes to ferrous form, which is green or blue colour (depending on the reducing power), called the Prussian blue in the reducing power assay. Higher reducing power is indicated by the higher absorbance of Fe^{2+} at 700 nm[28]. The result of reducing power assay is depicted in fig. 3, which clearly showed the increase in absorbance with the increase in the concentration

TABLE 1: LIST OF PHYTOCONSTITUENTS PRESENT IN SAE AND FAE

Phytoconstituents	SAE	FAE
Alkaloid	-	-
Glycoside	-	+
Steroids and terpenes	+	+
Flavonoids	-	+
Proteins	+	-
Carbohydrates and reducing sugar	-	+
Tannin and phenolic compounds	+	+
Saponin	+	+

'+' and '-' denotes the presence and absence of particular phytoconstituents, respectively

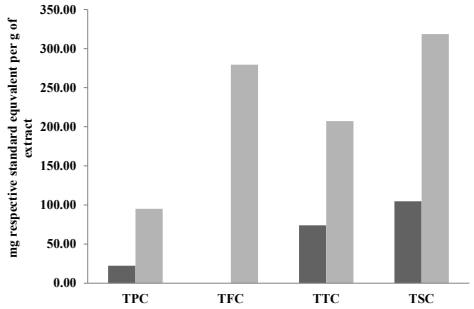


Fig. 1: Quantitative estimation of phytoconstituents in SAE and FAE

■ SAE, ■ FAE

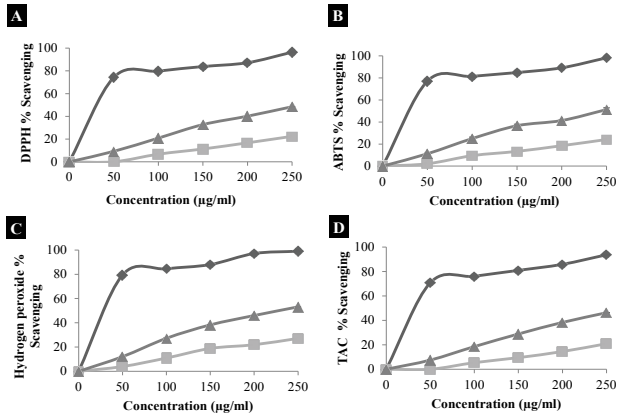


Fig. 2: Antioxidant study

(A) DPPH radical scavenging assay, (B) ABTS radical scavenging assay, (C) hydrogen peroxide assay and (D) phosphomolybdate assay (total antioxidant capacity) of ascorbic acid, SAE and FAE; —♦— ascorbic acid, —■— SAE, —▲— FAE; all the results are mean±SD (n=3)

TABLE 2: IC_{50} VALUES OF SAE AND FAE IN DIFFERENT ANTIOXIDANT ASSAY METHODS

Method	Test material	IC_{50} (µg/ml)	F-value	P-value
DPPH radical scavenging assay	SAE	549.47	7.4	0.000
	FAE	249.77		
ABTS radical scavenging assay	SAE	514.89	6.625	0.001
	FAE	234.40		
H_2O_2 radical scavenging assay	SAE	445.46	8.161	0.000
	FAE	219.76		
Phosphomolybdate assay	SAE	603.75	7.566	0.000
	FAE	258.59		
Reducing power	SAE	NA	18.558	0.000
	FAE			

Significance level, α=0.05

of extracts. The data for statistical evaluation was performed using SPSS version 20 (One-way-ANOVA), used to detect the significant difference between the extracts in antioxidant studies is depicted in Table 2. The data confirmed that there existed a significance difference between the antioxidant potency of SAE and FAE.

The results could be attributed to the presence of flavonoids and high TPC in FAE. Phenolic compounds and flavonoids in plants were reported to be the key phytoconstituents accountable for antioxidant activity[29]. Flavonoids are said to have tremendous scavenging potency. In addition to that, flavonoids also have ability to reduce lipid peroxidation of biological membranes[30]. On the other hand, extracts containing higher levels of TPC were reported to act as reducing agents and have the ability to inhibit, quench free radicals and terminate the radical chain reaction responsible for oxidative stress[31]. Free radical scavenging activity of any extract may be attributed to its phenolic content because, structurally phenols contain aromatic ring comprising of one or more hydroxyl substituent, that has the ability to scavenge free radicals by donating hydrogen atom[32], which supported scavenging activity of FAE and SAE due to the presence of phenolic content within them.

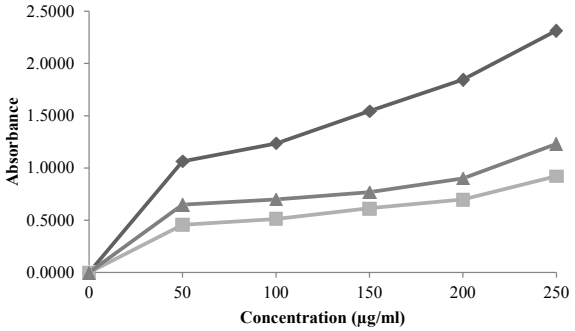


Fig. 3: **Reducing power assay of SAE and FAE along with standard ascorbic acid**
All the results are mean±SD (n=3); —♦— ascorbic acid, —■— SAE, —▲— FAE

Table 3 portrayed the zones of inhibition of SAE and FAE along with the positive control, *Streptomycin* and negative control, distilled water. SAE showed no zone of inhibition at concentration 100 mg/ml. FAE at the same concentration showed a zone of inhibition with *S. aureus*, *V. cholerae*, *A. baumanii* as shown in fig. 4. An increase in the concentration of the extract might result in the appearance of zone of inhibition with other bacteria. The accurate mechanism of action of phytoconstituents is not entirely explained thus far. But it is projected that the effectiveness of the medicinal plant extracts essentially depended on the extracting solvent used. Organic extracts proved to be more potent in terms of antimicrobial properties than the aqueous extracts[33]. Thus, in an earlier report, which included the antioxidant and antimicrobial study of methanol extract of the stem, showed zones of inhibition against all bacteria under test at 100 mg/ml concentration[34]. It is quite evident that evaluation of herbal activity on the whole depended upon the phytoconstituents present within it. So different solvent treatment might alter the pharmacological activity due to variations in phytoconstituents. This indicated the ability of the solvents to extract the metabolites present within the part of a plant tested according to their polarity or affinity of the metabolites towards the solvent used.

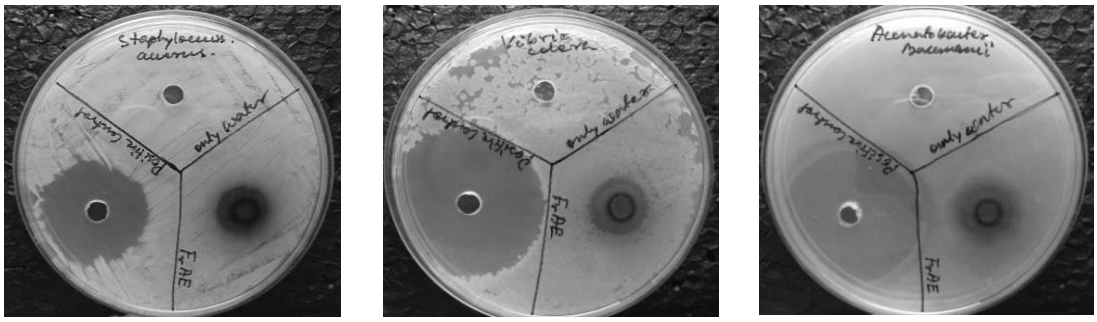
Flavonoids were reported to have potent antimicrobial property[35]. There were different mechanisms postulated by which tannin is responsible for producing antimicrobial activity. These mechanisms included inhibition of extracellular microbial enzymes, inhibition of oxidative phosphorylation in microbes, deprivation of substrates and iron required for microbial growth. Phenols have also been reported to show antimicrobial activities along with the flavonoids and tannins[36]. The presence of all the three phytoconstituents in FAE could be the reason for demonstrated antibacterial activity.

GC of FAE and SAE is depicted in figs. 5A and B, respectively. GC-MS report of FAE and SAE is

TABLE 3: ZONE OF INHIBITION OF SAE AND FAE ALONG WITH POSITIVE AND NEGATIVE CONTROLS

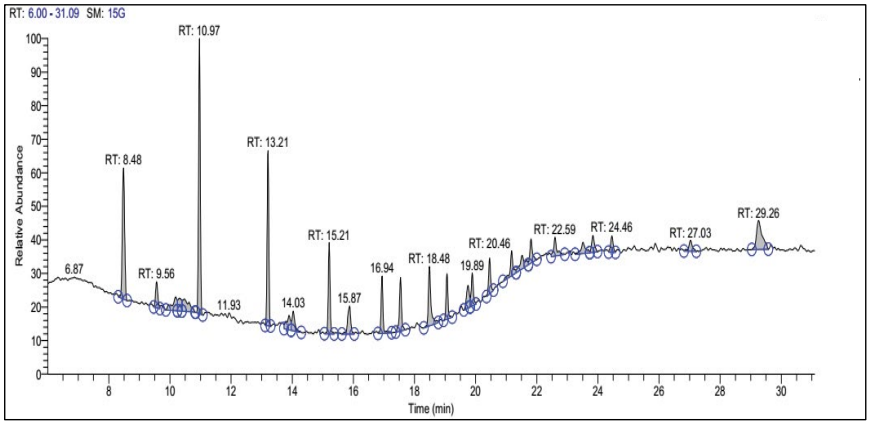
Test organism	Zone of inhibition ±standard deviation (mm)			
	Negative control	Positive control	SAE	FAE
*S. aureus**	0	23.33±1.15	0	8.67±0.58
*B. stereothermophillus**	0	9.83±1.15	0	0
P. aeruignosa	0	32.83±0.58	0	0
V. cholerae	0	34.00±2.37	0	10
E. coli	0	26.67±1.26	0	0
A. baumanii	0	29.67±1.15	0	7±1

All the results are mean±SD (n=3); Gram-positive bacteria are indicated by '*'. Rest are Gram-negative bacteria

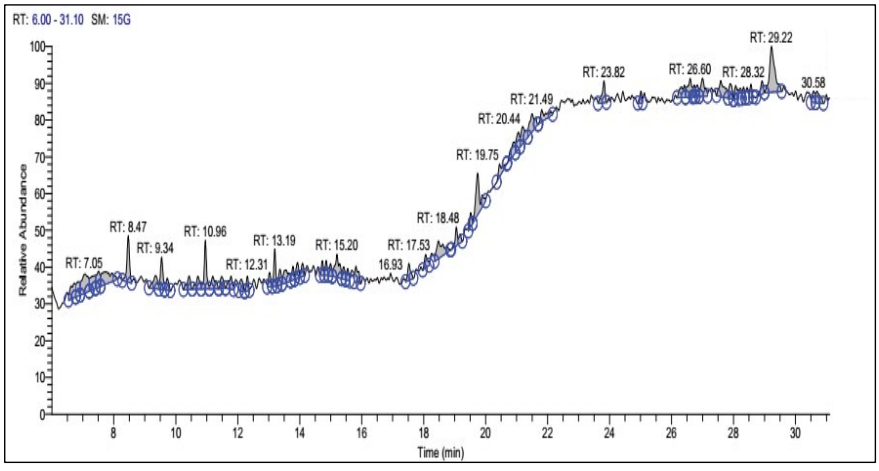


A **B** **C**

Fig. 4: Antimicrobial activity of FAE
Petriplates depicting zones of inhibition of FAE against (A) *S. aureus*, (B) *V. cholera* and (C) *A. Baumanii*



A.



B.

Fig. 5: GC-MS chromatogram of (A) FAE and (B) SAE

summerized in Tables 4. The MS results of FAE and SAE are shown in figs. 6 and 7, respectively. Compounds those were identified from FAE and SAE through GC-MS analysis have been summarised in Tables 4, and the GC chromatograms have been shown in fig. 5A and B, respectively. The MS results of FAE and SAE have depicted with respect to each compound that being identified as compared to NIST library shown in figs. 6 and 7, respectively. Respective structure of identified compounds with respect to their retention time has mentioned in the figs. 6 and 7. The GC-MS analysis of FAE and SAE shows the presence of a volatile organic compound like octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl in figs. 6 and 7 having antimicrobial property[37]. Other compounds that are used for the same purpose found in FAE are hexasiloxane,1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl (fig. 6), 1-monolinoleoylglycerol

trimethylsilyl ether (fig. 6), propanoic acid,2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)- (fig. 6)[38] and 13-docosenamide, (Z)- (fig. 6)[39]. This explains the antimicrobial property of FAE shows against *S. aureus*, *V. cholera* and *A. baumanii* (fig. 4) 1-Monolinoleoylglyceroltrimethylsilyl ether (fig. 6) and cyclononasiloxane, octadecamethyl (fig. 6) in FAE were known for their antioxidant property[39]. Both 1-monolinoleoylglyceroltrimethylsilyl ether (fig. 6) and cyclononasiloxane, octadecamethyl fig. 6 in FAE have antioxidant property, thus explaining the antioxidant property of FAE as illustrated in figs. 2 and 3 and Table 2. The potential of antioxidant property can be elucidated with the peak area % of these compounds embraced in the GC-MS analysis as presented in Table 4. More the peak area, more is the concentration of the compound in the extract. 1-Monolinoleoylglyceroltrimethylsilyl ether is a steroid that is used as an antiinflammatory, antiarthritic, diuretic and antiasthamatic agent[39]. Propanoic acid,2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)- and 1-monolinoleoylglycerol trimethylsilyl ether in FAE support as an antidiabetic agent[39-41]. Propanoic acid,2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)- has

TABLE 4: GC-MS RESULT OF FAE AND SAE

				FAE
RT	**MF**	**MW**	**PA %**	**Compound**
8.48	C10H30O5Si5	370.77	10.23	Cyclopentasiloxane,decamethyl-
9.56	C4H6N2O2	114	2.02	2,4-Imidazolidinedione,1-methyl
10.47	C6H7FO5	178.16	2.84	Furan-2-one,3,4-dihydroxy-5-[1-hydroxy-2-fluoroethyl]-
10.97	C12H36O6Si6	444.66	15.91	Cyclohexasiloxane,dodecamethyl
13.90	C12H38O5Si6	430.66	1.89	Hexasiloxane,1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl
18.48	C16H50O7Si8	578	5.15	Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl
19.75	C27H54O4Si2	498	2.19	1-Monolinoleoylglycerol trimethylsilyl ether
21.17	C18H54O9Si9	666	2.37	Cyclononasiloxane,octadecamethyl
21.52	C28H43NO6	489	2.31	(5á)Preganane-3,20á-diol,14á,18á-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diy1)]-,diacetate
23.51	C32H66O5Si4	642	1.70	Prosta-5,13-diel-1-oic acid,9,11,15-tris[(trimethylsilyn)oxy]-,trimethylsilylester,(5Z,9á,11á,13E,15S)-
23.84	C25H48O4	412	1.59	2,3-Dihydroxypropyl-cis-13-docosenoate
27.03	C27H42O4	430	1.37	Propanoic acid,2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)-
29.26	C22H43NO	337	5.45	13-Docosenamide,(Z)-
				SAE
8.47	C17H30O4Si2	354	3.35	3-(3-Hydroxyphenyl)-3-hydroxypropionic acid, ethyl ester, di-TMS
9.72	C20H26N2O2	326	0.98	Corynan-17-ol, 18,19-didehydro-10-methoxy-
11.19	C24H32O9	464	1.52	5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one,9,9a-bis (acetyloxy) 1,1a,1b,2,4a,7a,7b, 8,9,9a- decahydro-2,4a, 7b-trihydroxy-3-(hydroxymethyl)-1,1,6,8-tetramethyl-
13.02	C26H43NO6	465	0.97	Glycocholic acid
13.19	C16H50O7Si8	578	1.89	Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-
14.86	C10H30F2O3Si6	404.54	0.77	6,8-Difluoro-2,2,4,4,6,7,7,8,9,9-decamethyl-[1,3,5,2,4,6,7,8,9] trioxahexasilonane
15.55	C35H70O3	538	0.73	1,3-Dioxane, 5-(hexadecyloxy)-2-pentadecyl-, trans-
19.06	C25H34O7	446	1.64	(22S)-6à,11á,21-Trihydroxy-16à,17à propyl methylenedioxypregna-1,4-diene-3,20-dione
19.75	C42H64O2	600	4.66	psi.,.psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy-
20.44	C27H52O4Si2	496	1.19	9,12,15-Octadecatrienoic acid,2,3-bis[(trimethylsilyl)oxy]propyl ester, (Z,Z,Z)-
21.19	C36H69NO6Si3	696.32	2.08	Glycine,N-[(3à,5á,7à,12à)-24-oxo-3,7,12 tris[(trimethylsilyl)oxy] cholan-24-yl]-,methyl ester
23.82	C36H58	514	1.79	15,17,19,21-Hexatriacontatetrayne
26.60	C27H45Cl2NO2	482	2.11	Cholestane, 3,5-dichloro-6-nitro-, (3á,5à,6á)-
28.32	C29H56N2O10Si3	676	0.65	D-Glucopyranosiduronic acid,3-(5-ethylhexahydro-1,3-dimethyl2,4,6-trioxo-5-pyrimidinyl)-1-methylbutyl 2,3,4-tris-O-(trimethylsilyl)-, methyl ester
29.22	C45H86N2O2	686	6.66	Bis(cis-13-docosenamido)methane

RT: retention time, MF: molecular formula, MW: molecular weight, PA: peak area

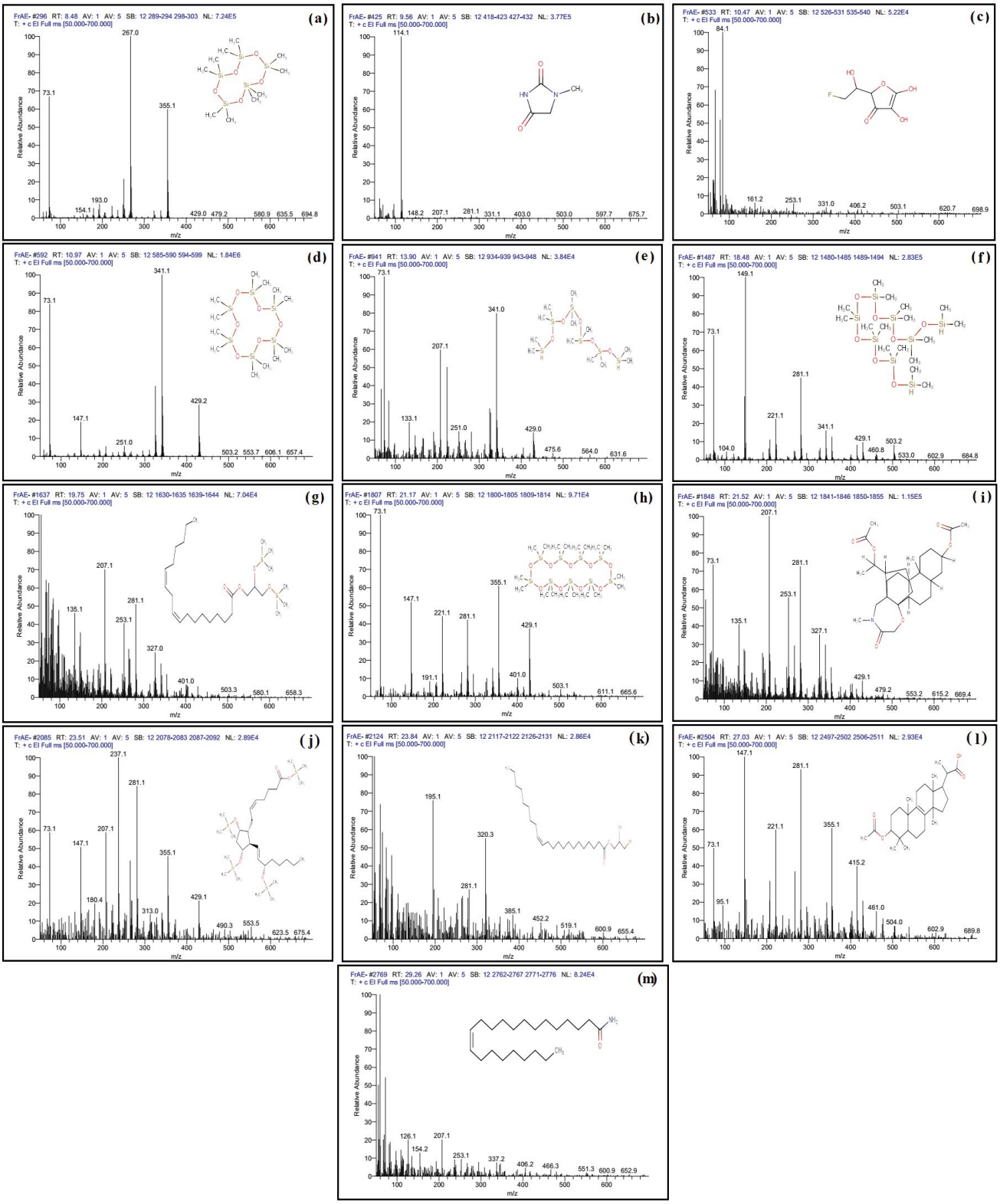


Fig. 6: MS of FAE

At retention time (min): (a) 8.48, (b) 9.56, (c) 10.47, (d) 10.97, (e) 13.90, (f) 18.48, (g) 19.75, (h) 21.17, (i) 21.52, (j) 23.51, (k) 23.84, (l) 27.03 and (m) 29.26

antitumor potentiality[39]. Glycine, N-[(3à,5á,7à,12à)-24-oxo-3,7,12 tris[(trimethylsilyl)oxy]cholan-24-yl]-, methyl ester of SAE depicted in fig. 7m has antibacterial and antiperspirant properties[39].

The experimental results of this study representing that both stem and fruits of this plant have *in vitro* antioxidant property but a comparison within them on basis of phytochemical screening indicated that parts

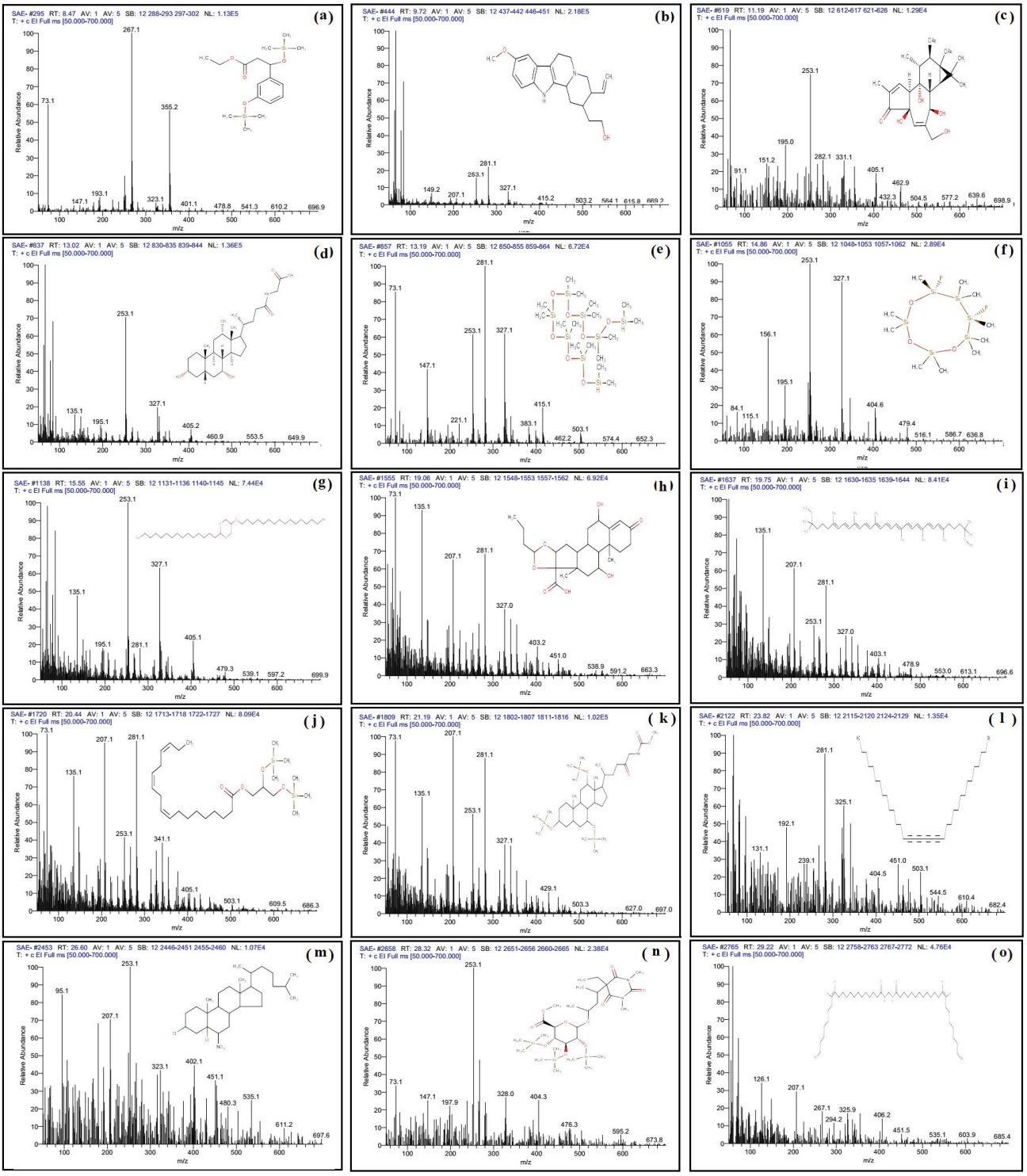


Fig. 7: MS of SAE

At retention time (min): (a) 8.47, (b) 9.72, (c) 11.19, (d) 13.02, (e) 13.19, (f) 14.86, (g) 15.55, (h) 19.06, (i) 19.75, (j) 20.44, (k) 21.19, (l) 23.82, (m) 26.60, (n) 28.32 and (o) 29.22

having flavonoids, tannin and phenolic content would exhibit diverse medicinal property. Throughout this experiment as compared to SAE, FAE showed better percent yield, presence of flavonoid, higher phenolic, tannin and saponin content (quantitatively) with greater *in vitro* antioxidant and antibacterial property. Even though evaluation through GC-MS also work out the compounds present in FAE with supportive research articles influence its better performance as compare to SAE. In this study the stem did not show any antibacterial property, which also supported the importance of flavonoid content in FAE. GC-MS

results showed few other constituents that can help FAE to prove useful as an antitumor, antiinflammatory, antiarthritic, diuretic and antiashmatic agents. Thus, it is necessary that FAE be further investigated for all the above uses and could prove to be with potential for the treatment of the above mentioned diseases.

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Conflict of interest:

Authors reported that there is no conflict of interest.

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