

Study of the Antibacterial Activity of the Extract from the Essential Oil of *Eucalyptus globulus* and *Rosmarinus officinalis* on Three Bacterial Strains

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Abstract

This work aims to contribute to the highlighting of the antimicrobial activity of essential oils contained in the leaves of two species of medicinals and aromatics plants: *Eucalyptus globulus* and *Rosmarinus officinalis*. Extraction by hydrodistillation was carried out, the yield obtained was rather low (0.132% for *Eucalyptus globulus* and 0.004% for *Rosmarinus officinalis*).

The Essential oil extracts of the two plants were used to highlighting of antibacterial activity of three pathogenic bacterial strains, *S. aureus*, *P. aeruginosa* and *E. coli*, using the aromatogramme method.

The results obtained showed antibacterial activity on all the strains tested. *E. coli* and *P. aeruginosa* were found to be the most sensitive while *S. aureus* were the most resistant. The minimum inhibitory concentrations were determined by the solid dilution method. The results of the tests carried out are almost negative.

Keywords: Medicinals plants, Essentials oils, *Eucalyptus globulus*, *Rosmarinus officinalis*, Hydrodistillation, Antibacterial activity.

1. INTRODUCTION

Plants are a huge source of complex chemical molecules used by man in perfumes, food, cosmetic and pharmaceutical industry. Aromatics plants are characterized by the biosynthesis of odorous molecules which constitute so-called essential oils (HE) known for a long time for their antimicrobial activities [1, 2].

The chemical composition of the essentials oils is quite complex, the terpene and aromatic compounds are the main constituents. It also contains, in low concentrations,

organic acids, ketones and volatile coumarins [3]. The nature of the chemical function of the majority compound (phenol, alcohol, aldehyde, ketone, etc.) plays a predominant role in the effectiveness of their biological activities and thus in the control of infectious diseases [4, 5, 6].

This led us to focus on the study of antimicrobial activities of aromatic plant extracts harvested in the region of El Kala (North East Algeria), an area very rich in medicinal flora. The choice of plants was based on two abundant species and widely used by the local population, namely: *Eucalyptus globulus* and *Rosmarinus officinalis*.

2. MATERIALS ET METHODS

2.1. Biological material

The plant material consists of the aerial parts of two very common medicinal plants in the region of El Kala (North East Algeria), *Eucalyptus globulus* and *Rosmarinus officinalis*. The leaves were collected in March 2013 in the area of El Kala. After drying at ambient temperature and away from sunlight, for 30 days in order to preserve the integrity of the molecules, the plant material of each of the two species is ground to a powder fine.

We extracted the essential oils from the leaves of the two plants by hydrodistillation. It has been brought to a boil a mixture of 136g of *Eucalyptus globulus* in 800ml of distilled water and a mixture of 100g of *Rosmarinus officinalis* with 900ml of distilled water [7].

The test of the antimicrobial activity is carried out by:

➤ **Microbial strains :**

Tests for the antimicrobial activity of essential oils of *Eucalyptus globulus* and *Rosmarinus officinalis* were carried out at the Microbiology Laboratory of El Tarf Hospital (Algeria). These strains were supplied by the Pasteur Institute of Algiers.

The bacteria used in this work are:

- ✓ *Escherichia coli* Gram negative ATCC 25922,
- ✓ *Pseudomonas aeruginosa* Gram negative ATCC 27853,
- ✓ *Staphylococcus aureus* Gram positive ATCC 25923.

➤ **Culture media :**

The culture of bacteria required the use of the following media:

- ✓ Mueller Hinton (MH) agar, the medium most used for susceptibility testing to antibacterial agents.
- ✓ Nutrient broths.

2.2. Methodology:

2.2.1. Extraction process:

The essential oil used for the determination of antibacterial activity was extracted from the leaves of the two selected species: *Eucalyptus globulus* and *Rosmarinus officinalis*, by hydrodistillation method.

The oil thus obtained is recovered and then treated with a desiccant, the petroleum ether to remove the remaining water which may have been retained in the oil and subsequently kept in a well-closed tube and shaded at low temperature (4-5 C°) [8, 9, 10].

The yield of essential oil (RHE) is defined as the ratio between the mass of the essential oil obtained after extraction (M') and the mass of the plant material used (M) [11]. It is given by the following formula:

$$RHE = M'/M.100$$

RHE: yield of essential oil.

M': mass of the essential oil obtained in grams;

M: mass of the powder used in grams.

2.2.2. Tests for antibacterial activity:

The antibacterial activity of the essential oils of *Eucalyptus globulus* and *Rosmarinus officinalis* is carried out by the technic of the Aromatogram or Disk methods (disk infusion).

For the determination of the bactericidal and bacteriostatic activity, we used 2 different concentrations.

➤ The Aromatogram or Disk methods

Preparation of inoculum:

From a pure culture of bacteria tested on isolation medium, a few well-isolated and perfectly identical colonies were scraped with a platinum loop or a sealed pasteur pipette [12, 13].

We then placed its bacteria in a medium containing 5ml of sterile physiological water at 0.9%.

After homogenization of the bacterial suspension, its opacity must be equivalent to 0.5Mc Farland. To adjust the inoculum, either we add the culture if it is too diluted or sterile physiological water, if it is too concentrated [14, 15].

Seeding:

The seeding is carried out 15 minutes after the preparation of the inoculum. The culture medium used is Mueller - Hinton.

The inoculation is carried out using a swab, which we have previously soaked in the bacterial suspension. Swab is discharged from the bacteria by friction on the whole of the agar surface contained in the petri dishes, with movements in tight grooves, from top to bottom (Figure 01) [16].

The operation is repeated twice, turning the Petri dish 60° each time, without forgetting to swivel the swab on itself. The seeding is finished by passing the swab over the periphery of the agar.

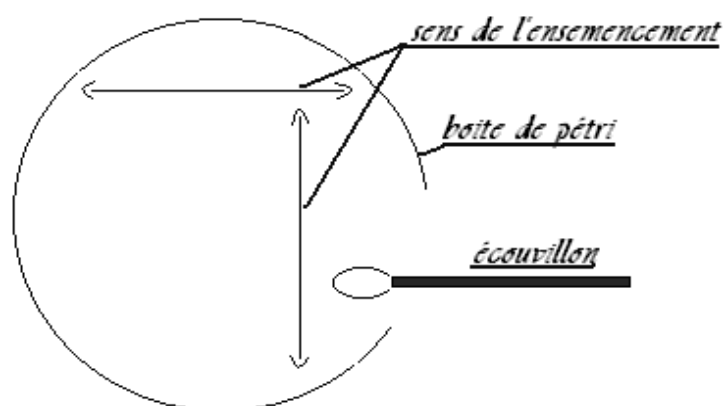


Figure 01: Swab seeding [17].

Preparation of aromatogram disks:

The disks are prepared from blotting paper, using a diameter of 5.5 mm, subsequently placed in a test tube and sterilized in an autoclave.

Once, the Mueller - Hinton agars are seeded, the discs soaked in the extracts of the essential oils of *Eucalyptus globulus* and *Rosmarinus officinalis* are deposited on the surface of the agar using a sterilized forceps [18, 19].

Dilutions of essential oils:

It consists in carrying out dilutions of aromatic extracts, and incorporating them into the molten and molten medium cooled to 45 ° C., and testing on these media the bacterial strains to be studied. This test gives an interval of values of the minimum inhibitory concentration of each strain. This method is used as an indicator of antibacterial activity [20, 21].

The concentrations of the aromatic extracts are prepared as follows [22]:

- ✓ A dilution of 1/10: put 1µl of essential oil with 9µl of ethanol at 60°.
- ✓ A dilution of 1/100: put 1µl from the first dilution with 9µl of ethanol at 60°.

Determination of Minimum Inhibitory Concentration (MIC):

The Petri dishes are inoculated with the same inoculum prepared for the aromatogram, with the aid of a clamp, the sterile disks are placed on the surfaces of the petri dishes. Three drops of each dilution are added to the sterilized disks. A control box is seeded without any extract [23].

The same seeding technique mentioned above is carried out for all the petri dishes. We used 10 petri dishes for each strains and each concentration.

Incubation and reading:

The petri dishes are incubated in a 37°C study for 24 hours. The results are observed the day after the experiment, by measuring the diameters of the clear halos around the disks, or the zones of inhibition, using a caliper.

3. RESULTATS AND DISCUSSION

3.1. Essential oil yield:

We recall that the essential oil was extracted from the aerial part (the leaves) of *Eucalyptus globulus* and *Rosmarinus officinalis* by hydrodistillation. We obtained a green colored oil with an aromatic odor of *Eucalyptus globulus* and a transparent and very fragrant oil for *Rosmarinus officinalis*. The yield obtained is 0.132% for Eucalyptus and 0.004% for Rosmarinus (Table 1).

Table 1 : Results of hydrodistillation

Plants	Color d'HE	Odour	Yield
<i>E. globulus</i>	Green	Aromatic	0.132 %
<i>R. officinalis</i>	Transparent	very aromatic	0.004 %

3.2. Antimicrobial activity:

Sensitivity to pure extracts:

Examination of the various Petri dishes revealed the presence of zones of inhibition ranging from 8-13 mm for *E. globulus* and from 10-17 mm for *R. officinalis*. The diameters of the zones of inhibition obtained are shown in Table 2.

Table 2 : Diameter of inhibition zones in (mm) due to essential oils

Microorganisms	Diameter of inhibition zones in (mm)	
	H E d' <i>E. globulus</i>	H E d' <i>R. officinalis</i>
<i>Escherichia coli</i> ATCC 25922	12 mm	17 mm
<i>Pseudomonas aeruginosa</i> ATCC 27853	13 mm	16 mm
<i>Staphylococcus aureus</i> ATCC 25923	8 mm	10 mm

The aromatograms of the *E.coli*, *P. aeruginosa* and *S. aureus* strains are shown in figures 2 and 3, it is clear that zones of inhibition caused by the essential oils of *E. globulus* and *R. officinalis*.



Figure 2 : Aromatogram of the *Eucalyptus globulus*.



Figure 3 : Aromatogram of the *Rosmarinus officinalis*.

Sensitivity to diluted extracts:

The results of the sensitivity of the bacterial strains to the diluted extracts of the essential oils extracted from *Eucalyptus globulus* and *Rosmarinus officinalis* are shown in Table 3.

Table 3 : Results of Minimum Inhibitory Concentrations (MIC) of *E. Globulus* and *R. officinalis*

Essential oils	Microorganisms	Dilutions	
		1/10	1/100
<i>E. globulus</i>	<i>E .coli</i>	-	-
	<i>P. aeruginosa</i>	-	-
	<i>S .aureus</i>	-	+
<i>R. officinalis</i>	<i>E .coli</i>	-	-
	<i>P. aeruginosa</i>	-	-
	<i>S .aureus</i>	-	+

(+) Presence of growth, (-) Lack of bacterial growth.

In our study, essential oils were extracted from the leaves of young twigs of *E. globulus* and *R. officinalis* by hydrodistillation. The yields obtained are more important for *E. globulus* than *R. officinalis*:

- ✓ 0.13% HE of *E. Globulus*, which is remarkable considering the amount of treated leaves which is only 136g. [18] worked on *Eucalyptus globulus* grown in Constantine, for 200g of *E. globulus* they obtained a yield of 2.5%.
- ✓ 0.004% oil of *R. officinalis* for an amount of treated leaves of 100g. [19] obtained from the aerial parts (leaves and flowers) a yield of 0.8% for the wild rosemary and 0.6% for the cultivated Rosemary at Tlemcen. This difference in the yield of essential oils is quite normal since it depends on several factors, namely the species and their chemotypes, geography, harvest period, extraction technique and leaf drying method [18].

The separation of essential oils after distillation is determined by its degree of solubility in water. What we noticed during the recovery stage of the essential oils from the hydrolyzate. The latter always contains droplets that we could not recover, which could distort the calculation of our yield.

We can say that the quantity of essential oils recovered in our study is quite important, given that the hydrodistillation technique has the disadvantage of being long and the resulting yields are relatively low [20].

The method of the aromatogram allowed us to demonstrate the antibacterial power of essential oils of *E. Globulus* and of *R. officinalis* with respect to the tested bacteria. Both oils show interesting antimicrobial activity for *R. officinalis* and low for *E. globulus*.

We tested the pure oils where we were able to obtain:

Strains tested with *E. globulus* extract, we observed zones of weak inhibitions, the *S. aureus* strain having a diameter of 8 mm, while for *E.coli* and *P. aeruginosa* the diameters of 12mm and 13mm respectively. The antibacterial activity of the extracts of the essential oil of *Eucalyptus globulus* on the three strains *S. Aureus*, *E. coli* and *P. aeruginosa* made by [18] represent diameters of the zones of greater inhibition: *S. aureus* 16mm, *E. coli* 16mm and *P. aeruginosa* 20mm.

The strains tested with the extract of *R. officinalis* showed an important zone of inhibition for the *E.coli* and *P. aeruginosa* strains; 17mm and 16mm respectively, with a small area for the *S. aureus* strain (10mm). The tests of the antimicrobial activity carried out by [19] show that the zone of inhibition of *E. coli* and *P. aeruginosa* is 24mm and 19mm respectively, whereas for *S. aureus* is 13.5mm.

The zones of inhibition observed for the two extracts with the two gram negative species *E. coli* and *P. aeruginosa* are larger than those of the gram positive *S. aureus*. This bacterial inhibition is mainly related to the chemical composition of the extracted essential oils. [20] showed the presence of α -pinene, 1,8-cineol, camphor, verbinone, borneol, camphor and verbinone in very high amounts in *R. officinalis*. These compounds have a proven high antibacterial capacity. The essential oil of *E. globulus* contains 1,8-cineol, citronellal, citronellol, citronellyl, p-cymenene acetate, eucamalol, limonene, linalool, β -pinene, γ -terpinene, α -terpinol, alloocimene and aromadendrene; having antimicrobial activity demonstrated by [21].

The dissemination disks method has indisputable disadvantages, the diffusion of essential oils into the agar can be limited due to its hydrophobic and volatile nature, the absorption capacity of the disks is also a major obstacle.

The results of the determination of the MIC by the method of dilution in a solid medium showed that the MICs are very low and that there is no difference between all the dilutions for the two extracts.

CONCLUSION

A large number of medicinal and aromatic plants contain chemical compounds with antimicrobial properties. Several works have focused on the essential oils extracted from these aromatic plants. The results indicate that they possess several biological properties. Therefore, the evaluation of such properties remains an interesting and useful task, particularly in finding new sources of natural antimicrobial agents.

In this study, we tried to evaluate in vitro the antibacterial activities of the essential oils of two medicinal plants belonging to the family Myrtaceae (*E. globulus*) and Lamiaceae (*R. officinalis*), very frequently used in Algeria and The El Kala region, for their therapeutic properties. The extraction by hydrodistillation of the essential oils made it possible to obtain relatively low yields; The yield of *E. Globulus* is higher than that of *R. officinalis*.

The antibacterial activity of essential oils, using the aromatogram method, demonstrated the antibacterial potency of these oils with respect to three bacterial species: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 (gram negative) and *Staphylococcus aureus* ATCC 25923 (gram positive).

This power is relatively low, with zones of inhibition varying between 8 and 17mm for the two extracts.

Determination of the minimum concentration of inhibition by the solid dilution method showed that the values obtained were very low and not measurable. As a result, there is no difference between the dilutions for the two extracts.

Finally, our results indicate that the essential oils of *E. Globulus* and *R. officinalis* have an interesting antibacterial activity and can be considered a natural antibiotic. To the development of the present study it would be interesting to carry out a more in-depth study on the essential oils of *E. Globulus* and *R. officinalis* in order to isolate, purify and identify compounds having antibacterial activity.

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