

COMPARATIVE STUDY FOR THE VOLATILE CONSTITUENTS OF *OCIMUM SANCTUM* AND *OCIMUM BACILICUM*

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ABSTRACT:

Ocimum sanctum and *Ocimum basilicum* belonging to lamiaceae family. The present study was designed to investigate comparative volatile content analysis in *Ocimum sanctum* and *Ocimum basilicum* using Gas chromatography -mass spectrometry (GC-MS) technique. Nine compounds were identified from the extracted volatile oil; which were identified as; hexadecanoic acid (8.37%), hepta - 9,10,11- trienoic acid (17.04%), octadecenoic acid (8.37%), 5-(hydroxymethyl) heptadecane (13.75%), eicosane aldehyde (37.36%) and octadecyl vinyl ether (15.12%), Benzene, 1, 2-dimethoxy-4-(2-propenyl)-, Isocaryophyllene and Eugenol among the major chemical constituents.

KEYWORDS:

Ocimum sanctum, *Ocimum basilicum*, Leaves, Lamiaceae, Methanolic, GC-MS and Phytochemicals.

INTRODUCTION

OCIMUM SANCTUM

The *Ocimum* genus is ranked highly among some of the amazing herbs for its tremendous medicinal potential. Previous studies indicate that there are a large number of species and cultivars that fall within this genus. Several authors recognize more than 60-150 species in the genus. Characterizations of each species in this genus (family *Lamiaceae*) are based on the leaves and habitat. The shape of the leaves on *Ocimum sanctum* and its close relatives varies in the size of the leaves, veins and petioles. The colors of the leaves vary from light green to dark green and sometimes almost black. Although colors vary in plants, the reasons for this, particularly in the case of basil, are not yet studied. The regular occurrence of interspecific hybridization within the genus has created taxonomic challenges, leaving very few publications on basil taxonomy that follow the International Code of Botanical Nomenclature. [1]

Ocimum sanctum (OS) or Tulsi extracts are used in Ayurvedic remedies for colds, headaches, stomach disorders, inflammation, heart disease, various poisonings and malaria. [2,3] Traditionally, *Ocimum sanctum L.* is taken in many forms, as herbal tea, dried powder or fresh leaf. Several recent studies of these extracts have suggested anti-inflammatory, antioxidant, immunomodulating, and anti-stress properties. In addition, it has been reported to have radioprotective and anticarcinogenic properties. [4,5] Several medicinal properties are attributed to *Ocimum sanctum L.* *Ocimum sanctum L.* is known as a general vitalizer and increases physical endurance. Various parts of *Ocimum sanctum L.* such as leaves, flowers, stems, roots, seeds, etc. are known to have therapeutic potential and have been considered by traditional health practitioners as expectorant, analgesic, anticancer, anti-asthmatic, antiemetic, diaphoretic, antidiabetic, antifertile, hepatoprotective, hypotensive, hypolipidemic, anti-stress medication. *Ocimum sanctum L.* has also been used to treat fever, bronchitis, arthritis, convulsions etc. [6]

Ocimum sanctum L. has been well documented for its therapeutic potentials and described as antiasthmatic and antikaphic drugs. Indian Materia Medica describe the use of aqueous, hydro-alcoholic and methanolic extract of *Ocimum sanctum* leaves in variety of disorders, like bronchitis, rheumatism and pyrexia. [6]

Figure 1: Plnt of *Ocimum sanctum* L.[7]

OCIMUM BACILICUM

O. basilicum has been widely reported in areas of agriculture, food, and pharmacology. Therefore, this review would shed more light on the various dimensions of *O. basilicum* for researchers. At the same time, the disappearance of *O. basilicum* in certain regions is increasing day by day, so it is important to raise awareness of the medicinal importance of this plant in order to prevent its extinction.[8] All of these things prompted us to write this report, which mainly focuses on the pharmaceutical perspective of *Ocimum basilicum*. [9]

Figure 3: Plant of *Ocimum basilicum*[10]

MATERIALS AND METHODS

Collection of the plant material

The leaves of *Ocimum sanctum* and *Ocimum basilicum* were collected from Rahuri Vidyapeeth, Ahmednagar, Maharashtra, India.

Isolation of essential oil:

Oils have been isolated from fresh leaves. The leaves were cut into small pieces and soaked in n-hexane for one hour and then gravity filtered. The extracts were then subjected to further filtration using a short column silica gel to remove impurities. The extracted volatiles are extracted with the Clevenger apparatus. [11]

The Clevenger apparatus, based on distillation, is the best method to determine the essential oil content of the fruit. [12–14] In general, an analysis procedure for orange fruit or peel essential oil involves two steps: extraction (hydrodistillation or steam distillation) and analysis (gas chromatography (GC), gas chromatography coupled to mass spectrometry (GCMS)). Although the second step takes only 1530 min, the extraction takes several hours. Extraction is often done by prolonged heating and stirring in boiling water. [15,16]

Gas Chromatography-Mass Spectroscopy Analysis (GC/MS)

Gas chromatography-mass spectroscopy analysis is a method that combines the features of gas, liquid chromatography and mass spectroscopy to identify different substances in a test sample. The gas chromatography-mass spectroscopy instrument consists of two parts: the gas chromatography (GC) part separates the chemical mixture into pulses of pure chemicals, and the mass spectrometer (MS) identifies and quantifies the chemicals. After the sample has passed the GC, the chemical pulses are passed on to the MS. [17,18]

Identification of Compounds

Interpretation of mass spectrum GC-MS was performed using the National Institute of Standard and Technology (NIST) database. [19] The mass spectrum of the unknown component was compared to the spectrum of the known components stored in the NIST library. [20]

RESULTS AND DISCUSSION

GC-MS analysis

Ocimum sanctum

The GC-MS chromatogram of the *Ocimum sanctum* methanolic extract showed three main peaks (Figure No. 3) and was identified after comparing the mass spectra to the NIST library, indicating the presence of three phytochemicals. From the results it was observed that the presence of benzene, 1,2-dimethoxy-4-(2-propenyl)- (synonym: methylisoeugenol), isocaryophyllene (synonym: caryophyllene) and eugenol (synonym: 2-methoxy-4-(2-Propenyl)phenol) were the main components in the extract. The phytochemicals that contribute to the medicinal properties of the plant leaves are listed in Table No. 1. Methyl Isoeugenol has the property of antifungal activity, nematocidal activity and anti-eating activity. Caryophyllene is known for its anti-inflammatory, cytotoxic, and antifungal activity. Eugenol is reported to possess antifungal, antiviral, disinfectant, antiparasitic, antioxidant, anticancer and antiinsect activities.

In addition, *Ocimum sanctum* also shows anticancerous, antifungal, antimicrobial, antifertility, hepatoprotective, antispasmodic, cardio protective, antiemetic, antidiabetic, analgesic, adaptogenic, and diaphoretic properties. The present communication deals with the GC-MS analysis of phytochemicals in the methanolic extract of the leaves of *Ocimum sanctum*.

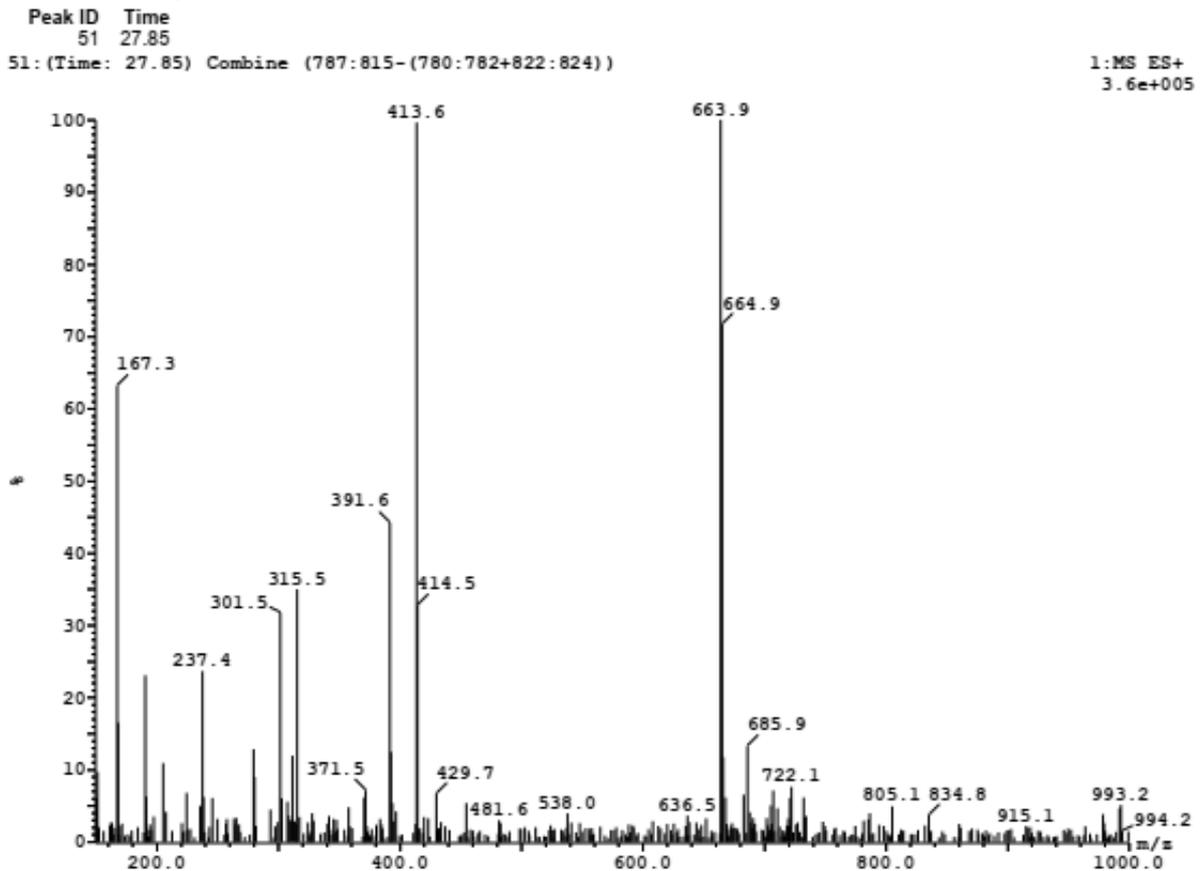


Figure No. 3: GC- MS chromatogram of the *Ocimum sanctum*

Table No.1: Chemical constituents and its Activity of some of the phytochemicals identified in the methanolic extracts of the Leaves of *Ocimum sanctum* by GC-MS

S. No	Retention Time	Name of the Compounds	Molecular Formula	Molecular Weight	Activity*
1	21.84	Benzene, 1, 2-dimethoxy-4-(1-propenyl) -	C ₁₁ H ₁₄ O ₂	178.2	Antibacterial, Nematicide Insect-attractant Perfumery, Flavour
2	22.16	Caryophyllene	C ₁₅ H ₂₄	204.3	Anti-tumor, Analgesic, Antibacterial, Anti-inflammatory, Fungicide
3	20.77	Eugenol	C ₁₀ H ₁₂ O ₂	164.2	Acaricide, Antibacterial, Anti-inflammatory, Antioxidant, Cancer Preventive, Antispasmodic, Antiviral, Insecticide

Ocimum basilicum

The result of GC-MS investigation showed that six peaks were identified from the chromatogram (Figure No. 4) of the methanolic leaf extract of *O. basilicum*. The peaks (1-6) indicate the presence of six phytochemicals (1-6) in the plant extract (Figure No. 4). The active principle, area of peak concentration (%), retention time (RT) molecular weight (MW) and molecular formula (MF) in the methanolic extract as identified through the NIST database is listed in Table 2. These compounds comprise mainly hydrocarbons, fatty acids, alcohols, esters and phenols. The composition of the extract comprises high amounts of eicosane aldehyde (37.36%), hepta-9, 10, 11- trienoic acid (17.04%), octadecyl vinyl ether (15.12%), 5- hydroxymethylheptadecane (13.75%) with low amounts of hexadecenoic acid (8.37%) and octadecenoic acid (8.37%). The results of the present study are in agreement with the findings.

The nature of phytochemicals and their biological activities of acknowledged compounds of the leaves of *O. basilicum* were presented in Table No. 2. The biological activities of the phytochemicals of *O. basilicum* mentioned in table 2 are based on phytochemical and ethnobotanical database by Jim Duke of the Agricultural Research service/USDA.

The phytochemicals reported in this extract may account for a variety of pharmacological actions. In fact, there is no study that can provide an apparent inspiration and be accurate on the mode of action of the plant's essential oils. Given the complexity of their chemical composition, it is very likely that each of the constituents of the essential oils as obtained from *O. basilicum* leaf has its own mechanism of action. However, owing to the unpredictability of amounts and profiles of the components of essential oils, it is likely that their different medicinal activities is not due to a single mechanism, but to several sites of action at the cellular level and involving different modes of action. Thus, present study using GC/MS analysis is the first step towards understanding the nature of active principles in this medicinal plant and this type of study will be helpful for further detailed study in line with the biochemical and phytochemical functions mentioned above.

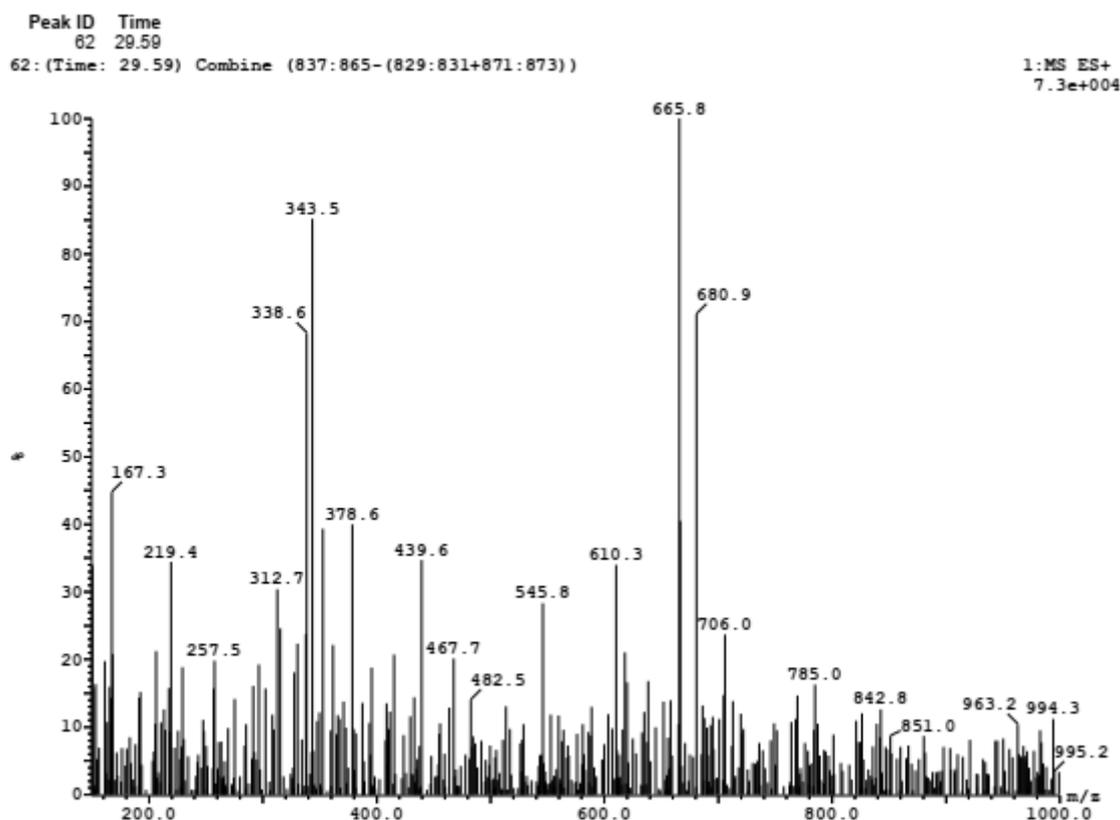


Figure No. 4: GC-MS chromatogram of *O. basilicum* leaves.

Table 2: Phytoconstituents identified in *Ocimum basilicum* leaves by GC-MS assay

Peaks	Compounds	Molecular formulae	Molecular weight	Retention time	Mass peak	Base peak	Percentage content
1	Hexadecanoic acid (Palmitic acid)	[C ₁₆ H ₃₂ O ₂] ⁺	256.48	16.46	67	43	8.37
2	Hepta-9, 10, 11- trienoic acid	[C ₁₇ H ₂₈ O ₂] ⁺	264.40	18.18	94	41	17.04
3	Octadecanoic acid (Stearic acid)	[C ₁₈ H ₃₆ O ₂] ⁺	284.48	18.31	75	41	8.37

4	5-(Hydroxymethyl)heptadecane	C ₁₈ H ₃₇ O ⁺	269.49	19.26	75	43	13.75
5	Eicosane aldehyde	[C ₂₁ H ₄₁ O] ⁺	309.59	20.77	162	55	37.36
6	Octadecyl vinyl ether	[C ₂₀ H ₄₀ O] ⁺	297.53	20.92	91	43	15.12

Table 3: Preliminary phytochemical screening of the plants [(+: present) (- : absent)]

Plant	Reducingsugar	Flavonoids	Alkaloids	Tanins	Terpenoids
<i>Ocimum Sanctum</i>	+ve	+ve	+ve	+ve	+ve
<i>Ocimum Basilicum</i>	+ve	-ve	+ve	-ve	+ve

CONCLUSION

The result of the GC-MS analysis showed that the *Ocimum sanctum* and *Ocimum basilicum* contained high amounts of eicosane aldehyde, hepta-9, 10, 11- trienoic acid octadecyl vinyl ether, 5-hydroxylmethyl heptadecane with low amounts of hexadecanoic and octadecenoic acid. However, the pharmacological effects of this plant may depend on the identified phytochemicals. Thus, there are need for further studies to isolate, spot and purify the specific phytochemical involved in preventing and treating ailments which may ultimately pave a way towards drug development.

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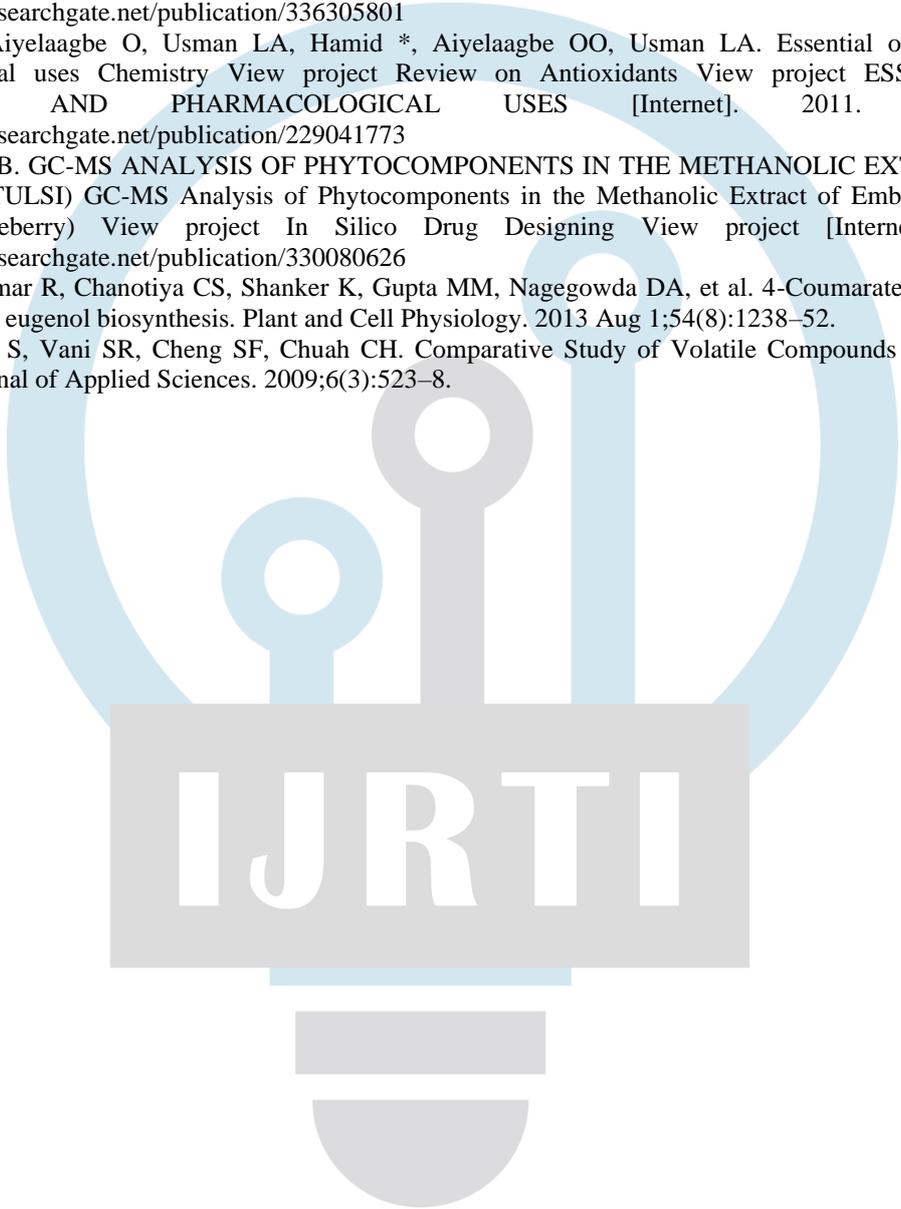
Conflict of interest

Authors declare that there is no conflict-of-interest to reveal.

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