International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 10-08-2021, Accepted: 25-08-2021, Published: 10-09-2021
Volume 6, Issue 5, 2021, Page No. 405-409

GC-MS analysis of phytocomponents in the methanolic extract of *Ocimum sanctum* seed

K Kavitha[1], S Ponne[2]
[1] Assistant Professer, Department of Foods and Nutrition, Vellalar College for Women, Thindal, Erode, Tamil Nadu, India
[2] Associate Professor, Department of Foods and Nutrition, Vellalar College for Women, Thindal, Erode, Tamil Nadu, India

Abstract
The present study is to investigate the methanolic extract of *O. sanctum* seed for its phytochemical screening using GC-MS. The methanolic extract of *O. sanctum* seed was subjected to GC-MS analysis on the instrument- THERMO MS DSQ II–TR, 5-MS capillary standard non - polar column and the GC-MS trace ultra-version 5.0 software was employed to obtain the peaks. The GC-MS analysis revealed different peaks with low and high molecular weight determining the presence of 54 phytochemical compound**s.** The presence of these compounds may be persuasive to have therapeutic impact.

Keywords: *O. sanctum* seed, GC-MS analysis, phytochemical compounds

Introduction
Plants provide variety of resources that contribute to the functional needs of both human being and animals (Seb, 2015) [2]. Plants have been utilized as therapeutic agents since time immemorial and the healing properties of herbal medicines have been recognized in ancient cultures (Girach, 2013) [1]. In the 20th century, the use of traditional medicine to treat health problems or diseases has increased due to their safety. About 80% of the world's population depends wholly or partially on traditional medicine for its primary health care needs (Kunwar and Adhikari, 2005) [3]. GC is a powerful tool only for the volatile constituents (otherwise derivatization of the analytes is required). Mass Spectroscopy is frequently used in conjugation with Gas Liquid Chromatography (GLC) and the combined operation provides a qualitative and quantitative identification of the many structurally complex components that are present in the plant extracts (Chowdhury *et al*, 2007; Lisa *et al*, 2008; Shafaghat *et al*, 2009; Suriyavathana and Rajan, 2012) [4, 5, 6, 7]. Among the 120 active compounds currently isolated from the higher plants are widely used in modern medicine today, and 80% show a positive correlation between their modern therapeutic use and the traditional use of the plants from which they are derived (Fabricant and Fransworth, 2001) [8]. Therefore, an attempt has been taken to determine the Phytochemical compounds present in the methanol extract of *O. sanctum* seed by Gas chromatography and Mass spectroscopy (GC-MS) technique.

Methodology
Collection and Identification of Plant materials
The *O. sanctum* seed were procured from the local market and cleaned for stones and impurities and was used for the current study.
The plant of *O. sanctum* was botanically identified and authenticated by the Botanical survey of India (BSI), Ministry of Environment and Forests, Government of India, Southern Region, Coimbatore.

Preparation of seed extracts
The *O. sanctum* seeds were extracted with methanol using Soxhlet extractor. The extract which is obtained is concentrated with rotary evaporator till dry powder was obtained. The final concentrated extract is analysed by using GC-MS.

Gas Chromatography–Mass Spectrometry (GC-MS) analysis of *O. sanctum* seed
The GC-MS has become an integral part in the analysis of chemical constituents of plant extracts.

GC-MS conditions
The methanolic extract of *O. sanctum* seed was subjected to GC-MS analysis on the instrument-THERMO MS DSQ II–TR, 5-MS capillary standard non - polar column and the GC-MS trace ultra-version 5.0 software employing the following conditions: RT x 5 MS column (30 x 0.25 mm ID x 1 µM df, composed of 100% Dimethyl poly diloxane). Initially oven temperature was maintained at 70ºC for 2 minutes, and the temperature was gradually increased upto 250ºC at 10 and 1 µL of sample was injected for analysis. Helium gas 99.995 % of purity was used as a carrier gas as well as an eluent. The flow rate of helium gas was set to 1 mL/min. The sample injector temperature was maintained at 250ºC and the split ratio is 10 throughout the experiment periods. The ionization mass spectroscopic analysis was done with 70 eV. The mass spectrum was recorded for the mass range 40-1000 m/z for about 40 minutes.
Identification of components was based on comparison of their mass spectra. As the compounds are separated, elusion through the column, were detected in electronic signals. As individual compounds are eluted from the gas chromatographic column, they entered the electron ionization detector where they were bombarded with a stream of electrons causing them to break apart into fragments. The fragments were actually charged ions with a certain mass. The m/z ratio obtained was calibrated from the graph obtained which was called as the mass spectrum graph which is the fingerprint of the molecule. The identification of compounds was based on the comparisons of their mass spectra with NIST Library 2008 WILEY8, FAME. Total GC running time is 40 min (Massada, 1996) [9]. Mass spectrum of individual's unknown compound was compared with the known compounds stored in the software database libraries. The name, molecular weight and structure of the components of the test materials were ascertained.

Results and Discussion

GC–MS chromatogram of the methanol extract of *O. sanctum* seed (Fig. 1) clearly showed fifty four peaks indicating the presence of fifty four phytochemical compounds and the Table 1 shows the compound name with its molecular formula, Retention time, Peak area and % Peak area.

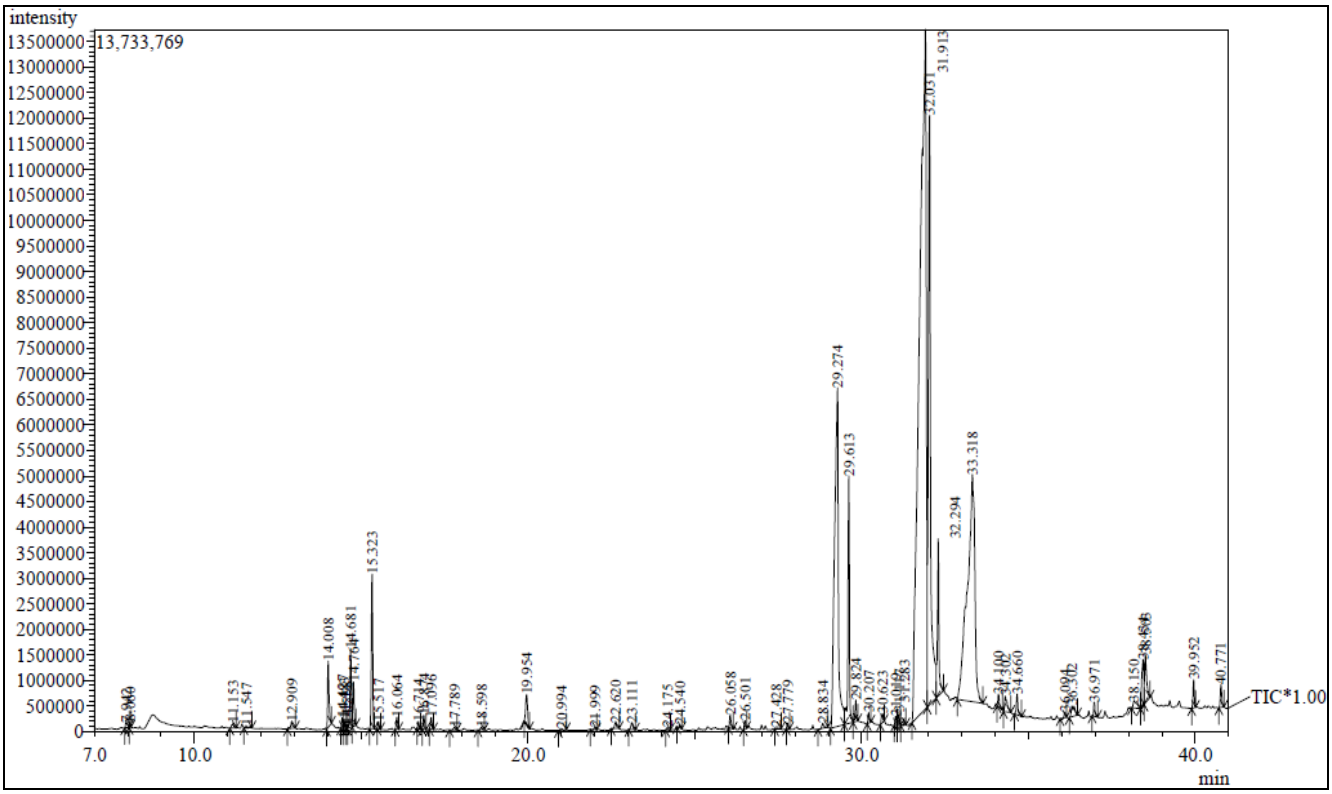

Fig 1: The GC-MS Chromatogram of methanol extracts of *O. sanctum* seed

The methanolic extract of *O. sanctum* seed showed fifty four peaks from the chromatogram of the extract. These peaks indicated the presence of fifty four compounds in the extract. This analysis revealed the presence of phytoconstituents belonging to the type - acids, esters, alcohols, ethers, phenols, hydrocarbon, terpene etc.

Table 1: Phytocomponents identified from the methanolic extract of *O. Sanctum* seed by GC-MS analysis

Peak	R. Time	I. Time	F. Time	Height	Height %	A/H	Components
1	7.942	7.908	8.042	102857	0.17	4.24	1-(Trimethylsilyl) oxy] propan-2-ol
2	8.060	8.042	8.100	109661	0.18	1.64	Tetraethyl silicate
3	11.153	11.058	11.250	85530	0.14	4.10	Borneol
4	11547	11.483	11.725	40401	0.07	4.60	Heptadecane
5	12.909	12.783	13.025	125635	0.21	4.49	Benzene, 1-methoxy-4-(1-propenyl)-
6	14.008	13.958	14.108	1296887	2.16	2.98	Eugenol
7	14.427	14.383	14.458	205768	0.34	2.59	Tricyclo[4.4.0.0(2,7)]dec-3-ene
8	14.483	14.458	14.525	171669	0.29	2.45	1-Tetradecanol
9	14.558	14.583	14.725	1532415	2.55	2.61	Cyclohexane, 1-Ethenyl-1-Methyl-2,4-bis
10	14.681	14.583	14.725	1532415	2.55	2.61	Cyclohexane, 1-ethenyl-1-me
11	14.764	14.725	14.842	865394	1.44	2.86	Benzene, 1,2-dimethoxy-4-(2-pr
12	15.323	15.250	15.392	3020465	5.03	2.82	Caryophyllene
13	15.517	15.475	15.575	139056	0.23	2.64	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-
14	16.064	16.017	16.125	236407	0.39	2.80	1,4,8-Cycloundecatriene, 2,6,6
15	16.714	16.675	16.775	361891	0.23	2.62	1,6-Cyclodecadiene, 1-methyl
16	16.874	16.825	16.942	828957	0.45	3.04	Naphthalene, Decahydro-4A-
17	17.096	17.042	17.167	738089	0.38	3.22	Alpha.-selinene
18	17.789	17.642	17.875	209709	0.09	3.70	Naphthalene, 1,2,3,5,6,8a-hexah
19	18.598	18.500	18.692	138646	0.09	2.51	Cyclohexanemethanol, 4-eth
20	19.954	19.883	20.042	2399726	0.96	4.14	(-)-5-oxatricyclo[8.2.0.0(4,6)]do
21	20.994	20.908	21.150	118737	0.05	3.66	2,4-dimethyl-3-cyclohexene-1
22	21.999	21.867	22.058	90877	0.07	2.27	10,10-dimethyl-2,6-dimethylenebicyclo
23	22.620	22.492	22.733	469142	0.19	4.14	Selina-6-en-4-ol
24	23.111	23.017	23.217	542923	0.22	4.05	Androstan-17-one, 3-ethyl-3-hydroxy-,
25	24.175	24.125	24.267	127577	0.08	2.71	6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-2-naphthalenol
26	24.540	24.458	24.608	32851	0.10	0.56	1-heptatriacotanol

27	26.058	26.008	26.150	858821	0.44	3.22	1-nonadecene
28	26.501	26.458	26.550	440249	0.26	2.77	3.alpha.,7.beta.-dihydroxy-5.beta.,6.bet
29	27.428	27.392	27.583	217351	0.07	5.34	2-aminoethanethiol hydrogen sulfate
30	27.779	27.742	27.833	287895	0.19	2.58	2,4a,8,8-tetramethyldecahydrocyclopro
31	28.834	28.692	28.983	360892	0.15	3.86	9-tetradecenal, (z)-
32	29.274	29.067	29.483	52551136	11.02	7.93	l-(+)-ascorbic acid 2,6-dihexadecanoate
33	29.613	29.483	29.742	15379470	8.05	3.18	Hexadecanoic acid, ethyl est
34	29.824	29.742	29.883	1203196	0.69	2.90	4-(3,5-di-tert-butyl-4-hydroxyphenyl)bu
35	30.207	30.167	30.275	569135	0.35	2.74	l-(+)-ascorbic acid 2,6-dihexadecanoate
36	30.623	30.558	30.675	674901	0.29	3.81	Hexadecanoic acid, ethyl ester
37	31.019	30.975	31.067	468457	0.29	2.68	Ethyl (9z,12z)-9,12-octadecadi
38	31.117	31.067	31.167	632264	0.44	2.39	9,12,15-octadecatrienoic acid, methyl est
39	31.283	31.167	31.325	223718	0.16	2.27	2,6,10-trimethyl,14-ethylene-1
40	31.913	31.508	31.967	187139061	22.12	14.08	9,12,15-octadecatrienoic acid, (z,z,z)-
41	32.031	31.967	32.233	56394924	19.20	4.89	Ethyl (9z,12z)-9,12-octadecadienoate
42	32.294	32.233	32.450	9784815	5.11	3.19	Octadecanoic acid, ethyl ester
43	33.318	32.867	33.633	69198226	7.39	15.58	Cholest-5-en-3-ol (3.beta.)-
44	34.100	34.058	34.217	641697	0.42	2.56	Ethyl (9z,12z)-9,12-octadecadienoate
45	34.302	34.250	34.475	1585955	0.51	5.17	Eicosanoic acid
46	34.660	34.583	34.792	1312878	0.68	3.21	Ethyl 14-Methyl-Hexadecanoate
47	36.094	35.950	36.142	264932	0.19	2.36	Octadecane
48	36.302	36.225	36.467	1447686	0.35	6.91	Hexadecanoic acid, 1-(hydrox
49	36.971	36.908	37.075	761427	0.46	2.77	6,11-dihydroxy-3,8-dimethoxy-1-meth
50	38.150	38.092	38.358	1006636	0.20	8.43	Ergost-5-en-3-ol, (3.beta.,24r)-
51	38.434	38.358	38.467	3755142	1.51	4.15	14-.beta.-h-pregna
52	38.503	38.467	38.642	4057304	1.57	4.30	12,15-octadecatrienoic acid, ethyl ester
53	39.952	39.892	40.008	1494788	0.92	2.72	2,6,10,14,18,22-tetracosahexaene, 2,6,1
54	40.771	40.708	40.858	1362327	0.73	3.10	Tetrapentacontane

Fifty four compounds were detected from the methanolic seed extract of *O. sanctum.* The results reveal the presence of 9, 12, 15-Octadecatrienoic acid, (Z, Z, Z)-(42.26%), Cholest-5-en-3-ol (3.beta.)- (15.63%), Ethyl (9z,12z)-9,12-octadecadienoate (12.73%), l-(+)-Ascorbic acid 2,6-dihexadecanoate (11.87%), Hexadecanoic acid, Ethyl Est (3.47%), Octadecanoic acid, Ethyl Ester (2.21%), Caryophyllene(1.92%), 12,15-Octadecatrienoic acid, ethyl ester (0.92%), Cyclohexane, 1-Ethenyl-1-Me (0.90%), Eugenol (0.87%), 14-.Beta.-h-pregna (0.85%), Benzene, 1,2-Dimethoxy-4-(2-PR(0.56%), (-)-5-Oxatricyclo [8.2.0.0(4,6)] DO (0.54%), Eicosanoic acid (0.36%), 2,6,10,14,18,22-Tetracosahexaene, 2,6,1 (0.34%), Hexadecanoic acid, 1-(Hydrox (0.33%), Tetrapentacontane (0.31%), Ethyl 14-methyl-hexadecanoate (0.30%), 4-(3,5-Di-tert-butyl-4-hydroxyphenyl)bu (0.27) and Ergost-5-En-3-Ol (3.Beta.,24R)- (0.23%), Benzene, 1-methoxy-4-(1-propenyl)- (0.21%), Tetraethyl silicate (0.18%), Borneol (0.14%), Heptadecane (0.07%), Naphthalene, 1,2,3,5,6,8a-hexah (0.09%), Cyclohexanemethanol, 4-eth (0.09%), 2,4-dimethyl-3-cyclohexene-1 (0.05%), 10,10-dimethyl-2,6-dimethylenebicyclo (0.07%), Selina-6-en-4-ol (0.19%), 6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-2-naphthalenol (0.08%), 1-heptatriacotanol (0.10%), 2-aminoethanethiol hydrogen sulphate (0.07%), 2,4a,8,8-tetramethyldecahydrocyclopro (0.19%), 9-tetradecenal, (z)-(0.15%), 2,6,10-trimethyl,14-ethylene-1 (0.16%), Ergost-5-en-3-ol, (3.beta.,24r)- (0.20%).

The spectrum profile of GC – MS confirmed the presence of 21 major components among 54 compounds with retention time (RT) 31.913, 33.318, 32.031, 29.274, 29.613, 32.294, 15.323, 38.503, 14.008, 14,681, 14.008, 38.434, 14.764, 19.954, 39.952, 34.302, 36.302, 40.771, 34.660, 29.824 and 38.150 respectively which is shown in Figure 1.

Table 2 describes the Mass spectrum and biological activities of phytocomponents identified by GC-MS in methanolic extract of *O. sanctum* seed.

Table 2: Mass spectrum and biological activities of phytocomponents identified by GC-MS in methanolic extract of *O. Sanctum* seed

S. No.	Name of the Compound	Activity*
1.	1-(Trimethylsilyl) oxy] propan-2-ol	Antibacterial activity
2.	Tetraethyl silicate	Antihistaminic activity
3.	Borneol	Chronotropic activity
4.	Heptadecane	Immunotoxicity, Carcinogens
5.	Benzene, 1-methoxy-4-(1-propenyl)-	Antimicrobial, antifungal activity
6.	Eugenol	Antimicrobial, antifungal Antioxidants, anti-inflammatory, antibacterial activity
7.	Tricyclo[4.4.0.0(2,7)]Dec-3-Ene	Antitumor, Antibacterial activity
8.	1-Tetradecanol	Reduction of myristic acid, Antimicrobial activity
9.	cyclohexane, 1-ethenyl-1-methyl-2,4-bis	Antifungal, Antibacterial, Antihyperlipedimic activity
10.	Cyclohexane, 1-Ethenyl-1-Me	Antifungal, Antibacterial, Antihyperlipedimic activity
11.	Benzene, 1,2-Dimethoxy-4-(2-Pr	Anticancer, Antioxidants Antimicrobial
12.	Caryophyllene	Antiasthmatic, Anticariogenic, Antiinflammatory, Antitumor, Antiulcer
13.	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-	Antioxidants, Antimicrobial activity
14.	1,4,8-Cycloundecatriene, 2,6,6	Antimicrobial activity**,** Anti-inflammatory
15.	1,6-Cyclodecadiene, 1-Methyl	Effective in treatment of psoriasis

16.	Naphthalene, Decahydro-4a-	Antifungal, Antimicrobial activity
17.	Alpha.-selinene	Antimalarial, Antiplasmodial, Antimicrobial and Cytotoxic Activity
18.	Naphthalene, 1,2,3,5,6,8a-Hexah	lung disorders, Antifungal, Antimicrobial activity
19.	Cyclohexanemethanol, 4-Eth	Immunomodulatory activity
20.	(-)-5-Oxatricyclo[8.2.0.0(4,6)]Do	Antimicrobial activity, optical activity
21.	2,4-Dimethyl-3-Cyclohexene-1	Antimicrobial, antioxidant activity
22.	10,10-Dimethyl-2,6-dimethylenebicyclo	Antimicrobial, antioxidant, antibacterial activity
23.	Selina-6-en-4-ol	Anticarcinogenic, antiphologistic antimicrobial, anti-inflammatory, cytotoxic activity
24.	Androstan-17-one, 3-ethyl-3-hydroxy-,	Antibacterial, free radical scavenging, insecticidal activity
25.	6-Isopropenyl-4,8a-Dimethyl-1,2,3,5,6,7,8,8a-Octahydro-2-Naphthalenol	Antimicrobial activity
26.	1-Heptatriacotanol	Antimicrobial activity, preservative
27.	1-Nonadecene	Antibacterial, anti-tubercular and cytotoxic, antiviral, antidiabetic activity
28.	3.alpha.,7.beta.-Dihydroxy-5.beta.,6.bet	Inhibited cholesterol gallstone formation effectively
29.	2-Aminoethanethiol Hydrogen Sulfate	Antioxidant and cytotoxic activity
30.	2,4a,8,8-Tetramethyldecahydrocyclopro	No activity
31.	9-Tetradecenal, (Z)-	Pesticides
32.	l-(+)-Ascorbic acid 2,6-dihexadecanoate	Antidiabetic, Antihypertensive, Antiinflammatory, Antimutagenic, Antitumor, Antiulcer
33.	Hexadecanoic Acid, Ethyl Est	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Antiandrogenic flavor, Hemolytic, Alphareductase inhibitor
34.	4-(3,5-Di-tert-butyl-4-hydroxyphenyl)bu	Ant oxidative, antioxidant activity
35.	l-(+)-Ascorbic acid 2,6-dihexadecanoate	Antidiabetic, Antihypertensive, Antiinflammatory, Antimutagenic, Antitumor, Antiulcer
36.	Hexadecanoic acid, ethyl ester	Anti-inflammatory activity, antifungal, antioxidant activity
37.	Ethyl (9z,12z)-9,12-Octadecadi	Antiarthritis**,** antihypertensive, antioxidant *activity*
38.	9,12,15-Octadecatrienoic acid, methyl ester	Hepatoprotective, nematicide, insectifuge antihistaminic, antiarthritic, anticoronary, antieczemic antiacne, 5-Alpha reductase inhibitor, Antiandrogenic
39.	2,6,10-Trimethyl,14-Ethylene-1	Anticancer, preventing oxidative cell damage
40.	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	Analgesic, antipyretic, anticonvulsant, antiseptic
41.	Ethyl (9z,12z)-9,12-Octadecadienoate	Antiarthritis**,** antihypertensive, antioxidant activity
42.	Octadecanoic Acid, Ethyl Ester	Antimicrobial activity, antifungal activity
43.	Cholest-5-En-3-Ol (3.Beta.)-	Antimicrobial, anticancer, antiarthritic, antiasthma, diuretic, anti-inflammatory
44.	Ethyl (9z,12z)-9,12-Octadecadienoate	Antiarthritis**,** antihypertensive, antioxidant activity
45.	Eicosanoic Acid	Anticancer, antioxidant activity
46.	Ethyl 14-methyl-hexadecanoate	Antibacterial, antidiabetic activity
47.	Octadecane	Antibacterial, antifungal activity
48.	Hexadecanoic Acid, 1-(Hydroxy-1-(Hydroxymethyl) Ethyl Ester	Hemolytic, pesticide, flavor, antioxidant
49.	6,11-Dihydroxy-3,8-dimethoxy-1-meth	Antifungal, antibacterial and antihyperalgesic activity
50.	Ergost-5-en-3-ol, (3.beta.,24r)-	anti-inflammatory effects
51.	14-.Beta.-h-pregna	-
52.	12,15-Octadecatrienoic acid, ethyl ester	Hepatoprotective, antihistaminic, hypocholesterolemic, antieczemic and cancer preventive
53.	2,6,10,14,18,22-Tetracosahexaene, 2,6,1	Antioxidant, anticancer, pesticide, sunscreen, perfumery, chemo preventive, Antimicrobial, Antiinflammatory activities.
54.	Tetrapentacontane	Antifungal, antioxidant and antibacterial activity

***Source:** Dr. Duke's Phytochemical and Ethnobotanical Databases

In the present study, the GC-MS analysis of the methanolic seed extract of *O. sanctum* showed the presence of twenty one major compounds. In terms of percentage amounts 9, 12, 15-Octadecatrienoic acid, (z, z, z) - (42.26 %), Cholest-5-En-3-Ol (3.Beta.)- (15.63 %), Ethyl (9z,12z)- 9,12-Octadecadienoate (12.73 %), l-(+)-Ascorbic acid 2,6-dihexadecanoate (11.87 %), Hexadecanoic Acid, Ethyl Est(3.47%), Octadecanoic acid, Eicosanoic acid, Ethyl Ester(2.21 %) and 6,11-Dihydroxy-3,8-dimethoxy-1-meth were predominant in the extract and have the property of antioxidant, analgesic, anti-pyretic, anti-convulsant, anti-septic, anti-arthritis, anti-hypertensive, anti-microbial, anti-fungal, anti-bacterial, anti-diabetic, anti-inflammatory, anti-mutagenic, anti-tumor, anti-ulcer, anti-antrogenic flavour, pesticide, haemolytic, alpha-reductase inhibitor and anti-cancer activity. Among the identified phytochemicals, l-(+)-Ascorbic acid 2,6-dihexadecanoate and 1-Nonadecene has anti-diabetic, anti-oxidant activity and also act as an anti-bacterial, anti-tubercular, cytotoxic, antiviral and more recently possess antihypertensive, anti-inflammatory, anti-mutagenic, anti-tumor and anti-ulcer property. It is also noted that the methanol extract was identified as a compound phytosterol Ergost-5-En-3-Ol, (3.beta. 24r) - which produce anti-inflammatory activity. From this, it was clear that the above compounds had anti-diabetic and antioxidant activity which is beneficial for reducing blood glucose level.

Conclusion

In the present study, fifty four phytochemical constituents have been identified from the methanol extract of seed of *O. sanctum* by Gas Chromatogram - Mass Spectrometry (GC - MS) analysis. The results of the GC-MS profile can be used as pharmacognostical tool and various phytochemicals

identified provoke the antidiabetic and antioxidant activity which potentiate hypoglycemic effect of *O. sanctum* seed.

Reference

1. Girach RD, Khan H, Ahmad M. Botanical identification of Thuhar, seldom used as Unani medicine. Hamdard Medicus,2013:96(1):27-33.
2. Seb THM. Medicinal plants- The chemical goldmines, a review, International Journal of Scientific Research Today,2015:1(1):34-41.
3. Kunwar RM, Adhikari N. Ethnomedicine of dolpa district Nepal: The plant their vernacular names and uses. Lyonia, 2005, 43-9.
4. Chowdhury JU, Nandhi NC, Nazrul Islam Bhuiyan. Chemical composition of leaf essential oil of *lantana camara L.* from Bangladesh. Bang. Bot,2007:36:193-194.
5. Lisa B, Francois H, Westhuizen V, Loots DT. Phytochemical contents and antioxidant capacities of two Aloe greatheadii var. davyana extracts. Molecules,2008:13:2169-2180.
6. Shafaghat A, Sadeghi H, Oji K. Composition and anti-bacterial activity of essential oils from leaf, stem and root of *Chrysanthemum Parthenium* (L) Bernh. from Iran. Nat.Prod.Comm,2009:4:859-860.
7. Suriyavanthana M, Rajan T.Chemical investigation of *Pseudarthria viscida* root by GC-MS analysis. Pharmacognosy Communications,2012:2(3):26-29.
8. Fabricant DS, Farnsworth NR. The value of plants used in traditional medicine for drug discovery. Environ. Health Perspect,2001:109(1):69-75.
9. Massada Y. Analysis of Essential Oils by Gas Chromatography and Mass Spectrometry John Wiley and Sons, New York, NY, USA, 1996.