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RESEARCH ARTICLE

CHEMICAL COMPOSITION AND ANTI BACTERIAL (ORAL ISOLATES) ACTIVITIY OF LEAF ESSENTIAL OIL OF *Ocimum gratissimum* L. GROWN IN NORTH CENTRAL NIGERIA

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ABSTRACT

Hydrodistilled leaves of *Ocimum gratissimum* L. yielded 1.25% (v/w) of essential oil. Investigation of the oil by GC and GC/MS revealed that the bulk of the oil was constituted by aromatic compounds (65.17%) with eugenol (61.9%) as the most abundant compound. Other notable constituents were cis-ocimene (8.2%), germacrene D (4.4%), β-pinene (1.6%), α-farnesene (1.6%), and camphor (1.5%). Antibacterial activity of the oil on oral bacterial isolates was investigated using agar diffusion method. The oil inhibited the growth of *Streptococcus mutans, Staphylococcus aureus, Staphylococcus epidermidis Lactobacillus casei, Streptococcus sanguis, Streptococcus salivarius,* and *Streptococcus pyogenes* while no activity was recorded against *Streptococcus viridans* and *Actinomyces israeli*.

INTRODUCTION

Ocimum gratissimum L. (family Lamiaceae) is an aromatic perennial herb wildely grown in Nigeria. It is commonly known as Scent leaf or "Nchu anwa" by the Igbos, "Effirin" by the Yorubas and "Dai doya tagida" by the Hausa (Orwa *et al.,*2009; Okoli *et al.,*2010). The plant is used as food spice (Okwu, 2006) and for the treatment of ailments such as; malaria, diabetes, respiratory and urinary tract infections, cough, fever, diarrhea, abdominal pains, pneumonia, conjunctivitis, oral wounds and tooth infection (Onajobi, 1986; Kokwaro, 1993; Ilori *et al* 1996; Aguiyi *et al.,*2000; Rabelo *et al.,*2003). Phytochemical investigations of the plants revealed the presence of alkaloids and flavonoids (Edeoga *et al.,*2006). Earlier works on its leaf essential oil revealed the presence of bioactive compounds such as, eugenol, thymol, camphor and linalool ((Rabelo *et al.,*2003; Lemos *et al.,*2005; Matasyoh *et al.,*2008)). Bioactivities such as; hypoglycaemic, antidiarrheal, antihelminthic, antimicrobial of the plant extracts

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traditional medicine (Onajobi, 1986; Dubey *et al.,*2000; Okoli *et al.,*2010; Oparoacha *et al.,*2010). Antihelminthic and antibacterial activity of Eugenol, a constituent of the oil has also been reported (Pessoa *et al.,*2002; Ali *et al.,*2005). Oral hygiene is very important to keep indigenous flora at check and exclude pathogens from the oral cavity. Dental plague leading to dental caries, periodontal diseases and dental-alveolar infection may result from unhygienic behaviours (Macfarlene and Samaranayake, 2001). The microbiota of the oral cavity of humans consists mostly of the genera *Streptococcus, Neisseria, Actinomyces, Veillonella and Lactobacillus* (Willey *et al.,*2008). *Streptococcus mutans* is highly implicated in dental caries. Other streptococci, lactobacilli and actinomycetes are also important agents of oral diseases.

It has been established that the composition pattern of essential oil is affected by geographical location which consequently influence their biological activities. It is on the basis of this that we investigated the constituents of leaf essential oil of north central Nigerian grown *Ocimum gratissimum*. Its activity on oral bacteria isolate was also established with a view to improve oral hygiene by its inclusion as herbal constituent of toothpastes.

MATERIALS AND METHODS

Plants Material

Fresh leaves of *Ocimum gratissimum* were collected from Ilorin, Ilorin West Local Government Area of Kwara State. Identification of the leaf was carried out at Herbarium of Plant Biology Department, University of Ilorin, where voucher specimens were deposited.

Oil Isolation

Pulverished leaves of *Ocimum gratissimum* (500g) was hydrodistilled for 3hrs in a Clevenger type apparatus according to the British Pharmacopoea Specification (1980). The resulting oil was stored under refrigeration until analysis.

Gas Chromatography

GC analysis was performed on an Orion micromat 412 double focusing Gas Chromatography system fitted with two capillary column coated with Cp-sil 5 and Cp-sil 19 (fused silica, 25m × 0.25mm × 0.15 film thickness) and flame ionization detector (FID). The volume injected was 0.2µl and the split ratio was 1:30. Oven temperature was programmed from 50-230℃ at 5℃/min, using hydrogen as carrier gas. Injection and detector temperatures were maintained at 200 and 250℃ respectively. Qualitative data were obtained by electronic integration of FID area percent without the use of correction factor.

Gas Chromatography /Mass Spectrometry

A Hewlett-Packard ITP5890A GC, interfaced with a VG analytical 70-250S double focusing mass spectrometer was used. Helium was the carrier gas at 1.2ml/min. The MS operating conditions were: ionization voltage 70eV, ion source 230^0C. The GC was fitted with a 25m×0.25mm, fused silica capillary column coated with CP-sil 5. The film thickness was 0.15μm.The GC operating conditions were identical with those of GC analysis. The MS data were acquired and processed by on-line desktop computer equipped with disk memory. The percentage compositions of the constituents of the oil were computed in each case from GC peak areas. The identification of the components was based on the comparism of retention indices (determined relative to the retention time of series of n-alkanes) and mass spectral with those of authentic samples and with data from literature ((Jennings and Shibamito, 1980; Adams, 1995; Joulain and Koenig 1998).

Isolation and Characterization of Bacteria

Organisms were isolated from the oral cavity of five volunteers who are students of University of Ilorin. Samples were obtained before mouth wash in the morning. Sterile distilled water (10ml) was used to rinse the oral cavity into sterile bottles. The bottles were sealed and laboratory analysis

(0.1ml) of the sample was used to inoculate nutrient agar plates by spread plate technique. Plates were incubated at 37 ^{0}C for 24 hours. Cultures were then observed for growth and subcultures of bacteria made to obtain pure cultures. Pure cultures were maintained on agar slants and stored in the refrigerator. Isolates were characterized in the microbiology laboratory of the University of Ilorin by the conventional microbiological method (Fawole and Oso, 2004).

Antibacterial Susceptibility and MIC Tests

The minimum inhibitory concentration was determined using the agar diffusion method described by Sartoratto *et al.,*2004. Inoculums were prepared with fresh cultures of bacteria, grown in nutrient broth for 18 hrs at 37^{o}C and standardized to McFarland scale 0.5. Twelve Wells (4mm each), were made on the Mueller-Hinton agar plates already seeded with bacteria by spread plate technique. The essential oil was diluted with Tween 80 to obtain concentrations ranging from 5.0% to 50.0% (v/v) and 0.1ml each was transferred to the wells. An aliquot (0.1ml) of Tween 80 was used as control. Plates were incubated at 37 ºC for 24h. Antibacterial activity was determined by clearance around loaded wells. MIC was defined as lowest concentration of oil that inhibited growth of bacteria.

RESULTS AND DISCUSSION

Pulverized leaves of *Ocimum gratissimum* on hydro-distillation afforded oil in the yield of 1.25% (v/w). The yield compared favorably with the yield obtained from the plants grown in other parts of the world (Viera *et al.,*2001; Matasyoh *et al.,*2008; Orwa *et al.,*2009). Table 1 shows the retention indices, relative percentages, mass spectra data and identities of the oil constituents. A total of 44 compounds representing 97.3% of the oil were identified from their retention indices and mass spectra data. Hydrocarbon and oxygenated monoterpenes constituted 13.4 and 7.0% of the oil, while the percentage composition of hydrocarbon and oxygenated sesquiterpenes were 8.6 and 2.8% respectively. The oil was characterized by the abundance of aromatic compounds representing abundant

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

S/N	Compound[a]	RI[b]	% Composition	Mass Spectra data
1	α-thujene	926	0.3	136,121,105,91
2	α-pinene	933	0.3	136,121,93,79,67
3	Sabinene	969	0.7	136,121,107,93,77
4	β-pinene	976	1.6	136,121,107,93,77
5	Myrcene	990	0.3	136,121,115,107,93
6	Limonene	1027	0.4	136,121,107,93,78
7	Benzyl alcohol	1028	0.7	108,91,79,73,65
8	1,8-cineole	1029	0.7	154,139,125,108,81
9	Cis-ocimene	1035	8.2	136,121,105,93,78
10	Trans-ocimene	1050	0.9	136,121,101,93,79,53
11	γ-terpinene	1057	0.6	136,121,105,93,77
12	Artemisia ketone	1062	0.3	136,121,91,83,69,55
13	Linalool	1098	0.3	139,121,97,67,43
14	Pinen-2-ol	1136	Tr	134, 111, 93, 79,55
15	Camphor	1140	1.5	135,119,109,95,69
16	Allo-ocimene	1142	0.3	136,121,105,91,67
17	Borneol	1163	0.6	121,110,95,81,67
18	Terpinene-4-ol	1176	0.3	154,136,125,111,43
19	α-terpineol	1188	0.3	136,121,107,93,81
20	Citronellol	1226	Tr	138,109,69,55,41
21	Neral	1238	0.8	135,119,109,95,69
22	Linayl acetate	1255	0.5	136,121,105,93,55
23	Geranial	1268	0.5	109,99,95,83,69,53
24	Borneol acetate	1284	0.9	136,121,108,95,67
25	Thymol	1290	Tr	150,135,91,77,65
26	β-elemene	1300	0.2	208,193,177,165,150
27	Eugenol	1354	61.9	164,149,137,131,121
28	Neryl acetate	1363	0.3	136,121,107,93,53
29	A-farnesene	1453	1.6	133,119,107,93,55,41
30	Ethylcinnamate	1460	0.5	147,133,119,105,91,7
31	Germacrene D	1480	4.4	204,161,147,133,105,91
32	Bicyclogermacrene	1494	0.5	121,107,93,79,67
33	β-bisabolene	1508	0.8	119,105,93,69,53
34	γ-cadinene	1513	1.1	161,119,105,91,79
35	Acetyl eugenol	1523	0.3	207,164,149,131,121
36	Elemecin	1553	0.2	208,193,177,165,150
37	Germacrene D-4-ol	1574	0.7	222,207,161,123,95,81
38	Spathulenol	1575	0.9	205,177,119,105,79,55
39	Caryophyllene oxide	1580	0.9	187,107,91,79,55
40	Goussonorol	1637	0.4	157,143,135,119,105
41	B-eudesmol	1647	0.2	93,79,67,59,41
42	Tetradecanoic acid	1720	0.8	220,171,115,60,57
43	Bisabolol oxide A	1744	0.1	238,220,202,154,134
44	Benzylbenzoate	1759	0.5	152,105,91,77,65,51
	Total		97.3	

[a]Compounds are listed in order of elution from Silica Capillary Column coated on CP-Sil 5; [b]Retention indices on fused Silica Capillary Column coated with CP-Sil 5

compound. Benzyl alcohol (0.7%), ethyl cinnamate (0.5%), benzyl benzoate (0.5%), gossonorol (0.4%), acetyl eugenol (0.3%) and elemicin (0.2%) were detected in significant proportions. Hydrocarbon monoterpenes that were found as major constituents were, cis-ocimene (8.2%) and β-pinene (1.6%). Sabinene (0.7%), γ-terpinene (0.6%), limonene (0.4%), α-thujene (0.3%), α-pinene (0.3%) and myrcene (0.3%) were found in significant proportions. The most abundant

Table 2. Bacterial Isolates and their relative occurrence

Isolates	Occurrence (no of individuals from which was isolated)	% Occurrence
Streptococcus pyogenes	5	100
Actinomyces Israeli	2	40
Streptococcus sanguis	5	100
Streptococcus salivarus	5	100
Streptococcus viridians	4	80
Staphylococcus epidermidis	5	100
Lactobacillus casei	3	60
Streptococcus mutans	4	80
Staphylococcus aureus	4	80

Table 3. Antibacterial Activities of leaf Essential Oil of *Ocimum gratisimum* L.

Isolates	Diameter zone of Clearance[c] (mm) at 50% (v/v)	MIC(%v/v)[d]
Streptococcus pyogenes	26.7±0.2	5.0
Actinomyces Israeli	-	-
Streptococcus sanguis	31.0±0.3	15.0
Streptococcus salivarus	24.7±0.1	5.0
Streptococcus viridians	-	-
Staphylococcus epidermidis	24.0±0.2	5.0
Lactobacillus casei	17.3±0.2	40.0
Streptococcus mutans	24.0±0.1	25.0
Staphylococcus aureus	26.3±0.2	5.0

[c]Each value represents the mean ($P < 0.5$) of diameters taken in three planes.
[d]MIC was obtained using agar diffusion method (Sartoratto et al, 2004).

were borneol acetate (0.9%), neral (0.8%), 1, 8-cineole (0.7%), borneol (0.6%) and linalyl acetate (0.5%). Isoartemisia ketone (0.3%), linalool (0.3%), terpine-4-ol (0.3%) and α-terpineol (0.3%) were also detected in significant amounts.

Predominant hydrocarbon sesquiterpenes were, germacrene D (4.4%), γ-cadinene (1.6%) and α-farnesene (1.1%). β- bisabolene (0.8%), bicyclogermacrene (0.5%), and β-elemene (0.2%) were also detected in appreciable quantities. Predominant oxygenated sesquiterpenes in the oil were, spathulenol (0.9%), caryophyllene oxide (0.9%) and germacrene D-4-ol (0.7%). β-eudesmol(0.2%) and bisabolol oxide A(0.1%) were detected insignificant proportions. With the abundance of eugenol in the oil, the oil is of eugenol chemotype. It is likened to leaf essential oils of Kenyan and Brazilian grown *O. gratissimum* with respected to the most abundant same vein, the oil differs from the oil obtained from Guinean grown *O. gratissimum* because it is of thymol chemotype.

Nine bacterial species, most of which are normal flora of the oral cavity of humans were isolated (Table 2). All the isolates were Gram positive and most belong to the genus Streptococcus. Members of the genus have been widely studied in relation to dental diseases and are known to cause dental caries (Selwitz *et al.,*2007). Actinomyces and Lactobacilli are also important agents of dental diseases (Lewis and Ismail, 1995). *Staphylococcus aureus* and *S. epidermidis* are normal flora of the skin and may get to the oral cavity from the exterior. *Streptococcus pyogenes, S. Sanguis, S. Salivarus and Staphylococcus epidermidis*, recorded 100% occurrence as they were isolated from all the samples. *S. aureus, Streptococcus viridians* and *Streptococcus mutans*

and *Actinomyces israeli* recorded 60 and 40% respectively. The variation can be explained as an indication of the level of the volunteers' oral hygiene.

The susceptibility pattern of bacteria isolated from the oral cavity of humans to *O. gratissimum* leaf essential oil as well as the minimum inhibitory concentration is presented in Table 3. Among the isolates, only *Streptococcus viridians* and *Actinomyces israeli* were not inhibited by the oil. Highest activity, represented by diameter zone of clearance around the loaded wells were recorded for *S. sanguis* while activity was detected at the lowest MIC of 5.0% (v/v) in *Streptococcus salivarus, S. pyogenes, Staphylococcus epidermidis* and *S. aureus*. Appreciable susceptibility also occurred with *Lactobacillus casei* and *Streptococcus mutans*.

Antimicrobial activities of essential oils are largely dictated by their chemical composition. The leaf essential oil of *O. gratissimum* has a great variety of phytochemicals that may be responsible for the significant part of the antibacterial activity (Table 1). Essential oils usually occur as complex mixtures and their bioactivity can generally be accounted for in terms of their major monoterpenoid components (Afolayan and Ashafa, 2009). Eugenol, which is the major component of the leaf essential oil of *O. gratissimum* L. used in this study, was reported to possess high antibacterial activity against *S. aureus* (Sartoratto *et al.,*2004; Tippayatum and Chonhenchob, 2007). Thymol, a phenolic constituent present in trace here, was reported to be active against *S. pyogenes* and *S. aureus* (Inouye *et al.,*2001). In addition, some other constituents that occur in minor quantities such as camphor, 1, 8-cineole as well as borneol may also contribute to the antimicrobial activity of the oil (Jung, 2009). Remarkable antibacterial effect comparable to those of commercial antiseptic products was obtained from leaf essential oil of *O. gratissimum* L. (Orafidiya *et al.,*2001). Leaf essential oil of *O. gratissimum* was also reported to be active against *Listeria monocytogenes* (Mbata and Saikia, 2005), *E. coli, Klebsiella sp., Proteus mirabilis, Morganella morgani* and *Enterobacter aerogenes* (Pereira *et al.,*2008).

Conclusion

This study has shown that the leaf essential oil of *Ocimum gratisimum* L. growing in North Central, Nigeria is a eugenol chemotype and is active against some oral bacteria that may cause dental caries if allowed to proliferate. Inclusion of the oil in toothpastes may contribute to public health by reducing the incidence of dental diseases.

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