

CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF FRUITS ESSENTIAL OIL OF *Ocimum gratissimum* (Linn). GROWN IN NORTH CENTRAL NIGERIA

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ABSTRACT

Fresh pulverized fruit of *Ocimum gratissimum* (Linn) was hydrodistilled to afford 0.5% v/w oil. The oil was analyzed by GC and GC-MS. The essential oil is characterized by high abundance of eugenol (53.7%), suspected to be principally responsible for the biological activity observed. Other principal constituents were 1,8-cineole (16.9%), β -caryophyllene (5.7%), allo-ocimene (5.5%), γ -muurolene (5.0), γ -humulene (4.8%) and β -selinene (3.2%). The *O. gratissimum* fruit oil was active against most of the tested bacteria: *B. megaterium*, *S. epidermidis*, *E. coli* with MIC of 25mgmol⁻¹

Keywords: *Ocimum gratissimum*, fruits, GC-MS, Antibacterial

INTRODUCTION

Scent plant *Ocimum gratissimum* L. (Lamiaceae) is an indigenous perennial shrub. It is about 1.9m in height with branched stems. It is also known as clove Basil or African Basil. *O. gratissimum* is common in tropical areas particularly India. It is also found in many parts of West Africa. In Nigeria, it is found on the savannah and Coastal areas. It is cultivated in Ceylon, South Sea lands and also within Nepal, Bengal, Chittagong and Deccan (Nadkarni, 1999). It is known by various names in different parts of the world. In India, it is called by its several vernacular names such as Vriddhutulsi (Sanskrit), Ram tulsi (Hindi), Nimma tulasi (Kannada) and in Nigeria, the plant is called "Effrinrinla" (Yoruba), "Ahuji" (Igbo) and "Daidoya" (Hausa). The plant is distinct for its phytochemical constituents which is of medicinal importance and has been exploited in traditional and clinical applications (Effrain *et al.*, 2003).

O. gratissimum has been used extensively in traditional medicine system in many countries. In the North East of Brazil, it is used for medicinal, condiment and culinary purposes. The flowers and the leaves of this plant are rich in essential oils, so it is used in preparation of tea and infusion (Rabelo *et al.*, 2003). In India, the whole plant has been used for the treatment of sunstroke, headache, influenza, as a diaphoretics, antipyretic and for its anti-inflammatory activity (Oliver *et al.*, 1980).

The plant is commonly used in folk medicine to treat different diseases such as upper respiratory tract infections, diarrhoea, headache, pneumonia, cold and catarrh, conjunctivitis, fever and diseases of the eye and skin (Adebolu and Salau, 2005).

O. gratissimum essential oils and extracts have been demonstrated as possessing antibacterial, antifungal, anti-diarrohea and anti-malarial properties (Offiah and Chikwendu, 1999; Saliu *et al.*, 2011; Terezinha *et al.*, 2006; Tchoumboungang *et al.*, 2005). It also possesses antioxidant (Akinmoladun *et al.*, 2007), antidiabetic (Mohammed *et al.*, 2007), anthelmintic (Pessoa *et al.*, 2002), anti-inflammatory (Sahouo *et al.*, 2003) and leishmania activity (Tania *et al.*, 2006).

The phytochemical constituents of *O. gratissimum* has also been investigated by various authors. Many active compounds have been isolated from the leaves and seeds such as engenol, bisaboline, thymol, and 1,8-cincole (Dubey *et al.*, 1997; Matasyoh *et al.*, 2007).

In continuation of our systematic study of the essential oils of Nigerian medicinal plants, we investigated the constituents of fruits essential oil of *O.gratissimum* and the antibacterial activity. To the best of our knowledge this is the first report on the fruits essential oil constituents of *O. gratissimum*, although many works has been carried out on the leaves and flowers.

MATERIALS AND METHODS

Plant Material

The fresh fruits of *O.gratissimum* were obtained in Ilorin town, Kwara State, North-Central Nigeria. Identification was carried out at the herbarium of Forestry Research Institute of Nigeria (FRIN) Ibadan, where voucher specimens were deposited. (Herbarium Voucher Number FHI 105250).

Essential Oil Isolation

Pulverized fruits were hydro distilled for 3 hrs in a Clevenger-type apparatus, according to the British Pharmacopoeia (1980) specifications. The resulting oil was collected, preserved in a sealed sample tube and stored under refrigeration until analysis.

Gas Chromatography Analysis

Gas chromatography (GC) analysis were performed on an orion micromat 412 double focusing gas chromatography system fitted with two capillary columns coated with CP-Sil 5 and CP-Sil 19 (fused silica, 25m x 0.25 mm, 0.1 µm film thickness) and flame ionization detector (FID). The volume injected was 0.2 µl and the split ratio was 1:30. Oven temperature was programmed from 50° - 230°C respectively. Qualitative data were obtained by electronic intergration of FID area percents without the use of correction factors.

Gas Chromatography-Mass Spectrometry Analysis

A Hewlett Packed (HP 5890A) GC interfaced with a VG Analytical 70-250S double focusing mass spectrometer was used. Helium was the carrier gas at 1.2 ml/min. The MS operating conditions were: ionization voltage 70 ev, ion source temperature 230°C. The GC was fitted with a 25 m x 0.25 mm. fused silica capillary column coated with CP-Sil 5. The film thickness was 0.15 µm. the GC

operating conditions were identical with those of GC analysis. The MS data were acquired and processed by online desktop computer equipped with disk memory. The percentage compositions of the oil were computed in each case from GC peak areas. The identification of the components was based on the retention indices (determined relative to the retention times of series of n-alkanes) and mass spectra with those of authentic samples and with data from Literature (Adams, 1995; Jennings and Shibamoto, 1980; Joulain and Koenig, 1988).

Antibacterial Activity

Antibacterial activities of the oil was carried out using disc diffusion method (Lalitha, 2004; Jogersen and Ferraro, 2009). Discs (8 mm each) were prepared from Whatman filter paper using perforating machine. The discs were wrapped in foil paper and sterilized in hot air oven at 150°C for 1 hr. The essential oil was diluted with Tween 80 to obtain 40, 20, 10, 5, and 2.5 mg/ml and discs were loaded by immersing in different concentration of oil.

The test bacteria were obtained from the collection of microorganisms of the Department of Microbiology, Faculty of Science, University of Ilorin, Nigeria. Ten species of bacteria comprising of five Gram-positive and five Gram-negative were used. The bacteria were *Bacillus cerceus*, *Bacillus megaterium*, *Streptococcus pyrogenes*, *Staphylococcus epidermides*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumonia*, *Serratia marcesceus* and *Pseudomonas aeruginosa*.

Fresh cultures of the test bacteria were prepared by growing on nutrient agar for 18hrs at $37\pm 1^\circ\text{C}$. Inoculum was prepared by transferring the individual bacteria into physiological saline and standardized to McFarland's scale 0.5. Bacteria cultures from the standardized inoculums were inoculated on Mueller-Hinton Agar plates by the spread plate technique. The essential oil laden discs were placed firmly on the surface of each inoculated plates with the aid of sterile forceps. Plates were incubated at 37°C for 24hrs and observed after growth for zones of inhibition. This was measured in triplicates with the aid of metric ruler and the mean was recorded to represent the inhibitory activity of the oil. The results were compared with the zone of inhibition produced by 100µg/ml of gentamicin. Minimum inhibitory concentrations (MIC) were determined. Activity Index (AI) was also determined as the mean inhibition zone of test oil divided by the mean inhibition zone of the standard drug (Singh *et al.*, 2002). Statistical analysis was performed using student t Test at P $\geq$ 0.05 (i.e. 5% probability level).

RESULTS AND DISCUSSION

The essential oil yield obtained from pulverized fruits of *O. gratissimum* on hydro-distillation was 1.5% (v/w). Table 1 shows the retention indices, relative percentage, mass spectra data and the identities of the essential oil constituents. A total of forty-six compounds representing 99.4% of the total essential oil were identified from their retention indices and mass spectra data.

Table .1: Chemical composition (%) of fruits essential oil of *Ocimum gratissimum* L.

S/N	Compound ^a	RI ^b	Percentage Composition	Mass spectral Data
1.	isoamyl acetate	876	tr	164 149 <u>131</u> 121 103
2.	tricyclene	922	tr	136 121 105 <u>93</u> 77
3.	α -thujene	926	tr	136 <u>121</u> 105 91 77
4.	α -pinene	933	tr	136 121 93 <u>79</u>
5.	camphene	947	tr	136 121 107 93 91
6.	(Is)-(l)-pinene	964	tr	137 123 109 103 9
7.	β -pinene	976	1.3	136 121 107 93
8.	myrcene	990	tr	136 121 115 107 93
9.	car-2-ene	1001	tr	136 121 105 93 79
10.	D-limonene	1027	tr	136 91 107 93 79
11.	benzyl alcohol	1028	tr	108 91 89 79 77 65
12.	1,8-cineole	1029	16.9	154 139 125 108 93
13.	cis ocimene	1035	tr	136 121 105 93 79
14.	allo-ocimene	1045	5.5	136 121 105 91 79
15.	γ -terpinene	1057	tr	136 121 105 91 79

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Table .1: Chemical composition (%) of fruits-essential oil of *Ocimum gratissimum* L. (contd.)

S/N	Compound ^a	RI ^b	Percentage Composition	Mass spectral Data
16.	iso artemisia	1062	tr	91 83 69 5 41
17.	fenchone	1085	tr	153 137 121 109 91
18.	linalool	1098	tr	139 121 109 97 93
19.	borneol	1162	tr	136 121 110 95 81
20.	terpine-4-ol	1175	tr	154 136 125 111 98
21.	α -terpineol	1188	tr	136 121 107 93 81
22.	nerol	1226	tr	139 121 111 93 81
23.	thymyl methyl ether	1233	tr	164 149 134 117 105
24.	neral	1238	0.7	135 119 109 99 95
25.	geraniol	1253	tr	139 123 111 93 81
26.	linalyl acetate	1255	tr	136 121 105 93 80
27.	geranial	1268	0.5	152 137 121 109 99
28.	eugenol	1354	53.7	164 149 131 121 103
29.	α -copane	1354	tr	204 189 161 145 133
30.	β -elemene	1391	tr	189 175 161 147 133
31.	α -caryophyllene	1435	tr	204 189 175 161 147
32.	trans- α -bergamotene	1435	tr	204 189 161 133 119
33.	γ -humulene	1452	4.88	234 189 161 147 134
34.	ethyl xinnamate	1461	tr	176 147 131 115 103
35.	γ -muurolene	1476	5.0	204 189 161 161 147

Table .1: Chemical composition (%) of fruits essential oil of *Ocimum gratissimum* L. (contd.)

S/N	Compound ^a	RI ^b	Percentage Composition	Mass spectral Data
36.	bicyclogermacrene	1480	tr	204 189 175 147 133
37.	β -selinene	1485	3.2	204 189 175 161 147
38.	α -selinene	1494	1	204 189 175 161 147
39.	β -sesquiphallandrene	1509	tr	204 161 147 133 120
40.	acetyl-eugenol	1523	tr	207 164 149 131 121
41.	1,6,10-dodecaqtrine- 3-ol-3,7,11-trimethyl	1534	0.7	234 147 131 115 103
42.	elemicin	1553	tr	208 193 177 165 150
43.	viridifloral	1589	tr	205 189 175 161 149
44.	β -bisabolene	1609	tr	204 189 176 161 147
45.	torreyol	1643	tr	204 161 133 119 105
46.	β -caryophyllene	1654	5.7	204 189 175 161 147
Total			99.4	

^aCompounds are listed in order of elution from Silica Capillary Column coated on CP-Sil 5; ^bRetention indices on fused Silica Capillary Column coated with CP-Sil 5

Hydrocarbon and oxygenated monoterpenes constituted 6.8 and 18.1% of the oil respectively, while the percentage composition of hydrocarbon sesquiterpenes was 20.8%. The essential oil was characterized by the abundance of eugenol (53.7%) as the main compound with other aromatic compounds; acetyl eugenol, elemicin, ethyl methyl ether in trace amounts.

Hydrocarbon monoterpenes that were found as the major constituents were allo-ocimene (5.5%) and β -pinene (1.3%). The predominant oxygenated monoterpene was 1, 8-cineole (16.9%). The notable oxygenated monoterpenes were neral (0.7%) and geranial (0.5%). Others such as fenchone, linalool, borneol, α -terpeneol, nerol, geraniol and linalyl acetate were detected only in trace quantities. The main hydrocarbon sesquiterpenes were β -caryophyllene (5.7%), γ -murolene (5.0%), α -humulene (4.8%), β -selinene (3.2%) and α -selinene (1.4%) which were detected in significant proportions.

The fruit essential oil of *O. gratissimum* in this study is a eugenol chemotype (Table 1). Earlier studies have reported essential oils of eugenol chemotype from the leaves of Nigeria grown *O. gratissimum* (Saliu *et al.*, 2011; Tania *et al.*, 2006), with the eugenol as the active principle responsible for the various therapeutic properties of the plant (Terezinha *et al.*, 2006).

Comparison of the compositional pattern of the fruits essential oil with the oil obtained from the leaves of *O. gratissimum* grown in Nigeria revealed significance qualitative and quantitative differences (Saliu *et al.*, 2011). Some principal constituents of the fruit essential oil in this study such as 1,8-cineole, γ -humulene, γ -muurolene, α and β -selimene and β -caryophyllene had not been detected in the leaf essential oil of *O. gratissimum*. On the other hand, germacrene D, camphor and A-farnesene which were major constituents of the leaf essential oil could not be identified in this study. The oil in this study is however similar to the leaf essential oil in respect to abundance of eugenol which makes the two oils eugenol chemotypes (Saliu *et al.*, 2011).

The antibacterial activities of the fruit essential oil of *Ocimum gratissimum* is presented in Table 2. All the bacteria tested were inhibited by the oil except *S. aureus* and *P. aeruginosa*. Among the susceptible bacteria, two Gram positives, namely *B. megaterium* and *S. epidermidis* and one Gram negative, *E. coli* were inhibited at 2.5 mg ml⁻¹ which was the minimum concentration tested, an indication that their minimum inhibitory concentration may be below 2.5 mg ml⁻¹. The other Gram positive bacteria also had significant susceptibility to the oil. *B. cereus* and *S. pyogenes* were inhibited at oil concentrations 5.0 and 10.0 mg ml⁻¹ respectively. *P. mirabilis* and *S. marcescens* had MIC of 10.0 mg ml⁻¹. This result indicates that the oil has a strong antimicrobial effect on both Gram positive and Gram negative bacteria.

Table 2: Anti-bacterial activity of fruit essential oil of *O. gratissimum* at varying Concentration

Bacteria	Mean diameter zone of inhibition (mm) ^a					
	100µg/disc gentamicin	2.5mg/ml	5mg/ml	10mg/ml	20mg/ml	40mg/ml
<i>B. megaterium</i>	15	13±0.4	15±0.5	16±0.3	17±0.4	18±0.4
<i>B. cereus</i>	13	-	11±0.5	12±0.2	13±0.4	14±0.3
<i>S. pyogenes</i>	12	10±0.2	11±0.3	12±0.1	13±0.4	15±0.6
<i>S. epidermidis</i>	10	-	-	12±0.1	15±0.4	15±0.5
<i>S. aureus</i>	14	-	-	-	-	-
<i>E. coli</i>	14	16±0.3	19±0.4	20±0.4	110.4	13±0.4

<i>P. mirabilis</i>	30	-	-	10±0.3	11±0.4	13±0.6
<i>K. pneumonia</i>	15	-	-	-	15±0.5	17±0.6
<i>S. marcescens</i>	12	-	-	11±0.3	12±0.4	14±0.5
<i>P. aeruginosa</i>	10	-	-	-	-	-

^a Each value represents mean of three measurements ± standard deviation

The activity index (AI) of the fruit essential oil of *O. gratissimum* revealed that the oil exhibited very high inhibitory activity against *B. megaterium* and *S. pyogenes* compared to gentamicin, the standard drug (Table 3). The activity index obtained for *B. cereus* and *S. epidermides* though lower, was appreciable and higher than that obtained for the Gram negative bacteria tested. This result supports earlier findings that the oil is more active against Gram positive than Gram-negative bacteria (Inouye *et al.*, 2001). Saliu *et al.*, (2011) also reported appreciable antibacterial activity of the leaf essential oil of *O. gratissimum* against Gram positive bacteria from oral cavity.

Table 3: Activity index of fruits essential oil *O. gratissimum* against gentamycin at 100µg/disc

Concentration of essential oil (mg/ml)	Gram-positive bacteria				Gram-negative bacteria			
	<i>B.m</i>	<i>B. c</i>	<i>S.p</i>	<i>S.e</i>	<i>E.c</i>	<i>P.m</i>	<i>K.p</i>	<i>S. m</i>
2.5	0.87	-	0.83	-	0.53	-	-	-
5.0	1.00	0.85	0.92	-	0.63	-	-	-
10.0	1.07	0.92	1.00	1.20	0.67	0.40	-	0.92
20.0	1.13	1.00	1.08	1.50	0.37	0.44	1.00	1.00
40.0	1.20	0.93	1.17	1.50	0.47	0.52	1.13	1.17

Key: *B.m* - *Bacillus megaterium*

B.c - *Bacillus cereus*

S.p - *Streptococcus pyogenes*

S.e - *Staphylococcus epidermides*

- E.c* - *Escherichia coli*
P.m - *Proteus mirabilis*
K.p - *Klebsiella pneumonia*
S.m - *Serratia marcescens*

In general essential oils occur as complex mixtures and their bioactivity is dependent on the major monoterpenoid components (Afolayan and Ashafa, 2009). Eugenol, the major component of the fruit essential oil of *O. gratissimum* in this study, had been reported to possess high antibacterial activity against *S. aureus* (Sartoratto *et al.*, 2004; Tippayatum and Chonhenchob, 2007). In addition, 1, 8-cineole which also occurs in appreciable quantity, together with some other constituents such as borneol has been reported to contribute to the antimicrobial activity of the oil (Jung, 2009). Antibacterial activity of the leaf essential oil of *O. gratissimum*, also a eugenol chemotype, against enteric bacteria such as *E. coli*, *Klebsiella sp.* and *Proteus mirabilis*, had been reported (Pereira *et al.*, 2004).

CONCLUSION

The results from this study showed that the fruit essential oil of *O. gratissimum* is of eugenol chemotype. The antibacterial activity of the oil against a number of Gram positive and Gram negative clinical bacteria has been demonstrated. The high abundance of eugenol in the oil may account for the therapeutic activity against some ailments. The knowledge of the minimum inhibitory activity of the oil could be employed in regulating its application.

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