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Antibacterial activity of the essential oils from the leaves of *Eucalyptus globulus* against *Escherichia coli* and *Staphylococcus aureus*

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ABSTRACT

Objective: To examine the *in vitro* antimicrobial activities of essential oil of the leaves of *Eucalyptus globulus (E. globulus)*. **Methods:** The essential oils of this plant were obtained by the hydrodistillation method. The inhibitory effects of this essential oil were tested against *Escherichia coli (E. coli)* and *Staphylococcus aureus* (*S. aureus*) by using agar disc diffusion and dilution broth methods. **Results:** The results obtained showed that essential oil of the leaves of *E. globulus* has antimicrobial activity against gram negative bacteria (*E. coli*) as well as gram positive bacteria (*S. aureus*). **Conclusion:** The encouraging results indicate the essential oil of *E. globulus* leaves might be exploited as natural antibiotic for the treatment of several infectious diseases caused by these two germs, and could be useful in understanding the relations between traditional cures and current medicines.

1. Introduction

The Myrtaceae family includes 140 genera and about 3800 species distributed in tropical and subtropical regions of the world[1]. Eucalyptus is one of the world's important and most widely planted genera[2]. It is a tall, evergreen tree, native to Australia and Tasmania, successfully introduced worldwide, now extensively planted in many other countries[3].It was introduced in Algeria in 1854 by Ramel[4]. Eucalyptus species are well known as medicinal plants because of their biological and pharmacological properties. In the international pharmacopeia, the most important and represented species, however, is *Eucalyptus globulus (E. globulus)* which is the main furnisher of essential oils[5]. These essential oils are in great demand in the market[5], since they find applications as anesthetic, anodyne, antiseptic, astringent, deodorant, diaphoretic, disinfectant, expectorant, febrifuge , fumigant, hemostat, inhalant, insect repellant, preventitive, rubefacient, sedative yet stimulant, vermifuge, for a folk remedy for abscess, arthritis, asthma, boils, bronchitis, burns, cancer, diabetes, diarrhea, diphtheria, dysentery, encephalitis, enteritis, erysipelas, fever, flu, inflammation, laryngalgia, laryngitis, leprosy, malaria, mastitis, miasma, pharygnitis, phthisis, rhinitis, sores, sore throat, spasms, trachalgia, worms, and wounds[6]. Sometimes their demand is also high in the soap and cosmetic industries[5].

The spread of drug resistant microbial pathogens is one of the most serious threats to successful treatment of infectious diseases[7].

Escherichia coli (E. coli) and *Staphylococcus aureus (S. aureus)* are two opportunistic pathogens that cause severe and life-threatening infections in immunocompromised patients[8]. The Gram-positive bacterium *S. aureus* is mainly responsible for post operative wound infection, toxic shock syndrome and food poisoning. The gram-negative bacterium *E. coli* is present in human intestine and causes lower urinary tract infection, coleocystis or septicemia[9]. Several studies have documented increasing resistance rates in *S. aureus* and *E. coli* to antibiotics[3,10-13].

The main objective of this study is to examine the antimicrobial activities of the Water-distilled extracts of *E. globulus* leaves on two clinically significant microorganisms, *E. coli* and *S. aureus* by means of the agar diffusion test and dilution broth method.

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2. Materials and methods

2.1. Plant material and essential oil extraction

Fresh plant leaves of *E. globulus* were collected during the flowering stage from the Djillali liabes University campus in Sidi Bel Abbes City, North West of Algeria. The plant leaves were identified by environmental sciences department, then were taken to the Biotoxicology Laboratory of Djillali liabes University for the extraction.

The fresh leaves were subjected to steam distillation using a Clevenger-type apparatus. Briefly, the plant leaves were completely immersed in water and heated to boiling, after which the essential oil was evaporated together with water vapour and finally collected after decantation. The distillate was isolated and dried in a Rota-vapor to giving greenish-yellow oil. The oil was stored at 4 ℃ until the antimicrobial screening[14,15]. The extraction yield of this essential oil was 1.2 % (w/w).

2.2. Bacterial strains

The essential oil was tested against two clinical isolated strains provided by the Laboratory of Medical Analysis, located in Dr. Hassani Abdelkader Hospital University Center (CHU) of Sidi Bel Abbes City, situated in the North West of Algeria for patients suffering from certain infectious diseases. The identity of the microorganisms used in this study (*E. coli* and *S. aureus*) was confirmed by standard biochemical tests and morphological studies[16,17].

2.3. Antimicrobial screening

Three methods were used to determine the antibacterial activity; agar disc diffusion method and dilution broth method.

The agar disc diffusion method was employed to determine the antimicrobial activities of the essential oil. Disc-assay was found to be a simple, cheap and reproducible practical method[18]. A suspension of each sample tested micro-organism diluted prior to 10-1, 10-2 and 10-3 (1 mL of 10^8 cells /mL) was spread on a solid agar medium in Petri dishes (Mueller-Hinton agar). Filter paper discs (6 mm in diameter) were soaked in 13 μL of the essential oil and placed on the inoculated plates and allowed to dry for 15 min, then incubated at 37 ℃ for 24 h. The diameters of the inhibition zones were measured in millimeters[19].

Dilution broth susceptibility assay[20] was used for the antimicrobial evaluation. Stock solutions of the essential oils were prepared in ethanol by mixing 1 ml of the extracts with 9 ml of alcohol in test tubes to obtain the mother solution, followed by successive dilutions at 10^{-2} and 10^{-3}. The control was prepared by mixing 1 mL of distilled water with 9 mL of alcohol solution. 1 mL of each dilution and 0.5 mL of tested culture strains are added to 8 mL of a nutrient broth, maintained after in a Marie bath at 37 ℃ under stirring for 24 h, then seeded by streaking the surface of the agar medium and incubated at 37 ℃ for 24 h.

3. Results

Figure 1 and 2 summarizes the microbial growth inhibition by the essential oil of *E. globulus*, which showed good antibacterial activities against the two tested organisms.

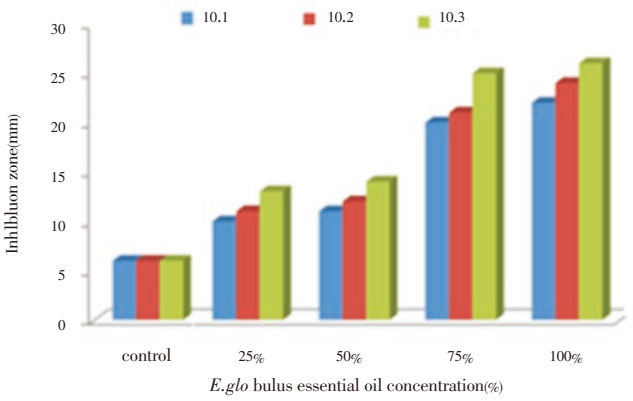

Figure 1. Antimicrobial activity evaluation of the essential oil *E. globulus* leaves against *E. coli* , using the agar disc diffusion method.

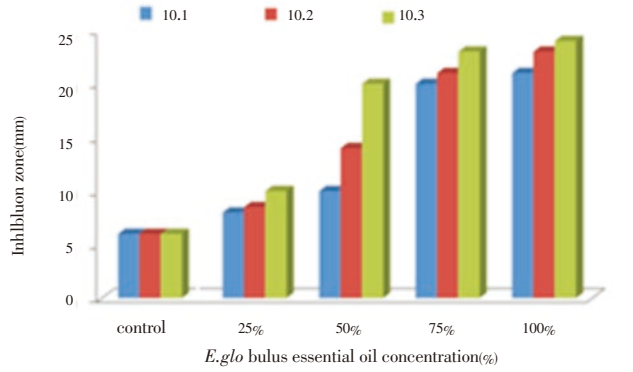

Figure 2. Antimicrobial activity evaluation of the essential oil *E. globulus* leaves against *S. aureus*, using the agar disc diffusion method.

Table 1
Antimicrobial activity evaluation of the essential oil of *E. globulus* leavesusing the dilution broth method against the two bacterial strains.

Microbial strains	Essential oil dilution				Control
	10^{-3}, 10^{-4}	10^{-5}	10^{-1}	10^{-2}	
E. coli	+	+	++	+++	+++
S. aureus	+	++	++	+++	+++

++: Comparable growth with that Control +; slow growth

The results revealed that the essential oil showed antibacterial activity with varying magnitudes, depending on the size of inoculums and the concentration of essential oil. Diameter of inhibition zone of essential oil of *E. globulus* leaves varied from 8 to 26 mm. The largest zone of inhibition was obtained for E.coli (10-3 dilution) with 100 % concentration of essential oil of *E. globulus* and the lowest for *S. aureus* (10^{-1} dilution) with 25 % concentration of essential oil leaves.

A more significant inhibition was seen with a higher essential oil concentration. At low concentrations, a very

limited inhibitory effect was observed on the growth of microorganisms in comparison with those controls.

With increasing essential oil leaves concentration, an obvious inhibitory effect on the growth of and *S. aureus*, was significantly increased.

Like previous test, the application of the dilution broth method confirms by its results shown in Table 1 the important antibacterial activity of the essential oil of E.globulus leaves on these two microbial strains.

In most cases, the essential oil of *E. globulus* leaves has the same inhibitory effect on both germs except for the dilution 10^{-3} where we see that the gram (−) bacterium *E. coli* was found to be more sensitive to the oil than the gram (+) bacterium *S. aureus*.

4. Discussion

The addition of essential oil leaves in broth culture inoculated with *S. aureus* and *E. coli* inhibited the growth of these organisms. The rate of inhibition was greater, on gram negative bacteria (*E. coli*) than that observed on gram positive bacterium (*S. aureus*). In most cases the size of inoculum and the concentration of essential oil leaves affected the growth/survival of the organisms.

These results are almost similar to those shown by other works on the antimicrobial activity of essential oil of *E. globulus* leaves as well as those of similar species[14,21-29], and confirms its traditional uses[26,30,31].

The growths of tested bacteria in high concentrations of essential oil leaves were highly inhibited, where it was considered that these organisms were sensitive to the oil.

Some authors have reported that gram-negative micro-organisms are slightly more sensitive to essential oils when compared to gram-positive[32-42]. The gram-positive and gram-negative microorganisms differ in several aspects other than with respect to the structure of their cellular walls, mainly with regard to the presence of lipoproteins and lipopolysaccharides in gram-negative bacteria that form a barrier to hydrophobic compounds[43,44].

Some researchers reported that there is a relationship between the chemical structures of the most abundant in the tested essential oil and the antimicrobial activity.

The antibacterial activity of Eucalyptus extracts has been due to the components such as 1,8-cineole, citronellal, citronellol, citronellyl acetate, p-cymene, eucamalol, limonene, linalool, β - pinene, γ -terpinene, α - terpinol, alloocimene and aromadendrene[45].

The essential oils from the leaf of *E. globulus* showed varying degrees of antibacterial activity against two clinical isolates. From the above experiment it can be inferred that extract suggest significant growth inhibiting effects on Gram-positive (*E. coli*) and Gram-negative bacteria (*S. aureus*). The efficacy of leaf oil of *E. globulus* against these microorganisms may provide a scientific ground for the application of the herb in the prevention and treatment of bacterial infections caused by various pathogenic bacteria such as Staphylococcus aureus and Escherichia coli, which have developed resistance to antibiotics. The incorporation of this oil into the drug formulations is also recommended. The results of this study present the herb as a good candidate to explore new alternative antibacterial agents to combat pathogenic microorganisms.

Conflict of interest statement

We declare that we have no conflict of interest.

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