Original Research Article

Effect of Harvest Time on Phytochemical Profile of *Citrus aurantifolia* Leaf Essential Oil Grown in North Central, Nigeria.

Abstract: Pulverized leaves of *Citrus aurantifolia* harvested in the morning (7a.m) and afternoon (2p.m) on the same day were separately subjected to hydro-distillation which yielded 0.4 and 0.5% (v/w) of the volatile oil respectively. Analyses of the oil harvested in the morning (7am) using GC and GC/MS revealed the predominance of oxygenated terpenes which constituted 58.3% of the oil. The principal constituents in the oil were; isolimonene (22.2%), neral (22.2%), citral (21.5%), caryophyllene (4.3%), and α-geranylacetate (4.1%). Furthermore, the leaf oil from the afternoon (2pm) harvest also showed predominance of oxygenated terpenes which constituted 57.7%. The principal constituents in the oil were; limonene (20.2%), neral (24.5%), citral (10.3%), caryophyllene (5.4%), and α-geranylacetate (3.3%). There was a slight compositional variation in the leaf essential oil obtained from morning and afternoon harvests.

Keywords: *Citrus aurantifolia*, Essential oil, Chemotype, Harvest Time

INTRODUCTION

Citrus aurantifolia belongs to Citrus family and a common name for edible fruits of this genus and sometimes related genera. Citrus is likely the most widely planted fruit for direct human consumption in the world[1].

Lime (*Citrus aurantifolia*) is a small shrub-like tree ranging from 3.5 to 9 m in height and 2.5 to 7.5 m in width. The fruit is typically round, green to yellow in color and about 3-6 cm in diameter, it is smaller, seedier, has a higher acidity, a stronger aroma, and a thinner rind than that of the Persian lime (*citrus latifolia*). It is value for its unique flavor compared other limes, with the key lime usually having a more tart and bitter flavor. The name comes from its association with the Florida keys where it is best known as the flavoring ingredient in key lime pie. It is also known as west Indian lime, bartender's lime. Omani lime or Mexican lime, the latter classified as a distinct race with a thicker skin and darker green colour[2].

Lime (*Citrus aurantifolia)* is a polyembryonic species with greenish yellow, smooth surfaced, thin-skinned fruits, and solid core at maturity with highly acidic juice[3]. Lime is used for the extraction of juice, preparation of squash, concentrates, beverages, and byproducts, such as citric acid and pectin[4]. Limes are also used for the preparation of beverages and pickles. In addition to vitamin C, other major classes of phytochemicals found in lime are limonoids, flavonoids, phenolic acids, coumarins, alkaloids and phytosterols[5].

Limes (*Citrus aurantifolia*) are popular for their attractive flowery, tart- tangy unique flavor, characteristic aroma, as well as other biologically active compounds. Citrus fruits are consumed globally in the form of fresh as well as processed juices. Recently, citrus fruits have been studied for various health benefits. This includes cardiovascular disease[6], obesity[7] and cancer[8-10].

Limes are popular for use in juice mixtures, carbonated beverages, and as a component of alcoholic drinks. In some countries, they are used in pickling, culinary, and medical applications. The juice (100 ml) supplies 110–140 KJ (26 Kcal) of energy, 50 mg of ascorbic acid (vitamin C) and a trace of dietary fiber. Furthermore, the most widely consumed product of citrus fruits is juice and it accounts for approximately more than 50 per cent of the total mass of the whole fruit[6]. Consumption of fruits and vegetables is associated with a lower risk of degenerative diseases including cancer, cardiovascular disease, cataracts, and brain dysfunction[11]. In addition, citrus species have been widely used in the ethnomedicine, with their broad range of bioactive ingredients, and have been found to possess anti-infection and anti-inflammatory properties[12].

Citrus fruits are known for their potential in prevention of cancer in an epidemiological survey[13]. Also the potential use of citrus flavonoids in cancer treatment has been suggested by some investigators[14]. Lime juice is being used by women as a barrier contraceptive, and there is a long reported history of African women douching with lime juice, lemon juice, vinegar or acidic soft drinks in the belief that it may prevent pregnancy and sexually transmitted diseases. However, data have suggested that this would have significant adverse effects on the genital mucosa, raising serious questions about the plausibility and safety of such a preventive approach[15].

It had been observed that the time of harvest, drying, location, season, nature of soil and age of plants greatly influences the yield of essential oil[16]. Thus, several factors affect the quality and quantity of essential oil in *Citrus aurantifolia*. For instance, from the research work carried out in University of Ngaoundere , Cameroon, showed that geographical location affects the leaf essential oil of *Citrus aurantifolia* both quantitatively and qualitatively[17].

Although, various factors have been noted to be responsible for the variation in the constituents of leaf of essential oil *Citrus aurantifolia* based on researches carried out in the past, but literature survey have shown that there is no report on the effect of harvest time on the phytochemical profile of *Citrus aurantifolia* leaf essential oil from North-central region of Nigeria. Thus, the aim of the research was to investigate variations in the yield and phytochemical composition of essential oils of fresh leaves of *Citrus aurantifolia* grown in North-central region of Nigeria.

EXPERIMENTAL

Plants Material: Fresh leaves of *Citrus aurantifolia* trees were harvested in the morning (7a.m) and afternoon (2p.m) from Ilorin, Ilorin West Local Government Area of Kwara State. Botanical identification was carried out at the herbarium of the Department of Plant Biology, University of Ilorin where voucher specimens was deposited.

Oil Isolation: Pulverized dried leaves of *Citrus aurantifolia* harvested at the morning and afternoon were hydrodistilled for three hours in a Clevenger type apparatus according to the British Pharmacopoea Specification[18]. The resulting oils were collected, preserved in a sealed sample tube and stored under refrigeration until analysis.

Gas Chromatography: GC analysis was performed on an orion micromat 412 double focusing gas Chromatography system fitted the two capillary column coated with Cp-sil 5 and Cp-sil 19 (fused silica, 25m x 0.25mm x 0.15 film thickness) and flame ionization detector (FID). The volume injected was 0.2 microlitre and the spilt ratio wwas 1:30. Oven temperature was programmed from 50-230oCat 5 degree/min, using hydrogen gas as carrier gas. Injection and detector temperature were maintained at 200 and 250oC respectively. Qualitative data were obtained by electronic integration of FID area percent without the use of correction factor.

Gas Chromatography /Mass Spectrometry: A Hewlett-Packard HP5890A GC, interfaced with a VG analytical 70-250s double focusing mass spectrometer was used. Helium was the carrier gas at 1.2ml/min. The MS operating conditions were: ionization voltage 70eV, ion source 230 ℃. The GC was fitted with a 25 m × 0.25 mm, fused silica capillary column coated with CP-sil 5. The film thickness was 0.15μm.The GC operating conditions were identical with those of GC analysis. The MS data were acquired and processed by on-line desktop computer equipped with disk memory. The percentage compositions of the constituents of the oil were computed in each case from GC peak areas. The identification of the components was based on the comparison of retention indices (determined relative to the

retention time of series of n-alkanes) and mass spectral with those of authentic samples and with data from literature [18-19].

RESULTS AND DISCUSSION

Quantities of essential oils obtained from fresh leaves of *Citrus aurantifolia* harvested in the morning and afternoon harvest during dry season were shown in Table 1 below.

Table 1: Volatile Oil Yield of Fresh *Citrus aurantifolia Leaves*

Days of Drying	Morning (%)	Afternoon (%)
Fresh	0.40	0.50

The hydrodistilled pulverized leaves (500g) of *Citrus aurantifolia* obtained from morning (7a.m) and afternoon (2p.m) harvests yielded 0.40 and 0.50 % (v/w) of essential oil respectively. Low amount of oil obtained from fresh leaves harvested in the morning could be attributed to the high moisture content in the leaves tissues. Consequently, the loss of water increases the oil obtained. This implied that time of harvest significantly affect the yield of the oils.

The chemical identities, kovat indices, percentage composition and mass spectra of oils obtained from *Citrus aurantifolia* fresh leaves harvested in the morning and afternoon are been shown in Table 2.

Table 2: Chemical composition of leaf volatile oil of *citrus aurantifolia* obtained from morning (7a.m) and afternoon (2p.m) harvests.

Compound[a]	KI[b]	Percentage Composition		Mass Spectra Data
		Morning (7am)	Afternoon (2pm)	
5-hepten-2-one, 6-methyl	985	3.5	--	43,69,41,108
Isolimonene	983	22.2	--	79, 93,77,91
Ocimene	1040	1.7	--	93,79,91,105
α – terpinolene	1088	Tr	--	93,79,65,105
Linalool	1228	1.1	--	71,93,121,136,
(R)-(+)-citronellal	1153	3.2	3.8	41,69,59,53
Geranial	1240	4.4	14.2	41,55,69,95
Neral	1270	22.2	24.5	69,84,94,152
Citral	1240	21.5	10.3	69, 84,94,109
citronellol acetate	1228	0.5	--	69,81,95,109
β –geranylacetate	1365	1.3	1.2	69,93,80,107
α – geranylacetate	1383	4.1	3.3	69,41,136,93
Caryophyllene	1418	4.3	5.4	93,105,120,133
α –caryophyllene	1454	0.5	0.6	93,107,121,147
β - farnesene	1458	Tr	tr	41,69,93,133
Isocaryophyllene	1407	tr	Tr	93,69,79,105
α –farnesene	1508	2.1	--	41,55,69,79,93
α -elemene	1430	0.8	--	121,93,107,147
caryophyllene oxide	1581	0.5	0.5	43,55,69,79,93
germacrene-D-4- β -ol	1574	tr	tr	81,43,105,123
α –bisabolol	1683	tr	tr	109,69,93,134
β -pinene	980	-	0.9	93,107,121,91
Limonene	1031	-	20.2	68,77,79,93
Decanal	1204	-	0.3	57,43,70,82,96
limonene epoxide	1147	-	0.4	43,55,67,79,94
(E)-ocimene	1508	-	2.7	41,93,107,79,69
Octadecanal	1357	-	tr	43,57,152,82,96
phytol	1949	-	tr	71,57,81,95,111
β -elemene	1375	-	0.5	81, 93,107,41
Total		**93.9**	**88.8**	
Hydrocarbon Monoterpenes		23.9	23.8	
Oxygenated Monoterpenes		58.3	57.7	
Hydrocarbon Sesquiterpenes		7.7	6.5	
Oxygenated Sesquiterpenes		0.5	0.5	
Non-Terpene		3.5	0.3	

a = compounds are listed in order of elution from silica capillary columns coated with Cp-sil5 and Cp-sil 19 KI[b]- Kovat Indices on fused DB-5capillary column

KEY: Tr-Trace Amount (<0.1)

Essential oils are usually characterized by monoterpenes and sesquiterpenes. In the Table, twenty-one (21) compounds from both morning (7a.m) and afternoon (2pm) were identified in the oils; the numbers represent 93.9 and 88.8% of the oil respectively.

Percentage composition of hydrocarbon monoterpenes in the essential oil of *Citrus aurantifolia* leaf obtained from morning (7a.m) and afternoon (2p.m) harvests are presented in the Table 2, In the Table, a total of three (3) compounds from both morning (7a.m) and afternoon (2p.m) harvests were identified; the numbers represent 23.9 and 23.8% of the oil respectively. Predominant hydrocarbon monoterpene in the oil obtained from morning harvest was isolimonene (22.2%). In the oil obtained from afternoon harvest, predominant hydrocarbon monoterpenes detected were; limonene (20.2%).

Furthermore, the oxygenated monoterpenes composition of *Citrus aurantifolia* leaf essential oil which is depicted in Table 2 shows that seven (7) and six(6) oxygenated monterpene compounds were identified morning (7a.m) and afternoon (2p.m) harvests represent 58.3 and 57.7% respectively. The principal oxygenated monoterpene in morning (7a.m) harvest were Neral (22.2%) and Citral (21.5) while in the afternoon harvested oil were (Geranial (14.2%), Neral (24.5%) and Citral (10.3%).

The composition of hydrocarbon sesquiterpenes in the leaf essential oil of *Citrus aurantifolia* obtained from morning (7a.m) and afternoon (2p.m) harvests depict that a total of six (6) compounds and five (5) compounds were identified in the oil respectively which also represent 7.7 and 6.5% respectively. Major hydrocarbon sesquiterpenes in the oil obtained from morning harvest were; Caryophyllene (4.3%) and α -Farnesene (2.1%). In the oil obtained from afternoon harvest, the major hydrocarbon sesquiterpenes detected was only caryophyllene (5.4%).

The number of oxygenated sesquiterpenes constituents of leaf essential oil of *Citrus aurantifolia* obtained from the morning harvest (7:00am) and in the afternoon (2:00pm) were a total of three (3) compounds each which represent 0.5 and 0.5% of the oil respectively. Oxygenated sesquiterpenes of appreciable quantity detected in the oil obtained from morning and afternoon harvest was caryophyllene oxide (0.5%) while α-bisabolol (tr) and germacrene-D-4-β-ol occur in trace amounts.

It has been established that the most active synthase catalyzes the formation of constituents in oil[20]. Thus, the most active mono- and sesquitepenoid synthase facilitate the

151 formation of monoterpenoids and sesquiterpenoids respectively in a plant[21]. The synthase
152 catalyzes carbocationic reaction which converts the precursors to intermediate ion by the
153 divalent metal ion-dependent ionization of the substrate[22]. The cationic intermediate
154 undergoes a series of cyclizations, hydride shifts or other rearrangements which is terminated
155 by deprotonation or the addition of a nucleophile to give various terpenic products[23].

156 Thus, the predominance of limonene, neral and citral in the oils implied that their
157 synthase are very active thereby mediate the formation of all monoterpenoids in the leaf oils
158 (Scheme 1). Furthermore, the prevalence of Caryophyllene in the oils depict that its synthase
159 facilitates the formation of all sesquiterpenoids in the oil (Scheme 2). The activity of these
160 synthase most likely tends to determine the compositional profile of essential oil obtained
161 from the leaf. Therefore, *Citrus aurantiifolia* leaf essential oils were of limonene/neral/ citral
162 chemotype.

163 Comparison of the composition patterns of the oils obtained in the morning and
164 afternoon harvests reveal that there were both qualitative and quantitative variations in the
165 constituents of the oils.

166 Qualitative variations reveal that; β-pinene, limonene, decanal, limonene epoxide,
167 octadecanal, and phytol were all absent in the leaf essential oil from the morning (7am)
168 harvest. Also, α-fernesene, α-elemene, linalool, citronellol acetate, isolimonene, ocimene, α-
169 terpinolene and 5-hepten-2-one,6-methyl were absent in leaf essential oil of *Citrus*
170 *aurantifolia* obtained from afternoon (2pm) harvest.

171 The quantitative variation also exists, for instance the percentage composition of
172 Geranial was 4.4 and 14.2% in the leaf essential oil obtained from morning and afternoon
173 harvests respectively; Citral was 21.5 and 10.3% in the in the leaf oil of *Citrus aurantiifolia*
174 harvested in the morning and afternoon respectively; Neral was 22.2 and 24.5% in the leaf
175 essential oil obtained from morning and afternoon harvests respectively. The percentage
176 composition of caryophyllene was 4.3 and 5.4% in the leaf oil of *Citrus aurantiifolia*
177 obtained from morning and afternoon harvests respectively.

178 However, the phytochemical profile of the oil harvested in the morning (7am) and
179 afternoon revealed the predominance of oxygenated terpenes which constituted 58.3 and
180 57.7% respectively. This correlate with the report on leaf oil obtained from Ikotun, Lagos
181 State, Nigeria (Lawal *et al.,* 2014). Furthermore, the predominance of limonene in the leaf

oils also correspond with existing literature data from earlier researches[24-26]. The variation in the phytochemical profile of the leaf oils can be attributed to different ecological and climatic conditions, age and nature of the plant.

CONCLUSION

There exists quantitative and qualitative variation in the leaf essential oil when *Citrus aurantifolia* was harvested at different time (morning and afternoon). The optimum percentage of essential oil was obtained from the morning (7am) harvest. Therefore, *Citrus aurantifolia* leaves flourishes more in the morning. The oils obtained from morning (7a.m) and afternoon (2p.m) harvests were found to have slightly different chemotypes and these may affect their biological activities.

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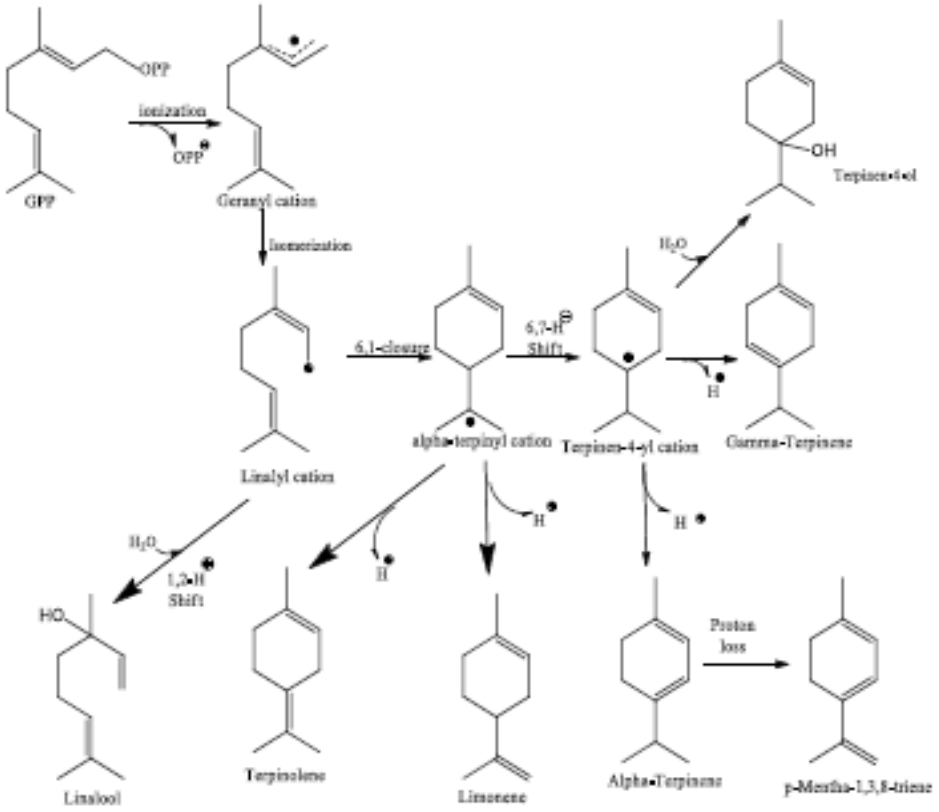
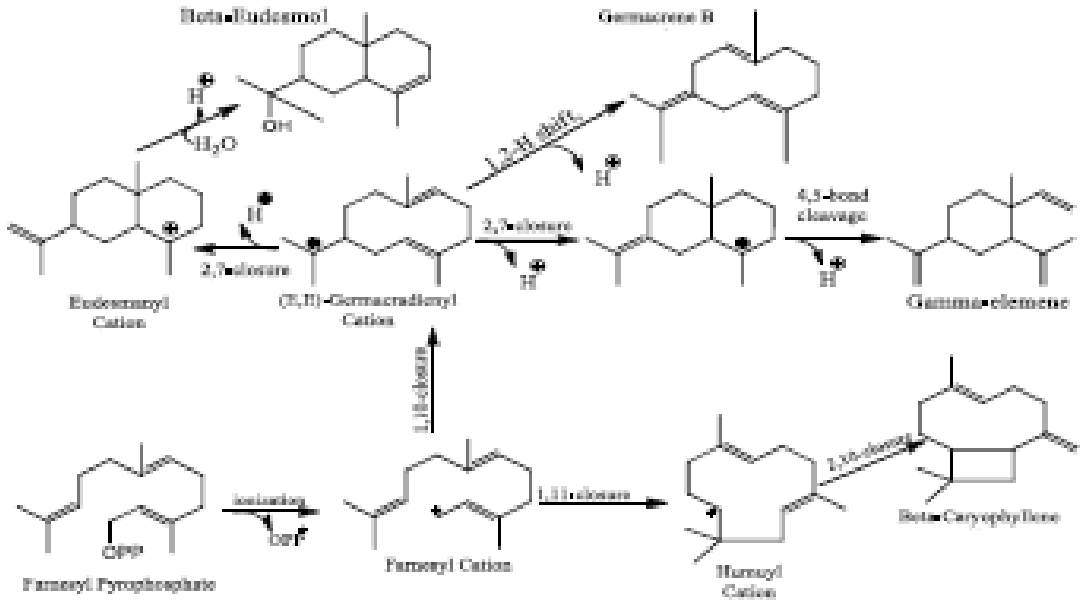


268
269 **Scheme 1: Limonene/Neral/ Citral synthase-mediated biosynthesis of monoterpenoids**



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271 **Scheme 2: Caryophyllene synthase-mediated biosynthesis of sesquiterpenoids**