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Essential Oil Chemotypes of Three Ocimum Species Found in Sierra Leone and Nigeria

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Abstract

Chemical composition of the essential oil of *Ocimum basilicum* L., *O. gratissimum* L. and *O. americanum* L. varieties found in Sierra Leone and Nigeria were determined by GC-FID and GC-MS. The antimicrobial test was evaluated by agar diffusion. The Sierra Leone and Nigeria *O. americanum* L. varieties were identified as the linalool chemotype and similarly, varieties of *O. gratissimum* L. from both countries are thymol chemotype. The high thymol content is consistent with the relative high antimicrobial activity of the *O. gratissimum* L oils. The Sierra Leone *O. basilicum* L. sample was established as the methyl eugenol chemotype while the Nigerian collection is predominantly methyl chavicol in composition. The high methyl eugenol content of the Sierra Leone collection is consistent with the observation of attraction of fruit flies to the distillate. To the best of our knowledge, there has been no report on essential oils of Ocimum species in Sierra Leone, hitherto.

Keywords: Chemotypes; Ocimum species; *O. basilicum* L; Lamiaceae

Introduction

The family Lamiaceae of which the genus Ocimum belongs is composed of diverse and rich source of essential oil containing plants. Ocimum contains between fifty to one hundred and fifty species of herbs and shrubs from the tropical regions of Asia, Africa, and Central and South America [1]. *O. gratissimum* L. is referred to as holy basil. In Sierra Leone, it is known as "Tea bush" (Krio) and "orgbethor" (Themne) while it is known as "efirin nla" in the Yoruba speaking culture of Nigeria. *O. basilicum* L. is also referred to as sweet basil. It is known as "Patmenji" (Krio) and "Sorow" (Themne) of Sierra Leone and "efinrin wewe" by the Yorubas. *O. americanum* L. (synonym of O. canum Sims) [2,3] is known as American basil or hoary basil. In Sierra Leone both the available varieties of *O. americanum* L and *O. basilicum* L. are used in culinary as "Patmengy" indiscriminately. The different varieties of *O. gratissimum* L. [implying different chemotypes] have been used extensively in the traditional system of medicine in many countries as had been reviewed [4-10]. The main issues of concern with the use of herbal drugs remain safety, validation of claims and standardisation of product. There exist the problems of significant variation in the content of Ocimum plants across and within species, with implication of varied biological activities. In spite of the popular use of the genus Ocimum in food and confectionery as spices and application in the treatment of gastrointestinal infections and conjunctivitis in Sierra Leone, the varieties of the plant growing in Sierra Leone have not been, hitherto, investigated. The present study therefore determined the essential oil constituents of the Ocimum species collected in Freetown, Sierra Leone compared with varieties growing in Ile-Ife, Nigeria in order to establish the specific chemotypes in the specific regions.

Experimental Materials

Plant materials

Aerial parts of cultivated *O. basilicum* (OBS), *O. gratissimum* (OGS) and *O. americanum* (OAS) at full flowering stage were collected from Krootown Road, Central Freetown, Sierra Leone while the Nigerian varieties *O. basilicum* (OBN), *O. gratissimum* (OGN) and *O. americanum* (OAN) were collected in Ile-Ife, Osun- State, Nigeria. The plants were identified at the Department of Botany, Faculty of Sciences, OAU, Ile-Ife, Nigeria where Voucher specimens were deposited.

Extraction of essential oils

Fresh or air-dried leaves of the plants were subjected to hydrodistillation using a Clevenger-type apparatus for 4 hours. The volatile oils were dried over anhydrous magnesium sulphate and stored in amber sealed vials at 4°C until analysis.

Analysis of the essential oils

The GC-MS analyses of the essential oils were carried out on Agilent 6890N coupled to a quadrupole MSD 5973. Column: DB-5MS (5% phenyl 95% dimethylarylene siloxane) 30 m × 0.25 mm × 1 mm film thickness (Agilent). Temperature program: from 50˚C (5 min) to 200˚C (10 min) at 2˚C/min. Injection temperature: 25˚C. Injection volume: 1.0 µL. Inlet pressure: 10.3 kPa PSI. Carrier gas: He, Linear velocity: 40 cm/sec. Injection mode: split (10:1). MS interface temp.:

230˚C; MS mode: EI at 70 eV; mass range: 30-400. The oil sample was dissolved in dichloromethane before injection.

The relative compositions of the essential oils were determined on Agilent 7890B coupled to FID and an autoinjector (Agilent G45138). Column: 19091J-413 HP-5 (5% phenyl methyl siloxane) 30 m × 0.32 mm × 0.25 mm film thickness (Agilent). Temperature program: from 50˚C (5 min) to 200˚C (10 min) at 2˚C/min. Injection temperature: 250˚C. Injection volume: 1.0 µL. Inlet pressure: 66.7 kPa. Carrier gas: He, Linear velocity: 40 cm/sec. Injection mode: split (50:1). FID temp.: 230˚C; H2 flow: 40 mL/min; air flow: 400 mL/min. The oil sample was dissolved in dichloromethane before injection.

Identification of the compounds

Compounds were identified by their retention indices determined using C8-C40 alkane standards and compared with literature data [11]. The identities were confirmed by the mass spectral data observed (Table 1) supported with library (National Institute of Standards and Technology).

NO.	m/z (abundance)	Compound
1	m/z 134 (35), M+. ; 119 (100); 91 (20)	p-Cymene
2	m/z 154 (100), M+. ; 139 (70); 43 (75)	1,8-Cineole (Eucalyptol)
3	m/z 136 (50), M+. ; 121 (48); 93 (100); 91 (60)	γ-Terpinene
4	m/z 152 (10), M+. ; 137 (2); 81 (100), 69 (46)	L-Fenchone
5.	m/z 154 (1), M+. ; 139 (3); 136 (20). ; 121(40); 93 (100); 71 (95)	Linalool
6.	m/z 152 (50), M+. ; 108 (40); 95 (100)	Camphor
7	m/z 154 (40), M+. ; 136 (20); 111 (90); 93 (75), 71 (100)	Terpinen-4-ol
8.	m/z 148 (100), M+.; 133 (20); 117 (30)	Methyl chavicol (Estragole)
9.	m/z 150(35), M+. ; 135 (100); 115 (12.5)	Thymol
10.	(m/z 164 (100), M+. ; 149 (30)	Eugenol
11	m/z 178 (100), M+. ; 163 (28); 147 (28)	Methyl eugenol
12	m/z 204 (12), M+. ; 189 (24); 161 (45); 133 (100)	β-Caryophylene (E)
13	m/z 204 (3), M+. ; 189 (5); 119 (100); 93 (82)	α-Bergamotene (trans)
14	m/z 204 (45), M+. ; 189 (45); 161 (49); 93 (47); 28 (100)	β-Selinene (β-Eudesmene)
15	m/z 204 (60), M+. ; 189 (100); 161 (37); 133 (45)	α-Selinene
16	m/z 220 (3), M+. ; 205 (10); 177 (23); 93 (90), 79 (100), 43 (70).	Caryophyllene oxide
17	m/z 222 (2), M+. ; 204 (36); 189 (10); 179 (10); 161 (70); 95 (100)	α-Muurolol
18.	m/z 204 (48); 189 (20); 161 (100); 119 (75)	α-Cadinol

Table 1: Observed Mass spectral features of the prominent components of the essential oils of *Ocimum* plants from Sierra Leone and Nigeria.

Antimicrobial Tests

Overnight broth cultures of the test organisms were diluted to give cell suspensions of 10^6 -10^7 CFU which were used to surface-inoculate nutrient agar (Oxoid, London) (bacteria) and Sabouraud dextrose agar (Oxoid, London) (*C. albicans*). The antimicrobial activity tests of the oils were performed using paper disc method with Whatman No. 1 paper of size 6 mm which were each saturated with the test neat essential oil and subsequently placed on the surface of the inoculated plates [12]. The plates were kept at 4˚C for 1 h to allow for diffusion before subsequent incubation at 37˚C for 24 h for bacteria and 25˚C for 48 h for *C. albicans*. The reference antibacterial agents were Streptomycin (1 mg/ml) or acriflavine (1%). The tests were carried out in duplicate and the average zones of inhibition were determined.

Results and Discussion

The yields of the essential oils from the six plants are 0.34% (OAS), 0.98% (OAN), 0.71% (OBS), 0.96% (OBN), 0.93% (OGS) and 0.84% (OGN). The results of the antimicrobial test of the oils are presented below. The compounds identified in the different oil samples are presented in Table 2.

The chemotyping of *Ocimum* plants had been defined in terms of the combination of all major components constituting more than 20% rather than a dominant single component of the essential oil of the plant [13]. In this respect, both the Sierra Leone and Nigeria *O. americanum* L. leaf materials were thus identified as the linalool chemotype, constituting 49.1% and 39.6% respectively, in the present study. This chemotype of *O. americanum* L. is consistent with those previously reported in Benin [14], Rwanda [15], Cameroon [6] and Brazil [16]. The high content of linalool in some varieties of *O.*

basilicum characterises the varieties as the best grade of commercial sweet basil for culinary use [17,18].

% Composition of Sierra Leone collections			% Composition of Nigeria collections			Compounds	Retention index
O. ame*	O. basil*	O. grat*	O. ame	O. basil	O. grat		
-	-	-	Trace	-	0.5	3-Hexen-1-ol	855.8
Trace	-	-	Trace	Trace	2.5	α-Thujene	924.1
Trace	-	Trace	0.2	Trace	0.7	α-Pinene	928.7
-	-	-	-	-	0.2	Camphene	940.9
-	-	-	-	-	0.5	Sabinene	967.1
Trace	-	-	Trace	0.3	1.9	β-Pinene	969.5
Trace	-	Trace	Trace	-	1.8	β-Myrcene	993.5
-	-	-	-	-	0.2	α-Phellandrene	1001.4
-	-	-	-	-	0.2	(+)-3-Carene	1006.7
Trace	-	Trace	0.2	0.5	1.9	α-Terpinene	1014.5
2.5	-	1.5	0.5	-	4.1	p-Cymene	1022.2
-	-	-	-	-	0.6	D-Limonene	1030.7
0.7	0.6	-	2.4	4.3	-	1,8-Cineole (Eucalyptol)	1033.3
-	-	-		-	0.5	β-Ocimene (Z)	1039.2
			1.5		0.2	β-Ocimene (E)	1049.1
1.2	-	0.8	0.5	-	10.8	Y-Terpinene	1058.3
0.6	-	0.8	-	-	-	Cis-Sabinene hydrate	1065.1
-	-	-	2.3	-	-	L-Fenchone	1084.8
-	-	-	-	-	0.2	Terpinolene	1085.7
-	-	-	-	-	1.2	P-Cymenene	1089.9
49.1	0.6	10.3	39.6	-	0.4	Linalool	1102.7
-	0.6	-	-	-	-	Fenchol (endo)	1120.1
10.4	-	5.7	0.2	-	-	Camphor	1144.7
6.3	0.8	3.2	7.5	0.1	1.3	Terpinen-4-ol	1175.1
0.7	-	-	0.5	-	-	α- Terpineol	1184.4
-	0.4	-	-	89.8	-	Estragole (Methyl chavicol)	1197.4
0.5	-	0.9	-	Trace	1.0	Methyl thymol	1235.0
-	-	-	0.8	-	-	Geraniol	1260.1
-	-	-	-	-	0.7	Unidentified	1270.8
-	-	-	0.2	-	-	Bornyl acetate	1283.3
5.6	-	60.5	-	-	42.2	Thymol	1294.4
-	-	1.7	-	-	0.6	Carvacrol	1300.5
1.0	-	-	-	-	-	Terpinen-4-ol acetate	1301.1

-	-	-	0.2	-	-	Unidentified	1329.7
3.4	-	-	18.0	-	0.5	Eugenol	1354.8
-	-	-	1.9	0.2	-	β-Elemene	1391.0
1.1	89.7	0.9	0.4	-	-	Methyl eugenol	1402.1
1.7	-	3.1	-	1.2	1.5	β-Caryophyllene (E)	1419.9
2.4	-	-	6.6	-	0.3	α-Bergamotene (trans)	1436.6
0.7	-	-	-	-	0.5	Humulene (α-Caryophyllene)	1453.4
-	-	-	0.5	-	-	(E) - β-Farnesene	1457.4
-	-	-	1.0	-	-	Y-Muurolene	1475.5
-	-	-	0.7	-	-	Germacrene D	1480.3
-	-	-	0.44	-	-	Bicyclogermacrene	1489.5
0.7	-	3.6	-	-	7.1	β-Selinene	1491.0
-	-	1.3	-	-	2.5	α -Selinene	1496.0
-	-	1.4	-	-	0.9	Unidentified	1499.5
-	-	-	0.3	-	-	α-Bulnesene	1507.8
1.3	0.9	-	0.9	-	1.7	Y-Cadinene	1509.6
-	-	-	-	-	1.2	α-selinene-7-epi	1515.4
-	-	-	0.2	-	0.2	δ-Cadinene	1519.1
-	-	-	0.4	-	-	β-Sesquiphellandrene	1521.4
-	0.9	-	0.3	-	-	Spathulenol	1578.5
0.6	0.9	3.8	-	-	3.2	Caryophyllene oxide	1583.1
-	-	-	-	-	0.6	Humulene epoxide II	1604.7
-	-	-	0.6	-	-	Unidentified	1609.4
1.2	0.7	-	-	-	-	Cubenol	1635.2
-	-	-	3.57	-	-	α-Muurolol	1641.1
7.7	3.3	-	2.3	-	-	α-Cadinol	1651.9
99.4 %	99.4 %	99.5%	95.7 %	96.4 %	94.3 %	TOTAL COMPOSITION	

Table 2: The composition of essential oils of Ocimum species collected from Sierra Leone and Nigeria. **Note:** *O. ame- O. americanum*, *O. basil. - O. basilicum*, *O. grat- O. gratissimum* *essential oil from air-dried leaves.

The Sierra Leone *O. basilicum* examined in the present study was clearly identified as the methyl eugenol chemotype (89.7%). This chemotype had been reported in Togo [19] and Turkey [20]. Methyl eugenol has been known in literature as a powerful insect attractant, [7,21-23], known to attract fruit flies from a distance as far as 0.8 km [7]. In the present study, attracted dead fruit insects (*Drosophilia melanogaster*) were found in the waste distillate (aqueous portion) drained into a bowl and left overnight. This observation underscores the potential use of methyl eugenol, or even this chemotype of *O. basilicum* co-formulated with an insecticide, as a powerful insecticidal product. On the other hand, the essential oil of the Nigerian *O. basilicum* variety was the methyl chavicol (89.8%) chemotype.

Both the Sierra Leone and Nigerian *O. gratissimum* L. varieties examined in this study are identified as the thymol chemotype having 60.5% and 42.2% thymol content respectively. This chemotype is widespread in West Africa (Nigeria [24], Cameroon [25], Togo [26] and Sao Tome [27]) although the presence of a Thymol-Cymene-Terpinene chemotype has been reported from the sub-region (Benin [14]). While *O. gratissimum* L. is the commercial source of eugenol in India [18,28], this component was not detected in the present material. The second most abundant constituent of Sierra Leone variety of L. is linalool (10.3%) which is a minor component in the Nigerian variety [0.4%] while the second most abundant constituent of the Nigerian variety is γ-terpinene (10.8%) which is only a minor component of the

Sierra Leone variety (0.8%). Caryophyllene oxide constitutes a prominent sesquiterpene in both varieties of *O. gratissimum* L. The predominant thymol content probably explains the superior antibacterial activities of both *O. gratissimum* collections in the antimicrobial test (Table 3).

Microorganism	OAN	OBS	OBN	OGS	OGN	Streptomycin	Acriflavine
S. aureus (NCTC 6571)	13	17	8.5	44	48	23	Nt
B. subtilis (NCTC 8236)	16	Nt	R	Nt	42	26	Nt
E. coli (ATCC 25922)	11.5	Nt	8	24	27	15	Nt
Ps. aeruginosa (ATCC 10145)	R	Nt	R	Nt	10	R	Nt
C. albicans	27	Nt	10	Nt	44	Nt	8

Table 3: The zones of inhibition (mm) of essential oils from Ocimum species collected from Sierra Leone and Nigeria (average of duplicate) compared with reference standards. Note: R=completely resistant, nt=not tested; OAN=*O. americanum* (Nigeria); OBS=*O. basilicum* (Sierra Leone); OBN=*O. basilicum* (Nigeria); OGS=*O. gratissimum* (Sierra Leone) and OGN=*O. gratissimum* (Nigeria).

Conclusion

Both Sierra Leone and Nigeria *O. americanum* L. varieties were identified as linalool chemotype. The Sierra Leone *O. basilicum* L. variety is the methyl eugenol chemotype, while the Nigerian variety is predominantly methyl chavicol. Both varieties of *O. gratissimum* L collected from Sierra Leone and Nigeria are of thymol chemotype.

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