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CHARACTERIZATION OF THE METHANOLIC EXTRACT OF LEAVES OF *TRICALYSIA SPHAEROCARPA* (DALZELL EX HOOK.F.) GAMBLE BY GC-MS

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ABSTRACT

Tricalysia sphaerocarpa is commonly known as wild coffee and its basianym was *Discospermum sphaerocarpum* Dalzell ex Hook. F. Gas Chromatography-Mass Spectrometry is an important technique used for metabolic profiling in plants and also used for the qualitative and quantitative estimation of organic compounds. Totally 30 chemical compounds were identified from the methanolic extract of the leaves of *Tricalysia sphaerocarpa*, among which fatty acid is the major group consists of 9 compounds. Eicosanoic acid was found to be present as the major compound with peak area 35.77% and retention time 21.865minutes, followed by octadecanoic acid (18.81%).

Keywords: GC-MS analysis, *Tricalysia sphaerocarpa*, *Discospermum sphaerocarpum*, Methanol extract.

INTRODUCTION

The genus *Tricalysia*, (Rubiaceae) comprises 50 species distributed in subtropical and tropical regions in Asia and Africa, of which 2 species has been reported form India [1]. Some of these used as folk fore medicine as sedative, emetic, malaria, yellow fever, skin diseases and also for urine disorders. Medicinal plants are important substances for the study of their traditional uses through the verification of pharmacological effects and can be natural composite sources that act a disease curing agents. So phytochemical investigation on the extract for their main phytocompounds is very vital. Hence in the present study, the methanolic extract of leaves of *Tricalysia sphaerocarpa* were screened for Gas Chromatography-Mass Spectrometry.

MATERIALS AND METHODS

Collection of Plant material

The plant of *Tricalysia sphaerocapa* was collected from the sacred grove of Thirumanikuzhi, of Cuddalore district, Tamil Nadu. The collected plant materials were botanically identified. The species confirmation was engaged at French Institute Herbarium (HIFP), Puducherry. The herbarium specimen was prepared and deposited at the Department of Botany, Kanchi Mamunivar Centre for Post Graduate Studies, Lawspet, Puducherry, for future reference.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

GC-MS analysis was performed with GC Clarus 500 Perkin Elmer equipment. Compounds were separated on Elite-1 capillary column (100% Dimethylpolysiloxane). Oven temperature was programmed as follows: isothermal temperature at 50ºC for 2min, then increased to 200ºC at the rate of 10ºC/min, then increased up to 280ºC at the rate of 5ºC/min held for 9 min. Ionization of the sample components was performed in the El mode (70 eV). The carrier gas was helium (1ml/min) and the sample injected was 2µl. The detector was Mass detector turbo mass gold-Perkin Elmer. The total running time for GC was 36 min and software used was Turbomass 5.2. Using computer searches on a NIST Ver.2.1 MS data library and comparing the spectrum obtained through GC –MS compounds present in the plants sample were identified [2-3].

Identification of Compounds

The individual compounds were identified from methanolic extracts based on direct comparison of the retention times and their mass spectra with the spectra of known compounds stored in the spectral database, NIST (Version year 2005).

RESULTS

The compounds were identified by GC-MS

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analysis enumerated with molecular formula, retention time, molecular weight and peak area% (Table 1; Fig.1). GC-MS analysis of an methanolic extract of leaves of *Tricalysia sphaerocapa* showed 30 compounds. Of which 9 compounds belongs to the group fatty acids, 4 belongs to aliphatic & aromatic bicyclics, 3 belongs to aromatic hydrocarbons, 2 belongs to aromatic nitriles, aliphatic aldehydes, aromatic ketones and aromatic dicarboxylic esters each and 1 compounds belongs to aromatic alcohols, phenolics, terpenoids, barbiturates, pyrimidinedione, aromatic ethers each. Among this, Eicosanoic acid was found to be present as the major compound with peak area 35.77% and retention time 21.865minutes followed by Octadecanoic acid with peak area 18.81% and retention time 20.093 minutes, followed by 9,12,15- octadecatrienic acid with peak area 12.32% and retention time 15.01minutes. 2,2-Dimethylindene,2,3-dihydro-, 2-(2-Hydroxyphenyl)buta-1,3-diene, 1,2,3,4,8,9-hexahydro-4,4,8-trimethyl-,(+)- was found to be as least quantity with the peak area 0.51% and retention time 22.882 minutes. Some of the important structure of phytocomponents was given below (Fig. 2).

Table 1. GC-MS Analysis of methanolic extract of leaf of *T. sphaerocarpa*

No.	Name of the compound	Molecular formula	Molecular weight	RT	Peak area %
Aliphatic & Aromatic bicyclics					
1	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, [1R-1α,2β,5α]-	$C_{10}H_{18}$	138	16.942	0.91
2	2,2-Dimethylindene,2,3-dihydro-	$C_{11}H_{14}$	146	22.882	0.51
3	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-	$C_{10}H_{18}$	138	16.942	0.91
4	2AH-Cyclobut[a]indene-2a-carboxylic acid, 1,2,7,7a-tetrahydro, methyl ester	$C_{13}H_{14}O_2$	202	13.456	2.89
Aromatic nitriles					
5	-Ethylbenzonitrile	C_9H_9N	131	13.456	2.89
6	2,5-Dimethylbenzonitrile	C_9H_9N	131	13.456	2.89
Aromatic ethers					
7	2,3,5,6-Tetrafluoroanisole	$C_7H_4F_4O$	180	13.863	1.05
Pyrimidinedione					
8	2,4(1H,3H)-Pyrimidinedione, 5(trifluoromethyl)-	$C_5H_3F_3N_2O_2$	180	13.863	1.05
Fatty acids					
9	Oleic acid	$C_{18}H_{34}O_2$	282	20.979	1.10
10	Octadecanoic acid	$C_{18}H_{32}O_2$	284	20.093	18.81
11	Nonadecanoic acid	$C_{19}H_{38}O_2$	298	20.979	1.10
12	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	18.176	10.41
13	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	18.176	10.41
14	9,12,15-Octadecatrienic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278	15.01	12.32
15	9,12-Octadecadienoic acid (Z,Z)-			19.977	11.54
16	Eicosanoic acid	$C_{20}H_{40}O_2$	312	21.865	35.77
17	Docosanoic acid	$C_{22}H_{44}O_2$	340	23.477	0.77
Aliphatic aldehydes					
18	9,17-Octadecadienal, (Z)-	$C_{18}H_{32}O$		19.977	11.54
19	1H-Benzimidazole, 5,6-dimethyl-	$C_9H_{10}N_2$	146	22.882	0.51
Aromatic hydrocarbons					
20	2-(2-Hydroxyphenyl)buta-1,3-diene			22.882	0.51
21	Phenanthro[3,2-b]furan-7,11-dione,1,2,3,4,8,9-hexahydro-4,4,8-trimethyl-, (+)-	$C_{19}H_{20}O_3$	296	23.274	1.62
22	(2-Methoxyphenyl)carbamic acid, naphthalene-2-yl ester	$C_{17}H_{15}O_3N$	281	23.129	1.38
Aromatic ketones					
23	Chrysene-1,7(2H,8H)-dione, 3,4,9,10-tetrahydro-2,8-dimethyl-	$C_{20}H_{20}O_2$	292	23.129	1.38
24	tert-Butyl(5-isoproply-2-methylphenoxy)dimethylsilane	$C_{16}H_{28}OSi$	264	23.129	1.38
Aromatic dicarboxylic esters					
25	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	23.216	1.42
26	Phthalic acid, di(2-propylpentyl ester)	$C_{24}H_{38}O_4$	390	23.216	1.42
Barbiturates					
27	cyclobarbital	$C_{12}H_{16}N_2O_3$	236	23.477	0.77
Terpenoids					

28	Squalene	$C_{30}H_{50}$	410	25.322	10.87
Phenolics					
29	4,6-Bis(1,1-dimethylethyl)-4´-methyl-1-1´-biphenyl-2-ol	$C_{19}H_{28}O$	272	23.274	1.62
Aromatic alcohols					
30	Dibenzo[a,c]phenazin-10-ol	$C_{20}H_{12}N_2O$	296	23.274	1.62

Fig 1. Gas Chromatography - Mass Spectrometry (GC-MS) Chromatogram of the methanolic extract of leaves of *T. sphaerocarpa*

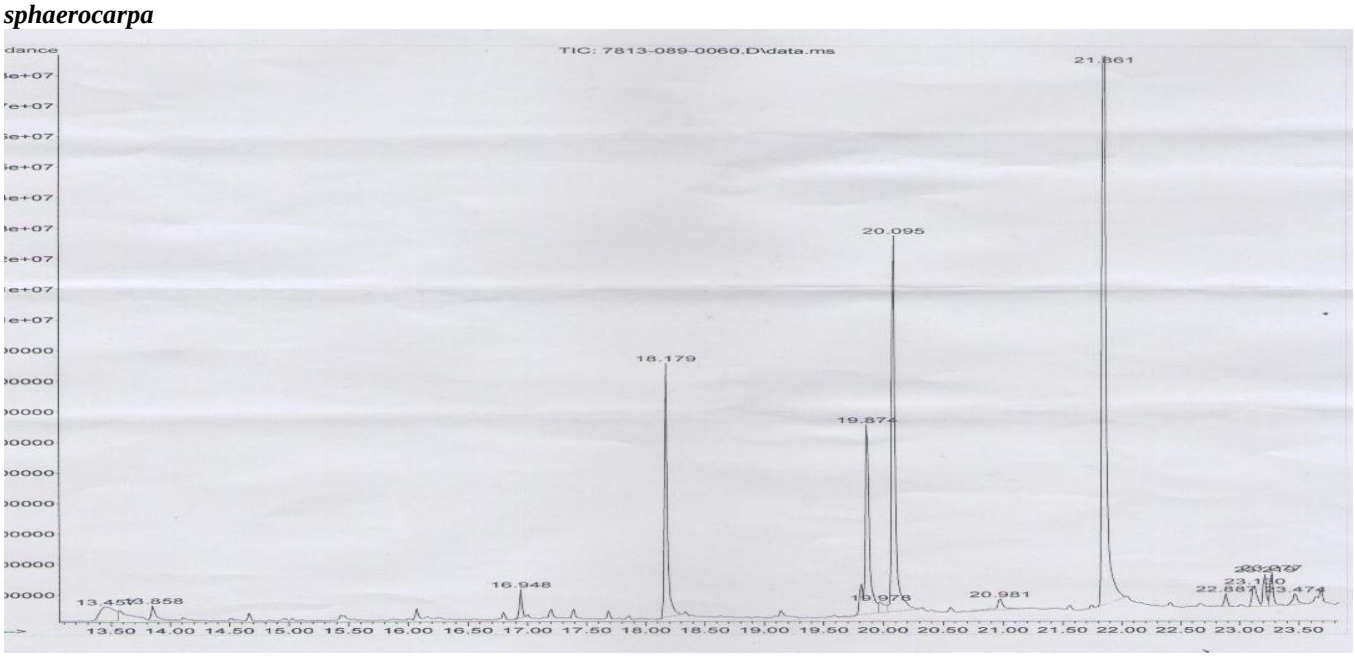
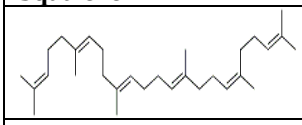
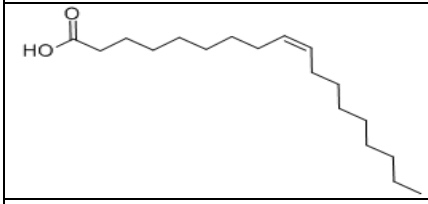
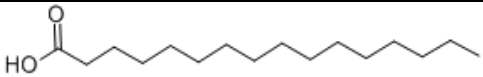
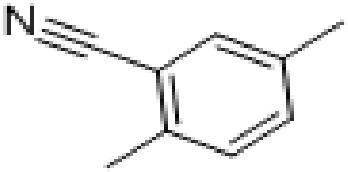
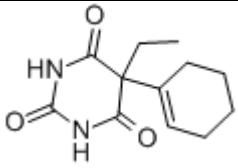
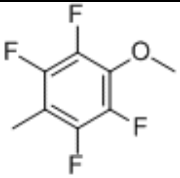
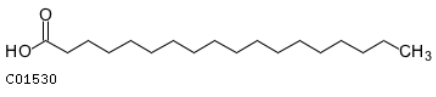
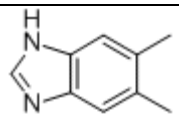
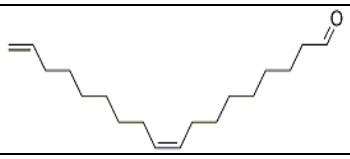


Fig 2. Structure of Some Important Phytocompounds

Squalene	**Docosanoic acid**	**Eicosanoic acid**
		
Oleic acid	**n-Hexadecanoic acid**	**2,5-Dimethylbenzonitrile**
		
2-Ethylbenzonitrile	**Cyclobarbital**	**2,3,5,6-Tetrafluoro-4-methylanisole**
		
Octadecanoic acid	**1H-Benzimidazole,5,6-dimethyl-**	**(9Z)-octadeca-9,17-dienal**
		

DISCUSSION

n-Hexadecanoic acid is also known as Palmitic acid. n-Hexadecanoic acid, Octadecanoic acid, 9,12-octadecadienoic acid (Z,Z)-, 9,12,15-octadecatrienoic acid , methyl ester (Z,Z,Z)- was already reported in the leaf of *Mallotus philippensis* [4]. 9,12,15-octadecatrienoic acid , n-Hexadecanoic acid, Octadecanoic acid was also reported in the methanolic extract of stem of *Tricalysia sphaerocarpa* [5]. Secondary metabolites in plant products are responsible for several biological activities in man and animals [6]. The active components usually interfere with growth and metabolism of microorganisms in a negative manner [7]. Phenolic compounds and steroidal compounds which are more effective in higher concentrations inhibiting the growth of all fungi[8]. The fatty acids being effective in the treatment of asthma, rheumatoid arthritis, inflammatory bowel diseases [9]. Esters are functionally used in the design of "Prodrugs" [10], terpenes are anti-allergic [11] and antimicrobial agents [12]. Squalene is the Triterpene compound showed activity against Antibacterial, Antioxidant, Antitumor, cancer preventive, Immunostimulant, Chemo preventive, Lipoxygenase inhibitor, pesticide [13].

CONCLUSION

From the present study, it was concluded that the plant *T. sphaerocarpa* are highly valuable in medicinal usage for the treatment of various human ailments along with the chemical constituents present in it. The compounds needs further research on toxicological aspects to develop a safe drug.

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