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Phytochemical Profile and Antioxidant Potential of Essential Oil from Fresh and Dried Leaves of *Ocimum gratissimum* Grown in North Central Nigeria

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Abstract

Post-harvest metabolic reactions in plant can be influenced by change in ambient temperature which affects the enzymes that catalysed the reactions. This study aimed at investigating the effect of drying at ambient temperature on the chemical constituents and free radical scavenging potential of essential oil from the leaf of *O. gratissimum* grown in North central Nigeria. Fresh leaves of *Ocimum gratissimum* (500g) were harvested and air dried for five consecutive days. Both fresh and dried leaves were separately subjected to hydrodistillation. Analyses of the oil using Gas Chromatography (GC) and Gas Chromatography – Mass Spectrometry (GC-MS) revealed the predominance of monoterpenes (27.3 – 8.4%) and sesquiterpenes (15.0–34.1%). Oils from the fresh and leaves dried for four to five days were predominated by γ -terpinene. A non-terpenic compound, precosene-I was the most abundant compound in the oil of leaves dried for one day. Oil from the leaves dried for two and three days were of β -selinene and thymol chemotypes respectively. Free radical scavenging potentials of the oils were separately determine using DPPH radical. Regardless of level of dryness, the oils exhibit more than 50% radical inhibitory potential at all concentrations. Nevertheless, oil from the fresh leaf scavenges DPPH radical better at IC₅₀.

Keywords: *Ocimum gratissimum*, Chemotype, DPPH, hydro-distillation, Antioxidant

INTRODUCTION

Reactive Oxygen Species (ROS) such as, singlet oxygen (O[•]), hydroxyl (•OH), peroxide (H₂O₂) and superoxide (O₂^{•-}) radicals are produced during physiological systemic metabolism in living cells (Haliwel, 1998). The radicals initiate many oxidation reactions in the cells, leading to oxidative stress. This condition causes pathophysiological aging and aggravation of various diseases and disorders such as, arthritis, hypertension, atherosclerosis and cancers (Valko *et al.*, 2007; Iqbal *et al.*, 2013). Various Synthetic drugs are been implicated in the treatment of the induced-oxidative stress, some of which are butylated hydroxylanisole (BHA), tertiary

butylhydroquinone (TBHQ), butylated hydroxyltoluene (BHT), alpha-tocopherol and ascorbic acid (Sultana *et al.*, 2007). Despite the efficacy of the drugs, they are known with several drawbacks such as; adverse reactions (anaphylaxis reaction, nausea, polydypsia, polyurea, obesity), high cost of purchase and non-accessibility to the users. Hence, this necessitated the need to search for cheaper, safer and readily available antioxidative agents from botanical source. Plant extracts with comparable antioxidant potentials to conventional drugs have essential oils that are obtainable from aromatic plants.

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The oils are devoid of the drawbacks associated with synthetic antioxidative agents. Notable among the oils, is oil from the leaf of *Argeratina adenophora* that was reported to have significant radical scavenging activity against DPPH and ABTS radicals. Its activity compared favourably with the standard, L-ascorbic acid and this was attributed to the presence of α -bisabolol and azulenone in the oil (Gopalakrishnan and Vasanthi, 2013). Essential oil from the Fruit, leaf, stem and flower of *Eucalyptus oleosa* exhibited similar activity against DPPH and ABTS radicals (Bouajila *et al.*, 2011). But oil from fruit showed greater activity with an IC₅₀ of 441.1 \pm 12.7mg/L against DPPH radical, than oils from other parts of the plant. This was attributed to predominance of eucalyptol in the oil (Bouajila *et al.*, 2011). Surprisingly, oil from leaf showed higher activity with an IC₅₀ of 31.0 \pm 0.6mg/L in the ABTS assay than the other oils. This may be an indication of the predominance of spathulenol and γ -eudesmol in the oil (Bouajila *et al.*, 2011).

The existence of different chemotypes of essential oil from leaf of *Ocimum gratissimum* has been established in different parts of the world. Oils from leaf of the Brazilian grown plant were of eugenol and methyl eugenol chemotypes (Vostrowsky, 1990; Silva *et al.*, 2004; Matasyoh *et al.*, 2007). However, only eugenol chemotype of the oil was found in Kenyan and Indian grown *O. gratissimum* (Benitez *et al.*, 2009; Shirwaika *et al.*, 2009). Thymol chemotype of the oil was discovered in the leaf of the plant grown in Benin republic (Kpoviessi *et al.*, 2012). γ -terpinene chemotype of the oil was also found in the leaf of South West-Nigerian grown *O. gratissimum* (Owokotomo *et al.*, 2012). Earlier work on the oil from the leaf of the plant from North central Nigeria also revealed the predominance of eugenol (Saliu *et al.*, 2011).

The chemotypic variations of oil from the leaf of various countries may be associated with

differences in environmental factors at various locations of the plant. These factors determine physiological conditions that dictate the terpene synthases required for the biosynthesis of terpenic constituents of the oil from their respective precursors. Hence, they could cause variations in phytochemical profile and biochemical activity of the oil. Changes in ambient temperature usually affect post-harvest metabolic reactions in plant by affecting the enzymes that catalysed the reactions. Therefore, influence the chemical constituent, biological and biochemical activities of the plant extracts. Apart from this, it determines the level of dryness of the harvested plant materials. It is on the basis, that this work aimed at investigating the effect of drying at ambient temperature on the chemical constituents and free radical scavenging potential of essential oil from the leaf of *O. gratissimum* grown in North central Nigeria.

Material and Methods

Collection of Sample

Fresh leaves of *Ocimum gratissimum* were collected from Sango Area, in Ilorin-East local Government, Kwara State. The sample was identified at the Herbarium of Forest Research Institute of Nigeria (FRIN), Ibadan where voucher specimen was deposited (FHI 108361).

Oil Isolation

Pulverized fresh and dried leaves (500g each) of *Ocimum gratissimum* were separately hydro-distilled for 3 hours according to the British Pharmacopoeia specification (British Pharmacopoeia, 1980). The resulting oil from each sample was collected, preserved in a sealed sample tube and stored under refrigeration (4°C) before analysis.

Gas Chromatography: An Orion micro mat 412 double focusing Gas chromatography system fitted with two capillary column

coated with Cp-sil 5 and Cp-sil 19 (fused silica, 25µm×0.25mm×0.15µm film thickness) and flame ionization detector (FID) was used. Qualitative data were obtained by electronic integration of FID area percent without the use of correction factors. The identification of the components was based on the comparison of retention indices (determined relative to the retention time of series of n-alkanes) with those of authentic compounds. The percentage compositions of constituents of the oil were computed in each case from GC peak areas (Kovats, 1958).

GC-MS Analysis: A Hewlett- Packard HP5890A GC, interfaced with a VG analytical 70-250s double focusing mass spectrophotometer was used. Helium was the carrier gas. The mass spectrometer data were acquired and proceed by on-line desktop computer equipped with disk memory. The identification of the components of the oils was based on the comparison of mass spectra with those of authentic compounds and with data from literature (Joulain and Konig, 1998; Adams, 2012).

DPPH Radical Scavenging Assay: The antioxidant activity of the oils was evaluated by DPPH radical scavenging assay, which was originally described by Jhursue (2001). 80% methanolic solution of the DPPH was prepared. Various volumes of the oils were mixed with 1 ml of 0.002% DPPH solution. After 30 min. incubation period at 30°C, the absorbance was read against a blank at 517 nm. Inhibition of free radical by DPPH in percent was calculated as follows:

$$\text{Inhibition} = 100 \left(\frac{A_{\text{Blank}} - A_{\text{sample}}}{A_{\text{Blank}}} \right);$$

Where;

A_{blank} is the absorbance of the control reaction (containing all reagents except the oil) and A_{sample} is the absorbance of the sample. The oil

concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentages against oil concentrations.

Data Analysis: The %Scavenging of DPPH values from the experiment were subjected to one way Analysis of variance (ANOVA). Where there was a significant difference, mean separation was done using Duncan Multiple Range Test at 5% level of significance. Statistical analysis was done using SPSS software, version 16.

Discussion of Results

Chemical Composition: Table 1 shows the yields (w/w) of essential oil from fresh and dried leaves of *Ocimum gratissimum*. Fresh leaves afforded oil in the yield of 0.30% (w/w). The yields from the dried leaves were in the range of 0.33-0.53% (w/w).

Table 1: Yield of Essential oil from fresh and dried leaves of *Ocimum gratissimum*

Sample	GF	GD1	GD2	GD3	GD4	GD5
% Yield (w/w)	0.30	0.45	0.51	0.53	0.33	0.45

Key: GD1= One day dried leaves; GD2 = Two days dried leaves; GD3 = Three days dried leaves; GD4 = Four days dried leaves; GD5 = Five Days dried leaves

The highest and lowest yields were from leaves dried for three and four days respectively. The yield increases from the fresh leaves up to the leaves dried for three days and subsequently decreased in the leaves dried four days. The yield subsequently increased in the leaves dried for five days (Figure 1).

The increased in the yields is attributable to loss of moisture content caused by increment in ambient temperature at the period of drying. The decreased in yield may also be due to the volatilization of some oil arising from increased ambient temperature during drying.

However, the yields compared favourably with the yields from the leaves of the plant grown in Togo and Kenya respectively (Matasyoh *et al.*, 2007). Surprisingly, the yields were lower than the yield from fresh leaves of the plant grown in south western Nigeria (Owokotomo *et al.*, 2012).

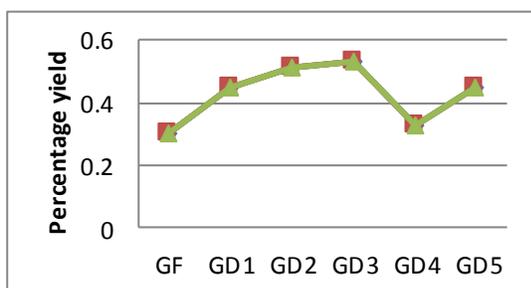


Fig. 1: Variations in the yield of essential oil from fresh and dried leaves of *O. gratissimum*

The identities, retention indices and percentage composition of constituents of essential oil from fresh and dried leaves of *Ocimum gratissimum* are presented in Table 2. A total of 17 - 25 compounds representing 90.0 - 99.2% of the oils, were identified from their mass spectra. The percentage composition of hydrocarbon and oxygenated monoterpenoids in the oils were in the range of 27.3- 48.4% and 10.0- 16.5% respectively. Hydrocarbon sesquiterpenoids constituted 15.0-34.1% of the oils. Meanwhile, 3.3- 17.9% of the oils were constituted by oxygenated sesquiterpenoids. Principal constituents of the oils were; α -thujene (6.8- 15.4%), γ -terpinene (10.0-17.5%), sabinene (2.2-6.0%), terpinolene (4.0-4.2%), thymol (9.2 - 14.2%), β -pinene (1.8-4.5%), terpinolene (0.9 - 6.2%), p-cymene (2.0- 8.0%), neo-allo-ocimene (1.1-8.4%), β -caryophyllene (4.8 - 10.1%), Precosene I (1.0 - 18.0%), β -selinene (12.5 - 27.5%), α -selinene (3.8 - 5.1%), caryophyllene oxide (3.3 - 11.1%), α -cadinol (0.6-4.9%), selina - 3.7-diene (1.0 - 3.4%) humulene (1.9 - 6.9%) and γ -selinene (11.1%) in the oil of the fresh leaf, methyl eugenol (6.8%) in the oil of the

leaves dried for a day, β -terpinene (8.1%) in the oil of the leaves dried for five days, β -thujene (7.4%) in the oil of the leaves dried for three days), carvacrol (13.6%) in the oil of the leaves dried for four days, spathulenol (4.0%) and cosmene (5.9%) in the oil of the leaves dried for two days. Camphene (0.6 - 1.2%), β -terpineol (0.6 - 1.5%), β -ocimene (0.7 - 1.3%), elemol (0.8 - 1.3%), humullene epoxide (0.5 - 1.4%), α -panasinsene (1.8 - 2.4%), eugenol (1.0 - 2.2%), acetophenone (1.7%) in the oil of the leaves dried for three days, 1,3,8-p-menthatriene (2.0%) in the oil of the leaves dried for a day, isothymol methyl ether (2.2%) in the oil of the fresh and α -himachallene (1.6%) in the oil of the leaves dried for two days were also detected in significant amounts. Compounds that were identified in appreciable quantities includes; durenol (0.4 - 0.6%), isoaromadendrene epoxide (0.6-0.8%), β -cadinene (0.9%) in the oil of the fresh leaves, umbellulone (0.8%) and γ - muurollene (0.4%) and aromadendrene oxide (0.8%) in the oil of the leaves dried for three days, isocaryophyllene (0.5%) and tau-cadinol (0.7%) in the oil of the leaves dried for five days, α -bergamotene (0.9%) in the oil of the leaves dried for four days.

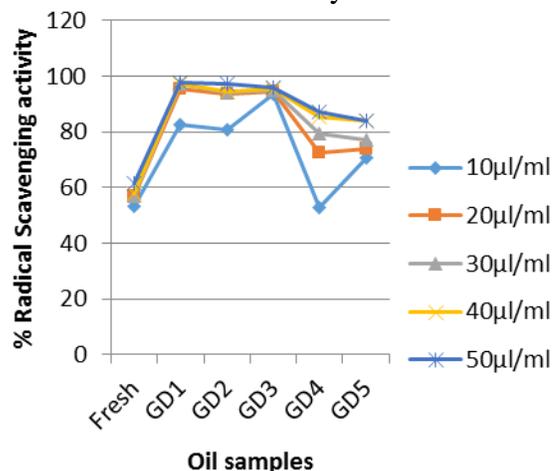


Figure 2: Variation in the Free Radical Scavenging Property of Fresh and Dried Leaves of *Ocimum gratissimum* against DDPH

Table 2: Chemical composition (%) of essential oils from fresh and dried leaves of *Ocimum gratissimum*

S/N	Compound	Retention Index	% Composition						Mass Spectra
			Fresh	GD1	GD2	GD3	GD4	GD5	
Page 3041									
1.	2 – hexanal	854	0.7	-	-	-	-	-	98, 83, 55 , 53,51
2.	Anisole	917	-	-	3.3	-	3.3	-	164, 149 ,135,119,105
3.	α – Thujene	931	10.6	15.3	15.4	-	6.8	-	136,121, 93 , 91, 79
4.	Camphene	953	0.6	-	-	1.2	-	-	136, 121, 93 , 79, 77
5.	Sabinene	976	5.6	6.0	2.2	2.5	-	-	136, 121, 93 , 91, 77
6.	β–thujene	-	-	-	-	7.4	-	-	136,121, 93 ,91,79
7.	β – Pinene	980	4.5	-	-	1.8	4.0	-	136, 121, 93 , 79, 69
8.	α – terpinene	1018	3.2	-	-	-	-	-	136, 121, 93 , 91, 79
9.	γ-terpinene	1062	14.7	-	-	10.0	17.5	16.8	136, 119, 93 , 79, 77
10.	Acetophenone	1065	-	-	-	1.7	-	-	204,180, 165 ,161,150
11.	β – terpinene	1071	-	-	-	-	-	8.1	136, 119, 93 , 91, 77
12.	Terpinolene	1088	0.9	4.0	6.2	-	-	-	136, 132, 93 , 91, 79
13.	β – Ocimene	1097	1.3	-	-	-	-	0.7	134, 121, 93 , 91, 79
14.	1,3,8-p-menthatriene	1111	-	2.0	-	-	-	-	134, 119 , 109, 105, 93
15.	Neo–allo–ocimene	1129	2.7	-	8.4	1.5	1.1	-	136, 134, 112 , 105, 93
16.	Cosmene	1134	-	-	5.9	-	-	-	139, 134, 119 , 105, 93
17.	β – terpineol	1144	-	0.6	-	1.5	0.9	0.8	154, 139, 71 , 55, 53
18.	Umbellulone	1171	-	-	-	0.8	-	-	150, 135, 108 , 91, 79
19.	P – Cymene	1206	4.3	-	-	3.4	5.3	10.5	139, 132, 117 , 91, 89
20.	Isothymol methyl ether	1235	2.2	-	-	-	-	-	164, 199, 134 , 119, 73
21.	Thymol	1290	10.3	10.9	-	14.2	-	9.2	150,135, 125 ,121,115
22.	Carvacrol	1298	-	-	-	-	13.6	-	150, 135 , 125, 115, 91
23.	Eugenol	1356	1.0	1.9	2.2	-	-	1.0	204,189, 164 ,149,131
24.	β-elemene	1375	-	-	-	-	2.3	-	189,175, 93 ,81,67
25.	β – cubebene	1390	0.6	1.7	-	1.1	-	0.7	204,189, 161 ,119,105
26.	Methyl eugenol	1401	-	6.8	-	-	-	-	204,189, 178 ,163,147
27.	Isocaryophyllene	1407	-	-	-	-	-	0.5	204, 189, 93 , 79, 67
28.	Aristolene epoxide	1416	-	-	-	2.0	-	-	220, 205, 109 , 96, 93
29.	β – Caryophyllene	1418	-	-	-	10.1	10.0	4.8	204,189, 133 , 120, 105
30.	α-bergamotene	1436	-	-	-	-	0.9	-	204,189, 119 ,107,73
31.	Precosene 1	1440	-	18.0	-	-	-	1.0	204,190, 175 ,160,147
32.	α – Himachalene	1447	-	-	1.6	-	-	-	209, 204, 93 , 80, 67

33.	Humulene	1454	2.7	6.9	-	3.6	1.9	-	204, 161, 93 , 80, 67
34.	γ -muurolene	1477	-	-	-	0.4	-	-	204,189, 161 , 133, 120
35.	γ -selinene	1484	11.1	-	-	-	-	-	204, 189, 105 , 93, 79
36.	β -Selinene	1485	-	-	27.5	13.8	12.5	15.0	204, 189, 105 , 93, 79
Page 3042									
37.	α -selinene	1494	3.8	-	-	-	5.1	-	204,189, 175 , 161, 147
38.	γ -Cadiene	1513	-	-	-	-	-	2.1	204,189, 161 , 133, 119
39.	Durenol	1516	-	-	-	0.4	-	0.6	149, 135, 119 , 105, 91
40.	β -cadinene	1518	0.9	-	-	-	-	-	204,189, 161 ,134,119
41.	Selina-3,7- diene	1534	-	6.4	-	-	-	1.0	204,189, 161 ,134, 122
42.	Elemol	1547	-	-	1.3	-	-	0.8	298, 283, 93 , 79, 67
43.	spathulenol	1576	-	-	4.0	-	-	-	220,207, 205 , 187, 177
44.	Isoaromadendrene epoxide	1579	-	0.6	0.8	0.8	-	-	220, 205, 91 , 79. 69
45.	Caryophyllene oxide	1581	4.0	4.5	8.0	11.1	3.3	7.7	220, 205, 79 , 69, 55
46.	Humulene epoxide	1606	-	0.5	1.4	-	-	0.8	220, 189, 109 , 96, 93
47.	Tau -cadinol	1640	-	-	-	-	-	0.7	204, 189, 161 ,147, 134
48.	α -cadinol	1653	1.0	1.9	3.5	0.6	-	4.9	222, 204, 95 , 81, 71
49.	Aromadendrene oxide	1702	-	-	-	0.8	-	-	220,205, 91 ,79,67
50.	α -panasinsen	1840	1.8	-	-	-	2.4	-	204,189, 161 , 133, 122
51.	Methoxy - Tetrahydrocannabinol	2475	0.7	1.2	4.1	-	-	-	328,313, 298 , 135, 105
TOTAL			90.0	91.2	99.2	91.8	91.9	90.7	

Table 3: Free Radical Scavenging Property and IC₅₀ of Essential Oil from fresh and dried leaves of *Ocimum gratissimum* against DPPH

Samples	% Radical Scavenging Property of the Oils					
	10 μ l/ml	20 μ l/ml	30 μ l/ml	40 μ l/ml	50 μ l/ml	IC ₅₀
Fresh	53.18 \pm 10.48	57.03 \pm 3.97	57.03 \pm 3.97	57.94 \pm 5.10	61.53 \pm 7.77	46.9
GD1	82.51 \pm 4.57	95.25 \pm 1.28	97.29 \pm 0.37	97.29 \pm 0.81	97.51 \pm 0.81	48.3
GD2	80.88 \pm 5.25	93.53 \pm 1.32	93.95 \pm 1.15	94.49 \pm 1.01	97.31 \pm 1.07	48.2
GD3	93.68 \pm 1.00	94.39 \pm 1.55	94.86 \pm 1.19	95.18 \pm 0.74	95.84 \pm 1.15	47.7
GD4	52.58 \pm 4.96	72.40 \pm 2.19	79.48 \pm 2.36	85.18 \pm 2.36	87.31 \pm 0.28	49.5
GD5	70.56 \pm 12.11	73.96 \pm 6.61	76.99 \pm 6.11	83.95 \pm 1.38	84.02 \pm 1.51	48.6

The results are mean \pm S.D of values in triplicate

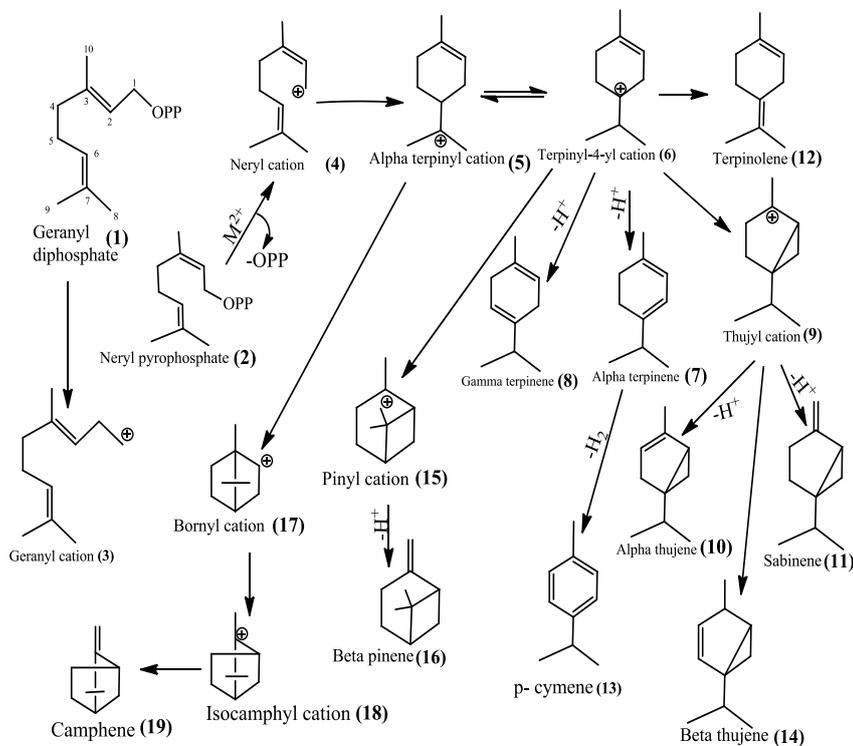
Comparison of the oils revealed that their phytochemical profiles varied significantly. Qualitatively, α -terpinene, isothymol methyl ether, γ -selinene and α -cadinene were found in the oil from the fresh leaves but were not detected in the oils of the dried samples. Oil of the leaves dried for three days were characterized by; α -thujene, acetophenone, umbellulone, aristolene epoxide, γ -muurolene and aromadendrene which were not identified in the oils from other samples. Cosmene and α -himachalene constituted oil of the leaves dried for two days but, were not found in other oils. The presence of carvacrol, α -bergamotene, β -elemene and β -terpineol were established in the oil of the leaves dried for four days but were not identified in other oils. γ -Terpinene and p-cymene were detected in the oils except oils from leaves dried for one, two and five days. Similarly, thymol and cubebene were found in the oils with the exception of oils from leaves dried for two and four days respectively.

Quantitatively, caryophyllene oxide and thymol were found in the oils but they were of greater abundance in the oil of leaves dried for three days than other oils. Similarly, oil from leaves dried for two days was richer in α -thujene, neo-allo-ocimene and β -selinene than other oils. The abundance of γ -terpinene, precosene I, β -seliene and thymol in the oils revealed that, the oils were of γ -terpinene, precosene I, β -selinene and thymol chemotypes. The chemotypic variations of the oils might be attributed to changes in ambient temperature during drying and this influences the physiological condition in the leaves. This condition dictates type of enzyme that mediates the biosynthesis of mono- and sesquiterpenoids from their respective precursors in plant. Interestingly, it has been established that enzymes of the most abundant mono- and sesquiterpenoids facilitate the transformations of their precursors to various terpenic products via cationic intermediates. The intermediates often undergo series of cyclisation, hydride and methyl shifts until the reaction is terminated by deprotonation or hydration to form several terpenoid. The reactions do proceed by cationic mechanism (Croteau, 1987; Wise and Croteau, 1999).

Reaction Mechanisms: The predominance of γ -terpinene, α -thujene and thymol indicated that the synthases of these compounds mediated the formation of all monoterpenoids in the oils where each of the compounds predominates (Reaction Scheme 1). In the scheme, geranyl pyrophosphate (**1**) and neryl pyrophosphate (**2**) ionized to geranyl and neryl cations (**3 and 4**) in the presence of a divalent metal ion. The electrophilic attack of the ion (**4**) on C₆ – C₇ double bond formed α -terpinyl cation (**5**). 6,7-hydride shift of the ion (**5**) gave terpinyl-4-yl cation (**6**) which deprotonated at C₁ by γ -terpinene synthase to form α -terpinene (**7**) in the oil of fresh leaves. Deprotonation of the ion at C₅ in the presence of γ -terpinene synthase formed γ -terpinene (**8**) in the oils with the exception of the oil from leaves dried for two and three days respectively. Electrophilic addition of the ion (**6**) to C₂ – C₃ double bond formed thujyl cation (**9**). Loss of proton by the ion (**9**) at C₄ formed α -thujene (**10**) in the oils except oil from the leaves dried for three and five days. The transformation of the ions (**9**) to the terpenoids (**10**) was aided by γ -terpinene synthase in the fresh and leaves dried for four days. However, α -thujene synthase mediated the formation of the compound (**10**) in the oils of leaves dried for one and two days. γ -terpinene, α -thujene and thymol synthases facilitated deprotonation of thujyl cation at C₁₀ to sabinene (**11**) in the oils from fresh and the leaves dried for one, two and three days respectively. Similarly, both γ -terpinene and α -thujene synthases catalyzed formation of terpinolene (**12**) in the fresh and the leaves dried for one and two days via deprotonation of terpinyl-4-yl cation (**6**) at C₇. γ -Terpinene and thymol synthases aided dehydrogenation of α -terpinene at C₄ and C₅ and formed p-cymene (**13**) in the oils from fresh and the leaves dried for three, four and five days. 3,4-hydride shift of thujyl cation (**9**) followed by deprotonation at C₅ formed β -thujene (**14**) in the leaves dried for three days. The formation of β -thujene was facilitated by thymol synthase. Folding of α -terpinyl cation (**5**) towards C₂-C₃ double bond formed pinyl cation (**15**). Deprotonation of the ion (**15**) at C₁₀ gave β -pinene (**16**) in the oils from fresh and dried leaves for three and four

days. γ -terpinene and thymol synthases mediated the formation of the compound (16). Folding of ion (5) towards C₂-C₃ double bond followed by its electrophilic attack via C₃

formed bornyl cation (17). Wagner Meerwein rearrangement of the ion (17) formed isocamphyl cation (18) that deprotonated at C₁₀ to produce camphene (19).



Reaction Scheme 1: Biosynthesis of predominant monoterpenoids in the oils from fresh and dried leaves of *Ocimum gratissimum*

The predominance of humulene and β - and γ -selinene in the oils revealed that their synthases facilitated the formation of all sesquiterpenoids in the oils where each of the compounds predominates (Scheme 2). In the scheme, the synthases in the presence of divalent metal ion ionized fernasyl pyrophosphate (20) to E, E- and E,Z-fernasyl cations (21 and 22). Electrophilic attack of the ion (21) on C₁₀-C₁₁ double bond via C₁₀ formed germacradienyl cation (23). The ion (23) subsequently deprotonated at C₁₂ to give germacrene A (24). Protonation of the compound (24) formed intermediate ion (25) that subsequently undergo electrophilic attack on C₂-C₃ double bond to form eudesmyl cation (26). Deprotonation of the ion (26) at C₄ formed α -selinene (27) in the oil of fresh leaves. This was mediated by α -selinene synthase. β -selinene (28) was formed via deprotonation of

the ion (26) at C₁₅ in the leaves dried for two, three, four and five days. The synthase of the compound (28) mediated its formation. γ -selinene (29) was formed by protonation of β -selinene at C₁₂ and subsequent deprotonation at C₁₀. The compound (29) which its synthesis was mediated by its synthase was detected in the oil of the fresh leaves. 10,11 and 1,10 hydride shift of germacradienyl ion formed an intermediate ion (30). Electrophilic attack of the ion on C₆-C₇ double bond formed cadinyl cation (31). Deprotonation of the ion at C₁₄ was catalysed by β -selinene synthase and produced γ -cadinene (32) in the oil of the leaves dried for five days.

Hydration of the ion (31) formed α -cadinol (33) in the oil from fresh and the leaves dried for one, two and three days. The formation of compound (33) was mediated by humulene, β - and γ -selinene synthases. Electrophilic attack

Antioxidant Activity: The free radical scavenging potential of the oils on DPPH is as presented in Table 3. The antioxidative activity of each of the oils was concentrated on dependent irrespective of their sources. The most active oils were those from fresh and the leaves dried for three and four days, with least activity at IC₅₀ values of 46.9 and 49.5% respectively. Interestingly, the radical scavenging activity of the oil also increased steadily from the oil of fresh leaves to the oil of the leaves dried for three days. Surprisingly, the activity decreased in the oil of the leaves dried for four days and later increased substantially in the oil of dried leaves for five days (Figure 2). Meanwhile, it has been reported that essential oil that bear γ -terpinene, terpinolene, β -pinene, thymol, carvacrol and α -thujene as their constituents exhibited significant free radical scavenging potential (Sonbol *et al.*, 1992; Song *et al.*, 2001 ; Tundis *et al.*, 2012; Sarrou *et al.*, 2013). The antioxidative potentials recorded in this study might be due to the presence of the compounds in the oils. However, the potential varied significantly which is attributable to differences in proportion of compounds that possess radical scavenging properties in the oils.

The variation in the activity of the oils at 50% inhibitory concentration shows that irrespective of length of dryness, they still possess free radical scavenging activities above 40% at IC₅₀. Oils from fresh and leaves dried for three and four days were the most with least active at IC₅₀ values of 46.9 and 49.5% respectively. Essential oil containing γ -terpinene, terpinolene, β -pinene, myrcene, thymol, carvacrol and α -thujene had been reported to show greater potential to scavenge free radicals (Sonbol *et al.*, 1992; Song *et al.*, 2001; Tundis *et al.*, 2012; Sarrou *et al.*, 2013). The activities of oils from this study can be linked to the synergistic actions of the compounds in the oils. Moreover, oil from the leaves dried for three days was the most active at IC₅₀ value of

10 μ l/ml and this is attributable to abundance of oxygenated mono- and sesquiterpenoids in the oils.

Conclusion and recommendations

Essential oil yields from fresh and dried leaves of *Ocimum gratissimum* increased with increase in days of drying except in the dried leaves for four days where the yield decreased. This implied that variations in ambient temperature at various days of drying significantly affected yields of oil from the leaves. The conditions also caused chemotypic variations in the oil as reflected from their antioxidative potentials that varied significantly. However, irrespective of the sources of the oils their anti oxidant potentials qualify them as suitable alternative to synthetic antioxidant for the treatment of oxidative stress.

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