Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN (E): 2320-3862
ISSN (P): 2394-0530
NAAS Rating 2017: 3.53
JMPS 2017; 5(3): 31-37

Received: 13-03-2017
Accepted: 14-04-2017

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GC-MS analysis of bioactive compounds in the entire plant parts of ethanolic extract of *Gomphrena decumbens* Jacq

P Yamuna, P Abirami, P Vijayashalini and M Sharmila

Abstract
The present investigation was carried out to determine the bioactive compounds present in entire plant of *Gomphrena decumbens* Jacq. By GC-MS analysis in the ethanolic extract of this plant, 28 bioactive compounds were identified. The major bioactive compounds are 1-Docosanol (36.93%); A: D-Neooleana-12, 14-diene, (3. xi. 5a) (25.27%); 9, 12, 15-Octadecatrienole acid (4.94%); Cholest-5-en-3-ol (3a) (4.46%); Stigmast- 5- en- 3-ol, (3a, 24S) (2. 98%); 9-octadecenoic acid (Z) (2. 92%); Lupan- 3- ol, acetate (2. 72%); Hexadeconoic acid (2.53%); Fucoxanthin (2.38%); Hexadecanoic acid, ethyl ester (1.91%); Phytol (1.39%); 4-chlorophenyl tert-pentyl sulphate (1.34%) and Ethyl9,12,15octadecatrienoate (1.11%).

Keywords: *Gomphrena decumbens*, GC-MS, ethanolic, Docosanol, Phytol

1. Introduction
India is a country rich in indigenous herbal resources which grow on their varied topography and under changing agro climatic conditions permitting the growth of almost 20000 plant species, of which about 2,500 are of medicinal value. So medicinal plants have been called part of nature's pharmacy. In early period of civilization human beings have been dependent on plants of their care and other needs. According to a WHO survey, 70–80% of the world population is dependent on plant based medicines.

India is the birth place of renewed system of indigenous medicine such as Siddha, Ayurvedha and Unani. Traditional systems of medicines are prepared from a single plant or combinations of number of plants. The green plants synthesize and preserve a variety of biochemical products, many of which are extractable and used as a chemical feed stocks or as raw material for various scientific investigations (Vinoth *et al.* 2011; Savithramma *et al.,* 2010) [1, 2]. From over 3, 00,000 species of higher plants to occur in nature, only about 2 percent have been screened so for. At least 12,000 compounds have been isolated so far; a number estimated to be less than 10% of the total. Furthermore, natural products, either pure compounds, or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. Knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies (Mojab *et al*., 2003) [3].

Gas Chromatography Mass Spectroscopy, a hyphenated system is a very compatible technique and the most commonly used technique for the identification and quantification purpose. The unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra. There are at least two significant advantages for using GC-MS in the analysis of herbal medicines. First with the capillary column, GC-MS has in general very good separation ability, which can produce a chemical fingerprint of high quality and secondly with the coupled mass spectral database, quantitative composition information of the herb investigated could be provided by GC-MS, which will be extremely useful for the further research for elucidating the relationship between chemical constituents in the herbal medicine and its pharmacology in further research. Hence the present study investigated that GC-MS analysis of bioactive compounds in the entire plant parts of ethanolic extract of *Gomphrena decumbens* Jacq.

Materials and Methods

Collection of Plant materials

The entire parts of *Gomphrena decumbens* Jacq. is collected from Arachalur, Erode district, Tamil Nadu, India and were authenticated by using local floras Gamble, Mathew and Botanical Survey of India, Southern Regional centre, Coimbatore, Tamil Nadu, The herbarium specimens are kept in the Herbarium of PG and Research Department of Botany, Vellalar College for Women, Erode (Tamil Nadu), India.

Preparation of plant extracts

Fresh plants were collected and air-dried at room temperature and then homogenized to obtain coarse powder. The powdered samples were extracted (Mukherjee, 2002) with the solvent ethanol by hot extraction using Soxhlet apparatus. The solvent free extracts were collected and stored in a vial (-4˚c) for further analysis.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Ethanolic extract of entire parts of *Gomphrena decumbens* Jacq. Were analyzed for the presence of different volatile compounds by Gas chromatography-Mass spectroscopy (GC-MS) technique. GC-MS analysis of some of the potent volatile constituents present in the extracts were performed at "The South India Textile Research Association (SITRA)", Coimbatore, Tamil Nadu, India. GC analysis of the extracts was performed using a GC-MS (Model; Thermo Trace GC Ultra Ver.5.0) equipped with a DB-35MS fused silica capillary column (30m length X outside diameter 0.25 mm X internal diameter 0.25 µm) 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 1µl; Injector temperature was 250oC; Ion source temperature was 200oC. The oven temperature was programmed from 70° to 200°C at the rate of 10°C/min, held isothermal for 1minutes and finally raised to 250°C at 10°C/min. Interface temperature was kept at 250oC. The relative percentage of each extract constituent 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 time and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures. NIST (Mc Lafferly, 1989) [9], (Stein, 1990) [10] library sources were also used for matching the identified components from the plant material.

Result

The GC-MS analysis of ethanolic extracts of entire parts of *Gomphrena decumbens* revealed the presence of twenty eight consituents. The GC-MS running time was 40.53 minutes. The GC-MS chromatogram is presented in Fig.1. The active principles with their Retention Time (RT), molecular formula, Molecular Weight (MW) and peak area are presented in the Table 1. The spectra of the compounds are matched with Wiley 9.0 and NIST libraries. The nature and uses of the phytoconstituents are presented in the Table 2 and molecular structure and its hit spectrum are depicted in the Table 3.

The identified compounds and its peak area are 1-Docosanol (36.93%); A: D-Neooleana-12, 14-diene, (3. xi. 5a) (25.27%); 9, 12, 15-Octadecatrienole acid (4.94%); Cholest-5-en-3-ol (3a) (4.46%); Stigmast-5-en-3-ol,(3a,24S) (2.98%); 9-octadecenoic acid(Z) (2.92%); Lupan-3-ol,acetate (2.72%); Hexadeconoic acid (2.53%); Fucoxanthin (2.38%); Hexadecanoic acid, ethyl ester (1.91%); Phytol (1.39%); 4-chlorophenyl tert-pentyl sulphate (1.34%); Ethyl9,12,15octadecatrienoate (1.11%); Diethoxymethylsilanol (0.99%); Nonanal (0.83%); Lycoxanthin (0.77%); 4,7-Methano-1H-indene,3a,4,5,6,7,7a-hexahydro-5-(2-propenyloxy) (0.75%); Neophytadiene 0.67%); Methanone, (1-hydroxyly clohexyl)phenyl (0.66%); (7-Bromo-3a,6,6-trimethyl-hexahydrobenzofuran-2-ylidene)-ethyl-amine (0.56%); 1,3-Bis(4-chlorobenzyl)-5,6-dihydrobenzo[f]quinazolin (0.50%); Cyclodecasiloxane, eicosamethyl (0.41%); Squalene (0.36%); Rhodopin (0.35%); 2-Amino-5-chlorobiphenyl (0.33%); Tetraethyl silicate (0.31%); Janoxepin (0.30%) and Phthalic acid, 5-methylhex-2-yl isobutyl ester (0.30%) were also obtained and some uses of these compounds are shown in table 2.

Among the identified compound, 9,12,15-Octadecatrienoic acid (Z,Z,Z)- and 9,12,15-Octadecatrienoic, methyl ester (Z,Z,Z)- which is a linoleic acid compound and reported to have an anti-inflammatory, anti-arthritic, hypocholesterolemic, hepatoprotective, anti-cancer, anti-histaminic, anti-acne, nematicide, insectifuge and anti-eczemic properties. Similarly, the presence of 9-octadecenoic acid was observed in the ethanolic root of *Plumbago zeylanica* by Ajayi *et al*., (2011) [11]. Hexadecanoic acid methyl ester is also known as palmitic acid ester. The fatty acid being effective in the treatment of antioxidant, pesticide anti-androgenic, nematicide, flavouring agent, hypocholesterolemic and lubricant. Pramitha and Sree Kumari (2016) [12] reported that ethyl acetate fraction of *Sargassum wightii* contains seventeen compounds and some of the major compounds detected were Bromoacetic acid, hexadecyl ester (94.98%),1,4-Eicosadiene (87.16%), Eicosane (73.97%), 6-Octadecenoic acid, (Z)- (72.17%), n-Hexadecanoic acid (62.97%), Benzene, 1,2-dimethoxy-4-(1-propenyl)- (62.92%), Stigmasta-5,24(28)-dien-3-ol, (3.beta)- (61.06%), Pyrrolo [1,2-a]pyrazine-1,4-dione, hexahydro-3(Phenyl methyl)-(56.62%) 2(1H)- Pyrimidinone, 4-amino-5-methyl-(41.97%) and 4-Methoxy-3Propoxy-Benzaldehyde (40.18%). GC-MS analysis of methanolic extract was done by standard protocol using the equipment Perkin-Elmer 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 (Sujayil and Dhanaraj, 2016) [13]. The GC-MS analysis revealed the presence of various compounds like n-Hexadecanoic acid, Hexadecanoic acid, methyl ester, Benzoic acid, D-Allose and Cytidine in the methanolic extract of *Evolvulus alsinoides*. These findings support the traditional use of *Evolvulus alsinoides* in various disorders.

In *Cassia auriculata* Rajkumar *et al.,* (2016) [14] revealed the presence of 8 compounds in GC-MS analysis. Some of the phyto-compounds Screened were Preganane-3,11,12,14,20-pentol,3,12,20triacetate 11-(hydroxyacetate),2-propenoic acid,3-(4-methoxyphenyl), ethyl ester,(E)-1-(2,6,6-trimethylcyclohex-1-en1-yl) undec-1-ene-3,6-dione. The phytochemical constituents of ethanol extracts of *Justicia carnea* leaf was analyzed using gas chromatography-mass spectrometry method (Otuokere *et al*., 2016) [15]. The ethanol leaf extracts of *Justicia carnea* was prepared by soxhlet extraction method and concentrated at 40°C using hot air oven. The concentrated ethanol extracts was subjected to

phytochemical analysis using GC-MS. GC-MS analysis showed the presence of six phytocompounds. The phytocompounds are isonicotinic acid N-oxide (2.58%), phosphinodithioic acid, diphenyl- (1.93%), hexadecanoic acid (10.50%), 2,2,3,3,4,4,5,5,5-nonafluoro-pentanoic acid methyl ester (73.19%), 9,12,15-octadecatrien-1-ol (9.33%) and 7H-purine, 7-benzyl-2,6-dichloro- (2.48%). The result of the GC-MS analysis showed that the ethanol extract of *Justicia carnea* contains many pharmacologically important bioactive compounds. Traditionally, *Justicia* species are used in the treatment of inflammation, gastrointestinal diseases, respiratory tract infection, rheumatism and arthritis. There is need for isolation of these phytocompounds for the control of diseases.The active principle molecular weight (Mw), concentration (%), molecular formula, Retention Time (RT) was presented in *Hyptis suaveolens* Linn (poit). It shows 25 compounds. This include Eucalyptol ($C_{10}H_{18}O$) 0.44%, Bicyclo [4.1.0]-3-heptene, 2-isopropenyl-5-isopropyl-7,7-dimethyl- ($C_{15}H_{24}$)0.07,Cyclobutaneacetonitrile,1-methyl-2-(1-methylethenyl)-($C_{10}H_{15}N$)0.05, Bicyclo [7.2.0]undec-4-ene, 4,11,11- trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]-($C_{15}H_{24}$) 2.44, 1,3,6,10-Dodecatetraene, 3,7,11-trimethyl-, (Z,E)-
(Adamantane, 1-(2-bromoethenyl)- ($C_{12}H_{17}$ Br) 0.15, 1,9-Decadiyne ($C_{10}H_{14}$) 0.03, Squalene ($C_{30}H_{50}$) 6.47,etc Identified by Mozhiyarasi and Anuradha (2017) [16].

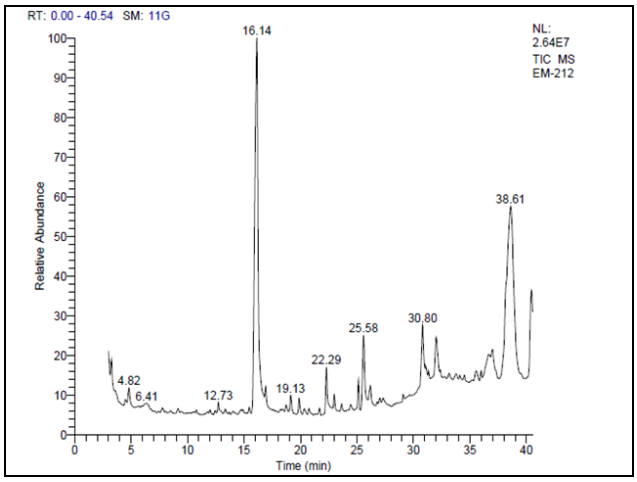


Fig 1: Chromatogram of the entire plant parts of ethanolic extract of *Gomphrena decumbens* Jacq**.**

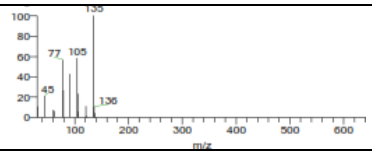
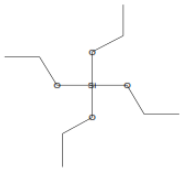
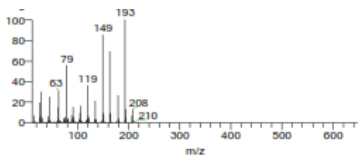
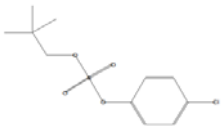
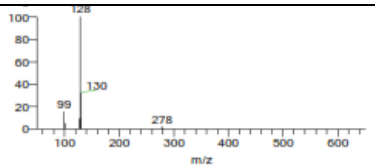
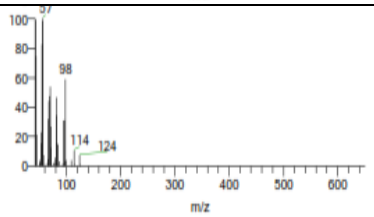
Table 1: GC-MS analysis of bioactive compounds in the entire plant parts of ethanolic extract of *Gomphrena decumbens* jacq.

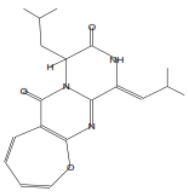
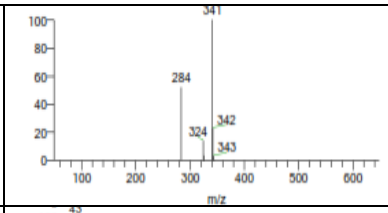
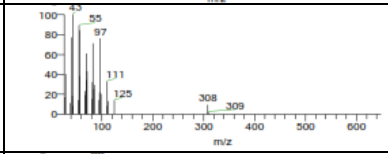
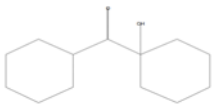
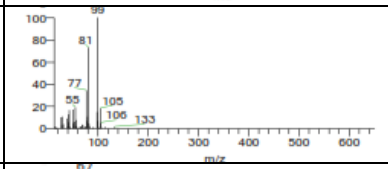
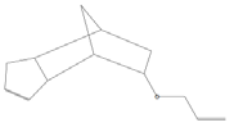
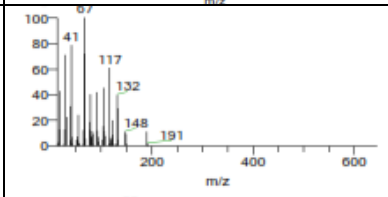
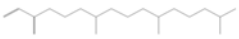
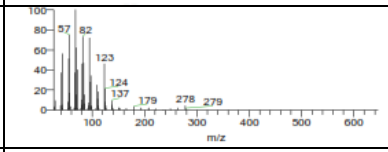
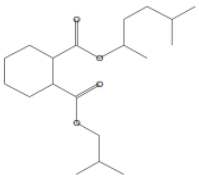
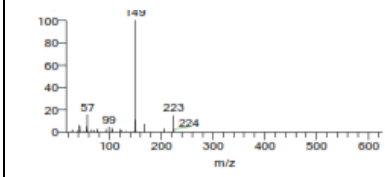
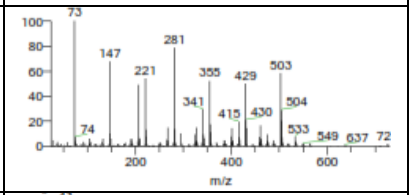
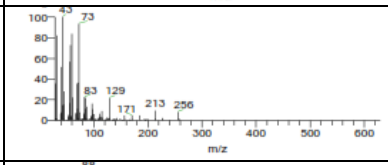
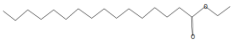
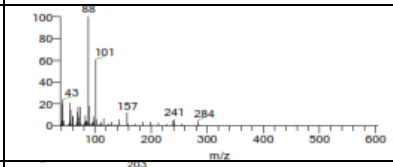
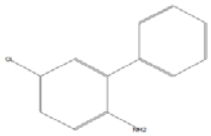
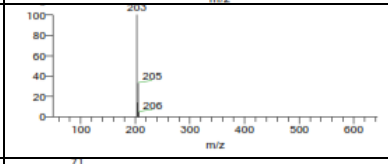
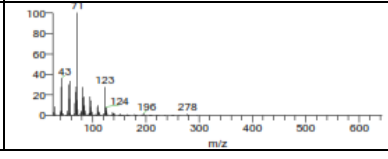
S. No.	Retention Time	Name of the compound	Molecular Formula	Molecular Weight	Peak Area
1	3.26	Diethoxymethylsilanol	$C_5H_{14}O_3Si$	150	0.99%
2	4.49	Tetraethyl silicate	$C_8H_{20}O_4Si$	208	0.31%
3	4.82	4-chlorophenyl tert-pentyl sulphate	$C_{11}H_{15}C_{10}$	278	1.34%
4	6.41	Nonanal	$C_9H_{18}O$	142	0.83%
5	9.18	Janoxepin	$C_{19}H_{23}N_3O_3$	341	0.30%
6	16.14	1-Docosanol	$C_{22}H_{46}O$	326	36.93%
7	16.92	Methanone,(1-hydroxyly clohexyl)phenyl	$C_{13}H_{16}O_2$	204	0.66%
8	19.13	4,7-Methano-1H-indene,3a,4,5,6,7,7a-hexahydro-5-(2-propenyloxy)-	$C_{13}H_{18}O$	190	0.75%
9	19.88	Neophytadiene	$C_{20}H_{38}$	278	0.67%
10	20.32	Phthalic acid, 5-methylhex-2-yl isobutyl ester	$C_{19}H_{28}O_4$	320	0.30%
11	21.69	Cyclodecasiloxane, eicosamethyl-	$C_{20}H_{60}0_{10}Si_{10}$	740	0.41%
12	22.29	Hexadeconoic acid	$C_{16}H_{32}O_2$	256	2.53%
13	22.98	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	1.91%
14	23.64	2-Amino-5-chlorobiphenyl	$C_{12}H_{10}ClN$	203	0.33%
15	25.13	Phytol	$C_{20}H_{40}O$	296	1.39%
16	25.58	9,12,15-Octadecatrienole acid,(Z,Z,Z)	$C_{18}H_{30}O_2$	278	4.94%
17	26.20	Ethyl9,12,15octadecatrienoate	$C_{20}H_{34}O_2$	306	1.11%
18	27.01	1,3-Bis(4-chlorobenzyl)-5,6-dihydrobenzo[f]quinazolin	$C_{26}H_0Cl_2N_2$	430	0.50%
19	27.33	(7-Bromo-3a,6,6-trimethyl-hexahydrobenzofuran-2-ylidene)-ethyl-amine	$C_{13}H_{22}BrNo$	590	0.56%
20	30.80	Cholest-5-en-3-ol(3a)-	$C_{27}H_{46}O$	386	4.46%
21	32	9-octadecenoic acid(Z)-	$C_{18}H_{34}O_2$	282	2.92%
22	33.15	Rhodopin	$C_{40}H_{58}O$	554	0.35%
23	35.51	Lycoxanthin	$C_{40}H_{56}O$	552	0.77%
24	35.65	Lupan-3-ol,acetate	$C_{32}H_{54}O_2$	470	2.72%
25	35.98	Squalene	$C_{50}H_{50}$	410	0.36%
26	37	Fucoxanthin	$C_{42}H_{58}O_6$	658	2.38%
27	38.61	A:D-Neooleana-12,14-diene,(3.xi.,5a)-	$C_{30}H_{48}$	408	25.27%
28	40.42	Stigmast-5-en-3-ol,(3a,24S)-	$C_{29}H_{50}O$	414	2.98%

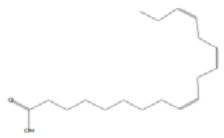
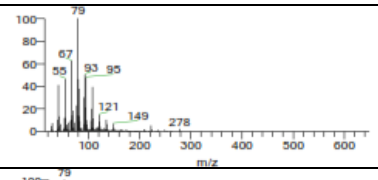
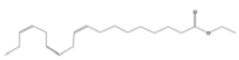
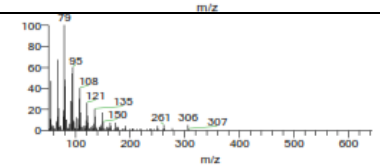
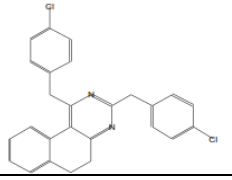
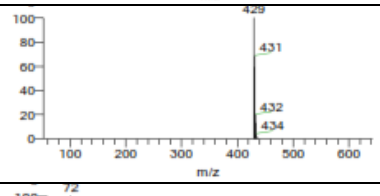
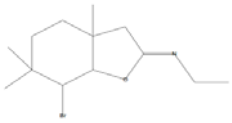
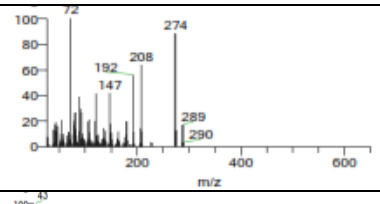
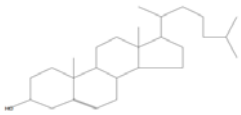
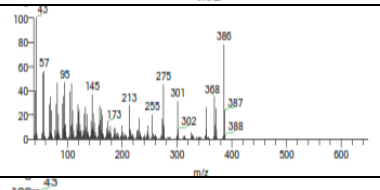
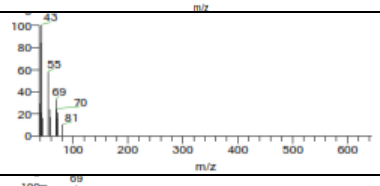
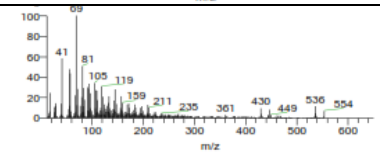
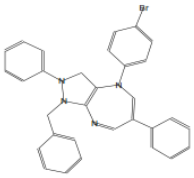
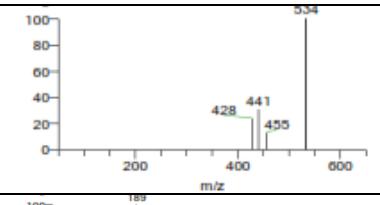
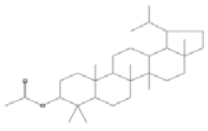
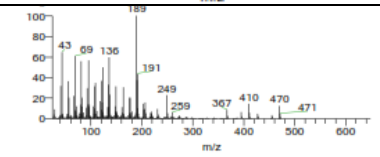
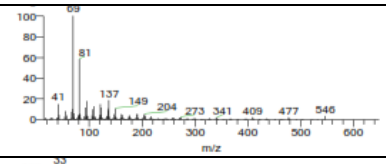
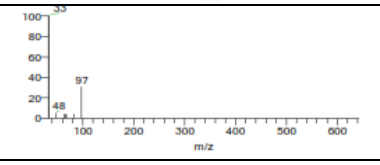
Table 2: Nature and the biological activities of phytoconstituents of the entire plant parts of ethanolic extract of *Gomphrena decumbens* Jacq.

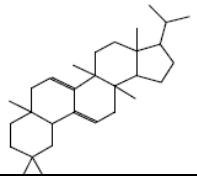
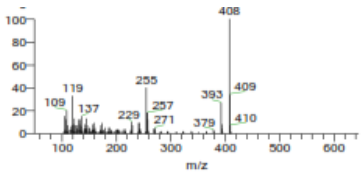
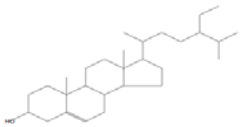
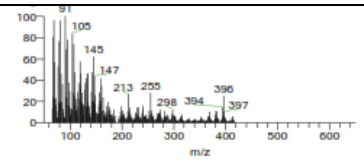
S. No.	Retention Time	Name of the Compound	Compound Nature	Uses
1	4.49	Tetraethyl silicate	Ethyl ester	Production of aerogel.
2	6.41	Nonanal	Colorless oily liquid	Used as the compound of perfume.
3	16.14	1-Docosanol	Saturated fatty acid	Emollient,Emulsifier, Thickener in cosmetics
4	16.92	Methanone, (1-hydroxyly clohexyl)phenyl	Ketone	Anticancer.
5	19.88	Neophytadiene	Hydrocarbons	Antipyretic, analgesic, anti-inflammatory, anti-microbial, Antioxidant.
6	22.29	Hexadeconoic acid	Saturated fatty acid	Anti –inflammatory, Hypocholesterolemic, Nematicide, Antioxidant.
7	25.58	9,12,15-Octadecatrienole acid,(Z,Z,Z)	Unsaturated fatty acid	Anti-inflammatory,Hypocholesterolenic,Cancer preventive, Hepatoprotective, Nematicide, Insectifuge, Antihistamininic, Antieczemic, Anticancer, 5-alpha reductase inhibitor, Antiandrogenic, anti-rthritic, Anti-coronary, Insectifuge.
8	26.20	Ethyl 9,12,15-octadecatrienoate	Linolenic acid	Cell viability.
9	32	9-octadecenoic acid(Z)-	Monounsaturated fatty acids	Emulsifying agent
10	33.15	Rhodopin	Carotenoids	Major compouds in phototrophic bacteria.
11	35.51	Lycoxanthin	Carotenoids	Antioxidant
12	35.65	Lupan-3-ol,acetate	Triterpenoids	Antiprotozoal, Antimicrobial, Antiinflammatory, Antitumour, Chemopreventive.
13	35.98	Squalene	Saturated fatty acid	Anti –inflammatory, Anti –bacterial, Antitumor, Immunostimulant, Chemopresentive, Cancer preventive, Lipogenase -inhibitor, Pesticide.
14	37	Fucoxanthin	Carotenoids	Antioxidant
15	40.42	Stigmast-5-en-3-ol,(3a,24S)-	Phytosterols	Reduce blood level of glucose, hypercholesterolemia

Table 3: The molecular structure and the molecular formula of the bioactive phytoconstituents of the entire plant parts of ethanolic extract of *Gomphrena decumbens* Jacq.

S. No.	Name of the compound	Molecular formula	Molecular structure	Hit Spectrum
1	Diethoxymethylsilanol	$C_5H_{14}O_3Si$		
2	Tetraethyl silicate	$C_8H_{20}O_4Si$		
3	4-chlorophenyl tert-pentyl sulphate	$C_{11}H_{15}C_{10}$		
4	Nonanal	$C_9H_{18}O$		

5	Janoxepin	$C_{19}H_{23}N_3O_3$		
6	1-Docosanol	$C_{22}H_{46}O$		
7	Methanone, (1-hydroxyly clohexyl)phenyl	$C_{13}H_{16}O_2$		
8	4,7-Methano-1H-indene,3a,4,5,6,7,7a-hexahydro-5-(2-propenyloxy)-	$C_{13}H_{18}O$		
9	Neophytadiene	$C_{20}H_{38}$		
10	Phthalic acid,5-methylhex-2-yl isobutyl ester	$C_{19}H_{28}O_4$		
11	Cyclodecasilaxone,eicosamethyl-	$C_{20}H_{60}O_{10}Si_{10}$		
12	Hexadeconoic acid	$C_{16}H_{32}O_2$		
13	Hexadeconoic acid, ethyl ester	$C_{18}H_{36}O_2$		
14	2-Amino-5-chlorobiphenyl	$C_{12}H_{10}ClN$		
15	Phytol	$C_{20}H_{40}O$		

16	9,12,15-Octadecatrienole acid,(Z,Z,Z)	$C_{18}H_{30}O_2$		
17	Ethyl-9,12,15-octadecatrienoate	$C_{20}H_{34}O_2$		
18	1,3-Bis(4-chlorobenzyl)-5,6-dihydrobenzo[f] quinazoline	$C_{26}H_{20}Cl_2N_2$		
19	(7-Bromo-3a,6,6-trimethyl-hexahydrobenzofuran-2-ylidene)-ethyl-amine	$C_{13}H_{22}BrNo$		
20	Cholest-5-en-3-ol(3a)-	$C_{27}H_{46}O$		
21	9-octadecenoic acid(Z)-	$C_{18}H_{34}O_2$		
22	Rhodopin	$C_{40}H_{58}O$		
23	1-Benzyl-2,6-diphenyl-4-(4'-bromophenyl)-2,3-dihydropyrazolo[3,4-b][1,4]diazepine	$C_{31}H_{25}BrN_4$		
24	Lupan-3-ol,acetate	$C_{32}H_{54}O_2$		
25	Squalene	$C_{50}H_{50}$		
26	Fucoxanthin	$C_{42}H_{58}O_6$		

27	A:D-Neooleana-12,14-diene,(3.xi.,5a)-	$C_{30}H_{48}$		
28	Stigmast-5-en-3-ol,(3a,24S)-	$C_{29}H_{50}O$		

Conclusion

The present study helps to predict the formula and structure of biomolecules of *Gomphrena decumbens* which can be used as drugs. It enhances the traditional usage of which possesses some known and unknown bioactive compounds. However, isolation of individual phytochemical constituents and subjecting it to pharmacological activity will definitely give fruitful results.

Acknowledgement

Authors thank to the South India Textile Research Association (SITRA), Coimbatore (Tamil Nadu), India for help in screening the GC-MS analysis.

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