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Evaluation of Phytochemical Constituents from Stems of *Memecylon umbellatum* Brum. F. by GC-MS Analysis.

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ABSTRACT

A valuable Indian ethnomedicinal plant, *Memecylon umbellatum* was investigated for GC-MS (Gas chromatography- Mass spectrophotometry) analysis to determine the chemical constituent present in various extracts of stem. Powdered stem plant materials was subjected to successive extract on with organic solvents such as petroleum ether, chloroform and ethanol by soxhlet extraction method. Totally, 20 different compound from chloroform extract, 11 different compounds from petroleum ether extract and 10 various compounds from ethanol extract were identified. All the compounds identified medicinally valuable for the treatment of various human ailments.in addition, all the phytochemical compounds were needed further investigation on toxological aspects for the development of new lead of therapeutic interest.

INTRODUCTION

Nearly, 95 % of plants used in traditional medicines are collected from forests and other natural sources. The plants collected from different sources show wide disparity in therapeutic values and also much variation in market rates. In the recent years, there has been greater expansion of indigenous drug industry in India. Consequently, the demand for the new material (medicinal plants) has enormously increased. According to latest estimate, there are about eight thousand licensed pharmacies of ISM in the country, engaged in the manufacture of bulk drugs to meet the requirement of people. The total annual requirement of the raw materials of these pharmacies was estimated to be thousands of quintals. This is presently met by cutting trees in the forest or uprooting herbs and shrubs either on nominal payment or unauthorized. Further, there is prime need to provide authentic or genuine drugs to manufacture standard medicine, as emphasized by earlier worker Singh and Ghouse [1]. The annual demand of the global market is $32 million of medicinal plants from developing countries. The herbal drug production in our country has been estimated to be rupees 4,000 crores in the year 2000. Out of 15,000 - 20,000 medicinal plants, our rural communities use 7,000 - 7,500 medicinal plants. About 130 pure compounds, which are extracted from 100 species of higher plants of Indian origin, are used throughout the world. India can play a major role for supplying the raw herbs, standardized extracted materials and pure compounds isolated from natural resources [2]. Traditional medicinal plants have been the origin and basis of pharmacology and therapeutics and played an important role in drug discovery. For these reason, ethnobotany and traditional medicine may still be a useful strategy and a valuable tool for future pharmaceutical research [3].

Several active compounds were discovered based on the ethnomedicines. Based on the ethnomedicinal information, a total of 122 compounds were identified and 80% of these compounds were used for the same (or related) ethnomedicinal purposes [4]. Further, it was discovered that these compounds were derived from only 94 species of plants [5]. Some of the prominent commercial plant-based medicinal compounds include colchicum, colchicine, betulinic acid, camptothecin, topotecan, CPT-11, 9-aminocamptothecin, delta-9-tetrahydrocannabinol, beta lapachone, lapachol, podophyllotoxin, etoposide, podophyllinic acid, vinblastine, vincristine, vindesine vinorelbine, docetaxel, paclitaxel, tubocurarine, pilocarpine, scopolamine among others. Recently, there has been a remarkable revival in the use of ethnomedicines in many western countries, partly as a result of rising interest in

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"plant derived drugs". Plants have also contributed valuable drugs for medicine on the basis of traditional practices on medicinal plants.

MATERIALS AND METHODS

Collection of plant materials

The plant materials (leaves and stem) of *Memecylon umbellatum* Burm.f., were collected from the Jamnamaruthur forest vicinity (Javadhu hills), Tiruvannamalai district, Tamil Nadu. The collected plant material was botanically identified and confirmed by the Botanist at Rapinat Herbarium, St. Joseph's College, Tiruchirappalli. The herbarium specimens were preserved and submitted to Department of Botany and Microbiology at A.V.V.M. Sri Pushpam College (Autonomous), Thanjavur District, Tamil Nadu for further reference (Voucher No. SM 003 & 004).

Preparation of the extracts

The collected plant materials (leaves and stem) were chopped into small pieces separately, shade-dried, and coarsely powdered using a pulverizor. The powders were subjected to successive extraction with organic solvents such as petroleum ether, chloroform and ethanol by Soxhlet method [6]. The extracts were then collected and distilled off on a water bath at atmospheric pressure and the last trace of the solvents was removed *in vacuo*. The solvent extracts were used for preliminary phytochemical screening, GC-MS analysis and antimicrobial studies while ethanol leaf extract alone was used for antidiarrhoeal and anti-ulcer activity.

Gas Chromatographic–Mass Spectrophotometric (GC-MS) Analysis

GC-MS analysis was carried out for all the three extracts (petroleum ether, chloroform and ethanol) of the leaves and stem of *M. umbelletum* at Indian Institute of Crop Processing Technology, (IICPT) Thanjavur, Tamil Nadu.

GC-MS analysis was performed with GC Clarus 500 Perkin Elmer equipment compounds were separated on Elite-1 capillary column (100% dimethyl-polysiloxane). Oven temperature was programmed as follows: isothermal temperature at 50ºC for 2 min., 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 EI 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 v.5.2. The individual constituents were identified by comparing their mass spectra with the spectra of known compounds stored in the spectral database (NIST version, 2005).

OBSERVATIONS AND RESULTS

The chemical constituents identified by the GC-MS analysis in various extracts of the stem of *Memecylon umbellatum* Burm.f, were enumerated along with molecular formula, retention time, molecular weight, peak area and given (Table 1-4). In petroleum ether extract, totally 11 compounds were identified, of which 9 compounds were belonged to aliphatic group and 2 compounds were belonged to aromatic groups (Table 1). In aliphatic group, fatty acids possess 9 compounds. In aromatic group, monoterpene and dicarboxylic acid possess single compound each. In aliphatic group, the fatty acid compound n-hexadecanoic acid was found the major constituent with the peak area of 39.12 % and retention time 16.20 followed by oleic acid with the peak area of 29.67% and retention time18.87, 9, 12-octadecanoic acid (Z,Z)- with the peak area of 5.97 % and retention time18.80, octadecanoic acid with the peak area of 4.82 % and retention time 9.23, 1-monolinoleoylglycerol trimethylsilyl ether with the peak area of 4.61 % and retention time 27.28, hexadecanoic acid, methyl ester with the peak area of 1.18 % and retention time 15.53, hexadecane with the peak area 0.52 % and retention time 8.88, undecane with the peak area of 0.45 % and retention time 4.89 and dodecane with the peak area of 0.34 % and retention time 6.23 respectively.

In aromatic type, monoterpene compound 1,5,heptadien-4-one, 3,3,6-trimethyl was recorded with the peak area 8.01% and retention time 3.35 while dicarboxylic acid compound 1,2-benzenedicarboxylic acid, diisooctyl ester was recorded with peak area 5.82% and retention time 24.87 respectively. In chloroform extract, totally 30 compounds were identified, of which 28 compounds were belonged to aliphatic groups and 2 compounds were belonged to aromatic groups (Table 2). In aliphatic groups, ester comprises single compound, fatty acids possess 25 compounds, fatty alcohol and triterpene possess single compound respectively. In fatty acids, oleic acid was found to be present with the peak area 41.46% and retention time 18.87 followed by n-hexadecanoic acid with the peak area 25.43% and retention time 16.21, octadecanoic acid with the peak area 11.41% and retention time 19.15, heptacosane with the peak area 4.74% and retention time 30.06, octacosane with the peak area 4.30% and retention time 26.89, eicosanoic acid with the peak area 2.56% and retention time 22.12, pentacosane with the peak area 1.42% and retention time 24.05, 4,18,12,16-tetramethylheptadecan-4-olide with the peak area

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1.09% and retention time 21.88, 1-hexadecanol with the peak area 0.64% and retention time 11.17, heptadecanoic acid with the peak area 0.62% and retention time 17.62, 9-nonadecene with the peak area 0.57% and retention time 13.69, tetradecanoic acid with the peak area 0.42% and retention time 13.39, 2-pentadecanone,6,10,14-trimethyl- with the peak area 0.36% and retention time 14.37, exadecanoic acid, methyl ester with the peak area 0.29% and retention time 15.46, phytol with the peak area 0.27% and retention time 18.37, 1-decane with the peak area 0.23% and retention time 6.10, tetradecane with the peak area 0.23% and retention time 8.86, 9, 12-octadecenoic acid, methyl ester, (E,E)- with the peak area 0.20% and retention time 17.95, 9-octadecenoic acid (Z)-, methyl ester with the peak area 0.20% and retention time 18.03, undecane with the peak area 0.19% and retention time 4.90, nonadecane with the peak area 0.13% and retention time 10.10, hexadecane with the peak area with the peak area 0.10% and retention time 11.27, hydroxylamine, 0-decyl- with the peak area 0.08% and retention time 6.23, tetradecane,2,6,10-trimethyl- with the peak area 0.08% and retention time 13.79 and tridecane with the peak area 0.05% and retention time 7.57 respectively. In aromatic group, alkylated phenol and alkaloid possess single compound each. In aromatic group, alkylated phenol compound namely phenol, 2,4-bis(1,1-dimethylethyl)- was found to be the major constituents with the peak area 0.80% and retention time 10.31 followed by alkaloid dascarpidan-1-methanol, acetate (ester) with the peak area 0.47% and retention time 20.64 respectively.

In ethanol extract, totally 5 compounds belonged to aliphatic groups were identified (Table 3). Among them, fatty acids possess 3 compounds while diterpene alcohols possess two compounds. The fatty acid oleic acid was found to be present as major constituent with the peak area 37.44% and retention time 18.76 followed by n-hexadecanoic acid with peak area 36.94 and retention time 16.12, octadecanoic acid with peak area 20.25% and retention time 19.10 respectively. Comparatively in total, 34 compounds were identified from various extracts of the stem of *M.umbellatum* (Table 4). Among them, 29 compounds were belonged to aliphatic and 4 compounds were belonged to aromatic groups. In aromatic groups, 16 compounds were belonged to alkane hydrocarbons, 2 compounds were belonged to diterpene alcohol, 12 compounds were belonged to fatty acids, and one compound belonged to fatty alcohol. In aromatic group, alkaloids, alkylated phenol, dicarboxylic acid and monoterpene compounds were represented by single compound each respectively. The compounds such as n-hexadecanoic acid, octadecanoic acid and oleic acid were present in all the three extracts. The compounds such as 1-monolinoleoylglycerol trimethylsilyl ether, 9, 12-octadecanoic acid (Z,Z)-, 1,2-benzenedicarboxylic acid, diisooctyl ester and 1,5,heptadien-4-one, 3,3,6-trimethyl were present only in petroleum ether extract. 1-decane, 1-hexadecanol, 1-tridecanol, 2-pentadecanone,6,10,14-trimethyl-, 4,18,12,16-tetramethylheptadecan-4-olide, 9, 12-octadecenoic acid, methyl ester, (E,E)-, 9-nonadecene,9-octadecenoic acid (Z)-, methyl ester, dascarpidan-1-methanol, acetate (ester), eicosanoic acid,heptacosane, heptadecanoic acid, hydroxylamine, o-decyl-, nonadecane, octacosane, pentacosane, phenol,2,4,bis(1,1-dimethylethyl)-, phytol, tetradecane, tetradecane,2,6,10-trimethyl-, tetradecanoic acid and tridecane were present only in chloroform extract.

Table 1: Identification of chemical constituents from petroleum ether extract of the stem of *M. umbellatum*

S. No.	Name of the compounds	Retention time	Molecular formula	Molecular weight	Peak area %
Aliphatic					
Alkane hydrocarbon					
1	Undecane	4.89	$C11H_{24}$	156	0.45
2	Dodecane	6.23	$C_{12}H_{26}$	170	0.34
3	Hexadecane	8.88	$C_{16}H_{34}$	226	0.52
Fatty acids					
4	Hexadecanoic acid, methyl ester	15.53	$C_{17}H_{34}O_2$	270	1.18
5	n-hexadecanoic acid	16.20	$C_{16}H_{32}O_2$	256	39.12
6	9, 12-octadecanoic acid (Z,Z)-	18.80	$C_{18}H_{32}O_2$	280	5.97
7	Oleic acid	18.87	$C_{18}H_{34}O_2$	282	29.67
8	Octadecanoic acid	19.23	$C_{18}H_{36}O_2$	284	4.82
9	1-Monolinoleoylglycerol trimethylsilyl ether	27.28	$C_{27}H_{54}O_4Si_2$	498	4.61
Aromatic					
Monoterpene					
10	1,5,heptadien-4-one, 3,3,6-trimethyl	3.35	$C_{10}H_{16}O$	152	8.01
Dicarboxylic acid					
11	1,2-benzenedicarboxylic acid, diisooctyl ester	24.87	$C_{24}H_{38}O_4$	390	5.82

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Table 2: Identification of chemical constituents from chloroform extract of the stem of *M. umbellatum*.

S. No.	Name of the compounds	Retention time	Molecular formula	Molecular weight	Peak area %
Aliphatic Acid					
1	Methoxyacetic acid, 4 tetradecyl ester	12.77	$C_{17}H_{34}O_3$	286	0.22
Alkane hydrocarbon					
2	Undecane	4.90	$C_{11}H_{24}$	156	0.19
3	1-decane	6.10	$C_{12}H_{24}$	168	0.23
4	Tridecane	7.57	$C_{13}H_{28}$	184	0.05
5	Tetradecane	8.86	$C_{14}H_{30}$	198	0.23
6	Nonadecane	10.10	$C_{19}H_{40}$	268	0.13
7	Hexadecane	11.27	$C_{16}H_{34}$	226	0.10
8	9-Nonadecene	13.69	$C_{19}H_{38}$	266	0.57
9	Tetradecane,2,6,10-trimethyl-	13.79	$C_{17}H_{36}$	240	0.08
10	2-Pentadecanone,6,10,14-trimethyl-	14.37	$C_{18}H3_{60}$	268	0.36
11	9, 12-Octadecenoic acid, methyl ester, (E,E)-	17.95	$C_{19}H_{34}O_2$	294	0.20
12	9-Octadecenoic acid (Z)-, methyl ester	18.03	$C_{19}H_{36}O_2$	296	0.20
13	4,18,12,16-tetramethylheptadecan-4-olide	21.88	$C_{21}H_{40}O_2$	324	1.09
14	Pentacosane	24.05	$C_{25}H_{52}$	352	1.42
15	Octacosane	26.89	$C_{28}H_{58}$	394	4.30
16	Heptacosane	30.06	$C_{27}H_{56}$	380	4.74
Aliphatic Acid					
1	Methoxyacetic acid, 4 tetradecyl ester	12.77	$C_{17}H_{34}O_3$	286	0.22
Alkane hydrocarbon					
2	Undecane	4.90	$C_{11}H_{24}$	156	0.19
3	1-decane	6.10	$C_{12}H_{24}$	168	0.23
4	Tridecane	7.57	$C_{13}H_{28}$	184	0.05
5	Tetradecane	8.86	$C_{14}H_{30}$	198	0.23
6	Nonadecane	10.10	$C_{19}H_{40}$	268	0.13
7	Hexadecane	11.27	$C_{16}H_{34}$	226	0.10
8	9-Nonadecene	13.69	$C_{19}H_{38}$	266	0.57
9	Tetradecane,2,6,10-trimethyl-	13.79	$C_{17}H_{36}$	240	0.08
10	2-Pentadecanone,6,10,14-trimethyl-	14.37	$C_{18}H3_{60}$	268	0.36
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14	Pentacosane	24.05	$C_{25}H_{52}$	352	1.42
15	Octacosane	26.89	$C_{28}H_{58}$	394	4.30
16	Heptacosane	30.06	$C_{27}H_{56}$	380	4.74
Fatty acids					
17	Hydroxylamine, O-decyl-	6.23	$C_{10}H_{23}NO$	173	0.08
18	1-Hexadecanol	11.17	$C_{16}H_{34}O$	242	0.64
19	Tetradecanoic acid	13.39	$C_{14}H_{28}O_2$	228	0.42
20	Hexadecanoic acid, methyl ester	15.46	$C_{17}H_{34}O_2$	270	0.29
21	n-Hexadecanoic acid	16.21	$C_{16}H_{32}O_2$	256	25.43
22	Heptadecanoic acid	17.62	$C_{17}H_{34}O_2$	270	0.62
23	Phytol	18.37	$C_{20}H_{40}O$	296	0.27
24	Oleic acid	18.87	$C_{18}H_{34}O_2$	282	41.46
25	Octadecanoic acid	19.15	$C_{18}H_{36}O_2$	284	11.41
26	Eicosanoic acid	22.12	$C_{20}H_{40}O_2$	312	2.56
Fatty alcohol					
27	1-Tridecanol	8.75	$C_{13}H_{28}O$	200	0.47
Triterpene					
28	Squalene	28.82	$C_{30}H_5O$	410	0.98
Aromatic Alkylate phenol					
29	Phenol,2,4,bis(1,1-dimethylethyl)-	10.31	$C_{14}H_{22}O$	206	0.80
Alkaloids					
30	Dascarpidan-1-methanol, acetate (ester)	20.64	$C_{20}H_{26}N_2O_2$	326	0.47

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Tables 3: Identification of chemical constituents from ethanol extract of the stem of *M. umbellatum*

S. No.	Name of the compounds	Retention time	Molecular formula	Molecular weight	Peak area %
Aliphatic Diterpene alcohol					
1	3,7,11,15-tetramethyl-2-hexadecen1-ol	14.36	$C_{20}H_{40}O$	296	3.32
2	Phytol	18.37	$C_{20}H_{40}O$	296	2.05
Fatty acids					
3	Oleic acid	18.76	$C_{18}H_{34}O_2$	282	37.44
4	n-Hexadecanoic acid	16.12	$C_{16}H_{32}O_2$	256	36.94

Table 4: Comparative analysis of chemical constituents identified by GC-MS in various extracts of the stem of *M. umbellatum*

S. No.	Name of the compounds	Petroleum ether	Chloroform	Ethanol
Aliphatic Alkane hydrocarbon				
1	1-decane	-	+	-
2	2-Pentadecanone,6,10,14-trimethyl-	-	+	-
3	4,18,12,16-tetramethylheptadecan-4-olide	-	+	-
4	9, 12-Octadecenoic acid, methyl ester, (E,E)-	-	+	-
5	9-Nonadecene	-	+	-
6	9-Octadecenoic acid (Z)-, methyl ester	-	+	-
7	Dodecane	+	-	-
8	Heptacosane	-	+	-
9	Hexadecane	+	+	-
10	Nonadecane	-	+	-
11	Octacosane	-	+	-
12	Pentacosane	-	+	-
13	Tetradecane	-	+	-
14	Tetradecane,2,6,10-trimethyl-	-	+	-
15	Tridecane	-	+	-
16	Undecane	+	+	-
Diterpene alcohol				
17	3,7,11,15-tetramethyl-2-hexadecen1-ol	-	-	+
18	Phytol	-	+	+
	Fatty acids			
19	1-Hexadecanol	-	+	-
20	1-Monolinoleoylglycerol trimethylsilyl ether	+	-	-
21	9, 12-octadecanoic acid (Z,Z)-	+	-	-
22	Eicosanoic acid	-	+	-
23	Heptadecanoic acid	-	+	-
24	Hexadecanoic acid, methyl ester	+	+	-
25	Hydroxylamine, O-decyl-	-	+	-
26	n-hexadecanoic acid	+	+	+
27	Octadecanoic acid	+	+	+
28	Oleic acid	+	+	+
29	Tetradecanoic acid	-	+	-
Fatty alcohol				
30	1-Tridecanol	-	+	-
Aromatic Alkaloids				
31	Dascarpidan-1-methanol, acetate (ester)	-	+	-
Alkylated phenol				
32	Phenol,2,4,bis(1,1-dimethylethyl)-	-	+	-
Dicarboxylic acid				
33	1,2-benzenedicarboxylic acid, diisooctyl ester	+	-	-
Monoterpene				
34	1,5,heptadien-4-one, 3,3,6-trimethyl	+	-	-

- = absent; + = present.

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DISCUSSION

In stem, totally 34 compounds were identified from various extracts by GC-MS analysis. The compounds such as n-hexadecanoic acid, octadecanoic acid and oleic acid were present in all the three extracts. The compounds such as 1-monolinoleoylglycerol trimethylsilyl ether, 9, 12-octadecanoic acid (Z,Z)-, 1,2-benzenedicarboxylic acid, diisooctyl ester and 1,5,heptadien-4-one, 3,3,6-trimethyl were present only in petroleum ether extract. 1-decane, 1-hexadecanol, 1-tridecanol, 2-pentadecanone,6,10,14-trimethyl-, 4,18,12,16-tetramethylheptadecan-4-olide, 9, 12-octadecenoic acid, methyl ester, (E,E)-, 9-nonadecene,9-octadecenoic acid (Z)-, methyl ester, dascarpidan-1-methanol, acetate (ester), eicosanoic acid,heptacosane, heptadecanoic acid, hydroxylamine, o-decyl-, nonadecane, octacosane, pentacosane, phenol,2,4,bis(1,1-dimethylethyl)-,phytol, tetradecane, tetradecane,2,6,10-trimethyl-, tetradecanoic acid and tridecane were present only in chloroform extract. Contrastingly, only four fatty acids namely octocosonoic acid, cerotic acid, ethyl palmitate, palmitic acid and butyric acid were reported from *Memecylon umbellatum*[7]. Biologically, fatty acids are reported to function as endogenous ACE and HMG-CoA reductase inhibitors, nitric oxide enhancers, β-blockers, diuretics, anti-hypertensive, and anti-atherosclerotic molecules[8] The presence of these phytochemicals in leaves and stem of *M. umbellatum* is a significant findings in the present study and form first report in Herbal Science[9].

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