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Comparative Study of Chemical Profiles of Leaf, Root and Seed Essential Oils of *Clausena anisata* (Willd.) Hook

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Clausena anisata (Rutaceae) leaf, root and seed essential oils from Nigeria were obtained by hydro distillation using Clevenger apparatus. The oils were analyzed by means of gas chromatography and gas chromatography-mass spectrometry. Eighty-one components were identified in the three plant parts. The oil yields were 0.8 % (leaf), 0.5 % (root) and 1.25 % (seed) v/w of the wet sample. The leaf oil reveals the presence of 26 components accounting for about 99.91 % of the whole volatiles. Sesquiterpenoids hydrocarbons are the most abundant (45.31 %), followed by hydrocarbon (42.03 %), oxygenated sesquiterpenoids (4.86 %) and oxygenated monoterpenoids (2.87 %). The major constituents in the leaf oil were 8-methylenedispiro[2.1.2.4]undecane (25.96 %), aromandendrene (14.90 %), germacrene D (12.98 %). Other notable constituents found are β-farnesene (5.59 %), patchoulane (3.84 %), β-bisabolene (3.37 %) and bisabol (3.24 %). Forty-five components were detected and identified in the root essential oil amounting to about 99 % of the whole volatiles and is characterized by large presence of sesquiterpenoids hydrocarbon (62.69 %) followed by monoterpenoids hydrocarbon (10.69 %), oxygenated monoterpenoids (8.01 %) and oxygenated sesquiterpenoids (7.39 %). The most abundant components are caryophyllene (19.21 %), Z-β-farnesene (11.18 %), aromandedrene (9.89 %), β-bisabolene (8.12 %) and (+)-nerolidol (4.56 %). In the seed essential oil, 21 compounds were identified accounting for about 99.76 % of the whole volatiles. The most abundant class of terpenoids are the oxygenated sesquiterpenoids (26.41 %), followed by monoterpenoids hydrocarbon (26.07 %). The main components of the seed essential oil are exaltone (26.41 %), limonene (9.72 %), oxirane tetradecyl (8.80 %), 4-methylcyclopentadecanone (8.80 %), *trans*-β-ocimene (6.16 %) and 1R-α-pinene (6.15 %).

Keywords: ***Clausena anisata*, Essential oil, GC-MS, Rutaceae, Hydrodistillation.**

INTRODUCTION

Clausena anisata (Willd.) Hook, is also called horse wood or maggot killer, They belong to the Rutaceae family, they are deciduous shrubs or small tree growing up to 4-10 m tall, the bark is smooth, grey-green in colour but becomes brownish and mottled with age [1]. It grows in the savannah or forest region of West Africa including Nigeria and Ghana. It is locally known and called 'Agbasa' by the Yoruba people of Nigeria [2]. The small white flowers have orange-yellow stamens [3]. The fruit are sweet and readily eaten by people and other animals.

The leaves, roots and seeds possess a pleasant odour on account of the essential oil and it's mainly used as a multi-purpose folk medicine. The various parts of these plants have been reported to be useful and effective against various ailment and diseases such as parasitic infections, especially flatworm infections, such as schistosomiasis, as well as in influenza, eye complaints and other respiratory ailments, heart disorder and hypertension; abdominal cramps; gastroenteritis and constipation, malaria, diabetics, pyrexia and fever, hepatic disease causing bad breath, rheumatism, boils, arthritis and other inflammatory conditions, headaches, toothaches, body pains, swollen gums, convulsion, mental disorders, impotencies [3-11].

A mixture of *C. anisata*; *Antraegle paniculata* and *Azadirachtha indica* is taken against gut disturbance in Nigeria and a concoction of the later called Agbo is used as antimalaria [3].

Traditional practitioner uses *C. anisata* against oral candidiasis and fungal infection of the skin in Tanzania [4]. It is also used against epilepsy and as an anticonvulsant in Temeke district of Darussalam; Tanzania [5]. *C. anisata* leaves are applied against high blood pressure in South Africa and some parts of Africa and Philippines burn the fresh leaves to obtain smokes used to repel mosquitoes [10]. The chemical composition of the leaves of this plant has been well studied. The leaf essential oils have been reported to consist of methylchavicol (estragole), ρ-anisaldehyde, anethole, *trans*-β-ocimene and caryophyllene as the major constituents [6,12].

Constituents of volatile oil had been the subject of several studies and various data from literature shows that it has no constancy neither with respect to the components or their percentage. The impact of environmental factors such as relative humidity, harvesting time and method of extraction, location, irradiance, photoperiod, soil structure and climate heavily influence the composition and quality of volatile oils [13]. It is on the basis stated above that led us to investigate the leaf, root and seed essential oil of *C. anisata* grown in Nigeria.

EXPERIMENTAL

The fresh matured leaves, roots and the seeds of *C. anisata* were collected from their natural habitat at the back of the premises of Kogi State University. Plant identification was done in the department of Botany, Kogi State University Anyigba. Voucher specimens were deposited in the herbarium of the Faculty of biological Sciences, Kogi State University Anyigba, Nigeria.

Oil isolation: 500 g of the fresh leaves, 150 g of the root and 250 g of the seeds were hydro distilled separately for 4 h using an all glass Clevenger apparatus according to European Pharmacopoeia (2008). Oils were collected and kept in the refrigerator without further treatment before GC-MS analysis.

Characterization: The chemical composition of the essential oil was analyzed using multi-dimensional gas chromatography coupled with gas chromatography-mass spectrometry (Shimadzu GC-MS-QP2010 PLUS, Japan) equipped with a non-polar and polar double capillary columns (25 m × 0.25 mm, film thickness 0.25 μm). 1.0 μL of the oil sample was injected using the split mode (split ratio 100:1) into GC and GC-MS using AOC20i auto injector for analysis. For GC/MS detection, an electron ionization system with ionization energy of 70 eV was used and the mass scanning range was 40-700 *m/z*. High purity Helium (99.99 %) was used as the carrier gas at a flow rate of 0.99 mL/min. The initial column temperature for the GC was set at 60 °C, heated at a rate of 3 °C/min to 250 °C and held isothermally for 3 min, the pressure was set at 56.2 Kpa; column flow at 0.99 mL/min and linear velocity of the column was 36.2 cm/sec. Ion source temperature of the GC-MS for these analyses was 200 °C, solvent cut time was 3 min. Each peak was then analyzed and assigned a number in the order that it was detected. The identification of the components was based on comparison of their retention indices with those of literature and further identification was made by comparison of their mass spectra with those of NIST library mass spectra database.

RESULTS AND DISCUSSION

The essential oil of *C. anisata* plant parts (leaf, root and seed) was investigated for its chemical components, the volatile oil imparted pleasant aromatic odour. The GC and GC-MS analyses of the essential oils hydro distilled from the leaf, root and seed of medicinal *C. anisata* plant from Nigeria produced a pale, clear and light-yellowish colour and revealed the presence of 81 components. The oil yield were 0.8 % (leaf), 0.5 % (root) and 1.25 % (seed) v/w of the wet sample. The chemical constituents identified by GC-MS were listed in order of their retention index, percentage composition, molecular formula and components as shown in Table-1. The leaf oil reveals the presence of 26 components accounting for about 99.91 % of the whole volatiles. Sesquiterpenoids hydrocarbons are the most abundant (45.31 %), followed by hydrocarbon (42.03 %), oxygenated sesquiterpenoids (4.86 %) and oxygenated monoterpenoids (2.87 %). The major constituents in the leaf oil were 8-methylenedispiro[2.1.2.4]undecane (25.96 %), aromandendrene (14.90 %), germacrene D (12.98 %). Other notable constituents found are β-farnesene (5.59 %), patchoulane (3.84 %), β-bisabolene (3.37 %) and bisabol (3.24 %).

Forty-five components were detected and identified in the root essential oil amounting to about 99.0 % of the whole volatiles and is characterized by a large presence of sesquiterpenoids hydrocarbon (62.69 %) followed by monoterpenoids hydrocarbon (10.69 %), oxygenated monoterpenoids (8.01 %) and oxygenated sesquiterpenoids (7.39 %). The most abundant components are caryophyllene (19.21 %), Z-β-farnesene (11.18 %), aromandendrene (9.89 %), β-bisabolene (8.12 %), (+)-nerolidol (4.56 %) and Z, E-α-farnesene (4.37 %).

In the seed essential oil, 21 compounds were identified accounting for about 99.76 % of the whole volatiles. The most abundant class of terpenoids are the oxygenated sesquiterpenoids (26.41 %), followed by monoterpenoids hydrocarbon (26.07 %). The main components of the seed essential oil are exaltone (26.41 %), limonene (9.72 %), oxirane tetradecyl (8.80 %), 4-methylcyclopentadecanone (8.80 %), *trans*-β-ocimene (6.16 %) and IR-α-pinene (6.15 %). Other notable compounds in the seed essential oil are *cis*-13-octadecenal (5.27 %) and methyl-11-octadecanoate (4.12 %). Comparing the components of essential oils in the three plant parts of *C. anisata* (leaf, root and seed) *trans*-β-ocimene was the only component common to it, artemesiatriene, aromandendrene, β-farnesene, caryophyllene and β-bisabolene were the constituents found in both leaf and root essential oil while 1R-α-pinene and limonene were both found in the essential oils of the root and seeds.

There was a difference in the earlier report [6,12] on *C. anisata* leaf essential oil of South West and North Central Nigeria grown samples which was reported to be composed of methyl chavicol (estragole), ρ-anisaldehyde, anethole, *trans*-β-ocimene and caryophyllene as the major constituents. In contrast the major components of the leaf essential oil of Nigerian grown sample in our study were 8-methylenedispiro [2.1.2.4] undecane (25.96 %), aromandedrene (14.90 %) and germacrene D (12.98 %). However, the presence of *trans*-β-ocimene and caryophyllene in both leave samples [6,12] and this present study sample present an interesting similarity.

Anethole, γ-cardinene and estragole found in the North-Central *C. anisata* leaf oil [12] were not detected in the present leaf, root and seed oils of our samples. The differences observed may be likely due to the impact of environmental factors such

TABLE-1
CHEMICAL PROFILES OF LEAVES, ROOTS AND SEEDS ESSENTIAL OILS OF *Clausena anisata*

S. No.	Retention index	Composition (%)			m.f.	Compound
		Leaves	Root	Seeds		
1	688		0.50		C_7H_{10}	2-Methylenebicyclo[2.1.1]hexane
2	733		0.159		C_7H_{10}	1,3-Dimethylenecyclopentane
3	804		O.15		C_7H_{10}	1,3-Cycloheptadiene
4	863			2.44	C_9H_{14}	3-Methylene-1,7-octadiene
5	896	0.37	0.19		$C_{10}H_{16}$	Artemesiatriene
6	910			0.97	C_9H_{12}	8-Methylenebicyclo[4.2.0]oct-2-ene
7	934		0.42		$C_{10}H_{16}$	1,2-Diisopropenylcyclobutane
8	943		1.29	3.07	$C_{10}H_{16}$	Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl
9	948		2.57	6.15	$C_{10}H_{16}$	1R-α-Pinene
10	958		1.32		$C_{10}H_{16}$	Ocimene
11	976	0.74	3.89	6.16	$C_{10}H_{16}$	*trans*-β-Ocimene
12	993		0.20		$C_{10}H_{16}$	Octatriene, dimethyl-
13	997	0.47			C_9H_{14}	1-Cyclohexyl-1-propyne
14	1011			0.97	$C_{10}H_{14}$	1,9-Decadiyne
15	1018		0.42	9.72	$C_{10}H_{16}$	Limonene
16	1023		0.20		$C_{10}H_{16}$	4-Methyl-3-(1-methylethylidene)-1-cyclohexene
17	1052		0.19		$C_{10}H_{16}$	Terpinolene
18	1067	0.78			$C_{12}H_{18}$	11-Methylene-tricyclo[4.3.1.1(2,5)]undecane
19	1067	0.78			$C_{12}H_{18}$	11-Methylene-tricyclo[4.3.1.1(2,5)]undecane
20	1076		0.15		$C_{11}H_{16}$	3-Methyl-3,4-divinyl-1-cyclohexene
21	1092		0.15		$C_{11}H_{16}$	1-Methyl-5,6-divinyl-1-cyclohexene
22	1115	12.90			$C_{12}H_{18}$	1-(1-Ethylvinyl)-1-(2-methylene-3-butenyl)cyclopropane
23	1116	0.37			$C_9H_{14}O$	Bicyclo[3.3.0]octan-2-ol, 7-methylene
24	1122		0.63		$C_{11}H_{18}$	Bicyclo[5.1.0]octane
25	1125		0.20		$C_{15}H_{24}$	(+)-Cycloisosativene
26	1136		0.63		$C_{10}H_{16}O$	Verbenol
27	1148	1.92			$C_{12}H_{18}$	1-Methyl-2-methylene-3,5-divinylcyclohexane
28	1150			0.98	$C_9H_{14}O$	3-Cyclohexene-1-propanal
29	1152	0.36			$C_9H_{14}O$	*cis*-8-Hydroxy-bicyclo (4, 3,0) non-3-ene
30	1169		0.65		$C_{10}H_{18}O$	6-Methyl-2-vinyl-5-hepten-1-ol
31	1174		1.63		$C_{10}H_{16}O$	β-Citral
32	1177		0.20		$C_{10}H_{15}Br$	1-Bromo-2,2,3,3-tetramethyl-1-(1-propynyl)cyclopropane
33	1180		0.65		$C_{10}H_{18}O$	*cis*-Myrtanol
34	1187	0.47			$C_{10}H_{14}O$	Spiro[bicyclo[3.3.0]octan-6-one-3-cyclopropane]
35	1215	25.96			$C_{12}H_{18}$	8-Methylenedispiro[2.1.2.4]undecane
36	1221		0.99		$C_{15}H_{24}$	Copaene
37	1228	1.62			$C_{10}H_{18}O$	Isogeraniol
38	1251		0.20		$C_{13}H_{2}0$	Bicyclo[2.2.1]heptane, 2-cyclopropylidene-1,7,7-trimethyl
39	1302		1.96		$C_{12}H_{20}O$	9-Methyl-5-methylene-8-decen-2-one
40	1318			0.98	$C_{12}H_{18}$	Spiro[2.9]dodeca-4,8-diene
41	1326		0.63		$C_{10}H_{1m8}O_2$	1,7,7-Trimethylbicyclo[2.2.1]heptane-2,5-diol
42	1328	0.46			$C_9H_{15}NO_2$	(2-Nitro-2-propenyl)cyclohexane
43	1331		0.88		$C_{10}H_{18}O_2$	5-(1-Hydroxy-1-methylethyl)-2-methyl-2-cyclohexen-1-ol
44	1344	2.35			$C_{15}H_{24}$	α-Bourbonene
45	1348		0.21		$C_{12}H_{20}O_2$	β-Terpinyl acetate
46	1349	0.78			$C_{10}H_{14}O_2$	4-Oxatricyclo [4.3.1.1(3,8)]undecan-5-one
47	1359		0.63		$C_{12}H_{18}O_2$	2-Heptenoic acid, 7-(methylenecyclopropyl)-, methyl ester
48	1386	14.90	9.89		$C_{15}H_{24}$	Aromadendrene
49	1393	3.84			$C_{15}H_{26}$	Patchoulane
50	1403		1.75		$C_{15}H_{24}$	α-Longipinene
51	1430		2.62		$C_{15}H_{24}$	*trans*-alpha-Bergamotene
52	1440	5.59	11.18		$C_{15}H_{24}$	β-Farnesene
53	1446		3.70		$C_{15}H_{24}$	β-Sesquiphellandrene
54	1452		4.68		$C_{14}H_{22}$	11,11-Dimethyl-spiro[2,9]dodeca-3,7-dien
55	1458		4.37		$C_{15}H_{24}$	α-Farnesene
56	1461			2.06	$C_{11}H_{20}O_2$	Sevinon
57	1468		1.63		$C_{13}H_{22}O$	4-(2,2-Dimethyl-6-methylenecyclohexyl) butanal
58	1471			2.00	$C_{13}H_{24}O_2$	Methyl 11-dodecenoate
59	1494	1.92	19.21		$C_{15}H_{24}$	Caryophyllene
60	1500	3.73	8.12		$C_{15}H_{24}$	β-Bisabolene
61	1509		0.87		$C_{12}H_{19}NO$	N-(4,6,6-Trimethylbicyclo[3.1.1]hept-3-en-2-yl)acetamide

62	1511	1.61			$C_{13}H_{22}O$	3,5,9-Trimethyl-deca-2,4,8-trien-1-ol
63	1515	12.98			$C_{15}H_{24}$	Germacrene D
64	1530	1.62			$C_{15}H_{26}O$	Viridiflorol
65	1564		4.56		$C_{15}H_{26}O$	+/-.-*trans*-Nerolidol
66	1579		0.66		$C_{15}H_{24}$	α-Caryophyllene
67	1580		0.87		$C_{15}H_{26}O$	α-Cadinol
68	1625	3.24			$C_{15}H_{26}O$	α-Bisabolol
69	1702			8.80	$C_{16}H_{32}O$	Oxirane, tetradecyl
70	1710		1.96		$C_{15}H_{26}O$	*cis*-Farnesol
71	1719	0.46			$C_{13}H_{20}N_2$	1,1-dicarbonitrile, 1-cyclohexyl-3-methyl-
72	1808			2.64	$C_{16}H_{30}O$	Z-11-Hexadecenal
73	1811		0.63		$C_{18}H_{26}O$	1,3-Bis-(2-cyclopropyl,2-methylcyclopropyl)-but-2-en-1-one
74	1924			0.90	$C_{19}H_{32}$	Z,Z,Z-1,4,6,9-Nonadecatetraene
75	1970			26.6	$C_{15}H_{28}O$	Exaltone
76	2007			5.27	$C_{18}H_{34}O$	*cis*-13-Octadecenal
77	2031			8.80	$C_{16}H_{30}O$	4-Methylcyclopentadecanone
78	2061	0.47		2.63	$C_{18}H_{32}O$	17-Octadecen-14-yn-1-ols
79	2085			4.12	$C_{19}H_{36}O_2$	Methyl-11-octadecenoate
80	2104			2.63	$C_{19}H_{36}O$	2-Methyl-Z,Z-3,13-octadecadienol
81	2483			2.06	$C_{23}H_{44}O_2$	Methyl (13E)-13-docosenoate

as relative humidity, harvesting time, method of extraction, location, irradiance, photoperiod, soil structure and climate which heavily influence the composition and quality of volatile oils [13].

It is worth mentioning that compounds such as sevinon, exaltone, verbenol, viridiflorol, oxirane tetradecyl, acetemide and patchoulane which were detected in the present study samples have not been reported previously as part of the constituents of the volatile oil of *C. anisata* plant parts.

Conclusion

The result obtained in this study showed that *C. anisata* possesses essential oils in all parts of the plants and that the oils were qualitatively and quantitatively different. The study represent the comprehensive analysis of the essential oil of *C. anisata* plants part grown in Nigeria. Exaltone, caryophyllene, β-farnesene, aromandendrene, germacrene D, limonene, 8-methylenedispiro[2.1.2.4]undecane were the dominant constituent of the leaf, root and seed essential oils of this medicinal plant. *trans*-β-Ocimene was found in the leaf, root and seed oils of *C. anisata* grown in Nigeria, artemesiatriene, aromandedrene, β-farnesene, caryophyllene and β-bisabolene were the constituents found in both leaf and root essential oil while 1R-α-pinene and limonene were both found in the essen-tial oils of the root and seeds. Further comprehensive work may be needed on the antioxidant; free radical scavenging and antimicrobial potentials of the leaves, root and seeds essential oil of *C. anisata*.

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