Original Article

GC-MS Analysis of Bioactive Components of Tubers of *Ruellia tuberosa* L. (Acanthaceae)

Rajendra kumar N[1], Vasantha K[1] and V.R. Mohan*[2]

[1]PG & Research Department of Botany, Government Arts College (Autonomous), Coimbatore, Tamil Nadu, India
[2]Ethnopharmacology Unit, Research Department of Botany, V. O. Chidambaram College, Tuticorin, Tamil Nadu, India

Address for Correspondence

Ethnopharmacology Unit Research Department of Botany, V.O. Chidambaram college, Tuticorin 628008. Tamil Nadu, India
E-mail: vrmohanvoc @gmail.com

ABSTRACT

The present investigation was carried out to determine the possible bioactive components of tuber of *Ruellia tuberosa* L. (Acanthaceae) using GC-MS analysis. Twenty five compounds were identified. The prevailing compounds in the ethanol extract of tuber of *R. tuberosa* were Lupeol (68.14%), Stigmasterol (8.89%), á-Sitosterol (3.99%), Sucrose (2.24%), Cholest-5-ene, 3-bromo-, (3á- (2.24%), Octadecane, 2-methyl- (2.10%), Nonadecane, 2-methyl- (1.93%), Eicosane, 2-methyl- (1.79%) Heptacosane (1.43%) and Heptacosane (1.29%).

Keywords: *Ruellia tuberosa,* Tuber, GC-MS, Bioactive compounds, Lupeol.

INTRODUCTION

Plants are used medicinally in different countries, and they are the source of many potent and powerful drugs. Plants have been an important source of medicine with qualities for thousands of years. Mainly on traditional remedies such as herbs for their history, they have been used as popular folk medicines[1]. It has been shown that *in vitro* screening methods could provide the needed preliminary observations necessary to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations[2]. Plants have great importance due to their therapeutic value and they are the major source of medicines which play an important role in the human history[3]. Plants synthesize primary metabolites (proteins, fats, nucleic acids and carbohydrates) by simple substances such as water, carbon dioxide, nitrogen and a number of inorganic salts in small amounts. These primary metabolites are transformed into secondary metabolites (alkaloids, steroids, terpenoids, saponins, flavonoids etc.,) that are used as drugs[4].

Ruellia tuberosa L. (Acanthaceae) is a tropical plant and widely distributed in Southeast Asia. In folk medicine, it has been used as antidiabetic, antipyretic, analgesic, anti hypertensive, thirst-quenching and antidotal agent[5]. Taking into consideration of the medicinal importance of *Ruellia*

tuberosa, in the present study, GC-MS analysis of ethanol extract of tuber of *Ruellia tuberosa* has been evaluated. This work will help to identify the compounds of therapeutic value.

MATERIALS AND METHODS

Collection of plant sample and extraction

Tubers of *Ruellia tuberosa* L. were collected from Government Girls Higher Secondary School campus, Barugur, Krishnagiri District, Tamil Nadu. With help of local flora, voucher specimen was identified and presented in the PG & Research Department of Botany, Government Arts College, Coimbatore, Tamil Nadu for further references. The tuber was cleaned, shaded dried and pulverized to powder in a mechanical grinder. Required quantity of powder was weighed and transferred to stoppered flask, and treated with ethanol until the powder is fully immersed. The flask was shaken every hour for the first 6 hours and then it was kept aside and again shaken after 24 hours. This process was repeated for 3 days and then the extract was filtered. The extract was collected and evaporated to dryness by using a vacuum distillation unit. The final residue thus obtained was then subjected to GC-MS analysis.

Ruellia tuberosa L

GC-MS Analysis

GC-MS analysis of these extracts were performed using a Perkin-Elmer GC Clarus 500 system and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with a Elite-I, fused silica capillary column (30mmX0.25mm 1D X 1 µMdf, composed of 100% Di methyl poly siloxane). For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1ml/min and an injection volume of 2µl was employed (split ratio of 10:1); Injector temperature 250°C; Ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min.), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 minutes. The relative % 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 Turbo mass.

Identification of 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.

RESULT AND DISCUSSION

The compounds present in the ethanol extract of tuber of *R. tuberosa* were identified by GC-MS analysis (Fig 1). The active

principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) in the ethanol extract of the tuber are presented in Table 1. Twenty five compounds were detected in ethanol extract of the tuber. The results revealed that Lupeol (68.14%), Stigmasterol (8.89%), á-Sitosterol (3.99%), Sucrose (2.24%), Cholest-5-ene, 3-bromo-, (3á- (2.24%), Octadecane, 2-methyl- (2.10%), Nonadecane, 2-methyl- (1.93%), Eicosane, 2-methyl- (1.79%) Heptacosane (1.43%) and Heptacosane (1.29) were found as the major compounds in the ethanol extract of tuber of *R. tuberosa* plant. Figures 2 and 3 show the structure of mass spectrum of *R. tuberosa*. Table 2 listed the various phytochemical constituents which contribute to the medicinal activity of ethanol extract of *R. tuberosa* tubers.

Among the identified phytochemicals, Tetradecanoic acid and n-Hexadecanoic acid have the property of antioxidant activity[6]. Squalene has the property of antioxidant. Recently squalene possesses chemopreventive activity against colon carcinogenesis. The results show that, reactive oxygen species-promising novel class of pharmaceutical for the treatment of rheumatic arthritis and possibly other chronic inflammatory diseases[7,8]. Stigmasterol is used as a precursor in the manufacture of semi synthetic progesterone, a valuable human hormone that plays an important physiological role in the regulatory and tissue rebuilding mechanisms related to estrogen effects, as well as acting as an intermediate in the biosynthesis of androgens, estrogens, and corticoids. It is also used as the precursor of Vitamin D_3[9,10]. Beta-sitosterol limits the amount of cholesterol entering the body by inhibiting cholesterol absorption in the intestines, therefore decreasing the levels of cholesterol in the body. It is helpful with benign prostatic hyperplasia (BPH), due to its anti-inflammatory effects and its ability to improve urinary symptoms and flow.

Thus, this type of GC-MS analysis is the first step towards understanding the nature of active principles in this medicinal plant and this type of study will be helpful for further detailed study. Further investigation into the pharmacological importance of *R. tuberosa* and their diversity and detailed phytochemistry may add new knowledge to the information in the traditional medicinal systems.

CONCLUSION

GC-MS method is a direct and fast analytical approach for identification of terpenoids and steroids and only few grams of plant material is required. The importance of the study is due to the biological activity of some of these compounds. The present study, which reveals the presence of components in tuber of *R. tuberosa* suggest that the contribution of these compounds on the pharmacological activity should be evaluated.

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Table 1. Components identified in the ethanol extract of tubers of *R. tuberosa*

S. No.	RT	Name of the compound	Molecular formula	MW	Peak area %
1	3.30	Bi-2-cyclohexen-1-yl	$C_{12}H_{18}$	162	0.08
2	3.79	Isooctanol	$C_8H_{18}O$	130	0.04
3	6.19	3-Undecene, 9-methyl-, (Z)-	$C_{12}H_{24}$	168	0.08
4	7.48	Sucrose	$C_{12}H_{22}O_{11}$	342	2.64
5	8.59	1-Undecene, 9-methyl-	$C_{12}H_{24}$	168	0.08
6	11.62	1, 2-Benzenedicarboxylic acid, diheptyl ester	$C_{22}H_{34}O_4$	362	0.06
7	13.03	Tetradecanoic acid, ethyl ester	$C_{16}H_{32}O_2$	256	0.08
8	15.15	1, E-11, Z-13-Octadecatriene	$C_{18}H_{32}$	248	0.10
9	15.23	E-2-Octadecadecen-1-ol	$C_{18}H_{36}O$	268	0.08
10	18.07	8-Methyl-6-nonenamide	$C_{10}H_{19}NO$	169	0.60
11	18.38	1-Iodo-2-methylnonane	$C_{10}H_{21}I$	268	0.27
12	19.80	Eicosane	$C_{20}H_{42}$	282	1.10
13	20.12	Hexadecanoic acid, 2,3-dihydroxypropyl ester	$C_{19}H_{38}O_4$	330	0.94
14	20.31	1, 2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	0.77
15	21.20	Heptacosane	$C_{27}H_{56}$	380	1.29
16	22.60	Octadecane, 2-methyl-	$C_{19}H_{40}$	268	2.10
17	23.86	8-Methyl-6-nonenamide	$C_{10}H_{19}NO$	169	0.44
18	23.99	Eicosane, 2-methyl-	$C_{21}H_{44}$	296	1.79
19	24.14	Squalene	$C_{30}H_{50}$	410	0.81
20	25.35	Nonadecane, 2-methyl-	$C_{20}H_{42}$	282	1.93
21	26.69	Heptacosane	$C_{27}H_{56}$	380	1.43
22	29.86	Cholest-5-ene, 3-bromo-, (3á)-	$C_{27}H_{45}Br$	448	2.24
23	30.33	Stigmasterol	$C_{29}H_{48}O$	412	8.89
24	31.50	Á-Sitosterol	$C_{29}H_{50}O$	414	3.99
25	33.33	Lupeol	$C_{30}H_{50}O$	426	68.14

Table 2. Activity of the components identified in the ethanol extract of tubers of *R. tuberosa*

S. No.	RT	Name of the compound	Molecular formula	MW	Peak area %	Nature of compound	**Activity
1	3.79	Isooctanol	C8H18O	130	0.04	Alcoholic compound	Antimicrobial
2	7.48	Sucrose	C12H22O11	342	2.64	Sugar moiety	Preservative
3	11.62	1, 2-Benzenedicarboxylic acid, diheptyl ester	C22H34O4	362	0.06	Plasticizer Compound	Antimicrobial Antifouling
4	13.03	Tetradecanoic acid, ethyl ester	C16H32O2	256	0.08	Myristic acid ester	Antioxidant, Cancer preventive, Nematicide, Lubricant, Hypocholesterolemic
5	18.07	8-Methyl-6-nonenamide	C10H19NO	169	0.60	Amide compound	Antimicrobial, Anti-inflammatory
6	18.38	1-Iodo-2-methylnonane	C10H21I	268	0.27	Iodine compound	Antimicrobial
7	20.12	Hexadecanoic acid, 2, 3-dihydroxypropyl ester	C19H38O4	330	0.94	Palmitic acid ester	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic, 5-Alpha reductase inhibitor
8	20.31	1, 2-Benzenedicarboxylic acid, diisooctyl ester	C24H38O4	390	0.77	Plasticizer Compound	Antimicrobial, Antifouling
9	23.86	8-Methyl-6-nonenamide	C10H19NO	169	0.44	Amide compound	Antimicrobial Anti-inflammatory
10	24.14	Squalene	C30H50	410	0.81	Triterpene	Antibacterial, Antioxidant, Antitumor, Cancer preventive, Immunostimulant, Chemo preventive, Lipoxygenase-inhibitor, Pesticide
11	29.86	Cholest-5-ene, 3-bromo-, (3á)-	C27H45Br	448	2.24	Steroid	Antimicrobial, Anticancer, Anti-inflammatory, Anti asthma, Diuretic, Antiarthritic
12	30.33	Stigmasterol	C29H48O	412	8.89	Steroid	Antimicrobial, Anticancer, Anti-inflammatory, Anti asthma, Diuretic Antiarthritic,
24	31.50	Beta Sitosterol	C29H50O	414	3.99	Steroid	Antimicrobial, Anticancer,Anti-inflammatory, Anti asthma, Diuretic Antiarthritic
25	33.33	Lupeol	C30H50O	426	68.14	Triterpenoid	Antimicrobial, Anticancer, Anti-inflammatory, Antioxidant, Antiarthritic

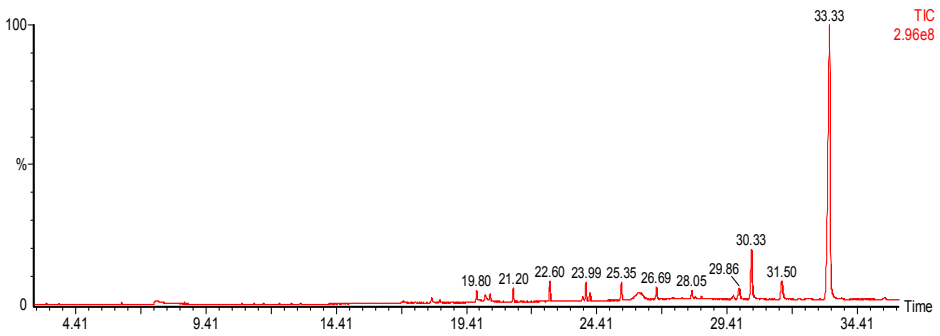
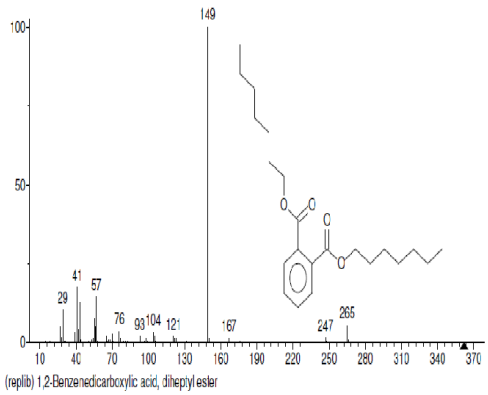
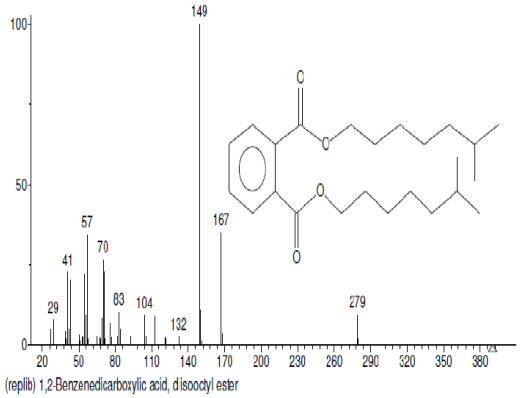


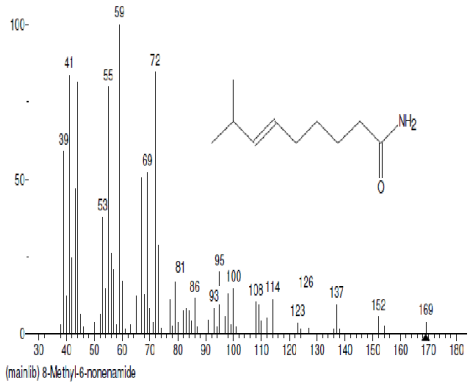
Figure 1. GC-MS Chromatogram of the ethanol extract of tuber of *R. tuberosa*



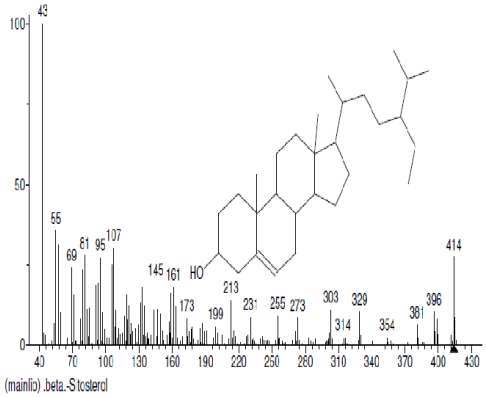
1,2-Benzenedicarboxylic acid, diheptyl ester



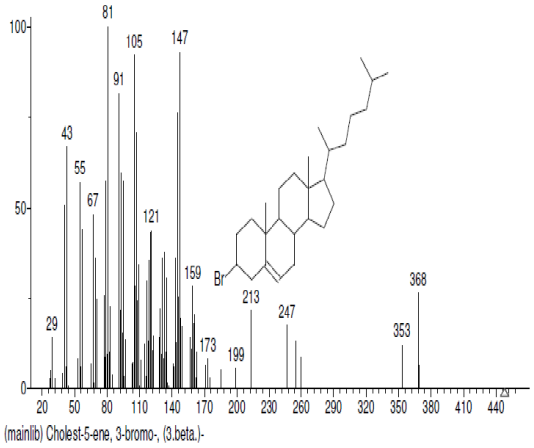
1,2-Benzenedicarboxylic acid, diisooctyl ester



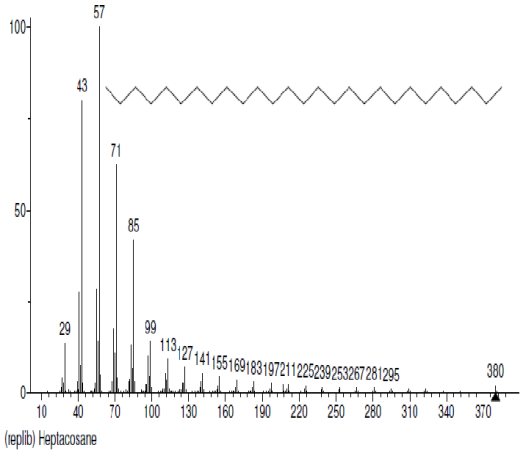
8-Methyl-6-nonenamide



Beta Sitosterol

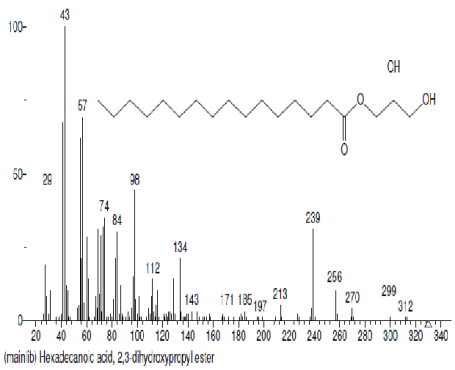


Cholest-5-ene, 3-bromo-, (3á)

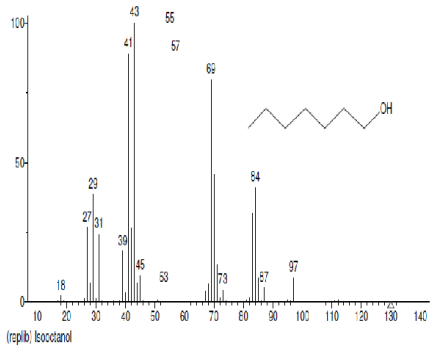


Heptacosane

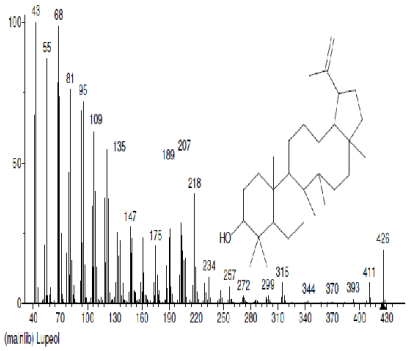
Figure 2. GC-MS Mass Spectrum of some compounds identified in the ethanol extract of tubers of *R. tuberosa*



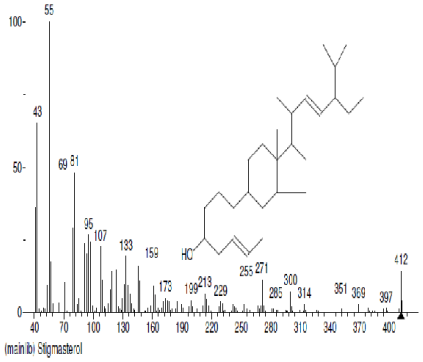
Hexadecanoic acid, 2,3-dihydroxypropyl ester



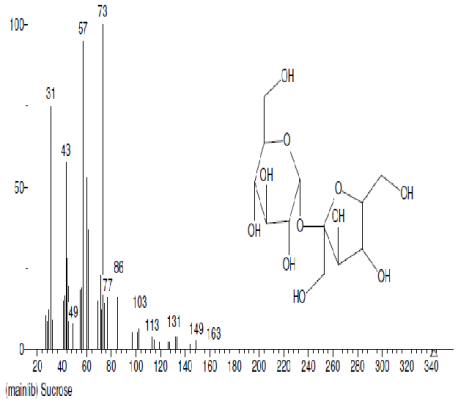
Isooctanol



Lupeo



Stigmasterol



Sucrose

Figure 3. GC-MS Mass Spectrum of some compounds identified in the ethanol extract of tuber of *R. tuberosa*