

Spectroscopic and chromatographic exploration of different phytochemical and mineral contents from *Syzygium alternifolium* (Wt.) Walp. an endemic, endangered medicinal tree taxon

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ABSTRACT

The present study is aimed to explore different phytochemical and mineral contents in endemic medicinal plant-*Syzygium alternifolium*. Different spectroscopic (UV-Vis, FT-IR, ICP-OES) and chromatographic techniques (GC-MS) are used to identify versatile phytochemical and mineral contents in different parts of *S. alternifolium* by qualitative and quantitative means. For qualitative phytochemical screening a total of 16 types of different primary and secondary metabolites are extracted in 07 different solvents, among them 15 types of metabolites out of 16 are identified in methanol extract. In quantitative analysis higher quantities of sugar i.e., 45.2 mg/g among primary metabolites of fruit and 451.20 mg/g of phenols among secondary metabolites from stem bark are quantified. FT-IR and GC-MS analysis of methanol extracts of plant reveals different functional groups and phytochemical compounds. In addition to these, mineral analysis through ICP-OES shows higher quantities of Potassium (43.60 mg/L) in fruit, Iron (2.186 mg/L) in stem bark among macro and micro elements respectively. The results of the present study reveal, *S. alternifolium* is potential source towards number of phytochemicals and different types of mineral contents. Hence, it becomes a treasure house for pharmaceutical as well as nutraceutical companies to prepare novel products.

INTRODUCTION

Since ancient times, usage of medicinal plants all over the world due its preservative and medicinal values as well as to convey flavour to food and medicines preparation. In recent past, there has been growing interest in crude extracts of medicinal plants to develop food additives and different medicine formulations (Omran and Esmailzadeh, 2009). Medicinal plants, in all aspects of life served as valuable starting material for development of herbal drugs, which are rich source of phytochemical compounds (Sasidharan *et al.*, 2011) and minerals (Ghani *et al.*, 2014) build up them through by different primary and secondary metabolic processes. Identification of plant phytochemicals through phytochemical screening is considered

to be an effective discovering method for phytochemical constituents of medicinal plants responsible for important physiological functions in living beings (Yamashita *et al.*, 2005). In addition to these, mineral nutrients are also needed for humans to maintain their health and proper organ function (Ballesta *et al.*, 2010).

According to World Health Organization most of the human population relies chiefly on medicinal plants for their daily ailments. In India, most of the people also depend on traditional herbal medicine systems and still explored number of medicinal plants for their therapeutic activities. Due to this, India is a dictum of botanical garden to the world and a goldmine of well recorded and traditionally well practiced knowledge of herbal medicine (Savithramma *et al.*, 2012). Various medicinal plants are also used as food along with their medicinal benefits, evaluating their mineral nutritional significance can help to understand the worth of medicinal plant species (Pandey *et al.*, 2006).

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For this, analyses of different phytoconstituents and mineral nutrients of medicinal plants was carried out by efficient scholars through utilization of several advanced analytical tools like- quantification and identification of specific functional groups of compounds solely by their absorption characteristics through UV-Vis and FT-IR spectroscopy respectively (Baeten and Aparicio, 2000). Identification of specific unique phytochemical compounds by combination of Gas chromatography (GC) data coupled with mass spectra (MS) (Hemalatha *et al.*, 2016) and quantification of different mineral elements by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) (Senila *et al.*, 2014). Now-a-days usage of these spectroscopic and chromatographic techniques in biology brought about for revolution in drug development, which explored more number of compounds and minerals present in medicinal plant parts, especially used in the preparation of herbal remedies to cure various diseases. Exploiting these techniques to identify the adulteration of drugs has become easy in the present scenario.

Phytochemicals are naturally occurring constituents in medicinal plants, which utilizes for defence mechanism to protect themselves, this specificity was precisely utilized to cure human diseases. These phytochemicals are mainly two groups, i.e., primary and secondary metabolites. Fatty acids, common sugars, proteins are included under primary metabolites; terpenoids, alkaloids, phenolic compounds, etc., are categorized under secondary metabolites. In recent past, analysis of phytochemicals by using UV-Vis spectroscopy, FT-IR and GC-MS techniques become firmly established as a key technological metabolic profiling of medicinal plants (Hemalatha *et al.*, 2016). UV-Vis and FT-IR spectroscopic tools have the capacity to quantify and analyze functional groups of phytochemicals, GC-MS has proven to be a powerful tool and had separation efficiency suitable for separation of phytochemicals (Kopka, 2006). The human body requires a number of minerals in order to maintain good health which influences the biochemical processes of metabolism. A number of minerals essential to human nutrition accumulate in different parts of plants as it accumulates minerals essential for growth conveying from the environment (Ajasa *et al.*, 2004). Study of the elements with respect to indigenous medicinal plant reveals that major and trace elements have significant roles in combating a variety of human ailments and diseases (Shirin *et al.*, 2010). ICP-OES is an analytical tool to identify and quantify number of mineral contents from medicinal plants. For this ascertainment of phytochemicals and mineral elements present in plants is imperative, because of the concentration and type of mineral presence is most often be stipulated on the label of a medicine for effective usage of drugs. The important components like quality, type and concentration of a mineral play pivotal role in the preparation of medicine and curing of different degenerative diseases. In a recent past many of the researchers quantify different mineral contents from medicinal plants with the help of ICP-OES technique (Senila *et al.*, 2014).

Syzygium alternifolium belongs to the family Myrtaceae and is locally known as mogi or adavinerudu. It is a deciduous

tree, grown in top hills of dry deciduous forest in Sri Venkateswara Wildlife Sanctuary of Chittoor and Cuddapah Districts of Eastern Ghats, Andhra Pradesh, India. Most of the researches and Andhra Pradesh State Biodiversity Board has categorized this plant as endemic, and globally endangered species as per the criteria of International Union for Conservation of Nature-Conservation Assessment and Management Plan (IUCN-CAMP) to these areas. Recently "The IUCN Red List of Threatened Species" categorized this plant under endangered state (Saha *et al.*, 2015).

Traditionally the plant parts like tender shoots, leaves, fruits were used for the treatment of dysentery, joint pains, burning sensations in the stomach (Savithramma and Sulochana, 1997), diarrhoea (Savithramma *et al.*, 2014a) and diabetes (Savithramma *et al.*, 2014b) by local and tribal people of Tirumala hills. Researchers proved that different parts of the plant having antimicrobial (Yugandhar *et al.*, 2015) and anticancer activity (Komuraiah *et al.*, 2014). Previously preliminary phytochemical screening and isolation of triterpenoids from stem bark was done by Reddy *et al.*, (2012), the flavonoid Eucalyptin 1 and a triterpinoid Epibetulinic acid 2 was done only by Komuraiah *et al.*, (2014) from leaf part of the plant. But there is no proper report on quantification of phytochemicals and mineral content validation in different parts of *S. alternifolium* so far.

Hence the present study is undertaken to explore different primary and secondary metabolites through qualitative phytochemical screening by using different solvents, quantification of these metabolites by UV-Vis spectroscopy, validation of functional groups by FT-IR, identification of different bioactive compounds by GC-MS by using crude methanol extracts and quantification of essential mineral nutrients by ICP-OES technique.

MATERIALS AND METHODS

Collection of plant material and Sample preparation

The healthy, fresh parts like stem bark, leaves and ripen fruits were collected from Nagatheertham area of Tirumala hills, parts of Eastern Ghats situated in Chittoor District of Andhra Pradesh, India, during the period between June to August of 2015, under the age of 08 to 09 older plants grown at black loamy soils situated at higher altitudes.

The plant was authenticated and cross checked by herbarium deposited (voucher no. 121) in Department of Botany, Sri Venkateswara University, Tirupati. The freshly collected plant parts were washed 2 to 3 times in running tap water and shade dried up to 10-15 days at room temperature.

A 50 g of finely grounded powders was extracted with 250 ml of different solvents like distilled water, benzene, chloroform, ethanol, hexane, methanol and petroleum ether by using soxhlet apparatus. These extracts are filtered through Whatman no. 1 filter paper and concentrated with the help of a rotary evaporator. The obtained residues were stored at 4⁰C for further screening of phytochemicals.

Qualitative phytochemical analysis

1 g of residues obtained above were re-suspended in 100 ml of their respective mother solutions. These solutions were used for preliminary phytochemical screening of different primary and secondary metabolites with the help of standard protocols (Harborne, 1998).

Quantitative phytochemical analysis

With the help of UV-Vis spectroscopy the quantitative analysis of different primary and secondary metabolites through standard protocols like proteins (Lowry *et al.*, 1951), starch (McCready *et al.*, 1950), sugar (DuBoise, 1956), lipids (Jayaraman, 1981), alkaloids (Trease and Evans, 2002), flavonoids (Chang *et al.*, 2002), tannins and phenols (Makkar *et al.*, 1993).

FT-IR analysis

Fourier-Transform Infra Red (FT-IR) spectra of methanol extracts were analyzed in the range of 4,000 to 500 cm⁻¹ with an ALPHA interferometer (ECO-ATR), Bruker, Ettlingen, Karlsruhe, Germany by KBr pellet method.

GC-MS analysis

GC-MS analysis of crude methanol extracts were carried out on GC-MS QP2010 Shimadzu (Japan) system comprising a gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument. The details of column used, carrier gas and maintenance of column temperature are followed by the method of Kumar *et al.*, (2012).

ICP-OES analysis

The Perkin Elmer 7000DV ICP-OES (Shelton, CT, USA) model was used for determination of Ca, K, Mg, P, B, Cu, Fe, Mn, Mo and Zn in digested *S. alternifolium* samples. For this 100 mg of dried plant powders were weighed in quartz vessels and digested with 1ml of 30% H₂O₂ and 7 ml of 70% HNO₃. The temperature was raised up to 170 °C for 10 min. in a muffle furnace for effective digestion of samples. The obtained solutions were filtered and made up to 25 ml with Milli-Q water.

Blank solutions were prepared for slot and the experiment was performed in triplicates (Babu and Savithamma, 2014).

RESULTS

Qualitative phytochemical analysis

In our studies the qualitative phytochemical screening of different parts of *S. alternifolium* reveals the presence of fatty acids, proteins, reducing sugars among primary metabolites. Alkaloids, anthocyanins, anthraquinones, coumarins, emodins, flavonoids, glycosides, leucoanthocyanins, lignins, phenols, saponins, tannins, triterpenoids among the secondary metabolites extracted with different solvents (Table 1). The highest number of phytochemicals were found in methanol extracts from all parts of the plant followed by distilled water and ethanol extracts. Distilled water and ethanol extracts yielded somehow same number of metabolites in different parts of the plant followed by benzene, chloroform, hexane and finally by petroleum ether. The metabolites like flavonoids and phenols obtained more repeatedly from the solvents like distilled water, benzene, chloroform, ethanol, hexane and methanol. Fatty acids, anthraquinones, coumarins and lignins were obtained very drastic in the extracts like, distilled water, benzene, chloroform, ethanol, methanol and petroleum ether.

Quantitative phytochemical analysis

According to qualitative phytochemical analysis (Table 1) more presence of phytochemicals in methanol/distilled water/ethanol extracts indicates ‘++’ were selected for quantification studies. Reducing sugars (starch and sugar), fatty acids (lipids), proteins among primary metabolites; alkaloids, flavonoids, tannins, phenols among secondary metabolites were subjected to quantification with the help of UV-Vis spectroscopy. When compare to all the studied plant parts, higher quantity of primary metabolite like sugar was present in fruit part of the plant followed by starch, proteins and lipids in leaf part of the plant. Among the secondary metabolites, higher quantity of phenols were quantified in stem bark followed by flavonoids in leaf part, alkaloids and tannins in stem bark of the plant (Table 2).

Table 1: Qualitative phytochemical analysis of different primary and secondary metabolites from different parts of *S. alternifolium*.

S. No	Phytochemical constituents	Benzene			Chloroform			Distilled water			Ethanol			Hexane			Methanol			Petroleum ether			
		S	L	F	S	L	F	S	L	F	S	L	F	S	L	F	S	L	F	S	L	F	
1.	Fatty acids	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	+	+	-	+	+	+	
2.	Proteins	-	-	+	-	+	-	++	++	++	++	+	+	+	-	-	++	++	++	-	-	-	-
3.	Reducing sugars	-	-	-	-	-	-	+	+	++	+	++	++	+	+	-	+	++	++	-	-	+	+
4.	Alkaloids	+	+	-	-	-	-	+	++	++	++	++	++	-	-	+	++	++	-	-	-	-	-
5.	Anthocyanins	-	-	+	-	-	-	++	++	+	++	+	+	-	-	++	++	+	-	-	-	-	-
6.	Anthraquinones	-	-	-	-	-	-	++	+	+	++	+	-	-	-	++	+	+	-	-	-	-	-
7.	Coumarins	+	+	+	-	-	-	+	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-
8.	Emodins	-	-	-	-	-	-	+	+	+	++	+	-	-	-	+	+	+	-	-	-	-	-
9.	Flavonoids	+	+	+	-	-	-	++	++	++	++	++	+	+	-	++	++	++	+	+	+	+	-
10.	Glycosides	-	-	-	-	-	-	+	++	+	++	+	+	-	-	+	+	+	-	-	-	-	-
11.	Leucoanthocyanins	-	-	-	-	-	-	++	++	+	++	++	+	-	-	++	++	+	-	-	-	-	-
12.	Lignins	-	-	-	+	+	+	-	-	-	+	+	-	-	-	+	+	+	-	-	-	-	-
13.	Phenols	+	+	+	+	+	-	++	++	++	++	+	-	-	+	++	++	++	-	-	-	-	-
14.	Saponins	-	-	-	+	-	+	+	+	+	+	+	-	-	-	+	+	+	-	-	-	-	-
15.	Tannins	-	-	-	+	+	+	++	++	++	++	++	++	-	-	++	++	++	-	-	-	-	-
16.	Triterpenoids	-	-	-	-	-	-	+	+	+	-	-	+	-	-	+	+	+	-	-	-	-	-

S: Stem bark, L: Leaf, F: Fruit

Table 2: Quantitative estimation of different primary and secondary metabolites from different parts of *S. alternifolium* (mg/g Dwt.)

S. No	Quantification of Phytochemicals	Concentration (mg/g)		
		Stem bark	Leaf	Fruit
1.	Proteins	4.81±0.05	5.72±0.07	4.25±0.04
2.	Starch	8.50±0.14	14.33±0.24	8.33±0.10
3.	Sugar	12.4±0.07	32.8±0.47	45.2±0.26
4.	Lipids	0.18±0.004	0.24±0.010	0.03±0.004
5.	Alkaloids	0.32±0.007	0.30±0.004	0.153±0.001
6.	Flavonoids	35.4±0.36	99.8±0.54	31.5±0.26
7.	Tannins	0.16±0.005	0.10±0.002	0.06±0.0003
8.	Phenols	451.20±0.84	374.40±0.50	325.03±1.84

Values are average of triplicates, ± indicates standard error

Table 3: FTIR analyses of functional groups from different parts of *S. alternifolium*.

S. No	Peak assignment (cm ⁻¹)			Corresponding functional group
	Stem bark	Leaf	Fruit	
1.	3338	3332	3338	O-H bond of alcohols or phenols
2.	2945	2928	2935	C-H stretch of alkanes
3.	2835	2842	2839	C-H stretch of aldehydes
4.		1689		C=O stretch of carboxylic acids
5.	1645	1615	1648	N-H bend of 1° amines
6.	1449	1450	1447	C-H bend of alkanes
7.		1377		C-H bend of alkenes
8.	1223	1217		C-N stretch of aliphatic amines
9.	1109	1103		C-N stretch of aliphatic amines
10.	1017	1018	1016	C-O stretch of alcohols, carboxylic acids, esters, ethers
11.	656	657	699	C-Br stretch of alkyl halides

FT-IR analysis

Qualitative phytochemical screening reveals that the methanol extract solubilises more number of metabolites when compare to other studied solvents. Due to this we use these crude methanol extracts for FT-IR and GC-MS studies. With the help of FT-IR, methanol extracts of different parts were used to identify functional groups of different phytochemicals in the range of 4,000 to 500 cm⁻¹ of the mid IR region [Figure 1; Table 3]. The FT-IR spectrum of methanol extracts of stem bark, leaf and fruit gives a number of absorption peaks and their corresponding functional groups like alcohols, aldehydes, aliphatic amines, alkanes, alkyl halides, amines, carboxylic acids, esters, ethers and phenols.

GC-MS analysis

Gas chromatography analyses in combination with mass spectra of methanol extracts were analyzed to know the different phytochemical compounds along with their molecular weight and molecular formula. The unknown spectrum gets from the plant [Figure 2] extracts were compared with the spectrum of known compounds stored in National Institute of Standard and Technology (NIST) library.

Nature of the chemical compound, retention time, area %, molecular weight, molecular formula, name and from which part of the plant they were separated was enumerated in Table 4. Nearly 40 types of different compounds were identified in all

the studied plant parts, among them Octamethylcyclotetrasiloxane (C₈H₂₄O₈Si₄) with 31.75%, Hexamethylcyclotrisiloxane (C₆H₁₈O₃Si₃) with 26.24% in stem bark, Diethoxydimethylsilane (C₆H₁₆O₂Si) with 45.26%, Acetaldehyde (C₆H₁₄O₂) with 5.67% in leaves, Diethoxydimethylsilane (C₆H₁₆O₂Si) with 26.04% and Zapotin (C₁₉H₁₈O₆) with 13.78% in fruit part of the plant shows highest peak areas represents as principal components and compounds like 1,5-Diphenyl-2H-1,2,4-triazoline-3-thione; iethoxydimethylsilane; Ethylidene diethyl ether; Furfural; Methylsilane; Phosphine oxide, bis(pentamethylphenyl) were repeated compounds in the studied plant parts.

ICP-OES analysis

The ICP-OES analysis of different parts of the plant shows highest concentration of potassium (43.60 mg/L) in upper part of the plant like fruits among macro elements and iron (2.186 mg/L) in lower part of the plant like stem bark among micro elements (Table 5).

When compared to all the parts of *S. alternifolium*, fruit part of the plant having higher number of elements with higher concentrations like potassium (43.60 mg/L), magnesium (10.03 mg/L), phosphorus (5.793 mg/L), copper (0.051 mg/L) and zinc (0.197 mg/L). This fruit part was followed by stem bark, having calcium (38.37 mg/L), iron (2.186 mg/L) molybdenum (0.131 mg/L) and leaf part having boron (0.458 mg/L) and manganese (0.584 mg/L) at higher concentrations.

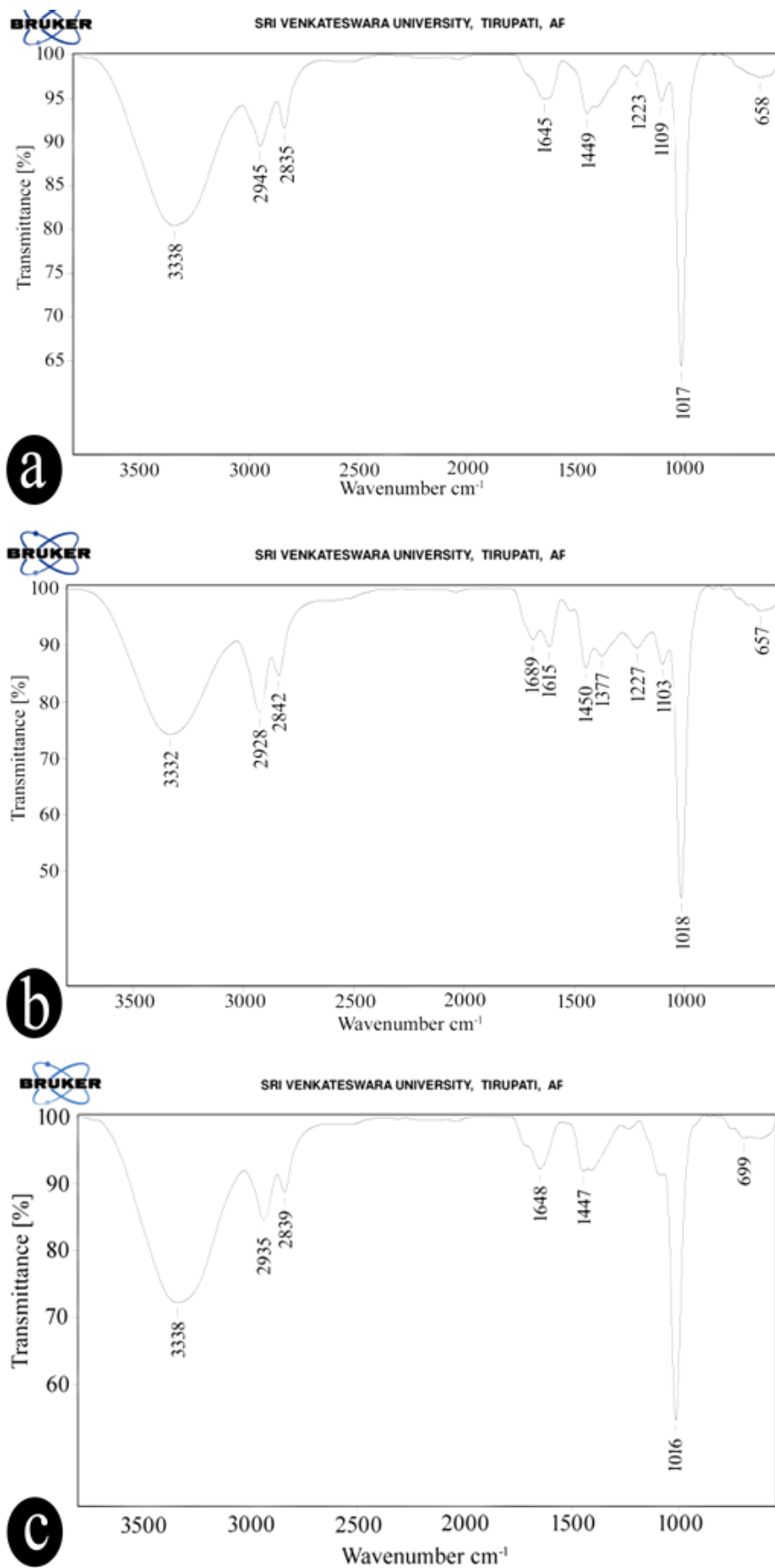


Fig. 1: FTIR spectral analyses for methanol extracts of *S. alternifolium* a: Stem bark extract b: Leaf extract c: Fruit extract.

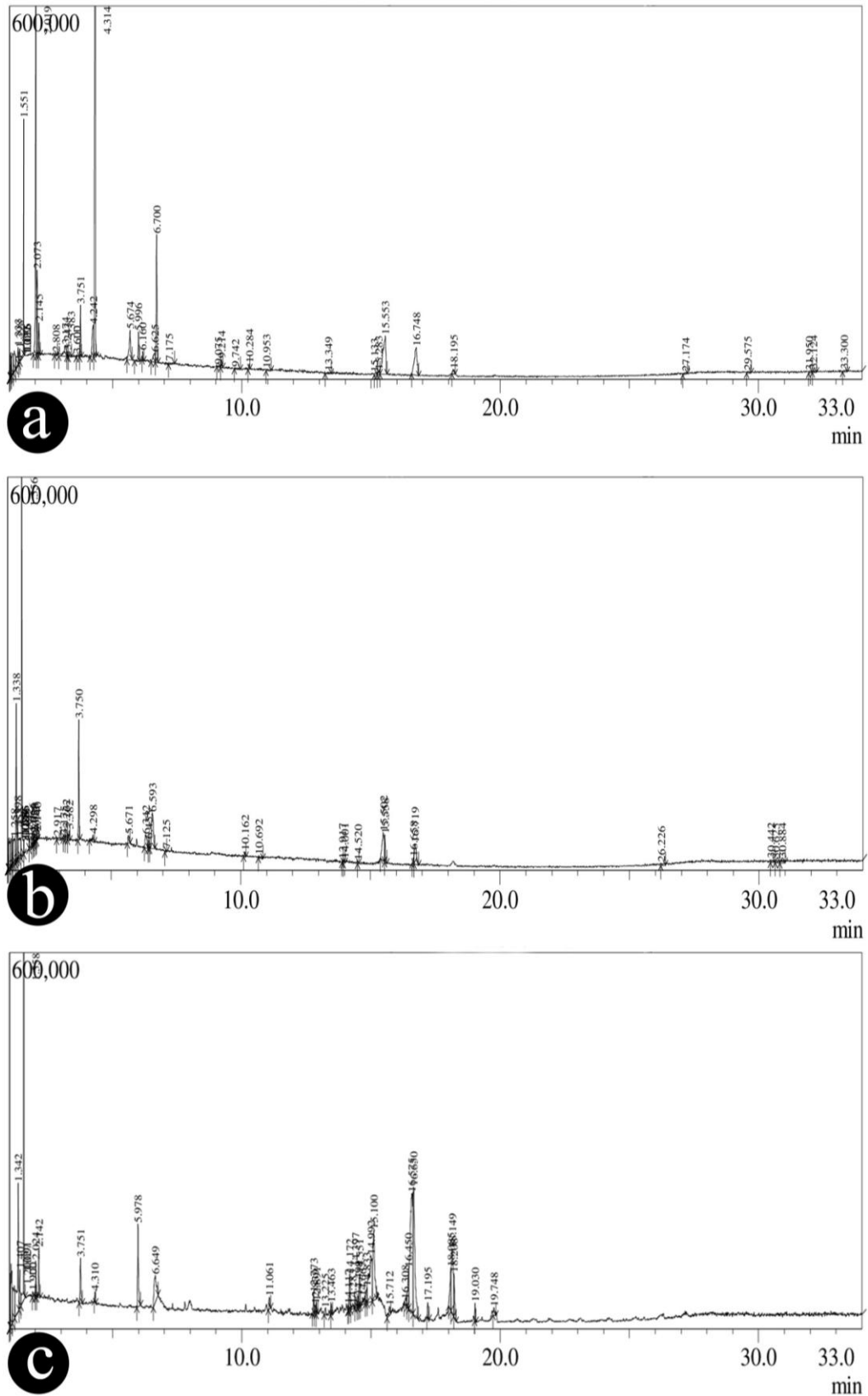
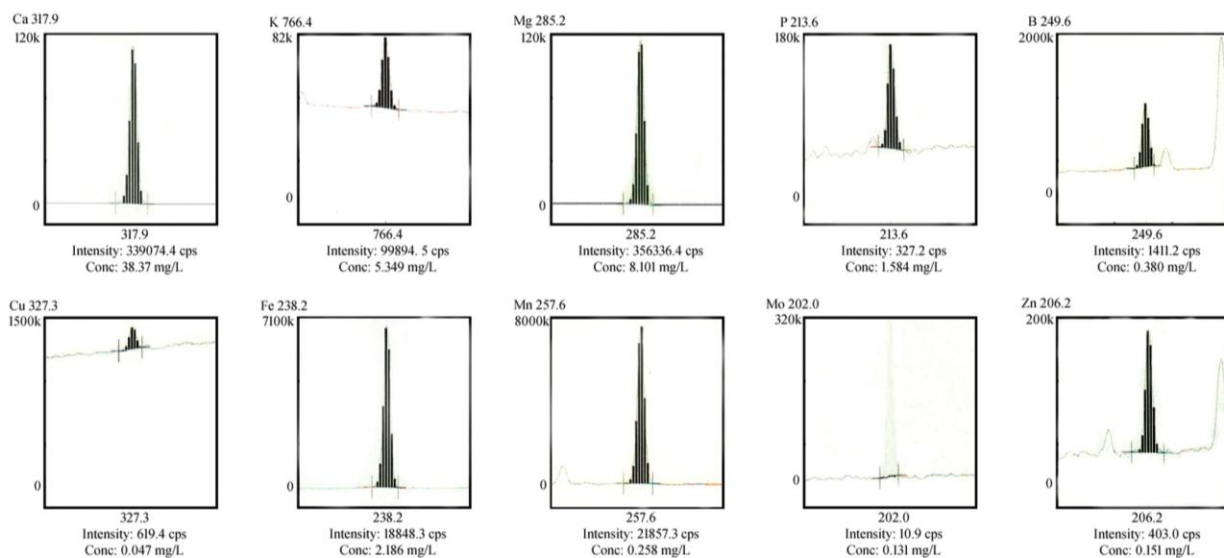
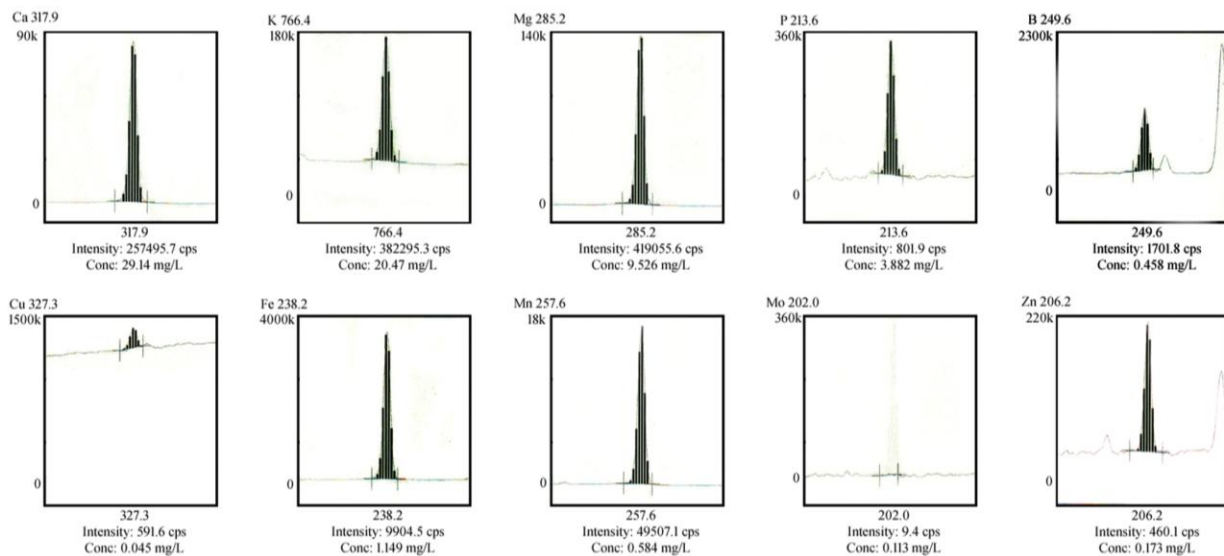


Fig. 2: GC-MS chromatographs of methanol extracts of *S. alternifolium* a: Stem bark extract b: Leaf extract c: Fruit extract.

Stembark



Leaf



Fruit

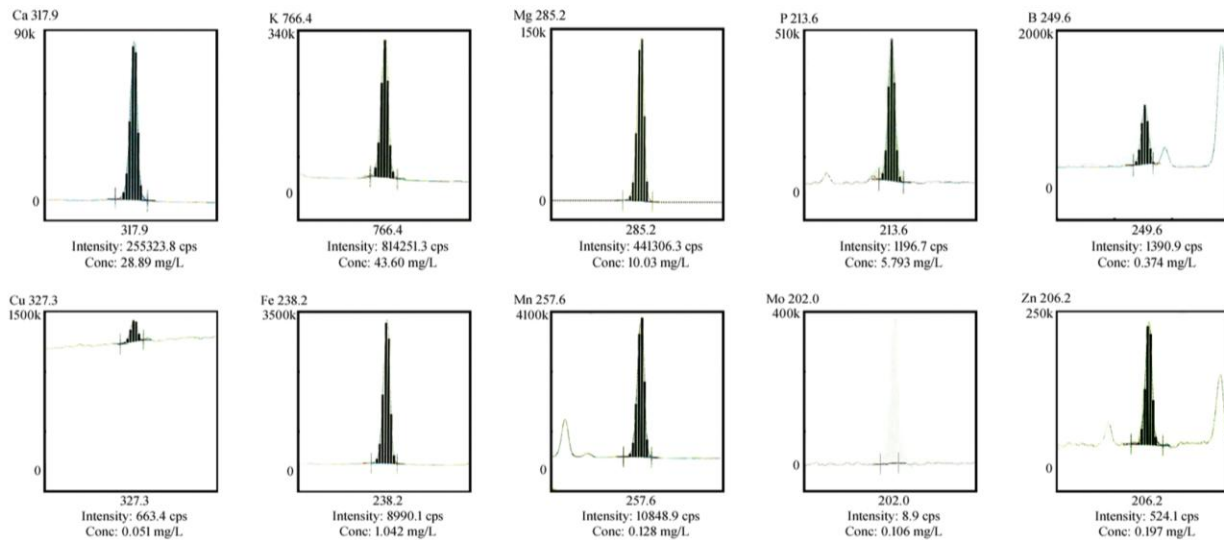


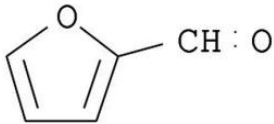
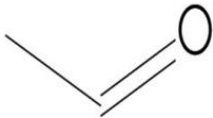
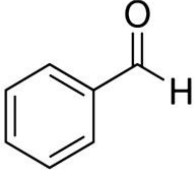
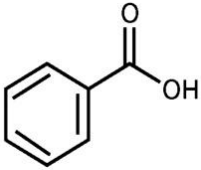

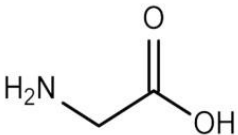
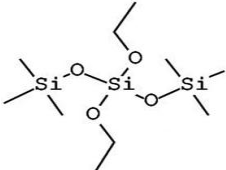
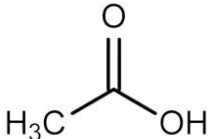
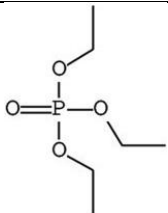
Fig. 3: ICP-OES spectral analyses for mineral constituents in different parts of *S. alternifolium*.

Table 4: GC-MS analyses for methanol extracts of different parts of *s. alternifolium*.

Nature of chemical compound	R. Time	Area %	Molecular formula	Molecular weight	Compound name	Plant part	
Alcohols/ Phenols	1.056	0.76	C ₄ H ₁₀ O	74	1-Butanol	Stem bark	
	5.996	1.14	C ₅ H ₆ O ₂	98	2-Furanmethanol		
	7.175	0.24	C ₅ H ₁₂ OSi	116	Silanol, allyldimethyl		
	27.174	0.19	C ₁₇ H ₁₄ O ₄	282	2-Hydroxy-3-(4-methoxybenzoyl)chrom-3-ene		
	33.300	0.20	C ₃ H ₆ O ₂	74	Formal glycol		
	1.966	0.62	C ₃ H ₈ O	60	Propol	Leaf	
	15.558	1.34	C ₁₉ H ₂₄ O	268	Phenol, 2-(1,1-dimethylethyl)-4-(1-methyl-1-phenylethyl)-		
	1.200	2.81	C ₅ H ₁₂ O	88	Methylpropylcarbinol	Fruit	
	14.992	5.74	C ₁₃ H ₂₄ O ₂ Si ₂	268	Benzene, 1-methyl-3,5-bis [(trimethyl-silyl)oxy]-		
	16.450	1.92	C ₁₉ H ₁₈ O ₆	342	Zapotin		
16.575	13.78	C ₁₉ H ₁₈ O ₆	342	Flavone, 2',5,6,6'-tetramethoxy-			
2.145	1.05	C ₅ H ₄ O ₂	96	Furfural	Stem bark		
3.243	0.20	C ₂ H ₄ O	44	Acetaldehyde			
6.160	0.21	C ₁₃ H ₂₂ O ₃ Si ₂	282	Benzaldehyde			
Aldehydes	1.458	2.88	C ₂ H ₄ O	44	Ethyl aldehyde	Leaf	
	1.842	1.70	C ₂ H ₄ O	44	Acetic aldehyde		
	2.140	0.29	C ₅ H ₄ O ₂	96	Furfural	Fruit	
	2.142	0.95	C ₅ H ₄ O ₂	96	Furfural		
	Alkanes	1.027	0.80	CH ₆ Si	46	Methylsilane	Stem bark
1.125		1.54	C ₆ H ₁₅ N ₃	129	1,3,5-Triazine, hexahydro-1,3,5-trimethyl-		
1.551		3.91	C ₆ H ₁₆ O ₂ Si	148	Diethoxydimethylsilane		
2.019		26.24	C ₆ H ₁₈ O ₃ Si ₃	222	Hexamethylcyclotrisiloxane		
3.174		1.41	C ₁₇ H ₃₈ O ₂ Si	302	Methyldodecyldiethoxysilane		
4.242		2.66	C ₈ H ₂₄ O ₄ Si ₄	296	Octamethylcyclotetrasiloxane		
4.314		31.75	C ₈ H ₂₄ O ₄ Si ₄	296	Octamethylcyclotetrasiloxane		
6.700		4.27	C ₁₀ H ₃₀ O ₅ Si ₅	370	Cyclopentasiloxane, decamethyl-		
9.075		0.18	C ₅ H ₁₁ NO ₂	117	2-Methylaminomethyl-1,3-dioxolane		
9.214		0.33	C ₁₂ H ₃₆ O ₆ Si ₆	444	Dodecamethylcyclohexasiloxane		
18.195		0.34	C ₁₂ H ₁₈ Si	190	(Z)-Trimethyl(1-methyl-2-phenylethenyl)silane		
1.556		45.26	C ₆ H ₁₆ O ₂ Si	148	Diethoxydimethylsilane	Leaf	
6.342		1.57	C ₁₀ H ₂₈ O ₄ Si ₃	296	Silicic acid, diethyl bis(trimethylsilyl) ester		
10.162		0.27	C ₆ H ₁₄	86	2,2-Dimethylbutane		
10.692		0.33	C ₃ H ₆ O ₂	74	1,3-Dioxolane		
30.884		0.57	C ₅ H ₁₂ O ₂	104	Propanal dimethyl acetal		
Alkenes		1.027	1.30	CH ₆ Si	46	Methylsilane	Fruit
		1.558	26.04	C ₆ H ₁₆ O ₂ Si	148	Diethoxydimethylsilane	
		12.891	0.33	C ₇ H ₁₆	100	Pentane, 2,2-dimethyl-	
		14.551	1.04	C ₁₂ H ₂₆	170	Decane, 3,7-dimethyl-	
	16.308	0.52	C ₆ H ₁₄	86	Butane, 2,2-dimethyl-		
	31.950	0.23	C ₁₅ H ₃₂ Si ₃	296	Cyclohexa-1,4-diene, 1,3,6-Tris(trimethylsilyl)-	Stem bark	
	1.258	2.97	C ₃ H ₅ Cl	76	1-Propene, 3-chloro-		
	1.398	2.10	C ₅ H ₈ O	84	3-Penten-2-one, (E)-		
	4.298	0.66	C ₂₀ H ₂₆ OSi	310	1-Pentene, 1,3-diphenyl-1-(trimethylsilyloxy)-		
	6.453	0.34	C ₁₀ H ₂₈ O ₄ Si ₃	296	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-		
Alkynes	6.593	4.56	C ₆ H ₁₅ O ₄ P	182	Triethyl phosphate	Leaf	
	30.625	0.46	C ₂₆ H ₂₆ OSi	382	3-Trimethylsilyloxy-1,3,5-triphenyl-4-pentyn-(E)-1-ene		
	18.085	2.03	C ₁₅ H ₃₂ Si ₃	296	1,4-Cyclohexadiene, 1,3,6-tris(trimethylsilyl)-	Fruit	
	9.742	0.24	C ₃ H ₄	40	Methylacetylene		
Alkyl halides	1.232	1.78	C ₃ H ₇ Cl	78	Propyl chloride	Stembark	
	11.061	0.42	C ₉ H ₁₉ I	254	Nonane, 1-iodo-	Fruit	
	13.463	0.36	C ₁₀ H ₂₁ I	268	1-Iododecane		
	14.397	0.58	C ₁₆ H ₃₃ BR	304	Hexadecyl bromide		
	14.667	0.69	C ₉ H ₁₉ I	254	1-Iodononane	Fruit	
	15.712	0.50	C ₉ H ₁₉ I	254	n-Nonyl iodide		
	Amines/Amides	2.808	0.16	C ₂ H ₅ NO ₂	75	Glycolamide	Stem bark
3.383		0.22	C ₉ H ₁₂ N ₂ O	164	2-Methylamino-N-phenyl-acetamide		
15.283		0.30	C ₁₂ H ₁₉ NO ₄ S	273	2,5-Dimethoxy-4-(methylsulfone)amphetamine		
1.025		1.31	C ₂ H ₇ N	45	Dimethylamine	Leaf	
1.133		1.92	C ₂ H ₄ FNO	77	Fluoroacetamide		
1.686		3.54	C ₁₀ H ₁₃ N ₅ O ₅	283	Guanosine		
2.092		0.21	C ₂ H ₅ NO	59	Acetamide		
2.917		0.39	C ₂ H ₅ NO ₂	75	Glycine		
3.175		0.24	C ₉ H ₁₃ N	135	Amphetamine		
3.262		1.10	C ₈ H ₉ NO ₂	151	p-Methoxybenzamide		
3.382	0.64	C ₁₀ H ₁₁ NO ₅	225	3-(3-Carboxy-4-hydroxyphenyl)-D-alanine			

	13.917	0.12	C ₁₆ H ₄₀ N ₂ SSi ₂	348	Bis[(1,1-dimethylpropyl)(trimethylsilyl)amino]sulfan	
	14.520	0.10	C ₃ H ₇ NO	73	Dimethylformamide	
	15.502	4.06	C ₁₄ H ₁₁ N ₃ S	253	1,5-Diphenyl-2H-1,2,4-triazoline-3-thione	
	30.442	0.37	C ₂ H ₅ NO ₂	75	Methyl carbamate	
	13.225	0.56	C ₁₁ H ₈ N ₂ O ₃	216	3-Benzoyluracil	
	14.283	0.44	C ₁₄ H ₁₉ C ₁ N ₄ O ₂	310	N-(4-Chlorophenyl)-N'-[2-(1-piperazinyl)ethyl]oxamide	Fruit
	19.748	0.41	C ₁₀ H ₁₂ C ₁₅ N ₂ O ₂ P	398	Bis(N,N-dimethylamino) pentachlorophenyl phosphate	
	13.349	0.22	C ₂₄ H ₃₈ O ₂ Si ₂	414	2,3-bis(trimethylsiloxy)-2,3-bis(4'-methylphenyl)butane	
	15.553	5.33	C ₁₄ H ₁₁ N ₃ S	253	1,5-Diphenyl-2H-1,2,4-triazoline-3-thione	Stem bark
	16.748	3.77	C ₂₂ H ₃₁ OP	342	1,3-bis((trimethylsilyl)ethynyl)tetrafluorobenzene	
Arenes	16.719	2.73	C ₂₂ H ₃₁ OP	342	Phosphine oxide, bis(pentamethylphenyl)-	Leaf
	14.833	0.78	C ₂₄ H ₁₆ N ₂	332	4,7-Diphenyl-1,10-diazaphenanthrene	
	15.100	5.00	C ₁₉ H ₂₄ O	268	Benzene, 1-methyl-3,5-bis[(trimethylsilyl)oxy]-	Fruit
	18.149	4.26	C ₁₆ H ₂₂ Si ₂	270	1,2-Diphenyltetramethyldisilane	
	18.208	1.72	C ₁₆ H ₂₂ Si ₂	270	Disilane,1,1,2,2-tetramethyl-1,2-diphenyl-	
	6.625	0.90	C ₁₆ H ₃₀ O ₄ Si ₃	370	Benzoic acid	Stem bark
	32.124	0.20	C ₂ H ₅ NO ₂	75	Carbamic acid, methyl ester	
Carboxylic acids	2.058	0.38	C ₃ H ₃ NO ₂	85	Acetic acid	Leaf
	5.978	2.71	C ₅ H ₆ O ₂	98	1,3-Butadiene-1-carboxylic acid	
	12.839	0.31	C ₈ H ₁₄ O ₃	158	n-Butyric acid anhydride	Fruit
Epoxides	1.900	0.26	C ₄ H ₈ O	72	trans-2-Butylene oxide	Fruit
	1.398	0.53	C ₆ H ₈ O ₂	112	Vinyl methacrylate	
	3.600	0.18	C ₃ H ₇ NO ₃	105	Nitric acid, isopropyl ester	
	3.751	1.42	C ₁₁ H ₁₂ O ₄	208	Terephthalic acid, ethyl methyl ester	Stem bark
	10.953	0.17	C ₁₆ H ₃₂ O ₄ Si ₂	344	bis[(t-butyl)dimethylsilyl] fumarate	
	15.133	0.18	C ₁₆ H ₂₆ O ₅ Si	326	Ethyl ester-.alpha.-o-ethyl-4-o-tms ether of vanillylman-delic acid	
	29.575	0.19	C ₁₀ H ₁₂ Cl ₅ N ₂ O ₂ P	398	Phosphorodiamidic acid, tetramethyl-, pentachlorophenyl ester	
Esters	1.083	3.00	C ₄ H ₆ O ₄	118	Ethanedioic acid, dimethyl ester	
	2.025	0.51	C ₉ H ₂₇ AsO ₃ Si ₃	342	Arsenous acid, tris(trimethylsilyl) ester	Leaf
	3.750	4.10	C ₇ H ₁₈ O ₃ Si	178	Methaneorthosiliconic Acid, triethyl ester	
	5.671	0.69	C ₁₀ H ₂₈ O ₄ Si ₃	296	Silicic acid	
	6.649	2.61	C ₆ H ₁₅ O ₄ P	182	Phosphoric acid	
	12.773	0.61	C ₁₂ H ₁₄ O ₄	222	Diethyl Phthalate	Fruit
	17.195	0.48	C ₂₅ H ₅₀ O ₂	382	Tetracosanoic acid, methyl ester	
	19.030	0.52	C ₂₃ H ₄₄ O ₂	352	Erucic acid methyl ester	
	1.333	1.22	C ₆ H ₁₄ O ₂	118	Ethylidene diethyl ether	
	2.073	3.07	C ₆ H ₁₈ O ₃ Si ₃	222	Cyclotrisiloxane, hexamethyl-	Stem bark
	5.674	2.05	C ₆ H ₁₈ O ₃ Si ₃	222	Dimethylsiloxane cyclic trimer	
	10.284	0.19	C ₃ H ₆ O ₂	74	Glycolformal	
	7.125	0.13	C ₄ H ₈ N ₂ O ₇	196	Dinitrodiglycol	
	14.001	0.21	C ₁₂ H ₂₂ O ₂ Si ₂	254	1,2-Benzenediol bis(trimethylsilyl) ether	
Ethers	26.226	0.37	C ₁₅ H ₂₀ O ₆	296	Methyl 2,3-O-[(4-Methoxyphenyl)ethylidene]-.beta.-D-ribofuranoside	Leaf
	1.091	3.00	C ₅ H ₁₂ O ₂	104	Ethoxymethyl ethyl ether	
	1.342	4.76	C ₆ H ₁₄ O ₂	118	Ethylidene diethyl ether	
	2.024	0.72	C ₆ H ₁₈ O ₃ Si ₃	222	Cyclotrisiloxane, hexamethyl-	Fruit
	3.751	1.31	C ₈ H ₂₂ O ₃ Si ₂	222	Disiloxane, 1,3-diethoxy-1,1,3,3-tetramethyl-	
	4.310	0.19	C ₈ H ₂₄ O ₄ Si ₄	296	Cyclotetrasiloxane, octamethyl-	
	14.117	0.21	C ₁₄ H ₃₁ BO	226	Borane, diethyl(decyloxy)-	
	1.185	1.92	C ₆ H ₁₂ O	100	2-Butanone, 3,3-dimethyl-	
	16.658	0.35	C ₂₈ H ₂₆ N ₂ O	406	4-Benzylamino-1,3-diphenyl-5,6,7,8-tetrahydro-quinolin-2(1H)-one	Leaf
Ketones	1.407	1.17	C ₃ H ₈ O	84	Methyl propenyl ketone	
	14.172	1.14	C ₁₃ H ₁₆ O ₂	204	1-Hydroxycyclohexyl phenyl ketone	
	14.499	0.35	C ₈ H ₁₆ O	128	4-Heptanone, 3-methyl-	Fruit
	16.650	7.44	C ₁₉ H ₁₈ O ₆	342	4H-1-Benzopyran-4-one, 2-(2,6-dimethoxyphenyl)-5,6-dimethoxy-	

Table 5: Structure and medicinal uses of compounds separated with GC-MS.

Name of the compound	Structure of the compound	Dr. Dukas medicinal use
Stem bark		
Furfural		Antiseptic, Flavor, Fungicide, Insecticide, Irritant, Pesticide.
Acetaldehyde		Addictive, Fungicide, Perfumery, Pesticide, Respirapalytic, Tyrosinase-Inhibitor
Benzaldehyde		Allergenic, Anesthetic, Antibacterial, Anticancer, Antimutagenic, Antiseptic, Antispasmodic, Antitumor, Candidicide, Flavor, Immunostimulant, Insecticide, Insectifuge, Motor-Depressant, Nematicide, Pesticide, Sedative, Termiticide, Tyrosinase-Inhibitor.
Benzoic acid		Allergenic, Anesthetic, Antibacterial, Antiotitic, Antipyretic, Antisalmonella, Antiseptic, Antiyeast, Choleric, Expectorant, Flavor, Fungicide, Insectifuge, Pesticide, Phytoalexin, Tyrosinase-Inhibitor, Uricosuric, Vulnerary.
Leaf		
Dimethylamine		Coleoptophile, Pesticide.
Glycine		Antiacid, Antialdosteronic, Antidote (Hypoglycin-A), Antiencephalopathic, Antigastric, Antiprostaitic, Antipruritic, Antisickling, Antiulcer, Cancer-Preventive, Neuroinhibitor, Uricosuric.
Silicic acid		Antidiabetic
Acetic acid		Acidulant Antibacterial, Antiotitic, Antisalmonella, Antivaginitic, Expectorant, Fungicide, Keratitigenic, Mucoytic, Osteolytic, Perfumery, Pesticide, Protisticide, Spermicide, Ulcerogenic, Verrucolytic.
Fruit		
Phosphoric acid		Acidulant, Additive, Flavor, Sequestrant.

DISCUSSION

Phytochemical screening tests were used for detection of different phytochemical compounds and discovery of new drugs subsequently may lead to the way of treatment to different diseases (Soares *et al.*, 2013). Among the qualitative phytochemical screening methanol extract reveals the higher number of phytochemicals, it may be due to the extracting ability and polarity difference of compounds found in plants (Hawaze *et al.*, 2012). Same type of results was found in the genus *S. cumini* (Bigoniya *et al.*, 2012), and *S. cordatum* (Sidney *et al.*, 2015) resulted more number of phytochemicals in methanol extract. Whereas in the case of quantification studies higher quantity of phenols were resulted in all the parts of the plant, it may be due to the selected plant is an intelligent one to accumulate these phenols to get protection from different biotic stresses and from different herbivore animals. This type of higher accumulation of phenols was found in the genus *S. cordatum* (Ndhala *et al.*, 2008). Primary metabolites are compounds that have essential roles associated with photosynthesis, respiration, growth and development. Secondary metabolites are those compounds synthesized by plants have a key role in protecting themselves from herbivores and microbial infections which have different therapeutic activities useful for mankind like, alkaloids isolated from *Sarcococca ruscifolia* having cytotoxic activity (Zhang *et al.*, 2015) and from *Alstonia scholaris* having antibacterial activity (Liu *et al.*, 2015). Flavonoids from *Cyclocarya paliurus* having antioxidant activity (Xie *et al.*, 2015) and *Chrozophora tinctoria* having anti-inflammatory activity (Abdallah *et al.*, 2015). Phenolic compounds possess a wide spectrum of biochemical activities such as antioxidant, antimutagenic, anticarcinogenic as well as ability to modify the gene expression (Nakamura *et al.*, 2003; Tapiero *et al.*, 2002). The tannin containing remedies is used as an antioxidant, antimicrobial and anti-viral purposes (Buzzini *et al.*, 2008; Koleckar *et al.*, 2008).

FT-IR is an analytical tool to detect different functional groups of plant material, which was used as a supportive tool in between qualitative phytochemical screening and GC-MS studies to enumerate different phytochemicals emerged from GC-MS. The functional groups appeared in FT-IR was correlated to qualitative phytochemical screening of methanol extracts and these studies pave a way for effective separation of different phytochemical compounds with the help of GC-MS. GC-MS is a hyphenated sensitive tool to separate group of chemical compounds into a single compound based on their molecular weight. Among the compounds separated in GC-MS instrument, the major compounds like Octamethylcyclotetrasiloxane is an organocyclic silicon compound used in the preparation of industrial silicone polymers and personal care appliances, it exhibits mild estrogenic activity in mice (He *et al.*, 2003). Hexamethylcyclotrisiloxane is a volatile methyl siloxane used for the preparation of liquid silicones and is an ingredient of lotions, fragrances and in skin care products (Wang *et al.*, 2009). Octamethylcyclotetrasiloxane and Hexamethylcyclotrisiloxane are found as major compounds in *S.*

alternifolium stem bark and which was also found in medicinal plants like *Olea europaea* (Mostafa *et al.*, 2011) and *Bauhinia acuminata* (Krishna *et al.*, 2015) respectively as major components. Acetaldehyde isolated from leaves is an important aldehyde group of organic compound occur widely in coffee, bread, fruits and produced by many of the medicinal plants, having different biological activities or properties like addictive, fungicide, perfumery, pesticide, respirapalytic, tyrosinase inhibitor (Dukes, 2013). Diethoxydimethylsilane is a flavonoid compound found in leaf and fruit parts of studied plant, having silicon as a major component and it act as excellent antimicrobial agent on different bacterial and fungal pathogens (Sihag *et al.*, 2014). This compound was also separated in leaf extracts of *Vitis vinifera* (Ceyhan *et al.*, 2012). Zapotin a natural chemo-preventive agent isolated from studied fruit part of the plant and also from *Casimiroa edulis* fruit having anticancer activity against human promyelocytic leukaemia HL-60 cells (Maiti *et al.*, 2007). Along with these, many of the compounds separated from plant parts having different biological activities when they were substituted in Dr. Duke's phytochemical and ethnobotanical database developed by Dr. Jim Duke of the Agricultural Research Service/USDA (Dukes, 2013) (Table 5).

The separated major compounds like Diethoxydimethylsilane, Acetaldehyde having antimicrobial activity and Zapotin having anticancer activity which were correlated to traditional uses of plant utilized by different ethnic groups with Dr. Duke's phytochemical and ethnobotanical database. Along with these, the minor compounds like Acetic acid, Benzaldehyde, Benzoic acid and Furfural having antimicrobial activity. Glycine acts as antiacid, antigastric and having antiulcer activity, Benzaldehyde and Glycine having anticancer activity, finally the Silicic acid having antidiabetic activity.

The quantification of minerals with ICP-OES reveals potassium among macro and iron among micro elements as higher concentrations at upper part of the plant like fruits and lower part of the plant like stem bark respectively. The same type of results was found in *Chrysanthemum indicum*, reveals higher concentrations of potassium in upper part and iron in lower part of the plant (Cui *et al.*, 2012). This may be due to, higher mobility of potassium among plant body through the xylem and deposited higher concentrations in fruits at the time of senescence or leaf falling stage. Whereas mobility of minerals like calcium, boron, iron and manganese are drastic and deposited at higher concentrations in lower parts of the plants like stem bark and leaves (Marschner *et al.*, 1996). Among the quantification of 10 mineral elements, fruit part of the plant having higher number of elements. This type of results was noticed in *Salvia sclarea* fruit part (Szentmihalyi *et al.*, 2009). Potassium is found especially high concentrations within plant cells, mixed diet and is highly concentrated in fruits (Bhaskarachary *et al.*, 2011). The tribal people of Tirumala hills eaten as ripen fruits as raw edible fruit and make into squashes and jellies used as food stuff (The Wealth of India, 1976), which may facilitates the tribal haleness. Consumption of wild edible plants containing minerals are needed

for different physiological functions of human body (Turan *et al.*, 2003), which play an important role in formation of active chemical constituents in medicinal plants and increasing the therapeutic action of prepared medicine (Adebajo *et al.*, 2013).

CONCLUSION

In the present study we prove that the *S. alternifolium* have characteristic phytochemical and mineral composition in different parts of the plant. Methanol solvent has best extractive ability to extract different primary and secondary metabolites from this plant, which pave a way for functional groups analysis by FT-IR and separation studies by GC-MS analyses. Existence of higher quantities of sugars among primary and phenols among secondary metabolites may conclude that the plant parts was rich source for energy utilization and may act as a good source towards antioxidants. GC-MS analysis of plant parts reveals different types of phytochemicals have biological activities, which may helpful to pharmacists to prepare novel drug formulations. ICP-OES analysis of plant revealed the measurable quantities of different mineral nutrients, consumption of this plant parts in different forms may useful to regulate different metabolic regulations in human beings. From these studies we explore that the plant *S. alternifolium* was not only important towards medicinally, but also for nutritionally and helpful to local people, who are utilized this plant as edible as well as for medicinal significances by more authentic way. Some members of local people endorse stem bark, leaf and fruits from the plant aggressively, due its high medicinal significance and somehow cut off the entire plant. Recent time's the plant fetches high index of forest fires, subjected to different types of diseases and faces many more threats from different ways. Finally we suggest this plant as a treasure house for pharma and nutraceutical companies to prepare novel drug and food formulations and utilize this plant parts as sustainable way not by in destructive way.

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