

EXTRACTION OF *ELIONURUS HENSII* K. SCHUM ESSENTIAL OIL IN A DOMESTIC SCALE IN THE “PLATEAU DES CATARACTES” (CONGO-BRAZZAVILLE)

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ABSTRACT

Elionurus hensii yields essential oils composed mainly of para-menthadienols in its stems and about 50 % aristo lone in its roots. These oils, which exhibit anti-microbial and anti-oxidant properties, were extracted by hydro distillation with a local extractor and was analyzed by gas chromatography (GC/FID and GC/MS).

To optimize this domestic process, a 2³ full factorial design is used to assess the effects and interactions of potentially influential factors. Stems yield 1-2% in essential oil. The extraction time and the degree of division of the plant material had negligible effects on the yield. Only the residual water content had a significant positive effect, together with the interaction between this content and the degree of division. The cumulated content of the four isomeric para-menthadienols present in the oil was insensitive to the effects and interactions of the factors. Roots yield 0.5 - 0.9 % in essential oil. The overall effects and interactions of factors on the yield and the aristo lone content were negligible.

Principal component analysis of samples obtained with the experimental design, and their radar plots, indicated a very strong resemblance between oils obtained from the same plant part, and a marked difference between those obtained from different plant parts.

Keywords: Experimental Design; Effects of Factor; Multivariate Analysis; P- Menthadienols; Aristo lone, *Elionurus Hensii*.

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1. INTRODUCTION

The genus *Elionurus* (Poaceae family), comprising some 20 species, is not well described in the literature. What work is reported concerns *Elyonurus muticus* [1], [2], [3], *Elionurus elegans* [4] and *Elyonurus viridulus* [5]. The essential oil derived from both the aerial parts (stems) and the below-ground parts (roots) of *Elionurus elegans* possesses antibacterial, antifungal and

antioxidant properties [4]. The methanol extracts of *Elyonorus muticus*, composed mainly of phenolic compounds, exhibit a high antioxidant activity [6].

Elionurus hensii, which is found in tropical and subtropical regions of South America (Brazil and Argentina), Africa (Republic of Congo, Gabon, DR Congo, Angola) and Australia [8], has been studied only in the Republic of Congo, where this wild plant grows abundantly on the “Plateau des Cataractes”.

In the first description we published in 2006 of the essential oils from different parts of the plant (roots, stems, leaves, flowers), we reported that the oils from the aerial parts were mainly composed of *p*-menthadienols, whereas the roots yielded an oil containing more than 40 % aristolone [9].

In a previous very detailed study of (i) the volatile components from stems and roots obtained by hydrodistillation and head-space SPME, and (ii) the phenolic compounds and flavonoids extracted with methanol, ethyl acetate and dichloromethane, the antioxidant activities of the extracts were demonstrated and compared [8].

Loumouamou *et al.* assess the seasonal variation in the composition of essential oils as a function of different harvest sites, and the impact of this composition on the biological activities [10], [11], [12].

However, the massive presence of *p*-menthadienol isomers points to possible antimicrobial properties, as in *Cymbopogon giganteus* from Burkina Faso [13], Cameroon [14], Benin [15], Mali [16], and Côte d’Ivoire [17].

Further, aristolone, a ketone that is very abundant in the roots, could either be isolated or gainfully used in its native state, or after conversion into its oxime: several studies have found this family of substances to have antitumor, antimicrobial, antioxidant, and antidepressant, anticonvulsive and antiviral properties [18].

We describe here the essential oil extraction from stems and roots of *Elionurus hensii*, to assess the effects of factors controllable at the artisanal level on the yield and quality of the products obtained.

The results of this work will support controlled artisanal production of this oil, which is of potential medicinal interest, by essential oil producers grouped in the OIBT project as part of the Republic of Congo anti-poverty programme [19].

2. MATERIALS AND METHODS

2.1. Plant Material

Elionurus hensii K. Schum is a perennial grass composed of culms 60–100 cm long, with strongly developed side branches forming blades 7–10 cm long, 2–3 cm wide that flower at maturity [20], [21].

In the Republic of Congo, where it grows under the lower-Congo climate, on clay-sand to sandy-moist soil, this plant, which does not exceed 1 m in height, presents upwardly branched culms, thin sinuous roots 4–8 cm long, rolled leaves 8–15 cm long and 2 cm wide, and a glabrous base.



Figure 1: Fresh Plant of Elyonurus Hensii (Stems, Leaves, Flowers, Roots,)

The samples studied were collected on the “Plateau des Cataractes” at Loufoulakari, Loukoko and Sese (District of Louingui, Pool Department, R Congo).

2.2. Extraction

In the Laboratory scale, the essential oils were obtained by steam distillation. Water and plant material (200 g of plant material for stems, or 135 g for roots.) were placed in a Clevenger apparatus 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.

The artisanal extraction is led in a cylindrical distiller (60 L, 4kg). The vapor resulting from the hydrodistillation passes in a pipe (2 cm diameter) crossing a (100 cm length, 30 cm broad and 30 cm height) reserve, filled with cool water circulating in opposite direction (figure 2).



Figure 2: Local equipment for hydrodistillation of essential oils (60 L)

2.3. Gaz Chromatography

GC analysis was performed on an Agilent GC 6890 instrument equipped with a split injector (280°C), a flame ionization detector (FID) and a DB-5 column (20m x 0,18mm x 0,18µm). The temperature program was 50°C (3.2 min) rising to 330°C (10°C /min). Dihydrogene was used as carrier at a flow rate 1ml/min.

2.4. Gaz Chromatography / Mass Spectrometry

GC/SM analysis was performed on Agilent GC 7890 /Agilent MS 5975 operating in EI mode (70 eV), equipped with a DB-5 column (20m x 0,18mm x 0,18µm). The temperature of injector was 280°C and helium was the carrier gas at 0.9 mL/min. The temperature program was 50°C (3.2 min) rising to 330°C (8°C /min).

The identification was carried out by calculating retention indices (RI) and comparing mass spectra with those in data banks [22], [23], [24], [25].

2.5. Modelling of Essential Oil Extraction (Hydrodistillation)

The variables influencing extraction yield were: time, temperature, condensation rate, the state of division of the plant material, the mass ratio of plant material to water, and water loss from plant material [26].

A model with six variables, even in the case of a first degree model, would need $2^6 = 64$ experiments [27]. For experimental convenience some variables were thus kept constant. We considered three variables: extraction duration, residual water content (X_2) and state of division of the plant material (X_3). These three factors offered the advantage of being easy to control, even in a small scale production unit. Extraction yield y and p - menthadiol content z depend on factors X_1 , X_2 and X_3 . Mathematically, this is expressed as y or $z = f(X_1, X_2, X_3)$ where y and z are the responses, $f(X)$ is the response function and X_1 , X_2 and X_3 are the factors taken into account.

The experiment is designed to determine the effects of certain factors on each response.

The two-level factorial design as developed by Davies [28] is well-suited to addressing this type of question, and has the advantage of needing only very elementary mathematical skills [29]. The general formula for a complete factorial plan with N experiments is $N = 2^k$, where k is the number of variables in the factorial. If $k = 3$, then $N = 2^k = 2^3 = 8$ experiments.

To construct the experiment matrix we define reduced variables x_i as:

$x_i = (X_i - X_0) / \Delta X$; X_0 is the base value, at the centre of the experimental domain (level 0), and ΔX is the variation step, i.e., the unit of variation of the variables. Table 1 give the two levels of the variables in steam extraction of *Elionurus hensii* stems.

Table 1: Levels of the variables in steam extraction of *Elionurus hensii* stems

Level	Extraction duration (h)	Residual water content	State of division (cm)
	X_1	X_2	X_3
Low (-1)	1.5	Low	< 5
High (+1)	3	High	10

The combination of these 3 variables and the 2 levels by variable lead to the following experimental design (table 2)

Table 2: Experimental design for essential oil extraction from *Elionurus hensii* stems

Run	X_1 (h)	X_2	X_3 (cm)
1	1.5	Low	< 5
2	3	Low	< 5
3	1.5	High	< 5
4	3	High	< 5
5	1.5	Low	10
6	3	Low	10
7	1.5	High	10
8	3	High	10

The domain of the study, with coded variables, becomes the domain (-1, +1) and the eight responses described by the experimental matrix are set up after randomisation (table 3).

Table 3: Experimental matrix for essential oil extraction from *Elionurus hensii* stems

Run	Extraction duration (h)	Residual water content	State of Division
	x_1	x_2	x_3
1	-1	-1	-1
2	+1	-1	-1
3	-1	+1	-1
4	+1	+1	-1
5	-1	-1	+1
6	+1	-1	+1
7	-1	+1	+1
8	+1	+1	+1

For a first degree model with interactions, the representative points of a three-variable experimental design are located in three-dimensional space. The corresponding response function is a first degree polynomial for each factor taken separately. It is notated:

$$y = a_0 + a_1x_1 + a_2x_2 + a_3x_3 + a_{12}x_1x_2 + a_{13}x_1x_3 + a_{23}x_2x_3 + a_{123}x_1x_2x_3$$

If the mathematical model associated with the factorial design is constructed with centred, reduced variables, the coefficients of the polynomial thus have very simple meanings: average a_0 , main effects a_i , and interactions a_{ij} , and a_{ijk} [27].

2.6. Statistical Treatment

Means, standard deviations and the usual graphs were obtained with Excel software. Multivariate analysis was performed on XLSTAT software (Addinsoft an add up of Excel Microsoft).

3. RESULTS AND DISCUSSION

3.1. Chemical Composition of Stem and Root Essential Oils of *Elionurus Hensii*

Previously works show that the essential oils from all the aerial parts were similar, and were mostly composed of oxygenated monoterpenes, in particular *p*-menthadienols, and that those from the below-ground parts contained some 40 % aristolone [9], [8]. In this study, all the above-ground parts were pooled to make a single sample, hereafter called “stems”; likewise for the below-ground parts, called “roots”.

Tables 4 and 5 show respectively chemical composition of stem and root essential oils from *Elionurus hensii*, table 6 recapitulates the main constituents of stem and root essential oils from Loufoulakari, Loukoko and Sese sites.

Table 4: Chemical composition of the essential oil from stems of *Elionurus hensii* (Loufoulakari site)

RI	RI _{LITT}	Constituents	%
838	839	2-Pentanone,4-hydroxy-4-methyl	0.1
921	926	Tricyclene	1.0
932	939	Pinene alpha	0.4
949	954	Camphene	2.8
988	990	Myrcene	0.1
1023	1024	Cymene -ortho	1.6
1028	1029	Limonene	6.4
1031	1031	Cineole 1,8	0.1
1089	1091	Cymenene para	0.3
1122	1122	Mentha-2,8-dien-1-ol<trans-para>	7.4
1137	1137	Mentha-2,8-dien-1-ol<cis-para>	4.9
1141	1141	Pinocarveol<tans->	0.6
1163	1164	Pinocarvone	0.3
1168		Bicyclo[3,3,0]oct-2-en-7-one,6-methyl	1.1

1189	1189	Mentha-1(7),8-dien-2-ol <trans-para->	11.1
1201		NI	4.2
1208	1200	Caranone<cis-4->	0.7
1209		Octylacetate	1.1
1219	1216	Carveoltrans	4.5
1231	1230	Mentha-1(7),8-dien-2-ol <cis-para->	14.1
1234	1229	Carveol <cis>	0.8
1245	1243	Carvone	3.2
1255	1252	Piperitone	0.4
1277	1271	Perillaaldehyde	0.3
1285	1285	Bornyleacetate	1.2
1292	1294	Undecanone-2	4.9
1307	983	NI	7.4
1320		NI	2.0
1332		NI	4.5
1434	1475	Néoclovène-beta	0.0
1495	1496	Tridecanone-2	2.1
1671	1666	Intermedeol	2.7
1698	1697	Pentadecanone-2	0.1
1764	1763	Aristolone	0.3
TOTAL			91.9

Table 5: Chemical composition on root essential oil (Loufoulakari site)

RI	RI _{LITT}	Constituents	% FID
838	839	2-Pentanone,4-hydroxy-4-methyl	0.6
921	926	Tricyclene	0.8
925	930	Thujene-alpha	0.3
932	939	Pinene-alpha	1.5
949	954	Camphene	2.7
972	975	Sabinene	0.3
988	990	Myrcene	0.6
1024	1024	Cymene<ortho->	0.4
1029	1029	Limonene	8.8
1032	1031	Cineole<1,8->	2.6
1084	1088	Terpinolene	0.1
1121	1122	Mentha-2,8-dien-1-ol <trans-para>	0.2
1136	1137	Mentha-2,8-dien-1-ol <cis-para>	0.1
1148	1146	Camphor	0.1
1181	1177	Terpinen-4-ol	0.2
1188	1189	Mentha-1(7),8-dien-2-ol<trans-para>	0.4
1195	1188	Terpineol-alpha	0.5
1219	1216	Carveol-trans	0.2
1230	1230	Mentha-1(7),8-dien-2-ol<cis-para>	0.4
1245	1243	Carvone	0.2
1255	1252	Piperitone	0.3

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1285	1285	Bornyleacetate	0.4
1292	1294	Undecanone-2	0.5
1391	1390	Elemene-beta	0.5
1399	1403	Methyleugenol	0.6
1412	1426	Cymene<2,5-dimethoxy para->	0.5
1415	1409	Gurjunene-alpha	1.0
1422	1423	Aristolene	0.8
1431	1429	Aristola-1(10),8-diene	1.3
1435	1433	Gurjunene-beta	10.0
1438	1472	Dauca-5,8-diene	0.6
1450	1442	Guaiene-alpha	0.2
1461	1473	Gurjunene-gamma	0.1
1464	1485	Selinene-beta	0.1
1475	1438	Muurola-3,5-dien <cis>	0.2
1493	1485	Selinene-beta	0.1
1495	1491	Valencene	1.2
1500	1498	Selinene-alpha	0.3
1516	1513	Cadinene-gamma	0.3
1520	1540	Amorphene-delta	0.2
1524	1522	Selinene- 7-epi-alpha	0.9
1575	1567	Maaliol	2.5
1581	1578	Spathulenol	0.4
1612	1600	Rosifoliol	0.4
1646	1542	Selina-3,7(11)-diene	0.3
1660	1653	Cadinol-alpha	0.2
1671	1666	Intermedeol	1.6
1721		NI	3.9
1769	1763	Aristolone	44.6
TOTAL			97.2

Table 6: Major constituents (***) of essential oils from stems (S) and roots (R) collected at the sites of Loufoulakari (sample 1), Loukoko (sample 2), and Sese (sample 3)

Sites de récolte	S1*	R1	S2	R2*	S3	R3	***
Camphene	2.8	1.1	4.9	2.6	2.3	0.8	I
Limonene	6.4	9.7	4.9	8.8	12.8	9.7	II
Cineole <1,8->	0.0	3.8	0.0	2.6	0.0	5.5	III
Mentha-2,8-dien-1-ol <trans para->	7.4	1.2	6.5	0.2	10.1	0.8	IV
Mentha-2,8-dien-1-ol <cis para->	4.9	0.8	4.7	0.1	6.3	0.5	V
Mentha-1(7),8-dien-2-ol <trans para->	11.1	2.3	11.2	0.4	12.2	1.1	VI
Carveol trans	4.5	0.8	3.9	0.2	5.1	0.4	VII
Mentha-1(7),8-dien-2-ol <cis para->	14.1	2.7	11.2	0.4	14.0	1.3	VIII
Carvone	3.2	0.8	3.2	0.2	14.0	0.4	IX

Undecanone -2	4.9	2.7	5.7	0,10	3.4	1.4	X
Gurjunene <beta->	0.0	4.6	0.0	2.2	0.0	6.2	XI
Maaliol	0.0	1.7	0.0	2.5	0.0	2.3	XII
Tridecanone 2	2.1	1.9	2.4	0.0	1.9	1.1	XIII
Intermedeol	2.7	2.7	2.2	1.6	2.0	1.5	XIV
Pentadecanone <2->	0.0	0.0	0.0	0.0	0.0	0.0	XV
Aristolone	0.0	42.2	0.0	44.6	0.0	41.8	XVI

* S1 means: stem of sample 1; R2: root of sample 2)

The essential oil from stems was composed of a large number of menthadienol isomers like in *Cymbopogon giganteus* [15], [16], [17], [30]. The identification of these different isomers is difficult. Garneau *et al.* [31] determined Kovats indices on two columns and recorded the mass spectra of six menthadienol isomers of known structures, which they synthesised. From these data, after analysis by gas phase chromatography coupled with mass spectrometry, we identified the isomers present in the essential oils studied. They were in decreasing order of content: *cis-p*-mentha-1(7),8-dien-2-ol, *trans-p*-mentha-1(7),8-dien-2-ol, *trans-p*-mentha-2,8(9)-dien-1-ol, *cis-p*-mentha-2,8(9)-dien-1-ol, and *trans-p*-mentha-1,8-dien-6-ol (carveol). Then came limonene, 2-undecanone, carvone, and 2-tridecanone.

These oils differed appreciably from those extracted from roots, which had lower essential oil contents, more *p*-cymene and intermedeol, and noteworthy levels of aristolone (more than 40 % against 3% in the aerial parts).

Aristolone was isolated and identified spectrally by NMR spectroscopy and mass spectrometry [10]

This difference can be visualised using a polar coordinate representation (radar plot). One notes that (i) the radar plot of the essential oil from stems differs from that of oil from roots, and (ii) those of oils from the same parts of the plant (stems or roots) are similar. Figure 3 shows radar plots of stem and root essential oils.

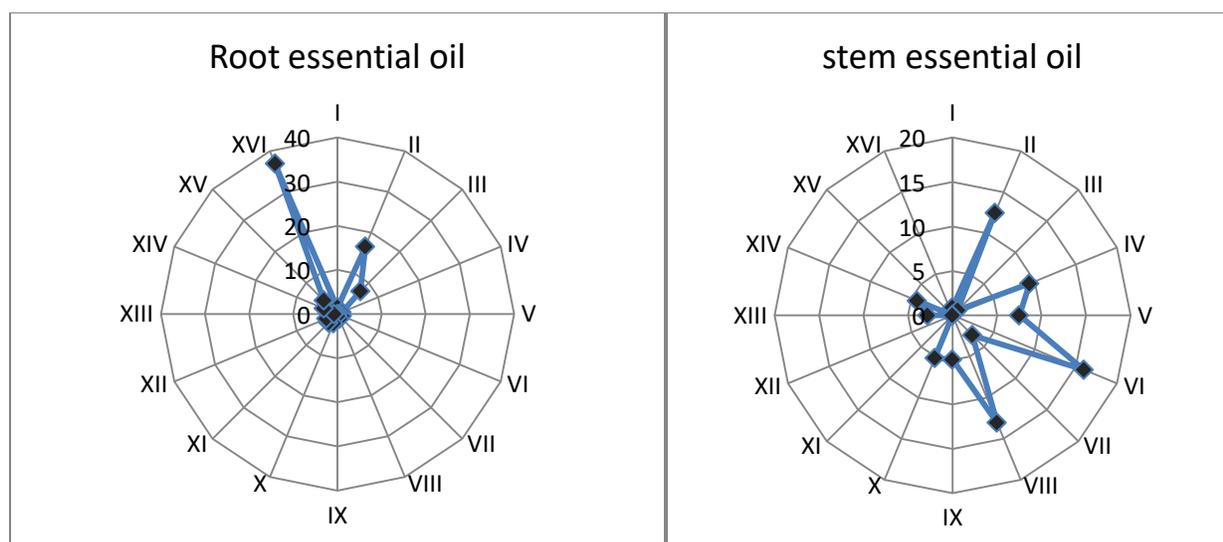


Figure 3: Radar- plots of Stem and root essential oil extracted from *Elionurus hensii*

3.2. Modelling of Essential Oil Extraction by Hydrodistillation from *Elionurus Hensii* in A Laboratory Scale

Steam distillation and water distillation are used to extract useful essential oils from aromatic plant resources.

Skaria *et al.* [32] distinguish three techniques: (i) hydro-distillation (the plant matter is partially or totally immersed in the distillation water), (ii) steam distillation (steam produced outside the extractor in a steam generator passes through the plant material, which is not in water) et (iii) vapo-hydro-distillation (steam is produced *in situ* in the extractor, where a grille separates the water from the plant material).

Only hydro-distillation and vapo-hydro-distillation can be used at artisanal scale.

3.2.1. Hydrodistillation of *Elionurus Hensii* Stems

The modelling of the extraction process is in principle complex, but can be simplified by a judicious choice of the factors to be studied. We selected three factors: extraction duration (h), X_1 ; residual water content, X_2 ; state of division (cm), X_3 (tables 1, 2 and 3).

Table 7 recapitulates the experimental matrix and the responses: essential oil content and cumulated *p*-menthadienol content.

Table 7: Experimental matrix and responses (essential oil content and cumulated *p*-menthadienol content).

Run	X_1	X_2	X_3	Essential oil yield (%)	<i>p</i> - menthadienol Content (%)
1	-1	-1	-1	1.0	47.9
2	+1	-1	-1	0.8	45.5
3	-1	+1	-1	1.6	53.1
4	+1	+1	-1	2.0	55.6
5	-1	-1	+1	0.4	45.9
6	+1	-1	+1	0.7	44.7
7	-1	+1	+1	2.1	50.2
8	+1	+1	+1	2.1	56.8

The mathematical equation representing the quantitative yield of the hydrodistillation of stems of *Elionurus hensii* generated by the model is:

$$y = 1.34 + 0.07x_1 + 0.61x_2 + 0.02x_3 + 0.04x_1x_2 - 0.001x_1x_3 - 0.17x_2x_3 + 0.10x_1x_2x_3$$

With:

$$a_0 = 1.34, a_1 = 0.07, a_2 = 0.61, a_3 = 0.02$$

$$a_{12} = 0.04, a_{13} = -0.001, a_{23} = -0.17$$

$$a_{123} = -0.10$$

This relation shows that the optimum should be located in the experimental domain or very close to it, as the values of an are not very high. The average yield of essential oil we can extract from the experimental set-up is $a_0 = 1.3\%$.

The influence of residual water content ($a_2 = 0.61$) is important in the response, the combined effect of residual water content and state of division of the plant material was favourable ($a_{23} = 0.17$). However, we note the very low a values for the other interactions, which can therefore be ignored in practice, especially for small-scale production units.

The mathematical equation representing the cumulated p -menthadienol content in the essential oils from stems of *Elionurus hensii* generated by the model is:

$$z = 49.95 + 0.686x_1 + 3.966x_2 - 0.563x_3 + 1.578x_1x_2 + 0.658x_1x_3 + 0.128x_2x_3 + 0.366x_1x_2x_3$$

The average content of cumulated p -menthadienols was 49.95% and the factor, residual water content in plant material, exhibits the most important effect in the response (+ 3.966). Extraction duration and state of division presents opposite effects: the first is positive (+0.686), the second, negative (-0.563) with a very close magnitude. All interactions are positive and x_1x_2 interaction was the most important (+1.578).

3.2.2. Hydrodistillation of the *Elionurus Hensii* Roots

Tables 8 and 9 give the levels of variation of the three variables, the experimental matrix, and the responses (essential oil yield and aristolone content).

Table 8. Levels of variation of variables for the extraction of essential oils from *Elionurus hensii* (roots)

Level	Extraction duration (h) X_1	Residual water content X_2	State of division (cm) X_3
Low (-1)	1.5	Low	< 5
High (+1)	3	High	10

Table 9: Experimental matrix and responses for the extraction of essential oils from *Elionurus hensii* (roots)

Run	X_1	X_2	X_3	Essential oil yield (%)	Aristolone Content (%)
1	-1	-1	-1	0.5	36.9
2	+1	-1	-1	0.9	38.9
3	-1	+1	-1	1.1	54.6
4	+1	+1	-1	0.8	36.7
5	-1	-1	+1	0.5	37.8
6	+1	-1	+1	0.8	37.3
7	-1	+1	+1	0.5	37.3
8	+1	+1	+1	0.9	50.0

The mathematical equation representing the quantitative yield of the hydrodistillation of roots of *Elyonurus hensii* generated by the model is:

$$y = 0.747 + 0.112x_1 + 0.0525x_2 + 0.0575x_3 - 0.0725x_1x_2 - 0.0375x_2x_3 + 0.0775x_1x_3 + 0.0975x_1x_2x_3$$

Average yield (0.747), effects of factors (0.0575 - 0.112) and effects of interactions (0.0375 – 0.0775) are less important in root essential oil extraction. The extraction duration presents the most important positive effect. Interaction effects are neglected.

The mathematical equation representing the aristolone content in essential oils of *Elionurus hensii* generated by the model is:

$$z = 41.181 - 0.456x_1 + 3.466x_2 + 0.568x_3 - 0.828x_1x_2 - 0.413x_2x_3 + 3.516x_{13} + 4.133x_1x_2x_3$$

Aristolone extraction is more sensitive to the effects of factors and interactions. With an average yield of 41.181 %, the extraction was impacted by the residual water content (3.466) and the interaction of extraction duration/state of division (3.516). The three- factor interaction lead to the most important effect on the response (4.133).

3.2.3. Characterisation of Oils During Extraction

The analysis of essential oils collected during the execution of the experimental design can yield information on how the different constituents of these oils are extracted in relation to the factors studied.

If light fractions are collected first during a distillation, we can expect to obtain oils rich in light fractions for short times and rich in heavy fractions for long times.

If by contrast all the constituents are collected at the steam temperature, the parts affected first by the steam will be collected together, and we can expect an oil that from the very first drop will have the average composition of the plant’s total essential oil.

The multivariate analysis of 16 samples from two experimental designs gave very interesting results.

Principal component analysis (PCA) carried out on the data given in Tables 11 and 12 indicates a very close correlation of variables, mainly around the first principal axis (F1) in the first principal plane (F1F2).

Table 10: Yield (%) and major component contents (Table 6 *) in essential oils from stems (S**) and roots (R***) of *Elionurus hensii* obtained in the execution of the experimental design

	%	I*	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI
S1**	0.4			0.													
	0	0.9	12.5	9	9.4	7.5	16.0	3.2	13.1	5.0	5.2	0.0	0.0	2.8	4.3	0.5	0.5
S2	0.6			0.													
	6	2.9	17.6	0	10.3	6.4	14.9	2.1	13.2	3.1	5.2	0.0	0.0	3.0	5.7	0.5	0.6
S3	2.1			0.													
	0	2.2	18.9	0	15.4	7.1	13.7	3.1	14.0	3,22	3.2	0.0	0.0	1.2	2.8	0.5	0.0
S4	2.1			0.													
	1	2.4	14.9	0	11.2	7.2	13.2	2.8	15.2	3,40	3.4	0.0	0.0	2.5	3.6	0.5	0.0
S5	0.9			0.													
	9	2.3	17.5	0	9.4	6.5	16.2	3.1	15.8	4,83	4.8	0.0	0.0	3.4	3.9	0.5	0.0

S6	0.8	0.															
	4	2.6	20.4	0	13.7	6.7	12.2	2.8	12.9	3,15	3.9	0.0	0.0	2.5	4.0	0.5	0.0
S7	1.5	0.															
	9	2.1	21.0	0	15.6	7.6	14.9	2.5	14.9	3,37	2.9	0.0	0.0	1.0	2.7	0.5	0.0
S8	2.0	0.															
	0	3.5	15.9	00	13.7	8.2	14.4	3.1	15.4	3,24	4.8	0.0	0.0	1.5	3.4	0.5	0.0
Mean	1,3	0.															
	4	2,4	17.3	0	12.3	7.2	14.5	2.8	14.3	3,74	4.2	0.0	0.0	2.2	3.8	0.5	0.1
SD	0.7	0.															
	0	0.7	2.7	3	2.4	0.6	1.3	0.4	1.1	0.7	0.9	0.0	0.0	0.9	0.9	0.0	0.2
R1***	0.5	7.															
	1	1.5	16.5	3	1.1	0.6	1.2	0.0	1.2	0.4	2.3	2.8	2.7	0.8	3.4	4.3	36.9
R2	0.9	5.															
	2	1.5	16.9	8	0.8	0.4	0.9	0.0	1.0	0.4	2.0	4.6	2.8	0.8	4.8	0.0	38.3
R3	1.0	6.															
	3	0.5	12.1	6	0.0	0.0	0.0	0.0	0.0	1.1	0.4	2.4	2.8	1.3	2.7	5.0	54.6
R4	0.7	5.															
	6	1.7	15.4	7	0.2	0.0	0.0	0.0	0.0	0.0	4.0	4.0	3.3	0.7	4.4	5.2	36.7
R5	0.5	8.															
	1	1.3	11.8	4	1.0	0.0	0.0	0.0	0.0	0.3	1.8	3.2	3.9	0.7	3.7	5.6	37.8
R6	0.8	5.															
	4	1.5	13.3	6	0.9	0.5	1.1	0.0	1.1	0.4	2.7	3.8	3.3	0,8	5.7	5.1	37.3
R7	0.4	5.															
	9	1.3	16.3	8	0.6	0.5	1.2	0.0	1.1	0.4	2.4	3.8	2.9	1.0	3.7	4.5	37.3
R8	0.9	5.															
	2	0.9	13.9	7	0.6	0.2	0.2	0.0	1.1	0.6	0.3	3.6	2.8	1.0	5.4	2.1	50.0
Mean	0,7	6.															
	5	1.3	14.5	4	0.6	0.3	0.6	0.0	0.7	0.5	2.0	3.5	3.1	0.9	4.2	4.0	41.1
SD	0,1	1.															
	9	0.2	1.6	2	0.2	0.2	0.5	0.0	0.5	0.1	0.9	0.3	0.4	0.1	0.9	1.4	5.5

This means that the analysis can be carried out with a much smaller number of variables (constituents of essential oils). Ten of the 16 variables used lie on the correlation circle. F1 contains 75,25 % of the information on the variability of the essential oils, and 83,14 % on F1F2. The study can therefore be limited to the first principal plane F1F2 (Figure 4).

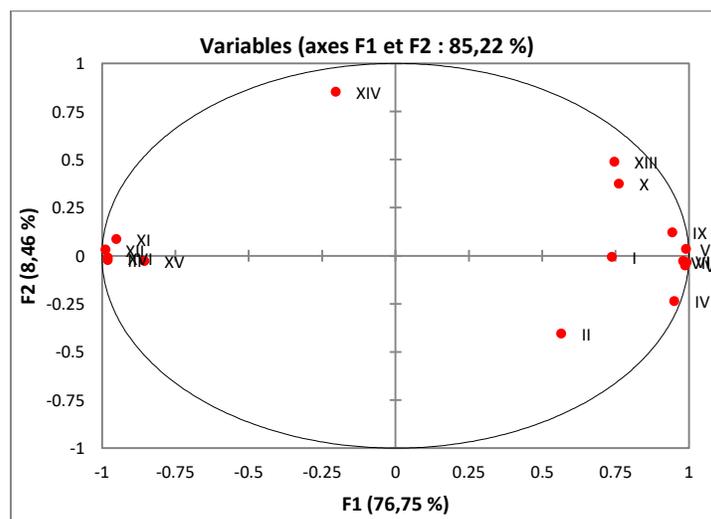


Figure 4: Correlation circle of variables in principal component analysis (PCA)

The distribution of individuals in the first principal plane F1F2 shows a clear separation between oils of the plant’s aerial parts and those of its below-ground parts, suggesting a close similarity of oils from the same plant part, and a marked difference in the oils from the two parts (Figure 5).

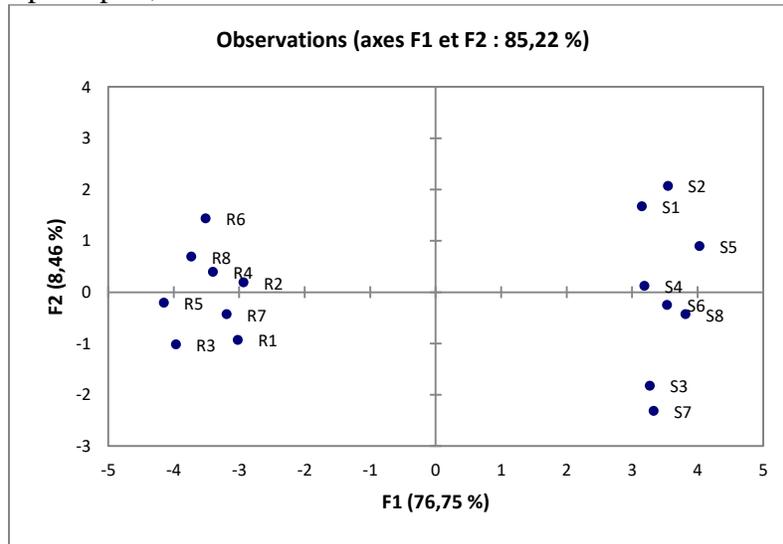


Figure 5: Distribution of individuals (essential oil samples) in principal component analysis (R: roots; S: stems)

Ascending Hierarchical Clustering (AHC) confirms the distribution into two groups each totally separate and homogeneous (oils from stems and oils from roots) (Figure 6, Table 11).

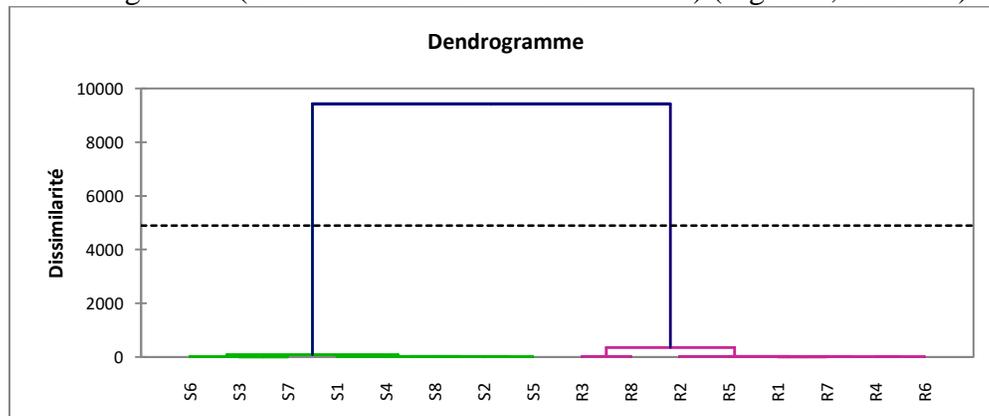


Figure 6: Classes generated by HAC of essential oils

Table 11: Distribution of individuals into classes by HAC

Classes	1	2
Individuals	S1	R1
	S2	R2
	S3	R3
	S4	R4
	S5	R5
	S6	R6
	S7	R7
	S8	R8

3.3. Modelling of the Artisanal Extraction (Hydrodistillation)

A local extractor, used in a domestic scale in *Elionurus hensii* essential oil extractions, was tested in following conditions (figure 2, tables 12 and 13).

Table 12: Levels of variation of variables in the artisanal extraction of essential oils from *Elionurus hensii* (aerial parts).

Niveau	Extraction duration (h) X_1	State of division X_2	Extraction water quantity (L) X_3
Low (-1)	1.5	chopped	20
High (+1)	3	Non chopped	30

Table 13: Experimental matrix and responses for the extraction of essential oils from *Elionurus hensii* (aerial parts).

Run	X_1	X_2	X_3	Essential oil yield (%)	<i>p</i> - menthadienol Content (%)
1	-1	-1	-1	0.8	52.1
2	+1	-1	-1	0.8	50.9
3	-1	+1	-1	0.7	48.2
4	+1	+1	-1	0.8	47.7
5	-1	-1	+1	0.6	48.5
6	+1	-1	+1	0.7	48.1
7	-1	+1	+1	0.8	49.5
8	+1	+1	+1	0.8	47.2

3.3.1. Response: Essential Oil Yield

The average of yield of essential oil extraction is 0.75. Figure 7 gives the values and the relative importance of the principal and interaction effects of the first order factor.

The principal effects b_1 , b_2 and b_3 are weak and of the same order of magnitude, the two first are positive and the third is negative. The effects of interactions 1-2 and 1-3 are nulls. On figure 8, the diagrams a, b, c and d the features in dotted strait lines has representing the principal effects, their slopes are identical (similar principal effects) and thus are parallel (absence of interaction effects). The diagrams e and f illustrate the existence of interaction effects. Then interaction x_2 - x_3 produces the most important effect on the extraction yield contenu de *p*-menthadienol and it is positive.

The mathematical expression of the first degree polynom associated to the full factorial design model selected here is:

$$y = 0.75 + 0.03 x_1 + 0.03 x_2 - 0.02 x_3 + 0.05 x_1 x_3$$

With a coefficient of regression $R^2 =$ which validates *a posteriori* the model.

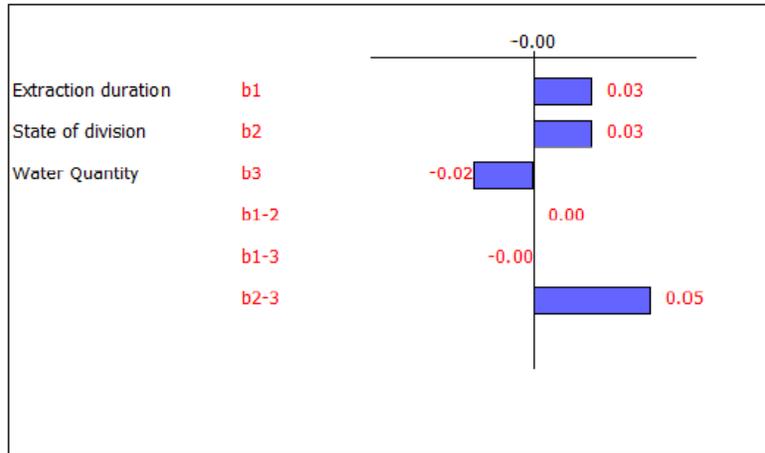


Figure 7: Graphic representation of the coefficient of factor effects (principal and interaction) on the yield of essential oil extraction.

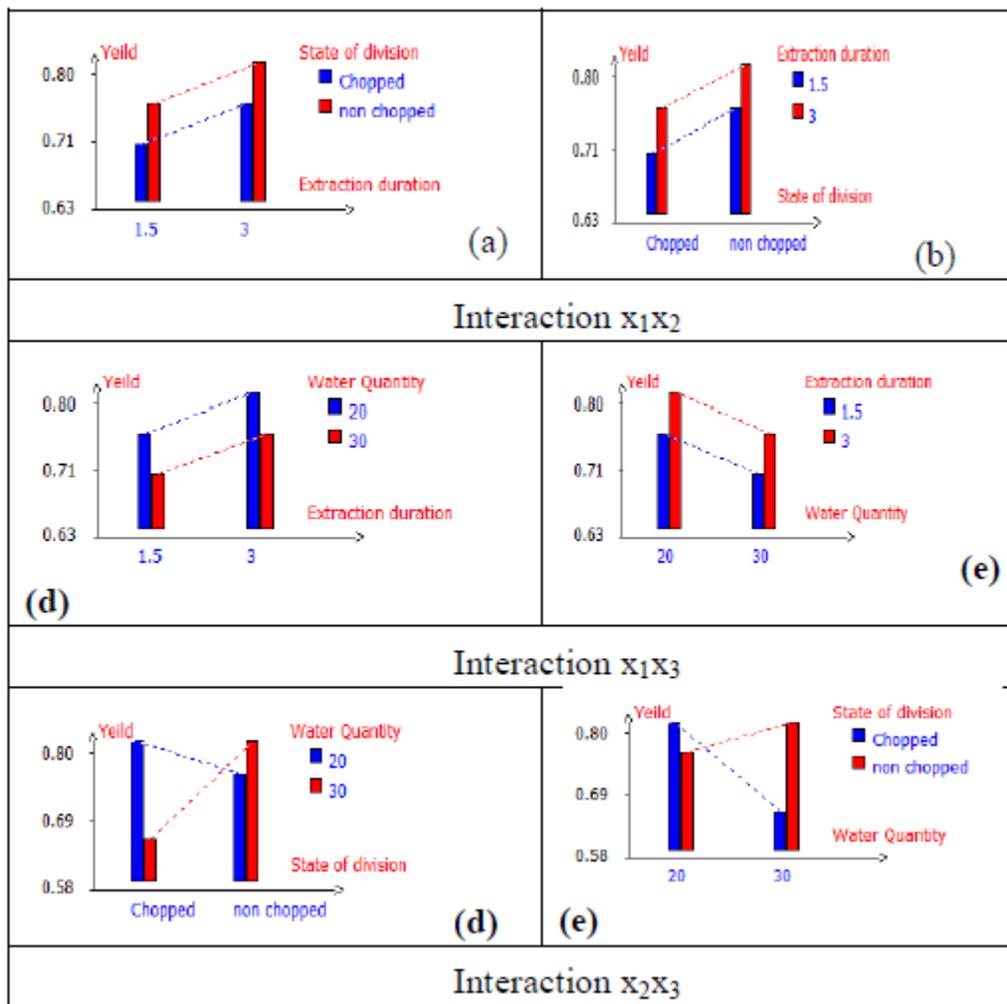


Figure 8: Graphic representation of factor interaction effects on the yield of essential oil extraction.

3.3.2. Response : P-Menthadienol Content

The statistics of the coefficients of the model leading to the coefficient of regression $R^2=0.958$ validate this last, which results mathematically in:

$$z = 40.0 - 0.055x_1 - 0.88x_2 - 0.70x_3 - 0.15x_1x_2 - 0.13x_1x_3 + 0.98x_2x_3 - 0.33x_1x_2x_3.$$

The average of the *p*-menthadienol content is of 40.0 %. The content of *p*-methadienols is more sensitive to the principal and interaction effects of the factors; these effects are overall negative. Only the interaction x_2x_3 is positive (figure 9).

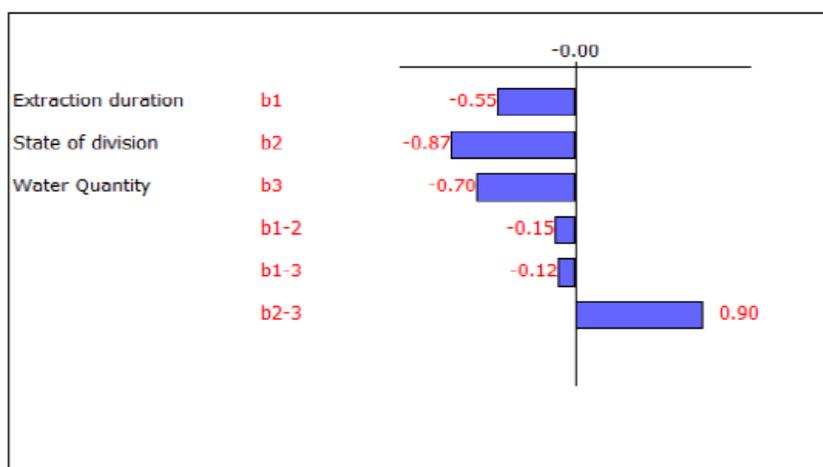


Figure 9: Graphic representation of the coefficient of factor effects (principal and interaction) on *p*-menthadienol content.

3.3.3. Composition of 8 Samples Extracted Via The Artisanal Scale

The composition of 8 samples extracted *via* the artisanal scale were closed similar according to the SD values of the major constituent contents and the representative radar plots of the essential oils (table 14, figure 10).

Table 14: Essential oil composition of samples extracted via the artisanal experimental design (Elionurus hensii stems).

Run (reference of the sample)	1 (03)	2 (04)	3 (05)	4 (06)	5 (07)	6 (08)	7 (09)	8 (10)	Mean (SD)
Tricyclene	1.0	1.0	1.2	0.8	1.0	1.1	0.6	0.9	
Pinene alpha	0.2	0.2	0.3	0.2	0.2	0.2	0.1	0.2	
Camphene	3.6	3.3	3.8	2.7	3.1	3.5	2.2	3.1	3.1 (0.4)
NI	0.3	0.3	0.2	0.3	0.4	0.5	0.2	0.5	
Cymene ortho	1.7	1.6	2.1	1.7	1.9	2.1	1.2	2.0	
Limonene	2.0	1.8	1.9	1.4	1.4	1.8	1.3	1.7	
Cineole 1,8	0.2	0.2	0.3	0.2	0.1	0.2	0.2	0.3	
Cymenene para	0.2	0.3	0.2	0.2	0.1	0.1	0.2	0.1	
Mentha-2,8-dien-1-ol trans	10.0	9.9	8.6	8.6	8.8	8.0	9.4	8.1	8.9 (0.7)

para										
Mentha-2,8-dien-1-ol cis										5.7 (0.3)
para	6.2	6.0	5.6	5.5	5.6	5.3	5.7	5.4		
Verbenoltrans	1.1	-	-	1.0	1.0	1.0	1.0	1.0		
NI	0.4	-	-	0.3		0.4	0.4	0.4		
Pinocarvone	0.4	0.3	1.3	0.4	0.3	0.3	0.3	0.3		
NI	1.6	-	-	1.8	1.5	1.5	1.4	1.7		
Mentha-1(7),8-dien-2-ol				17.5				17.3		17.8
trans para	18.3	18.1	18.2		17.6	18.1	17.7			(0.4)
Caranone cis-4	0.2	0.3	0.7	3.6	2.6	2.5	2.8	3.2		
Menthatriene 1,3,8 para	4.1	-	-	5.6	4.1	5.0	5.2	4.8		
Caranone trans-4	0.8	-	-	0.4	1.0	0.4	0.4	0.5		
Mentha-1(7),8-dien-2-ol				15.8						16.6
cis para	17.6	16.9		16.1	16.6	16.7	16.8	16.5		(0.5)
Carveol cis	0.5	-	0.7	0.6	0.9	0.8	0.8	1.0		
Carvone	5.1	-	5.1	5.6	5.0	4.8	4.9	5.2		
Piperitone	0.7	0.6	0.6	0.6	0.6	0.7	0.6	0.6		
Perilla aldehyde	0.7	0.3	0.3	0.4	0.4	0.4	0.3	0.4		
Bornyleacetate	2.1	2.0	0.6	0.8	0.7	0.7	0.6	0.4		
Undecanone 2	7.5	5.5	2.2	2.1	1.9	2.1	2.0	2.0		3.2 (2.0)
Tridecanone 2	2.6	2.6	2.4	1.0	3.1	2.8	2.7	3.1		
Selina-3,7(11)-diene	2.6	2.6	-	2.2	2.8	2.5	2.5	2.5		
TOTAL	91.1	73.8	71.9	8.7	82.5	83.7	81.8	83.3		

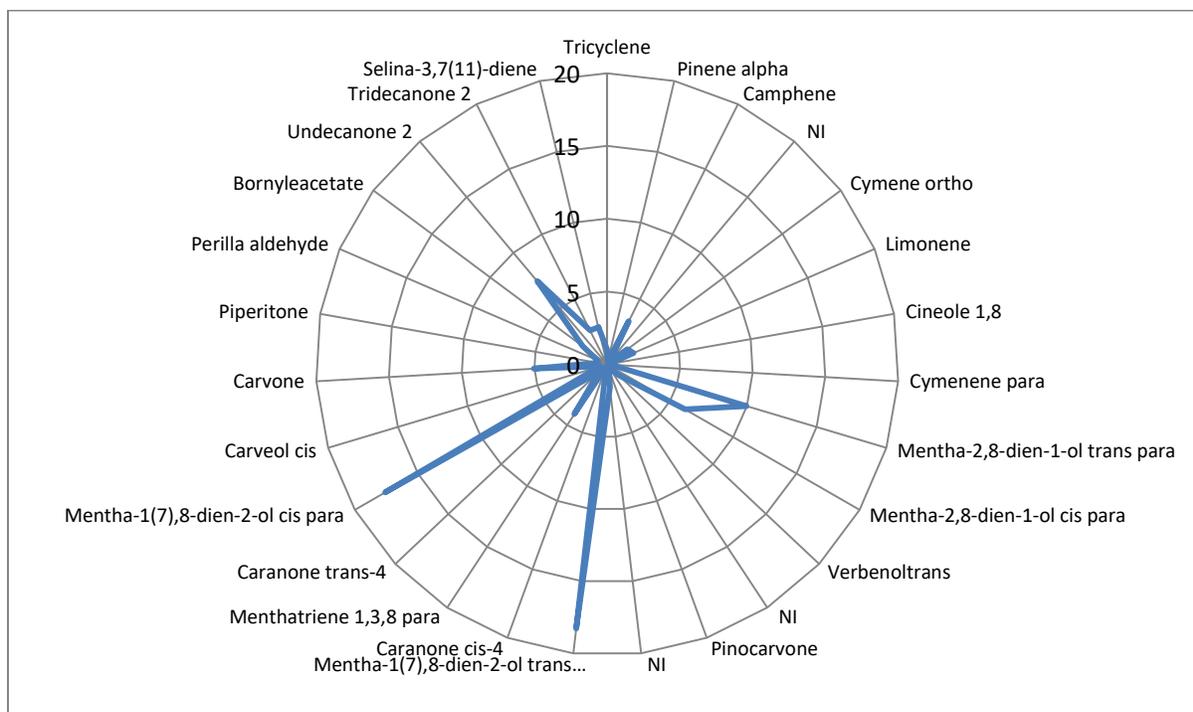


Figure 10: Representative radar plot of the essential oil extracted *via* the artisanal hydrodistillation from *Elionurus hensii* stems (similar radar plots for the 8 samples).

4. CONCLUSION

Hydrodistillation is used to extract useful essential oils from aromatic plant resources. Their efficiency depends on both the plant material and the extraction process. It is important to work with homogeneous plant material obtained in the most favourable conditions (collection of plant material when the content and composition of the essential oil is optimal)

The modelling of the extraction process is in principle complex, but can be simplified by a judicious choice of the factors to be studied. We selected three factors for the steam distillation and four factors for the water distillation. These were used as variables in a two-level factorial design. This design, involving a mathematical model in the form of a first degree polynomial, was used to calculate average response, and effects of factors.

The factor considered and the experimental domain selected have very weak influence on the extraction of the essential oil, which is on average 0, 75 %.

For the aerial part (stems, leaves and flowers) of *Elionurus hensii*, the values of the factor effects was close to 0. This means which one is very close to the optimum of extraction. The artisan can thus keep, the current extraction conditions. A finer research of optimum, with the actual extractor, will not produce any significant profit.

The effects of these same factors have impact more important on the contents of *p*-menthadienol, but non-significant on an artisanal scale.

The composition of the 8 samples resulting from the experimental design have a similar compositions; the factor studied do not have any impact on the quality of oils: nearly 50 % of *p*-menthadienols, which are responsible known biological properties of this plant.

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