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Comparative Study Of Four Ecotypes Of The Same Medicinal Plant (*Artemisia Herba-Alba* Asso); Yield And Antibacterial Activity

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Abstract

Artemisia herba alba is an medicinal and aromatic species very rich in secondary metabolites. In this work, the yield of essential oil extracted from the leaves of Artemisia herba alba and the antibacterial activity of its essential oils were studied. Samples of Artemisia herba alba from four ecotypes in western Algeria. Extraction by the hydro-distillation method showed that the white wormwood of ecotype of Rechaigua provided an average essential oil content 2.04% higher than that of the other ecotypes, Taassalet forest (1.53%), Ain d'heb (0.90%) and the forest of Belhacel (0.61%), This is the first work which consists in modifying the most used method to evaluate the antibacterial activity of essential oil on agar. This new technique using a cellulose ester membrane of 0.45um porosity between the agar and the bacterial mat has given us very good results. The inhibition halos obtained are very clear within a very homogeneous bacterial carpet. The analysis of the results from the study of the bacterial inhibitory power of essential oils from two study sites demonstrated a very pronounced bactericidal effect against Gram+ bacteria than Gram- bacteria. The diameters of inhibition of the first varying between 15 mm and 28.5 mm on the other hand in the second are between 6 mm and 22 mm.

The MIC obtained from essential oil of Artemisia herba-alba ecotypes, Rechaigua, Ain d'heb and Taassalet forest is 25ul against Staphylococcus aureus (ATCC 25 923) and Enterococus faecalis (ATCC 49452). It is 50ul for Escherichia coli (ATCC 25922) and Pseudomonas aeruginosa (ATCC 27853).However, the MIC of essential oil of Belhacel forest ecotype is 50ul to stop the growth of G+ bacteria and 75ul for G- bacteria. Also makes it possible to classify the four bacterial strains used according to their sensitivity to the essential oil of white wormood. Indeed, Pseudomonas aeruginosa is a resistant bacterium, Escherichia coli is sensitive, Enterococus faecalis is extremely sensitive, while Staphylococcus aureus is a very sensitive or extremely sensitive bacterium.

Keywords: *Artemisia herba alba, medicinal species, minimum inhibitory concentration, antibacterial activity, yield, cellulose ester membrane.*

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1. Introduction

Aromatic plants have been used since ancient times as well as in therapy in preserving and flavoring food, but only in the last decade scientific research has focused its interest on their essential oils and natural extracts as sources of antimicrobial compounds and antioxidants.(Tenore et al., 2011; Marangon and i Moura, 2011; Özcan and AL Juhaimi, 2015). *Artemisia herba-alba* presents a highly sought-after index for these pharmaceutical properties. Its essential oils has several properties, anti-cancer (Tilaoui et al., 2011; Khlifi, et al., 2013), anti-angiogenic (Jaouadi et al., 2015), hypotensive and diuretic (Zeggwagh et al., 2014) and antimicrobial (Fedhila et al., 2015), which have given it an application in many fields, therapeutic, cosmetic and in the food industry. Its chemical composition has been the subject of several phytochemical studies for economic interest. The performance of these oils is acceptable and can be profitable on an industrial scale. Indeed, the richness in essential oils of this plant constitutes an asset of valorization and exploitation of natural products with promising economic characteristics at the level of cosmetic and pharmacological industries.

For Algerian oil monoterpenes were the major components, essentially camphor, a- and ß thujones, 1,8-cineole and chrysanthenyl derivatives (Vernin et al., 1995 ; Dob, Benabdelkader, 2006) .In Tunisian oil oxygenated monoterpenes were found to be the major components of *A. herba-alba* oil extracted from aerial parts of plants originated from arid regions (Neffati et al., 2008). En pharmacopée traditionnelle, L'armoise blanche était reconnue depuis longtemps par les populations pastorales et nomades pour ses vertus purgatives. In traditional pharmacopoeia, white wormwood has long been recognized by pastoral and nomadic populations for its purgative properties.

The flavonoids detected in white wormwood also show a structural diversity ranging from common flavonoids (flavonoid glycosides and flavonols) to methylated flavonoids which are very unusual. Flavonoid glycosides include O-glycosides such as quercitin-3-glucoside and flavone C-glycosides which are rare in the genus Artemisia, as well as in all Asteraceae (Saleh et al., 1987; Salah, Jager, 2005). In addition to sesquiterpenes lactones and flavonoids, phytochemical analysis showed that the composition of essential oils of *Artemisia herba alba* Asso is rich in monoterpenes, pentacyclic triterpenes, santonins, coumarins and tannins (Mohamed et al., 2010).*Artemisia herba-alba* has traditionally been used in the treatment of

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diabetes, bronchitis, diarrhea, neuralgias and hypertension (Zouari et al., 2010). The review of the available scientific literature published on *Artemisia herba alba* showed that the antidiabetic effect of this plant was similar to that of repaglinide and insulin ordinary (Ribnicky et al., 2004; Tastekin et al., 2006)

For a long time, the technique of diffusion on agar was a good way to evaluate the biological inhibitory power and to detect the sensitivity of bacterial and fungal strains. Nevertheless, the halos of the diameters of inhibitions will sometimes be our clear and difficult to measure. It is in this context, we have proposed in this work for the first time a main modification on agar diffusion technique. Through this study, we made a comparative study of the bacterial inhibitory power and the essential oil yield of *Artemisia herba halba* in four regions with different ecological conditions.

2. Methods and Materials

2.1.Areas study location

The framework chosen for our study is placed in the geographical context of the high western steppe plains of Algeria. The study sites are located west of the capital of Algeria, it is the Tiaret region centered on the two Rechaiga and Ain dheb sites. Our first study site is located in the town of Rechaiga which is 56 km east of the Tiaret region at an altitude of 1110m (Table 1). The second area is that of Ain deheb which is located south of Tiaret region at 64 km at an altitude of 817m (Table 1). The second region of Relizane is located in the north-west of Algeria and is distinguished by the diversity of its landscapes and also by two mountainous reliefs (Fig.1). Two areas were chosen, the national forest of Taassalet which is in the town of Oued Esselem located 48 km east of Relizane and characterized by an Aleppo pine, the national forest of Belhacel located in the town of Belhacel is located 10 km north of the capital of the wilaya of Relizane with a dominance of the Eucalyptus stand.

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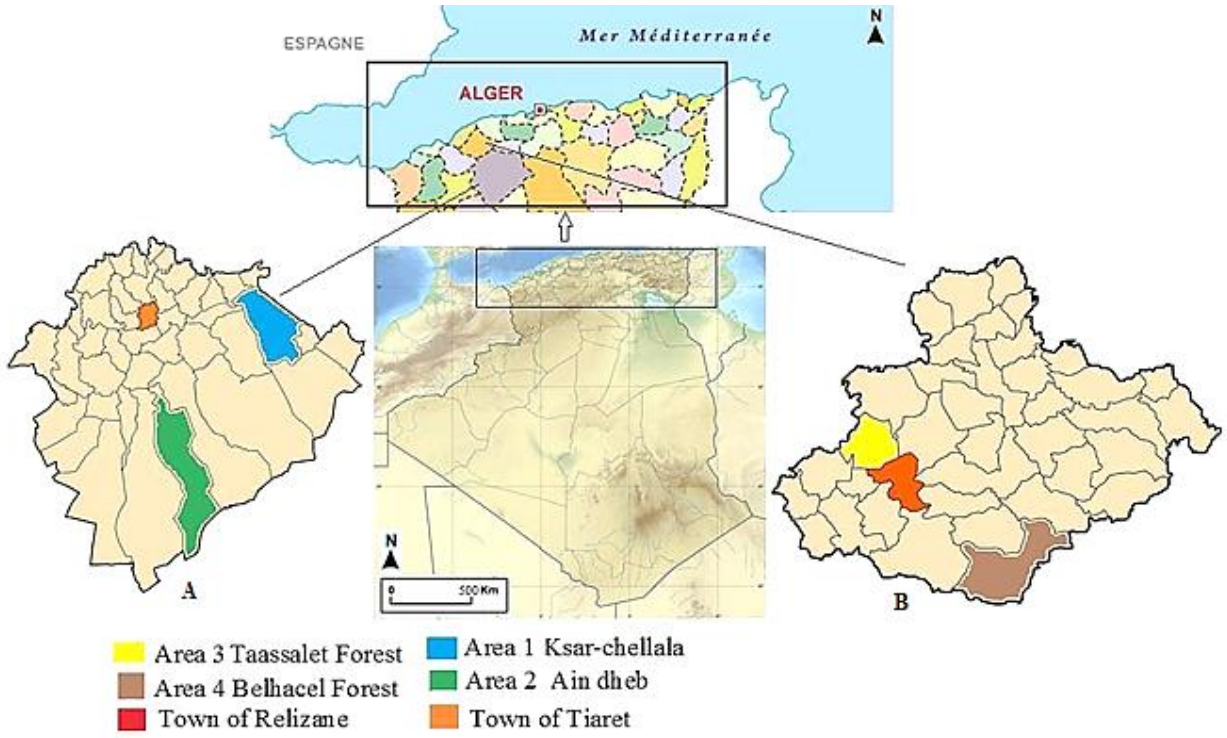

Figure 1. Areas study location ;A : Tiaret region ;B: Relizane region

The geographical coordinates of the four areas are presented in table 01.

Table 1. Geographical coordinates and nature of the soil of Areas study

Geographical coordinates	Altitude	Latitude	Longitude	Soil type
Area 1 (Rechaiga)	839 m	35°24’29″N	01°58’24″E	dolomite limestone
Area 2 (Ain dheb)	1110 m	34°50’40″N	01°32’57″E	Loamy, deep
Area 3 (Taassalet forest)	610 m	35° 50′ 26″ N	0° 29′ 56″ E	Clay-marl
Area 4(Belhacel forest)	435 m	35° 34′ 46″ N	0° 55′ 29″ E	Sandy clay, superficial

2.2.Climatic Characteristics

The climate of the Rechaigua region is of the continental type with rains concentrated in the winter period, with a drought period of nine months, extending from March to November. The average rainfall recorded for this region is 269 mm/year. The site of Ain dheb is characterized by a lower semi-arid climate with a dry period of five months from May to October. The national forest of Taassalet is a stand of Aleppo pine, with a hot and dry climate, with a slight mountain tendency. Winters are often rainy and sometimes snowy with an average annual rainfall of 200mm, sometimes snowy. The Belhacel state Forest is characterized by a precipitation rate of 350mm/year, the type of vegetation in this forest is a stand of Eucalyptus.

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2.3.Plant material

The samples of white wormwood were taken from our four study sites, in December 2019, which accompanies the flowering period characterized by a good yield of essential oil. The harvested plant material was freed from weeds and dried away from light and in a ventilated place for 20 days. The extraction method followed is that of hydro distillation. The extraction was done using a simple distillation assembly on the scale of the food science laboratory of the faculty of natural and life sciences at Ibn Khaldoun University in Tiaret. A flask with a capacity of 1 liter or bathes the vegetable matter, which is brought to a boil by means of a balloon heater. This flask is surmounted by a glass elbow connected to a cooler which is used to condense the water vapor containing the extracted essential oil. The distillate is recovered in an Erlen-meyer.

The decantation is carried out in a 500 ml separating funnel, in which the preceding mixture separates into two immiscible phases. The essential oil is recovered and stored at 4°C in glass tubes, hermetically sealed, to protect them from air, light and temperature variations, which are the main agents of degradation.

2.4.Extraction yield

According to the AFNOR standard (2000), the yield of essential oil (YHE) is defined as being the ratio between the mass of essential oil obtained after extraction in grams (M') and the mass of the plant material used in grams (M). The yield is expressed as a percentage, it is expressed by the following formula: YHE (%) = M'/M x 10 Study of the antibacterial activity of essential oil of *Artemisia herba alba.*

2.5. Bacterial strains studied

Four bacterial strains that are part of the ATCC collection were used in the antibacterial tests. They are provided by the Pasteur Institute of Algiers. These are two gram-positive bacteria, *Staphylococcus aureus* (ATCC 25923) and *Enterococus faecalis* (ATCC 49452) and two other gram-negative bacteria, *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853)

2.6.Preparation of the inoculum

From the boxes containing the bacterial strains, we prepared microbial suspensions for each species. Using a Pasteur pipette, we aseptically removed three pure and well-isolated colonies which are discharged into a tube containing 5 ml of McFarland. The enrichment lasts for 2 to 3 hours.

2.7.Measurement of the microbial load

The microbial load was determined using a UV spectrophotometer at a wavelength of 625 nm. After obtaining the bacterial suspensions, 5 ml of each suspension is transferred to a test tube containing 9 ml of physiological saline. The bacterial suspension is well homogenized, its opacity is equivalent to an optical density of 0.13 at 625 nm, which corresponds to a microbial load of 108 germs / ml.

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2.8.Principle of the Agar Diffusion Method

The diffusion method widely used in microbiology (antibiogram) is based on the diffusion of the antimicrobial compound in a solid medium. The effect of the antimicrobial product on the target is assessed by measuring an inhibition zone, and as a function of the inhibition diameter. The bacterial strain will be qualified as susceptible, intermediate or resistant. On dishes containing the agar medium (Muller Hinton) with a thickness of 7 mm well dried, 100 µl of the inoculum are introduced to obtain a uniform spreading in a sheet after inoculation. This step is carried out on five petri dishes for each extract and for the four bacteria studied. One of the petri dishes is a control and the other four dishes will be the subject of contact between bacteria and white wormwood extract (from area 01, 02, 03 or 04) which consists of aseptically depositing three discs in sterile wattman paper (6mm in diameter) soaked in essential oil emulsified in Tween 80 (V/V) and at different concentrations (25%, 50%, 75% and 100%) on the developing bacterial carpet. The Petri dishes are incubated at 370C for 24 hours. Each trial is repeated three times to minimize experimental error. After incubation at 37°C for 24 hours. The results are read by measuring the diameter of inhibition in mm.

2.9.Determination of the minimum inhibitory concentration (MIC)

In solid medium (Agar) The technique for determining MICs by direct contact in agar or liquid medium consists of dispersing the antimicrobial agent in a variable concentration in a homogeneous and stable manner in the culture medium of the germ studied (Drugeon and Garraffo, 1991). to determine the minimum concentration of essential oil which inhibits bacterial growth, five Petri dishes of agar media (Mueller-Hinton) were prepared. One presents the controls and in each of the other boxes is incorporated a concentration of essential oil of *Artemisia herba alba* (25%, 50%, 75% or 100%) emulsified in Tween 80 (V/V). Then, we followed the same steps as for the evaluation of the antibacterial activity. The MIC therefore corresponds to the lowest concentration of essential oil at which no bacterial growth is observed. Bacterial suspensions were seeded on these media. Finally, all five dishes were incubated at 37°C for 24 hours. These MIC determination steps are carried out for the essential oil of each ecotype studied.

2.10.Modification of the Agar Diffusion Method

We made one major change to the most commonly used disk streaming method. This modification consists of filtering the bacterial suspension through a 0.45um porosity cellulose ester membrane using a filtration ramp (0.22um porosity cellulose ester membranes can be used for spores such as Clostridia), then place it on the agar. So the membrane is between the agar and the bacterial culture. Then, a sterile wattman paper disc (6mm in diameter) soaked in essential oil is placed on the bacterial carpet in the course of development in each Petri dish. The bacterial suspension is aseptically filtered through a 0.45 um porosity cellulose ester membrane. This membrane is incubated on a selective medium, Tergitol agar, to isolate coliform bacteria. The bacteria-essential oil contact consists of aseptically depositing a disc (6mm in diameter) soaked in essential oil of different concentrations in the center of the membrane containing developing bacteria and then incubating the petri dishes containing the bacteria at 37 °C for 24 hours.

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2.11. Measurement of the zone of inhibition and sensitivity of the bacteria used

The inhibition diameter is measured in millimeters using a caliper. The sensitivity of the target bacteria to the different extracts is classified according to the diameters of the determined inhibition halos (Ponce, 2003). Thus, bacteria are considered non-sensitive or resistant if the diameter Ø <8 mm, sensitive or intermediate bacteria (9 < Ø < 14 mm), very sensitive bacteria (15 < Ø < 19 mm) and extremely sensitive bacteria (Ø > 20 mm).

3. Results

3.1.Organoleptic characteristics

The organoleptic characteristics of white wormwood essential oil from each site, namely appearance, color and smell, are shown in comparison with AFNOR standards in Table 2.

Table 2.Organoleptic characteristics of the essential oils obtained

	Appearance	Color	Odor
AFNOR			
EO of *A.herba a* of E1	Liquid	Light Brown	Very Camphoric
EO of *A.herba a* of E2	Mobile liquid	Pale yellow	Camphor
EO of *A.herba a* of E3	Liquid	Light Brown	Very Camphoric
EO of *A.herba a* of E3	Liquid	Light yellow	Camphor

Note : EO : Essential Oil **;** *A.herba a* **:** *Artémisia herba halba* ; E1:Ecotype of Rechaigua ; E2: Ecotype of Ain d'heb;E3: Ecotype of Taassalet fores ;E4: Ecotype of Belhacel forest .

The first two ecotypes that generated a good yield of essential oil both have a light brown oil color and a very camphorous smell. ANOVA of different essential oil yield values obtained in our four ecotypes studied gave a highly significant effect (P = 0.000001) between the four ecotypes at p < 0.001 and p < 0.005. However the effect was not significant for each ecotype. Indeed, the values obtained respectively are, P=0.0087, P=0.176, P=0.065 and P=0.036. The reading of the yield of essential oil extracted from the leaves of white wormwood shows that *Artemisia herba alba* from the Rechaigua site generated a higher yield than the other sites with a rate of 2.04%. The lowest value is recorded in the ecotype of site 4 of the Belhacel forest (0.61%) (Table 3).

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Table3. Average essential oil yield of the four ectypes studied

	Ecotype1	**Ecotype 2**	**Ecotype 3**	**Ecotype 4**
R1.P1	2.10 ± 0.981	1.33 ± 0.045	1.05 ± 0.010	0.60 ± 0.052
R1.P2	2.30 ± 0.032	1.10 ± 0.020	1.41 ± 0.110	0.50 ± 0.830
R1.P3	2.50 ± 0.410	0.80 ± 0.065	1.60 ± 0.105	0.50 ± 0.020
R2.P1	1.55 ± 0.133	0.85 ± 0.061	1.43 ± 0.028	0.67 ± 0.580
R2.P2	1.80 ± 0.180	0.95 ± 0.009	1.40 ± 0.085	0.69 ± 0.136
R2.P3	1.73 ± 0.830	0.80 ± 0.100	1.63 ± 0.015	0.72 ± 0.100
R3.P1	2.30 ± 0.010	0.80 ± 0.833	1.93 ± 0.110	0.70 ± 0.017
R3.P2	2.00 ± 1.005	0.75 ± 0.033	1.75 ± 1.005	0.55 ± 0.189
R3.P3	2.20 ± 0.075	0.86 ± 0.017	1.70 ± 0.005	0.63 ± 0.098

Note: **R:** Essential oil yield**; P:** Population

3.2.Antibacterial activity of EO

The analysis of the results obtained from the study of the antimicrobial power of essential oil extracted from the leaves of *Artemisia herba alba* showed that the essential oil of white wormwood from Ecotype of Taassalet forest generated zones of inhibition of bacteria greater than those measured for samples of the same plant from other ecotypes. The four extracted oils revealed a very high bactericidal activity against Gram+ bacteria than for Gram- bacteria (Fig.2 and 3,fig.3). Indeed, the averages of the inhibition halos of *Staphylococcus aureus* (Gram+) bacteria resulting from the effect of 25µL, 50µL 75µL 100µL of essential oil are respectively varied between 6 and 18mm, 9 and 22mm, 11 and 25mm, 17 and 28.5mm .However, the averages of the inhibition halos of *Pseudomonas aeruginosa*(Gram-) bacteria resulting from the same concentrations of essential oil are respectively 6 and 15mm, 9 and 19mm, 12 and 22mm.

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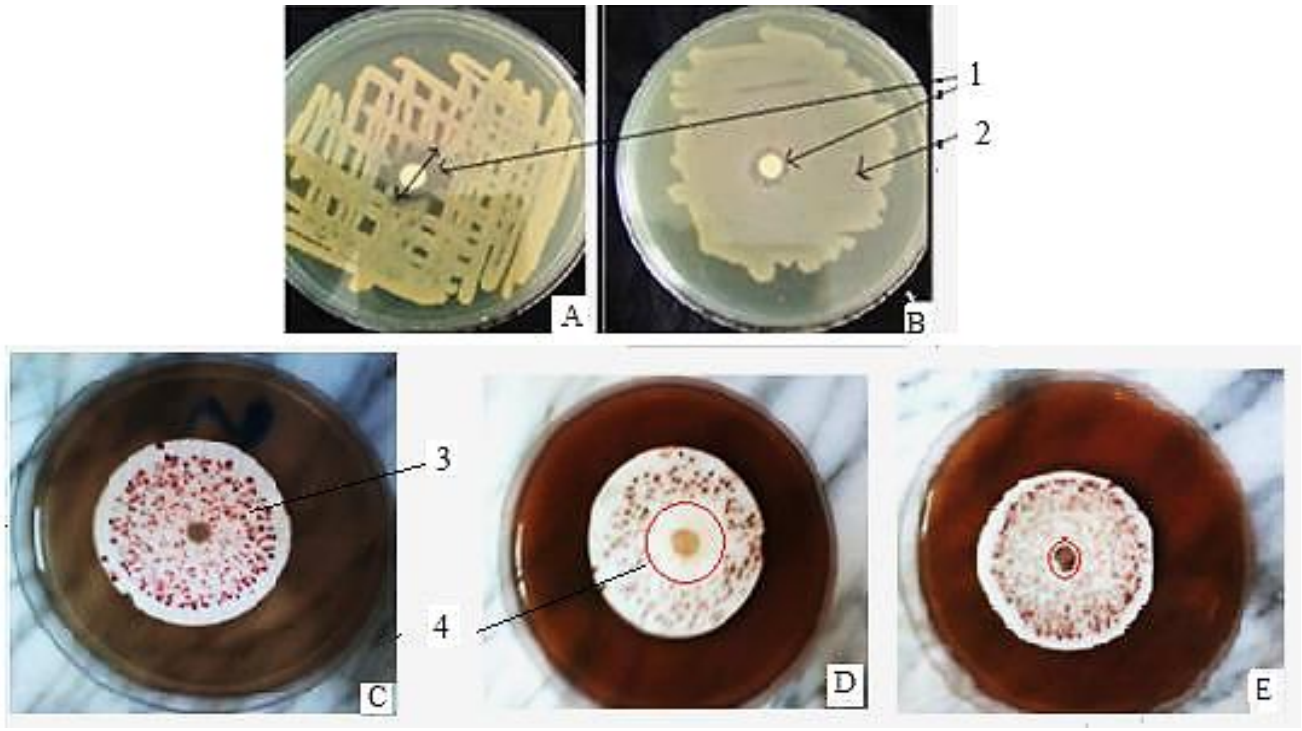

Figure 2. Halos of inhibitions; A, B: Agar diffusion method; 1: Halos of inhibition; 2: bacterial growth of *S. aureus*; C,D, E: new method (on filtration membrane) ; 3: bacterial growth of *E. coli*; 4: Halos of inhibition.

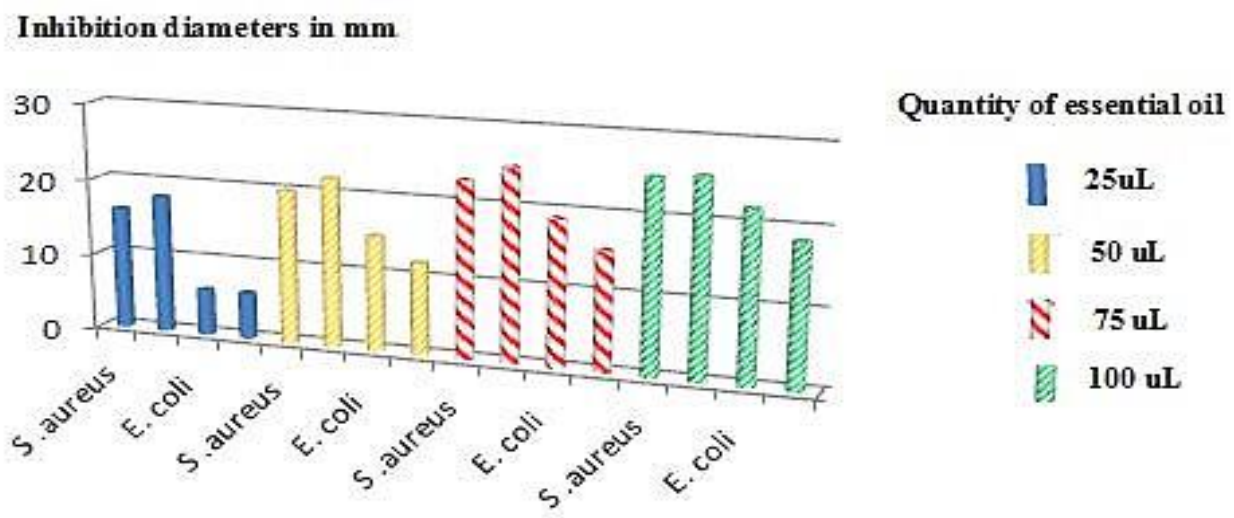

Figure 3. Diameters of the inhibition halos of the bacterial strains studied; Ecotype1

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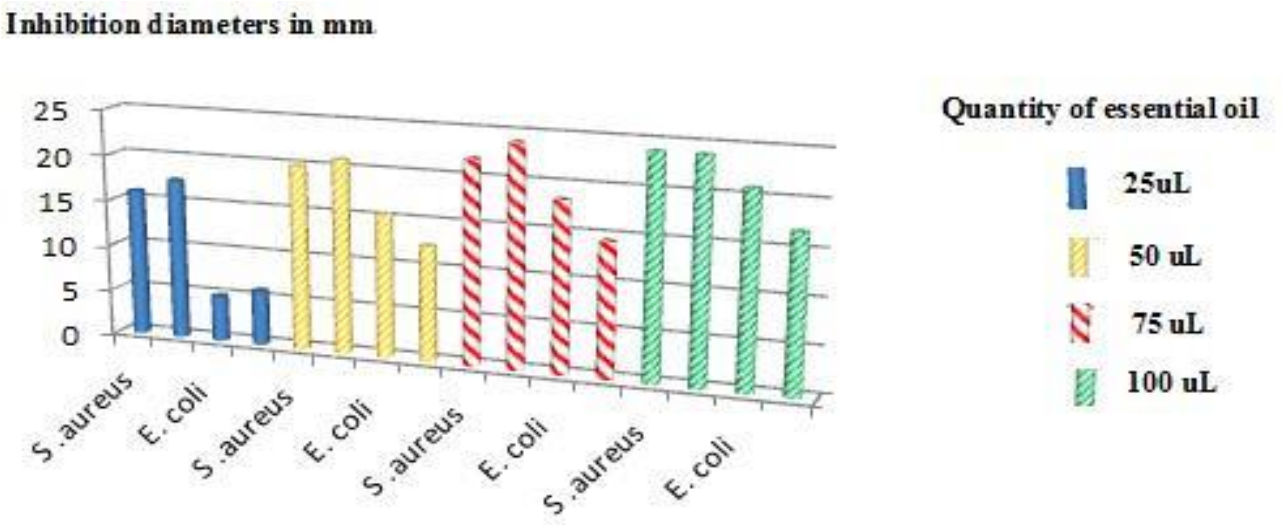

Fig. 4. Diameters of the inhibition halos of the bacterial strains studied; Ecotype2

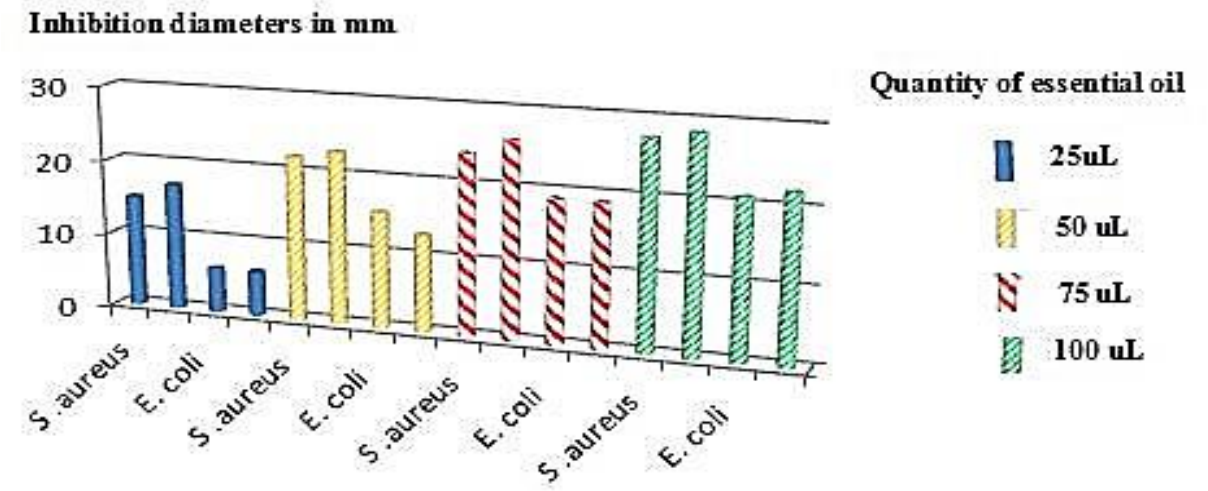

Figure 5. Diameters of the inhibition halos of the bacterial strains studied; Ecotype3

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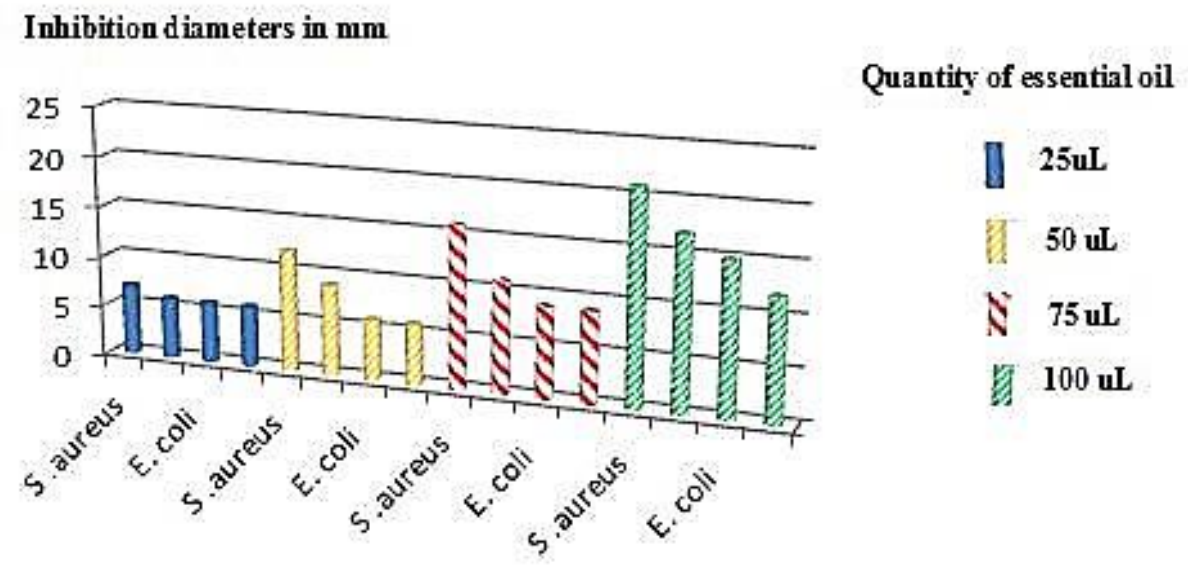

Figure 6. Diameters of the inhibition halos of the bacterial strains studied; Ecotype4

The strain *P. aeruginosa* (ATCC 27 853) showed great resistant against the different essential oils at a dose of 50uL with diameters varying between 6 mm and 13 mm and diameters wavering between 9 mm and 19 mm for the 75uL dose (fig.3). However, while the strain of *E. faecalis* (ATCC 49452) and *S.aureus* (ATCC25 923) were the most sensitive with inhibition diameters varying respectively between 9 mm-23mm and 12 mm-22 mm at a dose of 50uL and again with diameters fluctuating between 11 mm-26mm and 16 mm-24mm for the 75uL dose.

3.3.Minimum Inhibitory Concentration

The MIC of the essential oil of *Artemisia herba-alba* is 25ul for ecotypes 1,2 and 3 necessary to inhibit the growth of *S. aureus* and *E. faecalis* and it is 50ul for the two strains *E. coli* and *P. aeruginosa.* However, it takes 50ul of essential oil of ecotype 4 to stop the growth of *E.faecalis* and 75ul for *E. coli* and *P. aeruginosa* .

The MIC of the essential oils tested on the four bacterial microbial strains studied are presented in the Table 4 in uL

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Table 4. Minimum Inhibitory Concentration (MIC)of the essential oils tested

Q EO in µL	25µL				50 µL				75 µL				100 µL				Witness			
	E1	E2	E3	E4	E1	E2	E3	E4	E1	E2	E3	E4	E1	E2	E3	E4	E1	E2	E3	E4
***Staphylococcus aureus* ATCC 25 923**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
***Enterococus faecalis* ATCC 49452**	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
***Escherichia coli* ATCC 25922**	+	+	+	+	-	-	-	+	-	-	-	-	-	-	-	-	+	+	+	+
***Pseudomonas aeruginosa* ATCC 27 853**	+	+	+	+	-	-	-	+	-	-	-	-	-	-	-	-	+	+	+	+

***Note*:** E:Ecotype ; Q EO: Quantity of essential oïl in micro liter(ul) ; (+): Croissance ; (-) ; inhibition ; E1:Ecotype of Rechaigua ; E2: Ecotype of Ain d'heb;E3: Ecotype of Taassalet fores ;E4: Ecotype of Belhacel forest .

4. Discussion

4.1.Essential oil yield

The noted difference in essential oil yields of the different ecotypes is strongly attributed to variations in climatic, topographical and edaphic conditions characterizing the four study sites. We have found that the highest yield is produced by ecotypes that occur under drought and low rainfall conditions. Thus, the site has a drought period of nine months and with an average annual rainfall of 269 mm followed by area of the Taassalet forest, with a hot and dry climate and rainfall of 200 mm/year. The type of soil and the altitude are also two significant criterion factors in our study, it turned out that the extraction yield is important in site 1 which has a dolomite limestone soil at an altitude of 893 m.

Compared to provenances of the same species from other neighboring countries, the yield of essential oil provided by white wormwood from our study regions is similar to that of white wormwood from Tunisia (0.68%-1.93%) (Houari, 2009) and Morocco. (0.56% to 1.23%) (Ghanmi, 2010). The same infraspecific variation in white wormwood yield was noted in Spain (0.41% - 2.30%) for 16 samples from 4 provenances (Salido, 2004). from one stand to another or even from one individual to another. So, this difference in chemical composition can be an indicator to differentiate between two neighboring species that abound at the same altitudinal height (Ouyahia, 1990). Thus, Lawrence (1999) and Ghanmi (2010) reported that the EO of

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Artemisia mesatlantica is comparable in its chemical composition to *Artemisia herba alba* (Asteraceae) except for chrysanthenone, which is non-existent in mesatlantica. Commonly, white mugwort essential oil is known for its composition of monoterpenoids, especially oxygenated, such as 1,8 cineole, chrysanthenone, chrysanthenol, α/β thujones, davanone and camphor as major components. Chrysanthenone is thus present as a major constituent (47.71%) in most wormwood. It can be concluded that this variation or chemovariety may be due to endogenous factors, in particular the genetic polymorphism characterizing this species. This genetic polymorphism demonstrated by the use of ISSR molecular markers for the analysis of genetic material, carried out on a sample of 12 individuals from the south-east of the Tiaret region, this genetic analysis demonstrated that the *Artemisia herba alba* is characterized by a very marked genetic polymorphism, proven by a population of amplifications of 37 bands of different sizes, of which 78.4% of these bands were polymorphic (Maghni et al., 2016). Also this genetic variability is expressed by an anatomical variability(Maghni et al., 2018) and a morphological variability(Maghni et al., 2017). The dynamics of this morphological variability of *A. herba alba* is also related to variations in ecological conditions (Maghni et al., 2023).

4.2.Susceptibility of bacterial strains

The four bacterial strains used in this study are classified according to their inhibition diameters. Thus, the bacterium *Enterococus faecalis* (ATCC 49452) is extremely sensitive, *Staphylococcus aureus* (ATCC 25923) is considered as a very sensitive or extremely sensitive bacterium, *Escherichia coli* (ATCC 25922) coli is sensitive and finally the *Pseudomonas aeruginosa* strain (ATCC 27 853) as a resistant bacterium.

Locust activity and MIC The antibacterial activity of essential oils can be explained by the molecular interaction of the functional groups of essential oil components with the bacterial wall, which causes deep lesions. Generally essential oils are recognized by their natural components, such as monoterpenes, diterpenes and hydrocarbons with various functional groups. Several compounds are often cited as responsible for the antiseptic properties of essential oils: thymol, carvacrol, cinnamaldehyde, eugenol, 1,8-cineole, camphor and thujones (Hubert, 2006).

According to (Faleiro 2003) the relative action of thujones and eucalyptol (or 1,8-cineole) has been associated with their low water solubility and the ability to form hydrogen bonds, which limits their entry into Gram – which have inoperative hydrophobic pathways in the outer membrane. Thus, (Wan, 1998) linked the resistance of Gram-bacteria to their hydrophilic outer

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membrane which can block the penetration of hydrophobic compounds into the target cell membrane. According to Duke (998) the presence of β-thujone in the essential oil of Artemisia herba-alba gives it a strong antiseptic property in addition to other physiological characteristics; it is abortifacient, antibacterial, emmenagogue, insecticide and larvicide. In addition to this, other compounds of this oil have interesting biological activities. Camphor, for example, exhibits antibacterial, antidysenteric and also fungicidal activity (Tantaoui-Elaraki, 1993). The presence of camphor could potentially be a reason for the microbial hypersensitivity towards the essential oil of *Artemisia herba-alba* (Tabanca, 2001). This antibacterial activity of essentials oils could be explained by the molecular interaction of the functional groups of essential oil components with the bacterial wall, which causes deep lesions. We can therefore conclude that this activity may be the result of a synergistic effect between several compounds of this essential oil (Felice et al, 2004) The result obtained for the anti-aircraft activity of the essential oil of the four ecotypes of *Artemisia herba-alba* is very similar to other studies in which the G+ bacteria (*S. aureus* and *E. faecalis*) were the most sensitive. Some authors have explained that the presence of a significant content of oxygenated monoterpenes (thujones, camphene, camphor and 1,8-cineole) in the essential oil of *Artemisia herba-alba* may be responsible for its pronounced activity against *S.aureus*. Thus, Dorman (2000) and Oussalah (2007) demonstrated that *S. aureus* is most affected by monoterpene ketones such as thujones. On the other hand, Wan (1998) demonstrated that the majority of essentials oils tested for their antibacterial properties have a more pronounced effect against Gram + and that the resistance of Gram – is attributed to their hydrophilic outer membrane which can block the penetration of hydrophobic compounds in the target cell membrane.

We can explain the biological activity of an essential oil by the chemical composition of its essential oils, in particular the functional groups of the main compounds (alcohols, phenols, compounds terpenes and ketones) and their synergistic effects. Dorman & Deans (2000) have suggested that oils active against Gram-negative bacteria contain components of secondary metabolites that are small enough to pass to through proteins into outer membrane receptors and so on able to access the cytoplasmic membrane. In the case of Gram-negative bacteria, the proteins, which are found in the outer membrane, are deactivated before they reach the membrane cytoplasmic and cytoplasm (Fujisawa et al., 2009)

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5.Conclusion

We found intra-specific variability in essential oil yield within the same species. This variability may be due to the dissimilarities of exogenous ecological factors (topographic, climatic and edaphic) and also to endogenous factors such as the genetic polymorphism of individuals. These factors are all parameters that influence both the yield and the chemical quality of the essential oil. The multiple biological activities of *Artemisia herba alba* essential oil give it wide use in many food, pharmaceutical, cosmetic and even medical fields. These essential oils can also be the basis for the formation of pesticides and insecticides. Finally, we record as perspectives the determination of the chemical composition of white wormwood in the regions studied or on other species of genus of Artemisia because these aromatic and medicinal plants are rich in biomolecules active that they can be used to produce bio-pesticides or bio-fungicides. He is very interesting to test these essential oils on bacteria or pathogenic germs.

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