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New chemotype of essential oil of *Achillea santolina* L. collected from different regions of Algeria

Tayeb Berramdane¹ · Nadhir Gourine¹  · Isabelle Bombarda² · Mohamed Yousfi¹

Abstract

Chemical composition of essential oil (EO) obtained by hydrodistillation of the aerial parts of *Achillea santolina* L. was analysed using GC and GC–MS. Especially, this study involved a large scale investigation, using different regions in which, five flowering wild growing populations collected from the high plateau of Algeria were engaged. Unlike previous reports, the current investigation showed very high EO yields for this plant (up to 1.7% w/w “dw”). The main result of the current study was the occurrence of a new chemotype rich in camphor (39.54–67.86%) and 1,8-cineole (7.14–8.57%).

Keywords *Achillea santolina* L. · Algeria · Essential oil · Chemical composition · Chemotype · Yield

Introduction

The genus *Achillea* is one of the most important genres of the Asteraceae family and comprises 115 species, which are mainly distributed in Europe, Asia and North Africa [1]. There are about five species of *Achillea* which are widely distributed in Algeria; *A. ligustica* All., *A. leptophylla* M.B., *A. odorata* L., *A. santolinoïdes* Lag. and *A. santolina* L. [2].

The aerial parts of different species of the genus *Achillea* are widely used in folk medicine due to various purposes and pharmacological properties in various biological activities, such as, anti-inflammatory [3], antimicrobial [4, 5], anti-spasmodic [6], antiulcer [7], and antiradical activities [8, 9]. Furthermore, this plant is also used as treatment for cancerous cells [9, 10]. More specifically for *A. santolina*, the dried aerial parts and flowers of this plant were used traditionally as antidiabetic and as anti-inflammatory. It also used to relieve pain or dryness of the navel, stomach pain or gas and to relieve the symptoms of common cold [11]. Moreover, previous experimental investigations on *A. santolina*

confirmed different biological and antioxidant activities of this plant [8, 11–23].

To our best knowledge, *A. santolina* growing in Algeria did not exhibit any studies concerning the chemical composition of their essential oil. At the opposite side, and according to literature, there were only few articles which reported the chemical composition of the essential oil of *A. santolina* coming from different countries of origin: Egypt [14, 22–24], Jordan [25] and Iran [26–28]. These reports revealed a high variability of the essential oil of *A. santolina*, which suggests the presence of different chemotypes for this plant, or at least indicates the presence of large chemical polymorphism within the population of this plant. The current investigation aims to identify the possible presence of new chemotype for the Algerian *A. santolina* essential oils.

Materials and methods

Plant collection

The aerial parts of *A. santolina* L. (at their full flowering phenological stage) were collected at the end of May 2014 from five different areas of the high plateaus; three from the wilaya of El-Bayadh (Tousmoulina: R1, Mikther: R2, Bouzoulay: R3), and two from the wilaya of Laghouat (Lalmaya: R4 and Sidi Makhlouf: R5). More specifically, the geographical coordinates of the exact locations of these regions of collection and their altitudes, were summarized

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in Table 1. The plant was identified by Dr. Seridi abdel-kadir from Department of Agronomy, University of Laghouat. Voucher specimens have been deposited in the herbarium of the National Agronomic Institute of Algiers (N.A.I.), Algeria (Herbarium No. P: 11).

Essential oil extraction

The plant material samples were dried in a shade at ambient temperature; next they were carefully milled; after that they were hydrodistilled for 3 h using a Clevenger type apparatus. The essential oil samples were recovered from the distillate with diethyl-ether solvent and then dried overnight using anhydrous sodium sulfate Na_2SO_4 . After filtration the extract solutions, they were reduced at room temperature under light vacuum pressure using rotary evaporator (rotavap). Finally, the obtained EO samples were stored at (+4 °C) until analysis.

Essential oil analysis

Analysis was carried out by gas chromatography (GC) using two columns and by gas chromatography–mass spectroscopy (GC–MS).

Gas chromatography (GC)

For the first column (polar), a CP-Varian 3800 gas chromatograph was used with a flame ionization detector (FID), and a UB-Wax fused silica capillary column (60 m × 0.32 mm, 0.25 µm film thickness). Oven temperature was programmed from 50 to 250 °C at a rate of 3 °C min⁻¹ and held at 250 °C for 10 min. Injector and detector temperatures were set at 250 and 260 °C, respectively. Helium was the carrier gas at a flow rate of 1 ml min⁻¹. Splitting ratio 1:50.

For the second column (apolar), analytical GC was carried out in a Hewlett-Packard 6890 (Agilent Technologies, Palo Alto, CA, USA) gas chromatograph with a HP GC ChemStation Rev. A.05.04 data handling system, equipped with a single injector and flame ionization detection (FID) system. A graphpak divider (Agilent Technologies, part no. 5021-7148) was used for sampling to fused silica capillary column HP-5 (polydimethylsiloxane 30 m × 0.20 mm

i.d., film thickness 0.20 µm). Oven temperature program: 70–220 °C (3 °C min⁻¹), 220 °C (15 min); injector temperature: 250 °C; carrier gas: helium, adjusted to a linear velocity of 30 cm s⁻¹; splitting ratio 1:40; detectors temperature: 250 °C.

Gas chromatography–mass spectroscopy (GC–MS)

GC–MS was carried out in a Hewlett-Packard 6890 gas chromatograph fitted with a HP-1 fused silica column (polydimethylsiloxane 30 m × 0.25 mm i.d., film thickness 0.25 µm), interfaced with an Hewlett-Packard mass selective detector 5973 (Agilent Technologies) operated by HP Enhanced ChemStation software, version A.03.00. GC parameters as described above; interface temperature: 250 °C; MS source temperature: 230 °C; MS quadrupole temperature: 150 °C; ionization energy: 70 eV; ionization current: 60 µA; scan range: 35–350 units; scans s⁻¹: 4.51.

Components of each EO sample were identified by their linear retention indices on both UB-Wax and SPB-1 columns. Linear retention indices were calculated relative to linear homologous series of *n*-alkanes C₈–C₂₄. The identifications of the components were based on the comparison of their mass spectra with those of Wiley and NIST (National Institute of Standards and Technology) libraries, as well as by comparison of their retention indices with those of the values of a homemade database.

Statistical analysis

Cluster analysis

Cluster analysis was performed using AHC (Ward's technique) with Euclidean distance measure. The calculus was performed using two sets of data. The set of data which refer to the analysis of chemical composition of the essential oil of *A. santolina*, was composed of five different EO plant samples (from different regions of collection in Algeria). The total number of adopted variables was ten (representing simply the major identified components in all represented individuals, or the most influencing parameters that could make a difference i.e. some minor compounds).

Table 1 Characteristics of *Achillea santolina* L. accessions used in this study

Attributed region code	Origin		Latitude	Longitude	Altitude (m)
R1	El-Bayadh	Tousmouline	33°38'11.6"N	0°18'50.5"E	1191
R2		Mikther	33°47'33.0"N	1°00'30.22"E	1223
R3		Bouzoulay	34°01'43.8"N	0°57'36.6"E	1094
R4	Laghouat	Lalmaya	33°25'53.1"N	2°00'01.7"E	918
R5		Sidi Makhlouf	34°7'39.45"N	3°00'53.2"E	881

Principal component analysis (PCA)

The principal component analysis was performed using the same individuals and the same variables adopted for agglomerative hierarchical clustering method (as previously described).

Results and discussion

The essential oils isolated by hydrodistillation from plant samples harvested at their full flowering stage were obtained as pale yellow color. The obtained yields of the EO of the five different regions were different; they varied between 0.72 and 1.70% (Table 2). The determined yields of studied samples coming from different localities in Algeria revealed they were very rich in essential oil. The average calculated yield of these essential oils (expressed as mean \pm SD) was equal to $1.06 \pm 0.39\%$. These obtained values represented very high yields in comparison with the majority of previous reported works that dealt with the same plant material, but originating from different countries. More explicitly, the EO yield of the aerial parts of *A. santolina* from Jordan was found to be 0.18% [25]; for those of Iran (different localities) they were in the range of 0.10–0.60% [26, 27]. The EO yields of different parts of *A. santolina*; were also reported. They were identical for leaves, stems and flowers from Egypt (Sinai desert) with a yield values of 0.9% [14], but different for those coming from Iran: leaves (1.5%), stems (0.2%) and flowers (1.4%) [28].

For the current study, the most important and the highest yield was recorded for the region of R4 (1.7%), and the lowest one for the region of R5 (0.72%); both regions belonged to the wilaya of Laghouat. The regions R2 and R3 showed practically similar yield values. The same previous observation could be extended to the yields of both R1, and R5 regions. The comparison of the maximum obtained yield with those of literature (no matter the studied parts: aerial or specific) revealed that it was 1.88 (≈ 2) to 17 times higher than those of above mentioned reports [14, 25–28]. In fact, the mean yield value of all investigated samples (1.06%) was the highest one, in comparison with all of those reported previously, for the same plant material. Moreover, the lowest yield (R5) was slightly lower than the highest yield (0.9%) reported in literature [14].

When considering other species of *Achillea* as a purpose of yield comparison, the obtained yields values were also roughly equal or simply higher than those of other species rich in EO such as *A. filipondila* (0.76%), *A. pbrygia* (0.70%), and *A. odorata* (0.64–0.79%) [29].

The essential oil compositions of the *A. santolina* samples considered for this study were characterized by the presence of high amounts of oxygen containing monoterpenes

(60.56–78.84%) (Table 2). As matter of fact, the percentages of oxygenated monoterpenes were by far higher than monoterpenes hydrocarbons (2.34–10.64%). In this case the most representative oxygenated monoterpenes was exclusively camphor (39.54–67.86%). The percentages of total monoterpenes were varying from 65.05 to 91.82%. In another side, the percentages of oxygenated sesquiterpenes were most of the time very weak (max. 1.98%). In this context, no sesquiterpenes hydrocarbons were detected this time.

The results brought by the current investigation on the chemical composition of the essential oils of *A. santolina* were different from those previously reported in literature. In fact, the determined values belonged to a new chemotype rich in camphor (39.54–67.86%), with mean and standard deviation values of ($55.06 \pm 11.00\%$) and 1,8-cineole (7.14–8.57%) with (mean \pm SD = $7.70 \pm 0.65\%$); and contain a minor compound α -terpineol (1.24–4.16%), which was practically absent in almost all previous reports, exception is made for the plant coming from Egypt [23] where its value was in the range of our presented work (Table 3). Moreover, the obtained percentages of some other minor compounds such as α -pinene (1.05–2.52%) and camphene (1.29–4.28%), were slightly higher than those of previous reports dealing only with the aerial parts: (α -pinene: 0.0–0.6%; camphene: 0.0–1.2%).

In order to determine, if exists, the similarities and/or the differences within the chemical compositions of the studied EO samples representing different regions of collection in Algeria, two powerful methods of multivariate statistical analysis were employed: AHC and principal component analysis (PCA). Besides, these two statistical methods were also engaged in highlighting the occurrence of the new Algerian chemotype taking in consideration the comparison of current values study with those of literature.

First, let's study both similarities and difference among the investigated Algerian EO samples. The results of AHC analysis represented by the dendrogram of the Fig. 1, showed the presence of two clusters regrouping (R1, R2, R3) and (R4, R5), respectively. These result showed very good similarities among the three samples R1, R2 and R3, representing the region of El-Bayadh, and also for the samples R4 and R5, referring to the region of Laghouat, respectively. The difference between the two clusters R1, R2, R3 and R4, R5 is not obvious but existing (slight difference). R4 and R5 are exhibiting lower percentages of camphor (39.54%; 48.88%) in comparison with R1,2,3 samples (58.66–67.88%). This quick and general inspection excludes the idea of the presence of more than one chemotype among the studied samples (only variability that exists). It can be clearly observed from the Table 3, that the Algerian samples were quite different from the rest of the reported samples.

These above observations for the studied samples were also confirmed by the results of PCA (Fig. 2), and

Table 2 Essential oil composition of aerial parts of *Achillea santolina* L. from five different regions of collection in Algeria

No.	Components	LRI ^a	LRI ^b	Composition ^c					Identification
				El-Bayadh			Laghouat		
				Tousmoul- ine (R1)	Mikther (R2)	Bouzoulay (R3)	Lalmaya (R4)	Sidi Makhlouf (R5)	
1	Tricyclene	1011	919	0.24	0.27	0.26	–	–	MS, RI
2	α -Pinene	1022	929	2.21	2.52	1.64	1.68	1.05	MS, RI
3	Camphene	1061	942	3.28	3.47	4.28	2.89	1.29	MS, RI
4	β -Pinene	1102	970	0.98	1.06	0.79	0.98	–	MS, RI
5	Sabinene	1117	963	0.53	0.69	0.37	0.64	–	MS, RI
6	α -Terpinene	1184	1008	0.28	0.40	0.35	–	–	MS, RI
7	Limonene	1206	1022	0.57	0.69	0.45	0.58	–	MS, RI
8	1,8-Cineole	1218	1018	8.22	8.57	7.29	7.14	7.29	MS, RI
9	γ -Terpinene	1250	1046	0.68	0.52	0.63	–	–	MS, RI
10	<i>E</i> - β -ocimene	1277		0.41	–	0.34	–	–	MS, RI
11	<i>p</i> -Cymene	1287	1011	0.16	0.81	0.24	1.30	–	MS, RI
12	α -Terpinolene	1299	1076	0.24	0.21	0.24	–	–	MS, RI
13	<i>cis</i> -Sabinene hydrate	1510	1050	0.53	0.66	0.31	0.98	0.75	MS, RI
14	α -Campholenal	1493	1102	0.21	–	–	–	–	MS, RI
15	Camphor	1518	1118	60.38	58.66	67.86	39.54	48.88	MS, RI
16	<i>trans</i> -Sabinene hydrate	1544		0.19	–	0.67	–	–	MS, RI
17	Linalool	1550	1081	0.13	–	–	–	–	MS, RI
18	Pinocarvone	1563	1133	0.12	–	–	–	–	MS, RI
19	<i>cis</i> - <i>p</i> -Menth-2-en-1-ol	1577	1105	0.78	0.43	–	1.04	–	MS, RI
20	Bornyl acetate	1592		0.78	2.08	–	–	–	MS, RI
21	Terpinen-4-ol	1615	1157	2.45	4.32	–	3.02	1.23	MS, RI
22	<i>trans</i> - <i>p</i> -Menth-2-en-1-ol	1634		–	0.21	–	–	–	MS, RI
23	Myrtenal	1667	1163	0.21	0.24	–	2.97	–	MS, RI
24	α -Terpineol	1705		4.61	1.24	1.43	2.15	3.01	MS, RI
25	<i>cis</i> -Verbenol	1709		–	0.40	–	–	–	MS, RI
26	Borneol	1715	1133	0.34	0.22	–	1.65	2.30	MS, RI
27	Myrtenol	1794	1175	1.39	1.12	–	3.05	–	MS, RI
28	Caryophyllene oxide	1990		1.61	–	0.74	1.98	0.85	MS, RI
29	Caryophyllenol II	2230		0.18	–	–	–	–	MS, RI
30	<i>E,E</i> -farnesyl acetate	2279		0.11	0.23	–	0.78	0.84	MS, RI
Total				91.82	89.02	87.89	72.37	67.49	
Monoterpene hydrocarbons				9.58	10.64	9.59	8.07	2.34	
Oxygen containing monoterpene				78.84	75.41	76.58	60.56	62.71	
Total monoterpenes				88.42	86.05	86.17	68.63	65.05	
Sesquiterpene hydrocarbons				–	–	–	–	–	
Oxygen containing sesquiterpene				1.79	0.00	0.74	1.98	0.85	
Total sesquiterpenes				1.79	0.00	0.74	1.98	0.85	
Esters				1.61	2.97	0.98	1.76	1.59	
Essential oil yield % (w/w)				0.76	0.99	1.11	1.70	0.72	

Compounds listed in order to their elution on the UB-Wax column column

^aLinear retention indices on the UB-Wax column relative to C₈–C₂₄ *n*-alkanes^bLinear retention indices on the HP-5 column relative to C₈–C₂₄ *n*-alkanes^cPercentages obtained by FID peak-area normalization on UB-Wax column

Fig. 1 Dendrogram obtained from a cluster analysis of five different Algerian essential oil samples of *Achillea santolina* L. Samples are clustered using Ward's technique with a Euclidean distance measure

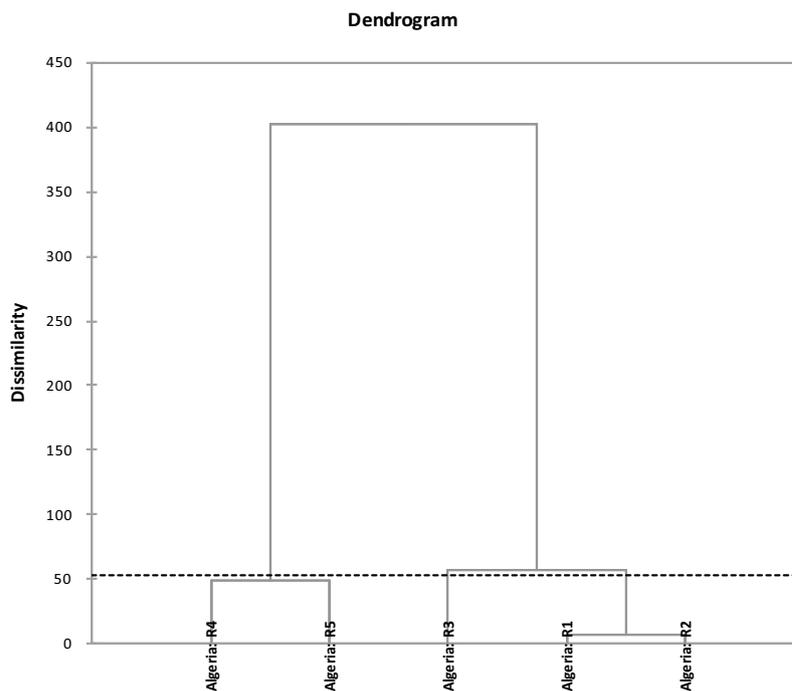
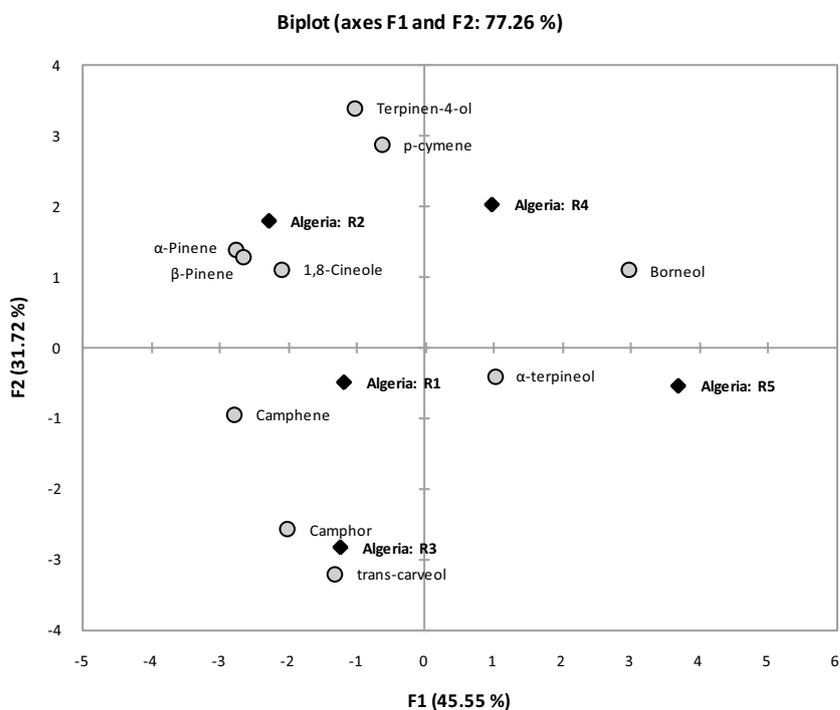


Fig. 2 Two dimensional plots on axes F1 and F2 using PCA of five different Algerian essential oil samples of *Achillea santolina* L.



which allowed us to discuss the similarities and the differences upon the chemical compositions. Loading factors for principal axes F1 and F2 (representing 77.26% of the total information), are given in Fig. 2. The F1 Axis, which represents 45.55% of the total information, is highly positively correlated with borneol (95.5%), and also highly negatively correlated with α -pinene (-89.1%), camphene

(-89.5%) and β -pinene (-85.7%). In addition this axis is also in good correlation with 1,8-cineole (Table 5).

In another hand, axis F2, which represents 31.72% of the total information, is highly negatively correlated with *trans-carveol* (-86.1%), and in good negative correlation with camphor (-69.1%). At the opposite, this axis is

strongly positively correlated with terpinen-4-ol (91.4%) and *p*-cymene (77.7%) (Table 5).

The values of the correlations of the compounds among each other and with the axis F1 and F2 were summarized in Tables 4 and 5.

As determined earlier by the AHC analysis, the samples R1–R5 (of Laghouat) were divided from the rest of the samples (R1, 2, 3 of El-Bayadh) by the first axis (F1). The PCA can provide more information's about the similarities and/or the differences between the samples. In fact, the samples studied herein this investigation can be distinguished mainly upon their composition variation relative to camphor “as major compound” and also relative to both terpinen-4-ol and borneol “as minor compounds”, among others.

The examination of the data of current study with those of literature (According to Table 3), confirmed the occurrence of a new chemotype (camphor/1,8-cineole) native to Algeria, which was distinguished only by two major compounds which were camphor (39.54–67.86%) and 1,8-cineole (7.14–8.57%); and might also be distinguished by the minor compound α -terpineol (1.24–4.16%), which was determined in relatively higher percentages in comparison with those of literature reports. In fact, in previous studies α -terpineol was not detected or at least was not detected at measurable percentages in most of case studies (Table 3).

Now let's try to identify (only by assumption) the possible existing chemotypes on the basis of the data provided by literature (Table 3). Upon the survey data presented in this table, the essential oils were distributed in eight chemotypes of *A. santolina* (Algerian samples are not included).

The first one is the Jordan chemotype (I) which enclosed four major compounds β -thujone/1,8-cineole/camphor/terpinen-4-ol, in which the percentages of camphor and 1,8-cineole were very close to each other's (17.6 and 17.5%, respectively). Before we proceed, let's mention that although they presented some similar percentages of fragranol (8.20–18.69% or $12.49 \pm 3.93\%$), the samples

Table 5 Correlations between variables and factors (F1, F2) obtained by PCA method

	F1	F2
α -Pinene	−0.891	0.376
Camphene	−0.895	−0.254
1,8-Cineole	−0.673	0.300
Camphor	−0.651	−0.691
Terpinen-4-ol	−0.329	0.914
Borneol	0.955	0.295
α -Terpineol	0.329	−0.106
<i>trans</i> -Carveol	−0.425	−0.861
β -Pinene	−0.857	0.343
<i>p</i> -Cymene	−0.204	0.777

from Egypt were presenting three different chemotypes (plant part was not considered).

The second chemotype found in Egypt that might be considered is: chemotype (II) rich in 1,6-dimethyl-1,5-cyclooctadiene (60.52%), fragranol (10.52%) and 1,8-cineole (8.64%) and β -thujone (8.96%), but doesn't contain fragranol-acetate.

The third chemotype (III) also from Egypt is very similar to the previous chemotype in almost all components, but the difference lies in lower percentage of 1,6-dimethyl-1,5-cyclooctadiene (14.6%), and the occurrence of new component fragranol-acetate with high amount (27.3%) found also in similar amounts in the next chemotype (IV). In this case, the major components were fragranol acetate (27.3%), 6-dimethyl-1,5-cyclooctadiene (14.6%), fragranol (8.2%) and 1,8-cineole (7.1%), β -thujone (8.4%) and camphor (6.7%).

The fourth chemotype (IV) belongs to both Egypt and Iran countries and was rich in fragranol acetate (28.4–51.7%) and fragranol (7.8–18.69%). In addition, this chemotype did not contain 1,6-dimethyl-1,5-cyclooctadiene, but presented relatively important content of terpinen-4-ol (5.89–7.1%), and doesn't contain β -thujone at all. Let's remind that the new

Table 4 Correlation matrix of the compounds of the essential oil of *Achillea santolina* L. obtained by PCA method

Variables	α -Pinene	Camphene	1,8-Cineole	Camphor	Terpinen-4-ol	Borneol	α -Terpineol	<i>trans</i> -Carveol	β -Pinene	<i>p</i> -Cymene
α -Pinene	1									
Camphene	0.615	1								
1,8-Cineole	0.871	0.277	1							
Camphor	0.369	0.656	0.417	1						
Terpinen-4-ol	0.693	−0.025	0.655	−0.355	1					
Borneol	−0.746	−0.918	−0.574	−0.835	−0.051	1				
α -Terpineol	−0.141	−0.434	0.062	−0.134	−0.085	0.275	1			
<i>trans</i> -Carveol	0.012	0.688	−0.136	0.785	−0.703	−0.651	−0.047	1		
β -Pinene	0.851	0.786	0.487	0.203	0.531	−0.707	−0.241	0.173	1	
<i>p</i> -Cymene	0.329	0.203	−0.031	−0.561	0.637	0.052	−0.464	−0.446	0.599	1

chemotype of Algeria did not contain neither 1,6-dimethyl-1,5-cyclooctadiene, nor fragransyl-acetate or β -thujone.

Furthermore, it was observed that the samples coming from Iran were presenting lot of dissimilarities (five possible chemotypes), and were characterized by very low contents of 1,8-cineole (0.0–0.77%). These chemotypes were somewhat unique: chemotype (V): camphor (10.27); chemotype (VI): camphor/borneol (15.61/9.34%); chemotype (VII): camphor/germacrene D (48.11/19.91%); chemotype (VIII): germacrene D/bicyclogermacrene (19.91/8.18%).

Finally, when referring also to Table 3, we could notice that the Jordan sample is quiet similar to the Algerian samples (close chemotypes), this could be explained simply by the presence of some percentage similarities among several minor components i.e. α -pinene, camphene, terpinen-4-ol, borneol, α -terpineol, *trans*-carveol, etc. In addition, and like the Algerian samples, the Jordan sample exhibited high percentages of 1,8-cineole and camphor, but the difference lies in their range variation values; for Jordan sample the percentage of 1,8-cineole was 17.6% which is higher (almost twice times) than the range recorded for the samples of Algeria (7.14–8.57%). Inversely the camphor which was present with almost the same percentage in Jordan sample (17.5%), is now very low than recorded for the Algerian samples (39.54–67.86%). In addition, the difference between the two chemotypes lies partially in the component terpinen-4-ol. For Jordan chemotype the percentage of this component is considerably higher than those of Algeria (7.0% vs. 0–4.32%). Finally, it was observed that this chemotype (Jordan) is characterized by the relatively highest content of *trans*-carveol (4.8%) in comparison with the rest of reported chemotypes (0.0–2.4%).

Conclusion

The analysis of chemical composition of essential oils of *A. santolina* aerial parts showed the occurrence of a new chemotype “camphor/1,8-cineole”, which was characterized by high amounts of two major compounds camphor (39.54–67.86%) and 1,8-cineole (7.14–8.57%).

For future perspectives, and upon the fact that the studied EOs revealed the presence of high amounts of oxygenated compounds or at least the presence of 1,8-cineole (Eucalyptol) in good amounts (besides the high EO yields), further studies involving the investigation of antimicrobial and/or antioxidant activities are strongly advised for this new chemotype coming from Algeria.

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