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The UV and FTIR Fingerprint of *Ocimum kilimandscharicum* Guerke Essential oil: A Eugenol-Rich Chemo Type

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Abstract

Ocimum kilimandscharicum Guerke, a member of the family Lamiaceae, is a valuable medicinal plant used both in traditional and modern medicine. It is a perennial aromatic undershrub with tremendous phytochemical polymorphism. The present study aims to assess the amount of eugenol in the essential oil (EO) of *O. kilimandscharicum*. Eugenol is one of the most popular phenolic compounds, which is naturally synthesized and extracted from the EO of different plant species. The fresh leaves and flowers of *O. kilimandsharicum* were used to extract EO using a hydrodistillation method. Ultraviolet (UV) and Fourier Transform Infrared (FTIR) spectrometry techniques were used to assess and quantify the chemical fingerprint of the EO and their main phytoconstituents. In this study, eugenol showed its peak absorbance to be around 282 nm in both the EO and pure eugenol spectra. The FTIR spectra of the EO and eugenol showed different functional groups determined by comparing the vibration frequencies in wave numbers of the EO and eugenol spectra with those of an IR correlation chart. Eugenol is a well-known phenolic compound with medicinal and economic value. The UV and FTIR spectra of the EO of *O. kilimandsharicum* proved the presence of a high amount of eugenol in the *O. kilimandscharicum* plant.

Keywords: *Ocimum kilimandscharicum* guerke, Medicinal plants, Essential oil, Eugenol, UV spectrometry, FTIR spectrometry.

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Ethical: This study follows all ethical practices during writing.
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1. Introduction

Phenylpropenes contain a large number of organic molecules, which plants produce for purposes such as defensive compounds against animal predators and microorganisms, structural components, growth, and floral attractants for the pollinators. In addition, these compounds have long been used by humans as food preservatives, food additives, medicinal agents, etc. Chavicol, t-anol, eugenol, isoeugenol, and methyl eugenol are well-known examples of phenylpropenes currently used for industrial and pharmaceutical purposes [1, 2].

Eugenol (4-allyl-2-methoxyphenol), with the molecular formula of C10H12O2, is an organic phenolic compound that is naturally synthesized and extracted from the essential oil of different plant species such as clove, basil, cinnamon, lemon balm, and nutmeg [3, 4]. This phytoconstituent is known for its medicinal value as an antiseptic [5], dental anesthetic [6], antioxidant [3, 7], anti-inflammatory [8], antiviral [9], antimicrobial [10], antibacterial, antifungal [11], anthelmintic [12,

13] etc. In addition, eugenol is used in the perfumery industry, food industry as a food additive [5] in clove-flavored cigarettes, dental and oral hygiene preparations, as an irritant, sensitizer, and in the production of local anesthetics [5, 14].

The genus *Ocimum* "L". is one of the most important genera of the family Lamiaceae, which includes a large number of medicinal aromatic plants. These plants are widely distributed through the tropical and warmer temperate regions of Asia, Africa, and Central and Southern America [15-17]. Due to their medicinal and economic importance, these plants are cultivated worldwide. The aromatic herbs, under- shrubs, and shrubs of genus *Ocimum*, including *O. kilimandscharicum*, produce essential oils (EOs) of different aromas and chemical profiles with tremendous value in pharmaceutical, perfume, and food industries [15, 18-21].

Ocimum kilimandscharicum is a perennial aromatic undershrub native to Central and East African countries, including Rwanda, Kenya, Tanzania, Uganda, Sudan, and Ethiopia [22]. It can be cultivated in flat and hilly areas. Due to its geographic distribution and abundance in African countries, *O. kilimandscharicum* is commonly called African blue basil. The plant has attracted attention due to its high camphor content and as an exotic plant.

Taxonomical classification of *Ocimum kilimandscharicum* Guerke
Kingdom: Plantae
Subkingdom: Tracheobionta
Division: Magnoliophyta
Subdivision: Spermatophyta
Class: Magnoliopsida
Subclass: Asteridae
Order: Lamiales
Family: Lamiaceae
Genus: Ocimum
Species: *O. kilimandscharicum* Guerke
Synonyms: *O. johnstonii* Baker , and *O. tortuosum* Baker.

Figure 1.
Ocimum kilimandscharicum Guerke.

O. kilimandscharicum Guerke is a valuable medicinal plant used both in traditional and modern medicine. The plant has neuro-protective [23], gastro-protective, digestive, antidiarrheal [24], carminative [25], antioxidant [26], antibacterial [26] antifungal [26], antipyretic [25], stimulant [25], anti-parasitic [27-29], wound-healing [30], and immunomodulatory [31] properties. The EO of *O. kilimandscharicum* is widely used in modern perfumery and the pharmaceutical industry. It is also used as a flavoring agent, in local application on sprains, in diarrheal medications, and in oral and dental preparations [25]. The EO has potent anti-oxidant [32, 33], anti-inflammatory [33], anti-cancer [33], and pesticidal qualities [34-36]. The variations in the chemical composition of EO of *O. kilimandscharicum* populations grown in different parts of the world have been widely investigated [37, 38]. The main components of the EO include camphor, 1, 8-cineol, α-pinene, linalool, limonene, eugenol, methyl chavicol, α- and β-bisabolene [39, 40]. The present study shows the Ultraviolet (UV) and Fourier Transform Infrared (FTIR) spectra of *O. kilimandscharicum* Guerke EO, which show very high amounts of eugenol.

2. Material and Methods

2.1. Plant Materials

The fresh leaves and flowers of *O. kilimandsharicum* were collected from the cultivated habitats in the medicinal and herbal garden of the Pharmacy Department of Lovely Professional University in February 2019. The fresh plant materials

were separated into two different parts. The first part was immediately processed for EO extraction, and the second part was labeled and stored in a refrigerator (–20°C) for future use.

2.2. Pharmacognostic Evaluation

In order to ensure the plant identity and prevent adulteration, the collected plant materials were subjected to pharmacognostic evaluation. *O. kilimandsharicum* is an evergreen, aromatic, perennial undershrub with simple ovate-oblong leaves, light purple or white-colored flowers, and ovoid-oblong black or brown-colored mucilaginous seeds. The EO is a light-yellow liquid with a strong odor of camphor (Bha12) (Figure 1). Histological studies of *O. kilimandsharicum* leaves were characterized by the presence of isobilateral lamina covered with a cuticle layer; glandular trichomes with multicellular heads containing essential oil (especially newly collected leaves); multicellular hair-covered trichomes; mid-rib arc-shaped vascular bundles consisting of xylem and phloem; and 3–4 layers of collenchymatous tissue present on the upper side of the vascular bundles (Figure 2).

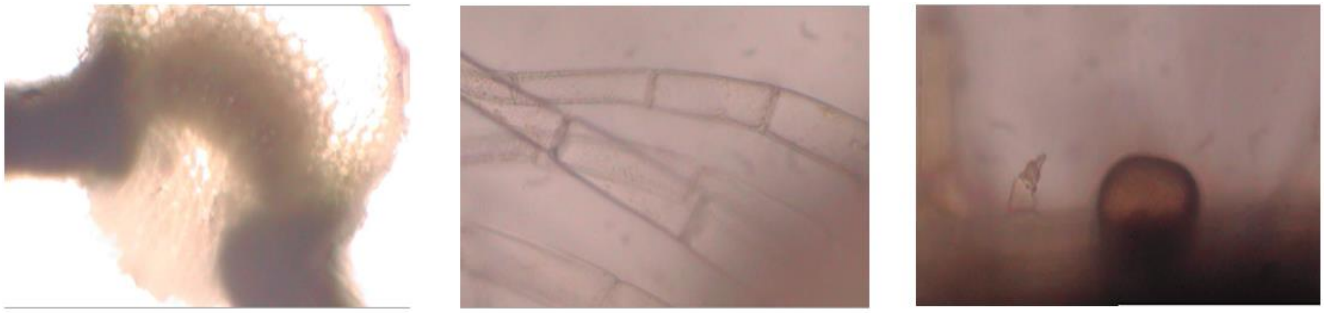

Figure 2.
Microscopic illustration of O. kilimandscharicum in 10X magnification A. leaf cross section, B. Hairy trichomes, and C. Glandular trichomes.

2.3. Essential oil Extraction

For the extraction of the EO, an accurately weighed amount of the plant material was subjected to hydrodistillation for 3 hours using a Clevenger-type apparatus, as previously described [26]. After completing the distillation process, the apparatus was allowed to cool and form clear yellow EO above the water inside the graded tube of the distillation apparatus. The condensed oil was collected and dried over anhydrous sodium sulfate and then stored in the dark at –20°C.

2.4. UV Analysis of Essential Oil

2.4.1. Preparation of Eugenol Stock Solution

The stock solution of eugenol (1 mg/ml) was prepared by dissolving the accurately measured eugenol in methanol. The standard working solutions of eugenol were diluted from prepared stock solutions. The sample solutions were prepared by diluting the EO in an appropriate solvent (methanol) at a ratio of 1:1200 [41]. To quantify the concentration of eugenol in the EO of *O. kilimandscharicum*, the eugenol standard curve was plotted (Figure 3).

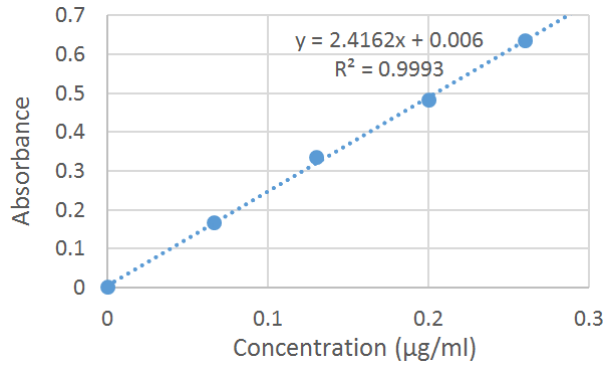

Figure 3.
Eugenol standard curve.

2.4.2. UV Scanning of Essential Oil

The molecular absorption Ultraviolet- visible (UV–Vis) spectra of the EO of plant materials were recorded in the 200–400nm region. A computer-operated spectrophotometer was used with a 1 cm optical path quartz cuvette. The data were acquired and processed using the software package (UV Probe, Ver. 2.61).). The same protocol was followed for the eugenol standards [42].

2.5. FTIR Analysis of Essential Oil

The FTIR spectra of the EO were acquired on a Shimadzu FTIR Spectrometer 8400S. The environmental conditions were maintained (25°C temperature and 30% humidity). The signal-to-noise ratio was 20000:1. Ten percent of the EO and reference chemicals and their specific solvents were deposited directly onto the Attenuated Total Reflection (ATR) sampling device without any treatment. The corresponding spectra were recorded in the range of 400–500 cm^{-1} with a resolution of 4 cm^{-1} and 12 scans for the sample and background. The velocity of the scan was 10 kHz with an interferogram size of 14,220 points. Before scanning each sample, the device was cleaned with 70% ethanol, and the background of air was taken. Shimadzu's IRsolution software was used to acquire spectra of samples and spectral manipulation [43, 44].

3. Result and Discussion

3.1. UV Analysis of Essential Oil

The UV spectra of standard eugenol and EO of *O. kilimandscharicum* are presented below (Figure 4 and Figure 5). In both spectra, eugenol has shown a peak absorbance at 282 nm. Based on the plotted standard curve of eugenol, the EO of *O. kilimandscharicum* contained 0.19 µg/ml of eugenol.

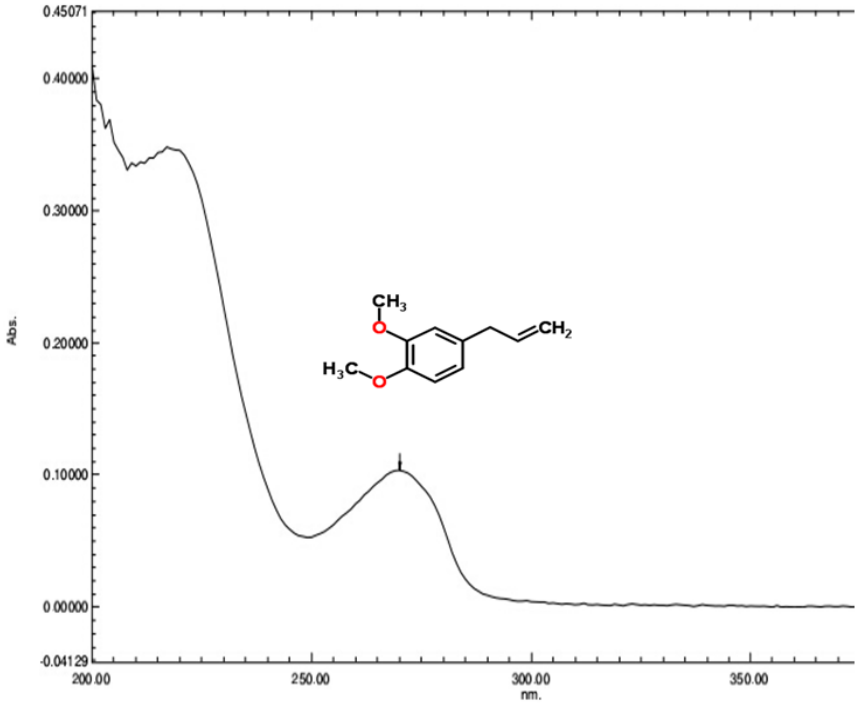

Figure 4.
The UV absorption spectrum of EO of O. kilimandscharicum.

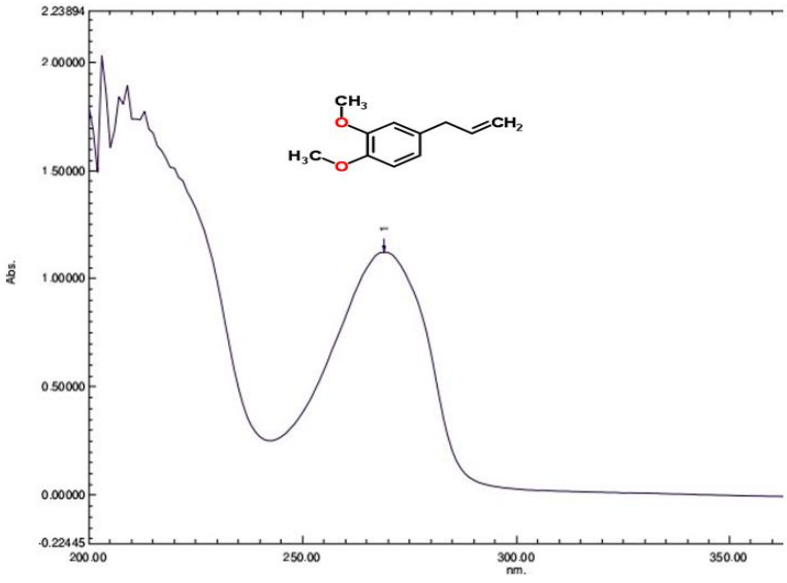

Figure 5.
The UV absorption spectrum of eugenol.

3.2. FTIR Analysis of Essential Oil

In this study, the FTIR absorption of EO, which was extracted from the leaves and flowers of *O. kilimandscharicum*, was investigated. The FTIR spectra of the EO and eugenol are shown below (Figure 6 and Figure 7). The presence of different functional groups was determined by comparing the vibration frequencies in wave numbers of the EO and eugenol spectra with those of an IR correlation chart (Table 1).

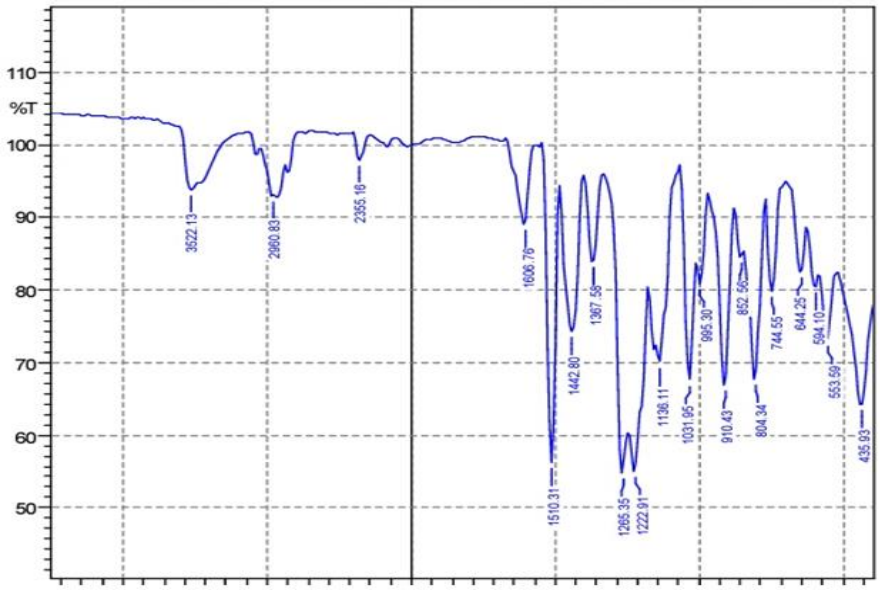

Figure 6.
FTIR spectrum of EO of O. kilimandscharicum.

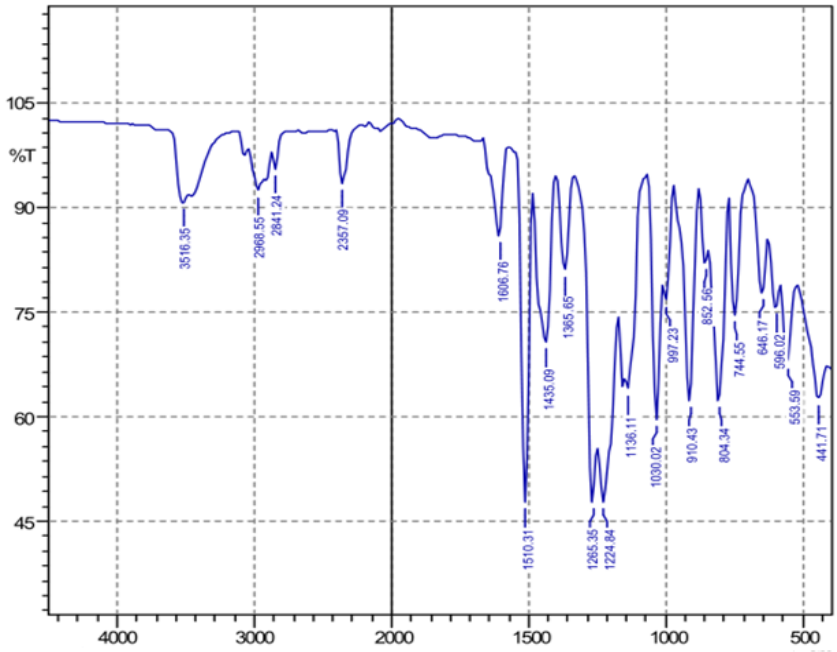

Figure 7.
FTIR spectrum of Eugenol.

Based on the recorded FTIR spectra, eugenol showed its signature peaks at 2968.55 cm^{-1}, 2841.24 cm^{-1}, 1435.09 cm^{-1}, and 441.71 cm^{-1}, which were completely and/or partially different from the one of EO, but the other peaks were the same for both the eugenol and the EO. In the FTIR absorption spectra of EO and eugenol, the occurrence of peaks in the region 3522.13 cm^{-1} and 3516.35 cm^{-1} indicate the presence of hydroxyl groups, which may correspond to alcohols. The presence of peaks within the regions 2968.55 cm^{-1} (C–H), 2355.16 cm^{-1}, 2960.83 cm^{-1}, 2357.09 cm^{-1}, 2841.24 cm^{-1}, 1367.58 cm^{-1}, 1442.8 cm^{-1}, 1365.65 cm^{-1}, 1435.09 cm^{-1} (CH3 group) plus a band at 744.55 cm^{-1}(-CH2 group), and 644.25 cm^{-1}–646.17 cm^{-1} is indicative of saturated aliphatic structures. The sharp peaks observed in the regions 1606.76 cm^{-1} and 1510.31 cm^{-1} may show the stretching of C=C of the aromatic moiety. The existence of halogenated aliphatic compounds may easily be determined from the peaks within the regions 553.59 cm^{-1}, 594.1 cm^{-1}, and 596.02 cm^{-1} [45].

Although the EO of most of the species of genus Ocimum, including *O. kilimandscharicum,* are valuable sources of eugenol, the main phytoconstituents for which they are known may vary [46, 47]. This phytochemical polymorphism even exists within the same plant populations [26, 38].

There could be several reasons for the profile polymorphism of *O. kilimandscharicum* EO viz, climate [48] or environmental factors [49-51], geographical zones [52-54], season [55-57], plant ontogeny [58, 59], harvesting time [60], plant chemo-type [26], stress factors [61, 62], genetic variability [26], etc.

Table 1.
IR correction chart.

Frequencies on FTIR Spectra (1/cm)		Assignment of bonds	Mode of Vibration
O. kilimandscharicum oil	**Eugenol**		
3522.13	3516.35	O-H	Stretching
-	2968.55	C-H (hydrocarbons)	Stretching
2355.16, 2960.83	2357.09, 2841.24	CH_3	Stretching
1606.76	1606.76	C=C	Stretching
1510.31	1510.31	C=C	Stretching
1367.58, 1442.8	1365.65, 1435.09	C-H (methyl)	Rocking
1265.35	1265.35	C-O/C-N	Stretching
1222.91	1224.84	C-N	Stretching
1030.95, 1136.11	1030.02, 1136.11	C-O/C-N	Stretching
995.3	997.23	=C-H	Bending
910.43	910.43	=C-H	Bending
804.34, 852.56	804.34, 852.56	C-H	Oop
744.55	744.55	-C-H (methylene)	Rocking
644.25	646.17	C-H	Bending
553.59, 594.1	553.59, 596.02	C-X	Stretching
435.93	441.71		

Several physicochemical techniques help assess and quantify the chemical fingerprint of EOs and their main phytoconstituents [63]. The UV and FTIR spectrometry techniques are two examples of tools currently used for these purposes [64, 65].

After identifying the main components of the EO, the UV spectrum could be used as a valuable tool for comparing and quantifying the desired phytoconstituents of the EO with the available standard. In this study, eugenol showed its peak absorbance around 282 nm in both the EO and pure eugenol spectra. Additionally, the EO contained 0.19 µg/ml of eugenol. Moreover, the availability of the FTIR fingerprint of every single chemotype would assist researchers in classifying the populations of a plant in specific clusters, based on the main phytoconstituents, and assess the purity of their EOs.

4. Conclusion

Ocimum kilimandscharicum is an important medicinal plant of the genus Ocimum, which shows a high level of phytochemical polymorphism. The EO of different plant populations possesses various types of phytoconstituents of medicinal and economic value. Eugenol is one of these components present in high amounts in the EO of *O. kilimandscharicum.* The UV and FTIR spectra of the EO confirmed this as the eugenol showed peak absorption at 282 nm in the UV region and an intense vibration frequency in the FTIR spectrum obtained. Thus, the UV and FTIR fingerprint of EOs may consider as a rapid and reliable tool for the preliminary assessment and quantification of different phytoconstituents in the EOs.

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