

Research Article

Characterization of Essential Oils of *Xylopi aethiopia* (Dunal) A. Rich for Afforestation of the Coastal Savanna at Pointe-Noire (Congo-Brazzaville)

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Abstract: The essential oil from fruits, leaves and stem bark of *Xylopi aethiopia* of Congo-Brazzaville was obtained by steam distillation and analyzed by CG on two columns with different polarities (polar and apolar) and by CG/SM. The essential oil from fruits was characterized by the presence of three constituents at levels of at least 10%. These were pinenes (alpha-+beta-) as major components (17%), 1, 8-cineole (13.3%) and sabinene (10%), all monoterpene hydrocarbons. The three most abundant oxygenated monoterpenes were trans-pinocarveol (8.2%), myrtenal (6.3%) and myrtenol (6.2%). The essential oil from leaves was characterized by the presence of pinenes (alpha-+beta-) as major components (39-60%). Sesquiterpenes came second, with caryophyllene the most abundant (6-18%). Oil from stem bark was made up of pinenes (27-57%), with beta-cubebene (11-14%) in second position and transpinocarveol (6%) and myrtenal (5%) jointly in third position.

Keywords: Bark, essential oil, fruits, leaves, *Xylopi aethiopia*

INTRODUCTION

Essential oils are a Non-Wood Forest Products (NWFPs). This FAO term designates all biological materials that are exploited other than timber and other woody raw materials. NWFPs include a broad diversity of useful products, including foods, spices, medical drugs, forage, essential oils, resins, gums, latex, tannins, dyes, cane, fibres, bamboos and all sorts of ornamental animal and plant products (CEE-FAO, 1999).

Essential oils are well-placed to enter in official trade statistics, but the potential value of NWFPs is difficult to estimate accurately and is indeed often under-estimated because such products are mostly used locally.

Xylopi aethiopia is a dense forest understory tree 15-30 m high and 60-75 cm in diameter, growing on river banks or marshland. It has a slender trunk with a buttressed base 50 cm to 1 m in diameter (Thomas, 1969). It is relatively tall and topped by a plume of branches and twigs spread out horizontally. It belongs to the Annonaceae family, subfamily Annonoideae, tribe Unoneae, subtribe Xylopineae. This forest tree is found in the Congo Basin, where it has many uses: Its

strongly peppery seeds and carpels are used as spices or condiments (Aubreville, 1959; Tchiegang and Mbougoung, 2005; Letouzey, 1982; Tisserant, 1950). *Xylopi aethiopia* is also used to treat scabies (Letouzey, 1982), asthma, stomach pains, rheumatism, malaria (Burkill, 1985; Fekam *et al.*, 2003), cough, bronchitis, dysentery, female sterility and abdominal pains (Anvam, 1998).

In Congo, where it is exploited essentially for firewood, *Xylopi aethiopia* is under strong human pressure. To ensure its sustainability, commercial use is envisaged by the production of essential oils for food and medicine through a programme of afforestation of poor coastal savannas in the region of Pointe-Noire. This option has been taken by the Coastal Forest Research centre (CRFL), Congo-Brazzaville, with extremely encouraging results (Fig. 1). However, such an endeavour implies acquiring basic knowledge of the forestry, chemistry and technology involved.

The study described here concerns the quantitative and qualitative characterization of essential oils of *Xylopi aethiopia* extracted from the fruits, leaves and bark of naturally-growing trees.

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Fig. 1: *Xylopia aethiopica* plantation in coastal savanna (Loandjili, Pointe Noire, Congo Brazzaville, 6 years old trees)



Fig. 2: Fruits of *Xylopia aethiopica* collected in the wild forest (Youbi, Pointe Noire, Congo-Brazzaville)

MATERIALS AND METHODS

Plant material: The fruits (Fig. 2), leaves and bark of *X. aethiopica* studied were collected wild in the Youbi forest, 100 km north of the town of Pointe-Noire.

The Youbi forest is located between latitudes 4°0'00'' and 4°30'00'' South and longitudes 11°30'00'' and 12°0'00''. Annual rainfall in the region is about 1400-1500 mm. The harsh dry season lasts from June to September and the rainy season from October to May. The samples were collected in September 2010.

Extraction of essential oils: The essential oils were obtained by steam distillation. Water and plant material (300 g of dried fruits) were placed in a 500 mL round-bottomed flask and boiled for 4 h. The organic phase of the resulting condensate was separated from the aqueous phase by extraction with diethyl ether. The organic phase was dried over sodium sulphate and the essential oil was recovered after evaporation of the solvent.

Determination of the chemical composition of the essential oils: After detailed analysis of the essential oil extracted from fruits (protocol 1), which identified more than 70 constituents, we undertook a systematic study of the essential oils from the different plant parts with a simplified experimental protocol (protocol 2).

Experimental protocol 1:

GC analysis: GC analysis was carried out using a Perkin-Elmer Auto System apparatus (Waltham, MA, USA) equipped with a dual Flame Ionization Detection (FID) system and the fused-silica capillary columns: Rtx-1 (polydimethylsiloxane) and Rtx-wax (polyethyleneglycol). The oven temperature was programmed from 60 to 230°C at 2°C/min and then held isothermally at 230°C for 45 min. Injector and detector temperatures were maintained at 250 and 280°C. Samples were injected in the split mode (1/50)

using helium as carrier gas (1 mL/min) and a 0.2 µL injection volume of pure oil. Retention Indices (RI) of compounds were determined relative to the retention times of a series of *n*-alkanes (C5-C30) (Restek, Lisses, France) with linear interpolation using the Van den Dool and Kratz (1963) equation and software from Perkin-Elmer.

GC-MS analysis: Samples were analyzed with a Perkin-Elmer turbo mass detector (quadrupole) coupled to a Perkin-Elmer Auto System equipped with the fused-silica capillary columns Rtx-1 and Rtx-wax. Carrier gas: helium (1 mL/min), ion source temperature: 200°C, oven temperature programmed from 60 to 230°C at 2°C/min and then held isothermally at 230°C (35 min), injector temperature: 280°C, energy ionization: 70 eV, electron ionization mass spectra were acquired over the mass range 35-350 Da, Identification of individual components was based:

- On comparison of calculated RI on polar and apolar columns, with those of authentic compounds or literature data
- On computer matching with commercial mass spectral libraries and comparison of mass spectra with those of our own library of authentic compounds or literature data. Components relative concentrations were calculated based on GC peak areas without using correction factors (König *et al.*, 2001; NIST (National Institute of Standards and Technology), 1999; Anonymous, 2005; Adams, 2001)

Experimental protocol 2:

GC analysis: GC analyses was performed on a Hewlett-Packard 6890 equipped with a split/splitless injector (280°C, split ratio 1:10), using DB-5 column (30 m×0.25 mm, df: 0, 25 µm). The temperature program was 50°C (5 min) rising to 300°C at rate of 5°C/min. Injector and detector temperature was 280°. Helium was used as carrier gas at a flow rate 1 mL/min. The injection of the sample consisted of 1.0 µL of oil diluted to 10% v/v with acetone.

GC-MS analysis: GC/MS was performed on a Hewlett-Packard 5973/6890 system operating in EI mode (70 eV), equipped with a split/splitless injector (280°C, split ratio 1:20), using DB-5 column (30 m×0.25 mm, df: 0.25 µm). The temperature program was 50°C (5 min) rising to 300°C at rate of 5°C/min. Injector and detector temperature was 280°. Helium was used as carrier gas at a flow rate 1 mL/min.

The identification was carried out by calculating retention indices and comparing mass spectra with those in data banks (Adams, 2001; Mc Lafferty and Stauffer, 1989).

Statistical treatment: Means, standard deviations and the usual graphs were obtained with Excel software.

RESULTS AND DISCUSSION

Essential oil contents in the different plant compartments: In preliminary work, we evaluated the factors determining the quantitative and qualitative production of this oil: duration of the extraction and state of conservation of the raw material (storage conditions). This study was carried out on fruits. It is recognised that the fruit of *X. aethiopica* is the part richest in essential oil (Ayedoun *et al.*, 1996; Karioti *et al.*, 2004). It was therefore in this part that we sought the characteristic essential oil of the plant, assuming a significant intra-tree variability in its oil. On a reduced number of samples we then evaluated the levels of essential oil according to its storage compartments in the plant (fruits, leaves and bark).

Figure 3 shows the curve for the essential oil extraction from fruits as a function of extraction time during steam distillation. We found that after 100 min, the mass of essential oil extracted was 9.2 g. Extrapolation of the curve $1/m = f(1/t)$ to $1/t = 0$ ($t = \text{infinity}$) gives $1/m = 104$ g, corresponding to a total mass of 9.6 g of essential oil contained in the fruits studied, indicating an extraction rate of $(9.2/9.6) 100 = 96\%$ (Fig. 4). There was no need to prolong the extraction beyond 240 min. Accordingly we set our extraction time to 4 h for the rest of the study.

The second parameter evaluated was the influence of the conservation of the raw material on extraction yield. We see in Fig. 5 that fruits conserved at ambient temperature (30-35°C) lost one third of their extractable essential oil after 1 week and half after 2 weeks. By contrast, oil loss was negligible when the fruits were stored at 4°C for 2 weeks.

Like the fruits, leaves and bark also contain essential oils, but in smaller amounts. Table 1 gives the data for four trees taken at random in the wild forest. We can see that fruits, which serve as the main essential oil storage compartment in *X. aethiopica*, had levels of up to 7%. Leaves were the second richest storage compartment, with levels of 0.2-0.3% and bark contained only 0.02-0.07% of essential oil.

In a given compartment, we found very high amplitude variations in essential oil levels: 2-7% for fruits, 0.1-0.3% for leaves and 0.01-0.07% for bark.

There seems to be a correlation between essential oil content in fruits and that in leaves (Fig. 6), fitting the equation:

$$y = 0.027x + 0.039 \text{ with } R^2 = 0.880$$

Confirmation of this relation using a larger number of samples would provide a way to predict fruit essential oil content simply from assay of oil in the leaves of young trees.

Chemical composition of the essential oils from different plant compartments:

Characterization of essential oils from fruits, leaves and bark:

Analysis on two columns with different

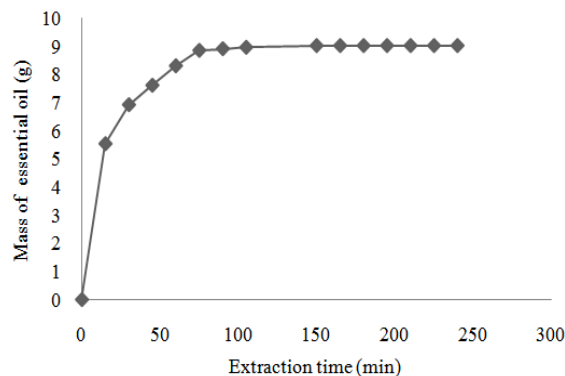


Fig. 3: Variation of the mass of extracted essential oil vs. extraction time

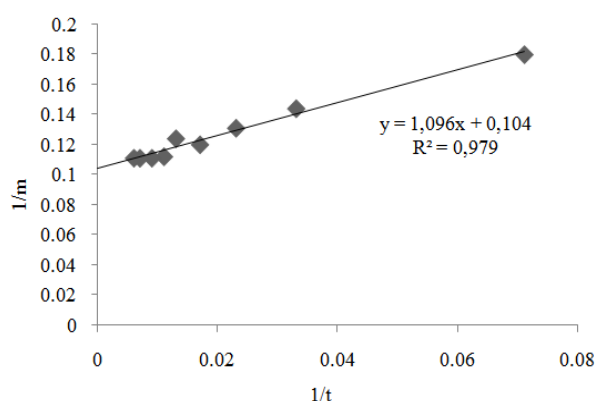


Fig. 4: Correlation plot: $(1/m)$ vs. $(1/t)$
m: Mass of essential oil; t: Extraction time

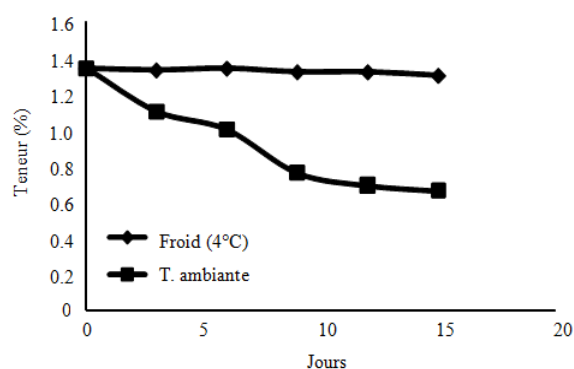


Fig. 5: Variation of extraction yield of fruit essential oil vs. conservation conditions

◆: Storage temperature 4°C; ■: Ambient temperature

Table 1: Essential oil yield (%) in different compartments of the tree

Trees	Fruits	Leaves	Bark
A1	7.43	0.26	0.013
A5	3.13	0.12	0.038
A11	2.21	0.12	0.068
A15	4.77	0.14	0.062
Mean	4.39	0.16	0.045
S.D.	2.29	0.07	0.030

S.D.: Standard deviation

Table 2: Chemical composition of essential oil extracted from the wild forest fruits of *X. aethiopica*

Constituents	RI (apol)	RI (pol)	Content (%)	RI lit (apol)
α -thujene	924	1021	0.5	922
α -pinene	933	1021	6.0	931
Camphene	945	1063	0.3	943
Thuja-2.4 (10) -diene	948	1125	0.2	946
Sabinene	972	1119	9.9	964
β -pinene	975	1107	11.5	970
Dehydro-cineole-1.8	982	1184	0.2	979
Mycene	986	1158	tr	979
α -terpinene	1011	1172	0.5	1008
p-cymene	1017	1259	0.5	1011
Cineole-1.8	1024	1208	13.3	1020
Limonene	1024	1193	1.1	1020
Ocimene- β -Z	1036	1220	tr	1024
Ocimene- β -E	1039	1236	0.1	1034
γ -terpinene	1051	1236	0.9	1047
Trans sabinene hydrate	1058	1454	2.9	1051
Camphenilone	1060	1454	0.1	1059
p-cymenene	1075	1414	0.1	1072
Terpinolene	1081	1268	0.3	1078
Cis sabinene hydrate	1087	1536	1.4	1083
Linalol	1087	1536		1081
Perillene	1087	1399	tr	-
β -thujone	1100	1424	0.1	1103
α -campholenal	1108	1473	1.7	1105
Nopinone	1112	1554	1.1	1109
Trans pinocarveol	1130	1636	8.2	1125
Cis-verbenol	1137	1661	3.9	1132
Sabina-cetone	1137	1606	0.9	1129
Pinocarvone	1145	1549	3.2	1136
δ -terpineol	1151	1650	0.2	1161
Mentha-1.5-diene-8-ol	1151	1701	0.2	1146
Isopinocampnone	1154	1523	tr	1153
Cryptone	1155	1647	0.6	1157
Terpinen-4-ol	1168	1587	2.1	1161
Myrtenal	1177	1608	6.3	1172
α -terpineol	1177	1674	2.4	1179
Myrtenol	1188	1771	6.2	1177
Carveol-trans	1202	1807	0.5	1196
Cuminaldehyde	1216	1749	0.4	1217
Pulegone	1219	1621	0.1	1217
Carvone	1219	1707	0.3	1222
Peryllaldehyde	1248	1750	0.1	1248
Bornyl-acetate	1279	1560	0.3	1269
Myrtenyl-acetate	1302	1616	0.1	1313
δ -elemene	1334	1456	tr	1337
α -cubebene	1349	1444	0.1	1350
α -copaene	1376	1477	0.3	1379
β -cubebene	1386	1497	0.1	1386
β -elemene	1386	1570	0.2	1388
Trans-caryophyllene	1416	1574	tr	1424
β -copaene	1426	1574	tr	1431
γ -elemene	1428	1615	0.1	-
α -humulene	1448	1638	tr	1456
Cis-muurolo-4 (14) .5-diene	1467	1747	tr	-
Muurolo- γ	1470	1662	0.1	1471
Germacrene-D	1475	1688	0.5	1480
β -selinene	1480	1697	tr	1483
Trans-muurolo-4 (14) .5-diene	1484	1797	tr	-
4-epi-cubebol	1485	1863	tr	1487
α -muurolo-4	1494	1697	tr	1496
γ -cadinene	1506	1729	0.3	1507
Cubebol	1506	1915	0.3	1509
Calamenene	1509	1801	0.1	1512
δ -cadinene	1514	1729	0.1	1515
Cadina-1-4-diene	1524	1751	tr	1523
α -calacorene	1528	1879	tr	1531
Germacrene-B	1550	1797	0.2	-
Caryophyllene oxyde	1569	1950	0.2	1576
Alismol	1612	2225	0.6	-
Manoyl oxyde	2002	2317	tr	1996
α -kaurene	2031	2334	0.1	2046
Total			91.7	
Monoterpene hydrocarbons			31.4	
Sesquiterpenes hydrocarbons			2.1	
Diterpenes hydrocarbons			0.1	
Monoterpenes oxygenated			57.0	
Sesquiterpenes oxygenated			1.1	

RI: Retention index; apol: Apolar column; pol: Polar column

Table 3: Majors constituents (>2%) of fruit essential oils of *X. aethiopica*

Constituents	RI (apol)	RI (pol)	Content (%)
α-pinene	933	1021	6.0
Sabinene	972	1119	9.9
β-pinene	975	1107	11.5
Cineole-1.8	1024	1208	13.3
Trans sabinene hydrate	1058	1454	2.9
Trans pinocarveol	1130	1636	8.2
Cis-verbenol	1137	1661	3.9
Pinocarvone	1145	1549	3.2
Terpinen-4-ol	1168	1587	2.1
Myrtenal	1177	1608	6.3
α-terpineol	1177	1674	2.4
Myrtenol	1188	1771	6.2

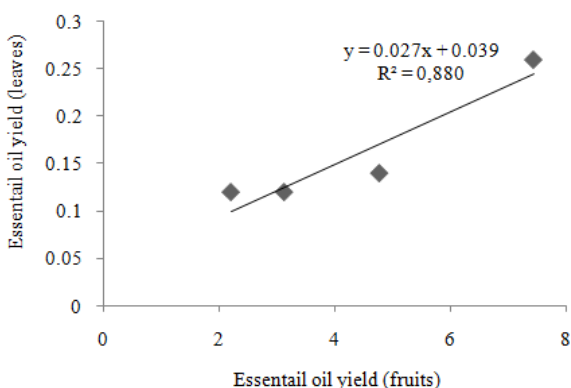


Fig. 6: Correlation between fruit essential oil vs. leaf essential oil yield (%) on a same tree

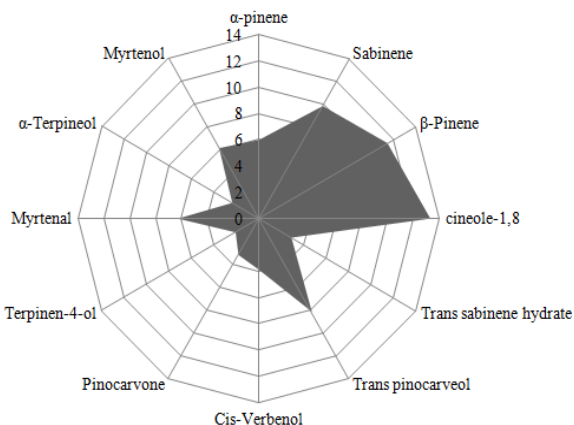


Fig. 7: « Radar plot » representation of fruit essential oils extracted from *X. aethiopica* growing in the wild forest

polarity (polar and apolar) of essential oil extracted from fruits of *X. aethiopica* (Table 2) identified 71 constituents, representing nearly 92% of the total essential oil, with 31.4% monoterpene hydrocarbons. 57.0% oxygenated monoterpenes, 3.2% sesquiterpenes and 1% diterpenes.

Of the 71 constituents of the essential oil, 16 present at levels of more than 1% totalled 81% and so

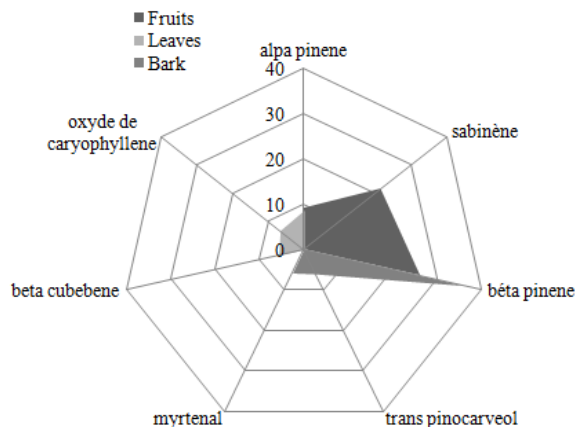


Fig. 8: « Radar plot » representation of fruit essential oil composition from natural forest *X. aethiopica* in the different compartments on the same tree (A1)

can be considered as characteristic of the oil from the fruits studied.

Three constituents were present at a levels of at least 10%: these were 1.8-cineole (13.3%) and sabinene (10.0%), pinenes (17.0%) in particular alpha-pinene (6%) and beta-pinene (11.5%), all monoterpenes hydrocarbons.

The three most abundant oxygenated monoterpenes were trans-pinocarveol (8.2%), myrtenal (6.3%) and myrtenol (6.2%).

Given this composition, we can hypothesise (Table 3) a monoterpene chemotype with pinenes as major constituents followed by oxygenated monoterpenes, in particular 1.8-cineole, trans-pinocarveol, myrtenal and myrtenol: % pinene (alpha- and beta-) >% 1.8-cineole >% trans-pinocarveol >% myrtenal >% myrtenol.

With only three constituents exceeding an individual content of 10%, we have an essential oil with a large number of constituents each present at rather low levels, giving a relatively complex radar plot (Fig. 6 and 7).

As an illustration, Table 4 gives the composition of essential oils extracted from fruits, leaves and bark limited to ten major components making up some 80% of the total composition of the essential oil extracted from fruits.

For a given tree, for example Tree 1, the essential oils extracted from the fruits, leaves and bark yield the following total radar plot (Fig. 8).

In sum, we find that *X. aethiopica* gives relatively complex essential oils, which vary in particular according to the plant compartment that produces them: fruits, leaves or bark.

Intra-tree variability of essential oils: A comparative examination of the compositions of essential oils from the different plant compartments (Table 4) shows that pinene (alpha- and beta-) occurred in all these compartments as by far the most abundant component.

Table 4: Yield and composition of fruit leaf and bark essential oils from 4 wild forest trees

RI _{exp}	RI _{calc}	Constituents	A1			A5			A11			A15		
			Fruits	Leaves	Bark	Fruits	Leaves	Bark	Fruits	Leaves	Bark	Fruits	Leaves	Bark
		Teneur HE (%)	7.43	0.26	0.01	3.13	0.12	0.04	2.21	0.12	0.07	4.77	0.14	0.06
926	932	Alpa pinene	9.16	8.72	7.83	8.70	9.82	12.78	4.76	8.44	5.77	7.43	13.31	5.98
967	969	Sabinene	21.56			25.13			23.57			40.09		
972	974	Beta pinene	26.19	31.89	36.01	25.31	36.29	44.61	13.06	30.77	22.16	19.55	47.14	22.25
1022	1025	1,8-cineole							9.19	14.01	6.56	5.71		
1024	1026	(Z)-beta-ocimene				9.05								
1136	1135	Trans pinocarveol			6.08							1.85		
1197	1195	Myrtenal	6.14		5.85	6.28		5.73	7.17			3.00		
1292	1292	Para cymen-7-ol												
1391	1387	β cubebene		5.76							11.38			
1431	1434	β caryophyllene					5.57			5.28			5.03	14.14
1486	1484	Germacrene-D												8.88
1588	1582	Oxyde de caryophyllene		6.75			12.84				5.48			11.55

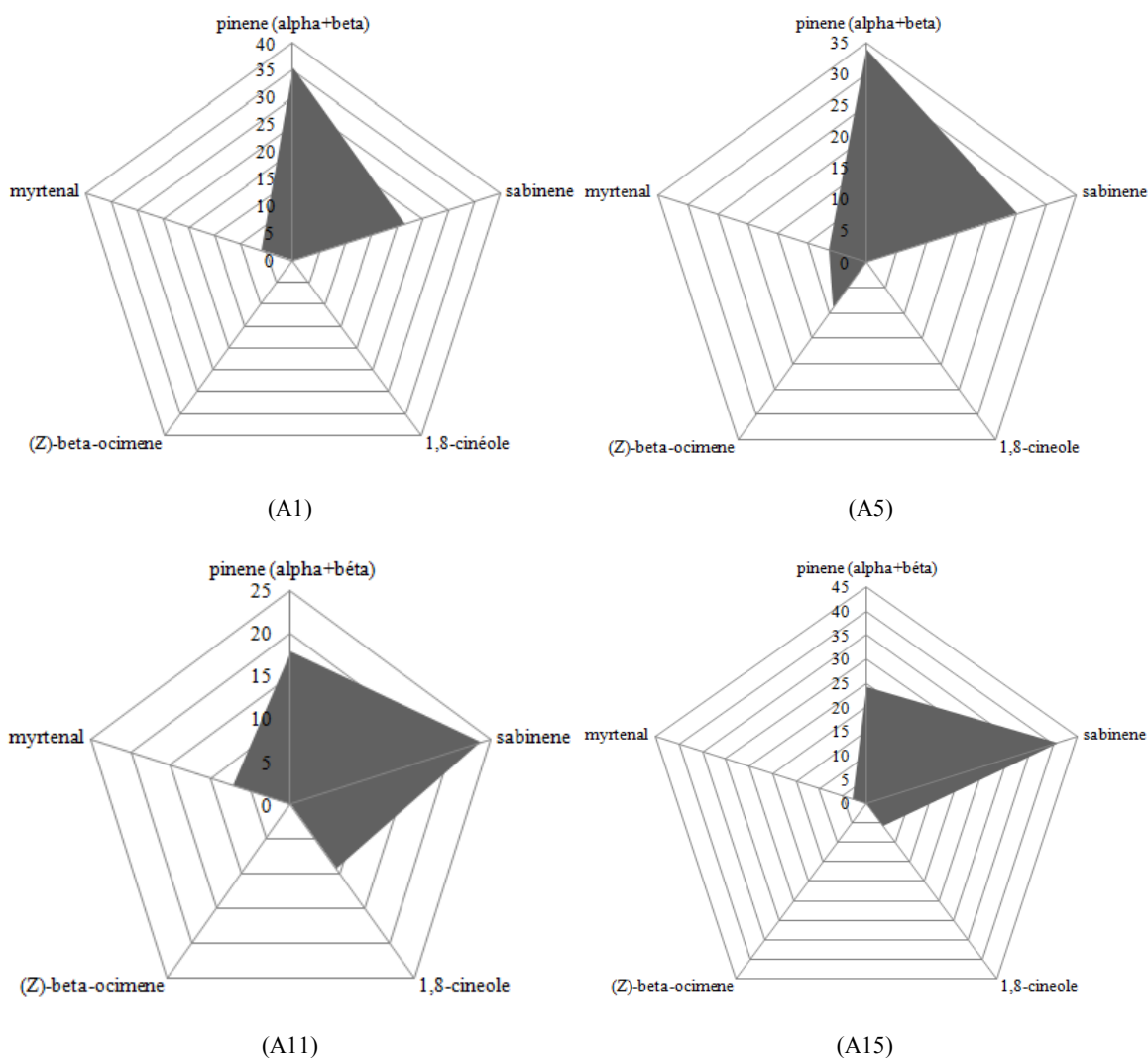


Fig. 9: « Radar plot » representation of fruit essential oil composition from different *X. aethiopica* trees (A1, A5, A11 et A15) growing in natural forest

Sabinene seems to be characteristic of the fruits, in which it occurred at fairly high levels (>20%) and was even the foremost major component in one of the four trees studied. It was entirely absent from leaves and bark.

Caryophyllene was more abundant in the leaves, either as the isomer beta-caryophyllene (5-9%), or in its oxidised form, caryophyllene oxide (5-6%). It was found in the bark of one tree among the four studied.

We note, however, that 1.8-cineole was almost absent from leaves except in Tree 11, where it was present in an appreciable amount (14%). Bark contained beta-cubebene at fairly high levels (11-14%) in two of the four trees.

The compounds Z-beta-ocimene (9%), trans-pinocarveol (6%) and germacrene D (11%) were also present in the essential oil profile of the trees studied.

Inter-tree variability of essential oils: The lowest inter-tree variability was found for essential oil extracted from fruits, which had a profile based on pinenes (alpha and beta) and sabinene (40-60%), one or the other predominating. The oxygenated monoterpenes, which were high in the total oil, came

third for individual content, with myrtenal leading (6-7%).

These oils displayed a quasi stable composition from one tree to another, as illustrated in Fig. 9.

Starting with Tree 1 as a basic morphogram (radar plot), we can see that: Z-beta-ocimene appeared in Tree 5 with levels well below 10% and the pinene/sabinene ratio was inverted and 1.8-cineole appeared in Tree 11 (likewise for Tree 15).

The oils extracted from the leaves contained practically no sabinene. Although the chemotype remained pinene-rich (39-60%), sesquiterpenes came second, with caryophyllene leading (6-18%). Oxygenated monoterpenes were totally absent.

Table 5: Variation of fruit essential oil composition of *Xylopiya aethiopica* vs. harvesting countries in Africa

Constituents	Congo							
	Brazzaville (a)	Nigeria (b)	Benin (c)	Côte d'Ivoire (d)	Cameroun (e)	Sudan (f)	Benin (g)	Egypt (h)
α -thujene							5.3	
Alpha pinene	14.620	5.57	11.6	17.77	3.38	1.88		0.2
Sabinene	20.150		3.6			1.59	41.8	
Beta Pinene	39.440	13.78	34.9	20.50	8.22	6.12	8.1	14.6
A			3.5					
Alpha phellandrene		12.36		5.63	1.41			
p-cymene	0.470	1.33				2.82		
Limonene	0.990						1.8	
Beta phellandrene	0.130				31.42			
1.8-cineole	2.760	6.90		7.44		5.42	23.8	16.3
(Z)-beta-ocimene	0.070		2.3					
B	0.390						2.7	
Linalol	0.400	1.22					3.1	
Trans pinocarveol	3.770							
Trans verbenol	1.110							
2-carene				2.95				
Terpinene-4-ol	0.97				1.54	11.30	1.1	23.4
Cryptone	0.320				1.01			
Myrtenol	0.150					2.94		
Myrtenal	4.890							
α -terpinene				2.10				
Verbenone	0.490					1.37		
L-pinocarveol						3.26		
α -terpineol					3.95	6.02	3.2	
β -myrcene				2.45				
Alpha copaene	0.270				3.70			
β -elemene			3.5		1.95	1.19		
Z-caryophyllene					1.68			
Beta caryophyllene			1.4					
β -duprezianene					1.11			
Cumic alcohol						2.29		3.3
C					1.86			
10-epi-zonarene					1.28			
Germacrene-D			3.8	3.17	5.34			
Z- γ -bisabolene					1.47			2.0
δ -cadinene					2.62			
Elemene δ						1.58		
Elemol			14.9			1.85		
Spathulenol						4.26		
Oxy. caryophyllene	0.230					1.10		
E					1.36			
Guaiol			1.8					
Cuminal								6.5
a-eudesmol			1.3					
P-eudesmol			1.3					
F						1.68		
G						2.35		
Cis- α - copaene-8-ol*						4.68		
I						1.48		
J						2.32		
13-epimanoyl oxide						4.62		
Kaur-16-ene						2.21		

a: This study; b: Olonisakin *et al.* (2007); c: Ayedoun *et al.* (1996); d: Konan *et al.* (2009) e: Noudjou *et al.* (2007); f: Elhassan *et al.* (2010); g: Noudogbessi *et al.* (2011); h: Karawya *et al.* (1979); A: p-mentha-3.8-diene; B: cis hydrate de sabinene; C: Dehydro aromadendrene; E: Epoxy-allo alloaromadendrene; F: Eudesma-3.7 (11) -diene; G: Eudesma-1.3-dien-11-ol; I: Eudesma.4-11 (13) -dien-12-ol; J: Eudesma.4-11 (13) -dien-2-ol

Table 6: Variation of leaf essential oil composition of *Xylopiya aethiopica* vs. harvesting countries in Africa

Constituants	Congo Brazzaville (a)	Côte d'Ivoire (b)	Benin (c)	Benin (d)
Alpa pinene	10.27	10.390	11.60	-
α -thujene	-	-	-	12.60
Sabinene	1.50	0.320	3.60	1.70
Beta pinene	32.50	16.010	34.90	46.10
p-mentha-3.8-diene	-	-	3.30	-
cis-meta- mentha-2.8-diene	-	-	-	1.20
2.6-dimethyl-2-heptanol	-	-	-	1.70
1.8-cineole	-	-	1.00	1.00
(Z)-p-ocimene	-	-	2.30	-
(Z)-beta-ocimene	2.00	-	-	-
Trans pinocarveol	2.50	-	-	-
Trans-sabinol	-	-	-	1.00
Camphene	-	1.680	-	-
Myrtenol	-	-	-	2.30
Myrtenal	1-8.34	-	-	-
2-carene	-	1.190	-	-
β -elemen	-	-	3.50	-
Beta cubebene	9.34	-	-	-
Beta caryophyllene	8.33	-	-	-
β -myrcene	-	0.103	-	-
Gamma elemene	2.50	-	-	-
Alpha humulene	1.83	-	-	-
Germacrene-D	2.90	3.110	-	3.80
NI	1.72	-	-	-
Elemol	1.45	-	14.90	4.20
Germacrene-B	1.49	-	-	-
NI	1.77	-	-	-
Guaiol	-	-	1.80	3.80
Oxyde de caryophyllene	3.88	3.220	-	-
Humulene-1.2-epoxyde	-	-	-	-
Torilenol	-	-	-	-
a-acorenol	-	-	-	1.40
Isospathulenol	1.75	-	-	-
Eremoligenol	-	-	-	1.00
α -cubebene	-	4.050	-	-
Velerianol	-	-	-	1.06
Alpha cadinol	3.41	-	-	-
Bulnesol	-	-	-	1.00
α -eudesmol	-	3.780	1.30	-
β -eudesmol	-	12.660	1.30	-

a: This study; b: Konan *et al.* (2009); c: Ayedoun *et al.* (1996); (d) Noudogbessi *et al.* (2011)

The oils extracted from tree bark were also made up of pinenes (27-57%). It was in this compartment that we found the greatest variability of the second most abundant constituent; in decreasing order, we had: trans-pinocarveol, myrtenal and beta-cubebene (11-14%).

Thus for example, for the four trees studied, we had widely different profiles outside pinene:

- **Tree 1:** Pinenes>trans-pinocarveol>myrtenal
- **Tree 5:** Pinenes>myrtenal
- **Tree 11:** Pinenes>beta-cubebene>1.8-cineole
- **Tree 15:** Pinenes>beta-cubebene>germacrene D> β -caryophyllene

In sum, this preliminary study indicates that the content and composition of essential oils varied over a relatively broad scale of values, in particular when they were extracted from a naturally-growing population of trees, here the Youbi forest at Pointe-Noire, Congo-Brazzaville.

For the same tree compartment, e.g., the fruits, we are struck by the high variability in the composition of their oils according to the harvesting location across Africa (Table 5 and 6).

The oils from Nigeria, Benin, Côte d'Ivoire (Gulf of Guinea countries) contain mainly pinenes/sabinene, like those of Congo-Brazzaville, but those of Sudan and Egypt (more continental countries) are made up of terpinene-4-ol (11.3 and 23.4%), 1.8-cineole (5.4 and 16.3%) and pinenes (8 and 15%).

CONCLUSION

Essential oils extracted from wild forest trees varied qualitatively and quantitatively to a noteworthy degree:

- Among the different plant storage compartments
- From one tree to another

The fruits were the main storage compartment for the essential oils, with levels of up to 7%.

The leaves contained on average 30 times less essential oil than the fruits and the bark 100 times less.

We note a linear correlation between the essential oil contents of the fruits and of the leaves. If this finding was confirmed by the study of a more representative sample, we could predict the oil content of young trees in nurseries by analyzing their leaves.

The oils in all the compartments were mostly made up of pinenes; sabinene seemed to be characteristic of fruits, beta-caryophyllene and its oxide of leaves and beta-cubebene of bark.

The results of this study give an overall picture of a complex essential oil composition, based on a constant monoterpene profile with pinenes as major constituents.

Ultimately, selection of individuals would be desirable, based on what essential oil content or major constituents are sought.

REFERENCES

- Adams, R.P., 2001. Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy. Allured Publishing, Carol Stream, IL.
- Anonymous, 2005. National Institute of Standards and Technology. NIST Chemistry WebBook. NIST Standard Reference Database, Gaithersburg. Retrieved from: <http://webbook.nist.gov/chemistry>.
- Anvam, Z.P.H., 1998. Extraction et analyse des huiles essentielles de trois espèces de la famille des annonacées du Cameroun. Faculté des Sciences, Yaoundé, pp: 27.
- Aubreville, A., 1959. La flore forestière de la Côte d'Ivoire. Deuxième édition revue, Tome troisième. Publication No. 15, Centre Technique Forestier Tropical, Nogent-sur-Marne, France, pp: 334.
- Ayedoun, A.M., B.S. Adeoti and P.V. Sossou, 1996. Influence of fruit conservation methods on the essential oil composition of *Xylopiya aethiopic* (Dunal) A. Richard from Benin. Flavor Frag. J., 11: 245-250.
- Burkill, H.M., 1985. The Useful Plants of West Tropical Africa. Families A-D. Royal Botanic Garden, pp: 44-51.
- CEE-FAO, 1999. Données statistiques des produits forestiers non-ligneux du Cameroun. Rapport Technique. Programme de partenariat CE-FAO (1998-2001).
- Elhassan, I.A., E.E. Elamin and S.M.H. Ayoub, 2010. Chemical composition of essential oil in dried fruits of *Xylopiya aethiopic* from Sudan. J. Med. Arom. Plants, 1(1): 24-28.
- Fekam, B.F., V. Ngouana, Z.P.H. Amvam, C. Menut, J.M. Bessiere, J. Gut and P.J. Rosenthale, 2003. Composition and anti-plasmodial activities of essential oils from some Cameroonian medicinal plants. Phytochemistry, 64: 1269-1275.
- Karawya, M.S., S.M. Abdel Wahab and M.S. Hifnawy, 1979. Essential oil of *Xylopiya aethiopic* fruit. Planta Med., 37(1): 57-59.
- Karioti, A., D. Hadjipavlou-Litina, M.L.K. Mensah, T.C. Fleischer and H. Skaltsa, 2004. Composition and antioxidant activity of the essential oils of *Xylopiya aethiopic* (Dun) A. Rich. (Annonaceae) leaves stem bark root bark and fresh and dried fruits growing in Ghana. J. Agr. Food Chem., 52(26): 8094-8098.
- Konan, N., B.A. Kouame, J.A. Mamyrbekova-Bekro, J. Nemlin and Y.A. Bekro, 2009. Chemical composition and antioxidant activities of essential oils of *Xylopiya aethiopic* (Dunal) A. Rich. Eur. J. Sci. Res., 37: 311-318.
- König, W.A., D.H. Hochmuth and D. Joulain, 2001. Terpenoids and related constituents of essential oils. University of Hamburg available from MassFinder 3.5, Hochmuth Scientific Software: Hamburg, Germany.
- Letouzey, R., 1982. Manuel de botanique forestière. Afrique Tropicale. Tome 2. CTFT Nogent-sur-Marne, pp: 134.
- Mc Lafferty, F.W. and D.N. Stauffer, 1989. The Willey NBS Registry of Mass Spectral Data. 2nd Edn., J. Wiley and Son, New York.
- NIST (National Institute of Standards and Technology), 1999. PC Version 1.7 of the NIST/EPA/NIH Mass Spectra Library. Perkin-Elmer Corp., Norwalk, CT, USA.
- Noudjou, F., H. Kouninki, T. Hance, E. Haubruge, S.T.N. Léonard, M.M. Pierre *et al.*, 2007. Composition of *Xylopiya aethiopic* (Dunal) A. Rich essential oils from Cameroon and identification of a minor diterpene: ent-13-epi manoyl oxide. Biotechnol. Agron. Soc., 11: 193-199.
- Noudogbessi, J.P., A.K. Natta, F. Avlessi, D.C.K. Sohounhloue, G. Figueredo and J.C. Chalchat, 2011. Chemical composition of the essential oils extracted from two annonaceae required in Beninese pharmacopeia. Aust. J. Basic Appl. Sci., 5: 34-40.
- Onlisanakin, A., M.O. Oladimeji and L. Lajide, 2007. Composition and antibacterial activity of steam distilled oils from *Xylopiya aethiopic* and *Syzgium aromaticum*. J. Eng. Appl. Sci., 2: 236-240.
- Tchiegang, C. and P.D. Mbougueng, 2005. Composition chimique des épices utilisées dans la préparation du Nah-poh et du Nkui de l'Ouest Cameroun. Tropicultura, 23: 193-200.
- Thomas, A., 1969. Les annonacées. Museum national d'Histoire Naturelle, Paris, pp: 371.
- Tisserant, P. Ch., 1950. Catalogue de la flore de l'Oubangui-Chari. Mémoire de l'Institut d'études centrafricaines, 2: 20.
- Van den Dool, H. and P.D. Kratz, 1963. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J. Chromatogr., 11: 463-471.