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Stem volatile oils composition of *Ocimum basilicum* L. cultivars and *Ocimum gratissimum* L. from Nigeria

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Abstract
The stems essential oils of *Ocimum basilicum* L. (Napoletano and sweet basil cultivars) and *O. gratissimum* L. (Lamiaceae) were analyzed for composition by gas chromatography-mass spectrometry (GC-MS). A total of thirty-four constituents were identified. Estragole (59.6% and 57.4%) dominated the *O. basilicum* stem oils; β-caryophyllene (13.9%) and β-bisabolene (15.7%) were also prominent components in the sweet basil oil. The main components of *O. gratissimum* stem oil comprised of β-carophyllene (15.3%), thymol (12.9%) and γ-terpinene (11.7%). Distinct chemical profiles were obtained for the volatile oils of *O. basilicum* and *O. gratissimum* species; however the oils of *O. basilicum* cultivars showed strong chemical similarity.

Keywords: Lamiaceae, *Ocimum basilicum*, *Ocimum gratissimum*, essential oils composition, estragole

1. Introduction

The genus *Ocimum* comprises about 150 species of herbs and shrubs from the tropical regions of Asia, Africa, Central and South America; and is considered as one of the largest genera of the Lamiaceae family [1]. The common *O. basilicum* L. is known as sweet basil, whereas *O. gratissimum* L. is referred as clove basil. There are many cultivars of basil which vary in their leaf color (green or purple), size (broad or narrow), flower color (white, red, purple) and aroma [2]. *Ocimum*s pecies contain a wide range of essential oils rich in phenolic compounds and an array of other natural products, including polyphenols, such as flavonoids and anthocyanins [3].*Ocimum* is used in food and confectionery as spices, and employed in the treatment of gastrointestinal infections and conjunctivitis, headaches and coughs [4].

The chemical composition of basil leaf oils have been the subject of considerable studies. There exist the problem of significant variation in the content of *Ocimum* plants across and within species, with implication of varied biological activities. Therefore, several chemotypes have been established from various phytochemical investigations, which include, estragole (methyl chavicol), linalool, methyl cinnamate, methyleugenol, eugenol and geraniol as major components of the oils of different chemotypes of *O. basilicum* [5-7]. Lawrence [8] found that the main constituents of the volatile oil of basil are synthesized via two distinct biochemical pathways: phenylpropanoids, likechavicol, eugenol and methyl eugenol (via the shikimic acid pathway), and terpenes, such as linalool, by the cytosolic mevalonic acid pathway. The occurrence of the eugenol and thymolchemotypes of *O. gratissimum* reported by Terezinha *et al.* [9] and Iwalokun *et al.* [10], respectively, were at variance with the geraniol-rich chemotype from Purdue [11]. Vieira *et al.* [12] also showed thateugenol, thymol, and geraniol were major constituents of twelve *O. gratissimum* accessions.

All parts of *O. basilicum* and *O. gratissmum* are characterized by strong aromatic fragrance when crushed, and hence the interest to investigate their essential oils. Thorough literature search has proved the paucity of essential oil composition of *O. basilicum* stems [13, 14], and there is no report on *O. gratissimum*. As an integral part of our systematic evaluation of the poorly studied aromatic flora of Nigeria [15], we here report the identification of volatile constituents of *O. basilicum* and *O. gratissimum* stems.

2. Materials and Methods

2.1 Plant Sample

The cultivated plant samples (Napoletano and sweet basil cultivars, and *O. gratissimum*) were collected from farms in Uyo Local Government Area, Akwa Ibom State, Nigeria, in the month of May 2017. The Samples were identified by a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, where voucherspecimens were deposited. The essential oils were obtained by hydrodistillation (4 h) of the fresh stems using a Clevenger-type apparatus in accordance with the British Pharmacopoeia [16]. The oils were dried over sodium sulfate and stored in refrigeration (4 °C) immediately after estimation of percentage yield.

2.2. Gas chromatography-mass spectrometry (GC–MS)

The volatile oils were subjected to GC-MS analysis on an Agilent system consisting of a model
7890 N gas chromatograph, a model mass detector Triple Quad 7000 A in EI mode at 70 eV (m/z range 40–600 amu) (Agilent Technologies, Santa Clara, CA, USA), and an Agilent Chem Stationdata system. The GC column was an HP-5 ms fused silica capillary with a (5% phenyl)-methyl polysiloxanestationary phase (30 m x 250 µm x 0.25 µm). The carrier gas was helium with a column head pressure of 9.7853 psi and flow rate of 1.2 mL/min. Inlet temperature and MSD detector temperature was 250 °C. The GC oven temperature program was used as follows: 50 °C initial temperature held for 5 min; increased at 6 °C/min to 190 °C for 20 min; increased 7 °C/min to 290 °C for 15 min; increased 7 °C/min to 300 °C for 10 mins. The sample was dissolved in dichloromethane, and 2 µL was injected (split ratio 10:1; split flow 12 mL/min).
The components were identified by comparison of their mass spectra with NIST 1998 library data of the GC-MS system as well as by comparison of their retention indices (RI) with the relevant literature data [17]. The relative amount of each individual component of the essential oil was expressed as the percentage of the peak area relative to the total peak area. RI value of each component was determined relative to the retention times of a homologous n-alkane series with linear interpolation on the HP-5 ms column.

3. Results & Discussion

The volatile constituents of *O. basilicum* cultivars and *O. gratissimum* stems are presented in Table 1. The yellow oils from the Napoletano, sweet basil, and *O. gratissimum* stems afforded yields of 0.13%, 0.12%, and 0.10% (v/w) respectively; a total of thirty-four oils' components were identified: sixteen (16), ten (10), and twenty two (22) constituents accounted for 97.3%, 96.3%, and 70.4% respectively. Estragole, β-elemene, and β-caryophyllene were common constituents of the three stem essential oils. Estragole (59.63% and 57.37%) was predominant in the *O. basilicum* stem oils, though β-caryophyllene (13.93%) and β-bisabolene (15.65%) were also identified as major components of sweet basil oil. The main compounds in *O. gratissimum* stem oil comprised of β-carophyllene (15.27%), thymol (12.87%), and γ-terpinene (11.74%). Generally, eachoil displayed a unique chemical profile, which differed both quantitatively and qualitatively. However, distinct chemical profiles were obtained for the volatile oils of *O. basilicum* and *O. gratissimum* species; *O. basilicum* cultivars showed strong chemical similarity. Estragole was representative of the phenylpropanoid class in *O. basilicum* stem oils; in *O. gratissimum* oil, thymol was the principal component of the oxygenated monoterpenoid, while γ-terpinene and β-caryophyllene respectively prevailed among the monoterpene and sesquiterpene hydrocarbons. The percentage compositions of classes of compounds in Napoletano, sweet basil, and *O. gratissimum* oils, respectively, were indicated as such: monoterpene hydrocarbon (0.0, 0.0 and 22.7%), oxygenated monoterpenes (11.9, 1.1 and 13.9%), sesquiterpene hydrocarbons (21.4, 35.3 and 26.1%), oxygenated sesquiterpenes (4.3, 2.4 and 4.0%), and phenyl propanoids (59.6, 57.4, and 3.8%). The hydrocarbon contents were relatively abundant in *O. gratissimum* (48.8%) compared with the Napoletano and sweet basil cultivars (21.4 and 35.3% respectively).
In contrast, Chalchat and Ozcan [13] reported high levels of dill apiole (50.07%) in *O. basilicum* stem oil, though with a relative low occurrence of estragole (15.91%). Likewise, linalool (33.89%), (*E*)-methyl cinnamate (8.94%), and β-cadinene (8.9%) were identified as major volatile constituents of stem oil of sweet basil 'cinnamon cultivar' [14].

Table 1: Composition of essential oils of *O. basilicum* cultivars and *O. gratissimum* stems

Compound	LRI[a]	Relative abundance (%)[b,c]			QI[d]
		Genovese basil	Sweet basil	*O. gratissimum*	
α-Thujene	933	-	-	1.0	91
α-Pinene	941	-	-	0.3	91
β-Pinene	982	-	-	1.5	93
p-Cymene	1028	-	-	6.2	95
1,8-Cineole	1034	5.9	1.1	-	96
γ-Terpinene	1063	-	-	11.7	95
Dehydro-*p*-cymene	1088	-	-	0.4	94
Terpinolene	1090	-	-	1.6	93
Linalool	1101	3.6	-	0.4	95
Camphor	1145	1.2	-	-	95
4-Terpineol	1179	-	-	0.6	93
α-Terpineol	1191	1.2	-	-	94
Sylvestrene	1194	-	-	0.4	91
Estragole (Methyl chavicol)	1196	59.6	57.4	3.8	97
Thymol	1292	-	-	12.9	93
α-Copaene	1377	-	-	1.3	94
β-Cubebene	1391	-	-	0.8	91
β-Elemene	1392	1.2	0.6	0.3	92
α-Bergamotene	1415	4.1	-	-	96
β-Caryophyllene	1419	5.6	14.0	15.3	97
γ-Gurjunene	1478	0.9	-	-	91
Germacrene D	1482	1.4	-	-	93

β-Selinene	1487	-	3.0	4.9	95
α-Selinene	1495	-	2.2	1.5	93
β-Bisabolene	1508	4.1	15.7	-	93
γ-Cadinene	1514	1.0	-	-	95
δ-Cadinene	1524	-	-	1.2	93
β-Sesquiphellandrene	1525	1.3	-	-	90
α -Humulene	1564	1.9	-	-	90
(*E*)-Nerolidol	1566	-	-	0.7	95
Caryophyllene oxide	1582	-	0.6	2.9	93
τ-Cadinol	1641	3.5	0.6	-	91
β-Eudesmol	1650	0.8	1.1	-	95
Aromadendrene oxide	1652	-	-	0.4	92
Total identified		97.3	96.3	70.4	

[a]LRI, Linear retention indices;[b]Order of elution on HP-5ms capillary column; [c]Identification by comparison of the mass spectral and retention index data; [d]QI, 'quality index', reflects the fit comparison of experimental mass spectrum and NIST library mass spectrum; - = not detected.

4. Conclusions

The essential oil constituents of *O. basilicum* cultivars and *O. gratissimum* stems have been identified. Estragole occurred as the principal component in *O. basilicum* oils, whereas β-caryophyllene, thymol, and γ-terpinene dominated the *O. gratissimum* oil.

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