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Research Article

ESTIMATION OF BIO-ACTIVE COMPOUNDS IN INDIAN DESERTED PLANT *EUPHORBIA CADUCIFOLIA* (DANDA THOR) BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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ABSTRACT

Objective: The investigation was carried out to determine the possible bioactive components of methonolic extracts of *Euphorbia caducifolia* (whole plant) using Gas chromatography-Mass spectrometry (GC-MS). Experimental design: All the samples were dried in shade till constant weight was achieved. They were then macerated to powder form with a mixer grinder. The powder was stored in air sealed polythene bags at room temperature before extraction. The chemical compositions of the methonolic extracts of *Euphorbia caducifolia* (whole plant) were investigated using Thermo G C 1300 and "TSQ 8000 "Triple quadrupole GC-MS MS SYSTEM with auto sampler Al 1310 Gas chromatography-Mass spectrometry, while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. Observation and Conclusion: GC-MS analysis of the extract reveals the identification of forty four compounds. This is the first report of identification of components from the whole plant of *E. caducifolia* by GC-MS. Most of the compounds in the list are bioactive and possess medicinal properties.

Key words: *Euphorbia caducifolia,* Gas chromatography-Mass spectrometry, bioactive components.

INTRODUCTION

Taking into consideration of the medicinal importance of this plant, the methonolic extracts of *Euphorbia caducifolia* (whole plant) were analyzed for the first time using Gas chromatography-Mass spectrometry (GC-MS). This work will help to identify the compounds of therapeutic value. GC-MS is one of the best techniques to identify the bioactive constituents of long chain, branched chain hydrocarbons, alcohols, acids, ester, steroids, phenolic compounds etc[1, 2, 3, 4].

Various plants are used in traditional treatments to cure variety of diseases. In the last few decades there has been an exponential growth in the field of herbal medicine. Natural products have been a source of drugs for centuries. In the present study methonolic extracts of *E. caducifolia* were analyzed by GC-MS technique to study the major and minor phyto-constituents of the vegetative parts of the whole plant.

The ecological life-history of "leafless" spurge, *E. caducifolia* Haines, which serves as an indicator of rocky and gravelly habitat in Rajasthan (India) is dealt with here. Root decoction is used as an effective abortifacient at initial stages[5] and dried stem is burnt to produce smoke, affected painful body part is kept in smoke for some time to relive pain[6]. There appear to be two forms in existence: one with red and the other with green inflorescence, which interbreed freely in nature resulting in different shades of red coloured inflorescence. The plant is suitably adapted to xerophytic conditions, but surprisingly the leaves are borne only in summer when there is acute shortage of water[7].

MATERIAL AND METHOD

Plant material: *Euphorbia caducifolia* were collected in the month of August 2015 from the Central Arid Zone Research Institute (CAZRI), Jodhpur (Rajasthan). Plants samples were identified and deposited in the herbarium, Department of Botany, University of Rajasthan, Jaipur. The collected plant materials were transferred immediately to the laboratory cleaned with water and selected plant parts were separately shade dried[8] until weight has been constant.

Preparation of plant extracts: The collected plant materials were shade dried till constant weight was achieved[9]. The plant material were powered with the help of grinder[10] and passed through 40mm meshes and stored in clean container for further use[11]. The dried powder material was extracted with methanol by using the Soxhlet apparatus[12] for 18 hours at a temperature not exceeding the boiling point of the respective solvent[13, 14]. The obtained extracts were filtered by using Whatman No. 1 filter paper and then concentrated at 40^{0}C by using an evaporator[15] and stored the residual extracts in refrigerator at 4^{0}C in small and sterile amber colour glass bottles[16] for subsequent use in the further antimicrobial, anti-fungal and phyto-chemical analysis. The extract contains both polar and non-polar phyto-components.

Gas chromatography-Mass spectrometry analysis: Gas chromatography-Mass spectrometry (GC-MS) analysis of these extracts was carried out by following the method[17]. The GC-MS analysis of the extracts was performed using a GC-MS **Thermo G C 1300 and "TSQ 8000 "Triple quadrupole GCMSMS SYSTEM with auto sampler Al 1310.** Gas Chromatography 1300 with a fused GC column TG-5MS AMINE. The column

length was 30 m with internal diameter; coated film 0.25µm with flow rate 10 ml/m in and the condition were as follows: PTV Temp. Program: 70 °C, hold 1.00 min, 10 °C/min to 280 °C, hold 15 min. Carrier gas helium flow rate 1ml/min, split ratio 1:50. GC is equipped with auto-sampler AI 1300 and sample volume was 1µ liter. The elutes were automatically passed into a mass spectrometer. GC mass Spectrum analysis was conducted using TSQ8000 with transfer line temperature 270°C and ion source temperature 230°C in EI mode. Mass scan time was 4 min with full Scan MS. The mass spectrum was also equipped with a computer fed NIST mass Spectra data library.

Identification of Components: Interpretation on mass spectrum of GC-MS was done using the database of National Institute of standard and Technology NIST-08 LIB[18, 19] and WILEY-8 LIB[20, 21] library sources were used for matching the identified components from the plant material having more than 62,000 patterns[22, 23]. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library[24, 25]. The name, molecular weight and structure of the components of the test materials were ascertained[26].

Table-1: Total Bio-active compounds of *Euphorbia caducifolia* by Gas Chromatography- Mass Spectrometry

S. No.	RT	Compound Name	Area	Area %	RSI
1.	4.46	Tetrazolo [1,5-b]pyridazine, 6-chloro-7-methyl-	71716	2.77	859
2.	4.61	1-Phenyl-3,4-dimethyl-4-nitroso-2-pyrazolin-5-one	40496	1.57	683
3.	5.06	Pyrazinamine, 3,4,5,6-tetrahydro-6-imino-N-phenyl-4-(phenylmethyl)-	56464	2.18	909
4.	5.21	8,11 Octadecadiynoic acid, methyl ester	23257	0.90	767
5.	5.25	Sydnone, 3(phenylmethyl)	37550	1.45	830
6.	5.35	Hydrazine, phenylsulfinyl	38604	1.49	797
7.	5.53	1-H Imidazole1ethanol, 2-methylàphenyl	54253	2.10	800
8.	6.24	Sydnone, 3(phenylmethyl) Benzene,1nitro2(pmethylphenoxy) 4fluoro	29507	1.14	812
9.	6.51	2,2,2Trichloro1 (2nitrophenylthioamino) ethanol	38275	1.48	787
10.	6.55	2,2-Diphenyl-1,3,6-trioxa-2-sila-cyclooctane	216070	8.36	731
11.	6.68	1,3,5Triazine,1,3,5tricyclohexylhexahydro	16352	0.63	789
12.	8.10	3,7-Decadien-2-one, 10-(3,3-dimethyloxiranyl)-4,8-dimethyl-, (E,E)-ñ-	26676	1.03	743
13.	9.54	Acetic acid, (2,5-dichloro-4-methoxyphenoxy)-, methyl ester	101191	3.91	782
14.	9.83	Manganese, tricarbonyl[(1,2,3,4,5-ü)-1-[(dimethylamino) sulfonyl]-2-(1- hydroxy-1-methylethyl)-2,4-cyclopentadien-1-yl]-	57707	2.23	715
15.	12.09	Diazene, 1-ethyl-2-phenyl-, 1-oxide, (Z)-	76739	2.97	734
16.	12.46	à-D-Xylofuranoside, methyl 5-O-methyl-	58169	2.25	622
17.	12.68	Pyrazinamine, 1,2,3,4Oxatriazolium,5(ethylamino)3phenyl,hydroxide, inner salt	32202	1.25	745
18.	13.00	Tetrazolo[1,5b] pyridazine, 8methyl	34575	1.34	817
19.	14.05	Benzenesulfonic acid, 2-nitro-, hydrazide	55935	2.16	620
20.	14.33	Propanedioic acid, (1-cyclohexen-1-ylmethyl)-, dimethyl ester	37512	1.45	529
21.	15.45	3-Hexene, 1-(1-ethoxyethoxy)-, (Z)-	94449	3.65	806
22.	15.88	Tricyclo[4.3.1.1(3,8)]undecane, 1-bromo-	34567	1.34	592
23.	17.45	1,3-Cyclopentanedione, 4-butyl-	36298	1.40	599
24.	17.78	Copper, bis(4chloro3,5cyclohexadiene1,2dione 2oximatoN2, O1) (pyridine)	47456	1.84	665
25.	18.82	5-Chloro-2,4-dithiahexane 2,2-dioxide	53845	2.08	623
26.	19.79	Oxetane, 2,2,3,3-tetramethyl-4,4-diphenyl-	42651	1.65	768
27.	19.82	1,1-(Phenylthio)-2-isopropyl-cyclobutane	62578	2.42	636
28.	20.18	4-Octene, 2,3,7-trimethyl-, [S-(E)]-	47879	1.85	703
29.	21.23	Chromium, tricarbonyl[(1,2,3,4,5,6ü) 1,3,5,7cyclo octatetraene]	42201	1.63	792
30.	22.52	Phthalic acid, decyl phenyl ester	71547	2.77	771
31.	23.20	Tricyclo[2.2.1.0(1,4)]heptan2one,6nitro	35882	1.39	706
32.	26.19	Cyclopentane, nonyl-	36721	1.42	803
33.	26.44	Methyl N-cyclohexyl-3-phenylpropanimidate	31363	1.21	689
34.	26.76	2Dodecen1ol, 12chloro	80740	3.12	796
35.	26.94	NVinylpyridiniumbromide	54809	2.12	825
36.	28.12	2,4-Dimethyl-6-phenyl-3-thioxo-5-oxo-2,3,4,5-tetrahydro-1,2,4-triazine	113011	4.37	577
37.	30.56	3,5,8Trimethyl1phenyl4,5,6,8tetrahydropyrazolo[3,4b][1,4]diazepin7 (1H)one#	55720	2.16	765
38.	30.72	4Methyl2,4bis(4'trimethylsilyloxyphenyl)pentene1	121940	4.72	740
39.	30.79	3 Phenyl4benzoyl7mercaptomethyl2,6dioxa3azabicyclo [3.3.0] 7octene	40035	1.55	806
40.	31.02	1H1,2,3Triazol1amine, N[(4methoxyphenyl) methylene]4,5dimethyl	99516	3.85	771
41.	31.56	Iron, tricarbonyl[(1,2,3,4ü) 7methylene1, 3,5cycloheptatriene]	71787	2.78	799
42.	31.61	Trimethyl [4(1,1,3,3,tetramethylbutyl) phenoxy]silane	104508	4.04	782
43.	31.75	2Butanone, 3chloro4hydroxy1,4diphenyl	38394	1.49	785
44.	31.80	4Methyl2,4bis(4'trimethylsilyloxyphenyl)pentene1	63560	2.46	784

Table-2: Major Bio-active compounds of *Euphorbia caducifolia* by Gas Chromatography- Mass Spectrometry

S. No.	RT	Compound Name	Area %
Major constituents			
1.	6.55	2,2-Diphenyl-1,3,6-trioxa-2-sila-cyclooctane	8.36
2.	30.72	4Methyl2,4bis(4'trimethylsilyloxyphenyl)pentene1	4.72
3.	28.12	2,4-Dimethyl-6-phenyl-3-thioxo-5-oxo-2,3,4,5-tetrahydro-1,2,4-triazine	4.37
4.	31.61	Trimethyl [4(1,1,3,3,tetramethylbutyl) phenoxy]silane	4.04
5.	9.54	Acetic acid, (2,5-dichloro-4-methoxyphenoxy)-, methyl ester	3.91
6.	31.02	1H1,2,3Triazol1amine, N[(4methoxyphenyl) methylene]4,5dimethyl	3.85
7.	15.45	3-Hexene, 1-(1-ethoxyethoxy)-, (Z)-	3.65
8.	26.76	2Dodecen1ol, 12chloro	3.12
9.	12.09	Diazene, 1-ethyl-2-phenyl-, 1-oxide, (Z)-	2.97
10.	31.56	Iron, tricarbonyl[(1,2,3,4ü) 7methylene1, 3,5cycloheptatriene]	2.78

Table-3: Minor Bio-active compounds of *Euphorbia caducifolia* by Gas Chromatography- Mass Spectrometry

S. No.	RT	Compound Name	Area %
Minor constituents			
1.	17.45	1,3-Cyclopentanedione, 4-butyl-	1.40
2.	23.20	Tricyclo[2.2.1.0(1,4)]heptan2one,6nitro	1.39
3.	13.00	Tetrazolo[1,5b] pyridazine, 8methyl	1.34
4.	15.88	Tricyclo[4.3.1.1(3,8)]undecane, 1-bromo-	1.34
5.	12.68	Pyrazinamine, 1,2,3,4Oxatriazolium,5(ethylamino)3phenyl,hydroxide, inner salt	1.25
6.	26.44	Methyl N-cyclohexyl-3-phenylpropanimidate	1.21
7.	6.24	Sydnone, 3(phenylmethyl) Benzene,1nitro2(pmethylphenoxy) 4fluoro	1.14
8.	8.10	3,7-Decadien-2-one, 10-(3,3-dimethyloxiranyl)-4,8-dimethyl-, (E,E)-ñ-	1.03
9.	5.21	8,11 Octadecadiynoic acid, methyl ester	0.90
10.	6.68	1,3,5Triazine,1,3,5tricyclohexylhexahydro	0.63

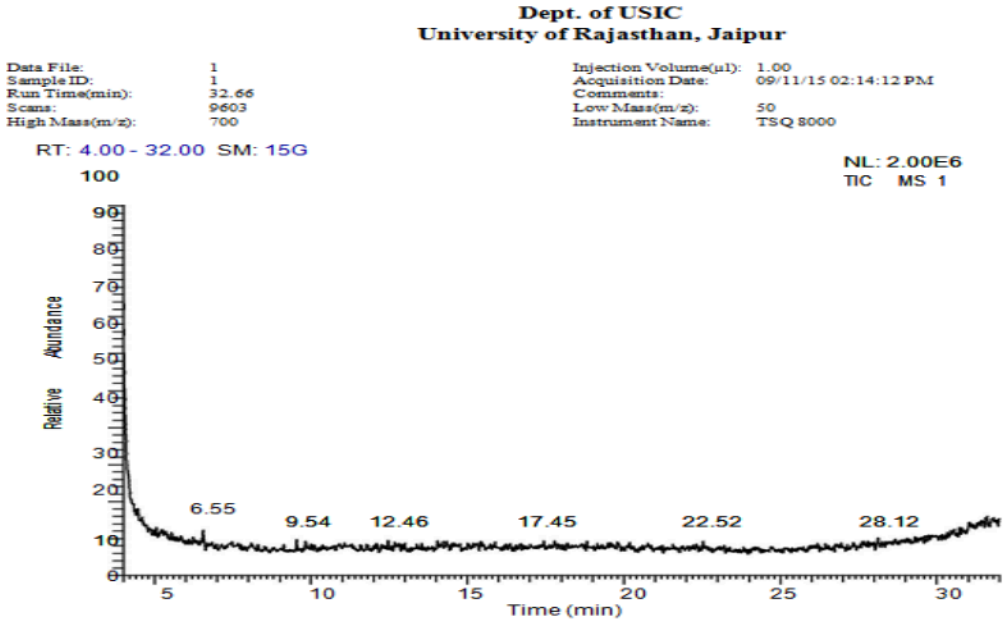
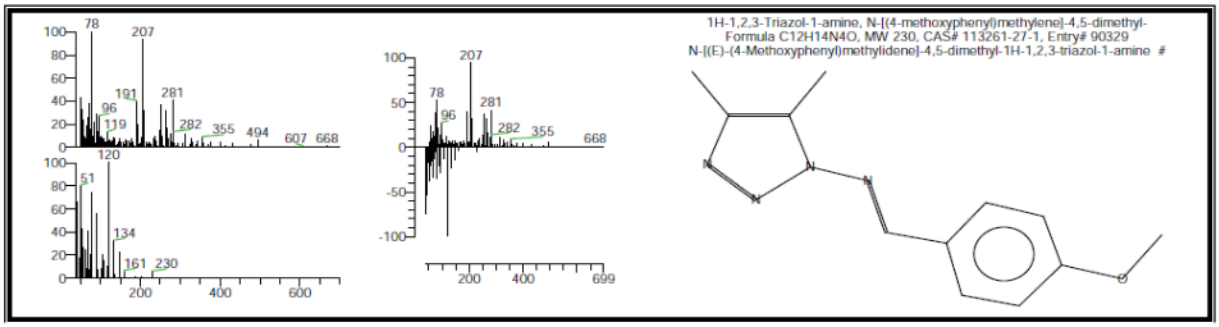
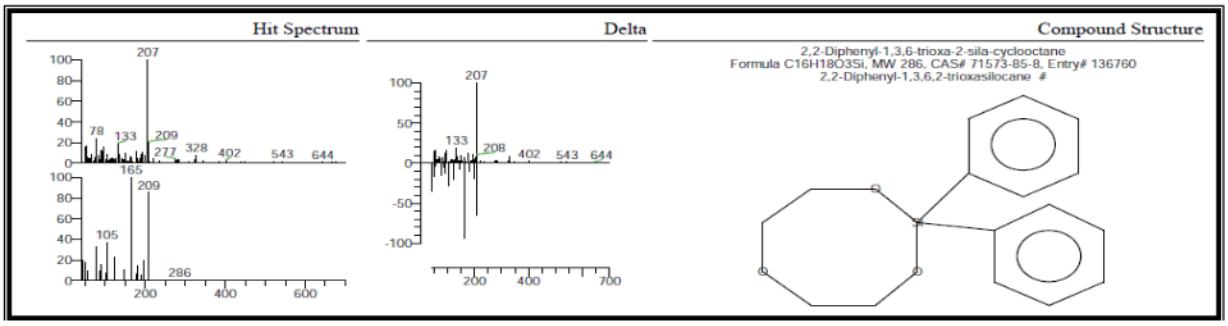


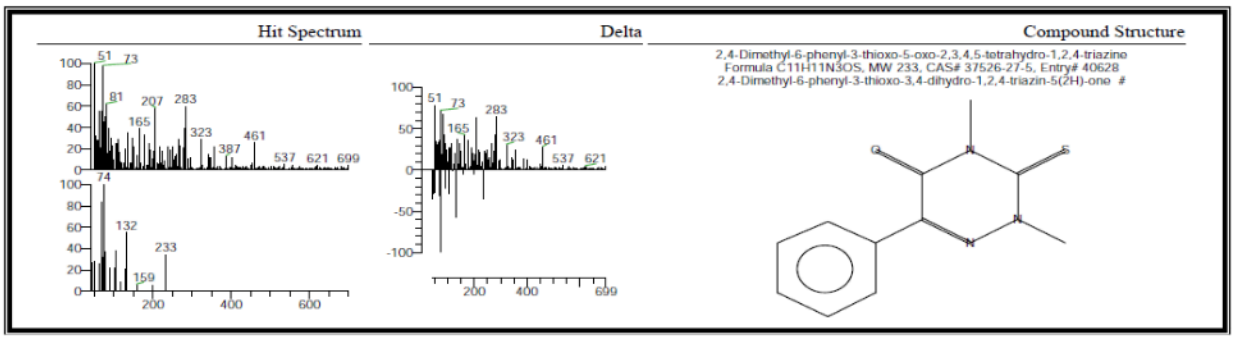
Fig. 1 Chromatogram of Methanolic extract of whole plant of *Euphorbia caducifolia* by GC-MS



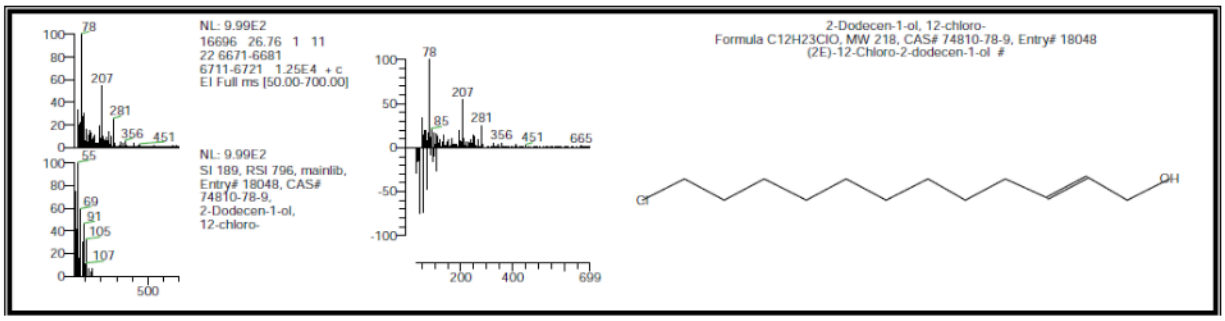
1H1,2,3Triazol1amine, N[(4methoxyphenyl) methylene]4,5dimethyl



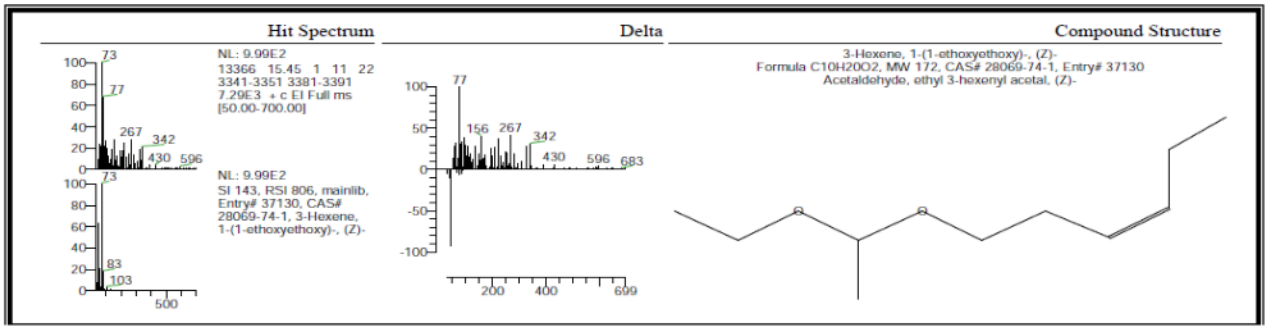
2,2-Diphenyl-1,3,6-trioxa-2-sila-cyclooctane



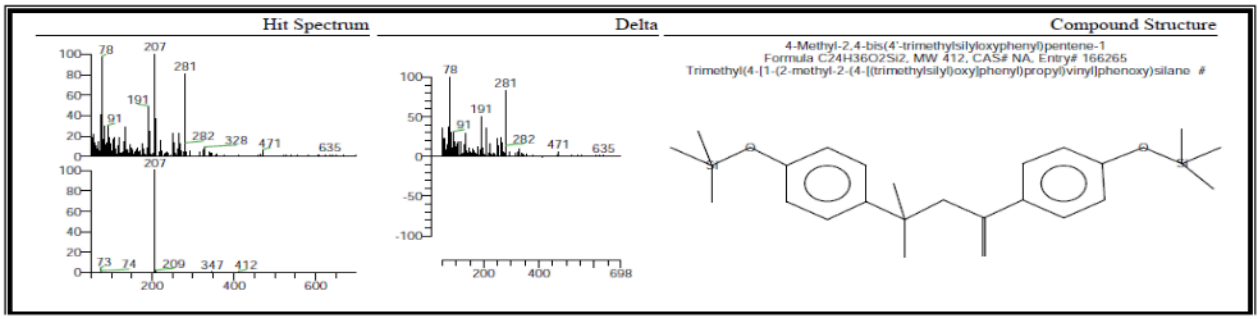
2,4-Dimethyl-6-phenyl-3-thioxo-5-oxo-2,3,4,5-tetrahydro-1,2,4-triazine



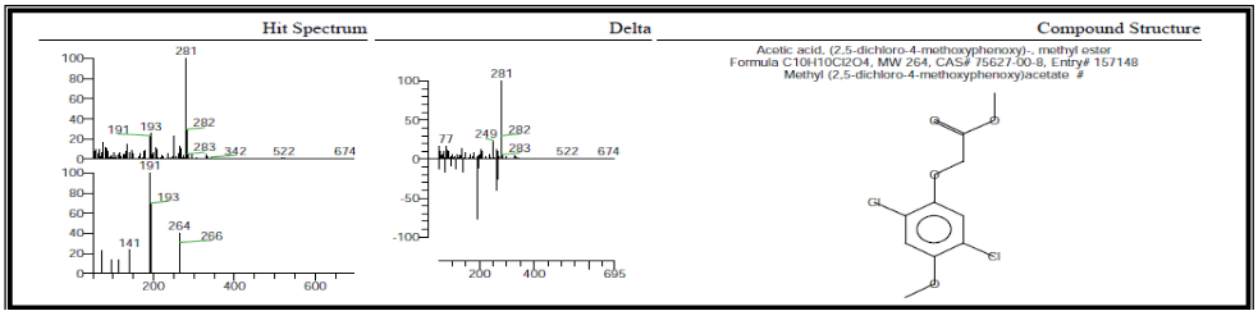
2Dodecen1ol, 12chloro



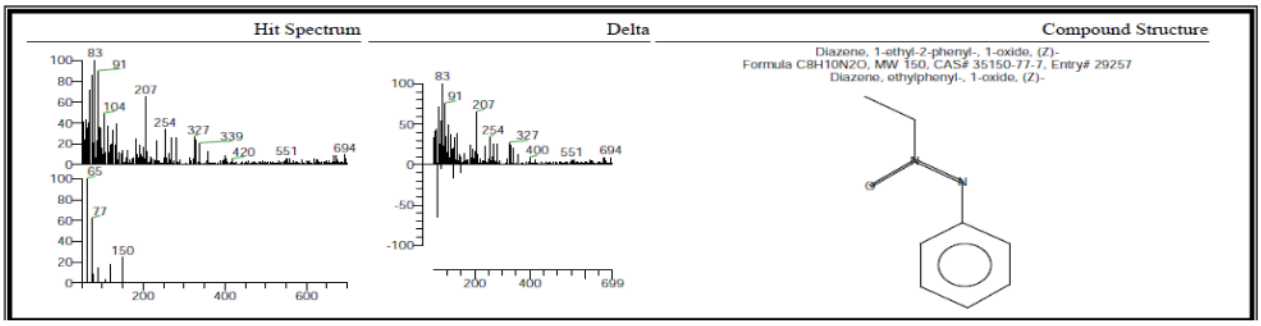
3-Hexene, 1-(1-ethoxyethoxy)-, (Z)-



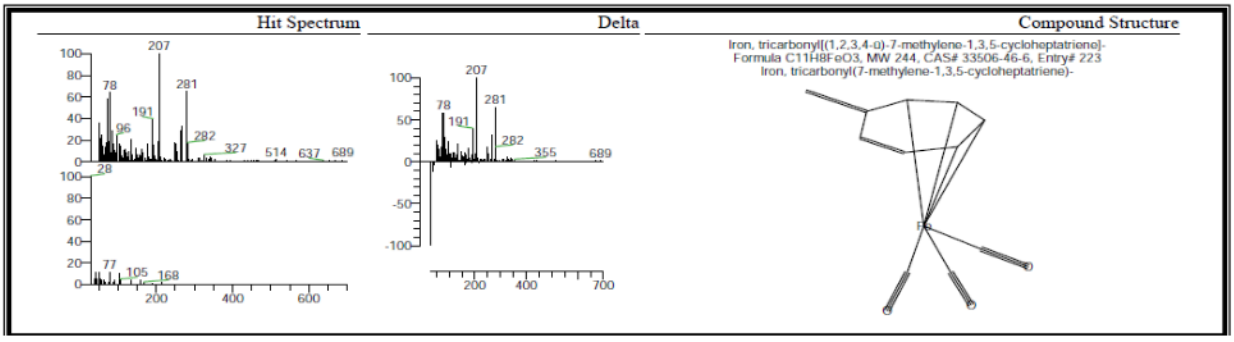
4Methyl2,4bis(4'trimethylsilyloxyphenyl)pentene1



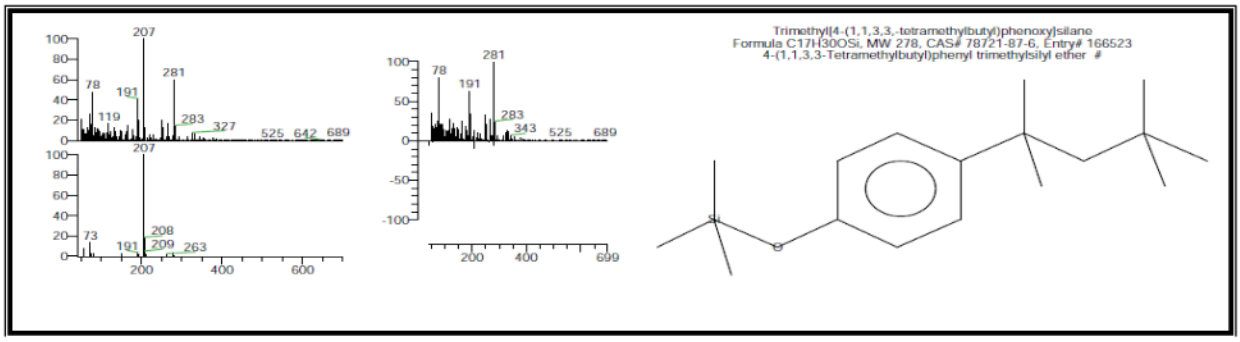
Acetic acid, (2,5-dichloro-4-methoxyphenoxy)-, methyl ester



Diazene, 1-ethyl-2-phenyl-, 1-oxide, (Z)-



Iron, tricarbonyl[(1,2,3,4ü) 7methylene1, 3,5cycloheptatriene]



Trimethyl [4(1,1,3,3,tetramethylbutyl) phenoxy]silane

Fig. 2 The best hit for the prevailing compounds in the chromatogram.

RESULTS AND DISCUSSION

The active principles with their retention time (RT), molecular formula (MF), molecular weight (MW) and concentration (%) in the methanolic extracts of the whole plant of *Euphorbia caducifolia* are presented in tables 1 followed by[27, 28]. The GC-MS analysis of the extracts showed the presence of phyto-components, the phyto-components of the above said plant extract are presented in Table-1 and the GC-MS chromatogram with peak area of each extract is also given[29] (figure-1). Totally 44 bio-active constituents were identified in the present study from the acetone extracts of the whole plant of *E. caducifolia* which including both major and minor constituents.

The major constituents were 2,2-Diphenyl-1,3,6-trioxa-2-sila-cyclooctane (8.36%); 4 Methy l2, 4 bis (4'trimethylsilyloxyphenyl) pentene1 (4.72%); 2, 4-Dimethyl- 6-phenyl-3- thioxo-5- oxo-2,3,4,5-tetrahydro-1,2,4-triazine (4.37%); Trimethyl [4(1,1,3,3,tetramethylbutyl) phenoxy] silane (4.04%); Acetic acid, (2,5-dichloro-4-methoxyphenoxy)-, methyl ester (3.91%); 1H1,2,3Triazol1amine, N[(4methoxyphenyl) methylene]4,5dimethyl (3.85%); 3-Hexene, 1-(1-ethoxyethoxy)-, (Z)- (3.65%); 2Dodecen1ol, 12chloro (3.12%); Diazene, 1-ethyl-2-phenyl-, 1-oxide, (Z)- (2.97%) and Iron, tricarbonyl [(1,2,3,4ü) 7methylene1, 3,5cycloheptatriene] (2.78%) (table-2). Minor constituents were 1,3-Cyclopentanedione, 4-butyl- (1.40%); Tricyclo [2.2.1.0 (1,4)] heptan 2 one, 6 nitro (1.39%); Tetrazolo[1,5b] pyridazine, 8 methyl (1.34%); Tricyclo [4.3.1.1(3,8)] undecane, 1-bromo- (1.34%); Pyrazinamine, 1,2,3,4 Oxatriazolium, 5(ethylamino) 3phenyl, hydroxide, inner salt (1.25%); Methyl N-cyclohexyl-3- phenylpropan imidate (1.21%); Sydnone, 3 (phenylmethyl) Benzene, 1nitro2 (p-methylphenoxy) 4 fluoro (1.14%); 3,7-Decadien-2-one, 10-(3,3-dimethyloxiranyl)-4,8-dimethyl-, (E,E)-ñ- (1.03%); 8,11 Octa decadiynoic acid, methyl ester (0.90%) and 1,3,5Triazine,1,3,5tricyclohexyl hexahydro (0.63%) (table-3). The best hit for the prevailing compounds in the chromatogram (Fig. 2).

The GC-MS chromatogram with peak area has shown in fig-1. The aim of the present study is to provide more information about the essential phyto-constituents of *E. caducifolia*. The results from the present investigation were very encouraging and indicates that this plant should be studied more extensively to explore its potential to use as plant medicinal nutritive.

CONCLUSION

Therapeutic mechanism of a plant can be better understood with a proper investigation of its active ingredients. In the present study, 44 components from the methanolic extracts of the whole plant of *E. caducifolia* were identified by GC-MS analysis. The presence of various bioactive compounds justifies the use of this plant for various ailments by traditional practitioners. These active principles provide inspiration for further investigation to achieve lead molecules in the discovery of novel herbal drugs. However, isolation of individual photochemical constituents and subjecting it to biological activity will definitely give fruitful results. It could be concluded that, *E. caducifolia* contains various bioactive compounds. So it is recommended as a plant of phyto-pharmaceutical importance. However, further studies are needed to undertake its bioactivity and toxicity profile.

There are so many type of genes and proteins present in desertic plants so it can grow easily in stress condition[30, 31]. At the cellular level, plant cell responds to these stresses by the activation of cascades of molecular mechanisms involved in stress perception, signal transduction and the expression of specific stress related genes and metabolites. A number of genes are induced by exposure to such condition, those that protect against environmental stresses directly[32].

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