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Composition and Acetyl Cholinesterase Inhibitory Properties of the Essential Oil of *Xylopia Aethiopica* (Dun) A. Rich (Annonaceae) Stem Bark from South East Nigeria

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Abstract
The chemical composition of the essential oil of *Xylopia aethiopica* stem bark from Nigeria was investigated by gas chromatography/mass spectrometry analyses. A total of 57 compounds were identified in the oil, thus representing 94.6% of the total oil and the major constituents include ϒ-terpineol (4.9%), benzaldehyde (4.9%), cyperene epoxide (5.8%), camphene (6.3%), α-guaiol (6.6%), α-thujene (9.5%) and tricyclene (16.5%). The monoterpene hydrocarbons and oxygenated monoterpene formed the main portion in the oil with 42.5% and 28.9% respectively. The acetyl cholinesterase inhibitory property of the essential oil was investigated comparing it with the standard drug Huperzine A. The oil recorded a significant inhibitory concentration against the enzyme acetyl cholinesterase at the minimum concentration of 5 ppm; an indication that the essential oil can be explored in treatment of cases related to memory improvement.

Key words: *Xylopia aethiopica*, essential oil, monoterpenoids, acetyl cholinesterase enzyme, Huperzine A.

Introduction

The genus *Xylopia* (Annonaceae) comprises many species, which occur in the tropics, especially Africa [1]. The West Africa pepper tree, *Xylopia aethiopica* which is one of the species, is a tree that can be found growing in the wild in Nigeria [2]. The tree has been used locally in herbal medicine as calmatives, antiemetic agents, stimulant, appetizer, and additive to other remedies for the treatment of skin infections, attacks of asthma, stomach-aches, rheumatism, and for the management of cough and fever [3].

Literature has revealed many studies of the fruits and seeds of *X. aethiopica* [4,5,6,7,8,9,10,11,12,13,14,15], but no detailed research has been reported on the stem bark essential oil from Nigeria. However, Karioti et al. in 2004 [16] characterized the essential oils from different parts of the plant including the stem bark growing in Ghana. Enzyme inhibition is an important aspect of pharmaceutical research which has led to the development of a wide variety of drugs that are useful for treating a number of diseases [17]. Cholinesterase is an enzyme of the body necessary for proper nerve function. The enzyme acetyl cholinesterase (AChE) present in nerve tissue, muscles and red blood cells catalyses the hydrolysis of acetylcholine (ACh) to choline and acetic acid. Compounds which block the action of the enzyme acetyl cholinesterase are called "Acetyl choline-sterase inhibitors". AChE inhibitors are the most effective approach to treat the cognitive symptoms of Alzheimer disease (AD) [8,18] and other possible therapeutic application in the treatment of Parkinson's disease, senile, dementia, and ataxia, among others [19]. This present study is a continuation of our effort targeted at characterizing and screening extracts from medicinal plants for the treatment of ailments related to memory loss in human beings [20]. This is the first report, to the best of our knowledge, on the acetyl cholinesterase inhibitory property of the essential oil of *X. aethiopica*.

Materials and Methods

The stem barks of the plant were collected in Ikot Abasi in Akwa-Ibom State, Southeast Nigeria. The sample was authenticated at the

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Forest Herbarium, Forest Research Institute of Nigeria (FRIN), where voucher specimens were deposited (FHI 107128).

Plant Preparation
The sample was air dried for 7 days and pulverized prior to extraction.

Isolation of Essential Oil
Pulverized sample (300g) was hydrodistilled for 4 hrs in an all glass Clevenger distillation unit, according to the British Pharmacopoeia specification [21].

Gas chromatography (GC) Analysis
Gas chromatographic analysis (GC) of the oil was carried out on an Orion Analytical Micromat gas chromatography fitted with a thermal conductivity detector (TCD). The separation was achieved by capillary columns of different polarities, CPSil-5 (25 m x 0.25 mm i.d), equivalent to OV 101, and CPSil-19 (25 m x 0.25 mm i.d) similar to BP10 with film thickness of 0.15µm. The column temperature was program-med from 50^{o}C to 230^{o}C at 3^{o}C/min. The injector and detector temperatures were maintained at 200^{o}C and 250^{o}C respectively. The carrier gas was hydrogen at a pressure of 0.5 bar and a flow rate of 1.20 mL/min.

Gas chromatography-Mass spectrometry (GC/MS) Analysis
Gas chromatography-Mass spectrometer (GC-MS) analysis of the essential oil was performed on a Hewlett-Packard Gas Chromatography (GC) HP5890A, interphased with a VG Analytical 70-250s double focusing mass spectrometer, operating at 70eV, with an ion source temperature of 230^{o}C. The GC was fitted with a 25m x 0.25mm i.d fused silica capillary column coated with CPSil-5. Helium was used as the carrier gas at a flow rate of 120mL/min.

The GC operating parameters were identical with those of the GC analyses. The mass spectral data were acquired and processed by an on-line desktop computer. The constituents were identified based on comparison of the retention times with those of the authentic samples, comparing their retention indices relative to the series of n-hydrocarbons, and by comparison of their mass spectra with published spectra and those of reference compounds from NIST 98 (Adams; Davies). The relative concentration of each constituent was calculated by integration of GC peak areas [22, 23].

Enzyme assay
Fresh sheep liver was obtained from a healthy sheep just after being slaughtered. A 1% homogenate w/v of the sheep liver was prepared in double distilled water at 0^{o}C. The cholinesterase inhibitory properties of the test compounds were done by colori-metric method but with some modifications. Three different concentrations (5, 10, 20 µg/0.1mL of propanone) of the essential oils were prepared separately in test tubes and the solvent propanone was allowed to evaporate. 0.1mL of the 1% sheep liver homogenate was pre-incubated with 5, 10, 20 µg of the essential oils, separately for 15 minutes at 37^{o}C in a thermostatic water bath. A positive control Huperzine A was adopted. The Huperzine A technical grade (obtained from Sigma Co. U.S.A) was also pre-incubated in the same manner described above with 0.1mL of 1% (w/v) homogenate at 5, 10, 20 µg amounts to allow inhibition of liver cholinesterase by test compounds.

Immediately after pre-incubation, 0.2 mL of 0.2% fast blue B (a diazotized product of 4-benzoylamino-2,5-dimethoxy aniline-$ZnCl_2$) in water was added followed by 0.1 mL of 0.01 M ethylacetoacetate substrate in propanone and the reaction mixture was again incubated for one minute for enzymatic reaction. The reaction mixture was made up to a total of 1.0 mL with distilled water prior to addition of substrate. The enzymatic incubation time was 1 minute after the addition of substrate. The enzyme activity was stopped at the end of exactly 1 minute by adding 4 mL of glacial acetic acid. The magenta colour developed was read at 540 nm with the aid of a UV spectrophotometer. The control enzyme reaction mixture was done the same way as the samples but without any essential oil. The experiment was conducted in triplicate at each dose level.

Results and Discussion

The bright yellow oil yield obtained from hydrodistillation of the stem bark of X. *aethiopica* was 0.50% (w/w). Fifty-seven constituents which accounted for 94.6% of the essential oil of *X. aethiopica* were identified (Table 1).

Table 1: Essential Oil Constituents of *Xylopia aethiopica* Stem Bark

S/N	Compound	RI	%
1.	tricyclene	933	16.5
2.	α-thujene	935	9.5
3.	α-pinene	943	1.6
4.	benzaldehye	947	4.9
5.	camphene	957	6.3
6.	sabinene	977	0.4
7.	β-pinene	981	2.9
8.	verbenene	993	3.1
9.	p-1-menthene	1017	0.2
10.	p-cymene	1021	0.1
11.	1,8-cineol	1030	0.2
12.	limonene	1031	0.5
13.	γ-terpinene	1057	0.2
14.	α-pinene epoxide (isomer)+	1077	0.5
15.	p-cymemene	1082	0.3
16.	terpinolene	1087	0.9
17.	dehydrosabinaketone	1099	0.3
18.	α-pinene epoxide (isomer)+	1113	0.6
19.	5,7-dimethyl-octa-1,6-diene	1119	T
20.	camphor	1129	0.7
21.	E-pinacarveol	1136	0.3
22.	menth-1,5-diene-8-ol	1142	0.6
23.	pinacarvone	1150	0.4
24.	isopinocamphone	1160	0.1
25.	Z-dihydrocarvone	1167	1.6
26.	terpinene-4-ol	1172	1.6
27.	myrtenal	1180	1.2
28.	γ-terpineol	1184	4.9
29.	myrtenol	1192	T
30.	p-isopropylbenzaldehyde	1220	3.3
31.	carvone	1223	0.2
32.	3-thujene-10-al	1231	3.9
33.	perilla aldehyde	1254	1.3
34.	isobornylacetate	1275	2.1
35.	E-pinocarvylacetate	1285	0.2
36.	α-cubebene	1352	0.2
37.	cyclosativene	1373	1.2
38.	α-copaene	1380	0.2
39.	β-elemene	1391	0.4
40.	cyperene	1405	0.3
41.	isocaryophyllene	1421	0.7
42.	selina-4(15),5-diene	1430	0.4
43.	selina-4(15),6-diene	1448	0.4
44.	rotundene	1461	0.2
45.	bicyclosesquiphellandrene	1489	0.1
46.	α-muurolene	1494	0.3
47.	cyperene epoxide	1523	5.8
48.	α-calacorene	1531	0.3
49.	elemol	1538	0.7
50.	caryophyllenoxide	1573	0.2
51.	α-Gusiol	1589	6.6
52.	1,10-di-epi-cubenol	1617	0.1
53.	cubenol	1632	0.6
54.	β-eudesmol	1641	0.6
55.	bulnesol	1656	0.6
56.	cyperotundone	1674	0.4
57.	cyperenal	1727	2.9
	TOTAL		**94.6%**

T = Trace amount (<0.1%)
RI= Kovat Indices
+ = correct isomer not identified

Table 2: Acetyl cholinesterase Enzyme Assay

Concentration (ppm)	Percentage inhibition of Huperzine A	Percentage inhibition of *X. aethiopica*
5	44.2	41.1
10	54.6	41.9
20	67.3	45.2

The main constituents of the essential oil were tricyclene (16.5%), α-thujene (9.5%), α-guaiol (7.6%) and camphene (6.3%). It consisted of 71.4% monoterpenoids out of which is 42.5% hydrocarbons, these are tricyclene (16.5%), α-thujene (9.5%), α-pinene (1.6%), camphene (6.3%), sabinene (0.4%), β-pinene (2.9%), verbenene (3.1%), p-menthene (0.2%), p-cymene (0.17%), 1,8-cineole (0.2%), limonene (0.5%), γ-terpinene (0.2%), p-cymememe (0.3%) and terpinolene (0.9%). The oxygenated monoterpene totalling 28.9% had γ-terpineol (4.9%), 3-thujene-10-al (3.9%) and p-isopropylbezaldehyde (3.0%) occurring in higher proportion among them. A simple aliphatic compound 5,7-dimethyl-octa-1,6-diene was found in trace amount. Sesquiterpenoids accounted for 23.2% with 4.7% sesquiterpene hydrocarbon and 18.5% for the oxygenated sesquiterpenes. The three oxygenated sesquiterpenes, α-guaiol (7.6%), cyperene epoxide (5.8%) and cyperenal (2.9%) were identified in substantial quantity.

Trans-m-menth-1(7),8-diene (30.7%), β-pinene (5.8%), α-pinene (9.7%), germacrene D (8.8%), cyperene (7.6%), and α-gurjunene (4.5%) were the prominent constituents of the same species from Ghana [16]. A close examination of the compositional features of the oils show some quantitative and qualitative variations. For example, tri-cyclene, α-thujene, benzaldehyde, camphene, verbenene, γ-guaiol which were present in significant amounts were not identified in the Ghanaian oil. α-pinene, β-pinene, p-cymene, γ-terpinene, terpinolene, camphor, E-pinacarveol, terpinene-4-ol, isobornylacetate, α-copaene, cyperene were identified as common constituents in the two volatile oils. In contrast, the Ghana essential oil had trans-m-mentha-1(17),8-diene (30.7%) as the major constituent.

The observed differences in chemical composition may be attributed to geographic and seasonal variations, effect of plant maturity at the time of oil production and probably the existence of chemotypic differences [24]. It has been reported that the quantitative composition and the relative proportion of oil component are widely influenced by the genotype, ontogenic development, environmental and growing conditions of a plant [25,26]. Essential oils have been found to be relevant in pharmaceuticals. Likewise, different plant families have been investigated for their AChE inhibitory activity; such as Amaryllidaceae [27,28] Boraginaceae [29], Chenopodiaceae [30], Laminaceae [31], and Liliaceae [32]. A study on the inhibitory effects of seven diterpenes isolated from the combined hexane and ethylacetate extract of the back of *Xylopia aethiopica* against the enzymes propyl endopeptidase (PEP) and thrombin have been reported [33]. It revealed that five of the compounds possessed dose-dependent inhibitory activity against PEP compared to positive control bacitracin.

The essential oil in the present study displayed varying acetyl cholinesterase inhibitory properties at the three tested concentrations (Table 2). The percentage inhibition of the oil was significant when compared to positive control Huperzine A at the lowest dose level of 5ppm. This is an indication of the probability of the effectiveness of *Xylopia aethiopica* in the

treatment of ailment related to memory impairment. The observed activities are similar when compared to essential oils from some plants reported; Dohi et al., 2009 [34], reported the AChE inhibitory activity of some commercial essential oils, of which *Artemisia dracunculus* L showed the most potent inhibitory activity of 58.0 µg/mL while that from the bark of *Peltophorum dasyrachis* Kurz ex Baker showed activity of 83.2 µg/mL [35]. However, there is still the need to investigate which of the constituents in the plant is responsible for the observed activity.

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