A COMPARATIVE ANALYSIS OF THE VOLATILE CONSTITUENTS FROM THE AIR-DRIED AND FRESH LEAVES OF *Baphia nitida* (LODD.) OBTAINED IN NIGERIA

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Abstract: The volatile constituents of the air-dried and fresh leaves (500 g) each of *Baphia nitida* grown in Nigeria was obtained by hydrodistillation using an all glass Clevenger-type apparatus. The extracted oils were analyzed using a gas chromatography coupled with mass spectrometry (GC-MS). The essential oils obtained were colourless with an herbal smell and a yield of 0.29% (v/w) in both cases. A total of sixteen constituents representing 99.99% of *B. nitida* oil were obtained from the air-dried leaves while six constituents representing 100% was gotten from the fresh leaves. Oleic acid (54.69%) was the main constituent of the air-dried leaves essential oil, followed by 2-Propanoic acid-3-[4-[[4-methoxyphenyl)methylene]amino]phenyl]-ethyl ester (17.65%), 2-Hydroyethyl Oleate (5.86%) and Palmitoleic acid (5.29%). The fresh leaves had methyl palmitate (33.63%) as the major constituent while methyl stearate (3.83%) was the least. Carboxylic acids (68.40%), Esters (26.99%), Hydrocarbons (3.04%) and aldehyde (1.56%) were the common classes in the air-dried leaves while Monoterpenoids (37.27%) and Esters (62.73%) were those in the fresh leaves. Oleic acid and methyl palmitate present as the major constituents in the oils justifies the traditional use of the plant against parasitic skin disease and as an anti-inflammatory agent.

Keywords: *Baphia nitida*, essential oil, Fabaceae, hydrodistillation, methyl palmitate

Introduction

The genus Baphia includes about 45 species, and they are commonly distributed globally especially within the coastal region of West Africa (Olowosulu and Ibrahim, 2006; Kato *et al.*, 2008). *Baphia nitida* Lodd., a member of the Fabaceae family is a leguminous, shrubby, hard wooded tree species found in the rain and secondary forests and on abandoned farmland from sea-level up to 600 m altitude (Omobuwajo *et al.*, 1992; Olowosulu and Ibrahim, 2006; Onyekwere *et al.*, 2014). It is commonly called African sandalwood, barwood, camwood while the Yoruba people of Nigeria call it Osun (Njoku *et al.*, 2014). It is well distributed in the eastern and western parts of Nigeria, especially in the villages where they are usually planted for provision of shade, ornamental purposes and as source of medicines and dye (Chibuisi *et al.*, 2015; Oli *et al.*, 2017). High quality red dye used to dye raffia and cotton textiles are derived from the bark and hardwood of the plant. *Baphia nitida* is a fast-growing smallish tree that can be as tall as 5 m. Leaves are simple, alternately arranged, oblong-elliptic, 10-15 cm long and strongly tipped. Flowers are bisexual, white, solitary or up to 4 grouped together on the main branch, faintly fragrant and inconspicuous (Wee, 1990).

The young leaves of *Baphia nitida* are used as vegetables, fodder for goats, the twigs and small branches are popularly used as chewing sticks while the pulp is sweet and silky (Soladoye, 1985; Etukudo, 2003). The plant has also been investigated for its medicinal activities. These include anti-inflammatory effects, antidiarrheal activities, analgesic activity, antimicrobial, antidiabetic, antibacterial, antifungal, anti-depressant and antioxidant activity (Opakunle, 1988; Poorter *et al.*, 2004; Olowosulu and Ibrahim, 2006; Adeyemi and Akindele, 2008; Akande, 2011; Agwa *et al.*, 2012). *Baphia nitida* produces a red gum which is very much used in folk medicine for treating various pathologies, including skin disorders, skin wounds and infected umbilical cords (Onwukaeme and Lot, 1991).

Furthermore, the leaves/bark of the plant have been used to cure enteritis, gastrointestinal problem, venereal diseases, asthma, arthritis, nosebleed, female sterility, painful menstruation, jaundice, constipation, parasitic skin diseases, bone fractures, sores, wounds, epilepsy, cardiac pain, joint pain, rheumatic complaints, diarrhea, anaemia, headache, ulcers and boils (Onwukaeme and Lot, 1992; Onwukaeme, 1995; Odugbemi and Akinsulire, 2006; Adeyemi *et al.*, 2006; Akande *et al.*, 2011; Konkon *et al.*, 2011; Mbanchta *et al.*, 2011; Agwa *et al.*, 2012: Okon *et al.*, 2013).

In addition, some of the phytochemicals identified from *B. nitida* leaves extracts includes; Baphianoside (Onyekwere *et al.*, 2014), isoflavonoids known as medicarpin and sativan (Arnone *et al.*, 1981; Omobuwajo *et al.*, 1992), tannins, flavonoids, terpenoids and saponins, glycosides, alkaloids, anthraquinones, cardiac glycosides and phlobatanins (Omobuwajo *et al.*, 1992; Onwukaeme, 1995; Kato *et al.*, 2008; Agwa *et al.*, 2012; Akinjogunla *et al.*, 2018; Ndukwe *et al.*, 2020). Also identified are 16-β-(β-D-gluco pyranosyl) lanost-1,5,11,15-tetraene-3–yl6-O-(3,4,5-trimethoxy cyclo hexanoyl)-βD-gluco pyranoside, 1-deoxynojirimycin (DNJ), 3-O-β-D-gluco pyranosyl-(DNJ), 6-O-β-D-gluco pyranosyl-(DNJ), 1-deoxy mannojirimycin, 1-deoxy allonojirimycin, 3-epi-fagomine, 2R,5R-dihydroxy methyl3R,4R-dihydroxy pyrrolidine (DMDP), 1- O-β-D-fructo furanoside of DMDP, 3-O-βD-gluco pyranosyl-DMDP, 1,4- dideoxy-1,4-imino-D-arabinitol, 3,9-dimethoxy-6aR,11aR-dihydro-6H-benzofuro(3,2-C)[1]benzopyran (homopterocarpin) and 2,4-dimethoxybenzaldehyde (Kato *et al.*, 2008; Chabbi *et al*, 2010; Ndukwe *et al.*, 2020). Onwukaeme, (1995) reported the isolation of isoflavonoids-flavonoids dimer santalins A and B and santarubins A, B and C, baphic acid, baphiin, deoxysantarubin, homopterocarpin, maackiain, pterocarpin and santal. Also, eleven known compounds which includes; isoafrormosin, 7,3´-dihydroxy-8,4´-dimethoxyisoflavone, pratensein, (+)-catechin, β-sitosterol, stigmasterol, friedelin, friedelin-3α-ol, lupeol, nonadecanoic acid, nonacosane and one new compound, 3-Prenyl-2-flavene (Baphiflavene) has been reported for the first time from *Baphia massaiensis* (Keroletswe *et al.*, 2018).

Similarly, Analysis of ethanol extract of *Baphia pubescens* leaves revealed the presence of alkaloids, flavonoids, saponin, carbohydrates, steroids, proteins and tannins (Anowi *et al.*, 2015). The composition of essential oil from the seed oil of *Baphia massaiensis* analysed with ^{1}H-NMR and GC-MS techniques was found to consist of palmitic acid methyl ester (7.3%), Linoleic acid methyl ester (49.0%), Linolenic acid methyl ester (36.7%), Stearic acid methyl ester (2.2%),

Gondoic acid methyl ester (2.1%), Behemic acid methyl ester (2.6%) and Lignoceric acid methyl ester (<0.1%) (Keroletswe *et al.*, 2017). Although, the Seed oil of *B. massaiensis* has been reported from Botswana (Keroletswe *et al.*, 2017) but there is no information on the essential oil constituents from the leaves of *Baphia nitida* in spite of its attractive traditional medicinal uses.

Sequel to the above, we report in this paper the essential oil constituents of the air-dried and fresh leaves of *B. nitida* from Nigeria for the first time as our contribution towards increasing the available literature on the essential oil of the leaves.

Materials and Methods

Sample collection and identification

The leaves of *Baphia nitida* were collected from a tree sample in Ibadan, Oyo State, Nigeria. Identification and authentication was done at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria where a voucher specimen with herbarium no. FHI 111868 was deposited.

Extraction of essential oil

The pulverized air-dried and fresh leaves of *B. nitida* (500 g) each were subjected to hydrodistillation in an all glass Clevenger-type apparatus separately for 3 h in accordance with established procedure (British Pharmacopoeia, 1980). The oils obtained were dried over anhydrous sodium sulphate (Na_2SO_4), stored in vials and kept inside a refrigerator until ready for analysis.

Gas chromatography-mass spectrometry (GC-MS) analysis of the leaf oil

GC-MS analysis of the oil was done on an Agilent model 7890A gas chromatograph coupled with a mass spectrometer 5975 that is equipped with a FID and fitted with a fused silica capillary HP-5 MS column (30 m x 0.32 mm id, film thickness 0.25 µm). The oven temperature was programmed from 80 – 240°C at the rate of 8°C/min. The ion source was set at 240°C and electron ionization at 70 eV. Helium was used as the carrier gas at a flow rate of 2 mL/min. Scanning range was 50 – 550 amu. Diluted oil in n-hexane (1.0 µL) was injected into the GC/MS spectrometer.

Identification of the constituents of the leaf oil

The individual components of the oil were identified on the basis of their retention indices (RI) determined by co-injection with reference to a homologous series of n-alkanes, under identical experimental conditions. Further identification was performed by comparison of their mass spectra with those from National Institute of Standards and Technology NIST (Data base 69) and the home-made MS library built up from pure substances and components of known essential oils, as well as by comparison of their retention indices with literature values (Adams, 2007).

Results and Discussion

The essential oils (0.29% v/w) each of air-dried and fresh leaves were found to be colourless oils with an herbal smell. The result of the chromatographic analysis done on the essential oils of air-dried and fresh leaves is shown in Figs. 1 and 2, respectively.

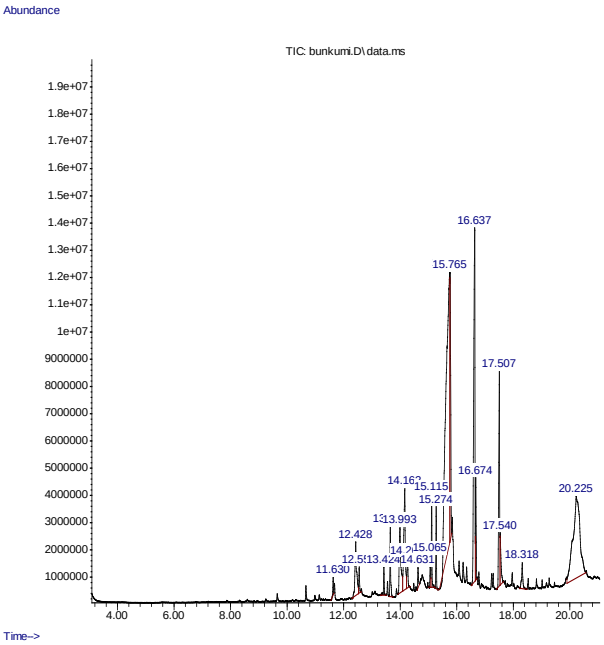

Fig. 1: Chromatogram of the air-dried leaves of*Baphia nitida* essential oil components

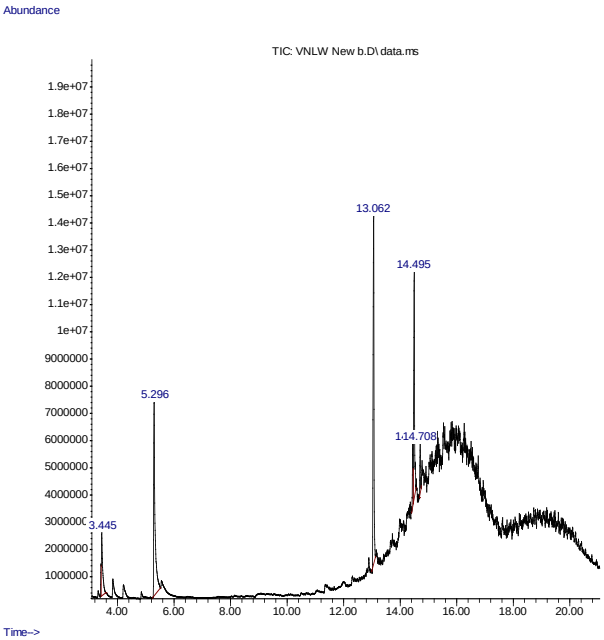

Fig. 2: Chromatogram of the fresh leaves of*Baphia nitida* essential oil components

From Table 1, a total of Sixteen (16) constituents were identified representing 99.99% of the essential oil from air-dried leaves of *B. nitida* with the following components; Oleic acid (54.69%) as the major constituent, followed by 2-Propanoic acid-3-[4-[[4- methoxyphenyl) methylene]amino]phenyl]-ethyl ester (17.65%), 2-Hydroyethyl Oleate (5.86%) and Palmitoleic acid (5.29%). The other constituents of the oil were n-Hexadecanoic acid (4.72%), Tetradecanoic acid (2.73%), 9,17-Octadecadienal, (Z)- (1.56%), Isobutyl myristate (1.51%), Isobutyl palmitate (1.49%), Cis-13-Octadecenoic acid (0.97%), Eicosane (0.89%), Nonadecane (0.64%), Octadecane (0.60%), Heneicosane (0.59%), 2-Hydroxyethyl myristate (0.48%) and Heptadecane (0.31%), classified as Carboxylic acids (68.40%), Esters (26.99%), Hydrocarbons (3.04%) and aldehyde (1.56%).

Table 1: Chemical composition of essential oil from the air-dried leaves of *Baphia nitida*

S/N	Name	KOVAT Index	Retention Time	% Composition	Molecular Formula
1	Heptadecane	1310	11.630	0.31	$C_{17}H_{36}$
2	Tetradecanoic acid	1787	12.430	2.73	$C_{14}H_{28}O_2$
3	Octadecane	1460	12.549	0.60	$C_{18}H_{38}$
4	Nonadecane	1800	13.425	0.64	$C_{19}H_{40}$
5	Isobutyl myristate	1781	13.649	1.51	$C_{18}H_{36}O_2$
6	Palmitoleic acid	1667	13.992	5.29	$C_{16}H_{30}O_2$
7	n-Hexadecanoic acid	1852	14.163	4.72	$C_{16}H_{32}O_2$
8	Eicosane	1600	14.263	0.89	$C_{20}H_{42}$
9	2-Hydroxyethyl myristate	2046	14.630	0.48	$C_{16}H_{32}O_3$
10	Heneicosane	1600	15.063	0.59	$C_{21}H_{44}$
11	Isobutyl palmitate	1650	15.273	1.49	$C_{20}H_{40}O_2$
12	Oleic Acid	1806	15.749	54.69	$C_{18}H_{34}O_2$
13	2-Hydroyethyl Oleate	1800	17.506	5.86	$C_{20}H_{38}O_3$
14	9,17-Octadecadienal, (Z)-	1992	17.539	1.56	$C_{18}H_{32}O$
15	Cis-13-Octadecenoic acid	1829	18.320	0.97	$C_{18}H_{34}O_2$
16	2-Propanoic acid-3-[4-[[4-methoxyphenyl)methylene]amino]phenyl]-ethyl ester	1987	20.225	17.65	$C_{19}H_{19}NO_3$
	Hydrocarbons			3.04	
	Carboxylic Acids			68.40	
	Esters			26.99	
	Aldehyde			1.56	
	Total			99.99	

Table 2: Chemical composition of essential oil from the fresh leaves of *Baphia nitida*

S/N	Name	KOVAT Index	Retention time	% composition	Molecular formula
1	Eucalyptol	650	3.440	8.62	$C_{10}H_{18}O$
2	Terpinen-4-ol	1135	5.296	28.65	$C_{10}H_{18}O$
3	Methyl palmitate	1764	11.063	33.63	$C_{17}H_{34}O_2$
4	Methyl linoleate	1796	14.444	4.37	$C_{19}H_{34}O_2$
5	Methyl oleate	1900	14.497	20.90	$C_{19}H_{36}O_2$
6	Methyl stearate	1826	14.706	3.83	$C_{19}H_{38}O_2$
	Monoterpenoids			37.27	
	Esters			62.73	
	Total			100.00	

From Table 2, a total of six (6) constituents were detected representing 100% of the essential oil from the fresh leaves with methyl palmitate (33.63%) as the major constituent followed by; terpinen-4-ol (28.65%), methyl oleate (20.90%), eucalyptol (8.62%), methyl linoleate (4.37%) and methyl stearate (3.83%), comprising Monoterpenoids (37.27%) and Esters (62.73%) as the classes of identified compounds.

A comparative analysis of the essential oil of both leaf samples showed Oleic acid (52.36%) and methyl palmitate (33.63%) as the major constituents present in the oil extracted from the air-dried and fresh leaves respectively. Furthermore, the quantitative amount of Oleic acid (52.36%) and methyl palmitate (33.63%) in the oils are noteworthy, since they have not been previously reported to be a major compound of *B. nitida*.

Similarly, both leaves contained esters with varying qualitative, quantitative amount and formulae. The esters found in the air-dried leaves are; 2-Propanoic acid-3-[4-[[4-methoxyphenyl)methylene]amino]phenyl]-ethyl ester (17.65%), 2-Hydroyethyl Oleate (5.86%), Isobutyl myristate (1.51%), Isobutyl palmitate (1.49%) and 2-Hydroxyethyl myristate (0.48%) while methyl palmitate (33.63%), methyl oleate (20.90%), methyl linoleate (4.37%) and methyl stearate (3.83%) were found in the fresh leaves. However, terpenoids are conspicuously missing in the air-dried leaves unlike the fresh leaves that contained both eucalyptol (8.62%) and terpinen-4-ol (28.65%).

Furthermore, Carboxylic acids (68.40%) and Hydrocarbons (3.04%) found in the air-dried leaves were absent in the fresh leaves. Also, comparison of the present result with previously analysed seed oil sample of *B. massaiensis* revealed some similarities with the fresh leaves components. Keroletswe *et al.*, (2017) reported seven constituents which accounted for 99.9% of oils and the current research identified six constituents too which accounted for 100% of the oil. In addition, methyl palmitate, methyl linoleate and methyl stearate reported in previous study (Keroletswe *et al.*, 2017) were also found to be present in the essential oil of the fresh leaves though with quantitative variation.

We found out that Oleic acid which is a common constituent of plant oils was detected as a major constituent (54.69%) of the oil from the leaves in the present work, but was not identified in previous study of the seed oil (Keroletswe *et al.*, 2017). Oleic acid has been previously reported to exhibit antibacterial, antifungal, antimicrobial properties and a potent anti-inflammatory activity, with beneficial effects on genes linked to cancer including allopathic and pest control potentials (Dilika *et al.*, 2000; Walters *et al.*, 2004; Agoramoorthy *et al.*, 2007; Costa *et al.*, 2020).

Nevertheless, the presence of ester and other esterified acids in previously reported data are in accordance with the current study though with structural differences (Keroletswe *et al.*, 2017).

From the fore-going, in spite of some similarities, there are qualitative and/or quantitative compositional variations in the volatile compounds present in *B. nitida* as well as other species from the same genus and this may be due to ecological factors, age of the plant, period of collection, handling procedure and climatic condition (Dai *et al.,* 2013; Inikpi *et al.*, 2014; Rehman *et al.*, 2016; Ibanez and Blazquez, 2019).

Conclusion

The study provides information on the essential oil constituents of the air-dried and fresh leaves from *B. nitida* which has been scarce in literature. The investigation revealed a total of twenty-two (22) constituents for both air-dried and fresh leaves of *B. nitida* as determined by GC-MS analysis. The presence of oleic acid and methyl palmitate as the major constituent in the oils justifies the traditional use of the plant against parasitic skin disease and as anti-inflammatory agent. Also, the identified chemical constituents from the plant are new additions to the chemical data base for the plant.

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Conflict of Interest

Authors declare that there is no conflict of interest related to this study.

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