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Chemical Compositions of *Dialium guineense* Willd. Leaf, Stem-bark and Fruit Essential Oils

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Authors' contributions

This work was carried out in collaboration between all authors. Author DOM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author OFK supervised the GC, GC-MS analyses. Authors DOM and OO managed the analyses of the study. Author CO and other authors' managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: We report chemical composition of three essential oils from leaf, stem bark and fruit parts of *Dialium guineense* [Fabaceae], which we studied. *D. guineense* is a Nigerian fruity plant utilized widely in treatment of jaundice, ulcer, eye and heart problems, as antimicrobial and anti-malaria in ethno-medicine, also as dietary supplement. Its fruits, which are edible, possess sweet to sour astringent flavorings enjoyed in beverages, for quenching thirst and refreshing.
Methodology: The volatile essential oils were obtained by hydro-distillation, using an all-glass apparatus adapted to British Pharmacopeia specifications and gave good yields of 0.06 to 0.10%, which we studied using GC and GC-MS.
Results: Twenty out of twenty-five compounds, representing 92.91% of the leaf oil were characterized, while eighteen and thirteen out of twenty-one and eighteen compounds were

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identified in stem-bark and fruit oils, representing 85.65% and 81.44% of each respectively. Most abundant compounds were cis-3-hexenyl butanoate in leaf, and trans-δ-9-octadecenoic acid in both stem-bark, and fruit essential oils.
Dominant classes of compounds (%) in leaf oil were esters (56.86), terpenoids (18.27) and acids (11.20); stem bark had mostly acids (57.41), esters (19.82) and terpenoids (3.05); most abundant in fruit oil were acids (65.79), hydrocarbons (8.56) and esters (3.42). Acids and esters dominated the 3 essential oils of *D. guineense*, with appreciable amounts of terpenoids and hydrocarbons in the three oils.
Conclusion: We present chemical compositions of three essential oils of *D. guineense* Willd a Fabaceae, which has not been reported in literature. Presence of high oxygenated compounds (esters, acids, alcohols and terpenoids) in the three oils, are important quality determining factor, as well as responsible for flavoring and pharmacological properties, some of these have been highlighted here. Results of our study reveal the rich chemical compositions of *D. guineense* essential oils, which have long been utilized for traditional purposes.

Keywords: Essential oils; hydro-distillation; Dialium guineense; Fabaceae; GC and GC-MS; esters; acids; terpenoids.

1. INTRODUCTION

Dialium guineense Willd., called black velvet tamarind, is a perennial flowering plant common in temperate and tropical areas, and grows in dense savannah forests, shadowy canyons and gallery forests. It naturally grows wild in bushes and forests on moist, sometimes brackish soils across Nigeria and West Africa. It is found from Senegal to Sudan along the southern border of the Sahel. In Ghana, it is seen along transition zones bordering high forest, in riverine forest of the savannah woodland, in coastal scrub, and in riparian vegetation [1]. The genus Dialium is in the family Leguminosae (Fabaceae). Its common names are “West African Velvet Tamarind”, “black velvet”, “velvet tamarind”, “tamarinier noir”, “kedebe”, “mako”, “meko”, “mekohi”, “yoyi”, “icheku”, “Tsamiyar Kurm”, “kosito”, “solam”, “solom”; In Nigeria it is called “awin” in Yoruba land [2,3].

D. guineense is an evergreen tree reaching up to 30 m in height; with densely leafy crown, which is shrubby with smooth grey bark. Its composite leaves are 5-13 cm long, with odd terminal leaflet usually two pairs of opposite or alternate leaflets. The lower pair is smaller. Its fruits are usually abundant, more or less circular and flattened, but sometimes almost globose, up to 2.5 cm in diameter, and is densely velvety and black- which may be responsible for its common names [1]. In Nigeria the tree flowers between September to October, and fruits from October to January [4]. Ripe fruits are available from January till May; but the peak period for harvest is between March and April [5].

The fruit when ripe is red, with a sweet-sour, astringent flavour similar to baobab, but sweeter. It is peeled and eaten raw. The thirst quenching, refreshing fruit pulp can also be soaked in water and drunk as a beverage. Leaves are edible but bitter. The wood is hard, durable, heavy, light brown, with a fine texture, hence used in constructions of vehicles, houses and flooring and also as firewood and charcoal [1]. The edible pulp of the fruit is a source of protein, minerals and ascorbic acid [6], which are chewed among some women in Southeast Nigeria to improve lactation and check genital infections [7]. Proximate and elemental analyses of fruit (pulp) of *D. guineese* shows the overall safety and health benefit of the plant [8], fruits are also dietary supplement for rural dwellers in Nigeria during dry season when fruits are scarce [9]. *D. guineese* with its various parts have ethno-medicinal properties, which are used against various diseases and ailments. Bark and leaves are utilized to cure stomach and pulmonary troubles, malnutrition, malaria fever, jaundice, ulcer, eye and heart treatment [8,10].

Results of antioxidant and antimicrobial studies, as well as molluscidal activities of the plant substantiate its popular and wide traditional applications in treatment of diverse ailments especially in the management of those caused by Vibrio spp. [11,12,13].

The phytochemical analyses of the leaf and bark of the plant showed presence of flavonoids, alkaloids, tannins and saponins at various concentrations while glycoside and phenol were conspicuously absent in the fruit [14,15].

We present results of our studies on compositions of volatile oil of the 'West African Velvet Tamarind' leaf, stem-bark and fruit, for the first time in literature.

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh growing samples of *Dialium guineense* Willd were collected from Ago-Iwoye, Ogun-State, Nigeria. The plants were authenticated at the herbarium, Department of Botany, University of Ibadan, Ibadan, where samples were deposited with voucher number UIH-22600.

2.2 Isolation of Essential Oils

The plant was separated into leaf, stem-bark, and fruit. Each part [300 g to 500 g] was crushed and hydro-distilled for 2.5 hours in an all glass Clevenger-type apparatus designed to British Pharmacopeia specifications, with 0.5 mL of distilled n-hexane (which was later removed by the analytical equipment) and the oils samples were stored in a refrigerator for the GC analyses. The essential oils were procured in 0.06 to 0.10% yields. Each of the oils had distinct characteristic pleasant smell.

2.3 Gas Chromatography [GC]

Each of the essential oils was subjected to GC analyses on GC-2010 [AOC-20i] gas chromatograph with column details [HP 5 ms 30 m length, 0.25 mm ID, 0.25 µm film thickness]. Column oven temperature was 60℃, injection temperature of 250℃, split injection mode, at 100.2 kPa; column flow of 1.61 mL/min and total flow of 6.2 mL/min; 1.0 split ratio; oven temperature programming was 60℃ (for 5 mins), and at the rate of 5^{o} /min till 140℃, 15°/min till 280℃.

2.4 Gas Chromatography-mass Spectrometry [GC-MS]

The GC-MS analyses were performed on GC-MS QP2010 Plus. Ion source temperature 200℃; interface temperature 250℃; solvent cut time 2.5 min; with relative detector gain mode and threshold 300°; scan MS ACQ mode; detector FTD; mass range of m/z 40-400.

2.5 Identification of Compounds

Identification of the essential oil compounds were based on their retention indices (determined with reference to homologous series of n-alkanes), along with comparison of their mass spectral fragmentation patterns by computer matching against in-built data, as well as in-house "Başer Library of Essential Oil Constituents" built up by genuine compounds and components of known oils, also on commercials like Adams 2007 [16]; Joulain and Koenig, 1998 [17].

3. RESULTS AND DISCUSSION

Each of the essential oils was procured by hydro-distillation from leaf, stem-bark and fruit of fresh *D. guineense* and was clear in appearance, with distinct characteristic odours. Oils were obtained in good yields of (w/w) 0.06% (leaf), 0.10% (stem-bark) and 0.06% (fruit) (Table 1). The three oils were analyzed using GC and GC– MS, with the identified compounds in each listed in Table 2.

Gas chromatograms revealed presence of twenty-five, twenty-one and eighteen compounds, which made-up the essential oils of leaf, stem-bark and fruits respectively. In the leaf oil, twenty out of twenty-five compounds, which constitute 92.91% of the leaf volatile oil, were identified and most abundant compound was cis-3-hexenylbutanoate. Its other nineteen compounds are shown in Table 2. The leaf essential oil consisted of 56.86% esters, 18.27% terpenoids, 11.2% acids, 3.34% hydrocarbons, 2.97% alcohols, with 0.27% sulfurate (Table 3). Five compounds representing 7.09% of leaf oil are yet to be identified.

Eighteen compounds were identified in the stem bark oil with trans-δ-9-octadecenoic acid (30.00%) as the most abundant compound (Table 2). The oil had 57.41% acids, 19.82% esters, 3.05% terpenoids, 2.38% alcohols, 1.45% hydrocarbon, 1.28% sulfurate and 0.26% ketone (Table 3). The three unidentified compounds constitute 14.33% of the stem oil.

Fruit oil contained eighteen compounds, the thirteen identified accounted for 81.44% of them, the most abundant being trans-δ-9-octadecenoic acid (32.70%). The five unidentified compounds represent 18.56% of the fruit oil. Classes of compounds in the fruit oil were 65.79% acids, 8.56% hydrocarbons, 3.42% esters, 1.41% aldehydes, 1.08% terpenoids, 0.67% sulfurate and 0.51% ketone (Table 3). Thirty-six compounds have been identified in the three oils.

The leaf, stem-bark and fruit oils have in common the following classes of compounds (%) respectively: Esters (56.86, 19.82, 3.42), acids (11.20, 57.41, 65.79) Terpenoids (18.27, 3.05,

1.08), hydrocarbons (3.34, 1.45, 8.56) and sulfurates (0.27, 1.28, 0.67). Alcohols are (2.97, 2.38) in leaf and stem oils respectively. Ketones are (0.26, 0.51) in stem and fruit oils respectively. Aldehyde (1.41) is only found in fruit oil (Table 3). Compounds found and common to the three oils are methylhexadecanoate (9.13, 3.56, 2.24), hexadecanoic acid (7.70, 16.53, 23.29) and octadecanoic acid (3.50, 10.88, 8.76). The sulfurates are also present in the three oils.

The presence in significant amounts of oxygenated compounds (esters, acids, alcohols and terpenoids) in these oils is an important quality-determining factor. They are known to have important flavoring and pharmacological properties, as well as being precursors in the acetate and shikimate biosynthesis of important metabolites [18].

Camphor has anti-inflammatory effect; it is an important essential oil component in lavandin, which is naturally utilized as a disinfectant, in household detergents. It has been reported for its ability to suppress sprouting in potato tubers during storage. Infusion of the whole plant is used against flatulence and digestive problems [19,20].

Linalool is known for treating rheumatism [19,21,22]; It is also used for indigestion and possesses emmenagogue properties [19,23, 24,25].

Natural products (*Melaleuca quinquenervia*) containing significant amount of nerolidol are known to be active as emmenagogues and stimulants. They are also used to treat skin infections, fever and pulmonary problems. They are effective antidotes against fish poisoning and are useful in boosting the immune system in case of chronic diseases such as cystits and glandular fever [19,26,27,28].

Hexadecanoic acid is in significant amount in lime (*Triphasia trifolia*) essential oil, leaf of which is utilized against skin diseases, diarrhea and colic. Cataplasm of fresh leaves is used against dandruff eruption [19,29].

Details on the mass spectrum (MS) and calculated RI of each unidentified compound are in Table 2. Our study reveals the chemical compositions of the essential oils and supports the vast uses of *D. guineense* in ethno-medicine. It is the first time essential oil composition of *Dialium guineense* Willd is reported.

Table 1. Essential oils procured from leaf, stem-bark and fruit of *Dialium guineense* Willd

Plant part	Weight of sample (g)	Weight of essential oil procured (g)	% Yield of essential oil procured	Physical examination of essential oil
Leaf	320	0.2	0.06	Acceptable leafy odour
Stem-bark	315	0.3	0.10	Sweet woody odour
Fruit	500	0.3	0.06	Pleasant fruity odour

Table 2. Chemical composition of essential oils from leaf, stem-bark and fruit of *D. guineense*

S/N	Identified compounds[a]	Calculated RI[b]	% TIC[c] in			Class of organic compound
			Leaf	Stem-bark	Fruit	
1	(4E)-4-hexenylacetate	784	3.50			Ester
2	2-ethylhexanol	1062	2.97			Alcohol
3	Nonanal	1117			0.67	Aldehyde
4	β-linalool	1119	4.14			Terpenoid
5	DL-camphor	1145			0.52	Terpenoid
6	Hexahydrothymol	1484			0.56	Terpenoid
7	Cis-3-hexenyl butanoate	1492	16.55			Ester
8	Decanal	1504			0.74	Aldehyde
9	Cis-3-hexenyl pentanoate	1526	1.17			Ester
10	4E-4-hexenyl hexanoate	1951	1.37			Ester
11	Copaene	1957	0.69			Terpenoid
12	2,3-heptanedione	1971		0.26		Ketone
13	Ethylcyclohexanone	1991			0.51	Ketone
14	(+)-trans-nerolidol	2355	3.40			Terpenoid
15	2,6-dimethyl-1,5-heptadiene	2361	1.42			Hydrocarbon

S/N	Identified compounds[a]	Calculated RI[b]	% TIC[c] in			Class of organic compound
			Leaf	Stem-bark	Fruit	
16	1,1,3-trimethylcyclopentane	2367		0.51		Hydrocarbon
17	2-ethylhexyl isohexyl sulfurate	2370		0.59	0.67	Sulfurate
18	Epiglobulol	2381	0.62			Terpenoid
19	tetradecanoic acid	2403			1.04	Acid
20	9E-9-icosene	2413		0.94		Hydrocarbon
21	2-ethylhexylhexylsulfurate	2415	0.27	0.69		Sulfurate
22	Diisobutylphthalate	2421	7.00		1.18	Ester
23	Methylhexadecanoate	2748	9.13	3.56	2.24	Ester
24	Hexadecanoic acid	2754	7.70	16.53	23.29	Acid
25	Nonyldichloroacetate	2762		0.57		Ester
	Ui[d e] (57,71,85,43,99,41,55,113, 42,56,70,69)	2764	1.05			
26	Methyl linoleate	2776	9.42	3.05		Terpenoid
27	Methyl trans-9-octadecenoate	2778	15.18	7.69		Ester
28	Methyl n-octadecanoate	2780	1.85	0.72		Ester
29	Trans-δ-9-octadecenoic acid	2784		30.00	32.70	Acid
30	Octadecanoic acid	2787	3.50	10.88	8.76	Acid
	Ui[d e] (93,105,57,85,71,43,41, 81,96)	2795		5.70		
31	n-heneicosane	2796	1.92		8.56	Hydrocarbon
32	Methyl-2-ethyl-2-methylicosanoate	2806		3.72		Ester
	Ui[d e] (57,71,85,43,99,41,113, 55,69,70)	2810	1.55		5.67	
	Ui[d e] (57,71,85,43,99,41,113, 55,69,56,70)	2823			2.05	
	Ui[d e] (57,71,85,43,99,113,41,55, 127,69,83,97,56,70)	2824	1.30			
	Ui[d e] (111,107,80,94,129,55, 105,110,81,95,57)	2832		6.15		
33	2,4-diethyl-1-heptanol	2836		2.38		Alcohol
	Ui[d e] (277,77,278,199,51,281, 56,95,41)	2838		2.48		
	Ui[d e] (57,71,85,43,99,41,113, 55)	2839	1.00		6.24	
34	di-n-octyl phthalate	2842	1.11	2.21		Ester
	Ui[d e] (57,71,85,43,99,83,55,82,4 1,69,82,113,97)	3156			3.42	
35	Dodec-9-ynyl trichloroacetate	3165		0.84		Ester

S/N	Identified compounds[a]	Calculated RI[b]	% TIC[c] in			Class of organic compound
			Leaf	Stem-bark	Fruit	
	Ui[d e] (57,71,85,43,99,113,83, 55,69,97,41,82)	3176			1.18	
36	Methyl-15-methylhexadecanoate	3179		0.51		Ester
	Ui[d e] (57,71,85,43,99,113,55, 69,83,97,41)	3228	2.19			
	Total (No) & % Identified		(20) 92.91%	(18) 85.65%	(13) 81.44%	
	(No) & % UI with fragmentations in MS		(5) 07.09%	(3) 14.33%	(5) 18.56%	

[a]See identification of compounds; [b]Retention Index (RI) calculated and determined with reference to homologous series of n-alkanes; [c]Total ion concentration in %; [d]Unidentified compound (Ui) with [e][m/e] values of base peak 1st stated, and other prominent ions in its mass spectrum (MS)

Table 3. Classes of compounds in leaf, stem bark and fruit of *D. guineense* essential oils

Plant part	Esters %	Acids %	Hydrocarbons %	Terpenoids %	Alcohols %	Aldehydes %	Ketones %	Sulfurates %	Total %
Leaf	56.86	11.20	3.34	18.27	2.97	-	-	0.27	92.91
Stem-bark	19.82	57.41	1.45	3.05	2.38	-	0.26	1.28	85.65
Fruit	3.42	65.79	8.56	1.08	-	1.41	0.51	0.67	81.44

4. CONCLUSION

The three essential oils procured in good yields (w/w) 0.06 to 0.10% by hydro-distillation from fresh *Dialium guineense* Willd., were clear in appearance, with distinct characteristic odours. Their GC, GC–MS analyses revealed thirty-six identified compounds in the three oils, where each of the oils had distinct characteristic chemical compositions different from the others. In the leaf oil, the 20 compounds identified constituted 92.91% of the oil, with the most abundant being cis-3-hexenylbutanoate. Eighteen (85.65%) out of twenty-one compounds were identified in the stem-bark oil, while in the fruit oil, thirteen of the eighteen compounds were identified, which accounted for 81.44% of the oil. Both stem and fruit oils had trans-δ-9-octadecenoic acid (30.00, 32.70% respectively) as the most abundant compound in them.

Classes of compounds (%) common to the leaf, stem-bark and fruit oils respectively were esters (56.86, 19.82, 3.42), acids (11.20, 57.41, 65.79), terpenoids (18.27, 3.05, 1.08), hydrocarbons (3.34, 1.45, 8.56) and sulfurates (0.27, 1.28, 0.67).

Presence of high amount of oxygenated compounds in the oils is important in determining quality, flavoring and pharmacological properties of the oil. This supports the many traditional applications of *Dialium guineense*.

This study presents the essential oil composition of leaf, stem-bark and fruits of *Dialium guineense* Willd for the first time.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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