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DRYING EFFECT ON THE ISOLATION, CHARACTERIZATION AND ANTIOXIDANT POTENTIALS OF LEAF ESSENTIAL OILS OF *Ocimum gratissimum* HARVESTED AT 10:00am IN A DAY

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ABSTRACT

Essential oils are volatile compounds found in special brittle secretory structures in plants, such as glands, they have several modes of actions as antioxidant, such as prevention of chain initiation and free radical scavenging. *Ocimum gratissimum* is an herbaceous plant which belongs to the Labiatae family used in the treatment of epilepsy, high fever, diarrhea, mental illness, management of the baby's cord, to keep the wound surfaces sterile and in the treatment of fungal infections, cold and catarrh. In this study, the antioxidant activity of *Ocimum gratissimum* in both fresh and dry leave is accessed. Pulverized fresh leaves (500g) of *Ocimum gratissimum* harvested at 10:00am in a day were air dried for five consecutive days and separately hydro-distilled for 3 hours. The oil yields range from 0.42-0.91% (w/w). Analysis of the oils using GC and GC-MS revealed the predominance of hydrocarbon sesquiterpenes (16.1-44.5%) and hydrocarbon monoterpenes (39.3-43.7)). The oils were predominated by α-thujene (14.6-24.5%), β-pinene(2.3-3%), α-terpinolene (9.3%), 1,3,8-p-menthatriene (1.8-3.5%), β-selinene (19.0-32.9%), Selina-3,7(11)-diene (5.6-12%), Isocarophillene (1.2-1.7%), β-Caryophyllene (8.5%), Humulene (2.3%), γ-cadinene (1.4%), E,Z-alloocimene (2.6-13.2%) and p-cymene (6.3-9.5%). α-phellandrene was detected in significant quantity (0.8%). The predominance of α-thujene and β-selinene in the oils shows that the oils were of α-thujene and β-selinene chemotypes. The free radical scavenging activities of oils were tested against2,2-diphenyl-1-picrylhydrazyl(DPPH). The oils were active against DPPH regardless of the level of dryness. The most and least active oils were the oils of leaves dried for four days (95.68±1.40) and two days (41.91±2.42) at 20µl/ml and IC_{50} of 4.29µl/ml and 20.05µl/ml respectively.

Keywords: Essential oils, *Ocimum gratissimum*, β-selinene, α-thujene, Antioxidants

INTRODUCTION

Essential oils (also called volatile or ethereal oils, because they evaporate when exposed to heat in contrast to fixed oils) are a mixture of odorous and volatile compounds found in special brittle secretory structures in plants, such as glands, secretory hairs, secretory ducts, secretory cavities or resin ducts (Ciccarelli *et al.*2008; Morone- Fortunato *et al.* 2010).They have several modes of actions as antioxidant, such as prevention of chain initiation, free radical scavengers, reducing agents, termination of peroxides, prevention of continued hydrogen abstraction as well as quenchers of singlet oxygen formation and binding of transition metal ion catalysts (Yildirim et al. 2000; Mao et al. 2006). The antioxidant capability of phenolic compounds is mainly due to their redox properties, which permit them to act as hydrogen donors, reducing agents, singlet oxygen quenchers as well as metal chelators (Kumar *et al.* 2005).

Ocimum gratissimum is an herbaceous plant which belongs to the Labiatae family. It is known by various names in different parts of the world. In India it is known by its several vernacular names, the most commonly used ones being Vriddhutulsi (Sanskrit), Ram tulsi (Hindi), Nimmatulasi (Kannada). The plant is called "effinrin-nla" by the Yoruba speaking tribe. It is called "Ahuji" by the Igbos, while in the Northern part of Nigeria, the Hausas call it "Daidoya" (Effraim *et al.*2003). It is an important herbal medicinal plant not only in Nigeria but also in the sub-Saharan Africa. In the southern part of the country, crude aqueous

extract of *O. gratissimum* is commonly used in the treatment of epilepsy, high fever and diarrhea (Effraim *et al.* 2003). In the Savannah areas, decoctions of the leaves are used to treat mental illness (Akinmoladun, 2007). It is used by the Igbo community of south eastern Nigeria in the management of the baby's cord, to keep the wound surfaces sterile. It is also used in the treatment of fungal infections, fever, cold and catarrh (Ijeh *et al.*2005).Considering the fact that *Ocimum gratissimum* is used in most local dishes/foods to achieve a variety of purposes, there is need to ascertain if its extract antagonizes or acts in synergy when used together with conventional antioxidant drugs. In addition, despite the fact that the various extracts of *O. gratissum* have been shown to possess high antioxidant activity and to be active against some bacteriatested *in vitro* (Nakamura et al. 2004; Silva et al. 2005; Lemos et al. 2005), effect of moisture on the antioxidant activities of the leaves of this spice is yet to be determined, and this was one of the motivations for this study. This study thus investigates the effect of moisture on the antioxidant potentials of the leaf essential oil of *O. gratissimum* and the compound composition of the leaf responsible for this activity.

MATERIALS AND METHOD

Plant Materials

Fresh leaves of *Ocimum gratissimum* were harvested at 10am at the Biological Park and Garden, University of Ilorin, Ilorin, Nigeria, and air dried for five consecutive days. The taxonomic identification of the plant was carried out at the Herbarium, Plant Biology Department, University of Ilorin, Ilorin, Nigeria where voucher specimens were deposited.

Isolation of Essential Oil

Pulverized fresh and dried (500g) leaves of *O. gratissimum* were separately hydro distilled for 3hrs, according to British Pharmacopoeia (1981) specification. The oil was collected and stored in air-tight containers at 4^0C prior to analysis.

Gas Chromatography-Mass Spectrometry Analysis

The oil was analyzed by GC/MS using an Agilent 5973EI mass selective detector coupled with an Agilent GC6890A gas chromatograph, equipped with a cross-linked 5% PH ME siloxane HP-5MS capillary column (30m x 0.32mm, épaisseur de film 0.25µm). Operating conditions: The carrier gas flow was 1.6 ml He/min, column pressure was 100Kpa. The injector and detector temperatures were 220°C and 250°C respectively. The column temperature was held at 60°C for 1 min, then raised from 60°C to 200°C at 10°C/min and held there for 5 min and from 200°C to 240°C at 10°C /min and held there for 6 min. The program was run in the splitless mode with a mass range of 50–400 u, and the scan interval was 0.5 s. Detector voltage was set at 1.5 kV. Identification of oil components was achieved on the basis of their retention indices RI, (determined with reference to a homologous series of normal alkanes), and by comparison of their mass spectral fragmentation patterns with those reported in the literature and stored on the MS library (NIST database). The concentration of the identified compounds was computed from the GC peak total area without any correction factor (**Djebaili *et al.* 2013**).

Determination of DPPH Radical Scavenging Activity

The free radical scavenging activity of essential oils from fresh and dried leaves of *Ocimum gratissimum harvested at 10:am in a day* was measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) using the method of Blois 1958; a method based on the reduction of a methanoic solution of the colored DPPH radical (Cao, 1997). DPPH evidently offers a convenient and accurate method for measuring the antioxidant potential. 0.1 mM solution of DPPH in methanol was prepared and 1 mL of this solution was added to 3mL of the extract suspension in water at different concentrations (10, 20, 30, 40 and 50µl). After 30 minutes of incubation, the absorbance of the mixture was measured at 517 nm. Methanol was used as reference material. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. All the tests were performed in triplicate and the average was calculated. The percentage reduction in absorbance was calculated from the initial and final absorbance of each solution (Shishoo*et al.*1999).

The percentage inhibition was calculated by comparing scavenging of DPPH radical using the formula:

% Scavenging of DPPH = ($Abs_{control}$-Abs_{test} sample)/$Abs_{control}$×100

Where $Abs_{control}$ = absorbance of the control (reacting mixture without the test sample) and, Abs_{test} sample = absorbance of reacting mixture with the test sample.

Data Analysis

The % Scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) values from the experiment were subjected to a 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.

RESULTS AND DISCUSSION

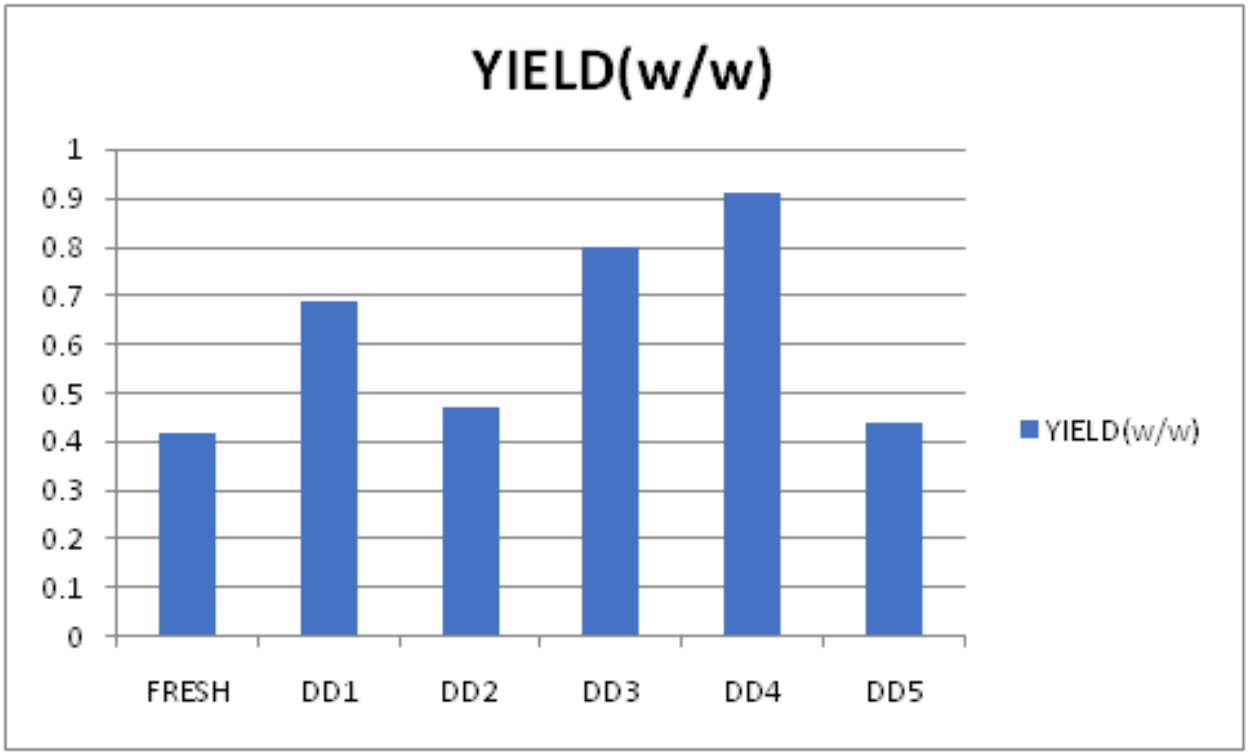

Figure 1: Variations in the yields of essential oils from fresh and dried leaves of *Ocimum gratissimum* harvested at 10:00am in a day

The variations in the oil of essential oils from fresh and dried leaves of *Ocimum gratissimum* harvested at 10:00am in a day are presented in figure 1. The yields ranged from 0.42- 0.91 (w/w). The dried leaves yielded more oils than the fresh samples**.** Meanwhile, highest yield was obtained from the leaves dried for four days. The lowest yield obtained from fresh sample can be linked to high moisture content. The yields increased from fresh to the leaves dried for one day and decreased in the leaves dried for two days. There was a steady increase for leaves dried for three and four days before decreasing in the leaves dried for five days.

Table 1: Chemical composition (%) of essential oils from fresh and dried leaves of *Ocimum gratissimum* harvested at 10:00am in a day

COMPOUNDS	KI	PERCENTAGE COMPOSITION						M.S DATA
		FRESH	DD1	DD2	DD3	DD4	DD5	
2-Hexenal, (E)-	854	1.3	-	-	-	-	-	98,83,**55**,50,0
α-Thujene	931	**14.6**	14.6	18.8	**24.5**	23.3	21.4	136,121,**93**,91,77
β-pinene	980	-	2.7	2.3	2.9	3.0	3.0	136,115,**93**,89,77
α-phellandrene	1005	0.8	-	-	-	-	-	136,105,**93**,77,65
α-terpinolene	1088	-	-	-	9.3	-	-	136,132,**132**,117,91
Linalool	1098	-	-	-	2.1	-	-	154,121,**71**,69,55
1,3,8-p-menthatriene	1111	2.5	1.8	3.5	-	2.8	2.6	134,130,**119**,91,77
E,Z-alloocimene	1129	13.2	2.6	8.8	7.0	-	7.3	136,130,**121**,91,79
2,4,6-octatriene,3,4-dimethyl	1132	-	-	-	-	8.8	-	136,130,**121**,105,77
E,E-cosmene	1134	-	2.9	-	2.7	-	-	134,128,**119**,105,93
Cis- β-terpineol	1142	0.7	-	-	1.3	-	-	154,111,**71**,69,55
p-cymene	1206	9.5	7.6	5.9	-	6.3	7.5	132,121,**117**,91,65
2,3,6-trimethyl-anisole	1209	0.4	-	-	-	-	-	160,155,**150**,135,91
p-cymen-7-ol	1287	1.7	1.9	-	-	-	-	150,140,**135**,119,105
Aristolene epoxide	1293	-	1.0	-	-	-	-	220,159,**123**,95,82
Carvacrol	1298	-	0.6	-	-	-	-	150,140,**135**,107,91
Phenol, 2-ethyl-4,5-dimethyl	1340	-	2.0	-	-	-	-	150,140,**135**,91,80
Thymol acetate	1355	-	4.3	-	-	-	-	192,150,**135**,91,77
Eugenol	1356	1.3	1.5	1.3	1.7	1.9	1.4	164,149,**103**,77,65
Carvacrol acetate	1371	0.6	0.7	-	-	-	-	192,150,**135**,81,69
β-cubebene	1390	2.8	1.9	2.4	-	2.2	2.2	204,189,**161**,105,81
Isocarophillene	1407	-	-	1.2	-	1.7	1.7	204,133,**93**,79,67
β-Caryophyllene	1418	-	-	-	8.5	-	-	204,189,**178**,133,120
Humulene	1454	-	-	-	2.3	-	-	204,121,**93**,80,67
Alloaromadendrene oxide(1)	1461	3.4	2.3	-	-	-	-	220,177,**91**,69,55
β-selinene	1485	-	**19.0**	**32.9**	-	**28.7**	**29.1**	204,161,**105**,93,67
Cubedol	1494	0.5	-	-	-	-	-	222,204,**194**,161,105
γ-cadinene	1513	1.4	-	-	-	-	-	204,189,**161**,119,91
β-cadinene	1529	2.3	-	-	-	-	-	204,189,**161**,147,105
Selina-3,7(11)-diene	1542	9.6	-	8.0	12.0	5.6	8.0	204,175,**161**,122,91
Norolidol(trans)	1564	-	-	-	0.7	-	-	222,93,**69**,55,50
Spatulenol	1576	-	2.6	1.9	2.7	2.1	1.7	220,205,**91**,79,55
Isoaromadendrene epoxide	1579	12.0	3.3	-	0.8	-	-	220,189,**93**,79,67
Caryophyllene oxide	1581	2.2	11.2	8.1	10.1	4.5	6.6	220,121,**79**,69,55
Epiglobulol	1588	1.6	-	-	-	-	-	222,189,**161**,109,93
Calarene epoxide	1592	0.8	0.8	-	0.8	1.4	-	220,177,**159**,133,119
1,2-epoxide-humulene	1606	-	1.7	-	0.9	-	-	220,138,**109**,96,67
tau-cadinol	1640	-	-	-	0.8	2.2	-	222,204,**161**,119,105
β-Eudesmol	1649	2.8	-	-	-	-	-	222,164,**149**,121,81
α-cadinol	1653	3.6	3.9	2.1	3.3	3.6	3.0	222,121,**95**,81,71
Abietic acid	2336	0.8	1.0	-	-	-	-	302,241,**201**,163,121
Gibberillic acid	2393	2.0	0.7	0.9	1.3	-	-	346,203,**135**,121,91
Methoxy-tetrahydrocannibol(THC)	2475	1.5	1.8	1.1	1.0	1.4	1.6	328,313,**328**,179,149
γ-costol	2533	-	-	-	0.7	-	-	220,202,**187**,121,79
2-[(acetylphenyl)carbamoyl]benzoic acid	2685	-	-	-	-	-	1.4	222,150,**135**,115,109
TOTAL		**93.9**	**94.4**	**99.2**	**97.4**	**99.5**	**98.5**	

a- Compounds are listed in order of elution from silica capillary column coated with Cp-Sil 5. b- Kovat retention indices on fused capillary column coated with Cp-Sil 5. Fresh: Fresh leaves, DD1: leaves dried for one day, Dd2: leaves dried for two days, DD3: leaves dried for three days, DD4: leaves dried for four days, DD5: leaves dried for five days

The Kovat retention indices, relative percentages and identities of constituents of essential oils from fresh and dried leaves of *Ocimum gratissimum* harvested at 10:00am in a day are shown in Table 1. A total of 15-33 compounds ranging 93.9- 99.5 % were identified from their mass spectra data. α-thujene was predominant in the oils from fresh and leaves dried for three days while β-selinene was the most predominant compound in the other oils. The predominance of these compounds reveal that the oils were of α-thujene and β-selinene chemotypes.

A total of 4-5 hydrocarbon monoterpenes representing 29.3-43.7% were identified from their mass spectra data. The major hydrocarbon monoterpenes in the oils were α-thujene (14.6-24.5%), β-pinene(2.3-3%), α-terpinolene (9.3%), 1, 3, 8-p-menthatriene (1.8-3.5%), E, Z-alloocimene(2.6-13.2%) and p-cymene (6.3-9.5%). α-phellandrene was detected in significant quantity (0.8%). 2-5 oxygenated monoterpene compounds representing 3.4-9.5% were identified from their mass spectra data. The major oxygenated monoterpenoids in the oils were linalool (2.1%), Cis-β-terpineol (0.7-1.3%), p-cymen-7-ol (1.7-1.9%), phenol, 2-ethyl-4,5-dimethyl (2.0%) and thymol acetate (4.3%). Carvacrol (0.6%) and carvacrol acetate(0.6-0.7%) were present in significant quantity while 2, 3, 6-trimethyl-anisole (0.4%) was present in minor quantity.

A total of 2-4 hydrocarbon sesquiterpene compounds representing 16.1-44.5% were identified from their mass spectra data. Major hydrocarbon sesquiterpenes in the oil were β-selinene (19.0-32.9%), selina-3, 7 (11)-diene (5.6-12%), isocarophillene (1.2-1.7%), β-caryophyllene (8.5%), humulene (2.3%) and γ-cadinene (1.4%). A total of 3-9 oxygenated sesquiterpenes compounds representing 11.3-26.8% were identified from their mass spectra data. Major oxygenated sesquiterpenes in the oils were caryophyllene oxide (4.4-11.2%), isoaromadendrene epoxide (12%), aristolene epoxide (1.0%), alloaromadendrene oxide-1(2.3-3.4%), spatulenol (1.7-2.7%), epiglobulol(1.6%), calarene epoxide(0.8-1.4%), 1,2-epoxide-humulene (0.9-1.7%), tau-cadinol (0.8-2.2%), β-eudesmol (2.8%) and α-cadinol(2.1-3.9%). cubedol (0.5%) and nerolidol (trans)(0.7%) were also detected in appreciable amounts.

Finally, a total of 3-5 non-terpenic compounds representing 3.3-12.1% were identified from their mass spectra data. Major compounds found in the oils were eugenol (1.3-1.9%), methoxy-THC (1.0-1.8%), 2-hexenal, (E)- (1.3%), 2, 4, 6-octatriene, 3, 4-dimethyl (8.8%), abietic acid (0.8-1.0%), gibberillic acid (0.7-2.0%) and 2-[(acetylphenyl) carbamoylbenzoic acid (1.4%).

Qualitatively, there are variations in the constituents of the oils. For instance, 2-hexenal, (E), α-phellandrene, cubedol, γ-cadinene, β-cadinene, epiglobulol and β-eudesmol were found in the oils obtained from the fresh leaves but were not detected in the oils from other samples. Also, aristolene epoxide, carvacrol, Phenol, 2-ethyl-4,5-dimethyl and thymol acetate that were present in the oils from leaves dried for one day, were not detected in the oils from other samples. Similarly, α-terpinolene, linalool, β-caryophyllene, humulene, norolidol(trans) and γ-costol that were present in the oils from leaves dried for three days, but were not detected in other oils. In addition, 2,4,6-octatriene,3,4-dimethyl was found only in the oils from leaves dried for four days. Meanwhile, 2-[(acetylphenyl) carbamoylbenzoic acid was present in the oils from leaves dried for five days but was not detected in the oils from other samples. Furthermore, p-cymen-7-ol, carvacrol acetate, alloaromadendrene oxide-(1) and abietic acid were found in the oils obtained from fresh leaves and leaves dried for one day only. Similarly, 1,3,8-p-menthatriene, p-cymene and β-cubebene were found in the oils obtained from all samples except leaves dried for three days. Also, E, E-cosmene and 1, 2-epoxide-humulene were found in the oils obtained from leaves dried for one and three days, but not identified in other. E, Z-alloocimene was found in the oils obtained from all but leaves dried for four days while Selina-3, 7(11)-diene was found in the oils obtained from all but leaves dried for one day. Similarly, Spatulenol was identified in the oils obtained from all but fresh leaves.

Quantitative variations were also observed in some of the constituents of the oil. For example, irrespective of period of drying, α-es

thujene, eugenol, caryophyllene oxide, α-cadinol and methoxy-THC were all identified in the oils obtained from the leaves, but present in varying quantities. α-Thujene was much more abundant in the oils except the oils from fresh and leaves dried for one and two days. Likewise, caryophyllene oxide was detected in higher quantities in the oils of leaves dried for one, two and three days than other samples. E, Z-alloocimene, p-cymene, β-cubebene and gibberillic acid were of greater abundance in the oils from fresh leaves than oils from other samples. Similarly, caryophyllene oxide, α-cadinol and methoxy-THC were of greater abundance in the oil from leaves dried for one day than oils from other samples. More so, 1, 3, 8-p-menthatriene and β-selinene were higher in the oil from leaves dried for two days than oils from other samples. α-thujene and Selina-3, 7(11)-diene were higher in the oil from leaves dried for three days than oils from other samples. Oils from the leaves dried for four days were richer in eugenol than other oils. The qualitative and quantitative variations may be attributed to change in weather conditions and increase in ambient temperature which may lead to the volatilization of the oil.

Table 2: Free radical scavenging activity of essential oils from fresh and dried leaves of *Ocimum gratissimum* harvested at 10:00am in a day against DPPH

	%Scavenging activity of*O. gratissimum* extracts*				
SAMPLES	**10µl/ml**	**20 µl/ml**	**30 µl/ml**	**40 µl/ml**	**50 µl/ml**
FRESH	84.52±1.47	91.84±1.43	92.07±1.33	90.86±1.24	87.94±1.92
DD1	82.58±1.51	83.15±1.68	94.37±1.47	93.32±0.45	91.82±1.21
DD2	73.36±5.21	41.91±2.42	68.17±4.07	78.87±1.98	71.82±5.01
DD3	63.25±2.66	71.88±2.58	71.19±0.03	74.80±1.34	75.05±1.97
DD4	91.82±1.25	95.68±1.40	91.80±0.70	92.97±1.26	93.44±1.55
DD5	67.38±3.39	90.91±1.62	86.24±4.69	73.25±2.34	71.31±0.89

*values expressed are means±S.D of three parallel measurements

The free radical scavenging activity of essential oils from fresh and dried leaves of *Ocimum gratissimum* harvested at 10:00amin a day against 2,2-diphenyl-1-picrylhydrazyl (DPPH) is shown in table 2. The change in color of DDPH solution at various concentrations from purple to yellow suggests the ability of these oils to act as donors of hydrogen atoms or electrons in transformation of DPPH into its reduced form DPPH-H. The antioxidant potentials were concentration dependent. The highest activity of the oil of the leaves dried for four days can be attributed to the greater amount of eugenol which had earlier been reported to possess antioxidant property (Joshi, 2013). In the table, at minimum concentration of 10µl/ml, samples dried for three days had the lowest percentage inhibition of 63.25 on the DPPH while samples dried for four days had the highest 91.82. At 20µl/ml, samples dried for two days had the lowest percentage inhibition while oils from samples dried for four days had the highest. At 30µl/ml, samples dried for two days had the lowest percentage inhibition while samples dried for one day had the highest. Oils from other samples had intermediate percentage inhibitions. At 40µl/ml samples dried for five days had the lowest percentage inhibition while samples dried for one day had the highest. At 50µl/ml, samples dried for five days had the lowest percentage inhibition while samples dried for four days had the highest. The dose response of oils from various harvests suggests a positive correlation between the concentrations of the oil and their antioxidant activity.

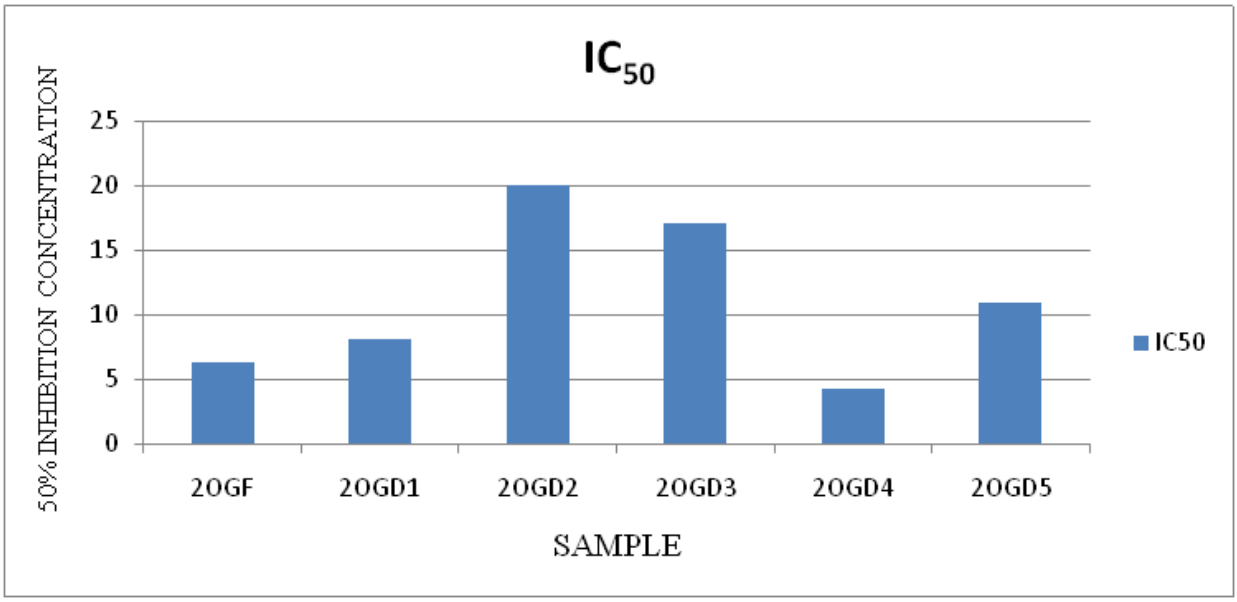

Figure 2: Variations in the 50% inhibition concentration of the essential oils from fresh and dried leaves of *Ocimum gratissimum* harvested at 10am in a day against DPPH

Figure 2 shows the 50% inhibition concentration (IC_{50}) of essential oils from fresh and dried leaves of *Ocimumgratissimum* harvested at 10:00am in a day against DPPH. Variations in the 50% inhibition concentration are shown in figure 3. The calculated 50% inhibition (IC_{50}) values were 6.29, 8.14, 20.05, 16.99, 4.29 and 10.93µl/ml for oils of leaves from FRESH, DD1, DD2, DD3, DD4 and DD5. Oils of leaves dried for four days have the highest Ic_{50} while Samples dried for two days has the lowest value. The lower the IC_{50} of oils the more effective they are as antioxidants. The most and least active oils were the oils of leaves dried for four days and two days at 20µl/ml and IC_{50} of 4.29µl/ml and 20.05µl/ml respectively.

Reaction Mechanisms

It has been established that terpene syntheses of the most abundant mono- and sesquiterpoids facilitates the transformation of their precursors [geranylpyrophosphate**(1)** and fernesylpyrophosphate**(19)]** to various cationic intermediate [linalyl **(4)**, geranyl **(2)**, fernasyl**(20)**, nerolidyl**(21)**, and humulyl**(26)** cations] in the presence of divalent metal ions. The cationic intermediates can be transformed by series of cyclization, rearrangement and hydride shift until the process is terminated by hydration, deprotonation, oxidation or reduction reactions to form several terpenic compounds (Degenhardt *et al.* 2009).

The predominance of α-thujene in the oils implies that, its synthase mediates the formation of all monoterpenoids from geranylpyrophosphate (1). The terpene synthase in the presence of a divalent metal forms geranyl cation (2), neryl cation (3) and linalyl cation (4**)** which can be transformed by series of cyclization, rearrangement and hydride shift to various cationic intermediates until the process is terminated by hydration, deprotonation, oxidation or reduction reactions to form several monoterpenoids, (Figure 3). In the figure, Hydration of linalyl cation gives linalool (7). Electrophillic attack of cationic intermediate (3) on C_6-C_7 gives α-terpinyl cation (8). C-7 deprotonation of terpinyl cation leads to terpenolene(9). 7,3-hydride shift followed by hydration of terpinyl cation and C-8 deprotonation gives cis- β- terpinenol(18). 1,7-hydride shift of α-terpinyl cation gives phellandryl cation (10), deprotonation of this cation at C-5 gives α-phellandrene (17). 6,7-hydride shift of α-terpinyl cation gives terpinen-4-yl cation (11). Electrophillic attack of terpinen-4-yl cation on C-2 gives thujyl cation (12) which undergoes C-4 deprotonation to give α-thujene (13). Folding **o**f α-terpinyl cation

towards C_2-C_3 double bond followed by an electrophillic attack on C-2 gives pinyl cation (14). C-4 and C-10 deprotonation of pinyl cation gives α-pinene (15) and β-pinene (16) respectively.

The predominance of β-selinene in the oils indicates that, its synthase mediates the formation of all sesquiterpenoids from fernasyl pyrophosphate **(19).** Theterpene synthases in the presence of divalent metal forms fernasyl**(20)** andnerolidyl cation **(21)** which can be transformed by series of cyclization, rearrangement and hydride shift to various cationic intermediates until the reaction is terminated by hydration, deprotonation, oxidation or reduction reactions to form several sesquiterpenoids (Figure 4). In the figure, Hydrolysis of fernesyl and nerolidyl cation forms fernesol**(23)** and nerolidol (**22**) respectively**.** Electrophillic attack of fernasyl cation **(20)** on C_{10}-C_{11} double bondgives humulyl cation (**26)** and E,E)-germacradienyl cation (**24).** Humulyl cation undergoes C-9 deprotonation to produce humulene **(27**) while 6,1-closure of (E,E)-germacradienyl cation followed by deprotonation at C-12 gives β-selinene (**28**). Electrophillic attack at C-2 on humulyl cation **(26)** gives caryophynyl cation **(30**), which in turn undergoes C-4 and C-15 deprotonation to form α-caryophyllene (**31**) and β-caryophyllene **(32**) respectively. 5,7-epoxidation of β-caryophyllene leads to the formation of caryophyllene oxide (**33**). Electrophillic attack at C-10 on nerodyl cation gives (Z,E)-germacradienyl cation (**25**), Which undergoes 6,1-closure followed by loss of proton at C-4 to form β-cadinene (**29**).

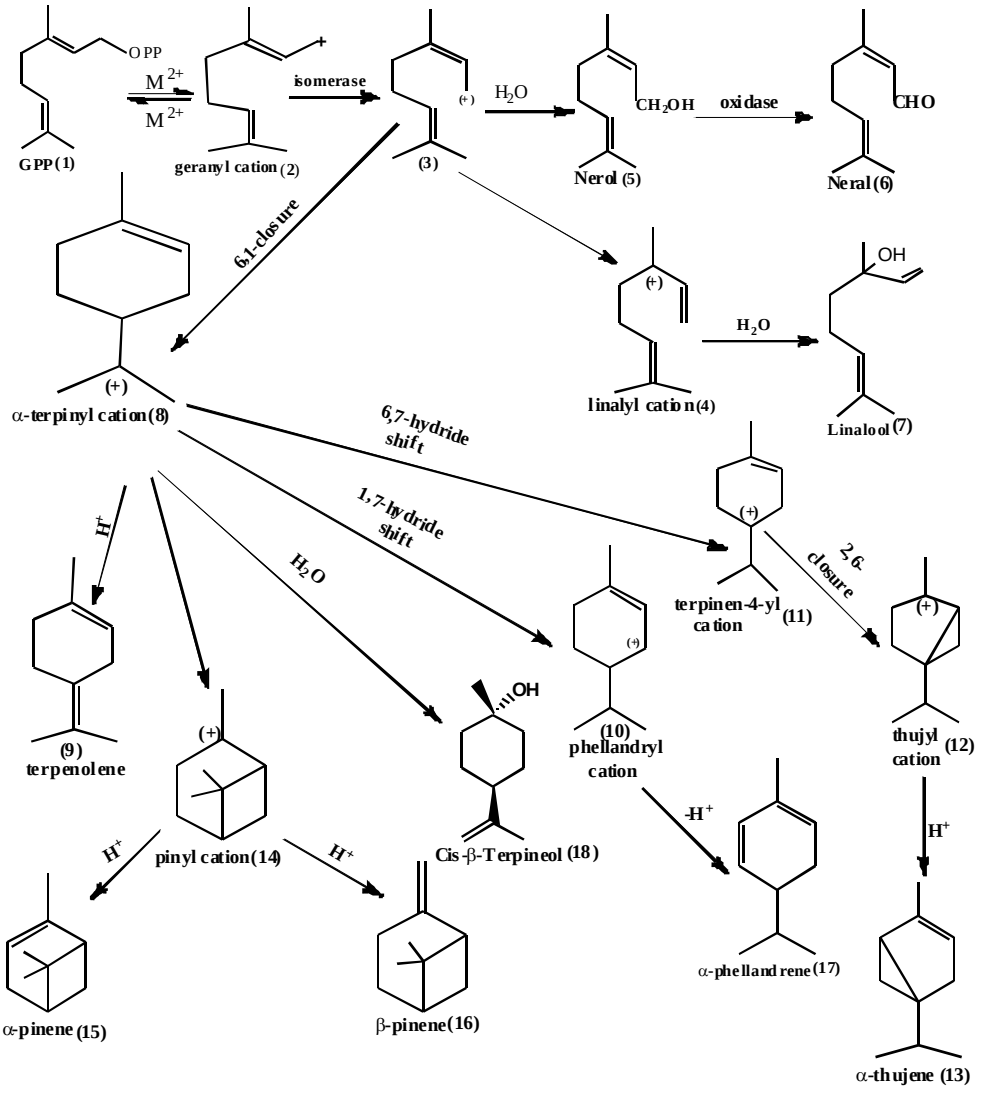

Figure 3: Biosynthesis of predominant monoterpenoids in oils from fresh and dried leaves of *Ocimum gratissimum* harvested at 10amin a day

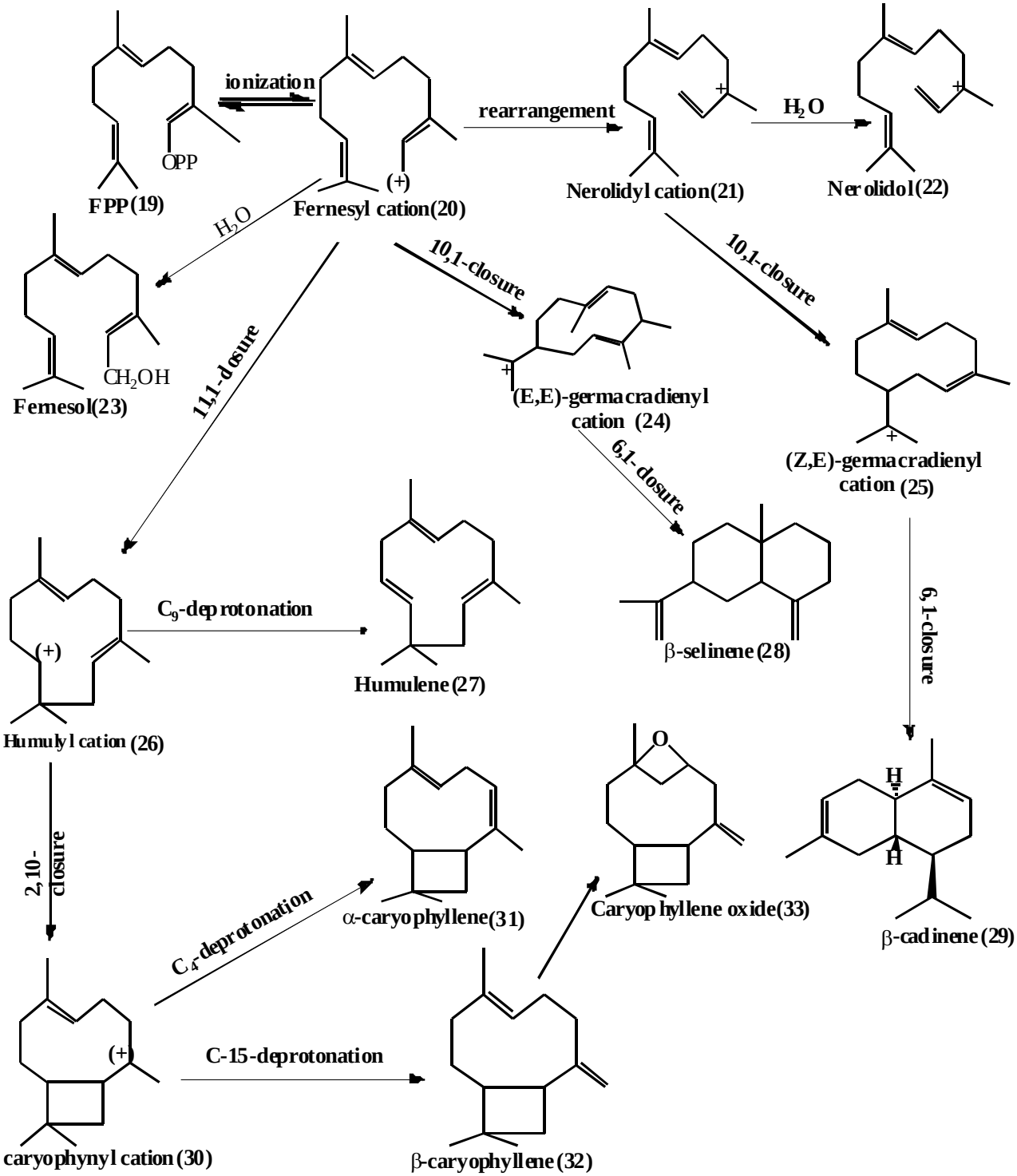

Figure 4: Biosynthesis of predominant sesquiterpenoids in oils from fresh and dried leaves of *Ocimum gratissimum* harvested at 10am in a day

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