Chemical Composition of *Ocimum kilimandscharicum* Guerke Essential Oil Cultivated in Phagwara, Punjab, India

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Abstract

The Lamiaceae family contains the important and well-known genus Ocimum, which comprises more than 150 species that spread widely. One of the well-known representatives of this genus, *Ocimumkilimandscharicum*, is a native plant of east Africa. Its common name, African blue basil, refers to the plant's geographical distribution and abundance in African countries. *O. kilimandscharicum* attracted much attention due to high camphor content. The primary phytoconstituents of *O. kilimandscharicum's* essential oil (EO) include camphor, 1, 8-cineol, a-pinene, linalool, limonene, eugenol, methyl chavicol, á- and â-bisabolene.

O. kilimandscharicum leaves and flowers were collected from the medicinal and herbal garden of Pharmacy department, Lovely Professional University (LPU), Phagwara, Punjab, India, in the month of March, 2019.The EO of fresh leaves and flowers was obtained by hydro-distillation. Shimadzu- QP2010 Ultra mass spectrometer with DB-5 (30m0.25mm internal diameter, 0.25m film thickness) fused silica capillary column was utilized for the GC-MS analysis to determine the composition of the EO.

In total, thirty compounds were identified in the EO obtained from the leaves and flowers of *O. kilimandscharicum*. The most abundant constituents were eugenol (74.28%), á-pinene (8.20%), and germacrene-D (5.13%). The result of GC- MS analysis in the present study described a "eugenol- á-pinene" chemo-type of the plant, which has not been recorded yet.

Keywords

Ocimum species; *O. kilimandscharicum;* Essential Oils; GCMS; Eugenol- a-PineneChemo-type

1. INTRODUCTION

The genus *Ocimum* is one of the main and medicinally known genera of Lamiaceae family, which includes more than 150 species and is able to grow widely (Doll *et al*., 2012). Different species of this genus have different pharm-acological and medicinal activities.For example,

antiemetic activity of *O. basilicum* and *O. gratissium* (Tchatchouang *et al*., 2017), neuro-protective (Singh *et al*., 2017), and anti-oxidant effects of *O. kilimandscharicum* (T. Joshi & Juyal, 2017), cardio protective (Sharma *et al*., 2001; Zainab A. H. AL-Mousawi, 2019), anthelmintic (Pessoa *et al*., 2002), antimicrobial and anti hyperglycemic activities of *O. gratissium* (Matasyoh *et al*., 2008), *O. basilicum* (Kaya *et al*., 2008) (Malapermal *et al*., 2017) and *O. sanctum*[6](Asha *et al*., 2001; Eswar *et al*., 2016; Malapermal *et al*., 2017) are well established. EO is mostly responsible for these activities of these plants (Pandey *et al*., 2014).

O. kilimandscharicum is a native plant of east Africa, and because of its geographical distribution and abundance in African countries, it is also known as African blue basil. The EO, obtained from the leavesof this plant has light yellow color with distinct and strong scent of camphor. EO of this plant hasantioxidant, anti-inflammatory and cancer controlling activity and the major constituents responsible for these effects are camphor, mixture of limonene, and 1, 8-cineol (Doll *et al*., 2012; Misra & Das, 2016).

2. MATERIALS AND METHODS

2.1. Plant materials

Fresh *O. kilimandsharicum* leaves and flowers were gathered in March 2019 from cultivated habitats in the pharmacy department's medicinal and herbal garden at Lovely Professional University (LPU), Phagwara, Punjab, India. The collected plant material was identified as *O. kilimandsharicum* leaves and flowers by Prof. Devendra Kumar Pandey, faculty staff of Botany department, LPU, Phagwara, Punjab, India. Herbarium sheets of the collected plant were prepared and the voucher specimens (No. LPU-15032017-LPU16032017-LPU17032017) were deposited as references in herbarium of plant biotechnology lab, LPU, Phagwara, Punjab, India. The collected fresh plant material was properly cleaned, freed of stem fragments, sliced into small pieces, and divided into two halves. The first portion was used right away to extract the EO, while the second portion was labeled and placed in a refrigerator (-20^{o}C) for later use.

2.2. Pharmacognostic evaluation

Pharmacognostical evaluation was performed on the plant materials that were collected. An evergreen aromatic perennial under shrub, *O. kilimandsharicum* has simple ovate-oblong leaves, light purple or white flowers, and ovoid-oblong, dark-colored, mucilaginous seeds (*Fig. 1*). The EO has a strong camphor scent and is a bright yellow liquid.

Fig.1: *O. kilimandscharicum* Guerke grown in medicinal and herbal garden of Pharmacy department, Lovely Professional University, Phagwara, Punjab, India.

2.3 Essential oil Extraction

A precisely weighted amount of the plant materials were hydro-distilled for three hours using a Clevenger type apparatus in order to extract the EO (Chaturvedi *et al*., 2018). After the distillation process was finished, the apparatus was left to cool for a time while waiting for the production of a clear yellow EO phase above the water inside the distillation apparatus' graded tube. In preparation for further investigation, the condensed oil was collected, dried over anhydrous sodium sulphate, and then kept in the dark at -20°C. The experiment was carried out three times.

2.4. GC- MS Analysis of Essential Oil

Shimadzu-QP2010 Ultra mass spectrometer with a DB-5 (30 m 0.25 mm internal diameter, 0.25 m film thickness) fused silica capillary column was utilized for the GC-MS analysis to determine the composition of the EO. The GC oven's temperature was held at 40°C for five minutes before rising to 300°C at a rate of 50°C per minute. With a flow rate of 1.24 ml/minute and a pressure of 66.7 kpa, helium gas was employed as the carrier while the injector temperature was maintained at 250 °C. Using an ionization energy of 70 eV, a split ratio of 1:10, and a mass scan range of 40–500 amu, the injection volume was 0.03 L neat. The retention index and mass spectral data from the MS Library search were compared with the literature in the presence of the key components' standards to identify the phytoconstituents of the EO based on their retention time (RT) (Chaturvedi *et al*., 2018).

3. RESULTS AND DISCUSSION

The EO yield of *O. kilimandscharicum* leaves and flowers collected from the medicinal and herbal garden of Department of Pharmacy, LPU, Phagwara, Punjab, India, was 0.8± 0.05.% (v/dw). The GC chromatogram of the EO of *O. kilimandscharicum* leaves and flowersis presented below (*Fig. 2*). Table 1 lists the chemical constituents of *O. kilimandscharicum* EO in the order in which they were eluted from the DB-5 column. A total of thirty compounds, or 100% of the total oil, were found in the EO of the plant's leaves and flowers. According to the GC chromatogram of the EO and the phytochemical profile, which was obtained from MS library search, the most abundant compounds were eugenol (74.28%), followed by á-pinene (8.20%) and germacrene-D (5.13%).

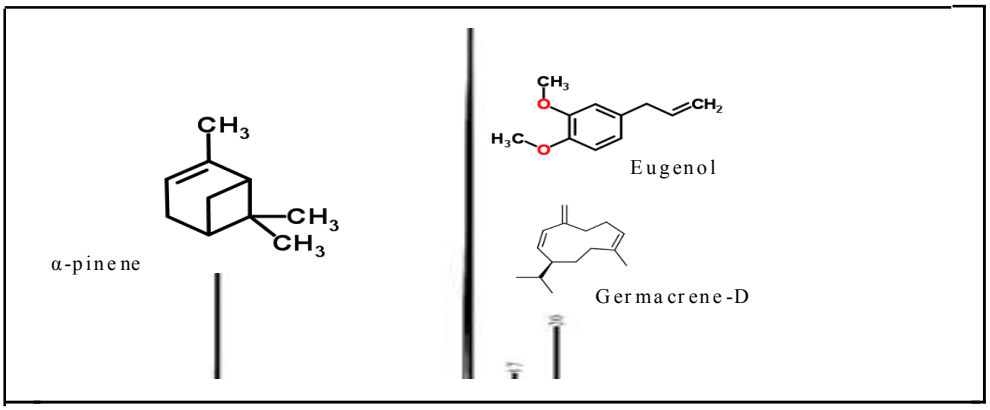

Fig.2: GC chromatogram of the EO of *O. Kilimandscharicum* leaves and flower

Table 1. The chemical profile of EO of *O. kilimandscharicum* leaves and flowers with their %age

Peak. No.	RT*	Compound Name
1	9.829	α-Thujene
2	11.692	Sabinene
3	12.430	β-Myrcene
4	13.492	α-terpinene
5	14.250	α-Pinene
6	14.683	β-Ocimene
7	15.190	¥-Terpinene
8	15.668	cis- β-Terpineol
9	16.903	Linalool
10	18.015	Neo-allo-Ocimene
11	20.037	(-)-4-Terpeniol
12	20.614	L-α-Terpeniol
13	26.573	Eugenol
14	27.223	α –Copaene
15	27.498	(-)-β-Bourbonene
16	27.683	Cyclohexane, 2,4 –di isopropenyl -1-methyl-1-vinyl
17	28.711	Caryophyllene
18	29.041	β-Copaene
19	29.890	Humulene
20	30.751	Germacrene-D
21	31.208	Germacrene-B
22	31.833	Dihydro-β-agarofuran
23	31.923	Delta-Cadinene
24	33.742	Germacrene D-4-ol
25	34.007	Diethyl phathalate
26	35.787	α-Cadinol
27	36.135	α-Cadinol
28	55.405	Tetratetracontane
29	55.684	Squalene
30	59.136	Tritetracontane

*RT: retention time

The result of the current study was very different from the previously published data. *O. kilimandscharicum*'s chemical composition was primarily dominated by camphor (64.9%), limonene (8.7%), camphene (6.4%), and (E)-ocimene (3.0%), according to Padalia and Verma's research (Padalia & Verma, 2011). In the other study, Lima et al. reported the chemical profile of *O. kilimandscharicum*, which was rich in camphor (51.81%), 1, 8- cineol (20.13%), and limonene (11.23%) (De Lima *et al*., 2014). According to the data documented by Rao et al. and Varinder Singh, camphor was the main phytoconstituents of *O. kilimandscharicum* EO (51.7%) (Rajeswara Rao *et al*., 2011; Singh, Krishan and Shri, 2014).The most prevalent compounds in the EO of *O. kilimandscharicum* leaves and flowers, according to a chemical profile by R. Joshi, were camphor (45.9%), 1, 8-cineol (14.6%), and limonene (8.1%)(R. K. Joshi, 2013). The EO composition of the plant growing in Indiana was examined in Charles and Simon's description of a linalool-camphor chemotype of *O. kilimandscharicum*. Linalool and camphor concentrations were 41.94–58.85% and 17.02–15.82%, respectively, in the EO of leaves and flowers (Charles & Simon, 2011). Similar to this, Oladipupo *et al*. reported that the main ingredients in *O. kilimandscharicum* leaf oil were methyl eugenol (53.9%) and -cadinene (16.2%) (Oladipupo *et al*, 2014). According to the findings of Ntezurubanza et al. found that the EO of *O. kilimandscharicum* growing naturally in Rwanda was abundant in 1.8-cineole (62%), limonene, and -pinene (Ntezurubanza *et al*., 1984).

The EOs enables plants to adapt the harsh environmental and ecological conditions and survive. The geoclimatic conditions, seasonal variability, plant chemotype, stress factors, genetic variability etc. are some well-known examples of influencing factors that could affect the quality and quantity of EO phytoconstituents.

The high content of eugenol in EO of *O. kilimandscharicum* leaves and flowers grown in specific geography could be justified by its various functional roles in nature especially to the chemical defense of the plantagainst the herbivores and parasitic bacteria and fungi (Obeng-Ofori & Reichmuth, 1997; Abd El- Baky & Shawky, 2016), because this compound act as an anti-microbial and anti animal toxin (Pavithra, 2014). Moreover, eugenol is an important floral attractant, which facilitate pollination (Yan *et al*., 2018). The antioxidant capacity of eugenol is thought to be one of the several reasons for production of EO with high eugenol content by *O. kilimandscharicum* (Gulçin, 2011; da Silva *et al*., 2018), because it helps the plant to adapt high temperature and radiation of the environment and survive.

4. CONCLUSION

According to the research done on *O. basilicum* varieties, these species have the capacity to produce and store phenylpropanoide derivatives, such as eugenol, in the peltate glandular trichomes on the surface of their leaves (Koeduka *et al*., 2006). Moreover, the number, dominance, and size of peltate and capitate glandular trichomes are factors that affect the EO yield of these plants (Maurya *et al*., 2019; Viña & Murillo, 2003; Telci *et al*., 2006;Verma *et al*., 2013). Moreover, the number, dominance, and size of peltate and capitate glandular trichomes are factors that affect the EO yield of these plants (Maurya *et al*., 2019; Via & Murillo, 2003; Telci *et al*., 2006; Verma *et al*., 2013). The presence of a group of enzymes (pinoresinollariciresinol reductase, isoflavone reductase, and phenylcoumaran)

that structurally belong to NADPH-dependent reductases and use coniferyl acetate and NADPH to form eugenol accounts for the ability of the aforementioned varieties to produce phenylpropenes (Koeduka *et al*., 2006). As mentioned before, *O. kilimandscharicum*is a variety species of *O. basilicum*so; the abundance of glandular trichomes along with a specific proteomic profile in *O. kilimandscharicum*might be the other valid reason behind high eugenol content of EO obtained from its leaves.

According to the recorded GCMS data of the EO of *O. kilimandscharicum* leaves and flowers, the tested plant could be considered as "eugenol- á-pinene"chemotype of *O. kilimandscharicum* that has not been reported yet.

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