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Occurrence of a new essential oil chemotype in *Elionurus hensii* K. schum from the plateau des cataractes Congo-Brazzaville

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Abstract
The essential oils extracted from the stems and roots of *Elionurus hensii* from “Plateau des Cataractes” present markedly different chemical profiles forming distinct chemotypes. A new chemotype for stem oil was also recently found in the same study area. This study aims tocharacterize the new chemotype and to propose objective criteria to distinguish between the three chemotypes. The essential oils of *Elionurus hensii* from sites at Loufoulakari, Nkama, Mpého, Loukoko and Sésé studied previously contained predominantly*p*-mentha-2, 8-dien-1-ol/*p*-mentha-1(7), 8-dien-2-ol (from stems), and aristolone and limonene (from roots). A third profile, with mentha-1, 3, 8-triene/*p*-mentha-1(7), 8-dien-2-ol/undecanone as major constituents, was recently found in stems from Kimbélé on “Plateau des Cataractes”. The essential oils of *Elionurus hensii* from “Plateau des Cataractes” present three chemotypes: two for stems and one for roots. The existence of these three chemotypes was confirmed by multivariate statistics (PCA, AHC) on all the data collected over ten years.

Keywords: *Elionurus hensii, p*-manthadienols, menthatrienes, limonene, aristolone, Congo basin

1. Introduction

Elionurus hensii K. Schum. belongs to the Poaceae family. The genus *Elionurus* comprises some 20 species spread over three geographical regions: Africa, America and Oceania. Only a few species have been scientifically studied, some of which are aromatic: *E. miticus* [1-4], *E. elegans* [5], *E. viridulus* [1, 6]. The essential oils extracted from *E. elegans* display antimicrobial activity and antioxidant properties. Methanol extract of *E. miticus*, rich in phenolic compounds, presentsa strong antioxidant activity [7]. Four species have been described in Congo-Brazzaville: *Elyonurus hensii* [8], *E. argentea* [9], *E. hirtifolius* and *E. brazzae* [10, 11]. *Elyonurus hensii*, an aromatic plant growing almost exclusively on “Plateau des Cataractes”, has been studied in depth over a ten-year times pan, for both the composition of its essential oils and their biological activity [12-17]. During this work, carried out for five different sites on “Plateau des Cataractes”. Two chemotypes were found; one from stems (aerial parts), rich in *p*-methadienols, and another from roots (underground parts), rich in aristolone. A new chemical profile was also recently found for essential oils of stems in samples collected at Kimbélé, a sixth site studied on the “Plateau des Cataractes”.

This work set out to characterize this new profile and seek objective criteria to distinguish between these three chemotypes of *Elionurus hensii* from the “Plateau des Cataractes”.

2. Materials and Methods

2.1 Study area

The “Plateau des Cataractes” is located in the District of Louingui, Department of “Le Pool”. The District of Louingui is located 100 km southwest of Brazzaville. It is bounded to the north by the District of Kinkala, to the northeast by the District of Mindouli and Congo-Kinshasa, to the south by the District of Boko, to the east by the district of Mbanza Ndounga, and to the west by the District of Loumo. The District of Louingui covers an area of 655 km^2 [18]. Louingui has a Sudano-Guinean climate characteristic of the lower Congo comprising: (i) a long dry season lasting 4-5 months, coinciding with a temperature and water vapor tension minimum, linked to the cold Benguela Current flowing along the coast of Angola and the lower Congo, and (ii) a long rainy season but with reduced rainfall in January and February, a period called the “small dry season”, which is of some agronomic importance.

Annual rainfall is fairly constant throughout the region studied, fluctuating between 1270 and 1350 mm. However, there is a small gradient east to west: Brazzaville (15°15'E) 1335 mm, Mouyondzi (13°57'E) 1236 mm. The monthly distribution of rainfall shows a difference between weather stations located in uplands (Mouyondzi, Boko) between 500 m and 600 m and those in valleys (Brazzaville, Kinkala) between 300 m and 450 m. The temperatures are very similar from one station to another, and their seasonal variations are small, but the temperatures are appreciably lower in the uplands than in the valleys. The daily temperature variation is small.

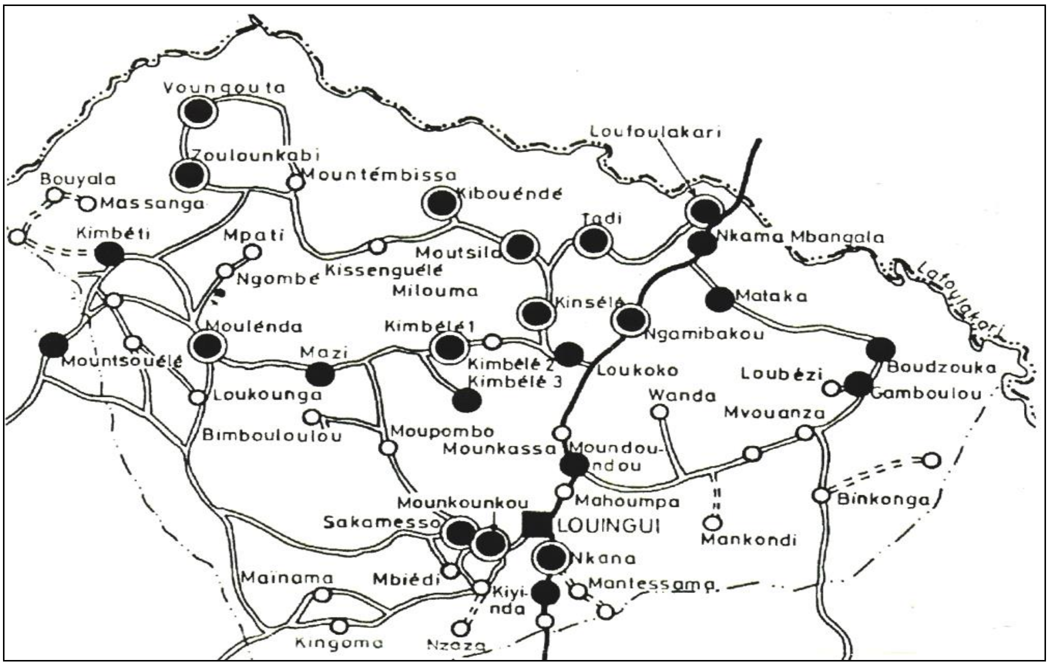


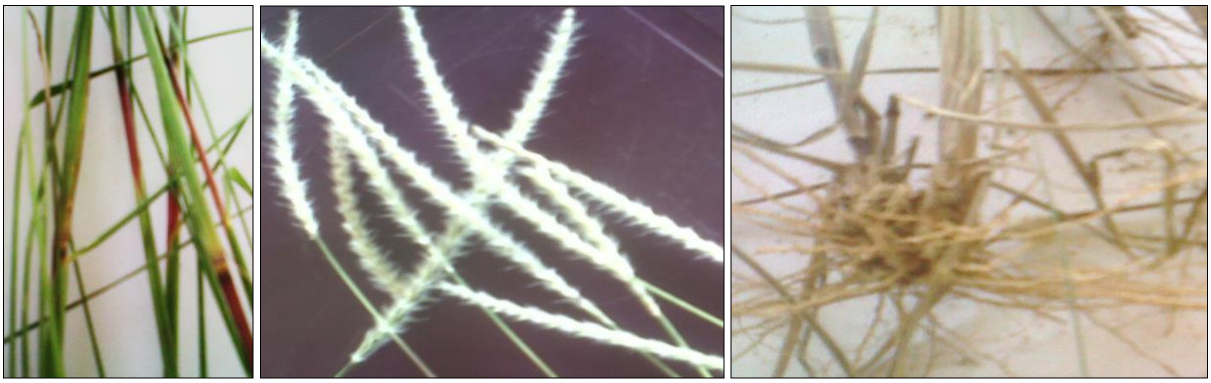
Fig 1: Administrative limits and routes of the district of Louingui

Louingui is an area with a predominantly hilly relief marked out by waterways. Altitude in the district ranges between 400 mand 600 m. The river network is dense, with some hundred waterways, the main ones being the Loufoulakari, Louingui, Moulenda, Loudzamou, Mamfoubou, and Mankonongo. The Districthas mainly clay soils, with patches of sandy, infertile soils. There are also some degraded soils (from leaching of organic constituents) resulting from reduction of forest cover, and hydromorphic soils rich in organic matter in mudflats and wooded areas. As regards vegetation, Louingui is dominated by shrubby savanna. Forests are receding, in particular owing to slash-and-burn agricultural practices [18].

2.2 Plant material

Elionurus hensii is a perennial grass with culms60–100 cm long; it has lateral off shoots with blades 7–10 cm long and 2–3 cm wide that flower when mature [19].

In Congo-Brazzaville, where it grows in a climate typical of the lower Congo on clay-sand to wet sand soils, this plant, which grows to a height of less than 1 m, presents slender culms branched at the top, and fine winding roots 4–8 cm long, curling leaves 8–15 cm long and 2 cm wide, and a non-villous base. The samples studied (specimen deposited at the “Institut National de Recherche en Sciences Exactes et Naturelles (IRSEN)”, Brazzaville) were collected on the “Plateau des Cataractes” at Loufoulakari, Nkama, Mpého, Loukoko, Séséand Kimbélé (District of Louingui, Department of Le Pool, Congo-Brazzaville).



Stems/leaves Young flowers Roots

Fig 1.1: Fresh Plant of *Elyonurus hensii* (Stems, leaves, flowers, roots)

2.3 Extraction of essential oils
2.3.1 In the laboratory
The essential oil was extracted using a Clevenger-type apparatus either by hydrodistillation (plant matter in the water) or by steam distillation (plant matter out of the water).
After distillation, the essential oil obtained was dried with sodium sulfate (Na_2SO_4) and weighed. The extraction yield is given by the formula:

$$Y_{EO} = (m_{EO} / m_d) \times 100,$$

Where
Y_{EO} is the essential oil recovered expressed as a percentage,
m_{EO} is the mass of essential oil obtained in grams, and
m_d is the mass of dried plant material in grams.

2.3.2 At pilot scale in the field station
The pilot extraction of essential oil was carried out at the Loukoko Rural Campus using a field distillation set-up. The distillation vessel was a square-ended box tank (50 cm × 50 cm × 100 cm) made of black steel sheet, and the cooling device was a pipe 2 cm in internal diameter fitted with an expansion chamber and running through a tank measuring 30 cm × 30 cm × 100 cm (Figure 2). The distillate was collected in a vessel that separated out the essential oil from the distillation water. The yield was calculated as described above.

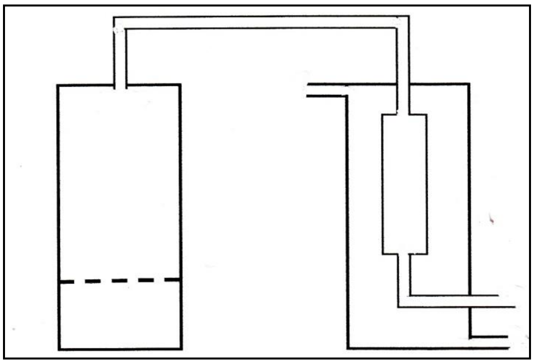


Fig 2: Distiller set-up for steam and hydrodistillation (Rural Campus of Loukoko).

2.4 Analysis of essential oils
2.4.1 Gas phase chromatography
The quantitative analysis of the essential oil was carried out with an Agilent model 6890 gas phase chromatograph equipped with a DB5 column (20 m × 0.18 mm × 0.18 µm). The oven temperature set at 50 °C for 3.2 minutes was ramped to 300°C at 10°C per minute. The injector temperature was 280°C. The apparatus was fitted with a flame ionization detector (FID) hydrogen (40 ml/min) / air (450 ml/min). The hydrogen carrier gas flow rate was 1 mL per minute. A mixture of *n*-alkanes (C8–C30) was analyzed in the same conditions to calculate retention indices using the equation of Van den Dool and Kratz [20]. The relative concentrations of the essential oil constituents were calculated from their respective peak areas, with no correction factor.

2.4.2. Gas phase chromatography in tandem with mass spectrometry
Qualitative analysis was carried out using an Agilent model 7890 gas phase chromatograph in tandem with an Agilent model 5975 mass spectrometer equipped with a DB5 column (20 m × 0.18 mm × 0.18 µm). The oven temperature was set at 50°C for 3.2 minutes and then ramped to 300°C at 8°C per minute. The injector temperature was 280°C. Ionization was by electron impact at 70 eV. The helium carrier gas flow rate was 0.9 mL per minute.
The components were identified by comparing their mass spectra and retention indices (RI) with those in the Adams [21], NIST [22], König [23] databanks and in our own (LEXVA http//lexva-analytique.com).

2.5 Statistics
Descriptive statistics and graphics were executed on Microsoft Excel 8.0. Multivariate analysis (principal components analysis (PCA) and ascending hierarchical clustering (AHC)) was performed on XLSTAT (www.xlstat.com).

3. Results and discussion
It had already been established that the essential oils of all the aerial parts (stems, leaves and flowers) were similar and were made up predominantly of oxygenated monoterpenes, in particular *p*-menthadienols, and those of the underground parts of about 50% aristolone [12]. In the present study, all the aerial parts were pooled in one sample referred to as “stems”; the underground parts formed the sample referred to as “roots”.

3.1 Fine characterization of essential oils of stems (chemotype 1) and roots (chemotype 2)
Samples (stems and roots) collected at three sites on the “Plateau des Cataractes” (Loufoulakari, Loukoko and Sésé) were extracted by hydrodistillation in a laboratory Clevenger apparatus and analyzed by gas phase chromatography. The results obtained are given in Table 1.
The essential oil of stems comprised a large number of menthadienol isomers like that from *Cymbopogon giganteus* [24-27]. Identifying these different isomers is not easy. Garneau *et al.* [28] determined Kovats indices on two columns and recorded the mass spectra of six isomers of menthadienols with known structures that they synthesized. Analysis by gas phase chromatography in tandem with mass spectrometry thus identified the isomers present in the essential oils studied as, in decreasing order of abundance: *cis*-*p*-mentha-1(7),8-diene-2-ol/ *trans*-*p*-mentha-1(7),8-diene-2-ol/ *trans*-*p*-mentha-2,8(9)-dien-1-ol, and *cis*-*p*-mentha-2,8(9)-dien-1-ol/*trans*-*p*-mentha-1,8-dien-6-ol (carveol). Then came limonene, 2-undecanone, carvone and 2-tridecanone, in much lower quantities. These oils differed appreciably from the essential oils extracted from the roots by their lower total contents, high amounts of *p*-cymene and intermedeol, and remarkable levels of aristolone (in general more than 40% against 3% for aerial parts); aristolone had previously been isolated and identified [14].

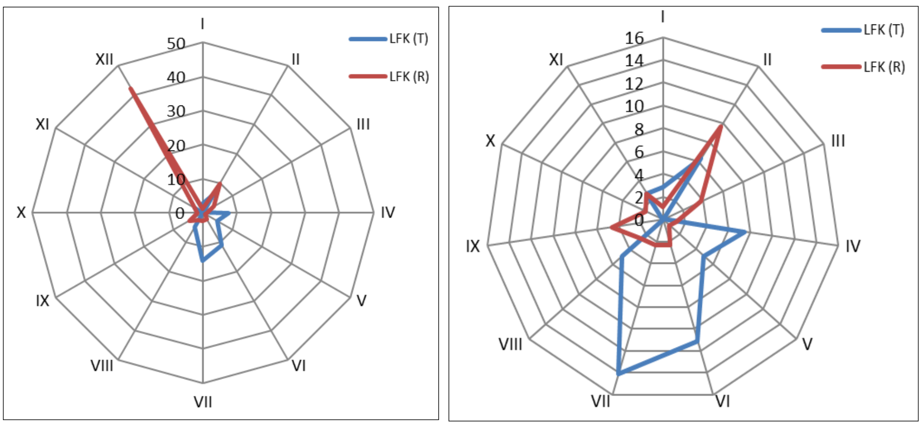
Table 1: Composition of essential oils of the stems and the roots of *Elionurus hensii* collected in Loufoulakari, Loukoko and Sésé.

RI_C	RI_{LITT}	Constituents	LFK (T)	LFK (R)	LKO(T)	LKO(R)	SSE (T)	SSE (R)	Code*
838	839	2-Pentanone, 4-hydroxy-4-methyl	0.0	0.0	0.0	0.0	0.1	0.1	
921	926	Tricyclene	0.1	0.8	1.7	0.8	0.8	0.3	
925	930	Thujene-alpha	0.0	0.2	0.0	0.2	0.0	0.40	
932	939	Pinene-alpha	0.3	0.7	0.3	1.5	0.4	0.9	

949	954	Camphene	2.8	1.1	4.9	2.6	2.3	0.8	I
972	975	Sabinene	0.0	0.2	0.0	0.3	0.0	0.3	
988	990	Myrcene	0.1	0.6	0.2	0.6	0.2	0.7	
1003	-	Verbénène	0.4	0.0	0.0	0.0	0.0	0.0	
1024	1024	Cymene<ortho->	1.6	0.70	0.4	0.2	1.6	0.4	
1029	1029	Limonene	6.4	9.7	11.2	8.8	12.8	9.7	II
1032	1031	Cineole<1.8->	0.1	3.8	0.0	2.6	0.4	5.5	III
1084	1088	Terpinolene	0.0	0.0	0.0	0.1	0.0	0.0	
1121	1122	Mentha-2.8-dien-1-ol <trans-para>	7.4	1.2	6.5	0.2	10.1	0.8	IV
1136	1137	Mentha-2.8-dien-1-ol <cis-para>	4.9	0.8	4.7	0.1	6.3	0.5	V
1168	-	Bicyclo[3.3.0]oct-2-en-7-one.6-methyl	1.1	0.3	0.8	0.0	0.8	0.0	
1188	1189	Mentha-1(7).8-dien-2-ol<trans-para>	11.1	2.3	11.2	0.4	12.2	1.1	VI
1195	1188	Terpineol-alpha	0.0	0.0	0.0	0.2	0.0	0.3	
1201	1229	Carveol cis	4.2	0.8	2.1	0.1	4.5	0.5	
1219	-	Carveol trans	4.5	0.8	3.9	0.2	5.1	0.4	
1230	1230	Mentha-1(7).8-dien-2-ol<cis-para>	14.1	2.3	11.2	0.4	14.0	0.3	VII
1245	1243	Carvone	3.2	0.8	3.3	0.3	3.6	0.4	
1255	1252	Piperitone	0.4	0.7	0.4	0.2	0.5	0.8	
1277	1271	Perilla aldehyde	0.3	0.0	0.2	0.0	0.0	0.0	
1285	1285	Bornyle acetate	1.2	0.3	1.6	0.3	1.0	1.2	
1292	1294	Undecanone-2	4.9	2.6	5.7	0.9	3.4	1.4	VII
1307	1300	*cis*-Pinocarvyl acetate	7.4	0.1	2.5	0.0	4.0	0.0	
1320	1318	Piperitenone	2.0	0.0	0.6	0.0	0.9	0.0	
1332	1335	Piperitenone oxide	4.5	0.0	1.5	0.0	2.0	0.0	
1374	1369	Methyl eugenol	1.4	0.0	0.5	0.0	0.5	0.0	
1391	1390	Elemene-beta	0.0	0.3	0.0	0.4	0.0	0.0	
1412	1426	Cymene<2.5-dimethoxy para->	0.0	0.4	0.0	0.0	0.0	0.0	
1415	1409	Gurjunene-alpha	0.0	4.6	0.0	1.0	0.0	0.0	IX
1422	1423	Aristolene	0.0	0.3	0.0	0.	0.0	0.5	
1431	1429	Aristola-1(10).8-diene	0.0	0.6	0.0	1.2	0.0	1.1	
1435	1433	Gurjunene-beta	0.0	4.6	0.0	10.0	0.1	6.2	
1438	1472	Dauca-5.8-diene	0.0	0.3	0.0	0.6	0.0	0.3	
1495	1496	Tridecanone-2	2.1	1.9	2.4	0.0	1.9	1.1	
1500	1498	Selinene-alpha	0.0	0.3	0.0	0.3	0.0	0.1	
1516	1513	Cadinene-gamma	0.0	0.2	0.0	0.3	0.0	0.1	
1524	1522	Selinene- 7-epi-alpha	0.0	0.4	0.0	0.9	0.0	0.7	
1575	1567	Maaliol	0.0	1.7	0.0	2.5	0.0	2.3	X
1581	1578	Spathulenol	0.0	0.2	0.0	0.4	0.0	0.5	
1612	1600	Rosifoliol	0.0	0.2	0.0	0.4	0.0	0.5	
1646	1542	Selina-3.7(11)-diene	0.0	0.0	0.0	03	0.0	1.5	
1671	1666	Intermedeol	2.7	2.7	2.2	1.6	2.0	1.5	XI
1698	1697	Pentadecanone-2	0.1	0.1	0.1	0.0	0.0	0.0	
1769	1763	Aristolone	0.0	42.2	0.0	44.6	0.0	41.8	XII
		Total	89.3	92.1	80.1	88.2	91.5	85.4	

* Code of constituents

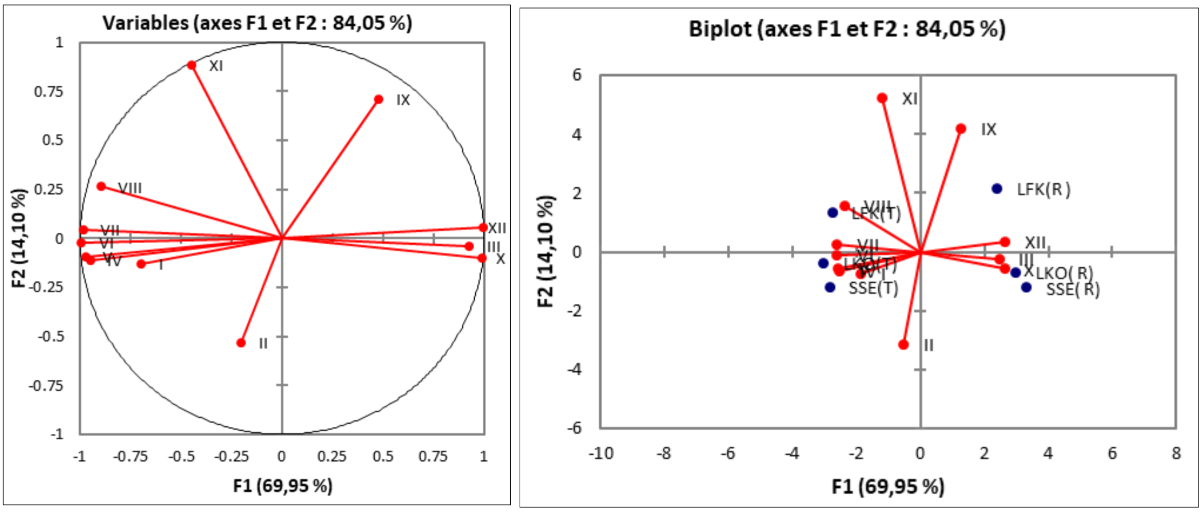
This difference can be visualized graphically on a radar plot. Fig.3 constructed from the Loufoulakari sample (LFK) shows a marked difference between the morphogram of the essential oil of stems and that of the essential oil of roots. If we remove aristolone which overwhelms the representation by its high content, we can more clearly see the difference in composition between these two types of oil.



(A). With aristo lone (B). With out aristo lone

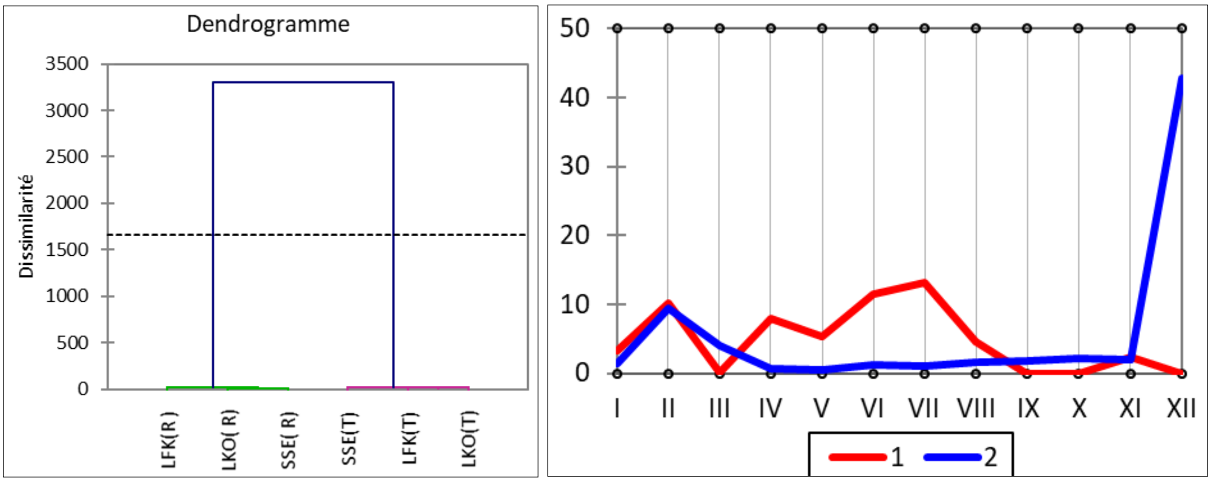
Fig 3: Characteristic radar plots of essential oils of the stems and of the roots of *Elionurus hensii*

We thus have two different chemotypes:
(i) Chemotype 1 for stem essential oil: characterized by the massive presence of paramethadienols with *trans*-and *cis*-*p*-mentha-1(7), 8-dien-2-ol (25%) predominating leading to the following overall profile:*p*-mentha-1(7), 8-dien-2-ol (22–26%)>*p*-mentha-2, 8-dien-1-ol (11–16%)> (*p*-mentha-1,8-dien-6-ol (carveol : 8–10%) > limonene (6–13%) > undecanone (3–5%) > camphene (2–5%) > intermedeol (2–3%) > tridecanone (2%).
(ii) Chemotype 2 for root essential oil: comprising about 50% aristolone. Followed by much lower levels of limonene (8–10%), beta-gurjunene (4–10%), and camphene (1–3%).
Principle components analysis. With 84% of information on the first principal plane confirms the existence of these two chemotypes and clearly indicates the constituents that contribute most to their composition: II, X, XI, XII for roots; I, IV, V, VI, VIII for stems (fig. 4). Likewise ascending hierarchical clustering(AHC) confirmed there weretwo chemotypes (5a) and showed their profiles (5b) Finally and through the cluster profile, AH Crevealed the following two chemotypes:(i) for the stems from Loufoulakari (LFK(T)), Loukoko (LKO(T)) and Sésé (SSE(T)): *p*-mentha-1(7).8-dien-2-ol,*p*-mentha-2,8-dien-1-ol,*p*-mentha-1,8-dien-6-ol (carveol), limonene and undecanone; (ii) for the roots from Loufoulakari (LFK(R)), Loukoko (LKO(R)) and Sésé (SSE(R): aristolone, beta-gurjunene and camphene.



(a) (b)

Fig 4: Circles of correlation (a) of the variables and distribution of the individuals (b) on the plane F1F2 of 3essential oil samples of *Elionurus hensii*from «plateaudes Cataractes ».



(a) Dendrogam (b) Profiles of classes. 1: stems; 2: roots

Fig 5: Dendrogram (a) and profiles ofclasses (b) of 3 essential oil samples of *Elionurus hensii*« plateaudes Cataractes ».

3.2 Occurrence of a new essential oil chemotype for stems of *Elionurus hensii* at the Kimbélé site on the "Plateau des Cataractes" (chemotype 3)

3.2.1. Characterization of the chemical profile of stem essential oils

A comparative study of essential oils extracted from the stems of plants collected at Loufoulakari (LFK), Nkama (NKM), Mpého (MPH), Loukoko (LKO) and Sésé (SSE) and at Kimbélé (KBL) all located on the "Plateau des Cataractes"(Fig. 1) identifyat least two chemical profiles: the first essentially comprising *p*-menthadienols and the second *p*-menthadienols/ *p*-menthatrienes.
The results obtained, given in Table 2, lead to the morphogram in Fig. 6, representing a sample from Loufoulakari and one from Kimbélé.
The essential oils extracted from the whole plant or stems by either hydrodistillation or steam distillation was identical. The mainly comprised *trans*- et *cis*-*p*-mentha-1(7), 8-dien-2-ol like all the other oils studied previously, in particular those from LFK, MPH and LKO (Table 2). However, we note the absence of *trans*-and*cis*-*p*-mentha-2, 8-dien-1-ol andthe occurrence of notable amounts of mentha-1, 3, 8-triene (8–

10%).

The difference between these two types of oil is visualized by the radar plot in Fig. 7. These two oils significantly share only *trans*-and*cis*-*p*-mentha-1(7), 8-dien-2-ols.

Table 2: Composition of stem sample essential oils from Loufoulakari, Mpého, Loukoko and Kimbélé on "Plateau des Cataractes"

Sample codes	LFK(T) *	NKM(T) *	MPH(T) *	LKO(T) *	KBL(T) *	KBL(T) *	KBL(T) **	Code ***
Essential oil yeild (%)	-	-	-	-	0.60	0.70	0.83	
Constituents (%)								
Tricyclene	0.96	0.95	1.14	1.73	1.24	1.93	1.59	
Alpha pinene	0.33	0.19	0.26	0.56	0.25	0.35	0.31	
Camphene	2.80	3.16	3.45	4.94	4.07	5.50	5.09	I
p- mentha-1,5,8 triène	0.00	0.44	0.36	0.00	0.21	0.74	0.25	II
p-cymene	1.55	1.87	1.81	4.21	2.12	3.51	2.35	III
Limonene	6.41	1.81	1.90	11.2	3.05	2.96	4.11	IV
Cineole <1.8->	0.12	0.21	0.14	0.0	0.60	0.56	0.54	V
Nonan-2-one	0.00	0.22	0.11	0.00	0.16	0.15	0.15	
p-cymenene	0.00	0.22	0.14	0.00	0.43	0.83	0.50	
NI	0.00	0.00	0.22	0.00	0.19	022	0.22	
Mentha-1,3,8- triene	0.45	0.00	0.00	0.00	8.64	8.43	10.00	VI
NI	0.00	0.00	0.00	0.00	5.69	5.71	5.94	VII
Mentha-2,8-dien-1-ol <trans para->	7.36	7.23	9.23	6.52	0.00	0.00	0.00	VIII
Mentha-2,8-dien-1-ol <cis para->	4.92	5.33	5.80	4.70	0.00	0.00	0.00	IX
Trans pinocarvol	0.55	0.94	0.00	0.75	0.77	0.75	0.80	
décahydronaphtalène	0.00	0.00	0.00	0.00	0.22	0.25	0.18	
6-methyl-bicyclo [3,3,0] oct-2-en-7-one	0.00	0.00	0.00	0.77	0.46	0.257	0.35	
Mentha-1(7),8-dien-2-ol <trans para->	11.10	14.88	15.3	11.2	18.55	16.81	17.08	X
Carveol trans	4.49	2.75	3.05	3.88	4.93	5.27	4.28	XI
octyle acetate	0.00	0.00	0.00	0.00	0.71	0.80	0.71	
cis caran-4-one	0.05	2.69	1.42	0.00	0.84	0.90	0.82	
NI	0.00	0.00	0.00	0.00	1.32	2.19	1.73	
Carveol cis	0.82	0.90	0.00	0.61	1.68	1.56	1.54	XII
NI	0.00	0.00	0.00	0.00	2.82	2.70	2.78	
NI	0.00	0.00	0.00	0.00	0.63	1.06	0.83	
Mentha-1(7),8-dien-2-ol <cis para->	14.11	14.86	15.39	11.21	14.78	12.78	13.42	XIII
NI	0.00	0.00	0.00	0.00	0.90	1.65	1.13	
Carvone	3.23	4.32	0.91	0.00	4.25	3.90	3.98	XIV
Piperitone	0.44	0.50	0.49	0.44	0.63	0.71	0.61	XIV
Perilic aldehyde	0.26	0.62	0.62	0.21	0.25	0.21	0.22	
Bornyle acetate	1.18	1.6	4.71	1.63	2.49	0.00	2.59	XV
Undecanonone-2	4.94	7.82	7.60	5.66	11.16	10.32	1068	XVI
Tridecanone 2	2.10	2.31	3.17	2.44	3.95	3.07	4.12	XVII
Intermedeol	2.72	1.67	1.47	2.17	0.00	0.00	0.00	XVIII
Pentadecanone <2->	0.11	0.00	0.00	0.07	0.00	0.00	0.00	XIX
Aristolone	0.34	0.00	0.00	0.024	0.00	0.00	0.00	XX
Beta panasinsene	0.00	0.00	0.00	0.00	1.08	0.55	0.80	

* HD : hydrodistillation ; ** VHD : steam distillation ; *** major constituent codes.

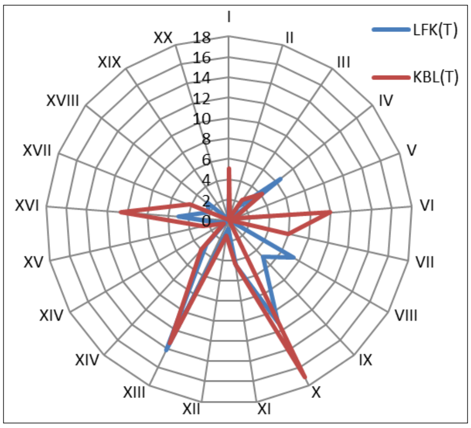


Fig 6: Radar plotrepresentations of stem sample essential oils from Loufoulakari

3.2.2. Distinguishing between the two stem oil chemotypes

Whole plants (stems + roots. Fig.7) were collected at the Kimbélé site for one year (2016).and 23 field extractions each of 6–12 kg of plant material were carried out using a 250 L pilot extractor (Fig. 3). The composition of the oils obtained, given in Table 2 allows the stability of the chemical profile (chemotype) to be evaluated. For the purpose of distinguishing between the different oils, composition was compared with that of oils of known *p*-menthadienol chemotype collected at Loukoko and Loufoulakari.

(A). **(B).** **(C).**

Fig 7: *Elionurus hensii*from Kimbélé site (whole Plants (a), adult flowers (b) and essential oil (c))

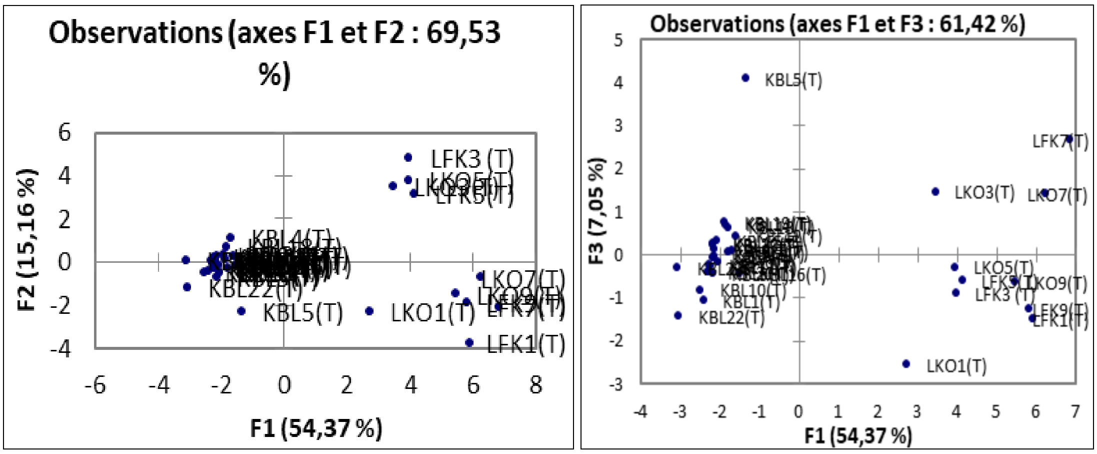
Table 3: Major component compositionof stem essential oils of *Elionurus hensii* from Kimbélé (KBL), Loukoko (LKO) and Loufoulakari (LFK)

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX	XX
KBL1(T)	5.50	0.74	3.52	2.96	0.56	8.43	5.71	0.00	0.00	16.90	5.27	1.56	12.80	3.91	2.50	10.32	3.07	0.00	0.00	0.00
KBL2(T)	4.82	0.34	2.78	3.05	0.67	9.40	6.11	0.00	0.00	13.80	5.28	1.94	13.80	4.41	2.50	9.51	2.82	0.00	0.00	0.00
KBL3(T)	5.90	0.25	2.35	4.11	0.54	9.98	5.94	0.00	0.00	17.10	4.23	1.54	13.40	3.98	2.60	10.68	4.12	0.00	0.00	0.00
KBL4(T)	3.98	0.00	1.83	2.63	0.52	9.61	6.12	0.00	0.00	18.30	4.89	0.00	14.60	4.53	2.60	11.32	3.55	0.00	0.00	0.00
KBL5(T)	1.75	0.00	0.97	6.00	2.16	5.46	3.70	0.00	0.00	13.80	3.27	3.01	10.50	3.54	2.60	12.04	5.46	0.00	0.00	0.00
KBL6(T)	5.69	0.38	2.72	3.98	0.65	9.15	5.94	0.00	0.00	16.80	5.14	1.89	12.80	3.85	2.50	10.5	3.05	0.00	0.00	0.00
KBL7(T)	5.41	0.40	2.85	3.59	0.6	9.31	6.17	0.00	0.00	17.60	5.16	2.00	12.90	3.95	2.50	10.37	3.42	0.00	0.00	0.00
KBL8(T)	4.94	0.39	2.81	2.58	0.54	9.55	6.26	0.00	0.00	18.10	5.31	2.00	13.70	4.08	2.40	10.94	3.28	0.00	0.00	0.00
KBL9(T)	5.40	0.54	3.15	3.01	0.51	8.58	5.63	0.00	0.00	17.40	5.26	1.87	13.60	4.17	2.30	10.5	3.63	0.00	0.00	0.00
KBL10(T)	5.90	0.67	3.59	3.35	0.61	8.46	5.69	0.00	0.00	17.30	5.25	1.78	13.10	3.86	2.40	10.51	3.68	0.00	0.00	0.00
KBL11(T)	5.45	0.42	3.1	2.75	0.51	9.02	6.04	0.00	0.00	18.20	5.44	2.08	13.50	3.98	2.50	10.46	3.38	0.00	0.00	0.00
KBL12(T)	4.09	0.35	2.21	4.03	0.72	8.59	5.90	0.00	0.00	18.10	5.29	1.86	14.40	4.12	2.00	9.61	3.80	0.00	0.00	0.00
KBL13(T)	5.62	0.40	2.78	4.38	0.99	8.71	5.78	0.00	0.00	17.80	5.09	1.82	13.70	4.04	2.40	9.95	3.19	0.00	0.00	0.00
KBL14(T)	5.01	0.16	2.28	3.89	0.87	9.08	6.12	0.00	0.00	18.20	4.93	1.82	14.40	4.10	2.40	9.76	3.44	0.00	0.00	0.00
KBL15(T)	5.01	0.40	2.35	3.92	0.82	8.79	5.74	0.00	0.00	17.50	4.83	1.75	13.70	3.92	2.50	11.10	3.67	0.00	0.00	0.00
KBL16(T)	4.43	0.60	2.52	3.48	0.61	7.35	4.84	0.00	0.00	16.90	4.80	1.5	14.20	3.97	2.30	8.63	2.92	0.00	0.00	0.00
KBL17(T)	5.28	0.54	3.1	3.88	0.81	7.37	5.21	0.00	0.00	17.60	5.26	1.57	13.80	3.95	2.50	10.46	4.00	0.00	0.00	0.00
KBL18(T)	4.07	0.21	2.12	3.05	0.60	8.64	5.69	0.00	0.00	18.70	4.93	1.68	14.80	4.24	2.50	11.16	3.95	0.00	0.00	0.00
KBL19(T)	3.53	0.29	2.5	3.77	0.77	7.43	5.11	0.00	0.00	17.90	4.91	1.65	13.90	4.36	2.50	11.55	4.40	0.00	0.00	0.00
KBL20(T)	4.62	0.39	2.69	4.42	0.90	7.63	5.19	0.00	0.00	17.60	4.91	1.32	13.40	4.18	2.60	11.50	3.93	0.00	0.00	0.00
KBL21(T)	6.07	0.46	3.12	3.05	0.57	8.16	5.56	0.00	0.00	18.40	5.04	1.85	13.60	4.00	2.60	10.97	3.09	0.00	0.00	0.00
KBL22(T)	5.92	1.14	4.55	4.21	0.95	5.98	4.50	0.00	0.00	17.10	4.8	1.39	12.40	3.80	2.70	11.06	3.5	0.00	0.00	0.00
KBL 23(T)	5.15	0.60	3.24	3.40	0.00	18.4	5.78	0.00	0.00	18.40	5.3	1.6	13.89	3.99	2.60	10.60	3.46	0.00	0.00	0.00
LKO1(T)	4.94	0.44	4.21	11.20	0.00	0.00	0.00	6.51	4.70	11.20	3.87	0.60	11.20	3.26	1.60	5.65	0.00	2.16	0.06	0.20
LKO3(T)	1.60	0.00	1.51	4.70	0.26	0.00	0.00	7.76	5.44	20.90	7.26	4.77	19.00	4.28	1.20	6.48	0.00	0.97	0.34	0.41
LKO5(T)	1.34	0.11	1.48	2.37	0.25	0.00	0.00	6.22	4.62	19.90	3.87	0.00	21.4	4.51	1.50	7.71	0.00	3.11	0.47	1.09
LKO7(T)	1.34	0.00	0.00	20.50	0.27	0.00	0.00	11.5	6.13	12.80	5.79	3.15	13.00	3.69	0.90	3.45	0.00	1.07	0.14	1.6
LKO9(T)	1.16	0.00	1.21	15.90	0.00	0.00	0.00	10.7	5.84	11.00	4.65	0.74	12.70	3.34	0.60	2.98	0.00	0.82	0.12	0.41
LFK1(T)	2.48	0.00	0.00	17.00	0.00	0.00	0.00	10.5	5.57	10.50	4.04	0.69	12.30	0.00	1.00	0.00	0.00	1.29	0.02	0.00
LFK3 (T)	1.09	0.00	0.00	2.21	0.25	0.00	0.00	6.1	4.52	20.80	7.46	0.00	18.80	4.47	1.70	0.00	0.00	3.35	0.3	0.31
LFK5(T)	1.60	0.00	0.00	3.21	0.35	0.00	0.00	5.32	3.85	18.70	5.71	0.00	16.50	4.08	2.00	0.00	0.00	5.04	0.00	0.6
LFK7(T)	1.25	0.00	0.00	19.30	0.00	0.00	0.00	16.2	6.39	12.90	1.89	5.36	13.50	3.45	1.10	0.00	0.00	3.19	0.00	0.68
LFK9(T)	2.64	0.00	0.00	16.20	0.1	0.00	0.00	9.06	1.63	11.00	4.33	0.7	12.70	3.02	1.10	0.00	0.00	0.01	2.15	0.98
Mean	4.03	0.41	2.65	5.94	0.65	8.86	5.60	8.89	4.87	16.64	4.94	1.91	14.06	3.97	2.11	9.63	3.60	2.10	45	0.70
SD	1.75	0.24	0.85	5.35	0.37	2.36	0.61	3.34	1.39	2.83	0.98	1.04	2.09	0.36	0.62	2.31	0.57	1.54	0.70	0.45

A PCA of 33 samples of which 23 from Kimbélé constructed on 20 variables (constituents) indicates on the first principal plane F1F2 (70% of information) a good separation of essential oils collected atKimbélé from the other oils studied (Fig.8a).

Inspection of the second principal plane F1F3 (61% of information) confirms this separation and indicates a clear grouping of the other oils studied in a second class corresponding to the chemotype previously identified [12].

The sample from Kimbélé (KBL) form a very tight group forminga new chemotype (Fig. 8b).



(a) (b)

Fig 8: Principal planes F1F2 and F1F3 in PCA of 33 samples including 23 of Kimbélé and built on 20 variables (components)

An AHC of 33 samples of which 23 from Kimbélé constructed on 20 variables (constituents) suggests by automatic truncation the occurrence of two classes corresponding to chemotype 3 (class 3) and chemotype 1 (class 2) presented in Fig.9 and Table 4; their profiles are visualized in Fig. 10.

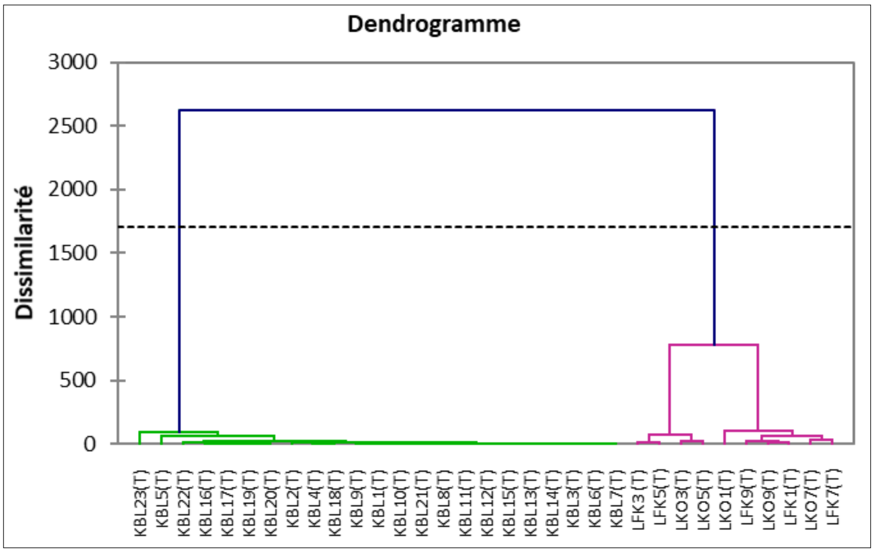


Fig 9: Dendrogram in HAC of 33 essential oil samples of *Elionurus hensii* from «plateaudes Cataractes »

Table 4: Classes of 33 essential oil samples of *Elionurus hensii* from «plateaudes Cataractes »

Classes	
1	2
KBL1(T) KBL2(T) KBL3(T) KBL4(T) KBL5(T) KBL6(T) KBL7(T) KBL8(T) KBL9(T) KBL10(T) KBL11(T) KBL12(T) KBL13(T) KBL14(T) KBL15(T) KBL16(T) KBL17(T) KBL18(T) KBL19(T) KBL20(T) KBL21(T) KBL22(T) KBL23(T)	LKO1(T) LKO3(T) LKO5(T) LKO7(T) LKO9(T) LFK5(T) LFK7(T) LFK9(T)

Finally. and through the cluster profile. AHC shows the following two chemotypes: (i) for Kimbélé stems (KBL (T)):*p*-mentha-1(7).8-dien-2-ol > undecanone 2 > mentha-1.3.8- triene > carvone > limonene > cineole. And (ii) for Loufoulakari (LFK (T)) and Loukoko (LKO (T)) stems:*p*-mentha-1(7),8-dien-2-ol > *p*-mentha-2,8-dien-1-ol>limonene>carveol>undecanone.

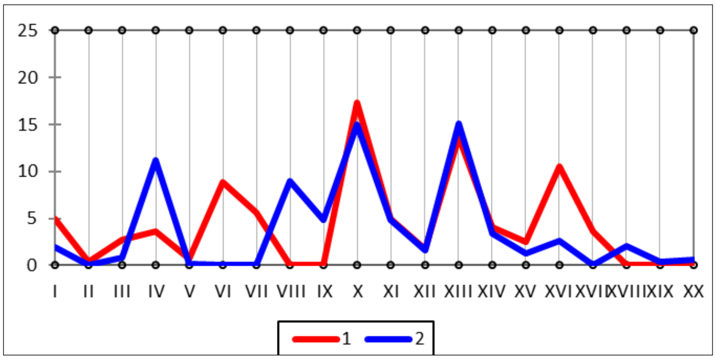


Fig 10: Profiles of the classes of 2 essential oil samples of *Elionurus hensii* stems from « plateau des Cataractes » ; profile 1: chemotype 3; profile 2: chemotype 1.

4. Discussion

To position this last chemotype in the 53 samples of *Elionurus hensii* from the« plateau des Cataractes » studied since the first publication on this species in 2006 [12]. We carried out a multivariate analysis of 53 samples constructed over the 20 most abundant constituents of these oils representing more than 70% of the total oil.

The representation of individuals on the first principal plane with 71% of the information clearly reveals the occurrence of three classes corresponding to three perfectly delimited chemotypes (Fig. 11); the biplot associating variables and individuals highlights the major constituents of these different chemotypes: chemotype 1 with VII, IX,XI, XIII,XIV, XIX as major constituents; chemotype 2 with II, III, VI, VIII, XV, XVII as major constituents, and chemotype 3.with IV, V, XII, XX, thus confirming the greater complexity of the stem oils (chemotypes 1 and 3) relative to those of the roots (chemotype 1).

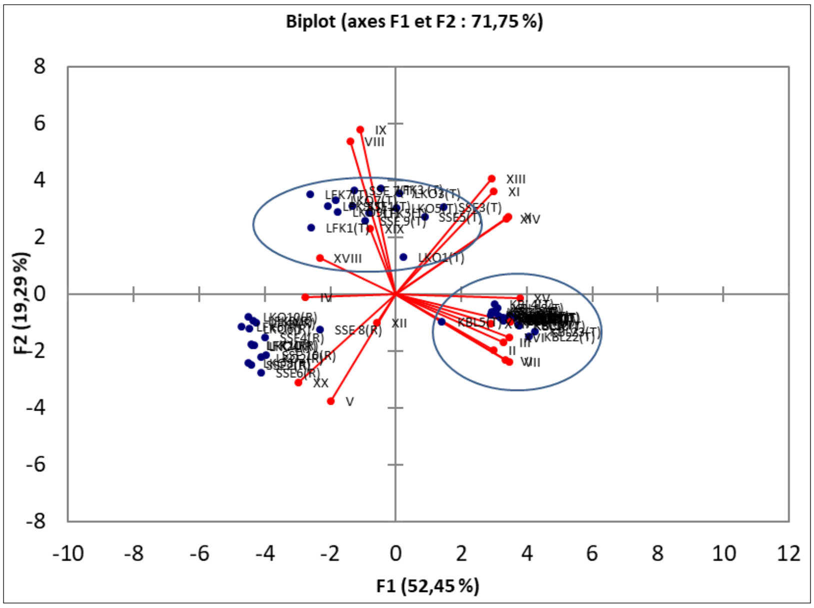


Fig 11: Variable correlation and individual distribution on the plane F1F2 in PCA of 53essential oil samples of *Elionurus hensii*from «plateaudes Cataractes ».

AHC distributes the samples perfectly into the different chemotypes. Separation into two classes distinguishes the stem oils from the root oils. Automatic classification into three classes distinguishes perfectly between the three chemotypes found: two chemotypes for the stem oils and one chemotype for the root oils.

Tableau 5: Distinguishing between the 3 chemotypes of 53 essential oil samples of *Elionurus hensii*from« plateau des Cataractes »

Repartition in 2 classes		Repartition in 3 classes		
1 (stems)	**2 (roots)**	**1 (chémotype 1)**	**2 (chémotype 3)**	**3 (chémotype 2)**
KBL1(T)KBL2(T)KBL3(T)KBL4(T) KBL5(T)KBL6(T)KBL7(T)KBL8(T) KBL9(T)KBL10(T)KBL11(T)KBL12(T) KBL13(T)KBL14(T)KBL15(T)KBL16(T) KBL17(T)KBL18(T)KBL19(T)KBL20(T) KBL21(T)KBL22(T)KBL23(T)LKO1(T) LKO3(T)LKO5(T)LKO7(T)LKO9(T) LFK1(T)LFK3 (T)LFK5(T)LFK7(T) LFK9(T)SSE1(T)SSE3(T)SSE5(T) SSE 7(T)SSE 9(T)	LKO2(R)LKO4(R) LKO6(R)LKO8(R) LKO10(R)LFK2(R) LFK4(R)LFK6(R) LFK8(R)LFK10(R) SSE2(R)SSE4(R) SSE6(R)SSE 8(R) SSE 10(R)	KBL1(T)KBL2(T)KBL3(T) KBL4(T)KBL5(T)KBL6(T) KBL7(T)KBL8(T)KBL9(T) KBL10(T)KBL11(T)KBL12(T) KBL13(T)KBL14(T)KBL15(T) KBL16(T)KBL17(T)KBL18(T) KBL19(T)KBL20(T)KBL21(T) KBL22(T) KBL23(T)	LKO1(T) LKO3(T) LKO5(T) LKO7(T) LKO9(T) LFK1(T) LFK3 (T) LFK5(T) LFK7(T) LFK9(T) SSE1(T) SSE3(T) SSE5(T) SSE 7(T) SSE 9(T)	LKO2(R) LKO2(R) LKO4(R) LKO6(R) LKO8(R) LKO10(R) LFK2(R) LFK4(R) LFK6(R) LFK8(R) LFK10(R) SSE2(R) SSE4(R) SSE6(R) SSE 8(R) SSE 10(R)

Sample codes: KBL: Kimbélé; LFK: Loufoulakari; LKO: Loukoko; SSE: Sésé

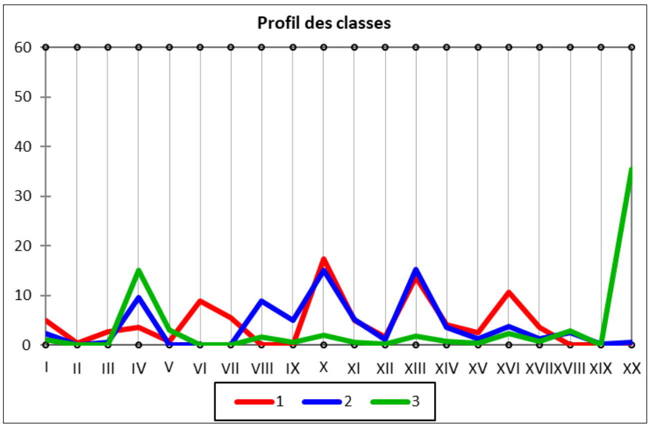


Fig 12: Profiles of the classes of 3 essential oil samples of Elionurus hensii from « plateau des Cataractes » : profile 1: chemotype 3 (stems); profile 2: chemotype 1 (stems) ; profile 3 : chemotype 2 (roots).

In all the 53 samples studied over ten years. AH Creveals two chemotypes for stem oils:(i) from Kimbélé (KBL(T)):%X ~%XII >%VI ~%VII >%V >%I >%IV >%III. (ii) from Loufoulakari (LFK) and from Loukoko (LKO): %X ~%XVII >%IV >%VIII >%XIV >%XVIII and one chemotype for root oil: %XX >%IV.

5. Conclusion

A study of the essential oils of *Elionurus hensii* from six sites on the "Plateau des Cataractes" over ten years found three chemotypes: two for stems and one for roots. The essential oilsfrom Loufoulakari, Nkama, Mpého, Loukoko and Sésé analyzedover these ten years mainly comprised *p*-mentha-2,8-dien-1-ol/*p*-mentha-1(7),8-dien-2-ol forstems and aristolone andlimonene for roots. For the first time a third profile. With mentha-1,3,8-triene/*p*-mentha-1(7),8-dien-2-ol /undecanone as major constituents was recently found for essential oil of stems from Kimbélé. The existence of these three chemotypes was confirmed by multivariate analysis (PCA and AHC).

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