Evaluation of Antibacterial Effect of Some Essential Oils by Contact and Volatile Methods

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ABSTRACT

Nowadays nosocomial infection is a major concern in the world and the situation is aggravated by the emergence of antibiotic resistance. Disinfection of hospital surfaces by air is one of the means used for decontamination. However, the agents used in this process are toxic to humans. In this respect, the search for new, less toxic, and natural alternatives is necessary. Among these alternatives, essential oils (EOs) known for their antibacterial effects and their volatile nature constitute a promising potential. In this context, the evaluation of the antibacterial activity of essential oils of some Moroccan plants (Oreganum compactum, Rosmarinus officinalis, Artemisia herba-alba, Thymus vulgaris, Lavandula officinalis, Cedrus atlantica, and Syzygium aromaticum) on three bacteria, S. aureus, K. pneumoniae, and E. coli was performed. Three classical methods have been used: the well method, micro-atmosphere, and micro-dilution. Most of the EOs tested show antibacterial activity on all three strains. Oreganum compactum EO was the most effective while A. herba-alba EO was the weakest. The EOs used in this study for their antibacterial effects could be good disinfectants.

Key Words: *Antibacterial, Essential oil, Disinfectant, Nosocomial disease.*

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INTRODUCTION

The hospital environment is largely contaminated by human or environmental microorganisms that are prevalent in both developed (7%) and developing countries (10%). These infections affect a very large number of patients and lead to a significant increase in mortality. According to WHO [1] estimates, about 15% of all hospitalized patients suffer from these infections which are also responsible for death in 4-56% of newborns, with an incidence rate of 75% in South-Eastern Asia and sub-Saharan Africa.[2, 3] This represents a concern in terms of health and loss of life, but also in terms of the additional cost of hospital care. [4-6]

The most dominant bacteria responsible for nosocomial infections in Moroccan hospitals are *Escherichia coli* (55%), followed by *Pseudomonas aeruginosa* (28%), *Staphylococcus aureus* (28%), and *Klebsiella pneumoniae* according to a prevalence study conducted between 2011 and 2013 by four Moroccan centers. [7, 8]

Each sector of activity (whether industry, hospital, or laboratories) must implement hygiene procedures that can produce or work in a healthy and secure environment. There are different methods and procedures for disinfection such as heat, steam, antisepsis, and chemicals. However, this disinfection remains incomplete for areas inaccessible to humans. Hence, there is a need for disinfection of surfaces by air (DSVA). Nowadays, this one is among the most used processes.

Chemicals used include sodium hypochlorite (NaClO), alcohols, hydrogen peroxide, and quaternary ammoniums, [9] which have been shown to be toxic to humans and the environment over time. Hydrogen peroxide (H_2O_2) is the most commonly used in automatic DSVA processes. It is known for its bactericidal, tuberculocidal, fungicidal, and virucidal effect with rapid efficacy, but it is more expensive than most other disinfectant assets and has no sporicidal effect at low concentrations. [10] In addition, H_2O_2 is a caustic product for skin and mucous membranes. In high doses, it may be responsible for

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digestive, neurological or acute respiratory distress syndrome. [11] It is, therefore, necessary to find more natural and less toxic alternatives.

Plants contain phytochemicals such as saponins, tannins, essential oils, flavonoids, and alkaloids which have preventive and curative characteristics. [12, 13] Essential oils (EOs) are good solutions to the toxicity problems posed by chemicals. They are also used for their effectiveness in removing teeth plaque. [14] These fragrant plant extracts are the subject of much scientific research in various fields including the medical field where they have demonstrated their antimicrobial potential. [15] Several studies have revealed that the essential oils of some plants inhibit the growth of different bacterial strains. [16] Unlike the vast majority of antimicrobial agents used for disinfection, these substances have little or no toxicity. Indeed, one of the most attractive features of EOs is that they are generally low-risk products. Their mammalian toxicity is low and has been relatively well studied experimentally and clinically because of their use as drugs. [17] As for some EOs, there are those containing aldehyde and phenolic components that may present some toxicity. The latter is important for the chemotherapeutic applications of EOs against different viruses, bacteria, and fungi. Although the biological impacts of the individual chemical components of EOs are identified, the toxicokinetics of their mixtures is much more complicated to evaluate. [17]

The traditional Moroccan pharmacopoeia is rich in medicinal plants, due to the diversity in the country climates and biotopes (desert, mountains, coastal areas, etc.). Morocco has a very significant flora distinguished by a large diversity of plants with 4200 species and subspecies. Besides, Morocco is characterized by a rich and diverse aromatic and medicinal flora with high levels of endemic plants. This endemic flora (22%) includes 879 species and subspecies classified into 55 families and 287 genera. This wealth of endemic species places Morocco in an important position among other Mediterranean countries. [18]

The EOs are complex natural mixtures of volatile secondary metabolites. They are extracted from plants by various processes, the most commonly used of which are hydro-distillation, solvent distillation or carbon hydroxide distillation. [15] These EOs are rich in terpenoid, alcohol compounds (e.g., menthol), acidic compounds, aldehydes, ketones (e.g. thymol) and phenols; among these, terpenes, trapezoids, and aromatic phenols (carvacrol, thymol, escarole, etc.) have major roles in the composition of different EOs. [19]

The antimicrobial or other biological activities of the EOs are directly correlated with the presence of these bioactive components. Besides, the antioxidant activity of essential oils has been brought about by several studies using flavonoids and terpenoids existing in these oils. It has been shown that EOs exhibit inhibitory activities against various broad-spectrum Gram-positive and Gram-negative bacterial pathogens. [20]

Recently, many Moroccan aromatic and medicinal plants have been extensively studied for their antimicrobial activities. Thyme and oregano are the most famous Moroccan medicinal plants. They are frequently used in traditional medicine for the treatment of many infections. Several scientific reports have evaluated the antimicrobial activity of different species of Thymus. [18]

Several studies have also confirmed that vapor phase EOs are more potent antimicrobials than liquid forms. Among these EOs, there are those of thyme, citrus, *Eucalyptus globulus*, tea tree, and lemongrass. [21]

To evaluate the antibacterial activity of volatile components of vapor phase EOs and to provide a rational approach to the development and implementation of novel biocidal agents, we have used the micro-atmosphere method. In this regard, the objective of this work was to evaluate the antibacterial activity of a certain number of essential oils extracted from Moroccan plants, on three bacteria responsible for nosocomial infections (*S. aureus*, *E. coli*, and *K. pneumoniae*) and highlight the essential oils which have an antibacterial effect in the vapor phase.

MATERIAL AND METHODS

Biological material:

This study focused on three species (*Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumonia*) available in the collection of the Laboratory of Physiopathology, Molecular Genetics, and Biotechnology.

The essential oils tested:

The essential oils tested (Table 1) were *Cedrus atlantica*, *Syzygium aromaticum*, *Artemisia herba-alba*, *Thymus vulgaris*, *Oreganum compactum*, *Rosemarinus officinalis*, and *Lavandula officinalis*. We tested also an essential oil is not Moroccan, clove, but used by the population.

These essential oils studied were obtained directly from leaves or aerial parts of the plant species. The plants were harvested at random, then washed and dried in a well-ventilated place at room temperature for ten days. The six essential oils were obtained by hydro-distillation for 3.5 h using the standard Clevenger apparatus. The oil was extracted from the distillate with hexane and dehydrated by passing through anhydrous sodium sulfate. After filtration, the solvent was distilled off under reduced pressure in a rotary evaporator at 35°C, and the pure oil was stored in a dark bottle at 4 °C until further use.

Table 1: Plant species used for the extraction of essential oils.

Family of the plant	Scientific name	Local name	Harvest site	Part of the plant used
Lamiaceae	*Lavandula officinalis*	Khozama	Fes	Air part
	Rosmarinus officinalis	Azir	Rabat	Air part
	Origanum compactum	Zaatar	Fes	Air part
Asteraceae	*Artemisia herba-alba*	Chih	Ouarzazate	Air part
Pinaceae	*Cedrus atlantica*	Larz	Rabat	Leaves
Lauraceae	*Thymus vulgaris*	Zaitara	Al Hoceima	Air part
Myrtaceae	*Syzygium aromaticum*	Koronfole	Indonesie	Buttons

Cultivation conditions:

Bacterial cultures and MIC tests were performed using liquid LB medium (lysogeny broth). Solid medium tests were performed on LB medium supplemented with 0.5% agar. In our investigation, all tests were repeated twice.

Characterization of strains

The count on the solid medium is expressed in CFU/ml according to the relation:

$$\text{UFC} = \frac{nomber\ of\ counted\ colonies}{Volume\ of\ inoculum\ \ (ml)} \times dilution\ of\ sample$$

Preparation of bacterial inocula

The one-night culture was used from stocks stored at -20 °C; 100 µl of the stock was added to a tube containing 5 ml of LB medium. The tubes were then incubated at 32 °C for 24 hours. After 24 hours, 50 µl of one-night culture was added to 5 ml of LB medium, and the tubes were incubated at 32 °C for 2 hours (day culture).

In view of the rapid growth of *E. coli*, the growth curve and the enumeration were carried out from 5 isolated colonies obtained by inoculating a Petri dish containing 20 ml of LB medium with agar by the streak method with 5 µl of the culture of *E. coli*. The dishes were incubated at 32 °C for 24 hours.

Enumeration and kinetics of growth:

In an Erlenmeyer flask containing 100 ml of LB medium, 1 ml of the bacterial culture of the day (in case of *S. aureus* and *K. pneumoniae*) or 3 to 5 colonies of *E. coli* were added. Erlenmeyer flasks were incubated at 32 °C with stirring at 250 rpm. At intervals of 90 min and for 9 hours, 1.5 ml was taken up in Eppendorf tubes for the measurement of the optical density at 600 nm. In parallel, decimal dilutions ranging from 10^{-1} to 10^{-7} were prepared. In a dish containing LB agar medium, 100 µl of the dilutions were spread out. The dishes were incubated at 32 °C for 24 hours.

Antibacterial activity

Antibiogram

An antibiogram test was performed to compare the effectiveness of the EOs on bacterial strains with 6 antibiotics, novobiocin, vancomycin, colistin sulfate, chloramphenicol, penicillin G, and tetracycline. To do this, 6 discs of Whatman paper soaked in each antibiotic were placed on the Petri dishes in which bacteria were growing to determine the sensitivity or resistance of a bacterium to antibiotics. Measurement of the diameter of the area of inhibition allowed assessing the antimicrobial effect of the product. A bacterial suspension (10^7 to 10^8 UFC/ml) of each strain was used for the tests of antibacterial activity until obtaining a sufficiently dense mat of confluent colonies.

For each strain, two LB agar plates were prepared: one for the test and one as a positive control. 400 µl of each bacterial suspension was added and mixed with 40 ml of the medium; then the medium was poured into boxes and allowed to solidify. Right after, six antibiotic discs per box were placed. Finally, the dishes were incubated at 32 °C for 18-24 h.

Well method

The well method is used to determine the antibacterial activity of the EOs to be tested. It rests on the migratory power of the EOs on a solid environment inside a Petri dish. The method consists of making wells filled with a quantity of essential oil on the surface of the agar seeded by the sprouts to be tested and measuring the diameters of inhibition in millimeters (mm) after incubation. The protocol followed is from Amit and Parul [22] with some modifications. For each strain, six soft agar plates of LB were prepared, five for the well test and one for positive control. After solidification and cooling, 5-mm wells were cut and 50 µl of each essential oil was deposited therein. The Petri dishes were then closed and left for the dissemination of oils for 30 minutes at room temperature. Then they were put in the oven at a temperature of 32 °C for 24 hours.

Micro-atmosphere technique

Based on the volatile nature of EOs, the micro-atmosphere technique was used to assess the antimicrobial activity of volatile compounds of EOs against the target bacteria. Inoculation of the culture was carried out like that of the well method. For this purpose, Whatman discs (5 mm in diameter) were soaked with 10 µl of each EO, placed in the middle of the lid of the Petri dish and not in direct contact with the inoculated agar. The dishes were then closed with Parafilm to prevent

vapor transfer between samples as well as the loss of volatile components of the EOs and incubated for 24 hours at 32 °C with the lid down. The absence of microbial growth resulted in a translucent zone on the agar.

Minimal Inhibitory Concentration (MIC)

The method of Chebaibi et al. [23] was used for **the** determination of the MIC with some modifications. In this study, three essential oils (*Syzygium aromaticum*, *Origanum compactum*, and *Artemisia herba-alba*) were chosen for the MIC determination. It's a micro-dilution technique to determine MIC values using resazurin as an indicator [24] of the bacterial growth.

Series of dilutions ranging from ½ to 256 were prepared in DMSO for *S. aromaticum* and *A. herba-alba* and from ⅓ to 1/1152 for *O. compactum*. First, 20 µl of DMSO or 0.2% agar solution was introduced to the first 10 wells. The dilution series of each oil were prepared by transferring 20 µl of EO of *A. herba-alba* (0.9 mg/ml) and *S. aromaticum* (0.95 mg/20 µl) and 10 µl of EO of *O. compactum* (1 mg/ml) to the first wells. Then, 20 µl of each strain and 160 µl were added at its wells containing EOs. For each analysis, the following controls were prepared:

- A positive control (column 11) including the LB medium and the bacterial strain.
- Positive control with DMSO (column 10) to find out what is the effect of DMSO on the bacterium containing DMSO, LB medium, and bacteria.
- Negative control (column 12) including LB medium alone without bacterial suspension.

The micro-plates were then incubated at 32 °C for 18-24 hours. For the revelation of the MIC, 30 µl of resazurin (0.2 mg/ml) was added to each well. MIC is defined as the lowest concentration of essential oil that does not produce a change in resazurin staining and corresponds to the absence of bacterial growth.

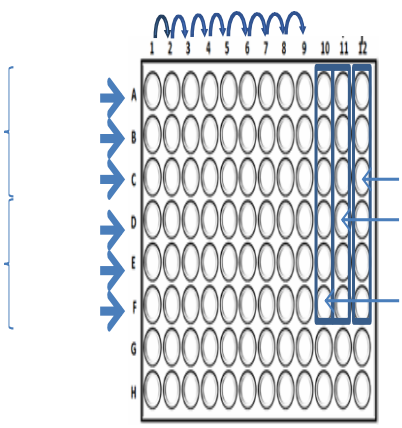

Figure 1: The protocol followed for micro-dilution.

Minimum Bactericidal Concentration (MBC)

MBC is the lowest concentration of EO which can eliminate more than 99.9% of bacterial growth. It defines the bactericidal effect of an EO. Wells lacking visible bacterial growth (no color change) from the micro-dilution are used for the determination of MBC. For this purpose, 100 µl of the contents of each well was spread on plates containing LB agar. Then, the dishes were incubated at 32 °C for 24 hours. The MBC of each EO was deduced from the first box devoid of bacteria with the minimum concentration.

RESULTS

Characterization of the strains:

Enumeration of *Staphylococcus aureus.*

The enumeration results of *S. aureus* revealed 10^{-5} dilution as the most reliable one for calculating the number of colonies.

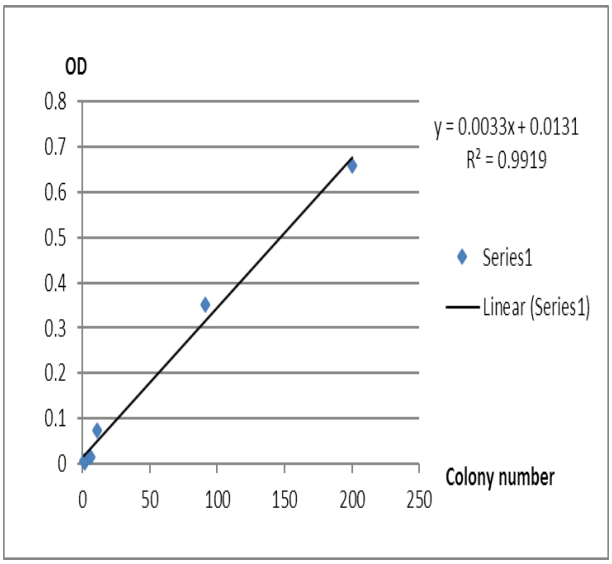

Figure 2: Optical density as a function of the number of colonies of *Staphylococcus aureus.*

Growth kinetics:

The study of growth curves showed similar growth kinetics between *S. aureus* and *K. pneumoniae*, characterized by a fairly long latency phase that lasted 270 min and an exponential phase lasting on average 120 min. While *E. coli* was differentiated with a short latency phase of 180 min and a rather long exponential phase of 540 min. The stationary phase was first established with *S. aureus* followed by *K. pneumoniae* and finally *E. coli*. In general, the growth of *E. coli* was much greater (OD_{600} of up to 1.48) than that of *S. aureus* and *K. pneumoniae* (OD averaging 0.7).

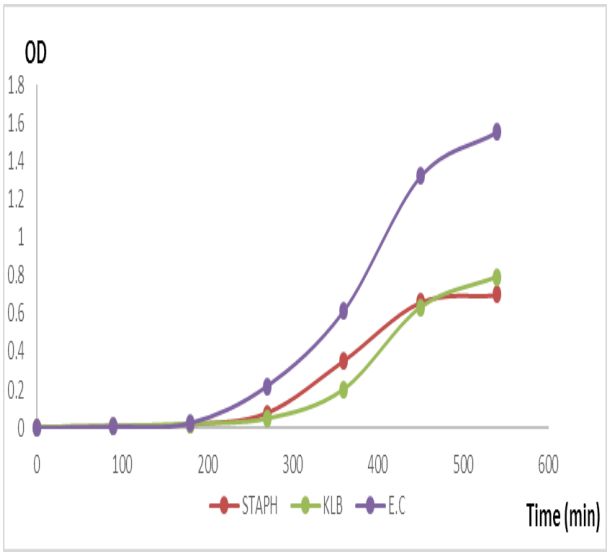

Figure 3: Growth curves of *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumonia.*

Antibacterial activity on solid medium:

Antibiogram

The results of the antibiogram (Table 2) showed some heterogeneity with a specificity of some antibiotics vis-à-vis bacterial strains. Chloramphenicol is positioned first with inhibition diameters ranging from 10 mm (*E. coli*) to 20 mm (*S. aureus*). Novobiocin was weak but specific to *K. pneumoniae*. Vancomycin and penicillin G were specific to *S. aureus* with inhibition diameters of 17 and 8 mm, respectively. Colistin sulfate was specific *to E. coli* with an inhibition zone of 11 mm. *K. pneumoniae* was the most resistant (4/6 ineffective antibiotics) followed by *E. coli* (sensitive to three out of six antibiotics) and *S. aureus* (sensitive to four out of six antibiotics).

Table 2: Resistance profile of the three strains tested.

Antibiotics	Bacteria		
	***Escherichia coli* (mm)**	***Staphylococcus aureus* (mm)**	***Klebsiella pneumoniae* (mm)**
Tetracycline	11	20	0
Novobiocin	0	0	4
Vancomycin	0	17	0
Colistin sulfate	11	0	0
Chloramphenicol	10	20	13
Penicillin G	0	08	0

Well method

Although all EOs showed an inhibitory effect, except that of cedar, this inhibition varied according to the strain tested. Indeed, *S. aureus* was the most resistant (3 out of 7 EOs were without effect) followed by *K. pneumoniae* (2 out of 7 were without effect). The most sensitive species to the effect of EOs was *E. coli* with zones of inhibition ranging from 10 mm (*S. aromaticum*) to 47 mm (*O. compactum).* The most effective EO was that of *O. compactum* with an inhibitory effect on the three strains and zones of inhibition of 34, 47, and 35 mm respectively for *S. aureus*, *E. coli*, and *K. pneumoniae* (Table 3).

Table 3: The diameter in mm of the inhibition zones of essential oils towards the three strains.

Bacteria	Oils						
	Thymus vulgaris	***Oreganum compactum***	***Rosemarinus officinalis***	***Artemisia herba-alba***	***Syzygium aromaticum***	***Lavandula officinalis***	***Cedrus atlantica***
S. aureus	10	34	9	0	8	0	0
E. coli	25	47	20	15	10	30	0
K. pneumoniae	7	35	17	7	10	0	0

The micro-atmosphere

From the results of the well diffusion test, five EOs were selected for the micro-atmosphere test, *R. officinalis*, *T. vulgaris*, *O. compactum*, *A. herba-alba*, and *S. aromaticum*. The results showed that only EO of *O. compactum* had a volatile effect, with inhibition diameters of 20, 8, and 30 mm respectively for *S. aureus*, *E. coli*, and *K. pneumoniae* (Table 4).

Table 4: Diameters of zones of inhibition of EO in the micro-atmosphere (mm).

Bacteria	Oils				
	Thymus vulgaris	*Oreganum compactum*	*Rosemarinus officinalis*	*Artemisia herba-alba*	*Syzygium aromaticum*
S. aureus	-	20	-	-	-
E. coli	-	8	-	-	-
K. pneumoniae	-	30	-	-	-

Study of antibacterial activity on liquid medium (micro-dilution)

MIC and MBC focused on three EOs (oregano, Artemisia, and clove). MIC was achieved by the micro-dilution method in the plate; the reading was made with resazurin (Fig. 4) in which pink and blue colors in the wells indicated positive and negative growth, respectively. Better solubility was observed with DMSO which at the same time has no effect on bacterial growth as shown by the positive control containing DMSO.

Determination of the MIC and MBC of *O. compactum*

The results presented in tables 5 and 6, shows that oregano EO had good activity on all the strains tested. It had bactericidal activity at very low concentrations, where the minimum inhibitory and bactericidal concentrations were very close to the MICs and MBCs of *K. pneumoniae* (0.34 and 0.34 µg/µl), *E. coli* (0.34 and 0.7 µg/µl), and *S. aureus* (0.17 and 0.7 µg/µl, respectively).

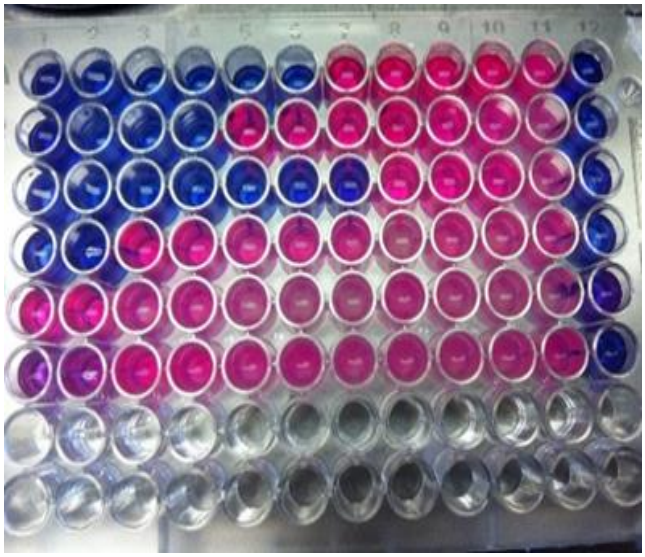

Figure 4: Result of the micro-dilution using oregano.

Table 5: Results of the MIC of *O. compactum*.

Bacteria	Dilutions								
	1/3	1/9	1/18	1/36	1/72	1/144	1/288	1/576	1/1156
E.coli	-	-	-	-	-	-	+	+	+
K. pneumonia	-	-	-	-	-	-	+	+	+
S. aureus	-	-	-	-	-	-	-	+	+

-: lack of growth +: positive growth

Table 6: Results of the MBC of *O. compactum*.

Bacteria	Dilutions			
	1/36	1/72	1/144	1/288
E. coli	-	-	+	
K. pneumonia	-	-	-	
S. aureus	-	-	+++	+++

Determination of the MIC and MBC of *A. herba-alba*, and *S. aromaticum*

Clove EO had antibacterial activity at low concentrations and a close MIC/MBC (0.37 µg/µl and 0.74 µg/µl, respectively). While sagebrush EO showed low inhibition with a very high MIC (5.62 µg/µl) that deviated from MBC (11.25 µg/µl) (Tables 7, 8, 9, and 10).

Table 7: Results of MIC for *A. herba-alba* and *S. aromaticum.*

Bacteria	Dilutions																	
	½		¼		1/8		1/16		1/32		1/64		1/128		1/256		1/512	
	C	A	C	A	C	A	C	A	C	A	C	A	C	A	C	A	C	A
E. coli	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	+
K. pneumoniae	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	+	+
S. aureus	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+

-: lack of growth **C: Clove (*Syzygium aromaticum*)**

+: positive growth **A: *Artemisia herba-alba***

Table 8: Results of MBC for *Artemisia herba-alba*.

Bacteria	Dilutions						
	½	¼	1/8	1/16	1/32	1/64	1/128
E. coli	-	-	-	-	-	+	
K. pneumonia	-	-	-	+	+	+	+
S. aureus	-	-	-	+++	+++	+++	+++

Table 9: Results of MBC for Clove.

Bacteria	Dilutions								
	½	¼	1/8	1/16	1/32	1/64	1/128	1/256	1/512
E. coli	-	-	-	-	-	-	-	+	+++
K. pneumonia	-	-	-	-	-	-	-	+++	
S. aureus	-	-	-	-	-	-	-	++	

Table 10: The MIC and MBC values of the three oils.

Bacteria	Oils					
	Oreganum compactum		*Syzygium aromaticum*		*Artemisia herba-alba*	
	MIC (µg/µl)	MBC (µg/µl)	MIC (µg/µl)	MBC (µg/µl)	MIC (µg/µl)	MBC (µg/µl)
E. coli	0,34	0,7	Nd	0,74	0,18	2,81
K. pneumonia	0,34	0,34	0,37	0,74	5,62	11,25
S. aureus	0,17	0,7	0,37	0,74	0,7	11,25

Nd: not determined

DISCUSSION

In order to evaluate the antibacterial activity of some essential oils, assessments on solid and liquid media, as well as vapor tests were performed on three bacteria known as the main causes of nosocomial infections: *S. aureus* (gram-positive), *E. coli*, and *K. pneumoniae* (gram-negative). Almost all essential oils (sagebrush, rosemary, thyme, cedar, lavender, oregano, and clove) showed low to very effective inhibitory properties. This is consistent with various studies including Ouedrhiri et al. [25] and Bouyahya et al. [26] The results revealed that oregano EO had a very strong antibacterial power with inhibition diameters of up to 34 and 47 mm. The results obtained with thyme and rosemary EOs were in agreement with those of López et al. [27] and Borugă et al. [28] Both EOs have shown moderate activity depending on the strain tested and the concentrations. Studies on clove EO showed contradictory results to ours. They showed that this oil has a moderate activity on *S. aureus* and *E. coli*. [27, 29] Lavender, on the other hand, showed a negative result with *K. pneumoniae* and *S.*

aureus and a high effect with *E. coli*. Studies on this oil have shown average activity on all three species. [30] The nature and provenance of the strain appear to play a role in the activity of essential oils. A study carried out by Imelouane et al. [31] on the activity of sagebrush essential oil on *S. aureus*, *E. coli*, and *K. pneumoniae* gave totally contradictory results, for the same bacterium; however, there was moderate to high activities. On the other hand, our results showed no effect of this oil on *S. aureus*, whereas it exerted a weak inhibition of the growth of *E. coli* and *K. pneumoniae*. Contrary to the results found by Derwich et al. [32], our tests on cedar EO showed zero activity for all the three bacterial strains. Numerous studies have demonstrated a difference in biological activities related to the difference and the heterogeneity of the composition of EOs and in particular their major constituents. [26]

The results of MIC and MBC showed in table 10 confirmed the strong activity of oregano EO. The ratio of MBC/MIC was in favor of a bactericidal effect on *E. coli* and *K. pneumoniae* and a bacteriostatic effect on *S. aureus*. Ouedrhiri et al. [25] published results attributing this strong activity to the majority of compounds of *O. compactum*; carvacrol (47.80%), γ- terpinene (17.25%), and thymol (15.74%). One study demonstrated the mode of action of carvacrol adhering to the membrane of gram-negative bacteria by attachment to membrane proteins and lipopolysaccharides through their functional groupings and thereby reaching the more vulnerable inner membrane. [33] The bactericidal effects were obtained with the essential oil of *S. aromaticum* on *K. pneumoniae* and *S. aureus* and the essential oil of *A. herba-alba* on *K. pneumoniae*, the latter having a little bacteriostatic effect on *E. coli* and *S. aureus*.

The tests carried out in liquid and solid media with different EOs on the three bacterial strains revealed resistance of the gram-positive species (*S. aureus*) compared to the two gram-negative species (*K. pneumoniae* and *E. coli*), except oregano EO that is effective for both gram-negative and gram-positive bacteria. Ouedrhiri et al. [25] and López et al. [27] have demonstrated a contrary effect by explaining their results by the structure of the outer membrane of gram-negative strains, which gives them some resistance.

Contrary to the results described above, the antibiogram showed resistance to gram-negative bacteria and the sensitivity of the gram-positive strain.

The last part of this work was devoted to the study of the antibacterial effect of the volatile fraction of essential oils by the micro-atmosphere method. Five essential oils were the subject of this test. Only the essential oil of oregano was effective. A study by Ghabraie et al. [34] showed antibacterial activity in the gas phase of EOs of oregano, clove, and rosemary. Studies on the volatile effect of EOs are still few.

CONCLUSION

Pathogen infection control has become a concern today, and the search for compounds that are effective and have the least resistance and toxicity is becoming critical. In this context, we are interested in Moroccan plants and more particularly in extracts in the form of essential oils that have a promising alternative against different types of pathogens. To this end, we evaluated the antibacterial activity of essential oils extracted from plants *T. vulgaris*, *O. compactum*, *R. officinalis*, *A. herba-alba*, and *L. officinalis* on three strains responsible for nosocomial infections; *K. pneumoniae*, *S. aureus*, and *E. coli*. Essential oils showed promising results with antibacterial effects against the three strains tested. But only one essential oil (EO of *O. compactum*) showed a volatile antibacterial effect on the strains tested. Our research will continue to develop more results for the remaining plants.

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