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The Bark Essential Oil Composition and Chemotaxonomical Appraisal of *Cedrelopsis grevei* H. Baillon from Madagascar

Miarantsoa Rakotobe[a], Chantal Menut[b,*], Hanitriniaina Sahondra Andrianoelisoa[c], Voninavoko Rahajanirina[a], Philippe Collas de Chatelperron[d], Edmond Roger[a] and Pascal Danthu[d]

[a]*Université d'Antananarivo, Faculté des Sciences and URP Forêts et Biodiversité. BP 853, Antananarivo, Madagascar*

[b]*ENSCM, Equipe Glycochimie, IBMM, UMR 5247, 8 Rue de l'Ecole Normale, 34296 Montpellier cedex 05, France*

[c]*Centre National de Recherches Appliquées au Développement Rural/Département de Recherches Forestières et Piscicoles (FOFIFA/CENRADERU/DRFP) and URP Forêts et Biodiversité, BP 904, Antananarivo, Madagascar*

[d]*CIRAD, URP Forêts et Biodiversité, BP 853, Antananarivo, Madagascar and Campus de Baillarguet, 34392 Montpellier cedex 05, France*

chantal.menut@univ-montp2.fr

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Cedrelopsis grevei is an aromatic and endemic tree of Madagascar. Its essential oil, resulting from its bark, and known under the name katrafay oil, is used in Madagascar in traditional medicine and in aromatherapy in the areas of the North. The chemical composition of this oil shows a great variability. This work proposed to study some determinants of this variability. Sampling was carried out tree by tree, in six representative sites of the distribution area of the species, followed by distillation and analysis by GC-MS. Twenty one samples were thus analyzed and 71 molecules were identified in at least one sample. A principal components analysis was performed considering the thirteen principal compounds identified in these essential oils. Four chemical patterns were distinguished. The first is characterized by eudesmane skeletons, mainly represented by selinenes (3.4-17.2%) and eudesmols (9.9-37.8% and 0-11.1% for the α– and γ– isomers, respectively); the second is rich in α-pinene (2.1-30.0%) and copaborneol (4.7-20.0%); the third is dominated by α-copaene (5.9-11.8%) and ishwarane (13.7-22.1%), and the last by cadinane skeletons (cadinenes 0.5-35.2%, T-muurolol 0-11.8% and α-cadinol 0-6.7%). Variable amounts of α-bisabolol were found in samples belonging to these four groups. Our study indicates a linkage between the zone of gathering and the essential oil chemical composition. There was not much variability between trees in the same zone, but there were big differences from one zone to another. These results make it possible to propose methods for sustainable collection and durable management of the species.

Keywords: *Cedrelopsis grevei*, katrafay, essential oil composition, individual variation, geographic variation, Madagascar.

Cedrelopsis grevei Baillon, belonging to the Rutaceae (previously classified in the Meliaceae, then the Ptaeroxylaceae), is an aromatic and endemic tree of Madagascar that can reach 15 m in height and over one meter in diameter. Its distribution area is broad and ecologically diversified. It corresponds with the spiny bush of the South, and with the xerophil and tropophil forests of the West of Madagascar [1,2] (Figure 1).

C. grevei is known under the Malagasy name katrafay. Its bark and leaves are very much used for their tonic, fortifying, anti-inflammatory, febrifuge and antalgic properties [3-6]. Such properties probably result from the presence of alkaloids, coumarins and chromones [7-10].

C. grevei essential oils are mainly produced from either the leaves or the barks. They are marketed

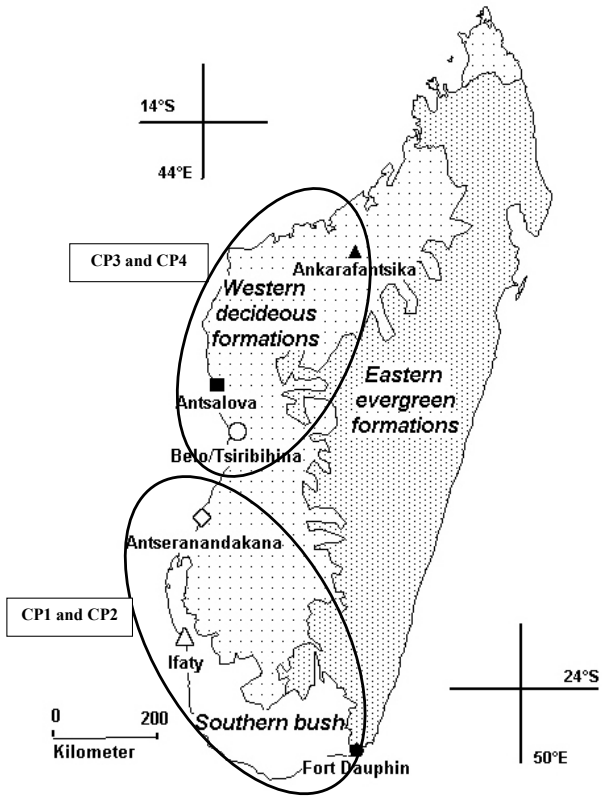

Figure 1: Location of the gathering sites of *C. grevei* samples.

locally and in the areas of the North (under the name of katrafay oils). The few works carried out on these essential oils have shown great variability in their composition. As far as the leaf oils are concerned, Menut *et al*. [11] reported that the main compounds were (*E*)-β-caryophyllene (42.5%), α-pinene (7.9%), α-humulene (6.4%), and (*Z*)-β-ocimene (5.3%). On the other hand, Gauvin *et al*. [12] identified *trans*-β-farnesene (35%), β-pinene (12.8%), hydrated *cis*-sesquisabinene (9.8%), and *ar*-curcumene (8.6%).

Other works carried out on the bark essential oils show the same variability. Cavalli *et al.* [13] mention the dominance of (*E*)-β-caryophyllene (9.3%), α-copaene (7.7%), and selinenes (10.3%) in one sample of commercial oil, and the dominance of ishwarane (1.0-17.4%), (*E*)-β-caryophyllene (1.3-12.5%), α-copaene (4.9-11%), β-elemene (0.2-9.6%), and selinenes (3.9-16.7%) in other commercial samples. Finally, Gauvin *et al*. [12] mention the presence of β-pinene (17.1%), *cis*-sesquisabinene hydrate (12.8%) and caryophyllene oxide (7.0%) as the main compounds in the bark essential oil of C. *grevei*.

In all cases, the oils were described as complex mixtures, dominated by sesquiterpenic structures, with only approximately 80% of identified components. Thus these authors point out a strong variability in the essential oils of *C. grevei*, without studying the determinants of this variability.

Is it linked to intrinsic (genetic) factors, as has been demonstrated for *Thymus vulgaris* [14] and *Hypericum perforatum* [15]? Are the environmental conditions (geography, habitat, native soils) the determinant features, as for *Melaleuca ericifolia* [16] and *Cinnamosma fragrans* [17], taking into account that the *C. grevei* distribution area is broad and ecologically diversified? Or is the situation more complex, as the one described for *Halfordia kendack* [18] and for *Ravensara aromatica*, species for which the pattern of bark oil variation is different from that of the leaf oils [19-20]?

Our objective was to find answers to these questions. Our study thus compared the chemical compositions of essential oils produced from *C. grevei* barks gathered from different points within the species distribution area. These points were representative of the different ecological contexts (Table 1). In order to obtain access to individual variability, each sample had to been taken from one single tree.

Hydrodistillation of 21 bark samples afforded essential oils with variable yields -0.01-1.0%- (Table 2). Their GC and GC-MS analyses allowed the identification of 71 compounds present in at least one sample.

Among these 71 volatiles, only eleven were found to represent more than 10% of the oil in at least one sample, taken individually or summed according to their structural linkage. They consisted of one monoterpene hydrocarbon (α-pinene), four sesquiterpene hydrocarbons (α-copaene, ishwarane, selinenes, cadinenes), and six oxygenated sesquiterpenes (copaborneol, T-muurolol, α-cadinol, α- and γ-eudesmols, α-bisabolol). These compounds, as well as caryophyllene and caryophyllene oxide, two major components previously mentioned [12,13] and which were found in noticeable amounts in almost all our samples (Table 2), were selected for Principal Component Analysis (PCA).

PCA pointed out four groups of variables characterizing four Chemical Patterns called respectively CP1, CP2, CP3 and CP4 (Figures 2 and 3, Table 2).

Table 1: Characteristics of *C. grevei* gathering sites analyzed in this study

Gathering site	Nb. of samples	Coordinates	Altitude (m)	Rainfall (mm)	Ecological context
Ankarafantsika	3 (K1-K3)	16°17'S, 46°49'E	140	1590	Dense, deciduous forest
Antsalova	1 (V1)	18°39'S, 44°11'E	60	1500	Semi-deciduous forest
Belo-sur-Tsiribihina	5 (B1-B5)	19°26'S, 44°35'E	57	815	Deciduous forest
Antseranandakana	3 (A1-A3)	20°58'S, 43°56'E	50	1000	Degraded dry forest
Ifaty	4 (I1-I4)	23°04'S, 43°37'E	10	390	Spiny xerophytic bush
Fort Dauphin (Andohahela)	5 (F1-F5)	24°47'S, 46°23'E	43	700	Spiny bush

Table 2: Chemical composition (relative percentages, extreme values are given for each group) and yields (mg/100g fresh material) of essential oils from bark of *C. grevei* collected in six regions in Madagascar (in bold: discriminate compounds for each CP).

	Chemical Pattern 1				Chemical Pattern 2		Chemical Pattern 3		Chemical Pattern 4	
	Belo/ Tsiribihina	Antseranan dakana	Ifaty	Fort Dauphin	Ifaty	Fort Dauphin	Antsa-lova	Commercial samples[a]	Belo/ Tsiribihina	Ankara-fantsika
Compound	B3	A1-A3	I1	F3,F5	I2-I4	F1,F2,F4	V1	C1-C3	B1,B2,B4,B5	K1-K3
α-pinene	0	0.3-2.1	0	11-18.1	**2.1-3.1**	**12.8-30.0**	0	0.3-0.7	0-2.7	0
α-copaene	0.3	1.3-3.4	3.7	0.4-1.7	1.9-3	1.1-5.1	**11.8**	**5.9-7.6**	0.1-6.3	1.6-13.8
β-caryophyllene	1.1	0-1.9	1.7	1.3-1.5	0.4-1.1	2.3-2.5	0.8	1.3-2.7	0.2-2.1	1.3-3.9
ishwarane	0	2.7-13.1	2.9	0	3.3-3.6	0	**13.7**	**19.1-22.1**	0	0
(α,β,δ)-selinene	**9.3**	**5.0-17.2**	**5.9**	**3.4-4.6**	1.1-4.5	2.2-4.4	1	0.7-1.6	0.1-5.6	0
(γ,δ)-cadinene	1.8	0-1	2.1	0.6-2	1.5-1.7	1.1-7.4	1	0.6-1.2	**0.5-5.1**	**12.6-35.2**
caryophyllene oxide	2.6	0-2.8	1.9	1.7-3.3	3.3-7.1	1.6-4.4	2.7	1.1-1.7	1.3-4.8	0.6-1
copaborneol	0	0	9.6	1.7-3.9	**12.3-20.0**	**4.7-6.3**	0	0	0-1	0
T-muurolol	13.7	0	0	0	0	0	1.1	0.4-1.9	**8.8-11.8**	**0-8.6**
γ-eudesmol	**0**	**2.4-11.1**	**7.9**	**7.3-8.2**	0-4.6	3-6.2	0.9	0.1-1	0	0
α-cadinol	0	0	1.1	0	0	0	0	0	**4.1-15.3**	**0-6.7**
α-eudesmol	**19.4**	**9.9-37.5**	**23.1**	**24-27.5**	7.6-11.3	3.9-5.6	0	0	0	0
α-bisabolol	0	0	7.1	5.6-5.7	**0**	**3.2-16.9**	1.0	0.2-0.6	**0-15.2**	**2.6-4.5**
Total	48.2	37.3-69.6	67.0	62.3-71.2	46.5-48.9	48.8-69	34.0	34.8-36.7	23.9-54.7	40.5-49.4
Oil yield (w/w, %)	0.3	0.1-0.2	0.6	0.04-0.05	0.5	0.02	0.06	un[b]	0.01-1.0	0.1

[a]: Commercial samples: BioAroma (C1), Homeopharma (C2), Bio'Mada (C3), [b]: unknown.

CP1 is composed of all the samples coming from Antseranandakana (A1-A3), of two samples from Fort Dauphin (F3, F5) and two lonely individuals (one from Ifaty, I1 and the second from Belo-sur-Tsiribihina, B3); it is characterized by eudesmane skeletons, mainly represented by selinenes (3.4-17.2%) and eudesmols (9.9-37.8% and 0-11.1% for the α– and γ– isomers , respectively). CP2, which gathers the last three Fort Dauphin samples (F1, F2 and F4), as well as the three other samples collected in Ifaty (I2-I4), is dominated by α-pinene (2.1-30.0%) and copaborneol (4.7-20%). CP3 containing the Antsalova sample (V1) is rich in α-copaene (11.8%) and ishwarane (13.7%). Finally CP4, which is constituted by all the samples coming from Ankarafantsika (K1-K3) and the four remaining samples from Belo-sur-Tsiribihina (B1, B2, B4 and B5) is dominated by cadinane structures, as cadinenes (0.5-35.2%), T-muurolol (0-11.8%) and α-cadinol (0-15.3%).

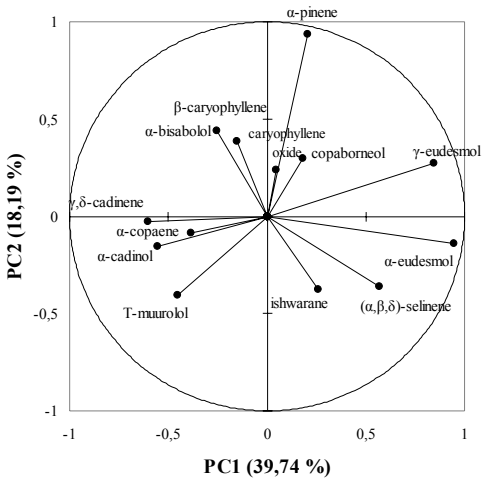

Figure 2: Circle of correlation of the thirteen examined variables by the two principal components (PC1/PC2).

The three commercial samples, which show similarities with the sample from Antsalova, have been classified in CP3 (Figure 3).

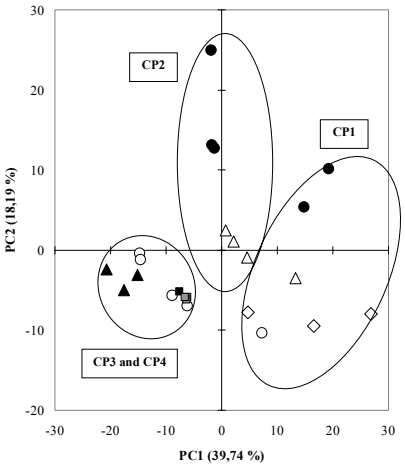

Figure 3: Graphical representation of the chemical pattern (CP) of the 21 individual essential oil samples from *C. grevei*, using PCA according to PC1-PC2. Trees from Belo-sur-Tsiribihina (B1-B5) are represented by white circles, those from Ifaty (I1-I4) by white triangles, those from Fort Dauphin (F1-F5) by black circles, those from Antseranandakana (A1-A3) by white rhomb, those from Ankarafantsika (K1-K3) by black triangles and that from Antsalova (V1) by a black square. Three supplementary points (grey square) represent commercial oils.

A comparison with previous results [12,13] shows globally a higher content of oxygenated sesquiterpenes, which results probably from a longer hydrodistillation process. Finally, *cis*-sesquisabinene hydrate, which was mentioned by Gauvin *et al*. as a major component of katrafay oil [12], was not present in our samples.

An agglomerative hierarchical clustering analysis has been computed on these data. No assumption was made concerning either the number of groups or the group structure. We used the single linkage criterion (smallest distance. i.e. nearest neighbour) applying to the matrix of the Euclidean distances (taking the thirteen variables into consideration) between the 21 individual samples.

The results of this single linkage clustering are graphically displayed in the dendrogram shown in Figure 4. Four clusters were identified, corresponding to the four Chemical Patterns already shown by PCA. It is worth noticing that the individual distribution of those four groups is linked to their origin. Thus all samples gathered in the south zone (Antseranandakana, Ifaty and Fort Dauphin) are distributed in the chemical patterns 1 and 2, whereas the samples from the northern zone (Antsalova, Belo-sur-Tsiribihina and Ankarafantsika) belong to CP3 and CP4 (Figure 1). The one exception is that of the sample B3 (Belo-sur-Tsiribihina), which was classified in CP1 owing to a high content of selinenes (9.3%) and α-eudesmol (19.4%), even if

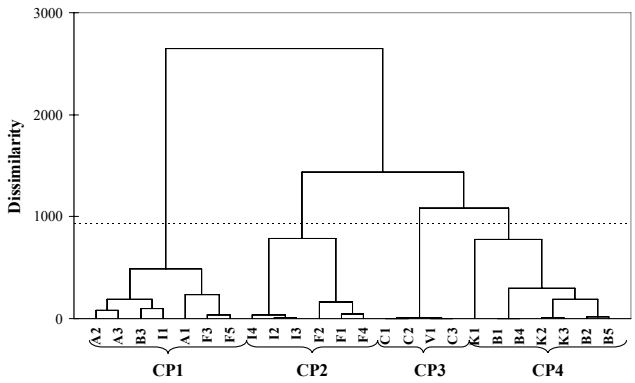

Figure 4: Single linkage dendrogram of the hierarchical clustering for Euclidean distances between the 20 individual samples of essential oil from *C. grevei* barks and the three commercial samples (C1, C2 and C3).

the proportion of α-cadinol brings it closer to the other samples of the same area, located in CP4. Finally, variable amounts of α-bisabolol were found in samples belonging to those four groups.

Likewise, even if the number of samples is small (maximum five samples per gathering zone), it appears that the essential oil composition variability between trees within the same zone is much weaker than that between zones.

Such a geographical variation mode of the essential oil composition has already been observed for other species, namely for *Cinnamosma fragrans* [17], which occupies, at least partly, the same distribution area. It is partly different from the distribution pattern found for *Halfordia kendack* (Rutaceae) for which the variation of leaf essential oil composition is not associated with geographic locality, altitudinal range or habitat [18]. It is also very different from the one found for *R. aromatica*, another Malagasy species, which shows a very strong individual variability of leaf oils, but a constancy of composition of the bark oils [19,20].

This can explain the fact that the commercial samples analyzed in this study are chemically close to the Antsalova sample though they come from Tuléar region (near Ifaty), unless the hydrodistillation process plays a prominent part in the chemical composition.

This very preliminary study shows the great variability in the composition of *C. grevei* oils, thus confirming previous work [12,13]. It shows that individual variability between trees within the same gathering zone could be less important compared with variability between sites (native soils), without

in any way giving an explanation for such determinism. These first conclusions must be verified, but they make it possible to propose the first steps to a comprehension of the variability in *C. grevei* essential oils with a view for the sustainable exploitation of the resource.

Experimental

Plant material and extraction: About 200 g of bark per tree was gathered from 21 trees of *C. grevei* in six different zones of the species distribution areas (Table 1). The collections were made at the end of the rainy season (April/May 2006) in order to rule out possible seasonal variations. Barks were gathered carefully to avoid damaging the tree cambium. On average, they were 2 to 3 mm thick. The collected barks were stored at ambient temperature, in a dry and shady place before use. They were cut into small pieces of about 1 cm² and submitted to hydro distillation for 14 h in a Clevenger type device. Because of the low oil content, the volatile compounds were trapped with 5 mL of a solution of tridecane (as internal standard) in *n*-hexane. The organic layers thus obtained were dried with anhydrous sodium sulfate before being stored in a cool and dark place until analyzed. The yields were calculated from the relative percentage (electronic integration measurements) of the internal standard and that of the other components of the mixture. Some botanical specimens of each sample have been deposited at the herbarium of the "Département de Recherches Forestières et Piscicoles" of the "Centre National de Recherches Appliquées au Développement Rural", Antananarivo (Madagascar).

Three commercial samples – two of which were bought by commercial companies in Madagascar (BioAroma and Homeopharma) and one in France (Bio'Mada) – were also analyzed for comparison.

Chemical analysis: The twenty essential oil samples extracted and the commercial samples were analyzed by gas chromatography (GC) and gas chromatography coupled with mass spectrometry (GC-MS).

Capillary gas chromatography was carried out using a Varian (model CP-3380) chromatographic system with a flame ionization detector (FID) equipped with a CP SIL 5 CB low bleed/MS 100% dimethyl polysiloxane capillary column (30 m x 0.25 mm, film thickness 0.25 μm). Oven temperature was programmed from 50 to 200°C at 5°C/min. The carrier gas was N_2, with a flow rate of 0.8 mL/min. Injector and detector were heated at 220 and 250°C, respectively. The injection volume was 1 μL for each sample in a split mode (1:10).

Gas chromatography-Mass spectrometry (GC/MS) analyses were performed using a Hewlett-Packard GC 5890A equipped with a HP-1 (cross linked methyl siloxane) fused column (30 m x 0.25 mm ; film thickness 0.25 μm) and interfaced with a quadrupole detector (Model 5972); temperature was programmed at 70-200°C (10°C/min); injector temperature, 220°C; temperature of connection parts, 180°C; carrier gas, helium; flow rate, 0.6 mL/min; injection type split, 1:10 (1 μL of a 10:100 *n*-pentane solution); ionization voltage, 70 eV; electron multiplier, 1400 eV; mass range, 35-300 amu; scan rate, 2.96 scan/s.

Component identification was carried out by comparison of retention data (determined relatively to the retention times of a series of *n*-alkanes) and mass spectra with those of spectrometric electronic libraries [21,22], literature data [23,24] and with the stored laboratory mass spectral library. Quantitative analysis of each oil component (expressed in percent) was carried out by peak area normalization measurements.

Data analysis: The distribution of the 21 oil samples was analyzed by principal component analysis (PCA) and by agglomerative hierarchical clustering analysis performed by XLSTAT 2007, considering 13 variables for each individual sample. The selected variables corresponded to the eleven major compounds representing more than 10% of the total composition of the essential oil in at least one sample [19] plus two components (β-caryophyllene and its oxide) identified as major components in the previous works. In order to compare our results with those previously produced in the literature, we included in our analysis three additional samples (commercial samples).

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