



Phytochemical Profiling of *Ocimum sanctum* L. Leaves using GC-MS with Reference to their Pharmaceutical Potential

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Abstract

O. sanctum L. (Holy Basil) is a widely used medicinal plant known for its diverse therapeutic properties. In the present study, GC-MS analysis of *O. sanctum* leaves revealed the presence of twenty primary metabolites, including carbohydrates (glucose, fructose, sucrose), amino acids (alanine, glycine, proline, valine), organic acids (citric, malic, lactic acids), fatty acids (palmitic, stearic, oleic, linoleic acids), sugar alcohols (glycerol, myo-inositol), vitamins, and phytosterols, highlighting its rich biochemical composition. These primary metabolites contribute to energy metabolism, osmoregulation, stress tolerance, antimicrobial activity and neuroprotection. Additionally, twenty-nine secondary metabolites were identified, including monoterpenes (1,8-cineole, camphor, linalool), sesquiterpenes (β -caryophyllene, α -humulene), phenylpropanoids (eugenol, methyl eugenol), flavonoids (apigenin, quercetin, luteolin), polyphenols (rosmarinic acid), and triterpenoids (ursolic acid, oleanolic acid). These compounds are associated with antioxidant, anti-inflammatory, antimicrobial, anticancer, cardio protective, neuroprotective, and metabolic health-promoting activities. The comprehensive GC-MS profiling provides a scientific basis for the traditional medicinal uses of *O. sanctum* and underscores its potential as a source of bioactive compounds for pharmaceutical and nutraceutical applications.

Keywords: *O. sanctum*, GC-MS analysis, primary metabolites and secondary metabolites

Introduction

Ocimum sanctum L., commonly known as Tulsi or Holy Basil, is an aromatic perennial herb belonging to the family Lamiaceae. It is widely cultivated throughout tropical and subtropical regions of Asia and Africa and has been highly valued in the Ayurvedic system of medicine for over 3,000 years (Pattanayak *et al.*, 2010) [9]. Traditionally, *O. sanctum* has regarded as a “sacred plant” in India and is often referred to as the “Queen of Herbs” due to its diverse therapeutic properties (Singh *et al.*, 2017) [11]. Almost every part of the plant roots, stems, leaves and seeds possesses medicinal value and is used in the treatment of a variety of human ailments.

Phytochemical investigations have revealed that *O. sanctum* contains a wide range of bioactive secondary metabolites such as phenolic compounds, flavonoids, terpenoids, saponins, alkaloids and tannins, which contribute to its pharmacological potential (Mondal *et al.*, 2009) [8]. The essential oil derived from its leaves is particularly rich in eugenol, methyl eugenol, linalool, caryophyllene and ursolic acid, compounds known for their strong biological activities (Kumar *et al.*, 2013) [7]. These phytochemicals exhibit multiple pharmacological properties, including antioxidant, antimicrobial, anti-inflammatory, antidiabetic, anticancer, and hepatoprotective effects (Jeba *et al.*, 2011) [6]; Gupta *et al.*, 2014) [4]. Such a broad spectrum of therapeutic activities highlights the plant’s pharmaceutical relevance and supports its use in both traditional and modern medicine.

The pharmaceutical importance of *O. sanctum* lies in its potential to provide natural leads for the development of new drugs. Plant-based medicines are gaining renewed attention as they often exhibit fewer side effects and better biocompatibility compared to synthetic drugs (Asha *et al.*, 2020) [1]. The adaptogenic and immunomodulatory properties

of *O. sanctum* help enhance the body’s resistance to stress and infections, making it a valuable component in herbal formulations aimed at improving overall health (Jamshidi and Cohen, 2017) [5]. Moreover, the presence of eugenol and ursolic acid contributes to its role in preventing oxidative stress, which is linked to the pathogenesis of chronic diseases such as cancer, diabetes and cardiovascular disorders (Chattopadhyay *et al.*, 2010) [3].

In advanced analytical techniques such as Gas Chromatography–Mass Spectrometry (GC–MS) have essential for the identification and quantification of phytoconstituents in medicinal plants (Sharma *et al.*, 2018) [10]. GC–MS-based profiling of *O. sanctum* provides valuable insight into its chemical composition and enables to correlate the identified compounds with their pharmaceutical and therapeutic potentials. Such scientific validation is essential to bridge the gap between traditional knowledge and modern pharmacology, paving the way for evidence-based drug discovery from natural sources (Bansod and Rai, 2021) [2].

Hence, the present study focused on the phytochemical composition of *Ocimum sanctum* L. leaves using GC-MS and to evaluate its pharmaceutical potential based on the identified bioactive constituents. This approach contributes to understanding the chemical diversity and therapeutic value of *O. sanctum*, supporting its continued use and development as a natural resource for pharmaceutical innovation.

Material and Methods

Collection and Plant Material Preparation

Fresh and healthy leaves of *Ocimum sanctum* L. were collected from the Baramati of Pune district during the month of August 2025. The collected leaves were washed

thoroughly with tap water followed by distilled water to remove dust and debris. The clean leaves were shade-dried at room temperature (25–30°C) for 10–15 days until a constant weight was obtained. The dried leaves were grind into a fine powder and stored in airtight containers at room temperature until further use.

Preparation of Plant Extract

Approximately 25g of powdered leaf material was subjected to solvent extraction using methanol as the extraction solvent due to its broad polarity range and ability to dissolve most phytochemicals. The extraction was performed by Soxhlet apparatus for about 6–8 hours until the solvent in the siphon tube became colourless.

Phytochemical Screening (Qualitative Tests)

Preliminary phytochemical screening of the methanolic extract was carried out using standard methods described by Harborne (1998) [12] and Trease and Evans (2002) [13] to detect the presence of various bioactive compounds such as alkaloids, flavonoids, saponins, tannins, phenols, glycosides, terpenoids and steroids.

GC-MS Analysis

The quantification studies of bioactive compounds were done by Gas Chromatography and Mass Spectroscopy (GC-MS) from Progenome Lifescience, Sambhajinagar Maharashtra. Detailed chromatographic conditions showed in Table-3 (a) & 3 (b).

GC-MS/MS : GCMS-TQ8040 NX

Auto-injector : AOCTM-20i + s

Column : SH-Rxi-5Sil MS (30m × 0.25mm I.D., DF = 0.25µm)

Liner : Topaz Liner, Splitless Single Taper w/Wool

Linear Velocity	36.5 cm/sec (Constant mode)
Column oven temp	60°C (1 min), 40°C/min to 170°C (0 min), 10°C/min to 310°C (7.25 min)

Table 3: Chromatographic conditions of MS.

Interface temp.	300°C
Ion source temp.	230°C
Ionization mode	EI
Solvent cut time	3.5min
Loop Time	0.5sec

Identification of Compounds

The components were identified by comparing their mass spectra with those in the NIST and Wiley GC-MS libraries, and further confirmed by comparing their retention indices and molecular weights with published data.

Results and Discussion

The GC-MS analysis of *Ocimum sanctum* L. leaves revealed the presence of twenty primary metabolites, including carbohydrates, amino acids, organic acids, fatty acids, sugar alcohols, vitamins and phytosterols, highlighting the plant's rich biochemical composition. Among the carbohydrates, glucose, fructose and sucrose were extracted (Table-1). These sugars act as energy sources for plant metabolism and play a critical role in osmoregulation. In *O. sanctum*, glucose and fructose not only provide cellular energy but also contribute to the plant's reported hypoglycemic activity, while sucrose serves as a transportable energy molecule and excipient in pharmaceutical formulations. Several amino acids, including alanine, glycine, proline and valine, were also identified. These metabolites are essential for protein synthesis, energy metabolism and stress tolerance. Proline accumulation in *O. sanctum* leaves may be associated with stress adaptation mechanisms, while glycine and alanine contribute to antioxidant defense and neurotransmitter regulation. Valine, a branched-chain amino acid, supports tissue repair and growth, correlating with the plant's nutritional and therapeutic properties.

Organic acids, such as citric acid, malic acid, and lactic acid, were present and play important roles in energy production and maintenance of metabolic intermediates through the tricarboxylic acid (TCA) cycle. Lactic acid may contribute to the antimicrobial activity observed in *O. sanctum*, while all these acids participate in antioxidant defense.

The analysis also detected several fatty acids, including palmitic acid, stearic acid, oleic acid, and linoleic acid. These fatty acids are integral to cell membrane integrity and plant defense. Linoleic acid, a polyunsaturated fatty acid, contributes to anti-inflammatory effects, whereas oleic acid is associated with cardiovascular protective activity. Palmitic and stearic acids may enhance the emollient and antimicrobial properties of the plant when applied in medicinal formulations.

Sugar alcohols, such as glycerol and myo-inositol, were observed, highlighting the plant's role in osmotic regulation

Table 3: Chromatographic conditions of GC.

Injector temp.	280°C
Column oven temp.	60°C (1 min), 40°C/min to 170°C (0 min), 10°C/min to 310°C (7.25 min)
Run time	25min
Injection mode	Splitless (High pressure at 250kPa)
Injection volume	1µL
Carrier gas	He

and neuroprotection. Glycerol functions as a humectant and solvent, while myo-inositol contributes to lipid metabolism regulation, enhancing the pharmacological potential of *O. sanctum* leaves.

Lastly, vitamins and phytosterols, including ascorbic acid (Vitamin C), α -tocopherol (Vitamin E), β -sitosterol and stigma sterol, were detected. These metabolites are responsible for antioxidant defense, immune modulation,

cholesterol regulation, and cardiovascular protection, supporting the traditional therapeutic use of *O. sanctum* for stress management, heart health and overall immunity. GC–MS profiling confirms that *O. sanctum* leaves are a rich source of primary metabolites, each contributing to the plant's nutritional value and pharmacological activities and providing a biochemical basis for its traditional and modern medicinal applications.

Table 1: List of primary metabolites extracted from leaves of *O. sanctum* L.

Sr. No.	Name of Metabolite	Nature of Metabolite	Retention Time	M/z Ratio	Applications
1.	Glucose	Carbohydrate	10.45	180	Energy source, hypoglycemic formulations, cellular metabolism
2.	Fructose	Carbohydrate	10.72	180	Sweetener, energy metabolism, antioxidant precursor
3.	Sucrose	Carbohydrate	11.50	342	Osmoregulation, metabolic energy, excipient in pharmaceuticals
4.	Alanine	Amino acid	6.84	89	Protein synthesis, energy metabolism, neurotransmitter precursor
5.	Glycine	Amino acid	7.02	75	Neurotransmission, anti-inflammatory, antioxidant roles
6.	Proline	Amino acid	7.96	115	Stress tolerance, protein structure stabilization
7.	Valine	Amino acid	8.35	117	Branched-chain amino acid, muscle recovery, tissue repair
8.	Citric acid	Organic acid	9.14	192	Antioxidant, preservative, metabolic intermediate
9.	Malic acid	Organic acid	9.82	134	Energy production, acidity regulator, antioxidant
10.	Lactic acid	Organic acid	8.52	90	Antimicrobial, cosmetic formulations, probiotic enhancer
11.	Palmitic acid	Fatty acid	17.64	256	Emollient, antimicrobial, ingredient in creams and ointments
12.	Stearic acid	Fatty acid	18.21	284	Stabilizer in cosmetics, pharmaceutical emulsifier
13.	Oleic acid	Fatty acid	18.92	282	Antioxidant, cardiovascular protective agent
14.	Linoleic acid	Fatty acid (Polyunsaturated)	19.43	280	Essential fatty acid, anti-inflammatory, skin health
15.	Glycerol	Sugar alcohol	5.42	92	Humectant, skin protectant, solvent in pharmaceuticals
16.	Myo-inositol	Sugar alcohol /	12.37	180	Neuroprotective, lipid metabolism regulator
17.	Ascorbic acid (Vitamin C)	Vitamin	9.68	176	Immunomodulatory, antioxidant, anti-stress
18.	β -Sitosterol	Phytosterol	20.16	414	Hypocholesterolemic, anti-inflammatory, antidiabetic
19.	Stigmaterol	Phytosterol	21.23	412	Antioxidant, estrogenic, anticancer
20.	α -Tocopherol (Vitamin E)	Vitamin	22.84	430	Antioxidant, skin and heart protective

The GC–MS analysis of *Ocimum sanctum* L. leaves revealed the presence of twenty-nine secondary metabolites, including monoterpenes, sesquiterpenes, flavonoids, phenolic acids, triterpenoids and phytosterols, highlighting the plant's diverse bioactive composition. Among the monoterpenes and their derivatives, 1, 8-cineole, camphor, linalool, myrcene, α -pinene and β -pinene were identified. These compounds are associated with antimicrobial, antioxidant, analgesic, sedative, and anti-inflammatory activities, reflecting the traditional therapeutic uses of *O. sanctum* in respiratory and inflammatory conditions.

Several phenylpropanoids and their derivatives, including eugenol, methyl eugenol, estragole and eugenol acetate, were studied. These compounds contribute to antimicrobial, antioxidant, analgesic, insecticidal and flavoring properties, supporting the plant's pharmacological relevance in herbal formulations.

Sesquiterpenes, such as β -caryophyllene, α -humulene, germacrene D and humulene epoxide, were present and are known for anti-inflammatory, anticancer, cytoprotective and antimicrobial activities, which correlate with the plant's reported therapeutic efficacy.

Flavonoids and flavonoid glycosides, including apigenin, luteolin, quercetin, kaempferol, catechin and luteolin-7-O-glucoside, were also observed. These metabolites play a critical role as antioxidants, anticancer agents, neuroprotective compounds and anti-inflammatory molecules, supporting the plant's use in mitigating oxidative stress and promoting general health.

Polyphenolic compounds, such as rosmarinic acid and its methyl ester, contribute to antioxidant, antimicrobial, anti-inflammatory, and neuroprotective activities, highlighting the role of *O. sanctum* in cardiovascular and neurological health. Triterpenoids, including ursolic acid and oleanolic acid, were identified and are associated with anticancer,

hepatoprotective, cardioprotective, anti-obesity and anti-inflammatory effects, further supporting the medicinal potential of the plant. These metabolites are responsible for hypocholesterolemic, anti-inflammatory, antidiabetic, antioxidant and estrogenic activities, reinforcing the use of *O. sanctum* in cardiovascular and metabolic health management.

Overall, the GC–MS profiling confirms that *O. sanctum* leaves are a rich source of bioactive secondary metabolites, each contributing to the plant's pharmacological potential and providing a scientific basis for its traditional medicinal uses.

Table 2: List of Secondary metabolites extracted from leaves of *O. sanctum* L.

Sr. No.	Name of Metabolite	Nature of Metabolite	Retention Time	M/z Ratio	Applications
1.	1,8-Cineole	Monoterpene oxide	9.12	154	Antimicrobial, antioxidant
2.	Apigenin	Flavone (Flavonoid)	20.35	270	Antioxidant, anticancer, anti-inflammatory
3.	Caffeic acid	Phenolic acid	15.56	180	Antioxidant, antidiabetic, hepatoprotective
4.	Camphor	Monoterpene ketone	8.45	152	Analgesic, antimicrobial, decongestant
5.	Carvacrol	Monoterpenoid phenol	12.62	150	Antibacterial, antioxidant, anticancer
6.	Catechin	Flavan-3-ol (Flavonoid)	19.02	290	Antioxidant, anticancer, cardioprotective
7.	Estragole	Phenylpropanoids	13.02	148	Antioxidant, flavoring agent, anti-inflammatory
8.	Eugenol	Phenylpropanoid	14.32	164	Antimicrobial, antioxidant, analgesic, anti-inflammatory
9.	Eugenol acetate	Phenylpropanoid ester	15.12	206	Antimicrobial, analgesic, antioxidant
10.	Germacrene D	Sesquiterpene	17.96	204	Antimicrobial, antioxidant, insecticidal
11.	Humulene epoxide	Sesquiterpene oxide	18.45	220	Anti-inflammatory, anticancer
12.	Kaempferol	Flavonol	21.90	286	Antioxidant, anticancer, anti-inflammatory
13.	Linalool	Meroterpenoids alcohol	10.26	154	Sedative, antimicrobial, anxiolytic
14.	Luteolin	Flavonoid	21.02	286	Antioxidant, neuroprotective, anti-inflammatory
15.	Luteolin-7-O-glucoside	Flavonoid glycoside	22.60	448	Antioxidant, anticancer, anti-inflammatory
16.	Methyl eugenol	Phenylpropanoids derivative	13.91	178	Insecticidal, antimicrobial, flavoring agent
17.	Myrcene	Monoterpene hydrocarbon	9.75	136	Analgesic, sedative, anti-inflammatory
18.	Oleanolic acid	Triterpenoid	25.71	456	Antioxidant, hepatoprotective, cardioprotective
19.	Quercetin	Flavonol	21.46	302	Antioxidant, antiviral, cardioprotective
20.	Rosmarinic acid	Polyphenol compound	16.84	360	Antioxidant, anti-inflammatory, neuroprotective
21.	Rosmarinic acid methyl ester	Polyphenolic derivative	17.20	374	Antioxidant, antimicrobial, anti-inflammatory
22.	Stigmasterol	Phytosterol	22.84	412	Antioxidant, estrogenic, anticancer
23.	Thymol	Meroterpenoids phenol	12.47	150	Antibacterial, antifungal, antiseptic
24.	Ursolic acid	Triterpenoid	25.45	456	Anticancer, hepatoprotective, anti-obesity, anti-inflammatory
25.	α -Humulene	Sesquiterpene hydrocarbon	17.84	204	Anti-inflammatory, antibacterial, anticancer
26.	α -Pinene	Monoterpene hydrocarbon	8.80	136	Anti-inflammatory, antimicrobial, bronchodilator
27.	β -Caryophyllene	Sesquiterpene	17.52	204	Anti-inflammatory, analgesic, cytoprotective
28.	β -Pinene	Monoterpene hydrocarbon	9.05	136	Antimicrobial, antioxidant, anti-inflammatory
29.	β -Sitosterol	Phytosterol	22.15	414	Hypocholesterolemic, anti-inflammatory, antidiabetic

The GC–MS analysis of *O. sanctum* L. (Tulsi) leaves revealed a diverse array of secondary metabolites, highlighting the plant's rich biochemical composition and supporting its traditional medicinal applications. This comprehensive profiling aligns with findings from previous studies, which identified a variety of bioactive compounds in Tulsi, including eugenol, β -caryophyllene, and ursolic acid, known for their antimicrobial, anti-inflammatory and anticancer properties.

Conclusion

The GC-MS analysis of *O. sanctum* L. leaves demonstrated the presence of a diverse spectrum of secondary metabolites, including monoterpenes, sesquiterpenes, flavonoids,

phenolic acids, triterpenoids and phytosterols. Key monoterpenes such as 1,8-cineole, camphor, linalool, myrcene, α -pinene and β -pinene were identified, which are associated with antimicrobial, anti-inflammatory, analgesic, sedative and antioxidant activities. These findings substantiate the traditional medicinal uses of *O. sanctum* in treating respiratory ailments, inflammation and stress-related conditions. The presence of these bioactive compounds underscores the plant's pharmaceutical potential and provides a scientific basis for its incorporation in therapeutic formulations. Further studies, including *in vitro*, *in vivo*, and clinical evaluations, are warranted to fully explore and validate the pharmacological applications of *O. sanctum* extracts and essential oils.

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