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GC-MS analysis of biologically active compounds in Leaves of *Calophyllum inophyllum* L.

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Abstract : Biologically active compounds in the leaves of *Calophyllum inophyllum* was made by GC-MS. A total of 17 compounds were identified from alcoholic extract of the *C. inophyllum* leaves. The major components of the leaves were 1,2-Benzene dicarboxylic acid, diisooctyl ester (28.11%), Androstan - $1\propto$ - 0l-17- one, 2, 3 – isopropylidenedioxy – 4 β-. methyl (21.63%),1-monolinoleoylglyceroltrimethylsilyl ether(11.80%), squalene (10.74 %), oleic acid (7.41 %), n-Hexa decanoic acid (4.57 %), 3, 7, 11, 15 Tetramethyl -2 hexadecen- 1 – 0l (t_R =14.81 min) (4.45 %), 9,12 octadecadienoic acid methyl ester,(E.E)-(2.59 %), octadecanoic acid (2.34 %), Benzene (1-methyldodecyl) (1.03 %) , 3, 7, 11, 15 – Tetramethyl – 2- hexa decen-0l(1.12%) (t_R = 15.43 min), phytol (1.00 %) were detected. The GC-MS analysis revealed that alcoholic extract of *C. inophyllum* are mainly composed of oxygenated hydrocarbons, Phenolic compounds and ketone group.
Key words: *Calophyllum inophyllum* – leaves – GC-MS -Biologically active components.

Introduction

The genus *Calophyllum* (Clusiaceae / Guttiferae) is composed of about 180 – 200 different species confined to the warm humid tropics of the world[1, 2]. The beauty of some plants is that they occur in the ethnopharmacy and folklore of more than one country and so we are able to make comparisons between the ways in which that the plant has been used medicinally and culturally[3]. Several species of *Calophyllum* genus are known to be used in folk medicine[4].

Extensive chemical investigation of the genus *Calophyllum* has resulted in the isolation of a wide variety of natural products, including xanthones, coumarins, biflavonoides, chalcones, benzoflurans and triterpenes[2,5-11].

The present investigation on biologically active phytochemical diversity of the leaves of *Calophyllum inophyllum* by GC-MS, nevertheless the species is highly appreciated as medicine worldwide[12-15].

Materials and Methods

Plant Material

Leaves of *C. inophyllum* L was collected during June 2009, from campus of Govt. Arts College (Autonomous), Kumbakonam, Thanjavur Dist, Tamil Nadu, India. A voucher specimen has been retained in the Department of Botany, Govt. Arts College (Autonomous), Kumbakonam – 612 001.

Extraction of chemicals from plant

20gm of shade dried plant powder was soaked in 50ml of absolute alcohol for overnight and then filtered through Whatmann filter paper No 41 along with 2gm of sodium sulfate to remove the sediments and traces of water in the filtrate. The filter paper is wetted with absolute alcohol before filtering. The filtrate is then concentrated by bubbling nitrogen gas into the solution and the volume is reduced to 1ml. The

extract contains both polar and non-polar phyto-components.

GC-MS Analysis

GC-MS analysis was carried out on a GC Clarus 500 Perkin Elmer System comprising a A OC-20 i auto sampler. Gas chromatograph interfaced to a Mass spectrometer (GC-MS) instrument employing with column Elite -1 fused silica capillary column 30 X 0.25mm X 1µmdf composed of 100% Dimethyl poly siloxane, operating in electron impact mode at 70eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml per minute with injection volume of 0.2µl was employed (split ratio of 10:1) injector temperature maintained at 250° C, with ion-source temperature 280°C. The oven temperature was programmed from 110° C(Isothermal for 2min) with an increase of 10°C per-minute to 200°C, then with 5°C increase per-minute up to 280°C ending with a 9min hold isothermal at 280°C. Mass spectral analysis was taken at 70eV: a scan interval of 0.5 seconds Mass scan fragments from 45 to 450 Da. Total GC running time is 36min and total MS running time is also 36min.

Identification of Compounds

Interpretation of Mass spectrum GC-MS was conducted using database of NIST (National Institute Standard and Technology-2005) having more than 62,000 patterns. The spectrum of the unknown component was compared with the known components stored in the NIST library. The Retention time, molecular formula, molecular weight and peak area with composition percentage of the test plant material was identified.

Table 1 Biologically active phyto-compounds of *C. inophyllum*

S. No	RT*	Name of the compounds	Molecular Formula	Molecular mass	Peak Area %
1	10.83	Cyclohexene,3-(1,5-dimethyl-4-hexenyl)-6-methylene-,[S-(R*,S*)]	$C_{15}H_{24}$	204	0.76
2	13.36	Azulene, 1,4-dimethyl-7-(1-methylethyl)-	$C_{15}H_{18}$	198	0.68
3	13.72	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	0.40
4	14.09	3-Trifluroacetoxypentadecan	$C_{17}H_{31}F_3O_2$	324	0.19
5	14.81	3,7,11,15-Tetramethyl-2-hexadecen-1-0l	$C_{20}H_{40}O$	296	4.45
6	15.43	3,7,11,15-Tetramethyl-2-hexadecen-1-0l	$C_{20}H_{40}O$	296	1.12
7	15.88	Benzene,(1-methyldodecyl)-	$C_{19}H_{32}$	260	1.03
8	16.59	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	4.57
9	18.92	Phytol	$C_{20}H_{40}O$	296	1.00
10	19.24	9,12-Octadecadienoic acid, methyl ester, (E,E)-	C19H34O2	294	2.59
11	19.32	Oleic acid	C18H34O2	282	7.41
12	19.66	Octadecanoic acid	C18H36O2	284	2.34
13	23.49	Phenol, 2,4-bis(1-phynylethyl)-	C22H22O	302	1.15
14	25.42	1,2-Benzenedicarboxylic acid, diisooctyl ester	C24H38O4	390	28.11
15	29.88	Androstan-1α-ol-17-one,23-isopropylidenedioxy-4β-methyl-	C23H36O4	376	21.63
16	30.04	Squalene	C30H50	410	10.74
17	30.77	1-Monlinoleoyglycerol trimethylsilyl ether	C27H54O4Si2	498	11.80

RT* - retention Time

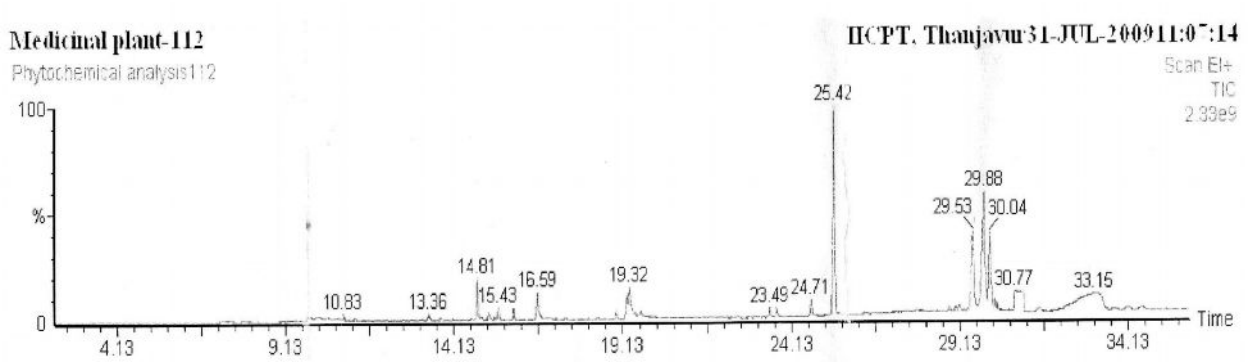

Fig 1 GC-MS Chromatogram of *C. inophyllum*

Results and Discussion

The results of the GC-MS analyses on the alcoholic extract of *C .inophyllum* are presented in Table. 1. The chromatogram of the analysis is shown in Fig 1.

A total 17 compounds were identified from the alcoholic extract of the leaves of *C. inophyllum*[16]. The identified compounds represented 99.97 % of the total extract. Of the total 17 compounds isolated, the following four phytochemicals recorded above 10 % level of peak area, squalene (10.74 %), 1-monolinoleoylglycerol trimethylsilyl ether (11.80 %), Androstan- 1α -0l-17- one, 2,3- isopropylidenedioxy - 4β - methyl- (21.63 %), 1-2- Benzene dicarboxylic acid, di isooctyl ester (28.11 %). While cyclohexene was first which came out from the column (t_R = 10.83 min) 1 – monolinoleoyl glycerol trimethyl silyl ether (t_R = 30.77 min.) was retained in the column for the longest of all. The following four components like cyclohexene, Azulene, 1, 4- dimethyl-7-(1-methyl ethyl), Benzene, (1-methyl dodecyl), squalene lacked in any oxygen in the molecule[17-20].

In total eight chemically differentiated compounds were identified in present investigation in which Oxygenated hydrocarbon dominated in position (5 compounds like S. No- 3, 8, 11, 12, 14, Table1), Alcoholic group compounds occupied the second position that is three compounds belong to this group (S. No 5, 6, 17, Table1), phenolic hydrocarbon (S. No. 9, 13, Table1) and Alkene (unsaturated compound) (S.No.2, 16, Table1), Ether compound (S.No.4, 17, Table1) recorded two compounds each kind during present investigation only one compound recorded in the following chemical groups Ketonic compound (S.No.15, Table1), Alicyclic compound (S.No.1, Table1) and Aromatic hydrocarbon (S.No.7, Table1). Similar works in C. inophyllum was done by various workers[2,7,8,11].

Conclusions

The GC-MS analysis revealed that alcoholic extract of *C. inophyllum* are mainly composed of oxygenated hydrocarbons, Phenolic compounds and ketone group.

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