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Research Article

Effect of afforestation of the coastal savannah of Pointe-Noire (Congo-Brazzaville) on the chemical composition of leaves and stem bark essential oils from *Xylopia aethiopica* (Dunal) A. Rich

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Xylopia aethiopica, domestication, pinene, caryophyllene, Congo-Brazzaville.

Abstract

Xylopia aethiopica from the Youbi natural forest was used to establish plantations on the coastal savannah in Pointe Noire, Congo-Brazzaville. Leaves and stem barks essential oils, were analysed by GC/MS for both natural forest and plantation. The oils from the natural forest all belong to the pinene chemotype (α pinene and β pinene), those from the plantation showed the presence of two chemotypes: pinene chemotype and caryophyllene chemotype (β caryophyllene and caryophyllene oxide). The pinene chemotype has a less complex chromatographic profile than the caryophyllene one. In *Xylopia aethiopica* endemic to Congo, domestication leads to a complexification of the chemical composition of the essential oil.

1. Introduction

Xylopia aethiopica is a dense forest understorey tree, 15 to 30 m high and 60 to 75 cm in diameter, growing on the banks of rivers or in swampy areas. It is also found in the Guinean savannah zone, along rivers. Several others are particular to the forest zone. Frequently, its bole is narrow with some buttresses at the base, 50 cm to 1 m high, relatively tall, and topped with a plume of horizontally spreading branches and twigs. It belongs to the family Annonaceae, subfamily Annonoideae, tribe Unoneae and subtribe Xylopineae [1].

The essential oil of *X. aethiopica* has antibacterial [2], antifungal [3, 4] and antiparasitic properties [5, 6]. Gözcü et al [7] demonstrated the activity of this oil on *Salmonella typhi*, *citrobacter sp*, *pseudomonas aeruginosa* and *serratia sp*. Anti-inflammatory properties of leaf essential oils have been recently reported [8].

In the Republic of Congo, *Xylopia aethiopica* is exploited in natural forests for the wood energy needs of large cities. This anthropic pressure, which only concerned forests close to large cities, has spread to remote forests, particularly on the edge of the Conkouati-Douli National Park in Kouilou. To ensure the sustainability of the species, commercial use of essential oils for food and medicine was envisaged through a programme of afforestation of the poor savannahs around the city of Pointe-Noire [9]. This programme was conducted by the former Centre de Recherche Forestières du Littoral (CRFL), now called the Institut National de Recherche Forestière (IRF) with encouraging results [10].

The characterisation of essential oils from natural forest stands used to reforest savannahs has been studied by our team [10]. The results, which served as a basis for comparison in the present study, showed the presence of pinenes (alpha + beta) as the majority compound in all compartments (fruits, leaves and stem bark) of the tree and in all trees studied.

The cultivation of *X. aethiopica* on poor savannahs should give rise to new reflections in order to evaluate the effect of this change of ecosystem on the chemical composition of essential oils. The objective of this study was to assess the effect of domestication of *X. aethiopica* on the chemical composition of leaves and stem bark essential oils.

2. Materials and methods

2.1. Plant material

Samples of leaves and stem bark of *X. aethiopica* were collected from 30 trees in a 6-year-old plantation in Youbi, in September 2010. The plant was identified at the Brazzaville National Herbarium by Dr Miabangana Edmond Sylvestre under the herbarium numbers J. Koechlin n°547; J. Koechlin n°1073; J. Trochain n°8386; J. Trochain n°8482; J. Trochain n°10228; J. Koechlin n°5518; J. Trochain n°10154; B. Descoings n°8272; Kami, T. n° 159; Cheek, M. n°14791. The plantation is located on a savannah included in the same study area carried out by our team and reported by Kama Niamayoua et al. [10].

The locality of Youbi is located 100 km from Pointe-Noire between latitudes 4°0'00'' and 4°30'00'' South and longitudes 11°30'00'' and 12°0'00'' East. The average annual rainfall in the region is in the order of 1400 - 1500 mm. The climate of the study area is tropical and humid, characterised by an alternating hot and rainy season and a cool and dry season. A severe dry season extends from June to September and a rainy season from October to May [11].

2.2. Extraction of essential oils

Water and plant material (300g of shade-dried leaves and dried bark), placed in a 500 mL flask, were boiled for 4 hours in a Clevenger type apparatus. The organic phase of the collected condensate is separated from the aqueous phase by extraction with diethyl ether. After drying the organic phase with sodium sulphate, the essential oil is recovered after evaporation of the solvent in the open air.

2.3. Determination of the chemical composition of essential oils

Qualitative analysis was carried out using an Agilent Gas Chromatograph, model 7890 coupled to an Agilent Mass Spectrophotometer (GC-MS), model 5975, equipped with a DB5 column (20m x 0.18mm x 0.18µm). The oven temperature is 50°C for 3.2 minutes and then increases to 300°C at a rate of 8°C per minute, the injector temperature is 280°C. The volume injected is 1 µL and ionisation is performed by electronic impact at 70 eV. The flow rate of helium, the carrier gas, is set at 0.9 mL/minute.

Quantitative analysis was carried out on an Agilent gas chromatograph, model 6890, equipped with a DB5 column (20m x 0.18mm x 0.18µm) fitted with a flame ionisation detector (FID). The oven temperature is 50°C for 3.2 minutes and then increases to 300°C at a rate of 10°C per minute, and the injector temperature is 280°C. The volume injected is 1 µL. The flow rate of hydrogen, the carrier gas, is 1mL/minute.

The spectra are then interpreted using Enhanced Data Analysis software and databases [12].

2.4. Statistical processing

Descriptive statistics led to the calculation of means and standard deviations and running graphs using Excel software.

3. Results and discussion

3.1. Leaves and stem bark essential oils yield

The essential oil content of plantation leaves is relatively identical to that obtained from forest trees, with a slightly higher average (0.21%) than that obtained in the forest (0.16%) [10], in the same study area (Table 1).

These yields remain slightly lower on average than those obtained [13] in Côte d'Ivoire, [14] in Benin [15] in Ghana (Table 2).

Studies reporting the essential oil content of *X. aethiopica* stem barks are quite rare. However, the contents obtained in the present work are on average 10 times lower than those obtained in Ghana [15].

Yields of stem bark essential oil are identical in both forests and plantations (Table 3).

Table 1. Comparative study of essential oil content (%) of plantation and forest leaves

Trees	Plantation (a)	Trees	Forest (b)
A2	0,24	A1	0,26
A7	0,27	A15	0,14
A9	0,13	A5	0,12
A13	0,21	A11	0,12
Moyenne	**0,21**		**0,16**
Ecart-type	0,06		0,07

(a) : Present works; (b) : [10].

Table 2. Comparative study of leaf essential oil content of *X aethiopica* in relation to the literature

Countries	Ecosystems	Yield (%)
Bénin [14]	Forest	0,30
Ghana [15]	Forest	0,46
Cote d'Ivoire [13]	Forest	0,25
Congo [10]	Forest	0,12-0,26
Congo (present work)	Plantation	0,13-0,21

Table 3. Comparative study of essential oil content (%) of plantation and forest stem bark

Plantation trees (a)	Yield (%)	Forest trees (b)	Yield (%)
A2	0,030	A1	0,013
A7	0,090	A15	0,062
A9	0,020	A5	0,038
A13	0,040	A11	0,068
Moyenne	**0,040**		**0,045**
Ecart-type	0,03		0,03

(a) : present work; (b): [10].

3.2. Chemical composition of leaf essential oils

The essential oils extracted from the leaves of the plantation trees show two main trends as recorded in Table 4, obtained from 4 randomly selected trees (A7, A8, A13 and A17). On the one hand, there are individuals with a high content of pinenes (alpha + beta) with 30 to 40%, representing half of the population studied, and on the other hand, those in which caryophyllenes (beta caryophyllene+ caryophyllene oxide) represent the majority compound with 28 to 31% for the other half of the population. Fig. 1 shows the molecular structure of the main compounds present in the leaves and stem bark essential oils of *X. aethiopica* from the Youbi area. With such a composition, we can hypothesise the existence, in plantations, of two chemotypes, one with

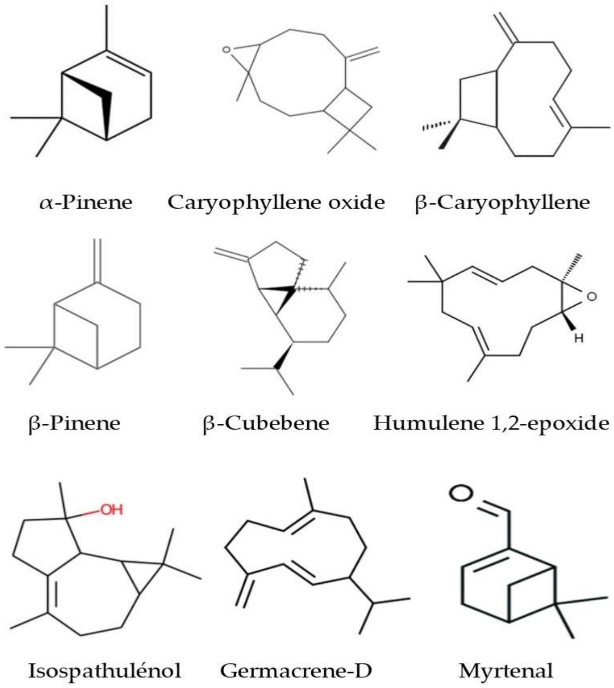

Figure 1. Molecular structure of the main compounds present in the leaves and stem bark essential oils of X. aethiopica from the Youbi area.

existence, in plantations, of two chemotypes, one with pinene and the other with caryophyllene. This leads to a "radar plot" representation illustrated by Figs 2 and 3.

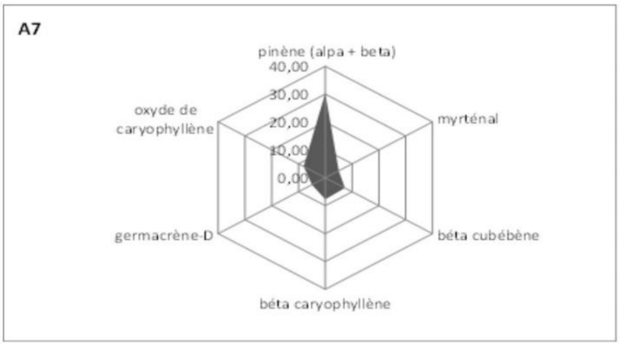

Figure 2. Radar plot of leaves essential oil of the pinene chemotype

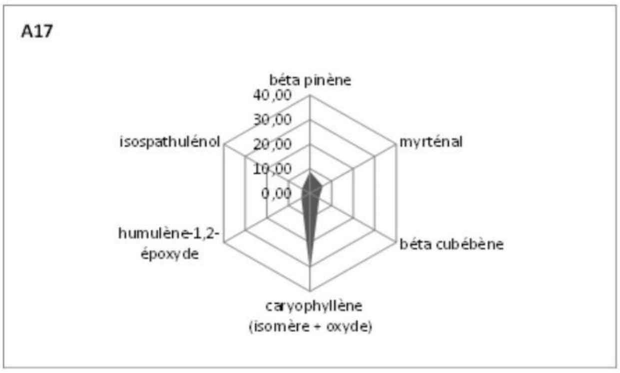

Figure 3. Radar plot of leaves essential oil of the caryo-phyllene chemotype

Table 4. Comparative study of the chemical composition of leaves essential oils of *X. aethiopica* from forest and plantation

IR	IRcalc	Constituants	Plantation (a)				Natural forest (b)			
			A7	A8	A13	A17	A1	A5	A11	A15
926	932	Alpa pinène	6,22	2,46	9,22	1,65	8,72	9,82	8,44	13,31
967	969	Sabinène					1,49	1,11	4,11	1,79
967	969	Béta pinène	24,10		32,9	8,91	31,89	36,29	30,77	47,14
1022	1025	1,8-Cinéole							14,01	
1197	1195	Myrténal	4,98	2,18	5,14	5,95	3,65	4,41	1,01	3,30
1391	1387	Béta cubébène	7,30	2,91	6,89	4,23	5,76	5,12	8,16	4,45
1431	1434	Béta caryophyllène	7,71		9,49	3,46	10,47	2,90	5,28	5,03
1485	1484	Germacrène-D	4,99		3,64		5,14		7,07	2,51
1564	1559	Germacrène-B		6,37						
1616	1608	Humulène-1,2-époxyde	1,59	3,56		4,00				
1588	1582	Oxyde de caryophyllène	8,12	28,85	5,32	28,33	6,75	4,96	1,44	2,87
1620	1611	Torilénol						5,04		
1632	1621	Isospathulénol	2,81	2,36	1,28	3,77				
Compounds classes										
Monoterpene hydrocarbons			30,32	2,46	42,12	10,56	42,1	47,22	43,32	62,24
Oxygenated monoterpenes			4,98	2,18	5,14	5,95	3,65	4,41	15,02	3,30
Sesquiterpene hydrocarbons			20,00	2,91	20,02	7,69	21,37	8,02	20,51	11,99
Oxygenated sesquiterpenes			12,52	34,77	6,6	36,1	6,75	10	1,44	2,87
Others										
Total identified			***67,82***	***42,32***	***73,88***	***60,30***	***73,87***	***69,65***	***80,29***	***80,40***

a : present work ; b: [10].

However, the work of [10] showed the presence of only one pinene chemotype in all the trees of the Youbi forest. The existence of the pinene chemotype in the leaf essential oil of *X. aethiopica* has also been reported in several countries including Benin [16, 15], Cote d-Ivoire [14], Cameroon [17], while in Ghana, [18] reports the predominance of trans-m-mentha-1(7),8-diene. Studies on the essential oil of *X. aethiopica* from plantations are rare. This is also the case for those reporting the presence of caryophylls as the majority compound in leaves essential oil as reported in the present study. Caryophyllene oxide has been reported at levels of 3.22% in the leaf essence of *X. aethiopica* from the forest in Côte d'Ivoire [14].

Figs. 4 and 5 show typical chromatograms of pinene and caryophyllene profiles. In plantations, Isospathulenol and humulene-1,2-epoxide are present although at low levels (1-4%), whereas they are absent in forest trees. The presence of Isospathulenol was reported by [19] in forest leaf oil from Benin at 3%. Germacrene D is only present in the pinene chemotype with 3 to 5%, it is absent in the caryophyllene chemotype. Several authors have observed the presence of germacrene D in the same proportion (2-7%) mainly in pinene chemotypes [15,

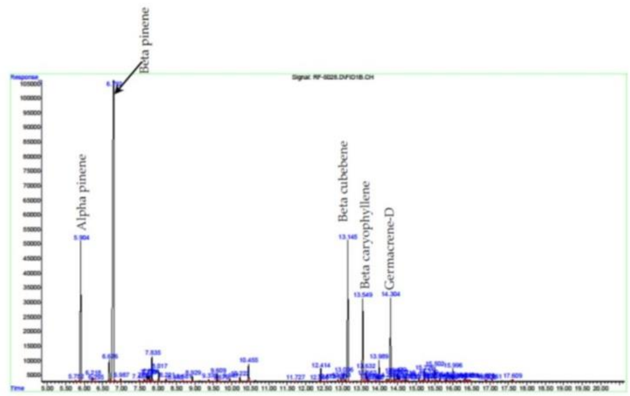

Figure 4. Chromatographic profile of pinene chemotype

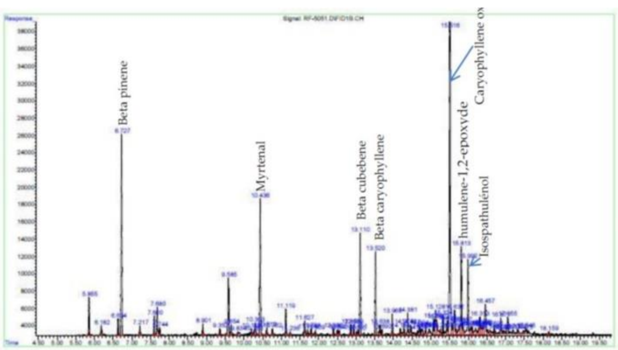

Figure 5. Chromatographic profile of caryophyllene chemotype

14].

Oxygenated hydrocarbons such as 1,8-cineole and torilenol have been reported in forest trees in 1 tree out of 4 at proportions of 14% and 5% respectively [10] while they are absent in plantations. The cultivation of *X. aethiopica*, a forest species, on a savannah seems to explain the variability observed in the chemical composition of plantation trees. The edaphic characteristics and the microclimate seem to be at the origin of the differences observed. Indeed, the studies carried out on both young and adult forest trees show the predominance of pinenes in all samples.

Pinenes (alpha+beta) and caryophylls (beta caryophyllene and caryophyllene oxide) are present in the leaves oil of all plantation trees in very different proportions depending on the chemotype.

While compounds such as myrtenal and beta-cubebene are present in all samples both in the forest [10] and in plantations in relatively identical proportions (2-8%) and can thus be considered as the most stable compounds in the study area, the leaves essential oils of *X. aethipica* from Benin are totally devoid of them [16, 19, 14].

Sabinene is totally absent from plantation leaves whereas it has been reported in the leaves of all forest trees, although at levels below 5%. It has also been reported in the oil of forest trees in Benin at a content of 14% [19].

3.3. Chemical composition of stem bark essential oils

The chemical composition of stem bark essential oils shows that 3 compounds representing almost 70% of the total essence can be considered as characteristic of stem bark oil (Table 5). These are: pinenes (alpha +beta), beta-cubebene and caryophyllenes (beta caryophyllene and caryophyllene oxide).

These essential oils show a predominance of pinenes (alpha +beta) in all the trees studied and at levels of 25 to 40% of the total oil composition. This is also shown in the work of [10] on the stem bark oils of forest from the same study area.

Beta-pinene remains the majority constituent with contents of up to 36%. This finding is similar to that of [20] on the stem bark essential oil of pepper in Benin with 10%, while the stem bark of the Ghanaian species is rather rich in trans-m-mentha-1(7),8-diene with 30% [18].

In plantations, hydrogenated sesquipertenes such as beta-caryophyllene, beta-cubebene and germacrene D are totally absent from the essential oil, whereas they are present in the stem barks essential oil of forest at quite high levels (2-14%).

The stem bark essential oils of plantation are richer in oxygenated hydrocarbons than those of forest trees. However, hydrocarbon sesquiterpenes are almost totally absent in plantations, whereas forest stem bark is rather well endowed with them, with contents of around 35%. Myrtenal is the second most important constituent with contents of up to 14% and is present in all samples, both in plantations and in forests. Trans-pinocarveol is also present in almost all trees in the study area, except in forest trees, where it is found in only 2 out of 4 trees and in proportions not exceeding 7%. The presence of these two oxygenated hydrocarbons (myrtenal and trans pinocarveol) was reported by [20] at rather modest proportions (2.85 and 5.42% respectively) in the stem bark of the Ghanaian pepper tree.

Humulene-1,2-epoxide and alpha cadinol, two oxygenated hydrocarbons were only identified in plantation samples at individual levels of 1-7%.

4. Conclusions

This study shows that the domestication of *Xylopia aethiopica* on the coastal savannahs of Congo Brazzaville has an influence on the chemical composition of the leaves essential oils of plantation trees. Indeed, the chemical composition of the plantation trees showed the presence of two chemotypes in the essential oils of the leaves, notably a pinene chemotype in 50% of the population studied and another with caryophyllene, whereas the oils of the natural forest trees are all pinene. The stem bark oils of the planted trees were identical to those of the natural forest trees, and all contained pinenes. This study constitutes a tool for predicting the quality of the oils that would be produced by the *Xylopia aethiopica* plantations that develop on the savannahs of the Congolese coast.

Authors' contributions

Conceptualization, J.B.B. and A.F.B.; Methodology, J.B.B. and A.F.B..; Software, J.B.B.; Validation, T.S., H.M. and R.K.N.; Formal analysis, J.C.C.; Investigation, J.B.B. and A.F.B.; Resources, R.K.N., J.B. B. and A.F.B.; Data curation, J.B.B.; Writing original draft preparation, J.B.B.; Writing review & editing,

Table 5. Comparative study of the chemical composition of stem bark essential oils of *X. aethiopica* from forest and plantation individuals

IR	IR$_{calc}$	Constituants	Plantation (a)				Natural forest (b)			
			A7	A8	A13	A17	A1	A5	A11	A15
926	932	Alpa Pinène	8,10	4,04	5,26	1,54	7,83	12,78	5,77	5,98
967	969	Béta Pinène	32,82	21,45	24,07	8,08	36,01	44,61	22,16	22,25
1021	1024	Limonène							6,56	
1136	1135	Trans Pinocarvéol	2,86	1,52	4,07	6,98	6,08	2,02		
1197	1195	Myrténal	7,25	4,04	10,87	14,22	5,85	5,73	1,76	1,29
1391	1387	Béta Cubébène					1,74	1,61	11,38	14,14
1431	1434	Béta Caryophyllène						1,17	3,86	8,88
1486	1484	Germacrène-D							3,62	11,55
1588	1582	Oxyde De Caryophyllène	2,90	3,43	2,36	5,96	3,23	3,43	5,48	2,48
1616	1608	Humulène-1,2-Epoxyde	4,07		3,67	1,29				
1620	1611	Torilénol		7,75						
1660	1652	Alpha Cadinol	1,21	7,51	3,80	2,28				
Compounds classes										
Monoterpene hydrocarbons			40,92	25,49	29,33	9,62	43,84	57,39	34,49	28,23
Oxygenated monoterpenes			10,11	5,56	14,94	21,2	11,93	7,75	1,76	1,29
Sesquiterpene hydrocarbons							1,74	2,78	18,86	34,57
Oxygenated sesquiterpenes			8,18	18,69	9,83	9,53	3,23	3,43	5,48	2,48
Others										
Total identified			***59,21***	***49,74***	***54,10***	***40,35***	***60,74***	***71,35***	***60,59***	***66,57***

a:present work; b: [10]

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Availability of data and materials

All data will be made available on request according to the journal policy.

Conflicts of interest

The authors declare no conflict of interest.

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