

Phytochemical and GC-MS Analysis of Hydro Ethanolic Leaf Extract of *Ocimum sanctum* (L.)

Atul Srivastava¹, Subhashini², Anand Kumar Keshari³, Ragini Srivastava^{1,*}

ABSTRACT

Background: The pharmacological efficiency of herbal drugs has shown to be effective as conventional pharmaceutical drugs. Preliminary screening of herbal extracts helps in analysing the bioactive compounds present. *Ocimum sanctum*, the queen of herbs keeps a spiritual importance in Indian culture and have important place in traditional medicinal system of India.

Objectives: The present study was implement to investigate the preliminary phytochemical screening and GC-MS analysis of leaf extract of *O. sanctum* to determine the phyto-constituents. **Materials and Methods:** Leaves collected were dried, crushed and mixed with ethanol and water and further applied for extract preparation. The prepare extract was used for phytochemical and GC-MS analysis. **Results:** In qualitative phytochemical analysis tannins, carbohydrate, quinine, anthraquinones, coumarins, phlobatanins, alkaloids, flavonoids, glycosides, terpenoids, diterpenes, phenols were found present while GC-MS analysis showed Eugenol, Cyclohexane, bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene, Oxatricyclo[8.2.0.0(4,6)]dodecane, 12-trimethyl-9-methylene, Tetracontane, Phytol were present in majority. **Conclusion:** The study concluded that *O. sanctum* leaf extracts contain many biological active compounds which could be exploited for a development of plant based drug.

Key words: Phenolics, Flavonoids, Herbal drugs, Phytochemical, GC-MS analysis.

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INTRODUCTION

Plants are potent and powerful biochemist with number of phytochemicals incorporated that prevents and treat several disorders.^[1] Traditional medicinal practitioners have been using medicinal plants and their parts as stem, leaves, bark, roots, seeds etc since long back to cure various ailments. Phytochemicals present in the plants having therapeutic benefits are considered to be “active ingredients” or “active component” of herbal medicines and provide the primary source for drug development.^[2] India, with rich flora has been called as the botanical garden of the world and is the largest producer of medicinal herbs. Medicinal herbs with active ingredients have been found to be powerful source as therapeutics ingredient. Hence, medicinal plants are being of great interest to the researchers in the field of biotechnology, as most of the pharmaceutical compounds are produced from the medicinal plants.

Ocimum sanctum commonly called as “holy basil”, “the Queen of herbs” and “Tulsi” is one of the holiest and most exquisite medicinal herbs of India.^[3] In India Tulsi, is renowned for its religious and spiritual sanctity, as well as for its important role as a natural and traditional herb for holistic health and herbal medicine.^[4] It is also regarded as “elixir of life” in Ayurvedic science due to its potentiality to promote longevity. *Ocimum* has a unique characteristic pharmacological feature

and have been known to have ethno-medicinal property.^[5] In the present pandemic COVID-19 scenario, *Ocimum* has been explored as a component in brew to act as an immunobooster. In the present study the phytochemical screening and GC-MS analysis of *O. sanctum* has been performed to explore its phyto constituents.

MATERIALS AND METHODS

Plant material and its collection

Young and fresh leaves of *Ocimum sanctum* (L.) for the experiment were collected from Botanical Garden of Banaras Hindu University, Varanasi. The specimen was identified and verified, by Department of Botany, Banaras Hindu University and a voucher number (Lamia.2019/1) was provided.

Preparation of crude extract

Extract was prepared by the established protocol of Keshari *et al.* 2017 with certain modification.^[6] Fresh and healthy young leaves collected from the Botanical Garden were washed under running water and further with deionised water and then dried in shade and then at 37°C in incubator. Dried leaves were further crushed with the help of mixer grinder. Alcoholic extract was prepared by Soxhlet apparatus

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using 5gms of crushed powered leaves in the Erlenmeyer flask containing 100ml of 70% ethanol. The mixture was applied to the apparatus for 24hr at a temperature not exceeding the boiling point of the solvents. This mixture was then heated for 2hr on magnetic stirrer at 70°C. The obtained extract was centrifuged at 5000 rpm for 15 min and the supernatant was further filtered using Whatmann filter paper No. 1. The prepared concentrated extracts were concentrated to dryness at 40°C under reduced pressure using a rotatory evaporator, and then dried extracts were stored at -18°C in air-tight screw-capped glass vials for GC-MS analysis.

Preliminary qualitative phytochemical analysis of extract

The preliminary screening was performed for the phytochemical analysis of the crude extracts to identify the various phytoconstituents using standard procedures as described by Keshari A *et al.* and Babu N. R. *et al.*^[6,7] Detection of tannins, phlobatanins, saponins, alkaloids, flavonoids, quinine, anthraquinones coumarins, sterols, glycosides, terpenoids, diterpenes, triterpenes, phenols, starch, carbohydrates, proteins was performed.

Gas Chromatography-Mass Spectrometry analysis of test extract

The ethanolic extract of *O. sanctum* was subjected for GC-MS analysis on a GC-MS Shimadzu GC-MSQP2010 Plus system equipped with RTX-5 m.s. capillary column (0.25 mm X 30 m X 0.25 µm). Helium gas (99.999%) was used as carrier gas with a constant flow rate of 16.3ml/min. Column flow rate was maintained 1.2ml/min. Column temperature was started at 50°C, held for 2 min, ramped to 250°C for 6 min and finally ramped at 280°C and held for 22 min. The extract was prepared as discussed above. Before applying for GC-MS analysis the extract was filtered with syringe filter. The sample was injected in a volume of 20µl.

Identification of phytocompound of GC-MS

Interpretation of mass spectrum GC-MS was performed using the database and the spectrum of the unknown components was compared with the spectrum of known components stored in the library. The name, molecular weight, and structure of the components of the test materials were ascertained. Compounds in the extract were identified using WILEY8.LIB and NIST14s.lib MS data library. The average peak area to the total areas was calculated for comparing relative percentage amount of each component.

Total phenolic and flavonoid content

Total phenolic and flavonoid content was determined by Folin-Ciocalteu and aluminium chloride method where gallic acid and quercetin was used as standard respectively. Total phenolic content of crude extract was determined according to the established protocol of Jan *et al.*^[8] Briefly reaction mixture was prepared by mixing 1ml of different concentration of test extract (100-1000µg/ml) with 5ml of Folin-Ciocalteu reagent (1:10 dilution). The mixture was kept for 5 min at room temperature and then 4ml of sodium carbonate (115µg/ml) was added. After 2 hr the absorbance was measured at 765nm by spectrophotometer (Orion Aquamate 8000 UV-VIS Thermo scientific). Calibration curve was prepared by mixing 1ml Gallic acid at different concentration (25-400µg/ml). Total phenolic content in *O. sanctum* was expressed as Gallic acid equivalent (GAE) mg/gms of the dry extract.

Total flavonoid content was measured with the aluminum chloride colorimetric assay. 1ml of different concentration of extract (100-1000µg/ml) or 1ml of standard quercetin solution (100-1000µg/ml) was taken into test tubes with 4ml of distilled water. Reaction mixture was prepared by mixing 0.3 ml of 5 % sodium nitrite solution was added into each,

0.3 ml of 10 % aluminum chloride (after 5 min) and 2 ml of 1 M sodium hydroxide. Finally, volume was made up to 10 ml with distilled water and mixed well. After 15 min absorbance was measured at 510nm by spectrophotometer (Orion Aquamate 8000 UV-VIS Thermo scientific spectrophotometer). The calibration curve was plotted using standard quercetin. The data of total flavonoids of *O. sanctum* were expressed as mg of quercetin equivalents (QE)/ 100 g of dry extract.

RESULTS AND DISCUSSION

In the present era medicinal plants are considered as rich sources of ingredients for new modern drugs. Many of the modern medicines are produced and synthesized from medicinal plants.^[9] The analysis and extraction of different plant material and their compounds play an important role in the development, modernization, use and quality control of herbal formulations.^[9] Pushpangadan and Bradu (1995) have reported more than 150 species of *Ocimum*.^[10] The phytoconstituents in medicinal herbs varies with species and according to the environmental condition they are grown. Till now the essential oil of *O. sanctum* has been only screened and herbal extract of leaves has not been explored for its phytochemical analysis.^[11] Hence the present study was undertaken to find out the bioactive compounds present in the ethanolic extract of *O. sanctum* by using Gas chromatography and Mass spectroscopy.

Various test to identify compounds facilitates their qualitative estimation study. The preliminary phytochemical screening of the leaf extract was mainly performed to recognize and identify the main bioactive phyto-compounds.^[12] The result showed the presence of biomolecule compounds such as tannin, carbohydrate, phlobatanins, flavonoid, terpenoids, triterpenes, glycosides, alkaloid, quinine, anthraquinine whereas compounds as protein, saponin, starch, sterols and diterpenes were absent as summarized in Table 1. Plant extract having flavonoid, terpenoids, coumarins are considered as a significant source of potential therapeutic compound for many ailment. Further the total flavonoid and phenolic compounds were quantified in the extract (Figure 1). The crude extract showed increased concentration of flavonoid and phenols with the

Table 1: Showing the phytoconstituents screened. + indicates the presence and - indicates absence of that phyto-constituent.

S. No	Compounds	Present or Absent
1	Tannins	+
2	Phlobatanins	+
3	Saponin	-
4	Alkaloids	+
5	Flavonoids	+
6	Quinine	+
7	Anthraquinones	+
8	Coumarins	+
9	Sterols	-
10	Glycosides	+
11	Terpenoids	+
12	Diterpenes	-
13	Triterpenes	+
14	Phenols	+
15	Starch	-
16	Carbohydrates	+
17	Proteins	-

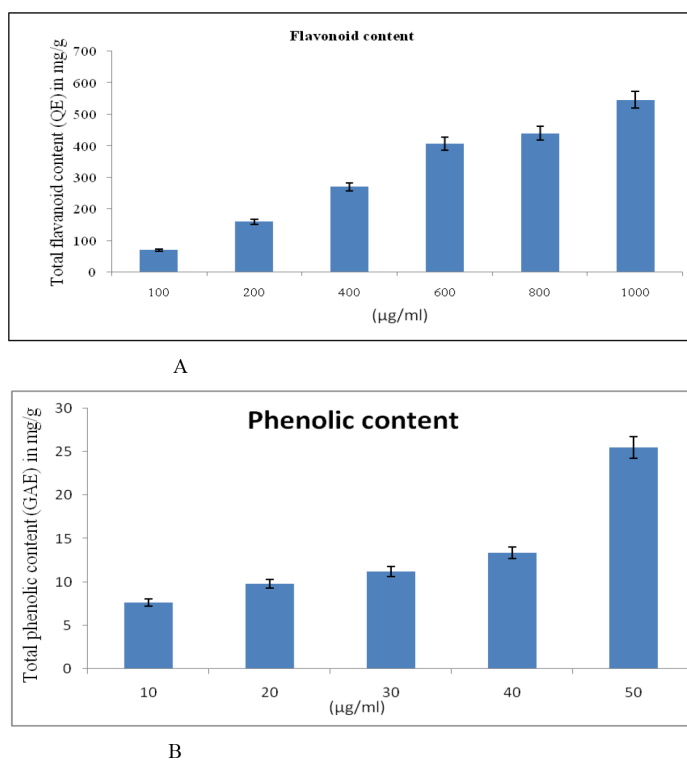


Figure 1: Showing the presence of total flavonoids (A) and total phenolic content (B) in extract of *Ocimum sanctum*.

increase concentration of *O. sanctum*. Both flavonoid and phenols due to its radical scavenging activity are considered to constitute the potent antioxidative property of herbal extract.^[13] Phenolic compound are supposed to exhibit significant free radical scavenging activity because of the presence of hydrogen or electron donating agent and metal ion chelating property.^[14] Similarly, flavanoids inhibit free radical mediated event by its chemical structure as these transfers electrons, chelate metal catalyst, activates antioxidant enzymes and inhibit oxidases.^[14] The result pertaining to GC-MS analysis of ethanolic extract of *O. sanctum* showed on a total of 40 identified compounds from the chromatogram as summarized in Table 2. The active principle (name), concentration (% peak area) and retention time (RT) in the ethanol extract was identified for different compounds. The GC-MS spectrum confirmed the presence of various components with different retention times as illustrated in Figure 2. The identified compounds comprise mainly hydrocarbons, fatty acids, alcohols, esters and phenols. The composition of the extract comprises major component as Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene- (24.22%), Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl) (21.59%), Oxatricyclo [8.2.0.0(4,6)] dodecane,12-trimethyl-9-methylene (7.27%) and Eugenol (7.01%). Among the identified phytochemicals, n-hexadecanoic acid, Octadecenoic acid and squalene (triterpene) have reported to exhibits the antioxidant, anti-inflammatory and antibacterial property.^[15,16] Eugenol, the main bioactive compound of *O. sanctum* has been found to exhibit antimicrobial, anti-inflammatory and anti-oxidative,^[17,18] while coapene (a tricyclic sesquiterpenes) has reported to act as anti-microbial and anti-oxidant property.^[19,20]

Table 2: GC-MS spectral analysis of ethanolic extract of *Ocimum sanctum*. (Please See Supplementary file).

Peak#	R. Time	Area	Area%	Name
1	7.843	141740	0.34	BICYCLO[2.2.1]HEPTAN-2-OL, 1,7,7-TRIMETHYL-, (1S-ENDO)-
2	11.085	2952103	7.06	Eugenol
3	11.343	231735	.55	Copaene
4	11.433	485878	1.16	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]-
5	11.559	9027246	21.59	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]-
6	12.082	10130388	24.22	BICYCLO[7.2.0]UNDEC-4-ENE, 4,11,11-TRIMETHYL-8-METHYLENE-, [1R-(1R*,4E,9S
7	12.657	601275	1.44	1,4,8-CYCLOUNDECATRIENE, 2,6,6,9-TETRAMETHYL-, (E,E,E)-
8	13.059	464226	1.11	1,6-CYCLODECADIENE, 1-METHYL-5-METHYLENE-8-(1-METHYLETHENYL)-, [S-(E,E
9	13.194	363823	.87	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha
10	13.302	470262	1.12	ALPHA.-SELINENE
11	13.476	155084	0.37	CYCLOHEXANE, 1-ETHENYL-1-METHYL-2,4-BIS(1-METHYLETHENYL)-, [1S-(1.ALPHA.,2.BETA.,4.BETA.)]- \$ \$ 2,4-DIISO, CYCLOPROP[E]AZULENE, Cycloheptane, 1,3,6,10-Cyclotetradecatetraene,
12	14.627	3039100	7.27	(-)-5-OXATRICYCLO[8.2.0.0(4,6)]DODECANE,,12-TRIMETHYL-9-METHYLENE-, [1R
13	15.040	140599	0.34	(1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene
14	15.731	273776	0.65	Isoaromadendrene epoxide
15	16.446	244474	0.58	2-((2R,4aR,8aS)-4a-Methyl-8-methylenedecahydronaphthalen-2-yl)prop-2-en-1-ol
16	16.698	737972	1.76	1,1,4,7-Tetramethyldecahydro-1H-cyclopropa[e]azulene-4,7-diol
17	18.010	224757	0.54	Neophytadiene
18	19.717	1299736	3.11	Ethyl 9-hexadecenoate
19	19.975	797570	1.91	HEXADECANOIC ACID, ETHYL ESTER
20	20.317	149544	0.36	9-Hexadecenoic acid, (Z)-, TMS derivative
21	20.556	473909	1.13	Palmitic Acid, TMS derivative

Continued...

Table 2: Cont'd

Peak#	R. Time	Area	Area%	Name
22	21.990	474438	1.13	Ethyl Oleate
23	22.059	229917	0.55	(E)-9-Octadecenoic acid ethyl ester
24	22.500	935411	2.24	Phytol, acetate
25	23.205	245778	0.59	Glycidyl oleate
26	23.419	229976	0.55	Glycidyl palmitate
27	26.013	422106	1.01	1,2-BENZENEDICARBOXYLIC ACID
28	26.914	381217	0.91	Eicosyl isopropyl ether, Hexadecyl isopropyl ether, Isopropyl tetradecyl ether
29	28.594	169550	0.41	2-methyloctacosane
30	30.587	619379	1.48	Squalene
31	31.684	211876	0.51	Hexatriacontane
32	32.902	259017	0.62	Heptadecane, 3-methyl-
33	34.040	617018	1.48	STIGMAST-5-EN-3-OL, OLEAT
34	34.314	422605	1.01	Hexatriacontane
35	38.041	1804127	4.31	Tetracontane
36	38.379	637256	1.52	Stigmast-5-ene, 3.beta.-(trimethylsiloxy)-, (24S)- \$\$ Silane, trimethyl[[[(3.beta.,24S)-stigmast-5-en-3-yl]oxy]-\$\$ Silane, trimethyl(stigma
37	39.831	358191	0.86	Dotriacontane, 1-iodo-
38	40.520	338846	0.81	Tetrapentacontane
39	43.616	774787	1.85	Hexatriacontane
40	45.303	283938	0.68	Phytyl palmitate

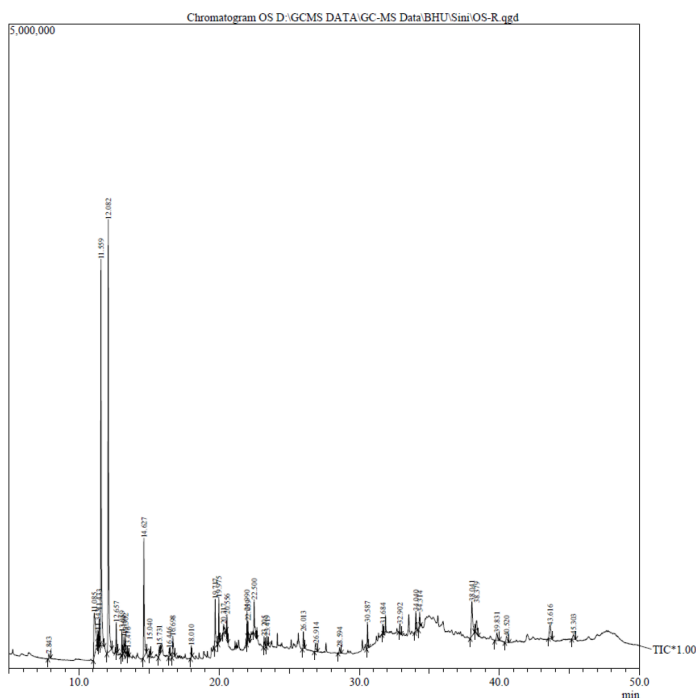


Figure 2: Showing the Chromatogram of Hydroethanolic leaf extract of *Ocimum sanctum*. (Please see supplementary file).

The presence of various bioactive compounds in the *O. sanctum* justifies the use of leaves extract for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents and subjecting it to the biological activity will definitely give prolific results. The presence of various bioactive compounds makes *O. sanctum* applicable in various pharmaceutical and industrial applications and therefore it may be recommended as a plant of phytopharmaceutical importance.

CONCLUSION

The occurrence of various bio-active compounds detected in the GC-MS analysis using the ethanolic extracts of *O. sanctum* justifies the use of leaves of Tulsi plant for various elements by traditional practitioner. However, isolation of individual phytochemical constituents and analyzing its biological activity would be beneficial and would open a new area of investigation of individual compounds and their pharmacological potency. From these results, it could be concluded that "*Ocimum sanctum*" contains various bio-active compound which could provide therapeutic in several disorders. Hence further research is needed to explore its biological activity which is ongoing.

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CONFLICT OF INTEREST

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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ABBREVIATIONS

GCMS: Gas chromatography and mass spectrometry; **UV-VIS:** Ultraviolet visible; **RT:** Retention Time; **GAE:** Gallic acid equivalent; **QE:** Quercetin equivalents; **nm:** nanometre; **NIST14s.lib MS:** National institute of standards and technology Mass spectroscopy library; **WILEY8.LIB:** Wiley online library.

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GRAPHICAL ABSTRACT



SUMMARY

The results obtained from phytochemical screening studies identified the presence of tannins, carbohydrate, quinine, anthraquinones, coumarins, phlobatanins, alkaloids, flavonoids, glycosides, terpenoids, diterpenes and phenols. In GC-MS analysis; 40 different compounds with 7 major components Eugenol, Cyclohexane, bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene, Oxatricyclo[8.2.0.0(4,6)]dodecane, 12-trimethyl-9-methylene Tetra contane, Phytol were found. These compounds possess important biological activity and strongly support the pharmacological potency of *Ocimum*.

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