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PHYTOCHEMICAL PROFILE OF FRUIT AND LEAF ESSENTIAL OILS OF *Thuja orientalis* GROWN IN NORTH CENTRAL NIGERIA

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Page | 2871

ABSTRACT

Fruit and leaf (500 g) of *Thuja orientalis* were separately hydrodistilled and yielded 0.20 and 0.14 % (w/w) of essential oils. GC and GC-MS analyses revealed that the oils were predominated by hydrocarbon monoterpenoids (57.0 and 43.6 %). Oxygenated monoterpenoids constituted 9.1 and 0.6 % of fruit and leaf oils. Percentage composition of hydrocarbon sesquiterpenoids in the fruit and leaf oils were 14.6 and 38.5 % respectively. Oxygenated sesquiterpenoids constituted 8.7 and 1.0 % of the fruit and leaf oils. The principal constituents of the fruit oil were; 2-carene (21.1 %), 3-carene (15.8 %), β -myrcene (8.0 %), D-limonene (5.5 %), terpinen-4-ol (7.8 %), γ -terpinene (4.7 %), β -caryophyllene (5.2 %), β -humulene (4.2 %) and cedrol (7.8 %). 3-carene (18.3 %), 2-carene (7.6 %), D-limonene (9.5 %), terpinylacetate (5.6 %), β -caryophyllene (14.2 %), germacrene D (7.1 %), α -caryophyllene (6.9 %) and β -myrcene (3.8 %) were the major compounds in the leaf oil. The predominance of 2-carene and 3-carene in the fruit and leaf oils indicated the oils were of 2-carene and 3-carene chemotype respectively.

Keywords: *Thuja orientalis*, bourbonene, sylvestrene, 3-carene, 2-carene.

INTRODUCTION

Thuja orientalis is a monoecious shrubs of 10-60 feet tall (Farjon, 2008). The plant is used in folk medicine for treatment of bronchial catarrh, enuresis, cystitis, psoriasis, high blood pressure, amenorrhea, bleeding, arthralgia and haemostatic (Baran, 1991; Zhu *et al.*, 2004; Biswas, 2011). Biological activities of the plant extracts such as; antibacterial (Chen *et al.*, 1989), antifungal (Mishra, 1992; Guileria and Kumar, 2006; Guleria *et al.*, 2008), antiviral (Offergelg 1992; Hassan *et al.*, 1996; Monica *et al.*, 2008), anticancer (Sunila, 2011) justify their use in folk medicine. Phytochemical studies of the plant led

to the isolation of a new labdane diterpene, 15-nor-14-oxolabda-8(17), 13(16)-dien-19-oic acid (Kim, 2013). Characterization of essential oils from fruit and leaf of Egyptian, Iranian and Indian grown *T. orientalis* revealed that the oils were of α -pinene chemotype (Nickavart, 2003; Ibrahim, 2004; Guleria *et al.*, 2008; Wajaht and Malipara, 2013).

However, the fruit oil was richer in α -pinene than the leaf oil. Major compounds in the fruit oil of Indian grown *T. orientalis* were; α -pinene, β -phellandrene, tricyclene, sabinene and 3-carene (Wajaht and Malipara, 2013).

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However, predominant compounds in the fruit oil of the plant from Iran includes; α -cedrol, α -humulene, germacrene D, β -caryophyllene, α -terpinolene, 3-carene, myrcene and α -pinene (Nickavar *et al.*, 2003). Furthermore, α -pinene, cyclofenchene, β -myrcene, β -pinene, trans- β -ocimene, 3-carene, 4-terpineol, α -humulene, ledol and 13-tetradecanal were the major constituents in fruit oil of the plant from South West Nigeria (Ololade *et al.*, 2014). Interestingly, β -selinene, β -caryophyllene, α -terpinylacetate, 4-terpineol, α -terpinene, cis-ocimene, β -myrcene, α -phellandrene, β -pinene, camphene, sabinene and α -pinene were the principal constituents of the fruit oil of the plant from Egypt (Ibrahim, 2004).

Leaf oil of Indian grown *T. orientalis* was predominated by; α -pinene, 3-carene, cedrol, caryophyllene, α -humulene, limonene, α -terpinolene and α -terpinyl acetate (Guleria *et al.*, 2008). Meanwhile, α -cedrol, elemol, α -humulene, thujopsene, β -cedrene, β -caryophyllene, bornylacetate, α -terpinolene, limonene, o-cymene, 3-carene, α -fenchene and α -pinene were the principal constituents of the oil from the leaf of the plant from Iran (Nickavart, 2003). Predominant compounds in the leaf oil of the plant grown in Egypt includes; benzylbenzoate, cedrol, α -longipinene, selinene, widdrene, β -caryophyllene, α -terpinylacetate, bornylacetate, linalylpropionate, terpinolene, limonene, α -phellandrene, β -pinene and α -pinene (Ibrahim, 2004). Variations in the composition pattern of the oils imply that, environmental factor and physiological conditions of the plants differ from one geographical location to another. Hence, caused variation in the phytochemical profiles of the oils (Nickavar *et al.*, 2003; Ibrahim, 2004; Guleria *et al.*, 2008; Wajaht and Malipara, 2013; Ololade *et al.*, 2014). It is based on these that, this work aimed at investigating the phytochemical profile of fruit and leaf essential oils of *Thuja orientalis* grown in north central Nigeria.

MATERIALS AND METHODS

Plant materials:

Fresh leaves and fruits of *Thuja orientalis*, were harvested from Mirabilis Hotel, Fate Road off University of Ilorin Road, Fate, Ilorin (Ilorin West Local Government Area), Kwara state, Nigeria. The plant was identified at the Herbarium of Plant Biology Department, University of Ilorin, Nigeria.

Oil Isolation:

Pulverized leaves and fruits (500 g) of *Thuja orientalis* were separately hydrodistilled for 3 hours in a Clevenger-type apparatus, according to the British Pharmacopoeia specification (1980). The oils were collected into screwed cap sample bottles and were preserved in a refrigerator at 4°C until analysis.

Gas Chromatography Analysis

GC analysis was performed on an Orion micromat 412 double focusing gas chromatography system fitted with two capillary columns coated with Cp-Sil 5 and Cp-Sil 9 (fused silica, 25 μ m x 0.25 mm, 0.15 μ m film thickness) and flame ionization detector (FID). The volume injected was 0.2 mL and the split ratio was 1:30. Oven temperature was programmed from 50 – 230 °C at 3 °C/min using hydrogen as carrier gas. Injection and detector temperature 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 spectrometer was used. Helium was used as the carrier gas at 1.2 mL/min. The MS operating conditions were: ionization voltage 70 eV, ion source, 250 °C. The GC was fitted with a 25 m x 0.25mm, fused silica capillary column coated with Cp – Sil 5. The film thickness was 0.15 μ m; 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 compositions of the oils 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 literature (Jennings and Shibamoto, 1980; Joulain and Kong, 1998; Adams, 2012).

RESULTS

Table 1: Chemical composition (%) of leaf and fruit essential oils of *Thuja orientalis*

S/N	Compounds ^a	RI ^b	% Composition		Mass Data	Spectra
			FRUIT	LEAF		
1	Tricyclene	926	-	1.0	136,121, 93 ,79,41	
2	α -Thujene	931	1.4	0.2	136,121, 93 ,77,41	
3	β -Myrcene	991	8.0	3.8	136, 93,69, 41 ,27	
4	2-Carene	1001	21.1	7.6	136,121,105, 93 ,41	
5	α -Phellandrene	1005	-	0.2	136,119, 93 ,77,41	
6	3-Carene	1011	15.8	18.3	136,121,105, 93 ,91	
7	O-Cymene	1020	-	0.3	134, 119 ,103,91,77	
8	D-Sylvestrene	1027	-	0.2	136,93,79, 69 ,41	
9	β -Phellandrene	1031	4.0	1.6	136,121, 93 ,77,41	
10	D-Limonene	1031	5.5	9.5	136,121, 93 ,79,68	
11	Cis- β -Ocimene	1040	-	0.5	136,121, 93 ,79,41	
12	γ -Terpinene	1062	4.7	0.4	136,121,105, 93 ,77	
13	Terpinen-4-ol	1177	7.8	0.3	154,136,121, 93 ,71	
14	α -Terpineol	1189	1.3	-	136,121, 93 ,81,59	
15	Bornyl acetate	1285	2.7	1.1	196,136,121, 95 ,43	
16	δ -Elemene	1339	-	0.5	204,161, 121 ,93,77	
17	α -Terpinylacetate	1354	-	5.6	136,121,93,68, 43	
18	β -Elemene	1375	-	1.6	204,161,121,93, 81	
19	α -Copaene	1376	3.5	0.2	204, 161 ,93,77,55	
20	γ -Elemene	1378	0.9	-	204, 121 ,93,79,41	
21	Geranylacetate	1383	0.9	-	154,121,93, 69 ,41	
22	β -Bourbonene	1384	-	0.3	204,161,123, 81 ,41	
23	β -Cubebene	1390	-	0.4	204, 161 ,120,105,91	
24	β -Cedrene	1418	0.8	-	204, 161 ,120,69,41	
25	β -Humulene	1440	4.2	-	204,147,121,107, 93	
26	β -caryophyllene	1454	5.2	14.2	204,161,133, 93 ,79	
27	α -Caryophyllene	1454	-	6.9	204,147,121, 93 ,41	
28	γ -Muurolene	1477	-	0.8	204, 161 ,119,93,79	
29	α -Muurolene	1480	-	0.6	204,161, 105 ,93,41	
30	β -Selinene	1485	-	0.5	204,161,105,93, 41	
31	Germacrene D	1490	-	7.1	204,161,93, 67 ,41	
32	α -Selinene	1494	-	0.5	189,175,161,147,133	
33	α -Amorphene	1506	-	1.2	204,161, 105 ,94,41	
34	δ -Cadinene	1524	-	3.6	204, 161 ,105,81,41	
35	Globulol	1576	0.9	-	222,161,109,81, 43	

36	Cedrol	1596	7.8	0.7	222,150,95,81,43
37	1,2-Epoxidehumulene	1606	-	0.3	147,138,109,67,43
38	t-Muurolene	1608	0.9	0.6	204,161,119,105,93
39	p-Tolymenthol	1967	-	0.3	109,93,79,69,55,43
Total Percentage (%)			97.4	90.9	
No of compounds			19	33	

a- Compounds are listed in order of elution from capillary column coated with Cp – Sil 5.

b- Retention Indices on fused capillary column coated with Cp – Sil 5.

Hydrodistilled fruits and leaves of *Thuja orientalis* afforded oils in the yield of 0.20 and 0.14 % (w/w) respectively. Table 1 shows the identities, Kovat indices and relative percentages of the constituents of fruit and leaf essential oils of *Thuja orientalis*. The oils were predominated by hydrocarbon monoterpenoids (57.0 and 43.6 %). Oxygenated monoterpenoids constituted 9.1 and 0.6 % of the oils. The percentage compositions of hydrocarbon sesquiterpenoids in the oils were 14.6 and 38.5 %. Oxygenated sesquiterpenoids constituents of the oils were 8.7 and 1.0 %. The most abundant compound in the fruit oil was 2-carene. Other principal constituents in the oil include; β -myrcene, 3-carene, d-limonene, γ -terpinene, terpinen-4-ol, cedrol, β -phellandrene, bornyl acetate, α -copaene and β -caryophyllene. γ -elemene, geranylacetate, β -cedrene, t-muurolene and globulol were also detected in appreciable quantities.

The predominant compound in the leaf oil was 3-carene. Other major compounds in the oil were; β -myrcene, 2-carene, d-limonene, terpinylacetate, β -caryophyllene, α -caryophyllene, germacrene D. Compounds that were present in appreciable quantities include; cedrol, β -phellandrene, bornyl acetate, tricyclene, α -amorphene and γ -muurolene. α -thujene, α -phellandrene, o-cymene, d-sylvestrene, γ -terpinene, terpinen-4-ol, α -copaene, β -bourbonene, β -cubebene and 1,2-epoxidehumulene were detected as minor constituents of the oil.

DISCUSSION

Comparison of the composition pattern of the fruit and leaf oils revealed that, α -terpineol, geranylacetate, β -cedrene, γ -elemene, β -humulene, globulol and cedrol existed in the fruit oil but were not found in the leaf oil of the plant. Similarly, tricyclene, α -thujene, α -phellandrene, sylvestrene, trans- β -ocimene, o-cymene, terpinylacetate, p-tolymenthol, β -elemene, β -bourbonene, β -cubebene, α -caryophyllene, γ -muurolene, germa crene D, β -selinene, α -muurolene, α -amorphene, δ -cadinene, cedrol, 1,2-epoxidehumulene, t-muurolene and α -selinene were detected in the leaf oil but were not identified in the fruit oil. Quantitatively, β -phellandrene, β -myrcene, terpinen-4-ol, α -copaene, cedrol and γ -terpinene were found in greater amounts in the leaf oil than the fruit oil. Meanwhile, the fruit oil was richer in d-limonene, β -caryophellene and 3-carene than the leaf oil. The existence of mono- and sesquiterpenoids as minor constituents of the leaf oil may be attributed to premature termination of their formation by the synthase that aided the transformation of their respective precursors.

The composition patterns of the oils also differ from fruit and leaf oils of the plant in other parts of the world. For instance; globulol, β -humulene, β -cedrene, geranyl acetate, t-muurolene, α -copaene, γ -elemene, and thujene were identified in the fruit oil but were not detected in the oils from Iranian, Egyptian and Indian grown *T. orientalis* (Nickavart, 2003; Ibrahim, 2004; Guleria *et al.*, 2008; Wajaht and Malipara, 2013). Similarly, tricyclene, o-

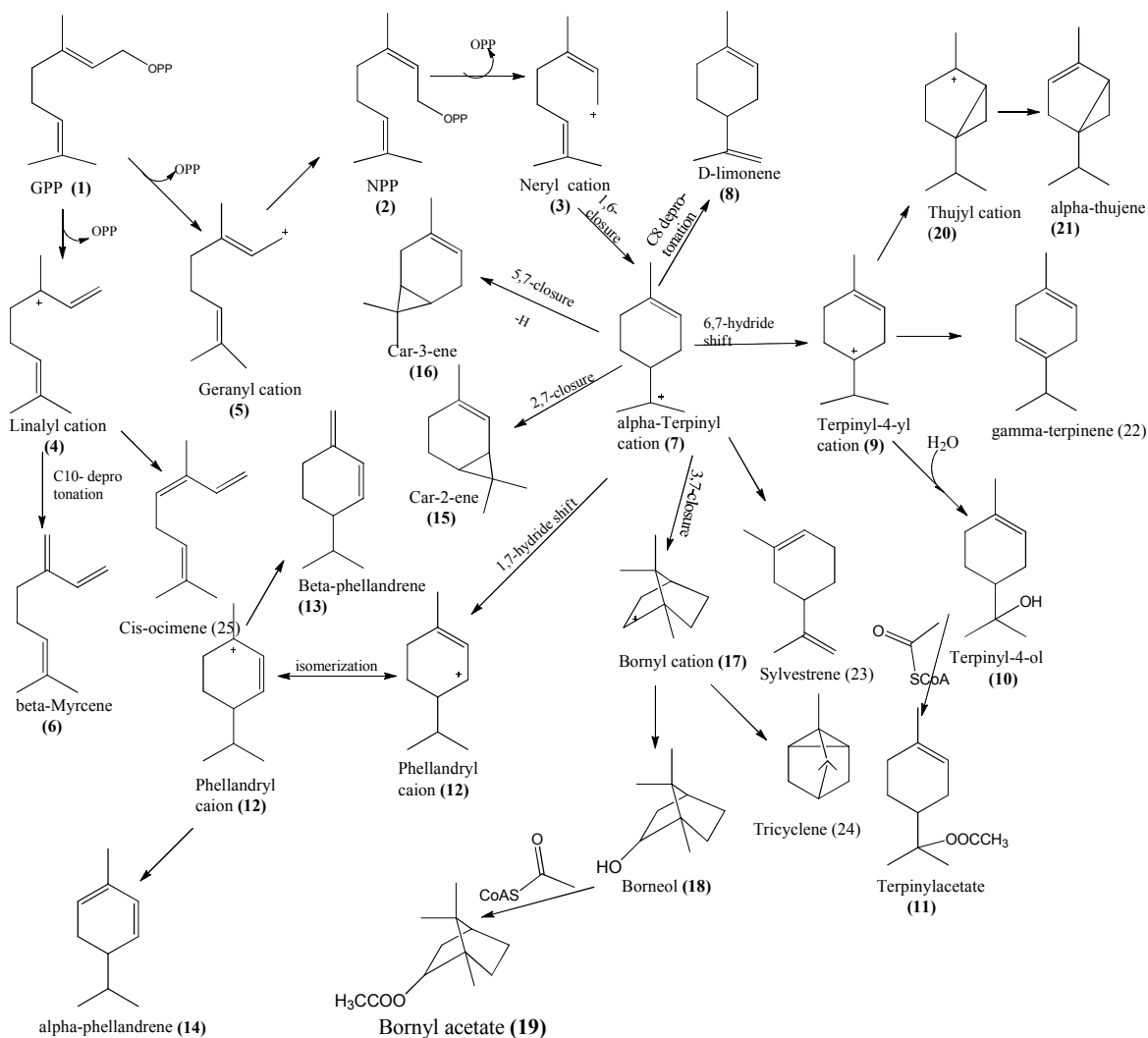
cymene, d-sylvestrene, cis- β -ocimene, δ -elemene, β -bourbonene, β -cubebene, α -caryophyllene, t-murolene, α -copaene, γ -murolene, α -murolene, α -amorphene, 1,2-epoxidehumulene, p-tolymenthol, 2-carene and β -phellandrene were detected in the leaf oil but were not detected in the oil of the plant grown in Iran, Egypt and India. The fruit and leaf oils were of 2-carene and 3-carene chemotype respectively. However, the leaf and fruit oils of Egyptian, Iranian and Indian grown plants were of α -pinene chemotypes (Nickavart, 2003; Ibrahim, 2004; Ololade *et al.*, 2014). Interestingly, α -pinene was not identified in the fruit and leaf oils indicating that the physiological conditions of the plant did not favour its biosynthesis in the fruit and leaf of the plant at its location.

The variations in composition patterns of the oils can be attributed to differences in environmental and physiological conditions of the plant at various locations. These factors determine the activity of enzyme that mediates the transformation of terpenoids precursors to their respective compounds. Interestingly, the enzymes of the most abundant mono- and sesquiterpenoids normally facilitate the transformation of the precursors (neryl, geranyl and farnesyl pyrophosphates) to various cationic intermediates (geranyl, linalyl, neryl, farnesyl, nerolidyl and humulyl cations) in the presence of divalent metal ion (Wise *et al.*, 1998; Degenhardt *et al.*, 2009). The ions usually undergo series of cyclization; hydride and methyl shifts followed by deprotonation, dehydrogenation and hydration to form various terpenoids via cationic mechanism.

Reactions Mechanism

The predominance of 2-carene and 3-carene in the fruit and leaf oils indicated that their

synthases facilitated the transformation of their precursors to all monoterpenoids in the oils (**Reaction Scheme 1**). In the scheme, the synthases aided the ionization of geranyl (**1**) and neryl pyrophosphates (**2**) to their respective cationic intermediates (neryl (**3**), linalyl (**4**) and geranyl (**5**) cations). Deprotonation of the ion (**4**) at C₁₀ and C₃ formed β -myrcene (**6**) in the oils and Cis-Ocimene (**25**) in the leaf oil. Electrophilic attack of the neryl cation (**3**) on the C₆-C₇ double bond formed α -terpinyl cation (**7**). Deprotonation of the ion (**7**) at C₈ formed limonene (**8**) in the oils. 6,7 hydride shift of the ion (**7**) gave terpinyl-4-yl cation (**9**). The ion (**9**) was hydrated and formed terpinen-4-ol (**10**) in the oils. Acylation of the compound (**10**) produced terpinyl acetate (**11**) in the leaf oil. Deprotonation of the ion (**9**) on C₆ formed γ -terpinene (**22**) in the oils. 6,7- and 1,6-hydride shifts of the ion (**7**) formed phellandryl cation (**12**). Deprotonation of the ion (**12**) at C₁₀ and C₃ formed β - phellandrene (**13**) in the oils and α -phellandrene (**14**) in the leaf oil. Deprotonation of the ion (**7**) at C₁ and C₅ followed by electrophilic attack on the deprotonated carbons formed 2-carene (**15**) and 3-carene (**16**) in the oils. Electrophilic attack of the ion (**7**) on C₂-C₃ double bond formed bornyl cation (**17**) which got hydrated to form borneol (**18**). The compound (**18**) got acetylated and formed bornyl acetate (**19**) in the oils. Loss of proton by the ion (**20**) at C₃ formed α -thujene (**21**) in the oils. Deprotonation of the ion (**17**) on C₄ followed by electrophilic attack of the ion on the deprotonated ion formed Tricyclene (**24**). Hydrogenation of the α -terpinyl cation followed by 6-7, 5-6 and 4-5 hydride shifts and 3-4 methyl shifts with deprotonation at C₄, and subsequent dehydrogenation at C₇- C₈ formed Sylvestrene (**23**).



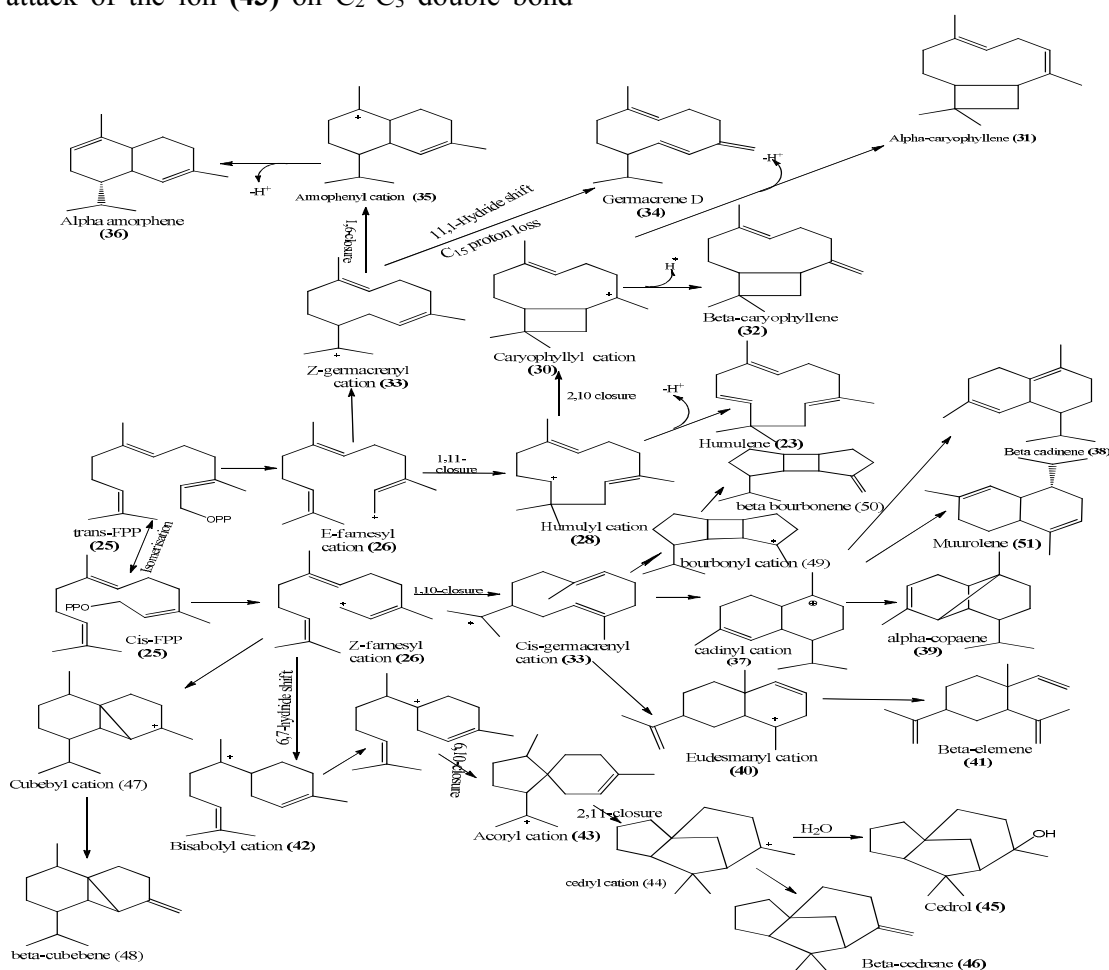
Reaction Scheme 1: Biosynthesis of Monoterpenoids catalysed by 2-carene and 3-carene synthases.

The predominance of cedrol and β -caryophyllene implied that their synthases mediated the formation of all sesquiterpenoids in the oils from their precursor (farnesyl pyrophosphate) via cationic mechanisms (**Reaction scheme 2**). In the scheme, the synthases facilitated the ionisation of farnesyl pyrophosphate (25) to farnesyl (26) and nerolidyl (27) cations. Electrophilic attack of the ion (26) on C₁₀-C₁₁ double bond formed humulyl cation (28) which subsequently deprotonated at C₉ and formed humulene (29) in the fruit oil. Electrophilic attack of the ion (28) on C₂-C₃ double bond formed caryophyllyl cation (30). Deprotonation of the

ion (30) at C₄ and C₁₅ formed α -caryophyllene (31) in the leaf oil and β -caryophyllene (32) in the oils. Electrophilic attack of the ion (26) on C₁₀ and C₁₁ double bond formed germacreanyl cation (33). The ion (33) undergo 10,11 and 1,10 hydride shifts followed by delocalisation of pie bond from C₂-C₃ to C₂-C₁ with subsequent deprotonation at C₁₅ formed germacrene D (34) in the leaf oil. 11,10 and 10,1 hydride shifts followed by electrophilic attack of the ion (30) on the C₆ and C₇ double bond formed armophenyl cation (35). Deprotonation of the ion (35) at C₈ formed α -amorphenone (36) in the leaf oil. 11,10- and 10,1- hydride shifts followed by electrophilic

attack of the ion (33) on the C₆-C₇ double bond formed cadinyl cation (37). Deprotonation of the ion (37) at C₃ formed cadinene (38) in the leaf oil. Deprotonation at C₃ of the ion (37) followed by change in the geometry of the hydrogens on C₁ and C₆ produced muurolene (51). Electrophilic attack of the ion (37) on C₂-C₃ double bond with loss of proton at C₄ formed α -copaene (39) in the oils. 2, 7 closure of germacrenyl cation (33) followed by delocalisation of pie bond from C₆-C₇ to C₅-C₆ and deprotonation at C₁₂ produced eudesmanylyl cation (40). Deprotonation of the ion (40) at C₁₅ followed by C₄-C₅ sigma bond cleavage formed β -elemene (41) in the leaf oil. Electrophilic attack of Z- farnesyl cation (26) on C₆-C₇ double bond produced bisabonyl cation (42). 6, 7 hydride shift in the ion (42) followed by its electrophilic attack on the C₁₀-C₁₁ double bond formed acoryl cation (43). Electrophilic attack of the ion (43) on C₂-C₃ double bond

formed cedryl cation (44). Hydration of the ion formed cedrol (45) in the oils. Deprotonation of the ion (44) at C₁₅ formed β -Cedrene (46) in the fruit oil. Electrophilic attack of farnesyl cation (26) on C₁₀-C₁₁ double bond formed germacra-dienyl cation (33). 10, 11 and 1, 10 hydride shifts followed by electrophilic addition of the ion on C₆-C₇ double bond via C₆ formed cadinyl cation (37). 6,7-hydride shift of the ion followed by its electrophilic attack on C₂-C₃ double bond formed cubeyl cation (47). Deprotonation of the ion at C₁₅ formed beta- cubebene (48) in the leaf oil. 10,11 and 1,10 hydride shifts followed by electrophilic addition of the resulting ion on C₆-C₇ double bond and formed a cationic intermediate. Electrophilic addition of ion on C₂-C₃ double bond via C₂ gave bourbonyl cation (49) that deprotonated at C₁₅ and formed β -bourbonene (50) in the leaf oil.



Nig. J. Pure & Appl. Sci. Vol. 29 (Issue 2): 2871-2879

Reaction Scheme 2: Biosynthesis of Sesquiterpenoids catalysed by cedrol and β -caryophyllene synthases

CONCLUSION

Fruit of the *Thuja orientalis* afforded more essential oil than the leaf. This implied that, the fruit is richer in oil secretory organ than the leaf. The phytochemical profiles of the oils varied significantly, which may cause variations in their biological activities. Interestingly, this study revealed the existence of new chemotypes of the oils in Nigeria that may be more biologically active than the existing oil chemotypes.

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