

Provenance effect on the yield, chemical composition and antimicrobial activity of the essential oils of *Artemisia herba alba* in the Eastern of Morocco

Ourid Btisam^{1,2}, Satrani Badr^{1,*}, Ghanmi Mohamed¹, Ghadraoui Lahsen², Fatmi Mariam^{1,3}, Mounyr Balouiri², Kerdouh Benaissa¹, Bakkali Yakhlef Salah Eddine⁴

¹Centre de Recherche Forestière, Haut Commissariat aux Eaux et Forêt et à la Lutte contre la Désertification, Rabat, (MAROC)

²Faculté des Sciences et Techniques, Fès, (MAROC)

³Faculté des Sciences, (KÉNITRA)

⁴Direction de l'Enseignement, de la Formation et de la Recherche, Ministère de l'Agriculture et de la Pêche Maritime, Rabat, (MAROC)

E-mail: badrsat@yahoo.fr

ABSTRACT

As part of the valorization of medicinal and aromatic plants in Morocco, we studied the effect of the provenance on the yield, chemical composition and antimicrobial activity of the essential oils of *Artemisia herba alba*. The essential oils obtained by hydrodistillation from branches were analyzed by GC and GC/MS. The most abundant compounds identified vary according to their origins: α -thujone (17,52 to 48,91%), chrysanthenone (26,64 to 48,11%), 1-4 cineole (0,32 to 9,7%), artemisia alcohol (1,83 to 8,65%), camphor (0,04 to 6,68%) and α -pinene (2,21 to 6,42%). The influence of the provenance on the antimicrobial activity of these essential oils was also highlighted.

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KEYWORDS

Artemisia herba alba;
Essential oils;
Chemical composition;
Antimicrobial activity;
Provenance.

INTRODUCTION

Morocco, by its diversity phyto-economic arising from its geographical location and its Mediterranean bioclimatic moods, reveals an appreciable wealth in terms of aromatic and medicinal plants (4500 plant species, 940 genera and 135 families)^[6]. Also, Morocco occupies the second rank among the Mediterranean countries for its richness in endemic plants^[8], and is one of the countries that are major producers and suppliers of medicinal and aromatic plants and their derivatives. Among the 7000 spe-

cies and under existing species 537 are endemic and 1625 are rare or threatened^[6].

In fact, the aromatic and medicinal plants constitute natural resources with high value, which can play an important role in the socio-economic development of certain economically disadvantaged regions. Besides, it is also noted the great trend or desire to return to nature and plants despite the important development of chemistry, biochemistry and the organic analysis in the therapeutic and medical areas.

Among the multitude of aromatic and medicinal

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plants existing in Morocco figure the genus *Artemisia* which belongs to the family of Asteraceae. This is one of the most pervasive genera and the most studied of this family, it contains a variable number of species reaching 400 species spread over the five continents^[32, 24, 46, 18]. The infusions of these species have been used as an analgesic, antispasmodic, anti-diarrhea or as diuretic agent^[12, 9], while several extracts and essential oils of these species have shown biological activities, such as the antimicrobial activity^[42, 18, 4], antioxidant^[16, 24, 28, 25], anti-inflammatory^[19] and insecticide^[34].

In Morocco, the genus *Artemisia* is represented by thirteen species. Among the most important, there is the *Artemisia herba alba* which is a genuine mine of natural and very interesting molecule. This *Artemisia herba alba* is also a great economic interest in Morocco. Two countries share the international market for essential oils of this specie: Morocco and Tunisia. But Morocco holds the biggest share, 90% of the world market^[31]. In Morocco, a single route of industrial exploitation of the *Artemisia herba alba* is currently used: the production of essential oil for the perfumery of high range. The consumption of the local market in the form of botanical remains negligible as compared to the first form of industrial exploitation. However, *Artemisia herba alba* is distinguished by four main chemical races, camphor, α -thujone, α,β -thujone and α -thujone and camphor^[8]. The product that perfumers look for is the one that matches the chemotype “ α -thujone and camphor”. The concentrations of these constituents in the essential oil of *Artemisia herba alba* must range between: 30-36% for α -thujone and 33-38% for the camphor. In addition, the regions of the east, the Middle Atlas, High Atlas and the Anti-Atlas foothills constitute the main areas of aquifers of the *Artemisia herba alba* which are still not exploited. The area is estimated to be of 1.5 million ha.

In fact, several works have been carried out on the essential oils of the *Artemisia herba alba* and their biological activities^[2, 18, 44, 3, 4].

However, no work has been done to identify and map the different chemical breeds and essential oils of the *Artemisia herba alba* in the region of eastern Morocco. This is the objective of our work which

is to characterize the chemical map of the five provenances to determine the origin of chemotype searched by the industrialists as well as the evaluation of their antibacterial and antifungal power on various micro-organisms for a better valorisation and conservation of the resource.

MATERIALS AND METHODS

Materials

Plant material

Samples of aerial parts of *Artemisia herba alba* were harvested in June 2013 from five region of eastern Morocco. It is about origins of the reserve Aswiwinia (Beni Matthar), Hassi Al Abyad (Jerrada), Oued Ogba (SIBE Acherrakh), Oued Al Kharroub (Dam Machraa Hammadi) and Oued Asla (Al Ayoun east).

Microorganisms studied

The antimicrobial activity of the *Artemisia herba alba* oils was evaluated on eleven isolated strains. The microorganisms used were as follows:

- Bacteria : *Bacillus subtilis* (gram+), *Escherichia coli* (gram-), *Micrococcus luteus* (gram+) et *Staphylococcus aureus* (gram+).
- Fungi : *Aspergillus niger*, *Penicillium digitatum* et *Penicillium expansum*.
- Wood rot fungi: *Gloeophyllum trabeum*, *Poria placenta*, *Coniophora puteana* and *Coriolus versicolor*.

The four pathogenic bacteria are chosen for their high antibiotic resistance and toxicity in humans. They are frequently encountered in many infections in Morocco and pose a clinical and therapeutic problem. The three selected fungi are agents of decay in common food and fruits and can be toxic and pathogenic for humans and animals. The four wood rot fungi used in this work are responsible for brown and white rot of wood. They are the most important wood-destroying fungi. They were chosen for the considerable damage they cause in buildings, wood in contact with the soil (poles and railways) or buildings (bridges)^[38, 22]. Bacterial strains are lots of American Type Culture Collection ATCC, they are deposited by subculture on nutrient agar for 24 hours

in the dark at 37°C. Mold and wood decay fungi belong to the collection of Mycotheque of Microbiology Laboratory in Forestry Research Centre, Rabat, Morocco. They are regularly maintained by subculture on nutriment medium PDA (*Potato Dextrose Agar*).

METHODS

Extraction of essential oils

The extraction of essential oils was performed by hydrodistillation in a Clevenger type apparatus^[11]. Three distillations were carried out by boiling 300g of fresh plant material with 2 liters of water for three hours. The average oil contents were calculated and expressed in ml/100g. The oil obtained was separated from the distillate water and dried with anhydrous sodium sulphate then it was stored at 4 °C for further use in bioassay and composition determination.

Chromatographic analysis

Chromatographic analysis of *Artemisia herba alba* essential oils was performed on a gas Chromatographer with electronic pressure control, type Hewlett Packard (HP 6890) equipped with a HP-5MS capillary column (30 m x 0.25 mm, film thickness 0.25 µm), a FID detector set at 250°C and using a H₂/Air mixture, and a *split-splitless* injector set at 250 °C. The injection mode was split (split ratio: 1/50, flow rate: 66 ml min) and the injected volume was about 1 µl. Nitrogen was used as carrier gas with a flow rate of 1.7 ml.min. The column temperature was programmed from 50 to 200 °C at a heating rate of 4°C.min, during 5 min. The apparatus was controlled by a "ChemStation" computer system.

The identification of the components is based on the comparison of their mass spectra (GC/MS), respective with spectra of the library NIST 98, of the bibliography^[1] and on the basis of calculation of Kovats indices (KI). Indeed, the index system is based on a notion of relative retention. It compares the retention of whatever product to that of a linear alkane. This system is applicable in gas chromatography to all compounds on all columns. By defini-

tion, it assigns an index of 800 to the linear alkane in C₈ (n-octane), 1000 to C₁₀ linear alkane (n-decane), and this, whatever the stationary phase, the length of column, the flow rate or the temperature. The KI are determined by injecting a mixture of C₉ to C₂₄ alkanes in the same operating conditions^[29]. They are calculated from the following equation:

$$Ik = \left[\frac{TR_x - TR_n}{TR_{n+1} - TR_n} + n \right] \times 100$$

Where in TR_x is the retention time of the solute x, TR_n and TR_{n+1} are the retention times of linear alkanes to n and n + 1 carbon atoms and which frame the peak of the solute. The retention index KI or a compound A is independent from the flow rate, of the column length and of the injected amount (within a certain limit). The retention index of a compound A depends on the stationary phase and temperature.

In general, the technique of KI is widely used to identify the usual essential oils compounds, but it is insufficient to determine the total chemical composition. The IK tables specific to each product are proposed in the literature. They were developed using analyzes on different types of columns. These benchmark indices are compared to those calculated from our samples.

Microbiological procedure

The minimum inhibitory concentrations (MIC) of the essential oils were determined according to the method reported by Remmal and al., 1993, also by Satrani and al., 2001. Because of the essential oil immiscibility with water and, therefore, to the cultural environment, an emulsification was realized thanks to an agar solution at 0.2%. It allowed to obtain, in the middle, a homogeneous distribution of essential oils and to make the higher maximum of compound/germ contact. Dilutions are prepared at 1/10^e, 1/25^e, 1/50^e, 1/100^e, 1/200^e, 1/300^e and 1/500^e in this agar solution.

In test tubes, containing each 13.5 ml of solid environment TSA (*Tryptic Soy Agar*) for bacteria, and the PDA (*Potato Dextrose Agar*) for fungi, sterilized at the autoclave during 20 min at 121°C and cooled at 45°C, we add aseptically 1.5 ml of each dilution so as to obtain the final concentrations of 1/100, 1/250, 1/500, 1/1000, 1/2000, 1/3000 and 1/

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5000 (v/v). We shake the tubes to disperse properly the essential oil in the cultural environment before pouring them into Petri dishes. Witnesses, containing the cultural environment and agar solution at 0.2% alone, are equally prepared.

This latter is presented in the form of culture broth of 24 hours for bacteria and in the form of a suspension in physiological water of spores resulting from a culture of 7 days in the PDA (*Potato Dextrose Agar*) for 3 fungi. The seeding is done, for these latters, by the fragments deposition of 1cm of diameter, taken from the periphery of a mycelia mat, and originating from a 7 days culture in the malt extract. The incubation is done at 37°C during 24 h for bacteria, and at 25°C during 7 days for fungi. Each test is repeated three times.

RESULTS AND DISCUSSION

Yield in essential oils of *Artemisia herba-alba* of five provenance

The yield of essential oils of *Artemisia herba alba* of the oriental of Morocco harvest obtained in June varies from $0.84 \pm 0.01\%$ to $2.03 \pm 0.002\%$. The best teneur in essence is obtained from the provenance of Oued Asla, followed by that of Oued Alkharroub, the reserve Aswiwinia, Oued Ogba and finally Hassi Al Abyad. Either of the respective rates of $2.03 \pm 0.002\%$, $1.61 \pm 0.001\%$, $1.15 \pm 0.083\%$, $0.99 \pm 0.017\%$, $0.84 \pm 0.017\%$.

The levels in essential oil of different provenances studied remain relatively high compared with those obtained by Imelouane and al., 2010 for the region of Taforalt (East of Morocco) and Ghanmi et al., 2010 for the region of Guercif, which are respectively 1% and 1.23%. This variation in the essential oil yields has been noted in the south of Spain in a study on the white wormwood crops in four different localities in which the yield ranged from 0.41% to 2.30%^[40]. It is also noted that the essential oil yields of the provenance Oued Asla, Oued Alkharroub and the reserve Aswiwinia are relatively high compared to some plants that are exploited industrially as a source of essential oils^[15].

This observed variation for the essential oil

yields of the *Artemisia herba alba* of five provenances of the oriental of Morocco can be attributed to climatic and edaphic conditions specific to each provenance. In fact, the sites of the north (Oued Al Kharrub and Oued Asla) are distinguished by a mild Mediterranean climate influenced by the mountains and the dams Mohammed V and Machraa Hammadi. The precipitations reach more than 400 ml on average per year. In contrast, the southern zone (reserve Aswiwinia, Oued Agba and Hassi Al Abyad) is influenced by a continental climate sensitive to Saharans factors. The rainfall does not exceed an average of 100 ml per year. In general, the soils of different sites are skeletal and stony.

Chemical map of the essential oils of the *Artemisia herba alba* of the five provenances

The chromatographic analyzes of the essential oils of the five provenances of the *Artemisia herba alba* of the oriental of Morocco are reflected in TABLE 1. The number of compounds identified differs from one provenance to another. Twenty two compounds for the provenance Oued Al-Khurub, twenty and a compound for that of Oued Asla, twenty compounds for the site of the reserve Aswiwinia. For the other two provenances of Hassi Al Abyad and Oued Ogba, the number of constituents detected in their essential oils is respectively 19 and 17 constituents.

The counting of chromatographic analyzes of the essential oils of five sources of white wormwood has shown their wealth in monoterpenes compounds (56%) compared to sesquiterpenes (20%).

The major constituents of the essential oil of *Artemisia herba alba* vary according to the source and reading of the results shows that the essential oils of the five origins are similar in quality, but different in quantity.

In fact, the essential oils of *Artemisia herba alba* of three sources: reserve Aswiwinia, Hassi Al Abyad and Oued Ogba are rich in α -thujone, a dominant compound to more than 40%. Hassi Alabyad has the highest percentage of this constituent with 48.91%, followed by Oued Aghba with 48.27% and finally reserve Aswiwinia with 40.19%. Furthermore, we note the presence of chrysanthenone with rates of

TABLE 1 : Chemical Composition of the essential oil of *Artemisia herba alba*

N°	IK	Constituants		P1	P2	P3	P4	P5
1	924	α -thujene	C ₁₀ H ₁₆	0,32	0,21	0,43	0,41	0,48
2	932	α -pinene	C ₁₀ H ₁₆	2,8	2,21	3,07	5,71	6,42
3	1002	α -phellandrene	C ₁₀ H ₁₆	1,14	1,14	0,78	0,69	0,85
4	1012	1-4 cineole	C ₁₀ H ₂₀	9,7	8,33	5,62	3,71	0,32
5	1062	Cis- sabinene hydrate	C ₁₀ H ₂₀ O ₂	0,26	0,3	-	-	-
6	1080	Artemisia alcohol	C ₁₀ H ₁₈ O	4,46	5,35	4,43	8,65	1,83
7	1101	α - thujone	C ₁₀ H ₁₆ O	40,19	48,91	48,27	24,18	17,52
8	1124	chrysanthenone	C ₁₀ H ₁₄ O ₂	29,15	26,64	27,7	43,35	48,11
9	1134	Iso-3-thujanol	C ₁₀ H ₁₈ O	0,14	0,16	0,17	0,17	1,74
10	1141	camphor	C ₁₀ H ₁₆ O	1,49	0,04	1,14	2,95	6,68
11	1147	Neoiso-3-thujanol	C ₁₀ H ₈ O	0,86	0,98	0,94	0,52	0,64
12	1164	3-thujanol	C ₁₀ H ₁₈ O	-	-	-	-	0,34
13	1169	Artemisyl acetate	C ₁₂ H ₂₀ O ₂	-	-	-	0,06	-
14	1174	Terpinene-4-ol	C ₁₀ H ₁₈ O	0,18	0,44	0,06	0,16	0,37
15	1214	Dihydro myrcenol acetate	C ₁₀ H ₂₀ O	0,42	0,33	-	0,38	0,06
16	1219	Cis-hydrate acetate de sabinène	C ₁₂ H ₂₀ O ₂	-	-	-	0,24	0,65
17	1228	Nor-davanone	C ₁₅ H ₂₄ O ₂	0,35	0,79	0,30	0,34	0,36
18	1235	Trans chrysanthenyl acetate	C ₁₂ H ₁₈ O ₂	0,79	1,12	0,8	0,37	0,35
19	1244	carvotanacetone	C ₁₀ H ₁₆ O	0,58	-	-	0,71	1,15
20	1277	Trans-ethyl chrysanthemumate	C ₁₂ H ₂₀ O ₂	1,54	1,43	1,56	0,84	0,57
21	1335	δ -elemene	C ₁₅ H ₂₂	2,99	0,17	3,94	1,8	1,14
22	1385	Trans acetate de myrtanol	C ₁₂ H ₂₀ O ₂	0,4	0,56	0,35	-	-
23	1429	Cis-thujopsene	C ₁₅ H ₂₄	-	-	-	0,61	-
24	1522	δ -cadinene	C ₁₅ H ₂₄	0,05	0,37	0,12	0,61	0,17
25	1537	α -cadinene	C ₁₅ H ₂₄	-	-	-	0,14	0,27
		Total %		96,32	99,48	99,7	96,6	83,34

P1: Dam Aswiwinia; P2: Hassi Alabyad; P3: Oued Ogba; P4: Oued Alkharroub; P5: Oued Asla; IK: indice de Kovalts; %: poucentage, -: absent

29.15% for reserve Aswiwinia, 27.7% for Oued Ogba and 26.64% for Hassi Alabyad. Other compounds are also identified, but at less important levels such as the 1-4 cineole, Artemisia alcohol, δ -elemene and α -pinene (TABLE 1).

In addition, the essential oils of two provenances of Oued Alkharroub and Oued Asla have shown very important rates in chrysanthenone with a percentage of 43.35% and 48.11%, respectively. Thus the presence of α -thujone which is as important as the previous compound with a rate of 24.18% for the provenance of Oued Alkharroub and 17.52% for the provenance of Oued Asla. We also note the presence of other minority constituents which are not devoid of importance like: α -pinene, Artemisia alcohol, camphor and 1-4cineole (TABLE 1).

According to these results, we have noted that the composition of the essential oil of *Artemisia herba alba* of the three provenances: Reserve Aswiwinia, Hassi Alabyad and Oued Ogba is dominated by the α -thujone followed by the chrysanthenone with percentages higher than the 40% these results show that α -thujone is the chemotype characteristic of the three provenances of the oriental of Morocco.

However, Imelouane et al., 2010 have noted the absence of α -thujone in the samples of the *Artemisia herba alba* of the region of Taforalt (east of Morocco). Ghanmi et al., 2010, were able to observe a low rate of 4.4% in α -thujone in the essential oils of the *Artemisia herba-alba* in the region of Guercif. Also, Benjilali et al., 1980, have already reported a

rial strains were inhibited by the essential oils of *Artemisia herb alba* Oued Ogba, Oued Alkharoub and Oued Asla. Only the bacterial strain *Escherichia coli* was inhibited at the low concentration of 1/1000 v/v related to the essential oil of Oued Alkharoub. As for the essential oil of Hassi Alabyad, *Staphylococcus aureus* has shown more resistance whereas *Bacillus subtilis* and *Micrococcus luteus* were inhibited from 1/250 and *Escherichia coli* from 1/500 v/v. In contrast, the strains *Bacillus subtilis* and *Micrococcus luteus* have been resistant to the essential oil of the reserve Aswiwinia.

As for the molds, they were all inhibited at the concentration 1/500 v/v of the essential oils of the five provenances. The most sensitive germ is *Aspergillus niger* whose growth has been inhibited at 1/1000 v/v for the essential oil from the reserve Aswiwinia.

Similarly the wood rot fungi have shown great sensitivity to the essential oil of *Artemisia herba alba* of five sources. The most sensitive fungi are *Coniophora puteana*, *Poria placenta* and *Gloeophyllum trabeum* whose growth has been inhibited to 1/3000 v/v of the essential oil from reserve Aswiwinia and at 1/2000 for the provenance Hassi Alabyad. It is also noted that *Coriolus versicolor* and *Poria placenta* were inhibited at 1/3000 v/v for the essential oil of Oued Alkharoub.

The essential oils of Oued Alkharoub and Oued Asla are more active against the bacteria while those of the reserve Aswiwinia and Hassi Alabyad are more effective against fungi of wood rot.

According to our results, we found that the wood rot fungi are more vulnerable to the essential oil of *Artemisia herba alba* than bacteria, which is also in agreement with the investigations of several authors who have shown that, for the essential oils of several aromatic and medicinal plants, fungi are more sensitive than bacteria^[17,30,45]. Some other work also show the antimicrobial power of the *Artemisia herba-alba* harvested in the regions of Taforal^[23] and Guercif^[18].

The strong antibacterial and antifungal activity of essential oils of *Artemisia herba-alba* seems mainly due to its richness in ketones terpene (α -thujone, chrysanthenone, camphor). In fact, these are

known for their antimicrobial activity^[14, 13, 35, 36, 37]. The rate in compounds terpenes in our essential oils and their interactions would be responsible for the significant inhibiting power against bacteria and fungi.

In general, the antimicrobial activity of essential oils of *Artemisia herba alba* can be explained not only by the major compounds but also by the synergy between all the volatile constituents even the minor ones. In fact, according to several studies^[20, 27, 43, 10, 47, 45] have reported that the reactions of synergy between the different compounds can be the origin of an activity much more than the predictable activity of the major compounds.

CONCLUSION

The results obtained in this work allow us to conclude that the yield, the chemical composition and antimicrobial activity of essential oils of *Artemisia herba alba*, collected in the regions of the east of Morocco, vary according to the harvesting regions.

Comparing the performance in essential oil of *Artemisia herba alba* of the five provenances studied, we found that the Oued Asla has provided the best performance with 2.03% compared to other provenances. The chromatographic and spectrometric analyzes have revealed the existence of two chemotypes one at α -thujone for the provenances of the reserve Aswiwinia, Hassi Alabyad and Oued Alkharoub and the other to chrysanthenone for the samples collected at Oued Ait Makhoulouf and Oued Asla provenances.

The bioassay data have shown that the essential oils of the eastern regions are effective against the bacteria, molds and wood rot fungi. These fungus were more vulnerable to the essential oils of the *Artemisia herba alba* from the east of Morocco. This set of data can be taken into account in any investigation on the possibilities for the exploitation of this essence in the areas of food industry, perfumery, pharmaceutical fields and wood preservation.

REFERENCES

- [1] R.P.Adams, Identification of essential oil compo-

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- nents by gas chromatography/mass spectrometry, 4th Edition, Allured Publishing Corporation, Carol Stream, IL, (2007).
- [2] A.Akrout; Etude des huiles essentielles de quelques plantes pastorales de la région de matmata (Tunisie), Institut des Régions Arides, **62**, 289-292 (1999).
- [3] Al J.Figuigui, M.Benjelloun, L.Elghadraoui, A.Zaim; Effet acridicide de deux plantes aromatiques et médicinales sur la survie des adultes de *Schistocerca gregaria*, AFPP – Dixième conférence internationale sur les ravageurs en agriculture Montpellier, (2014).
- [4] Al J.Shuneigat, Al S.Sarayreh, Al M.Qudah, Al Y.Sarairah, Al A.Qtaitat, Al I.Tarawneh; GC-MS analysis and antibacterial activity of the essential oil isolated from wild artemisia herba-alba grown in South Jordan, British Journal of Medicine & Medical Research, **5(3)**, 297-302 (2015).
- [5] R.Belhatab, L.Amor, J.G.Barroso, L.G.Pedro, A.C.Figueiredo; Essential oil from *Artemisia herba-alba* Asso grown wild in Algeria: Variability assessment and comparison with an update literature survey, Arabian Journal of Chemistry, **7**, 243–251 (2014).
- [6] A.Benabid; Flore et écosystème du Maroc, Evaluation et préservation de la biodiversité, Ibis Press, Paris, (2000).
- [7] B.Benjlali, H.Richard; Etude de quelques peuplements d'Armoise blanche du Maroc *Artemisia herba-alba*, Rivista Italiana E.P.P.O.S., 69-74 (1980).
- [8] B.Benjlali, S.Zrira; Plantes aromatiques et médicinales atouts du secteur et exigences pour une valorisation durable, Actes éditions Institut agronomique et vétérinaire Hassan-II Rabat Maroc, (2005).
- [9] D.Benjumea, S.Abdala, Hernandez F.Luis, Pérez P.Paz, Martín D.Herrera; Diuretic activity of *Artemisia thuscula*, An endemic canary species, J.Ethnopharmacol., **100**, 205-209 (2005).
- [10] S.Burt; Essential oils: Their antibacterial properties and potential applications in foods, Int.J.Food Microbiol., **94**, 223-253 (2004).
- [11] J.F.Clevenger; Apparatus for volatile oil determination: description of New Type Clevenger, Am Perf Ess Oil Review, 467-503 (1928).
- [12] V.Darias, L.Bravo, E.Barquín, Martín D.Herrera, C.Fraile; Contribution to the ethnopharmacological study of the Canary Island, J.Ethnopharmacol., **15**, 169-193 (1986).
- [13] H.J.D.Dorman, S.G.Deans; Antimicrobial agent from plants: antimicrobial activity of plant volatile oils, J.Appl.Microbiol., **88**, 308-316 (2000).
- [14] A.J.Duke; Phytochemical database, USDA-ARS-NGRL (ed), Beltsville Agricultural Research Center, Beltsville, Maryland, (1998).
- [15] P.Edward, E.Claus, E.T.Varro, R.B.Lyn; Pharmacognosy, 6th Edition, LEA and Febiger (ed): 184-187.EPPOS, **18**, 3-6 (1987).
- [16] El K.F.Massry, El A.H.Ghorab, A.Farouk; Antioxidant activity and volatile components of Egyptian *Artemisia judaica* L, Food Chem., **79**, 331-336 (2002).
- [17] P.Franchomme; L'aromatologie à visée anti-infectieuse, Phytomédecine, **1 et 2**, 25-45 (1981).
- [18] M.Ghanmi, B.Satrani, A.Aafi, M.R.Ismaili, H.Houti, El H.Monfalouti, K.H.Benchakroun, M.Aberchane, L.Harki, A.Boukir, A.Chaouch, Z.Charrouf; Effet de la date de récolte sur le rendement, La composition chimique et la bioactivité des huiles essentielles de l'armoise blanche (*Artemisia herba alba*) de la région de Guerçif (Maroc oriental), Phytothérapie, **8**, 295-301 (2010).
- [19] T.Guardia, A.O.Juarez, E.Guerreiro, J.A.Guzmán, L.Pelzer; Anti-inflammatory activity and effect on gastric acid secretion of dehydroleucodin isolated from *Artemisia douglasiana*, J.Ethnopharmacol., **88**, 195-198 (2003).
- [20] R.C.Gueldener, D.M.Wilson, A.Heidt; Volatile compounds inhibiting *Aspergillus flavus*, J.Agric.Food, **33**, 413-419 (1985).
- [21] M.Haouari, A.Ferchichi; Essential oil of *Artemisia herba-alba* from southern Tunisia, Molecules, **14**, 1585-1594 (2009).
- [22] R.P.Hartwig, C.Wilkinson; Mold and Insurance, Insurance Information Institute, **1(4)**, (2003).
- [23] B.Imelouane, El A.Bachiri, M.Ankit, K.Khedid, J.P.Wathelet, H.Amhamdi; Essential oil composition and antimicrobial activity of *Artemisia herba alba* Asso grown in Morocco, Banat's Journal of Biotechnology, **1(2)**, (2010).
- [24] U.J.Jung, N.I.Baek, H.G.Chung, M.H.Bang, J.S.Yoo, T.S.Jeong, K.T.Lee, Y.J.Kang, M.K.Lee, H.J.Kim, J.Y.Yeo, M.S.Choi, The anti-diabetic effects of ethanol extract from two variants of *Artemisia princeps* Pampanini, Mice Food and Chemical Toxicology, **45**, 10, 2022-2029 (2007).
- [25] A.Kadri, I.B.Chobba, Z.Zarai, A.Békir, N.Gharsllah, M.Damak, R.Gdoura; Chemical constituents and antioxidant activity of the essential oil from aerial parts of *Artemisia herba-alba* grown in Tunisian semi-

- arid region, *African Journal of Biotechnology*, **10**(15), 2923-2929 (2011).
- [26] K.S.Kim, S.Lee, Y.S.Lee, S.H.Jung, Y.Park, K.H.Shin, B.K.Kim; Anti-oxidant activities of the extracts from the herbs of *Artemisia apiacea*, *J.Ethnopharmacol.*, **85**, 69-72 (2003).
- [27] M.Kivanc, A.Akgul; Effect of some essential oil components on the growth of food borne bacteria and synergism with some food ingredients, *Flavour Fragrance J.*, **3**, 95-98 (1988).
- [28] S.Kordali, R.Kotan, A.Mavi, A.Cakir, A.Ala, Yildirim; Determination of the chemical composition and antioxidant activity of the essential oil of *Artemisia dracunculus* and of the antifungal and antibacterial activities of Turkish *Artemisia absinthium*, *A.dracunculus*, *Artemisia santonicum*, and *Artemisia spicigera* essential oils, *J.Agric.Food.Chem.*, **53**, 9452-8 (2005).
- [29] E.Kováts; Gas chromatographic characterization of organic substances in the retention index system, *Adv.Chromatogr.*, **1**, 229-247 (1965).
- [30] M.Lahlou; Methods to study phytochemistry and bioactivity of essential oils, *Phytother Res.*, **18**, 435-48 (2004).
- [31] B.M.Lawrence; A review of the world production of essential oils, *Perf, Flav*, **10**, 1-16 (1985).
- [32] E.D.McArthur; Sagebrush Ecosystem Symposium, US University, Logan, 14-22 (1979).
- [33] National institute of standards and technology, PC Version of the NIST/EPA/NIH Mass Spectra Library, (2008).
- [34] M.Negahban, S.Moharrampour, F.Sefidkon; Fumigant toxicity of essential oil from *Artemisia sieberi* Besser against three stored-product insects, *J.Stored Products Res.*, **43**, 2, 123-128 (2007).
- [35] P.L.Olliaro, R.K.Haynes, B.Meunier, Y.Yuthavong; Possible modes of action of the artemisinin-type compounds, *Trends Parasitol*, **17**, 122-6 (2001).
- [36] M.Oussalah, S.Caillet, L.Saucier, M.Lacroix; Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: *E.coli* O157:H7, *Salmonella thyphimurium*, *Staphylococcus aureus* and *Listeria monocytogenes*, *Food Control*, **18**, 414-420 (2007).
- [37] O.Pelkonen, K.Abass, J.Wiesner; Thujone and thujone-containing herbal medicinal and botanical products: Toxicological assessment, *Regulatory Toxicology and pharmacology*, **65**, 100-107 (2013).
- [38] G.D.Penanster; La santé des forêts : Une préoccupation majeure, Un suivi efficace, *Bulletin De Liaison Des Sylviculteurs Bretons*, **65**, (2008).
- [39] A.Remmal, Tantaoui A.Elaraki, T.Bouchikhi, K.Rhayour, M.Ettayebi; Improved method for determination of antimicrobial activity of essential oils in agar medium, (1993).
- [40] S.Salido, L.R.Valenzuela, J.Altarejos, M.Nogueras, A.Sanchez, E.Cano; Composition and infraspecific variability of *Artemisia herba-alba* from southern Spain, *Biochemical Systematics and Ecology*, **32**, 265-277 (2004).
- [41] B.Satrani, A.Farah, M.Fechtal, M.Talbi, M.Blaghen, A.Chaouch; Composition chimique et activité antimicrobienne des huiles essentielles de *Saturja calamintha* et *Saturja alpina* du Maroc, *Ann Fals Exp.Chin.*, **94**(956), 241-50 (2001).
- [42] W.N.Setzer, B.Vogler, J.M.Schmidt, J.G.Leahy, R.Rives; Antimicrobial activity of *Artemisia douglasiana* leaf essential oil, *Fitoterapia*, **75**, 192-200 (2004).
- [43] J.D.Thomson, J.C.Chalchat, A.Michet, Y.B.Linhart, B.Ehlers; Qualitative and quantitative variation in monoterpene co-occurrence and composition in the essential oil of *Thymus vulgaris* chemotypes, *J.Chem.Ecol.*, **29**(4), 859-880 (2003).
- [44] M.Tilaoui, Ait H.Mouse, A.Jaafari, R.Aboufatima, A.Chait, A.Zyad; Chemical composition and antiproliferative activity of essential oil from aerial parts of a medicinal herb *Artemisia herba-alba*, *Revista Brasileira de Farmacognosia Brazilian Journal of Pharmacognosy*, **21**(4), 781-785 (2011).
- [45] Viuda M.Martos, Ruiz Y.Navajas, Fernandez J.lopez, Perez J.A.Alvarez; Antibacterial activity of different essential oils obtained from spices widely used in Mediterranean diet, *Int.J.Food Sci.Technol.*, **43**, 526-531 (2008).
- [46] Y.Yin, F.Y.Gong, X.X.Wua, S.Yuna, Y.H.Lia, T.Chena, Q.Xu; Anti-inflammatory and immunosuppressive effect of flavones isolated from *Artemisia vestita*, *J.Ethnopharmacol.*, **120**, 1, 1-6 (2008).
- [47] A.Zahiri, D.Baudoux; Huiles essentielles chémotypées et leurs synergies: Aromathérapie scientifique, Luxembourg: Edition Inspir Développement, (2005).
- [48] A.Zaim, El L.Ghadraoui, A.Farah; Effets des huiles essentielles *Artemisia herba-alba* sur la survie des criquets adultes d'*Euchorthippus albolineatus* (Lucas, 1849), *Bulletin de l'institut scientifique, Rabat, section Sciences de la Vie*, **34**(2), 127-133 (2012).