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RESEARCH ARTICLE

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

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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.

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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

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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

For 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 |
| Dihydroo 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 chémo-type 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%) (Boutekedjiret *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%) (Boutekedjiret *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 | PC | | |
| <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 | 2.4 |
| <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 | 2.4 |
| <i>E. coli BLSE</i> | 9±0 | 9±0 | 10±1 | 10±0.5 | 0 | 32 | IPM | 4.8 |
| <i>C. koseri</i> | 8±0.5 | 9±1 | 10±0.5 | 11±0 | 0 | 30 | CIP | 2.4 |
| <i>A. baumannii</i> | 8±0 | 8±0 | 8±0 | 9±0.5 | 0 | 26 | CAZ | 4.8 |
| <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 | 4.8 |
| <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 | 2.4 |
| <i>S. aureus</i> | 12±1 | 12±0.5 | 15±0.5 | 19±1 | 0 | 22 | VA | 4.8 |

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.

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