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Effect of Collection Time on Chemical Composition and Antibacterial Activity of Flower Essential Oil of *Ocimum canum* (Sims) Grown in Nigeria

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Abstract: Flowers (1000 g) of *Ocimum canum* harvested at different times (7 am, 10 am, 1 pm, 4 pm, and 7 pm) in a day were separately hydrodistilled and yielded 0.19 - 0.27% (w/w) of essential oils. GC and GC-MS analyses revealed that the oils were predominated by oxygenated monoterpenoids (51.2-74.4%). Hydrocarbon monoterpenoids constituted 6.2-10.2% of the oils. Percentage composition of hydrocarbon and oxygenated sesquiterpenoids in the oils were in the range of 1.3-22.0%. The most abundant constituent of the oils was linalool (40.5-58.7%). Other principal constituents were as follows: Limonene (0.6 –7.5%), terpinen-4-ol (1.4–5.6%), eugenol (4.4–8.9%), geranyl acetate (0.2–4.9%), α-trans-bergamotene (3.2–9.4%) and (*E)*-isoeugenol (4.1–5.5%). The predominance of linalool in the oils showed that the oils were of linalool chemotype. Antibacterial activity of the oils was evaluated against *Staphylococcus aureus and Escherichia coli* using agar diffusion method. Irrespective of the time of collection of the flower, the oils were found to be active against the tested organisms. However, they are more active on *Escherichia coli* than *Staphylococcus aureus*. The activity of the oils on the organisms was concentration-dependent.

Keywords: *Ocimum canum*; Chemotype; linalool; terpene synthase; antibacterial activity.

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INTRODUCTION

Ocimum canum is an annual, odorous herb with several folkloric uses (1). They are used in treating conjunctivitis and headaches (2). The aerial parts of the plant are used as febrifuge and as an ingredient of many remedies of cold and sinusitis. An infusion of its leaves is used as a diaphoretic (3, 4). Biochemical and biological activities of the plant extracts account for their use in herbal medicine (5-7). The presence of phenolics, flavonoids, tannins and terpenoids that were established in the plant extracts is responsible for the activities exhibited by the plant (8).

The existence of linalool, eugenol, *trans*-methylcinnamate, methyl chavicol, eucalyptol, and camphor chemotypes of leaf oil of *O. canum* have been reported in Rwanda, Nigeria, Sao Tome, Brazil, India, and Cameroon (5, 9-14). Similarly, linalool, *cis*- and *trans*- piperitol chemotypes of flower oil of the plant grown in Rwanda and Burkina-Faso have been discovered (9, 15).

Variation in the chemotypes of the oils is attributable to environmental and physiological factors at various locations of the plant that bears the essential oils (5). These factors determine the activity of the enzyme that facilitates the biosynthesis of terpenoid constituents of essential oils from their respective precursors in plants (16). Hence, the factors could cause a variation in phytochemical profiles and biological activities of the oils. At a particular plant location, the factors could vary from time to time in a day. It is on the basis of this that this work aimed at monitoring the effect of collection time on the chemical composition and antibacterial activity of essential oil from flower of the plant on *Escherichia coli* and *Staphylococcus aureus*.

MATERIALS AND METHODS

Sample Collection and Preparation

Flowers of *O. canum* were harvested from its mature plant at park and garden of University of Ilorin, Ilorin, Kwara State Nigeria. The harvests were carried out at various times in a day (7 am, 10 am, 1 pm, 4 pm, and 7 pm). Identification of the plant was done in the herbarium of Plant Biology Department, University of Ilorin, Ilorin where a voucher sample was deposited (UILH/001/608). The harvested samples were separately pulverized. The test organisms were obtained from the culture collection of the Department of Microbiology, University of Ilorin. The organisms were isolated and characterized by colonial, microscopic, and biochemical techniques in a previous study (17).

Isolation of the Oil

1000 g of each of the pulverized flowers of *Ocimum canum* was hydro-distilled for 3 hours in a Clevenger-type apparatus, according to the British Pharmacopoeia specification (18). The resulting oil from each sample was collected, preserved in a sealed sample tube, and stored under refrigeration until analysis.

Gas chromatography (GC) analysis

Essential oil from each of the harvests was diluted in n-hexane by 1000-fold and subjected to GC analysis. The GC analyses 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, 25 m x 0.25 mm, 0.15µm 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 at 3 °C/min using hydrogen as a carrier gas. Injection and detector temperatures were maintained at 200 °C and 250 °C, respectively. Qualitative data were obtained by electronic integration of FID area percent without the use of correction factors.

Gas Chromatography – Mass Spectrometry (GC/MS) Analysis

A Hewlett – Packard HP 5890A GC, interfaced with a VG analytical 70-250s double focusing mass spectrometers was used. The MS operating conditions were: ionization voltage 70 eV, ion source and transfer line temperature was maintained at 230 ^{o}C. The GC operating conditions were identical with those of GC analyses. The MS data were acquired and processed by on-line desktop with a computer equipped with disk memory. The percentage composition of the oils' constituents were computed in each case from GC peak areas. The identification of the components was based on comparison of 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 the literature (19-21).

Antibacterial Assay (Agar Diffusion Method)

Antibacterial activity of the oils was determined using agar diffusion method described by Sartoratto *et al.* (22) Inoculums were prepared with the fresh cultures of bacteria, grown in nutrient broth for 24 hours at 37 ^{o}C and standardized to McFarland scale 0.5. Five wells (4 mm) were made on the Mueller-Hinton agar plates already seeded with bacteria by spread plate technique using flame sterilized Cork Borer. The essential oils were diluted with Tween 80 to obtain 25%, 50%, and 100% (v/v) concentrations and 0.1 mL each was transferred to separate,

appropriately labelled wells**.** An aliquot (0.1 mL) of Tween 80 was used as negative control while streptomycin served as positive control. Plates were incubated at 37 °C for 24 hrs. Antibacterial activity was determined by clearance around loaded wells. MIC was determined as lowest concentration of oil that inhibited growth of bacteria.

RESULTS AND DISCUSSION

Fresh flowers of *Ocimum canum* afforded 0.19-0.27% (w/w) of essential oil. The yield increased from 0.19% (w/w) in 7 am harvest to 0.21% (w/w) in 10 am harvest. It subsequently decreased to 0.20% (w/w) in 1 pm harvest. The yield later increased from 0.24% (w/w) in 4 pm harvest to 0.27% (w/w) in 7 pm harvest. Variations in oil yields from various harvests signified that the time of harvest affects the yield.

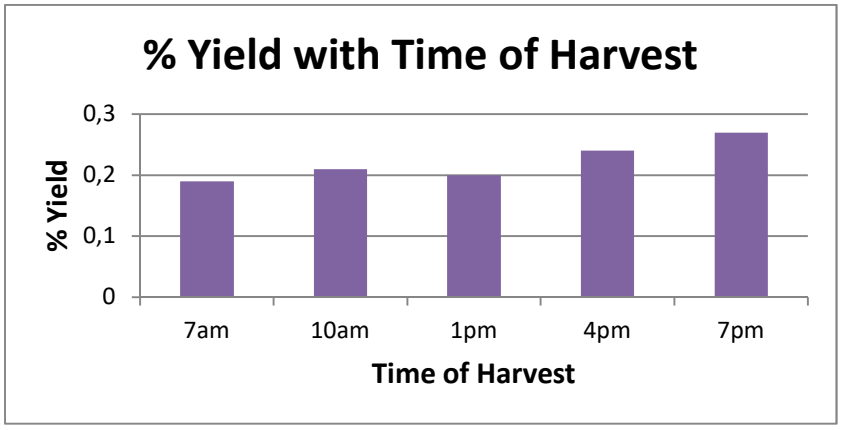


Figure 1: Yield of Oils from *Ocimum canum.*

Table 1: Chemical Composition (%) of Flower Essential Oil of *Ocimum canum.*

Compound[a]	RI[b]	RI[c]	% Composition	Mass Spectral Data			
			7 am	**10 am**	**1 pm**	**4 pm**	**7 pm**	
α-pinene	937	939	0.2	0.3	0.4	-	-	136,121,**93**,91,77
Car-2-ene	1001	1002	2.2	0.4	0.3	0.2	-	136,121,105,**93**,41
Car-3-ene	1009	1011	2.4	1.2	2.2	-	-	136,121,105,**93**,91
α-terpinene	1018	1015	-	-	0.3	0.4	-	136,**121**,105,93,77
p-cymene	1024	1024	1.3	-	-	0.3	0.4	134,**119**,103,91,77
o-cymene	1026	1026	-	0.4	-	-	-	134,**119**,105,91,77
Sylvestrene	1027	1030	-	0.3	0.5	-	-	136,121,107,**93**,68
Limonene	1031	1029	1.4	0.6	0.6	-	7.5	136,121,93,79,**68**
β-phellandrene	1031	1029	-	0.6	-	0.6	-	136,121,**93**,79,77
Eucalyptol	1033	1031	2.9	1.2	2.2	0.5	1.5	154,139,108,81,**43**
cis-β-ocimene	1040	1050	-	1.0	1.7	1.1	1.0	136,121,**93**,79,41
γ-terpinene	1062	1059	-	0.9	0.7	0.8	0.7	136,121,105,**93**,77
m-cymenene	1082	1082	-	0.5	-	0.6	0.6	134,119,103,91,77
α-terpinolene	1088	1088	0.5	0.3	0.6	-	-	136,121,105,**93**,79
Linalool	1098	1096	48.1	55.4	40.5	68.1	58.7	154,121,93,**71**,41
Octylacetate	1121	1112	2.4	-	-	-	-	187, 169,157,109,43
Lavandulol	1166	1163	-	-	0.1	0.5	0.5	136,123,93,**69**,41
Terpinen-4-ol	1177	1174	1.4	5.5	5.6	3.5	5.2	154,136,111,93,**71**
α-terpineol	1185	1188	-	0.4	-	0.5	0.4	136,121,93,81,**59**
Nerol	1228	1229	-	-	0.2	0.2	-	154,121,93,**69**,41
Geranial	1270	1268	1.6	1.8	0.6	0.9	1.1	152,109,**69**,53,41
Geranyl-formate	1300	1298	-	-	-	0.2	-	182,136,93,**69**,41
α-cubebene	1351	1351	0.1	0.2	0.1	-	-	204,161,119,**105**,41
Eugenol	1356	1359	8.7	8.9	6.6	4.4	4.8	**164,**149,131,103,91
Nerylacetate	1365	1361	-	-	1.5	-	-	154,121,93,**69**,41
β-elemene	1375	1374	1.8	1.1	1.0	0.7	0.5	204,161,121,**93**,81
α-copaene	1376	1376	1.9	-	0.8	-	-	204,**161**,119,105,91
Geranyl acetate	1383	1382	-	0.2	-	4.9	-	154,121,93,**69**,41
β-cubebene	1390	1388	0.2	0.2	0.2	-	-	204,**161**,105,91,41
β-caryophyllene	1418	1417	2.8	1.4	-	-	-	204,161,133,93,**41**
α-*trans*-bergamotene	1436	1435	-	-	9.4	3.2	5.3	204,161,119,**93**,41
α-guaine	1439	1439	1.7	0.8	0.8	0.5	0.6	204,161,**105**,93,41
(Z)-β-farnesene	1443	1442	2.3	0.3	-	0.2	-	204,133,93,69,**41**
(E)-isoeugenol	1447	1451	-	-	5.5	4.1	-	**164**,149,131,93,77
α-caryophyllene	1454	1454	0.2	0.2	-	-	-	204,161,133,**93**,79
(E)-β-farnesene	1458	1456	-	-	-	0.1	-	204,161,93,**69**,41
γ-gurjunene	1473	1471	-	0.2	0.2	-	-	204,**161**,119,91,41

β-chamigrene	1475	1473	-	-	1.0	-	-	204,**189**,105,93,41
γ-muurolene	1477	1475	1.2	0.3	1.7	-	0.8	204,**161**,119,91,41
Germacrene D	1480	1479	1.5	1.4	1.7	-	1.5	204,**161**,119,105,41
α-selinene	1494	1494	-	-	-	0.5	-	204,**189**,161,133,41
Bicyclogermacrene	1494	1500	-	0.9	0.8	0.2	0.4	204,161,**121**,93,41
β-himachalene	1499	1500	-	-	0.2	-	-	204,134,**119**,105,41
α-bulnesene	1505	1509	2.4	1.3	1.2	0.4	0.6	204,189,**107**,93,41
Amorphene	1506	1511	-	0.5	1.7	0.8	0.8	204,161,**133**,105,41
γ-cadinene	1513	1513	0.4	1.7	0.7	1.0	1.4	204,**161**,119,91,41
β-sesquiphellandrene	1521	1522	-	0.5	0.4	0.8	0.7	204,161,93,**69**,41
cis-nerolidol	1534	1563	-	-	1.2	0.2	-	204,161,136,69,**41**
Germacrene B	1560	1561	1.6	-	-	0.5	0.4	204,161,**121**,93,41
trans-nerolidol	1564	1532	0.3	-	-	0.2	-	204,161,105,**69**,41
Spathulenol	1576	1578	0.2	-	-	-	-	220,205,119,91,**43**
Caryophyllene oxide	1581	1583	-	0.2	-	-	-	219,161,121,79,**41**
β-eudesmol	1649	1650	0.2	0.2	0.1	-	-	222,204,189,149,**59**
Bisabolol	1683	1685	0.2	-	-	-	-	204,119,109,69,**43**
Patchoulene	1793	1756		0.8				204,161,**107**,93,79
TOTAL			**92.1**	**92.4**	**93.7**	**98.8**	**97.7**	
No of Compounds			**30**	**37**	**36**	**33**	**25**	

[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; [c]:- Retention Indices from literature.

Identities, retention indices, and percentage composition of the constituents of flower essential oils of *Ocimum canum* harvested at 3 hours interval in a day (7 am, 10 am, 1 pm, 4 pm, and 7 pm) is presented in Table 1. In the table, compounds 25-37 representing 92.1-98.8% of the oils were identified from their mass spectra. Hydrocarbon monoterpenoids constituted 6.2-10.2% of the oils while percentage composition of oxygenated monoterpenoids was 51.2-74.4%. Meanwhile, 1.3-22.0% of the oils was hydrocarbons and oxygenated sesquiterpenoids.

The most abundant constituent of the oil was linalool (40.5-68.1%). Other major constituents were limonene (0.6 – 7.5%), terpinen-4-ol (1.4 – 5.6%), eugenol (4.4 – 8.9%), geranyl acetate (0.2 – 4.9%), α-*trans*-bergamotene (3.2 – 9.4%) and (*E)*-isoeugenol (4.1 – 5.5%). Car-2-ene (0.2 – 2.2%), car-3-ene (1.2 – 2.4%), eucalyptol (0.5 – 2.9%), *cis*-β-ocimene (1.0 – 1.7%), octylacetate (2.4%), neryl acetate (1.5%), β-elemene (0.5 – 1.8%), α-copaene (0.8 – 1.9%), β-caryophyllene (1.4 – 2.8%), δ-guaine (0.5 – 1.7%), (*Z*)-β-farnesene (0.2 – 2.3%), γ-muurolene (0.3 – 1.7%), germacrene D (1.4 – 1.7%), α-bulnesene (0.4 – 2.4%), γ-cadinene (0.7 – 1.7%), *cis*-nerolidol (0.2– 1.2%) and germacrene B (0.4 – 1.6%) were also detected in appreciable quantities.

Comparison of the oils revealed that there were variations in their composition patterns. Qualitatively, octyl acetate and bisabolol in the oil from 7 am harvest were not detected in the oils from other harvests. Similarly, oil from 10 am harvest bears *p*-cymene, caryophyllene oxide, and patchoulene that were not found in other oils. Furthermore, the presence of neryl acetate, β-himachalene and β-chamigrene were established in the oil of 1 pm harvest but did not exist in other oils. Oil from 4 pm harvest also had geranyl formate, *(E)*-β-farnesene, and α-selinene that were not detected in other oils.

Eucalyptol, *cis*-β-ocimene, γ-terpinene, β-sesquiphellandrene, bicyclogermacrene and amorphene occurred in the oils except for the oil from 7 am harvest. Similarly, limonene, γ-muurolene and germacrene D were detected in the oils except oil of 4 pm harvest. 2-carene was found in the oils with the exception of the oil of 7 pm harvest. Absence of some compounds in the oils may be due to unfavorable physiological conditions of the plant at the time of harvest that did not favor their biosynthesis.

Quantitatively, linalool was more abundant in the oil of 4 pm harvest than other oils. Similarly, terpinen-4-ol was of greater abundance in the oil of 1 pm harvest than other oils. Geranial and eugenol were more abundant in the oil of 10 am harvest than other oils. β-Elemene was detected

in higher quantity in the oil of 7 am and 10 am harvests than other oils. However, β-cubebene and δ-cadinene were found in higher quantities in the oils of 4 and 7 pm harvests than other oils.

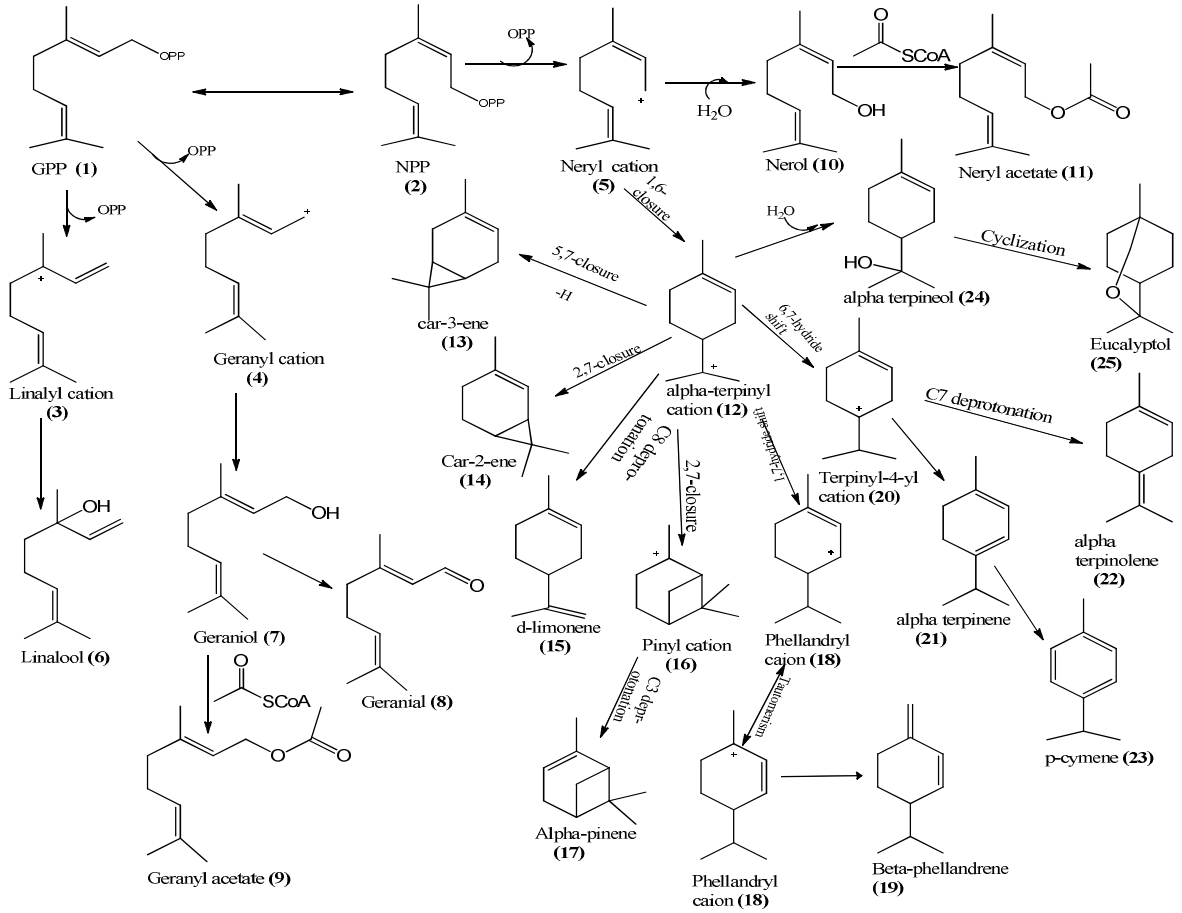
Terpinen-4-ol and eugenol occurred in appreciable quantities in the oils but more abundant in the oils from 7 am and 1 pm harvests, respectively. δ-Guaine was more abundant in the oil of 7 am harvest than other oils. Quantitative variations in the oil constituents are attributable to difference in activity of synthases that mediate the formation of the compounds from their respective precursors (23).

The biosynthesis of terpenoids is usually catalyzed by the synthase of the most abundant mono- and sesquiterpenoids in the presence of a divalent metal ion via cationic intermediates. These intermediates subsequently undergo series of reactions such as; hydride shift, cyclization, and other rearrangements until the reaction is terminated by proton loss or hydration to form various terpenoids (21, 23). The reactions do proceed by cationic mechanism.

Reaction mechanisms

The predominance of linalool in the oils implied that its synthase mediates the transformation of geranyl and neryl pyrophosphates to all monoterpenoids in the oils via cationic intermediates (Figure 1). In the figure, linalool synthase aided the transformation of geranyl **(1)** and neryl pyrophosphates **(2)** to geranyl **(4)** and neryl **(5)** cations. Isomerization of each of the ions (**4** and **5**) formed linalyl cation **(3).** Hydration of linalyl and geranyl cations produced linalool **(6)** in the oils. Hydration of geranyl cation that was oxidized and acetylated gave geranial **(8)** and geranyl acetate **(9)** in the oils from 10 am and 4 pm harvests, respectively**.** Neryl cation **(5)** was hydrated to nerol **(10)** in oil from 1 pm and 4 pm harvests and subsequent acetylation of nerol formed neryl acetate **(11)** in oil from 1 pm harvest. Electrophilic attack of the ion **(5)** on C_6-C_7 double bond produced α-terpinyl cation **(12)**. Deprotonation of the ion **(12)** at C_5 and C_1 followed by its electrophilic attack on the deprotonated carbons formed 3-carene **(13)** in oil from 7 am to 1 pm harvests and 2-carene (14) in oils from 7 am to 4 pm harvests. Deprotonation of α-terpinyl cation at C_8 **(12)** gave limonene **(15)** in the oils except oil from 4pm harvest. Folding of the ion **(12)** towards C_2-C_3 double bond followed by its electrophilic attack via C_2 produced pinyl cation **(16).** Deprotonation of the ion (**16**) at C_4 formed α-pinene **(17)** in the oils with the exception of oils from 4 pm and 7 pm harvests. 1, 6-hydride shifts of the ion **(12)** followed by delocalization of C2 – C3 π electrons to C1 – C2 formed phellandryl cation **(18).** Deprotonation of the ion **(18)** at C_{10} formed β- phellandrene **(19)** in oils from 10 am and 4 pm harvests. 6, 7-hydride shift of the ion **(12)** gives terpinyl-4-yl cation **(20).** Subsequent deprotonation of the latter **(20)** at C_1 and C_7

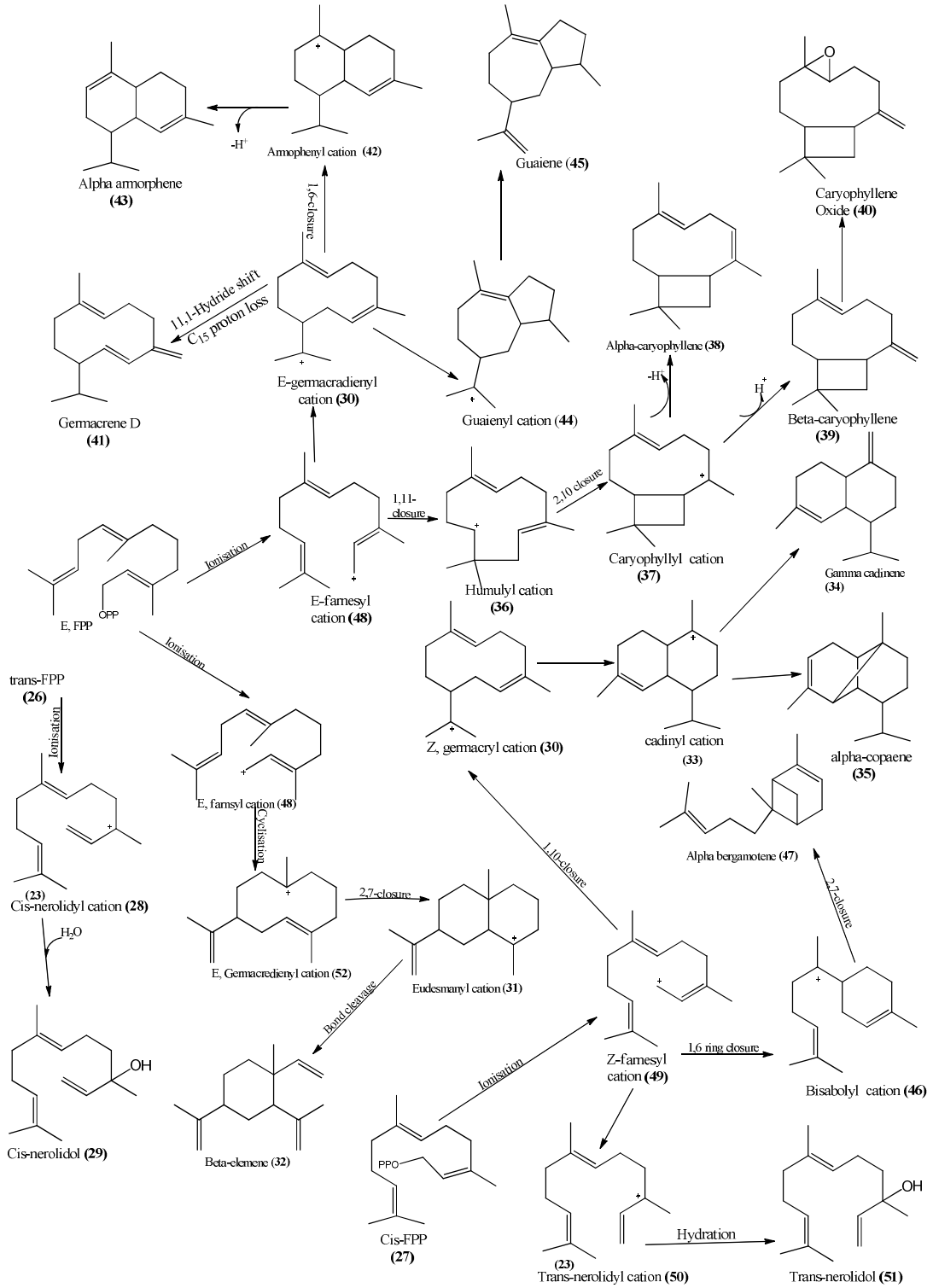
produced α-terpinene **(21)** in oils from 1 and 4pm harvests and α-terpinolene **(22)** in oils from 7 am, 10 am and 1 pm harvests respectively. Dehydrogenation of α-terpinene **(21)** at C_4 and C_5 formed p-cymene **(23)** in oil from 10am harvest. Hydration of α-terpinyl cation (**12**) formed α-terpineol **(24)** in oils from 10am, 4pm and 7pm harvests. Nucleophilic attack of hydroxyl oxygen of α-terpineol on C2-C3 double bond and subsequent protonation of the product formed eucalyptol (**25**) in the oils.



Reaction Scheme 1: Biosynthesis of monoterpenoids mediated by linalool synthase.

The abundance of β-caryophyllene, γ-cadinene and α-*trans*-bergamotene in the oils of 7 am, 10 am and 1-7 pm harvests implied that their synthases mediated the biosynthesis of all the sesquiterpenoids in the oils, where each of the compounds predominated (reaction scheme 2). In Scheme 2, the synthases catalyzed the ionization of *cis*- and *trans*-farnesyl pyrophosphates **(27** and **26)** to their respective cationic intermediates **(48** and **49).** Isomerization of the ions **(48, 49)** formed *cis*- and *trans*- nerolidyl cations **(50, 28)**. Hydration of the ions **(50, 28)** formed *cis*-nerolidol **(51)** in oils of 1 pm and 4 pm harvests and *trans* nerolidol in oils from 7 am and 4 pm

harvests (**29**). Electrophilic attack of the ion **(48)** on C_{10}-C_{11} double bond via C_{10} formed (Z)-germacrenyl cation **(30)**. Delocalization of the pi electrons from C_6-C_7 to C_6-C_5 followed by electrophilic addition of the *(E)*-germacrenyl cation (**52**) on C_2-C_3 double bond via C_2 formed eudesmanyl cation **(31)**. Bond cleavage at C_4-C_5 followed by deprotonation at C_{15} gave β-elemene **(32)** in the oils**.** Hydride shift followed by electrophilic attack of the ion **(30)** on the C_6-C_7 double bond via C_6 formed cadinyl cation **(33)**. Deprotonation of the ion at C_3 produced ϒ-cadinene **(34)** in the oils. Electrophilic attack of the ion **(33)** on the C_2-C_3 double bond via C_2 followed by deprotonation at C_4 formed α-copaene **(35)** in oils from 7 am and 1 pm harvests. Electrophilic attack of *(E)*-farnesyl cation **(48)** on C_{10}-C_{11} double bond via C_{11} formed humulyl cation **(36).** Subsequent electrophyllic attack of the ion (**36**) on C_2-C_3 double bond via C_2 formed caryophyllyl cation **(37)**. Deprotonation of the ion **(37)** at C_4 and C_{15} form α-caryophyllene **(38)** and β-caryophyllene **(39)** respectively in the oils from 7 am and 10 am harvests. Epoxidation of the compound **(39)** at C_6-C_7 double bond produced caryophyllene oxide **(40)** in oil from 10 am harvest. C_{11}-C_{10} and C_{10}-C_1 hydride shifts followed by delocalization of C_2-C_3 pi electron to C_1-C_2 and subsequent deprotonation at C_{15} of the ion **(30)** formed germacrene D **(41)** in the oils with the exception of oil from 4 pm harvest. C_{11}-C_{10} and C_{10} -C_1 hydride shifts followed by electrophilic attack of the ion **(30)** on the C_6 and C_7 double bond via C_6 formed armophenyl cation **(42)**. Deprotonation of the ion **(42)** at C_8 formed α-amorphene **(43)** in the oils except oil from 7 am harvest. Electrophyllic attack of the *(E)*-farnesyl cation (**49**) on C_6-C_7 double bond formed bisabolyl cation **(46).** Electrophyllic attack of the ion **(46)** on the C_2-C_3 double bond via C_2 followed by deprotonation at C_4 produced α-bergamotene **(47)** in the oils from 1-7 pm harvests.



Reaction Scheme 2: Biosynthesis of Sesquiterpenoids mediated by β-caryophyllene, γ-cadinene, and α-*trans*-bergamotene synthases.

Table 2: Antibacterial Activity of Fruit Essential Oil of *Ocimum Canum* Harvested at Different Hour in a Day.

Oil samples	Organisms	Activity as Diameter Zone of Clearance Around Bacterial Colony (mm)				
		25%	50%	100%	Streptomycin	Tween 80
A	*S. aureus*	-	-	12.2	15.6	0
	E. coli	-	-	15.2	12.4	0
B	*S. aureus*	-	14.8	18.9	16.8	0
	E-coli	-	24.1	28.2	28.1	0
C	*S. aureus*	-	14.7	18.1	16.7	0
	E-coli	-	24.0	24.9	24.1	0
D	*S. aureus*	-	14.6	15.6	14.7	0
	E-coli	-	26.1	31.6	30.5	0
E	*S. aureus*	-	14.0	16.0	17.0	0
	E-coli	-	13.0	19.1	9.9	0

KEY: A= Essential oil from 7 am harvest. B= Essential oil from 10 am harvest. C= Essential oil from 1 pm harvest. D= Essential oil from 4 pm harvest. E= Essential oil from 7 pm harvest. *S. aureus- Staphylococcus aureus. E-coli- Escherichia coli.*

The oils inhibited the test organisms at 50 and 100% concentrations irrespective of the time of collection (Table 2). No activity was however obtained at 25% oil concentration. Activity of the oils compared favorably with that of streptomycin in most of the tests except for sample collected at 7 am where no inhibition occurred at 50% concentration. Also, Tween 80, which was the solvent used for dilution, had no inhibitory activity on the test organisms. Judging by the diameter zone of inhibition, *Escherichia coli,* which is a Gram negative bacteria, was more susceptible to the antibacterial effect of the oils compared to the Gram positive *Staphylococcus aureus*. This is an indication that the essential oils of *O. canum* may be more active on Gram negative rather than Gram positive organisms. Since activity discriminate based on cell wall type, the mechanism of inhibition by the oil can be likened to that of the β lactam antibiotics which inhibits cell wall synthesis. Bassole *et al.* (15) and Jeferson *et al.* (12) also reported higher antibacterial activity of oils of *O. canum* on *E. coli* compared to *S. aureus*.

CONCLUSION

Essential oil yields from flowers of various harvests differ significantly. This implied that environmental factors and physiological conditions of plants that determine essential oil yield varied at various times of harvest of the flowers, hence, causes differences in their yields. The most abundant monoterpenoid in the oils was linalool of which its synthase aided the formation of all monterpenoids in the oils. However, the composition patterns of monoterpenoids in the oils varied appreciably due to changes in the activity of linalool synthase as determined by environmental and physiological conditions of the plant at various times of harvests. Similarly, β-caryophyllene, γ-cadinene, and α-*trans*-bergamotene were the most abundant sesquiterpenoids in oils from various harvests. Hence, their synthases mediated the biosynthesis of all sesquiterpenoids in the oils where each of the compounds predominates. Variations in sesquiterpenoids profile in the oils are attributable to differences in activity of the synthases.

The oils were active against *Escherichia coli* and *Staphylococcus aureus*. Furthermore, it was noticed that the essential oils of *O. canum* showed higher inhibitory activity against the test organism than the positive control (streptomycin). Although, they were more active against *Escherichia coli* (gram negative) than *Staphylococcus aureus* (gram positive) bacteria, concentration and modelling of such oil with inhibitory activity into antibiotics can serve as an intervention to the ever growing problem of antibiotic resistivity.

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Türkçe Öz ve Anahtar Kelimeler

Nijerya'da Yetiştirilen *Ocimum canum* (Sims) Çiçek Esansiyel Yağının Kimyasal Bileşimi ve Antibakteriyel Aktivitesi Üzerine Toplama Zamanının Etkisi

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Öz: Bir günün farklı zamanlarında (7 öö, 10 öö, 1 ös, 4 ös, 7 ös) hasat edilen *Ocimum canum* çiçekleri (1000 g) ayrı ayrı hidrodistilasyona tabi tutulmuş ve %0,19-0,27 (w/w) esansiyel yağ vermiştir. GC ve GC-MS analizleri, yağların oksijenli monoterpenoidler (%51,2-74,4) içerdiğini göstermiştir. Hidrokarbon monoterpenoidleri yağların %6,2-10,2'sini oluşturmuştur. Hidrokarbon ve oksijenli seskiterpenoidlerin yağlardaki yüzde bileşimi %1,3-22,0 arasında değişmektedir. Yağlarda en baskın bileşen linalooldür (%40,5-58,7). Diğer birincil bileşenler şöyle sıralanabilir: Limonen (%0,6-7,5), terpinen-4-ol (%1,4-5,6), eugenol (%4,4-8,9), geranil asetat (%0,2-4,9), α-trans-bergamoten (%3,2-9,4) ve *(E)*-izoeugenol (%4,1-5,5). Linaloolün yağlarda baskın olması, yağların linalool kemotipinde olduğunu göstermiştir. Yağların antibakteriyel aktivitesi *Staphylococcus aureus ve Escherichia coli* bakterilerine karşı agar diffüzyon yöntemi ile değerlendirilmiştir. Çiçeğin toplandığı zamandan bağımsız olarak, yağların test edilen organizmalara karşı aktif olduğu bulunmuştur. Ancak, bunlar *Staphylococcus aureus*'a nazaran *Escherichia coli*'ye karşı daha aktiftir. Yağların organizmalar üzerindeki aktivitesi derişime bağlıdır.

Anahtar kelimeler: *Ocimum canum*; kemotip; linalool; terpen sentaz; antibakteriyel aktivite.

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