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Determination of antibacterial actions of leaf fundamental oils of *Eucalyptus globulus* and *Eucalyptus camaldulensis*

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Abstract

The antibacterial activities of essential oils from leaves of two *Eucalyptus* species *(globulus* and *camaldulensis)* was determined against *Staphylococcus aureus* Gram (+) and *Escherichia coli* Gram (-) bacteria. The inhibiting activity was evaluated by three methods: aromatogramme, microatmosphere and germs in suspension. Results demonstrated of the leaf essential oils of the two species showed an excellent inhibitory effect on *S. aureus* than that of *E. coli*. These data would indicate the potential usefulness of the two *Eucalyptus* species as a microbiostatic, antiseptic or as disinfectant agent.

Keywords: Antibacterial activity, essential oil, *Eucalyptus globulus*, *Eucalyptus camaldulensis*.

INTRODUCTION

The ancient-Egyptians were familiar with many medicinal herbs and were aware of their usefulness in treatment of various diseases (Abu-Shanab et al., 2004). Phytomedicines derived from plants have shown great promise in the treatment of infectious diseases including viral infections (Cowan, 1999). Single and poly herbal preparations have been used through out history for the treatment of various diseases. Many studies have been carried out to extract various natural products for screening antimicrobial activity but attention has not been focused intensively on studying the combinations of these products for their antimicrobial activity (Belaiche, 1979; Abu-Shanab et al., 2004; Adwan et al., 2006; Al-Bayati, 2008). The *Eucalyptus* (Myrtaceae) are used to control several diseases derived from microbial infections. The development of bacterial resistance to presently available antibiotics has necessitated the need to search for new antibacterial agents. The gram positive bacterium such as *Staphylococcus aureus* is mainly responsible for post operative wound infections, toxic shock syndrome, endocarditis, osteomyelitis and food poisoning (Benayache et al., 2001). The gram negative bacterium such as *Escherichia coli* is present in human intestine and causes lower urinary tract infection, coleocystis or septicaemia (Benhassaini et al., 2003; Benjilali et al.,1986 The main objective of the present study was to investtigate the effects of essential oil of *Eucalyptus globulus* and *Eucalyptus camaldulensis on S. aureus* and *E. coli.*

MATERIALS AND METHODS

Plant material and isolation of essential oils

Fresh leaves were collected during the flowering stage of *E. camaldulensis* and *E. globulus* . *E. camaldulensis* leaves came from the "Nador-Mennaour" forest in the El bordj region located in Mascara willaya (city North West of Algeria). While *E. globulus* leaves were collected at the Mustapha Stambouli University campus in Mascara City. The essential oils of fresh plant leaves obtained by hydrodistillation procedure.

Microorganisms

The tests organisms were obtained from the Microbiology Department, Laboratory of Medical Analysis at Dr Yessaâd Khaled hospital in Mascara city. The two bacterial strains *Escherichia coli* and *S. aureus* were isolated and identified by classical methods (Menghini et al., 1987) . *S. aureus* was isolated in the urine of a patient, and *E. coli* from a patient's coproculture.

The organisms were identified by cellular, cultural and biochemical characteristics. All species of *Staphylococcus* are Gram positive cocci. On nutrient agar they tend to be white, circular, entire, convex colonies. On blood agar *S. aureus* showed hemolysis of the agar in the area around the colony. Additional biochemical tests useful in separating the *Staphylococcus* species include catalase, coagulase, growth and fermentation of mannitol salt, and resistance or susceptibility to the antibiotic novobiocin. They were chosen for their high frequencies of food contamination and their pathogenicites.

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Table 1. Antibiogram results of *S. aureus* and *E. coli* with traditional antibiotics.

Antibiotic	*S. aureus*		*E. coli*	
	inhibition zone Diameter (cm)	Results	inhibition zone Diameter (cm)	Results
Ampicillin	0	R	0	R
Amoxicillin	1.5	I	2.8	S
Chloramphenicol	2.7	S	0	R
Doxycycline	0	R	1.4	I
Erythromycin	1.4	I	2.4	I
Nitroxoline	2.1	I	1.2	R
Pristinamycine	2.6	S	0	R

R= resists, I = intermediary resistance, S = sensitive

Table 2. Zone of inhibition in centimeters of *S. aureus* and *E. coli* against *E. camaldulensis* and *E. globulus* essential oil

Microbial Species		***Staphylococcus aureus***					
The dose		**1 µl**	**2 µl**	**5 µl**	**7.5 µl**	**10 µl**	**20 µl**
Inhibition zone diameter (cm)	*Eucalyptus. Camaldulensis*	0	0	1.4	1.9	2	3
	Eucalyptus globulus	0	0	1.6	2.5	2.9	4
Microbial Species		***Escherichia coli***					
Inhibition zone diameter (cm)	*Eucalyptus camaldulensis*	0	0	1	1.2	1.5	1.8
	Eucalyptus globulus	0	0	1.3	1.5	2	2.4

Determination of the antibacterial activity of the essential oils

Aromatogram test

This technique is an *in-vitro* method of measuring the antibacterial power of chemotyped essential oils. To seed one ml of the bacterial inoculum in the petri dishes previously melted with Mueller-Hinton agar. Six disks of blotting paper (0.7 cm diameter) aseptically impregnated with 1, 2, 5, 7.5, 10, 20 l of essential oils were deposited on the agar surface. After a latency period at 37°C for 16 - 18 h, the inhibition zone surrounding the disks was measured (Boland et al., 1991; Franchomme, 1999; Euzéby, 1998; Farah et al., 2001; Cimanga et al., 2002).

M**icroatmosphere test**

This technique describes the diffusion of volatile essential oils components in closed Petri dishes to monitor the growth of the test organisms. A disc, 2.5 cm in diameter, was impregnated with 50 l essential oils and deposited in the center of the Petri dish which contains the bacteria in agar previously dried under a hood for 15 - 20 min. and incubated to 37° C for 16 - 18 h (Gamal and Sabrin, 2007; Gocho, 1991; Harkental et al., 1999; Iserin, 1997).

Broth dilution method

The suspension of test organisms (*S. aureus, E. coli*) were diluted to 10^{-1}, 10^{2}, 10^{-3} and 10^{-4} mg/ l in a nutrient broth containing 1% Tween 80.Essential oils were added at concentrations of 10, 50, and 100 l. Microbial growth was taken by taking the optical density at 600 nm at different incubation times of 10 min, 2, and 24 h. To determine the minimum bactericidal concentration (MBC), fresh culture from the broth are inoculated into Mueller-Hinton agar plates, incubated for 24 h and were observed for bacterial growth (Kouokam et al., 2002; Larrondo et al., 1995; Le Minor and Richard, 1993; Levine, 1987).

Ampicillin, Amoxicillin, Chloramphenicol, Erythromycin, Nitroxoline and Pristinamycine were used as controls in the tests conducted.

RESULTS AND DISCUSSION

The antibiotic sensitivity testing

The results of the antibiogram (Table 1), showed that the *S. aureus* strain was sensitive to Chloramphenicol and Pristinamycine, resistant to Ampicillin and Doxycycline, has intermediate resistance to the Amoxicillin, Erythromycin and Nitroxoline. On the other hand, *E. coli* was sensitive to Chloramphenicol, resistant to Ampicillin, Doxycycline and Pristinamycine and intermediate resistance to Erythromycin and Nitroxoline.

Table 2 shows that the growth of both test organisms were not inhibited at low concentrations (1 and 2 µl) of eucalyptus oil from *E. camaldulensis* and *E. globulus*. Increasing amount of essential oil however, gave a distinct zone of inhibition. These results are similar to those found by (Trivedi and Hotchandani, 2004; Farah et al., 2001; Gamal and Sabrin, 2007; Nair et al., 2008).

Our results shown in Photo 1 and 2, we see the zone of inhibition of the vapors of *Eucalyptus* essential oils against *S. aureus* and *E. coli*. The vapor of essential oils of *Eucalyptus* inhibited the growth of the both bacteria. The zone of inhibition is interpreted as the antibacterial activity of the essential oils from the leaves of both *E.*

Photo 1. Microatmosphere of *S. aureus* (*E. camaldulensis* left and *E. globulus* right).

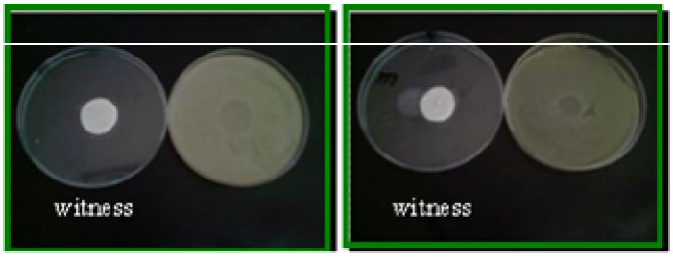

Photo 2. Microatmosphere of *E. coli* (*E. camaldulensis* left and *E. globulus* right).

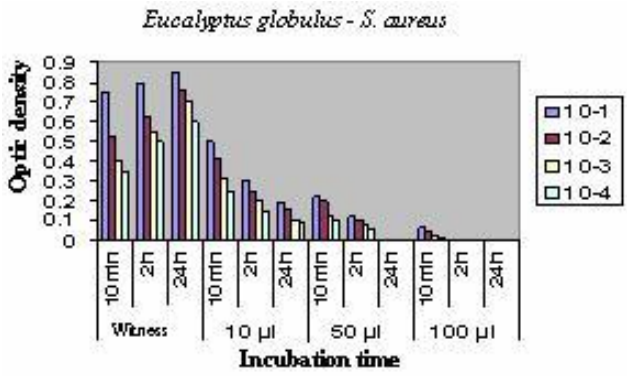

Figure 1. The optical density evolution values of *S. aureus* treated with the *E.globulus* essential oil.

globulus and *E. camaldulensis.* Iserin (1997), Inouye et al. (2001) and Cimanga et al. (2002) and some other researchers reported that the antimicrobial activity of an essential oil is linked to its chemical composition. The functional groups of some compounds found in most plant materials alcohol, phenols, terpenes and ketones are associated for its antimicrobial characteristics (Farah et al., 2001; Alma et al., 2004; Sartorelli et al., 2006; Braca et al., 2008; Mohammadreza,2008; Maksimovi et al., 2008 etc.). Pibiri (2006) has demonstrated in his PhD thesis that the tested essential oils of *Satureia Montana, Thymus, Origanum vulgaris* and cinnamon bark in gaseous phase do have a lethal effect on two strains *S. aureus* and *Pseudomonas aeruginosa*, even in small doses.

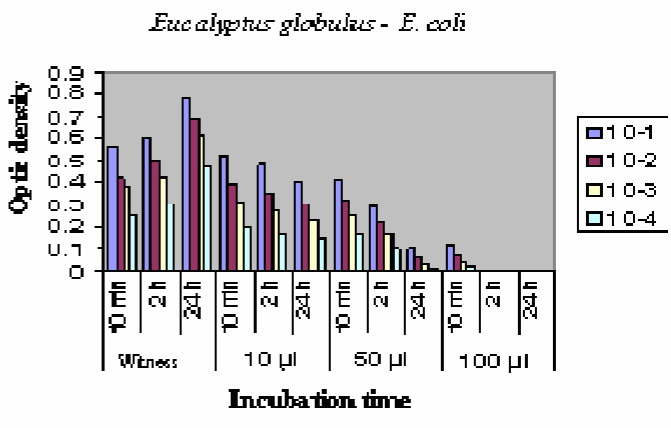

Figure 2. The optical density evolution values of *E. coli* treated with the *E. globulus* essential oil.

The essential oil constituents of the genus *Eucalyptus* (Myrtaceae) had been well characterized (Batista-Pereira et al., 2006). *Eucalyptus* species produce numerous volatile compounds in large amounts, especially isoprenoids (here referred to as terpenes), which are accumulated in glands abundantly distributed throughout the leaf parenchyma and bark (Rakotonirainy and Lavédrine, 2005; Moleyar, and Narasimham, 1986).

The chemical compositions of the leaf oils of various *Eucalyptus* species had been reported (Singh et al., 2000). The major component was 1,8-cineole, but a main contributor for the bioactivity was assumed to be α-terpineol, which showed eight-fold higher activity than 1,8-cineole against *S. aureus*. 1,8-Cineole had not been reported as an active principle in other eucalyptus oils (Inouye et al., 2001).

It has already been shown that the antimicrobial activity of volatile compounds results from the combined effect of direct vapor absorption on microorganisms and indirect effect through the medium that absorbed the vapor (Trivedi and Hotchandani, 2004). A significant contribution of the volatile compounds through agar absorption was reported for *E. coli* (Haggag, 1996).

Presented in Figure 1 and 2 are the inhibition of the growth of *S. aureus* and *E. coli* by the action of the essential oil from the leaves of *E. globulus* measured in terms of optical density at different concentration of the essential oil and broth dilution. There has been a remarkable decrease in the bacterial growth rate with increasing concentration of the essential oil and longer period of incubation. The growth of *S. aureus* (Figure 1) was totally inhibited when 50 l of the oil was used at 2 hours of exposure. At a higher concentration of 100 l of the oil, a 10 min exposure inhibited the growth of the bacteria for all the different amount of broth dilution.

On the other hand, Figure 2 shows that growth of *E. coli* was inhibited at 100 l with an incubation period of 2 h. Shown in Figure 3 and 4 are the inhibition of the growth of the two test organisms exposed to the essential oils of

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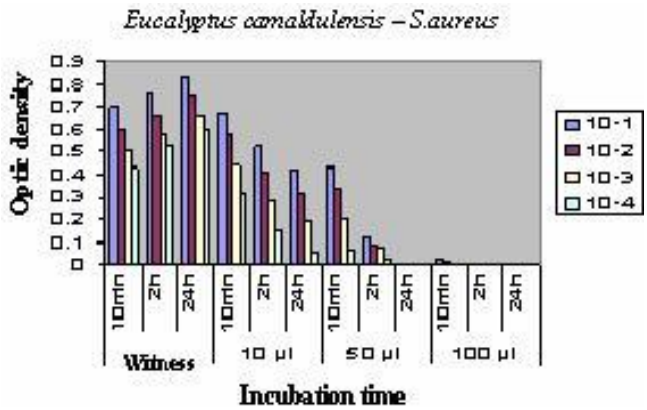

Figure 3. The optical density evolution values of *S. aureus* treated with the *E. carnaldulensis* essential oil.

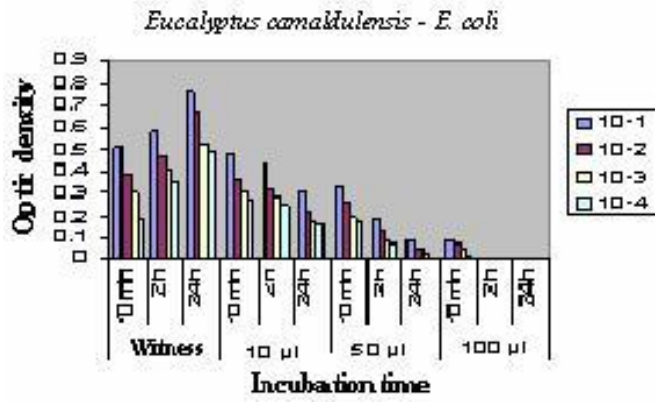

Figure 4. The optical density evolution values of *E. coli* treated with the *E. carnaldulensis* essential oil.

E. camaldulensis measured in terms of optical density. A decrease in the colonies of two species was marked with increasing concentration of *E. camaldulensis* essential oil. Like that of *E. globulus*, the inhibitory action of the *E. camaldulensis* essential oil is much remarkable in large doses as 50 and 100 l and at long incubation time of about two or 24 h, during which, we are witnessing a total inhibition of the two test organisms.

The results of the study revealed that essential oil of eucalyptus species used in this study has antibacterial activity against Gram-positive as well as Gram-negative bacteria resistant to commonly used antimicrobial agents.

Conclusion

The present study confirmed antimicrobial properties of essential oils from *E. globulus* and *E. camaldulensis* that showed significant growth inhibition for *S. aureus* and *E. coli* tested whose the problem relates to the emergence of strains that possess multiple resistance to a range of antibiotics, thereby making them difficult to treat. The encouraging results indicate the *E. globulus* and *E. camaldulensis* 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.

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