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Citrus Essential Oils of Nigeria Part IV: Volatile Constituents of Leaf Oils of Mandarins (*Citrus reticulata* Blanco) From Nigeria

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Abstract: The chemical composition of hydrodistilled oils obtained from the leaves of six *Citrus reticulata* Blanco (mandarin) cultivars grown in Nigeria were examined by GC and GC/MS, the result of their chemical composition were further submitted to cluster analysis. Fifty seven constituents were characterized accounting for 88.2 - 96.7% of the total oils. Sabinene, γ-terpinene, *p*-cymene, δ-3-carene and (*E*)-β-ocimene were observed in great variability in all the oils. Other constituents include linalool, myrcene, terpinen-4-ol and *cis*-sabinenehydrate. In addition, limonene, terpinolene, β-pinene, and α-pinene were also detected in appreciable concentrations. β-sinensal and α-sinensal were isolated by preparative GC and characterized by one- and two-dimensional NMR techniques.

Keywords: *Citrus reticulata*; Rutaceae; essential oil; sabinene; δ-3-carene; linalool.

1. Introduction

Citrus reticulata Blanco represents an assemblage of cultivars; they differ morphologically from Satsuma mandarins in having a more upright growth habit and generally with small flowers and fruits [1]. The name 'Mandarin" was given to the *C. reticulata* by the Portuguese who brought it from China and named it to reflect the country of origin [2]. Mandarin petit grain oils are known to contain

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methyl-*N*-methyl anthranilate as the major component [3-4]. This compound is considered as an important constituent of mandarin petit grain oil because it does not discolor or form condensation products in the presence of aldehydes. However, attempts to produce mandarin petit grain oil by distillation of leaves and twigs of mandarin trees occasionally resulted in essential oils without a trace of methyl-*N*-methyl anthranilate. Indeed, more recent studies have shown that the leaf oils of new varieties of mandarin contain mostly hydrocarbons and linalool.Thymol and or terpinen-4-ol, were found in some varieties of mandarin leaf oil [2]. Three major chemotypes, sabinene/linalool, linalool/γ-terpinene and methyl N-methylanthranilate were distinguished for the leaf oil of *Citrus reticulata* Blanco, in addition two major chemotypes, limonene and limonene / γ-terpinene were also distinguished for the peel oils [5]. In today's food and beverage market, *Citrus* flavored products are highly popular. The aroma elements from the *Citrus* oils are widely used in the creation of natural flavors. The commercial importance of these products leads to extensive scientific study resulting in a broad base knowledge of their chemical composition [2].

In Nigeria, the National Horticultural Research Institute (NIHORT), Idi-ishin Ibadan is responsible for cultivation and breeding of new cultivars and these are made available to the *Citrus* farmers. Despite government huge investment on *Citrus* breeding, there is no comprehensive data on the composition of *Citrus* essential oils of Nigeria origin.

Although the essential oil contents of some *Citrus* species grown in Nigeria have been examined data are not available on the volatile constituents of the majority of *Citrus* cultivated in Nigeria. As a consequence of this and in order to further explore already existing commercial crops; a deep study on Nigerian *Citrus* leaf oils has been carried out.

The present paper is the forth of a series devoted to the analysis of the leaf essential oils of various taxonomic groups and cultivars grown in the Orchard of NIHORT. In particular, the paper describes the composition of the oils extracted from the leaves of six mandarin cultivars: Dancy, Algeria, Clementine, King, Queen and Satsuma.

2. Materials and Methods

2.1. Plant Material

The leaves were obtained from cultivars growing in the orchards of the National Horticultural Research Institute (NIHORT) Idi Ishin Ibadan-Nigeria. Fresh matured leaves (300g each) were subjected to hydrodistillation for 4h in a modified Clevenger apparatus [6].

2.2 Gas Chromatography-Mass Spectrometry

The Gas chromatography (GC) analyses of the oils were performed on an Orion Analytical Micromat 412 double column Gas Chromatograph (GC) fitted with flame ionization detector (FID). Two capillary columns of differing polarities, CPSil-5 (25m x 0.25mm i.d.) equivalent to OV 101 and CPSil-19 (25m x 0.25mm i.d.) similar to BP10 (film thickness 0.15μm), were used. Oven temperature was programmed from 50^{o}C to 230^{o}C at 3^{o}C/ min. Injector and detector temperatures were maintained at 200^{o}C and 250^{o}C respectively. The carrier gas was hydrogen. GC-MS analyses were conducted out on a Hewlett-Packard Gas Chromatograph (GC) HP5890A interfaced with a VG Analytical 70-250s double- focusing mass spectrometer operating at 70eV with an ion source temperature of 230^{o}C. The GC w as fitted with a 25m x 0.25 i.d. fused silica capillary column coated with CPSil- 5. Helium was the carrier gas.

The constituents of the leaf essential oils were identified by comparison of their mass spectral pattern and Kovat's Indices (KI) with those data for original samples in library files built up from reference standard and authentic sesquiterpenoids. Confirmation of the identification of the constituents was by matching our MS and KI data with those published in the literature [7-10].

2.3 Numerical Cluster Analysis

The 87 *Citrus* samples were treated as operational taxonomic units (OTUs). The percentage composition of thirteen main essential oil components [α-pinene, sabinene, β-pinene, myrcene, δ-3-carene, α-terpinene, *p*-cymene, limonene, (*E*)-β-ocimene, γ-terpinene, terpinolene, linalool, and terpinen-4-ol] was used to determine the chemical relationship between the different *Citrus* leaf oil samples by cluster analysis using the NTSYSpc software, version 2.2 [11]. Correlation was selected as a measure of similarity, and the unweighted pair-group method with arithmetic average (UPGMA) was used for cluster definition.

3. Results and Discussion

Hydrodistillation of the leaves gave between 0.1% and 0.4% slightly yellow volatile oil. Comprehensive GC and GC-MS analyses of the oils afforded 31-46 constituents accounting for 88.2 - 96.7% of the total oils. Table 1 displayed the list of volatile constituents identified in the leaf oils of the six cultivars; they are arranged in the order of elution on a Cpsil-5 column; along with their retention (Kovat's) indices.

A large number of compounds were found common to all the *Citrus* leaf oils, while few were identified only in oils of certain varieties. Sabinene, γ-terpinene, *p*-cymene, δ-3-carene and (*E*)-β-ocimene were observed in great variability in all the oils. Other constituents include linalool, myrcene, terpinen-4-ol and *cis*-sabinenehydrate. In addition, limonene, terpinolene, β-pinene, and α-pinene were also detected in appreciable concentrations. Methyl *N*-methyl anthranilate, which has been described as an important constituent of Mandarin essential oil, was not identified in these oils.
Constituents of the oil samples were submitted to cluster analysis based on correlation and using the unweighted pair-group method with arithmetic average (UPGMA). The dendrogram (Figure 1) obtained from the cluster analysis show some apparent chemical clusters. Reading from the top of the tree down, there are six clusters which include sabinene/δ3-carene cluster ([N]-1 through [N]-3), sabinene-rich cluster ([14]-1 through [5]-32), linalool-rich cluster([14]-13 through [5]-24), limonene-rich cluster ([14]-8 through [5]-39), β-pinene-rich cluster([14]-11 through [14]-26), and γ-terpinene-rich cluster: [N]-6 through [5]-38.

Dancy, Algeria, and Clementine cultivars of the present study constitute sabinene/δ3-carene (sabinene as the major component) chemotype. To our knowledge, this is the first time of reporting this chemotype. Furthermore, Satsuma cultivar of our samples ([N]-6 and Kowano ([14]-25), San huhong chu ([14-34), kara ([5]-22), Guyanan ([5]-38) all from France and an unidentified cultivar [13] of *C. reticulata* Blanco from Nigeria constitute γ-terpinene-rich chemotype. However, King and Queen cultivar of our sample were closely related to the sabinene chemotype. As a direct consequence, the citrus samples of this present study can be classify into three chemotypes namely sabinene/δ3-carene (Dancy, Algeria, and Clementine), γ-terpinene-rich chemotype (Satsuma) and sabinene rich chemotype (King and Queen).

Table 1. Volatile Constituents of mandarin leaf oils of Nigerian *Citrus reticulata* Blanco.

RI	**Compound**	**% Composition**					
		1	**2**	**3**	**4**	**5**	**6**
933	α-Thujene	0.6	0.5	0.4	0.5	0.3	1.9
941	α-Pinene	2.2	2.3	1.9	1.9	1.1	3.9
973	Sabinene	34.4	34.7	39.7	33.3	22.6	1.4
978	β-Pinene	2.3	2.3	2.3	2.5	1.4	7.5
988	Myrcene	4.7	4.4	4.4	3.6	2.7	1.3
1002	α-Phellandrene	0.9	0.6	0.8	-	0.4	0.1
1011	δ3-Carene	11.5	11.0	11.6	0.2	7.3	t
1014	α-Terpinene	1.2	0.7	0.5	0.6	0.7	0.8
1016	*p*-Cymene	0.3	0.3	0.4	0.6	0.8	27.4
1024	Limonene	3.2	1.3	1.3	1.3	2.6	4.4
1029	(Z) - β- Ocimene	0.4	2.7	3.1	-	0.3	-
1038	(E)- β- Ocimene	9.3	9.4	9.1	19.7	5.9	3.2
1052	γ-Terpinene	1.9	1.0	0.7	2.6	1.1	32.8
1053	*cis*-Sabinenehydrate	1.2	4.2	3.4	0.9	0.3	-
1063	*cis*-Linalool oxide	-	0.3	0.2	-	-	-
1077	*p* - Cymenene	-	-	-	-	-	3.9
1083	Terpinolene	2.7	2.0	2.4	0.8	1.7	2.5
1086	Linalool	8.9	4.2	5.0	2.4	18.0	4.5
1104	*cis*-P-Menth-2-en-1-ol	t	-	0.2	0.4	-	-
1120	*trans*-P-Menth-2-en-1-ol	t	-	-	-	-	-
1129	Citronellal	0.6	-	0.4	2.2	3.0	-
1165	Terpinen - 4- ol	3.5	2.0	2.0	6.4	7.7	0.5
1175	α- Terpineol	0.3	0.2	0.3	0.3	1.0	0.3
1194	*trans*- Piperitol	t	t	t	0.2	1.8	-
1212	Nerol	-	-	-	-	-	t
1214	Citronellol	0.3	0.5	0.2	0.7	1.2	-
1217	Neral	t	-	0.3	0.8	4.1	t
1232	Piperitone	-	-	-	-	-	t
1236	Geraniol	t	-	0.2	0.2	1.3	t
1240	Linalyl acetate	-	-	t	-	-	-
1245	Geranial	0.1	-	0.3	1.0	5.4	t
1268	Thymol	-	-	-	-	0.3	t
1279	Carvacrol	-	-	-	-	-	t
1337	Citronellyl acetate	-	-	-	0.1	-	-
1342	Neryl acetate	t	0.4	-	0.2	0.4	t
1360	Geranyl acetate	1.6	-	0.2	0.2	-	t
1380	α - Copaene	t	-	-	-	-	-
1391	β-Elemene	-	0.2	-	0.8	0.3	0.5
1391	β- Cubebene	t	-	-	-	-	-
1402	Sesquithujene	t	-	-	-	-	-
1423	β- Caryophyllene	1.5	2.5	1.2	0.7	0.2	0.4
1431	β - Copaene	t	-	-	-	-	-
1437	Sesquisabinene	t	-	-	-	-	-
1449	(E)-β - Farnesene	0.2	0.1	0.2	-	-	-
1450	Sesquisabinene B	0.2	-	-	-	-	-

Table 1 Continued

1456	α - Humulene	t	0.2	tr	0.6	0.1	0.2
1478	Germacrene D	t	-	-	-	-	-
1503	α - Farnesene	t	-	-	-	-	-
1499	(*E, E*)- α-Farnesene	0.3	0.6	0.2	0.1	-	0.6
1505	Germacrene A	-	-	-	-	-	t
1510	Cubebol	t	-	-	-	-	t
1517	β-Sesquiphellandrene	0.3	-	-	-	-	-
1522	δ - Cadinene	-	t	-	-	-	t
1541	Elemol	0.1	-	0.2	-	0.4	t
1547	Caryophyllene oxide	-	-	t	-	t	-
1553	*trans*-Nerolidol	t	t	0.1	-	-	t
1554	Germacrene B	-	-	-	-	t	-
1566	3E, 7E-4, 8,12- Trimethyl-trideca1,3,7,11-tetraene	-	-	-	-	t	t
1572	Germacrene D	-	-	-	-	-	t
1579	Caryophyllene oxide	-	t	0.2	0.1	0.2	t
1599	Viridifloral	t	-	-	-	-	
1602	Humulene epoxide	-	-	t	-	-	-
1618	γ - Eudesmol	-	-	-	-	-	t
1629	T - Muurolol	t	-	-	-	-	-
1631	T- Cadinol	-	-	t	-	-	t
1643	α - Cadinol	-	-	t	-	-	t
1676	β - Sinensal	1.6	1.5	1.6	0.7	0.8	-
1727	α - Sinensal	1.6	1.4	2.0	1.5	0.3	-
2090	Phytol	-	0.4	t	t	0.1	-

a= Elution order and retention indices on a Cpsil-5 column (See Experimental)

t = trace.1 = Dancy, 2 = Algeria, 3 = Clementine, 4 = Kings, 5 = Queen, 6 = Satsuma

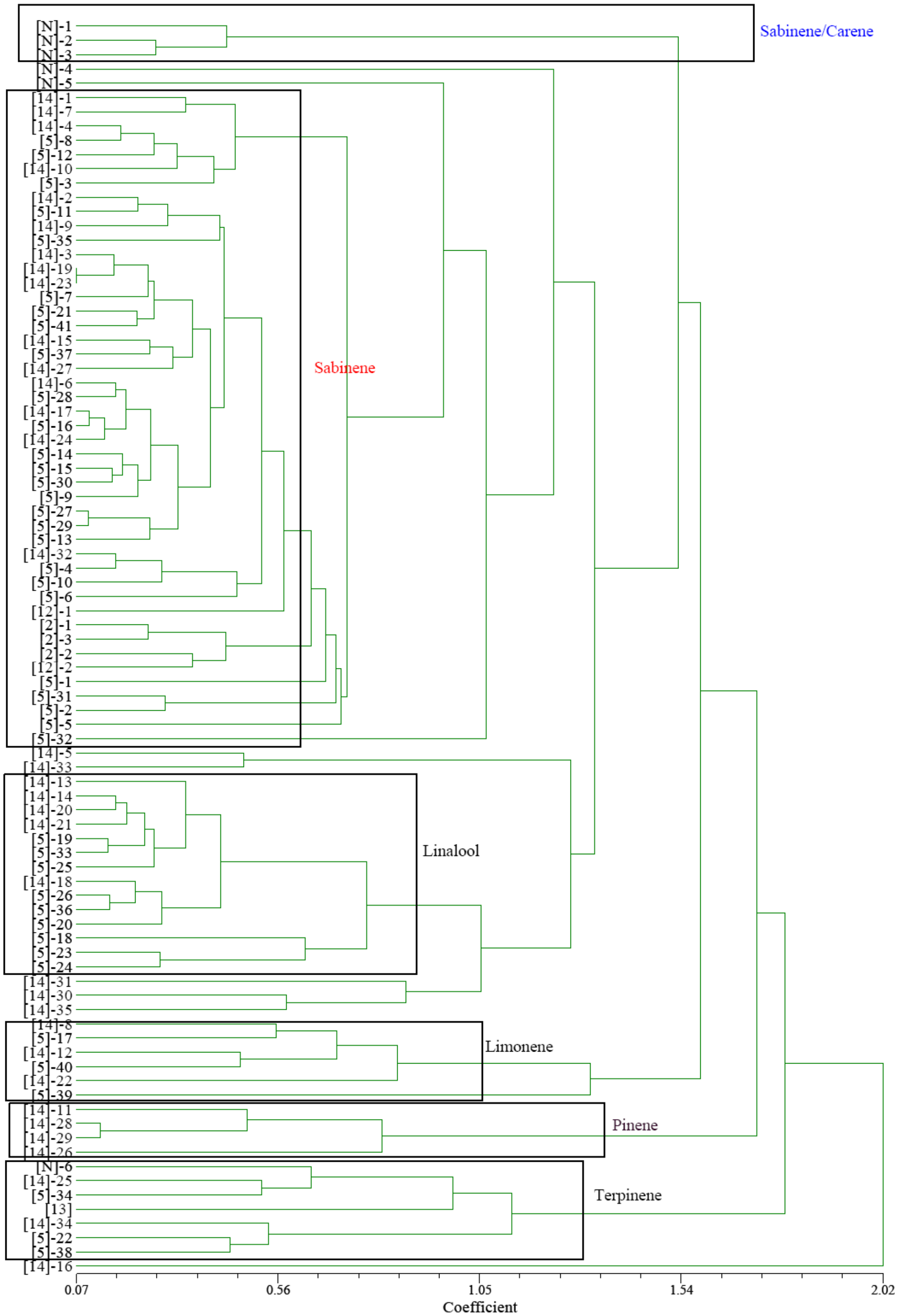

Figure 1. Dendrogram obtained by cluster analysis of the percentage composition of essential oils from *Citrus* leaf essential oil samples, based on correlation and using the unweighted pair-group method with arithmetic average (UPGMA). [N] = citrus samples of present study, [5], [12], [13], and [14] = citrus samples of previous studies in references 5, 12, 13, and 14.

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