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Chemical Constituents of Essential Oil of *Ehretia cymosa* Thonn

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Authors' contributions

This work was carried out in collaboration between all authors. Authors ALO and IAO designed the study. While author IAO wrote the first draft of the manuscript and managed literature searches. Authors CON and ROO performed the hydrodistillation supervised by author ALO. In addition, author IAO managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The chemical constituents of essential oil obtained by hydrodistillation of the leaves of *Ehretia cymosa* are being reported. The essential oils were obtained by hydrodistillation in a Clevenger-type apparatus and analyzed by gas chromatography (GC-FID) and gas chromatography coupled with mass spectrometry (GC-MS). The components of the essential oils were identified by comparison of their mass spectra (MS) data and linear retention indices (LRI) with literature data. The essential oil content was 0.32% (v/w), calculated on a dry weight basis. The leaves oil of *E. cymosa* comprised mainly of sesquiterpene hydrocarbon compounds represented mainly by *trans*-α-bergamotene (15.2%), α-curcumene (14.5%) and β-cedrene (14.0%). The chemical compositions of the essential oil from the leaves of the plant are being reported for the first time.
Aims: The aim of this study was to report the volatile constituents identified from hydrodistilled oil from the leaves of *Ehretia cymosa*.
Study Design: The study design involves the collection of the leaves sample and hydrodisitillation

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of essential oil from the plant material as well as subsequent analysis of the oil for its chemical composition.
Place and Duration of Study: Mature leaves of *E. cymosa* were collected from Amuoloko area, Akanran, Ibadan, Oyo State, Nigeria, in May 2015.
Methodology: About 600 g of air-dried plant sample was pulverized and the hydrodistillation was carried out according to the British Pharmacopoeia specifications to obtained colourless oil. The chemical constituents of the hydrodistilled oil were analyzed by using the techniques of gas chromatography (GC-FID) and gas chromatography coupled with mass spectrometry (GC-MS).
Results: The major constituents of the essential oil were sesquiterpene hydrocarbons represented mainly by *trans*-α-bergamotene (15.2%), α-curcumene (14.5%) and β-cedrene (14.0%).
Conclusion: The chemical constituents of essential oil of *E. cymosa* are being reported for the first time. The oil contained large amount of sesquiterpene compounds.

Keywords: Ehretia cymosa; hydrodistillation; sesquiterpenes; trans-α-bergamotene; α-curcumene; β-cedrene.

1. INTRODUCTION

Recent attempts aimed at the characterisation of the chemical constituents and biological activities of the essential oils from plants growing in Nigeria have produced interesting results which have been published [1,2]. The main classes of compounds identified in these essential oils were monoterpenes, sesquiterpenes, alcohols, phenylpropanoids, esters and fatty acids. In addition, some of the studied volatile oils have exhibited biological activities such as antimicrobial, phytotoxicity, insecticidal and cytotoxicity [3,4].

Ehretia cymosa is a deciduous shrub or small tree, only growing up to 7 m tall in the western parts of its range, but can sometimes reach heights of 20-25 m recorded from Guinea to Southern Nigeria. It is a glabrous shrub with ovate leaves and white flowers. The fruit is black, ovoid to globose drupe, 2-6 mm long. The greyish brown wood has alternate darker and lighter bands. The tree has a number of uses, supplying medicines, food and wood for the local population. It has potential for use as an ornamental. The fresh leaves are sometimes used in sauces. *Ehretia cymosa* are used in traditional folklore medicine for treatment of diarrhea [5]. The leaves have been used for treatment of measles (viral diseases) among the people of Southern Western Nigeria [6] and also used as febrifuge, laxative and pain-killer as well as amelioration of paralysis, epilepsy, convulsions and spasm [7]. Sap from the fresh leaves is a mild laxative for children. The bark decoction is used to regulate menstrual cycle [7] and pneumonia [8]. The use of the plant for the control of venereal diseases [9], epilepsy, dry cough, malaria, tonsils, mental problems, asthma, typhoid, wounds, and aphrodisiac [10,11] have been reported.

Extracts from *E. cymosa* showed significant antibacterial effects on both gram-negative and gram-positive bacteria [12]. The extract displayed strong antioxidant activity and also exhibited inhibitory activity against *Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis and Staphylococcus aureus* [13]. Various extracts of the plant were reported to possess significant antihyperglycemic and antioxidant [13] potentials. A previous phytochemical screening conducted on the extract of the plant revealed the presence of alkaloids, saponins, glycosides, terpenoids, anthraquinones, phenolics and flavonoids in varying quantity [14].

It is well known that several *Ehretia* plants have been studied for their biological activities such as antibacterial [15,16], antioxidant [17], anti-venom [18], stimulation of reproductive function [19], and histamine release inhibitory [20]. However, little information is known about the biological potentials of *E. cymosa*. In addition, Ehretia plants have been studied for their non-volatile components [18,19,21-25]. However, there is lack of information on the volatile constituents from members of this genus including *E. cymosa*. This arouses our interest in this research into the volatile compositions of this relatively unexploited flora of Nigeria.

2. MATERIALS AND METHODS

2.1 Plant Sample

Mature and fresh leaves of *E. cymosa* were collected from a location in Amuloko area, Akanran, Ibadan, Oyo state, Nigeria, in May

2015. The plant sample was authenticated by Mr. Soyewo of the Herbarium Forestry Research institute of Nigeria (FRIN), Ibadan, Nigeria where a voucher specimen FHI 110189 was deposited for future reference.

2.2 Hydrodistillation of the Oil Sample

Pulverized air-dried leaves (600 g) of *E. cymosa* were subjected to hydrodistillation in a Clevenger-type glass apparatus for 3 h in accordance with the British Pharmacopoeia specification [26]. Briefly, 600 g of the pulverized sample were carefully introduced into a 5 L flask and distilled water was added until it covers the sample completely. Hydrodistillation was carried out in an all glass Clevenger-type distillation unit designed according to the specification. The volatile oil distilled over water and was collected in the receiver arm of the apparatus into a clean and previously weighed sample bottle. The oil was kept under refrigeration at 4℃ until the moment of analysis.

2.3 Gas Chromatography (GC) Analysis

GC analysis of the oil was carried out on a Hewlett Packard HP 6820 Gas Chromatograph equipped with a FID detector and HP-5MS 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. Retention indices (RI) value of each component was determined relative to the retention times of a homologous *n*-alkane series with linear interpolation on the HP-5MS column. The relative amounts of individual components were calculated based on the GC peak area (FID response) without using correction factors.

2.4 Gas Chromatography- Mass Spectrometry (GC-MS) Analysis

GC-MS analysis of the oil was 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 70eV. 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 the Constituents of the oil Sample

The identification of constituents 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 [27] 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 [28].

3. RESULTS AND DISCUSSION

The yield of essential oil was 0.32% (v/w) calculated on a dry weight basis. The oil sample was light yellow in colouration. Table 1 indicates the chemical constituents present in the oil, their percentages as well as retention indices on HP-5MS column. The sesquiterpene hydrocarbons (85.4%) and oxygenated monoterpenes (11.6%) represent the abundant classes of compounds identified in the oil. Monoterpene hydrocarbons and oxygenated sesquiterpene compounds were not detected in the oil sample. All the 12 constituents of the essential oil were identified in amount above 2%. The main constituents of the oil which are *trans*-α-bergamotene (15.2%), α-curcumene (14.5%) and β-cedrene (14.0%) belong to the sesquiterpene hydrocarbon class of compound. There were significant quantities of β-ylangene (9.5%), β-damascenone (9.3%), methyl salicylate (8.9%), isocaryophyllene (7.5%) and β-bisabolene (7.1%). Essential oil with high content of *trans*-α-bergamotene and α-curcumene was recently reported [29].

The phytoconstituents of volatiles from some other *Ehretia* plant revealed that the main compounds identified in the ethanol leaves extract of *E. abyssinica* were octadecenamide (5.77%), lucenin (5.46%), docosane (3.75%) and nonacosane (3.75%). The extracts displayed significant antibacterial activity [15]. The hexane extract of *E. laevis* was found to be dominated by hydrocarbons such as docosane, 1-tridecene, tetradecane among others [30]. A comparative

Table 1. Chemical constituents of essential oil of *E. cymosa*

Compounds [a]	RI (Cal.)	RI (Lit.)	Percent composition
Linalool	1100	1095	2.7
trans-Sesquisabinene hydrate	1100	1098	3.4
Methyl salicylate	1190	1197	8.9
o-tert-Butylphenol	1272	1273	3.0
β-Ylangene	1372	1374	9.5
β-Damascenone	1386	1388	9.3
β-Cedrene	1410	1413	14.0
β-Caryophyllene	1419	1417	4.9
trans-α-Bergamotene	1430	1432	15.2
Isocaryophyllene	1461	1461	7.5
ar-Curcumene	1485	1481	14.5
β-Bisabolene	1506	1505	7.1
Total			100.0
Monoterpene hydrocarbons			-
Oxygenated monoterpenes			11.6
Sesquiterpene hydrocarbons			85.4
Oxygenated sesquiterpenes			-
Non-terpenes			3.0

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

analysis of the compositional patterns of volatiles of *E. cymosa* with other *Ehretia* species grown elsewhere revealed a marked variation in their chemical constituents. The hydrocarbons compounds present in *E. abyssinica* and *E. laevis* were not identified in *E. cymosa* which consisted mainly of terpene compounds.

The present study may represent the first of its kind aimed at the characterization of volatile compounds of *E. cymosa*. This result could contribute positively to the chemotaxonomic study of volatiles of *Ehretia* plants grown all over the world. The biological activities of an essential oil may be due to the effect of the major compound(s) or synergy between the major and some minor compounds present in the oil [3,4]. Referring to literature, for example, *trans*-α-bergamotene was reported to possess anti-rhinoviral, anti-ulcer and anti-viral activities [31] while essential oil containing a sizeable proportion of ar-curcumene has shown antioxidant potential [32]. In additional, the anti-termite activity of β-cedrene has been reported [33]. It may be postulated that the components of the essential oil of *E. cymosa* may contribute to biological activity the plant might posses.

4. CONCLUSION

The chemical constituents of essential oil of *E. cymosa* are being reported for the first time. The oil contained large amount of sesquiterpene compounds of which *trans*-α-bergamotene, α-curcumene and β-cedrene occurred in higher quantity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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