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Chemical profiles and brine shrimp toxicity of volatile oils hydrodistilled from stem bark and heartwood of *Ceiba pentandra* Linn.

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Abstract
Ceiba pentandra (Linn.) Gaertn. (Bombacaceae) is an important plant among the family of flowering plants. Ethnobotanical surveys have shown that the extracts from the plant possess significant medicinal properties. Because of the dearth of information on the essential oils (EOs) of the plant, the present study aim to investigate the chemical profile and brine shrimp toxicity of the EOs extracted from its stem bark and heartwood. The oils were analyzed by gas chromatography coupled with mass spectrometry. A total of 28 and 22 constituents were identified in the stem bark and heartwood oils equivalent to 96.7% and 76.8% of the whole EOs respectively. The stem bark oil was dominated by sesquiterpene hydrocarbons principally β-caryophyllene (28.7%), β-elemene (18.5%), α-muurolene (7.8%), and α-humulene (4.2%) while the heartwood oil was dominated by non-terpenes mainly 2-ethoxyacetate (11.3%) and nonanal (7.3%) along with α-eudesmol (21.1%) as the only major oxygenated sesquiterpene. The stem bark and heartwood oils exhibited good brine shrimp lethality with LC_{50} of 25.9 and 89.8 µg/mL, respectively. The chemical profiles and brine shrimps toxicity of these oils are been reported for the first time.

Keywords: *Ceiba pentandra*, essential oils, chemical profile, brine shrimp toxicity

1. Introduction

Substances of natural products have been a source of inspiration in new drugs discovery, which are able to prevent or treat many diseases. Interestingly, most of the drugs we use today are the product of extensive investigation of chemical profiles and therapeutic properties of plants. Plants are known to possess many medicinal values due to the presence of bioactive constituents in them known as phytochemicals. Phytochemicals are plant-derived chemicals that play a vital role in human health by decreasing the risk of cell damage which is one of the major causes of multiple health challenges of man. Examples are alkaloids, flavonoids, saponins, steroids, phenolics, tannins, glycosides, terpenes etc. Essential oils which are generally composed of terpenes, obtained from different plant organs by steam distillation or any suitable mechanical process without heating are also important natural products with a wide range of bioactivity [1]. Examples of essential oils of commercial importance are *Eucalyptus radiata* oil used as expectorant and antiviral agent against cold, flu and respiratory congestion in children, *Salvia sclarea* oil an antispasmodic, aphrodisiac and relaxant, *Lavandula stoechas* oil a neurotoxin if taken internally but when used as a topical spray it controls *Pseudomonas* infections [2]. Beside terpenes, essential oils can also contain non-terpenes like alkanes, alcohols, aldehydes, carboxylic acids, esters, ethers, apocarotenoids, phenylpropanoids, heterocyclic and aromatic compounds, which are important materials in perfumery, cosmetic, sanitary and pharmaceutical industries [3]. Literature showed that essential oils and their components are widely used as medicine for both animals and humans [4].

Ceiba pentandra (Linn.) Gaertner (commonly known as silk cotton tree) is an important plant among the Bombacaceae with many medicinal and pharmacological properties. Virtually, all the plant parts (leaves, stems, roots, gum, spine, flower, heartwood and seed) are used in traditional medicine as remedy for diabetes, dysentery, insect bite, arthritis, mental disorder, constipation, fever, dizziness, peptic ulcer, leprosy, edema, diarrhea, and head ache to mention few [5]. Among the pharmacological activities reported in the literatures are anti-inflammatory [6], angiogenesis [7], anti-diarrheal [8], antihelmintic [9], antihypertensive [10], antimicrobial [11],

antioxidant [12], hepatoprotective [13], hypoglycaemic [14], hypolipidemic [15], antiulcerogenic [16] and cytotoxicity [17].
Although, previous researchers have work extensively on the extracts from various organs of the plant [8, 11, 18], the essential oils from the stem bark and the heartwood have not yet been inspected. Thus, the present study was undertaken to examine the chemical profiles and brine shrimp toxicity of the volatile oils extracted from stem bark and heartwood of *Ceiba pentandra*.

2. Materials and Methods

2.1 Extraction of Essential Oils

The plant was collected at the boundary of Department of Chemistry, University of Ibadan (7° 26' 42.3" N/3° 53' 35.7" E), Nigeria and authenticated at Forestry Research Institute of Nigeria where a voucher specimen (FHI 112962) was deposited. The plant materials were air dried and pulverized before subjecting a known quantity to hydrodistillation for 3 h using an all glass Clevenger-type apparatus according to British Pharmacopeia specification (1980) [19]. The oils extracted were free of moisture by drying over anhydrous sodium sulfate, then stored in sealed glass vials and kept under refrigeration at 4 ℃ prior to analysis.

2.2 Gas Chromatography/Mass Spectrometry (GC-MS)

The GC-MS analysis of the oils was carried out using Schimadu GC-MS-QP2010 equiped with ZB-5 fused silica capillary column (Phenomenex, Torrance, CA, USA) with a (5% phenyl) polymethylsilosane stationary phase and film thickness of 0.25 µm. The GC-MS was operated at the electron impact mode of 70 eV, scan range 40-400 a.m.u. and scan rate 3.0 scans/s. Both the injector and the ion source temperature were 260 ºC. The GC oven temperature was programmed for 50 ℃ and then increased at the rate of 2 ℃/ min to 260 ℃. The carrier gas was helium with the column head pressure of 552 kPa and film thickness of 1.37 mL/min. A 5% w/v solution of the sample in CH_2Cl_2 was prepared and 0.1 µL was injected with a splitting mode of 30:1.
Identification of the essential oil components was based on their retention indices determined by reference to *n*-alkanes homologous series and by comparison of their mass spectral fragmentation pattern with those reported in the literature [20] and our own data base library [21].

2.3 Brine Shrimp Lethality Assay

The brine shrimp lethality assay was first proposed by Micheal *et al* in 1956 [22]. The assay is an important bioassay that predicts ability of natural products (including essential oils) to kill cancer cells in cell cultures [23]. Besides, it is also capable of detecting a broad spectrum of bioactivity present in a plant extracts [24] including pesticidal activity [25]. This assay is based on the ability of the essential oil to kill laboratory cultured brine shrimp (*Artemia salina* larvae). The assay was developed with three serial dilutions (1000 ppm, 100 ppm and 10 ppm) and in triplicate, in which 10 active larvae were introduced into 0.5 mL of each concentration making 30 shrimps per dilution. The survived larvae were counted after 24 h and the observed values were subjected to probit regression analysis [26] to evaluate the toxicity of the oils in terms of lethal concentration (i.e., LC_{50}) which is the concentration that kills 50% of the larvae.

3. Results and Discussion

3.1 Chemical constituents of *Ceiba pentandra* essential oils

Essential oils extracted from both stem bark and heartwood of *C. pentandra* were colorless with the percentage yield of 0.14 w/w and 0.09 w/w respectively. The GC-MS analysis results of the oils are given in Table 1. The essential oil constituents are listed in order of their elution from ZB-5 fused capillary column. A total of 28 compounds were identified in the stem bark oil equivalent to 98.8% of the whole oil. The volatile oil was mainly terpenes, dominated by sesquiterpene hydrocarbons (75.6%). The most prominent constituents were β-caryophyllene (28.7%), β-elemene (18.5%), α-muurolene (7.8%), caryophyllene oxide (4.8%) and α-humulene (4.2%). β-Caryophyllene and caryophyllene oxide are used as food and cosmetic additive. Both compounds are approved by European Food Safety Authority and Food and Drug Administration as flavoring substance in food [27]. Previous studies also showed that they possess antioxidant [28, 29], anticancer [30, 31], anti-inflammatory [32, 33] and analgesic properties [34, 35]. Also, α-humulene has demonstrated reasonable cytotoxicity against cell lines [27, 36]. On the other hand, 22 components were identified in the heartwood oil which was equivalent to 76.8% of the total oil. The stem bark oil was largely non-terpenes (40.5%). The most abundant constituents were α-eudesmol (21.1%), 2-ethoxyacetate (11.3%) and nonanal (7.3%).
There was no report on the essential oils from stem bark and heartwood of *C. pentandra* but the floral essential oil showed sabinene (20.8%), (*E*, *E*)-α-farnesene (20.3%), 2-hexenal (8.9%), α-pinene (7.1%), α-copaene (5.5%) and 1,8-cineole (5.3%) as the most abundant components [37]. Chemical profiles of volatile oils from other Bignoniaceae plants have been investigated. Venkatesh *et al*., (2015) [38] gave account of the leaf and stem bark essential oils of *Cullenia exarillata* Robyns where palmitic acid (41.5%) and myristic acid (11.0%) were reported as the most prominent constituents in the stem bark oil which were not found in the present study. *Eriotheca longitubulosa* A. Robyns floral essential oil was dominated by α-farnesene (28.0%), germacrene-D (25,7%), *E*-β-ocimene (9.9%) and toluene (6.9%) [39], while the floral oil of *Adansonia digitata* L. contained appreciable quantity of 3-methylbutanol (16.3%), dimethyl disulfide (10.3%) and benzaldehyde (7.8%) as major compounds [37].

Table 1: Chemical constituents of *Ceiba pentandra* Essential oil

Constituents [a]	RI	Percentage composition	
		Stem bark	Heartwood
Methylcyclohexane	718	-	0.4
2-Methyl-2-pentanol	723	-	1.9
3-methyl-3-pentanol	744	-	1.1
1-Methylcyclopentan-1-ol	793	-	1.2
Hexanal	799	0.7	2.5
Octane	800	-	0.8
Styrene	890	-	0.7
Nonane	900	1.5	-
2-Ethoxyethyl acetate	903	-	11.3
α-Pinene	930	-	3.2
3-Octanone	983	0.8	-
2-Pentylfuran	987	-	0.7
1-Octanal	1002	-	0.8
Nonanal	1103	1.1	7.3
Decanal	1204	-	2.5
2-Undecanone	1290	-	0.8
β-Bourbonene	1380	0.7	-
β-Elemene	1386	18.5	-
Vanillin	1391	1.7	-
Geosmin	1410	-	0.6
β-Caryophyllene	1416	28.7	
Geranyl acetone	1444	0.8	4.5
(*E*)-β-Farnesene	1449	1.1	-
α-Humulene	1452	4.2	-
allo-Aromadendrene	1457	0.5	-
γ-Muurolene	1471	1.5	-
Germacrene D	1477	0.7	-
ar-Curcumene	1477	-	1.2
Bicyclogermacrene	1492	2.3	-
α-Muurolene	1495	7.8	-
γ-Cadinene	1509	1.6	-
Cubebol	1511	1.0	-
δ-Cadinene	1514	0.8	-
α-Bulnesene	1515	-	2.5
Germacrene-D-4-ol	1573	2.4	-
Caryophyllene oxide	1575	4.8	-
10-*epi*- γ-Eudesmol	1628	-	0.8
τ-Cadinol	1638	0.9	-
α-Eudesmol	1651	-	21.1
α-Cadinol	1652	1.0	-
Phytone	1837	1.9	-
(5*E*,9*E*)-Farnesyl acetone	1904	-	3.0
Methyl palmitate	1921	1.4	-
Methyl stearate	2121	1.4	0.9
1-Docosanol	2484	1.0	7.0
Pentacosane	2500	0.8	-
Total Identified		28	22
Percentage identified		98.8	76.8
Monoterpene hydrocarbons		-	3.2
Oxygenated monoterpenes		0.8	4.5
Sesquiterpene hydrocarbons		75.6	3.7
Oxygenated sesquiterpenes		10.1	24.9
Non terpenes		12.3	40.5

RI- Retention determined with respect to a series of *n*-alkanes on a ZB-5 column; [a]-Order of elution from a ZB-5 fused silica capillary column;

3.2. Brine shrimp lethality of *Ceiba pentandra* essential oil

The result of the brine shrimp lethality assay is given in Table 2. The mortality rate increases as the concentration increases. Negative control was likewise subjected to the same procedure to validate the test method and no mortality was observed, indicating the solvent has not contributed to the activity of the oil samples. The concentration that kills 50% of the larvae (LC_{50}) was calculated from the figures recorded from survived and dead shrimps after 24 h. According to Meyer *et al*. (1982) [25] who classified plant phytochemicals using their LC_{50} values as highly toxic when the value is less than 100 µg/mL, toxic for values between 100 and 500 µg/mL, moderately toxic when less than 1000 and greater than 500 µg/mL and non-toxic if greater than 1000 µg/mL. Thus, both the stem bark oil (LC_{50} = 25.9 µg/mL) and the heartwood oil (LC_{50} = 89.8 µg/mL) showed high cytotoxic effect. Caryophyllene oxide, β-caryophyllene and α-humulene identified in appreciable quantity in the stem bark oil are known to possess cytotoxic effect against tumor cells [30, 31, 36] and their presence as dominant constituents in essential oils has been reported to improve the cytotoxic activity of the oil. This might have contributed to the higher toxicity of the stem bark oil. Besides, the toxicity of the stem bark may also be attributed to the synergistic effect of other minor constituents. Apart from the fact that brine shrimp lethality assay can predict the cytotoxicity and anti-tumor properties of plant extracts [40], it also helps to envisage the pharmacological activity of the plant extracts [41]. High lethal concentration indicates high pharmacological activity [22].

Table 2: Brine shrimps lethality activity of *Ceiba pentandra* Essential oil

Essential oils	LC_{50}	LC limit	UC limit	CL
Stem bark	25.92	16.35	40.91	0.1363
Heartwood	89.82	67.63	107.12	0.1843

LC-Lower confidence, UC-Upper confidence, LC_{50}- Lethal concentration, CL-Confidence limit

4. Conclusions

The essential oil hydrodistilled from *Ceiba pentandra* L. stem bark and heartwood were dominated by sesquiterpene hydrocarbons and non-terpenes respectively. The result from this study suggests both oils could possess anti-tumor and other pharmacological activities. Hence, the oils should be tested against human cell lines and more bioassays should be carried out to validate their bioactivity.

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