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GC-MS ANALYSIS OF *CONVOLVULUS ARVENSIS*

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ABSTRACT

The investigation was carried out to determine the qualitative and quantitative analysis of phytochemical screening and possible chemical components of *C. arvensis* aerial parts and roots by GC-MS. The aerial parts and roots showed the presence of as sugar, tannins, flavonoids, steroid, terpenoids and alkaloids. A total of 31 compounds, which were the major part of the extracts, were identified by matching mass spectra with a mass spectrum library (NIST 2.0 f). GC-MS analysis of acetone aerial parts extracts lead to identification of nine compounds while methanol aerial parts extract identification of seven compounds. Three compounds were identified from methanol roots extract. There were seven compounds identified from acetone roots extract and four compounds identified from aquatic roots extract.

Key Words:- *Convolvulus arvensis*, GC-MS, Phytochemical.

INTRODUCTION

Convolvulus arvensis is a long lived deep rooted weed belongs to the family Convolvulaceae. It is commonly known as European bindweed, bindweed, creeping jenny, morning glory, and devil's guts. It has at least 84 common names (Arora M and Malhotra M, 2011).

Aerial parts of *C.arvensis* are used as laxative, wound healing, anti-spasmodic and anti haemorrhagic, anti-angiogenetic effect. It is still used in Turkey as a vegetable and condiment; in Arabic-speaking areas, the roots and leaves are used as an anti-hemorrhagic and laxative (Austin DF, 2000). The plant contains Saponins, Alkaloids, and Polyphenolic compounds Flavonoids and Tannins (Kaur M & Kalia AN, 2012). It has stimulant, antibacterial (Ali A *et al*., 2013), anti-oxidant (Elzaawely AA and Tawata S, 2012), anticancer (Kaur M, Kalia AN 2012), (Sadeghi-Aliabadi H *et al*., 2008) and stimulatory effect on the immune system (Bowait ME *et al*., 2010).

TLC and HPLC were applied to carry out qualitative and quantitative determination of polyphenolic compounds in the plant, such as coumarins and phenolic acids. It was identified and confirmed that umbelliferon and scopoletin were present in coumarin fraction. In the fraction of phenolic acids the occurrence of protocatechuic ,caffeic, chlorogenic, gentisic, p-coumaric, p-hydroxybenzoic, p-hydroxyphenylacetic, ferulic, vannilic and salicylic acids were present (Todd F *et al*., 1995).

The aerial parts, roots, and flowers of *C. arvensis* were investigated for their secondary metabolites. Eleven flavonoids were detected, namely Kaempferol and its 3-O-β-D-glucoside, 7- O-β-D-glucoside, 3-O-α-L-rhamnosyl, 7-O-β-D-glucoside, 3-O-rutinoside,7-O-rutinoside, 3-O-α-L-rhamnoside and 3-O-β-D-galactorhamnoside as well as Quercetin and its 3-O-α-L-rhamnoside and 3-O-rutinoside. Four coumarins reported are 7- hydroxycoumarin (umbelliferone); 6,7-dihydroxycoumarin (esculetin); 6-methoxy-7-hydroxycoumarin (scopoletin) and 6-methoxycoumarin 7-O-glucoside (scopoletin7-O-glucoside). Amino acids and free sugars were also detected (Joseph M, 2013). Qualitative and quantitative assay of the content of different fractions of aerial and root parts of *C. arvensis* were studied by GC/MS technique.

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MATERIALS AND METHODS

Plant material

Aerial parts and roots of *C. arvensis* were collected during September 2012, from around AL-Mustansiryia University of Baghdad/Iraq. Thailand and authenticated visually according to a taxonomic method by Professor Ali Al-Musawi / Herbarium of the College of Sciences, Department of Biology, University of Baghdad/ Iraq.

Extraction

1. Acetone extraction

Samples, previously aerial parts or roots, were Soxhlet extracted with acetone (Panreac, Barcelona, Spain) for 6 h. The extract was filtered using Whatman No.1 filter paper and the residue was removed. It was again filtered and sterilized through 0.22 µ micro filters.

2. MeOH extract

One hundred gram of aerial parts or roots was used for extraction. Methanol 95% extract were prepared by percolation. Extraction time was fixed for 72 h. The methanol extract was filtered using Whatman No.1 filter paper and the residue was removed. It was again filtered and sterilized through 0.22 µ micro filters.

3. Aqueous extract

The fresh raw material (32 gm was mixed in distilled water (290 ml) at a concentration of 0.16 g/mL using a commercial blender for 5 minut . The mixture was put in water bath at (35-40°C) for thirty minutes, allowed to cool, and shaking for thirty minutes. The extract was filtered using Whatman No.1 filter paper, allowed to cool. The filtrate was centrifuged at 2000/min for 15 min., at 4OC. The supernatant was filtered through 0.22 µ micro filters.

Screening of Phytochemical Components:

Phytochemical components were analyzed qualitatively. Detection of Steroids, Terpenes, Sugar, Saponins, Flavonoids, Alkaloids and Tannins were done according to (Harborn JB 1984).

GC-MS analyses

1. Acetone and methanol extract

GC-MS analyses were done according to (12 Tokuşoğlu, Ö. Ünal, M. K. and Yıldırım, Z. 2003) with some modifications. Gas Chromatography – Mass Spectrometry GC-MS analysis used an Agilent 6890 GC system coupled with an Agilent 5973N MSD operating at 70 eV, ion source temperature 200 °C, in lit temperature 200 °C; split injection (1 µl injection volume, split ratio, 50:1). Capillary column (HP-5MS 30 m x 0.25 mm ID x 0.25 µm film, Agilent J & W, USA) was used; oven: 100 °C/min ; 275 °C at 10 °C/min for 20 min; transfer line temp.: 220 °C. Carrier gas helium; constant flow rate 1 ml/min; data acquisition by Agilent GC/MSD Chem-Station Version D.02.00.

2. Aquatic extract

Gas Chromatography – Mass Spectrometry GC-MS analysis used an Agilent 6890 GC system coupled with an Agilent 5973N MSD operating at 70 eV. Operating conditions for analysis of aquatic extract are summarized below.

Carrier gas, helium with a flow rate of 0.7 mL/min; column temperature, 5 min at 60°C, 60- 290°C at 3°C/min and finally 5 min at 290°C; injector temperature, 250 °C; detector temperature, 290°C , Volume injected, 1 µL; Split ratio, 1:53. The MS operating parameters were as follows: ionization potential, 70 eV; ion source temperature; 290°C; quadrupole 100°C, Solvent delay 4.0 min, scan speed 2000 amu/s and scan range 30-600 amu, EV voltage 3000 volts (Delazar A *et al*., 2009).

Identification of components

Identification of components of *C. arvensis*, the acetone, methanol and aquatic extract was based on direct comparison of the retention times and mass spectral data with those for standard compounds, and computer matching with the (NIST mass spectral search program for the NIST/EPA/NIH mass spectral library version 2.0 f / 2008).

RESULTS AND DISCUSSION

Screening of Phytochemical Components

The qualitative analysis of the extracts from the aerial parts of *C. arvensis* showed the presence of phytochemical constituents such as sugar, tannins, flavonoids, steroid, terpenoids and alkaloids. At the same time, the phytochemical constituent, like saponins, were absent from methanol and chloroform extracts, Table (1). Preliminary phytochemical analysis of roots showed similar feature to aerial parts analysis (Table 2).

GC-MS analyses

GS-MS chromatogram of different parts extracts study showed 30 peaks in *C. arvensis*. The fragmentation patterns of the peaks were compared with that of the library of compounds. The active principles of acetone aerial parts extract with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in (Table 3), (Fig. 1). Nine compounds were identified in acetone aerial parts

extract by GC-MS. The major components present was 1,2-Ethanediamine, N'-ethyl-N,N-dimethyl- (36.010%). Mass spectra from full scan analysis of components were showed in (Fig. 2).

Seven bioactive phytochemical compounds were identified in the methanol aerial parts extract of *C. arvensis*, (Table 4). The highest peak area (%) of 48.427 was obtained by 2,3-Butanediol, 1,4-dimethoxy- with retention-time 6.278 min. flowed by Octasiloxane, 1, 1, 3, 3, 5, 5, 7, 7, 9, 9, 11, 11, 13, 13, 15, 15-hexadecamethyl- (40.870%) with retention-time 18.972 min (Fig. 3, 4).

In the present investigation a variety of compounds have been detected in methanol roots extract including (Fig. 5): Cyclohexasiloxane, dodecamethyl- (0.169%), Octacosane (0.217%), Octasiloxane, 1, 1, 3, 3, 5, 5, 7, 7, 9, 9, 11, 11, 13, 13, 15, 15-hexadecamethyl- (4.543%),Triphenylphosphine oxide (23.435%), (Table 5). Mass spectra from full scan analysis of components were showed in (Fig. 6).

Chromatogram of *C. arvensis* acetone roots extract by GC-MS was presented in (Fig. 7). Of the seven compounds identified, the most prevailing compounds were 1,2-Ethanediamine, N'-ethyl-N,N-dimethyl- compound (41.738%). Other compounds were showed in (Table 6). Mass spectra from full scan analysis of components were showed in (Fig. 8).

As is apparent in Table (7), 4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene)tyramine; 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- and 6-Thiadodecane, 1-[1-cycloazapropyl]- were perfectly separated from *C. arvensis* aquatic roots extract. Their amounts (%) were: 0.067%, 0.287% and 0.312% respectively. Chromatogram of acetone roots extract by GC-MS was presented in (Fig. 9) and mass spectra from full scan analysis of components were showed in (Fig. 10).

The present work and detection of compounds is in consonance with the work reported by other scientists in the same plant (Kaur M and Kalia AN, 2012). Cuscohygrine and calystegines have been previously reported from *C. arvensis* roots. Calystegins showed significant inhibitory activity towards β-glucosidase and α-galactosidase (Molyneux RJ *et al*., 1993) Aerial parts of the plant contains polyphenolic compounds flavonoid and tannins (Faraz M *et al*., 2003).

It has antioxidant Activity. Thirteen saponins were isolated and identified from Calendula officinalis, *C. arvensis* and Hedera helix. Mutagenic and antimutagenic activities of these products were investigated using a modified liquid incubation technique of the Salmonella/microsomal assay (Elias R *et al*., 1990). Flavonoids glycosides like are well distributed in the leaves: Kaempferol3-mono- glycosides and Quercetin 3-mono or di- glycosides (Yusuf M *et al*., 2002).

Nine glycosidase activities were detected in isolated cell wall of cultured *C.arvensis* cells (19 Pierrot, H *et al*., 1997). Acidic ethyl acetate extract of leaves contains phenolic compounds including p-hydroxybenzoic acid, syringic acid, vanillin, benzoic acid and ferulic acid and Flavonoid like rutin (Elzaawely AA and Tawata A., 2012). Water extract contains primarily proteins and polysaccharides, it inhibit the tumor growth and angiogenesis in chick embryo and improved lymphocyte (Meng XL *et al*., 2002).

Extracts of the plant largely comprised of proteoglycan molecules (PGMs), in combination with proper nutrition, it enhances the immune system's ability to maintain good health (Nicholas Calvino DC 2002). Phytol is an extremely common terpenoid, found in all plants esterified to Chlorophyll to confer lipid solubility. Phytol is commonly used as the basic raw materials for the manufacture of synthetic forms of vitamin E (Netscher T, 2007) and vitamin K1 (Daines AM *et al*., 2003).

Table 1. Preliminary phytochemical analysis of aerial parts of *C. arvensis*

Phytochemical	methanol	Acetone	Aqueous	chloroform
sugar	+	+	-	+
Tannins	+	+	+	+
Alkaloids	+	+	-	+
Flavonoids	+	+	+	+
Saponins	–	+	-	–
Steroids	+	+	+	+
Terpenes	+	+	+	+

Table 2. Preliminary phytochemical analysis of roots of *C. arvensis*

Phytochemical	methanol	Acetone	Aqueous	Chloroform
sugar	+	–	-	+
Tannins	+	+	+	+

Alkaloids	+	+	+	–
Flavonoids	+	+	+	+
Saponins	+	+	+	+
Steroids	+	+	+	+
Terpenes	+	+	+	+

Table 3. Composition of *C. arvensis* acetone aerial parts extract

No.	Compound	Retention time (min)	Amount (%)	Chemical formula	Molecular weight	Synonyms
1	1,2-Ethanediamine, N'-ethyl-N,N-dimethyl-	1.557	36.010%	$C_6H_{16}N_2$	116	Ethylenediamine, N'-ethyl-N,N-dimethyl- N'-Ethyl-N,N-dimethylethylenediamine N,N-Dimethyl-N'-ethylethylenediamine
2	2-Pentanone, 4-hydroxy-4-methyl-	2.152	1.609 %	$C_6H_{12}O_2$	128	Acetonyldimethylcarbinol Diacetone alcohol Diketone alcohol Tyranton
3	2,3-Butanediol, 1,4-dimethoxy-	5.906	3.795 %	$C_6H_{14}O_4$	150	.1,4-Dimethoxy-2,3-butanediol
4	Cycloheptasiloxane, tetradecamethyl-	8.530	0.152 %	$C_{14}H_{42}O_7Si_7$	518	2,2,4,4,6,6,8,8,10,10,12,12,14,14-Tetradecamethylcycloheptasiloxane
5	Cyclooctasiloxane, hexadecamethyl-	10.507	0.456 %	$C_{16}H_{48}O_8Si_8$	592	Hexadecamethylcyclooctasiloxane 2,2,4,4,6,6,8,8,10,10,12,12,14,14,16,16-Hexadecamethylcyclooctasiloxane
6	Cyclononasiloxane, octadecamethyl-	12.239	0.162 %	$C_{18}H_{54}O_9Si_9$	666	Octadeamethyl-cyclononasiloxane
7	Cyclodecasiloxane, eicosamethyl-	13.788	0.256 %	$C_{20}H_{60}O_{10}Si_{10}$	740	2,2,4,4,6,6,8,8,10,10,12,12,14,14,16,16,18,18,20,20 Icosamethylcyclodecasiloxane
8	Phytol	15.080	0.140 %	$C_{20}H_{40}O$	296	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- trans-Phytol 3,7,11,15-Tetramethyl-2-hexadecen-1-ol
9	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	15.211	0.159 %	$C_{16}H_{50}O_7Si_8$	578	1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexadecamethyloctasiloxane

Table 4. Composition of *C. arvensis* methanol aerial parts extract.

No.	Compound	Retention time (min)	Amount (%)	Chemical formula	Molecular weight	Synonyms
1	2,3-Butanediol, 1,4-dimethoxy-	6.278	48.427 %	$C_6H_{14}O_4$	150	1,4-Dimethoxy-2,3-butanediol
2	2,5-Dimethylhexane-2,5-dihydroperoxide	6.884	0.283 %	$C_8H_{18}O_4$	178	Hydroperoxide, (1,1,4,4-tetramethyl-1,4-butanediyl)bis-
3	Cycloheptasiloxane, tetradecamethyl-	8.524	1.290 %	$C_{14}H_{42}O_7Si_7$	518	2,2,4,4,6,6,8,8,10,10,12,12,14,14-Tetradecamethylcycloheptasiloxane

4	Cyclooctasiloxane, hexadecamethyl-	10. 507	0.436 %	$C_{16}H_{48}O_8Si_8$	592	Hexadecamethyl-cyclooctasioxane Hexadecamethylcyclooctasiloxane 2,2,4,4,6,6,8,8,10,10,12,12,14,14,16,16-Hexadecamethylcyclooctasiloxane
5	n-Hexadecanoic acid	13.599	0.131 %	$C_{16}H_{32}O_2$	272	Hexadecanoic acid n-Hexadecoic acid Palmitic acid
6	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	15.080	0.266 %	$C_{14}H_{44}O_6Si_7$	504	1,1,3,3,5,5,7,7,9,9,11,11,13,13-Tetradecamethylheptasiloxane
7	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	18.972	21.848 %	$C_{16}H_{50}O_7Si_8$	578	1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexadecamethyloctasiloxane

Table 5. Composition of *C. arvensis* methanol roots extract

No.	Compound	Retention time (min)	Amount (%)	Chemical formula	Molecular weight	Synonyms
1	Cyclohexasiloxane, dodecamethyl-	6.404	0.169%	$C_{12}H_{36}O_6Si_6$	444	Dodecamethylcyclohexasiloxane 2,2,4,4,6,6,8,8,10,10,12,12-Dodecamethylcyclohexasiloxane
2	Octacosane	10.908	0.217%	$C_{28}H_{58}$	394	n-Octacosane
3	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	17.314	4.543	$C_{16}H_{50}O_7Si_8$	578	1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexadecamethyloctasiloxane
4	Triphenylphosphine oxide	18.863	23.435%	$C_{18}H_{15}OP$	278	Phosphine oxide, triphenyl- Triphenyl phosphorus oxide (C6H5)3P=O Triphenylphosphanoxid Triphenylphosphanoxide

Table 6. Composition of *C. arvensis* acetone roots extract

No.	Compound	Retention time (min)	Amount (%)	Chemical formula	Molecular weight	Synonyms
1	1,2-Ethanediamine, N'-ethyl-N,N-dimethyl-	1.562	41.738%	$C_6H_{16}N_2$	116	Ethylenediamine, N'-ethyl-N,N-dimethyl- N'-Ethyl-N,N-imethylethylenediamine N,N-Dimethyl-N'-ethylethylenediamine
2	Cyclohexasiloxane, dodecamethyl-	6.398	0.330%	$C_{12}H_{36}O_6Si_6$	370	Dodecamethylcyclohexasiloxane 2,2,4,4,6,6,8,8,10,10,12,12-Dodecamethylcyclohexasiloxane
3	Cycloheptasiloxane, tetradecamethyl-	8.529	0.473%	$C_{14}H_{42}O_7Si_7$	398	2,2,4,4,6,6,8,8,10,10,12,12,14,14-Tetradecamethylcycloheptasiloxane
4	Cyclooctasiloxane, hexadecamethyl-	10.513	0.419%	$C_{16}H_{48}O_8Si_8$	592	Hexadecamethyl-cyclooctasioxane Hexadecamethylcyclooctasiloxane 2,2,4,4,6,6,8,8,10,10,12,12,14,14,16,16-

						Hexadecamethylcyclooctasiloxane
5	Cyclononasiloxane, octadecamethyl-	12.239	0.369%	$C_{18}H_{54}O_9Si_9$	666	Octadeamethyl-cyclononasiloxane 2,2,4,4,6,6,8,8,10,10,12,12,14,14,16,16,18,18-Octadecamethylcyclononasiloxane
6	Methyl abietate isomer	14.096	0.247%	$C_{21}H_{32}O_2$	358	Methyl abieta-7,13-dien-18-oate
7	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	15.216	0.687%	$C_{16}H_{50}O_7Si_8$	578	1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexadecamethyloctasiloxane

Table 7. Composition of *C. arvensis* aquatic roots extract

No	Compound	Retention time (min)	Amount (%)	Chemical formula	Molecular weight	Synonyms
1	4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene)tyramine	1.548	0.067 %	$C_{16}H_{14}N_2O_4$	298	no synonyms
2	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	4.486	0.287 %	$C_6H_8O_4$	144	3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one
3	6-Thiadodecane, 1-[1-cycloazapropyl]-	6.035	0.312 %	$C_{13}H_{27}NS$	229	no synonyms.
4	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	18.86	6.069 %	$C_{16}H_{50}O_7Si_8$	578	1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexadecamethyloctasiloxane

Fig. 1. Chromatogram of *C. arvensis* acetone aerial parts extract by GC-MS

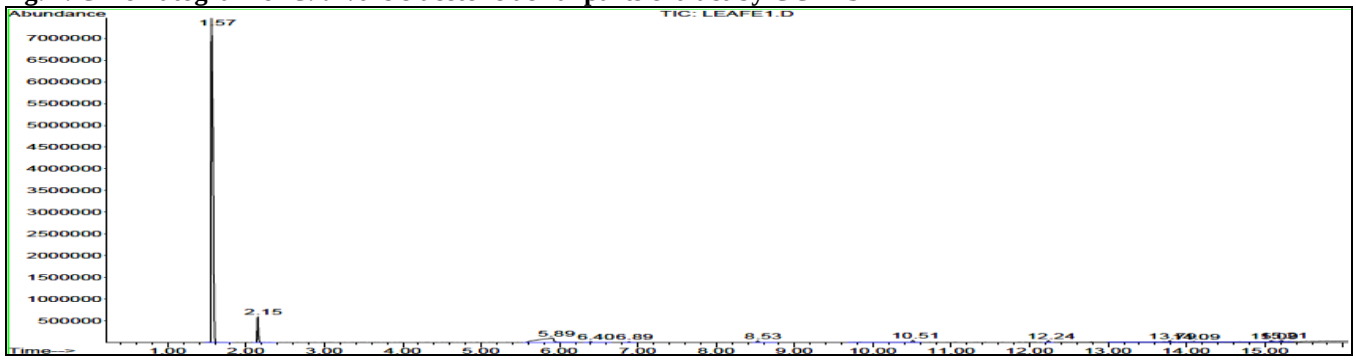
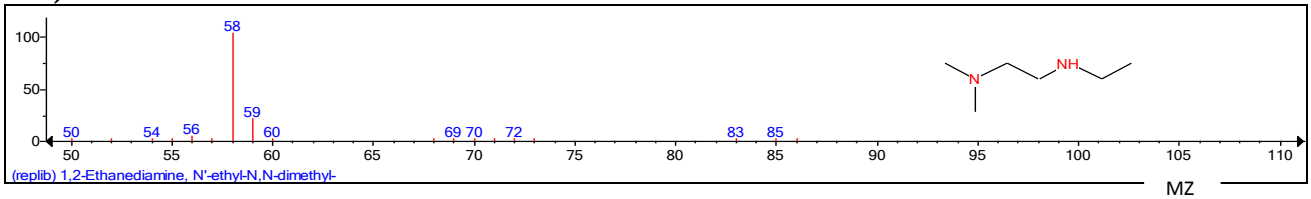
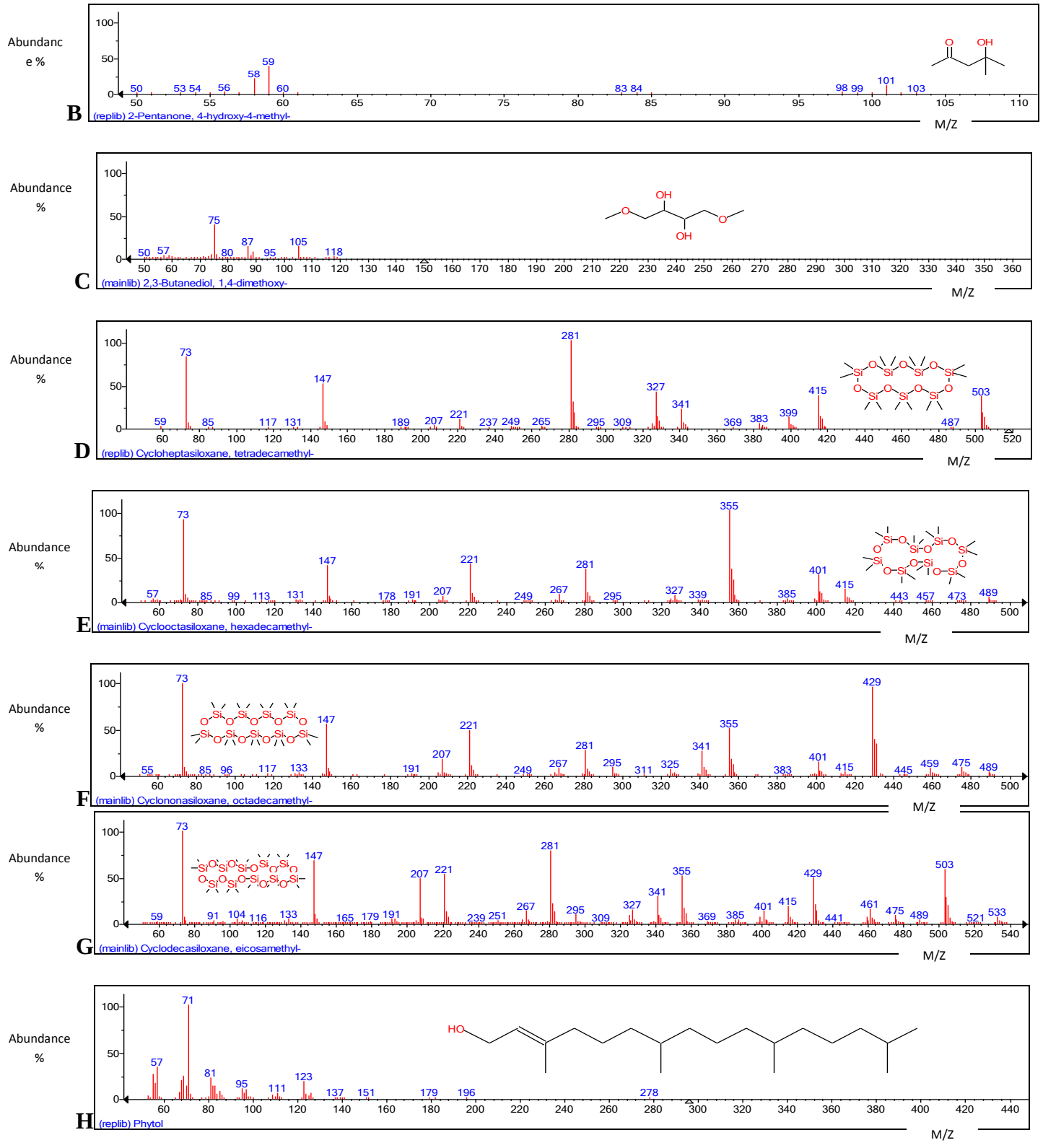


Fig.2. Mass spectra from full scan analysis by GC/MS of (A) 1,2-Ethanediamine, N'-ethyl-N,N-dimethyl- (B) 2-Pentanone, 4-hydroxy-4-methyl- (C) 2,3-Butanediol, 1,4-dimethoxy- (D) Cycloheptasiloxane, tetradecamethyl- (E) Cyclooctasiloxane, hexadecamethyl- (F) Cyclononasiloxane, octadecamethyl- (G) Cyclodecasiloxane, eicosamethyl- (H) Phytol (K) Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl- extracted from aerial parts (acetone extract).



A


Abundanc
e %
B
(replib) 2-Pentanone, 4-hydroxy-4-methyl-
M/Z
Abundance
%
C
(mainlib) 2,3-Butanediol, 1,4-dimethoxy-
M/Z
D
(replib) Cycloheptasiloxane, tetradecamethyl-
M/Z
E
(mainlib) Cyclooctasiloxane, hexadecamethyl-
M/Z
F
(mainlib) Cyclononasiloxane, octadecamethyl-
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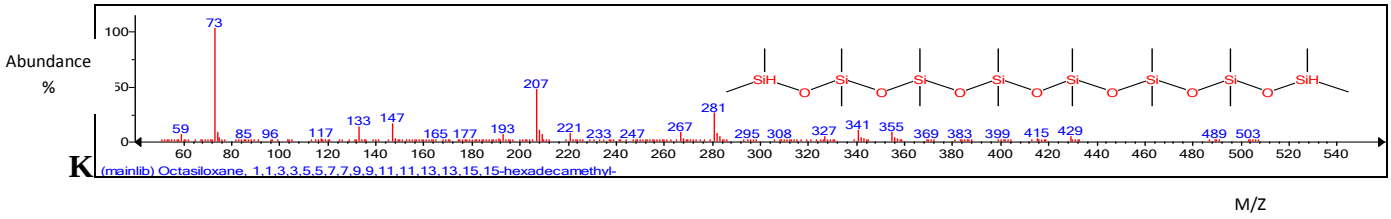


Fig. 3. Chromatogram of *C. arvensis* methanol aerial parts extract by GC-MS.

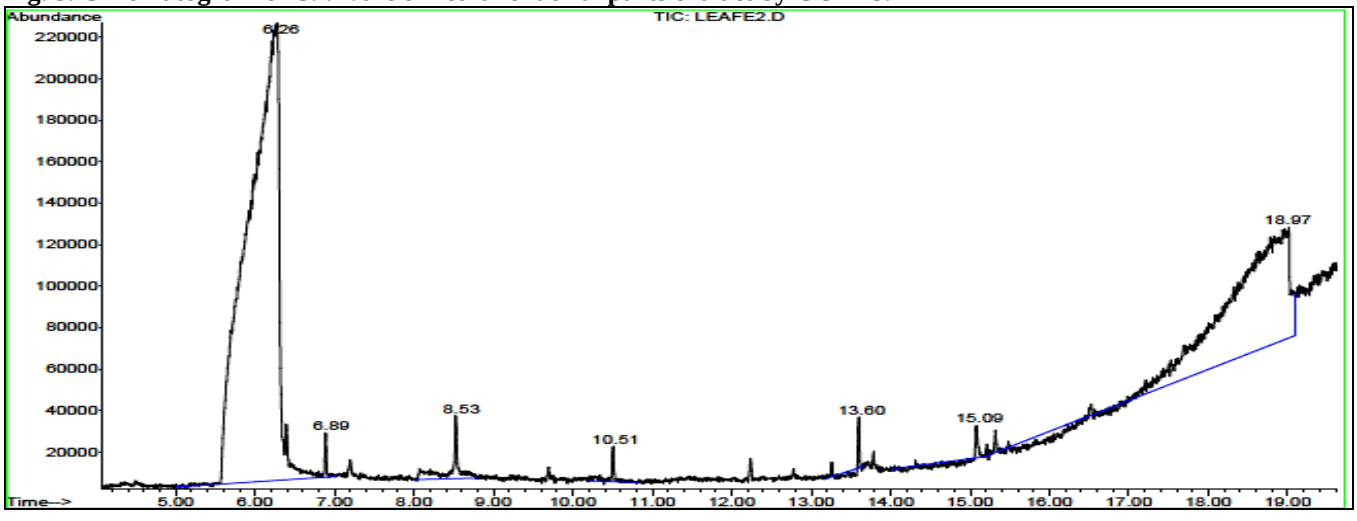
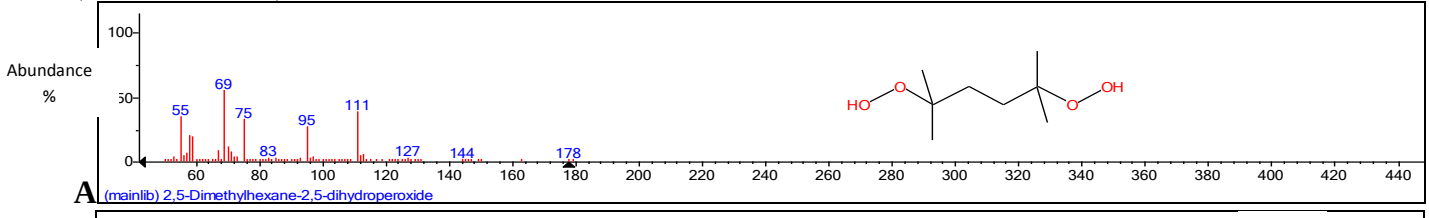
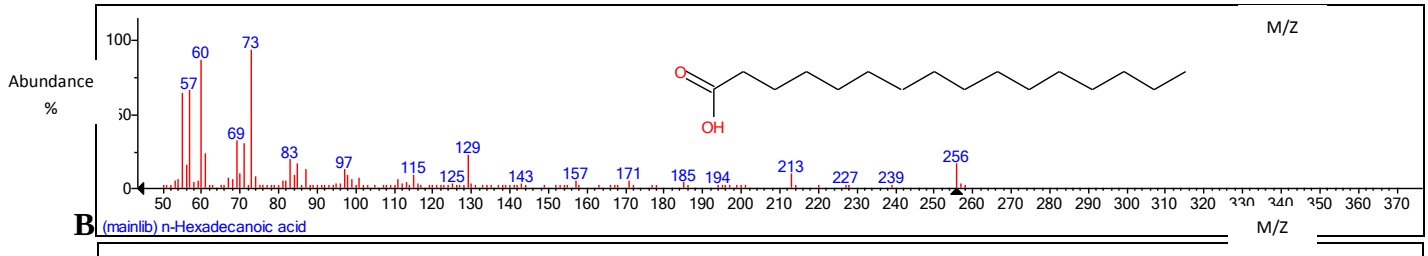


Fig. 4. Mass spectra from full scan analysis by GC/MS of (A) 2,5-Dimethylhexane-2,5-dihydroperoxide (B) n-Hexadecanoic acid (C) Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl- extracted from aerial parts (methanol extract).





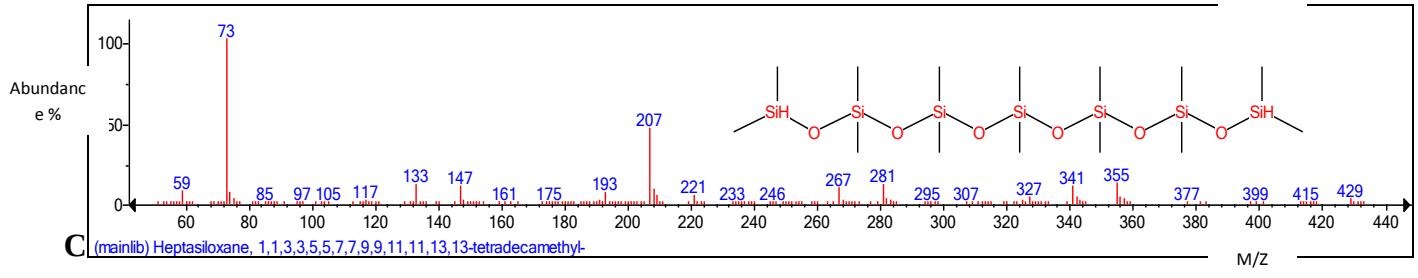


Fig. 5. Chromatogram of *C. arvensis* methanol roots extract by GC-MS.

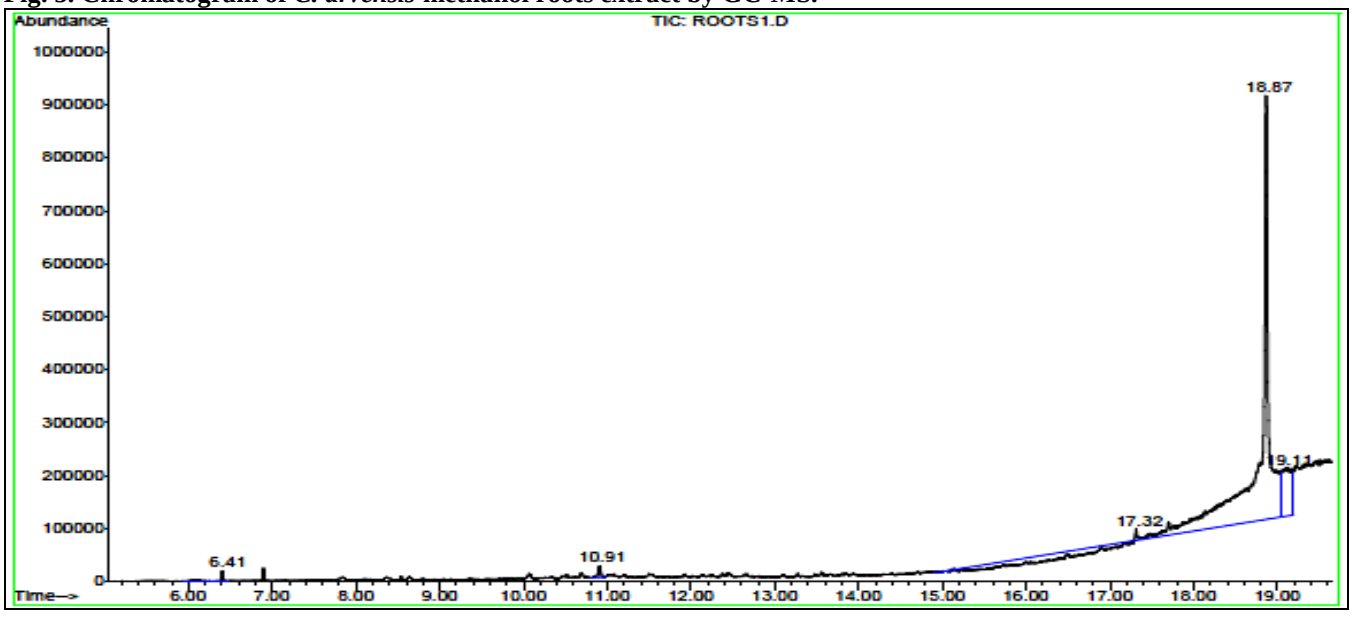


Fig. 6. Mass spectra from full scan analysis by GC/MS of (A) Octacosane (B) Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl- (C) Triphenylphosphine oxide extracted from roots (methanol extract).

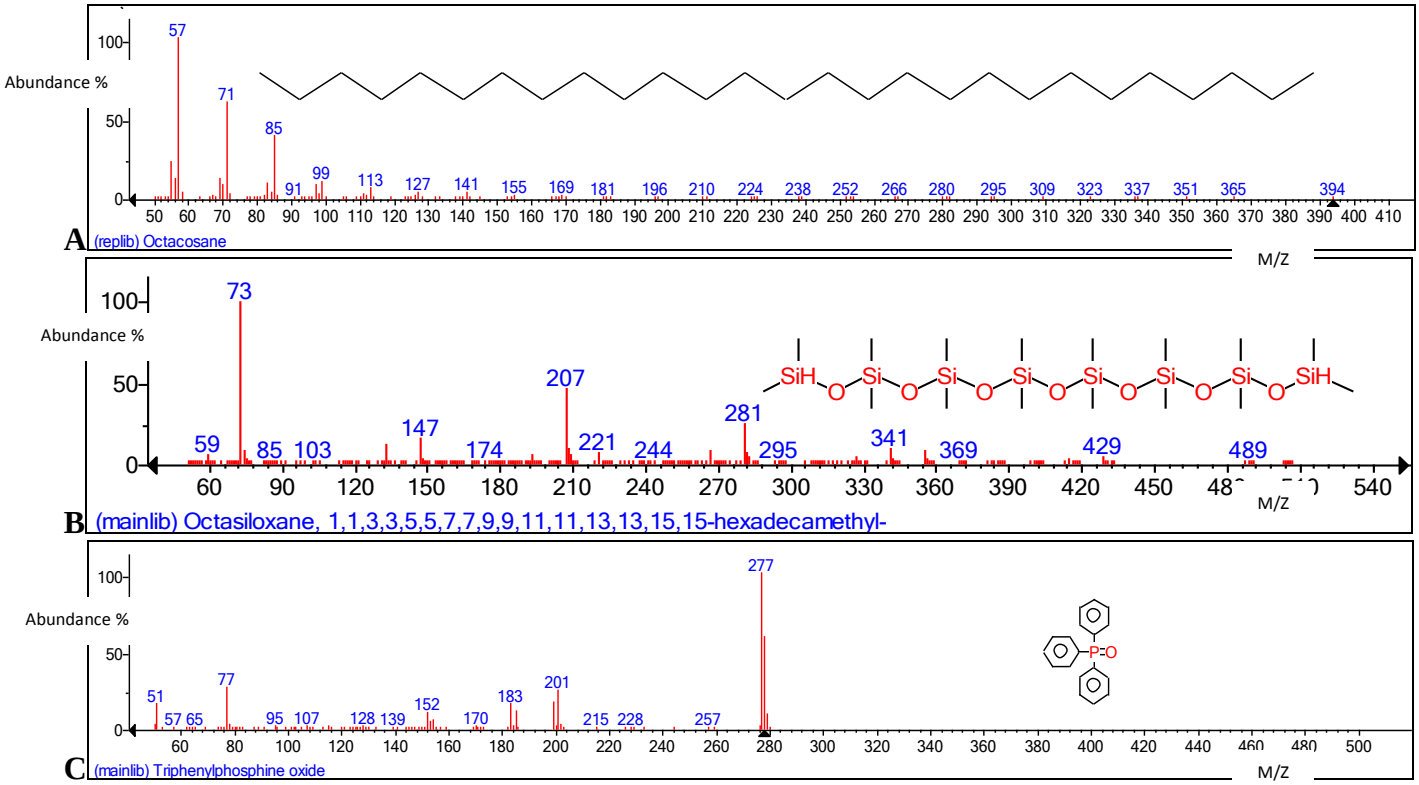


Fig. 7. Chromatogram of *C. arvensis* acetone roots extract by GC-MS.

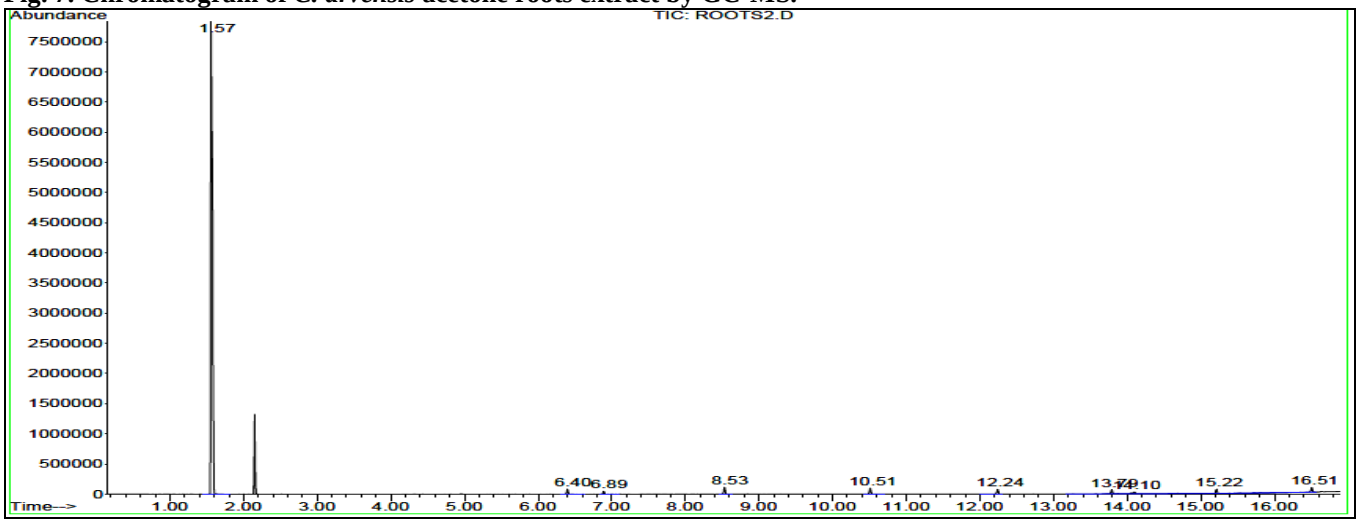


Fig. 8. Mass spectra from full scan analysis by GC/MS of Methyl abietate isomer extracted from roots (acetone extract).

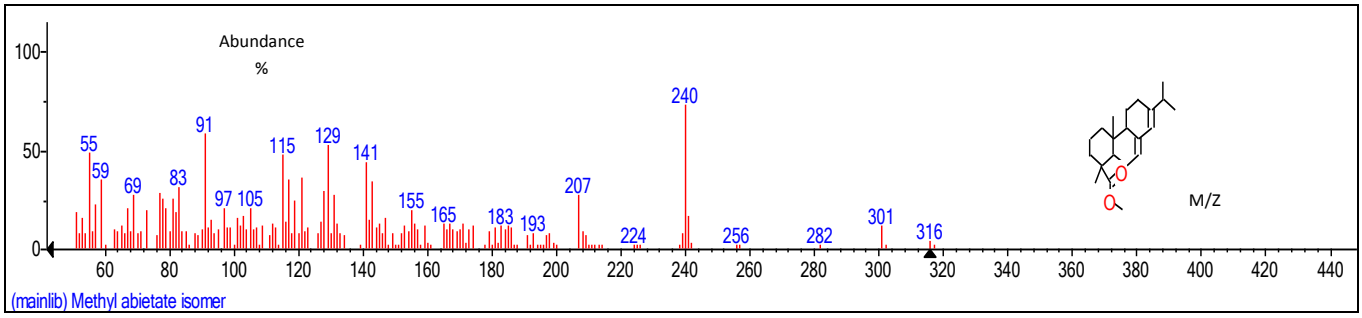


Fig. 9. Chromatogram of *C. arvensis* aquatic roots extract by GC-MS.

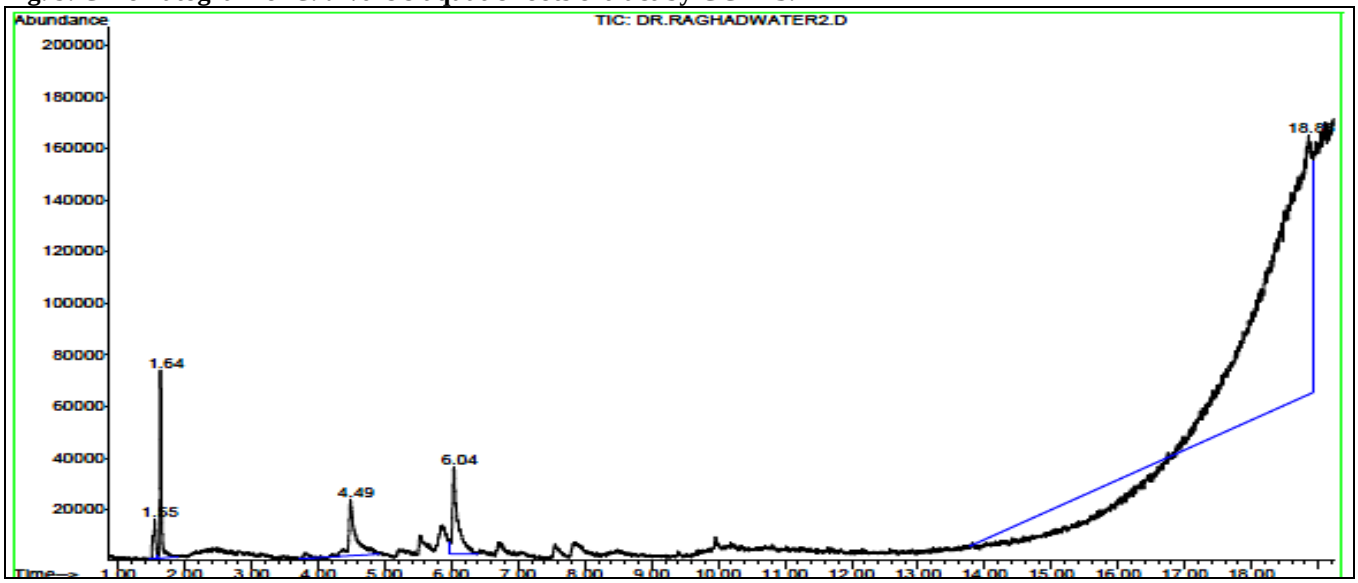
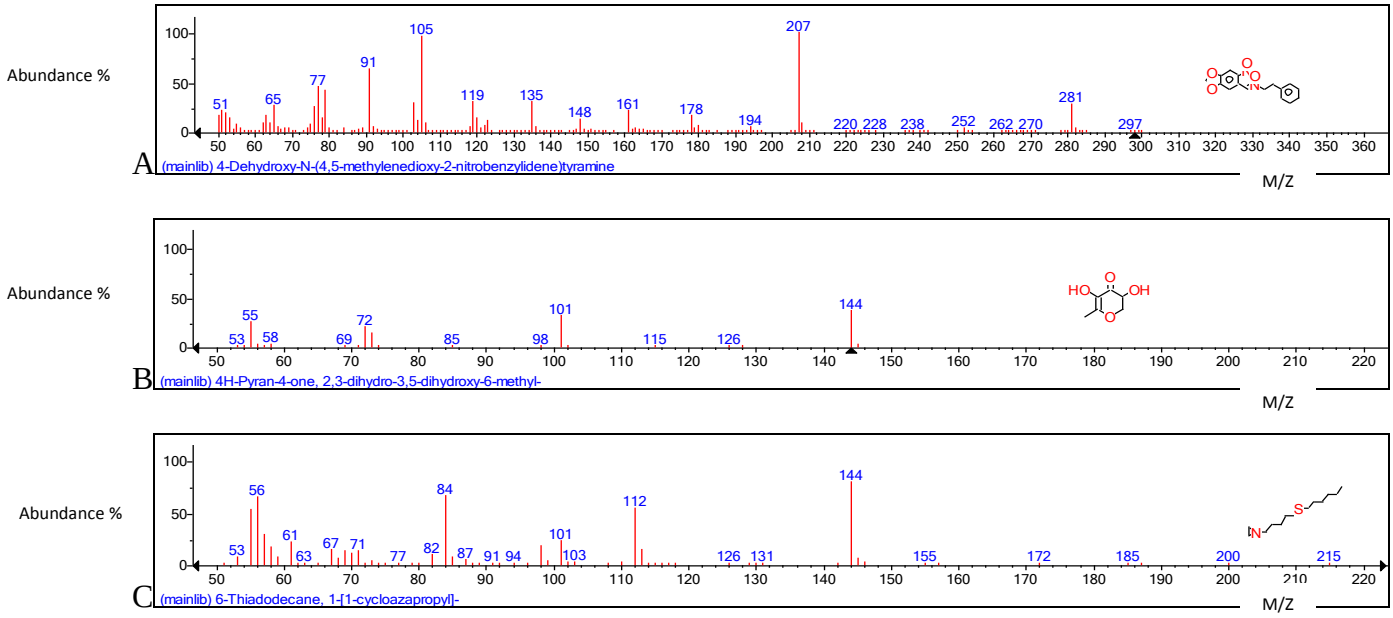


Fig 10. Mass spectra from full scan analysis by GC/MS of (A) 4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene)tyramine (B) 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (C) 6-Thiadodecane, 1-[1-cycloazapropyl]- extracted from roots (aquatic extract)



CONCLUSION

The GC-MS analysis of *C. arvensis* showed a highly complex profile, containing approximately 31 components. This study may be useful to explore the pharmacological and biosynthetic activity of the plants further. However, isolation of individual phytochemical constituents from other parts of plant and subjecting it to pharmacological activity will definitely give fruitful results.

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