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Comparative analysis of essential oils of *Citrus aurantifolia* Swingle and *Citrus reticulata* Blanco, from two different localities of Lagos State, Nigeria

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Abstract
The chemical composition of essential oils hydrodistilled from the leaves of *Citrus aurantifolia* Swingle and *Citrus reticulata* Blanco from Nigeria were being reported. The main compounds of *C. aurantifolia* from Ijanikin was characterized by large amounts of caryophyllene oxide (32.2%), caryophylla-3(15),7(14)-dien-6-ol (30.0%), α-pinene (7.9%) and 2,6-dimethyl-1,5,7-octatrien-3-ol (7.3%) while the Ikotun sample was rich in limonene (44.7%) and geranial (38.2%). The oils of *C. reticulata* from Ijanikin had its main compounds as pinocarvone (22.7%), *trans*-pinocarveol acetate (20.0%), β-thujone (12.8%) while citronellal (38.1%), (*Z*)-β-ocimene (25.9%), linalool (14.5%) and limonene (12.2%) were the major constituents identified in Ikotun sample. The present oil samples may represent new chemical forms of the respective plant.

Keywords: Citrus aurantifolia, Citrus reticulata*, Rutaceae, monoterpenes, sesquiterpenes*

1. Introduction
In continuation of our research into the volatile compositions of Nigerian flora [1], we report the chemical compounds identified in the leaf oils of *Citrus aurantifolia* Swingle and *Citrus reticulata* Blanco collected from two different locations within Lagos State, Nigeria. The Key lime, *C. aurantiifolia,* is a species with a globose fruit; 2.5–5 cm in diameter that is yellow when ripe but usually picked green commercially. It is smaller and seedier, with a higher acidity, a stronger aroma, and a thinner rind. Extracts of the plant are known to possess antioxidant [2, 3], anti-cholinesterase [3], anti-tuberculosis [4], antibacterial [5] and cytotoxic [6] activities. Some of its phytochemical constituents were 5-geranyloxypsoralen, 5-geranyloxy-7-methoxycoumarin, palmitic acid, 5,7-dimethoxycoumarin, 5-methoxypsoralen, 5,8-dimethoxypsoralen, 5,7-dimethoxycoumarin, 3-methyl-1,2-cyclopentanedione, 1-methoxy-cyclohexene, corylone and umbelliferone [4]. Others were apigenin, rutin, quercetin, kaempferol and nobiletin [3]. Furthermore, the limonoids identified are limonexic acid, isolimonexic acid, and limonin [7].
Citrus reticulata, mandarin is an evergreen tree growing up to 4.5 m by 3 m. The flowers are hermaphrodite (have both male and female organs) and are pollinated by Apomictic, insects. The plant is self-fertile. *Citrus reticulata* exhibited inhibitory effects on pulmonary fibrosis [8], modulate blood cholesterol profile and increase bone density of ovariectomized rats [9], possess antioxidant effect [10, 11] and antimicrobial [11]. It is a source of natural compounds such as citruscridone [12], citriolide-A [13], isolimonexic acid methyl ether, in addition to the previously isolated limonin, deacetylnomilin, obacunone and ichangin [14]. The marginal antimalarial activity of isolimonexic acid methyl ether is reported [14]. It has phenolic [11] and flavonoids among its components [15].
Literature information on the chemical constituents of their essential oils revealed a great variability which may have been due to several factors, among them the particular varieties studied, the geographical location, season and environmental factors, such as soil type and climate, genetic factors processing and extraction method and the part of the plant used to extract the oil.

2. Materials and methods

2.1 Plant Materials

Fresh leaf samples of *C. aurantifolia* and *C. reticulata* were collected from private gardens at Ikotun and Ijanikin, both in Lagos State, Nigeria. Botanical identification of the plant samples were carried out at Department of Botany, University of Lagos, Akoka-Yaba, Lagos, Nigeria. Voucher specimens (LUH 5787 and LUH 6057) for *C. aurantifolia* and (LUH 5787 and LUH 6057) for *C. reticulata* from Ikotun and Ijanikin, respectively were deposited in the Herbarium of the University.

2.2 Extraction of essential oils

Air-dried and pulverised (250 g each) of plant materials were subjected to separate hydrodistillation using Clevenger-type apparatus for 3 h in accordance with the British Pharmacopoeia specification [16]. The distilled oils were preserved in a sealed sample tube and stored under refrigeration until analysis. Oils were pall yellow in colouration.

2.3 Gas Chromatography (GC) analyses

GC analysis of the oils were carried out on a Hewlett Packard HP 6820 Gas Chromatograph equipped with a FID detector and DB-5 column (60 m x 0.25 mm id, film thickness was 0.25 µm) and the split ratio was 1:25. The oven temperature was programmed from 50 °C (after 2 min) to 240 °C at 5 °C/min and the final temperature was held for 10 min. Injection and detector temperatures were maintained at 200 °C and 240 °C, respectively. Hydrogen was the carrier gas at a flow rate of 2 mL/min. An aliquot (0.5 µL of the diluted oil) was injected into the GC. Peaks were measured by electronic integration. A homologous series of *n*-Alkanes were run under the same conditions for determination of retention indices.

2.4 Gas chromatography-Mass spectrometry (GC-MS) analyses

GC-MS analyses of the oil were performed on a Hewlett Packard Gas Chromatography HP 6890 interfaced with a Hewlett Packard 5973 mass spectrometer system equipped with a DB-5 capillary column (30 m x 0.25 mm id, film thickness 0.25 µm). The oven temperature was programmed from 70-240 ºC at the rate of 5 ºC/min. The ion source was set at 240 ºC and electron ionization at 70eV. Helium was used as the carrier gas at a flow rate of 1mL/min. The scanning mass range was 35 to 425 amu. Diluted oil in *n*-hexane (1.0 µL) was injected into the GC/MS.

2.5 Identification of compounds

The identification of constituents was performed on the basis of retention indices (RI) determined with reference to the homologous series of *n*-Alkanes, under identical experimental conditions, co-injection with standards (Sigma-Aldrich, St. Louis, MO, USA) or known essential oil constituents, MS library search (NIST 08 and Wiley 9th Version), and by comparing with MS literature data [17].

Table 1: Chemical composition of *C. aurantifolia* and *C. reticulata* leaf oils

Compounds [a]	RI[a]	Percentage composition			
		C. aIj	***C. aIk***	***C. rIj***	***C. rIk***
(*E,E*)-2,4-Hexadienal	923	5.7	-	-	-
α-Thujene	929	-	0.3	0.6	-
α-Pinene	941	7.9	-	3.1	-
Sabinene	976	-	0.2	-	-
β-Pinene	979	1.1	0.2	0.8	-
Myrcene	986	0.1	1.6	-	-
β-Phellandrene	1020	-	-	3.8	-
Limonene	1028	-	44.7	-	12.2
(*Z*)-β-Ocimene	1042	-	1.3	4.2	25.9
2,6-dimethyl-1,5,7-Octatrien-3-ol	1045	7.3	-	-	-
(*E*)-β-Ocimene	1048	2.1	-	4.5	-
γ-Terpinene	1072	0.4	-	1.2	0.9
Terpinolene	1089	1.0	-	1.0	-
Linalool	1101	0.2	1.3	4.2	14.5
β-Thujone	1123	-	-	12.8	-
trans-Sabinol	1140	0.4	-	2.6	-
trans-Pinocarveol	1147	-	-	5.9	-
Isoborneol	1156	-	-	5.0	-
Citronellal	1159	-	4.0	-	38.1
p-Mentha-1,5-dien-8-ol	1161	-	0.8	-	-
Pinocarvone	1164	-	-	22.7	-
trans-α-Bergamotol	1171	2.3	-	-	-
Terpinen-4-ol	1176	-	-	-	1.5
Geranial	1251	-	38.2	-	-
Guaniol	1257	-	1.2	-	-
Citronellyl formate	1276	-	-	-	1.2
Bornyl acetate	1285	-	-	1.5	-
trans-Pinocarveol acetate	1305	-	-	20.0	-
Citronellyl acetate	1358	-	-	-	1.4
Eugenol	1365	-	-	0.8	-

Neryl acetate	1369	-	4.7	-	-
trans-α-Bergamotene	1419	1.0	-	-	-
cis-α-Bisabolene	1504	1.7	-	-	-
Caryophyllene oxide	1582	32.2	-	-	-
δ-Cadinol	1667	-	-	0.3	-
Caryophylla-3(15),7(14)-dien-6-ol	1670	30.0	-	-	-
Guaiyl acetate	1724	-	-	0.6	-
6,10,14-Trimethyl-2-pentadecanone	1832	2.0	-	-	-
Total		**96.5**	**96.7**	**96.2**	**92.7**
Monoterpene hydrocarbons		**12.6**	**46.7**	**19.8**	**39.0**
Oxygenated monoterpenes		**0.6**	**50.2**	**75.5**	**56.7**
Sesquiterpene hydrocarbons		**2.7**	**-**	**-**	**-**
Oxygenated sesquiterpenes		**67.6**	**-**	**0.9**	**-**
Non-terpene		**13.0**	**-**	**-**	**-**

[a] Retention indices to C9-C24 *n*-alkanes on the DB-5 column; *C. aIj* - *C. aurantifolia* from Ijanikin ; *C. aIk* - *C. aurantifolia* from Ikotun; *C. rIj* - *Citrus reticulata* from Ijanikin; *C. rIk* - *Citrus reticulata* from Ikotun

3. Results & Discussion

The identities and the percentage composition of the each of the components were summarized in Table 1 in order of their elution on a DB-5 column. Sixteen and twelve compounds representing 96.5% and 96.9% of the total oil contents were identified in the oils of *C. aurantifolia* from Ijanikin and Ikotun respectively. Oxygenated sesquiterpene compounds (67.6%) predominated in Ijanikin sample while oxygenated monoterpenes (50.2%) and monoterpene hydrocarbons (46.7%) were the class of compounds present in Ikotun sample. Caryophyllene oxide (32.2%) and caryophylla-3(15),7(14)-dien-6-ol (30.0%), α-pinene (7.9%) and 2,6-dimethyl-1,5,7-octatrien-3-ol (7.3%) were the main compounds of Ijanikin sample, which were not identified in Ikotun oil. However, limonene (44.7%) and geranial (38.2%) were the major constituents of Ikotun sample. A great variability was observed in the comparison of the present oil compositions with literature data (Table 2). The major sesquiterpene compounds of Ijanikin sample, i.e. caryophyllene oxide and caryophylla-3(15),7(14)-dien-6-ol were not reported previously to be of significant quantities in *C. aurantifolia* oils [18-22]. The geranial/limonene contents of Ikotun sample competes favourable with previous data [19, 20].

Nineteen and eight compounds representing 96.2% and 95.7% of the total oil contents were respectively identified in *C. reticulata* from Ijanikin and Ikotun samples. Oxygenated monoterpenes (75.5% and 56.7% respectively) and monoterpene hydrocarbons (19.6% and 39.0% respectively) were the main class of compounds present in the oils. Pinocarvone (22.7%), *trans*-pinocarveol acetate (20.0%) and β-thujone (12.8%) were the major constituents of Ijanikin oil while the Ikotun oil comprised mainly of citronellal (38.1%), (*Z*)-β-Ocimene (25.9%), linalool (14.5%) and limonene (12.2%).

Table 2 revealed the summary of major compounds identified in the essential oils of *C. reticulata* [23-41]. The results indicated great variability in the oil compositions which led to the delineation of about ten chemical classes. The present chemical forms of essential oils of *C. reticulata* namely pinocarvone/trans-pinocarveol acetate/β-thujone and citronella/(*Z*)-β-Ocimene linalool/limonene were not described previously in the literature.

Table 2: Summary of major constituents of *Citrus aurantifolia* and *Citrus reticulata* in literature

Parts	**Origin**	**Major constituents**	**References**
Citrus aurantifolia			
Leaf	Nigeria	limonene (33.7%), geraniol (21.5%), nerol (6.1%), β-pinene (5.8%)	18
Fruit	Nigeria	limonene (43.1%), ß-pinene (14.2%)	19
Fruit	Cuba	limonene (40.4%), α-terpineol (12.7%), γ-terpinene (9.5%), terpinolene (8.7%)	21
Peel	India	neral and geranial (7.8%), geraniol (7.3%)	22
Peel	Malaysia	β-pinene (28.4%) and limonene (39.3%)	20
Leaf	Malaysia	geranial (19.4%), limonene (16.4%) and neral (11.4%)	20
Citrus reticulata			
Chemical Forms I			
-	Spain	limonene (74.7%) and γ-terpinene (15.7%);	23
Fruit	Nigeria	limonene (67.0%)	24
Peel	Burundi	limonene (84.8%), γ-terpinene (5.4%)	25
Peel	Uganda	limonene (85.9 and 86.9%), y-terpinene (6.0 and 5.4%)	26
Peel	Uganda	limonene (55.6%)	28
Peel	Uruguay	limonene (75.26–96.23%)	30
Peel	Thailand	*d*-limonene (62.39%) and γ-terpinene (14.06%).	32
Peel	Cuba	limonene (78.3%) and γ-terpinene (7.2%)	33
Peel	Tunisia	limonene (48.85%)	34
Peel	Tunisia	limonene (87.1%), γ-terpinene (1.5%)	35

Peel	India	l-limonene (92.4%), γ- terpinene (2.6%) and β-phellandrene (1.8%)	36
Peel	India	limonene (87.45%)	36
Peel	China	limonene (80.2%)	41
Chemical form II			
Leaf	China	γ-terpinene (53.0%) and linalool (16.1%)	24
Chemical form III			
Leaf	China	limonene (77.22%), β-myrcene (3.7%), β-phellandrene (2.75%), ocimene (2.61%)	27
Chemical form IV			
Leaf	Italy	methyl N-methyl anthranilate (50%), γ-Terpinene (23.9–28.5%) ,(7.2–12.6%)	29
Chemical form V			
Leaf	Turkey	sabinene (42.5%) and γ-terpinene (3.8%)	31
Chemical form VI			
Unopen flower	China	linalool (4676%), β-pinene (9.20%)	37
Half-open flower	China	linalool (50.43%), β-pinene (7.83%),	37
Opened flower	China	linalool (47.74%), β-pinene (6.59%0, indole (4.99%)	37
Chemical form VII			
Young leaf	China	linalool (47.22%), β-terpiene (14.38%)	38
Chemical form VIII			
Mature leaf	China	β-terpiene (21.83%), linalool (19.49%), (E) β-ocimene (8.97%)	38
Chemical form IX			
Fruit	India	limonene (46.7%), geranial (19.0%), neral (14.5%)	39
Chemical form X			
Leaf	Iran	linalool (23.47%), sabinene (38.91%), (E) β-ocimene (7.44%)	40
Flower	Iran	linalool (24.91%), sabinene (36.33%), (E)-nerolidol (7.51%), limonene (7.46%)	40

- Part not known

4. Conclusions

It could be seen that there was a variation within the chemical compositions of the studied oil samples which may attributable to the different ecological and climatic conditions as well the nature of the plants in the different parts of the world.

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