



ISSN: 0975-833X

RESEARCH ARTICLE

CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF *ARTEMISIA HERBA ALBA HUGUEII* ESSENTIAL OIL FROM SOUTH OF MOROCCO

*Majdouli Karima, Soro N'Dédianhoua K., Elazzouzi Hanane, Benmachou Joihara, Drioiche Aziz and Zaïr Touriya

Department of Chemistry, Laboratory of Bioactive Molecules and Environment, University of Sciences Moulay Ismail, BP 11201. Zitoune, Meknès, Morocco

ARTICLE INFO

Article History:

Received 09th January, 2015
Received in revised form
25th February, 2015
Accepted 27th March, 2015
Published online 28th April, 2015

Key words:

Artemisia herbaalba,
Chemical composition,
Antibacterial activity,
Cis-thujone,
Camphor,
Phytochemical screening,
CMI.

ABSTRACT

Bacteria are often involved in cases of food poisoning and the abusive use of chemical antibacterial agents in medication leads to selection of resistant bacterial strains. Indeed essential oils appear to be a good alternative to antibiotics' use. Empirically known for centuries, their anti-infective efficiency has been now scientifically demonstrated in vitro and in vivo. This work aims to study chemical composition and antibacterial power of *Artemisia herba alba hugueii*'s essential oils (EO). EO are obtained by hydrodistillation of *A. herba alba hugueii* buds harvested in June 2012 from TATA region (southern Morocco). EO content is about 4.2 ml per 100 g of dry matter. Chemical composition of *A. herba alba hugueii*'s EO was determined by gas chromatography coupled with mass spectrometry (GC / MS). Forty-five compounds were identified with cis-thujone (35.06%), camphor (32.79%) and trans-thujone (6.83%) as majority ones. Oxygenated monoterpenes (85%) constitute the most abundant group among the whole identified compounds (91.72%). Phytochemical screening of *A. herba alba hugueii*'s leaves was performed by staining and precipitation reactions. These tests revealed the high content of phenolic compounds (flavonoids and tannins), sterols and triterpenes. These secondary metabolites have a wide range of biological activities and they probably are at the basis of *A. herba alba*'s medicinal properties. Antibacterial activity of essential oils was tested in vitro on five clinical bacterial isolates. Evaluation of antibacterial power was performed by disk-diffusion method and minimum inhibitory concentration (MIC) was determined by macrodilution method on liquid medium. The study of antibacterial effect showed that *A. herba alba hugueii*'s EO has an inhibitory effect against nine of the fifteen tested strains. This bioactivity is mainly due to the richness of this essence in cis-thujone and camphor known to be effective against microbial agents.

Copyright © 2015 Majdouli Karima et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

Infectious diseases constitute a serious public health problem in developing countries where they are the main cause of high mortality rates and in industrialized countries where misuse of antibiotics led to broad-spectrum resistance of bacteria (Aafi et al., 2002). Therefore, the use of natural substances, especially medicinal and aromatic plants (MAP) becomes a very important and interesting field to search new and more effective antibacterial products. Among vegetable products, essential oils which are terpenes and phenolic-rich substances have several effective biological properties. Therefore they constitute an indispensable source of bioactive molecules. Today, modern medicine uses the healing properties of essential oils and their constituents. Indeed, many volatile compounds are today common ingredients of pharmaceutical

preparations. Due to its geographical position, Morocco is a natural setting that offers genuinely a full range of Mediterranean and Saharan bioclimates and promotes a rich and varied flora with a marked endemism. It ranks second among Mediterranean countries (Benjilali et al., 2005).

In this study we were interested in *Asteraceae*'s family and particularly *Artemisia*'s genus. This genus is known for its richness in essential oils (EO). It is found throughout the Mediterranean area and frequently develops in clayey steppes, rocky and earthy pastures of low mountain's. This genus is characterized by a large number of species recorded in it about four hundred species on five continents (Vajs et al., 2004). In Morocco, it is represented by 12 species and the most important is: *A. herba-alba* (white wormwood), known as desert wormwood (Shih: Arabic, Izri Tamazight). The species is found in Oriental regions, Eastern Rif, Middle Atlas, High Atlas and Saharan Anti-Atlas (Aafi et al., 2002). It has been widely used for a long time in Moroccan traditional medicine

*Corresponding author: Majdouli Karima,
Department of Chemistry, Laboratory of Bioactive Molecules and Environment, University of Sciences Moulay Ismail, BP 11201. Zitoune, Meknès, Morocco.

for the treatment of various diseases (Aziz *et al.*, 2012). Decoction of its leaves is used to treat diabetes, bronchitis, abscess, diarrhea and serves as an anthelmintic (Akrouit 2004; Ahmed *et al.*, 1990; Boriky *et al.*, 1996; Laid *et al.*, 2008). EO from *Artemisia*'s leaves is known for its regulatory properties of the menstrual cycle (Akrouit *et al.*, 2001). It is used in cosmetic and perfume industries.

Several studies focus on the chemical composition of *A. herba-alba*'s EO which represents a great socio-economic interest for the country. This EO is intended mainly for export to the international market. In general, white wormwood had been the subject of several studies in Morocco, Spain, Tunisia (Akrouit *et al.*, 2004) and Algeria (Vernin *et al.*, 1995). In recent decades, studies on herbaceous *A. herba lba* have characterized some chemical constituents such as flavonoids (Salah *et al.*, 2005; Salch *et al.*, 1987), sesquiterpenes lactones (Ahmed *et al.*, 1990; Boriky *et al.*, 1996; Laid *et al.*, 2008), mono- and sesquiterpenes in EO. Chemical composition of white wormwood's EO, from several parts of the world has already been studied and revealed a high degree of polymorphism which led to the definition of several chemotypes. In Morocco, 16 chemotypes were identified (Lamiri *et al.*, 1997) with monoterpenes as the major ones. In Tunisia (Houari *et al.*, 2009), ten compounds, with a content greater than 10% were identified. The main components are eucalyptol, thujones, chrysenthenone, camphor, borneol, chrysanthenyl acetate, sabinyle acetate, davanon and its derivatives. *Artemisia herba-alba*'s EO from Algeria, is essentially characterized by the presence of camphor, α - and β -thujones, eucalyptol and chrysanthenyl derivatives (Dob *et al.*, 2006; Vernin *et al.*, 1995). In the Jordanian species, α -thujone and β -thujone, and santolinol are the main compounds (Hudaib *et al.*, 2006). In Spain there is a predominant presence of camphor, eucalyptol, p-cymene and davanon (Salido *et al.*, 2004).

Artemisia's EO have been widely used for medicinal purposes for many years. And several species of this genus are known to have various biological activities (Abou El Hamd *et al.*, 2010). In fact these plants are able to produce the main secondary metabolites of medical importance (Ambasta, 1986; Priscila *et al.*, 2007). Antimicrobial activity of *Artemisia*'s EO was the subject of many studies. Indeed, several authors have shown that these EOs have significant potential as antimicrobial agents (Mighri *et al.*, 2009). Research conducted on *A. herba alba*'s EO, rich in oxygenated monoterpenes, shows that it has a large antimicrobial potency (Amri *et al.*, 2013). It is also worth noting that, *Artemisia annua*'s EO, strongly inhibit the growth of Gram positive bacteria (Juteau *et al.*, 2002). Thus our purpose is the chemical study of *A. herba alba hugueii* and the evaluation of its EO's antibacterial power against fifteen bacterial strains.

MATERIALS AND METHODS

Material

Plant material

Samples composed of aerial parts (stems, leaves and flowers) of *A. herba alba hugueii* were collected in June 2013 from Tata

area (Moroccan Anti-Atlas). Identification of this species *A. herba alba hugueii* was performed in the laboratory of Botany and Plant Ecology at Scientific Institute of Rabat by Professor IBN TATOU MOHAMED.

Bacterial strains

To evaluate antibacterial power of *A. Herba alba hugueii*, we used in this study, fifteen pathogenic bacteria, Gram-negative (nine) and Gram-positive (six) (Table 1) isolated from patients suffering from different infections: food poisoning, typhoid fever, urinary tract infections, abscesses and pus. This antibacterial activity was evaluated qualitatively and quantitatively by diffusion method on solid medium and macrodilution method.

Table 1. Test bacteria

Gram-négative pathogenic Bacteria	Gram-positive pathogenic bacteria
<i>Acinetobacter baumannii</i> (<i>A. baumannii</i>)	<i>Enterococcus faecalis</i> (<i>E. faecalis</i>) <i>Staphylococcus xylosum</i> (<i>S. xylosum</i>)
<i>Citrobacter koseri</i> (<i>C. koseri</i>)	<i>Staphylococcus aureus</i> BLACT (<i>S. aureus</i> BLACT)
<i>Escherichia coli</i> ESBL (<i>E. coli</i> ESBL)	<i>staphylococcus aureus</i> STAIML/ MRS/ <i>mecA/ HLMUP/ BLACT</i> (<i>S. aureus</i> MRS)
<i>Escherichia coli</i> wild (<i>E. coli</i>)	<i>Streptococcus agalactiae</i> (<i>St.</i> <i>agalactiae</i>)
<i>Enterobacter aerogenes</i> ESBL (<i>E. aerogenes</i>)	<i>Streptococcus acidominimus</i> (<i>St.</i> <i>acidominimus</i>),
<i>Klebsiella pneumoniae</i> <i>ssp pneumoniae</i> (<i>K. pneumoniae</i>)	
<i>Proteus mirabilis</i> (<i>P. mirabilis</i>)	
<i>Pseudomonas aeruginosa</i> (<i>P. aeruginosa</i>)	
<i>Raoultella ornithinolytica</i> (<i>R. ornithinolytica</i>)	

Methods

Phytochemical Study

Determination of moisture

For determination of water content, gravimetric or weighing method was used. The principle consists in determining lost water mass of samples taken after a 24-hour stay in the oven at the temperature of 103°C. We introduced in the oven at 103°C for 24 h, three weighed melting-pots containing (three) masses (M1, M2, M3) equal to 5g of plant material, after cooling, the samples were reweighed. Weigh difference is used to calculate the water content.

Essential oils extraction

Essential oils extraction was performed by steam distillation of dry matter in a Clevenger type apparatus according to the technique described by Simard *et al* (Simard *et al.*, 1988). Raw material (100 g) was immersed in a two-liter flask fitted with a Clevenger apparatus. This mixture was boiled for three hours to produce steam which permit to extract essential oils (volatile substances). The produced vapor is condensed into a refrigerant. The condensates (EO + water) are separated by decantation and EO was dried over magnesium sulfate and then stored at 4 °C until use. We performed three trials by the same manner to determine the average yield of EO.

Essential oils analyses and chemical composition identification

Chromatographic analysis of two samples from *A. herba-alba hugueii*'s EO was performed on a gas chromatograph type Thermo Electron (Trace GC Ultra) coupled to a mass spectrometer type Thermo Electron Trace MS system (Thermo Electron: Trace GC Ultra, Polaris Q MS), fragmentation is performed by electron impact intensity 70 eV. The chromatograph is equipped with a DB-5 column (5% phenyl-methyl-siloxane) (30m x 0.25mm x 0,25µm film thickness), a flame ionization detector (FID) supplied by a gas mixture of H₂ / Air. The column temperature is programmed at a rate of 4 ° C / min from 50 to 200 ° C for 5 min. The injection mode is split (leakage ratio: 1/70, flow ml / min), the carrier gas used was nitrogen with a flow rate of 1ml / min. Identification of the chemical composition of *A. herba alba hugueii*'s EO was performed based on the comparison of their calculated Kovats indices (KI) with those of the reference compounds known in the literature (Kovats 1965; Adams, 2007). This was supplemented by a comparison of indices and mass spectra with different references (Adams, 2007; National Institute of Standards and Technology, 2014). Kovats indices compare retention time of a product with the one of a linear alkane of even carbon number. They are determined by injecting a mixture of alkanes (standard C₇-C₄₀) in the same operating conditions.

Phytochemical Screening

Phytochemical screening's aim is to characterize different families of secondary metabolites in plant material. In this study, we conducted investigations with aqueous and ethereal extracts. Characterization tests are based on precipitation of complexation reactions and by the use of specific reagents of each compounds family. Phytochemical tests are conducted using the usual methods described mostly by Harborne (Harborne1998)

- Compounds belonging to flavonoids are highlighted by the reaction to cyanidin.
- Characterization of tannins is carried out with ferric chloride reagent.
- Research of alkaloids is performed using general reagents for alkaloids' characterization. Two reagents are used: Dragendorff and Mayer reagent.
- Research of saponosides is based on the observation of a constant foam after agitation of aqueous solutions.
- Research of sterols and terpenes was carried out by Liebermann – Burchard reaction.

Antibacterial activity

Evaluation of antibacterial activity by diffusion method on solid medium

Diffusion method on solid medium (disk method or aromatogramme) based on the same principle of antibiotics susceptibility testing. Bacterial suspension (10⁸ CFU / ml) inoculated on agar medium surface (Mueller-Hinton) in a Petri dish with a cotton swab. Filter paper discs (6 mm in

diameter) loaded with 5µl, 10 µl, 15µl and 20µl of pure essential oil are placed on the inoculated Petri dishes and antibiotics disks are deposited as positive controls. A disk containing physiological saline is used as negative control. Incubation is done for 24 hours at 37°C. Inhibition halos around the disks were measured in millimetre using a transparent ruler.

Evaluation of antibacterial activity by macrodilution method in liquid medium

The basic protocol used in our experiment is the one reported by Billerbeck (Billerbeck, 2007). The following modifications were made according to experimental conditions: concentration range includes nine EO's dilutions which are 1/2, 1/4, 1/8, 1/16, 1/32, 1/50, 1/64, 1/80, 1/128 (Oussou *et al* 2004), and Trypase soy agar is replaced by Mueller Hinton agar. Minimum Inhibitory Concentration (MIC) is determined for bacteria that were sensitive to the tested EO. Due to the immiscibility of EO in water and therefore in the culture medium, emulsification was carried out with 40% of DMSO to foster germ/compound contact.

RESULTS AND DISCUSSION

Phytochemical study

Artemisia herba alba hugueii (from Southern Morocco) Essential oil yield

A. herba alba hugueii (from southern Morocco) essential oil average yield calculated based on dry matter is about 4.28%. This EO content is higher than those of 18 samples of white wormwood from southern Tunisia (0.68% -1.93%) obtained from 18 sources (houari *et al.*, 2009). This EO yield is also greater than those obtained in other studies on *A. herba alba* from 16 Spanish samples (0.41% - 2.30%) collected from four sources (Salido S., *et al*, 2004). The recorded content of *A. herba alba*'s EO from Guercif region (Morocco) harvested in June is about 1.23% (Ghanmi *et al.*, 2010). We find that EO yields obtained from other *Artemisia* species: *A. Cana* (1.3%)? *A. Afrigida* (1.5%) (Lopes-Lutz *et al.*, 2008), still inferior to the yield of *A. herba alba* in our study. This relatively higher yield could be profitable on an industrial scale. This difference observed between white wormwood's EO yields can be attributed to many factors such as: growth stage or vegetative stage of harvest, soil, climatic and edaphic conditions of the region, extraction technique, etc. (Fellah *et al.*, 2006).

Chemical Composition of *A. herba alba hugueii*'s essential oil (Southern Morocco)

Analysis of *A. herba-alba hugueii*'s EO from Tata region by GC-MS (see Table 2), has revealed 45 components. Monoterpenes (6.91%) and oxygenated monoterpenes (83.3%) are the the most abundant groups of all identified compounds (91.72%). Among the 45 identified compounds, six: cis-thujone (35.08%), trans-thujone (6.83%), camphor (32.79%), α-Fenchone (4.22%), borneol (2.20%), β pinene (1.22%) can be considered as the main constituents, representing approximately 83.5% of the total EO composition. Indeed, this

composition is different from the one of M'sila region EO (Algeria), which is dominated by camphor (19.4%), trans-pinocarveol (16.9%), chrysanthenone (15.8 %) and trans-thujone (15%) (Vernin *et al.*, 1995), and that of Matmata (Tunisia) mainly composed by cis-thujone (43.85%), trans-acetate sabinyle (17.46%), trans-thujone (10.10%), 1,8-cineole (3.30%), chrysanthenone (2.32%) and acetate chrysanthenyle (3, 93%) (Akrouit 2004). For white wormwood from Jaen (Spain province), davanon (18.1%), p-cymene (13.5%), 1,8-cineole (10.2%) and chrysanthenone (6.7%) appear as the main compounds. Lamiri *et al.* (Lamiri *et al.*, 1997) reports that in Moroccan *Artemisia herba alba*, five chemotypes with camphor were determined.

Table 2. Composition of *A. herba alba hugueii* Essential Oil from southern Morocco "

Compounds	Pourcentage	KI
Z-salvene	0.10	856
Tricyclene	0.31	926
α -pinene	0.22	939
α -fenchene	4.22	952
β -pinene	1.22	979
δ -2-carene	0.04	1002
α -terpinene	0.03	1017
p- cymene	0.85	1024
1,8- cineole	0.90	1031
Santolina alcohol	0.10	1040
γ -terpinene	0.20	1059
Terpinolene	0.04	1088
Cis- thujone	35.08	1102
Camphenol<6->	0.08	1113
Trans -thujone	6.83	1114
Sabina ketone <Dehydro->	0.87	1120
dihydro-linalool	0.04	1135
Camphor	32.79	1146
Tujanol <neois-3->	0.04	1151
Sabina ketone	0.30	1159
Pinocarvone	0.50	1164
Borneol	2.20	1169
Terpinen-4-ol	0.98	1177
Thuj-3-en-10-al	0.06	1184
Myrtenal	0.53	1195
Myrtenol	0.67	1195
Terpineol< γ ->	0.37	1199
Dihydro carveol <neois->	0.07	1228
Cumin aldehyde	0.37	1241
Piperitone	0.36	1252
chrysanthemyl acetate <cis->	0.06	1265
Isobornyl acetate	0.17	1285
Sabinyl acetate <trans->	0.02	1290
Carvacrol	0.08	1299
Guaiacol <p-vinyl->	0.25	1309
Piperitenone	0.03	1343
Isolodene	0.11	1376
Germacrene D	0.05	1481
zingiberene < α ->	0.05	1493
Cadinene < γ ->	0.03	1523
Spathulenol	0.18	1578
Copaen-4- α -ol< β ->	0.05	1590
Sesquithuriferol	0.10	1605
Allohimachalof	0.08	1662
Himachal-4-en-1- β -ol <11- α H->	0.09	1699
Total monoterpenes	6.91	
Total oxygenated monoterpenes	83.5	
Total sesquiterpenes	0.24	
Total oxygenated sesquiterpenes	0.5	
Total C9	0.57	
Total	91.72	

a. Compounds are listed in their elution order on DB-5 column. Kovats indices (KI) are relative to C₇-C₄₀ n-alkanes.

In Jordan the main constituents were cis and trans thujone (16.2 and 8.5% respectively) thus the plant was calling a thujone chemotype one (Hudaib *et al.*, 2006). The most common chemotype regroup cineole and camphor and has been observed in Moroccan and Spanish samples (Lamiri *et al.*, 1997; Feuerstein *et al.*, 1988). Benjilali (Benjilali *et al.*, 1980) identified four main chemotypes related to origin of the plant (*A. herba alba*): cis-thujone and camphor, camphor, cis-thujone and trans-thujone. Other compounds are also reported with lower rates (around 1%) such as 1,8-cineol, camphene and alcohol santolina). According to the same author, Moroccan species is toxic due to the high levels of cis and trans-thujone although these compounds have deworming properties.

Other studies on *A. herba alba* revealed the presence of other major compounds such as cis-thujone acetate (25.6 to 40.9%) (Boutekdjiret *et al.*, 1992; Fleisher *et al.*, 2002; Lawrence, 1995), trans-thujone (44%) and davanone (18.1 to 51.2%) (European Pharmacopoeia 2008; Satrani *et al.*, 2001), chrysanthenone (54.5%) (Boutekdjiret *et al.*, 1992), 1,8-cineole (3-50%) (Feuerstein *et al.*, 1986; Salido *et al.*, 2004), cis- chrysanthenol (24.5 to 30%) (Feuerstein *et al.*, 1988) and cis-chrysanthenyl acetate (69%) (Fleisher *et al.*, 2002). Previous studies have shown that camphor is the main component of Algerian and Spanish white wormwood with percentages between 15 and 68% (Feuerstein *et al.*, 1988; Fleisher *et al.*, 2002). Presence of davanone is also mentioned (37.71%) in samples grown in Errachidia Morocco (Moumni *et al.*, 2013). It seems clear that EO composition white wormwood is highly variable. Therefore, what specifically characterizes Asteraceae's family is the chemical polymorphism especially in *Artemisia* species. This variation or chimiovariety may occur from one plant population to another or even from one individual to another and may be due to external factors such as sunlight, soil nature and components, temperature, altitude, etc. and endogenous factors such as genetic ones. These factors are parameters that influence both the yield and the chemical quality of essential oil (Collin *et al.*, 2005).

Phytochemical screening

The phytochemical screening results showed that the leaves of *A. herba alba hugueii* (from southern Morocco) are rich in sterols, triterpenes (terpenoids), flavonoids and tannins. Saponins are found in trace (Table 3). Organic and aqueous extracts of *A. herba alba hugueii* are free of alkaloids, anthocyanins, leucoanthocyanes and mucilage. These results are similar to those obtained by different researchers on *Artemisia herba alba*'s leaves (Aljaiyash *et al.*, 2014; Makhloufi *et al.*, 2014). Terpenoids are potentially endowed with anti-inflammatory, antimycotic and analgesic properties (Bennett *et al.*, 2003). Flavonoids through their antiradical and chelating activity are endowed with antioxidant, anti-antihypercholesterolemia and anti-aggregation platelet properties (Bennett *et al.*, 2003). Tannins are antimicrobial, antifungal (Jedlicka *et al.*, 2005). The presence of these chemical principles in aerial parts of *Artemisia herba alba*, could justify its various uses in Moroccan traditional pharmacopoeia.

Table 3. Result of phytochemical study of *A. herba alba hugueii* from Southern Morocco

Chemical group	Detection Test		Results
Tannins	Total tannins	FeCl ₃	+++ Black-green
	Gallic tannins	StiasnyReagent	+++ Black-Blue
Polyphenols	Catechic tannins	FeCl ₃ + sodium acetate	- No precipitate
	Flavonoïds	Flavonones	Cyanidinreaction
Anthocyanes		Acido-basique reaction	-
Leucoanthocyanes		Cyanidin reaction without magnesium ships	-
Alkaloids		Reagent Dragendorff	- Precipitate
	Triterpenes and sterol		Reagent Mayer
		Liebermann-Burchardreaction	+++ brownish ring and greenish supernatant
Mucilage		precipitationreaction	- Flaky interface
saponosids		Foam test	+

+++ = abundant - = absent + = weak reaction

Antibacterial activity

In our investigations, antimicrobial activity of *Artemisia herba alba*'s EO was evaluated by observing the inhibitory power at different concentrations against bacteria. Results are summarized in Table 4.

follows: resistant bacteria, less than or equal to 8 mm; susceptible bacteria, diameter between 8 and 14 mm; very susceptible bacteria, diameter between 14 and 20 mm and extremely susceptible, diameter greater than or equal to 20 mm (Duraffourd *et al.*, 1986; Hersch-Martinez, *et al.*, 2005; Ponce *et al.*, 2003).

Table 4. Antibacterial activity and Minimal Inhibitory Concentrations of *A. herba alba hugueii*'s Essential oil

Bacteria	Diameters of inhibition Zone (DZI) in (mm)					ATB	MIC in µl EO/ml H ₂ O)
	5(µl/disk)	10(µl/disk)	15(µl/disk)	20(µl/disk)	NC		
<i>E.coli</i>		No activity			0	16	GM
<i>K. pneumoniae</i>		No activity			0	34	IPM
<i>P. mirabilis</i>	8±0	9±0.5	9±0	9±0	0	42	TPZ
<i>R. ornithinolytica</i>		No activity			0	13	GM
<i>P. aeruginosa</i>	9±0.5	9±0	9±0	9±1	0	30	CAZ
<i>E. coli BLSE</i>	9±0	9±0	10±1	10±0.5	0	32	IPM
<i>C. koseri</i>	8±0.5	9±1	10±0.5	11±0	0	30	CIP
<i>A. baumannii</i>	8±0	8±0	8±0	9±0.5	0	26	CAZ
<i>St. Acidominimus</i>		No activity			0	19	AX
<i>S. aureus MRS</i>	8±1	9±0	10±0	11±0	0	19	VA
<i>E. faecalis</i>	0	7±0	8±1	8±0.5	0	16	GM
<i>st. Agalactiae</i>		No activity			0	20	CTX
<i>st. Xylosus</i>	8±0	9±0	9±0.5	10±1	0	16	K
<i>S. aureus</i>	12±1	12±0.5	15±0.5	19±1	0	22	VA

ATB : Antibiotics, NP :Negative Control, PC : Positive Control, GM:Gentamicine 500, IPM: Imipeneme, TPZ: Piperacilline + Tazobactam – Tazocilline, CAZ: Ceftazidime, CIP: Ciprofloxacine, AX; Amoxiline, VA: Vancomycine, CTX : Céfotaxime, K: Kanamycine.

In fact, *A. herba alba hugueii*'s essential oil showed an inhibitory effect against nine bacterial strains while six strains were highly resistant to that EO. Based on the diameters of inhibition zone (DZI) in mm, the results were appreciated as

Indeed *A. herba alba hugueii*'s essential oil presents a good inhibitory activity against the tested germs in vitro. However, the microorganisms studied did not show the same sensitivity towards the tested essential oil. Indeed, *S. aureus* strain

BLACT (DZI = 19 mm: 20µl / disc) showed greater susceptibility compared to strains *P. Mirabilis* (9mm), *P. Aeruginosa* (9mm), *E. coli* ESBL (10mm), *C. koseri*, (11mm) *A. baumannii*, (9mm), *S. aureus* MRS (11mm), *E. faecalis* (8mm) and *St. xylosus* (10 mm). However wild *E. coli* strains, *K. pneumoniae*, *R. ornithinolytica*, *St. acidominimus*, *st. Agalactiae*, *E. faecalis* were resistant towards this EO. We report in Table 4, minimum inhibitory concentrations (MIC) of *A. herba alba hugueii*'s essential oils obtained by activity against *Staphylococcus aureus*. Indeed, it has been shown that *Staphylococcus aureus* is the most affected by the monoterpene ketones as thujone (Oussalah et al., 2007, Dorman et al., 2000; Tantawi-Elaraki et al., 1993). The presence of an oxygen in terpenoids's structure increases their bacteriostatic properties.

These results are consistent with those described in the literature for a camphor-rich essential oil of *Artemisia herba alba* (14.5%) which showed strong activity against *S. aureus* (Charchari et al. 1996). Other studies have shown that *Artemisia*'s essential oils rich in camphor and 1,8-cineole are very active against microbial agents (Kordali et al., 2005). direct contact method in liquid medium. MIC is determined for the most susceptible bacterial strains. Indeed, *A. herba alba hugueii*'s essential oil showed an inhibitory effect against most of the bacterial strains investigated. It is important to note that EO efficacy is inversely proportional to the value of its MIC against a given strain.

Thus, microbiological results show *E. coli* BLSE, *A. baumannii*, *S. aureus* MRS and *S. aureus* are inhibited from the same threshold concentration of essential oil: 4.8µl / ml, the same observation is made for *P. aeruginosa*, *P. mirabilis*, *C. koseri* and *St. Xylosus* but at a lower concentration 2.4µl / ml. High content of oxygenated monoterpenes (thujone, camphor) in *A. herba alba hugueii*'s essential oil may be responsible for its pronounced.

Conclusion

In order to valorize medicinal plants from TATA region, our choice fell on *Artemisia herba alba hugueii* whose essential oil extracted from aerial parts is rich in monoterpene compounds, major products are cis-thujone (35.06%), camphor (32.79%) and trans-thujone (6.83%). Phytochemical screening showed the richness of secondary metabolites, including phenolic compounds which are natural antioxidant substance with great interest in pharmacology. From antibacterial tests, it appears that *A. herba alba hugueii*'s EO has significant antibacterial power over most of the tested pathogens. These results are very promising for the use of *A. herba alba hugueii*'s aromatic fraction as active ingredient in pharmaceutical antiseptic preparations or to fight against microbial infections.

REFERENCES

- Aafi, A., Taleb, M.S. and Fechtal, M. 2002. Espèces remarquables de la flore du Maroc. Rabat : Édition MCEF.
- Abou El-Hamd H. Mohamed, 2010. Chemical Constituents and Biological Activities of *Artemisia herba-alba*. *Rec. Nat. Prod.*, 4(1): 1-25.
- Adams, R.P. 2007. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. 4th edition Allured Publishing Corporation.
- Ahmed, A. A., Abou-El-Ela, M., Jakupovic, J., Seif El-Din, A.A., Sabri, N. 1990. Eudesmanolides and other constituents from *Artemisia herba-alba*. *Phytochemistry*, 29(11): 3661-3663.
- Akrouf, A. 2004. Etude des huiles essentielles de quelques plantes pastorales de la région de Matmata (Tunisie). In : Ferchichi A. (comp.), Ferchichi A. (collab.). Réhabilitation des pâturages et des parcours en milieux méditerranéens . Zaragoza : CIHEAM. p. 289-292 (Cahiers Options Méditerranéennes; n. 62).
- Akrouf, A., Chemli, R., Chreif, I., et al. 2001. Analysis of the essential oil of *Artemisia campestris* L. *J. Flav Fragr* 16: 337-9
- Aljaiyash, A.A., Gonaid, M.H., Islam, M., and Chaouch, A. 2014. Antibacterial and cytotoxic activities of some Libyan medicinal plants. *J. Nat. Prod. Plant Resour.*, 4 (2): 43-51.
- Ambasta, S.P. 1986. The useful plants of India. Publications & Information Directorate. CSIR, New Delhi 1986, 55-56
- Amri, I., De Martino, L., Marandino, A., Lamia, H., Mohsen, H., Scandolera, E., De Feo, V., Mancini, E. 2013. Chemical composition and biological activities of the essential oil from *Artemisia herba-alba* growing wild in Tunisia *Nat Prod Commun*; 8(3):407-10.
- Aziz, M., Karim, A, El Ouariachi, E.M. et al. Relaxant, 2012. Effect of Essential Oil of *Artemisia herba-alba* Asso. on Rodent Jejunum Contractions. *Scientia Pharmaceutica*; 80(2):457-467.
- Benjilali, B. and Richard D.H., 1980. Etude de quelques peuplements d'armoise blanche du Maroc (*Artemisia herba alba*); *Rivista Italiana EPPOS* 62: 69-74.
- Benjilali, B. and Zrira, S. 2005. Plantes aromatiques et médicinales atouts du secteur et exigences pour une valorisation durable. Actes éditions Institut agronomique et vétérinaire Hassan-II Rabat Maroc
- Bennett, R.N., Mellon, F.A., Foidl, N., Pratt, J.H., Dupont, M.S., Perkins, L. and Kroon, P.A. 2003. Profiling glucosinolates and phenolics in vegetative and reproductive tissues of the multi-purpose trees *Moringa oleifera* L. (horseradish tree) and *Moringa stenopetala* L. *J Agric Food Chem.*, 4;51(12):3546-3553.
- Billerbeck, V.G. 2007. Huiles essentielles et bactéries résistantes aux antibiotiques. *Phytothérapie*. 5, 249-253.
- Boriky, D., Berrada, M., Talbi, M., Keravis, G., Rouessac, F., 1996. Eudesmanolides from *Artemisia herba-alba*. *Phytochemical*. 43(1): 309-311.
- Boutekdjiret, C., Charchari, S. and Bélabbès, R. 1992. Contribution à l'étude de la composition chimique de l'huile essentielle d'*Artemisia herba-alba* Asso. *Rivista-Italiana- EPPOS* 3: 39-42
- Charchari, S., Dahoun, A., Bachi, F. and Benslimani, A. 1996. Antimicrobial activity in vitro of essential oils of *Artemisia herba-alba* Asso and *Artemisia judaica* L. from Algeria. *Riv Ital EPPOS* 18: 3-8
- Collin, G., Graneau, F.X. and Zaya, P., 2005. Huile essentielle – De la plante à la commercialisation – Manuel pratique, chapitre 1, Éd. Corporation Laseve, UQAC, Chicoutimi, Canada. <http://corpolaseve.uqac.ca/manuel/index.html>

- Dob, T. and Benabdelkader, T., 2006. Chemical composition of the essential oil of *Artemisia herba-alba* Asso grown in Algeria. *J Essen Oil Res.*, 18(6): 685-690.
- Dorman, H.J.D. and Deans, S.G. 2000. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *J. Appl. Microbiol.*, 88: 308-316
- Duraffourd, C. and Lapraz, J. C., 1986. Cuadernos de Fitoterapia Clínica (Ed.). Masson, Mexico, p. 86.
- Fellah, S., Romdhane, M., Abderraba, M. 2006. Extraction et étude des huiles essentielles de la *Salvia officinalis*.l cueillie dans deux régions différentes de la Tunisie. *J.Soc.Alger.Chim.*, 16(2) :193-202.
- Feuerstein, I., Danin, A., Segal, R., 1988. Constitution of the essential oil from an *Artemisia herba-alba* population of Spain. *Phytochemistry* 27(2): 433-434.
- Feuerstein, I., Muller, D., Hubert, K., Danin, A. and Segal, R., 1986. The constitution of essential oils from *Artemisia herba alba* populations of Palestinel and Sinai. *Phytochemistry*, 25(10): 2343-2347.
- Fleisher, Z., Fleisher, A., Nachbar, R.B., 2002. Chemovariation of *Artemisia herba-alba* Asso. Aromatic plants of the Holy Land and the Sinai Part XVI. *J Ess Oils Res.*, 14: 156–160
- Ghanmi, M., Satrani, B., Aafi, A., Isamili, M.R., Houti, H., El Monfalouti, H., Bencheqroun, K.H., Aberchane, M., Harki, L., Boukir, A., Chaouch, A., Charrouf, Z., 2010. 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 Guercif (Maroc oriental). *Phytothérapie* 8 : 295 – 301
- Haouari, M. and Ferchichi, A. 2009. Essential Oil Composition of *Artemisia herba-alba* from Southern Tunisia. *Molecules* 14(4):1585-1594.
- Harborne J.B., *Phytochemical Methods A Guide to Modern Techniques of Plant Analysis*, Ed. Springer, 1998.
- Hersch-Martinez, P., Leanos-Miranda, B.E. and Solorzano-Santos, F. 2005. Antibacterial effects of commercial essential oils over locally prevalent pathogenic strains in Mexico. *Fitoterapia*, 76 : 453–457
- Hudaib, M. and Aburjai, T., 2006. Composition of the essential oil from *Artemisia herba-alba* grown in Jordan. *J Essen Oil Res.*, 18(3): 301-304.
- Jedlicka, A. and Klimes, J. 2005, Determination of water and fat solubles vitamins in different matrices using HPLC. *Chem. Pap*, 59 (3) : 202 – 222.
- Juteau, F., Masotti, V., Bessière, J.M., Dherbomez, M. and Viano, J. 2002. Antibacterial and antioxidant activities of *Artemisia annua* essential oil. *Fitoterapia.*, 73(6): 532–535
- Kordali, S., Cakir, A., Mavi, A., Kilic, H. and Yildirim, A. 2005. Screening of Chemical Composition and Antifungal and Antioxidant Activities of the Essential Oils from Three Turkish *Artemisia* Species. *J. Agric. Food Chem.*, 53 : 1408–1416
- Kováts, E. 1965. Gas chromatographic characterization of organic substances in the retention index system. *Adv Chromatogr.*, 7: 229–47.
- Laid, M., Hegazy, M.-EF. and Ahmed, A.A. 2008. Sesquiterpene lactones from Algerian *Artemisia herba-alba*. *Phytochem Letters* 1(2): 85-88
- Lamiri A, Bélanger A, Berrada M, Zrira S. and Benjilali B. 1997. Polymorphisme chimique de l'armoise blanche (*Artemisia herba-alba* Asso) du Maroc. Actes Editions IAV Rabat, p. 69-79.
- Lawrence, B.M. 1995. Armoise oil Natural Flavor and Fragrance Materials In: Perfumer and Flavorist (Ed.), Essential Oils 1992–1994. Allured Publishing Corporation, Carol Stream, IL 179-180
- Lopes-Lutz, D., Alviano, D.S., Alviano, C.S. and Kolodziejczyk, P.P., 2008. Screening of chemical composition, antimicrobial and antioxidant activities of *Artemisia essential oils*. *Phytochemistry*, 69(8): 1732–1738
- Makhloufi, A., Bouyahyaoui, A., Seddiki, N., Benlarbi, L., Mebarki, L. and Boulanouar, A., 2014. Phytochemical screening and anti-Listerial activity of essential oil and crude extract from some medicinal plants growing wild in Bechar (south west of Algeria). *Inter. J. of Phytotherapy*, 4 ((2): 95-100.
- Mighri, H., Hajlaoui, H., Akrouf, A. Najjaa, H. and Neffati, M., 2009. Antimicrobial and antioxidant activities of *Artemisia herba-alba* essential oil cultivated in Tunisian arid zone. *Comptes Rendus Chimie*, 13(3) : 380-386
- Moumni, M., Elwatik, L., Kassimi, A. and Hormani Bakali, A., 2013. Etude comparative des rendements en huile essentielle d'*Artemisia herba alba* asso à état sauvage et domestique à Errachidia (Sud-est du Maroc). ScienceLib Editions Mersenne: Volume5, N°130103.
- National Institute of Standards and Technology <http://webbook.nist.gov/chemistry/>
- Oussalah, M., Caillet, S., Saucier, L. and Lacroix, M. 2007. 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.
- Oussou, K.R., Kanko, C., Guessend, N., Yolou, S., Koukoua, G., Dosso, M., YTN'guessan, Figueredo, G. and Jean-Claude Chalchat, 2004. Activités antibactériennes des huiles essentielles de trois plantes d'***** e. C.R. Chimie 7 : 1081-1086.
- Pharmacopée européenne, 2008. Huiles essentielles. Aetherolea (01): 2098
- Ponce, A.G., Fritz, R., del Valle, C. E. and Roura, S.I., 2003. Antimicrobial activity of essential oils on native microbial population of organic Swiss Chard. *Lebensmittel-Wissenschaft und-Technologie*, 36 : 679–684.
- Priscila, I.U., Mariama, T.N.S., Luiz, C.D.S., Luciano, B. and Fernandes, A.J. 2007. Antibacterial activity of medicinal plant extracts. *Braz J Microbiol.*, 38:717-719.
- Salah, S.M., Jäger, A.K., 2005. Two flavonoids from *Artemisia herba alba* Asso with in vitro GABAa-benzodiazepine receptor activity. *J Ethopharmacol.*, 99: 145 33.
- Salch, N. A. M., El-Negoumy, S. I. and Abou-Zaid, M.M., 1987. Flavonoids from *Artemisia judaica*, *Artemisia monosperma* and *Artemisia herba-alba*. *Phytochemistry*, 26(11): 3059–3064.
- Salido, S., Altarejos, J., Nogueras, M. and Sánchez, A., 2001. Chemical Composition of the Essential Oil of *Artemisia herba-alba* Asso ssp. *valentina* (Lam.) Marcl., *J. of Essential Oil Research*, 13(4): 221-224

- Salido, S., Valenzuela, L.R., Altarejos, J., Nogueras, M., Sanchez, A., Cano, E., 2004. Composition and infraspecific variability of *Artemisia herba-alba* from southern Spain. *Biochemical Systematics and Ecology*, 32: 265–277
- Satrani, B., Farah, A., Fechtal, M., 2001. Composition chimique et activité antimicrobienne des huiles essentielles de *Saturja cala-mintha* et *Saturja alpina* du Maroc. *Ann Fals Exp Chim.*, 94(956): 241–250
- Simard, S., Hachey, J.M., Colin, G.J., 1988. The variation of the essential oil composition with the extraction process, the case of *Thuja occidentalis* L and *Abies balsamea* L. *J. Mill Wood Techn.*, 8(4): 561-573
- Tantaoui-Elaraki, A., Ferhout, H. and Errifi, A. 1993. Inhibition of the fungal asexual reproduction stages by three Moroccan essential oils. *J. Essent. Oil Res.*, 5: 535-545
- Vajs, V., Trifunovi, S., Janackovic, P., Sokovic, M., Milosavljevic, S., Tecevic, V., 2004. Antifungal activity of davanone-type sesquiterpenes from *Artemisia lobelii* var. *conescens*. *J. Serb. Chem. Soc.*, 69(11): 969–972
- Vernin, G., Merad, O., Vernin, G.M.F., Zamkotsian, R.M., Parkanyi, C. 1995. GC–MS analysis of *Artemisia herba-alba* *Asso* essential oils from Algeria. In: Charalambous G, editor. *Food Flavors: Generation, Analysis and Process Influence*. Elsevier Science BV; Amsterdam: 147–205.
