



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

**T. C. Ogunyemi<sup>1\*</sup>, C. M. Ekuma<sup>1</sup>, T. O. Akintoye<sup>2</sup> and S. T. Ogunyemi<sup>3</sup>**

<sup>1</sup>Department of Chemistry, Federal Polytechnic of Oil and Gas, Bonny Island, Nigeria.

<sup>2</sup>Department of Industrial Chemistry, University of Benin, Nigeria.

<sup>3</sup>Department of Biochemistry, Ekiti State University, Ado-Ekiti, Nigeria.

### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author TCO designed the study, performed the statistical analysis and wrote the protocol. Authors CME, TOA and STO managed the analyses of the study and literature searches. All authors wrote the first draft of the manuscript, read and approved the final manuscript.*

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### **ABSTRACT**

*Citrus aurantifolia* essential oils are volatile phytochemicals obtained from various part of the plant which has found wide range of domestic, medicinal and industrial applications. The research seeks to investigate the effect of time of harvest on the yield and phytochemical composition of *Citrus aurantifolia* leaf oil. 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) revealed the predominance of oxygenated terpenes which constituted 58.3% of the oil. The principal constituents were; isolimonene (22.2%), neral (22.2%), citral (21.5%), caryophyllene (4.3%), and  $\alpha$ -geranyl acetate (4.1%). Furthermore, the leaf oil from the afternoon (2pm) harvest also showed predominance of oxygenated terpenes which

\*Corresponding author: E-mail: [charlestolulope@gmail.com](mailto:charlestolulope@gmail.com);

constituted 57.7%. The principal constituents in the oil were; limonene (20.2%), neral (24.5%), citral (10.3%), caryophyllene (5.4%), and  $\alpha$ -geranyl acetate (3.3%). This study established that there was compositional variation in the leaf essential oil obtained from the different time of harvests.

**Keywords:** *Citrus aurantifolia*; essential oil; chemotype; harvest time.

## 1. INTRODUCTION

*Citrus aurantifolia* belongs to Citrus genus which are cultivated in several part of the world especially subtropical or tropical region such as Nigeria [1]. It is commonly known as lime in Nigeria. It is a small polyembryonic shrub-like tree of about 3.5 to 9 m tall. The leaves are yellow-green to dark green, winged petioles and long blades with edges [2]. The fruit is typically round, green to yellow in color which contain a few white pointed seeds with highly acidic juice [3]. *Citrus aurantifolia* are popular for their attractive flowery, tart-tangy unique flavor, characteristic aroma, as well as other biologically active compounds. It is used for juice extraction, preparation of squash, concentrates, beverages, byproducts (citric acid, pectin) and other phytochemicals such as limonoids, flavonoids, phenolic acids, coumarins, alkaloids and phytosterols [4,5]. The plant is used in traditional medicine for treatment of several diseases such as cold and stomach ailment since it exhibits biological activities, such as antimicrobial activity against several pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas spp*, *Aspergillus niger* and *Candida albicans* [6-9]. In West Africa, it is an essential ingredient in the most herbal concoctions [2].

Furthermore, its essential oils which are essence obtained from various part of *Citrus aurantifolia* plant are used in the food industry to give flavor to drinks and foods [10]. It is also used in drugs preparation, soaps, perfumes, hair cream, body oil and other cosmetics as well as for home cleaning products [11]. Studies on the essential oil of *C. aurantifolia* showed that it had an excellent inhibitory action against *Phaeoramularia angolensi*, *Aspergillus niger*, *Aspergillus parasiticus*, its aflatoxins, and *Candida albicans*. It's antifungal activity could be attributed to the presence of monoterpenes which suggest that the oil may be potent for preserving food and feeds from toxigenic fungal growth alongside aflatoxin contamination [6,12]. Previous research had showed that *C. aurantifolia* oil can be used for prevention of

cancer especially human colon cancer [13]. Further studies on the effect of essential oil of *C. aurantifolia* on weight gain in mice showed that there was reduction in both the amount of food intake and body weight. The weight loss was attributed to the fact that *C. aurantifolia* can possibly promote anorexia which plays significant role on weight loss [14].

Researchers have observed that time of harvest, drying, location, season, nature of soil and age of plants influence the large intraspecific chemical variation displayed by constituent of *C. aurantifolia* essential oils [15-16]. For instance, research within Cameroon has shown that geographical location affects the leaf essential oil of *Citrus aurantifolia* both quantitatively and qualitatively [12].

However, literature surveys have shown that there is no report on the effect of harvest time on the phytochemical composition of *Citrus aurantifolia* leaf essential oil from Nigeria. Thus, the aim of the research was to investigate the influence of time of harvest on the yield and phytochemical profile of essential oils from fresh *Citrus aurantifolia* leaves grown in North-central region of Nigeria.

## 2. EXPERIMENTAL

**Plants Material:** Fresh leaves of Ten (10) *Citrus aurantifolia* trees from Ilorin, Ilorin West Local Government Area of Kwara State were harvested in the morning (7a.m) and afternoon (2p.m) within the month of November. 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 (500 g) 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 [17]. 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 with two capillary columns 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 split ratio was 1:30. Oven temperature was programmed from 50-230°C at 5 degree/min, using hydrogen gas as carrier gas. Injection and detector temperature were maintained at 200 and 250°C 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 1.2 ml/min. The MS operating conditions were: ionization voltage 70eV, ion source 230°C. The GC was fitted with a 25 m x 0.25 mm, fused silica capillary column coated with CP-sil 5. The film thickness was 0.15  $\mu$ m. 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].

### 3. 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.

The hydrodistilled pulverized leaves of *Citrus aurantifolia* obtained from morning (7a.m) and afternoon (2p.m) harvests yielded different quantities of essential oil. Lower 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 shown in Table 2.

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

A total of three (3) hydrocarbon monoterpene compounds were identified from both morning (7a.m) and afternoon (2p.m) harvests; the numbers represent 23.9 and 23.8% of the oil respectively. The predominant hydrocarbon monoterpene in the oil obtained from morning harvest was isolimonene. Isolimonene is an isomer of d-limonene. In the oil obtained from afternoon harvest, the predominant hydrocarbon monoterpene detected was limonene. Furthermore, seven (7) and six (6) oxygenated monoterpene 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 and Citral while the afternoon harvested oil were Geranial, Neral and Citral.

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;  $\beta$ -caryophyllene and  $\alpha$ -Farnesene. In the oil obtained from afternoon harvest, the major hydrocarbon sesquiterpenes detected was  $\beta$ -caryophyllene. The numbers of oxygenated sesquiterpenes constituents of leaf essential oil of *Citrus aurantifolia* obtained from the morning (7a.m) and afternoon harvest (2p.m) were a total of three (3) compounds each which represent 0.5 and 0.5% of the oil respectively. Oxygenated sesquiterpenes of appreciable

**Table 1. Volatile oil yield of fresh *Citrus aurantifolia* leaves**

Days of drying	Morning (%)v/w	Afternoon (%)v/w
Fresh	0.40	0.50

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

Compound <sup>a</sup>	KI <sup>b</sup>	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
β -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
<b>Total</b>		93.9	88.8	
Hydrocarbon		23.9	23.8	
Monoterpenes				
Oxygenated Monoterpenes		58.3	57.7	
Hydrocarbon		7.7	6.5	
Sesquiterpenes				
Oxygenated		0.5	0.5	
Sesquiterpenes				
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<sup>b</sup>-Kovat Indices on fused DB-5capillary column

KEY: Tr-Trace Amount (<0.1)

quantity detected in the oil obtained from morning and afternoon harvest was caryophyllene oxide while α-bisabolol and germacrene-D-4-β-ol occur in trace amounts.

Comparison of the composition patterns of the oils obtained in the morning and afternoon harvests reveal that there were both qualitative and quantitative variations in the constituents of the oils. This study revealed that; β-pinene,

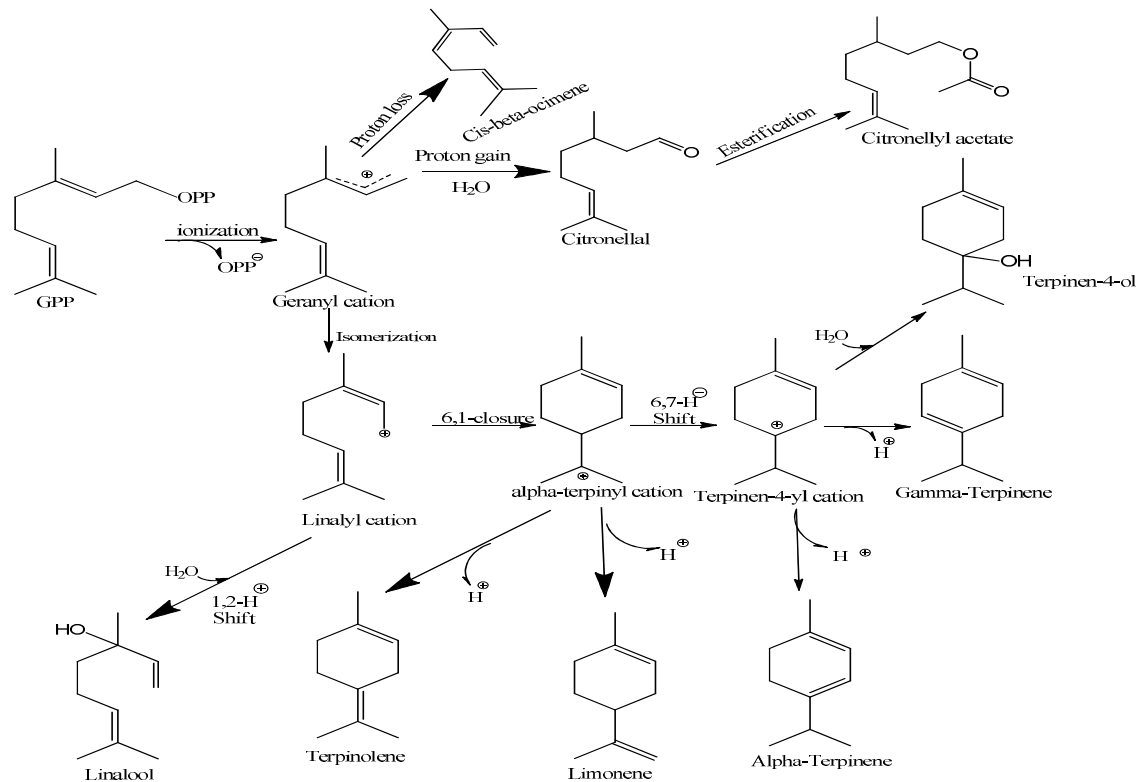
limonene, decanal, limonene epoxide, octadecanal, and phytol were all absent in the leaf essential oil from the morning (7am) harvest. Also, α-farnesene, α-elemene, linalool, citronellol acetate, isolimonene, ocimene, α-terpinolene and 5-hepten-2-one, 6-methyl were absent in leaf essential oil of *Citrus aurantifolia* obtained from afternoon (2pm) harvest. Quantitative variation also exists within the oil samples, for instance the percentage composition of Geranial was 4.4 and

14.2% in the leaf essential oil obtained from morning and afternoon harvests respectively; Citral was 21.5 and 10.3% in the leaf oil of morning and afternoon harvest respectively; Neral was 22.2 and 24.5% in the leaf essential oil obtained from morning and afternoon harvests respectively. The percentage composition of caryophyllene was 4.3 and 5.4% in the leaf oil of *Citrus aurantiifolia* obtained from morning and afternoon harvests respectively.

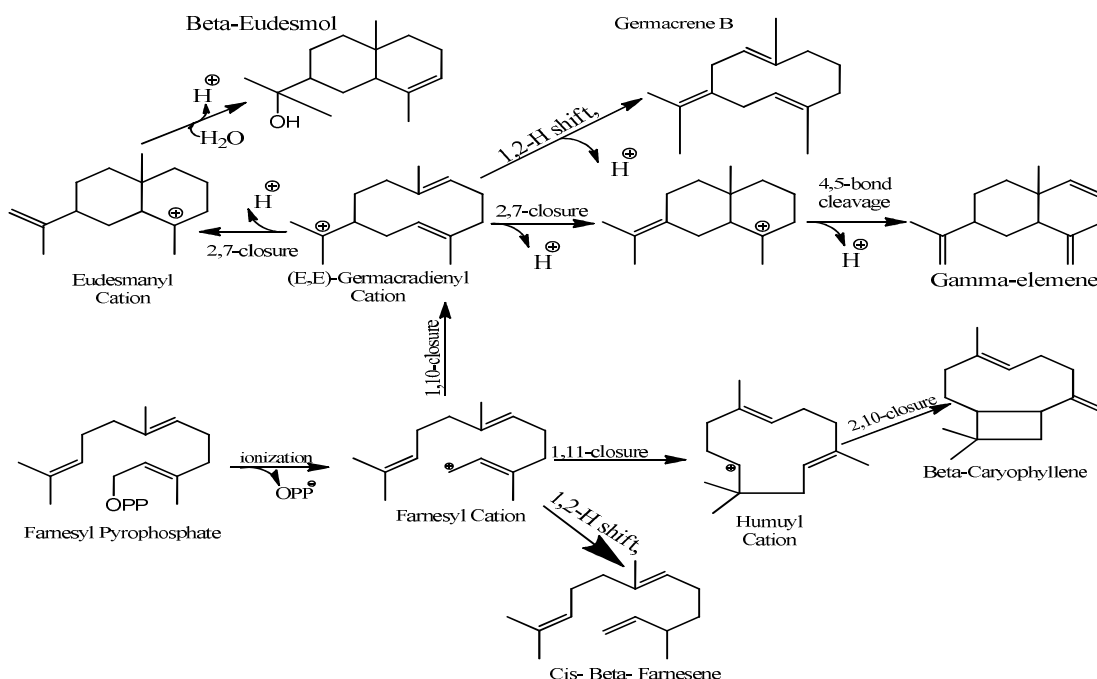
It has been established that the most active synthase catalyzes the formation of constituents in oil [18]. Thus, the most active mono- and sesquiterpenoid synthase facilitate the formation of monoterpenoids and sesquiterpenoids respectively in a plant by catalysing carbocationic reaction which converts the precursors to intermediate ion by the divalent metal ion-dependent ionization of the substrate [19-20]. The cationic intermediate undergoes a series of cyclizations, hydride shifts or other rearrangements which is terminated by deprotonation or the addition of a nucleophile to give various terpenic products [21]. Thus, the predominance of limonene, neral and citral in the oils implied that their synthase are very active

thereby mediate the formation of all monoterpenoids in the leaf oils (Scheme 1). Furthermore, the prevalence of Caryophyllene in the oils depict that its synthase facilitates the formation of all sesquiterpenoids in the oil (Scheme 2). The activity of these synthase most likely tends to determine the compositional profile of essential oil obtained from the leaf. Therefore, *Citrus aurantiifolia* leaf essential oils were of limonene/neral/ citral chemotype.

The phytochemical profile of the oil harvested in the morning (7am) and afternoon revealed the predominance of oxygenated terpenes which constituted 58.3 and 57.7% respectively. This corresponds to the report on leaf oil obtained from Ikotun, Lagos State, Nigeria [22]. Furthermore, the predominance of limonene in the leaf oils also correspond with existing literature data from earlier researches [22-24] such as the leaf oil from Italy which contained limonene,  $\beta$ -pinene,  $\gamma$ -terpinene, and citral as the major compounds [16,25]. 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.



**Scheme 1. Limonene/Neral/ Citral synthase-mediated biosynthesis of monoterpenoids**



**Scheme 2. Caryophyllene synthase-mediated biosynthesis of sesquiterpenoids**

The potential biological activity of *C. aurantifolia* essential oils reported by previous researchers may be ascribed to the activity of the major chemical constituents of these essential oils in a individualistic or synergistic mechanism and its lipophilicity to penetrate targeted cells [26-28]. Furthermore, the predominance of constituent such as limonene, neral and citral in *C. aurantifolia* leaf oil indicate its prospects to serve as a source of these bioactive compounds for industrial applications.

#### 4. 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.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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