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Research Article

GC-MS Analysis of Ethanolic Extract of Leaf of *Elaeocarpus tuberculatus* Roxb.

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ABSTRACT

To detect the bioactive compounds of ethanolic extract of *Elaeocarpus tuberculatus* Roxb. leaves using GC-MS analysis. Thirty compounds were identified from the test plant. Some of the bioactive compounds screened include Lupeyl acetate, Phytol, 9,12,15-Octadecatrienoic acid,(Z,Z,Z)-, 3-Eicosane, Neophytadiene, Docosane, Dodecanoic acid and 1,2-Benzenedicarboxylic acid. The compounds were identified by comparing with retention time and peak area and by interpretation of mass spectra. From the result it can be concluded that the bioactive compounds have many applications like anti-microbial, antioxidant, anti-cancer and anti-inflammatory properties.

Keywords: Lupeyl acetate, Phytol, Neophytadiene, Docosane.

INTRODUCTION

Cure of any debilitating human ailments and diseases may be found among the world's flora in nature's pharmacy and there are multitudes of potential useful bioactive substances to be derived from plants[1]. Plants are endowed with diverse range of secondary metabolites whose roles within plants are elusive. However, most of them have pharmacological activities and these have been exploited to provide medicinal drugs[2]. The current trend is to isolate and characterize the individual phytochemical components with the aim of producing an analogue of increased bioactivity. In recent years, the major secondary plant metabolites are of potential medicinal interest that has been extensively investigated as a source of medicinal agents in drug discovery[3].

The family Elaeocarpaceae has been reported to be used in traditional medicines particularly in India. A noteworthy chemical feature of Elaeocarpaceae is their ability to elaborate a series of oxygenated steroids or cucurbitacins and ellagic acid derivatives which abound in this family, hold some potential as a source of cytotoxic agents[4-6]. Other principles of interest in Elaeocarpaceae are indolizidine alkaloids, which have attracted a great deal of interest on account of their ability to inhibit the enzymatic activity of glucosidases because of a structural similarity with glucose; hence there is some potential to explore it further in the treatment of Human Immunodeficiency Virus, diabetes and cancer. A small number of indolizidine alkaloids have emerged that have therapeutic indices favoring their introduction into clinical practice[7]. All these pharmacological events together lend considerable support to the view that Elaeocarpaceae plants would be worthy screening thoroughly for cytotoxic agents. One can reasonably expect the discovery of molecules of chemotherapeutic value in this large family.

Elaeocarpus tuberculatus Roxb. is a majestic tree about 80 ft. high and 7 ft. in girth distributed from South and East Asia through Malaysia to Australia and Pacific Islands. In India the species is widely available in the Western Ghats particularly common in Nilgiri, Palni and Annamalai hills. Decoction of the stem bark is used as a remedy for rheumatism, indigestion and biliousness. Rudraksa beads or seeds are used as a treatment for rheumatism, typhoid fever and epilepsy, controls heartbeat, stress, anxiety, depression, palpitation[8]. Review of literature reveals that information on the GC-MS analysis of [9,10] of the *Elaeocarpus serratus* plant extract. Despite the many uses of *E.tuberculatus*, there is no data showing the chemical composition of its ethanol extracts. Hence this work was carried out to profile chemical compounds from ethanolic extract of *E. tuberculatus*.

MATERIALS AND METHODS

Plant material and extraction

The leaves of *Elaeocarpus tuberculatus* Roxb. were collected from Upper Palani Hills of Western Ghats (Kodaikanal Forest Division), India and were authenticated at Botanical Survey of India (BSI), Southern Circle, Coimbatore, India and the herbarium of Voucher specimen number BSI/SRC/5/23/2011-12/Tech.239 has been deposited at the PG and Research Department of Botany, Vellalar College for Women, Erode (Tamil Nadu), India. Fresh leaves were collected and air-dried at room temperature. The dried material was then homogenized to obtain coarse powder and stored in air-tight bottles for further analysis. The shade dried, powder leaf was extracted with ethanol solvent by hot

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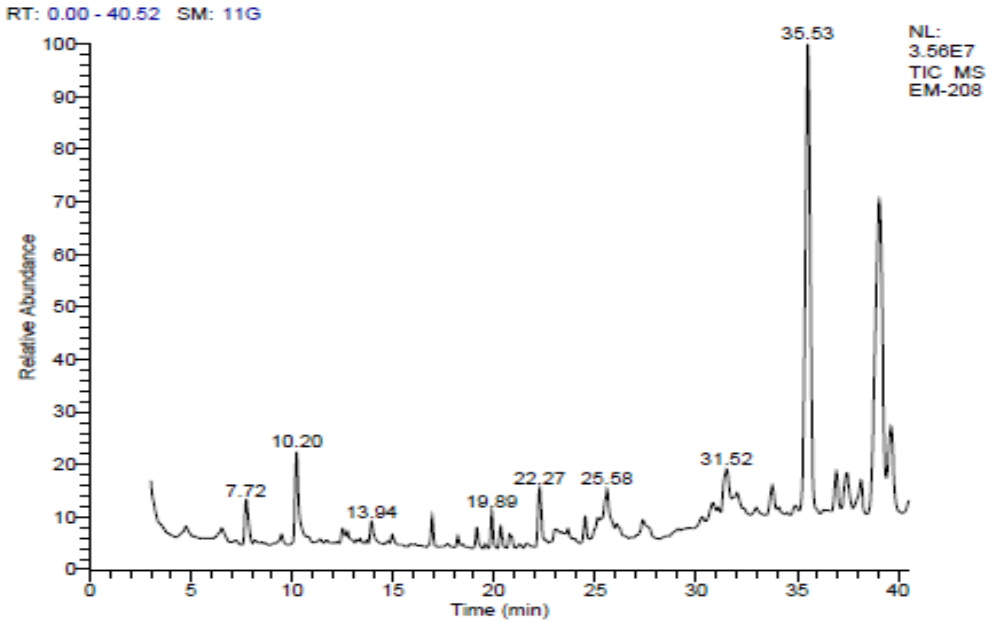

Figure 1: GC-MS Chromatogram of ethanolic extract of leaf of *Elaeocarpus tuberculatus*

extraction using soxhlet apparatus collected and stored in a vial for further analysis[11].

GC-MS Analysis

Ethanolic extract of leaf of *Elaeocarpus tuberculatus* were analyzed for the presence of different compounds by Gas chromatography-Mass spectroscopy technique. GC-MS analysis of some of the potent volatile constituents present in the extracts was performed at The South India Textile Research Association (SITRA), Coimbatore (Tamil Nadu), India. GC analysis of the extract was performed using a GC-MS (Model; Thermo Trace GC Ultra) equipped with a DB-5MS fused silica capillary column (30m length x outside diameter 0.25mm x internal diameter 0.25µm) 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 2µl; Injector temperature 250ºC; Ion source temperature 200ºC. The oven temperature was programmed from 80° to 200°C at the rate of 10°C/min., held isothermal for 1min and finally raised to 260°C at 10°C/min. Interface temperature was kept at 250ºC. Total GC run time was 40.52 min. The relative percentage of the each extract constituents 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 indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures. NIST, WILEY[12,13] library sources were also used for matching the identified components from the plant material.

RESULT

The bioactive compounds present in the ethanolic extract of leaf of *Elaeocarpus tuberculatus* were identified by GC-MS analysis (Fig.1). Thirty compounds were detected in the ethanolic extract of leaf of *E. tuberculatus*. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) in the ethanol extracts of leaf of *E. tuberculatus* are presented in Table 1 and the total running time was 40.52 minutes. The spectra of the compounds are matched with Wiley 9.0 and NIST libraries.

The most prevailing major compounds were α-Amyrin (31.08%) and Lupeyl acetate (24.86%). Among the identified compounds Taraxasterol (4.36%), 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (3.80%), 1,2,3-Benzenetriol (3.17%), 1,4-Bis(3,5-dibromo-2-thienyl) benzene (2.78%), 2-Butyl-2,3-dihydro naphtho[2,3-b]furan-4,9-dione (2.77%), Lanosta-9(11), 24-dien-3-ol,acetate,(3á)- (2.46%), 2-(2-Chlorophenyl)aniline (2.17%), 9-Octadecene,1,1'-[1,2-ethane diylbis (oxy)] bis-, (Z,Z)-(1.89%), Tricosane (1.85%), C-CAM-3-(1-propyl) Ether (1.84%), Naphthalene (1.80%), Neophytadiene (1.26%), Cyclopentane, ethyl- (1.13%), Dodecanoic acid (1.07%), 1,1'-Bicyclohexyl-1,1'-diol (1.06%), Docosane(1.04%), 4-(2'-Methoxyethylidene) and minor components were 4-(2'-Methoxyethylidene) cyclopentene (0.96%), Phenol, 2-chloro- (0.91%), Dodecane,5,8-diethyl-(0.90%), Phytol(0.90%), 5-hydroxy-indolyl-acetic acid (0.86%), 2,4-Pentanedione,3-(2-propenyl)-(0.74%),1-Bromo-1,4,4a,5,8,8a-hexahydronaphthalene (0.71%), Phthalic acid, hept-4-yl isobutyl ester (0.66%), 1,2-Benzenedicarboxylic acid (0.61%), 3-Eicosyne(0.46%),Vitamin-2,3-(18) and O-epoxide (0.43%) possess many biological properties.

DISCUSSION

Table 1: GC-MS analysis of the ethanolic extract of leaf of *Elaeocarpus tuberculatus.*

S. No.	RT	Name of the compound	Molecular formula	Molecular weight	Peak area %
1.	4.73	Phenol, 2-chloro-	C_6H_5ClO	128	0.91
2.	6.51	Cyclopentane, ethyl-	C_7H_{14}	98	1.13
3.	7.72	Naphthalene	$C_{10}H_8$	128	1.80
4.	7.81	Dodecane, 5,8-diethyl-	$C_{16}H_{34}$	226	0.90
5.	9.46	1,2-Benzenedicarboxylic acid	$C_8H_6O_4$	166	0.61
6.	10.20	1,2,3-Benzenetriol	$C_6H_6O_3$	126	3.17
7.	12.74	Zingiberene	$C_{15}H_{24}$	204	1.47
8.	13.92	Dodecanoic acid	$C_{12}H_{24}O_2$	200	1.07
9.	16.93	1,1'-Bicyclohexyl-1,1'-diol	$C_{12}H_{22}O_2$	198	1.06
10.	19.13	1-Bromo-1,4,4a,5,8,8a-hexahydronaphthalene	$C_{10}H_{13}Br$	212	0.71
11.	19.89	Neophytadiene	$C_{20}H_{38}$	278	1.26
12.	20.32	Phthalic acid, hept-4-yl isobutyl ester	$C_{19}H_{28}O_4$	320	0.66
13.	20.77	3-Eicosyne	$C_{20}H_{38}$	278	0.46
14.	22.27	2-Butyl-2,3-dihydronaphtho[2,3-b]furan-4,9-dione	$C_{16}H_{16}O_3$	256	2.77
15.	23.66	2-(2-Chlorophenyl)aniline	$C_{12}H_{10}ClN$	203	2.17
16.	24.49	5-hydroxy-indolyl-acetic acid	$C_{10}H_9NO_3$	191	0.86
17.	25.13	Phytol	$C_{20}H_{40}O$	296	0.90
18.	25.58	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278	3.80
19.	27.36	4-(2'-Methoxyethylidene)cyclopentene	$C_8H_{12}O$	124	0.96
20.	30.82	Docosane	$C_{22}H_{46}$	310	1.04
21.	31.50	1,4-Bis(3,5-dibromo-2-thienyl)benzene	$C_{14}H_6Br_4S_2$	554	2.78
22.	32.05	2,4-Pentanedione, 3-(2-propenyl)-	$C_8H_{12}O_2$	140	0.74
23.	33.78	C-CAM-3-(1-propyl) Ether	$C_{32}H_{35}ClN_2O_4$	546	1.84
24.	34.90	Vitamin-2,3-(18)O-epoxide	$C_{31}H_{46}O_3$	466	0.43
25.	35.55	á-Amyrin	$C_{30}H_{50}O$	426	31.08
26.	36.94	9-Octadecene, 1,1'-[1,2-ethanediylbis(oxy)]bis-,(Z,Z)-	$C_{38}H_{74}O_2$	562	1.89
27.	37.47	Lanosta-9(11),24-dien-3-ol, acetate, (3á)-	$C_{32}H_{52}O_2$	468	2.46
28.	38.18	Tricosane	$C_{23}H_{48}$	324	1.85
29.	39.10	Lupeyl acetate	$C_{32}H_{52}O_2$	468	24.86
30.	39.67	Taraxasterol	$C_{30}H_{50}O$	426	4.36

In recent years GC-MS studies have been increasingly applied for the analysis of medicinal plants as this technique has proved to be a valuable method for the identification of non-polar components and volatile essential oil, fatty acids, lipids and alkaloids. GC-MS analysis of ethanol extracts of leaf of *Elaeocarpus tuberculatus* revealed the presence of α- amyrin, a pentacyclic triterpenol that belongs to the group of ursane and oleanane series. It has a chemical structure similar to that of a steroid and is extremely useful in prevention or treatment of many diseases, particularly those in which oxidative and inflammatory stress plays a key role in pathogenesis. Similarly, Sandos *et al*.,[14] investigated the anti-inflammatory and antinociceptive, antioxidant, anti-pruritic, gastroprotective and hepatoprotective effects of α, β-amyrin, from the resin of *Protium heptaphyllum*.

Lupeyl acetate is one of the major compounds detected and noted for its potent anti-microbial, anti-inflammatory, anti-tumour, antiprotozoal, chemo preventive and anti-skin cancer activity. Taraxasterol is a natural triterpene which has many important pharmacological actions including anti-cancer activity was identified in the present study. Similarly, Keïta *et al*.,[15] demonstrated the presence of lupeyl acetate and taraxasterol in the roots and aerial parts of two varieties of *Vernonia galamensis*. The compound 9,12,15- Octadecatrienoic acid, (Z,Z,Z)- is a linolenic acid is widely used as an anti-inflammatory, hypochloesterolemic, cancer preventive, anti-acne, hepatoprotective, nematicide, insectifuge, anti-histaminic and anti-eczemic. Similar compound was detected by Velanganni and Kadamban[16] in the leaf of *Mallotus philippensis*.

Phytol is one among the twenty nine identified compounds in the present study. Phytol is a diterpene and is widely used as an anti-microbial, antioxidant, anti-tumor, anti- arthritic, anti-cancer, immuno stimulatory, anti-diabetic, chemo preventive, pesticide and diuretic agent and has sunscreen properties[17]. Similarly, Mohan *et al.*[18] reported the occurrence of phytol in the aerial parts of *Kirganelia reticulata.* The compound 3-Eicosane is an alkane noted for its potent anti-tumor activity against the human gastric SGC-7901 cell line[19]. Likewise, Sivasubramanian and Brindha[20] detected the presence of 3-Eicosane in the aerial parts of *Centratherum punctatum*. Neophytadiene, a terpenoid compound has antipyretic, anti-inflammatory, anti-microbial and antioxidant

activity[21]. It is a fatty acid-related compound and plays an important role in competitive inhibition of lipoxygenase or cyclooxygenase in an inflammation reduction, resulting in decreased production of prostaglandins and leukotriene's[22]. Similarly, the compounds like 3-Eicosane and Neophytadiene are present in the ethanolic leaf extract of *Glochidion* ellipticum[23]. Further in the present study,Vitamin-2,3-(18) O-epoxide a vitamin compound having analgesic, anti-diabatic, anti-inflammatory, antioxidant, anti-dermatitic, anti-leukemic, anti-tumor, anticancer, hepatoprotective and antispasmodic activity[24].

CONCLUSION

The GC-MS technique is the first step towards understanding the nature of active principles in the medicinal plants. However, isolation of individual phytochemical constituent and subjecting it to biological activity will definitely give fruitful results. It could be concluded that, Elaeocarpus *tuberculatus* containing various bioactive compounds could be recommended as plant of pharmaceutical importance. However, further studies are needed to undertake its bioactivity and toxicity profile.

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