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International Journal of Current Research
Vol. 5, Issue, 12, pp.4070-4074, December, 2013

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

GC-MS ANALYSIS FOR BIOACTIVE COMPOUNDS IN THE METHANOLIC LEAF AND ROOT EXTRACTS OF *HYPOCHAERIS RADICATA* L. (ASTERACEAE)

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ARTICLE INFO

Article History:
Received 09th September, 2013
Received in revised form
18th October, 2013
Accepted 20th November, 2013
Published online 25th December, 2013

Key words:
Hypochaeris radicata,
Asteraceae, GC-MS analysis,
Leaf and root parts.

ABSTRACT

The present study was aimed at to investigate the bioactive compounds from the leaf and root extracts of *Hypochaeris radicata* using GC-MS analysis. 11 compounds from leaf and 9 compounds from root extracts were identified. The major chemical constituents in leaf and root extracts are phytol, acetate (19.22%), hexadecanoic acid, methyl ester (17.37%), 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)- (16.74%) and phytol (13.60%) and Urs-12-en-3-ol, acetate, (3.beta.) (43.86%) and 1-Benzazirene-1-carboxylic acid, 2,2,5a-trimethyl-1a-(3-oxo-1-butenyl) perhydro-, methyl ester (30.31%). The bioactive compounds in the methanolic leaf and root extracts of the species, *H. radicata* exhibited the phytochemical importance and hence its therapeutic significance.

INTRODUCTION

Plants have been used in treating human diseases for thousands of years. Even today, bioactive compounds from plants continue to play a major role in health care benefits (Karuppasamy *et al.,* 2012). In future, phytomedicines are needed to standardize the plant constituents. GC-MS analysis for bioactive components is the more appropriate technique to identify the new phytochemicals of medicinal importance which have higher activity against many diseases (Gopalakrishnan, 2011; Selvamangai and Anusha, 2012; Janakiraman *et al.,* 2012). *Hypochaeris radicata* L. (Asteraceae) is an edible perennial herb found in the forest margins of Nilgiris, the Western Ghats around the altitude of 2000m above msl. The whole plant is used traditionally for anticancer, anti-inflammatory, anti-diuretic, hepatoprotective, antioxidant (Jamuna *et al.,* 2012), antibacterial (Jamuna *et al.,* 2013), antifungal (Jamuna *et al.,* 2012) and antidiuretic properties. This species is being used for medicinal purpose in Meghalaya (Tynsong *et al.,* 2006) and also used as food for ruminants in British Columbia (Cheryl *et al.,* 2007). Despite these importances, no major works have been carried out in this species on the aspect of phytochemical compounds. To address this lacuna, the present study was carried out to evaluate the phytochemical compounds present in the methanolic extracts of leaf and root parts of *H. radicata* using GC-MS analysis.

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MATERIALS AND METHODS

Collection of plant material

The plant material of *H. radicata* were collected from Kattabettu, Nilgiris, the Western Ghats, India. The authenticity of the plant was confirmed in Botanical Survey of India, Southern Circle, Coimbatore by referring the deposited specimen. The voucher number is BSI/SRC/5/23/2010-11/Tech.153.

Preparation of extract

The fresh leaves and roots of *H. radicata* were shade-dried and pulverized to powder. About 50g powdered plant materials were extracted with 250ml of methanol (60-80°C). The solvent present in the extracts were condensed under room temperature.

GC-MS analysis

Preparation of extract

1µL of the methanolic leaf and root extracts of *H. radicata* were employed separately for GC-MS analysis.

Instruments and chromatographic conditions

GC-MS analysis was carried out on a GC-MS 5975C (AGILENT) instrument employing the following conditions: - column DB-5ms Agilent (30m X 0.25mm, 0.25µm film thickness), operating in electron impact mode at 70eV. Helium (99.9995%) was used as carrier gas at a constant flow of

1.51mL/min in the split mode (split ratio-10:1), injector temperature 240°C, ion source temperature 200°C. The oven temperature was programmed from 70°C (hold time for 2 min) with an increase of 10°C/min to 300°C/min ending with a 9 min isothermal. Mass spectrum was taken at 70eV, scan range of 40-1000m/z and a scan interval of 5mins.

Data analysis

Identification of bioactive compounds from methanolic extracts of leaf and root parts of *H. radicata* was based on the molecular structure, molecular mass and calculated fragmentations. The mass spectrum of the unknown compound was compared with the spectrum of the known components stored in the National Institute Standard and Technology (NIST11.LIB (Stein, 1990)) library, having more than 62,000 patterns.

RESULTS

The compounds present in the methanolic leaf and root extracts of *H. radicata* were presented in Figs. 1 and 2.

The active principles with their retention time (RT), molecular formula, molecular weight, peak area (%), nature of the compound, activity and IUPAC name in the methanolic extracts of leaf and root parts were presented in Tables 1 and 2. In the present investigation, the methanolic leaf extract showed the presence of 11 compounds namely, docosanoic acid 1-methyl-butyl ester, N-methyl-N-acetyl-3,4-methylenedioxy benzylamine,N'-2-(2-cyanopropyl)-N,N-dimethylformamidine, phytol, acetate, 2-hexadecene, 3,7,11,15-tetramethyl-, (R-(R*,R*-(E)))-, 1,13-tetradecadiene, 1,4-eicosadiene, hexadecanoic acid, methyl ester, 9,12-octadecadienoic acid, methyl ester, 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)- and phytol, and the root extract exhibited 9 compounds namely, undecane, hexadecanoic acid, methyl ester, 9,12-octadecadienoic acid (Z,Z)-, methyl ester, 5- acetamido-4,7-dioxo-4,7-dihydrobenzofurazan, methyl (5-hydroxy-1H-benzimidazol-2-yl) carbamate, hexahydro pyridine, 1-methyl-4-(4,5-dihydroxyphenyl)-, 2(1H) Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-, 1-benzazirene-1-carboxylic acid, 2,2,5a-trimethyl-1a-(3-oxo-1-butenyl) perhydro-, methyl ester and urs-12-en-3-ol, acetate, (3.beta.).

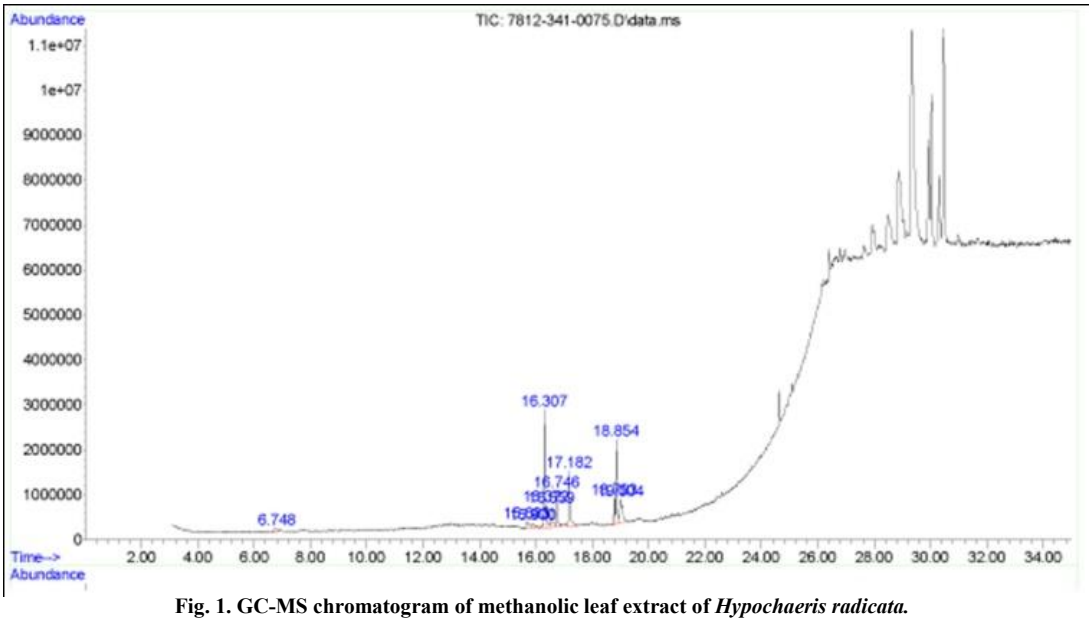

Fig. 1. GC-MS chromatogram of methanolic leaf extract of *Hypochaeris radicata.*

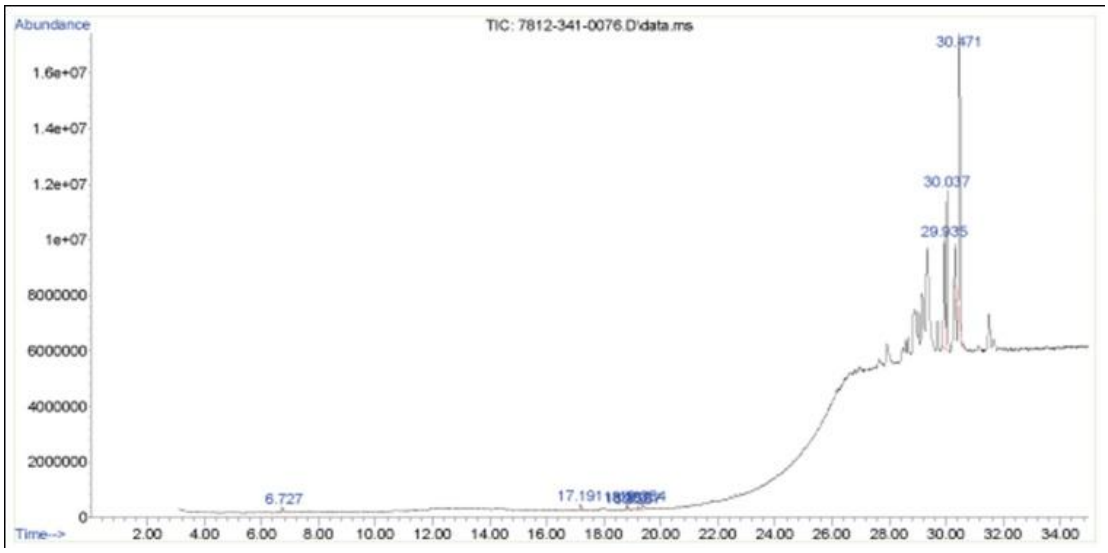

Fig. 2. GC-MS chromatogram of methanolic root extract of *Hypochaeris radicata.*

Table 1. Activity of bioactive compounds identified in the methanolic leaf extract of *Hypochaeris radicata.*

S. No	Name of the compound	Retention time (min)	Molecular formula	Molecular weight (Da)	Peak area (%)	Nature of the compound	Activity	IUPAC Name
1.	Docosanoic acid 1-methyl-butyl ester	6.747	$C_{27}H_{54}O_2$	410.716492	2.00	-	-	2-Pentanyl docosanoate
2.	N-methyl-N-acetyl-3,4-methylenedioxybenzylamine	15.678	-	-	3.89	-	-	-
3.	N'-2-(2-Cyanopropyl)-N,N-dimethylformamidine	15.896	$C_7H_{13}N_3$	139.198196	2.39	-	-	N-(2-cyanopropyl)-N,N'-dimethylmethanimidamide
4.	Phytol, acetate	16.303	$C_{22}H_{42}O_2$	338.56768	19.22	Oleic acid	Antitubercular activity against mycobacterium tuberculosis H37Rv at 500µg/mL by BACTEC460 radiometric susceptibility assay.	(2E,7R,11R)-3,7,11,15-Tetramethyl-2-hexadecen-1-yl acetate
5.	2-Hexadecene, 3,7,11,15-tetramethyl-, (R-(R*,R*-(E)))-	16.375	$C_{20}H_{40}$	280.5316	6.64	Aliphatic hydrocarbons	Antimicrobial, anti-inflammatory.	2-Hexadecene, 3,7,11,15-tetramethyl-Phytene-2
6.	1,13-Tetradecadiene	16.564	$C_{14}H_{26}$	194.356201	6.15	-	The metabolism of plant could be controlled by gene engineering.	1,13-Tetradecadiene
7.	1,4-Eicosadiene	16.753	$C_{20}H_{38}$	278.5157	8.32	Alkene Compound	No activity reported.	(4E)-icosa-1,4-diene
8.	Hexadecanoic acid, methyl ester	17.189	$C_{17}H_{34}O_2$	270.4507	17.37	Palmitic acid ester (methyl palmitate)	Antioxidant, hypercholesterolemic, pesticide.	Methyl palmitate
9.	9,12-Octadecadienoic acid, methyl ester	18.786	$C_{19}H_{34}O_2$	294.4721	3.68	Linolenic acid	Hepatoprotective, antihistaminic, hypocholesterolemic, antieczemic.	methyl (9Z,12Z)-octadeca-9,12-dienoate
10.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	18.859	$C_{19}H_{32}O_2$	292.455994	16.74	Linolenic acid	Antiinflammatory, hypocholesterolemic cancer preventive, hepatoprotective, nematicide insectifuge, antihistaminic, antieczemic, antiacne, 5-alpha reductase inhibitor antiandrogenic, antiarthritic, anticoronary.	methyl (9Z,12Z,15Z)-octadeca-9,12,15-trienoate
11.	Phytol	19.004	$C_{20}H_{40}O$	296.531006	13.60	Diterpene	Anticancer, antioxidant, anti-inflammatory, diuretic, antimicrobial, use in vaccine formulations.	(2E,7R,11R)-3,7,11,15-Tetramethyl-2-hexadecen-1-ol

Table 2. Activity of bioactive compounds identified in the methanolic root extract of *Hypochaeris radicata*

S. No	Name of the compound	Retention time (min)	Molecular formula	Molecular weight (Da)	Peak area (%)	Nature of the compound	Activity	IUPAC Name:
1.	Undecane	6.732	$C_{11}H_{24}$	156.3083	1.23	-	Anti-fungal agents, transducer for immunosensor and its method of production. Carcinogens, enzyme inhibitors, solvents.	Undecane
2.	Hexadecanoic acid, methyl ester	17.189	$C_{17}H_{34}O_2$	270.450714	1.51	Palmitic acid ester (methyl palmitate)	Antioxidant, hypercholesterolemic, pesticide.	methyl hexadecanoate
3.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.801	C19H34O2	294.47206	0.71	Linoleic acid	Anti inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide insectifuge, antihistaminic, antieczemic, antiacne, 5 alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary.	methyl (9Z,12Z)-octadeca-9,12-dienoate
4.	5-Acetamido-4,7-dioxo-4,7-dihydrobenzofurazan	18.859	$C_8H_5N_3O_4$	207.143	0.86	-	-	N-(4,7-Dioxo-4,7-dihydro-2,1,3-benzoxadiazol-5-yl)acetamide
5.	Methyl (5-hydroxy-1H-benzimidazol-2-yl) carbamate	19.208	$C_9H_9N_3O_3$	207.06	0.50	-	-	Methyl (6-hydroxy-1H-benzimidazol-2-yl)carbamate
6.	Hexahydropyridine, 1-methyl-4-(4,5-dihydroxyphenyl)-	19.353	$C_{12}H_{17}NO_2$	207.12	1.78	-	-	4-(1-Methyl-4-piperidinyl)-1,2-benzenediol
7.	2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-	29.940	$C_{15}H_{22}O$	18.334	19.23	-	-	6-Isopropenyl-4,8a-dimethyl-3,5,6,7,8,8a-hexahydro-2(1H)-naphthalenone
8.	1-Benzazirene-1-carboxylic acid, 2,2,5a-trimethyl-1a-(3-oxo-1-butenyl) perhydro-, methyl ester	30.042	$C_{15}H_{23}NO_3$	265.34802	30.31	-	-	Methyl 2,2,6-trimethyl-1-((1E)-3-oxo-1-buten-1-yl)-7-azabicyclo(4.1.0)heptane-7-carboxylate
9.	Urs-12-en-3-ol, acetate, (3.beta.)	30.477	$C_{32}H_{50}O_3$	482.7376	43.86	-	-	(8a-formyl-4,4,6a,6b,11,12,14b-heptamethyl-2,3,4a,5,6,7,8,9,10,11,12, 12a,14,14a-tetradecahydro-1H-picen-3-yl) acetate

The methanolic leaf and root extracts of *H. radicata* showed highest peak area of 19.22% and 43.86% respectively were obtained by phytol, acetate (oleic acid) and urs-12-en-3-ol, acetate, (3.beta.) with retention time, 16.303 and 30.477 (min) respectively. The lowest peak area of 2% and 0.5% were obtained by docosanoic acid 1-methyl-butyl ester and methyl (5-hydroxy-1H-benzimidazol-2-yl) carbamate with retention time 6.747 and 19.208 (min) respectively.

DISCUSSION

In the present study, the GC-MS analysis of the methanolic leaf extract of *H. radicata* showed the presence of 11 compounds. The mass spectra and structures of four major compounds are presented in Figs. 3-6.

They are phytol, acetate (peak area, 19.22%), hexadecanoic acid, methyl ester (peak area 17.37%), 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)- (peak area 16.74%) and phytol (peak area 13.60%). The compounds phytol, acetate is reported to have antitubercular activity. Hexadecanoic acid, methyl ester has the property of antioxidant, hypercholesterolemic, pesticide (Antara and Amla, 2012; Jagadeeswari, *et al.,* 2012). 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)- found to have antiinflammatory, hypocholesteroleimic, cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic, antieczemic, antiacne, antiarthritic and anticoronary properties (Ravi Kumar *et al.,* 2012). The compound, phytol which was identified in the leaf extract of *H. radicata* having anticancer, antioxidant and anti-inflammatory properties. In earlier report said that it have ability of curing the arthritis (Ravi Kumar *et al.,* 2012). The methanolic root extract of *H. radicata*

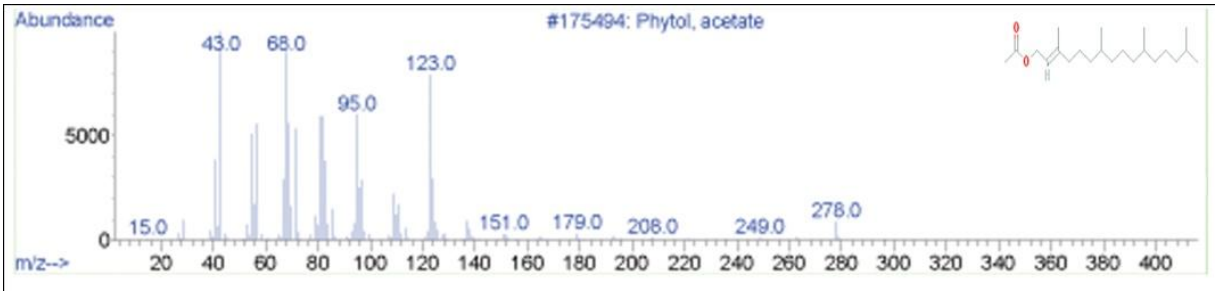

Fig. 3. Mass spectrum of phytol, acetate (RT: 16.303)

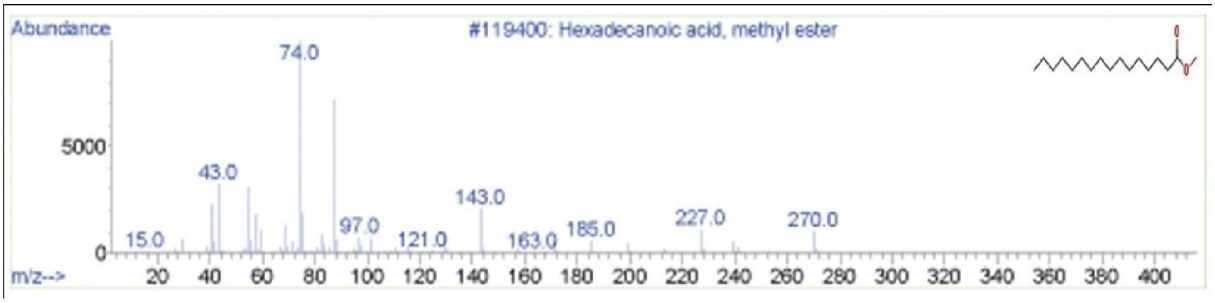

Fig. 4. Mass spectrum of hexadecanoic acid, methyl ester (RT: 17.189)

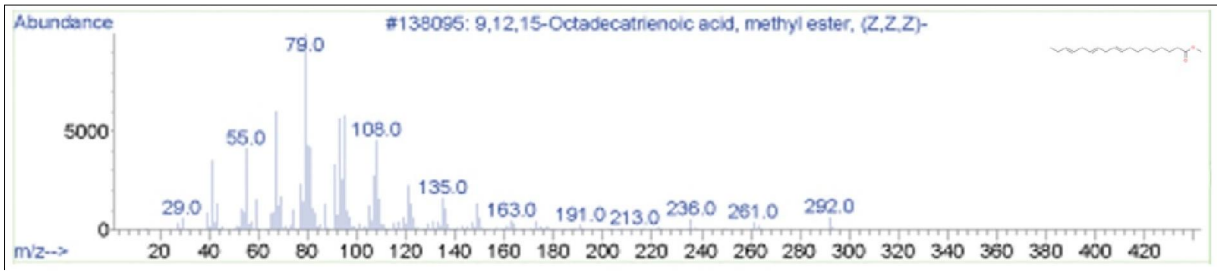

Fig. 5. Mass spectrum of 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)- (RT: 18.859)

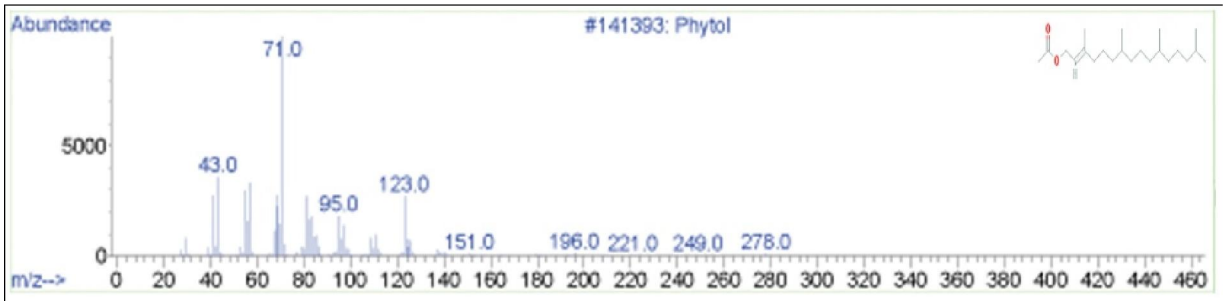

Fig. 6. Mass spectrum of phytol (RT: 19.004)

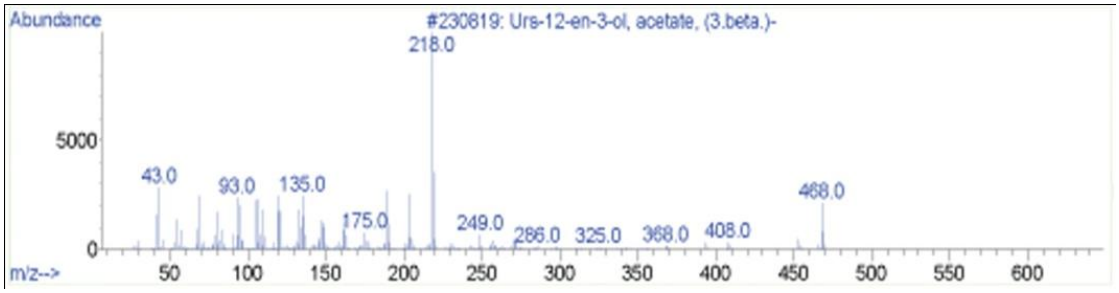

Fig. 7. Mass spectrum of Urs-12-en-3-ol, acetate, (3.beta.) (RT: 30.477)

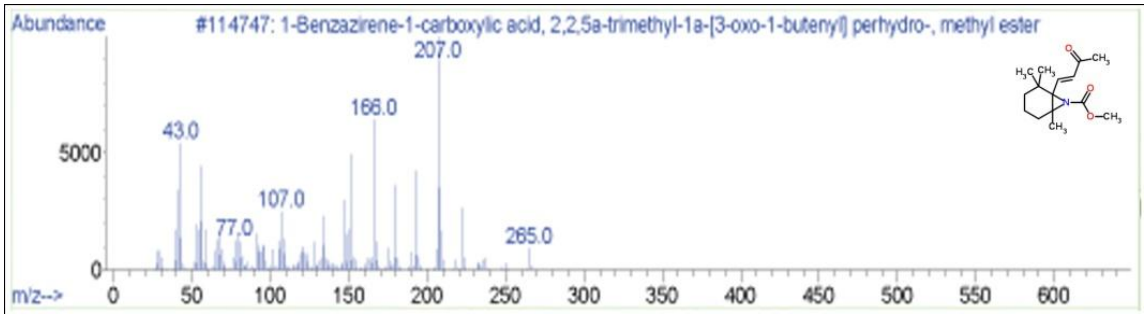

Fig. 8. Mass spectrum of 1-Benzazirene-1-carboxylic acid, 2,2,5a-trimethyl-1a-[3-oxo-1-butenyl] perhydro-, methyl ester (RT: 30.042)

showed the presence of 9 compounds. Of them, only two compounds which are present in leaf extract also were identified for its nature and activity. Hexadecanoic acid, methyl ester (peak area 1.51%) and 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)-(peak area 0.71%). For other major compounds reported in the root extract were Urs-12-en-3-ol, acetate, (3.beta.) (peak area 43.86%) and 1-Benzazirene-1-carboxylic acid, 2,2,5a-trimethyl-1a-(3-oxo-1-butenyl) perhydro-, methyl ester (peak area 30.31%) (Fig. 7, 8). For these compounds, the therapeutic properties could not be reported.

Conclusion

In the present study, 11 and 9 compounds were identified from the methanolic leaf and root extracts of *Hypochaeris radicata* respectively by using GC-MS analysis. Presence of various bioactive compounds in the extracts justifies the use of this plant for various ailments by traditional medicinal practioners. Further investigation into isolation of pure compounds and pharmacological studies were needed to give fruitful results. From the above results, it could be recommended as a plant of phytopharmaceutical importance.

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