Antimicrobial and Antioxidant Activities of the Essential Oils of Eucalyptus Globulus and Rosmarinus Officinal is from Algeria (Constantine)

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Abstract:

This comparative study aims to examine the biological activity of essential oils extracted from the leaves of two medicinal plants (*Eucalyptus globulus* and *Rosmarinus officinalis*) harvested in the Constantine region. The essential oils were obtained through hydrodistillation, and the yield obtained from *E. globulus* (2.32%) is higher compared to that of *R. officinalis* (1.04%). Antimicrobial study of the essential oils using the direct contact method revealed inhibitory activity on the growth of the tested microorganisms. The antioxidant activity of the essential oils, evaluated by the DPPH reduction method, showed significant activity for all products but remained lower compared to the standard antioxidant used, BHT. The activities can be ranked in descending order as follows: BHT >*E. globulus* essential oil >*R. officinalis* essential oil. This work demonstrates that enhancing the biological activities of plants can contribute to their valorization.

Keywords: Antimicrobial activity, Antioxidant activity, Hydrodistillation, Essential oïl, *Eucalyptus globulus*, *Rosmarinus officinalis*.

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Introduction

Since the dawn of humanity, plants have provided sustenance, shelter, and warmth to humans. They have also played a crucial role in healing various ailments. Currently, science confirms the diverse virtues of aromatic and medicinal plants, their essential oils, and their crude extracts. These natural resources find extensive applications in the food industry, cosmetics, perfumery, and are also incorporated into the composition of numerous medications in the form of creams, capsules, and suppositories… (J. Kaloustianand F. Hadji, 2013).

Algeria, due to its geographical position, boasts a wide variety of plant species. Among the aromatic and medicinal plants that constitute its vegetal cover (N. Benabadj et *al*, 2007); particular attention is given to the Myrtaceae family, which encompasses over 5650 species organized into 130 to 150 genera. Additionally, the Lamiaceae family, which includes more than 200 genera and 7,000 species (M. Antonio, 2017), is extensively utilized as a global source of spices and extracts with potent antimicrobial properties. Our choice is focused on the species *Eucalyptus globulus* and *Rosmarinus officinalis* originating from the Constantine region (Didouche Mourad). The leaf extracts of these plants have been widely used in traditional medicine for centuries as anti-inflammatory, antiseptic, and burn treatment agents. Several recent studies have highlighted the antioxidant and antimicrobial properties of their essential oils and methanolic extracts (Pintore et *al*, 2001; Ahmad et *al*, 2001; Kamal Fadili, 2015; Mishra et *al*, 2010).

The aim of this study is to extract bioactive molecules from the leaves of two medicinal plants, *Eucalyptus globulus* and *Rosmarinus officinalis*, and to conduct a comparative analysis of their essential oils regarding their biological activities, specifically antibacterial, antifungal and antioxydant activities.

1. Materials and Methods

1.1. Preparation of plant material

The plants used in this study are *Eucalyptus globulus* from the Myrtaceae family and *Rosmarinus officinalis* from the Lamiaceae (Labiatae) family. The plants were collected during the flowering period in March 2019 in the Didouch Mourade region (Constantine province) (Figure 1). The leaves of both plants were carefully chosen, ensuring they were free from any impurities, and then dried in the shade at room temperature. The aerial parts of the plants, weighing 50 g and previously air-dried, were subjected to hydro distillation for a period of 3 hours using a Clevenger-type apparatus. The resulting oils were then dried using anhydrous sodium sulfate and stored at 4°C until they were subjected to biological activities.

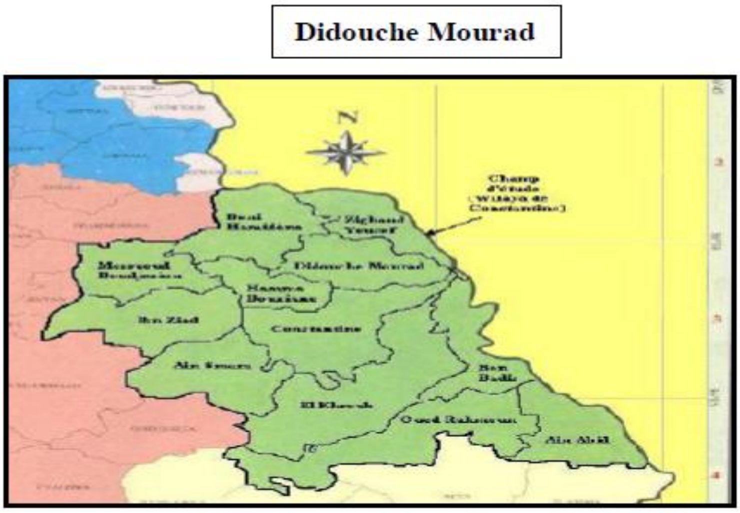

Figure 1: Geographical location of the study area Didouche Mourad (Constantine)

(Mk.Kholladi, 2005).

2.2. Bacteria and Fungi strains

The antibacterial tests were carried out using reference strains: *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) and a strain for the antifungal test *Aspergillus Niger* (ATCC 2CA936).

2.3. Antibacterialactivity

The disc diffusion method was employed to evaluate the antibacterial activity of the essential oils. Inoculums containing 2.0 × 10^6 CFU/mL of bacteria and 10^7 CFU/mL of yeast were evenly spread on Muller-Hinton agar and Sabouraud dextrose agar (SDA), respectively. Small sterile paper discs measuring 6 mm in diameter were saturated with 10 µl of various oil dilutions (1/2, 1/5, and 1/10 v/v) in Dimethyl sulfoxide (DMSO) from Sigma-Aldrich. These impregnated discs were subsequently positioned on the surface of Petri dishes with bacterial or yeast inoculation, with the Petri dishes measuring 90 mm in diameter. After 24 hours of incubation at 37°C for bacteria and 7 days of incubation at 28°C for fungi, the diameter of the inhibition zones was measured. Gentamicin (10 µg/mL from Sigma Aldrich), Nystatin (100 µg/mL), and DMSO were used as positive and negative controls, respectively. The measurements of the inhibition zones were recorded in millimeters. All the growth inhibition tests were conducted in triplicate. The strains were cultured under standard conditions, with bacteria incubated in Mueller-Hinton broth (MHB) at 37°C for 24 hours, and yeasts incubated in Sabouraud dextrose agar (SDA) at 28°C for 48 hours.

2.4. Free radical scavenging assay

The DPPH radical scavenging ability of *Eucalyptus globulus* and *Rosmarinus officinalis* oils was determined using a slightly modified method described by Bounihi. (Que et *al*, 2006). In this method, 1.5 ml of various sample dilutions was mixed with 1.5 ml of a 0.004% methanol solution of DPPH (2,2-diphénylpicrylhydrazine). After 30minute incubation at room temperature, the remaining absorbance was measured at 517 nm for all samples. Methanol was used as a blank, and a control was prepared by combining methanol with the DPPH solution. The inhibition ratio (in percentage) was calculated using the following equation:

% inhibition = [(control absorbance – sample absorbance) / control absorbance] × 100%.

Butylated hydroxytoluene (BHT) was used as a positive control.

3. Results and Discussions

The hydrodistillation of the aerial parts of *Eucalyptus globulus* and *Rosmarinus officinalis*oils provided essential oil yields in the range of (2.32% and 1.4%) (w/w)**(Figure 2)**.The essential oil yields obtained are very close to those of D. Habiba et *al*, 2010 (2.5%) for *E. globulus*, and B. lamia et *al*, 2016 (1.06%) and N. prakriti et *al*, 2018 (1.15%) for *R. officinalis*.

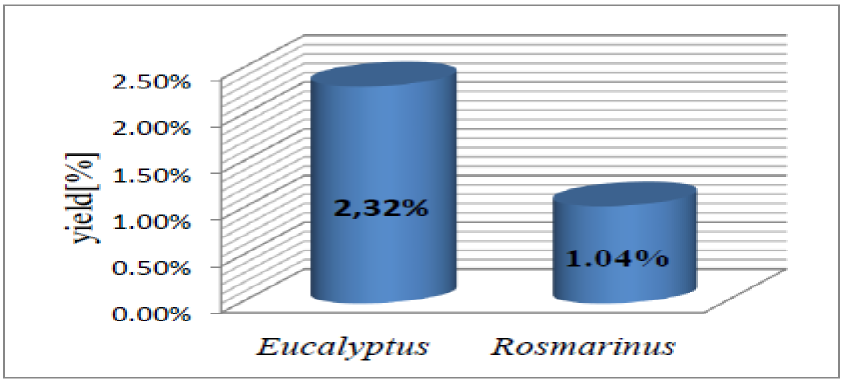

Figure 2: Yields of obtained essential oils.

The difference observed between the yields of the two plants may be due to various factors that come into play. Among them, we can mention the drying duration, soil type, chemical composition, climate, and others.

Table**1** shows results regarding the antimicrobial activity of the two studied plants. The antimicrobial activity of the essential oils was evaluated using a disk diffusion test, which provides varying diameters depending on the tested strain.

Table 1: Antimicrobial activity of Essential Oils using the agar diffusion method.

	Inhibition zone indiameter (mm)							
	E. globulus			*R. officinalis*			Gentamicine	Clotrimazol
	Dilution							
Microorganisms	1/2	1/5	1/10	1/2	1/5	11/10	/	/
S. aureus ATCC 25923	25.33	20.66	15.33	222.33	13.66	11	27	/
E. coli ATCC 25922	19	9.5	-	14	7.66	-	23	/
*A.niger*ATCC 2CA936	11.16	9.33	-	10	-	-	/	15

The results obtained have shown that both EOs (Essential Oils) possess antimicrobial activity against the selected microorganisms in the conducted protocol. However, it should be mentioned that the antimicrobial activity varies depending on the dilutions and the nature of the strain itself.*S. aureus*strain was the most sensitive bacterium to the EOs, which inhibited its growth at a low concentration of about 1/10 (v/v) (**Figure 3**). This inhibition increased with the concentration, reaching an inhibition zone nearly equal to that of the positive control (Gentamicin) at a concentration of 1/2 (v/v). On the other hand, the *E. coli* strain showed resistance to the 1/10 (v/v) dilution with both Eos (**Figure 4**). Furthermore, the fungus *A. niger* exhibited sensitivity only to the 1/2 (v/v) dilution of *R. officinalis* EO and showed sensitivity starting from the 1/5 (v/v) concentration for *E. Globules* (**Figure 5**).

The results obtained in this study are consistent with those of (Pintore et *al*, 2001; S. Santoyo et *al*, 2004; Burt, 2004), who reported that the EOs of *E. globulus* and *R. officinalis* exhibited higher antimicrobial activity against *S. aureus* (Gram-positive) compared to *E. coli* (Gram-negative), with the least sensitive microorganism being the fungus *A. niger*. Therefore, the mechanism of action of EOs is primarily linked to the structure of microorganisms' cell walls but also related to the compositions of the EOs.

(Jordan et *al*, 2013; Inouye et *al*, 2001) mention that the main component of both EOs was 1,8-cineole, but it was presumed that terpineol and α-pinene were the main contributors to the bioactivity, and their activities were eight times higher than that of 1,8-cineole.

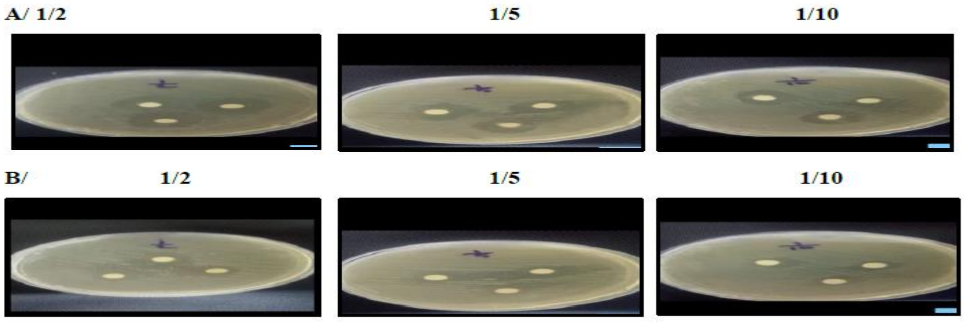

Figure 3: Antimicrobial activity by *E. Globulus*against *S. aureus* (a) and by *R.officinalis*against *S. aureus* (b) using disk diffusion method.

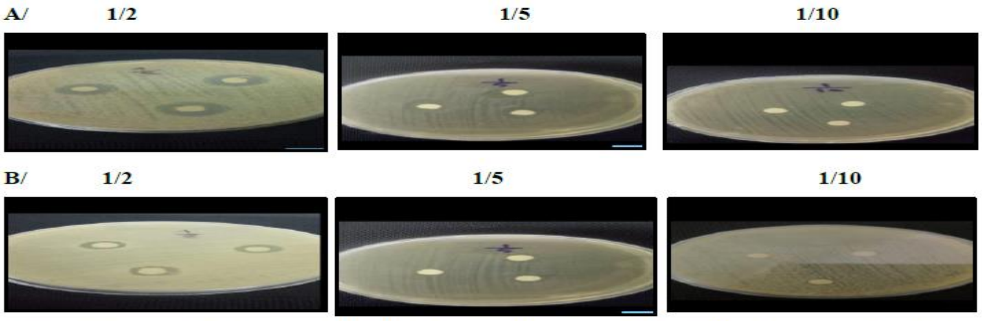

Figure 4: Antimicrobial activity by *E. Globulus*against *E. coli* (a) and by *R.officinalis* against *E .coli*(b) using disk diffusion method.

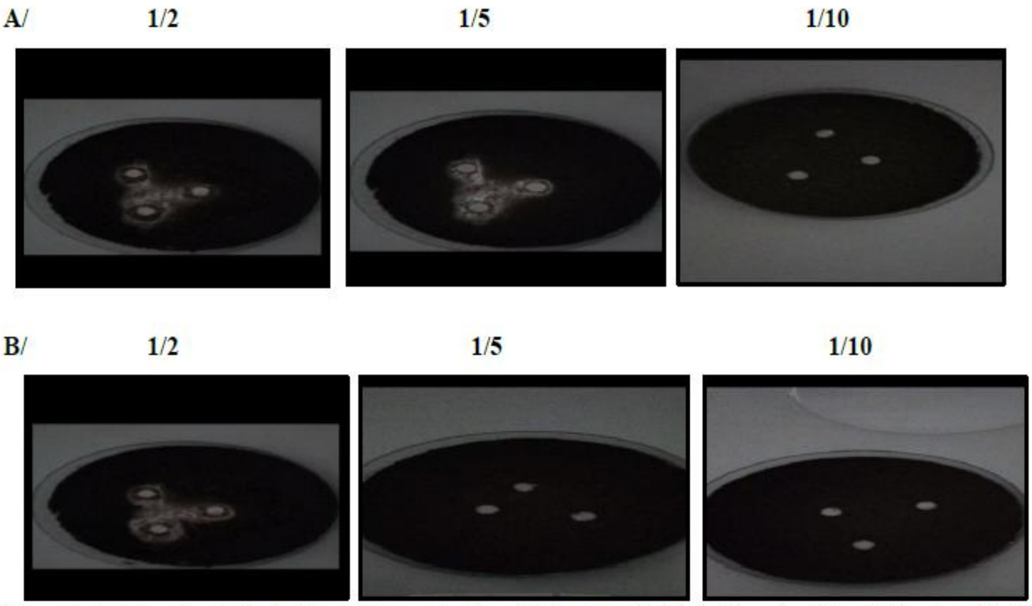

Figure 5: Antifungul activity by *E. globulus*against*E. coli* (a) and by *R.officinalis* against *E .coli*(b) using disk diffusion method.

The antioxidant activity of the essential oils was evaluated using the DPPH test. The measurement of absorbance (or optical density OD) was carried out by spectrophotometry at 517 nm (**Table 2**).

Table 2: IC_{50} values of BHT and essential oils.

Samples	**IC_{50} (mg/ml)**	
E. globulus	0.127	(R^2=0.974)
R. officinalis	0.245	(R^2=0.982)
BHT	0.004	(R^2=0.989)

This test allows us to conclude that both EOs have significant antioxidant activity. The strong activity of these essences has also been reported by (Wang et *al*, 2008 and Mishra et *al*, 2010). However, the EO of *E. globulus* was more effective in scavenging DPPH free radicals than that of *R. officinalis*, with IC_{50} values of 0.127mg/ml and 0.245mg/ml, respectively (**Table2**). (Prakriti et *al*, 2018) mentioned that the essential oil of *Eucalyptus globulus* has a better antioxidant potential compared to the essential oil of *Rosmarinus officinalis*. This strong activity of these essences can be explained by the presence of Carvacrol among the components of the EO of *E. globulus* and γ-terpinene in the EO of *R. officinalis*. Indeed, both of these constituents have already demonstrated their strong antioxidant power in previous studies by (Kulisic et *al*, 2004; Tepe et *al*, 2005, 2007; Rubert and Barrata, 2000).

These activities are lower than those of BHT used as a reference antioxidant (IC_{50}=0.004 mg/ml). This low IC_{50} value indicates a strong antioxidant activity, and it is consistent with the literature (Mansouri et *al*, 2011; Abdellaoui M, 2011) who found IC_{50} values of (2.25±2.51μg/ml and 4.21±0.04μg/ml) respectively.

Conclusions

This work was conducted as part of the evaluation of the antioxidant and antimicrobial activity of essential oils extracted by hydrodistillation from the aerial parts of *Eucalyptus globulus*and *Rosmarinus officinalis*. The extraction yields are higher for the essential oil of *E. globulus* than for the essential oil of *R. officinalis.* The direct contact method shows that both essential oils of *Eucalyptus globulus* and *Rosmarinus officinalis* exhibit significant antibacterial effects on the growth of *S. aureus* with inhibition diameters of (25.33mm) and (22.33mm) respectively at a dilution of 1/2 v/v. They also show moderate antibacterial activity against *E. coli*, with inhibition diameters of (19mm) for *E. globulus* EO and (14mm) for *R. officinalis* EO. However, these oils have been found to be almost inactive against *A. niger* with inhibition diameters of (11.66mm) and (10mm) at a dilution of 1/2 v/v for *E. globulus* EO and *R. officinalis* EO respectively. There was no activity observed at the dilution of 1/10 v/v for both oils. The antimicrobial activity of the oils, according to the literature, could be partly attributed to their main components (terpinene-4-ol and α-pinene). The method used to determine the antioxidant power of the essential oils from both plants is the DPPH radical scavenging effect. Both tested essential oils exhibited significant antioxidant activity. However, these activities were found to be lower than those of BHT used as the reference antioxidant. In conclusion, our results indicate that the extracts from both plants have interesting antimicrobial activity. Additionally, they demonstrated good antioxidant activities, which supports and justifies their use in food and traditional medicine for treating various pathologies.

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