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Leaf Oil Composition of *Croton zambesicus* Muell. Arg. Growing in Southwestern Nigeria: Essential Oil Chemotypes of *C. zambesicus*

Moses S. Owolabi*, Lawal A. Oladipupo, Labunmi Lajide, Rebecca M. Hauser, and William N. Setzer

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Moses S. Owolabi
Department of Chemistry, Lagos State University, PMB 001, Ojo, Lagos, Nigeria

Lawal A. Oladipupo
Department of Chemistry, Lagos State University, PMB 001, Ojo, Lagos, Nigeria.

Labunmi Lajide
Department of Chemistry, University of Technology, Akure, Ondo, Nigeria

Rebecca M. Hauser
Department of Chemistry, University of Alabama in Huntsville, Huntsville, AL 35899, USA

William N. Setzer
Department of Chemistry, University of Alabama in Huntsville, Huntsville, AL 35899, USA

Correspondence:
Farukh S. Sharopov
Department of Chemistry, Lagos State University, PMB 001, Ojo, Lagos, Nigeria.
E-Mail: sunnyconcept2007@yahoo.com

ABSTRACT

The leaf essential oil of *Croton zambesicus* growing in Odogbolu, Ogun State, Nigeria, was obtained and analyzed by GC-MS. The leaf oil was dominated by the sesquiterpenoids β-caryophyllene (8.8%) and caryophyllene oxide (21.7%), the monoterpenoids linalool (6.2%) and camphor (5.9%), and the diterpenoid*ent*-trachyloban-3-one (8.1%). A numerical cluster analyses has revealed at least two chemotypes of *C. zambesicus*, a monoterpene-rich chemotype, dominated by β-pinene and limonene, and a sesquiterpenoid-rich chemotype, dominated by β-caryophyllene and caryophyllene oxide.

Keywords: Essential oil composition, gas chromatography-mass spectrometry, caryophyllene oxide, numerical cluster analysis.

1. Introduction

Croton zambesicus Muell Arg. (Euphorbiaceace) (syn *C. amabilis* Muell. Arg. *C. gratissimus* Burch) is a large shrub or a small tree of 16 m high of fringing forest and savanna from Gambia to the southern part of Nigeria. *C. zambecius*is an ornamental tree grown in villages and towns in Nigeria. It is a Guineo – Congolese species widely spread in tropical Africawith a scaly bark, silvery leaves and has an attractive appearance [1]. It has reputation of conferring protection to ward off evil influences and commonly called, *àjẹ́ kòbàlé*, (Yoruba), whichmeans 'witches do not dare to perch on it', the plant enters an incantation for the placation of witches [2]. Ethnobotanically, the leaf decoction is use in traditional medicine for the treatment of several ailments, for example hypertension, diabetics, urinary tract infections, malaria, gonorrhea arthritis, and impotence have been reported [3-5]. Non-volatile extracts of the plant are known to possess anti-diabetic, vesorelaxant, anti-diabetic, antimalarial, antimicrobial [6] and aperient [7] properties. Earlier investigators have demonstrated the antimicrobial [8, 9] and antiplasmodial [10] properties of crude extracts of the leaf and stem of *C. zambesicus*. Previous studies on the composition of the essential oils from the leaves of *C.zambesicus*found the oils to be rich in either monoterpenes (limonene and β-pinene) [11-13] or sesquiterpenoids (β-caryophyllene, caryophyllene oxide, and germacrene D) [14, 15]. Despite the achievements of these previous studies and on the successful isolation of some important phytochemical constituents of *C. zambesicus* [7, 16-19], there is a need to further characterize the chemotypes of *C. zambesicus* based on its volatile constituents. In this work, we present the leaf essential oil composition of *C. zambesicus* from southwestern Nigeria, and characterize the chemotypes of *C. zambesicus* leaf oils.

2. Materials and Methods

2.1 Plant Material

The fresh leaves of the *Croton zambesicus* Muell. Arg. (Euphorbiaceae) were collected May, 2012, from Odogbolu, in Ogun State, Nigeria, and authenticated at Botany Department, University of Lagos. Voucher specimen is deposited at the herbarium of the University of Lagos with voucher specimen LUH 4760.A sample (500 g) of *C. zambesicus*was dried inthe laboratory for 7 days, reduced to powder and subjected to hydrodistillation in a Clevenger-type apparatus for 4 h. The yield of oil was 0.54% on a dry weight basis. The oil was dried over anhydrous sodium sulfate and stored in a sealed vial under refrigeration prior to analysis.

2.2 Gas Chromatographic / Mass Spectral Analysis

The volatile oil sample was subjected to GC-MS analysis on an Agilent system consisting of an Agilent model 6890 gas chromatograph, an Agilent 5973 mass selective detector (EIMS, electron energy = 70 eV, scan range = 40-400 amu, and scan rate = 3.99 scans/sec) and an Agilent Chemstation data system. The GC column was a HP-5ms fused silica capillary with a (5% phenyl)-methyl polysiloxane stationary phase, film thickness 0.25 µm, length 30 m, and internal diameter of 0.25 mm. The carrier gas was helium with a column head pressure of 7.07 psi and a flow rate of 1.0 mL/min. Inlet temperature was 200 °C and MSD detector temperature was 280 °C. The GC oven temperature program was used as follows: 40 °C initial temperature, hold for 10 min, increased at 3 °C/min to 200 °C, increased 2 °C/min to 220 °C. The sample was dissolved in dichloromethane and a splitless injection technique was used. Identification of the constituents of the volatile oil was achieved based on their retention data (retention indices) determined with reference to C_{10}-C_{40}*n*-alkane homologous series, and by comparison of their mass spectral fragmentation patterns with those reported in the literature [20] and stored on the MS library [NIST database (G1036A, revision D.01.00) / ChemStation data system (G1701CA, version C.00.01.08)]. The chemical composition of *C. zambesicus*essential oil is summarized in Table 1.

2.3 Numerical Cluster Analysis

Six*Croton zambesicus*samples were treated as operational taxonomic units (OTUs). The percentage composition of the 17 major essential oil components (limonene, caryophyllene oxide, β-caryophyllene, β-pinene, linalool, α-copaene, γ-terpinene, germacrene D, α-humulene, α-pinene, *ent*-trachyloban-3-one, borneol, camphor, sabinene, humulene epoxide II, *p*-cymene, and alloaromadendrene) was used to determine the chemical relationship between the different *C. zambesicus* essential oil samples by cluster analysis using the NTSYSpc software, version 2.2 [21]. Correlation was selected as a measure of similarity, and the unweighted pairgroup method with arithmetic average (UPGMA) was used for cluster definition.

3. Results and Discussion

Analysis of the essential oil resulted in the identification of 58 components comprising 93.9% of the total volatile oil (Table 1). The major components were caryophyllene oxide (21.7%),β-caryophyllene (8.8%), *ent*-trachyloban-3-one (8.1%), linalool (6.2%) and camphor (5.9%), with unidentified components consisting of (6.1%). There are at least two chemotypes of *C. zambesicus* (see Figure 1): A monoterpene-rich chemotype, dominated byβ-pinene and limonene (samples from Khartoum, Sudan [11], Ilorin, Nigeria [13], and Yaounde, Cameroon [12]), and a sesquiterpenoid-rich chemotype, dominated by β-caryophyllene and caryophyllene oxide (samples from Doba, Chad [14], Cotonou, Benin [15], and from Odogbolu, Nigeria, this present study). Notable, also, are the presence of diterpenoids from the sesquiterpenoid-rich samples from Benin [15] and Nigeria (this work) and the apparent absence of diterpenoids from the monoterpene-rich chemotype. The wide variation in chemical compositions for this plant is important considering its use in traditional medicine.

Table 1: Chemical composition of *Croton zambesicus* essential oil.

RI[a]	Compound	%[b]		RI[a]	Compound	%[b]
936	α-Thujene	0.1±0.0		1428	β-Copaene	0.4±0.0
941	α-Pinene	0.8±0.1		1437	α-Guaiene	0.3±0.0
954	Camphene	0.3±0.0		1444	Aromadendrene	0.2±0.0
976	Sabinene	1.4±0.2		1453	α-Humulene	2.1±0.0
979	β-Pinene	2.9±0.2		1460	Alloaromadendrene	1.3±0.0
982	1-Octen-3-ol	2.6±0.2		1467	*cis*-Muurola-4(14),5-diene	tr
993	Myrcene	0.2±0.0		1477	*trans*-Cadina-1(6),4-diene	0.9±0.0
1024	*p*-Cymene	0.5±0.0		1481	Germacrene D	0.3±0.0
1028	Limonene	0.5±0.1		1483	γ-Selinene	0.2±0.0
1031	1,8-Cineole	1.2±0.0		1490	β-Selinene	0.2±0.0
1059	γ-Terpinene	tr[c]		1495	*epi*-Cubebol	1.0±0.0
1073	*cis*-Linalool oxide (furanoid)	0.1±0.0		1501	α-Muurolene	0.3±0.0
1089	*trans*-Linalool oxide (furanoid)	0.2±0.0		1516	Cubebol	1.4±0.0

1101	Linalool	6.2±0.2		1524	δ-Cadinene	0.5±0.0
1114	1-Octen-3-yl acetate	tr		1552	(*Z*)-Caryophyllene oxide	1.1±0.1
1138	*trans*-Pinocarveol	0.2±0.0		1583	Caryophyllene oxide	21.7±0.9
1143	Camphor	5.9±0.2		1594	Salvial-4(14)-en-1-one	0.7±0.2
1165	Borneol	0.3±0.0		1610	Humulene epoxide II	2.2±0.1
1172	Menthol	0.4±0.0		1629	1-*epi*-Cubenol	3.8±0.2
1176	Terpinen-4-ol	0.9±0.0		1643	τ-Muurolol	0.7±0.1
1190	α-Terpineol	0.4±0.0		1646	α-Muurolol (= Torreyol)	0.5±0.0
1194	Myrtenol	0.1±0.1		1655	α-Cadinol	1.2±0.2
1196	Myrtenal	0.2±0.0		1656	Pogostol	1.5±0.1
1208	Verbenone	0.1±0.0		1804	Unidentified sesquiterpenoid	1.7±0.2
1337	δ-Elemene	0.4±0.0		1836	Unidentified sesquiterpenoid	1.1±0.0
1349	α-Cubebene	tr		1860	Unidentified sesquiterpenoid	1.3±0.1
1375	α-Copaene	3.8±0.1		2176	Unidentified diterpene	0.8±0.2
1383	β-Bourbonene	1.4±0.0		2189	Unidentified diterpenoid	1.1±0.1
1390	β-Cubebene	0.1±0.0		2217	*ent*-Trachyloban-3-one	8.1±0.8
1392	β-Elemene	0.3±0.0		2240	*ent*-Trachyloban-3β-ol	0.9±0.1
1398	Cyperene	0.3±0.0		2253	Isopimara-7,15-dien-3β-ol	1.9±0.1
1419	β-Caryophyllene	8.8±0.1			Total Identified	93.9

[a] RI = "Retention index" determined on an HP-5ms column based on a series of *n*-alkanes.
[b] Composition (average ± standard deviation) based on three separate analyses.
tr = "trace" (< 0.05%).

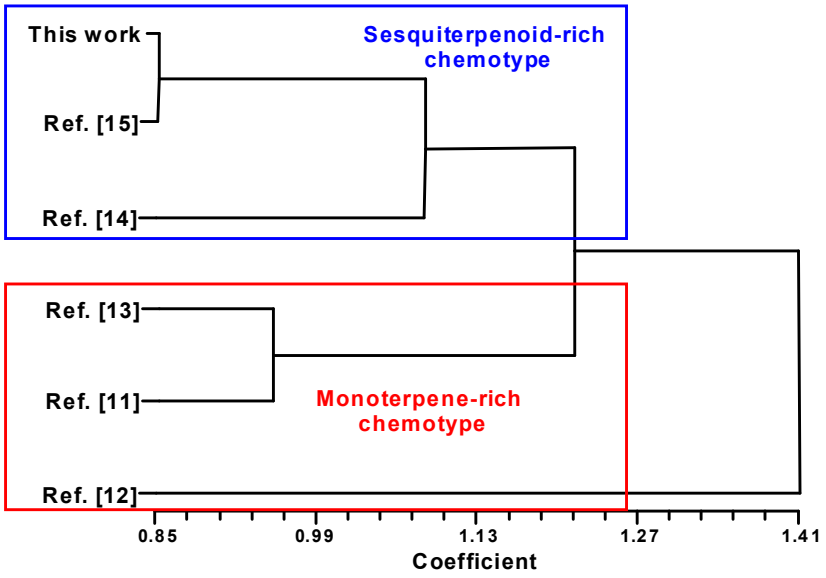

Fig 1: Dendrogram obtained by cluster analysis of the percentage composition of essential oils from *Croton zambesicus* samples, based on correlation and using the unweighted pair-group method witharithmetic average (UPGMA).

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