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# CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF THE ESSENTIAL OIL OF *ARTEMISIA HERBA–ALBA* ASSO. FROM DJELFA

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## Abstract

**Description of the subject:** In order to search for an alternative to antibiotics due to drug resistance and problems related to immunodeficiency, the use of medicinal plants, in the case *Artemisia herba-alba* Asso., as the main source of new natural bioactive molecules has become indispensable.
**Objective:** The aim of the present investigation was to enhance our diverse and rich national heritage and to study the antibacterial effect of *Artemisia herba-alba* Asso. essential oil on different bacterial species.
**Methods:** The aerial parts of *Artemisia herba-alba* Asso. were collected on April 2012 from Djelfa. Dried leaves were subjected to the hydrodistillation using a Clevenger apparatus. The composition of the essential oil was studied by GC-MS. The antibacterial activity was evaluated using the agar diffusion method against six microbial strains. The minimum inhibitory concentration (MIC) was determined using the agar dilution method.
**Results:** The essential oil was characterized as having a high content of camphor (37.5%) followed by chrysanthenone (10.38 %), 1, 8-cineole (8.6 %) and α-thujone (7.03%). The latter showed good activity against all the bacteria tested, with the exception of *Pseudomonas aeruginosa*, which proved to be resistant. The minimum values of the inhibitory concentration (MIC) varied between 0.10 mg. $ml^{-1}$ and 0.84 mg. $ml^{-1}$.
**Conclusion:** The essential oil of *Artemisia herba-alba* Asso. growing in Algeria may be a potential source of natural antibacterial agents in pharmaceutical industry in order to find possible alternative to antibiotics.
**Keywords**: *Artemisia herba-alba* Asso; essential oil ; Camphor ; antibacterial activity; minimum inhibitory concentration.

# COMPOSITION CHIMIQUE ET ACTIVITÉ ANTIMICROBIENNE DE L'HUILE ESSENTIELLE D'*ARTEMISIA HERBA-ALBA* ASSO. DE DJELFA.

## Résumé

**Description du sujet :** Dans le but de rechercher une alternative aux antibiotiques à cause de la résistance aux médicaments et aux problèmes liées à l'immunodéficience, le recours aux plantes médicinales, en occurrence l'espèce *Artemisia herba-alba* Asso. comme source principale de nouvelles molécules bioactives naturelles est devenu indispensable.
**Objectif :** Valorisation du patrimoine algérien diversifié et riche en étudiant l'activité antibactérienne de l'huile essentielle d'*Artemisia Herba-alba* Asso vis-à-vis des souches testées.
**Méthodes :** Les parties aériennes d'*Artemisia herba-alba* Asso. Ont été récoltées en avril 2012 à Djelfa. Les feuilles séchées ont été soumises à l'hydrodistillation, en utilisant un appareil de type Clevenger. La composition de l'huile essentielle a été analysée par GC-MS. L'activité antibactérienne a été évaluée par la méthode de diffusion dans l'agar contre six souches microbiennes. La concentration minimale inhibitrice (MIC) a été déterminée en utilisant la méthode de dilution en gélose.
**Résultats :** L'huile essentielle a été caractérisée comme ayant un important contenu de camphre (37, 5%), suivi par la chrysanthénone (10, 38%), 1, 8-cinéole (8,6%) et l'α-thujone (7,03%). Cette dernière a montré une bonne activité contre toutes les bactéries testées, à l'exception de *Pseudomonas aeruginosa* qui s'est révélée résistante. Les valeurs minimales de la concentration inhibitrice (MIC) varié entre 0,10 mg. $ml^{-1}$ et 0,84mg. $ml^{-1}$.
**Conclusion :** L'huile essentielle d'*Artemisia herba-alba* Asso. de la région de Djelfa peut être une source potentielle d'agents antimicrobiens naturels dans l'industrie pharmaceutique comme une alternative aux antibiotiques.
**Mots clés** : *Artemisia herba-alba* Asso; Huile essentielle; Camphre; Activité antibactérienne; Concentration minimal inhibitrice.

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## INTRODUCTION

In the last few years, due to the misuse of antibiotics and an increasing incidence of immunodeficiency-related diseases, the development of microbial drug resistance has become more and more of a pressing problem [1]. Recently, natural products from medicinal plants represent a fertile ground for the development of novel antibacterial agents [2]. Plants essential oils have come more into the focus of phytomedicine [2, 3]. It is important to develop a better understanding in basic research applications, especially, of the anti-microbial activity of essential oils [4]. The Mediterranean region is relatively rich with plants (between 15.000 and 20.000 species) [5]. Algeria, a North African country with a large variety of soils (littoral, steppe, mountains and desert) and climates, possesses a rich flora (more than 3.000 species and 1.000 genders) [6].

In this context and in order to enhance our diverse and rich national heritage, we are interested in the study of *Artemisia herba-alba* Asso. greenish-silver perennial herb, grows 20 to 40 cm in height and belongs to the daisy family Asteraceae [7]. In Algeria, this plant commonly known as "white wormwood", in arbaic as "Chih" and in Franch as "Armoise blanche" [8]. It has been used in folk medicine by many cultures since ancient times, to treat colds, coughing, bronchitis, intestinal disturbances, diarrhea, neuralgias arterial hypertension and/or diabetes [9, 10, 11]. Many researchers have reported various biological and/or pharmacological activities of *Artemisia herba-alba* Asso. essential oil as an anti-microbial, antioxydant, antidiabetic, antileshmanial, anthelmintic and antispasmodic agent [6, 12-16]. The present study determined the variations in chemical composition, oil yield and antibacterial collected during flowering stage of growth from Djelfa.

## MATERIAL AND METHODS

### *1. Plant material*

The aerial parts of *Artemisia herba-alba* Asso. (120 tufts) were collected from Djelfa, a city with a semi- arid climate, located right in the heart of the steppe zone, 300 km south of Algiers. It is the last town before the Saharan Atlas and the desert. (coordinates; UTM: latitude 34° 40′ 00″ N, longitude 3° 15′ 00″ E; Elevation: 1000 m), at the flowering stage (April 2013). After been harvested, the fresh vegetable matter was first weighted and then dried on the shadow, until weight stability (10 days). Then the leaves and flowers, were separated from stems. The plant was identified by Dr. Djaballah Fatima Botanist in INRF (Institut National de Reservation Forestière) of Djelfa.

### *2. Barterial strains*

Antibacterial activities of *Artemisia herba-alba* Asso. essential oil were tested gainst five strains of bacteria: *Pseudomonas aeruginosa* ATCC12228, *Staphylococcus aureus* ATCC 25923, *Klebsiella pneumoniae* (ATCC 13883), *Bacillus cereus* (ATCC, 11778) and *Escherichia coli* (ATCC125922). Microorganisms were obtained from the culture collection of the Military specialized hospital in Bouchaoui (Algeria).

### *3. Essential oil extraction*

Air-dried leaves were submitted to hydrodistillation for 3 h, using a Clevenger type apparatus [17], according to the European Pharmacopoeia [18]. The oil yield was expressed v/w vs. dry matter. The essential oil was dried over anhydrous sodium sulphate, filtered and stored in a sealed vial in the dark at +4 °C before analysis and antibacterial activity.

### *4. Mass spectrometry analysis of Artemisia herba-alba Asso. essensial oil*

The oil was analysed by gaz schromatography-mass spectrometry (GC-MS) using a Hewlett Packard 6890 mass selective detector coupled with a Hewlett Packard 6890 gas chromatograph equipped with a 30 m x 0.25 mm HP-5 MS; 5% (Phynel-Methyl Siloxane) column with 0.25 µm film thickness. The gaz schromatography- mass spectrometry operating parameters were as follows [19]:

-The vector gas used is Helium with a flow rate of 1.4 ml / min.
-The analysed sample volume was 0.1 µl

-The temperature of the injector (split mode): 250°C.
-The column temperature was programmed from 35 to 85°C at rate 20°C/min, increased from 85 to 300C° at rate of 5°C/min and finally held at this temperature for 10 min.
-Ionisation potential, 70 eV.
-Ionisation current, 2 A.
-Ion source temperature, 200°C.

The Linear retention indices (RI) for all the compounds were determined using n-alkanes mixture ($C_8$-$C_{17}$) as standards.

Identification of individual compounds was performed by matching their mass spectral fragmentation patterns with corresponding data (NIST 05 and Wiley 275 mass spectra libraries), as well as by comparison of the fragmentation patterns of mass spectra with those reported in the literature Adams (1995). The percentage composition was computed from the GC peak areas.

## 5. *Antibacterial Activity*

The in vitro antibacterial activity of *Artemisia herba-alba* Asso. essential oil against the microorganisms employed and its activity potentials were qualitatively and quantitatively assessed by the presence or absence of inhibition zones, zone diameters and MIC values. Two techniques were used to test the antibacterial activity of *Artmisia herba-alba* Asso.:

### *5.1. Agar diffusion method*

Antibacterial activitiy of *Artemisia herba-alba* Asso. essential oil was assessed using the paper disk agar diffusion method according to Imelouane and *al*. [19] with some modifications:

The density of bacterial cultures required for the test was adjusted to 0.5 Mc Farland standards to achieve an inoculum of approximately $10^6$ CFU (colony-forming unit)/ ml.

Culture suspension of the tested bacteria (200 µl) was spread in three directions on 4 mm thick Mueller Hinton agar (4 mm thick).

Absorbent disk (Whatman disk No. 4 of 6 mm diameter) containing 15 µl of essential oil were applied on the surface of the Petri dishes (90 mm) inoculated with different microbial strains.
Petri dishes were then incubated for 24 h at 37°C.
Negative control was prepared using a disk impregnated with sterile water.

Antibacterial activity was evaluated by measuring the diameter (mm) of the growth inhibition zones including the 6mm disk and expressed by adopting the estimate of Djeddi and *al*. [20]:

-Extremely sensitive strain : Diameter ≥ 20mm
-Very sensitive strain : 15 mm ≤ Diameter ≤ 19 mm
-Sensitive strain : 9 mm ≤ Diameter ≤ 14 mm
-Not sensitive : Diameter ≤ 8 mm

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### *5.2. Agar dilution method*

The minimum inhibitory concentration (MIC) of the tested essential oil was determined using the agar dilution method approved by Bansod and Rai [21] with the following modification:

Tween-80 was incorporated into the agar after autoclaving to enhance oil solubility. Briefly, Petri plates containing various concentrations of essential oil 0.003 to 2.5 % (v/v) were inoculated with each tested strain. Plates were dried at 35°C prior to inoculation with 1–2 ml spots containing approximately $10^5$ CFU of each organism. Uninoculated plates containing essential oil served as negative control.Tween-80 was used as a positive growth control. Test and control plates were then incubated for 24 h for pathogenic bacterial strains at 37°C Plates were evaluated for the presence or the absence of colonies after incubation.

For each treatment, the absence of colonies on all plates tested was considered as an inhibitory effect. The lowest concentration of essential oil required to completely inhibit the growth of the tested microorganism was designated as the MIC [22].

## 6. *Statistical analysis*

All experimental measurements were performed in triplicate. Data are presented as mean values ± SD SD using Excel 2013.

## RESULTS

### 1. Chemical composition

Hydrodistillation of the leaves of *Artemisia herba-alba Asso*. yielded yellow liquid oil with a strong penetrating pleasant herbaceous odor characteristic of the plant. The oil yield was 0.8 %.

The chemical composition of the oil was investigated using GC/ FID and GC/MS techniques. The percentages and the retention indices of the identified components are listed (Table 1) in the order of their elution on the HP-5MS column. From the data obtained, 31compounds were identified, representing 92, 84 % of the oil.

The chemical classes'distributions of *Artemisia herba-alba* Asso essential oil could be separated into four classes (Fig. 1 and Table 1). These were monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, and others.

Oxygenated monoterpenes dominated the chemical composition of the investigated oils (77.78%) and they were represented by camphor (37.50%). chrysanthenone (10.38%) 1.8-cineole (8.6%). α-thujone (7.03%). borneol(3.35%) and bornyl acetate (2.52%) as the principal components.

Moreover, monoterpene hydrocarbons were represented by 11.29% of the total oil. Among these compounds, Camphene (6.0%). α-pinene (1.16%) and ρ-cymene (0.48%) were the most important.
The sesquiterpenes are present in smaller quantities. germacrene D (1.07%) and spathulenol (1.31%) were the main ones.

Table1: Chemical Composition of *Artemisia herba-alba* Asso.essential oil by gaz schromatography- mass spectrometry

| RI | Compound | % |
|---|---|---|
| 934 | α-pinene | 1.16 |
| 950 | Camphene | 6.00 |
| 974 | Sabinene | 0.90 |
| 990 | 1-octen-3-ol | 0.27 |
| 1017 | α-terpipene | 0.26 |
| 1025 | para-Cymene | 0.48 |
| 1033 | 1.8-cineole | 8.60 |
| 1058 | γ-Terpinene | 0.30 |
| 1074 | α-thujone | 7.03 |
| 1091 | α-Terpinolene | 0.26 |
| 1107 | Filifolone | 1.04 |
| 1119 | β-Thujone | 1.74 |
| 1124 | Chrysanthenone | 10.38 |
| 1153 | Camphor | 37.50 |
| 1156 | Sabina ketone | 1.07 |
| 1162 | Pinocarvone | 1.79 |
| 1169 | Borneol | 3.35 |
| 1178 | Terpinene-4-ol | 1.07 |
| 1206 | Verbenone | 0.23 |
| 1229 | Cis-carveol | 0.25 |
| 1237 | E-ocimenone | 0.34 |
| 1244 | Carvone | 0.15 |
| 1275 | Bornyl acetate | 2.52 |
| 1287 | Carvacrol | 0.70 |
| 1298 | γ- Elemene | 0.12 |
| 1313 | Bicycloelemene | 0.17 |
| 1393 | Germacrene D | 1.07 |
| 1418 | Germacrene B | 0.55 |
| 1444 | Bicyclo- germacrène | 0.33 |
| 1457 | Delta-cadinene | 0.22 |
| 1465 | Spathulenol | 1.31 |
| Oxygenated monoterpenes | | 77.78 |
| Monoterpene hydrocarbons | | 11.29 |
| Sesquiterpenes hydrocarbons | | 3.77 |
| Total | | 92.84 |

RI: Retention indices calculated against n-alkanes on the HP 5MS column. Compounds are listed in order of their elution from HP 5MS column. % Percentages obtained by peak-area normalization.

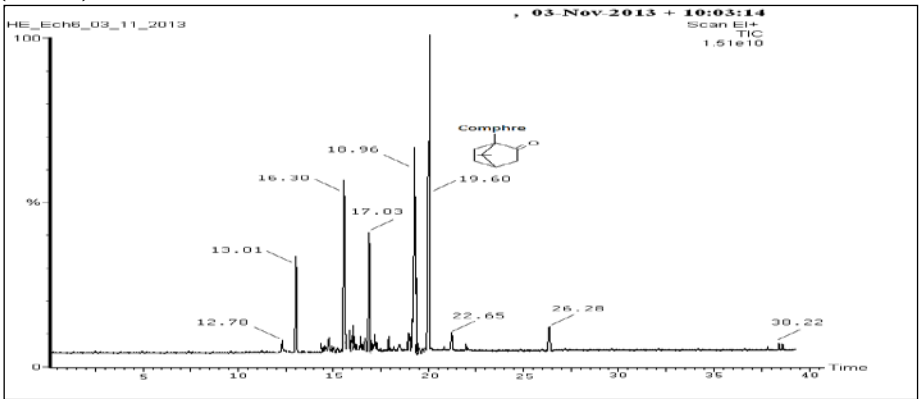


Figure 1: Chromatogram of chemical Composition of *Artemisia herba-alba* Asso.essential oil by gaz schromatography- mass spectrometry

## 2. Antibacterial Activity

The in vitro antibacterial activity of *Artemisia herba-alba* Asso. essential oil against the microorganisms employed and its activity potentials were qualitatively and quantitatively assessed by the presence or absence of inhibition zones, zone diameters and MIC values.

Figure 2 reports the inhibition zone of essential oil determined of Gram positive and Gram negative bacteria using the diffusion technique on solid media. The data shows that this oil had variable antibacterial activity against all tested strains. The inhibition zones were in the range of 15-33 mm. Gram positive bacteria were shown to be more sensitive to the *Artemisia herba-alba* Asso. essential oil. The data indicated, adopting the estimate of Djeddi and *al.* [20], that Gram-positive *Staphylococcus aureus and Bacilluscereus* were extremely sensitive to the tested oil with the strongest inhibition zone (33.00±0.45mm and 21.00±0.43mm) respectively. Indeed, Gram-negative strains also displayed variable degree of susceptibility against investigated oil. adopting the estimate of Djeddi and *al*. [20], *Escherichia coli and Klebsiella pneumoniae* were very sensitive to the tested oil . Maximum activity was observed against *Escherichia coli* with inhibition zones (19.00±0.55). Modest activity was observed against *Klebsiella pneumoniae* (15±0.54 mm). *Pseudomonas aeruginosa* was considered resistant since no inhibition zone was observed. It is known to have high level of intrinsic resistance to virtually all known antibacterials and antibiotics due to a combination of a very restrictive outer membrane barrier which is highly resistant even to synthetic drugs.

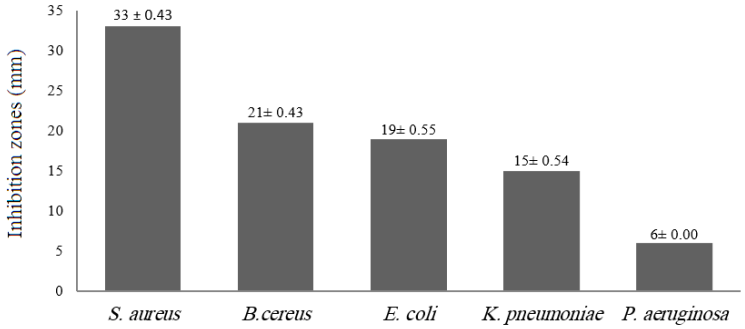


Figure 2: Antibacterial activity of A*rtemisia herba_alba* Asso. essential oil.

The results of minimum inhibitory concentration (MIC) of *Artemisia herba-alba* Asso. essential oil, evaluated by agar dilution method, was reported in Table 2.

Table 2: Minimal inhibitory concentration (MIC) of essential oil

| *Artemisia herba-alba* Asso. | | | | |
|---|---|---|---|---|
| [C] (%) | [C] (mg/ml) | Gram (-) | | Gram (+) |
| | | *k.p.* | *E.c.* | *S.a.* |
| 2.5 | 13.45 | - | - | - |
| 1.25 | 6.72 | - | - | - |
| 0.6 | 3.36 | - | - | - |
| 0.3 | 1.68 | - | - | - |
| 0.15 | 0.84 | - | - | - |
| 0.07 | 0.42 | + | + | - |
| 0.03 | 0.21 | + | + | - |
| 0.015 | 0.1 | + | + | - |
| 0.007 | 0.05 | + | + | + |
| 0.003 | 0.02 | + | + | + |

| Microorganisms | MIC (mg/ml)* |
|---|---|
| *Klebsiella pneumoniae* | 0.84± 0.01 |
| *Escherichia coli* | 0.84± 0.01 |
| *Staphylococcus aureus* | 0.1 ± 0.01 |

*Data are presented as mean values ± SD, MIC: Minimal Inhibitory Concentration.

The data indicate that the oil exhibited varying levels of antibacterial activity against the investigated pathogens. The inhibitory properties of the oil were observed within a range of concentrations from 0.1 to 0.84 mg/ml, with maximum activity was observed against *Staphylococcus aureus.*

## DISCUSSION

*Artemisia herba alba* Asso. essential oil from Djelfa was characterized as having a high content of camphor (39.5%) followed by chrysanthenone (10, 38 %), 1, 8-cineole (8,6 %) and α-thujone (7,03%). For further comparison, the composition of *Artemisia herba-alba* Asso. essential oil dominated by Camphor was found in Morocco (Taforalt, Machraa) [19, 23], Algeria (Méchrea, Nord sahara, M'sila, Djelfa, Bejiai, Saida) [24-30] and Iraq (Karbala desert) [31].

On the other hand, the major component of this oil differed from that reported by several authors for A. herba -alba oils, in which α- and β-thujone [6,13, 32, 33, 34 ] , Cis-chrysanthenyl acetate [36,37], davanone [38,39,40] , 1,8-cineole [38,41] and chrysanthenone [42] were found to be the most abundant components.

The chemical composition of *Artemisia herba-alba* Asso. essential oils shows a large inter-species variability and, within the same species. Various compositions dominated either by a single component (α-thujone, camphor, chrysanthenone or trans-sabinyl acetate) or characterized by the occurrence, of two or more of these compounds at appreciable contents [33].

Chemical variability of *Artemisia herba-alba* Asso.seems to depend on the genetic characteristics of the plant [43], geographical locations, consequently different climatic conditions under which it has grown [13, 25], part of the plant, phenological stage, and the method used to obtain the essential oil [44, 47]. In fact, these factors influence the plant's biosynthetic pathways and, consequently, the relative proportion of the main characteristic compounds [48].

The essential oil was evaluated for antibacterial activity against pathogenic strains. It was found to be active against all tested bacteria with the exception of *Pseudomonas aeruginosa* which proved to be resistant. The activity of the oil varies with its concentration as shown in Table 2.

The essential oils evaluated in this work have a great variety of phytochemicals that could be considered as responsible for a larger or smaller part of the antibacterial activity. The antibacterial activity of *Artemisia herba-alba* Asso.essential oil would be related to its oxygenated monoterpenes components [49] which constitute about 77.78% of the oil. Research into the antibacterial actions of monoterpenes suggests that they diffuse into and damage cell membrane structures [50]. Indeed, it was shown that monoterpenes hydrocarbons and oxygenated monoterpenes in essential oils are able to destroy cellular integrity resulting in respiration inhibition and permeability alteration [51]. Besides, the most abundant component in essential oil of *Artemisia herba-alba Asso.*, camphor, has been reported to exhibit bacteriostatic activity against *Pseudomonas aeruginosa* [19], and this compound is a major constituent in a number of antibacterial essential oils [52, 53].

The major components of *Artemisia herba-alba* Asso. essential oils, such as the monoterpenoids thujone, camphor, 1–8 cineole, camphene, are known for their potential antibacterial properties against both gram-positive and gram-negative bacteria [54-56].In addition, other minor components such as borneol(4.88%) have been also reported to have antibacterial potential [13]. In other studies, α-pinene, has been known to exhibit antibacterial activity against the bacterial strains (*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis*) [1]. In fact, the biological effectiveness of essential oil is related to their different chemical constituents (major, minor and their mutual ratios) acting either synergistically or antagonistically with major components [57, 58].

In general, the antibacterial activity of the essential oils tested was more pronounced against Gram-positive than against Gram-negative bacteria [59].
This generally higher resistance among Gram-negative bacteria, according to Lambert [59], Harris [61] and Bezic and *al.* [62], could be ascribed to the structure of the cell wall of gram-negative bacteria primarily made up of a lipopolysaccharide that blocks the penetration of hydrophobic compounds and prevents the accumulation of essential oils in the membrane of target cells.

The absence of this barrier in Gram-positive bacteria allows the direct contact of the essential oils hydrophobic constituents with the phospholipid bilayer of her cell membrane, where they bring about their effect, causing either an increase of ion permeability and leakage of vital intracellular constituents, or impairment of the bacterial enzyme systems [63, 64].

Although the antibacterial activity of essential oils from many plant species has been extensively surveyed, their antibacterial mechanism has not been reported in great detail. According to Burt [65], given the large number of different groups of compounds present in essential oils, the antibacterial activity of essential oil is most likely not attributable to a specific mechanism but to several mechanisms related to various targets in the cell. Since the active antibacterial compounds of essential oils are terpenes, it seems reasonable that their mode of action might be similar to that of phenolic compounds. Most of the studies on the mechanism of phenolic compounds focused on their effects on cellular membranes, altering its response to antibacterial challenge. These effects may develop as a result of membrane depolarization by altered ion transport or through changes in the membrane structure, inhibition of energy (ATP) generation by interference with glucose uptake or inhibition of enzymes involved in oxidative or substrate level phosphorylation. Increases in cytoplasmic membrane permeability appear to be a consequence of the loss of the cellular pH gradient, decreased ATP levels and, loss of the proton motive force, which lead to cell death [66].

The antibacterial activity and, consequently, the minimum inhibitory concentration of essential oils can be influenced by the growing region of the plant, the extraction method used, the plant part used (leaf or whole plant), the method of preparation of the raw material (fresh or dry), the type of organism and the cultivation conditions(incubation time, temperature, oxygen), the culture medium, the concentration of the test substance and the solvents used to dilute the oil, among other factors [65, 67-73].

## CONCLUSION

The essential oil of A*rtemisia herba-alba* Asso. issued from Djelfa has confirmed its antibacterial power that it can be useful in pharmaceutical industry as an alternative of antibiotics for the prevention and the treatment of diseases caused by the micro-organisms tested especially *Staphylococcus aureus* and *Bacillus cereus*, which showed an extreme sensitivity. Further toxicological and clinical studies are required to prove the safety of the oil.

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