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PHYTOCHEMICAL DIVERSITY ON THE LEAVES OF METHANOL EXTRACT OF *HYPTIS SUAVEOLENS* L (POIT.) FROM CUDDALORE (CHIDAMBARAM) DISTRICT, TAMIL NADU, INDIA

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ABSTRACT

Hyptis suaveolens L (Poit.) leaves of Methanol Extract obtained by Solvent extraction by maceration process were analyzed by gas chromatography mass spectroscopy (GC-MS). Thirty five components were identified in the leaf extract. The major components were Phenanthrenemethanol (26.19%), Podocarp (8.68%), 9-Octadecynoic acid (8.16%), Palustric acid (8.05%), n-hexadecanoic acid (7.23%), bicycle (3.1.1) hept-2-ene, 2, 2'-(1, 2-ethanediyl) bis(6, 6-dimethyl (6.75%), 7-isopropyl-1, 1, 4a- trimethyl-1, 2, 3, 4, 4a, 9, 10, 10a-octahydrophenanthrene (6.36%), benzaldehyde, 2-hydroxy-6-methyl (6.02%), 3, 7, 11, 15-tetramethyl-2-hexadecen-o1 (4.61%). The compositions of Methanol Extract varied qualitatively and quantitatively.

INTRODUCTION

Hyptis suaveolens L (Poit.) and commonly known as American mint belongs to the family Lamiaceae. It is a weed of waste places, plantation crops, forest margins, road sides and becoming abundant in fallow ground. It prefers a wet tropical climate, less common in regions with a seasonal wet/dry regime (Waterhouse and Mitchell, 1998). The genus *Hyptis* comprising more than 300 species, exhibits a major morphological diversity found in various tropical and subtropical regions of the world including Tamil Nadu. Most of them originate from tropical America. This specie is quite aromatic and is frequently used in treatments of gastrointestinal infections, cramps, and pain, as well as skin infections (Correa, 1931). The plant is used in the southern Sahara to treat asthma and malaria, cereals conservation (Adjanohoun *et al*., 1986) and to repel mosquitoes (Seyoum *et al*., 2002). The plant showed antibacterial and antifungal activities (Goun *et al*., 2003; Zollo *et al*., 1998). Despite those intensive works done to investigate the chemical composition of *Hyptis species* Methanol Extract all over the world. But there is no published report in the literature about the chemical composition of *Hyptis suaveolens* L (Poit.) Methanol Extract from Cuddalore (Chidambaram) district of Tamil Nadu, India. So, an attempt has been taken to investigate the chemical components of Methanol Extract obtained from the leaves of *Hyptis suaveolens* L (Poit.) grown widely in Cuddalore (Chidambaram) district of Tamil Nadu.

MATERIALS AND METHODS

Plant Material

The fresh plant organ (Leaves) of *H. suaveolens*, (L), poit. Were collected from Cuddalore (Chidambaram 11°25.19N and 079°20.57E), district of Tamil Nadu, India. The voucher specimens were preserved in the Department of Botany, Annamalai University, Annamalai Nagar, Tamil Nadu, India.

Preparation of Extract

Solvent extraction by maceration process 25gms of the powder of plant material was transferred into different conical flask (250ml). The conical flask containing 100ml of Methanol. The conical flask containing plant powder and solvent was shaked it well for 48 hours by mechanical shaker. The extracts were

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filtered using Whatmann filter paper No.1. The filtrates were evaporated to dryness using water bath. The obtained extracts were stored at 4°c in air tight bottle until further use.

GC-MS Analysis

The Methanol Extract from leaves of *H. suaveolens* L, (Poit) were analyzed by 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 TurboMass Ver 5.2.0. (Adams 2009).

Identification of the Compounds

Interpretation on mass spectrum 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.

RESULTS

Methanol Extract from the leaves of *H. suaveolens* L, (Poit) were analyzed by GC-MS. Tables 1, 2 and figure 1 reported the composition of the leaves extract of *H. suaveolens* L, (Poit). The major constituents of the leaf Methanol Extract were Phenanthrenemethanol (26.19%), Podocarp (8.68%), 9-Octadecynoic acid (8.16%), Palustric acid (8.05%), n-hexadecanoic acid (7.23%), bicycle (3.1.1) hept-2-ene, 2, 2'-(1, 2-ethanediyl) bis (6, 6-dimethyl (6.75%), 7-isopropyl-1, 1, 4a-trimethyl-1, 2, 3, 4, 4a, 9, 10, 10a-octahydrophenanthrene (6.36%), benzaldehyde, 2-hydroxy-6-methyl (6.02%), 3, 7, 11, 15-tetramethyl-2-hexadecen-o1 (4.61%).

DISCUSSION

The study reveals that composition of the extract differs from the earlier reports and may, therefore be treated as different chemotypes. On the basis of aforementioned fact, it may be concluded that *H. suaveolens*, growing widely in Cuddalore (Chidambaram) in Tamil Nadu, may be utilized as a source for the isolation of natural Phenanthrenemethanol (26.19%), Podocarp (8.68%), 9-Octadecynoic acid (8.16%), Palustric acid (8.05%), n-hexadecanoic acid respectively. The high concentration of 9-Octadecynoic acid (8.16%), n-hexadecanoic acid (7.23%) in leaf oil make it potentially useful in the medicines because they exhibit antitumor and antioxidant activities (Dr. Duke online database). However, further study has to be conducted for its confirmation. It is worth noting that the methanol extract of *H. suaveolens* L, (Poit) has been reported to be used in folk medicine in the treatment of asthma and malaria, cereals conservation and to repel, larvicidal, adulticidal activities of mosquitoes.

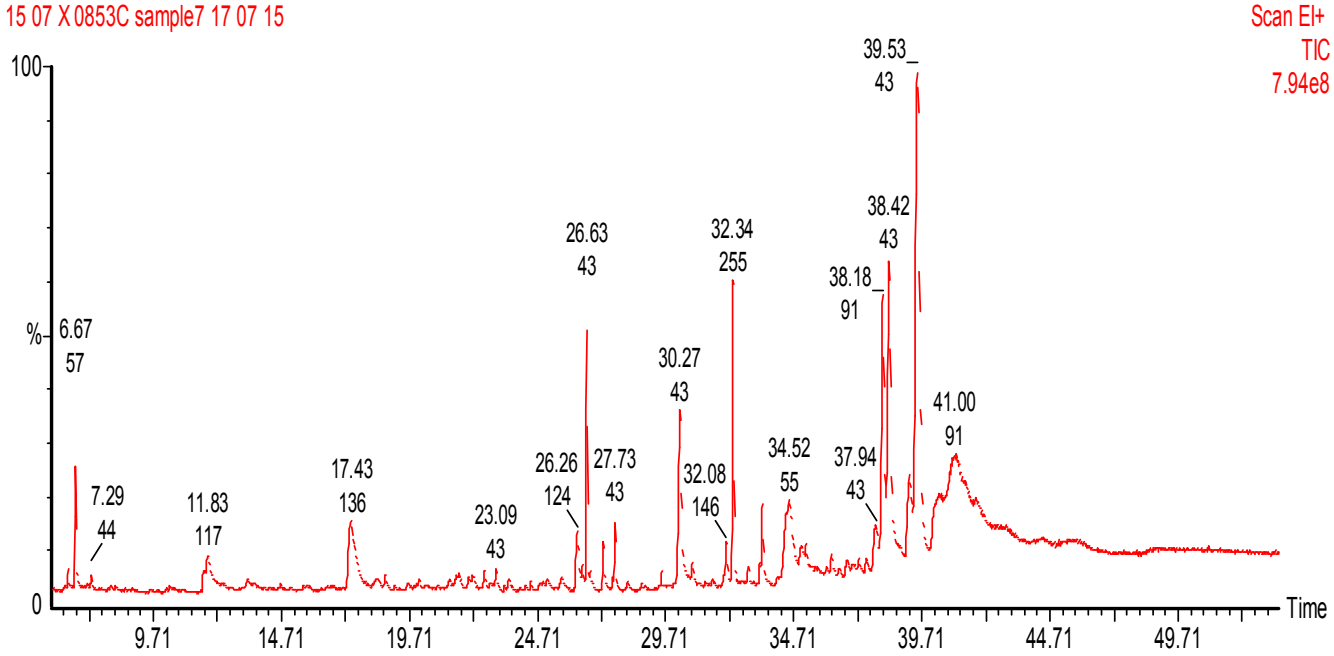

Figure 1 Chromatogram obtained by the GC/MS with the methanol extract of *Hyptis suaveolens* from Cuddalore (Chidambaram) District of Tamil Nadu

Table 1 shows the components identified by GC/MS in methanol extract of *Hyptis suaveolens* from Cuddalore (Chidambaram) District of Tamil Nadu

S.No	Peak Name	Retention time	Peak area	%Peak area	Molecular Formula	Molecular weight
1.	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	6.39	2324825	0.4473	$C_{10}H_{16}$	136
2.	1-Hexanol, 2-ethyl	6.67	13374762	2.5733	$C_8H_{18}O$	130
3.	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)	7.29	1557035	0.2996	$C_{10}H_{16}$	136
4.	2-Methoxy-4-vinylphenol	13.38	2333362	0.4489	$C_9H_{10}O_2$	150
5.	Benzaldehyde, 2-hydroxy-6-methyl-	17.43	31319364	6.0258	$C_8H_8O_2$	136
6.	D-Allose	18.40	2859936	0.5502	$C_6H_{12}O_6$	180
7.	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	18.76	1501346	0.2889	$C_{11}H_{16}O_2$	180
8.	Caryophyllene oxide	20.08	918202	0.1767	$C_{15}H_{24}O$	220
9.	Megastigmatrienone	21.28	1549830	0.2982	$C_{13}H_{18}O$	190
10.	2-Cyclohexen-1-one, 4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-, [R-[R*,R*-(E)]]-	22.04	1791654	0.3447	$C_{13}H_{20}O_2$	208
11.	Caryophyllene oxide	22.63	2422736	0.4661	$C_{15}H_{24}O$	220
12.	Acetic acid, 1-methyl-3-(1,3,3-trimethyl-bicyclo[4.1.0]hept-2-yl)-propenyl ester	22.21	1328335	0.2556	$C_{16}H_{26}O_2$	250
13.	Bergamotol, Z-à-trans-	23.09	3506784	0.6747	$C_{15}H_{24}O$	220
14.	2-Cyclohexen-1-one, 4-(3-hydroxybutyl)-3,5,5-trimethyl-	23.60	2485758	0.4783	$C_{13}H_{22}O_2$	210
15.	7-Acetyl-2-hydroxy-2-methyl-5-isopropylbicyclo[4.3.0]nonane	24.45	964935	0.1857	$C_{15}H_{26}O_2$	238
16.	Tetradecanoic acid	25.08	2196184	0.4225	$C_{14}H_{28}O_2$	228
17.	1-(3-Isopropylidene-5,5-dimethyl-bicyclo[2.1.0]pent-2-yl)-ethanone	25.64	3127642	0.6017	$C_{12}H_{18}O$	178
18.	Phenethyl alcohol, 2,5-dihydroxy-à-methyl-, (-)-	26.25	8884598	1.7094	$C_9H_{12}O_3$	168
19.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	26.63	23972436	4.6122	$C_{20}H_{40}O$	296
20.	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	26.77	340007	0.0654	$C_{20}H_{40}$	280
21.	2,5,5,8a-Tetramethyl-6,7,8,8a-tetrahydro-5H-chromen-3-one	28.24	1680942	0.3234	$C_{13}H_{20}O_2$	208
22.	4,6,6-Trimethyl-2-(3-methylbuta-1,3-dienyl)-3-oxatricyclo[5.1.0.0(2,4)]octane	29.55	1936521	0.3726	$C_{15}H_{22}O$	218
23.	n-Hexadecanoic acid	30.27	37609840	7.2360	$C_{16}H_{32}O_2$	256
24.	4-Oxazolecarboxylic acid, 4,5-dihydro-2-phenyl-, 1-methylethyl ester	32.07	5940534	1.1429	$C_{13}H_{15}NO_3$	233
25.	7-Isopropyl-1,1,4a-trimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene	32.34	33090166	6.3664	$C_{20}H_{30}$	270
26.	8a (2H)- Phenanthrenol, 7- ethenyldodecahydro-1,1,4a,7 – tetramethyl - , acetate, [4as – 4aά, 4bά, 7ά, 8aά, 10aά)]	32.93	2583766	0.4971	$C_{22}H_{36}O_2$	332
27.	Phytol	33.49	14259571	2.7435	$C_{20}H_{40}O$	296
28.	9-Octadecynoic acid	34.52	42458432	8.1689	$C_{18}H_{32}O_2$	280
29.	Octadecanoic acid	35.01	8478155	1.6312	$C_{18}H_{36}O_2$	284
30.	Androst-5,7-dien-3-ol-17-one	36.17	2843629	0.5471	$C_{19}H_{26}O_2$	286
31.	(3E,5E,7E)-6-Methyl-8-(2,6,6-trimethyl-1-cyclohexenyl)-3,5,7-octatrien-2-one	37.55	1848176	0.3556	$C_{18}H_{26}O$	258
32.	Bicyclo[3.1.1]hept-2-ene, 2,2'-(1,2-ethanediyl)bis[6,6-dimethyl-	38.18	35129152	6.7587	$C_{20}H_{30}$	270
33.	Podocarp-7-en-3á-ol, 13á-methyl-13-vinyl-	38.42	45133100	8.6835	$C_{20}H_{32}O$	288
34.	1-Phenanthrenemethanol, 1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-7-(1-methylethyl)-, [1S-(1à,4aà,10aá)]-	39.53	136137648	26.1925	$C_{20}H_{30}O$	286
35.	Palustric acid	41.00	41869200	8.0555	$C_{20}H_{30}O_2$	302

Table 2 Activity of phyto-components identified in *Hyptis suveolens* by GC/MS from Cuddalore (Chidambaram) District of Tamil Nadu

S.No	R/T	Name of the Compound	Activity**
1	35.01	Octadecanoic acid,	Anti tumour
2	34.52	9-Octadecynoic acid	Antipreventive, Faviur, Fungicide, Pesticide, Perfumery, Anti-inflammatory, Hypocholesterolemic, Cancer preventive ect.
3	30.27	n-Hexadecanoic acid	Antioxidant, Hypocholeserolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic.
4	34.52	9-Octadecynoic acid	Hypocholesterolemic, 5-Alpha reductase inhibtor, Antihisaminic, Insectifuge, Antieczemic, Antiacne.
5	20.08	Caryophyllene oxide	Anti-tumor, Analgesic Antibacterial, Anti-inflammatory, Sedative, Fungicid.

**Source: Dr.Duke's phytochemical and Ethnobotanical databases [Online database].

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