

Qualitative phytochemical screening and GC-MS analysis of *Ocimum sanctum* L. leaves

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ABSTRACT

*The investigation was carried out to determine the qualitative analysis of phytochemical screening and possible chemical components *Ocimum sanctum* L. leaves GC-MS. GC-MS analysis of hydroalcoholic extract lead to identification of 10 compounds. This analysis revealed that contains *Ocimum sanctum* L. leaves mainly Eugenol, Caryophyllene.*

Keywords: *Ocimum sanctum* L., GC-MS analysis, Eugenol, Caryophyllene.

INTRODUCTION

Plant is man's friend in survival, giving him food and fuel and medicine from the days beyond drawn of civilization [1]. Plant continue to be a major source of medicine, as they have throughout human history [2]. *Ocimum sanctum* commonly known as Tulsi in Hindi and Holy Basil in English a popular herb was used for this study. The herb is found throughout the semitropical and tropical parts of India. *Ocimum sanctum* Linn. is a 30-75 cm high erect herb which is grown practically in every part of India. Leaves are 2.5 – 5 cm long and 1.6 – 3.2 cm broad, elliptical, oblong obtuse. Inflorescence is verticillate and flowers are in racemes 15-20 cm long in close whorls. Odour and taste are aromatic and sharp [3]. The use of this herb has been reported in Indian Traditional Systems of Medicine and its modern applications are receiving wide spread attention day by day. It has been observed that tulasi has antioxidant, antibiotic, antiatherogenic, immunomodulatory, anti-inflammatory, analgesic, antiulcer, chemopreventive and antipyretic properties [4].

Ocimum sanctum leaves have been shown to exert hepatoprotective effect in the models of predictable hepatotoxicity like paracetamol and carbon tetrachloride induced liver damage in rats [5]. *Ocimum sanctum* is being used on common cold, cough, fever, as stimulant and antihelminthic. It is also used as mosquito and insect repellent. Sanctum leaf extract have

stimulatory effects on physiological pathways of insulin secretion, ethanolic extract of *O. sanctum* mediated a significant reduction in tumour cell size and an increase in life span of mice having sarcoma-180 solid tumours [6]. Benzene extract of *O. sanctum* in albino rat decreases the total sperm count and sperm motility [7]. Animal research has verified that extracts of Tulsi leaves prevented changes in plasma level of the stress hormone corticosterone induced by both acute and chronic noise stress. Hence, the present investigation was carried out to determine the possible chemical components from *Ocimum sanctum* L. leaves by GC-MS.

MATERIALS AND METHODS

Collection and identification of plant material

Ocimum sanctum L. collected from Thanjavur district of Tamil Nadu. The botanical identify of the plant was confirmed by Dr. John Britto Rapinet Herbarium, St. Joseph's College, Tiruchirappalli.

Plant sample extraction

Leaves were cleaned, shade dried and pulverized to powder in mechanical grinder. Required quantity of powder was weighed and transferred to Stoppard flask, and treated with hydroalcohol (70% v/v) until the powder is fully immersed. The flask was shaken every hour for the first 6 hrs and then it was kept aside and again shaken after 24 hrs. This process was repeated for 3 days and then the extract was filtered. The extract was collected and evaporated to dryness by using vacuum distillation unit. The final residue thus obtained was then subjected to GC-MS analysis.

Phytochemical screening

Chemical test were carried out on the aqueous extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara [8], Trease and Evans [9] and Harbone [10].

GC-MS analysis

GC-MS analysis of these extracts was carried out by following the method of Hema *et al.* [11]. GC-MS analysis were performed using a Perkin-Elmer GC clauses 500 system and Gas Chromatograph interfaced to a mass spectrometer (GC-MS) equipped with a Elite-1, fused silica capillary column (30 m × 0.25 mm ID × 1 μ df, composed of 100% Dimethyl poly siloxane). For GC/MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1 ml/min and an injection volume of 2 μl was employed (Split ratio of 10:1) injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min) with an increase of 10°C/min to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 minutes. The relative % amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was a Turbo mass.

Identification of components

Interpretation of mass spectrum of GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of

the known component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials was ascertained.

RESULTS AND DISCUSSION

The qualitative analysis of the extracts from the leaf sample of *Ocimum sanctum* showed the presence of phytochemical constituents such as tannins, saponin, flavonoids, steroid, terpenoids and cardiac glycerides. At the same time, the phytochemical constituent like phlobatannin were absent (Table 1). The compounds present in the hydroalcoholic extracts of *Ocimum sanctum* were identified by GC-MS analysis (Figure 1). The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in Table 2. Ten compounds were identified in hydroalcoholic extract by GC-MS. The major components present in leaves of *Ocimum sanctum* were Eugenol (43.88%), Caryophyllene (26.53%), Cyclopentane, Cyclopropylidene-(1.02%), Cyclohexane, 1,2,4-triethenyl (15.31%), octadecane, 1,1-dimethoxy-(2.04%) and Benzene methanamine, N,N,a,4-tetramethyl-(2.04%). Phytochemical constituents which contribute to the medicinal activity of the hydroalcoholic extract of *Ocimum sanctum*. The leaves contains eugenol and caryophylline are considered mainly to be responsible for various antimicrobial properties. Eugenol is the main constituent and it is responsible for its repellent property. The presence of eugenol attributes to its anti-oxidative property and is also thought to be responsible for inhibition of lipid peroxidation [3]. This property helps in maintaining good health and in preventing the changes occurrence of heart diseases as well as most of the other biochemical diseases because oxidative stress is the hallmark of such diseases [12]. Eugenol is the major components found in the whole plant of *Ocimum sanctum* which is being used for the pharmacological work.

Saponins are generally regarded a antinutrients but are also believed to be useful in human diet for controlling cholesterol. Its presence in this plant therefore could suggest that the plant is of medicinal value. There is evidence of the presence of saponins in traditional medicine preparations [13, 14]. Where oral administrations might be expected to lead to hydrolysis of glycoside from terpenoid (and obviation of any toxicity associated with the intact molecules). Tannins are astringent, bitter plant polyphenols that either bind and precipitate or shrink proteins, tannins are distributed all over the plant kingdom. Tannins have traditionally been considered antinutritional but it may be employed medicinally in antidiarrheal, hemostatic and antihemorrhoidal compounds. Its presence in the plant suggest it to be of medicinal value because tannins have shown potential antiviral [15], antibacterial and antiparasitic effects [16].

Table 1. Qualitative analysis of the phytochemical screening of leaf sample of *Ocimum sanctum*

Plant	Alkaloids	Tannin	Saponin	Steroid	Phlobatannin	Terpenoid	Flavonoid	Cardiac glyceride
<i>Ocimum sanctum</i> leaf	+	+	+	+	-	+	+	+

In the present study, ten compounds have been identified from hydroalcoholic extract of *Ocimum sanctum* leaves by Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The presence of various bioactive compounds justifies the use of the plant leaves for various ailments by

traditional practitioners. However, isolation of individual phytochemical constituents and subjecting it to pharmacological activity will definitely give fruitful results.

Figure 1. Chromatogram of *Ocimum sanctum* leaves by GC-MS

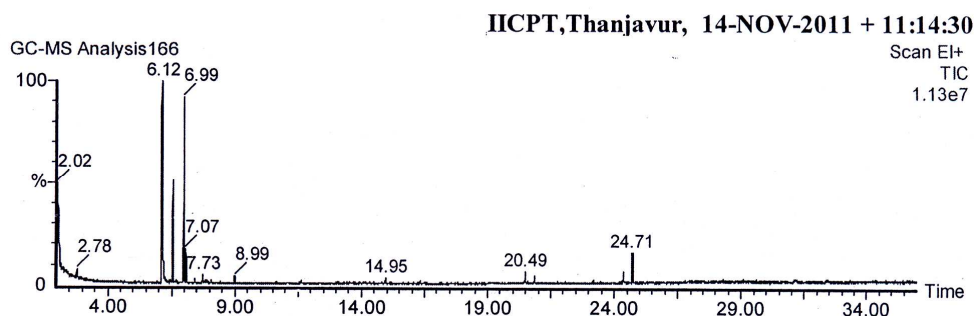


Table 2. Components identified in the *Ocimum sanctum* leaf extract

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1.	6.12	Eugenol	C ₁₀ H ₁₂ O ₂	164	43.88
2.	6.56	Cyclohexane, 1,2,4-triethenyl-	C ₁₂ H ₁₈	162	15.31
3.	6.99	Caryophyllene	C ₁₅ H ₂₄	204	26.53
4.	7.73	10-Heptadecen-8-ynoic acid, methyl ester, (E)-	C ₁₈ H ₃₀ O ₂	278	1.02
5.	8.99	Cyclopentane, cyclopropylidene-	C ₈ H ₁₂	108	1.02
6.	14.95	Z,Z-4,16-Octadecadien-1-ol acetate	C ₂₀ H ₃₆ O ₂	308	1.02
7.	20.49	Benzene methanamine, N,N-a,4-tetramethyl-	C ₁₁ H ₁₇ N	163	2.04
8.	20.85	3',8,8'-trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4,4'-tetrone	C ₂₈ H ₂₅ NO ₇	487	1.02
9.	24.36	Octadecane, 1,1-dimethoxy-	C ₂₀ H ₄₂ O ₂	314	2.04
10.	24.71	Pentanedinitrile, 2-methyl-	C ₆ H ₈ N ₂	108	6.12

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