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Comparison of the Chemical Composition of the Essential Oils from the Fresh and Dried Leaves of *Greenwayodendron suaveolens* (Engl. & Diels)

Oseyemi O. Olubomehin 1[1], Odunayo C. Atewolara-Odule 2[1], Abdulrazaq O. Ogunmoye 3[1]

[1]*Department of Chemical Sciences, Faculty of Science, Olabisi Onabanjo University, P.M.B. 2002, Ago-Iwoye, Ogun State, Nigeria*

Correspondence
Oseyemi Omowunmi Olubomehin, Department of Chemical Sciences, Faculty of Science, Olabisi Onabanjo University, P.M.B. 2002, Ago-Iwoye, Ogun State, Nigeria.
Email:olubomehin.oseyemi@oouagoiwoye.edu.ng

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Abstract:

Introduction: *Greenwayodendron suaveolens* (Engl. & Diels) is a tree with a straight regular trunk, dark bark as well as a small crown with horizontal branches. Its bark and roots are used for vermifuges, and aphrodisiac while the leaf is used as a pain-killer, and a cure for dysenteries and fevers.

Aims: This work sets out to identify and compare the essential oil constituents of the fresh and dried leaves of *Greenwayodendron suaveolens*.

Materials and Methods: *Greenwayodenron Suaveolens* leaves were collected at the biology farm of Olabisi Onabanjo University Ago-Iwoye, Ogun State and extracted by hydro-distillation using Clevenger apparatus. The qualitative and quantitative analyses of the wet and dried leaf essential oils were performed using Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS).

Results: From the GC-MS analysis, twenty-eight (28) compounds were identified in the fresh sample, while the dried sample had fourteen (14). The major components were: Caryophyllene, 20.85% and 22.13%; α-Zingiberene, 16.55% and 17.00%; Aromandendrene, 9.24% and 11.14%; Humulene, 9.08% and 9.92%; Caryophyllene oxide, 5.68% and 4.29% for the fresh and dried samples respectively. Curcumene, 20.89% occurred only in the dried sample.

Conclusion: This study presents the results of the essential oil composition of the fresh and dried leaf samples of *Greenwayodendron suaveolens* which will be useful in research. The presence of sesquiterpenes as the major components of both oils justifies the traditional use of the plant in treating pains, headaches and inflammations.

To Keywords: *Greenwayodendron suaveolens,* Clevenger apparatus, hydrodistillation, essential oil.

All co-authors agreed to have their names listed as authors.

1. INTRODUCTION

Greenwayodendron suaveolens (Engl. & Diels) Verdc, belongs to the Annonaceae family. It is widespread in West and Central Africa in such countries as Côte d'Ivoire, Gabon, Angola, Cameroon, Nigeria, Tanzania, Democratic Republic of Congo and Uganda [1]. *Greenwayodendron suaveolens* is a tree of the high-forest reaching to about a height of 20 m in Southern Nigeria [2] with some of its common vernacular names in Nigeria including 'ẹ́wáé' in Edo, 'Eleku' in Isekiri, and 'Agudugbu/Awuje' in Yoruba [3]. The dark bark and straight regular trunk of this tree, as well as the small crown with horizontal branches, make it easy to recognize. The wood is pale brown, fine-grained, of good quality used for furniture and construction work. It is often valued for firewood and is very important in pharmaceutics research because of the antifungal, bacteriostatic, and especially cytostatic capabilities of some chemical constituents of the leaves and bark [4, 5]. The fruit which has a very long fruiting season is green when immature, turns almost black when, ripe is widely consumed by many animals [6]. The roots, leaves and barks of this plant are used in traditional medicine for the treatment of fever, rheumatism pains, oedema, swollen glands, headache, stomach ache, constipation, hernia, facilitation of childbirth, fertility, anthelmintic, aphrodisiac and as ornamentals [7, 8]. In Cameroon and Gabon, the bark is applied to scarification for the treatment of malaria. In Democratic Republic of Congo, pounded bark is used in a mixture with other plants to make arrow poison while in Nigeria, the leaf has been recorded to be taken internally for menorrhagia; the fruit is equally edible [9]. Suaveolindole obtained from *Greenwayodendron suaveolens* was found to possess significant in vitro antibacterial activity against the Gram-positive bacteria; *Bacillus subtilis, Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus,* [10]. However, there has been no report of the essential components of the leaf and hence this research works sets out to do this.

2. MATERIALS AND METHODS

2.1 Plant materials

Fresh leaves (1 kg) of *Greenwayodendron suaveolens* were collected at Olabisi Onabanjo University, Ago-Iwoye. The plant was identified in the University's Department of Plant Science and Applied Zoology and authenticated at the herbarium of the Forestry Research Institute of Nigeria (FRIN), Ibadan by Mr. A. S. Odewo where a voucher specimen with FHI number 111865 was deposited.

2.2 Isolation of Essential Oils

The fresh (500 g) and dried (250 g) leaves were hydro-distilled in an all glass Clevenger-type apparatus for four hours (4 h) in each case. The oils obtained were dried over anhydrous sodium sulphate (Na_2SO_4), stored in vials and kept inside a refrigerator until ready for analysis.

2.3 Gas chromatography (GC)

The essential oils were subjected to GC analyses on an Agilent technology 7890 model with splitless mode and HP-5973 at 70eV with mass range m/z 40-420. The column was HP-5MS (30 x 0.32 mm, 0.25 µm film thickness) and the inlet pressure was 100.2 kPa. Helium was used as carrier gas at a flow rate of 1 mL/min. The GC oven temperature was programmed at 60°C (held for 0 min), heated to 140ºC at 3°C/min with a final hold time of 10 min at 280ºC. Injector and detector temperatures were fixed at 200°C and 250°C respectively.

2.4 Gas chromatography-mass spectrometry (GC-MS)

The GC-MS analyses were performed on an Agilent technology 7890 with splitless injector interfaced to a 5973 and 7683 mass selective detector operated at 70 eV with a mass range of m/z 40-450. The oven temperature was programmed from 60-280°C (hold for 5 minutes) at a rate of 3°C/min. The same operations and temperature programmings were used as for GC. FID chromatogram was used to calculate the relative percentage amounts of the separated compounds.

2.5 Identification of components

Identification of the essential oil components was based on their retention indices (determined with reference to a homologous series of n-alkanes) and by comparison of their mass spectral fragmentation patterns in computer matching against in-built data (NIST database/ Chemstation data system) with data previously reported in the literature [11] and the use of pherobase.

3. RESULTS

From Table I, a total of twenty-eight (28) compounds were detected representing 98.02% of the essential oil corresponding to 67.73% sesquiterpenes, 13.79% oxygenated sesquiterpenes, 6.21% terpenes, 3.02% heterocyclic compounds, 5.79% hydrocarbons and 1.48% esters. From Table II, a total of fourteen (14) compounds were detected representing 100% essential oil composition comprising 88.90% Sesquiterpenes, 8.32% Oxygenated sesquiterpenes, 1.80% Terpenes and 0.98% Hydrocarbons.

Table I: Chemical composition of essential oils of the fresh leaves of *Greenwayodendron suaveolens*

Compd No.	RT Min	RI	Identified Compounds	% Composition	Molecular Formula	MS [a,b]
1.	3.304	1040	β.-Ocimene	3.48	$C_{10}H_{16}$	93[a],41,53,77,79, 91,105,121,136[b]
2.	6.257	1280	(-)-Bornyl acetate	0.66	$C_{12}H_{20}O_2$	95[a],43,93,121,136 ,154,196[b]
3.	7.264	NA	Acetic Acid,1,7,7-trimethyl-bicyclo [2.2.1]hept-2-ylester	0.82	$C_{12}H_{20}O_2$	95[a].43,93,108,121 ,136,154,196[b]
4.	9.690	1451	Caryophyllene	20.85	$C_{15}H_{24}$	93[a],41,55,69,79, 91,106,120,147, 161,189,204[b]
5.	9.816	1428	α-Bergamotene	1.38	$C_{15}H_{24}$	93[a],41,55,69,77, 91,107,161,204 [b]
6.	10.211	1456	Humulene	9.08	$C_{15}H_{24}$	93[a],41,80,121,147 ,204 [b]
7.	10.354	1440	Aromandendrene	9.24	$C_{15}H_{24}$	41[a],55,69,79,91, 105,119,133,147, 161,189,204[b]
8.	10.508	1473	γ,-Muurolene	0.73	$C_{15}H_{24}$	161[a],41,55,69,79,93 105,119,133, 189,204 [b]
9.	10.633	1439	(E)-β-Famesene	3.82	$C_{15}H_{24}$	41[a],69,93,120,133 ,161,204[b]
10.	10.726	1464	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4aα,7α,8aβ)]-	2.06	$C_{15}H_{24}$	41[a],55,67,81,93,105 121,133,147,161, 189,204 [b]
11.	10.914	1493	α-Zingiberene	16.55	$C_{15}H_{24}$	93[a],41,69,119,161 ,204[b]
12.	11.029	1519	β-Bisabolene	0.40	$C_{15}H_{24}$	69[a],41,93,109,161 ,204 [b]
13.	11.281	1530	β-Sesquiphellandrene	1.50	$C_{15}H_{24}$	69[a],41,55,71,93, 109,120,133,161, 204 [b]
14.	11.750	NA	Patchoulane	0.58	$C_{15}H_{26}$	41[a],29,55,67,79, 91,107,121,206 [b]
15.	12.099	NA	Cytosine	1.46	$C_4H_5N_3O$	41[a],69,83,111[b]
16.	12.288	1579	Caryophyllene Oxide	5.68	$C_{15}H_{24}O$	43[a],41,55,69,79,93, 109,121,220[b]
17.	12.591	NA	Bicyclo[3.1.1]heptane 2,6,6-trimethyl-,[IR-(1.α.,2.β.,5 α.)]-	2.61	$C_{10}H_{18}$	55[a],41,67,69,82,96 ,123,138[b]
18.	12.677	1605	Humulene epoxide II	1.36	$C_{15}H_{24}O$	109[a],43,55,67,96, 123,138,220[b]
19.	12.768	1611	Zingiberenol	4.81	$C_{15}H_{26}O$	69[a],41,93,119,161 ,204,222[b]
20.	13.083	NA	Oxirane, 3-butenyl	1.56	$C_6H_{10}O$	67[a],31,41,54,68,79 ,83,98 [b]
21.	13.152	998	2-Carene	1.92	$C_{10}H_{16}$	93[a],79,91,105,121 ,136[b]
22.	13.398	NA	Neointermedeol	1.46	$C_{15}H_{26}O$	43[a],67,71,81,136, 161,189,204,222[b]
23.	13.455	NA	9-(1-Methylethylidene)-bicyclo[6.1.0]nonane	1.69	$C_{12}H_{20}$	41[a],55,67,79,81, 93,107,121,136, 148, 164 [b]
24.	13.558	NA	(E)-2,5-Dimethyl-1,6-octadiene	0.50	$C_{10}H_{18}$	41[a],55,69,82,109, 138[b]
25.	13.655	1036	Z-Ocimene	0.81	$C_{10}H_{16}$	93[a],41,53,79,105,

						121,136 [b]
26.	13.764	1680	α-Bisabolol	0.48	$C_{15}H_{26}O$	43[a],69,95,109,119 ,161,204 [b]
27.	13.850	NA	γ-Curcumene	1.55	$C_{15}H_{24}$	119,93,105,134, 161,204 [b]
28.	18.033	NA	Cyclohexane,1,5-diethenyl-2,3-dimethyl- ,(1.alpha.,2.beta.,3.beta.,5 .beta.)-	0.99	$C_{12}H_{20}$	107[a],41,55,67,79, 93,121,136,149, 160[b]

Key:RT- Retention time in minutes, RI-Kovat values from literature, NA-Kovat values from literature not available,
MS [ab]a=base peak,b=Molecular ion peak.

Table II: chemical composition of essential oils of the dried leaves of *Greenwayodendron suaveolens*

Compd No.	RT Min	RI	Identified Compounds	% Composition	Molecular Formula	MS [a,b]
1.	9.679	1451	Caryophyllene	22.13	$C_{15}H_{24}$	93[a],41,69,79,105,120,133,147,161,175,189,204[b]
2.	9.833	1428	α-Bergamotene	3.49	$C_{15}H_{24}$	93[a],41,55,69,77,91,107,119,133, 147,161,189,204[b]
3.	10.205	NA	Humulene	9.92	$C_{15}H_{24}$	93[a],41,69,77,80,107,121, 147,204[b]
4.	10.348	1438	Aromadendrene	11.14	$C_{15}H_{24}$	41[a],55,69,79,91,105,119, 133,147,161,189,204[b]
5.	10.737	1485	Curcumene	20.89	$C_{15}H_{22}$	119[a],41,56,105,132,145,202[b]
6.	10.920	1493	α-Zingiberene	17.00	$C_{15}H_{24}$	93[a],41,69,77,105,119, 161,204[b]
7.	11.046	1506	β.-Bisabolene	1.12	$C_{15}H_{24}$	69[a],41,93,109,119,135, 161,204[b]
8.	11.292	1526	β-Sesquiphell-andrene	1.86	$C_{15}H_{24}$	69 [a],41,55,77,93,109,120, 133,161,204[b]
9.	12.288	1592	Caryophyllene oxide	4.29	$C_{15}H_{24}O$	43[a],41,55,69,79,93,109, 121,220[b]
10	12.591	NA	Bicyclo[3.1.1]heptane 2,6,6-trimethyl-,[IR-(1.α.,2. β.,5 α.)]-	1.80	$C_{10}H_{18}$	55[a],41,67,69,82,96,123,138[b]
11	12.768	1611	zingiberenol	2.81	$C_{15}H_{26}O$	69[a],41,55,93,119,137,161, 204, 222[b]
12	13.449	1470	9-epi-(E)-Caryophyllene	1.35	$C_{15}H_{24}$	91[a],41,55,69,79,105,119,133,147,161,189,204[b]
13.	13.850	NA	7-epi-trans-sesquisabinene hydrate	1.22	$C_{15}H_{26}O$	119 [a],41,55,69,82,93,105, 161,204,223[b]
14.	13.970	1580	4,8, 12-trimethyltrideca-1,7,11-tetraene	0.98	$C_{16}H_{26}$	69 [a],41,81,95, 107,218 [b]

Key:RT- Retention time in minutes, RI-Kovat values from literature, NA-Kovat values from literature not available,
MS [ab]a=base peak,b=Molecular ion peak

4. DISCUSSION

Major compounds found in the essential oil samples include: Caryophyllene, 20.85% and 22.13%; α-Zingiberene, 16.55% and 17.00%; Aromandendrene, 9.24% and 11.14%; Humulene, 9.08% and 9.92%; Caryophyllene oxide, 5.68% and 4.29% in the fresh and dried oils respectively, while Curcumene, 20.89% was found in the dried sample only. Trace amounts of hydrocarbons; 5.79% and 0.98% and β- bisabolene; 0.40% and 1.12% were found in the fresh and dried samples while the fresh sample had a small amount of heterocyclic compounds (3.02%) and esters (1.48%) which were absent in the dried sample. Caryophyllene which is the major constituent of both essential oils has been found to be present in large amounts in the essential oils of many different spice and food plants, such as oregano, thyme, rosemary, cinnamon, curry and black pepper [12]. Analysis of *Artemisia annua* growing wild in Bulgaria shows that its essential oil contains 24.70% of caryophyllene [13], Curry leaves contain up to 35.00% [12], *Cannabis sativa* has up to 35.00% [14]. It is a dietary phytocannabinoid approved by US-FDA which improves renal functions, lowers the nephrotoxictiy of molecules like cisplatin and reduces creatinine [15]. Caryophyllene binds to the CB2 receptor in peripheral tissues and as a non psychoactive CB2 receptor, it modulates immune responses, inhibit inflammation and oedema formation and exhibit antinociceptive effects, also it is a potential target for the treatment of atherosclerosis and osteoporosis [14]. Aromandendrene which is another major constituent has been found as one of the major constituents of *Eucalyptus microtheca* leaf oil (aromadendrene, 18.31%), from Semnan province [16], and in *Eucalyptus sargentii* (aromadendrene, 6.45%), from Isfahan province [17]. Humulene, a constituent of both oils known to possess analgesic, anti-inflammatory, appetite depressant, and anti-cancer properties, have also been found to be present in the essential oils of aromatic plants such as *Salvia officinalis* [18], *Lindera strychnifolia* [19], up to 29.9% of the essential oils of *Mentha spicata* [20], 10.00% of the leaf oil of *Litsea mushaensis* [21], and (31.80 and 12.90%) in the leaves and stem, respectively of *Eclipta prostrata* [22]. The dried essential oil of *G. suaveolens* contains 20.89% of curcumene which is one of the main constituents of turmeric root responsible for its anti-inflammatory properties.

4. CONCLUSION

This study provides information on the essential oil constituents of the fresh and dried leaves of *Greenwayondendron suaveolens* which has been scarce in literature. The presence of the sesquiterpenes as the major components of the fresh and dried oils justifies the traditional use of the plants in treating pains, headaches and inflammations.

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COMPETING INTERESTS

“Authors have declared that no competing interests exist.”

AUTHORS’ CONTRIBUTIONS

This work was carried out in collaboration among all Authors: ‘Author OO’ designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. ‘Author OC’ managed the analyses of the study. ‘Author AO’ managed the literature searches and type setting. ‘All authors read and approved the final manuscript.

CONSENT

Not Applicable

ETHICAL APPROVAL

Not Applicable

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