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Combined analysis of *Xylopia aethiopica* trunk bark oil by chromatographic and spectroscopic techniques.

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Abstract

The chemical composition of four oil samples of *Xylopia aethiopica* isolated from trunk bark harvested in three Ivoirian forests was investigated by chromatographic (GC with retention indices) and spectroscopic techniques (MS, ^{13}C NMR). Thirty compounds accounting for 90.2-98.2% of the oil have been identified. *X. aethiopica* stem bark produced a monoterpene-rich essential oil, whose composition was dominated by β-pinene (37.0-61.6%) and α-pinene (13.0-19.3%). Two samples out of four contained also 3,3-dimethyl-1-vinylcyclohexene (up to 6.4%) and 4,4-dimethyl-2-vinylcyclohexene (up to 12.7%).

Keywords: Xylopia aethiopica, trunk bark oil, chemical composition, ^{13}C NMR, Côte d'Ivoire.

1. Introduction

Xylopia aethiopica (Dunal) A. Rich. is an evergreen, aromatic tree, growing up to 20 m high with a clear straight bole, to 75 cm in diameter with smooth grey bark. It is a native to the lowland rainforest and moist fringe forests in the savanna zones of Africa widespread in the Region from Senegal to Sudan. The fruit is a slightly hooked cylindrical pod reaching 2-3 mm in width.

Xylopia aethiopica has a wide variety of application; the very odorous roots of the plant are employed in West Africa in tinctures, administered orally to expel worms and other parasitic animals from the intestines, or in teeth-rinsing and mouth-wash extracts against toothaches. The fruits, which are a common ingredient of African traditional cough medicines and are also used as a spice and flavouring agent [1]. In Ivory Coast, the leaves are employed to treat bronchitis and dysentery [2].

The composition of *X. aethiopica* fruit oil from various countries (Guinea, Mali, Ghana, Benin, Nigeria, Cameroon, Soudan and Egypt) has been investigated extensively. It is a monoterpene-rich oil, the major components being in most cases β-pinene, sabinene, cineole or terpinen-4-ol while germacrene D is the major reported sesquiterpene [3-9]. Leaf oils contained α- and β-pinene as major components, accompanied by various sesquiterpenes, such as β-eudesmol, β-elemol, α-cadinol or germacrene D [6, 9]. Finally, root bark and stem bark oil samples from Ghana differed markedly, their compositions being dominated by trans-mentha-1(7),8-diene (30.4% and 30.7%, respectively) and β-copaene-4 α-ol (13.3%, root bark oil) or germacrene D (8.8%, stem bark oil) [6].

Concerning Ivory Coast, a few articles reported about the composition of *X. aethiopica* essential oils. A fruit oil sample exhibited a very usual composition dominated by β-pinene (20.5%), α-pinene (17.8%), 1,8-cineole (7.4%), and α-phellandrene (5.6%). Leaf oil contained mostly two monoterpene hydrocarbons, β-pinene (16.0%) and α-pinene (10.4%), as well as the oxygenated sesquiterpene β-eudesmol (12.7%) [10]. We recently reported on the chemical composition of 48 essential oil samples isolated from the leaves of *X. aethiopica* harvested in six Ivoirian forests. Statistical analysis allowed the distinction of two groups on the basis of the β-pinene (up to 61.1%) and germacrene D (up to 28.7%) contents. Oils of Group I (38 oil samples) contained predominantly β-pinene, while those of Group II (10 samples) were characterized by the association of β-pinene and germacrene D. The leaves collected in the four inland forests produced β-pinene-rich oils (Group I), while the oil samples belonging to Group II were isolated from leaves harvested in forests located near the littoral [11]. In contrast,

the major components of a root oil sample were identified as 3,3-dimethyl-1-vinylcyclohexene and 4,4-dimethyl-2-vinylcyclohexene. The former was reported for the first time as a natural compound and the later was a new compound [12]. Ivoirian *X. aethiopica* root oil differed drastically from Ghanaian root oil [6].

In the present study, we report on the chemical composition of trunk bark oil of *X. aethiopica*, investigated for the first time. Qualitative and quantitative analysis of individual components has been carried out by chromatographic (GC with retention indices) and spectroscopic techniques (MS, ^{13}C NMR).

2. Materials and methods
2.1 Plant Material and Essential Oil Production
Trunk bark of *X. aethiopica* was collected in South-Eastern Côte d'Ivoire, in Petit Yapo forest (near Agboville, samples B1 and B2, May 2009), in the Ahoué forest (near Alépé, sample B3, June 2009) and in the Yapo Abbé forest (near Adzopé, sample B4, July 2009). Plant material has been authenticated by Professor L. Aké Assi, from the CNF, Abidjan. A voucher specimen has been deposited at the herbarium of the CNF, Abidjan, reference LAA 13159. Fresh material (around 0.6-1.4 kg) was separately submitted to hydrodistillation in a Clevenger-type apparatus for 3 hours. Yields (w/w) were calculated on the fresh weight basis.

2.2 Analytical GC
GC analysis was carried out using a Clarus 500 Perkin Elmer apparatus equipped with FID and two fused-silica capillary columns (50 m x 0.22 mm, film thickness 0.25 μm), BP-1 (polydimethyl siloxane) and BP-20 (polyethylene glycol). The oven temperature was programmed from 60-220 °C at 2 °C/min and then held isothermal at 220 °C for 20 min; injector temperature: 250 °C; detector temperature: 250 °C; carrier gas: helium (0.8 mL/min); split: 1/60. The relative proportions of the oil constituents were expressed as percentage obtained by peak area normalization, without using correction factors. Retention indices (RI) were determined relative to the retention times of a series of *n*-alkanes with linear interpolation (« Target Compounds » software from Perkin Elmer).

2.3 GC-MS Analysis
Oil sample B4 was analyzed with a Perkin-Elmer TurboMass detector (quadrupole), directly coupled to a Perkin-Elmer Autosystem XL, equipped with a fused-silica capillary column (60 m x 0.22 mm i.d., film thickness 0.25 μm), Rtx-1 (polydimethylsiloxane). Carrier gas, helium at 1 mL/min; split, 1/80; injection volume, 0.2 μL; injector temperature, 250 °C; oven temperature programmed from 60 °C to 230 °C at 2 °C/min and then held isothermal (45 min); Ion source temperature, 150 °C; energy ionization, 70 eV; electron ionization mass spectra were acquired over the mass range 35-350 Da.

2.4 ^{13}C NMR Analysis
NMR spectra were recorded on a Bruker AVANCE 400 Fourier Transform spectrometer operating at 100.63 MHz for ^{13}C-NMR, equipped with a 5 mm probe, in CDCl$_3$, with all shifts referred to internal TMS. ^{13}C-NMR spectra of the oil samples were recorded with the following parameters: pulse width = 4 μs (flip angle 45°); acquisition time = 2.7 s for 128K data table with a spectral width of 25 000 Hz (250 ppm); CPD mode decoupling; digital resolution = 0.183 Hz/pt. The number of accumulated scans was 2000-3000 for

each sample depending of the available amount of oil (when available, 40-50 mg of oil in 0.5 mL of CDCl$_3$).

2.5 Identification of Components
Identification of the individual components was based: (i) by comparison of their GC retention indices (RIs) on polar and apolar columns, determined relative to the retention times of a series of *n*-alkanes with linear interpolation with those of reference compounds (ii) on computer search using digital libraries of mass spectral data and comparison with literature [13-18] (iii) on comparison of the signals in the ^{13}C NMR spectra of essential oils with those of reference spectra compiled in the laboratory spectral library, with the help of a laboratory-made software. In the investigated samples individual components were identified by NMR at content as low as 0.3-0.4% [19-22].

3. Results & Discussion
Hydrodistillation of the fresh trunk bark collected on four individual trees of *X. aethiopica* produced a clear essential oil with yields (0.06-0.11%, *w/w*, calculated on fresh weight basis). The four oil samples have been analyzed by Gas Chromatography in combination with retention indices [GC(RI)] and by ^{13}C NMR, following a computerized method developed at the University of Corsica [19-22]. Sample B4 has been analyzed also by Mass Spectrometry (GC-MS). Thirty compounds, accounting for 91.6-98.7% of the whole composition of the samples, have been identified (Table 1). The oils were made up predominantly of monoterpenes accounting for 78.8-92.7%, whereas the sesquiterpenes represented 5.1-9.7%. Although all the samples were qualitatively similar, a quantitative chemical variability was observed, with a significant variation of the contents of the major components, β-pinene (37.0-61.6%) and α-pinene (13.0-19.3%). Five other components were present at appreciable contents: camphene (up to 3.5%), *p*-cymene (up to 3.5%) and cyperene (up to 3.5%), as well as (Z)-β-ocimene (up to 3.9%) and sabinene (up to 4.3%). By contrast, oxygenated compounds are poorly represented: bornyl acetate (0.1-2.1%), τ-cadinol (0.4-1.8%), terpinen-4-ol (0.5-1.4%), 1,8-cineole (0.3-1.3%), linalool and α-terpineol (up to 1.1% respectively). It should be pointed out the occurrence in all the samples, at variable contents (0.1-1.4%) of *endo*-5-methoxypatchoul-3-ene, a sesquiterpene oxide first isolated from *X. aethiopica* root oil [12]. Similarly, the vinylcyclohexenes, 3,3-dimethyl-1-vinylcyclohex-1-ene (up to 6.4%) and 4,4-dimethyl-2-vinylcyclohex-1-ene (up to 12.7%) also previously found as natural compounds of *X. aethiopica* root oil [12] were identified in the samples B3 and B4 from Petit Yapo forest. In contrast, these two compounds were absent from the sample B2 and represented just 0.4% in sample B1. The two samples from Petit Yapo forest differed substantially from the others.

Although the composition of the four oil samples was not extremely complex, it could be pointed out the crucial role of ^{13}C NMR in the identification of various components. Indeed, identification of the vinylcyclohexene isomers (the major components of *X. aethiopica* root oil) [12] and that of *endo*-5-methoxypatchoul-3-ene, also isolated from the same root oil sample [12] was achieved by comparing the ^{13}C NMR chemical shift values in the trunk bark oil samples and in the root bark oil. ^{13}C NMR appeared also useful in the identification of limonene, cineole and β-phellandrene which were overlapped on the apolar column used to carry out GC-MS analysis. In a similar way,

rotundene is a tricyclic sesquiterpene hydrocarbon bearing the bicyclo[3.2.2]nonane substructure instead of the bicyclo[3.2.1]octane substructure of the *endo*-5-methoxypatchoul-3-ene. This compound was first isolated from *Cyperus scariosus* essential oil [23]. Its identification was suggested by MS and confirmed by ^{13}C NMR, in agreement with the data reported by Joulain and König [14].

4. Conclusions
From these results, it may be concluded that trunk bark of *X. aethiopica* produces a monoterpene-rich essential oil, the composition of all investigated samples being dominated by β-pinene and α-pinene. The unusual 3,3-dimethyl-1-vinylcyclohex-1-

ene and 4,4-dimethyl-2-vinylcyclohex-1-ene have been observed at appreciable content in two samples out of four. By comparison, oil samples isolated from leaves of the same trees contained also β-pinene (37.0-61.6%) and α-pinene (11.4-16.0%) as main components accompanied by germacrene D (14.0-24.6%) and (E)-β-caryophyllene (4.0-6.8%) [24]. It could be pointed out that the trunk bark oil from Ivoirian *X. aethiopica* differed drastically from the stem bark and root bark oils from Ghana, that exhibited *trans-m*-mentha-1(7), 8-diene as major component [6]. Finally, ^{13}C NMR was confirmed as a powerful tool in the analysis of essential oils.

5. Tables and Figures

Table 1: Chemical composition of four trunk bark oil samples from *Xylopia aethiopica*.

Compounds	RIa	RIp	B1	B2	B3	B4
α -Thujene	920	1024	**0.4**	**0.5**	**0.4**	**0.3**
α -Pinene	927	1022	**13.0**	**19.3**	**13.5**	**14.3**
Camphene	940	1068	**3.5**	**1.2**	**0.2**	**0.3**
Sabinene	962	1121	**3.7**	**4.3**	**0.9**	**1.3**
β-Pinene	969	1113	**45.8**	**61.6**	**37.0**	**37.3**
Myrcene	976	1160	0.4	-	**1.1**	**0.7**
3,3-Dimethyl-1-vinylcyclohex-1-ene	980	1154	0.1	-	**6.1**	**6.4**
4,4-Dimethyl-2-vinylcyclohex-1-ene	984	1167	0.3	-	**10.9**	**12.7**
α -Phellandrene	994	1165	**0.6**	0.2	**2.6**	**2.1**
p-Cymene	1008	1270	**1.1**	0.2	**3.5**	**0.4**
Limonene*	1017	1200	**1.9**	**1.1**	**2.3**	**1.8**
1,8-Cineole*	1017	1208	**1.2**	**1.3**	**0.5**	-
β -Phellandrène*	1017	1210	**1.5**	**1.2**	**2.1**	**2.0**
(Z)- β -Ocimene	1022	1232	**3.9**	**1.2**	**2.0**	**3.1**
(E)- β -Ocimene	1033	1248	**1.0**	**0.3**	0.3	0.4
γ-Terpinene	1044	1244	0.3	0.2	**0.4**	**0.4**
Linalool	1079	1540	**1.1**	0.1	**0.6**	**0.8**
Terpinen-4-ol	1157	1596	**1.4**	**0.5**	**0.7**	**0.6**
α -Terpineol	1167	1695	**0.7**	0.2	**1.1**	**0.7**
Bornyl acetate	1265	1577	**2.1**	0.2	0.1	0.1
α -Copaene	1371	1489	0.1	0.1	**0.8**	0.2
β -Elemene	1384	1586	0.2	0.2	0.3	**0.4**
Cyperene	1396	1525	**1.0**	**2.1**	**1.4**	**3.5**
(E)- β -Caryophyllene	1413	1593	**0.7**	0.3	**2.3**	**0.8**
Rotundene	1453	1634	0.2	0.3	0.1	**0.4**
Germacrene D	1472	1704	**1.9**	**0.8**	**1.0**	**2.5**
β -Selimene	1486	1713	0.1	0.1	**2.8**	0.2
δ-Cadinene	1509	1752	0.2	0.2	**0.6**	**0.5**
endo-5-Methoxypatchoul-3-ene	1540	1778	**1.4**	**0.5**	0.1	**0.4**
τ-Cadinol	1620	2160	**1.8**	**0.5**	**0.4**	**0.6**
Total			91.6	98.7	96.1	95.5

Order of elution and percentages of individual components are given on apolar (BP1) capillary column except for compounds indicated by an asterisk, percentage on polar (BP20) column. Compounds with percentages in bold letters have been identified by ^{13}C NMR.

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