Journal of Materials and Environmental Sciences ISSN : 2028-2508 CODEN : JMESCN

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Essential oil variation in wild-growing populations of *Artemisia herba-alba Asso* collected from Morocco: Chemical composition and multivariate analysis

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Received 06 Jan 2017, Revised 06 May 2017, Accepted 10 May 2017

Keywords

- ✓ Artemisia herba-alba Asso;
- ✓ Essential oil;
- ✓ Multivariate statistical analysis ;
- ✓ GC/MS

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1. Introduction

Abstract

The essential oils of nine Artemisia herba-alba Asso (AHA) samples growing in different areas of Morocco were investigated by gas chromatography coupled to mass spectrometry detector (GC/MS). Their qualitative and semi-quantitative analysis confirmed the high degree of variability in chemical composition between specimens. Chemical variability in essential oil composition was estimated using hierarchical cluster analysis (HCA) and principal component analysis (PCA). The results of these two multivariate statistical analysis methods led to the classification of the nine AHA populations into three main groups which represents the following chemotypes: camphor (Ain Aghbal, Tizi Lafaka, Lakhtatba, Talezzarte), α -thujone (Bni Boufrah, Boudnib), davanone (Es-Samara), camphor/davanone (Ighrem) and α -thujone /camphor (Tata).

Artemisia herba-alba Asso (AHA), commonly known as white wormwood (Chih in Arabic), is a small shrubby aromatic plant native to the Mediterranean basin (northern Africa, Iberian Peninsula, Middle East) and western Asia [1-3]. This species develops in arid and semi-arid climates [4]. In Morocco, AHA is the most common aromatic plant and found in the eastern and northern areas, the middle and High Atlas, the saharan Anti-Atlas and the Sahara [5]. This plant is widely used in the traditional medicine to treat diabetes, bronchitis, diarrhea, gastric disturbances, abdominal cramps, hypertension and neuralgias [2,6,7] and the essential oil of this species was known for its therapeutic disinfectant, anthelminthic and antispasmodic virtues [5]. In perfumery and cosmetology, essential oils (EOs) of AHA originating from some regions of Morocco are very appreciated [2].

Concerning the EOs chemical composition of AHA growing all over the world, several type of oils were reported in the literature [8,3]. In each of these types, one or more constituents predominate (camphor, α - and/or β -thujone, 1,8-cineole, chrysanthenone, chrysanthenyl acetate, davanone, etc.). In Morocco, various compositions were observed (at least seven chemotypes), dominated either by a single component (camphor, thujone, davanone, chrysanthenone, chrysanthenyl acetate) or characterized by the occurrence, at appreciable content, of two or more of these compounds (thuione / camphor, 1.8-cineol / camphor) [2,9-11]. The chemical composition of AHA essential oil is thus very complex and subject to a high number of variables (seasonal and maturity variation, geographical origin, genetic variation, growth stages, part of plant utilized, isolation techniques employed, harvesting method and postharvest drying and storage) [12-17]. Therefore, knowing the exact constituents of an essential oil is fundamental, in order to be certain of its quality and to be able to explain its properties and warn of its potential toxicity. In this context, EOs composition of AHA belonging to various geographical locations and climate conditions of Morocco (Figure 1) were investigated. As far as we know, the composition of the EOs of AHA which occur in these nine different sites, have never been reported or are less studied in the literature [18,22]. Consequently, the aim of this work is to analyze the EOs of these AHApopulations in order to find out to which chemotype they belong. The natural variation of EOs between AHA populations was assessed using a statistical approach with Hierarchical Cluster Analysis (HCA) and Principal Component Analyses (PCA). Furthermore, the knowledge of the chemical composition of this plant permits the

evaluation of its potential value and warning of any toxicity related for example to the presence of relatively high doses of α - and β -thujones [19,20].



Figure 1: Moroccan map and collection sites of Artemisia herba-alba.

2. Experimental

2.1. Reagents and material

The aerial parts of *AHA* were collected, during May at flowering stage (Harvest time: 2013-2016), from nine natural habitats of Morocco (Talezzarte, Ain Aghbal, Tizi Lafaka, Lakhtatba, Bni Boufrah, Boudnib, Ighrem, Tata and Es-Samara). Within each population, more than ten individual plants were sampled at random. The geographic and climate data of studied sites are shown in table 1.

Sampling location	Region	Latitude	Longitude	Altitude (m)	MAP (mm)	MWT (°C)	MST (°C)
Ain Aghbal	Oujda	34° 54' 46"N	2° 06' 49" W	259	350	12	25
Tizi Lafaka	Oujda	34° 55' 06"N	2° 08' 03" W	259	350	12	25
Lakhtatba	Oujda	35° 05' 23"N	2° 18' 22" W	1	350	12	25
Talezzarte	Oujda	34° 47' 42"N	2° 34' 23" W	796	334	10	25
Bni Boufrah	Alhoucima	35° 01' 00"N	40° 03' 00" W	121	349	11.9	25
Boudnib	Errachidia	32° 00' 46"N	03° 46' 32"W	954	119	8.5	31
Ighrem	Ouarzazate	29° 27' 45"N	09° 40' 30"W	2173	112	11	32
Tata	Tata	29° 40′ 00″ N	75° 50′ 00″ W	676	100	-	-
Es-Smara	Layoune	26° 45′ 26″ N	11° 41′ 54″ W	460	138	14	25

Table 1: Geographical and climate data of natural habitats of AHA populations.

Notes: MAP : Mean Annual Precipitation; MWT : Mean of Winter Temperature ; MST : Mean of Summer Temperature *Source* : http://www.geomondiale.fr/noms_geographiques/name.php?uni=-40137&fid=3970&c=morocco

Chemicals, such as *n*-hexane for GC-MS, anhydrous sodium sulfate, α -pinene, β -pinene, camphene, 1,8-cineol, α -thujone, camphor and borneol acetate, were purchased from SOMAPROL, Casablanca, Morocco.

2.2. Essential oil isolation

The *AHA* samples were placed in the shady room to be naturally dried until stability of their weight (10 days). The fully-dried samples were cut into pieces before distillation. Then, 100 g of plant material in 300 mL of water were subjected to hydrodistillation using a modified Clevenger-type apparatus until level stability of the essential oil (2 to 3 hours). After extraction, anhydrous sodium sulfate was used to remove water trace and the essential oil was stored in an airtight glass container in a refrigerator at 4°C until the analysis. The oils were obtained in yields ranging from 0.3% to 1.7% (w/w) (Table 2).

		DIC	% Peak area							Identification		
Compounds	KI [*] (min)	KI	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>	methods ^d
Tricyclene	4.44±0.06	953	0.32±0.01	0.28±0.09	0.29±0.03	0.35±0.03	0.07±0.01	0.14±0.04	0.27±0.08	0.09±0.01	0.09±0.03	MS
α-pinene	4.62±0.06	971	0.65 ± 0.07	0.44±0.11	0.30 ± 0.05	0.57±0.11			0.26±0.09	0.09±0.01	0.15±0.03	MS/RT
Camphene	5.04±0.05	998	6.12±0.48	6.08±2.29	6.42±0.95	7.04±0.21	1.06±0.10	0.35±0.06	3.22±0.40	1.99±0.04	1.17±0.19	MS/RT
Trans-verbenol	5.25±0.04	1008	0.21±0.05	0.07 ± 0.03		0.11 ± 0.02	0.24±0.03				$0.59{\pm}0.02$	MS
Sabinene	5.46 ± 0.04	1020	$0.29{\pm}0.07$	0.21 ± 0.02		0.22 ± 0.04	0.25±0.03	4.02±0.01	$0.19{\pm}0.04$	1.20±0.09		MS
Thujene	5.46 ± 0.04	1021			0.11 ± 0.03						0.08 ± 0.03	MS
β-Pinene	5.57±0.04		$0.20{\pm}0.08$	$0.22{\pm}0.08$	$0.22{\pm}0.05$	$0.38{\pm}0.09$		0.08 ± 0.03	$0.36{\pm}0.03$	0.15±0.02	0.22±0.05	MS/RT
β-Myrcene	5.65 ± 0.03	1033							0.35 ± 0.05		1.73±0.12	MS
(+)-4-carene	6.21±0.03	1069	0.18 ± 0.01	0.23±0.10	$0.18{\pm}0.09$	$0.31{\pm}0.07$		$0.18{\pm}0.08$	$0.30{\pm}0.10$	0.25±0.05		MS
β-phellandrene	6.30±0.03	1092	0.29±0.05	$0.42{\pm}0.15$	0.31 ± 0.10	$0.42{\pm}0.10$				0.15±0.02	0.47 ± 0.04	MS
1,8-Cineol	6.74±0.03	1103	2.28±0.46	5.82±1.70	5.16±0.58	3.36±0.02	2.17±0.10	0.62±0.11	8.75±0.39	0.53±0.07	7.16±0.64	MS/RT
γ-terpinene	7.04 ± 0.02	1119	$0.26{\pm}0.05$	$0.20{\pm}0.07$	0.21 ± 0.05	0.17 ± 0.04	$0.12{\pm}0.04$	0.47 ± 0.08		0.30 ± 0.00		MS
cis-Sabinenehydrate	7.52±0.03	1148		$0.34{\pm}0.13$	0.43 ± 0.02	$0.39{\pm}0.06$	$0.19{\pm}0.03$	0.32 ± 0.09	0.64 ± 0.10	0.11 ± 0.03		MS
UC	8.07 ± 0.02	1184							1.01 ± 0.14		4.65±0.19	
α-thujone	8.45±0.02	1207	13.52 ± 2.10	7.74±1.04	$2.82{\pm}0.60$	9.78±0.54	47.38±1.86	75.41±1.71	5.08 ± 0.58	49.74±0.79	3.19±0.64	MS/RT
β-thujone	8.61±0.02	1219	4.01±0.78	5.13±1.33		3.38±0.16	8.91±0.71	10.49±0.91	2.91±0.051	8.23±0.60		MS
(3E)-2.6-Dimethyl-1.3.5- heptatriene	9.03±0.02	1246	18.28±1.50	11.98±1.20	9.55±1.05							MS
α-isophoron	9.36±0.02	1266	0.25 ± 0.05	0.18 ± 0.06						0.75±0.01		MS
Camphor	9.53±0.02	1278	49.84±5.49	53.72±5.29	58.78±3.52	59.82±1.95	10.05 ± 0.24	4.55±0.58	32.62±0.77	30.74±0.08	12.89 ± 0.80	MS/RT
Borneol	9.58±0.01	1281	1.70 ± 0.20	1.96±0.39	9.92±0.47	0.12 ± 0.02	5.21±1.12					MS/RT
Pinocarvone	9.81±0.01	1296	1.61±0.15	1.66±0.13	0.91 ± 0.17	1.27±0.19	0.74±0.10	$0.19{\pm}0.04$	0.21 ± 0.04	0.66±0.17		MS
α-terpineol	9.91±0.02	1303	0.23 ± 0.04	0.22 ± 0.04	$0.16{\pm}0.01$	$0.19{\pm}0.02$		0.09 ± 0.02	$0.64{\pm}0.10$	0.22 ± 0.03	0.71±0.19	MS
Sabinoketone	9.99±0.01	1308					0.33±0.12			0.41 ± 0.04		MS
α -thujenal	10.11 ± 0.01	1316	0.25±0.03	$0.24{\pm}0.08$	$0.20{\pm}0.02$	0.25 ± 0.08	0.55±0.11	0.38 ± 0.07		0.33 ± 0.04		MS
(-)-Mertynal	10.45 ± 0.01	1338	0.22 ± 0.03	0.29 ± 0.09	0.25 ± 0.09	$0.24{\pm}0.09$	$0.19{\pm}0.04$	0.37 ± 0.02	$0.42{\pm}0.11$	0.26±0.05		MS
UC	10.55 ± 0.02	1344					0.83±0.19	0.07 ± 0.02	2.82 ± 0.49		1.26±0.49	
(-)Verbenone	10.81 ± 0.01	1363	0.63±0.10	0.41 ± 0.08	0.28 ± 0.09	0.41 ± 0.12						MS
Borneol acetate	11.09 ± 0.01	1382	1.13±0.26	1.42 ± 0.26	1.52 ± 0.18	1.34±0.16	0.08 ± 0.01		$0.71 {\pm} 0.04$	0.25±0.08	0.21±0.07	MS/RT

Table 2: Chemical constituents identified in the essential oils of AHA collected from different regions of Morocco

Cogaene	11.85 ± 0.02	1331	0.10 ± 0.04	0.08 ± 0.05	0.07 ± 0.01	0.11 ± 0.05	0.23±0.08		0.33 ± 0.04		0.48 ± 0.09	MS
UC	11.96 ± 0.02	1338	0.60±0.10	0.34±0.10	$0.29{\pm}0.01$	0.35 ± 0.04		$0.49{\pm}0.04$	0.37±0.10			
Caryophyllene	12.84±0.02	1396							0.19±0.07		0.21±0.09	MS
UC	13.68 ± 0.04	1563		0.29±0.08	0.16±0.04		2.91±0.74		0.68±0.20		1.12±0.42	
Germacrene D	13.76±0.01	1573	0.68 ± 0.08	0.75±0.11	$0.70{\pm}0.03$	0.72±0.17	1.24±0.34		0.46±0.11	0.37±0.10	1.21±0.45	MS
Germacrene B	13.99 ± 0.02	1593		0.32±0.13	0.53±0.13	0.53±0.13	1.08 ± 0.47	0.21±0.05	0.99±0.20			MS
Epiglobulol	14.38 ± 0.01	1618					2.43±0.20				1.48 ± 0.22	MS
Davana ether	14.61±0.01	1632					0.28±0.04		6.39±1.26		0.93±0.15	MS
Trans-Nerolidol	14.79±0.01	1655					0.44±0.08		0.95±0.04		4.12±0.49	MS
UC	14.96±0.01	1691	0.35±0.09	0.20±0.11	0.28 ± 0.04	$0.19{\pm}0.08$	3.24±0.27				1.05±0.13	
Davanone	15.13±0.01	1719							12.26±0.89		41.40±3.18	MS
(-)-Spathulenol	15.19±0.01	1721	1.27±0.39	0.77±0.17	$0.49{\pm}0.08$	0.68±0.06	1.02 ± 0.11		3.71±0.96		2.09±0.44	MS
1H-Cycloprop[e] azulene	15.26±0.01	1729	0.68±0.12	0.53±0.05	1.09±0.33	0.39±0.07	2.76±0.29			0.46±0.06	3.31±0.43	MS
α -Bisabolene epoxide	15.36±0.01	1741	0.23±0.02	$0.29{\pm}0.08$	$0.29{\pm}0.04$		$0.90{\pm}0.16$			0.73±0.16	1.76 ± 0.20	MS
UC	15.65 ± 0.03	1778					0.73±0.20		0.52±0.13		2.50±0.43	
Monoterpene hydroca	arbons		8.31	8.08	8.04	9.46	1.50	5.16	4.69	4.22	3.91	
Oxygenated monoter	penes		75.63	79.02	80.43	80.66	76.04	92.42	51.98	91.48	24.75	
Sesquiterpene hydroc	carbons		1.38	1.17	1.06	1.18	1.47	0.49	1.35	0.37	1.90	
Oxygenated sesquiter	rpenes		1.95	1.62	2.11	1.60	8.01	0.21	24.30	0.46	53.33	
Unidentified compou	nds		19.48	12.99	10.28	0.54	7.71	0.56	5.40	0.75	10.58	
Total identified			86.64	89.89	91.64	92.90	87.02	98.28	82.32	96.53	83.89	
Essential oil yield	(%)		0.70	0.82	0.80	1.18	0.30	1.65	0.81	1.07	0.34	

Notes: ^a UC : Unidentified Compound. ^b RT : Retention Time. ^c RI: Retention Indices. ^d Identification methods : MS (by comparison of MS data with those stored on the NIST147 computer library). MS/RT (by comparison of MS data and retention times with those of authentic standards). $\underline{1}$: Ain Aghbal; $\underline{2}$: Tizi Lafaka; $\underline{3}$: Lakhtatba; $\underline{4}$: Talalezzart; $\underline{5}$: Bni Boufrah; $\underline{6}$: Boudnib; $\underline{7}$: Ighrem; $\underline{8}$: Tata; $\underline{9}$: Es-Samara. Boldface marked compounds were chosen for HCA and PCA statistical analyses.

2.3. Qualitative and semi-quantitative analysis of AHA EOs

The analysis was performed using a gas chromatograph (Shimadzu GC-2010) equipped with a fused-silica capillary column (5% phenyl methyl siloxane, 30 m x 0.25 mm, 0.25 um film thickness) coupled with a mass spectrometer detector (GC-MS-QP2010). The helium as a carrier gas was adjusted to a constant pressure of 100 KPa. The oven temperature was set initially at 50°C (maintained for 1 minute), followed by a gradient of 10°C/min up to 150°C (maintained for 1 minute), and then programmed to 250°C at 20°C/min (maintained for 1 minute). The temperatures of injector, transfer line and ion source were set at 250°C, 250°C and 200°C respectively. For the qualitative and semi-qualitative analysis (Table 2), solutions containing 1µL of the samples diluted in hexane (50 mg/g) were injected in split mode (split ratio = 25-100:1) and the GC–MS system was operated in scan mode. Mass spectra were recorded at 70 eV (electron impact ionization mode) with a m/z range of 40-350 a.m.u (rate and solvent delay were 5s/scan and 4.5 minutes respectively). Identification of the essential oil constituents was accomplished based on the comparison of retention times with those of authentic standards and by comparison of their MS data with those stored on the National Institute of Standards and Technology (NIST147) computer library. A LabSolutions (version 2.5) was used for the data collection and processing. Table 2 gives the relative percentage of each component of studied EOs according to their GC peak areas without correction factors. All experiments were carried out in triplicate and data was expressed as the mean \pm SD. Retention indices were obtained by coinjection with a mixture of linear hydrocarbons, C8-C24, and by the equation of Van Den Dool & Kratz [21].

2.4. Statistical analysis

The oil components with percentage higher than 5 % of the total oil were subjected to a hierarchical cluster analysis (HCA) and analyses principal component analysis (PCA) using SPSS v22.0 software. In the case of HCA, the dendrogram (tree) was produced using the Ward's method of hierarchical clustering with squared Euclidean distance between oil samples.

3. Results and discussion

3.1. Extraction and Chemical composition of essential oils

The essential oils of nine Artemisia herba-alba samples belonging to various geographical locations and climate conditions of Morocco (Figure 1) were obtained by hydrodistillation method in a yield ranging from 0.3% to 1.7% (Table 2). The composition of the essential oils was investigated using GC-MS technique and the identification of their constituents was accomplished based on the comparison of retention times with those of authentic standards and by comparison of their MS data with those stored on the National Institute of Standards and Technology (NIST147) computer library. The chemical components which represent 82.32 to 98.28% of the total oil compositions and the chromatograms of the studied oils are shown in table 2 and figure 2 respectively. Of all of the identified compounds, monoterpenes constituted the highest percentage (51.98 to 92.42%). The only exception being a sample collected in Es-Samara where the oxygenated sesquiterpenes prevail (53.33%). The hydrocarbon monoterpenes contribute generally in the oils composition with a relatively low percentage of about 1.50 to 9.46%. The oil of Bni Boufrah is curiously very poor in this class of terpenes. The data also show that the major components were found to be camphor, α -thujone and dayanone respectively for AHA collected from (Ain Aghbal/Tizi Lafaka/Lakhtatba/Talezzarte), (Bni Boufrah/Boudnib) and (Es-Samara). Oil compositions of Tata and Ighrem were characterized by the occurrence, at appreciable or high content, of two of these compounds. Some of these chemotypes (camphor, α -thujone, and davanone) are already known in other Moroccan areas: the camphor type has been found in Anti-Atlas, High Atlas and region of Nador (Northeastern Morocco) [9,22], and the α -thujone type in Anti-Atlas and High Atlas [9,23,24]. Other chemotypes were also encountered in the literature: β -thujone [9], β -thujone/Camphor [9], Chrysanthenone/Camphre [22,25] and Verbenol/Bisabolone oxide/Farnesene epoxide [26]. In other Mediterranean areas, the camphor type has been found in Algeria and Tunisia [1,27,28], the davanone type in Spain [3] and the α -thujone type in Algeria and Tunisia [6,27]. Chrysanthenone . Pinocarvone and trans-sabinyl acetate types have been found in Tunisia [28], β -thujone type in Tunisia and Iran [29,30], 1,8-Cineole type in Spain [**3**], β - α -thujone in Jordan [31,32] and Camphor/ Piperitone in Irag [33].

Finally, it is interesting to note that the great similarity between EOs of *AHA* samples collected from Bni Boufrah (northern Morocco) and Boudnib (east-south of Morocco) or Tata (southern Morocco) despite they grew under different edaphic and climatic conditions. Such a close similarity of oil composition in plants can be explained by the similarity of the genetic factors.

3.2. Chemical variation in essential oils composition

The oil components with percentages higher than or equal 5% of the total oil were subjected to Hierarchical cluster (HCA) and principal component analysis (PCA) in order to investigate the similarity and relationship between EOs composition of *AHA* populations.



Figure 2: GC–MS Total Ion Chromatogram (TIC) of the essential oils of *AHA* collected from different localities of Morocco

From a dendrogram produced by HCA (Figure 3), the *AHA* populations can be classified into two main clusters in distance of 25 units. Samples connected by a shorter distance are more similar than those connected by a longer distance. In the closer distances (~9 units), the examined populations were divided into three groups (Gr 1 to 3). The first group (Cluster I), represented by four samples originating from Eastern Morocco (Ain Aghbal, Tizi Lafaka, Lakhtatba, and Talezzarte), was rich in camphor (35.46-52.26%) and had a relatively moderate levels of (3E)-2,6-Dimethyl-1,3,5-heptatriene (12.21-16.70%) and α -thujone (2.54-12.58%). These populations in close proximity have relatively similar climate and soil, leading to similar oil contents. The second group (Cluster I) contains the samples of Ighrem (east-south of Morocco) and Es-Samara (Moroccan sahara; extreme south of Morocco) and was characterized by a significant to a high percentage of davanone (12.26-41.40 %) and had a relatively low level of α -thujone (3.19-5.08%). In the third group (Cluster II), there are three samples. Bni Boufrah (northern Morocco), Boudnib and Tata (east-south of Morocco). This group was rich in α -thujone (47.38-75.41%) and had a significant to moderate level of camphor (4.55-30.74%). The relative abundance of these two components was reversed compared to the first group.

For PCA analysis, it revealed that the first two principal components represented 74.91% of the phytochemical variance (Table 3). Figure 4 shows the graphical representation of PCA. As shown in Tables 3 and 4, the first principal component (PC1) accounted for 43.79% of the total variance and positively correlated with camphor (0.804) which was predominant in oils of *AHA* collected from Ain Aghbal, Tizi Lafaka, Lakhtatba, and Talezzarte. On the other hand, PC1 correlated negatively with α -thujone (-0.953) which was predominant in oils of *AHA* collected from Bni Boufrah and Boudnib. The second principal component, accounting for an additional 31.12% of total variance, was negatively correlated with davanone (- 0.858) which exists in moderate to large amount in oils of *AHA* collected from Ighrem and Es-Samara respectively. These PCA data lead to the classification of the *AHA* into three main groups which represents the following chemotypes : camphor (Ain Aghbal, Tizi Lafaka, Lakhtatba, Talezzarte), α -thujone (Bni Boufrah, Boudnib) and davanone (Es-Samara). The *AHA* samples of Ighrem and Tata represent two other chemotypes which are respectively the camphor/davanone and the α -thujone /camphor. This classification confirmed the HCA results.



Figure 3: Dendrogram of nine Artemisia Herba-Alba populations produced by the hierarchical cluster analysis



Figure 4: 2D graphical representation of principal component analysis of chemical compositions oils of Artemisia Herba-Alba collected from different regions of Morocco. (<u>A</u>) PCA distribution of variables. (<u>B</u>) PCA distribution of samples

I		Initial E	igen values	Extra	Extraction Sums of Squared loadings				
Components	Total	% of variance	Cumulative %	Total	% of variance	Cumulative %			
1	3.941	43.794	43.7942	3.941	43.794	43.794			
2	2.801	31.120	74.914	2.801	31.120	74.914			
3	0.911	10.117	85.031						
4	0.750	8.338	93.370						
5	0.454	5.049	98.419						
6	0.101	1.120	99.539						
7	0.025	0.277	99.815						
8	0.017	0.185	100.000						
9	2.506E-16	2.785E-15	100.000						

 Table 3: Principal components data based on oil components of Artemisia Herba-Alba with percentages higher than or equal 5%

Table 4 : Components matrix

Compounds	Components						
Compounds	1	2	3				
α-thujone	-0.953	0.257	0.006				
β-thujone	-0.870	0.352	0.245				
Camphene	0.826	0.478	0.239				
Camphor	0.813	0.467	0.266				
Dimethylheptatriene	0.567	0.550	-0.045				
Davanone	0.121	-0.858	-0.339				
Davana_ether	0.177	-0.701	0.454				
Cineol	0.664	-0.682	0.021				
Borneol	0.352	0.411	-0.631				

3.3. Potential value and toxicity

Regarding the potential value of this plant, the extracted oils can be used in perfumery and cosmetology since they are rich in oxygenated terpenes and poor in hydrocarbon terpenes. The essential oil of *AHA* collected from Tata is interesting in perfumery because the *AHA* oils which are preferred by perfumers internationally, contain 30 to 35% of thujone and 34-45% of camphor [**2**]. In flavor industry and in aromatherapy, the oils containing α -thujone cannot be used due to the toxicity of this compound [19,20]; the maximum content of α -thujone tolerated in beverages is 10 mg/kg [34]. Finally, these oils are a source of products, such as camphor, α -thujone and davanon, which are interesting in fine organic synthesis [35-37].

Conclusion

The present study reports the chemical profiles of EOs extracted from nine *AHA* populations that were collected from different contrasting habitats in Morroco. GC/MS analysis of these oils allowed the identification of thirtyseven compounds and confirmed the high degree of intraspecific variability in chemical composition between specimens occurring in separate geographic localities. According to the multivariate statistical analysis (HCA and PCA), the *AHA* samples may be characterized by the concentration of major components of their volatile oils and could be classified into three main groups which represents the following chemotypes : the camphor (Talazzert, Ain Aghbal, Tizi Lafaka and Lakhtatba populations); the α -thujone (Bni Boufrah, Boudnib and Tata populations); the davanone (Es-Samara population); the camphor/davanone (Ighrem population) and the α thujone /camphor (Tata population). Geographically, the populations growing in habitats located far from each other had in general different essential oil composition due to the difference of the climate, soil and biotic factors. Concerning the potential value of this plant, the oils can be used in perfumery and cosmetology and as a source of products (camphor, α -thujone and davanon) for organic synthesis. In flavor industry and in aromatherapy, the oils containing α -thujone cannot be used due to the toxicity of this compound. **Acknowledgements-**The authors are grateful to Professor M. Affila and Dr. M. Azzaoui for providing *AHA* originating from Bni Boufrah and Es-Samara respectively.

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