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PHYTOCHEMICAL ANALYSIS OF *LINDERNIA MADAYIPARENSE* EXTRACTS BY GC-MS.

S. UMAKRITHIKA[1*], P. K. MANNA[2], K. KANNAN[2], M. K. M. ABDUL LATIFF[1]

1. Department of Pharmaceutical Chemistry, Swamy Vivekanandha College of Pharmacy, Tiruchengode.
2. Department of Pharmacy, Annamalai University, Chidambaram.

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Abstract: Background: However, lindernia species were considered as weeds, they occupy a most important place in the traditional system of medicine used worldwide especially in China. Linderniaceae is a family having high traditional medical summary with less pharmacognostical, phytochemical and biological profile. **Objective:** This study was designed to identify and characterize the phytochemical profile of different crude extracts of newly identified whole plant *Lindernia madayiparense*, using gas chromatography–mass spectrometry (GC- MS). **Method:** Powdered plant material was extracted and evaluated for various *in-vitro* pharmacological activities. Furthermore the potent extracts were analyzed by GC- MS. **Results:** The obtained Total ion chromatogram of potent extracts revealed the different types of having small and moderate phytochemicals under the classification of alkaloids, glycosides, terpenoids and phenols in major and minor amount. **Conclusions:** The study gives a detailed insight about the phytochemical profiles of three crude extracts of *Lindernia madayiparense.* So the chemical entities found are may be responsible for the pharmacological activities that probably will act as lead molecules for furthermore drug development process.

Keywords: GC-MS, Phytocomponents, *Lindernia madayiparense*, Ethanol, Aqueous, Pet-ether.

Corresponding Author: S. UMAKRITHIKA

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INTRODUCTION

The medicinal plants quoted in various traditional system of medicines habituated worldwide, becomes a major resource for discovery of a great number of bioactive substances having many therapeutic uses with less or no side effects. It has been estimated that 74% of the pharmacologically active, plant-derived components were discovered after the ethnomedicinal uses of the plants started to be investigated.[1] Another important way of discovering new medicinal plants and lead compounds is the phylogenetic approach, in which a number of closely related species of plants, assumed to contain related chemical compounds are screened for their biological effects.[2] The active principles are extracted from the plants and purified for therapeutic utility and characterized by different specific techniques. Presently, a modern phytochemical screening technique to characterize the active constituents of pharmaceutical significance mainly involves chromatographic examination which is used to identify the phyto chemicals of crude drug based on the use of major chemical constituents as markers and to estimate the amount of the major classes of constituents. Gas chromatography combined with mass spectroscopy (GC-MS) is one of the most precise methods to identify the various secondary metabolites present in the plant extract mainly small polar molecules.

One of the plant families with a high traditional medical summary with less pharmacognostical, the phytochemical and biological profile was linderniaceae. Plants belonging linderniaceae are one of the major source of herbal preparations in several traditional medicines practised in various nations including China and India. Linderniaceae is a family of flowering plants in the order Lamiales, which comprises of around 13 genera and 195 species from around the world, commonly in the neotropics.[3] There are 22 species reported so far in India, in that 18 species were recorded in the checklist of angiosperms of Kerala.[4] During the floristic exploration in Madayipara, Kannur district, a new plant species '*Lindernia madayiparense*,' was discovered by the scientists from the M.S. Swaminathan Research Foundation, Kalpetta in May 2012.[5]

MATERIALS AND METHOD:

The GC-MS analysis was performed at 'The South India Textile Research Association' (SITRA), Coimbatore, Tamil Nadu, India

Plant material:

Wild crafted plant, *Lindernia madayiparense* was collected during its flowering season in the month of October to December, 2013 in Kannur District, Kerala, India. The plant material was identified and authenticated by botanist Mr. P. Biju, Assistant Professor, Government College, Kasaragod, Kerala, India.[6]

Preparation of plant extracts

The powdered whole plant material, *Lindernia madayiparense* was subjected to two different extraction procedures, i.e. Decoction and continuous hot extraction process. By these methods, aqueous extract, petroleum ether extract and ethanol extract were obtained. All the extracts were concentrated, dried and preserved at 8°C until use. [7-10]

Preparation of sample

Petroleum ether, aqueous and ethanol extracts of *Lindernia madayiparense* were used for GC/MS analysis. The sample was prepared by dissolving lyophilized extract of *Lindernia madayiparense* in methanol at 1 mg/ml of concentration. The volume of 1.0 µl of each sample was injected into the GC-MS system for analysis of possible active phytoconstituents.

Instruments and chromatographic conditions

Petroleum ether, aqueous and ethanol extracts of the plant, *Lindernia madayiparense* was separately injected into the Gas chromatography unit. The instrument used for GC-MS analysis was Thermo GC – Trace Ultra Ver.5.0, Thermo MS DSQ II, a gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument equipped with an autosampler. 1 µl of each extract of the plant was injected into GC. The injector temperature was maintained at 260^{0}C. The detector used was thermal conductivity detector which was maintained at 260^{o}C. The pressure of the carrier gas, helium was kept at 10 psi and maintained the flow rate at 1.0 ml/min. The oven temperature was set at 70^{o}C and raised to 260^{o}C with a gradual increment of 6^{o}C per min. The injected extracts were eluted in the DB-35 MS capillary standard non-polar column of 30 m long, 0.25 mm inner diameter and the film thickness of 0.25 µm. The eluted constituents were detected by flame ionization detector, and the GC chromatogram was recorded. The mass spectra of compounds in samples were obtained by electron ionization (EI) at 70 eV and the detector operated in scan mode from 20 to 600 atomic mass units (amu).[11] The chromatogram a plot of intensity against retention time was recorded by the software attached to it. The compounds found in the extract were matched with the National Institute of Standard and Technology (NIST) library.

RESULTS:

The spectrum obtained from the GC-MS gave the chemical proforma of individual phytochemicals present in the extracts.

The Total ion chromatogram (TIC) of Aqueoues extract of *L. madayiparense* was shown in fig. 1 .The major constituents identified on Aqueous extract were (1RS,2SR,3RS,4SR)-3-(2-propenyl)-1,2,4-cyclopentanetriol(61.42), Synaptogenin B(4.88) and Di-(2-ethylhexyl)phthalate(10.44)

and 20 minor components were identified along with Arbutin and Phytol which were tabulated in Table. 1.

The TIC of ethanol extract of *L. madayiparense* was shown in fig. 2 .The major constituents identified on Ethanolic extract were Hexadecanoic acid, ethyl ester (14.40) and Ethyl 6,9,12-hexadecatrienoate (29.18) and 23 minor components which were tabulated in Table. 2.

The TIC of petroleum ether extract of *L. madayiparense* was shown in fig. 3 .The major constituents identified on Petroleum ether extract were 11-Heneicosanone, Hexadecane, Octadecane, 2-Pentadecanone, 6,10,14-trimethyl, Ethyl 2-dichloromethylhexanoate which were tabulated in Table. 3.

Discussion:

GC-MS is applicable for solids, liquids and gaseous samples. Injected sample is converted into a gaseous state and isolated based on their retention time. Then the molecules are analyzed by mass spectroscopy on the basis of mass by charge ratio. Generally, flavonoids were decomposed during GC-MS analysis.

The potent crude extracts of *L. madayiparense* were selected for GC-MS analysis depends upon the information provided by the *in vitro* screening methods of diverse pharmacological activities. The petroleum ether extract have showed potent anti-cancer activity against Human Lung Carcinoma Cell Lines (A-549) and Human Hepatocellular Carcinoma Cell Lines (HepG-2) and aqueous and ethanol extracts have high antioxidant and anthelmintic activity.

The awareness in correlating the phytochemical constituents with its pharmacological activity keeps on growing in the pharmaceutical and herbal industry. So, the effective plant extracts were analysed to explore the chemical profile of phytoconstituents. [12] Results revealed pet ether extract has major amount of volatile compounds belongs to hydrocarbon and alcohol family which may cause toxicity to the A549 and Hep-G cancer cell lines.[13, 14] Aqueous and ethanol extracts contains steroid glycosides, terpenoids and alkaloids in major amounts which act by various mechanisms involving in anthelmintic and antioxidant activity.[15, 16]

CONCLUSION

Since the crude extracts from *L. madayiparense* exhibited marked anti-cancer, anthelmintic and anti-oxidant activities, our current investigation was to explore the GC- MS profile of potent aqueous, ethanol and petroleum ether extracts of *Lindernia madayiparense* which were not reported yet.

The study reveals that the major and minor bioactive compounds of each extract of *L. madayiparense*. The reported biological activities and the identified secondary metabolites support the medicinal application of the *L. madayiparense* which in turn recommended the plant for the resource of new plant-based drugs.

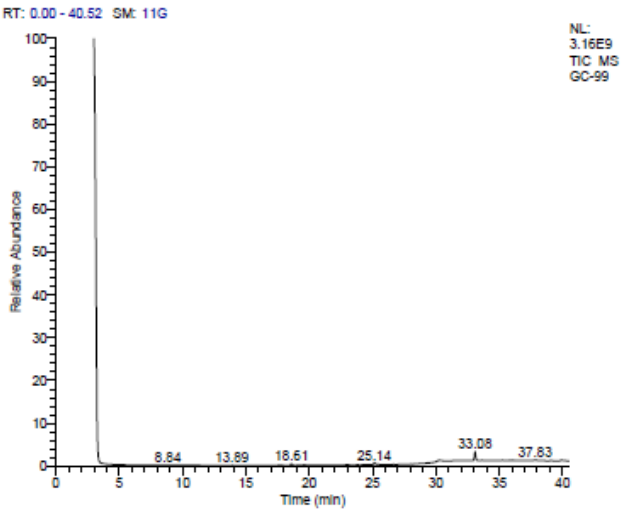

Fig. 1. Total ion chromatogram of Aqueous extract of *Lindernia madayiparense*

Table.1 Identified phytocomponents in TIC of Aqueous extract of *Lindernia madayiparense*

S. No	RT	IUPAC name	Molecular Formula	Molecular weight	Peak Area
1	3.09	(1RS,2SR,3RS,4SR)-3-(2-propenyl)-1,2,4-cyclopentanetriol 2-o-ethoxyethyl ether	C12H22O4	230	61.42
2	7.55	Pentacosanoic acid, 2,10-dimethyl-, methyl ester, [S-(R*,S*)]-	C28H56O2	424	0.35
3	8.84	Hexadecanoic acid, ethyl ester	C28H56O2	284	0.84
4	10.82	Benzene, (1-hexadecylheptadecyl)	C39H72	540	0.42

5	17.68	Dimethyl 5,6,7,8-tetramethyl-2-(2',3',4',5,6'-pentamethylphenyl)heptalene-3,4-dicarboxylate	C31H36O4	472	0.53
6	18.61	Neophytadiene	C20H38	278	2.19
7	19.22	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C20H40O	296	0.38
8	19.6	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C20H40O	296	1.34
9	21.68	10-Heneicosene	C21H42	294	0.45
10	23.04	6-amino-2-methoxy-4-(prop-2-ynyloxy)pyrimidine	C8H9N3O2	179	0.97
11	23.81	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	C17H24O3	276	0.44
12	24.2	1,4-Dioxaspiro[4.5]decane, 6-methylene	C9H14O2	154	0.38
13	25.14	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]] (Phytol)	C20H40O	296	3.92
14	26.65	Octadecanoic acid, ethyl ester	C20H40O2	312	0.57
15	30.26	Synaptogenin B	C30H46O4	470	4.88
16	31.55	(2RS)-1,3,8-trimethyl-4-propyl-5-ethyl-2-(1-hydroxyethyl)-7-methoxycarbonylethyl-6,.gmma.-methylenecarbonyl-porphine	C36H42N4O4	594	0.85
17	31.88	3-n-Pentadecyl-2,4-dinitrophenol	C21H34N2O5	394	0.79
18	33.08	Di-(2-ethylhexyl)phthalate	C24H38O4	390	10.44
19	33.67	Methyl 5-oxo-3,4-dinor-2,3-secocholestan-2-oate	C26H44O3	404	0.66
20	34.18	(2RS)-1,3,8-trimethyl-4-propyl-5-ethyl-2-(1-hydroxyethyl)-7-methoxycarbonylethyl-6,.gmma.-methylenecarbonyl-porphine	C36H42N4O4	594	0.42
21	37.83	(E)-á-iodo-à-nitrostilbene	C14H10INO2	351	1.6

22	39.06	[1,1':2',1''-Terphenyl]-3',4'-dicarboxylic acid, 5',6'-diphenyl-,dimethyl ester	C34H26O4	498	0.48
23	39.93	N,N-Dimethyl-4-(2,2,2-trifluoro-1-hydroxyethyl)aniline	C10H12F3NO	219	1.42

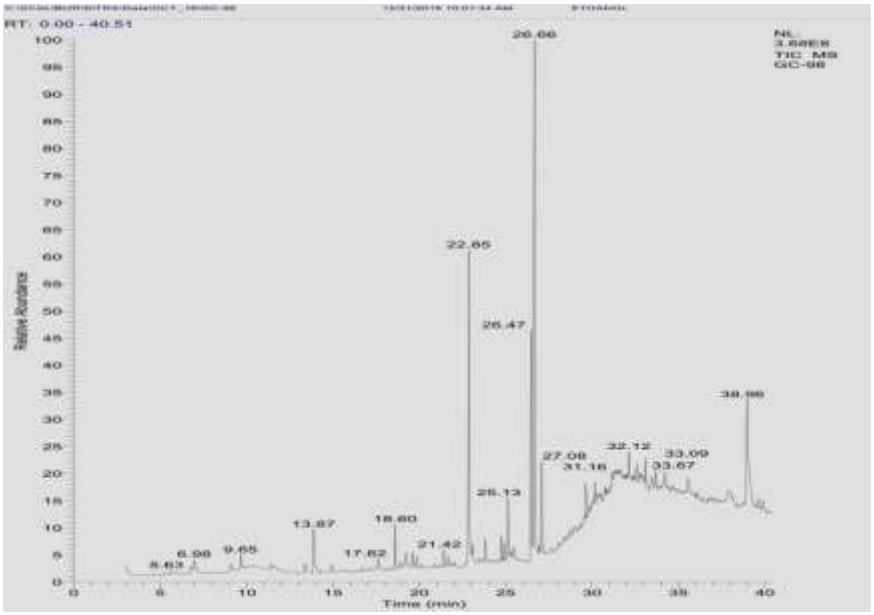

Fig. 2 Total ion chromatogram of Ethanolic extract of Lindernia madayiparense

Table.2. Identified phytocomponents in TIC of Ethanol extract of *Lindernia madayiparense*

S.No	RT	IUPAC Name	Molecular Formula	Molecular Weight	Peak Area
1	7	Acetic acid, 2-ethylhexyl ester	C10H20O2	172	1.86
2	9.67	Cyclotetradecane	C14H28	196	1.11
3	10.31	Methyl 4-hydroxypentanoate	C6H12O3	132	0.88
4	11.45	5,5'-difluoro-2,2'-(propane-1,3-diyldiimino)bis(benzyl alcohol)	C17H20F2N2O2	322	0.90

5	13.9	2-tert-Butyl-4-isopropyl-5-methylphenol	C14H22O	206	2.86
6	17.62	1-Hexadecanol (CAS)	C16H34O	242	0.89
7	18.6	Neophytadiene	C20H38	278	1.44
8	19.23	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl	C20H40O	296	1.47
9	21.66	N-[3'-Cyano-6'-(3''-methyl-5''-oxo-1''-phenyl-2''-pyrazolin-4''-yl)-4'-phenylpyridin-2'-yl]benzamide	C29H21N5O2	471	1.12
10	22.87	Hexadecanoic acid, ethyl ester	C18H36O2	284	14.40
12	23.82	7,9-di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2, 8-dione	C17H24O3	276	1.69
13	24.74	6-Iodoacetoveratrone	C10H11IO3	306	1.29
14	25.47	Tetradecanal	C20H40O	296	2.88
15	26.66	Ethyl linoleate	C14H28O	212	0.92
16	27.06	Ethyl 6,9,12-hexadecatrienoate	C20H36O2	308	29.18
17	29.65	Methyl 19-methyl-eicosanoate	C18H30O2	278	4.50
18	30.14	2-Bromo-1-(2',2'-bis(methoxycarbonyl)-12'-tridecenyl)-5,5-bis(methoxycarbonyl)-3-methylenecyclohexene	C22H44O2	340	1.02
19	32.59	3,4,6,7,12,12b-Hexahydro-2-methoxy-4-(4'-bromophenyl)indolo[2,3-a]quinolizine	C28H41BrO8	584	3.42

20	33.09	Phthalic acid, 2-ethylhexyl pentadecyl ester	C28H48O7	496	4.05
21	33.44	5-(Ethoxycarbonyl)-7-[(4-fluorophenyl)amino]thieno[3,2-d][1,3]diazepine	C22H21BrN2O	408	0.85
22	33.67	3á-Acetoxy-2'-cyclohexyl-2'',3'',4'',5'',16á,17á-hexahydro-2'H- 5à-androstano[16,17-e]furo[3'',4''-c][1',2']oxazin-2''-one	C31H52O4	488	1.88
23	35.56	2,2-bis(Hydroxymethyl)propane-1,3-diyl -bis(2'-hydroxybenzoate)	C31H47NO5	513	0.83
24	37.92	[1]Benzothieno[2',3'-3,4]thieno[3'',2''-7,8]cycloocta[1,2-b:5,6-b']diquinoxaline	C19H20O8	376	1.81
25	38.96	3-Hexene, 1-(1-methoxyethoxy)	C28H32O4Si4	544	0.69

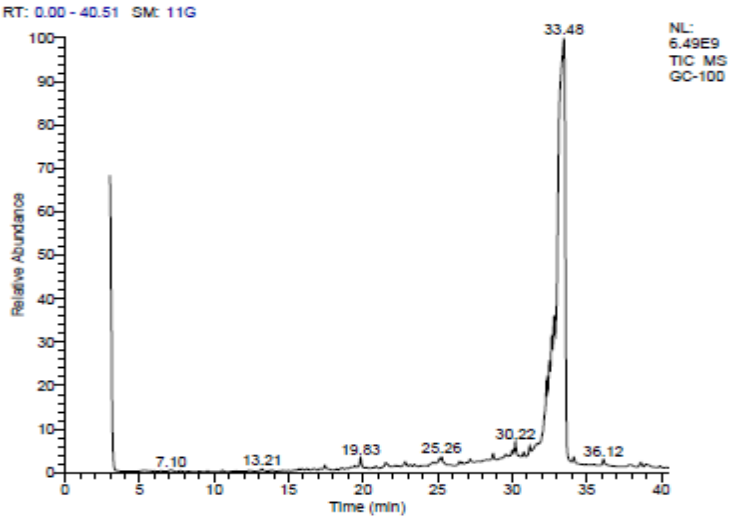

Fig. 3 Total ion chromatogram of Petroleum ether extract of *Lindernia madayiparense*

Table. 3. Identified phytocomponents in TIC of Petroleum ether extract of *Lindernia madayiparense*

S. No	RT	IUPAC name	Molecular Formula	Molecular weight	Peak Area
1	3.05	3-Methyl-6-isopropylcyclohex-3-en-1-one	C10H16O	152	1.16
2	5.29	11-Heneicosanone	C21H42O	310	0.23
3	7.10	1,2,3,4-Tetramethyl-5-methylenecyclopenta-1,3-diene	C10H14	134	0.23
4	13.21	Hexadecane	C16H34	226	0.31
5	17.44	Octadecane	C18H38	254	0.48
6	19.83	2-Pentadecanone, 6,10,14-trimethyl-	C18H36O	268	0.94
7	20.89	Ethyl 2-dichloromethylhexanoate	C9H16Cl2O2	226	0.21
8	21.50	Eicosane	C20H42	282	0.68
9	23.43	Heneicosane	C21H44	296	0.21
10	24.71	7-Methoxy-4-phenyl-2,5,8(1H)-quinoneone	C16H11NO4	281	0.26
11	25.26	2-(2-Carboxyvinyl)[4](1,1')ferrocenophane	C17H18FeO2	310	0.98
12	36.47	Hexadecanoic acid, butyl ester	C20H40O2	312	0.35
13	27.19	Triacontane	C30H62	422	0.40
14	28.72	2-(n-Hexyl)-1,1'-binaphthyl	C26H26	338	
15	29.57	Octadecanoic acid, butyl ester	C22H44O2	340	0.27
16	30.22	Hexanedioic acid, bis(2-ethylhexyl) ester	C22H42O4	370	1.39
17	30.77	5,10-dimethyl-6,8-	C20H16	256	0.29

		bisdehydropentatridecafulavalene			
18	31.18	Octacosane	C28H58	394	0.46
19	32.31	Tetramethyl ester of 4-(2-Cyano-1-methylethenyl)-7b,11a-dihydrobenzo[a]pyrrolo[1',2':3,4]pyrimido[6,1,2cd]pyrrolizin-8,9,10, 11-tetracarboxylic acid	C28H23N3O8	529	1.35
20	33.48	2-Hydroxy-6-methylbenzaldehyde oxime	C8H9NO2	151	82.13
21	34.15	Octacosane	C28H58	394	10.57
22	36.12	Nonacosane	C29H60	408	0.58
23	37.94	[2,2](3,6)Phenanthrenophanediene	C32H20	404	0.34
24	38.63	Nonacosane	C29H60	408	0.39
25	46.14	13-Docosenamide, (Z)-	C22H43NO	337	0.38

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