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# STUDY OF THE ANTIBACTERIAL ACTIVITY OF ESSENTIAL OILS OF CERTAIN PLANTS ON ISOLATED BACTERIA OF MAMMARY MILK IN ALGERIA

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**ABSTRACT**

The infection or inflammation of the mammary gland represents a dominant pathology in dairy cattle. The aim of this work was to study *in vitro* the antimicrobial activity of essential oils of some plants on bacteria which were isolated from mammary milk. The essential oils of *Thymus vulgaris*, *Rosmarinus officinalis*, *Artimisia herba alba* and *Myrtus communis* were obtained by hydro distillation of the aerial part. They were tested on strains of staphylococci that were multi-resistant to antibiotics which are isolated from mammary milk. The essential oils contents are between a low value of 0.3% for *Myrtus communis* and a relatively high value of 2.5% for *Thymus vulgaris*. The different essential oils tested revealed interesting antimicrobial activities. The strongest antibacterial activity was observed for the essential oil of *Thymus vulgaris* (9-52 mm), while the oils of *Rosmarinus oficinalis*, *Artimisia herba alba* and *Myrtus communis* showed moderate antimicrobial activities (9-19.5 mm). We also determined the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and the MIC / MBC ratio of *Thymus vulgaris* on the three multi-resistant strains of staphylococci; it turned out that MIC ranged between 0.01 and 0.016 µg / ml and MBC ranged between 0.021 and 0.078 µg / ml. In the conclusion, Thymus vulgar is revealed a bactericidal effect on the strains tested.

Keywords: Antibacterial activity; aromatherapy; essential oil; mastitis; medicinal plants.

## INTRODUCTION

In dairy cattle breeding, mastitis or inflammation of the mammary gland represent a dominant pathology [1,2]. The use of anti-infectious in veterinary medicine is essential for the treatment of bacterial infections and for the control of secondary infections in the case of viral attacks [3]. However, their improper and often reckless use can be complicated by the presence of residues in food products of animal origin on one hand which contributes to the selection of multi-resistant pathogenic strains on the other hand [4]. Today, the rapid development of these resistances strongly limits the therapeutic interest of these molecules [5,6]. The need to find new anti-infectious agents and alternative therapeutic or preventive strategies is currently one of the major challenges of pharmaceutical research. Consequently, the use of essential oils, prebiotics, probiotics or even antimicrobial peptides could play an increasingly important role in the management of antibacterial resistance in veterinary medicine and reduce its impact on public health [7,6].

However, noting the lack of information on this approach. Elsewhere, controlled trials are nevertheless carried out, but are not published or are published in inaccessible journals. In Algeria, no similar work has been carried out in veterinary medicine. Paradoxically, there are more information in human medicine where plant extracts have a recognized action in the treatment of various infectious diseases [8,9], but these references are of little use to us in veterinary medicine and especially in the case of mastitis. In addition, the use of such substances in veterinary therapy implies not only that the product would be effective and less expensive but also it would not be a source of residues in food products that are potentially dangerous for the health of animals or the consumers.

During 2012-2013, we launched a survey of 60 cattle breeders from districts in the central region of Algeria, in order to know their treatment behavior facing pathologies [10]. A frequent resort to phytotherapy and aromatherapy has appeared with the use of plants, their extracts and a dozen different oils, in various ways. First, in vitro study of the germs responsible for diseases in animals would be interesting in order to seek a practical alternative to conventional treatments. This present work aims to seek an interesting alternative to propose instead of antibiotics in the treatment of bovine mastitis. It aims to evaluate the antimicrobial activity of essential oils in vitro from four plants: *Rosmarinus officinalis*, *Thymus vulgaris*, *Artimisia herba alba* and *Myrtus communis* on bacterial strains isolated from clinical and subclinical mastitis that are resistant to most antibiotics used in the treatment of animal pathologies.

## MATERIALS AND METHODS

### Biological Materials

### Bacterial strains

Various bacterial strains isolated from both clinical and subclinical mastitis have been evaluated for their *in vitro* sensitivity to antibiotics. They are distributed as follows: 10 strains of coagulase-positive staphylococcus, 35 strains of staphylococcus coagulase,-negative and 35 species of the enterobacterial group. Following the antibiotic, we only chose multi-resistant bacteria: Resistance to more than two antibiotics. The in vitro test was carried out on the latter. There are 5 of them.

### Antibiotics

The twenty-one 21 antibiotics tested are selected from the molecules currently active on staphylococci (compared with 12 different antibiotics) and enterobacteria (16 different antibiotics). Most of them are used in the treatment of mastitis in lactation and/or off lactation. The antibiotic disks tested are cited in Table 1.

### Tested Plants

### Selection of pants

With the aim of seeking an alternative to therapeutic failures by antibiotics encountered in the field in the treatment of bovine mastitis, our choice is based on a new approach not yet adopted in Algeria: Phytotherapy. However, But, as a first step, *in vitro* testing is essential before any clinical trial.

The purpose of this in vitro evaluation is to investigate a real activity of extracts from certain plants traditionally used in medicine against the germs most frequently responsible for the disease, including *staphylococcus aureus*.

In this study, we selected plants based on the following criteria:

- Traditionally used in the treatment of diseases of microbial origin.
- The availability of these plants.
- Their potential for secondary metabolites: phenolic compounds with essential oils.
- These plants must be non-toxic due to the protection of the final product, which is the milk and health of the animal (the cow).

Four plants were chosen. The latter have proved to be the most used in traditional medicine. The plants used in this work are found on the market throughout the year, for their major importance and daily use in Algerian cuisine. They were purchased in dried form, four (4) plants were used as a secondary metabolite source; the plants were bought from a Maamoura herbalist in the wilaya of Laghouat. For *Myrtus communis*, it was taken from the mountains of Jijel (East of Algeria).

**Table 1. The different disks of tested antibiotics**

| Famiy | Antibiotic | Abbreviation | Concentration |
|---|---|---|---|
| β. Lactamines | Penicillin G | P | 6 |
| | Ampicillin | AMP | 10 |
| | Amoxicillin | AM | 30 |
| | Oxacillin | OX | 1 |
| | Amoxicillin + Clavulanic Acid | AMC | 10 |
| Cephalosporins | Cefotaxime | CTX | 30 |
| | Cefoxitine | FOX | 30 |
| | Cefodiazin | Cz | 30 |
| | Ceftiofur | XNL | 30 |
| Aminoglycosides | Clindamycin | CM | 2 |
| | Gentamicin | G | 10 |
| | Neomycin | N | 30 |
| Quinolones | Flumequine | FT | 30 |
| | Chloramphenicol | C | 30 |
| | Nalidixic acid | NA | 30 |
| Macrolides | Erythromycin | E | 15 |
| | Enrofloxacin | ENR | 5 |
| Lincosamides | Vancomycine | VA | 30 |
| Association sulfamides diaminopyrimidines | Triméthoprim Sulfaméthoxazol | SXT | 1,25 – 23,75 |
| Tétracyclines | Tetracycline | TE | 30 |
| Polypeptides | Colistine | CL | 50 |

**Table 2. The regions of collection of the used plants and the tested parts of the plants**

| Name of the plants | Family | Common names | Regions of the collection | | Collected and tested part | Medical use |
|---|---|---|---|---|---|---|
| | | | Willaya | Localion | | |
| *Rosmarinus officinalis* | Labiate | Rosemary | Laghouat | Maamoura | Aerial part | Stimulation of brain activity, improved memory, against aging and to relieve abdominal pain [11]. |
| *Thymus vulgaris* | Labiate | Thym | Laghouat | Maamoura | Aerial part | Treatment of respiratory diseases (colds, flu, angina) and gastric disorders [12]. |
| *Artimisia herba alba* | Asteraceae | Armoise blanche | Laghouat | Aflou | Aerial part | Ease digestion; relieve abdominal pain, against seizures [13]. |
| *Myrtusco mmunis* | Myrtacae | Myrte | Jijel | Jijel | Aerial part | Against bronchitis, Muco-purulent catarrhes, pulmonary tuberculosis, rhinorrhea; hypoglicemic effect [12]. |

**Table 3. Growth mediums and the used reagents during the study**

| Growth mediums and the reagents | Utilization |
|---|---|
| **Solid growth mediums** | |
| Mueller-Hinton(MH) | Antibiogram (sensitivity of bacteria to plant extracts |
| **Liquid growth mediums** | |
| Bouillon nutritif (BN) | Bacterial enrichment |
| Mueller-Hinton (MH) | CMI |
| **Reagents** | |
| DMSO | dilution of HEs |
| Sterile physiological water: NaCl (9 g/l) | To prepare and dilute bacterial suspensions. |

**Products**

**Growth medium**

The following table shows the culture medium used to conduct our *in vitro* test.

**Methods**

**Essential oil preparation**

We used the aerial part: leaves and stalks (dried) of plants. The HES were extracted using the hydrodistillation method with the installation of "Clivenger". In total, we used 200 grams of each plant in one liter of distilled water for 03 hours at 100 [14].

**Performance calculation**

The yield of HE is determined in relation to the initial plant weight. It was calculated as the ratio between the mass of essential oil obtained and the mass of the plant material to be treated.

RHE (%) = MHE / MS .100.

R: Performance in fixed extracts in g /100g of dry matter. MHE: Quantity of the extract recovered, expressed in grams. MS: quantity of of the dry vegetable matter used for extraction, expressed in grams.

**Antibacterial activity evaluation method**

Testing of antibacterial activity of HEs was carried out using two methods:

- ✓ The solid medium diffusion method, as a first step in selecting strains with significant sensitivity.
- ✓ The liquid dilution method, to determine minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC), for active extracts.

**Protocol for the evaluation of antibacterial activity by disk dissemination method**

For the testing of antimicrobial activity, pre-cultures were prepared. Bacteria were enriched with a nutritive broth incubated for 24 hours at 37°C; then the bacteria were put into cultivation on a nutrient agar. Although it is recognized as reliable and reproducible, it is mainly used as a preliminary step for further studies, as it provides access to essentially qualitative results. The technique used is a modification of the Hayes and Markovic method [15].

It is also called the technique of the antibioaromatogram. In this method, we used filter paper disks with a diameter of 06 mm, impregnated with HE and deposited on the surface of gelose medium according to the technique adopted by Jacob et al., [16] and Tharib et al., [17]. After incubation, the results were read by measuring the diameter of inhibition in mm.

## Inoculum Preparation

From a pure 24-hour culture on an isolation mediium, we scraped some well isolated and perfectly identical colonies with a platinum anise. We then unloaded it in 9 ml of sterile physiological water while looking for a bacterial suspension with an optical density of 0.08 MacFarlend to 1 Mac Farlend. The sowing process took place within 15 minutes of the preparation of the inoculum.

## Sowing

We dipped a sterile swab into the bacterial suspension to load it to the maximum; and then we scrubbed the swab over the entire dry MH from top to bottom in stripes. We repeated the operation 3 times by turning the box 60º each time and ended up with a swab over the agar's peripheral.

## Application of the Disks

Disks are deposited on the surface of the Muller Hinton agar (MH) medium after sowing the strain studied (*Staphylococci aureus*) to T 37°C for 24 h on the boxes on which antibiotics are deposited. The 9 cm diameter Petrie boxes were flown with the MH medium and were subsequently inoculated with a freshly prepared pure suspension. We then imbibed 06 mm diameter sterile Watman paper disks with 5 µl HE of different plants tested and deposited them on the surface of the sown agar. The set was incubated for 24 hours at 37°C [18].

## Reading Results

Accurately measuring the diameter of the inhibition zones using a sliding foot on the bottom of the petry box. The results are expressed by the diameter of inhibition and are symbolized by signs based on the sensitivity of the strains to HE [19] as follows:

- Not sensitive (-) or resistant: less than 8mm
- Sensitive (+): diameter between 9 and 14mm.
- Very sensitive (++): diameter between 9 and 14mm.
- Extremely sensitive (+++):diameter more than 20mm.

## Determination of the MIC

This technique consists of inoculation, by a standardized inoculum, of a decreasing concentration range in HE. After incubation, the range observation allows access to the Minimal Inhibiting Concentration (MIC), which is the lowest concentration of HE capable of suppressing bacterial growth [20].

This technique of determining the MIC by direct contact in agar or liquid medium consists of dispersing the extract solution to variable concentration in a homogeneous and stable manner in the medium in the presence of a tested germ [21]. For our trial we chose the method described by Lahlou [22]. First, HEs were diluted in DMSO (an emulsifier) beforehand. A suspension of each germ is prepared in sterile physiological water and adjusted to108 cells/ml. From each suspension, 100µL. are deposited on the medium plus extracts at different concentrations.

To calculate MIC by successive dilution in MH-box, we incubated a medium with a dilution of increasing doses of the extracts to be tested.

The Minimum Bactericide Concentration (MBC) is the lowest HE concentration capable of killing more than 99.9% of the initial bacterial inoculum (less than 0.01% of survivors). It defines the bactericidal effect of an essential oil.

The MBC was determined in solid medium.

The same concentration range, carried out by the liquid macro dilution technique, is used to determine the MIC and MBC of the HE to be tested.

Using a calibrated platinum cove, we seeded in parallel striations on agar boxes (Muller Hinton). The series of tubes starting from the one that determined the MIC. Tests are made in the control tube and in each of the tubes without bacterial lips and deposited "in line" on Muller Hington agar. Seeded boxes are incubated 24 hours at 37°C, according to Sériys' recommendations [23].

The MBC (%, v/v) of the essential oil is derived from the first bacterium-free box. Each experiment is performed three times in three successive experiments. Read: The MIC was read as follows: concentration for which there is no visible bacterial culture [24].

### Statistical Study

Statistical analysis was performed using Statistica (Version 10, Stat Soft France, 2003). Experimental results were expressed by an average of three repetitions ± standard deviation. In order to bring out the comparative aspect, the ANOVA test was used. The difference between samples is considered significant if the probability $p < 0.05$.

## RESULTS AND DISCUSSION

### Antibiogram Results of Selected Strains

In this work, only the results of the antibiogram of the strains tested with the extracts of the plants are presented (Table 4). We have noted that all strains on which the aromatogram was made have shown multiple resistances to antibiotics.

The bacterial strains tested are multi-resistant strains, i.e. resistant to more than two antibiotics.

*In vitro* testing of the efficacy of extracts from certain plants would be very interesting since a huge problem will be solved: multi-resistance.

**Table 4. Antibiogram results of strains tested against plant extracts**

| Strain | Code | P | OX | FOX | AMC | ENR | VA | SXT | CM | GM | TE | N | E | R | I | S |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SCN | **53** | R | R | S | R | S | R | S | R | S | R | S | R | 7 | 0 | 5 |
| SCN | **43** | R | R | R | S | I | R | S | R | S | S | S | R | 6 | 1 | 5 |
| SCP | **82** | R | R | R | R | S | R | S | R | S | S | S | R | 7 | 0 | 5 |
| SCN | **39** | R | R | S | R | S | S | S | S | S | S | S | R | 4 | 0 | 8 |
| SCN | **46** | R | R | I | R | S | R | S | R | S | R | S | R | 7 | 1 | 4 |

**R: Resistance, *I: Intermediate, *S: Sensitive*

**Table 5. Performance of extraction of HEs from *Rosmarinus officinalis*, *Thymus vulgaris*, *Artimisia herba alba*, and *Myrtus communis***

| | Weight (g) | Performance (%) |
|---|---|---|
| *Thymus vulgaris* (TV) | 200 | 2.5 |
| *Rosmarinus officinalis* (RO) | 200 | 1.1 |
| *Artimisia herba alba* (AHA) | 200 | 0.98 |
| *Myrtuscommunis* (MC) | 200 | 0.3 |

**The Extraction Efficiency of Essential Oils**

The performance results of HE hydrodistillation are reported in Table 5.

The highest yield is *Thymus vulgaris* (2.5%) and the lowest yield is Artemisia herba-alba (0.98%). For the armoise species, the yield found in our experiment is similar to that of the white Armoise yield recorded in Spain (0.41% - 2.30%) for 16 samples from 4 sources [25]. The yield of *Rosmarinus officinalis* is 1.1%; that is a lower return than reported by Ayadi et al. [26] in the region of Sidi Bouzid in Tunisia (1.35%).All extractions for dried plant parts produced significantly low yields. This decline is likely related to evaporation of volatile compounds during prolonged drying [27].

The yield of these secondary metabolites is different from one botanical family to another, from one species to another, and even between plants of the same species. In addition, this difference in HE content may be linked to several factors such as the geographic area of collection, climate, pedoclimatic conditions, extraction technique, stage of development and season [28,29,30].

It may be significantly higher in other Anthimed Asteraceae such as the Armoise [31].

**Antimicrobial Activities**

**Vincent method results (Disk method)**

The results of the HE antimicrobial evaluation by the disk method are given below (Table 6). In this table, the values in (mm) of the diameters of the inhibitions zones are included, representing the magnitude of the halo formed by bacteria destroyed by antimicrobial activity of HE. The disk diffusion method demonstrated the antimicrobial power of isolated HEs from four plants; the satisfactory positive result is validated for a halo of inhibition with a diameter greater than 15 mm, as recommended by Billerbek [32].

**Table 6. The diameters in millimeter, of the zones of inhibition of HE**

| Strains | Diameter in mm des HE (5 µl/disk) | | | |
|---|---|---|---|---|
| | TV | RO | AHA | MC |
| **39** | 31.25 | 9 | 6 | 16.5 |
| **53** | 39.87 | 6 | 13.25 | 6 |
| **82** | 45 | 10.5 | 8 | 6 |
| **46** | 47.5 | 6 | 6 | 6 |
| **43** | 52 | 11 | 11.75 | 19.5 |

In the test of antimicrobial activity of HE, the results obtained by the disk method show a wide variation in the diameter of the inhibition zones ranging from 0 to 52 mm, depending on the strains. The strains studied did not show the same sensitivity to HE. The essential oil of *Thymus vulgaris* proved to be 100% effective.

The activity of this oil is higher than that of the other three plants (Fig. 1).

According to Conner [33], Thyme and Romarin HEs show high inhibitory capacity against staphylococcus sp with inhibition zones of up to 52 mm.

This is revealed by an inhibition zone greater than 50 mm, regardless of the bacterial strain tested. Therefore, these germs showed a high sensitivity to this type of oil.

In the second position, the HE of *Rosmarinus officinalis* revealed 60% activity with inhibition zones between (9 and 11 mm). This result is similar to those of Benikhlef [34] and Frouhat and Lahcini [35], which showed that HE of *Rosmarinus officinalis* has less antimicrobial activity against *Staphylococcus aureus* with an inhibition diameter of 09 mm.

In the same vein, Skocibusic et al., [36] reported that Romarin HE has antimicrobial activity against Staphylococcus.

*Myrtus comminus* HE showed some efficacy against multi-resistant bacterial strains such as strains (39.43). However, this oil appears to be effective only with 40% of the strains, based on the results of Skocibusic et al., [36].

The results obtained from AHA HE inhibitions areas (8 mm -13.25 mm) are lower than those of the Mighri et al. study [37] (22.3 mm).

We found that HES studies did not inhibit all bacterial species.

The results of antimicrobial activity of essential oils by the disk method show a variation in the diameters of the inhibitions zones ranging from 9 mm to 52 mm depending on the species tested.

Our results show antibacterial activity in the extracts of the tested plants and are consistent with those of the literature. EHs contain terpenes that have stimulating, antibacterial, and sedative properties [38].

Because essential oils are phenol, they often have anti-infectious roles against mastitis [39].

Much research has shown that positive Gram bacteria have a high sensitivity to essential oils than negative Gram bacteria [40]; The cell wall structure of positive Gram bacteria, however, makes them more sensitive to the action of these oils than that of negative Gram strains that limit the passage of substances [41]. These explanations could be the causes of sensitivity of the staphylococcal multiresistant strains (Gram positive) tested in this work.

We find that some of the HEs used do not inhibit the growth of bacterial strains studied such as AHA and MC.

Oussalah et al. [42] reported that the difference in HES antibacterial activities may be related to concentration, nature and content, functional groupings, compound configuration, and possible synergistic interaction.

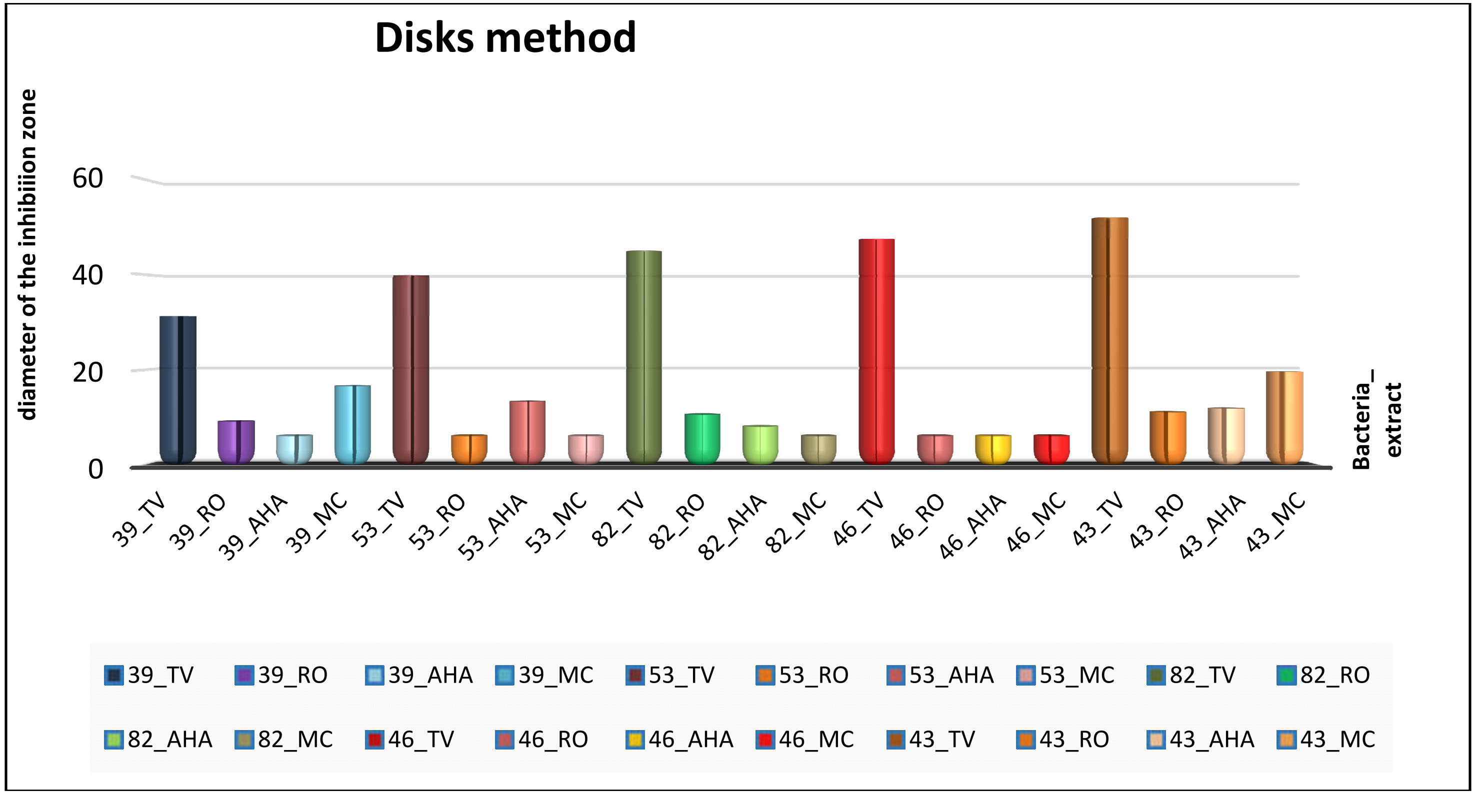


**Fig. 1. Zones of inhibitions of essential oils in the tested bacterial strains**
**TV: Thymus vulgaris,*RO: Rosmarinus officinalis, *AHA: Artimisia herba alba,*
** MC: Myrtus communis*

**Table 7. The minimum inhibitory concentration (MIC) values of *Thymus vulgaris* HE**

| | Dilutions of the CMI, the concentrations of the extract | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Dilutions | $\frac{1}{100}$ | $\frac{1}{50}$ | $\frac{1}{33.33}$ | $\frac{1}{25}$ | $\frac{1}{20}$ | $\frac{1}{16.66}$ | $\frac{1}{14.25}$ | $\frac{1}{12.5}$ | $\frac{1}{10}$ | $\frac{1}{6.66}$ |
| Concentration $\mu g/ml$ | 0.005 | 0 .010 | 0 .016 | 0.021 | 0.026 | 0.031 | 0.036 | 0.042 | 0.052 | 0.078 |
| Strains | | | | | | | | | | |
| 46 | + | - | - | - | - | - | - | - | - | - |
| 82 | - | - | - | - | - | - | - | - | - | - |
| 43 | + | + | - | - | - | - | - | - | - | - |

**+: presence of growth, *- : absence of growth*

**Table 8. The minimum bactericidal HE concentration values of *Thymus vulgaris* on strains: (43, 46, 82)**

| | Dilutions of the MIC, the concentrations of the extract | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Dilutions** | $\frac{\mathbf{1}}{\mathbf{100}}$ | $\frac{\mathbf{1}}{\mathbf{50}}$ | $\frac{\mathbf{1}}{\mathbf{33.33}}$ | $\frac{\mathbf{1}}{\mathbf{25}}$ | $\frac{\mathbf{1}}{\mathbf{20}}$ | $\frac{\mathbf{1}}{\mathbf{16.66}}$ | $\frac{\mathbf{1}}{\mathbf{14.25}}$ | $\frac{\mathbf{1}}{\mathbf{12.5}}$ | $\frac{\mathbf{1}}{\mathbf{10}}$ | $\frac{\mathbf{1}}{\mathbf{6.66}}$ |
| concentration µg/ml | **0.005** | **0 .010** | **0 .016** | **0.021** | **0.026** | **0.031** | **0.036** | **0.042** | **0.052** | **0.078** |
| 46 | **+** | **+** | **+** | **+** | **+** | **+** | **+** | **+** | **-** | **-** |
| 82 | **+** | **+** | **+** | **-** | **-** | **-** | **-** | **-** | **-** | **-** |
| 43 | **+** | **+** | **+** | **+** | **+** | **+** | **+** | **+** | **+** | **-** |

**+: presence of growth, *- : absence of growth*

In this case, the investigation should now be expanded over time and space and new alternatives based on better use of plant biodiversity, which are also capable of creating and promoting new high-value products through simple and accessible biotechnologies.

**The Results of MIC and MBC**

This technique of direct contact MIC determination in agar or liquid mediim consists of dispersing the antimicrobial agent in variable concentration homogeneously and stable in the germ medium studied [2,43].

This technique, which is highly reliable and reproducible for water-soluble antimicrobial agents, poses a problem of diffusion and homogeneity of dispersion with essential oils that have very low solubility in aqueous culture medium.

This problem has been partly solved by the use of essential oil emulsions in solutions of different detergents such as DMSO [43,20].

For MIC, we chose the most active HE, that is, *Thymus vulgaris* against the most sensitive multi-resistant bacteria of this HE, and we eliminated the other HEs.

The results of MIC, MBC and the values of the MBC/MIC report are illustrated in the Tables 7, 8 and 9.

In Table 7, the MIC of our essential oils are reported in solid-medium studies with inhibitions diameters greater than 12 mm (arbitrary choice). MIC are inversely proportional to the diameter of the inhibition zones, obtained using the disk method.

The results of the HE MIC tests extracted from *Thymus vulgaris*, tested on the three strains of S. the most sensitive of this oil. In strain 82 (SCN), we noticed the absence of a growth-related disorder with dilutions tested. In this case, there is a total absence of growth even with a low concentration of HES.

Our study found that even with low concentrations of MIC (0.005-0.010) the thyme inhibited all tested strains, then our results are lower than the results of Moulay et al. [44] the study of which reported the results of MIC:(0.033 _0.05) and that of which of Benabed [45] which reported results of MIC of 1,016, and also that of Alessandra et al. [46] that reported MIC results: (0.005 this concentration may inhibit growth of *Staphylococc aureus*.

We also noted that the MIC of *Thymus vulgaris* essential oils on the three strains tested ranged between 0.01 and 0.016µg/mL and CMBs ranged between 0.021 and 0.078 µg/mL.

Our MBC result: (0.021_ 0.078 µg/ml) is lower than that of Benabed [45] which reported results of (1.016 µg/ml); this concentration can kill bacterial strains.

Aligiannis et al. [47] proposed a classification of HES of plant material on the basis of the MIC results, as follows:

Strong inhibition: MIC less than 0.5;
Moderate Inhibition: MIC ranges from 0.6 to 1.5;
Low inhibition: MIC greater than 1.6.

Thus, according to this classification, there is a strong inhibition with the essential oil of *Thymus vulgaris* on the different strains tested.

**Table 9. CMB/CMI report values**

| Strains | CMI | CMB | CMB/CMI | Effet |
|---|---|---|---|---|
| **46** | 0.010 | 0.052 | 0.19 | B.cide |
| **82** | 0.005 | 0.021 | 0.238 | B.cide |
| **43** | 0.016 | 0.078 | 0.20 | B.cide |

**B.cide: bactericidal effect*

The differences observed for MIC values can be explained by the presence of antibacterial compounds in plant HES at different concentrations, but also by the choice the used technique. In order to evaluate the activity of our essential oils in terms of bactericides or bacteriostats, the MBC/MIC report was calculated by Oussou et al. [48].

The MBC/MIC report defines the bacteriostatic or bactericidal character of an HE. According to Moroh et al. [49] and Oussou et al. [48], when the MBC/MIC activity ratio of an antimicrobial substance is less than or equal to four (≤ 4), the antimicrobial substance is classified as a bactericidal substance and if the MBC/MIC ratio is greater than four (> 4), then this HE is called a bacterial antimicrobial substance bacteriostatic.

The MBC/MIC ratios of *Thymus vulgaris* HE are all 4-inferior and therefore considered to be bactericides, the HE of *Thymus vulgaris* has a bactericidal effect on the strains [47].

HE inhibits the growth of all bacterial strains used in this study. To measure its inhibition potential, MIC tests were conducted on the three multi-resistant bacterial strains. These tests were performed by the liquid microdilution technique, except for three strains to determine the MIC and solid to determine the MBC.

## CONCLUSION

Evaluation of the antibacterial activity of essential oils showed effective and strong activity against the tested bacterial strains, including the results of the extract of *Thymus vulgaris*et and of *Rosmarinis officinalis*. However, less important results were obtained for the essential oils of *Artimisia herba alba* and *Myrtus communis*. By determining the MIC and MBC of *Thymus vulgaris* which showed high activity on miltiresistant antibiotic strains, we found that *Thymus vulgaris* has a bactericidal effect on the tested strains. Obtained data confirmed the possible use of some essential oils extracts in the clinical management of the pathology as phytotherapic natural agents. Further studies about the toxicity, the concentrations and the interest of other plant compounds have to be investigated.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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