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Original Article

# Comparative study of the antibacterial potential of natural and commercial oils of *Rosmarinus officinalis* L

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**ABSTRACT**

The article tries to shed light on the antibacterial power of rosemary oils to see if there is a possibility of making drugs but also to compare this power with that existing in commercial oils of rosemary and to check if these formulas really contain oils rosemary. This study consisted of the extraction of the essential oil from the studied plant followed by preliminary phytochemical tests and then by TLC analyzes to determine exactly the different compounds that characterize the species and then by the antibacterial study on the plants diluted essential oils (synthesized and commercial). Phytochemical tests have shown that essential oils contain many chemicals and active ingredients that give rosemary a wide range of indications : The TLC of the raw extract shows stains, indicating the presence of flavone, hydroxyflavone, Dihydroxyflavone, flavanones, methoxyflavones and flavonols ; they are compounds of paramount importance in the therapeutic effects enjoyed by rosemary. The bacteriological study on essential oils (extract and commercial) by the process represented on the concentrated disk of essential oils shows good antibacterial activity on the fixed stem ; the zone of inhibition is of 08 and 06 mm for *Escherischia. coli* and 10.09 and 08 mm for *Staphylococcus aureus* and 17, 14, 13.11 mm for *Klebsiela sp*. Essential oils extracted from rosemary from Algeria will be of some economic interest in the Algerian pharmaceutical industry. The negative test of commercial rosemary essential oil compared to the positive tests of the rosemary extract (just 10 mm diameter at concentration D1 for *Klebsiella. sp* and zero results at all other concentrations with the other strains including those that reacted to the rosemary extract tested ) provides information on the counterfeiting that characterises this field.. The study confirms the interest we must bring to this plant, not only for its characteristics in the perfume industry, but especially for its undisputed medical virtues.

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## 1. Introduction

The discovery of the healing properties of plants was certainly fortuitous. In wanting to feed on plants, primitive man found medicine. But after what harmful experiences did he discover the virtues of plants and can he differentiate the results? He was helped by the animals he knew how to use [3].

Nearly two thousand essential oils are known today, nearly 200 of which are the subject of important international commercial transactions. A hundred of them are in common use and a dozen serve as a first for important syntheses [17]. To better know the therapeutic virtues of a plant, it is necessary to analyze the chemical composition of its active ingredients that will allow us to fight against infections produced by bacteria or certain other pathogens [20]. It has been found that the extract of leaf of several plants have an important antimicrobial activity against human pathogens [4, 23] Many oils have been defined as antibacterial [8]. Essential oils have important antibacterial activities and can successfully replace antibiotics that show their inefficiencies against resistant microorganisms [7]. *Rosmarinus officinalis* is one of the most widely used medicinal plants around the world. Extracts of essential oils of this plant are widely used in traditional medicine for centuries against a multitude of ailments. Today, Rosemary

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has entered modern medicine [16]. Their spectrum of action is very broad because they act against a wide range of bacteria, including those that develop resistance to antibiotics. This activity is also variable from one essential oil to another and from one bacterial strain to another [19]. Bacteria that are resistant to certain antibiotics can sometimes be inhibited by essential oils[22]. To benefit from the benefits of these essential oils of rosemary and other plants certain industries and without going through the modern pharmacopoeia have put on the market preparations boasting of being essential oil of rosemary with all the virtues that we know him so far, our study confirms the antibacterial action of essential oils of rosemary on certain bacterial strains and sets the essential objective of demonstrating whether these commercial preparations really have the reputation they are supposed to have.

Among the plants with perfume with an industrial character, Rosemary occupies a place of choice and its essential oil is most appreciated.

The purpose of this work is to obtain secondary metabolites, by the hydro-distillation method, and subsequently the phytochemical and biological study of *Rosmarinus officinalis* L.

In this study the method used for the extraction of the essential oil of the studied plant followed by preliminary phytochemical tests and then TLC analyzes and an antibacterial study on diluted essential oils (synthesized and commercial) were described.

## 2. Materials and Methods

### *2.1. Plant material*

The choice of the medicinal plant was on the rosemary whose scientific name is "*Rosmarinus officinalis* L". The aerial part will serve as raw material for the extraction of essential oils.

The plant was harvested whole (stems and leaves) in the region of El Hamma with the coordinates Latitude: 35° 27' 29.39" N

Longitude: 7° 04' 34.20" on March 2022 (full bloom).

The rosemary's sample is cleaned of dust and impurities, and then the aerial part was dried for 15 days, in a well-ventilated room at a temperature not exceeding 35° C in the shade to avoid photosensitivity. After drying, the plant material is crushed in an electronic mill, the powder is weighed up to 200g.This powder will be used for the extraction of essential oil.

### *2.2. Drying and Extraction of oil*

The simple hydro-distillation was used for the extraction of the oil. It consists to plant's direct immersion in a still filled with water which is then brought to a boil. The heterogeneous vapors are condensed on a cold surface and the essential oil separates by difference in density [6]. The extraction of the essential oils was carried out by hydrodistillation for one hour, at a temperature of 100 ° C., of the dried aerial parts of the plant in a Clevenger type apparatus according to the European Pharmacopoeia [13]. Water vapor enriched with volatile constituents is condensed and then decanted at 20 ° C. This operation is followed by the calculation of yields made according to the AFNOR standard [1].

The vapors charged with water and oil pass through an elbow acting as a small column, and condense in a refrigerant which will be maintained in a slightly inclined position.

The distillate flows dropwise and then, collected in a separating funnel located at the end of the refrigerant tube ; the balloon is fed with aromatic water (the water that remains after recovery of the essential oil) so as to maintain its constant water level. The recovery is by separate fraction at different time intervals up to 5 hours of extraction.

100 of vegetable matter requires 1 liter of water under a temperature of 100° C during 5 hours

### *2.3. Phytochemical tests*

Consist in detecting the different families of existing compounds in the studied parts of the plant by qualitative characterization reactions. These reactions are based on precipitation or staining by reagents specific to each family of compounds : tannins, polyphenols, anthocyanins, leucanthocyanins, coumarins, alcaloids, reducing compounds and flavonoids

### *2.4. Antibacterial activity*

The anibacterial activity of Rosemay's oil is evaluated by the aromatogram method which allows determining the sensitivity of the different bacterial species to the essential oil given.

The aromatogram is a qualitative method to test the antimicrobial activity of a substance against a particular microorganism. This method has been prepared using essential oils of *Rosmarinus officinalis*. The strains used were *staphyloccocus aureus* (ATCC 25923), *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella sp*. (ATCC 25922). Little sterilised disks of blotting paper saturated with with 10 µL of these EOs were placed on the surface of a Müeller Hinton plate count agar previously spreaded with *the strain* inoculum. After a latency period at 37°C±1 for 24 h, the diameter of the inhibition halo against the strain was measured with a caliber [27]

Generally, micro-organisms tested (in this study : *Escherichia coli, Staphyloccocus aureus, pseudomonas aeruginosa* and *Klebsiella sp*) will be classified as susceptible, intermediate or resistant, depending on the

diameter of the zone of inhibition [28]. The aromatogram technique was applied in order to evaluate the activity antimicrobial essential oils. This test is performed by depositing a sterile disk of 6 mm diameter impregnated with an amount of essential oil on an agar medium previously inoculated with a microbial culture. After incubation, the reading of results are measured by the diameters of the inhibition zones in millimeters [2,11]. After 24 hours, the diameters of the zones of inhibition of the essential oils appeared are measured for the two types of essential oils (extracted and commercial) and the four types of bacteria (*Escherichia coli*, *Klebsiella sp*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*) with four concentrations of essential oils from the most concentrated to the least concentrated according to four formulas : undiluted, 25%, 50% and 75% dilution with water according to a personal approach.

### *2.5. Thin layer chromatography*

Rosemary oils were subjected to TLC analysis following the method of [20]. Glass plates were coated with a slurry of silica gel to 300 pm thickness and after air drying were sensitized by heating at 105°C for about one hour. TLC separation was effected in standard TLC tanks with filter paper. Plates were developed in benzene-methanol (80 : l, v : v). After visualization under UV light, the components were developed by spraying the plates with a fresh 5% solution of vanillin in ethanolic acid (1 g of vanillin in 10 ml concentrated sulfuric acid, then diluted to 50 ml with absolute ethanol) and then heated at 105°C for five minutes. The intensities of the spots were noted as high, moderate, low, or trace. The patterns of the spots and the colors produced by the spray reagent were also recorded and the major components identified.

### *2.6. Statistical analysis*

The inhibition diameters for the bacterial strains and the concentrations tested for the extracted rosemary oil were subjected to a one-factor analysis of variance using SPSS version 25 software and the homogeneous groups were deduced.

## 3. Results and Discussion

### *3.1. Phytochemical Screening of Rosmarinus officinalis L:*

The results obtained from phytochemical tests are summarized in Table 1.

In view of these results, we deduce that the plant (*Rosmarinus officinalis* L) sampled in the region of El Hamma Khenchela, Algeria like other species of the labiate family [8], is rich in various secondary metabolites. Positive with tannins, anthocyanin, coumarin, alkaloids, reducing compound and flavonoids, which explains the interest and the particular attention carried by the researchers through the scientific studies with this plant. these results are consistent with those obtained by [15] who worked on a species of the same "labiate" family as rosemary "Teucrium polium".

**Table 1.** The results of tube reactions of the aerial part of rosemary

| Search | Reaction | Search | Reaction |
|---|---|---|---|
| The tannins | Appearance of a black blue coloration (+) | The Anthocyanins | Blue color (+) |
| Polyphenols | Red color (-) | leucanthocyanans | A fluorescent yellow color (+) |
| The coumarines | A fluorescent yellow color (+) | Alkaloids | A precipitation (+) |
| Reducing compounds | A blue-green layer for sulfuric acid and a red-brown layer for acetic acid. (+) | Flavonoids | The appearance of a yellow color (+) |

### *3.2. Results of chromatographic checks*

The resulting chromatograms comprise a series of spots, the identification of the compounds was based on the comparison of the Rfs and UV-observed colors of the spots appeared on TLC.

The spots appear in different colors (green, yellow, purple...). All this high number of spots and various colors is an indication of the presence of several types of chemical substances according to [9]. All results are grouped in the table 2.

The TLC of the raw extract of rosemary has the following tasks: (Red, green, yellow, blue, yellow, purple) which indicates the presence of (Flavone, Hydroxyflavone, Dihydroxyflavone, Flavanones, methoxyflavones, flavonols. These results agree with those obtained by [27], who worked on the same plant "(*Rosmarinus officinalis* L" as we do, these results demonstrate the great richness of our oil in various compounds, these last give the oil remarkable therapeutic virtues.

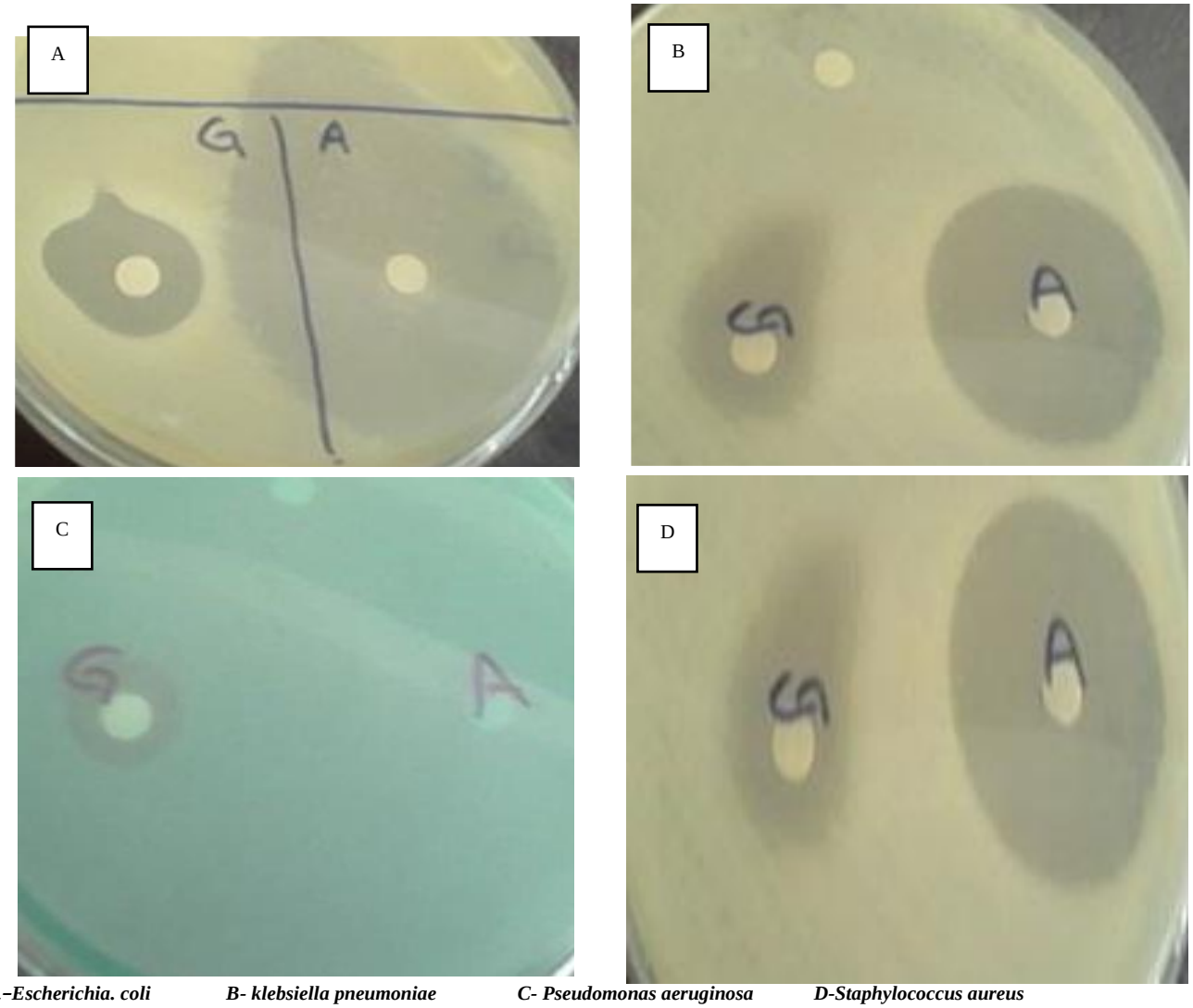
**Table 2.** Chromatographic Control Outcomes

| | | | | **Revelations under UV light lamdaa = 254 nm** | | |
|---|---|---|---|---|---|---|
| | | **System** | **Number of tasks** | **colour** | **Front report (FR)** | **Probable compound type** |
| **Methanol raw extract** | 01 | Ethyl acetate / formic acid / glacial acetic acid / water | 03 | Red<br>Green<br>Yellow | 0,86<br>0,85<br>0,62 | Flavone [10,12, 20]<br>Hydroxyflavone<br>Dihydroxyflavone [10,12, 20] |
| | 03 | Chloroform / acetic acid / methanol / water | 03 | Blue<br>Yellow<br>Violet | O.75<br>0,71<br>0,51 | Flavanones, Methoxyflavones<br>Flavonols [10,12, 20] |

Rf = the distance between the centre of the task and the starting point / distance between the solvent front and the starting point.

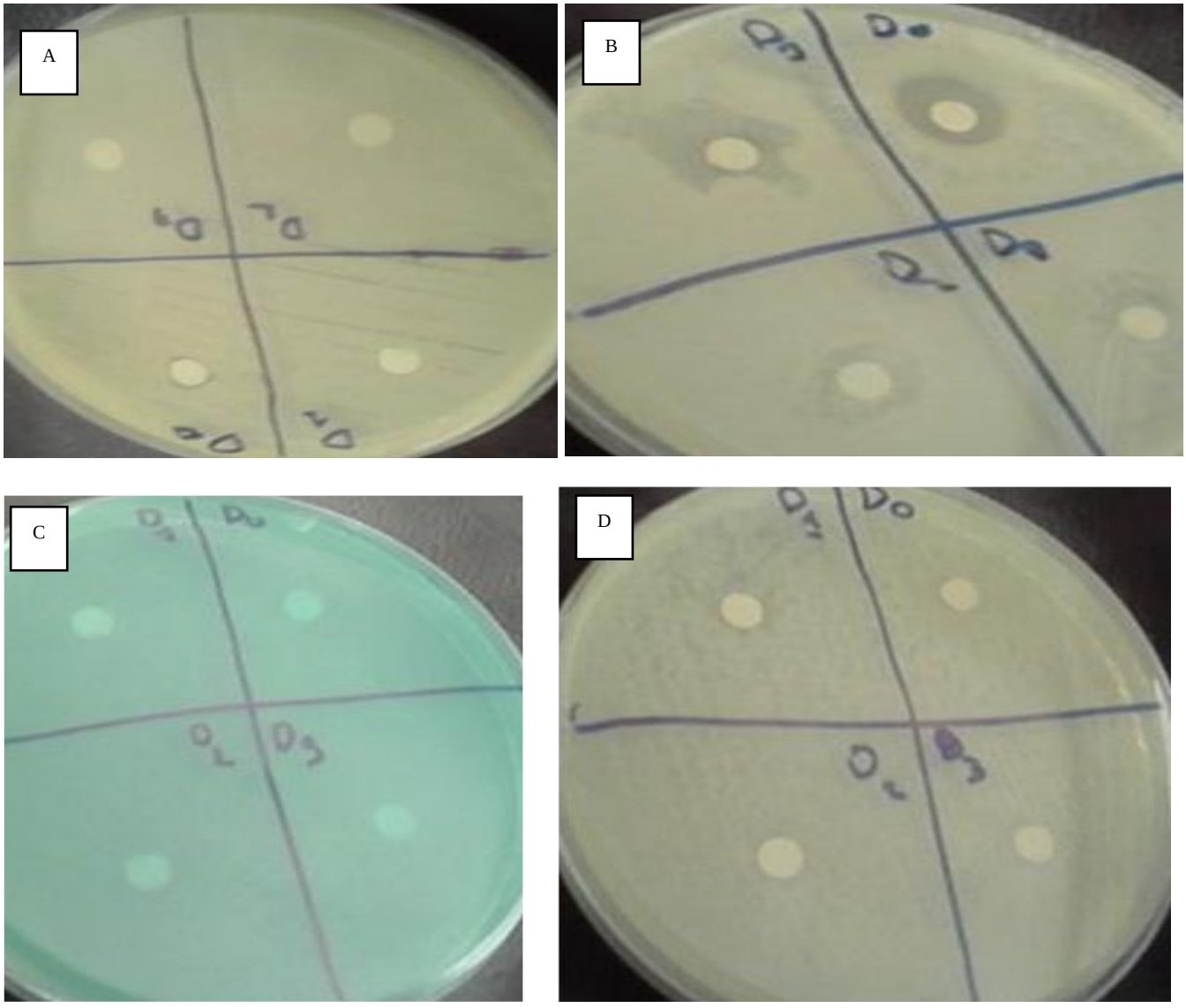
### *3.3. Results of the antibiogram*

The results are shown in the figure below.



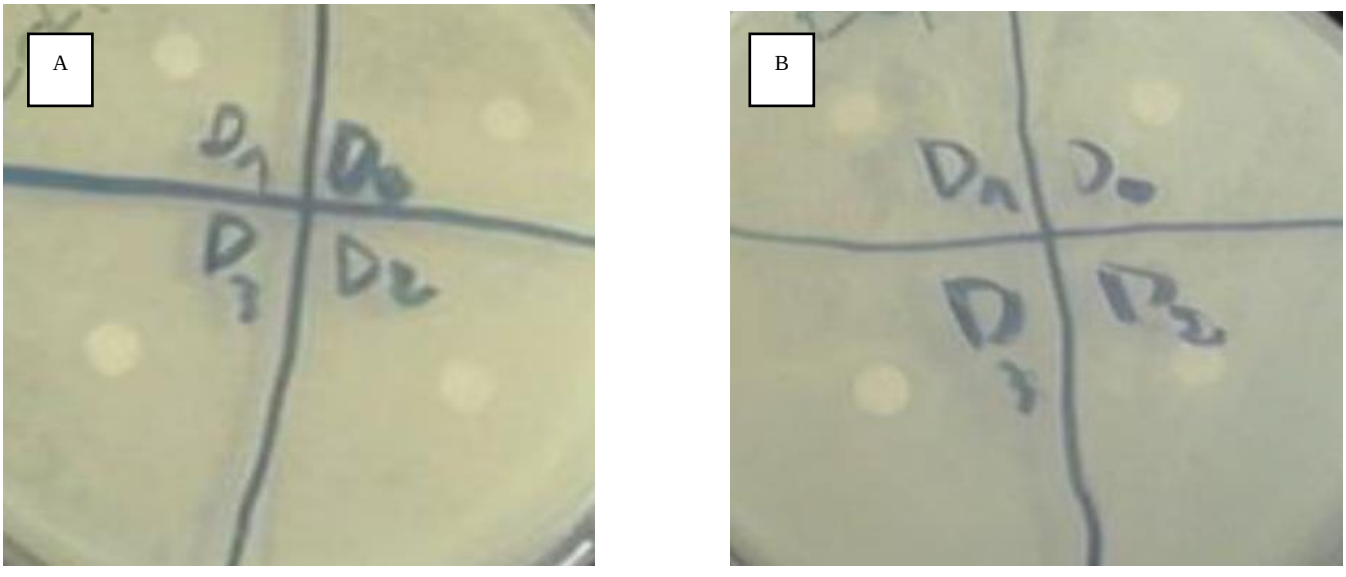

***A–Escherichia. coli*** ***B- klebsiella pneumoniae*** ***C- Pseudomonas aeruginosa*** ***D-Staphylococcus aureus***

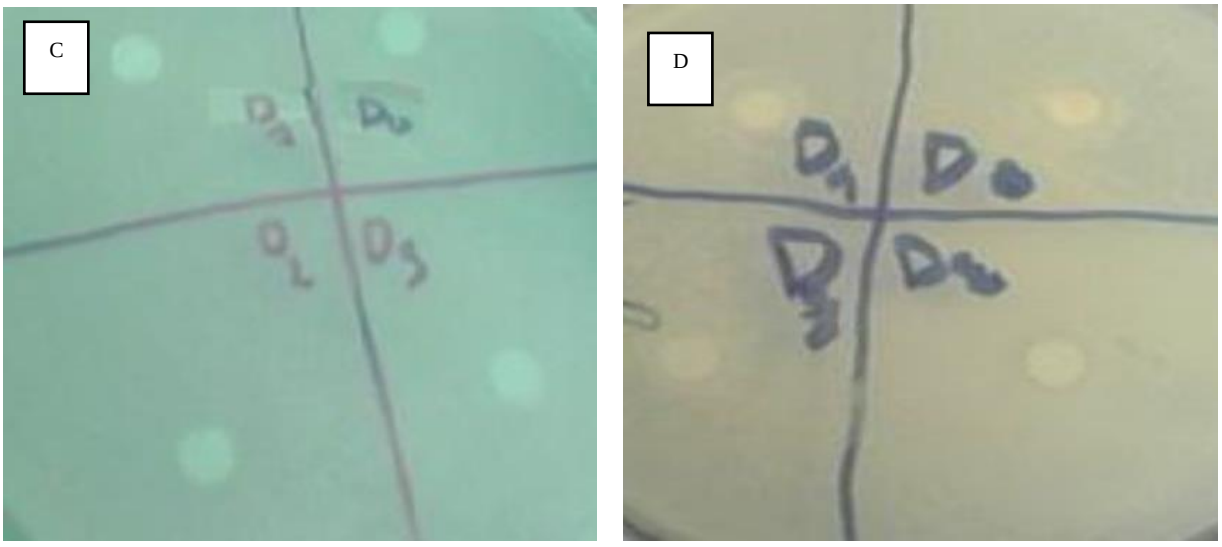
**Figure 1.** Positive and negative controls of antibacterial activity




***A–Escherichia. coli*** ***B- klebsiella pneumoniae*** ***C- Pseudomonas aeruginosa*** ***D-Staphylococcus aureus***

**Figure 2.** Antibacterial effect of the essential oil extracted from *Rosemarinus officinalis*






***A–Escherichia. coli*** ***B- klebsiella pneumoniae*** ***C- Pseudomonas aeruginosa*** ***D-Staphylococcus aureus***

**Figure 3.** Antibacterial effect of commercial essential oil of *Rosemarinus officinalis*

**Table 3.** Results of the antibacterial activity of the essential oil of *Rosemarinus officinalis*

| **Bacterial strains** | **The diameter of the inhibition zone (mm)** | | |
|---|---|---|---|
| | Amoxicillin | Gentamicin | DMSO |
| ***Echerichia.coli*** | 46±0.58 | 20±1.53 | - |
| ***Staphylococcus aureus*** | 32±1.15 | 20±0.00 | - |
| ***Pseudomonas aeruginosa*** | - | 13±1.53 | - |
| ***Klebsiella.sp*** | 12±2.00 | 27±2.08 | - |

**Table 4.** Results of Antibacterial Activity of Antibiotic Amoxicillin and Gentamycin

| | **Diameter in (mm)** | | | | |
|---|---|---|---|---|---|
| | **Concentration** | ***E. coli*** | ***S. us aureus*** | ***P.s aeruginosa*** | ***Klebsiella . sp*** |
| **Rosemary essential oil extracted** | D0 | 08 ±0.58 | 10 ±2.0 | - | 17 ±2.5 |
| | D1 | 06 ±0.58 | 09 ±2.0 | - | 14 ±0.0 |
| | D2 | - | 08±0.0 | - | 13 ±2.08 |
| | D3 | - | - | - | 11 ±1.15 |
| **ommercial rosemary essential oil** | D0 | - | - | - | - |
| | D1 | - | - | - | 10 ±0.0 |
| | D2 | - | - | - | - |
| | D3 | - | - | - | - |

### 3.4. DISCUSSION

Amoxicillin has been very active on strains tested with inhibition diameters (46, 32, 12 mm) while they exhibit a resistance to the action of gentamycin. A resistance that can be explained by the intense use of this antibiotic (Gentamycin)

The purpose of the study was to shed light on the antibacterial power of rosemary oils to see if there is a possibility of making drugs but also to compare this power with that existing in commercial oils of rosemary and to check if these formulas really contain oils rosemary

For the extracted essential oil The negative results obtained for the *Pseudomonas aeruginosa* strain in the preceding table can be explained by the fact that the sample used (essential oil) has a really insignificant inhibitory effect on the strain tested. The absence of the effect of the oil extracted on the strain (*pseudomonas aeruginosa*) can be explained by the low concentration of polyphenols in the sample. This is in agreement with the results obtained by [6] on the aerial part.

The results of the test also show that the extracted essential oil has an acceptable antibacterial activity on the three species (*Escherichia coli, Staphyloccocus aureus, Klebsiella sp*) with however a certain gradient of increasing efficiency: with a zone of inhibition of 08 and 06 mm for *E. coli* and 10.09 and 08 mm for *Staph .au* and 17, 14, 13.11 mm) for *Kle.sp*, this decreasing gradient is in the direction *klebsiella sp, staphyloccocus aureus* and Escherichia *coli.* The results confirm those obtained by [8] but with larger diameters (between 15 and 17 for *Staphylococcus aureus* and *Escherichia coli*). Therefore, these results supported by those of other authors confirm the action of rosemary on certain bacterial strains**.** [5] found during his work on rosemary that his essential oils are effective against the *Escherichia coli* strain and *Staphylococcus aureus* which are strains sensitive to the

essential oil while the *Pseudomonas aenuginosa* is resistant which concords exactly with the results obtained by this study. The inhibition zone increases as the sample concentration increases. However, the best inhibitions remain closely related to the highest concentration as indicated by the analysis of variance and the homogeneous groups mentioned below

**Table 5.** ANOVA and homogeneous groups for reaction of *Escheichia coli* to different concentrations of *Rosmarinus officinalis* extracted oil (diameter)

| | Sum of squares | df | Medium square | F | Sig. |
|---|---|---|---|---|---|
| Intergroups | 149,667 | 3 | 49,889 | 299,333 | **0,000** |
| Intragroups | 1,333 | 8 | 0,167 | | |
| Total | 151,000 | 11 | | | |

| Oil1 | N | 1 | 2 | 3 |
|---|---|---|---|---|
| D2 | 3 | ,0000 | | |
| D3 | 3 | ,0000 | | |
| D1 | 3 | | 6,3333 | |
| **D0** | 3 | | | 7,6667 |

**Table 6 .** ANOVA and homogeneous groups for reaction of Staphyloccocus aureus to different concentrations of Rosmarinus officinalis extracted oil (diameter)

| | Sum of squares | df | Medium square | F | Sig. |
|---|---|---|---|---|---|
| Intergroups | 188,250 | 3 | 62,750 | 31,375 | **,000** |
| Intragroups | 16,000 | 8 | 2,000 | | |
| Total | 204,250 | 11 | | | |

| Oil1 | N | 1 | 2 |
|---|---|---|---|
| D3 | 3 | ,0000 | |
| D2 | 3 | | 8,0000 |
| D1 | 3 | | 9,0000 |
| D0 | 3 | | 10,0000 |

Table 7 ANOVA and homogeneous groups for reaction of *Klebsiella sp* to different concentrations of *Rosmarinus officinalis* extracted oil (diameter)

| | Sum of squares | df | Medium square | F | Sig. |
|---|---|---|---|---|---|
| Intergroups | 56,000 | 3 | 18,667 | 6,222 | **,017** |
| Intragroups | 24,000 | 8 | 3,000 | | |
| Total | 80,000 | 11 | | | |

| Oil1 | | 1 | 2 |
|---|---|---|---|
| D3 | 3 | 11,3333 | |
| D2 | 3 | 13,3333 | 13,3333 |
| D1 | 3 | 14,0000 | 14,0000 |
| D0 | 3 | | 17,3333 |

The pure oil that is to say the least diluted had the most marked effects, it goes perfectly with the results of [8] who claim that the bacteria tested are very sensitive to pure oil. On the other hand, these germs have a relative sensitivity with regard to the dilutions ½ and / or ¼ of the essential oils of the rosemary harvested from the studied stations. Jordán et *al*. (2013) report that a high proportion of α-pinene increases the effectiveness of rosemary essential oils against Staphylococcus aureus, while the presence of 1.8 cineole, as the most abundant compound, decreases considerably. The effectiveness of these oils. The sensitivity of microorganisms may vary according to the germ tested because an essential oil may be bactericidal with respect to certain strains, bacteriostatic with respect to others or have no effect [19]. Previous studies on the essential oil of *Rosmarinus officinalis* reveal antimicrobial activity and indicate a similarity with the results obtained in this work [23, 26, 13, 24, 20, 5]. Gram- bacteria are more resistant than Gram +, this is due to the structural differences of their outer membranes as advanced by [7].
Commercial essential oil had no inhibitory activity on all three strains (*E. coli*, *Staph .au*, *pseudo*) and for all concentrations. An apparent limiting activity for the strain (Kle.sp) with a zone of inhibition equal to 10 mm.

## 4.CONCLUSION

The bacteriological study on essential oils (extracted and commercial) showed that concentrated essential oils present good antibacterial activity of extracted oils on well-defined strains. Essential oils extracted from Algerian rosemary will be of economic interest for the Algerian pharmaceutical industry. Negative tests for a commercial essential oil of rosemary compared to the positive tests of our extract provide information on the counterfeit that knows this sector.
The TLC of the raw extract of rosemary presents the tasks, indicating the presence of (Flavone, Hydroxyflavone, Dihydroxyflavone, flavanones, methoxyflavones, flavonols, these compounds are of paramount importance in the therapeutic effects that possesses Rosemary.

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