

Journal of Essential Oil Bearing Plants

ISSN: 0972-060X (Print) 0976-5026 (Online) Journal homepage: http://www.tandfonline.com/loi/teop20

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To cite this article: L.A. Usman, J.O. Akolade, B.O. Odebisi & B. Olanipekun (2016) Chemical Composition and Antibacterial Activity of Fruit Essential Oil of Xylopia aethiopica D. Grown in Nigeria, Journal of Essential Oil Bearing Plants, 19:3, 648-655, DOI: 10.1080/0972060X.2014.935073

To link to this article: https://doi.org/10.1080/0972060X.2014.935073

Published online: 25 May 2016.

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Taylor & Francis
Taylor & Francis Group

ISSN Print: 0972-060X
ISSN Online: 0976-5026

Chemical Composition and Antibacterial Activity of Fruit Essential Oil of *Xylopia aethiopica* D. Grown in Nigeria

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Received 17 April 2014; accepted in revised form 23 October 2014

Abstract: Qualitative and quantitative analyses of the chemical constituents of fruit essential oil of *Xylopia aethiopica* and its antibacterial activity were investigated. Pulverized fruit of *X. aethiopica* was hydro-distilled in a Clevenger type apparatus. The resulting oil was characterized by Gas chromatography-Mass spectrometry (GC-MS) and its antibacterial activity was evaluated using agar diffusion method. Hydro-distilled *X. aethiopica* fruits yielded 1.3 % v/w of essential oil. Investigation by GC-MS revealed that bulk of the oil was constituted by hydrocarbon monoterpenes (44.0 %). The major constituents of the oil were; Eugenol (12.2 %), acetyleugenol (7.0 %), 1,8-cineole (6.8 %), sabinene (6.7 %), *cis*-ocimene (6.2 %), β-caryophyllene (5.6 %), γ-terpinene (5.3 %), α-pinene (4.3 %), β-linalool (4.3 %), α-terpineol (4.1 %), pinocarveol (3.7 %), terpinene-4-acetate (3.6 %), α-thugene (3.6 %), β-phellandrene (3.5 %) and benzylbenzoate (3.3 %). The oil showed various degree of inhibitory effect against three gram positive bacteria; *Streptococcus pyogenes, Staphylococcus aureus, Bacillus subtilis* and four gram negative bacteria; *Enterobacter aerogenes, Klebsiella pneumoniae, Escherichia coli, Serratia marcescens,* while no activity was recorded against *Proteus mirabilis.* The calculated activity index compared favourably with the standard gentamycin antibiotic used as control. Data from this study showed that aromatic compounds predominates the fruit essential oil of *X. aethiopica* growing in North Central Nigeria and the oil is active against a broad spectrum of clinical bacterial isolates.

Key words: *Xylopia aethiopica,* fruit essential oil, chemical constituents, eugenol, antibacterial activity.

Introduction

Xylopia aethiopica (Annonaceae; Dunal A. Rich.) is a widely distributed plant in West, Central and Southern Africa. It is wildly grown in Nigeria, where it is commonly known as "Eni awoka" by the Yorubas [1,2]. *X. aethiopica* is an aromatic tree with fairly smooth grey brown bar, which can reach 20 m and 60-75 cm in height diameter respectively [3,4,5]. In sub-saharan Africa, the dried fruits are used as spice and carminative by natives [6,7,8,9]. The plant dried fruits is used in ethnomedicine solely or in decoctions with other plants for treatment of several ailments like cough, bronchitis, skin disease, stomach ache, stomach ulcer, hernia, dysentery, and cholera [1,10,11].

The therapeutic properties of extracts from the plant parts such as antimicrobial and antioxidant activities have been established by various re-

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searchers [12,13]. Phytochemical investigations of the *X. aethiopica* fruits revealed the presence of reducing sugar, glycosides, flavonoids, saponins, tannins, phytosterols, anthraquinones, alkaloids and phenols [14,15]. Insecticidal, antimicrobial, antioxidant and cytotoxic potentials of the plant essential oil have also been documented [5,8,12,16].

β-Pinene, a hydrocarbon monoterpene is widely reported as the most abundant constituent of fruit essential oil of grown *X. aethiopica* from South Western Nigeria, [7], and many other African [5,18,19].

In some reports, other hydrocarbon monoterpenes like α-pinene [9], sabinene [20], β-phellandrene [21], the sequiterpene Germacrene D [6] as well as monoterpenoids such as I,8-cineole [22], linalool [23] and 4-terpineol predominates among up to 71 constituents as quantified by El- Hassan *et al*. [24].

It has been established that composition pattern of essential oil could be affected by the geographical and agroclimatic conditions [25] and our previous works showed this variations may consequently influence their biological activity [26]. It is on the basis and in continuation of our systematic study of essential oils of Nigerian medicinal plants, that we investigated the chemical composition and the antibacterial activity of fruit essential oil of North Central Nigerian grown *X. aethiopica*.

Material and methods

Plant material

Xylopia aethiopica fruits were collected from Ilorin, Ilorin West Local Government Area of Kwara State. Identification of the fruit was carried out at Herbarium of Plant Biology department, University of Ilorin, where voucher specimens were deposited.

Oil isolation

Pulverized fruits of *Xylopia aethiopica* (500 g) were hydrodistilled for 3 hr in a Clevenger type apparatus according to the British Pharmacopoea Specification [27]. The resulting oil was collected, preserved in a sealed sample tube and stored under refrigeration until analysis.

Gas chromatography

GC analysis was performed on an Orion micromat 412 double focusing Gas chromatography system fitted with two capillary column crated with Cp-sil 5 and Cp-sil 19 (fused silica, 2 5 m x 0.25 mm 0.15 μm film thickness) and flame ionization detector (FID). The volume injected was 0.2 μl and the split ratio was 1:30. Oven temperature was programmed from 50-230°C at 5/min, using hydrogen as carrier gas. Injection and detection temperatures were maintained at 200°C and 250°C respectively. Qualitative data were obtained by electronic integration of FID area percent without the use of correction factor.

Gas chromatography-Mass spectrometry

A Hewlett-Packard ITP5890A GC, interfaced with a VG analytical 70-250S double focusing mass spectrometer was used. Helium was the carrier gas at 1.2 ml/min. The MS operating conditions were: ionization voltage 70 eV, ion source 230°C. The GC was fitted with a 25 m 0.25 mm, fused silica capillary column coated with CP-sil 5. The film thickness was 0.15 μm. The GC operating conditions were identical with those of GC analysis. The MS data were acquired and processed by on-line desktop computer equipped with disk memory. The percentage compositions of the oils were computed in each case from GC peak areas.

The identification of the components was based on the comparison of retention indices (determined relative to the retention time of series of n-alkanes) and mass spectral with those of authentic samples and with data from literature [28,29,30].

Antibacterial sensitivity test

Antibacterial activity of the oil was carried out using agar diffusion method as described by Saliu *et al.*[26] with slight modification. Eight bacteria isolates comprising of three gram-positive; *Streptococcus pyogenes, Staphylococcus aureus, Bacillus subtilis* and five gram negative bacteria; *Klebsiella pneumoniae, Escherichia coli, Serratia marcescens, Proteus mirabilis, Enterobacter aerogenes* were obtained from the collection of microorganisms of the Department of Microbiology, Faculty of Science, University of Ilorin. Inoculums were prepared with fresh cultures of bacteria, grown in nutrient broth for 18 hr

at 37°C and standardized to McFarland scale 0.5. Wells (10 mm each) were made on the Mueller-Hinton agar plates already seeded with bacteria by spread plate technique. The essential oil was diluted withTween 80 to obtain five concentrations ranging from 0.25 to 4 % (v/v) in a two-fold dilution series corresponding to 2.5, 5, 10, 20, 40 mg/ml. Aliquots (100 µl) of each concentration was transferred to the wells (0.25 - 4 mg/well or % v/v) and gentamycin, a standard drug was used as control. Plates were then incubated at 37°C for 20 hr. All tests were carried out in duplicates and antibacterial activity was determined by measuring zone of inhibition around loaded wells. The activity index (AI) was calculated as the mean inhibition zone of test oil divided by the mean inhibition of the standard drug [31].

Results

Pulverised fruit of *Xylopia aethiopica* on hydrodistillation afforded oil in the yield of 1.3 % v/w. Table 1 shows retention indices, relative percentages and identities of the constituents of the oil. A total of 37 compounds that represent 98.7 % of the fruit oil were identified from their retention indices and mass spectra data. Hydrocarbon and oxygenated monoterpenes constituted 38.4 % and 29.3 % of the oil. Percentage composition of hydrocarbon sesquiterpenes and aromatic compounds were 5.6 % and 25.4 % respectively, while oxygenated sequiterpenes were quantified in trace amounts.

Sabinene (6.7 %), *cis*-ocimene (6.2 %), β-transocimene (6.2 %), β-caryophyllene (5.6 %) and γ-terpinene (5.3 %) were the most abundant

Table 1. Chemical composition (%) of fruit essential oil of *Xylopia aethiopica*

No.	Compounds	RI [b]	% Composition	Mass Spectra data
1	α-Thujene	923	3.6	136,121,105,91
2	α-Pinene	931	4.3	136,121,93,79,67
3	Camphene	946	tr	136,121,107,93,67
4	Sabinene	969	6.7	136,121,107,93,77
5	β-Pinene	974	tr	136,121,107,93,77
6	Myrcene	988	tr	136,121,115,107,93
7	2-Carene	1001	2.6	136,105,79,65,41
8	β-Phellandrene	1026	3.5	136,121,107,77,67
9	Limonene	1026	tr	136,121,107,93,78
10	1,8-Cineole	1029	6.8	154,139,125,108,81
11	*cis*-Ocimene	1034	6.2	136,121,105,93,78
12	β-*trans*-Ocimene	1046	6.1	136,105,79,53,41
13	*allo*-Ocimene	1046	tr	136,121,93,7743
14	γ-Terpinene	1057	5.3	136,121,105,93,77
15	β-Linalool	1097	4.3	139,109,80,67,43
16	Pinocarveol	1137	3.7	134, 119, 92, 70,55
17	Borneol	1163	tr	121,110,95,81,67
18	Terpinen-4-ol	1176	5.8	154,136,125,111,43
19	α-Terpineol	1187	4.1	136,121,93,81,59
20	*cis*-Carveol	1228	1.0	134,109,83,55,41
21	Thymyl methylether	1233	tr	192,150,135,91,65
22	Neral	1237	tr	135,119,109,95,69
23	Cinnamic aldehyde	1265	2.9	131,103,91,78,74
24	Geranial	1267	tr	109,99,95,83,69,53
25	Borneol acetate	1284	tr	136,121,108,95,67

table 1. (continued).

No.	Compounds	RI [b]	% Composition	Mass Spectra data
26	Terpinen-4-acetate	1327	3.6	154, 136, 86, 71, 43
27	Eugenol	1353	12.2	164,149,137,131,121
28	α-Copaene	1374	tr	204,189,161,119,5
29	β-Elemene	1393	tr	121,105,93,79,67,55
30	β-Caryophyllene	1417	5.6	147,133,119,105,91,79
31	Ethylcinnamate	1461	tr	147,133,119,105,91,79
32	Germacrene D	1480	tr	204,161,147,133,105,91
33	Acetyl eugenol	1525	7.0	207,164,149,131,121
34	Elemicin	1553	tr	208,193,177,165,150
35	Viridiflorol	1590	tr	205,189,161,149,109
36	Torreyol	1645	tr	204,161,133,119,105
37	Benzylbenzoate	1759	3.3	152,105,91,77,65,51
	Total		98.7	

[a] Compounds are listed in order of elution from Silica Capillary Column coated on CP-Sil 5

[b] Retention indices on fused Silica Capillary Column coated with CP-Sil 5

Tr are compounds quantified in trace amount (< 0.1 %)

hydrocarbon monoterpenes in the oil. The most abundant oxygenated monoterpene was 1,8-cineole (6.8 %). Terpinen-4-ol (5.5 %), β-linalool (4.3 %), pinocarveol (3.7 %), terpinene-4-acetate (3.6 %), *cis*-carveol (1.0 %) existed in appreciable quantity. Six sesquiterpenes were identified, all in trace quantity except β-caryophyllene (5.6 %), a hydrocarbon sequiterpenes. Eugenol (12.2 %), the most abundant constituent of the oil, its derivative acetyl eugenol (7.0 %) and other aromatic compounds such as benzylbenzoate (3.3 %) and cinnamic aldehyde (2.9 %) were also quantified in significant amount.

`The antibacterial potentials of the fruit oil was investigated using agar diffusion method and the degree of susceptibility of the microorganisms to the bactericidal effect of the oil was measured by the clearance zones of inhibition in millimeter (mm) around the wells as shown in Table 2.

The oils showed various degrees of inhibitory activity against all test bacteria isolates except *Proteus mirabilis* which was not sensitive to the oil. The sensitivity of the gram positive bacteria to the lowest concentration (0.25 mg/well or % v/v) of the oil assayed decreases in the following order; *S. aureus* >*S. pyogenes* >*B. subtilis*, while the gram negative bacteria was *K. pneumoniae* >*Enterobacter aerogenes* >*S. marcescens* > *E. coli*. The activity index was ≥ 63 % for all susceptible isolates.

Discussion

The quantitative composition of the North Central Nigerian grown *X. aethiopica* fruit oil differs from that of other locations earlier documented in literature. β-Pinene, the notable constituent of most *X. aethiopica* fruit oil from different parts of the world and which may constituted up to 40 % of the oil [32] was only found in trace amount (< 0.1 %). However, the oil was significantly constituted by other hydrocarbon monoterpenes that predominates in some other studies such as α-pinene (4.3 %) and sabinene (6.7 %) as well as that monoterpenoids 1,8-cineole (6.8 %) and α-terpinenol (5.8 %). Also, Karioti *et al.*[6] reported a Germacrene D chemotype of fruit essential oil of *X. aethiopica* grown in Ghana, the sesquiterpene was only detected in trace amount in the North Central Nigerian oil studied.

Variations in the composition pattern of the fruit oils can be attributed to factors ranging from geographical to agro-climatic conditions and this may

Table 2. Antibacterial activity of fruit essential oil extracted from *Xylopia aethiopica*

	***X. aethopica* fruit essential oil**					**GC**	**% AI**
Concentration in mg/ml	2.5	5	10	20	40	10	
Concentration in mg/well (% v/v)	0.25	0.5	1	2	4	1	
Gram Positive Bacteria	Zone of Inhibition (mm)						
Streptococcus pyogenes	20^{++}	24	27	30	32^{+++}	37^{+++}	73
Staphylococcus aureus	20^{++}	22	23	28	30^{+++}	34^{+++}	68
Bacillus Subtilis	18^{++}	20	22	25	27^{++}	31^{+++}	71
Gram Negative Bacteria	Zone of Inhibition (mm)						
Klebsiella pneumoniae	26^{++}	30	34	37	39^{+++}	39^{+++}	87
Serratia marcescens	19^{++}	21	23	27	30^{+++}	14^{+}	164
Escherichia coli	16^{++}	20	22	28	30^{+++}	35^{+++}	63
Enterobacter aerogenes	12^{+}	18	20	25	27^{++}	24^{++}	83
Proteus mirabilis	-	-	-	-	-	-	-

$^{+}$ Highly resistant micro-organisms, having a weak overall sensitivity
$^{++}$ Microorganisms with a medium over-all sensitivity
$^{+++}$ Highly sensitive microorganisms
GC = Gentamycin
AI = Activity index
mm = Millimeter

subsequently affects their biological activity [26]. Studies by Fleischer *et al.*[16] on antimicrobial potentials of the essential oil of Ghananian grown *X. aethiopica* fruit, a Gemacrene D chemotype showed that all tests microorganism isolates used in the study except *E. coli* were sensitive to the oil., whereas phenolic extract of the fruit significantly inhibits the gram negative coliforms [13].

The essential oil evaluated in this study was more active against tested Gram positive than Gram negative bacteria. This result agrees with many other studies on *X. aethiopica* phytoextracts [7,13,16]. Non-susceptibility and resistance observed among Gram-negative bacteria have been attributed to the presence of an outer membrane made of lipopolysacharride covering the cell wall, which do restricts diffusion of hydrophobic compounds such as terpenes [33]. Whereas, the absence of such membrane in Gram positive bacteria may allow easy permeation by antimicrobial agents that interferes with cell mechanisms inhibiting growth or stimulating cell death.

The biological activity of a plant extract is defined by its phytochemical constituents. For instance, phenolic extracts have shown high degree of activity against broad spectrum of microorganisms [13]. Phenolic compounds consist of hydroxyl group(s) that allows hydrophilic interaction coupled to an aromatic ring containing delocalized electrons. This electronic structure stimulates disruption of lipopolysaccharide membranes, interacts with microbial enzymes and cell cytosolic substrates such as metal ions [34,35].

Essential oils usually occur as complex mixtures and their antimicrobial activities are sometimes dictated by their aromatic terpenoid (phenolic) components. The phenolic hydroxylgroup of aromatic terpenoids has been demonstrated as essential for action against pathogenic microbes [34]. Our previous investigation revealed susceptibility of oral isolates of gram positive bacteria *S. aureus* and *S. pyogenes*to eugenol chemotypic

(61.9 %) essential oil extracted from the leaves of *Ocimum gratissimum* [26]. Bioactivities of eugenol (clove oil), an aromatic terpenoid derived principally from the general *Eugenia*, have also been reported against gram negative bacteria [36]. These studies correlate with the observed activity of the oil under investigation in this study on the susceptible tested bacteria isolates including *E. coli*. The oil consists of six different phenolic compounds about 25.4 % of the oil, which includes eugenol (12.2 %), the predominant constituent of the oil.

Basak *et al.*[37] reported that the bioactivity of essential oil may not only be attributed to the effect of the main constituents of such oil but also to the synergistic/antagonistic effects of the different components that make up the oil. This effect may also be responsible for the variations in microbial susceptibility pattern. Okigbo *et al.*[7] reported that *Proteus mirabilis* was susceptible to the ethanolic extract of Nigerian grown *X. aethiopica* fruit. However, the essential oil extract used in this study showed no activitiy against *P. mirabilis*. Thus, variations in antibacterial activities could be attributed to nature, composition and concentration of extracts. Other delineating factors include methods of extraction, strain of bacteria isolates, dispersing solvents and antimicrobial assay techniques.

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