Optimization of the extraction of the \( p \)-menthadienol isomers and aristolone contained in the essential oil from *Elyonurus hensii* using a \( 2^3 \) full factorial design

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**Abstract**

The aim of this study was to optimize the extraction of \( p \)-menthadienol isomers and aristolone from the essential oil of *Elyonurus hensii* by hydrodistillation. The study of the seasonal variation in the chemical composition has shown that the plant material has been subject to a natural selection regarding the biosynthesis of the \( p \)-menthadienol isomers: during periods of water stress, the extracts are rich in \( cis \) and \( trans \)-\( p \)-mentha-1(7),8-dien-2-ol and poor in \( cis \) and \( trans \)-\( p \)-mentha-2,8-dien-1-ol. Regarding the modeling, eight experiments were carried out by considering three easily interpretable factors (the extraction duration, the residual water content and the state of the division of the plant material). The average yield was 1.33% for the aerial part and 0.74% for the roots. The residual water content is the most important factor, which significantly influences the average yield of the essential oil and the content of the major constituents. Regarding the aerial part, a low residual water content of the plant material varies the essential oil yield (from 0.40% to 2.11%) and the content of \( cis \) and \( trans \)-\( p \)-mentha-2,8-dien-1-ol (from 15.87% to 23.24%). At the root level, the samples that have a very low residual water content provide extracts richer in aristolone. The combined effects of the extraction duration, the state of division, and the residual water content influence greatly the extraction of aristolone (from 36.68% to 54.55%). However, these interactions are more complex and difficult to assess.

**KEYWORDS**

8-dien-1-ol, 8-dien-2-ol, aristolone, \( cis \) and \( trans \)-\( p \)-mentha-1(7), \( cis \) and \( trans \)-\( p \)-mentha-2, *Elyonurus hensii*, modeling

**1 | INTRODUCTION**

*Elyonurus hensii* is found in the tropical and subtropical regions of South America (Brazil and Argentina), Africa (Congo Republic, Gabon, DR Congo, Angola), and Australia (Yang et al., 2013). *Elyonurus hensii* has been studied in only the Republic of Congo, where this plant grows spontaneously on the “Plateau des Cataractes”. It is usually used by local people as a théiforme drink to relieve pain, which justifies its vernacular name “tikoni” (pain).

The first description of the chemical profile of the essential oils from different parts (roots, stems, leaves, and flowers) showed that the oils from the aerial parts were mainly rich in \( p \)-menthadienol...
isomers, and the main ones are cis and trans-p-mentha-1(7),8-dien-2-ol, cis and trans-p-mentha-2,8-dien-1-ol. The essential oil of the roots is rather rich in sesquiterpene compounds, and aristolone, with a content of approximately 40%, is the major compound. (Silou, Loubaki, Figuéredo, & Chalchat, 2006). Studies of the essential oils of species of the genus Elyonurus are very limited; however, it appears that Elyonurus hensii is the only species of the genus that is rich in p-menthadienol isomers. However, these isomers are present at significant contents in the oils of Cymbopogon giganteus and Cymbopogon densiflorus. Indeed, the essential oil of Cymbopogon giganteus from the Ivory Coast is characterized by high contents of cis and trans-p-mentha-2,8-dien-1-ol (8.7% and 18.4%, respectively) and cis and trans-p-mentha-1(7),8-dien-2-ol (16% and 15.7%, respectively), and this oil presents antimicrobial properties (Boti et al., 2006). Moreover, the antimicrobial properties were also observed on the essential oil of Cymbopogon giganteus from Burkina Faso (Menut et al., 2000), Cameroon (Jirovetz, Buchbauer, Eller, Ngassoum, & Maponmetsem, 2007), Benin (Ayedoun, Souhoumhoué, Menut, Lamaty, & Béssière, 1999), and Mali (Sidibe, Chalchat, Garry, & Hamara, 2001); these species produce essential oils rich in p-menthadienol isomers. The essential oil from the flowers and leaves of Cymbopogon densiflorus of Zambia is also rich in p-menthadienol isomers, and it contains 22.4% trans-p-mentha-2,8-dien-1-ol and 11.1% cis-p-mentha-1(7),8-dien-2-ol. The oil of the Brazilian plant presents a similar composition (Boelens, 1994; Chisowa, 1997). The literature reports several uses of Cymbopogon densiflorus: the crushed leaves are used as a treatment for rheumatism in Gabon, the flower head is smoked in a pipe as a cure for bronchial affections in Malawi, and the plant sap is used in the Congo-Brazzaville, where it is also given as a treatment for asthma and to calm fits (Akhila, 2010). Manifestly, the essential oil of Cymbopogon densiflorus exhibits biological activities. The massive presence of p-menthadienol isomers in the essential oil of Elyonurus hensii points also to possible antimicrobial properties. Aristolone is also an interesting constituent since it is likely to induce an antalgic activity (Tian-Shung, Amoou, Damu, & Kuo, 2004).

In a previous study of Elyonurus hensii from Congo-Brazzaville, (1) the volatile components from the stems and the roots were obtained by hydrodistillation and head-space solid phase microextraction (HS-SPME), and (2) for the extracts obtained with methanol, ethyl acetate and dichloromethane, the antioxidant activities of the extracts were demonstrated and compared. The essential oil of Elyonurus hensii did not have significant properties of DPPH and ABTS for either the stems or the roots. However, the solvent extracts are effective antioxidants according to in vitro assays. The authors conclude that the extracts of Elyonurus hensii have potential as natural additives for the food and pharmaceutical industries (Yang et al., 2013).

The use of experimental designs can improve the yields of essential oil. They also optimize the extraction of the major constituents of the essential oils by organizing the steam distillation with a minimum number of experiments to be performed (Silou, Malanda, & Loubaki, 2004; Silou et al., 2009). Taking into account the scientific interest that seems to represent the isomers of p-menthadienol and aristolone, we have tried to optimize the extraction of these major constituents by using a 2² full factorial design. We have used a simple model of the first degree which gives the representation of the response function based on variables, which allows us to evaluate the influence of different factors studied on the contents of the aristolone and p-menthadienol isomers.

# MATERIAL AND METHODS

## 2.1 Plant material

The plant material consisting of the aerial parts (stems, leaves, and flowering tops) and the roots was harvested. The samples were collected on the “plateau des cataractes” (District of Louingui, Pool Department, Republic of Congo) at three sites, Loufoulacari (L), Campus rural (C), and Sese (S), and during different period of the year 2015, February (f), may (m), july (j), november (n), and december (d). These harvest periods include periods of drastic reductions in rainfall, the dry season (May and June), and periods of heavy rainfall, the rainy season (November and December). The precipitation levels on the “plateau des Cataractes” are an average of 2 mm in the dry season and 250 mm in the rainy season. Moreover, the sites of Loufoulakari and rural Campus are on a clay ground, whereas the Sese site is located on a sandy soil.

For the determination of the chemical composition, the plant material was dried in the shade for 8 days and then subjected to steam distillation. Indeed, from the sixth day, the loss of water reached a plateau, as is the case for many species of the Poaceae family (Silou et al., 2004).

## 2.2 Extraction of the essential oil

After drying the plant material, the essential oil has been obtained by steam distillation using a Clevenger-type apparatus (Clevenger, 1928). The heating temperature was fixed at 100°C, which allowed a constant flow of condensation. Each time, 300 g of vegetable material, consisting either of roots or stems, leaves, and young flowers was placed in a flask with 500 ml of water and subjected to distillation for 3 hr. The organic phase from the distillation was separated from the aqueous phase by extraction with diethyl ether. The organic phase was placed in a flask with 500 ml of water and subjected to distillation for 3 hr. The organic phase from the distillation was separated from the aqueous phase by extraction with diethyl ether. The organic phase obtained was then dried over anhydrous sodium sulfate to remove traces of water, and the essential oil was recovered after the evaporation of the diethyl ether on a rotary evaporator. The samples of essential oil were stored in a refrigerator before submitting them to GC-FID and GC-MS analysis.

## 2.3 Determination of the chemical composition

### 2.3.1 Analysis by gas chromatography (GC)

The quantitative analysis of the essential oil was carried out using an Agilent gas chromatograph model 6890 equipped with a DB5 MS column (20 m × 0.18 mm; 0.18 μm). The oven temperature was...
programmed to 50°C for 3.2 min, then heated to 300°C at a rate of 10°C/min. The temperatures of the injector and the flame ionization detector (FID) were maintained at 280°C. The essential oils were diluted in acetone to 3.5% (v/v) and injected in split mode (1/60); hydrogen was used as the carrier gas (1 ml/min), and the injection volume was 1 μl. At the same time, a solution of n-alkanes (C8-C30) was analyzed under the same conditions to calculate retention indices (RI) using the Van den Dool and Kratz equation (Van Del Dool & Kratz, 1963). The relative concentrations of the compounds were calculated from the peak area obtained by gas chromatography without using correction factors.

2.3.2 | Analysis by coupling gas chromatography and mass spectrometry (GC-MS)

Qualitative analysis was performed using an Agilent gas chromatograph model 7890 coupled to an Agilent mass spectrometer model 5975 equipped with a DB5 MS column (20 m × 0.18 mm; 0.18 μm). The oven temperature was 50°C and remained constant for 3.2 min; then, it was increased to 300°C at a rate of 8°C/min. The injector temperature was 280°C. Ionization was obtained by electron impact at 70 eV, and the electron multiplier was maintained at 2200 eV. The temperature of the ion source was 230°C. Mass spectral data were acquired in the scan mode in the range m/z 33–450. The flow of carrier gas (helium) was set at 0.9 ml/min; compound identification was made by comparison of their spectra and RI with those of libraries such as Adams (2012), Nist (2008), and König, Hochmuth, and Joulain (2001) and were incorporated in the laboratory.

2.4 | Modeling of essential oil extraction by hydrodistillation

The variables influencing extraction yield were as follows: time, temperature, condensation rate, the state of division of the plant material, the mass ratio of the plant material to water, and water loss from the plant material (Denny, 1991). A model with six variables, even in the case of a first-degree model, would require 2^6 = 64 experiments (Goupy, 2001). For experimental convenience, some variables were thus kept constant. We considered three variables: the extraction duration (X1), the residual water content (X2), and the state of the division of the plant material (X3). These three factors offered the advantage of being easy to control. Extraction yield (y), p-menthadienol isomers, and aristolone contents (Z) depend on factors X1, X2, and X3. Mathematically, this is expressed as y or z = f(X1, X2, X3), where y and z are the responses, f(X) is the response function, and X1, X2, and X3 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 (1954) is well-suited to addressing this type of question (Ortigosa, 1993). 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 = 2^3 = 2^3 = 8 experiments.

To construct the experiment matrix, we define reduced variables X, as the following:

\[ x_i = (X_i - X_0)/\Delta X; \]
\[ X_0 \] is the base value at the centre of the experimental domain (level 0), and \( \Delta X \) is the variation step, that is, the unit of variation in the variables. Table 1 gives the two levels of the variables in the steam extraction of Elyonurus hensii.

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 randomization. The combination of these three variables and the two levels by variable lead to the following experimental design (Table 2).

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

\[ y = a + a_1 x_1 + a_2 x_2 + a_3 x_3 + a_12 x_1 x_2 + a_13 x_1 x_3 + a_23 x_2 x_3 + a_{123} x_1 x_2 x_3 \]

If the mathematical model associated with the factorial plan is constructed with centered, reduced variables, the coefficients of the polynomial thus have very simple meanings: average ai, main effects ai, and interactions aijk (Goupy, 2001).

3 | RESULTS

3.1 | Chemical composition

The essential oil of the aerial part (stems, leaves, and flowering tops) obtained with the samples collected from the Loufoulakari site is mainly rich in oxygenated monoterpenes whose contents range between 50.81% and 71.59%, with the following major compounds: the isomers of p-menthadienol: trans-p-mentha-2,8-dien-1-ol (5.32%–16.22%), cis-p-mentha-2,8-dien-1-ol (3.85%–6.39%), trans-p-mentha-1(7),8-dien-2-ol (10.45%–20.83%), and cis-p-mentha-1(7),8-dien-2-ol (12.30%–18.87%). The limonene is the only monoterpene hydrocarbon found at appreciable content (2.21%–19.28%). These results are in agreement with those obtained by Silou et al. (2006); Yang et al. (2013). According to the period of harvesting the samples, the contents of all the p-menthadienol isomers are between 38% and 50%.

| TABLE 1 | Levels of the variables in the steam extraction of Elyonurus hensii |
|----------|-----------------|-----------------|-----------------|
| Level    | Extraction duration (hr) X1 | Residual water content* X2 | State of division (cm) X3 |
| Low (−1) | 1.5              | Low             | <5              |
| High (+1) | 3               | High            | 10              |

*The residual water content is low when the distillation is carried out with the plant material dried during 8 days and high when the fresh material is distilled. For the aerial part, the residual content varies from 62% (high) to 4% (low); for the roots, it varies from 35% to 6%.
TABLE 2  Experimental matrix for essential oil extraction from *Elyonurus hensii*

| Run | X₁ | X₂ | X₃ | Y | Z |
|-----|----|----|----|---|---|
| 1   | −1 | −1 | −1 | Y₁ | Z₁ |
| 2   | +1 | −1 | −1 | Y₂ | Z₂ |
| 3   | −1 | +1 | −1 | Y₃ | Z₃ |
| 4   | +1 | +1 | −1 | Y₄ | Z₄ |
| 5   | −1 | −1 | +1 | Y₅ | Z₅ |
| 6   | +1 | −1 | +1 | Y₆ | Z₆ |
| 7   | −1 | +1 | +1 | Y₇ | Z₇ |
| 8   | +1 | +1 | +1 | Y₈ | Z₈ |
| Level -1 | 1.5 | low | <5 | |
| Level +1 | 3 | high | 10 | |

and these contents are comparable with those of the essential oils of *Cymbopogon giganteus* from Benin (Ayedoun et al., 1999), Ivory Coast (Boti et al., 2006), and Mali (Sidibe et al., 2001). As the *Cymbopogon giganteus* and *Cymbopogon densiflorus*, it appears that *Elyonurus hensii* is an interesting species for the production of essential oil rich in p-menthadienol isomers.

The essential oil obtained from the roots is rather rich in oxygenated sesquiterpenes. These compounds represent between 37.23% and 66.39% of all the essential oil, and the main sesquiterpene compound is aristolone (18%-48%). Monoterpenes hydrocarbon is also present in large quantities, they represent 13–35% of all the essential oil, and the main compound is the limonene whose yield ranges between 9% and 30% (Table 3).

3.2 | Evolution of the content of the main major constituents: natural selection

For all three sites, Loufoulakari (L), Campus rural (C), and Sese (S), it is observed that the behavior of the five main major constituents is identical for the aerial parts. During periods of significant rainfall decreases, May (m) and July (j), the cis and trans-p-mentha-1(7),8-dien-2-ol isomers are produced in large quantities (on average, the content is of the order of 18%), and the content of the trans-p-mentha-2,8-dien-1-ol isomer (around of 5%) decreased in the same period for the Lm, Cm, Sm, Lj, Cj, and Sj samples (Figure 1). Moreover, for the three sites, the results show that the type of soil does not influence the production of the main major constituents. In the zone of "plateau des Cataractes" where the harvests were performed, the average temperature is 22°C (min 15°C, max 26°C) during the dry season and 28°C (min 23°C, max 33°C) during the rainy season (Samba Kimbata, 1978). It is, therefore, possible to consider that the factors related to the sunlight and to the water stress have an impact on the quantitative production of these isomers. The drastic decrease in rainfall is already an important factor influencing the yield of essential oil, and in this case, the composition of essential oil is also affected. There is a natural selection in the biosynthesis of isomers favoring the synthesis of cis and trans-p-mentha-1(7),8-dien-2-ol during periods of water stress. The separation of the isomers by conventional methods of chemistry is not always easy, these results show that it just to extract the essential oil from the plant material harvested during the water stress period for obtaining extracts enriched in cis and trans-p-mentha-1(7),8-dien-2-ol and poor in cis and trans-p-mentha-2,8-dien-1-ol. This is one of the first possible steps in the separation of four isomers of p-menthadienol.

In the roots, on all three sites, the chemical profile is primarily aristolone. We also find a certain selectivity of the plant in the production of two major compounds. In February and December, for all three sites, the content of aristolone is at the maximum, whereas that of limonene is low. In general, it seems that the production of aristolone comes at the expense of limonene. (Figure 2).

3.3 | Modeling of the extraction

Water distillation is used to extract essential oil. The modeling of the extraction process is in principle complex but can be simplified by the judicious choice of the factors to be studied. We selected three factors: the extraction duration (hr), X₁; the residual water content, X₂; and the state of division (cm), X₃.

3.3.1 | Aerial part

The essential oil from the aerial part is rich in the p-menthadienol isomers. The experimental matrix and the essential oil yields and p-menthadienol isomers content are given in Table 4.

The mathematical equation representing the quantitative yield generated by the model is as follows:

\[ Y = a₀ + a₁x₁ + a₂x₂ + a₃x₃ + a₄x₁x₂ + a₅x₁x₃ + a₆x₂x₃ + a₇x₁x₂x₃ \]

with:

\[ a₀ = 1.33, a₁ = 0.06, a₂ = -0.61, a₃ = -0.04, a₄ = 0.01, a₅ = -0.01, a₆ = -0.04, a₇ = 0.001, a₈ = -0.17, a₉ = 0.10 \]

The average yield of the essential oil that we can extract is \( a₀ = 1.33 \)% for 8 experiments on a scale ranging from 0.40% to 2.11%. In the experimental domain considered, the factors of the coefficients are low, reflecting the weak influence of factors chosen on the essential oil yield. However, there is an influence of the residual water content (\( a₂ = -0.61 \)) on the yield of the essential oil. The negative sign of the coefficient indicates that a high water content in the plant material does not favor the extraction of essential oil, thus confirming the observations made on modeling the extraction of the essential oils of *Eucalyptus citriodora* (Silou et al., 2009) and of *Cymbopogon citratus* (Silou et al., 2004).

Concerning the cis and trans-p-mentha-2,8-dien-1-ol the mathematical equation generated by the model is as follows:

\[ Z₃ = b₀ + b₁x₁ + b₂x₂ + b₃x₃ + b₄x₁x₂ + b₅x₁x₃ + b₆x₂x₃ + b₇x₁x₂x₃ \]

where:

\[ b₀ = 19.47, b₁ = -0.14, b₂ = -2.03, b₃ = -0.87, b₄ = 1.22, b₅ = 0.18, b₆ = -0.93, b₇ = -0.34 \]
TABLE 3 Chemical composition of the essential oil of the aerial part and the roots of *Elyonurus hensii* (site Loufoulakari)

| Components               | RI lit | RI cal | February | May | July | November | December |
|--------------------------|--------|--------|----------|-----|------|----------|----------|
|                          |        |        | Aerial part | Roots | Aerial part | Roots | Aerial part | Roots | Aerial part | Roots | Aerial part | Roots |
| Tricyclene               | 926    | 922    | 0.82      | 0.34 | 0.78 | 0.22     | 0.47     | 0.10     | 0.35     | 0.35 | 0.89     | 0.30  |
| α-thujene                | 930    | 925    | -         | -    | 0.21 | -        | -        | 0.12     | -        | 0.02 | -        | 0.22  |
| α-Pinene                 | 939    | 933    | 0.43      | 0.72 | 0.14 | 0.46     | 0.09     | 0.19     | 0.25     | 0.78 | 0.42     | 0.61  |
| Camphene                 | 954    | 950    | 2.48      | 1.09 | 2.41 | 0.91     | 1.6      | 0.64     | 1.25     | 1.24 | 2.64     | 0.95  |
| Sabine                   | 975    | 973    | 0.07      | 0.19 | -    | 0.16     | -        | -        | 0.06     | 0.05 | 0.21     | 0.15  |
| β-Pinene                 | 979    | 980    | -         | -    | -    | -        | -        | -        | -        | -    | 0.21     | -     |
| Myrcene                  | 990    | 989    | 0.29      | 0.54 | -    | 0.85     | -        | 0.45     | -        | 0.48 | 1.05     | 0.51  |
| α-Phellandrene           | 1002   | 1005   | -         | 0.04 | -    | 0.02     | -        | -        | -        | -    | 0.21     | -     |
| ortho-Cymene             | 1024   | 1025   | 1.38      | 0.75 | 2.22 | 0.93     | 1.89     | 0.59     | 0.95     | 1.11 | 1.36     | 0.37  |
| Limonene                 | 1029   | 1029   | 16.99     | 9.73 | 2.21 | 2.28     | 3.21     | 11.4     | 19.28    | 30.4 | 11.98    | 12.22 |
| 1,8-Cineol               | 1031   | 1032   | -         | 3.83 | 0.25 | 3.7      | 0.35     | 1.56     | -        | 4.51 | 0.16     | 2.39  |
| γ-Terpinene              | 1059   | 1057   | -         | 0.07 | -    | -        | -        | -        | -        | -    | -        | -     |
| Terpinolene              | 1088   | 1085   | -         | 0.01 | -    | 0.22     | -        | -        | 0.06     | 0.27 | -        | -     |
| para-Cymene              | 1091   | 1091   | 0.13      | 0.04 | 0.59 | 0.12     | 0.6      | -        | 0.36     | 0.26 | 0.21     | -     |
| Linalool                 | 1098   | 1101   | -         | -    | -    | 0.14     | -        | -        | -        | -    | -        | -     |
| Menthatriene <1,3,8-para-> | 1110  | 1111   | 0.07      | -    | -    | -        | -        | -        | -        | -    | 0.41     | -     |
| *trans* -p-Mentha-2,8-dien-1-ol | 1122 | 1125   | 10.47     | 1.2  | 6.1  | 1.17     | 5.32     | 0.65     | 16.22    | 2.15 | 9.06     | 1.16  |
| *cis*-Limonene oxide     | 1132   | 1136   | 0.13      | -    | -    | -        | -        | -        | 0.27     | 0.19 | 0.12     | -     |
| *cis*-p-Mentha-2,8-dien-1-ol | 1137  | 1137   | 5.57      | 0.85 | 4.52 | 0.8      | 3.85     | 0.29     | 6.39     | 1.63 | 6.12     | 0.76  |
| *trans* -Pinocarveol     | 1139   | 1141   | 0.44      | -    | 0.81 | -        | 0.79     | -        | -        | -    | -        | -     |
| Camphor                 | 1146   | 1148   | -         | 0.08 | 0.26 | 0.11     | 0.31     | -        | 0.10     | -    | -        | -     |
| Pinocarvone              | 1164   | 1163   | 0.25      | 0.08 | 0.50 | 0.11     | 0.52     | -        | 0.25     | 0.13 | 0.29     | -     |
| Terpinen-4-ol           | 1177   | 1182   | 0.08      | 0.25 | -    | 0.62     | -        | 0.20     | -        | 0.5   | -        | -     |
| *trans* -p-Mentha-1(7),8-dien-2-ol | 1189 | 1192   | 10.45     | 2.24 | 20.83 | 3.77   | 18.67    | 1.73     | 12.89    | 3.49 | 10.93    | 1.48  |
| α-Terpineol             | 1190   | 1196   | -         | 0.47 | -    | 0.98     | -        | -        | -        | 1.22 | -        | 0.37  |
| 3-methyl-Buten-2-ol.     | 1197   | 1201   | 2.69      | -    | 5.48 | -        | 4.74     | -        | -        | -    | -        | -     |
| Caranone <cis-4->       | 1200   | 1209   | 1.18      | 0.18 | 0.64 | -        | -        | -        | -        | 0.58 | 0.09     | 0.99  |
| *Trans* Carveol         | 1229   | 1221   | 4.04      | 0.07 | 7.46 | 1.34     | 5.71     | -        | 1.83     | 1.6  | 4.33     | -     |
| *cis*-p-Mentha-1(7),8-dien-2-ol | 1232  | 1230   | 12.3      | 2.69 | 18.81 | 2.94   | 16.46    | 1.31     | 13.52    | 3.23 | 12.66    | 1.6   |
| *cis*-Carveol           | 1229   | 1233   | 0.69      | 0.82 | -    | -        | -        | -        | 5.36     | -    | 0.7      | 0.63  |
| Carvone                 | 1243   | 1245   | 3.16      | 0.76 | 4.47 | 1.33     | 4.08     | 0.71     | 3.45     | 1.48 | 3.02     | 0.44  |
(Continues)
| Components                  | February | May  | July | November | December |
|----------------------------|----------|------|------|----------|----------|
| Aerial part Roots Aerial part Roots Aerial part Roots Aerial part Roots Aerial part Roots Aerial part Roots Aerial part Roots |
| Piperitone                 | 1.04     | 1.07 | 0.37 | 0.86     | 0.07     |
| Perilla aldehyde           | 0.44     | 0.46 | 0.66 | 1.06     | 0.37     |
| Borneolacetate             | 0.29     | 0.29 | 0.11 | 0.08     | 0.03     |
| Limonene <dioxide-2->      | 0.03     | 0.03 | 0.03 | 0.03     | 0.03     |
| Undecane <A-2->            | 0.03     | 0.03 | 0.03 | 0.03     | 0.03     |
| Elemente <beta->           | 0.03     | 0.03 | 0.03 | 0.03     | 0.03     |
| Methyl Eugenol             | 0.03     | 0.03 | 0.03 | 0.03     | 0.03     |
| Caryophyllene <E->         | 0.34     | 0.34 | 0.34 | 0.34     | 0.34     |
| Caryophyllene oxide <E->   | 0.26     | 0.26 | 0.26 | 0.26     | 0.26     |
| Intermedeol                | 2.39     | 2.39 | 2.39 | 2.39     | 2.39     |
| Total                      | 82.08    | 88.84| 98.39| 92.2     | 95.67    |
The cumulated average content of cis and trans-p-mentha-2,8-dien-1-ol is \( a_0 = 19.47\% \) for all eight experiments on a scale of 15.87\% to 23.24\%. The effects of the main factors are negative with a large weight to the residual water content \( a_2 = -2.03\): the maximum content of cis and trans-p-mentha-2,8-dien-1-ol is obtained when the amount of water in the plant material is low. The duration of the extraction has very little impact on the content because its coefficient factor is low. The combination of the extraction duration and the residual water content \( a_{12} = 1.22\) seems to have an important influence on the content of cis and trans-p-mentha-2,8-dien-1-ol.

The content of cis and trans-p-mentha-1(7),8-dien-2-ol can be modeled by the following expression:

\[
Z = 28.87 - 0.78x_1 - 0.07x_2 - 0.32x_3 - 0.93x_1x_2 + 0.96x_1x_3 + 0.56x_2x_3 + 0.76x_1x_2x_3
\]

The cumulated average content of cis and trans-p-mentha-1(7),8-dien-2-ol is 28.87\% for all eight experiments on a scale of 25.11\% to 32\%. The coefficients of the factors are generally low; the content of cis and trans-p-mentha-1(7),8-dien-2-ol is weakly influenced by the factors chosen. The residual water content \( a_3 = -0.07\), which has a strong influence on the content of cis and trans-p-mentha-2,8-dien-1-ol, does not influence the content of cis and trans-p-mentha-1(7),8-dien-2-ol. Furthermore, the combined effect of the extraction duration and the residual water content \( a_{12} = -0.93\) is unfavorable for the extraction of cis and trans-p-mentha 1(7),8-dien-2-ol, whereas this interaction increases the content of cis and trans-p-mentha-2,8-dien-1-ol. The combined effect of the extraction duration and the state of division \( a_{13} = 0.96\) positively influences the content of cis and trans-p-mentha 1(7),8-dien-2-ol. Finally, the residual water content is the most important influencing factor and determines the contents of cis and trans-p-mentha-2,8-dien-1-ol and cis and trans-p-mentha 1(7),8-dien-2-ol.

### 3.3.2 | Roots

The essential oil yield of the roots (Table 4) is low compared to that of the aerial part. The average yield that could be extracted is 0.74\% for the eight experiments, with a range of 0.49\% to 1.02\%.

The aristolone content of essential oil from the roots can be modeled by the following expression:

\[
Z = 41.10 - 0.52x_1 - 3.53x_2 + 0.50x_3 + 0.76x_1x_2 - 3.58x_1x_3 - 0.47x_2x_3 + 4.07x_1x_2x_3
\]

The content of aristolone is more sensitive to the effects of the factors and their interactions. With an average content of 41.10\% for...
eight experiments and a range of 36.68% to 54.55%, the extraction of aristolone is impacted by the residual water content with a large negative weight ($a_2 = -3.53$). The effects of the interactions are also important; the combined effects of the duration of extraction and the state of division have a large negative weight ($a_{13} = -3.58$). Additionally, the combined effects of the duration of extraction, the residual water content, and the state of division led to the most significant effect on the response ($a_{123} = 4.07$). In the latter case, the interpretation of this interaction is very complex. However, if we consider the mathematical model in which we neglect the factors and interactions that have a minor influence, we obtain the following equation:

$$Z = 41.10 - 3.53X_2 - 3.58X_1X_3 - 4.07X_1X_2X_3$$

In the selected experimental area, we find that when the duration of distillation and the residual water content are low and the state of division is strong, the average content of aristolone ranges from 41.10% to 52.28%, with an increase of 11.18%. The lowest content of aristolone is obtained when the duration of extraction and the state of division are at high levels and when the residual water content is low, which corresponds to a variation in the average content of 41.10% to 36.98% (a decrease of 4.12%). However, this interpretation must integrate the fact that the distillation time and the state of division are weak influences.

### 4 | CONCLUSION

The essential oil of the aerial part is rich mainly in cis and trans-p-mentha-2,8-dien-1-ol, cis and trans-p-mentha-1 (7), 8-dien-2-ol. The essential oil obtained from the roots is rich in aristolone and limonene. The content of these components varies depending on the harvest period. The residual water content is the most important factor that significantly influences the average yield of the essential oil and the content of the major constituents. Regarding the aerial part, a low residual water content of the plant material increases (1) the essential oil yield and (2) the content of cis and trans-p-mentha-2, 8-dien-1-ol.

For the roots, the samples that have a very low residual water content should provide extracts richer in aristolone. The combined effects of the extraction duration and the state of division, on the one hand, and the extraction duration, the state of division and the residual water content, on the other hand, greatly influence the extraction of aristolone. However, these interactions are complex and difficult to assess.

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### CONFLICT OF INTEREST

None declared.

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