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Chemical Composition of the Leaf Essential Oils of *Croton zambesicus* Müll.-Arg. Grown in Lagos, South-West Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author ALO managed and supervised authors HGG and RG in the process of collection of plant sample and extraction of the oils. Author IAO designed the study, managed the literature searches and wrote the final draft of the manuscript while the author GF carried out the GC and GC-MS analyses. All authors read and approved the final manuscript.

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ABSTRACT

The chemical composition of the leaf essential oil of *Croton zambesicus* Müll.-Arg., collected from Agbara-Lagos, Nigeria, was analysed by means of Gas chromatography-

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flame ionization detector (GC-FID) and Gas chromatography coupled with Mass spectrometry (GC-MS). Sixty constituents accounting for 98.9% of the total oil contents were identified from the oil sample. The classes of compounds identified in the oil were monoterpene hydrocarbons (35.3%), oxygenated monoterpenes (22.9%), sesquiterpene hydrocarbons (32.4%) and oxygenated sesquiterpenes (5.6%). The oil was dominated by β-pinene (15.1%), β-caryophyllene (12.6%), germacrene D (10.9%), camphor (7.3%), linalool (7.0%), sabinene (6.4%) and α-pinene (5.2%).

Aims: The aim of the research is to investigate the volatile constituents from *C. zambesicus* harvested in Lagos, Nigeria.

Study Design: Extraction of essential oil from the air-dried leaf samples of *C. zambesicus* and investigation of its chemical constituents.

Place and Duration of Study: Leaf samples of *C. zambesicus* were collected from Agbara, Lagos, on April 2011.

Methodology: Air-dried and pulverized leaves were hydrodistilled in a Clevenger-type apparatus to obtained pale yellow volatile oil whose chemical constituents was analyzed by GC and GC/MS.

Results: A total of sixty compounds were identified, amounting to 98.9%of the total oil contents. The major were compounds β-pinene (15.1%), β-caryophyllene (12.6%), germacrene D (10.9%) and camphor (7.3%). Variations in compositional pattern were observed between this result and the previous studies.

Conclusion: The literature about the *C. zambesicus* indicates a high variability in the chemical composition of the essential oils.

Keywords: Croton zambesicus; β-pinene; β-caryophyllene; germacrene D; camphor; linalool.

1. INTRODUCTION

Croton zambesicus Müll.-Arg., (family *Euphorbiaceae*) is an ornamental plant grown in Nigeria and widely spread in tropical Africa. It is a large shrub or small tree up to 16 to 25 ft. high [1]. The leaves are green, firmly membraneous and penninerved. Flowering usually occurs at the beginning of dry season. The plant is commonly known as koriba in Hausa and Ajekofole in Yoruba [2,3]. Traditionally, the plant is used in the treatment of urinary infection, malaria and dysentery [4]. Ethnobotanically, the leaf decoction is used in Benin as anti-hypertensive and urinary infections [5], and in parts of Niger Delta region of Nigeria the plant is use as antidiabetic and malarial remedy [6,7], while the Yorubas of western Nigeria use it traditionally for the treatment of Cancer [8]. The roots are used as anti malarial, febrifuge and antidiabetic by the Ibibios of Niger Delta region of Nigeria [9].

The ethanolic leaf extract has been reported to possess antiplasmodial [6,7], antidiabetic and hypolipidemic [7,10-12], anti-inflammatory, analgesic and antipyretic activities [13], while the root extract has been reported to possess antimalarial [9], anticonvulsant [14,15], antiulcer [14], anti-inflammatory, analgesic and antipyretic [16] and kidney-protective activities [17]. The root extract and fractions have also shown immune stimulatory, cytotoxicity against HeLa cell line [18], antileishmanial [18] and anticoagulant [19] activities. A previous study has reported on the antimicrobial properties of the leaf and stem extracts [20]. Extracts of *C. zambesicus* were reported to have potential as insecticidal [21] and possesses nephroprotective [22], anti-atherogenic and anti-ischemic potentials [23] and profertility [24] properties, enhance the functions of the liver [25] and increase reproductive indices in female African fish [26].

Phytochemcial compounds isolated from this plant includes diterpenes with vascular and vasoerelaxant activities [27,28], lupeol, betulinic acid, betulin, lupenone, diterpene ent kaurane-3,16,17-triol and vitexin [29]; quercetin-3-O-*p*-6-*O* (p-coumaroyl) glucopy-ranoside-30-methyl ether, helichrysoside-30-methyl ether, along with kaempferol-3-O-*p*-600 (p-coumaroyl) glucopyranoside, tiliroside and apigenin-6-C-glucoside, isovitexin [30] as the antioxidant constituents from the leaf of the plant. A report also showed that volatile oil of *C. zambesicus* exhibited vasorelaxant activity [31].

Previous studies have shown that volatile oil from the leaves of *Croton zambesicus* contained different compounds under the classes of monoterpenes, sesquiterpenes and diterpenes [32-38].

In continuation of our extensive research on the volatile constituents of Nigerian flora as they are made available [39], we report the chemical constituents of the leaf essential oil of *C. zambesicus* growing in Lagos, South-West Nigeria.

2. MATERIALS AND METHODS

2.1 Plant Collection

Fresh leaves of *C. zambesicus* were collected on April 2011, from Agbara, Lagos State, Nigeria. The plant was identified and authenticated by Mr Ugbogu O.A and Mr Soyewo L.T. of Plant Taxonomy Department, Forestry Research Institute (FRIN), Ibadan, Nigeria, where voucher specimen (FHI NO 109584) was deposited for future reference.

2.2 Isolation of Volatile Oils

The air-dried (under laboratory shade) plant sample (700g) was pulverized and hydrodistilled using a Clevenger-type apparatus in accordance with the standard procedure [40] for 4h. The distilled oil was collected over water and stored in well capped bottles prior to analysis.

2.3 Gas Chromatography (GC) Analysis

GC analysis was accomplished with a HP-5890 Series II instrument equipped with a HP-Wax and HP-5MS capillary columns (both 30m x 0.25mm, 0.25µm film thickness), working with the following temperature program: 60ºC for 10min, rising at 5ºC/min to 220ºC. The injector and detector temperatures were maintained at 250ºC; carrier gas nitrogen (1mL/min); detector dual, FID; split ratio 1:30. The volume injected was 0.5µL. The relative proportions of the oil constituents were percentages obtained by FID peak-area normalization without the use of response factor.

2.4 Gas Chromatography-mass Spectrometry (GC/MS) Analysis

GC-EIMS analysis was performed with a Varian CP-3800 gas-chromatograph equipped with a HP-5MS capillary column (30mx0.25mm; film thickness 0.25µm) and a Varian Saturn 2000 ion trapmass detector. Analytical conditions: injector and transfer line temperature 220ºC and 240ºC respectively; oven temperature programmed from 60ºC -240ºC at 3ºC/min; carrier gas was helium at a flow rate of 1mL/min.; injection of 0.2µL (10% hexane solution); split ratio 1:30. Mass spectra were recorded at 70eV. The acquisition mass range was 30-300 m/z at a scan rate of 1scan/sec.

2.5 Identification of the Constituents

Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their linear retention indices (LRI) relative to the series of n hydrocarbons, and on computer matching against commercially available spectral. Further identifications were also made possible by the use of home made library mass spectra built up from pure substances and components of known oils and MS literature data [41,42]. Moreover, the molecular weights of all the identified substances were confirmed by GC-CIMS, using MeOH as CI ionizing gas.

3. RESULTS AND DISCUSSION

The pale yellow oil from hydrodistillation of *C. zambesicus* was obtained in a yield of 0.29% (v/w), calculated on a dry weight basis. Table 1 revealed the identities and percentage composition of the sixty components (98.9% of total oil content) present in the oil. The main chemical classes of compounds identified in the oil were monoterpene hydrocarbons (35.3%), oxygenated monoterpenes (22.9%), sesquiterpene hydrocarbons (32.4%) and oxygenated sesquiterpenes (5.6%). The major oil constituents were β-pinene (15.1%), β-caryophyllene (12.6%), germacrene D (10.9%), camphor (7.3%), linalool (7.0%), sabinene (6.4%) and α-pinene (5.2%). Minor components includes myrcene (2.2%), limonene (2.3%), 1,8-Cineole (2.3%) and α-humulene (2.1%).

Literature information on *C. zambesicus* indicates a high variability in the chemical composition of the essential oils of leaves, stem bark, roots, flowering tops within the same plant species from different collections. Table 2 indicates the major compounds previously identified in the oil of *C. zambesicus*. Ubiquitous mono- and sesquiterpenes as defined for other results [32-38] were also identified in the oil sample, though the major compounds differs considerably from each other. However, when compared with leaf samples from previous results, notable diterpenes such as isopamara-7, 15-dien-3*β*-ol, *ent*-trachylobane, sandaracopimaradiene, kaurene ent-trachyloban-3-one and ent-trachyloban-3β-ol which are characteristics of the leaves oils of other reports [31,32] were conspicuously absent in this study. In addition, longiolene identified from leaf sample analysed in Benin [32], neral and *ent*-trachyloban-3-one obtained from previous Nigerian samples [34,38] were not identified in the present investigation. This may be attributed to different ecological and climatic conditions between the regions, as well as the nature of the plant and the processing method [39].

Table 1. Percentage composition of constituents of the leaf oil of *C. zambesicus*

Compounds[a]	LRI (Cal.)	RI (Lit.)	Percentage composition
(*E*)-3-Hexen-1-ol	854	857	0.4
1-Hexanol	875	863	Tr
n-Nonane	900	900	0.2
Santolina triene	910	906	Tr
α-Thujene	931	924	1.2
α-Pinene	940	932	5.2
Camphene	954	946	1.8
Thuja-2,4(10)-diene	958	953	Tr
Banzaldehyde	962	952	Tr
1-Heptanol	970	958	Tr
Sabinene	977	969	6.4

Compounds[a]	LRI (Cal.)	RI (Lit.)	Percentage composition
β-Pinene	980	974	15.1
3-Octanone	988	979	Tr
Myrcene	993	988	2.2
α-Phellandrene	1005	1002	Tr
δ-3-Carene	1011	1008	Tr
α-Terpinene	1018	1012	0.3
p-Cymene	1027	1020	0.2
Limonene	1031	1024	2.3
1, 8-Cineole	1034	1026	2.3
(Z)-β-Ocimene	1041	1032	Tr
(E)-β-Ocimene	1047	1044	Tr
γ-Terpinene	1062	1054	0.5
cis-Sabinene hydrate	1070	1065	0.3
cis-Linalool oxide (furanoid)	1075	1067	Tr
1-Nonen-3-ol	1083	1088	Tr
Terpinolene	1089	1086	0.8
Linalool	1099	1095	7.0
n-Nonanal	1103	1100	Tr
1-Octen-3-yl-acetate	1113	1110	0.3
cis-*p*-Menth-2-en-1-ol	1122	1118	0.1
3-Octyl acetate	1125	1124	Tr
cis-*p*-Mentha-2,8-dien-1-ol	1138	1133	Tr
trans-Pinocarveol	1140	1135	Tr
cis-Verbenol	1142	1137	Tr
Camphor	1144	1141	7.3
Menthone	1155	1148	0.1
Pinocarvone	1163	1160	0.2
Borneol	1166	1165	0.5
Menthol	1174	1167	1.6
Terpinen-4-ol	1178	1174	1.1
p-Cymen-8-ol	1184	1179	Tr
α-Terpineol	1190	1186	1.1
Methyl salicylate	1192	1190	0.3
n-Decanal	1205	1201	Tr
Verbenone	1208	1204	0.3
β-Cyclocitral	1218	1217	Tr
Nerol	1228	1227	0.2
3-methyl-3-hexen-1-yl Butanoate	1236	1232	0.1
3-methyl butanoic acid Hexyl ester	1243	1244	Tr
Carvone	1245	1239	Tr
Piperitone	1253	1249	Tr
Bornyl acetate	1285	1287	Tr
Carvacrol	1298	1298	Tr
Undec-10-en-1-al	1300	1299	Tr
Hexyl tiglate	1332	1330	Tr
δ-Elemene	1340	1335	0.6
α-Cubebene	1351	1345	Tr
α-Ylangene	1372	1373	Tr
Linalool isobutanoate	1374	1373	Tr

Compounds[a]	LRI (Cal.)	RI (Lit.)	Percentage composition
α-Copaene	1376	1374	1.6
β-Bourbonene	1384	1387	1.1
β-Elemene	1392	1389	0.2
Cyperene	1398	1398	0.2
Isocaryophyllene	1405	1408	Tr
β-Ylangene	1414	1419	Tr
β-Caryophyllene	1418	1417	12.6
β-YGurjunene	1432	1431	0.3
α-Guaiene	1439	1437	0.3
Aromadendrene	1441	1439	0.2
α-Humulene	1455	1452	2.1
allo-Aromadendrene	1461	1458	0.8
γ-Muurolene	1477	1478	0.4
Germacrene D	1480	1484	10.9
Valencene	1492	1496	0.2
epi-Cubebol	1494	1493	0.4
trans-β-Guaiene	1500	1502	0.1
Germacrene A	1503	1508	0.2
(E,E)-α-Farnesene	1508	1505	Tr
Cubebol	1515	1514	0.6
δ-Cadinene	1524	1522	0.4
trans-Cadina-1(2),4-diene	1533	1533	Tr
α-Cadinene	1538	1537	Tr
Germacrene B	1556	1559	0.2
(E)-Nerolidol	1565	1561	tr
Germacrene-D-4-ol	1574	1574	0.1
Caryophyllene oxide	1581	1582	1.5
Isoaromadendrene epoxide	1592	1594	0.2
Humulene epoxide II	1606	1608	0.1
1,10-di-*epi*-Cubenol	1614	1618	0.4
Epoxy alloaromadendrene	1632	1639	0.9
Caryophylla-4(14),8(15)-dien-5α-ol	1636	1641	Tr
epi-α-Cadinol	1641	1638	0.2
epi-α-Muurolol	1643	1640	0.1
α-Cadinol	1652	1652	0.4
Intermedeol	1667	1665	0.7
Khusinol	1680	1679	Tr
Occidentalol acetate	1682	1681	Tr
Pentadecanal	1717	1715	1.4
Total			98.9
Monoterpene hydrocarbons			35.3
Oxygenated monoterpenes			22.9
Sesquiterpene hydrocarbons			32.4
Oxygenated sesquiterpenes			5.6
Non-terpenes			1.7

[a] *Elution order on HP-5MS capillary column; LRI (Cal.) Retention indices on HP-5MS capillary column; RI (Lit.) Literature retention indices (see Materials and Methods); Tr, Trace amount, <0.1%*

Table 2. A summary of major constituents of the oils of *Croton zambesicus*

Origin (Part)	Major constituents	References
Nigeria (L)	Limonene (40.3%), γ-terpinene (12.1%), β-pinene (10.4%) and neral (9.4%)	34
Nigeria (L)	Caryophyllene oxide (21.7%), β-caryophyllene (8.8%), *ent*-trachyloban-3-one (8.1%) and linalool (6.2%)	38
Benin (L)	Caryophyllene oxide (19.5%), β-caryophellene (10.8%), copaene (6.3%), linalool (6.1%) and *β*-pinene (5.2%)	32
Central African Republic (L, Rt)	Linalool (34.6% and 9.9%) and β-caryophyllene (11.9% and 9.9%)	35
Saudi Arabia[a]		35
Cameroon (L, St, Rt)	Linalool (33.8%), limonene (19.2%), β-pinene (18.9%), α-pinene (8.7%), β-caryophyllene (15.8%) and spathulenol (14.0%)	36
Benin (L)	Longifolene (0.4-26.4%), caryophyllene oxide (2.9-25.9%), ent-trachyloban-3-one (1.4-28.0%) and ent-trachyloban-3β-ol (0.2-6.4%)	31
Sudan (Fr)	*m*-Cymene (21.56%), linalool (7.21%) and *p*-mentha-1-en-8-ol (5.28%)	33
Sudan (FLT)[b]	Pinene, limonene, menthol, carvone, thymol, α-humulene and *cis*-nerolidol	37

[a] *Plant part not known;* [a] *Quantitative data not available; L Leaves; St Stem; Rt Roots; FLT Flowering tops*

4. CONCLUSION

The chemical constituents of essential oil obtained from the leaf of *C.zambesicus* from Nigeria were being reported. The result indicated that both qualitative and quantitative variation exists between the present results and previous analysis from other parts of the world. This may be attributable to factors such as environmental conditions and the nature of the plant samples.

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CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interest exists.

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