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CHEMICAL COMPOSITION OF ESSENTIAL OIL OF OCIMUM GRATISSIMUM- L BY GC-MS ANALYSIS

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ABSTRACT

Ocimum gratissimum L, (clove basil, African basil) growing wild in India belongs to family Lamiaceae (mints).[1] Basil is possibly native to India. Ocimum gratissimum L is widely used for many pharmacological purposes in folk medicine, Ayurvedic, Homoeopathic, siddha, unani and Allopathic,[2,3] streams of medicine to treat diseases like upper respiratory tract infections, diarrhoea, headache, ophthalmic, skin diseases, pneumonia, and also as a treatment for cough, fever, and conjunctivitis (Corrêa 1932, Onajobi 1986). The essential oil of Ocimum gratissimum L exihibits considerable antibacterial and antifungal activities.[3,4] The aim of this study is to analyse and study the chemical composition of essential oil of leaf of Ocimum gratissimum L through G.C and G.C-M.S. The shade dried leaf of Ocimum gratissimum L was subjected to hydrodistillation using clevengers apparatus and analysed through GC-MS and found some different compounds.[4] The monoterpenoids dominated over sesquiterpenoids in the essential oil contents of leaf of Ocimum gratissimum L collected from surroundings of Hyderabad, Telangana by the authors. The major components among them were Eugenol (54.6%), α-Bulnesene (17.2%), thymol (5.2%), β-caryophyllene (5.0%), γ-terpenene (3.1%) and p-cymene (2.1%). This report also suggested the dominance of eugenol as the major constituent in essential oil of Ocimum gratissimum L as in earlier investigations.

KEYWORDS: Ocimum gratissimum L, Labiate, essential oil, Eugenol, Therapeutic effects, Pharmacognosy, Alternative and complementary medicine.

INTRODUCTION

Ocimum gratissimum L is a herbaceous plant of the Labiatae family. The plant is indigenous to tropical areas especially India and it is also found in West Africa.[1,2,3] In Nigeria, it is found in the Savannah and coastal areas. It is cultivated in Ceylon, South Sea Islands, and also within Nepal, Bengal, Chittagong and Deccan (Nadkarni, 1999).[3,4] It is known by various names in different parts of the world. In India it is known by its several vernacular names, the most commonly used ones being Vriddhutulsi (Sanskrit), Ram tulsi (Hindi), Nimma tulasi (Kannada).[4,5] In the southern part of Nigeria, the plant is called "effinrin-nla" by the Yoruba speaking tribe. It is called "Ahuji" by the Igbos (Effraim et al., 2003). It is called ''Elah (Elaa)'' in part of Urhobo (Eboh and Ekundina, 2012). In the Northern part of Nigeria, the Hausas call it "Daidoya" (Effraim et al., 2003).[6,7] Ocimum gratissimum L is a small erect plumb plant with many branches usually not more than 1 meter (Vierra and Simon, 2000) to 2 meters high with a taproot and many adventitious side rootlets. The leaves are simple, opposite or whorled with several oil glands and possess a peculiar scent smell due to its composition of volatile essential oils.[6,7,8] It produces an inflorescence that is capitate and reduces apical dominance while increasing branching. The flowers are zygomorphic, bilobal and bisexual with five petals and sepals and four stamens.[9,10] The gynecium has two carpels ascending from the ovary. The fruit has a group of four nutlets each with a brown seed that has scanty or no endosperm (Okujagu et al., 2005; Odebiyi and Sofowora, 1978).[10,11] It is commonly called African basil or shrubby basil. Ocimum gratissimum L is commonly used in traditonal medical practice for the treatments of such ailments as mental illness (Abdulrahman, 1992), epilepsy (Sofowora, 1993), high fever (Oliver, 1980), diarrhea (Oliver, 1980; Sofowora, 1993).[11,12] It is also used for pneumonia, cough, and conjunctivitis (Correa, 1932; Onajobi, 1986). The leaves of the plant are used culinarily in salads, soups, pastas, vinegars and jellies in different parts of the globe.

The essential oil of Ocimum gratissimum L contains eugenol and shows some evidence of antibacterial activity.[13,14] A polyherbal preparation of a water extract obtained from the leaves of Gongronema latifolia, Vernonia amygdalina and Ocimum gratissimum showed analgesic activity.[9] The essential oil has potential for use as a food preservative, and is toxic to Leishmania.[11] Extracts of the leaves are documented to possess antidiabetic

properties,[12,13] anti-hyperlipidemic effect[15] and recently, it was shown to improve heamatological variables in experimental diabetes mellitus via its well reported antioxidant property.[16,17] Ocimum gratissimum L is commonly used in folk medicine to treat diseases like upper respiratory tract infections, diarrhoea, headache, ophthalmic, skin diseases, pneumonia, and also as a treatment for cough, fever, and conjunctivitis (Corrêa 1932, Onajobi 1986).[18,19] The essential oil of Ocimum gratissimum L exihibits considerable antibacterial and antifungal activities. The aim of the present study was to analyse the essential oil components of Ocimum gratissimum L by G.C and GC-MS and to compare the active principles present with already reported in literature.

EXPERIMENTAL

Plant material

The aerial parts of plant, Ocimum gratissimum L were collected during the month of December 2017 from surroundings of Hyderabad. The plant material was identified by the authors and its herbarium sheet was deposited at the post graduate laboratory, Department of Chemistry, Gokul Group of institutions, Hyderabad. The collected aerial parts of plant, Ocimum gratissimum L was brought to the lab at Gokul Group Of institutions, Hyderabad, washed throughly with detergent to remove the dirt present on the aerial parts and separated the leaves and shade dried for 4-6 days.

Chemical Reagents

All chemicals used in the present study were of analytical grade and obtained from Sigma Co. (St. Louis, MO, USA).

Essential oil extraction

The shade dried aerial parts of Ocimum gratissimum L plant collected (1Kg) was subjected to hydrodistillation (1500 mL distilled water +500 g plant material, in 3000 mL round bottom flask) in a Clevenger apparatus for 3hrs using distilled water. The round bottom flask was kept on a heating mantel, the heat maintained is between 80^0C and 100^0C and the heating system is monitored by thermometer arranged to the round bottom flask. The essential oil was traped by n-hexane and separated from the aqueous layer using a 100mL capacity separatory funnel. The collected essential oil was dried over anhydrous sodium sulphate and filtered using a Whatman filter paper no. 40. The extracted essential oil was stored at 4°C in dark brown 5mL capacity sample vial until analysis. The yield of the oil was found to be 0.5% (w/w) in relation to the dry weight.

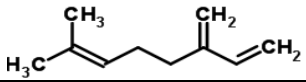
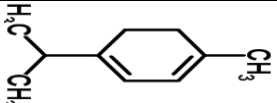
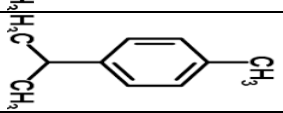
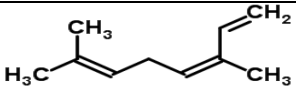
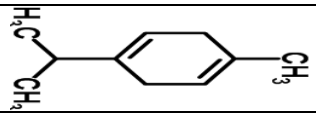
GC and GC-MS analysis

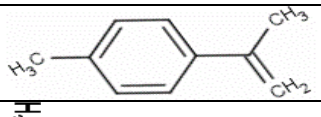
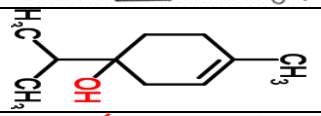
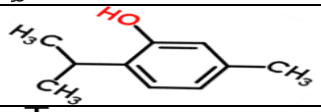
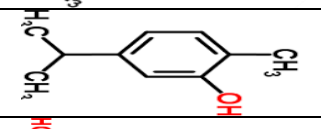
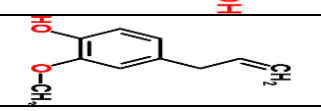
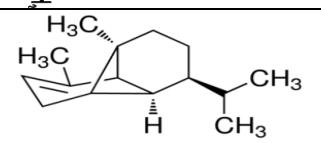
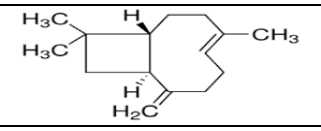
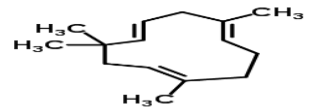
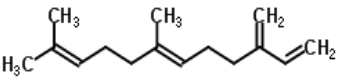
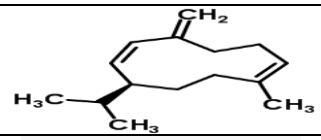
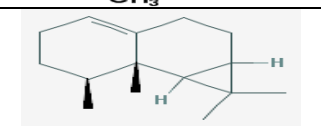
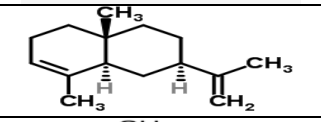
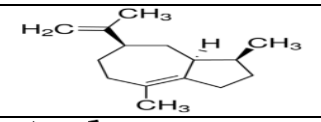
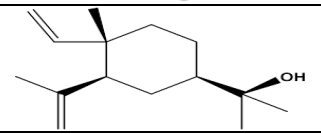
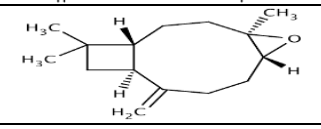
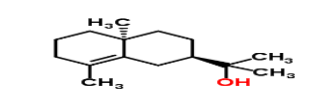
GC analysis was carried out in Agilent Technology 6890N Gas Chromatograph data handling system equipped with a spilt/splitless injector using N2 as carrier gas. The column was HP-5 capillary column (30m x 0.32mm, 0.25µm film thickness) and temperature program was used as follows: initial temperature of 60^0C (hold: 2min) programmed at a rate of 3^0C/min to a final temperature of 220^0 C (hold: 5min). The temperature of injector was maintained at 210^0 C. The GC-MS analysis was performed by Perkin Elmer Clarus 500 Gas Chromatograph equipped with a spilt/splitless injector (split ratio 50:1) data handling system. The column was an Rtx®-5 capillary column (60 mm x 0.32 mm, 0.25µm film thickness). Helium was used as carrier gas at a flow rate of 1.0mL/min. The GC was interfaced with Perkin Elmer 500 Mass Detector operating in EI+ mode. The mass spectra was recorded over 40-500amu and revealed the Total Ion Current chromatograms. The temperature program remained the same as in GC. The temperatures of injector and transfer line were kept at 210^0C & that of the ion source at 200^0C.

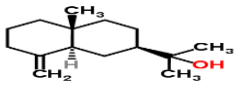
Identification of constituents was done on the basis of retention index (RI, determined with reference to homologous series of n-alkanes C_8–C_{25} under identical experimental conditions on BP-1 column), MS library search NIST 08 MS Library (Version 2.0 f; Thermo Fisher Scientific Austria), and WILEY MS 9th Edition (Thermo Fisher Scientific Austria), and by comparing with the MS literature data. The relative amounts of individual components were calculated based on the GC peak area (FID response) without using a correction factor.

RESULTS AND DISCUSSION

Table: 1 Chemical composition of essential oil of Ocimum gratissimum L.

Peak. No.	R.T	R.I	Compound Identified	Molecular formula	structure	% Composition
1	6.13	995	Myrcene	$C_{10}H_{16}$		0.34
2	8.23	1016	α-terpinene	$C_{10}H_{16}$		0.30
3	9.21	1051	p-cymene	$C_{10}H_{14}$		2.11
4	10.42	1052	β-Ocimene	$C_{10}H_{16}$		0.49
5	11.67	1062	γ-terpenene	$C_{10}H_{16}$		3.06

6	12.98	1087	p-cymenene	$C_{10}H_{12}$		0.26
7	13.87	1176	Terpin-4-ol	$C_{10}H_{18}O$		0.24
8	15.00	1286	thymol	$C_{10}H_{14}O$		5.20
9	16.28	1294	Carvacrol	$C_{10}H_{14}O$		0.28
10	17.76	1355	Eugenol	$C_{10}H_{12}O_2$		54.6
11	18.98	1373	α-Copaene	$C_{15}H_{24}$		0.11
12	20.12	1415	β-caryophyllene	$C_{15}H_{24}$		5.05
13	22.34	1453	α-Humulene	$C_{15}H_{24}$		0.31
14	23.90	1456	E-β-Farnesene	$C_{15}H_{24}$		0.33
15	24.98	1480	Germacrene D	$C_{15}H_{24}$		0.62
16	26.12	1490	β-Gurjunene	$C_{15}H_{24}$		1.03
17	27.54	1494	α-Selinene	$C_{15}H_{24}$		0.45
18	28.15	1503	α-Bulnesene	$C_{15}H_{24}$		17.2
19	29.00	1524	Elemol	$C_{15}H_{26}O$		1.22
20	29.95	1575	Caryophyllene oxide	$C_{15}H_{24}O$		0.84
21	31.35	1580	γ-Eudesmol	$C_{15}H_{26}O$		0.67

22	32.78	1593	β-Eudesmol	$C_{15}H_{26}O$		0.94
					Total	95.65

The GC-MS analysis of essential oil of Ocimum gratissimum L showed the presence of 22 components and the identified components are presented in Table-1. A total of 95.65% of compounds were identified. In the oil mixture monoterpine contents predominated (65.4%) over sesquiterpine components (15.7%). The major components are Eugenol (54.6%), α-Bulnesene (17.2%), thymol (5.2%), β-caryophyllene (5.0%), γ-terpenene (3.1%) and p-cymene (2.1%). in the oil composition. A previous study reports on the essential oil contents of Ocimum gratissimum L showed the dominance of monoterpine, eugenol as the major constituent.[8-10] The present report is also in agreement with the earlier studies, even though the quantity of eugenol varies.

The quantitative and qualitative divergence may be due to the geographical, climatic, and soil conditions, which in turn may affect the composition and other secondary metabolites of the plants.[42,43] The composition of the essential oil often changes between different plant parts. The difference in the complex composition of essential oils of one kind may sometimes be difficult to assign to specific chemotypes. The formation of essential oils depends on the tissue differentiation (secretory cells and excretion cavities, etc.) and on ontogenetic phase of the respective plant.[44] Individual plants also showed variation in the percentage of chemical components depending on the part of the plant from which the oil was extracted.[45] Moreover, oil constituent was extremely variable, and individual constituents were not affected by intralant location of the leaves, plant age, or geographic site.[46] This limits their taxonomic value but possibly enhances their ecological significance as a defense adaptation to herbivores.[47]

CONCLUSION

In conclusion, nevertheless, there are an almost uncountable number of single substances and a tremendous variation in the composition of essential oils. In our present study all the components identified with the percentage is compared to the earlier investigations according to literature. The monoterpinoids dominated over sesquiterpenoids in the essential oil contents of Ocimum gratissimum L extracted from Surroundings of Hyderabad by the authors. The major components are Eugenol (54.6%), α-Bulnesene (17.2%), thymol (5.2%),

β-caryophyllene (5.0%), γ-terpenene (3.1%) and p-cymene (2.1%).The main sesquterpine factor was β-caryophyllene in the oil composition. This report also suggests the dominance of eugenol as the major constituent in essential oil of Ocimum gratissimum L as in earlier investigations.

The healthy look and fitness has become a religion for present generation, so is the mindless gulping of supplementary pill to sustain and boost their system. In fact, according to medical consultation they could even prove to be fatal for life. Instead if they choose the natures products, they would not only benefit immensely, but also can cut cost to by those bitter pills to great extent. In the developing countries, increased cost of medication and their side effects are of great concern to general public, opening new channels of pharmacological investigations focusing on natural medication and diverting human trends toward natural cure. Such vitalizer is freely available in nature is ubiquitous ‘Tulsi ‘or Holy Basil. It contain phytochemicals working together these compounds process antioxidant, antibacterial and immune enhancing properties that maintain good health status. So, it can be concluded that Ocimum gratissimum L or Tulsi is a traditionally and clinically proved medicinal herb for both its application and efficacy.

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