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NOTE

GC/MS Analysis of Volatile Compounds of the Essential Oil of Leaves of *Ocimum sanctum* Growing in Hisar, India

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Ocimum sanctum also addressed as *Ocimum tenuiflorum* is a sacred plant in Hindu culture and known as Tulsi in Hindi and Holy Basil in English. In this study, volatile constituents of *Ocimum sanctum* were extracted using hydrodistilation and their chemical constituents were identified and quantified by using GC-MS. Analysis of the essential oil have allowed to identify 13 components, but methyl chavicol (34.12 %), γ-muurolene (32.44 %) and β-caryophyllene (24.33 %) are the main component of *Ocimum sanctum*.

Keywords: Holy basil, Essential oil, Methyl chavicol, γ-Muurolene, β-Caryophyllene, *Ocimum sanctum*, Hydrodistillation.

The genus *Ocimum* belonging to the Lamiaceae family comprises annual and perennial herbs and shrubs native to tropical and subtropical regions of Asia, Africa and South America[1]. It's comprises more then 150 species and is considered as one of the largest genera of the Lamiaceae family. Characterization of each species in this genus are based on the leaves and habitat[2]. The shape of the leaves in *Ocimum sanctum* and its close relative varies in size of leaves, vein and petioles. The colour of the leaves varies from bright green to dark green and sometime almost black. Though, the colours in plant vary but the regions behind it, especially in basil are not being known yet. Regular occurrence of the inter specific hybridization with the genus, have created taxonomic challenges, leaving less publications on basil taxonomic which follows the international code of Botanical nomenclature[2,3]. The difficulties in identification of species can be optimized by combined analyses of morphological traits, essential oil composition and molecular markers. Most members of this family such as Hyptis, Thymus, Origanum, Salvia and Mentha species are considered economical useful because of there basic natural characteristics as essential oil producers.

The individual spices within the genus *Ocimum* have been observed to show my significant variation in the aromatic characters as well as morphological features. Such observation have been attributed to the abundant crosspollination that occurs within this genus resulting considerable degrees of variations in the genotypes, hence diversity in growth characteristics, leaf size, flower colour, physical appearance and aroma. Consequently, high diversity of species, subspecies, varieties and chemo types are evident in this genus, each having distinct aromatic characters, morphological features and chemical composition in essential oil distillates.

Essential oils are fragrant, highly concentrated essences of plants which are considered to exemplify the soul or life force plant. Essential oils are approximately 75-100 times more concentrated then dried herbs[4]. Essential oils are generally extracted by distillation, solvent extraction, cold pressing, maceration or supercritical carbon dioxide extraction[5,6]. It has been reported that the quality and quantity of essential oil produced by plants depends on various factors such as seasonal variation[7], method of harvest[8], leaf development stage[9], climate and soil type[10]. Essential oil's combined effect, which depends on the desired application is more powerful then that of individual oil. This is commonly known as synergistic blend. Essential oil exhibits many usages such as in medicinal applications[6-11], as perfume for herbal toiletries, aromatherapy treatment[11] and also perfume industry[6,7]. *Ocimum sanctum* has been extensively used in Ayurvedic system of medicine for various aliments including capability of lowering plasma glucose[12]. Essential oils and herbals extract have attracted a great deal of scientific research interest due to their potential as natural flavours. A scientifically based guides has been developed to access the safety of naturally occurring mixtures based on chemical composition, particularly essential oils, for their intended use

flavours ingredients[13]. This research reports the essential oil composition of *Ocimum sanctum* obtained by hydrodistilation and analyzed by GC-MS.

The fresh leaves of *Ocimum sanctum* were collected from Azad Nagar, Hissar (Haryana) in March 2009 and stored overnight in refrigerator.

General procedure: The leaves were cut into small peaces and subjected to hydrodistilation over a 2 h period at 100 °C in an all glass standard cleavanger apparatus for oil lighter then water. The essential oil obtained was dried over anhydrous magnesium sulphate.

Detection method: Analysis of the sample was carried out using Schimadsu quadrupole 2010 GC-MS instrument. Helium was used as the carrier gas and the temperature programming was set with initial oven temperature at 80 °C and held for 4 min and increased to 260 °C at the rate of 5 °C per min and then increased with 3 °C per min up to 300 °C and held at this temperature for 10 min. Sample was injected with splitless mode. Mass spectra were recorded over 50-600 *m/z* range with solvent cut time 3.5 min. The total running time for a sample is 60 min.

The essential oil of *Ocimum sanctum* was subjected to detailed GC-MS analysis in order to determine its chemical constituents. The yield of oil was 1.2 %. Analysis of the oil indicated *Ocimum sanctum* to be composed of 13 components (Table-1). The major components of *Ocimum sanctum* were methyl chavicol (34.13 %) (Fig. 1a), γ-muurolene (32.44 %) (Fig. 1b) and β-caryophyllene (24.33 %). The other important components identified were β-selinene (4.02 %), α-copaene (1.77 %) and caryophyllene oxide (1.01 %). The constituents of O*cimum sanctum* were characterized by a high content of aromatic compounds. Essential oil found in *Ocimum sanctum* belongs to a variety of groups including monoterpene hydrocarbons, sesquiterpene hydrocarbons (*e.g.* α-copaene, β-caryophyllene), oxygenated sesquiterpenes (*e.g.* caryophyllene oxide) and aromatic compounds (*e.g.* methyl chavicol, eugenol).

However, literature review showed variation between chemical compositions, depending of location, seasonal variation and stages of developments. Eugenol is the main component of *Ocimum sanctum* grown in Bangladesh[14], Cuba[15], Germany[16]. Detailed morphological characters developed[17] can be used as reference to classify various types of *Ocimum sanctum.*

TABLE-1
CHEMICAL CONSTITUENTS OF THE ESSENTIAL OIL OF *Ocimum sanctum*

No.	Chemical constituents	Molar mass	Composition (% area)
1	Methyl chavicol	148	34.13
2	Eugenol	164	0.48
3	α-Copaene	204	1.77
4	β-Elemene	204	0.06
5	β-Caryophyllene	204	24.33
6	γ-Muurolene	204	32.44
7	α-Humulene	204	0.56
8	β-Selinene	204	4.02
9	δ-Cadinene	204	0.64
10	Caryophyllene oxide	220	1.01
11	Hexadecane	226	0.17
12	Limonene	268	0.21
13	Oleoyl chloride	301	0.18

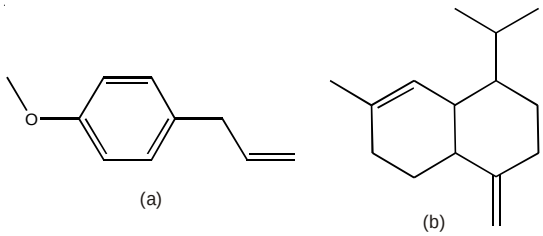

Fig. 1. (a) Methyl chavicol (34.13 %) (b) γ-muurolene (32.44 %)

Conclusion

The aim of this study was to describe the chemical composition of essential oils of O*cimum sanctum* from Hisar. The essential oils, obtained from leaves by hydrodistillation, were analyzed by gas chromatography-mass spectrometry (GC/MS). Thirteen eight compounds were identified and were characterized as Methyl chavicol (34.13 %), γ-muurolene (32.44 %), β-caryophyllene (24.33 %), β-selinene(4.02 %), α-copaene (1.77 %), caryophyllene oxide (1.01 %), δ-cadinene (0.64 %), α-humulene (0.56 %), eugenol (0.48 %), limonene (0.21 %), oleoyl chloride (0.18 %), hexadecane (0.17 %) and β-elemene (0.06 %).

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