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Domestication of the endemic plant of Morocco and high added value: *Artemisia herba alba*

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- ✓ Chemotype.

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Abstract

This study is based on the collection of *Artemisia herba alba* from seven provenances belonging to three distinct regions : the Oriental (5 stations), the High Atlas (1 station) and the Middel Eastern Atlas (1 station) to study the possibility of domestication of this plant in relation to the chemical profile of its essential oil (EO). The The extraction of essential oil (EO) from the aerial part of this domesticated plant was performed by hydrodistillation and analysed by GC/MS. The EO yield of Artemisia herba alba from the 7 provenances is 0.8%, 0.8%, 1.1%, 0.9%, 1.4%, 1.5% and 1.6% respectively. The EO of provenance P1 is dominated by Trans-acetate-Sabinene hydrate β-thujone and cis-chrysanthenol (23.66%, 23.12% and 11.96% respectively) EO of P2, P3, P4, P5 and P6 are characterized by the predominance of two oxygenated monoterpenes : Camphor (15.64%, 23.88%, 33.46%, 45.42% and 39.94% respectively) and chrysanthenone (19.6%, 18.18%, 12.97%, 12.37% and 18.26% respectively). Finally, the EO of P7 is characterized by the predominance of three oxygenated monoterpenes: cis-thujone (31.75%), β-thujone (29.06%) and Camphor (20.45%). This work has made it possible to identify three chemotypes: one to β-thujone and trans-acetate-hydrate in Middle Eastern Atla; the other with camphor and chrysanthenone in Oriental and finally one with cis-thujone, β-thujone and camphor in the High Atlas.

1. Introduction

The genus Artemisia includes important medicinal plants which are currently the subject of a phytochemical attention because of their biological and chemical diversity. In Morocco, it is represented by twelve species; six of which are endemic namely: *Artemisia. herba-alba, Artemisia. atlantica* var. *maroccana, Artemisia flahautii, Artemisia. mesantlantica, Artemisia. Negrei* and *Artemisia ifranensis* However, the species *A. herba-alba* or "Chih" is the most abundant and the most exploited. This is a species that is widely used in traditional medicine. Indeed, the infusions of this species are used as analgesic, antiplasmodial, antidiarrheal or as a diuretic [1] [2]. While several extracts and essential oils of this plant have shown biological activities, such as antimicrobial activity [3], antioxidant [4] [5] [6], anti-inflammatory [7] and insecticide [8].

In Morocco, white sagebrush occupies about 2.5 million hectares. The pastoralism of the great rangelands finds an irreplaceable pasture there. It is also recognized by its oil content which is considered the most important among the other species. Its essence is intended for cosmetology and perfumery industry. Two countries share the international market for this oil: Morocco and Tunisia. But the big part goes to Morocco, which holds 90% of the world market [9].

Despite its economic, ecological and ethnobotanical importance, this endemic plant in Morocco is disappearing due to drought conditions and pressure from farmers. The example of the forests of Jerrada and Debdou speaks. In addition, this plant still lives in the wild and rare the scientific works that are interested in its domestication and cultivation. In order to contribute to its domestication and cultivation, a series of seven nursery trials were conducted to evaluate the effect of domestication combined with the provenance on the chemical composition of its essence.

In this work, we studied the species *Artemisia herba-alba* collected from seven provenances belonging to three distinct regions: The Oriental (five stations), the High Atlas (one station) and the Middle Eastern Atlas (one station). Knowing that the domestication of spontaneous aromatic and medicinal plants (AMP) is very recent in Morocco, we investigated in this study the possibilities of domestication of this plant in relation to the chemical profile of its essential oil (EO). Thus, we extracted the essential oils from the aerial part of domesticated *Artemisia herba-alba* and determined their yield and chemical composition.

2. Material and Methods

2.1. Plant material

Surveys held in 2014 allowed to collect the basic plant material of *Artemisia herba alba* from seven provenances belonging to the following regions: The Oriental (five stations), the Middle Eastern Atlas (one station) and the High Atlas (one station). **Table 1** establishes the list of studied provenances. The collected seedlings were multiplied vegetatively at the forest nursery of Azrou (Regional directorate of water and forests and the fight against the desertification of the Middle Atlas-Meknes). All measures have been taken to maintain this unique collection.

Table 1: Origins of *Artemisia herba alba* samples studies

No of Provenance	Name of Provenance	Geographic area
P1	Ouled Ait Makhlouf	Middle Eastern Atlas
P2	Reserve Aswiwinia	Oriental
P3	Hassi Albyad	Oriental
P4	Oued Agba	Oriental
P5	Oued Alkharoub	Oriental
P6	Oued Asla	Oriental
P7	Amskroude	High Atlas

For the realization of this study, samples from this collection and corresponding to the aerial parts (stems, leaves and flowers) of *Artemisia herba alba* domesticated were taken in 2016.

2.2. Extraction of essential oil

The extraction of EO from the aerial part of the plant (stems, leaves and flowers) domesticated was performed by hydrodistillation in a Clevenger-type apparatus (Clevenger 1928) [10]. We performed three distillations by boiling 200 g of vegetal material cut with pruning shears and introduced into a 2 liter flask containing 1 liter of water. The extraction time is of the order of 3 hours on average. The essential oil obtained is dehydrated with anhydrous sodium sulphate and then stored at a low temperature (below 4 °C) and in the dark before use.

2.3. Determination of moisture content

The determination of the moisture content is carried out by weighing 25 g of each sample and placed in the oven at 60 ° C for 48 to 60 hours. After cooling, the average weight loss is calculated and the moisture content is determined by the following Equation (1):

$$\%mc = \frac{W_w - W_d}{W_w} \times 100 \qquad (1)$$

with %mc: moisture content, Ww: wet weight and Wd: dry weight

2.3. Essential oil yield calculation

The EO yield is expressed in ml of the distillate per 100 g dry matter according to the following Equation (2):

$$Yld\,(\%) = \left[\frac{V}{W_d} \times 100\right] \pm \left[\frac{\Delta V}{W_d} \times 100\right] \qquad (2)$$

With

Yld%: Yield of EO (ml/g)

V:Volume EO collected (ml)

ΔV: Reading on Error

W_d:dry plant weight

2.3. Analysis of essential oil by Gas chromatography coupled with Mass Spectrometry (GC/MS)

The analysis were carried out on a gas chromatograph with electronic pressure regulation Hewlett Packard type (HP 6890 series), equipped with an HP-5 capillary column (30 m × 0.25 mm) with a film thickness of 0.25 µm, a FID detector set at 250 ° C and powered by a mixture of gas H2 / Air and a split-splitless injector set at 250 ° C The injection mode is split type (leak report: 1/50, flow rate: 66 ml / min). The gas used was nitrogen with a flow rate of 1.7 ml/ min. The column temperature is programmed from 50 to 200° C at a rate of 4 ° C / min. The device is controlled by a computer system type "HP ChemStation", managing the operation of the device and following the evolution of chromatographic analysis.

The identification of the constituents was carried out based on their Kovats Index (KI) and gas chromatography-mass spectrometry (GC-MS). The latter is carried out on a Hewlett-Packard type gas chromatograph (HP 6890 series) coupled with a mass spectrometer (HP 5973 series). Fragmentation is done by electron impact in a field of 70 eV. The column used is an HP5MS capillary column (30 m × 0.25 mm), the film thickness is 0.25 µm. The temperature of the column is programmed from 50 to 250 ° C at 4 ° C/ min. The carrier gas is helium whose flow rate is set at 1.5 ml / min. The injection mode is split (leak report: 1/70 flow rate 112 ml / min). The device is connected to a computer system that manages a NIST 98 mass spectrum library.

3. Results and discussion

3.1. Yield of essential oils of Artemisia herba alba

The EO of *Artemisia herba alba* domesticated obtained by hydrodistillation is a viscous liquid, limpid of a deep yellow color with strong odor characteristic of juniper (Table 2).

Analysis of **Table 2** shows the followings conclusions:

- The average yield of essential oil in the seven provenances studied is 1.1%;

- Samples of white sagebrush from provenance : P7, P6 and P5 recorded the best EO yields : 1.6%, 1.5% and 1.4% respectively ;
- An intermediate EO content was obtained at the P3 provenance level of 1.1% ;
- The same yield was almost observed in three provenances namely: P1, P2 and P4 with respective rates of : 0.8%, 0.8% and 0.9%

Table 2: Average yields of EO obtained by provenance.

No of Provenance	Name of Provenance	Geographic area	Yield (%)
P1	Ouled Ait Makhlouf	Middle Eastern Atlas	0.8
P2	Reserve Aswiwinia	Oriental	0.8
P3	Hassi Albyad	Oriental	1.1
P4	Oued Agba	Oriental	0.9
P5	Oued Alkharoub	Oriental	1.4
P6	Oued Asla	Oriental	1.5
P7	Amskroude	High Atlas	1.6

Compared with several provenances of wild white sagebrush studied in Morocco, the Amskroude locality seems to have the highest EO yield (1.6%). Indeed, this value is higher than that obtained by Zaim et al. (2012) [11] in the region of Ouarzazate is 1.2%. Moreover, Ghanmi et *al*. (2010) [12] showed that the rate EO yield of the wild species *Artemisia herba alba* varies according to the harvest period in the Guercif region; it is between 0.56% and 1.23%. The yield obtained in this study is also higher than that reported in Jordan by Hdaiba & Aburjai (2006) 1.3% [13]. Paradoxically, the EO content of our samples of domesticated white sagebrush from the localities: Oued Ait Makhloof, Reserve Aswiwinia, and Oued Agba are comparable to those made in Algeria, whether 0.2% to 0.95% (Bezza et *al*,. 2010 ; Belhattab et *al*., 2012 ; Bouzidi, 2016) [14] [15] [16].

3.2. Chemical composition of the EO of the studied species

The constituents of the EO extracted from domesticated *Artemisia herba alba* were identified based on gas chromatography coupled with mass spectrometry in combination with Kovats Index (KI). **Table 3** presents these constituents in the order of their elution.

Chromatographic analysis of EO of domesticated *Artemisia herba alba* allowed to globally identify seventy-five constituents from all provenances. However by provenance, the volatile compounds identified in the EO of the provenances: P1, P2, P3, P4, P5, P6 and P7 are respectively: 47; 58; 62; 53; 55; 43 and 31 constituents. They represent approximately between 99.18 to 99.91% of EO.

The results obtained show that the combination of volatile compounds of this species is variable, in terms of diversity and concentration **(Table 3)**. In fact, the EO resulting from the provenance P1 is dominated by three compounds: Trans-Sabinene hydrate acetate, β-thujone and cis-chrysanthenol (23.66%, 23.12% and 11.96 respectively) (**Figure 1**).

They are accompanied by other minor constituents which are not without importance as: Neo-3-acetate thujanol (4.37%), camphor (3.32%), chrysanthenone (2.84%), γ- terpineol (2.31%); limonene-aldehyde (2.13%) or davanone (0.56%). Relatively similar observations were made by Satrani et *al*. (2016) with samples collected in the region of Imouzzer Marmoucha whose Artemisia alcohol, 6-camphenone, ß-thujone, cis- and Trans-Sabinene hydrate acetate are major compounds. However, quite different results were obtained by Benjilali & Richard (1980) [17]. The latter, working on 48 plants of the High and

Middle Atlas showed the dominance of camphor (>50%) in 18 samples from the Souss region. As for the EO of the provenances: P2, P3, P4, P5 and P6, they are characterized by the predominance of two oxygenated monoterpenes namely: Camphor (15.64%, 23.88%, 33.46%, 45.42%) % and 39.94% respectively) and chrysanthenone (19.6%, 18.18%, 12.97%, 12.37% and 18.26% respectively) (**Figure 2**).

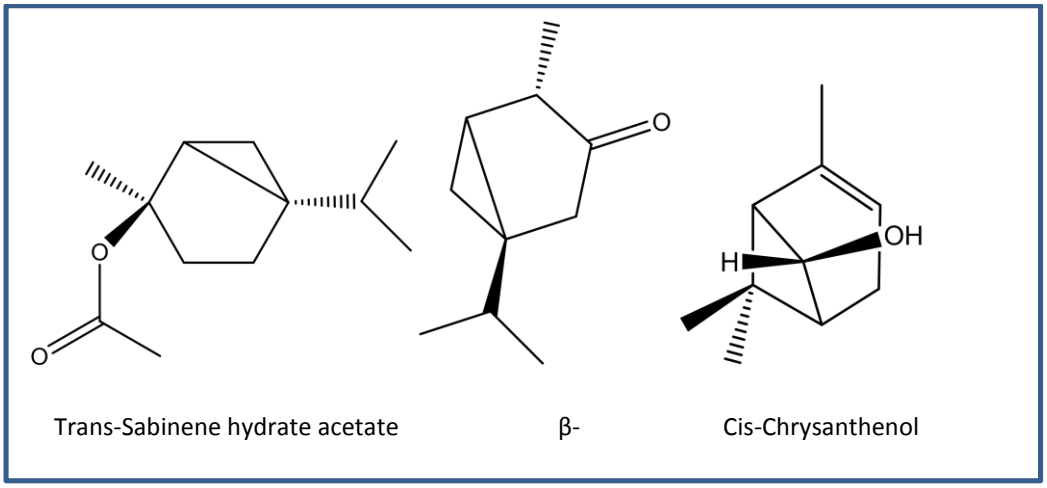

Figure 1: The chemical structure of the majority components of provenance P1

Some monoterpene are present with relatively high percentages such as cis-thujone (4% to 13.68%), 3-thujanol (1.76% to 4.18%) and cis-chrysanthenol (1.55% to 4%). 16%). Other constituents are minor such as e-β-Damascenone (0.4% to 5.9%); cis-acetate carvyl (0.37% to 5.76%); camphene (0.63% to 5.01%); β-thujone (0.32% to 1.61%) or even davanone (0.22% to 0.96%). This result is almost similar to that found by Zaim et *al* (2012) [11]. The latter reported that *A.herba alba* EO from the Ouarzazate region is dominated by chrysanthenone (28.10%) and camphor (26.67%). Lekhal S. et *al*. (2016) also found that the main constituents of *A.herba alba* EO in Djefla in Algeria are: camphor (39.5%), chrysanthenone (10.38%), 1,8-cineole (8.6%) and α-thujone (7.03%) [18].

Finally, the chromatographic analysis of the EO extracted from the samples from the P7 provenance showed the predominance of three oxygenated monoterpenes: cis-thujone (31.75%), β-thujone (29.06%) and camphor (20, 45%). Other constituents are present but with low concentrations such as: 3-thujanol (4.14%), 1,8-cineole (1.86%), neo-3-acetate thujanol (1.54%), β -thujaplicin (1.32%) or davanone (0.23%). Relatively different observations were made by researchers. Bouzidi et *al*. (2016), working on EO of *A.herba alba* from Algeria, have shown that the major components of this oil are camphor (29.81%), cyclopentadiene, 1, 2, 5.5 tetramethyl (15.58%), chrysanthenone (8.21%) [16]. In addition, Dahmani-Hamzaoui & Baaliouamer (2010) mentioned camphor (49.3%) in the EO of the northern Algerian Sahara as a major component. Chrysanthenone (3.2%) being minor in these extracts [19].

However, some minor chemical compounds in the EO of the provenances studied have been described by other authors as the major constituents of the EO of *A.herba-alba*. This is the case of davanone (Benjilali et *al*., 1982) and 1.8 cineol (Salido et al., 2014) [20] [21].

In conclusions, it seems that the species *A.herba-alba* is characterized by an important intraspecific variability in the chemical profile of its essential oils. Thus, the present study revealed the existence of three chemotypes: one with β-thujone and trans-acetate-hydrate in Middle Eastern Atlas; the other with camphor and chrysanthenone in Oriental and finally one with cis-thujone, β-thujone and camphor in the High Atlas. We note the presence of camphor in the sagebrush of the seven provenances studied.

Table 3: Chromatographic analysis (GC/MS) of the studied *Artemisia herba alba* samples from seven provenances. KI: Kovats index-: Trace. P1: Oued Ait Makhlouf (Middle Eastern Atlas). P2: Reserve Aswiwinia (Oriental). P3: Hassi Al Abyad (Oriental). P4: Oued Agba (Oriental). P5: Oued Al Kharroub (Oriental). P6: Oued Asla (Oriental). P7: Amskroute (High Atlas).

KI	Constituents	P1	P2	P3	P4	P5	P6	P7
900	Santolina triene	-	-	-	-	0.48	-	-
917	2.5-Diethenyl-2-methyl-tetrahydrofuran	-	-	-	0.19	0.27	-	-
928	α-pinene	0.12	0.65	0.28	0.64	0.25	0.23	-
943	Camphene	0.61	0.63	0.93	3.89	5.01	2.76	0.93
966	Sabinene	0.9	0.15	0.1	0.32	0.13	0.08	0.2
971	β-pinene	-	0.27	0.18	0.31	0.29	-	-
982	Cis-pinane	-	0.45	0.25	-	0.19	0.29	-
988	Myrcene	0.21	0.41	0.23	1.74	0.2	0.28	-
1001	δ-2-carene	-	0.08	0.14	0.08	0.14	-	-
1005	δ-3-carene	-	0.11	0.53	0.23	0.1	0.09	0.1
1016	p-cymene	0.41	1.02	2.67	1.49	0.55	0.64	0.61
1026	1.8 cineol	0.59	3.59	-	3.4	2	1.26	1.86
1044	(E)-β-Ocimene	-	0.31	0.12	0.41	1.12	0.15	0.28
1064	γ-terpinene	-	-	-	-	1.77	-	-
1077	Artemisia alcohol	0.18	0.3	0.19	0.24	0.19	0.19	-
1099	cis–thujone	2.93	13.68	7.66	11.43	4	9.6	31.75
1114	β –thujone	23.12	1.61	0.79	0.34	0.32	0.65	29.06
1124	Chrysanthenone	2.84	19.6	18.18	12.97	12.37	18.26	1.57
1136	Cis -β -dihydro-terpineol	1.77	0.14	-	-	0.09	-	-
1145	Camphor	3.32	15.64	23.81	33.46	45.42	39.94	20.45
1152	β-Pinene oxide	-	-	-	-	-	-	0.3
1158	Cis-chrysanthenol	11.96	4.16	2.1	3.45	1.55	1.67	0.86
1162	3-thujanol	-	-	2.49	1.76	5.2	4.18	4.14
1172	Terpinene -4-ol	0.22	1.81	1.6	1.16	1.36	1.16	0.83
1176	Iso-pinocampheol	-	0.24	0.32	0.17	-	0.14	0.09
1181	Thuj-3-en-10-al	-	0.65	0.66	0.5	0.22	0.39	-
1189	α-terpineol	-	0.49	0.76	0.45	0.67	0.43	0.81
1200	γ-terpineol	2.31	0.37	0.7	-	0.26	0.28	0.11
1214	Dihydro-myrcenol-acetate	1.23	-	-	-	-	-	0.08
1220	cis-acetate-hydrate Sabinene	0.22	0.12	0.18	0.26	0.22	0.34	0.26
1226	Nordavanone	0.28	0.19	0.23	0.14	0.11	0.17	0.08
1234	E-Ocimenone	-	0.41	0.24	0.1	0.19	0.26	-

Table3: (continued) Chromatographic analysis (GC/MS) of the studied *Artemisia herba alba* samples from seven provenances. KI: Kovats index-: Trace. P1: Oued Ait Makhlouf (Middle Eastern Atlas). P2: Reserve Aswiwinia (Oriental). P3: Hassi Al Abyad (Oriental). P4: Oued Agba (Oriental). P5: Oued Al Kharroub (Oriental). P6: Oued Asla (Oriental). P7: Amskroute (High Atlas).

KI	Constituents	P1	P2	P3	P4	P5	P6	P7
1204	p-cymene-9-ol	1.15	0.59	1.56	1.01	0.25	0.62	-
1214	Dihydro-myrcenol-acetate	1.23	-	-	-	-	-	0.08
1220	cis-acetate-hydrate Sabinene	0.22	0.12	0.18	0.26	0.22	0.34	0.26
1226	Nordavanone	0.28	0.19	0.23	0.14	0.11	0.17	0.08
1234	E-Ocimenone	-	0.41	0.24	0.1	0.19	0.26	-
1245	Car-3-en-2-one	-	0.5	0.88	0.07	0.43	1.46	0.22
1255	Trans-Sabinene hydrate acetate	23.66	0.1	1.62	1.54	0.77	1.46	0.17
1263	cis-Chrysanthenyl acetate	0.58	1.94	-	-	-	-	-
1275	Thujanol-Acetate-Neo-3	4.37	-	0.06	0.06	0.43	0.98	1.54
1289	p-cymen-7-ol	0.84	-	0.13	-	-	0.11	0.18
1296	3-thujanol acetate	-	-	0.13	0.09	-	0.73	-
1306	iso-verbenol acetate	-	1.39	1.69	1.24	0.35	1.45	-
1314	δ- terpinyl acetate	1.76	2.55	2.68	1.87	0.72	0.09	0.17
1326	Limonene- aldehyde	2.13	-	-	-	-	-	-
1333	δ-Elemene	1.2	0.31	0.37	0.39	0.18	1.62	-
1344	α- terpinyl acetate	1.22	1.14	0.86	1.07	1.43	4.38	-
1349	Acetate de thymol	0.84	-	-	-	-	0.15	-
1373	Cis-acetate carvyl	0.25	5.76	5.03	0.41	1.85	0.37	0.12
1383	E-β-Damascenone	0.68	5.86	5.9	4.17	2.68	0.4	0.55
1390	β-Elemene	-	-	0.28	-	-	0.2	-
1405	Sesquithujene	-	0.23	0.23	0.12	0.14	-	-
1416	E-Caryophyllene	0.18	-	0.08	-	-	-	-
1434	γ-Elemene	-	0.56	0.58	0.36	0.34	-	-
1457	Sesquisabinene	0.55	0.1	0.1	0.32	0.09	-	0.24
1464	9 -epi- E –caryophyllene	-	0.08	-	-	-	-	
1476	β-thujaplicine	0.96	1.8	0.99	1.26	0.75	-	1.32
1490	Cis-β-Guaiene	0.96	0.6	0.24	0.41	0.21	-	0.47
1511	γ-cadinene	0.17	0.2	0.2	0.17	0.11	-	-
1549	Elemol	0.12	0.11	0.2	-	-	-	-
1557	Trans-ether-cadinene	-	0.34	0.13	0.5	0.27	-	-
1568	α-Caryophyllene Alcohol	-	0.11	0.16	-	0.1	-	-

Table3: (continued) Chromatographic analysis (GC/MS) of the studied *Artemisia herba alba* samples from seven provenances. KI: Kovats index-: Trace. P1: Oued Ait Makhlouf (Middle Eastern Atlas). P2: Reserve Aswiwinia (Oriental). P3: Hassi Al Abyad (Oriental). P4: Oued Agba (Oriental). P5: Oued Al Kharroub (Oriental). P6: Oued Asla (Oriental). P7: Amskroute (High Atlas).

KI	Constituents	P1	P2	P3	P4	P5	P6	P7
1577	trans-Sesquisabinene hydrate	1.1	2.91	3.07	2.33	1.04	0.61	0.33
1587	Davanone	0.56	0.48	0.96	0.22	0.31	0.36	0.23
1594	Cubeban-11-ol	-	0.62	0.91	0.46	0.6	-	-
1608	Humulene epoxide II	0.51	0.76	1.04	0.46	0.19	0.13	-
1623	10-epi-γ-eudesmol	0.29	0.28	0.3	0.12	-	-	-
1635	Cis-caden-4-en-7-ol	0.33	0.79	0.75	0.56	0.32	0.27	-
1658	α –eudesmol	-	0.09	0.35	0.18	-	-	-
1677	Elemol acetate	0.48	-	0.14	-	-	-	-
1684	α-bisabolol	0.28	0.13	0.26	-	-	-	-
1748	α-Bisabolol oxide A	0.1	0.14	0.17	0.08	-	-	-
1813	Iso-Longifolol acetate	0.11	-	-	-	-	-	-
1864	cis-acid–Thujopsene	0.24	-	-	-	0.4	-	-
1889	5Z-9E-farnesylacetone	-	-	0.08	-		-	-
1942	(3E)-A-cembrene	0.34	0.76	0.27	0.4	0.19	0.2	-
1967	(3Z)-A-cembrene	-	0.24	0.3	-			-
1986	Manool oxide	-	0.64	0.71	-	0.64	0.44	-
2011	13-epi-Manool oxide	-	-	0.14	-	-	-	-
		99.18	99.19	98.89	99	99.46	99.47	99.91

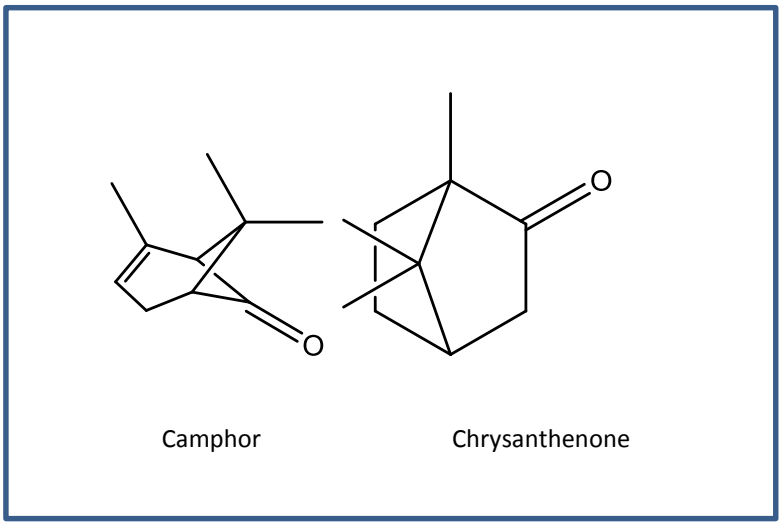

Figure 2: The chemical structure of the majority components of other provenances (P2-P3-P4-P5-P6)

This chemical polymorphism associated with the presence of several chemical compounds could be the origin of a biological activity of EO of this plant, with broad spectrum against a given pathogen.

Conclusion

The domestication of high value MAPs added is a niche that enhances the process of production, labeling and certifying the product and so generating a high added value. It is a sustainable way to supply the local and international market with quality products with traceability, which is not offered by wild species, whose chemical composition is heterogeneous and often unknown to sellers. Indeed, the MAPs are characterized by a great genetic and chemical variability which constitutes, moreover, an unavoidable factor of fight against the desertification and the climatic changes, by consuming few water resources.

In this sense, the EO obtained by hydrodistillation of the aerial part of domesticated *Artemisia herba alba* have different yields and chemical compositions. However, the yield of essential oil from the studied regions (Oriental, Middle Eastern Atlas and High Atlas) is acceptable and can be profitable on an industrial scale. The present work has also identified three distinct chemotypes in the species of domesticated *Artemisia herba alba* namely: The chemotype with β-thujone and trans-acetate-hydrate in Eastern Middle East with an average yield in EO of the order of 0.8%, the camphor and chrysanthenone chemotype in Oriental with an EO yield of about 1.14% and finally the cis-thujone, β-thujone and camphor chemotype in the High Atlas with an EO yield of 1.6%. It is difficult at the moment to attribute these variations to a given factor. Moreover, it would also be interesting to study whether the differentiation of chemotypes is the result of genetic determinism at the plant level.

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