ANALYSIS OF ESSENTIAL OIL OF THE NIGERIAN XYLOPIA AETHIOPICA (ANNONACEAE) -I- COMPOSITION OF DISTILLED-EXTRACTED DRIED WHOLE FRUITS.

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ABSTRACT: The essential oil isolated from dried whole fruits of Xylopia aethiopica was analysed by means of GLC, LSC and GC-MS. The oil sample was isolated by distillation-in a Likens-Nickerson-typeapparatus after pre-soaking the fruits in n-pentane-diethyl ether-(extraction) (1:1) for 48 h. The oil sample comprised mainly monoterpenes, with sabinene, - α - and βpinene, 1,8-cineole, terpinen-4-ol, a-terpineol and linalool being the most predominant compounds.

INTRODUCTION:

Xylopia aethiopica (Dun.) A. Rich. - synonyms: Annona aethiopica Dun. and X. eminii Chev.α family Annonaceae, is referred to as Ethiopian, Guinea or Negro pepper and as spice tree, or as African cubeb. The X. aethiopica fruits are fragrant and spicy, possessing a very strong and persistent odour. Its odour has been reported to resemble that of cinnamon.(1) All parts of the plant are aromatic and yield essential oils. The fruits are used for flavouring food; they give it a mildly hot and spicy note. Between 100 and 150 Xylopia species are reported to be distributed throughout the tropical regions of the world, particularly in Africa.(1) Some of these species, including X. aethiopica, are used as a spice substitute for black pepper (Piper nigrum).

X. aethiopica is also an important medicinal plant; it is included in many remedies in West Africa, particularly as a carminative, a stimulating additive to other remedies (1). It is used as an antitussive in Nigeria (1), as an anti-inflammatory (1); its active constituents are thought to be kaurene diterpenes (1). The fruits together with leaves of Anogeissus leiocarpus are used as good remedy for malaria fever (1). Formulations of X. aethiopica are said to be much used in Europe and have now been shown to possess antimalarial activity (1); other medicinal uses (2) as well as biological activities of this plant and of other species of the same genus have also been described.(3)

Alkaloids (4), diterpenes (1) and tannins (1) have been reported in Xylopia species of different geographical origin. A fixed oil, and an essential oil have been isolated (5,6). Cuminaldehyde has been isolated from the seeds (5).

In this paper, a report is given on the analysis of essential oil composition of dried whole fruits of X. aethiopica of Nigerian origin, which was isolated by distillation-extraction method.

EXPERIMENTAL

Plant material

A 3 kg sample of dried whole fruits of X. aethiopica (Dun.) A Rich. Annonaceae) was purchased from a local market at Ile-Ife (Nigeria). A voucher specimen was deposited at the Herbarium of the Department of Botany of the Obafemi Awolowo University (Ile-Ife).

Isolation of essential oil

A thawed frozen sample of dried whole fruits was pre-soaked in n-pentane-diethyl ether (1:1) for 48h. The air-dried residual marc was subsequently soaked in 95% ethanol, for another 48 h. The two resulting extracts were concentrated under reduced pressure at ca. 0°C and then subjected, each to distillation-extraction for 4 h using a Likens-Nickerson-type apparatus (7). These afforded sample XYLO A and a marc oil, respectively. The marc oil was kept for further analyses.

Liquid-solid chromatography (LSC)

To facilitate the analysis of the essential oil, 20µ of the oil sample XYLO A were pre-fractionated by LSC over silica gel, using a 3 ml Baker-10 disposable solid phase extraction column; by elution with 10 ml of redistilled n-pentane and 10 ml of redistilled diethyl ether in succession. This afforded two fractions (containing the hydrocarbons and the oxygen-containing components, respectively) which were each concentrated under reduced pressure at 0°C to 1 ml; 1.0 µl of each fraction was used for GC analysis.

Gas-liquid chromatography (GLC)

The oil sample and the fractions obtained by LSC were analyzed by GLC, using two capillary columns of different polarities in a dual channel gas chromatograph (Packard model 439) equipped with FIDs and connected with Shimadzu C-R3A chromatographic data processors. GC conditions were as follows; WCOT columns: fused silica, 60 m x 0.25 mm i.d., coated with Durabond-DB 1, film thickness 0.25µm (column A) and 50 m x 0.23mm i.d., coated with CP-Wax 52cb, film thickness 0.22 µm (column B); oven temperature : programmed, 45-240°C at 3°C/min, and subsequently isothermal at 240°C for 30 min; injector and FID temperature: 200°C; carrier gas : nitrogen; linear gas velocities : 15 cm/s and 11.5 cm/s for columns A and B, respectively. The samples were injected using the split sampling technique, ratio ca 1:100; sample size 1.0µl. The percentage composition of the oil samples was computed from the GC peak areas without using

correction factors.

Gas-liquid chromatography/Mass spectrometry (GC-MS)

Data were obtained on a gas chromatograph, Packard model 438A equipped with a fused silica column, 50 m x 0.22 mm i.d., coated with CP-Sil 5cb, film thickness 0.13 µm and interfaced with a Finnigan MAT 700 ion trap detector (ITD; software version 3.0). Operating conditions were as follows; GC oven temperature : as under GC; transfer line 250°C; carrier gas : helium, 100 kPa; injection by the split sampling technique, ratio ca 1:40; scan range : 40-350u scan time : 1 s.

Identification of components

Identification of the oil components was accomplished by comparison of their GC retention times on the two columns as well as their mass spectra with corresponding data of authentic compounds or of components of reference oils; some mass spectra were compared with those of the ITD computer data library (US National Institute of Standards and Technology and/or with spectral data given in the literature.

RESULTS AND DISCUSSION:

The distillation-extraction procedure was chosen to isolate the 'ideal oil' because previous research works had shown that the oil obtained by this method usually resemble the exact oil as it existed in the intact plant (8,9).

Preliminary GC analyses showed that the composition of the oil sample XYLO A, was complex. In addition to the advantages of the LSC procedure discussed in details in previous papers (q) such as the enrichment of minor components in each fraction, improved GC analysis, separation of components which will otherwise not be separated due to co-elution on the two columns with one component or the other that has the same GC-retention times; and lastly it enables the identification by GC-MS, especially of minor components which co-elute with others and have mixed mass spectra.The pre-fractionation showed that both the hydrocarbons and the oxygen-containing compounds contributed to the odour characteristics of the total oil, the former fraction had a spicy and pungent odour, while the latter had a sweet and fragrant note.

The identified components and their percentages have been listed in Table I, in order of their elution on the Durabond-DB 1 column. Many components, 73 of which are listed in Table I, were well resolved on both GC columns during the oven temperature programme; four components were eluted during the prolonged, isothermal elution, at 240°C for 30 min. These additional components were found to be diterpenes, and they occurred only in trace amounts in the oil sample.

The XYLO-A sample consisted mainly of monoterpenes (71%), with sabinene (37%) and β-pinene (20%) being the major components. The oxygen-containing monoterpenes constituted up to 18% of the sample, the main ones being 1,8-cineole and terpinen-4-ol (up to 11%). Other classes of compounds present in the oil sample included sesquiterpene hydrocarbons (up to 4%) and oxygen-containing sesquiterpenes (up to 7%). The identified components represented about 90% of the oil sample. The unidentified components below 0.25% have not been included in Table 1; the same is true for identified components present in trace amounts in the oil sample analysed. These included pentylbenzene, camphene, trans-ß-ocimene, citronellol, piperitone oxide, calarene, α-humulene, trans-methylisoeugenol, α-muurolene, t-cadinene, calacorene, α-cadinene (isomer) and α-santalol.

The literature data on the percentage composition of the essential oils from X. aethiopica were scarce and incomplete. Thus a comparison could only be made with a lot of caution, also because various isolation procedures had been utilized and/or different morphological parts had been employed. Monoterpene contents which have been described for the oils varied widely, from 10% to 90%. Unlike some oils in which β- pinene was reported to be most prominent (4), or in which sabinene was reported to be completely absent (10), sabinene dominated the oil sample analysed in the present study.

The results of the analysis of XYLO-A were more or less similar to those obtained by Lamaty et al (2) for the oil from seeds of the same species growing in Cameroon. For example, sabinene (24%) and β- pinene (17%) were the major components, and terpinen-4-ol (13%), α-terpineol (10%) and 1,8-cineole (2%) occurred in substantial amounts; while numerous other components were present as traces in their oil, as in XYLO-A. Their results were qualitatively and quantitatively different from those obtained for Egyptian (10) and other Nigerian (5,6) fruit oils. In contrast to the reported composition of the Cameroon seed oil (2) listed above, the Egyptian fruit oil (10) possessed 1,8-cineole as a major constituent. The results also differed from those obtained by Ekundayo et al (6) for the fruit oil of the same species collected in Nigeria, in which the furanoid (E)- and (Z)- linalool oxides were detected. The percentage composition of their oil was not published, but a summary of the components detected was given in a recent review (6); the oil was reported to possess a fragrant and leafy odour, which may be typical of fresh plant material.

The results described in the literature and in this paper suggest that the composition of the oils may be affected by the geographical origin of the plant material. They may also indicate the occurrence of at least two chemotypes of X. aethiopica in Nigeria, one of which had some similarities to that found in Cameroon. However, further studies are required, to clarify this point since the oils in these cases might have been isolated from different morphological parts and/or forms of the plant material by different methods.

The wide variation found in the percentage composition of the essential oils of X. aethiopica described in the literature may not necessarily only be attributed to such factors as the difference in geographical origin, the morphological part as well as the form of the plant material (e.g. fresh, dried, frozen etc.) investigated, and/or the occurrence of chemical varieties, but also to the method of isolation.

The findings of this study suggest that a conservative point of view should be taken when chemical varieties are described, based only on one method of isolation or on the examination of one of the morphological parts of a plant.

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Table I. Percentage composition of the distilled-extracted essential Oil from dried whole fruits of Xylopia aethiopica.

Component	XYLO-A[a] %
α -Thujene	0.3
α -Pinene	9.6
Sabinene	37.3
β -Pinene	20.4
β -Myrcene	tr
α -Phellandrene	tr
α -Terpinene	0.4
p-Cymene	1.8
Limonene)	
1,8-Cineole[b])	8.3
β Phellandrene)	
cis-B-Ocimene	tr
γ -Terpinene	0.9
trans-Sabinene hydrate	0.3
Terpinolene	tr
Linalool[b])	
cis-Sabinene hydrate)	0.6
2-Phenylethanol)	
α -Campholenaldehyde	tr
cis-p-Menth-2-en-1-ol	0.3
Pinan-3-one	0.5
Allo-ocimene (isomer)	tr
trans-Sabinol)	
Camphor)	0.7
Pinocarveol (isomer))	
β -Thujone)	0.4
Epoxypinane (isomer)	tr
Menthone	tr
Pinocarvone	0.4
β -Terpineol	tr
trans-Carveol	tr
Menthol	tr
trans-p-Menth-2-en-1-ol	0.4
Terpinen-4-ol	3.0
α -Terpineol[b]	0.7
Myrtenol	1.0
cis-Chrysanthenol	0.4
Cuminaldehyde	0.9
Piperitol (isomer)	tr
Cumin alcohol	tr
γ -Elemene	1.2
α -Copaene	tr

Component	XYLO-A[a] %
β -Elemene	1.6
α -Gurjunene	0.9
β -Caryophyllene)	
trans- α -Bergamotene)	tr
α - Cubebene	tr
γ - Muurolene	tr
Germacrene-D	tr
β -Selinene)	
Isolongifolene)	tr
α -Selinene	tr
α -Cadinene	0.4
Cadina-1,4-diene	tr
Elemol)	
α -Cadinol (isomer))	0.7
(E)-Nerolidol	0.3
Spathulenol	0.3
β -Caryophyllene expoxide	0.5
OCST (M_r 220)	tr
β -Oplopenone	0.3
δ -Cadinol	0.6
Epicubenol	0.7
OCST (M_r 220)	0.3
T-Cadinol	0.5
α -Eudesmol	0.9
β -Eudesmol	0.3
OCST (M_r 220)	0.3
OCST (M_r 220)	tr
Spathulenol (isomer)	1.6
Manoyl oxide	0.3
DTHC (M_r 272)	tr
OCDT (M_r 286)	tr
Grouped components	
MTHCs (monoterpene hydrocarbons)	70.7
OCMTs (oxygen-containing monoterpenes)	18.3
STHCs sesquiterpene hydrocarbons	4.1
OCSTs oxygencontaining sesquiterpenes	7.0
DTHC +OCDTs Interpene hydrocarbons + oxygen containing diterpenes.	0.3

a = distilled-extracted oil from pentane-diethyl ether pre-soaked fruits;
b = constituted the main proportion of the co-eluted components;
tr = trace (< 0.25%);