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Chemical Composition of Essential Oils from the Leaves, Seeds, Seed-pods and Stems of *Bixa orellana* L. (Bixaceae)

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Authors' contributions

This work was carried out in collaboration between all authors. Author AOGA designed the study and wrote part of the manuscript. Author AA isolated the essential oils. Author OAL managed the analyses of GC and GC/MS and wrote part of the manuscript. Author IAO managed the literature searches and wrote the final draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of the present study is to report the volatile compounds identified in the essential oils obtained by hydrodistillation from the various parts of *B. orellana*.
Study Design: The design includes the extraction of essential oil from air-dried leaves, seeds, seed-pods and stems samples of *B. orellana* and the determination and identification of the chemical constituents of the oils.
Place and Duration of Study: Mature leaves, seeds, seed-pods and stems of *B. orellana* were collected along LASU-Isheri Road in Alimosho Local Government Area, Lagos State, Nigeria, in May, 2014.
Methodology: Air-dried and macerated leaves, seeds, seed-pods and stems of *B. orellana* were

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hydrodistilled in a Clevenger-type apparatus to obtained colourless volatile oil whose chemical constituents was analyzed by GC-FID and GC/MS.
Results: A total of twenty-one different compounds were identified, out of which a range of 9–20 constituents were identified, accounting for 91.4–98.1% of the oil samples that are predominantly sesquiterpenes (40.8 – 94.9%), except for the seed oil with a higher amount of monoterpenes (45.0%). The leaf oil was characterized largely by α-guaiene (49.3%). The seed oil had α-pinene (29.1%) and β-pinene (14.5%). α-Guaiene (25.0%), spathulenol (12.0%) and caryophyllene oxide (9.7%) were the significant compounds of the seed-pot. The main constituents of the stem oil were caryophyllene oxide (38.8%), β-caryophyllene (22.3%) and myrtenyl acetate (12.5%).
Conclusion: The present study shows that the composition of *B. orellana* essential oils differed from each other and from data reported previously from other parts of the world.

Keywords: Bixa orellana; Bixaceae; essential oil; α-guaiene; caryophyllene oxide; α-pinene; β-caryophyllene.

1. INTRODUCTION

Bixa orellana L. (Bixaceae) commonly called annatto is an evergreen tropical shrub or small tree that is native to Brazil, Central and South America. But now grows in many tropical countries of the world [1,2]. It grows between 3 to 5 m tall as a shrub, but as a tree can sometimes rise to 10 m in height [1-3]. The leaves (*ca* 10 to 20 cm long and 5 to 10 cm wide) are simple, alternate, ovate to heart-shaped with attractive pink bisexual flowers and extended petioles. The seed (0.3–0.5 cm long and 0.2-0.3 cm broad) with a reddish aril has various shapes [4]. This plant was reported to have medicinal uses ranging from treatment of fevers to dysentary and cancers [1-4]. *Bixa orellana* also possesses hypocholesterolemic effect [5], antioxidant and antibacterial activities [6], antimicrobial potentials [7] and acts as natural preservatives [8]. In addition, anti-inflammatory [9,10], antihistamine [11], antimalarial [12], and mutagenic and cytotoxic activities [1] of the plant have been documented.

Bixa orellana aqueous extract was found to contain acetic acid (19.9%) as its major compound [10]. The main pigments of annatto have been identified as bixin, isobixin, norbixin and geranyl geraniol [4]. Moreover, apocarotenoids such as methyl (7Z, 9Z, 9Z')-apo-6'-lycopenoate,methyl (9Z)-apo-8'-lycopenoate, methyl (all-*E*)-apo-8'-lycopenoate, methyl (all-E)-8-apo-beta-carotene-8'-oate have been isolated from the seed of the plant [13]. Other phytochemical compounds present in *B. orellana* includes isobixin, β-carotene, cryptoxanthin, lutein, zeaxanthin, orellin, bixein, bixol, crocetin, ishwarane, ellagic acid, salicylic acid, threonine, tomentosic acid, tryptophan, and phenylalanine [4]. A dichloromethane extract of the air-dried leaves of *B. orellana* afforded ishwarane (with gastrointestinal, analgesic and antifungal activities), phytol, polyprenol, stigmasterol and sitosterol [14]. The terpenes, farnesyl acetone, geranylgeranyl octadecanoate and geranylgeranyl formate were also isolated from the plant [15]. Several antimalarial compounds such as ellagic acid, maslinic acid, inositiol, arjunolic acid and ursolic acid were also characterized from the root culture of *B. orellana* [16]. Furthermore, 9¢-cis-norbixin, all-trans-norbixin, zeaxanthin, methyl (9Z)-60-oxo-6,50-diapocaroten-6-oate and lutein were some of the phytochemicals of *B. orellana* with many possessing antifungal and antimalarial actions [17].

The main compounds of the volatile seed oil of *B. orellana* were (*Z*,*E*)-farnesyl acetate (11.6%), occidentalol acetate (9.7%), spathulenol (9.6%) and ishwarane (9.1%) [18]. Ishwarane was the major compound in the leaf oil [19]. The essential oil from *B. orellana* seeds which displayed antileishmanial activity against *Leishmania amazonensis* were found to have ishwarane (18.6%) and geranylgeraniol (9.1%) as the major components [20]. In addition to major terpenes such as -pinene, β-pinene, germacrene B, valencene, -elemene, spathulenol and β-humulene, several alcohols, aldehydes, ketones, esters and carboxylic acids were identified from different extracts of *B. orellana* [21-23]. The essential oils of *B. orellana* were reported to posse's mosquito repellency and larvicidal activities [24].

The aim of the present study is to report the volatile compounds identified in the essential oils obtained by hydrodistillation from the various

parts of *B. orellana*. This is in continuation of an extensive study on the essential oils from aromatic and medicinal plants from the flora of Nigeria origin [25-28].

2. MATERIALS AND METHODS

2.1 Plant Materials

Fresh plant materials of *B. orellana* were collected along LASU-Isheri Road (6°36′38″N 3°17′45″E) in Alimosho Local Government Area, Lagos State, Nigeria, in May, 2014. The identification of *B. orellana* was carried out at the Department of Botany, University of Lagos, Nigeria. A voucher specimen (LUH 5997) was deposited at the University Herbarium.

2.2 Oil Isolation

Air-dried and crushed samples of leaves (200 g), seeds (50 g), seed-pods (75 g) and stem (100 g) of *B. orellana* were separately subjected to hydrodistillation in a Clevenger-type glass apparatus for 3 h in accordance with the British Pharmacopoeia specification [29]. The distilled oil was preserved in a sealed sample tube and stored under refrigeration until analysis.

2.3 GC Analyses

GC analyses of the oils were carried out on a Hewlett Packard HP 6820 Gas Chromatograph equipped with a FID detector and DB-5 column (60 m x 0.25 mm id), film thickness was 0.25 µm and the split ratio was 1:25. The oven temperature was programmed from 50℃ (after 2 min) to 240℃ at 5℃/min and the final temperature was held for 10 min. Injection and detector temperatures were 200℃ and 240℃, respectively. Hydrogen was the carrier gas. An aliquot (0.5 µL of the diluted oil) was injected into the GC. Peaks were measured by electronic integration. A homologous series of *n*-alkanes (C_6 – C_{24}) were run under the same conditions for determination of retention indices.

2.4 Gas Chromatography- Mass Spectrometry

GC-MS analyses of the oils were performed on a Hewlett Packard Gas Chromatography HP 6890 interfaced with Hewlett Packard 5973 mass spectrometer system equipped with a HP 5-MS capillary column (30 m x 0.25 mm id, film thickness 0.25 µm). The oven temperature was programmed from 70- 240℃ at the rate of 5℃/min. The ion source was set at 240℃ and electron ionization at 70 eV. Helium was used as the carrier gas at a flow rate of 1 ml/min. Scanning range was 35 to 425 amu. Diluted oil in *n*-hexane (1.0 µL) was injected into the GC/MS.

2.5 Identification of Compounds

The identification of compounds was performed on the basis of 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 NIST 08 Libraries (on ChemStation HP) and Wiley 9th Version 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 [30,31]. The relative amounts of individual components were calculated based on the GC (HP-5MS column) peak area (FID response) without using correction factors.

3. RESULTS AND DISCUSSION

The hydrodistilled essential oils form the leaves, seeds, seed-pods and stems of *B. orellana* were pale yellow coloured and were obtained in yields of 0.21%, 0.65%, 0.17% and 0.11% (w/w) respectively. The percentage composition of the oils from the different organs of *B. orellana* and the components identified are listed in Table 1 in order of their elution on a DB-5 column. A total of twenty-one different compounds were identified, out of which a range of 9–20 constituents were identified, accounting for 91.4–98.1% of the oil samples that are predominantly sesquiterpenes. The leaf oil with 83.3% sesquiterpenes hydrocarbons was characterized by large amounts of α-guaiene (49.3%), along with guaiol (8.1%), valencene (7.7%) and β-elemene (5.9%). The seed-pod oil (45.8%) and stem oil (38.8%) of oxygenated sesquiterpenes have quantitatively significant compounds such as α-guaiene (25.0%), spathulenol (12.0%) and caryophyllene oxide (9.7%), and caryophyllene oxide (38.8%), β-caryophyllene (22.3%) and myrtenyl acetate (12.5%), respectively. However, the seed oil with highest amount of monoterpene hydrocarbons (45.0%) had α-pinene (29.1%), β-pinene (14.5%), germacrene D (10.0%) and spathulenol (9.0%) as its major compounds.

Table 1. Chemical composition of essential oils of *Bixa orellana*

Compounds[a]	RI[b]	RI[c]	Percentage composition			
			Leaf	Seed	Seed-Pod	Stem
3-Methylpentanol	856	852	-	-	2.0	-
α-Pinene	938	937	0.2	29.1	-	-
β-Pinene	981	979	-	14.5	-	-
Myrcene	994	992	-	1.4	-	-
1,8-Cineole	1034	1034	-	-	-	3.8
Linalool	1101	1101	-	-	-	2.1
Sabina ketone	1155	1156	-	-	-	3.7
Myrtenol	1203	1201	-	-	-	2.0
δ-Elemene	1338	1338	1.3	3.4	-	-
Myrtenyl acetate	1339	1341	-	-	-	12.5
α-Cubebene	1352	1349	0.1	-		
α-Copaene	1376	1376	0.8	-	-	-
β-Elemene	1389	1389	5.9	1.4	-	-
α-Terpinyl propionate	1389	1405	4.9	1.4	-	-
β-Caryophyllene	1426	1426	2.8	1.1	4.1	22.3
α-Gurjunene	1438	1436	0.5	2.2	-	-
α-Guaiene	1444	1439	49.3	5.5	25.0	-
α-Humulene	1461	1461	1.5	-	-	4.5
β-Cadinene	1474	1471	-	3.0	-	-
β-Chamigrene	1476	1475	-	1.2	-	-
allo-Aromadendrene	1481	1478	3.8	1.4	6.7	-
Germacrene D	1486	1483	-	10.0	-	2.8
β-Selinene	1491	1489	-	-	3.1	-
Valencene	1515	1511	7.7	-	-	-
δ-Cadinene	1522	1519	1.1	0.9	-	-
α-Panasinsene	1528	1525	3.6	0.6	1.5	-
Trimethyl hydroquinone	1539	1541	-	7.5	3.2	-
Spathulenol	1581	1579	1.3	9.0	12.0	-
Caryophyllene oxide	1589	1588	-	-	9.7	38.8
Cedrol	1601	1597	-	1.0	-	-
Guaiol	1629	1626	8.1	-	-	-
Globulol	1635	1632	-	2.5	6.6	-
Daucol	1642	1637	-	-	3.6	-
α-Cadinol	1654	1652	-	-	7.0	-
Bulnesol	1668	1665	2.2	-	-	-
Juniper camphor	1695	1691	-	1.0	6.9	-
Monoterpene hydrocarbons			0.2	45.0	-	-
Oxygenated monoterpenes			-	1.4	-	24.1
Sesquiterpene hydrocarbons			83.3	27.3	40.4	29.6
Oxygenated sesquiterpenes			11.6	13.5	45.8	38.8
Non-terpenes			-	7.5	5.2	-
Total identified			95.1	98.1	91.4	92.5

[a]Elution order on HP-5MS column; [b]RI (Lit.) - Literature retention indices; [c]RI (Cal.) - Retention indices relative to n-alkanes on HP-5MS column; - Not identified

Comparing the studied oil samples and reports from other countries on the compositions of essential oils of *B. orellana*, there are several major compositional variations [18-20,22,24]. Its noteworthy to mention that ishwarane, a major compound of the leaf and seed oils of *B. orellana* [18-20] was not present in the Nigerian oil sample. In addition, some compounds such as farnesyl acetate, occidentalol acetate and geranylgeraniol that are characteristics of the seed oils were not identified in the present study [18,20]. Furthermore, several alcohols, aldehydes, ketones, esters and carboxylic acids which characterized the water extracts of *B. orellana* [21-23] were not present in the Nigerian sample. This may be attributed to some factors

such as ecological and climatic conditions, nature and age of the plant, handling procedures, period of collection etc. However, the content of spathulenol competes favourably with previous studies [18,20]. But, the amounts of α-pinene, β-pinene, β-caryophyllene, myrtenyl acetate, germacrene D, caryophyllene oxide and guaiol in this study are significant higher when compared with previous studies [18,20,22].

4. CONCLUSION

The chemical composition of essential oils of *B. orellana* grown in Nigeria is been reported for the first time. The compositional patterns of the Nigerian samples were found to differ from the previous studies.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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