IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 11, Issue 6 Ver. IV (Nov. - Dec.2016), PP 40-45
www.iosrjournals.org

Phytochemical and Antibacterial Activity of The Essential Oil of *Artemisia herba-alba* from Morocco

Zineb Benziane Ouaritini [1]*, Marwa Chraibi[2], Kawtar Fikri Benbrahim[2], Rabab Ez-zriouli, Smahane Boukhira[3], Najoua Darkaoui[1], and El Houssine Derwich [4]

[1]*Biotechnology Laboratory and Natural Resources Preservation, Faculty of Sciences Dhar El Mehraz, Sidi Mohamed Ben Abdellah University, Fez 30000, Morocco.*
[2]*Laboratory of microbial biotechnology, Sidi Mohamed Ben Abdellah University, Faculty of Science and Technology, Fez 30000, Morocco.*
[3]*Neuroendocrinology laboratory and nutritional climatic environment , Sidi Mohamed Ben Abdellah University, Faculty of Sciences Dhar El Mehraz, Sidi Mohamed Ben Abdellah University, Fez 30000, Morocco.*
[4]*Agri Laboratory and Food Sanitary, Faculty of Sciences Dhar El Mehraz, Sidi Mohamed Ben Abdellah University, Fez 30000, Morocco.*

Abstract: *The extraction of essential oils from the aerial part of Artemisia herba-alba is obtained by hydrodistillation and analyzed by gas chromatography coupled with mass spectrometry (GC/MS) for determining their chemical composition. Their antibacterial activity was studied in vitro on two bacterial strains: Salmonella typhi and Staphylococcus aureus. The essential oil yields of the studied plant were 0.21 for fresh aerial part and 0.25% for dry aerial part. The major component of the essential oil from dry aerial part of Artemisia herba-alba was the α-terpineol (47.33%), myrtenyl acetate (22.22%) and chrysanthenyl acetate (20.55%). While the major compounds of essential oil from fresh aerial part was the Borneol (35.68%), α-terpineol (33.36%), and δ-cadinol (12.07%). The bacterial strains tested were found to be sensitive to essential oils studied and showed a very effective bactericidal activity with minimum inhibitory concentration (MIC) ranging from 0.125 to 0.5 mg/ml.*

Keywords: *Artemisia herba-alba, fresh and dry aerial part, essentiel oil yields, essentiel oil composition, GC/MS, Borneol, α-terpineol.*

I. Introduction

Morocco exports the equivalent of 250 million dirhams in medicinal and aromatic plants to the US and the European Union. Essential oils relate to it only about 165 million dirhams and is estimated that this potential can be doubled. *Artemisia* and *Rosmarinus* are the two species dominating the aromatic and medicinal landscape and are subject to significant business transactions, for the essential oil extracted from the plants (Benjilali and Zrira, 2005; USAID, 2005).

The essential oils in *Artemisia herba-alba* are subject to several studies in Morocco (Benjilali et al., 1982 ; Lawrence, 1993); in Spain (Salido et al., 2004) and Algeria (Vernin et Merad, 1994 ; Vernin et al., 1995). In fact, the essential oils contained in the leaves of *Artemisia herba-alba* are known for their regulatory properties on the menstrual cycle and as a remedy for many diseases such as diabetes, bronchitis, abscesses and diarrhea (Akrout et al., 2001).

The constituents of the essential oils are active against a wide range of bacteria, yeasts and fungi (Kuda et al., 2004 ; Derwich et al., 2009 ; Derwich et al., 2010). They have a very wide spectrum action and inhibit the growth of bacteria as well as mold and yeasts. Their antimicrobial activity is mainly based on their chemical composition, and in particular the nature of their major volatile compounds. In vitro, the microbicidal effect of certain essential oils has even been found higher than that of antibiotics. Indeed, it is recognized that the antimicrobial activity of essential oils is ranked in descending order according to the nature of their major compounds: Phenol> alcohol> aldehyde> ketone> oxide> hydrocarbons> esters (Franchomme, 1981 ; Akrout et al., 2001 ; Baser et al., 2002).

Artemisia herba-alba is used against several diseases including enteritis and intestinal disorders. The essential oil of this plant was tested against various bacteria which cause intestinal disorders, as well as in rabbits, to determine the antispasmodic activity of this extract. The essential oil of *Artemisia herba-alba* showed antibacterial activity against several bacteria such as *Escherichia coli*, *Shigella sonnei* and *Salmonella typhi* (Setzer et al., 2004). In addition to diabetes, the aqueous extract of *Artemisia herba-alba* is traditionally used in Jordan as an antidote against the venoms of several types of snakes and scorpions (Twaij and Al-Badr, 1988) and in North Africa to heal bronchitis, abscess, diarrhea, and as an anthelmintic (Gharabi et al., 2008).

The essential oils of *Artemisia herba-alba* also presents an antioxidant activity (El-Massry et al., 2002 ; Kim et al., 2003 ; Kordali et al., 2005), anti-inflammatory (Guardia et al., 2003) and insecticidal activitie (Zain et al., 2012).

In the light of this work we have determined, the chemical composition, the yield and antibacterial activity of essential oil of the dry and fresh aerial part of *Artemisia herba-alba.*

II. Materials and Methods

Plant Material

Artemisia herba-alba is a plant belonging to the Asteraceae family, which grows in the Oriental Morocco rif, Middle Atlas, High Atlas, Anti-Atlas and the Saharan Atlas. In this work, we studied the essential oils of the aerial parts of *Artemisia herba-alba* collected according to Afnor norm in Middle Atlas from the Guigou region in May 2015 (Afnor, 2000), this area is a Moroccan rural village in the province of Fez Boulemane region located at 35 km at southeast of Ifrane.

Essential oil Extraction

The fresh aerial part of *Artemisia herba-alba* was kept at 4°C in refrigerator, while the dried aerial part of *Artemisia herba-alba* were shade dried (25 days) at room temperature (23-24°C) until crisp and immediately hydro-distilled for 3h according to the method recommended in the British Pharmacopoeia (Adams, 2007). The oil was dried over anhydrous sodium sulfate and stored in the refrigerator (4°C).

Gas Chromatography-mass Spectrometry Analysis (GC/MS)

GC/MS analyses were performed on a Thermo Fischer capillary gas chromatograph directly coupled to the mass spectrometer system (model GC ULTRA S/N 210729). HP-5MS non polar fused silica capillary column (60 m × 0,32 mm, 0,25 µm film thickness) was used under the following conditions : oven temperature program from 40°C (2min) to 260°C at 2°C/min and the final temperature kept for 10 min ; injector temperature, 250°C ; carrier gas He, flow rate 1 ml/min ; the volume of injected specimen was 1 µl of diluted oil in hexane ; splitless injection technique ; ionization energy 70eV, in the electronic ionization mode ; ion source temperature 200°C ; scan mass range of m/z 40-650 and interface line temperature 300°C. The constituents of essential oil were identified in comparison with their specters of mass with those gathered in a library of (NIST-MS) type and with those reported in the literature (Pala-Paul et al., 1999 ; Derwich et al., 2009).

Antibacterial Activity

In recent years , there has been target interest in biologically active constituents, isolated from plant species for the elimination of pathogenic micro-organisms, due to the resistance that these micro-organisms have built against antibiotics (Essawi and Srour, 2000) because the plant constituents are ecologically safe compounds (Lee et al., 2005).

The essential oils from fresh and dried aerial parts were screened against one gram-negative bacteria (*Salmonella typhi*) and another gram-positive one (*Staphylococcus aureus*). The minimal inhibitory concentration (MIC) was determined only with micro-organisms that displayed inhibitory zones. MIC was determined by dilution of the essential oils in dimethyl sulfoxide (DMSO) and pipetting 0.01 ml of each dilution into a filter paper disc. Dilutions of the studied essential oils within a concentration range of 0.08-1.56 mg/ml were also carried out. MIC was defined as the lowest concentration that inhibited the visible bacterial growth (NCCLS, 2006). The bacterial plates were incubated at 37°C and the zone of inhibition measured in mm after 24h, 48h and 72h of growth. A control experiment was set up by using an equal amount of sterile distilled water in place of different extract concentrations. Many screening reports, using disc diffusion and dilution techniques, have established an antimicrobial activity of *Artemisia herba-alba* (Dorman and Deans, 2000 ; Baser et al., 2002).

Determination of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC)

The minimal inhibitory concentration was determined in 96 well-microplate using the microdilution assay according to the protocol previously described by Chraibi et al. (2016). Bacteriological agar at 0.15 % (w/v) was used as an emulsifier of the essential oil in the culture medium. Hence, the essential oil was serially diluted in Muller Hinton broth supplemented with agar to obtain final concentrations ranging between 8% and 0.007% (v/v). The 12^{th} well was considered as growth control (free-essential oil control). Then, 50 µL of bacterial inoculum, previously prepared and adjusted to 0.5 McFarland, were added to each well to reach the final concentration of 10^6 CFU/mL. After incubation at 37° C for 24 h, 10 µL of resazurin were added to each well as bacterial growth indicator. After further incubation at 37 °C for 2 h, the bacterial growth was revealed by coloration changing from purple to pink (Iscan et al., 2002). Experiments were carried out in duplicate.

III. Results and Discussion

Chemical composition

The constituents of the fresh and dry aerial part's essential oils *Artemisia herba-alba* from Morocco are listed in order of their elution on the HP-5MS column (Figures 1, 2).

In essential oils of fresh aerial part, five most abundant volatile compounds were identified (Table 1). Borneol (35.68%) was found to be the major compound followed by α-terpineol (33.36 %) and γ-Cardinol (12.07%), while the Myrtenyl acetate and Eugenol were found in minority. The most abundant components found in the dry aerial part were α-terpineol (47.33%), Myrtenyl acetate (22.22%) and Chrysanthenyl acetate (20.55), Camphor and Eugenol were less abundant. The essential oil yield of *Artemisia herba-alba* collected from Guigou (Morocco) was 0.25% for dry aerial part and 0.21% of fresh aerial part. It is relatively higher than that of other plants industrially exploited as a source of essential oils such as *Pistacia vera* (0.1 %) (Tsokou et al., 2007). However, the yield obtained remains relatively low compared to that of other *Artemisia* species, such as *A. haussknechtii* (2.1% [mL/100 g]) (Jalali et Sereshti, 2007) and *Artemisia sieberi* (1.7% [mL/100 g]) (Ghasemi et al., 2006).

The chemical compostion of *Artemisia* essentials oils of Guigou (Morocco) is different from essential oils of Matmata (Tunisia) which consists mainly on the α-thujone (43,85%), the trans-sabinyl acetate (17.46%) and the β-thujone (10.10%) accompanied by the 1,8-cineol (3.3%), chrysanthenone (2.32%) and the chrysanthenyl acetate (3.93 %) (Akrout, 1999). It is widely different from that of the M'sila region (Algeria) which is dominated by Camphor (19.4%), trans-Pinocarveol (16.9%), Chrysanthenone (15.8%) and β-thujone (15%) (Charchari et al., 1996). As for essential oil of *Artemisia herba alba* in Jordan, it has the α and β-thujones as major compounds (16.2 and 8.5% respectively), followed by santolina alcohol (13.0 %), Artemisia ketone (12.4%), acetate of trans-sabinyl (5.4%), D-germacrene (4.6%), α-eudesmol (4.2%) and acetate of caryophyllene (5.7%) (Hudaib and Aburjai, 2006). Previous studies have shown that camphor is the main component of *Artemisia herba-alba* in Algeria, Spain and Israel with a percentage between 15 and 68% (Feuerstein et al., 1988 ; Vernin et al., 1995 ; Fleicher et al., 2002). Other studies have revealed the presence of other major compounds such as acetate of the α-thujone (25,6 to 40,9%) (Boutekedjiret et al., 1992 ; Lawrence, 1995 ; Fleisher et al., 2002), β-thujone (44%) and davanon (18,1 to 51,2%) (Satrani et al., 2001), the chrysanthenone (54.5%) (Boutekedjiret et al., 1992), 1,8-cineole (3-50%) (Feuerstein et al., 1986 ; Salido et al., 2004), the cis-chrysanthenol (24.5 to 30%) (Feuerstein et al., 1988) or acetate cis-chrysanthenyl (69%) (Fleisher et al., 2002). The presence of davanon in samples from Spain was also mentioned (Salido et al., 2001 ; Salido et al., 2004). All this shows that the chemical composition of the essential oil of *Artemisia herba-alba* varies depending on its place of harvest.

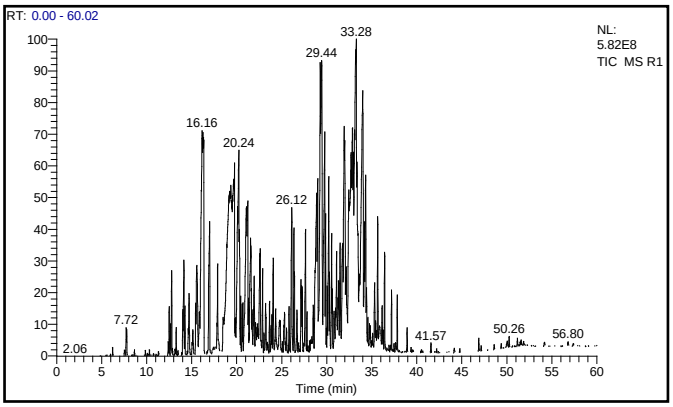

Fig. 1: Chromatogram of dry aerial part from *Artemisia heba-alba*

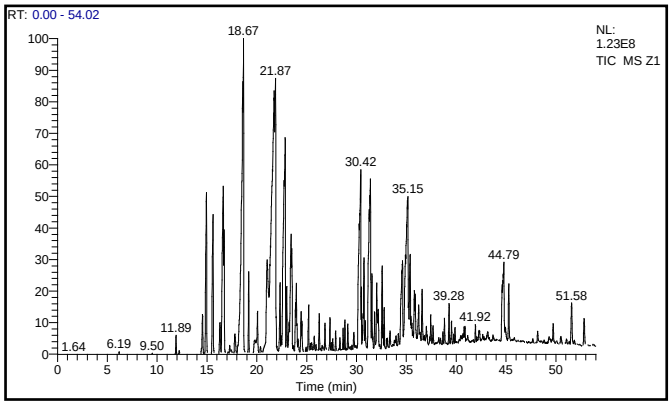

Fig. 2: Chromatogram of fresh aerial part from *Artemisia heba-alba*

Table 1: Chemical composition of dry aerial part's essential oil from *Artemisia heba-alba*

Peak	*RT (min)	Air %	Compound
1	16,16	8.82	Camphor
2	20,24	47.33	α- terpineol
3	26,12	20.55	Chrysanthenyl acetate
4	29,44	22.22	Myrtenyl Acetate
5	33,28	1.08	Eugenol

Table 2: Chemical composition of fresh aerial part E.O. from *Artemisia heba-alba*

Peak	*RT (min)	Air %	Compound
1	18.67	35.68	Borneol
2	21.87	33.36	α-Terpineol
3	30.42	9.19	Myrtenyl acetate
4	35.15	9.7	Eugenol
5	39.28	12.07	δ-cadinol

Table 3: Comparison of the chemical composition of essential oils of dry and fresh aerial parts of *Artemisia herba-alba*

	Compound	Essentiel oil yields (%)
Fresh aerial part from *Artemisia heba-alba*	Borneol α- terpineol Myrtenyl acetate Eugenol δ- cadinol	0.25 %
Dry aerial part from *Artemisia heba-alba*	Camphor α- terpineol Chrysanthenyl acetate myrtenyle Acetate de Eugenol	0.21 %

Antibacterial activity

The essential oil extracted from dry and fresh aerial part from *Artemisia herba-alba* was used in the present study to investigate their antibacterial potential. One gram-positive bacteria (*Staphylococcus aureus*) and a gram-negative one (*Salmonella typhi*) were used. The results obtained and screening of antibacterial activity of essential oil of dry and fresh aerial part from *Artemisia herba-alba* are summarized in (Table 4).

Table 4: Antibacterial activity of dry and fresh aerial parts from Moroccan *Artemisia heba-alba*

Essential oils	Dry aerial part		fresh aerial part	
	S. aureus	*S. typhi*	*S. aureus*	*S. typhi*
*Inhibition zone diameters: mm (Disc diffusion assay)	25 12	8 8	13 11	7 8
**MIC (mg/ml)	0.125 0.25	0.125 0.125	0.125 0.5	0.5 0.25

*Disc diameter 6 mm average of two consecutive trials
**MIC: Minimal Inhibitory Concentration, concentration range: 0.125-0.5 mg/ml.

The data indicated that *Staphylococcus aureus* was the most sensitive tested strain to the oil of dry aerial part with the highest inhibition zone diameter (25 mm). The dry aerial part was, in general found to be

more active against tested *Staphylococcus aureus* bacteria with inhibition zone diameters of 25-12mm. Very low activities were observed against *Salmonella typhi* with inhibition zones of 7-8mm.

Tested essential oils were found to be active against *Staphylococcus aureus* at a minimal inhibitory concentration (MIC) of 0.125-0.25 mg/ml for the dry aerial part's essential oil and 0.125-0.5 mg/ml for the fresh one. Concerning *Salmonella typhi*, the essential oil from *Artemisia*'s dry aerial part was found to be more active; in fact this tested essential oil showed MIC values of 0.125 and 0.25-0.125 respectively for *Salmonella typhi* and *Staphylococcus aureus*. The component of this oil, α-terpineol, has been known to exhibit antimicrobial activity against several bacterial strains *(Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus, Staphylococcus intermedius* and *Bacillus subtilus* (Sivropoulou et al., 1997). The antimicrobial activities, in general have been mainly explained through terpenes with aromatic ring and phenolic hydroxyl groups able to form hydrogen bonds with active sites of the target enzymes, although other active terpenes, as well as alcohols, aldehydes and esters can contribute to the overall antimicrobial effect of essential oils (Belleti et al., 2004). Pinene-type monoterpene hydrocarbons (α-pinene-type and β-pinene) are well known chemical having antimicrobial potentials (Dorman and Deans, 2000). Furthermore, enantiomers of α-pinene, β-pinene and limonene have a strong antibacterial activity (Magiatis et al., 1999 ; Filipowicz, 2003). The antimicrobial activity of essential oils is known to be beneficial in the treatment of different diseases.

IV. Conclusion

The present study was conducted to investigate the composition of essential oil of aerial part from *Artemisia herba-alba* from Morocco and in vitro evaluation of its antibacterial activity. The essential oil obtained from dry and fresh aerial part was characterized by GC-MS. Five most abundant volatile compounds were identified, Borneol (35.68%) were found to be the major compound followed by α-terpineol (33.36 %) and γ-Cardinol (12.07%), while the Myrtenyl acetate and Eugenol were in minority. The most abundant components found in the dry aerial part were α-terpineol (47.33%), Myrtenyl acetate (22.22%) and Chrysanthenyl acetate (20.55). Camphor and Eugenol are less abundant. The bacterial gram-negative tested strain (*Salmonella typhi*) and the Gram-positive tested one (*Staphylococcus aureus*) were found to be sensitive to essential oils studied and showed a very effective bactericidal activity with minimum inhibitory concentration (MIC) ranging from 0.125 - 0.25 for dry and 0.125 to 0.5 mg/ml for fresh aerial part's essential oils.

References

[1] Adams R.P. Identification of essential oil components by gas chromatography/mass spectrometry. *Allured Publ. Corp* 2007; 4: 69-351.

[2] Afnor. Huiles essentielles. Echantillonnage et méthodes d'analyse (tome 1) - Monographies relatives aux huiles essentielles 2000; (tome 2. volumes 1 et 2) mars.

[3] Akrout A. Etude des huiles essentielles de quelques plantes pastorales de la région de Matmata (Tunisie). Institut des Régions Arides 1999; 62: 289-92.

[4] Akrout A., Chemli R and Cherif I. Analysis of the essential oil of *Artemisia campestris* L. *J. Flav. Fragr* 2001; 16: 337-9.

[5] Baser K.H.C, Demirci B and Demirci F. Composition and antimicrobial activity of the essential oil of *Achillea multifida*. *Planta Med* 2002; 68(10): 941-3.

[6] Benjilali B., Sarris J and Richard H. Nouveaux chémotypes d'*Artemisia herba-alba*. *Sci. Aliment* 1982; 2: 515-27.

[7] Benjilali B and Zrira S. Plantes aromatiques et médicinales atouts du secteur et exigences pour une valorisation durable. Actes éditions Institut agronomique et vétérinaire Hassan-II Rabat Maroc 2005.

[8] Boutekedjiret C., Charchari S and Belabess R. Contribution à l'étude de la composition chimique de l'huile essentielle d'*Artemisia herba-alba* Asso. *Rivista-Italiana-EPPOS* 1992; 3: 39-42.

[9] Charchari S., Dahoun A., Bachi F and Benslimani A. In vitro antimicrobial of essential oils of *Artemisia herba-alba* and *Artemisia judaica* from Algeria. *Rivista-Italiana-EPPOS* 1996; 18: 3-6.

[10] Chraibi, M., Fikri-Benbrahim, K., Ou-yahyia, D., Balouiri, M., and Farah, A. Radical scavenging and disinfectant effect of essential oil from Moroccan *Mentha pulegium*. *Internat. J. Phar. Pharmac. Sci.* 2016; *8*(9): 116-119.

[11] Clevenger F. Apparatus for volatile the determination of volatile oil. J. *Am. Pharm Asso* 1928; 17, 346.

[12] Derwich E., Benziane Z and Boukir A. Chemical Composition and Insecticidal Activity of Essential Oils of three Plants Artemisia sp: *Artemisia herba-alba, Artemisia absinthium* and *Artemisia pontica* (Morocco). *Electronic J. Environ. Agric. Food Chem.* 2009 a; 8(11): 1202-1211.

[13] Derwich E., Benziane Z and Boukir A. Chemical Composition and Antibacterial Activity of Leaves Essential Oil of *Laurus nobilis* from Morocco. *Australian J. Basic Appl. Sci.* 2009 b; 3(4): 3818-3824.

[14] Derwich E., Benziane Z and Boukir A. Antibacterial activity and Chemical Composition of the leaf essential oil of *Mentha rotundifolia* from Morocco. *Electronic J. Env. Agric. Food Chem. 2010*. 9(1): 19-28.

[15] Dorman H.J.D and Deans S.G. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *J. Appl. Mic.* 2000; 88: 308-316.

[16] El-Massry K.F., El-Ghorab A.H and Farouk A. Antioxidant activity and volatile components of Egyptian *Artemisia judaica* L. *Food Chem* 2002; 79: 331-336.

[17] Essawi T and Srour M. Screening of some Palestinian medicinal plants for antibacterial activity. *J. Ethnopharmacol* 2000; 70: 343-349.

[18] Feuerstein I., Danin A and Segal R. Constitution of the essential oil from an *Artemisia herba-alba* population of Spain. *Phytochem* 1988. 27(2): 433-4.

[19] Feuerstein I., Muller D., Hubert K., Danin A and Segal R. The constitution of the essential oils from *Artemisia herba-alba* populatiosn of Israel and Sinai. *Phytochem* 1986; 25(10): 2343-7.

[20] Fleisher Z., Fleisher A and Nachbar R.B. Chemovariation of *Artemisia herba-alba* Asso. Aromatic plants of the Holy Land and the Sinai. Part XVI. *J. Essential Oil Res.* 2002; 14(3): 156-160.

[21] Franchomme P. L'aromatologie à visée anti-infectieuse. Phytomédecine 1981; 1: 25-47.

[22] Ghasemi E., Yamini Y and Bahramifar N. Comparative analysis of the oil and supercritical CO_2 extract of *Artemisia sieberi*. *J. Food Eng.* 2006. 79(1): 306-11.

[23] Ghrabi Z Sand RL. Artemisia herba-alba. A guide to Medicinal Plants in North Africa 2008; 49.

[24] Guardia T., Juarez A.O., Guerreiro E., Guzman J.A and Pelzer L. Anti-inflammatory activity and effect on gastric acid secretion of dehydroleucodin isolated from *Artemisia donglasiana. J. Ethnopharmacol* 2003; 88: (195-198).

[25] Guven K., Celik S and Uysal L. Antimicrobial activity of *Centaurea* species. *Pharmac. Biol.* 2005; 43: 67-71.

[26] Hudaib M.M and Aburjai T.A. Composition of the essential oil from *Artemisia herba-alba* grown in Jordan. *J. Ess. Oils Res.* 2006 18(3): 301-4.

[27] Iscan G., Demirci F., Kirimer N., Kurkcuoglu M and Baser K. Antimicrobial screening *Mentha piperita* essential oil. *J. Agric. Food. Chem* 2002; 50 : (3943-3946).

[28] Jalali H.M and Sereshti H. Determination of essential oil components of *Artemisia haussknechtii* Boiss. Using simultaneous hydrodistilllation-static headspace liquid phase microextraction-gas chromatography mass spectrometry. *J. Chromatogr A* 2007; 1160 (1-2): 81-9.

[29] Kim K.S., Lee S., Lee Y.S., Jung S.H., Park Y., Shin K.H and Kim B.K. Anti-oxidant activities of the extracts from the Herds of *Artemisia herba-alba. J. Ethnopharmacol* 2003; 85 : 69-72.

[30] Koneman E.W., Allen S.D., Janda W.M,. Schreckenberger P.C and. Inn W.C. Color atlas and textbook of diagnostic microbiology haladelphia. *Lippincott-Raven Publ* 1997; 13: 785-856.

[31] Kordali S., Kortan R., Mavi A., Cakir A., Ala A and Yildirim A. 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*, *Artemisia drunculus*, *Artemisia santonicum* and *Artemisia spicigera* essential oils. *J. Agric. Food. Chem* 2005; 53 : 9452-9458.

[32] Lawrence B.M. Armoise oil. Natural flavor and fragrance materials. In: Perfumer and Flavorist (Ed.), Essenential Oils 1988-1991. Allured Publishing Corporation, Carol Stream, II 1993; 52-4.

[33] Lee H.C., Cheng S.S and Chang S.T. Antifungal property of the essential oils and their constituents from *Cinnamomum osmophloeum* leaf against tree pathogenic fungi. *J. Sci. Food Agric.* 2005; 85(7): 2047-2053.

[34] NCCLS. National committee for clinical laboratory standards. Methods For dilution Antimicrobial Susceptibility Tests for Bacteria that Grow aerobically, 7th ed, Approved Standard M7-A7, Wayne 2006; Pennsylvania.

[35] Pala-Paul, J., Petrez-Alonso M.J. Velasco-Negueruela A. Ramos-V. zquez, P. Gomez-Contrera F and Sanz J. Essential oil of *Santolina rosmarinifolla* L. ssp. *rosmarinifolia*: first isolation of capillene, a diacetylene derivative. Flav. Fragr. J 1999; 14:131-134.

[36] Salido S, Valenzuela L.R and Altarejos J. Composition and intraspecific variability of *Artemisia herba-alba* from southern Spain. *Biochem. Syst Ecol.* 2004; 32:265-77.

[37] Satrani B., Farah A., Fechtal M. Composition chimique et activité antimicrobienne des huiles essentielles de *Satureja calamintha* et *Satureja alpina* du Maroc. *Ann. Fals. Exp. Chim* 2001 ; 94(956) : 241-50.

[38] Setzer W.N., Vogler B., Schmidt J.M., Leahy J.G and Rivers R. Antimicrobial activity of *Artemisia douglasiana* leaf essential oil. *Fitoterapia* 2004; 75: 192-200.

[39] Tsokou A., Georgopoulou K., Melliou E., Magiatis P and Tsitsa E. Composition and Enantiomeric Analysis of the Essential Oil of the Fruit and the Leaves of *Pistacia vera* from Greece. *Molecules* 2007; 12: 1233-1239.

[40] Twaij H.A and Al-Badr A. Hypoglycaemic activity of *Artemisia herba-alba*. *J. Ethnopharmacol* 1988; 24 (2-3): 123-126.

[41] USAID. Filière des plantes aromatiques et médicinales, note de synthèse. Chemonics Int. 2005; Inc. 608(5): 43-01.

[42] Vernin G and Merad L.O. Mass spectra and Kovats indices of some new cis-chrysantenyl esters found in the essential oil of *Artemisia herba-alba*. *J. Ess. Oils Res* 1994. 6(4): 437-48.

[43] Vernin G, Merad O, Vernin G.M.F., Zamkotsian R.M and Parkanyi C. GC-MS analysis of *Artemisia herba-alba* Asso essential oil from Algeria. *Dev. Food. Sci* 1995; 37:147-205.

[44] Zaim A., El Ghadraoui L and Farah A., Effets des huiles essentielles d'*Artemisia herba-alba* sur la survie des criquets adultes d'*Euchorthippus albolineatus* (Lucas, 1849). *Bulletin de l'institut scientifique* Rabat 2012; 34(2): 127-133.