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GC-MS analysis of bioactive compounds in the plant parts of methanolic extracts of *Momordica cymbalaria* Fenzl

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Abstract
Plants are excellent sources of secondary metabolites that have been used for the treatment of human diseases. The plant *Momordica cymbalaria* contains different types of phytochemicals like phenols, steroids, flavonoids, alkaloids and tannins. The present investigation was carried out to determine the bioactive compounds present in the different plant parts of *Momordica cymbalaria* by Gas chromatography- Mass spectroscopy (GC-MS) technique. Chromatograms and bioactive compounds of *in vivo* leaf, root and *in vitro* leaf callus methanolic extracts of *Momordica cymbalaria* are derived by GC-MS technique. 37 compounds were found in leaf methanolic extracts, 20 compounds *in vitro* leaf callus methanolic extracts and 18 compounds in root methanolic extracts. These are important bioactive compounds n- Hexadecanoic acid, 9-octadecenoic acid methyl ester, Cholesterol, Cholestanol, 5β-cholestane-3α,7α,12α,24α,25-pentol TMS, 1-Heptatriacotanol, Vitamin E, Ethyl iso-allocholate, Stigmasterol, B-sitosterol, Lupeol, Diethyl Phthalate, Propanoic acid,2-(3-acetoxy-4,4,14-trimethyl and rost-8-en-17-yl), 1H-pyrrole-2,5,dihydro-1-nitroso, d-Mannose, Spirost-8-en-11-one,3-hydroxy, (3β,5α,14 β,20β,22 β,25R) these are determined from *in vivo* leaf, root and *in vitro* callus explants. These identified compounds possess biological activities which can act against the incurable human diseases.

Keywords: Gas chromatography-mass spectroscopy (GC-MS), biomolecules, *In vitro, In vivo*, methanolic extract

1. Introduction

Human beings consuming foods contain different types of minor, major components and bioactive molecules like carbohydrates, peptides, antioxidants, lipids and glucosinolates. Plants produced vegetables and fruits are containing some useful bioactive molecules, these are act as antioxidants in human body which are helpful in skin damage control, oxidative damage cell control and prevent the cancer cell formation.

According to the Roessner and Beckles, (2009) [1], the diversity of plant bioactive compounds derived from the infinite combinations of fundamental functional groups or carboxylic groups such as alkyls, hydroxyls, alcohols, steroids, aldehydes, benzyl rings that originate compounds with peculiar chemical and physical characteristics such as solubility, melting point, and reactivity.

Plants produced Bioactive molecules are compounds having pharmacological or toxicological effects. Vitamins, minerals and nutrients were obtain pharmacological or toxicological effects when taken in high doses. In plants Vitamins, minerals and nutrients are generally not mentioned as bioactive compounds. Plants produced secondary metabolites are termed as bioactive compounds or bioactive molecules. Hence, a definition of bioactive compounds in plants referred as secondary plant metabolites obtaining the pharmacological or toxicological effects in human beings and animals. According to Gomathi *et al.*, 2015 [2], "Bioactive molecules" are compounds that occur in nature, part of the food chain, capable of interacting with one or more compounds of living tissue, exerting a synergistic effect on human health.

According to korhonen, (2002) [3], to detect the bioactive compounds in plants must perform the extraction or separation techniques and recovery techniques by taking bio accessibility measurements. These bioactivity measurements like *In vivo, In vitro* are based on the bioactive components interacts with biomolecules. This interaction generates an metabolites.

Extraction is the first step of any role on the final result of the study. Extraction methods are also called as "sample preparation techniques". Now-a-days modern chromatographic techniques and spectrometric techniques make bioactive compound analysis easier than past days.

These techniques success is depends on the nature of the plant, extraction methods and input parameters. Plant parts properties, temperature, extraction time and the solvents used as the important factors, which are affecting the extraction processes. Extraction process is the essential and it has to appropriately done for the next conducted separation, identification and characterization of biologically active compounds. Biologically active compounds can be extracted from plant material by various scientific extraction methods.

Most of these methods depend on the extraction power of the various solvents used and the application of heat and mixing. Commonly used scientific methods include soxhlet, maceration, reflux extraction and hydro distillation to obtain the crude extract, which is then concentrated using a rotary evaporator (Azmir *et al*., 2013) [4].

After extraction process, identification and characterization of the derived compounds is important. Plant extracts usually occur as a combination of different types of phytochemicals or biologically active compounds of different polarities. Separation in the process of identification and classification of biologically active compounds still remains a major challenge. The most commonly used separation techniques for bioactive compounds are Thin layer chromatography (TLC), column chromatography (GCMS, LCMS) flash chromatography and HPLC, should be used to obtain pure compounds. The pure compounds are then used for the determination of structure and biological activity.

Phytochemical screening assay, Fourier transform infrared spectroscopy (FTIR) and Immunoassay, which use monoclonal antibodies (MAbs) are the non-chromatographic techniques. Gas chromatography Mass spectroscopy, is most commonly used and compatible technique for the identification and quantification of biomolecules. In this study we performed GCMS, to analysis methanolic plant extracts of *Momordica cymbalaria* Fenzl.

The present investigation has helped to identify seventy five (75) bioactive chemical constituents from *in vivo* leaf, root and *in vitro* leaf callus methanolic extracts of *Momordica cymbalaria* by using GC-MS technique. Furthermore, these screened potential bioactive compounds can be effectively used for biomedical and therapeutic applications.

2. Materials and Methods

2.1 Collection of plant material

Momordica cymbalaria plants were collected from Jammikunta crop fields, Telangana State during monsoon season. The plants were maintained and grown in the medicinal harbour at Department of Biotechnology, Kakatiya University, Telangana State, India.

2.2 Preparation of extracts

Momordica cymbalaria leaves, roots were extracted from healthy grown field plants and *in vitro* leaf callus were taken for this experiment. The leaves and roots were rinsed under running tap water for 15mins, washed with 10% tween 20 (liquid soap) for 5mins, then washed 4 times with double steriled distilled water. These sterilized leaves, roots and also *in vitro* leaf callus were kept for shade dried for 10 days. Then they were ground to coarse powder using motor and pestle. These powders (each 10 gr) were extracted separately with methanol solvent (each 50 ml) by using cold maceration technique. Later the extracts were filtered through a Whatman filter paper and concentrated using rotary evaporator and subsequently subjected for gas chromatography mass spectroscopy (GCMS) technique using standard methods protocols. The extracts was stored in a refrigerator at 4°C for further use.

2.3 GC-MS analysis

The methanolic extracts of *Momordica cymbalaria* were analyzed for the presence of different volatile compounds by the technique of gas chromatography mass spectroscopy (GCMS). GCMS analysis of biologically active compounds present in the methanolic extracts of *Momordica cymbalaria* were performed at the Sophisticated Analytical Instrument Facility (SAIF) in IIT Bombay, Maharashtra, India. GCMS analysis of extracts was performed by using a GC-MS (GC Model: Agilent 7890A GC System, Mass Spectrometer: The Accu TOFGcV JMS- T100GcV from JEOL India Pvt. Ltd Japan). It is equipped with HP5 column (30 m in length x 0.25mm in diameter x 0.25mm film thickness). Mass range of the spectrometer was set to 35-750amu. The oven temperature was set to 280°C and Detector temperature was set to 250 °C. Helium gas was used as carrier gas and the flow rate of Helium gas was set to 1ml/min. For GCMS detection, an electron ionization system with an ionization power of + 70eV was used. The time from injection (start time) to the time at which the eruption occurred is referred to as the retention time (RT).

2.4 Identification of Bioactive compounds

The identity of the bio constituents present in the methanolic plant extracts was assigned by the comparison of their retention time (RT) and mass spectra fragmentation patterns with those stored on the NIST Library database.

3. Results and Discussion

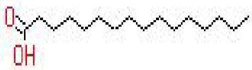
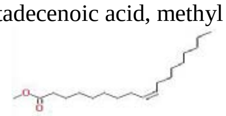
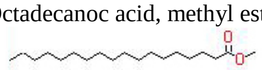
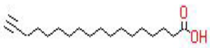
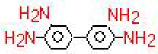
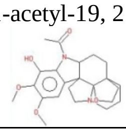
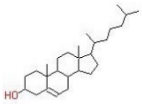
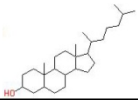
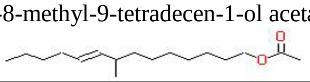
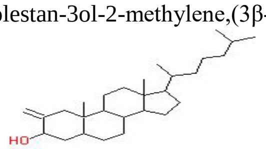
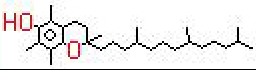
The GCMS analysis of *Momordica cymbalaria* methanolic extracts of three explants (leaf, root and *in vitro* leaf callus) revealed the presence of bioactive compounds. Active principles with their retention time (RT), molecular formula, molecular weight (MW), molecular structure and peak area percentage are tabulated in tables 1, 2 and 3.

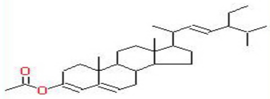
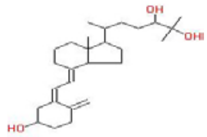
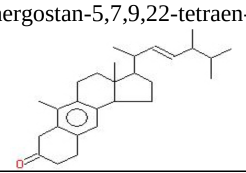
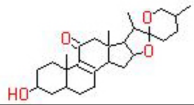
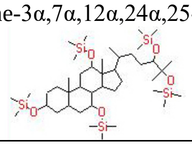
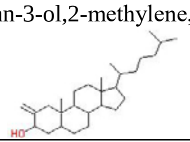
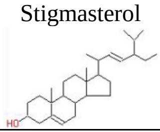
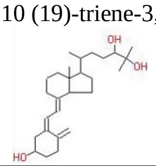
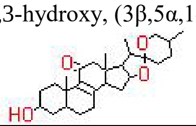
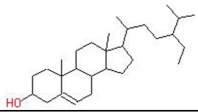
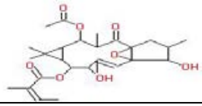
3.1 Extraction of bioactive compounds

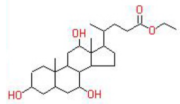
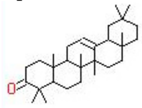
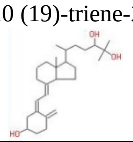
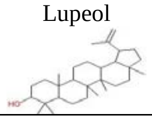
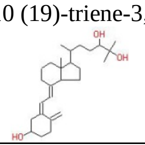
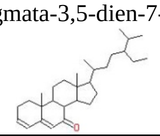
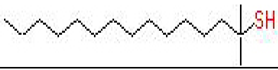
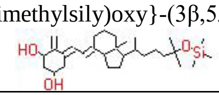
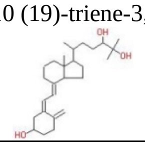
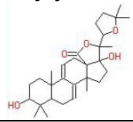
Methanol leaf extract of *Momordica cymbalaria* revealed the presence of thirty seven chemical constituents. The GCMS chromatogram of leaf methanolic extract is presented in fig 1. The identified compounds in the leaf extract (Table 1) are n-Hexadecanoic acid, it is reported to have an anti-inflammatory, antioxidant activities and also used as Pesticide, Nematicide (Jisha *et al*., 2016) [5], 9-octadecenoic acid, methyl ester, it is reported to have anti cancer properties (Syeda *et al*., 2011; Hema *et al*., 2011) [6, 7], Octadecanoic acid, methyl ester, it is showing anti-microbial properties (Gehan *et al*., 2009) [8], 17- Octadecynoic acid, it is reported to have anti- hypersensitive properties ((Fadeyi *et al*., 2009) [9], 3,3-Diaminobenzidine, Aspidospermidin, 17-ol,1-acetyl-19, 21-epoxy-15,16-dimethoxy, Cholesterol, it is used as pivotal constituent of cell membranes, steroid hormones and for the function of the hedgehog protein (Herz and Bock, 2002) [10], Cholestanol, it is reported to have anti-tumor and antioxidant properties (Jinu *et al*., 2015) [11], E-8-methyl-9-tetradecen 1-ol acetate, Cholestan-3ol-2-methylene, (3β-3α) it is reported as antioxidant (Israa Adnan Ibraheam *et al*., 2017)

[12], Vitamin E, which is very useful bioactive compound used as antiageing, antitumour, antidiabetic, anticancer, analgesic, antidermatitis, hepatoprotective, anti-inflammatory activities (Devi and Muthu., 2015) [13], Octacosanoic acid, methyl ester, it is reported to have antibacterial activities (Zheng *et al*., 2005) [14], Stigmasterol,3,4-dedihydro,acetate (ester), 9,10-secocholesta-5,7,10(19)-triene-3,24,25-troil, Anthiaergostan-5,7,9,22-tetraen-3-one, Ethyl iso-allocholate, it is reported to have antimicrobial properties (Malathi *et al*., 2016) [15], Spirot-8-en-11-one,3-hydroxy-(3β-5α,14β,20β,22β,25R), it is reported to have an anti inflammatory, estrogenic and progesterogemic effects (Hussein *et al*., 2016) [16], 5β-cholestane-3α,7α,12α,24α,25-pentol TMS, it is used in primary biliary cirrhosis disease (Kaarlagnis *et al*., 1983) [17], Cholestan-3-ol,2-methylene,(3β-5α), it is used as antioxidant (Israa Adnan Ibraheam *et al*., 2017) [12], Stigmasterol, it is used as antiviral, cancer preventive (Ponnamma and Manjunath *et al*., 2012) [18] and also reported to have anti inflammatory and anti osteoarthritic properties (Gabay *et al*., 2010) [19], B-sitosterol, it is reported to have anticancer, antimicrobial, antidiabetic, antifertility and antioxidant properties (Shirishkumar *et al*., 2014) [20], 2-Butenoic acid, 2-methyl,2-(acetyloxy)-1,1a,2,3,4,4,6,7,10,11,11a-decahydro-7,1dihydroxy, 1, 1, 3, 6, 9, pentamethyl-4a,7a-epoxy, Methyl traicontanoate, 4, 4, 6a, 6b, 8a, 11, 11, 14 Octamethyl-1, 4-4a, 5, 6, 6a, 6b, 7, 8, 9, 10, 11, 12, 12a, 14, 14a, 14b-octadecahydro-2H-picen-3-one, Lupeol, it is reported to have an antitumor, antioxidant, chemopreventive, antiarthritic (Rajendra kumar *et al*., 2014, Maruthupandian *et al*., 2011) [21,22], anti cancer (Saleem., 2009) [23], anti-inflammatory (Geetha *et al*., 2001) [24] effects. Stigmata-3,5-dien-7-one, it is reported to have antiinflammatory properties (Park *et al*., 2016) [25], Tert-Hexadecamethiol, it is used as enzyme activator (Rajendran *et al*., 2017) [26] and Lanosta-7,9 (11)-dien-18-oic acid,22,25-epoxy-3,17,20-trihydroxy-y-lactone-(3β), it is used in induction of apoptosis in human promyelocytic leukemia HL 60 cells (Gonzalez *et al*., 2002) [27].

Table 1: GC-MS of bioactive compounds present in the methanolic extracts of leaves derived from *in vivo* grown plants of *Momordica cymbalaria* Fenzl.

S. No	RT	Compound name and Structure	Formulae	Mwt	Area%	Biological activity
1	21.77	n- Hexadecanoic acid 	$C_{16}H_{32}O_2$	256	3.78%	Anti-inflammatory, Antioxidant, Pesticide, Nematicide, Inhibitor
2	23.98	9-octadecenoic acid, methyl ester 	$C_{19}H_{36}O_2$	296	0.38%	Anti-cancer
3	24.39	Octadecanoc acid, methyl ester 	$C_{19}H_{38}O_2$	298	1.14%	Anti-microbial
4	24.79	17-octadecynoic acid 	$C_{18}H_{32}O_2$	280	0.79%	Anti-hypertensive
5	27.48	3,3-Diaminobenzidine 	$C_{12}H_{14}N_4$	214	4.69%	Used in dyes &stains
6	28.19	Aspidospermidin-17-ol,1-acetyl-19, 21-epoxy-15,16-dimethoxy 	$C_{23}H_{30}N_2O_5$	414	0.74%	No activity
7	29.57	Cholesterol 	$C_{27}H_{48}O$	388	1.87%	Used as pivotal constituent of cell membranes
8	29.91	Cholestanol 	$C_{27}H_{48}O$	388	1.76%	Anti tumor, Antioxidant activity
9	30.13	E-8-methyl-9-tetradecen-1-ol acetate 	$C_{17}H_{32}O_2$	26	1.00%	No activity
10	30.87	Cholestan-3ol-2-methylene,(3β-3α) 	$C_{28}H_{48}O$	400	0.66%	Antioxidant
11	31.16	Vitamin E 	$C_{29}H_{50}O2$	430	5.00%	Antiageing, antitumour, antidiabetic, anticancer,
12	31.50	Octacosanoic acid, methyl ester	$C_{29}H_{50}O_2$	430	14.1%	Antibacterial

						
13	31.77	Stigmasterol,3,4-dedihydro,acetate (ester) 	$C_{13}H_{48}O_2$	452	1.07%	No activity
14	32.17	9,10-secocholesta-5,7,10(19)-triene-3,24,25-troil 	$C_{27}H_{44}O_3$	416	0.27%	Involved in the regulation of calcium metabolism
15	32.48	Anthiaergostan-5,7,9,22-tetraen-3-one 	$C_{28}H_{40}O$	392	3.01%	No activity
16	32.91	Ethyl iso-allocholate 	$C_{26}H_{44}O_5$	436	0.75%	Antimicrobial
17	33.54	Spirot-8-en-11-one,3-hydroxy-(3β-5α,14β,20β,22β,25R) 	$C_{27}H_{40}O_4$	428	3.81%	Anti-inflammatory, estrogenic and progesterogemic effect
18	33.87	5β-cholestane-3α,7α,12α,24α,25-pentol TMS 	$C_{42}H_{88}O_5Si_5$	812	3.44%	Used in primary biliary cirrhosis disease.
19	34.24	Cholestan-3-ol,2-methylene,(3β-5α) 	$C_{28}H_{48}O$	400	0.60%	Antioxidant
20	34.76	Stigmasterol 	$C_{29}H_{48}O$	412	3.11%	Antiviral, Cancer preventive, Anti-inflammatory, Anti osteoarthritic
21	35.16	9,10 secocholesta-5,7,10 (19)-triene-3,24,25-triol (3β,5Z,7E) 	$C_{27}H_{44}O_3$	416	1.40%	Involved in the regulation of calcium metabolism
22	35.80	Spirost-8-en-11-one,3-hydroxy, (3β,5α,14 β,20 β,22 β, 25R) 	$C_{27}H_{40}O_4$	428	0.50%	Anti-inflammatory, estrogenic and progesterogemic effects
23	36.19	Ethyl iso-allocholate 	$C_{26}H_{44}O_5$	436	2.23%	Antimicrobial
24	36.76	B-sitosterol 	$C_{29}H_{50}O$	414	1.02%	Anticancer, antimicrobial, antidiabetic, antifertility, antioxidant
25	36.98	2-Butenoic acid,2-methyl,2-(acetyloxy)1,1a,2,3,4,4,6,7,10,11,11a-decahydro7,1dihydroxy,1,1,3,6,9, pentamethyl-4a,7a-epoxy 	$C_{37}H_{76}O$	490	1.20%	No activity
26	37.37	1-Heptatriacotanol	$C_{37}H_{76}O$	536	4.50%	Antimicrobial

						
27	37.67	Ethyl iso-allocholate 	$C_{26}H_{44}O_5$	436	0.64%	Antimicrobial
28	38.18	4,4,6a,6b,8a,11,11,14Octamethyl-1,4-4a,5,6,6a,6b,7,8,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one 	$C_{30}H_{62}O_2$	424	2.43%	No activity
29	38.46	Methyl traicontanoate 	$C_{31}H_{62}O_2$	466	18.2%	No activity
30	38.99	9,10-Secocholesta-5,7,10 (19)-triene-3,24,25,triol,(3β,5Z,7E) 	$C_{27}H_{44}O_3$	416	1.34%	Involved in the regulation of calcium metabolism
31	39.31	Lupeol 	$C_{30}H_{50}O$	426	0.36%	Antitumor, antioxidant, chemopreventive, antiarthritic, Anti-cancer, Anti-inflammatory
32	39.62	9,10,secocholeste-5,7,10 (19)-triene-3,24,25-triol (3β,5Z,7E) 	$C_{27}H_{44}O_3$	416	0.37%	Involved in the regulation of calcium metabolism
33	40.32	Stigmata-3,5-dien-7-one 	$C_{29}H_{46}O$	410	2.74%	Anti-inflammatory
34	41.06	Tert-Hexadecamethiol 	$C_{16}H_{34}S$	258	2.77%	Enzyme activator
35	42.06	9,10,secocholeste-5,7,10 (19)-triene-1,3-diol-25-{(trimethylsily)oxy}-(3β,5Z,7E) 	$C_{30}H_{52}O_3Si$	488	0.97%	No activity
36	42.86	9,10,secocholeste-5,7,10 (19)-triene-3,24,25-triol, (3β,5Z,7E) 	$C_{27}H_{44}O_3$	416	3.40%	Involved in the regulation of calcium metabolism
37	43.32	Lanosta-7,9 (11)-dien-18-oic acid,22,25-epoxy-3,17,20-trihydroxy-y-lactone-(3β) 	$C_{30}H_{44}O_5$	484	1.13%	Used in induction of apoptosis in human promyelocytic leukemia HL-60 cells

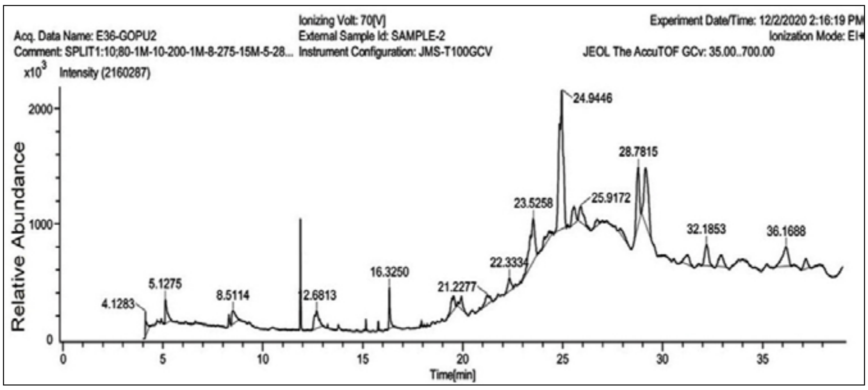
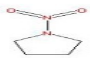
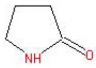
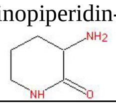
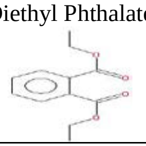
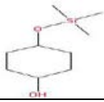
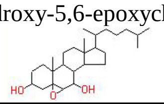
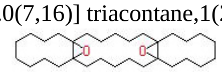
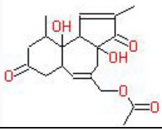
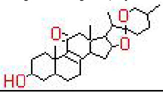
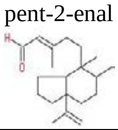
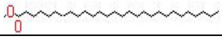


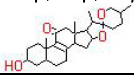
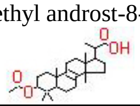
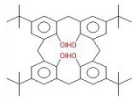
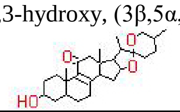
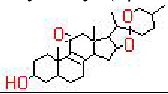
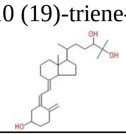
Fig 1: Chromatogram (GC-MS) of methanolic leaf extract of Momordica cymbalaria Fenzl.

3.2 Bioactive compounds analysis from of *in vitro* leaf callus

In vitro leaf callus methanol extracts of *Momordica cymbalaria* revealed the presence of twenty chemical compounds. The GCMS chromatogram of *In vitro* leaf callus methanolic extract is presented in fig 2. The identified compounds in the *In vitro* leaf callus extracts (Table 2) are Pyrrolidine,1-nitro, 2, pyrrolodinone, it is used as Strong solubilizing agent, used in oral medications (Strickley, 2004) [28]. 3- Aminopiperidin-2-one, Diethyl Phthalate it is reported to have an anti carcinogenic effect and it is used in skin treatments and Cosmetic products (Duty, S.M *et al*., 2003) [29], Cyclo hexanol,4- [(trimethylsily)oxy],cis, it is used as antioxidant (Hussein *et al*., 2016) [16], n-Hexadecanoic acid, 3,7-Dihydroxy-5,6-epoxycholestane, Tri cyclo [20.8.0.0(7,16)] triacontane,1(22),7(16)-diepoxy, Benz(e) azulene-3,8-dione, 5[(acetyloxy)methyl]-3a, a, 6a, 7, 9, 10, 10dihydroxy- 2, 10-dimethyl (3aα, 6aα, 10β, 10aβ, 10bβ).(+), Spirost-8-en-11-one,3-hydroxy, (3β,5α,14 β,20 β,22 β,25R), 5-(7a-Isopropenyl-4,5-dimethyl-octahydroinden-4-y)-3-methyl-pent-2-enal, Octacosanoic acid, methyl ester, Propanoic acid,2-(3-acetoxy-4,4,14-trimethyl androst-8-en-17-yl) it is reported to have an anti microbial, antitumor effects (Azhar Abdumeer Sosa *et al*., 2016) [30], Pentacyclo [19.3.1.1 (3,7) 1 (9,13) 1 (15,19)], Octacosa1(25), 3, 5, 7 (28), 9, 11, 13 (21), 15, 17, 19 (26), 21, 23-dodecanene-25,26, 27, 28- tetrol, 5, 11, 17, 23 – tetrakis (1,1).

Table 2: GC-MS of bioactive compounds present in the methanolic extracts of *in vitro* leaf callus derived from in vivo grown plants of *Momordica cymbalaria* Fenzl.

S. No	RT	Compound name and Structure	Formulae	Mwt	Area%	Biological activity
1	4.12	Pyrrolidine,1-nitro- 	$C_4H_8N_2O_2$	116	1.36%	No activity
2	5.11	2, pyrrolodinone 	C_4H_7NO	85	2.39%	Strong solubilizing agent, used in oral medications.
3	8.51	3- Aminopiperidin-2-one 	$C_5H_{10}N_2O$	114	0.21%	It is possessing antimycobacterial activity.
4	11.87	Diethyl Phthalate 	$C_{12}H_{14}O_4$	280	3.52%	Anti-carcinogenic, Used in skin treatments and Cosmetic products
5	12.68	Cyclo hexanol,4- [(trimethylsily)oxy],cis 	$C_9H_{20}O_2Si$	188	2.22%	Anti-oxidant
6	16.32	n-Hexadecanoic acid 	$C_{16}H_{32}O_2$	256	2.46%	Anti-inflammatory, Antioxidant, Pesticide
7	19.53	3,7-Dihydroxy-5,6-epoxycholestane 	$C_{27}H_{46}O3$	418	1.65%	No activity
8	19.93	Tri cyclo [20.8.0.0(7,16)] triacontane,1(22),7(16)-diepoxy 	$C_{30}H_{52}O_2$	444	1.90%	No activity
9	21.22	Benz(e)azulene3,8dione, 5[(acetyloxy)methyl]-3a,a,6a,7,9,10,10dihydroxy-2,10-dimethyl (3aα,6aα,10β,10aβ,10bβ).(+) 	$C_{19}H_{24}O_6$	348	1.95%	No activity
10	22.23	Spirost-8-en-11-one,3-hydroxy, (3β,5α,14 β,20 β,22 β,25R) 	$C_{27}H_{40}O_4$	428	10.3%	Anti-inflammatory, estrogenic and progesterogemic effects
11	23.52	5-(7a-Isopropenyl-4,5-dimethyl-octahydroinden-4-y)-3-methyl-pent-2-enal 	$C_{20}H_{32}O$	288	25.2%	No activity
12	24.94	Octacosanoic acid, methyl ester 	$C_{21}H_{32}O$	438	2.89%	Antibacterial
13	25.56	Spirost-8-en-11-one,3-hydroxy,	$C_{27}H_{40}O_4$	428	1.33%	Anti-inflammatory,

		(3β,5α,14 β,20 β,22 β,25R) 				estrogenic and progesterogemic effects
14	28.78	Propanoic acid,2-(3-acetoxy-4, 4,14-trimethyl androst-8-en-17-yl) 	$C_{27}H_{42}O_4$	430	7.67%	Anti-microbial, Antitumor effects
15	29.14	Pentacyclo[19.3.1.1(3,7)1(9,13) 1(15,19)]Octacosa-1(25),3,5,7 (28),9,11,13(21),15,17,19 (26),21,23-dodecanene-25, 26,27,28-tetrol,5,11,17, 23-tetrakis(1,1) 	$C_{44}H_{56}O_4$	648	11.5%	No activity
16	31.22	Spirost-8-en-11-one,3-hydroxy, (3β,5α,14 β,20 β,22 β,25R) 	$C_{27}H_{40}O_4$	428	2.08%	Anti-inflammatory, estrogenic and progesterogemic effects
17	32.18	Spirost-8-en-11-one,3-hydroxy, (3β,5α,14 β,20 β,22 β,25R) 	$C_{27}H_{40}O_4$	428	3.55%	Anti-inflammatory, estrogenic and progesterogemic effects
18	32.88	Ethyl iso-alcocholate 	$C_{26}H_{44}O_5$	436	2.51%	Antimicrobial
19	36.16	9,10-Secocholesta-5,7,10 (19)-triene-3,24,25-triol (3β,5Z,7E) 	$C_{27}H_{44}O_3$	416	5.19%	Involved in the regulation of calcium metabolism
20	37.15	1-Heptatriacotanol 	$C_{37}H_{76}O$	536	1.73%	Antimicrobial

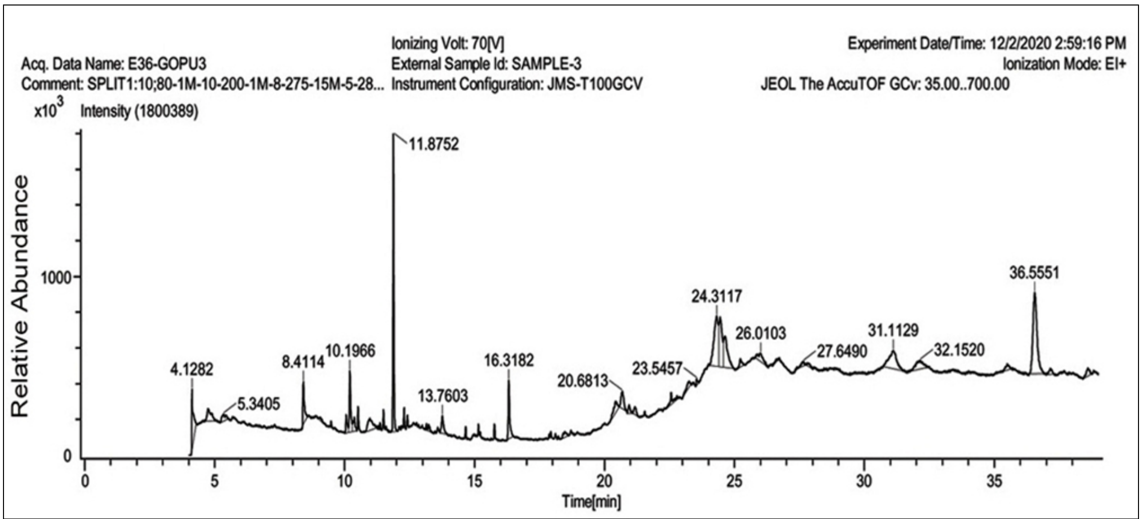


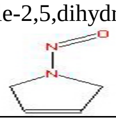
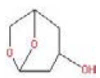
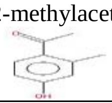
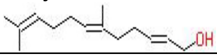
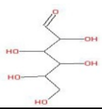
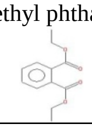
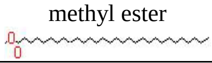
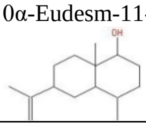
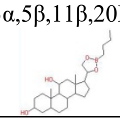
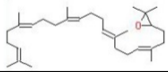
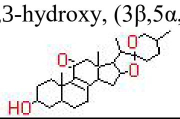
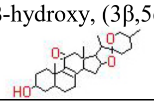
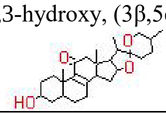
Fig 2: Chromatogram (GC-MS) of methanolic in vitro leaf callus extract of Momordica cymbalaria Fenzl.

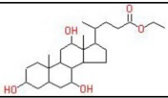
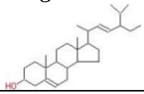
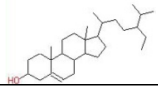
3.3 Bioactive compounds analysis from root methanolic extract

Methanol root extract of *Momordica cymbalaria* revealed the presence of thirty seven chemical constituents. The GCMS chromatogram of root methanolic extract is presented in fig 3. The identified compounds in the root extracts (Table 3) are 1H-pyrrole-2,5,dihydro-1-nitroso it is reported to have an antibacterial, antiviral, anti convulsant&analgeric (Azhar Abduameer Sosa *et al*., 2016) [30] effects. 1,6-Anhydro-2,4-dideoxy-β-D-ribo-hexopyranose, 4-Hydroxy-2-methylacetato phenone, 9-Octadecenal, it is reported to have an antimicrobial, anti-inflammatory (Subavathy *et al*., 2016) [31] effects. 6,11-Dimethyl-2,6-10-dodecatrien-1-ol, (Jisha *et al*., 2016) [5] reported that it is having antimicrobial activity. d-Mannose, it is reported to having the capacity of Urinary tract infection prevention (Bojana kranjcec *et al*., 2013, Domenici *et al*., 2016) [32,33]. Diethyl phthalate, n-Hexadecanoic acid, Triacontanoic acid, methyl ester, it is reported that using as antibacterial agent (Sermakkani M and Thangapandian V., 2012) [34]. 5β,7βH,10α-Eudesm-11-en-1α-ol, Pregnane-3,11,20,21-tetrol,cyclic 20,21-(butyl boronate), (3α,5β,11β,20R)-, it is reported to have anti-inflammatory properties (Rajendran *et al*., 2017) [26], Oxirane,2,2-dimethyl -3- (3, 7, 12, 16, 20-pentamethyl-3,7,11,15,19—

heneicosapentanyll), Spirost-8-en-11-one, 3-hydroxy, (3β, 5α, 14 β, 20 β, 22 β, 25R) it is reported to have an anti inflammatory, estrogenic and progesterogemic effects (Hussein *et al*., 2016) [16]. Ethyl iso-allocate it is used as an antimicrobial agent ((Malathi *et al*., 2106) [15]. Stigmasterol it is reported to have an antiviral, Cancer preventive (Ponnamma and Manjunath *et al*., 2012) [18], anti inflammatory, anti osteoarthritic (Gabay *et al*., 2010) [19] effects. β –sitosterol it is reported to have an anticancer, antimicrobial, antidiabetic, antifertility, antioxidant (Shirishkumar *et al*., 2014) [20] effects.

Table 3: GC-MS of bioactive compounds present in the methanolic extracts of root derived from *in vivo* grown plants of *Momordica cymbalaria* Fenzl.

S. No	RT	Compound name and Structure	Formulae	Mwt	Area%	Biological activity
1	4.12	1H-pyrrole-2,5,dihydro-1-nitroso 	$C_4H_6N_2O$	98	3.84%	Antibacterial, Antiviral, anti Convulsant & analgeric
2	4.74	1,6-Anhydro-2,4-dideoxy-β-D-ribo-hexopyranose 	$C_6H_{10}O_3$	130	2.30%	No activity
3	8.41	4-Hydroxy-2-methylacetato phenone 	$C_9H_{10}O_2$	150	2.55%	No activity
4	10.06	9-Octadecenal 	$C_{18}H_{34}O$	266	1.24%	Antimicrobial, anti-inflammatory
5	10.19	6,11-Dimethyl-2,6-10-dodecatrien-1-ol 	$C_{14}H_{24}O$	208	3.69%	Antimicrobial
6	10.96	d-Mannose 	$C_6H_{12}O_6$	180	2.65%	Urinary tract infection prevention
7	11.87	Diethyl phthalate 	$C_{12}H_{14}O4$	222	11.4%	Anti-carcinogenic, Used in skin treatments and Cosmetic products
8	16.31	n-Hexadecanoic acid 	$C_{16}H_{32}O_2$	256	3.75%	Anti-inflammatory, Antioxidant, Pesticide, Nematicide, Inhibitor
9	20.68	Triacontanoic acid, methyl ester 	$C_{31}H_{62}O_2$	466	2.19%	Antibacterial
10	22.56	5β,7βH,10α-Eudesm-11-en-1α-ol 	$C_{15}H_{26}O$	222	1.27%	No activity
11	23.54	Pregnane-3,11,20,21-tetrol, cyclic 20,21-(butyl boronate), (3α,5β,11β,20R)- 	$C_{25}H_{43}BO_4$	418	0.34%	Anti-inflammatory
12	24.31	Oxirane,2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15,19—heneicosapentanyll) 	$C_{30}H_{50}O$	426	9.85%	No activity
13	24.63	Spirost-8-en-11-one,3-hydroxy, (3β,5α,14 β,20β,22 β,25R) 	$C_{27}H_{40}O_4$	428	6.05%	Anti-inflammatory, estrogenic and progesterogenic effects
14	26.0	Spirost-8-en-11-one,3-hydroxy, (3β,5α,14 β,20β,22 β,25R) 	$C_{27}H_{40}O_4$	428	1.90%	Anti-inflammatory, estrogenic and progesterogenic effects
15	32.15	Spirost-8-en-11-one,3-hydroxy, (3β,5α,14 β,20β,22 β,25R) 	$C_{27}H_{40}O_4$	428	2.87%	Anti-inflammatory, estrogenic and progesterogenic effects
16	35.49	Ethyl iso-allocate	$C_{26}H_{44}O_5$	436	0.93%	Antimicrobial

						
17	36.55	Stigmasterol 	$C_{29}H_{48}O$	412	15.1%	Antiviral, Cancer preventive, Anti-inflammatory, Anti osteoarthritic
18	38.60	β –sitosterol 	$C_{29}H_{50}O$	414	1.19%	Anticancer, antimicrobial, antidiabetic, antifertility, antioxidant

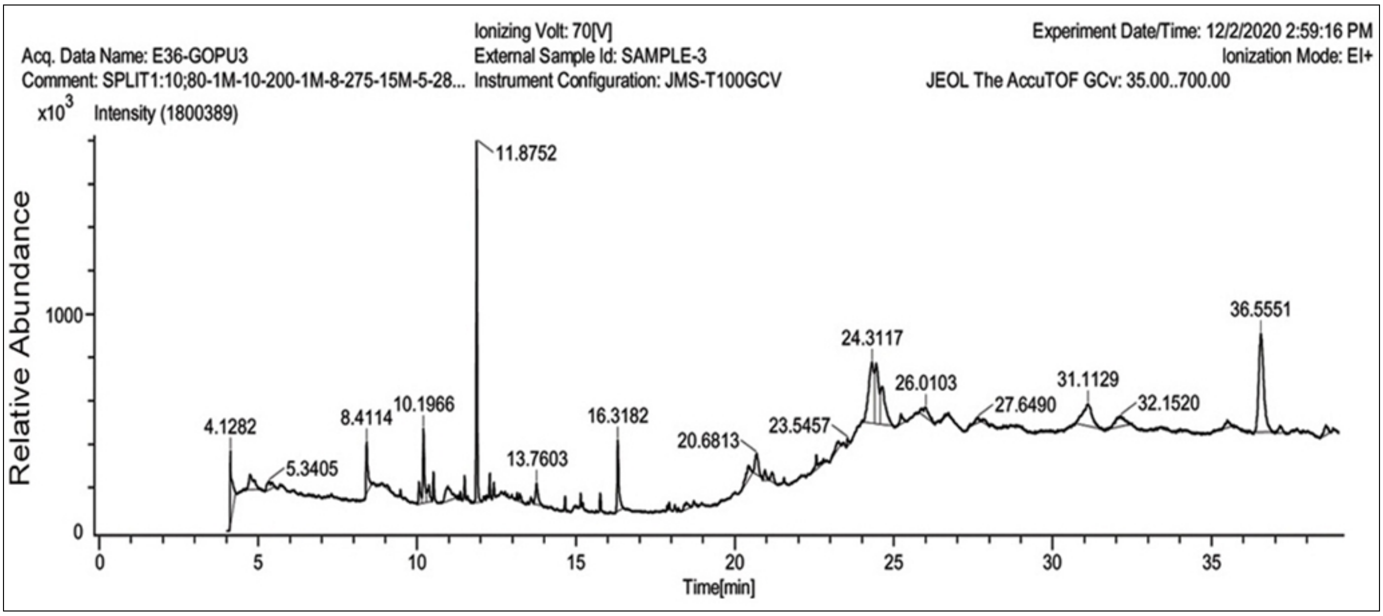


Fig 3: Chromatogram (GC-MS) of methanolic root extract of Momordica cymbalaria Fenzl.

4. Conclusion

The present investigation helps to identify seventy five bioactive chemical constituents from leaf, root and *in vitro* leaf callus methanolic extracts of *Momordica cymbalaria* Fenzl. by GCMS technique. Our study enhances the traditional usage of which possesses some known and unknown bioactive compounds. Identified bioactive compounds may subjecting to pharmaceutical area is useful to making of new drugs.

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