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GC - MS Analysis of the Ethanolic Extracts of the Leaf and Bark of *Symplocos cochinchinensis* (Lour.) Moore ssp. *Laurina*

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ABSTRACT

The therapeutic property of medicinal plants is due to the presence of certain bioactive components. These components when characterized using GC-MS can be very helpful in preparing a synthetic drug with the same property. Furthermore, GC-MS analysis can also reveal the presence of certain components in the herbal formulations which might have adverse impact on humans. The present study was conducted to identify and characterize the phytoconstituents present in the ethanolic extracts of the leaf and bark of *S.cochinchinensis* by GC-MS. The compounds were identified by comparing the peak area and their retention time with that of literature and by interpretation of mass spectra of GC-MS using the database of National Institute Standard and Technology (NIST) library. Nearly 25 phytoconstituents were identified. The prevailing compounds were n-Hexadecanoic acid, Hexadecanoic acid methyl ester, Benzoic acid, D-Allose and Cytidine. Many of these compounds are already in use by the industries for various applications like antioxidant, anti-inflammatory, anti microbial, pesticide, cancer prevention etc. Hence this analysis suggests that the ethanolic extracts of the leaf and bark of *S. cochinchinensis* can be subjected to further research to evaluate their therapeutic potential.

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INTRODUCTION

Modern medicine focuses much on the traditional practices for the discovery of new and effective drugs. Herbs are one of the major sources of compounds which can be characterized to form new drugs for a diverse range of diseases. Chemical formulations or synthetic drugs with structure similar to the natural active component are under intensive research since these synthetic compounds may have lesser side effects and more therapeutic value. According to the World Health Organization, WHO, in 2008, more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. (Pierangeli *et al*., 2009). The advanced technology has made it possible and feasible to isolate and characterize the individual components present in a particular plant extract. So if a plant is supposed to have a certain therapeutic property, even if the plant has not been studied extensively and if sufficient evidence exists for its use in traditional medicine, the active component in that plant can be characterized and analysed for its therapeutic value. In higher plants these bioactive

compounds or phytochemicals are found in vegetables, fruits, flowers, leaves and roots that act as a defense system against diseases or to be more accurate protect against diseases.(Krishnaiah *et al*., 2009). So, the current research is on the search for novel phytochemicals present in the various parts of the medicinal plants and their characterization. A knowledge of the chemical constituents of the plants is desirable not only for the discovery of therapeutic agents but also because, such information may be of great value in disclosing new sources of economic phytocompounds for the synthesis of complex chemical substances and for discovering the actual significance of folkloric remedies. (Milne *et al*.,1993).

Mass spectroscopy coupled with Gas chromatography is normally used for the direct analysis of phytoconstituents in traditional medicine and medicinal plants. Gas chromatography has a wide range of applications. It can quantitate the materials present in a sample even at very low concentration. It is a simple, sensitive and effective tool used for the qualitative and quantitative analysis of mixtures, purification of compounds etc. In the recent years, GC-MS studies have been increasingly applied for the analysis of medicinal plants, as the technique has proved to be a valuable method for the analysis of non polar components and volatile essential oil, fatty acids, lipids (Jie *et al*.,1988) and alkaloids (Betz *et al*.,1997). In the analysis of herbal medicines, GC-MS has at least two advantages: 1.With the capillary column, GC-MS, has in general a very good separation ability which can produce a chemical fingerprint of high quality. 2. With the coupled mass spectroscopy and the corresponding mass spectral database, the qualitative and relatively quantitative composition information of the herb could be investigated by GC-MS (Sermakanni *et al*., 2012). These data will be extremely useful for further research with the medicinal plants.

India, renowned for its traditional medicinal practices is a treasure groove of many medicinal plants. In olden days, people supplemented their diets with the medicinal herbs, in such a way that they did not have the need for any specific treatment against diseases. But now, we tend to borrow concepts from those traditional practices and try to develop medical formulations from those herbs. Among the most frequently used herb in the Ayurvedic formulation, the most common is LODHRA. This is actually *Symplocos racemosa* but at times when it was unavailable two other species viz., *S.paniculata* and *S. cochinchinensis* were also used as substitutes. (Pooja Singh *et al*., 2015).

The latter species are not extensively studied. But they contain bioactive compounds and are proved to cure many ailments. Previous research on the methanolic and ethanolic extracts of the leaf and bark of *S.cochinchinensis* has shown the presence of various phytoconstituents like phenols, flavonoids and alkaloids. (Archana *et al*., 2015). The aqueous methanol extract was found to possess antioxidant activity. (Sunil *et al*., 2012). So, the present study was done to characterize the phytocomponents of the ethanolic water extract of the leaf and bark of *S. cochinchinensis* by GC-MS analysis. The characterization of the volatile organic matter of the leaf and bark of *S. cochinchinensis* is done for the first time. This work will help in the identification of compounds that may be used by the body or that may possess certain therapeutic value.

MATERIALS AND METHODS

Plant source and preparation of the plant extracts

The leaves and bark of *Symplocos cochinchinensis* (Figures 1,2) were collected from the Western Ghats, Nilgiris, India in the month of June 2015. The plant parts were authenticated by Dr.Chelladurai, Research officer, Central council for research in Ayurveda and Sidda. The leaves and bark were shade dried and powdered. The ethanolic extracts of the dried powders were prepared. 10 g of the dried powder was dissolved in 100 ml of 1:2 ratio of ethanol – water. The contents were stirred well and left for 48 hours at room temperature. The filtrate collected after cold percolation was used for further analysis.

GC MS Analysis

GC MS analysis was carried out on Shimadzu 2010 plus comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column RTX 5Ms (Column diameter is 0..32mm, column length is 30m, column thickness 0.50µm), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1.73 ml /min and an injection volume of 0.5 µI was employed (split ratio of 10:1) injector temperature 270 ºC; ion-source temperature 200 ºC. The oven temperature was programmed from 40 ºC (isothermal for 2 min), with an increase of 8 ºC/min, to 150ºC, then 8ºC/min to 250ºC, ending with a 20min isothermal at 280ºC. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 51.25min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a Turbo Mass Ver 5.2.0 (Srinivasan *et al*., 2013).

Identification of components

Interpretation on GC MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained (Dr. Dukes, 2013).

RESULTS

In the present study twenty five chemical constituents have been identified from the ethanolic extract of the bark of *S.cochinchinenis* by Gas Chromatogram-Mass spectrometry (GC-MS) analysis. The prevailing compounds were n-Hexadecanoic acid, Diethyl Phthalate, Heptadecanoic acid, Hexadecanoic acid, methyl ester, 2-Methoxy-4-Vinylphenol, Hexadecanol and 1-Octadecanol (Table 1 and Fig 1). The biological activity of the bark extract is represented in the Table 2. ((Dr. Dukes, 2013).

Similarly twenty five chemical constituents have been identified from the ethanolic extract of the leaf of *S. cochinchinensis* by Gas Chromatogram- Mass spectrometry (GC-MS) analysis. The prevailing compounds were n-Hexadecanoic acid, Hexadecanoic acid, methyl ester, Benzoic acid, D-Allose and Cytidine (Table 3 and Fig 2). The presence of various bioactive compounds justifies the use of plant extract for various ailments by traditional practitioners. The biological activity of the leaf extract is represented in the Table 4. ((Dr. Dukes, 2013). Comparisons of the phytocompounds present in the leaf and bark extracts is given in Table 5.

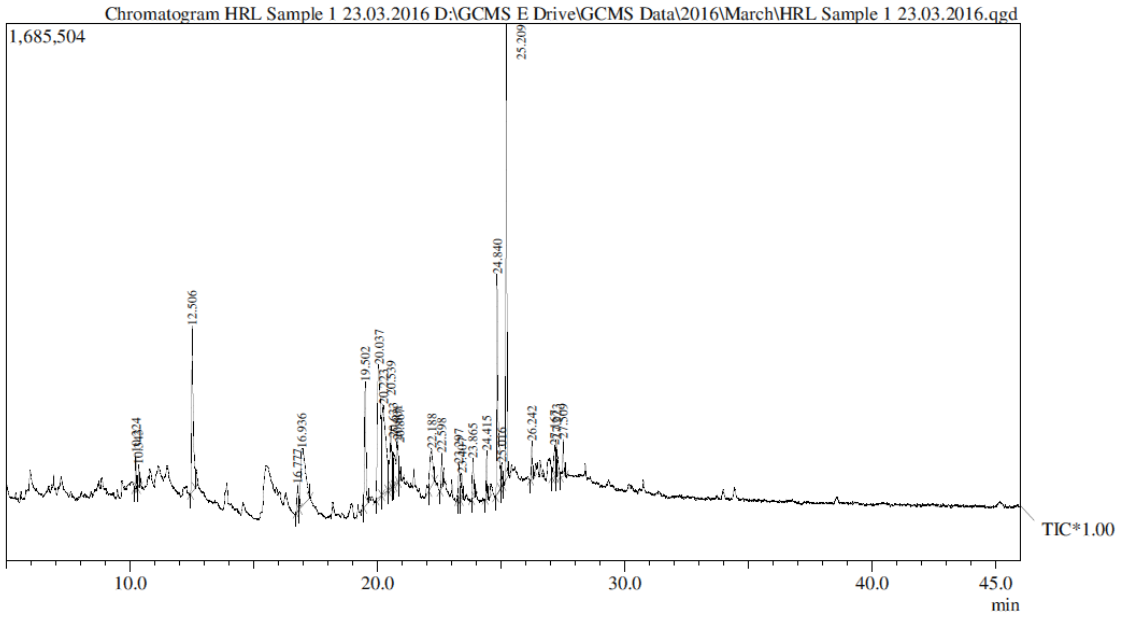

Fig: 1 GC MS chromatogram of ethanolic extract of the bark of *S.cochinchinensis*

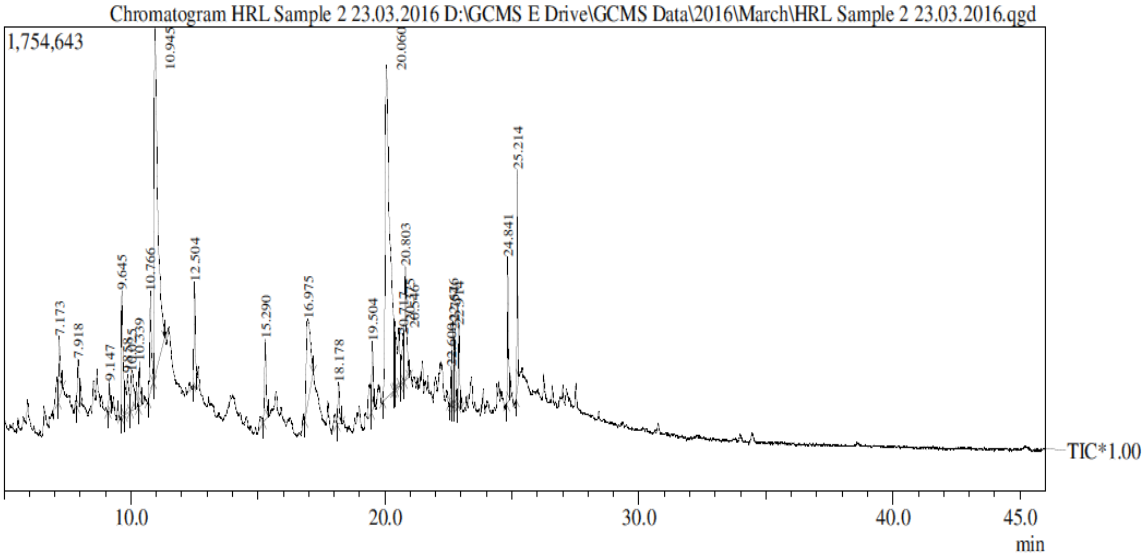

Fig: 2 GC MS chromatogram of ethanolic extract of the leaf of *S.cochinchinensis*

Table 1 Phytocompounds identified in the ethanolic extract of the bark of *S.cochinchinensis* by GC-MS study.

Peak	R.Time	Area%	Name of the compound	Molecular formula	Molecular Weight
1	10.224	0.80	1-Tridecene	$C_{13}H_{26}$	182
2	10.343	0.70	Hydroxylamine, O-decyl	$C_{10}H_{23}NO$	173
3	12.506	8.61	2-Methoxy-4-Vinylphenol	$C_9H_{10}O_2$	150
4	16.777	1.23	Cycloheptasiloxane, Tetradecamethyl	$C_{14}H_{42}O_7Si_7$	518
5	16.936	8.87	Beta.-D-Glucopyranose, 1,6-Anhydro	$C_6H_{10}O_5$	162
6	19.502	5.18	Diethyl Phthalate	$C_{12}H_{14}O_4$	222
7	20.037	13.59	Ethyl .Alpha.-d-Glucopyranoside	$C_8H_{16}O_6$	208
8	20.223	10.52	Methyl .Beta.-d-Galactopyranoside	$C_7H_{14}O_6$	194
9	20.539	4.34	Benzoic Acid, 2,6-Bis(Trimethylsil)	$C_9H_{10}O_2$	150
10	20.633	1.82	2-Butanone, 4-(4-Hydroxy-3-Metho	$C_{11}H_{14}O_3$	194
11	20.801	3.65	(4,4-Dimethyl-2,4,5,6-Tetrahydro-1H-inden-2-	$C_{13}H_{18}O_2$	206
12	20.867	0.81	Bacteriochlorophyll-C-Stearyl	$C_{52}H_{72}MgN_4O_4$	840
13	22.188	3.50	2-Propenal, 3-(4-Hydroxy-3-Methoxyphenyl)	$C_{10}H_{10}O_3$	178
14	22.598	1.29	3-Phenyl-2-[(2,3,4-Trimethoxy-6-Nit	$C_{24}H_{21}N_3O_6S$	479
15	23.297	1.59	E-6-Octadecen-1-ol Acetate	$C_{20}H_{38}O_2$	310
16	23.407	1.07	2-Pentadecanone, 6,10,14-Trimethyl-	$C_{18}H_{36}O$	268
17	23.865	1.48	1-Hexadecanol	$C_{16}H_{34}O$	242
18	24.415	1.42	Hexadecanoic Acid, Methyl Ester	$C_{17}H_{34}O_2$	270
19	24.840	10.17	n-Hexadecanoic Acid	$C_{16}H_{32}O_2$	256
20	25.016	1.03	Dibutyl Phthalate	$C_{16}H_{22}O_4$	278
21	25.209	12.33	Heptadecanoic Acid, Ethyl Ester	$C_{19}H_{38}O_2$	298
22	26.242	1.40	1-Octadecanol	$C_{18}H_{38}O$	270
23	27.167	1.90	Cyclopropanebutanoic Acid, 2-[[2-[[2-[(2-pent	$C_{25}H_{42}O_2$	374
24	27.223	1.26	Ethyl Oleate	$C_{20}H_{38}O_2$	310
25	27.509	1.44	Ethyl Pentadecanoate	$C_{17}H_{34}O_2$	270

Table 2 Activity of some of the phytocomponents identified in the ethanolic extract of bark of *S.cochinchinensis* by GC-MS.

S.No	Compound name	Nature of Compound	Biological activity**
1	n-Hexadecanoic acid	Palmitic acid	Antioxidant, Nematicide, 5-Alpha-Reductase-Inhibitor, Flavor, Hemolytic, Hypercholesterolemic, Pesticide Antialopecic, Antiandrogenic,Antifibrinolytic
2	Diethyl Phthalate	Plasticizer compound	Antimicrobial, Antifouling
3	Heptadecanoic acid	Margaric acid	Antioxidant, anti fungal, surfactant
4	Hexadecanoic acid, methyl ester	Fatty acid ester	Antioxidant, Hypocholesterolemic, Antiandrogenic, Hemolytic, Alpha Reducatase inhibitor.
5	2-Methoxy-4-Vinylphenol	Phenol	Antioxidant, Antifungal
6	Hexadecanol	Fatty alcohol	Antiviral, diuretic, antianemic, insecticide
7	1-Octadecanol	Fatty alcohol	Anaphylactic (antidote=Neostigmine) Antitumor (Nasopharynx) Decrease Norepinephrine Production Increase Natural Killer (NK) Cell Activity Increase natural killer cell activity Increase NK Cell Activity

**Dr.Duke's Phytochemical and Ethno botanical Databases, Phytochemical and Ethnobotanical Databases. https://phytochem.nal.usda.gov/phytochem/search/list.

Table 3 Phytocompounds identified in the ethanolic extract of the leaf of *S.cochinchinensis* by GC-MS study.

Peak	R.Time	Area%	Name of the compound	Molecular formula	Molecular weight
1	7.173	1.82	2,4-Imidazolidinedione, 3-Methyl	$C_4H_6N_2O_2$	114
2	7.918	1.34	4-Heptanone, 2-Methyl-	$C_8H_{16}O$	128
3	9.147	0.78	Levoglucosenone	$C_6H_6O_3$	126
4	9.645	3.46	4H-Pyran-4-one, 2,3-Dihydro-3,5-Dihydroxy-6-	$C_6H_8O_4$	144
5	9.858	2.30	Benzoic Acid	$C_7H_6O_2$	122
6	10.025	2.00	Cytidine	$C_9H_{13}N_3O_5$	243
7	10.339	1.03	4-Methyl-2-Oxopentanenitrile	C_6H_9NO	111
8	10.766	3.13	2,3-Dihydro-Benzofuran	C_8H_8O	120
9	10.945	20.92	5-Hydroxymethylfurfural	$C_6H_6O_3$	126
10	12.504	2.53	2-Methoxy-4-Vinylphenol	$C_9H_{10}O_2$	150
11	15.290	2.47	1,1'-Bicycloheptyl	$C_{14}H2_6$	194
12	16.975	6.00	D-Allose	$C_6H_{12}O_6$	180
13	18.178	1.18	Cyclohexene, 1-Methyl-3-(Formylmethyl)	$C_9H_{14}O$	138
14	19.504	1.51	Diethyl phthalate	$C_{12}H_{14}O_4$	222
15	20.060	26.50	Beta.-D-Glucopyranoside, methyl	$C_7H_{14}O_6$	194
16	20.375	1.61	Tricyclo[2.2.1.0(2,6)]Heptan-3-OL	$C_7H_{10}O$	110
17	20.546	3.06	Benzoic Acid, 2,6-Bis Trimethylsil	$C_{16}H_{30}O_4Si_3$	370
18	20.717	2.09	Cyclohexanol, 4-[(trimethylsilyl)oxy]-, cis-	$C_9H_{20}O_2Si$	188
19	20.803	3.88	2-Cyclohexen-1-OL, 2,4,4-Trimethy	$C_{16}H_{24}O_2$	248
20	22.600	0.68	N-[3-(5-Furan-2-YL-[1,3,4]Oxadiazol-	$C_{26}H_{19}N_3O_3$	421
21	22.676	2.03	2(4H)-Benzofuranone, 5,6,7,7A-tetr	$C_{11}H_{16}O_3$	196
22	22.754	1.71	6-(3-Hydroxy-But-1-Enyl)-1,5,5-Trim	$C_{13}H_{22}O_3$	226
23	22.914	1.70	2-Cyclohexen-1-one, 4-Hydroxy-3,5,5-Trimeth	$C_{13}H_{18}O_3$	222
24	24.841	2.91	n-Hexadecanoic Acid	$C_{16}H_{32}O_2$	256
25	25.214	3.38	Hexadecanoic Acid, Ethyl Ester	$C_{18}H_{36}O_2$	284

Table 4 Activity of some of the phytocomponents identified in the ethanolic extract of the leaf of *S.cochinchinensis* by GC-MS.

S.No	Compound name	Nature of Compound	Biological activity**
1	n-Hexadecanoic acid	Palmitic acid	Antioxidant, Hypocholesterolemic nemaicide, pesticde, Anti-androgenic flavor, hemalytic, 5-Alpha reductase inhibitor
2	Hexadecanoic acid, methyl ester	Fatty acid ester	Antioxidant, Hypocholesterolemic, Antiandrogenic, Hemolytic, Alpha Reducatase inhibitor.
3	Benzoic acid	Benzen	Arachidonic acid-Inhibitor, Increase Aromatic Amino Acid Decarboxylase Activity and Inhibit Production of Uric Acid
4	D-Allose	Aldohexose sugar	Alcohol-Dehydrogenase-Inhibitor, Anticancer (Duodenum), Antidote (Diazepam), Antidote (Digoxin), Antileukotriene-D4, Circulatory-Depressant, CNS-Depressant and Coronary-Dilator
5	Cytidine	Nucleoside molecule	Glutamatergic antidepressant drug

**Dr.Duke's Phytochemical and Ethno botanical Databases, Phytochemical and Ethnobotanical Databases. https://phytochem.nal.usda.gov/phytochem/search/list.

Table 5 Phytocompounds present both in the leaf and bark ethanol extracts of *Symplocos cochinchinensis*

		Bark extract		Leaf extract			
S.No.	**Phytoconstituents**	**Rt**	**Area %**	**Rt**	**Area%**	**Mol.Formula**	**Mol.wt.**
1.	2- methoxy -4-vinylphenol	12.506	8.61	12.504	2.53	$C_9H_{10}O_2$	150
2.	Diethyl Phthalate	19.502	5.18	19.504	1.51	$C_{12}H_{14}O_{14}$	222
3.	Benzoic acid, 2,6- Bis (Trimethylsil)	20.539	4.34	20.546	3.06	$C_9H_{10}O_2$	150
4.	n-Hexadecanoic acid	24.840	10.17	24.841	2.91	$C_{16}H_{32}O_2$	256

DISCUSSION

The knowledge of the phytocomponents in a medicinal plant is very essential to analyse their therapeutic value. Such precise qualitative analysis can be obtained by Gas chromatography coupled with mass spectroscopy (GC-MS). (Cong *et al.*, 2007). In the present study, an elaborative analysis of the ethanolic extract of the leaf and bark of *S.cochinchinensis* using GC-MS was done. The study has revealed the presence of 25 compounds. The compounds identified possess various biological properties. For instance, n-hexadecanoic acid – palmitic acid (R/T,2.91) is an antioxidant, hypocholestrolemic, pesticide, hemolytic, 5 –α reductase inhibitor and also has lubricant properties.

Hexadecanoic acid has been reported as a component in the alcohol extract of the leaves of *Kigelia pinnata* (Grace *et al.*,2002) and *Melissa officinalis* (Sharafzadeh *et al.*,2011). Similar results were also seen in the ethylacetate extract of *Goniothalamus umbrosus* (Siidig Ibrahim *et al.*,2009), *Cleistanthus collinus* (Parasuraman *et al.*,2009) and *Euphorbia longum* leaves (Devi *et al.*, 2009). n-hexadecanoic acid was present in *Caesalpania sappan* ethanol extract and was found to be effective against acetaminophen induced nephrotoxicity and oxidative stress in male albino rats. (Sarumathy *et al.*, 2011). n-hexadecanoic acid, hexadecanoic acid, Phytol, 9,12,15 Octadecatrienoic acid and squalene were identified in the ethanol leaf extract of *Aloe vera* (Arunkumar *et al.*, 2009) and *Vitex negundo* (Praveen Kumar *et al.*, 2010)

Major bioactive compound methyl ester of hexadecanoic acid was isolated from the leaves of *Annona muricata* and it was proved to have antifungal potentials.(Abubacker *et al.*,2013). Another bioactive compound revealed in GC-MS analysis of the ethanolic leaf extract of *S.cochinchinensis* is D-Allose . In previous studies

D-Allose is proved to inhibit the growth of cancer cells at G1 phase through specific thioredoxin interacting protein induction without exerting appreciable effect on normal cells. Benzoic acid was found in the ethanol extract of the leaf and bark of *S.cochinchinensis*. It has various medicinal uses. Benzoic acid is a constituent of Whitfield"s ointment which is used in the treatment of fungal diseases like Tinea, ringworm and athlete's foot.(Charles Owens Wilson *et al*.,2004). Benzoic acid was used as an expectorat, analgesic and antiseptic in the early 20th century. (Benjamin Lilliard, 1919). Certain phytoconstituents identified in the ethanol extract of the leaf and bark of *S.cochinchinensis* have other practical applications also. For example, 2-methoxy-4-vinylphenol is an aromatic substance used as a flavouring agent. (WHO,2007). It is one of the compounds responsible for the natural aroma of buckwheat. (Janes D *et al*.,2008).

The GC-MS analyses of the ethanolic extracts of the leaf and bark of *S.cochinchinensis* revealed many compounds with proven antioxidant or antitumor properties namely, n-hexadecanoic acid ethyl ester, D-allose, 1- Octadeacanol, heptadeacanoic acid etc. This is a novel study since the GC-MS characterization of these bioactive compounds in the ethanolic extracts of the leaf and bark of *S.cochinchinensis* has not yet been reported. These findings suggest that both the leaf and bark extracts of *S.cochinchinensis* can be used for further studies.

CONCLUSION

Medicinal plants possess certain bioactive compounds which might be in varying concentrations or may not be easily isolated. The aim of modern research is to identify the compound and purify and characterize them, so that it might be possible to develop synthetic analogues in case, the plant species is not widely available. Unique qualitative and quantitative patterns from a GC-MS analysis will often help to identify the bioactive compounds in the herbal extracts. GC-MS is a fast and direct analytical approach requiring only a few grams of the plant material. In the present study, the GC-MS analysis of the ethanolic extracts of the leaf and bark of *S. cochinchinensis* was done. This is the first report for the plant species. Various bioactive compounds were revealed. These compounds have proven therapeutic values. So further research is required to evaluate the potential therapeutic value of the bioactive compounds present in the leak and bark of *S.cochinchinensis.*

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