

COMPOSITION AND INTRASPECIFIC CHEMICAL VARIABILITY OF ESSENTIAL OIL FROM *Rhanterium adpressum* **Coss. & Dur.**

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ABSTRACT. The intraspecific chemical variability of *Rhanterium adpressum* essential oils was assessed for samples collected in four different localities of southern Algeria. Essential oils of leaves and flowers were obtained by Hydro-distillation and analysed by GC-MS. Monoterpene hydrocarbons represent the major class (40 to 53%), followed by oxygenated monoterpenes (22-47%). The main components are α pinene (11-29%), β-myrcene (17-28%), linalool (20-31%), α-terpineol (21-24%), α-cadinol (9-17%) and β-eudesmol (9-20%). The results of both methods of principal component analysis and hierarchical ascendant classification revealed the presence of three chemical classes characterizing a spatial chemical variation and also between organs in the studied samples (groups I, II and III): linalool/α-terpineol, αpinene/β-myrcene and α-pinene/β-myrcene/α-terpineol.

Keywords: *Rhanterium adpressum*, essential oils, chemical groups, intraspecific chemical variation

INTRODUCTION

The genus *Rhanterium*, from *Inulae*tribe, is species of arid environments. They are branched subshrubs, 40 to 60 cm high, with alternate leaves, small, whole and slightly serrated. These plants consist of numerous branches with heterogamous capitula and yellow flowers, females, ligulate, uniseriate and tridentate; the florets are hermaphroditic with five serrations [1]. It is in 1855 that the species *Rhanterium adpressum* was first described in *Bulletin de la Société botanique de France*. Cosson E. described it as an endemic species of Algeria and Morocco. Its general characteristics were presented in the study conducted by Wiklund [2].

New studies have explored the biochemical aspects of *R. adpressum*, first, by evaluating its chemical composition of terpenoids, phenols and lipids, and then, by investigating its biological potential on microbial agents and enzymes [3-7]. In general, the extracts obtained from different parts of this plant have shown promising biological activities, thus, further work on this species is required.

Terpenoids, especially monoterpenes, are considered characteristic elements of volatile compounds secreted by arid regions plants. In the study conducted by Elhouiti

et al. [3], composition variations of essential oils extracted from different samples of *R. adpressum*, collected over different periods, was noticed.

In order to deepen the knowledge of chemical components of the essential oils of leaves and flowers of *R. adpressum*, and to highlight the existence of chemical groups, a detailed study was carried out on plant samples from different regions of southern Algeria. In this study, and for the first time, the spatial intraspecific chemical variability of essential oils of *R. adpressum*, extracted from leaves and flowers, was assessed to reveal differences in one of the responses of plants of the same species to different environmental conditions.

MATERIALS AND METHODS

Collection sites of plant material

Thirty-five samples of *R. adpressum* were collected and identified by Pr. Yousfi M. from four regions in southern Algeria (Laghouat, Zelfana, Ouargla and El Golea) in May 2010 (Table 1). The plant samples are divided into two types: leaves (20 samples) and flowers (15 samples).

Region	Altitude	Latitude	Longitude
Laghouat	788 m	38°48'00.00" N	$2^{\circ}52'00.00"$ E
Zelfana	354 m	$32^{\circ}23'46.70''$ N	$4^{\circ}13'3440''$ E
Ouargla	137 m	$31^{\circ}57'46.72"$ N	5°20'31 17" E
El-Golea	398 m	$30^{\circ}35'45.99"$ N	2°52'54.73" E

Table 1. Geographic locations of sampling sites.

Extraction of the essential oils

Leaf and flower samples were air dried, in shade, at room temperature. The essential oils were obtained by Hydro-distillation using a Clevenger apparatus for 5 hours. The obtained oils were dried with anhydrous sodium sulphate $(Na₂SO₄)$ in order to remove all traces of water, then filtered and stored in the dark, at 4°C until analysis.

The operating conditions of the GC/MS

GC/MS analysis was performed using an Agilent Technologies gas chromatography 7890A equipped with a HP5MS capillary column $(30m \times 0.25mm \times 0.25\mu m)$, and a mass detector MS 5975C VL MSD operated in EI mode. Helium was used as a carrier gas at a flow rate of 1 mL/min, split 50:1. The oven temperature program was as follows: 2 min at 80°C; from 80 to 200°C at 5°C/min; 5 min at 200°C; then from 200 to 260°C at 20°C/ min; followed by 5 min at 260°C. Detector and inlet temperatures were 280°C.

The constituents of the essential oils were identified by comparing their mass spectra and retention indices, to the co-injection of reference compounds, as well as the mass spectra of the constituents of two libraries, Wiley and NIST (National Institute of Standards and Technology), comparing their linear indices with those of the literature [8-12].

Statistical analysis

Hierarchical Cluster (HCA) and principal component (PCA) analysis of chemical data were performed with XLStat 2014.5.03 and Minitab 17 software. For HCA, Ward's linkage method was used to determine the distance between clusters and Euclidean distance for their agglomeration. A correlation matrix was employed tocalculateprincipal components. Ombrothermic diagrams were generated from climate data downloaded from WorldClim version 2 [13]. Pearson correlation of these climatic data with the percentages of terpenes families was performed using R language.

RESULTS AND DISCUSSION

Chemical composition

Chromatographic analysis of the essential oils allowed the identification of 41 chemical components (representing between 65 and 82% of total oil) for flowers (Table 2) and leaves (Table 3), and for all samples monoterpene hydrocarbons make the major class (40-53%), followed by oxygenated monoterpenes (22-47%), oxygenated sesquiterpenes (15-38%) and sesquiterpene hydrocarbons (7-26%). The major components in the essential oils are α-pinene (11-29%), $β$ -myrcene (17-28%), linalool (20-31%), α-terpineol (21-24%) α-cadinol (9-17%) and β-eudesmol (9-20%). It should be noted that variations in contents and percentages of the components have been recorded, depending on the month of collection and the part of the plant used for the extraction [3-4].

Multivariate analysis of chemical composition

Hierarchical Ascendant Classification (HAC) and Principal Component Analysis (PCA) of chemical data were carried out on thirty-five essential oils of *R. adpressum*, from leaves (20 samples) and flowers (15 samples), collected from four different regions in Algeria.

Multivariate analysis was based on 20 major chemical components representing between 52-72% of the total composition of *R. adpressum* essential oils. PCA results (where F2 explains 17.31% of the variation) and HAC results have, on one hand, well separated between leaf and flower essential oils and, on the other hand, excluded any correlation of this variability with the sampling region. PCA has been used to study and visualize correlations between variables based on similarities and chemical differences. With PCA score chart (Fig. 1), which represents 49.36% of the total variance in the dataset, three chemical groups were formed. These groups reveal intraspecific variability between leaf and flower extracts. Thus, two chemical groups characterize the general composition of the leaves and the third of the flowers.

HAC also revealed the presence of three classes, (Fig. 2) separated this time according to dissimilarity indices. This aggregation method displays the different relationships between the essential oils samples, as a dendrogram calculated on the basis of Ward's minimum variance method.

PCA and HAC showed 3 chemical classes (group I, II and III) in all *R. adpressum* oil samples. The first class comprises 13 leaf samples (F9, F10, F12, F21, F22, F8, F19, F20, F6, F2, F3, F4, F5), distributed in the four regions. The chemical composition of this class is dominated by linalool/ α -terpineol (M = 52%, SD = 3.56%).

 $-$ _{0.4}

 -6.4

4.76 0.31 2.06 - 0.43 -- 1.02 5.6 0.84 **10.26**

4.88 - -5

 \circ 0.38 5.96 0.49 4.32 10.61 **23.36**

-1.6

0.2 3.44 0.13 5.6 5.54 **15.76**

0.31 0.14 **14.28**

0.55 - **22.42** -0.39

0.22 0.25 **30.83**

0.57

0.47 0.14 **31.82** 0.27 0.67 0.31 0.55 1.57 0.45 1.04 0.35 0.78 **5.99** 0.5 0.61 - 0.19 0.54 3.56 0.26 0.15 4.67 **10.48**

0.36 0.11 **25.17** 0.5 0.34 0.21 0.42 1.92 0.45 0.8 0.32 0.66 **5.62** 0.23 0.53 - 0.15 0.36 3.5 0.22 0.15 2.76 **7.9**

0.25 - **26.14** 0.19 0.34 0.18 0.45 0.82 0.21 1.03 0.44 0.81 **4.47** 0.39 0.71 - 0.23 0.49 3.96 0.28 0.22 4.53 **10.81**

0.38 - **24.67** - 0.22 - 0.54 0.98 1.7 0.92

0.53 0.1 **18.04** 0.43 0.22 0.22 0.47 1.68 0.4 0.57 0.56 0.12 **4.67** 0.43 - 0.77 0.2 0.61 5.06 0.15 0.24 0.26 **7.72**

0.68 0.25 **19.73** 0.2 0.47 0.19 0.39 1.13 0.49 1.24 0.36 0.9 **5.37** 0.48 1.17 0.21 0.29 0.38 7.54 0.36 6.77 7.59 **24.79**

0.41 0.19 **26.15**

0.31 0.2 **25.02** - 0.47 0.18 0.51 11.22 0.62 1.52 0.17 0.17 **14.86** 0.28 - 1.79 0.51 0.42 0.16 0.11 0.11 4.78 **8.16**

0.32 0.21 **34.33** 0.14 0.36 0.21 0.11 1.46 1.19 1.82 0.24 0.15 **5.68** 0.24 - 1.66 0.35 0.43 7.24 0.54 0.29 4.9 **15.65**

0.26 0.25 **23.07** 0.28 0.38 0.21 0.41 1.89 1.19 1.08 0.29 0.72 **6.45** 0.33 1.39 - 0.33 0.46 8.55 0.48 0.3 5.5 **17.34**

0.17 0.2 **22.6** 0.29 0.57 0.16 0.31 1.99 0.71 0.85 0.32 0.86 **6.06** 0.49 1.24 - 0.28 0.41 5.07 0.51 0.36 5.36 **13.72**

0.26 0.3 **24.52** 0.36 0.68 0.23 0.49 2.76 1.15 0.75 0.37 0.73 **7.52** 0.6 1.01 - 0.25 0.49 6.65 0.41 0.61 4.97 **14.99**

Methyl-Eugenol *trans*-Carveol

trans-Carveol

Methyl-Eugenol

Oxygenatedmonoterpenes

Oxygenatedmonoterpenes

β-Caryophyllene α-Humulene α-Amorphene Germacrene D

B-Caryophyllene

 18.57 0.29 0.23 0.18 0.3 2.01 0.44 0.8 0.47 1.08 **5.8** 0.39 - 0.93 0.24 0.52 5.28 0.43 0.36 6.65 **14.8**

 0.41 0.12 5.86 0.33 0.35 0.7 0.22 0.23 **8.22** 0.16 0.59

 0.17 0.15 0.35 1.94 0.43 1.66 0.12 0.13 **4.95** 0.3 - 1.39 0.18 0.3 5.85 0.3 1.38 7.13 **16.83**

 $\overline{0}$.

 -6.4

0.53 0.45 1.47 0.23 0.19 **3.77** 0.26 0.89 0.27 0.19 0.24 7.19 0.28 5.78 4.56 **19.66**

Bicyclo-Germacrene

Bicyclo-Germacrene

δ-Cadinene Germacrene B Caryophyllene Oxide

Caryophyllene Oxide

trans-2-Carene

trans-2-Carene

Sesquiterpenehydrocarbons

Sesquiterpenehydrocarbons

Viridiflorole **Spathulenol** Bisabolol Oxyde Dihydro-*Cis*-α-8, ol

Dihydro-Cis-a-8, ol Bisabolol Oxyde

Globulol α-Cadinol Isospathulenol α-Eudesmol β-Eudesmol

Isospathulenol

 -0.7

1.31 0.45 1.63

15.144

31

Oxygenatedsesquiterpenes

Oxygenatedsesquiterpenes

Fig. 1.Diagram of PCA scores of variation in chemical composition between different samples (F: Leaves, FL: Flowers).

Fig. 2. Dendrogram of the samples using Ward's method and Euclidean distance to calculate dissimilarity indices.

The second class also includes 13 samples; all of flowers (FL34, FL37, FL35, FL36, FL28, FL29, FL30, FL32, FL33, FL25, FL23, FL26, FL27). This class is characterized by the dominance of α -pinene/ β -myrcene (M = 57%, SD = 3.44%).

The third class comprises 9 samples (F13, F14, FL31, F16, F18, F15, F17, F1, FL24) and is characterized by high percentages of α -pinene/ β -myrcene/ α -terpineol (M = 64%, $SD = 3.33\%$).

Oxygenated components (monoterpenes and sesquiterpenes) have high percentages. They dominate the chemical composition of group I (71%), while in group II, the percentage of chemical components of these essential oils are more homogeneous, despite the relatively high percentages of α-pinene and β-myrcene. In group III, the percentage of oxygenated components is much lower compared to the first two groups, with four major components (84%) α -pinene, β-myrcene, α -terpineol and linalool.

These results clearly demonstrate that the essential oils of leaves belong to a chemical group different from that of flower essential oils. This difference has been revealed previously by Elhouiti et al. [3], in this study, the effect of *R. adpressum* leaves and flowers EOs was assessed by the biological activities on mycotoxigenic fungi of the genus *Fusarium*. Eos from flowers have been shown to be very active against fungal growth and mycotoxin production. This activity might be due to the major components of the essential oil which are known for their important antimicrobial activity [14].

The variability of chemical composition of secondary metabolites is generally related to different environmental conditions. In the ombrothermic diagrams in Fig. 3, Group II is distinguished from the other two groups by thermal amplitude of 23°C with an average temperature of 23°C and a total annual precipitation up to 109 mm. More drought was observed in the collection points of group I with a thermal amplitude of 24 °C and a total annual precipitation of 40 mm. Group III is much closer to group 02 but with less annual precipitation (82 mm). It has been shown that temperature directly affects the emission rate of volatile compounds and also their concentrations in tissues. This variation is also affected by changes in atmospheric $CO₂$, oxidative stress and other abiotic stresses [15].

Fig. 3. Ombrothermal diagrams of precipitation and temperature averages for all collection points for corresponding groups.

Relationship between the percentage of chemical composition families of *R. adpressum* essential oils in different samples groups with the climatic variables; annual minimum temperature, maximum annual temperature and annual precipitation was shown in Fig. 4.

Fig. 4. HeatMap of Pearson correlation between R. adpressum essential oils families and climatic variables. The correlation increases in red and decreases in blue.

Monoterpene hydrocarbons correlated to 42% and 43% with minimum annual temperature and maximum annual temperature respectively, while the other three families correlated negatively with these two parameters (between 12 and 33%). On the other hand, oxygenated monoterpenes, sesquiterpene hydrocarbons and oxygenated sesquiterpenes correlated positively with annual precipitation at 20%, 34% and 18% respectively, noting that monoterpene hydrocarbons correlated negatively with this parameter (44%).

On other species of the genus *Rhanterium*, the analysis of chemical composition of essential oils from Algerian *R. suaveolens* allowed the identification of 20 components, representing 98.01% of the total composition of the EO. Monoterpenes are the majority compounds (48.25%), followed by sesquiterpenes (37.97%). The chemical composition of these oils is characterized by major compounds such as β-pinene (3.21%), βcaryophyllene (5.17%), β-cadinol (5.61%) and caryophyllene oxide (24.82%) [16].

Chemical diversity and richness of monoterpenes was also observed in *R. epapposum* essential oils. For flower oils 47 constituents, representing 89.91%, were identified. For leaves oils, 34 components representing 94.86%; and 16 components, 76.35%, for stem oil. Hydrocarbon and/or oxygenated monoterpenes are the majority families in all these oils [17].

CONCLUSION

In this study, the variation observed in the chemical composition of *R. adpressum* essential oils, distributed in four localities of arid climatic character, made it possible, in a general way, to show the chemical behaviour of this species towards the bioclimatic variations of its habitat. The variability of the composition of these essential oils was found to be spatial and also between organs of the plant from which the oils were extracted. These results may be explained by the different mechanisms of production and regulation that govern the secretion of these compounds.

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