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# Chemical composition of essential oil from peels of *Citrus Aurantifolia* L. grown in South-West Nigeria

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#### Abstract

The citrus peels commonly treated as agro-industrial waste are the potential source of valuable secondary plant metabolites and essential oil. A pulverized peels *C. aurantifolia* L. was subjected to hydrodistillation for 3 hours and yielded 0.75% (w/w).

Characterization of the oil by Gas Chromatography-Mass Spectrometry showed that the oil was predominated by oxygenated monoterpenoids (36.3%). Monoterpenoids hydrocarbon constituted (34.4%) of the oil. Percentage composition of hydrocarbon and oxygenated sesquiterpenoids in the oil were in the range of 7.4%-7.0%. The most abundant constituent of the oil was D-limonene (21.3%). Other principal constituents were as follows p-cymene (7.1%), cis-p-mentha-1 (7), 8-dien-2-ol (5.8%), caryophyllene oxide (4.7%),  $\alpha$ -terpineol (4.2%),  $\beta$ -pinene (3.7%),  $\beta$ -bisabolene (3.4%), (-)-myrtenol (3.1%),  $\alpha$ -bergamotene (2.7%). The predominance of D-limonene in the oil showed that the oil was D-limonene chemotype.

Keywords: Citrus aurantifolia L; Essential oil; D-limonene; Chemotype; Terpene synthase

#### 1. Introduction

The genus citrus belonging to the Rutaceae or Rue family, comparises of about 140 genera and 1,300 species. *Citrus Sinensis* (Oranges), *Citrus Paradisi* (Grape fruits), *Citrus Jambhiri* (Rough lemon), *Citrus reticulate* (Tangerine), *Citrus grandis* (Shaddock), *Citrus aurantium* (sour orange), *Citrus medica* (Citron) and *Citrus aurantifolia* (Lime) are some important fruits of genus citrus. Citrus are well known as one of the world's major fruit crops that are produced in many countries with tropical or subtropical climate Brazil, USA, Japan, China, Mexico, Pakistan and countries of the Mediterranean region are the major citrus producers worldwide. The global citrus production in the year 2016 was approximately 124.3 million tons [1]. With orange (*Citrussinensis*) being the largest among all the citrus crops. The global production of oranges for the year 2018/2019 has been estimated to increase by approximated of 6.3 million to 54.3 million tons [2]. The important varieties cultivated commercially are oranges (61% of total), Mandarin (22% of total), Lime and Lemon (11% of total), and Grape (6% of total) [1].

Citrus fruits and their by-products are of high economic and medicinal value because of their multiple uses, such as in the food industry, cosmetic, and folk medicine [3]. In addition to large scale consumption as fresh fruit, the citrus are mainly process in the industry, left over after juice extraction such as peels, seeds and pulps corresponding to about 50% of the raw processed fruits which can be used as a potential source of valuable by-products [4]. Specifically, the citrus peels commonly treated as agro-industrial waste, and are a potential source of valuable secondary plant metabolites and essential oils [5].

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*Citrus aurantifolia L*. is a very popular and valued citrus species in the Gulf region due to its nutritional qualities, distinct flavor, and health benefits. Various parts of the plant are used in traditional medicine to treat cataract, cold, sore throat, fever, chest pain, earache, headache, stomach ailments, and edema, and it is considered an antiseptic, anthelmintic, mosquito repellent, anti-scurvy, astringent, digestive, and appetite stimulant, among others [6].Lime juice and its essential oil are also commonly used in the food, drug, and cosmetic industries because of their medicinal properties and fragrance. The traditional and pharmacological uses of *Citrusaurantifolia L*. plants are attributed to the presence of secondary plant metabolites including flavonoids, coumarins, and terpenoids [7-9]

Phytochemical investigation of the C. aurantifolia L. (peels) in Nigeria led to the isolation of D-limonene (29.22%) as the major compound with beta-pinene (23.65%), alpha-pinene (4.32%), lemonol (3.68%), beta-linalool (3.41%), alpha and beta citra 2.74% and 1.71% respectively [10]. D-limonene (63.35%) as the principal compound with 3,7-dimethyl-2,6octadien-1-ol (7.07%), geraniol (6.23%), E-citral (4.35%), Z-citral (3.29%) and beta-ocimene (2.25%) as other prominent constituents were isolated from the *C. aurantifolia L* (leaves) in Eastern Oman [11]. D-limonene (57.84%) was also isolated from the *C. aurantifolia L* (leaves) in North-Central Nigeria as major compound with neral (7.81%), linalool (4.75%), sulcatone (3.48%), isogeraniol (3.58%), citrolnella (2.19%), linalool-isobutyrate (2.19%), cis-betaocimene (2.09%) and beta-pinene (1.19%) as other notable compounds [12]. It is worth noting that there is a great variation in the chemical composition of citrus oil due to differences in origin, climate, season, age, plant part, genetic background and method of extraction [13]. All these exogenous and endogenous factors could affect the DNA of the aromatic plants leading from chemotypes to different genotypes [14]. The part of the plant used to obtain essential oils should be considered an endogenous factor because is related to the anatomical and physiological characteristics of the plants. For example, the essential oils are located in the peels, roots and leaves of *Citrus* genus of the Rutaceae, Moreover, the location and nature of the secretory structures vary from species to species. A number of papers have reported on the variability in the chemical composition of essential oils extracted from different parts of the plant and geographical location [15]. It was on this basis we aimed to determine the chemical composition of essential oil from peels of *Citrus* aurantifolia L. grown in south-west Nigeria.

# 2. Material and methods

### 2.1. Sample Collection

The *Citrusaurantifolia L.* (Lime) fruits were collected at Egbeda farm Okeho Kajola Oyo State. The plant was identified at the Herbarium of plant Biology Department, university of Ilorin, Ilorin, Nigeria. Where voucher specimens was deposited.

#### 2.2. Oil Isolation

Pulverized peels of *Citrusaurantifolia L*. were hydro-distilled for 3 hours in a Clevenger-type apparatus, according toBritish pharmacopoeia specification [16]. The resulting oil were collected, preserved in a seal sample tube and stored under refrigeration untilanalysis.

#### 2.3. GC-MS Analysis

Analysis of the oil was carried out using GC (Agilent 190915) couple with a quadruple focusing mass spectrometer (433 HP-5). Helium was used as the carrier gas at a flow rate of 1.5 ml/min. The GC was filled with a 30 mm x 0.25 mm fused silica capability column coated with phenyl methyl siloxane in split ratio of 1:50. The film thickness was 0.25  $\mu$ m. Oven temperature which was initially at 100 °C for 5min and then programmed to 150 °C at a rate of 40 c/min for 8min and increased to 250 °C at a rate of 20 °C /min. The MS operating conditions were as follows: Transfer line temperature, 300 °C, ionization potential, 70 ev. The percentage composition of the oils was computed in each case from GC peak areas. The identification of the components was done 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 [17-19].

# 3. Results

Pulverized peels of *Citrus aurantifolia L* on hydrodistillation afforded oil in the yield of 0.75%(w/w). Forty four chemic al compounds were identified using gas chromatography-mass spectrometry (GC/MS).

S/N	Compounds <sup>a</sup>	RI <sup>b</sup>	% Composition	Mass Spectra Data
1	Thujene	897	0.3	136,121,105,93,79
2	Camphene	943	0.1	136,121,107,93,79
3	β-Pinene	943	3.7	136,121,107,93,77
4	<i>α</i> -Pinene	948	0.5	136,121,105,93,77
5	β-Myrcene	958	0.5	136,121,107,93,79
6	D-Limonene	1018	21.3	136,121,107,79,68
7	Limonene oxide	1031	1.1	152,137,108,94,79
8	P-Cymene	1042	7.1	134,119,103,91
9	(+)-Nopinone	1047	0.4	138,95,88,67
10	Linalyl alcohol	1082	1.5	136,93,71,65
11	Pinocarvone	1114	0.7	150,81,69,53
12	Pinocarveol	1131	1.3	134,119,109,92
13	Cis verbenol	1136	0.9	150,137,109,94,81
14	Terpinen-4-ol	1137	1.9	154,136,93,71,69
15	Isoborneol	1138	0.6	154,136,110,95,71
16	Fenchol	1138	0.5	154,139,81,69
17	Mentha-2,8-dien-1-ol	1140	0.6	152,134,119,109
18	α-Terpineol	1143	4.2	154,140,59,43
19	$\beta$ -Terpineol	1158	0.1	154,140,59,43
20	Trans-linalool oxide	1164	0.9	149,137,59,43
21	Cis-ocimene	1164	0.9	150,135,91,79
22	Citral	1174	0.8	152,137,84,69
23	Citronellol	1179	1.0	156,138,81,69
24	(-)-Carvone	1190	1.6	150,135,82,67
25	(-)-Myrtenol	1191	3.1	152,134,79,67
26	Cis-p-mentha-1(7),8-dien-2-ol	1201	5.8	152,109,81,69
27	Trans-p-mentha-1(7),8-dien-2-ol	1201	2.6	152,109,81,69
28	Trans-carveol	1206	2.4	152,137,109,84
29	Nerol	1228	0.4	154,139,93,69
30	Geraniol	1228	0.4	154,139,93,69
31	Perillol	1261	1.4	152,134,105,68
32	Geranyl acetate	1352	2.1	196,153,80,69
33	Alloaromadendrene	1386	0.3	204,189,119,105
34	$\alpha$ -bergamotene	1430	2.7	204,189,119,107

**Table 1** Chemical composition (%) of essential oil from peels of Citrus aurantifolia. L

36	$\alpha$ -selinene	1474	0.3	204,189, 121,79,67
37	Caryophyllene	1494	0.2	204,189,79,69
38	$\beta$ -bisabolene	1500	3.4	204,189,79,69
39	Caryophyllene oxide	1507	4.7	220,205,109,79
40	$\beta$ -chamigrene	1507	0.2	204,189,175,161
41	Globulol	1530	0.7	222,204,189,81
42	Farnesene epoxide	1540	0.6	205,187,119,105
43	$\alpha$ -bisabolol	1625	0.4	204,189,109.93
44	Santalol	1668	0.6	220,202,94,79
Total			85.1%	
	Monoterpenoids Hydrocarbon		34.4%	
	Oxygenated Monoterpenoids		36.3%	
	Sesquiterpenoids Hydrocarbon		7.4%	
	Oxygenated Sesquiterpenoids		7.0%	

<sup>a</sup>Compounds are listed in order of elution from Silica Capillary Column coated on CP-Sil5; <sup>b</sup> retention indices on fused Silica Capillary Column coated with cp-sil5

#### 4. Discussion

Table 1 shows the retention indices, relative percentages, mass spectra data and identities of the constituents of the oil. A total of 44 compounds that represent 85.1% of the peels oil are identified from their retention indices and mass spectra.

Oxygenated and hydrocarbon monoterpenoids constituted (36.3%) and (34.4%) of the oil while the percentage composition of oxygenated and hydrocarbon sesquiterpenoids were (7.0%) and (7.4%) respectively.

Oxygenated monoterpenoids that were found in significant proportion were as follow; cis-p-mentha-1 (7), 8-dien-2ol (5.8%),  $\alpha$ -terpineol (4.2%), (-)-myrtenol (3.1%), trans-p-mentha-1 (7), 8-dien-2-ol (2.6%), trans-carveol (2.4%), geranyl acetate (2.1%), terpinen-4-ol (1.9%), (-)-carvone (1.6%), linalyl alcohol (1.5%), perillol (1.4%), pinocarveol (1.3%), limonene oxide (1.1%) and citronellol (1.0%). Trans-limonene oxide (0.9%), cis-verbenol (0.9%), citral (0.8%), pinocarvone (0.7%), isoborneol (0.6%), menthe-2,8-dien-1-ol (0.6%), fenchol (0.5%), geraniol (0.4%), nerol (0.4%),(+)norpinone (0.4%) and  $\beta$ -terpineol (0.1%) existed as minor constituents of the oil.

Monoterpenoids hydrocarbon constituted D-limonene (21.3%) as the major constituent while p-cymene (7.1%) and  $\beta$ -pinene (3.7%) were found in significant proportions. Cis-ocimene (0.9%),  $\alpha$ -pinene (0.5%),  $\beta$ -myrcene (0.5%), thujene (0.3%) and camphene (0.1%) existed as minor constituents of the oil.

Sesquiterpenoids hydrocarbons that were found in significant proportions were  $\beta$  -bisabolene (3.4%) and  $\alpha$  - bergamotene (2.7%). Farnesene (0.3%), alloaromadendrene (0.3%),  $\alpha$ -selinene (0.3%), caryophyllene (0.2%) and  $\beta$ - chamigrene (0.2%) existed as minor constituents of the oil.

Oxygenated monoterpenoids that are found in principal constituents are caryophyllene oxide (4.7%) while globulol (0.7%), farnesene epoxide (0.6%), santalol (0.6%) and  $\alpha$ -bisabolol (0.4%) existed as minor constituents of the oil.

Predominance of D-limonene (21.3%) in the oil showed that the oil was D-limonene chemotype, this compared favorably with peels oil from Nigeria. Leave oil only gave higher quantity of d-limonene [10, 11].

Comparison of the composition pattern of the peels oil of *C. aurantifolia* with that of northern Nigeria showed that  $\beta$ -pinene that constituted principal constituent in northern Nigeria peels oil only found in significant proportion in the oil. P-cymene (7.1%) and cis-p-mentha-1 (7), 8-dien-2-ol (5.8%) that were found in significant proportion of the oil were

not detected in northern Nigeria peels oil [10]. The variation in both qualitative and quantitative analysis can be attributed to different part of the plant utilized and geographical location [10-12].

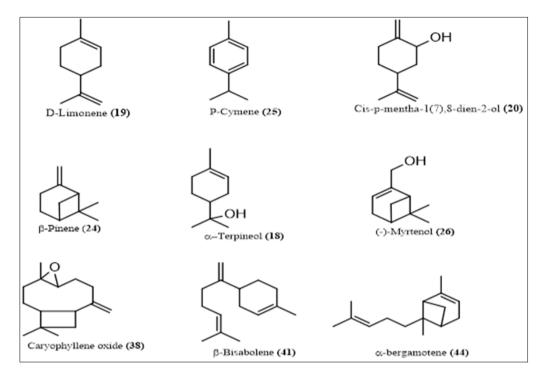


Figure 1 Major compounds in the peels essential oil of C. aurantifolia L grown in south-west Nigeria

The chief constituents of essential oil are monoterpenoid and sesquiterpenoids which may be inform of hydrocarbon and oxygenated. It has been established that the enzymes of the most abundant monoterpenoids and sesquiterpenoids facilitate the transformation of their precursor (geranyl pyrophosphate/ farnesyl pyrophosphate) to various cationic intermediates (linalyl, geranyl, neryl, farnesyl, nerolidyl and humullyl cations) in the presence of divalent metal ions [20,21]. The ions subsequently undergo series of cyclizations, hydride shifts and other rearrangements until the reaction is terminated by proton loss or hydration to give various terpenic products [22].

#### 4.1. Reaction Mechanism 1

The predominance of d-limonene, p-cymene,  $\alpha$ -terpineol and  $\beta$ -pinene in the oil implied that its synthase facilitate the transformation of geranyl and nervl pyrophosphate (GPP/NPP) to all monoterpenoids in the oil through cationic intermediate (Reaction Scheme 1). In the scheme, the synthases aided the transformation of geranyl (1) and nervl pyrophosphate (2) to geranyl (5) and neryl (4) cation. Isomerization of each of the ions (5 and 4) formed linalyl cation (3). Hydration of geranyl cation (5) and acetylation gave geraniol (7) and geranyl acetate (8) in the oil. Neryl cation (4) was hydrated to nerol (11). Oxidation of nerol (11) produced citral (12). Electrophilic attack of the ion (4) at  $C_2-C_3$ followed by deprotonation at  $C_{10}$  gave  $\beta$ -myrcene (10). Electrophilic attack of the ion (4) on  $C_6$ - $C_7$  double bond produced  $\alpha$ -terpinyl cation (13). Deprotonation of terpinyl cation at C<sub>8</sub> gave d-limonene (19) in the oil. Folding of the ion (13) towards C<sub>2</sub>-C<sub>3</sub> double bond followed by its electrophilic attack via C<sub>2</sub> produced pinyl cation (15). Deprotonation of the ion (15) at C<sub>4</sub> formed  $\alpha$ -pinene (23). Deprotonation of the ion (15) at C<sub>10</sub> formed  $\beta$ -pinene (24). Deprotonation at C<sub>10</sub> followed by hydration of  $\alpha$ -pinene gave myrtenol (26). Wagner-meerwein rearrangement of the pinyl cation (15) followed by 2, 3-methyl shift formed fenchyl cation (17) and its hydration gave fenchol (28). 6, 7-hydride shift of the ion (15) gave terpinyl-4-yl cation (14). Subsequent deprotonation of the ion (14) at  $C_1$  and dehydrogenation of the ion (14) at  $C_4$ - $C_5$  formed p-cymene (25) in the oil. 2, 6- cyclization of the ion (14) formed thujyl cation (16). Deprotonation of the ion (16) at  $C_{10}$  gave thujene (27). Electrophilic attack on d-limonene at  $C_2$ - $C_3$  double bond followed by hydration produced cis-p-mentha-1 (7), 8-dien-2-ol (20). Deprotonation of d-limonene at C<sub>4</sub> followed by hydration gave trandcarveol (21). Oxidation of trans-carveol (21) produced carvone (22).

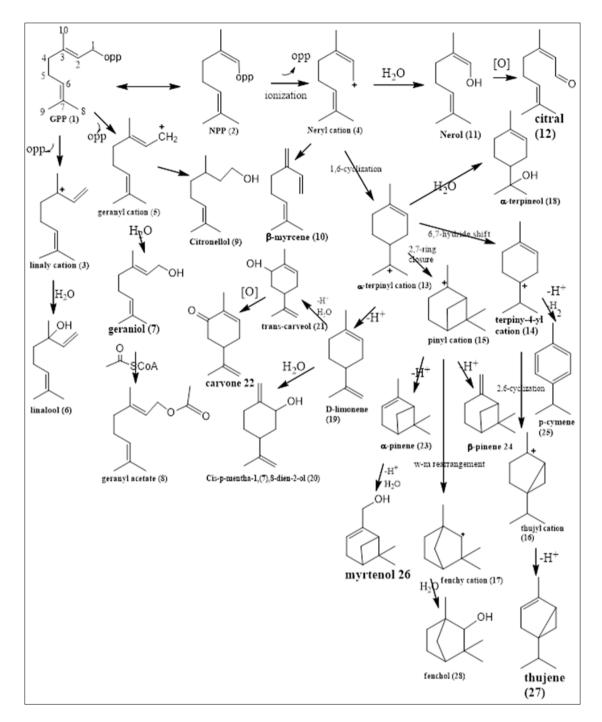


Figure 2 Biosynthesis of monoterpenoids catalyzed by d-limonene, p-cymene,  $\alpha$ -terpineol and  $\beta$ -pinene synthase

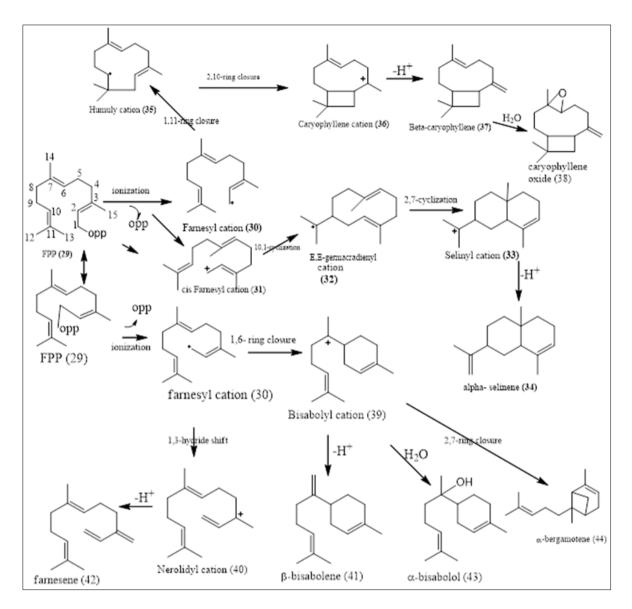


Figure 3 Biosynthesis of sesquiterpenoids catalyzed by caryophyllene oxide,  $\beta$ -bisabolene and  $\alpha$ -bergamotene synthase

#### 4.2. Reaction Mechanism 2

The abundance of caryophyllene oxide,  $\beta$ -bisabolene and  $\alpha$ -bergamotene in the oil implied that their synthases facilitate the biosynthesis of all sesquiterpenoids in the oil. In the reaction scheme 2, the synthase catalyzed the ionization of farnesyl pyrophosphate (29) to the cationic intermediate (30). Electrophilic attack of the ion (30) on C<sub>10</sub>-C<sub>11</sub> double bond gave humullyl cation (35). The ion (35) undergoes electrophyllic attack on C<sub>2</sub>-C<sub>3</sub> double bond to formed caryophylene cation (36). The ion (36) is deprotonated at C<sub>15</sub> to gives  $\beta$ -caryophyllene (37). 5,7-epoxidation of  $\beta$ -caryophyllene leads to the formation of caryophyllene oxide (38). Electrophilic attack of the ion (31) on C<sub>10</sub>-C<sub>11</sub> double bond formed germacrenyl cation (32). The cation (32) also undergoes 2, 7- ring closure to formed  $\beta$ -selinyl cation (33). Deprotonation of the ion (40) at C<sub>15</sub> formed farnesene (42). 1, 6-ring closure of the ion (30) gave bisabolyl cation (39). Deprotonation of the ion (39) at C<sub>14</sub> gave  $\beta$ -bisabolene (41). Hydration of the ion (39) gave  $\alpha$ -bisabolol (43). The ion (39) undergoes 2, 7-ring closure to formed 4-bisabolol (43).

# 5. Conclusion

Comparison of the composition pattern of the peels oil of C. aurantifolia L. with that of northern Nigeria showed that  $\beta$ pinene that constituted principal constituent in northern Nigeria peels oil only found in significant proportion in the south-west Nigeria oil. P-cymene and cis-p-mentha-1 (7), 8-dien-2-ol that were found in significant proportion of the south-west Nigeria oil were not detected in northern Nigeria peels oil. The variation in both qualitative and quantitative analysis can be attributed to geographical location. Therefore, geographical location of the plant has influence on the chemical composition of essential oil. The predominant of d-limonene in the oil showed that the oil was d-limonene chemotype. The essential oil of C. aurantifolia L. peels could be used in food preservative and flavoring due to high percentage of well-known antimicrobial compounds with  $\beta$ -pinene, limonene and caryophyllene oxide.

#### **Compliance with ethical standards**

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#### Disclosure of conflict of interest

There is no conflicts of interest.

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