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GC-MS ANALYSIS OF BIOACTIVE COMPOUNDS IN THE PLANT OF METHANOLIC LEAF EXTRACT OF *ELAEOCARPUS TUBERCULATUS* ROXB.

Botany

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ABSTRACT

The GC-MS analysis of the methanolic extract of leaf of *Elaeocarpus tuberculatus* revealed the presence of 40 bioactive compounds. The major bioactive compounds and its peak area are Phenol, 3-methoxy-2,4,6-trimethyl (59. 280 %); Oleic Acid (11. 335 %); Phenol, 3- methoxy- 2, 4, 6-trimethyl (3. 330 % and 1. 308 %); 9, 12- Octadecadienoic acid (Z, Z) (3. 018 %); Phenol, 4- methoxy-2, 3, 6- trimethyl (2.555 %); n-Hexadecanoic acid (1. 88 %) and Glycerol 1-palmitate (1. 096 %).

KEYWORDS

GC-MS, *Elaeocarpus tuberculatus,* methanolic extract of leaf, bioactive compounds, 59.280 %.

INTRODUCTION

Plants are used as therapeutic measures since time immemorial in planned (Ayurveda, Sidhha and Unani) and unorganized (folk, tribal, native) modes of traditional medicine. At that time there was no synthetic medicines, they have been using only the herbal medicines to treat all diseases. The main reason was that the synthetic drugs have many side effects that often lead to serious complications. From this we can understand that plants are rich in medicinal properties and they are very useful in human health and wellbeing. But still the many medicinal plants and their medicinal properties are unidentified. Recently many of the research were being carried out in medicinal plants. The development of herbal medicine was done by the primary screening of the compounds in the plant extracts. Among 4, 00, 000 plant species only 6% of the plants are studied for their biological activity and only few have been phytochemically investigated. This shows that the investigation is needed for many medicinal plants with pharmacological properties.

Gas Chromatography Mass Spectroscopy, a hyphenated system is a very compatible technique and the most commonly used technique for the identification and quantification purpose. The unknown organic compounds in a complex mixture can be determined by matching the spectra with reference spectra which will be extremely useful for the further research for elucidating the relationship between chemical constituents of medicinal plants and its pharmacology in further research. Hence the present study was planned to explore phytochemicals in the methanolic extract of leaf of *Elaeocarpus tuberculatus* through GC-MS analysis.

MATERIALS AND METHODS

Collection of Plant materials

In the present study the tree species of *Elaeocarpus tuberculatus* (commonly known as 'Rudraksh') was selected and twigs were collected (from Upper Palani Hills of Western Ghats, Kodaikanal Forest Division, India) and was authenticated at Botanical Survey of India, Southern Circle, Coimbatore, India. The plant leaf materials were dried separately under shade and powdered and finally stored in a closed container for further screening.

Preparation of plant extracts

The powdered leaf samples were extracted (Mukherjee, 2002) with the solvent methanol by hot extraction using Soxhlet apparatus. The solvent free extracts were collected and stored in a vial (-4˚c) for further analysis. The methanolic leaf extract of *Elaeocarpus tuberculatus* was analyzed for the presence of different volatile compounds by Gas chromatography-Mass spectroscopy (GCMS) technique.

GC-MS analysis of some of the potent volatile constituents present in the extract was performed at "The South India Textile Research Association (SITRA)", Coimbatore, Tamil Nadu, India. GC analysis of the extracts was performed using a GC-MS (Model; Thermo Trace GC Ultra Ver.5.0) equipped with a DB-35MS fused silica capillary column (30m length X outside diameter 0.25 mm X internal diameter 0.25 µm) and gas chromatograph interfaced to a Mass Selective Detector (MS-DSQ-II) with XCALIBUR software. For GC-MS detection, an electron ionization system with ionization energy of -70eV was used. Helium gas was used as a carrier gas at a constant flow rate of 1ml/min and the sample injected was 1µl; Injector temperature was 250°C; Ion source temperature was 200°C. The oven temperature was programmed from 70° to 200°C at the rate of 10°C/min, held isothermal for 1minutes and finally raised to 250°C at 10°C/min. Interface temperature was kept at 250°C. The relative percentage of each extract constituent was expressed as percentage with peak area normalization.

Identification of components

The identity of the components in the extract was assigned by the comparison of their retention time and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures. NIST (Mc Lafferly, 1989), (Stein, 1990) library sources were also used for matching the identified components from the plant material.

RESULTS

GC-MS analysis of the methanolic leaf extract of *Elaeocarpus tuberculatus* shows the presence of forty compounds. The GC-MS analysis of the test plant is presented in Figure 1. The active principles with their retention time (R.T.), molecular formula, molecular structure, molecular weight (MW), and peak area are presented in Table 1. The most prevailing major compounds and its peak area were Phenol, 3-methoxy-2,4,6-trimethyl (59. 280 %); Oleic Acid (11. 335 %); Phenol, 3- methoxy- 2, 4, 6- trimethyl (3. 330 % and 1. 308 %); 9, 12- Octadecadienoic acid (Z, Z) (3. 018 %); Phenol, 4- methoxy-2, 3, 6- trimethyl (2.555 %); n- Hexadecanoic acid (1. 88 %) and Glycerol 1-palmitate (1. 096 %).

Among the identified bioactive compounds, the following compounds have some therapeutic properties viz., Phenol, 3-methoxy-2,4,6-trimethyl- Antioxidant, anticancer, anti inflammatory and sex hormone activity; Oleic Acid - Antibacterial activity; Phenol, 3- methoxy- 2, 4, 6- trimethyl- antioxidant activity; 9, 12- Octadecadienoic acid - anticancer, Anti-inflammatory, antiandrogenic, cancer preventive, dermatitigenic, irritant, anti leukotriene-D4, hypocholesterolemic, 5-alpha reductase inhibitor, anemiagenic, insectifuge, flavor; n-Hexadecanoic acid - Anti-inflammatory, Antioxidant, hypo cholesterolemic nematicide, pesticide, anti androgenic flavor, hemolytic, 5-Alpha reductase inhibitor, potent mosquito larvicide and Glycerol 1-palmitate - anticonvulsant, anticancer activities, antiviral, anti-inflammatory, Painkiller, antihypertension, antibacterial, antimycobacterial, antifungal, antioxidant and antidepressant.

DISCUSSION

The methanolic leaf extract of *Elaeocarpus tuberculatus* shows the presence of forty compounds. Similarly number of reports supported the present study. Hariprasad and Ramakrishnan (2011) reported that the Gas chromatography-Mass spectrometry (GC-MS) analysis of *Rumex vesicarius* was carried out in four solvents like ethyl acetate extract (EE), ethyl alcohol extract (EAE), chloroform extract (CE), and hexane extract (HE)) to identified the phytochemical constituents. In hexane 61 compounds, chloroform 56 compounds, ethyl alcohol 49 compounds and ethanol extract 45 compounds were identified. Further, Sundari Ponnusamy *et al*. (2018) selected alcoholic seed extract of *Gauzuma ulmifolia* for GC-MS analysis. Totally sixteen

compounds were characterized. The major constituents includes D-Asarinin (65.02 %), 2, 6-Bis (3, 4- methylene dioxyphenyl)- 3, 7-dioxabicyclo (3.3.0) octane (20. 12 %), 1- Dodecanone, 2- (imidazol-1- yl)- 1- (4-methoxy phenyl) (7.54 %) and o-Anisic acid, tridec-2-ynyl ester (3.33 %).

GC-MS analysis of aqueous extract of *Aegle marmelos* by Vardhini *et al*. (2018) and the eluted compounds were 4-Acetoxy-3-methoxyacetophenone; Undecanoic acid, 10-methyl, methyl ester; Phytol; Corynan-17-ol, 18, 19- didehydro, 10- methoxy, acetate (ester); Hexadecanoic acid, 2-butoxy-,butyl ester and Flavone. The GC-MS analysis of Bioactive Phytocompounds in Methonalic leaf extract of *Cassia alata* results showed 13 peaks (Kavipriya and Chandran, 2018). The retention time (RT) of all these thirteen peaks indicate the presence of functional group such as 1-Butanol, 3-methyl-1,6-Anhydro-.beta.-D-glucopyranose (levoglucosan), 3-O-Methyl-dglucose, Oxirane, 10-Methyl-E-11-tridecen-1-ol propionate, l-(+)-Ascorbic acid 2,6-dihexadecanoate, (R)-(-)-14-Methyl-8-hexadecyn-1-ol, Oleic Acid , Vitamin E acetate and 1,2-Bis(trimethylsilyl) benzene. Similar work was also carried out by Aslam *et al* (2015) on *Clinacanthus nutan* and Arunachalam *et al* (2015) have reported the GC-MS analysis results on *Morinda tinctoria.*

CONCLUSION

The present study was focused on characterization of bioactive compounds of methanolic extract of leaf of *Elaeocarpus tuberculatus* by GC MS analysis and 40 compounds identified are responsible for new valuable components with various pharmacological actions. Each chemical compound can be extracted individually and can be used in clinical trials to check efficacy, and to develop a novel drug from a crude drug.

Figure 1: GC-MS Chromatogram of the methanolic extract of leaf of Elaeocarpus tuberculatus

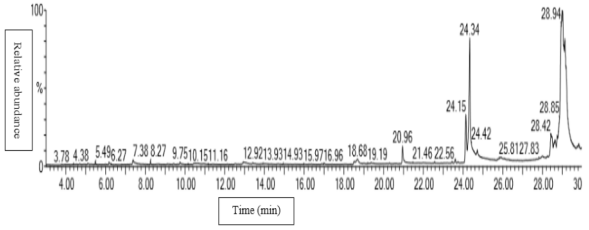
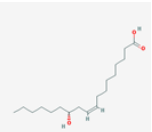
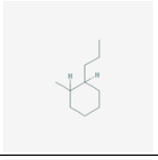
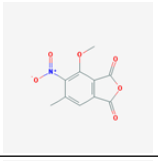
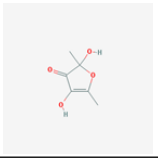
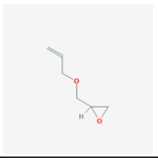
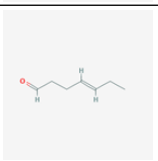
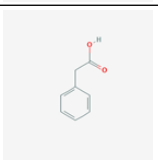
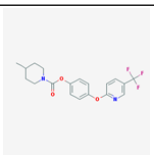
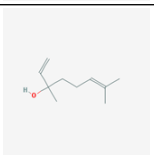
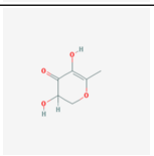
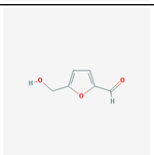
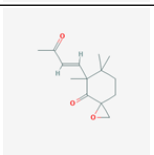
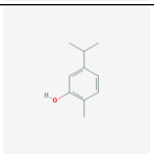
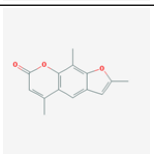
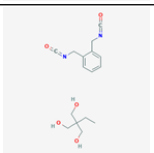
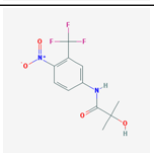
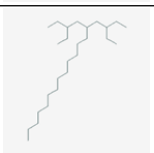
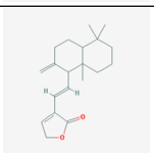
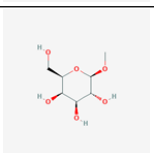
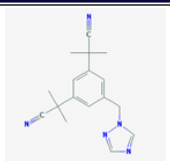
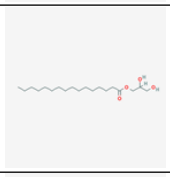
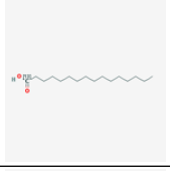
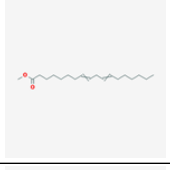
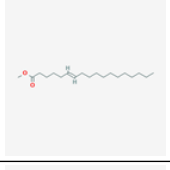
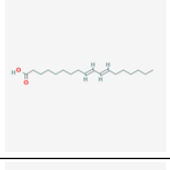
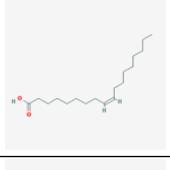
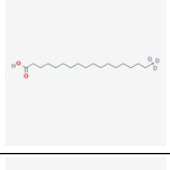
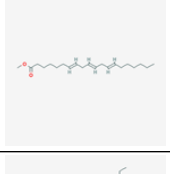
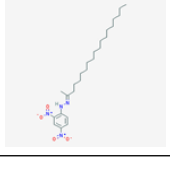
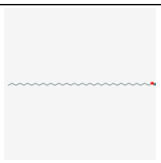
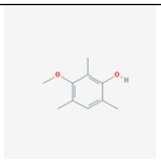
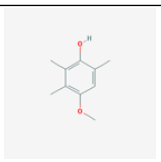
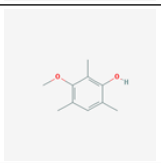
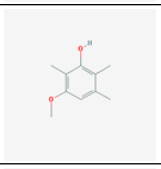
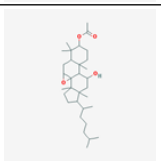
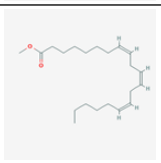
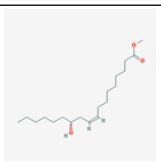


Table 1: Bioactive compounds identified in the methanolic extract of leaf of *Elaeocarpus tuberculatus* by GC-MS

S. No.	R.T.	Compound name	Molecular Formula	Molecular Weight	Molecular Structure	Peak area %
1.	3.048	Ricinoleic acid	$C_{18}H_{34}O_3$	298.461		0.318
2.	3.339	Cyclohexane, 1-methyl-2-propyl-	$C_{10}H_{20}$	140.27		0.151
3.	3.539	4-Methoxy-6-methyl-5-nitroisobenzofuran-1,3-dione	$C_{10}H_7NO_6$	237.167		0.123
4.	3.804	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	144.126		0.190
5.	3.979	Oxirane, [(2-propenyloxy)methyl]-	$C_6H_{10}O_2$	114.144		0.153
6.	4.039	Octadecanal, 2-bromo-	$C_{18}H_{35}BrO$	347.381		0.120
7.	4.379	4-Heptenal	$C_7H_{12}O$	112.172		0.149
8.	4.709	Phenylacetic acid	$C_8H_8O_2$	136.15		0.177

9.	5.099	4-Methylpiperidine-1-carboxylic acid, phenyl ester	$C_{19}H_{19}F_3N_2O_3$	380.367		0.134
10.	5.489	Linalool	$C_{10}H_{18}O$	154.253		0.154
11.	6.175	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6 methyl	$C_6H_8O_4$	144.126		0.269
12.	7.380	5-Hydroxy methyl furfural	$C_6H_6O_3$	126.111		0.770
13.	7.690	5,6,6-Trimethyl-5-(3-oxobut-1-enyl)-1oxaspiro[2.5]octan-4-one	$C_{14}H_{20}O_3$	236.311		0.128
14.	8.265	Phenol, 2-methyl-5-(1-methylethyl)-	$C_{10}H_{14}O$	150.221		0.153
15.	9.751	Trioxsalen	$C_{14}H_{12}O_3$	228.247		0.208
16.	10.531	1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)	$C_{16}H_{22}N_2O_5$	322.361		0.282
17.	10.631	Hydroxyflutamide	$C_{11}H_{11}F_3N_2O_4$	292.214		0.124
18.	10.666	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366.718		0.119
19.	10.766	Villosin	$C_{20}H_{28}O_2$	300.442		0.108
20.	12.987	à-D-Galactopyranoside, methyl	$C_7H_{14}O_6$	194.183		0.653

21.	13.417	Anastrozole	$C_{17}H_{19}N_5$	293.374		0.135
22.	18.510	Neophytadiene	$C_{20}H_{38}$	278.524		0.286
23.	18.695	Glycerol 1-palmitate	C19H38O4	330.509		1.096
24.	20.955	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	257.422		1.888
25.	23.451	8,11-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294.479		0.131
26.	23.607	6-Octadecenoic acid, methyl ester, (Z)-	$C_{19}H_{36}O_2$	296.495		0.307
27.	24.147	9,12-Octadecadienoic acid (Z,Z)-	$C_{19}H_{36}O_2$	280.452		3.018
28.	24.337	Oleic Acid	$C_{18}H_{34}O_2$	282.468		11.335
29.	24.707	Octadecanoic acid	$C_{18}H_{36}O_2$	287.502		0.441
30.	25.917	7,10,13-Eicosatrienoic acid, methyl ester	$C_{21}H_{36}O_2$	320.517		0.830
31.	26.093	2-Nonadecanone 2,4-dinitrophenylhydrazine	$C_{25}H_{42}N_4O_4$	462.635		0.294

32.	27.998	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366.718		0.427
33.	28.243	1-Heptatriacotanol	$C_{37}H_{76}O$	537.014		0.114
34.	28.438	Phenol, 3-methoxy-2,4,6-trimethyl-	$C_{10}H_{14}O_2$	166.22		3.330
35.	28.624	Phenol, 4-methoxy-2,3,6-trimethyl-	$C_{10}H_{14}O_2$	166.22		2.555
36.	28.744	Phenol, 3-methoxy-2,4,6-trimethyl-	$C_{10}H_{14}O_2$	166.22		1.308
37.	28.989	Phenol, 3-methoxy-2,4,6-trimethyl-	$C_{10}H_{14}O_2$	166.222		59.280
38.	29.574	7,8-Epoxylanostan-11-ol, 3-acetoxy-	$C_{32}H_{54}O_4$	502.78		0.330
39.	29.764	8,11,14-Eicosatrienoic acid, methyl ester, (Z,Z,Z)	$C_{21}H_{36}O_2$	320.517		0.225
40.	29.824	9-Octadecenoic acid, 12-hydroxy-, methyl ester, [R-(Z)]-	$C_{19}H_{36}O_3$	312.494		0.403

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