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Research Article

Antibacterial Activity of Essential Oil and Aqueous Extract of *Eucalyptus globulus* Against Methicillin Resistance *Staphylococcus aureus* and Methicillin Sensitive *Staphylococcus aureus*

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ABSTRACT

Objective: Our work was focused on the stady of antibacterial activity of essential oil and aqueous extract of *Eucalyptus globulus* leaves, plant that is widespred in the Algerian traditional pharmacology.

Methods: The essential oil of this plant was obtained by hydrodistillation method and aqueous by decoction in volume of destilled water. The extracts were subjected to screening of their possible antibacterial activity in vitro against eleven strains of methicillin sensitive *Staphylococcus aureus* and eight strains methicillin resistance *Staphylococcus aureus* isolated of different hospitalized patients, using agar disc diffusion method. Both minimum inhibitory concentration and minimum bactericidal concentration by agar dilution method.n Results: The essential oil has demonstrated a considerable antibacterial activity against all strains tested with best inhibition zone equal to 16,0±1,41mm for methicillin sensitive *Stahylococcus aureus* and 15,5±0,70mm for methicillin resistance *S. aureus*. Studied aqueous extact showed a good antibacterial activity higher than essential oil of some plant, when the best inhibition zone was 31,0±0,70mm for methicillin sensitive *S. aureus* and 20,5±0,70mm for methicillin resistance *S. aureus*. Conclusion: The results obtained showed that aqueous extract and essential oil of *Eucalyptus globulus* may be constitute a naturel antibiotic to exploited for raising problems of infectious diseases caused by *Staphylococcus aureus*.

Keywords: Essential oil, aqueous extract, *Eucalyptus globulus*, *Staphylococcus aureus*, hydrodistillation

INTRODUCTION

Aromatic and medicinal plant which push in the whole world have therapeutic virtues, because they produce certain bioactives molecules which react with other organisms in the environment, inhbiting bacterial or fungal growth. The molecules that can inhibit pathogens and have little toxicity to host cells are considered candidates for developing new antimicrobial drugs[13,19]. Today, treatements by plants comeback on the first plan because the efficacy of medicaments such as antibiotics, considered as almost universel resolution of infectious diseases decreasing, in reason of usage general of this chemical agents and prescript on a large scale and sometimes inappropriate have causing the strong adaptability of bacterial strains and selection of multiresistants strains which cause a public health problem[10]. *Staphylococcus aureus* is one of opportunistic pathogens that cause severe and life threatening infections in immunocompromised patients. The Gram-positive Bacterium *S. aureus* is mainly responsible for post-operative wound infection, toxic shock syndrome and food poisoning[2,6]. The *Eucalyptus*, a native genus from Australia belongs to *Myrtaceae* family and comprises about 900 species and subspecies[15,17]. *Eucalyptus* species are also know to contain bioactive products that display antibacterial, antifungal, analgestic, antioxidative and anti-inflamatory effects[9]. This research was conducted to evaluate the antibacterial activity of essential oil and aqueous extract of *Eucalyptus globulus* leaves were done twenty *S. aureus* strains isolated of different hospitalized patients by disc diffusion method and determination of minimum inhibitory concentration and minimum bactericidal concentration by used agar dilution method.

MATERIALS AND METHODS

plant material

Fresh leaves of *Eucalyptus golobulus* were collected from the region El- Kala (north east Algeria) during march 2013. Leaves were air-dried at room temperature (20-25°C) for one week and then stored in cloth paper bags.

Microbial strains

The essential oil and aqueous extract of *E. golobulus* were tested against twenty strains of *Staphylococcus aureus*. These microorganisms were clinical isolates from different hospitalized patients having various infections. A pathologic source and antibiotic resistance of microorganisms was represented in table 1.

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Table 1: pathologic sources and antibiotic resistance of microorganisms selected

Microorganisms	Age	sex	Sources	Antibiotic resistance
S. aureus ATTC	-	-	ATCC29223	Susceptible
S. aureus12	56	Female	Urine	P
S. aureus34	45	Female	Urine	P
S. aureus01	35	Male	Urine	P, Fos, K
S. aureus60	25	Male	Urine	P
S. aureus25	45	Male	Urine	Ox, P, E, RP
S. aureus37	69	Female	Wound	OX, P, Gn, K, TE
S. aureus49	47	Male	Wound	Ox,Fc, P
S. aureus04	53	Male	Urine	P, Fos
S. aureus52	69	Female	Wound	P, Gn, OFX, K, TE
S. aureus28	27	Male	Urine	P, Fos, K
S. aureus27	17	Male	Urine	P, Fos, K
MRSA45	55	Female	Wound	OX, P, FC, FOX
MRSA58	45	Female	Wound	OX, P, Fc, FOX, Gn, AK, OFX, K, E, TE, RP
MRSA47	70	Male	Urine	OX, Fc, P, FOX, K
MRSA40	42	Female	Wound	OX, Fc, P, FOX, Gn, AK, OFX, K, CD, Fos, TE, RD, RP
MRSA05	35	Female	Wound	OX, Fc, P, FOX, Gn, C, K, E, CD, Fos, TE, RD, RP
MRSA 41	52	Female	Wound	OX, Fc, P, FOX, Gn, AK, OFX, K, E, TE, RP
MRSA59	61	Male	Wound	OX, P, FOX, OFX, K, E, TE
MRSA23	54	Female	Wound	OX, Fc, P, FOX, Gn, AK, OFX, K, FosTE, RP

S. aureus : Staphylococcus aureus, MRSA : Methicillin Resistance *Staphylococcus aureus*

P : Penicillin, OX : Oxacillin, FOX : Cefoxitin, E : Erythromycin, Fc : Fusidic Acid, Fos : Fosfomycin, TE : Tetracyclin, RD : Rifampicin, RP : Pristinamycin, CD : Clindamycin, Gn : Gentamycin, AK : Amikacine, OFX : Ofloxacine, C : Chloromphenicol, K : Kanamycin

Extraction of aqueous extract

Ten grams of leaves powder were boiled with 200ml of disllited water for 20min with an occasional stirring. The decoction preparation was then filtred through a muslin cloth followed by filtration paper. The extract was kept at 4°C[12].

Extraction of essential oil

The essential oil was extracted by hydrodistillation method using a Clevenger type apparatus for 2h. The oil was conserved at 4°C until antibacterial activity testing[1,14]. Yield was calculated according to dry weight of the plant materials by using following formula[18]:

$$‰\text{Yield} = \frac{\text{weight of oil}}{\text{weight of dried powder}} \text{x}100$$

Sensitivity test of essential oil and aqueous extract

The agar disc diffusion method was employed for determination of antibacterial activity of *E. globulus* essential oil and aqueous extract. The inoculums were suspended in sterile saline water and diluted according to 0.5 Mac Farland standard and then spread on a solid agar medium in Petri dishes (Mueller Hinton agar). Two filter discs (6mm in diameter) was deposited on the agar surface then impregnated by 5 and 10ql of essential and two discs by 5 and 10ql of aqueous extract and another by 10ql of dimethylsulfoxid (DMSO) used as a negative control. The Petri dishes were incubated at 37°C for 24h[3,4,20,22].

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) by agar dilution method

minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of extracts was determined by agar dilution method described by Mayachiew and Davahastin. (2008). Different concentrations of extracts (1000,2000,3000,4000,6000,8000,10000ųg/ml) were tested; 1ml of each concentration were mixed with 9ml of Mueller Hinton medium to obtain final concentrations (50, 100, 200,300,400,500 ųg/ml) and poured into sterilized Petri dishes. Immediately after solidification the dishes were spot inoculated with 10ql of suspension containing 10^6CFU/ml of bacterium. The inoculated dishes were incubated at 37°C for 24h. The MIC represent the lowest concentration inhibit any growth visible after 24h of incubation at 37°C. Furthermore, the MBC represent the lowest concentration of extract inhibit any growth visible after 5 days of incubation at 37°C[18,16].

RESULTS

The essential oils of *Eucalyptus globulus* leaves obtained have pale yellow color, and aromatic smell spicy with a density less than of water. The percentage yield was 2.25‰ for 100g of powder leaves. The antibacterial activity of essential oil and aqueous extract of *E. golobulus* was represented in table 2. According to the width of the inhibition zone diameter expressed in mm, results were

Table 2: Antibacterial activity of *Eucalyptus globulus* essential oil and aqueous extract using disc diffusion method.

Bacterial strains	DMSO (10ql)	Oxacillin	Essential oil		Bacterial sensitivity		Aqueous extract		Bacterial sensitivity	
			5ql	10ql	5ql	10ql	5ql	10ql	5ql	10ql
S. aureus ATTC29233	00,0±0,00	-	09,5±0,70	12,00±0,00	+	+	17,50±0,00	19,00±1,41	++	++
S. aureus12	00,0±0,00	18,00±0,70	10,0±1,41	13,00±1,41	+	+	15,50±0,70	17,00±0,00	++	++
S. aureus34	00,0±0,00	14,00±0,00	10,5±2,12	12,50±0,00	+	+	13,50±0,70	15,00±0,00	+	++
S. aureus01	00,0±0,00	13,00±1,41	09,00±0,00	14,00±1,82	+	++	16,00±0,00	17,00±0,00	++	++
S. aureus60	00,0±0,00	17,00±0,00	09,50±0,70	13,00±0,00	+	+	14,00±0,00	15,50±0,70	++	++
S. aureus25	00,0±0,00	10,00±1,41	10,00±0,00	16,00±1,41	+	++	14,00±1,41	19,00±1,41	++	++
S. aureus37	00,0±0,00	00,0±0,00	13,5±0,50	16,00±1,41	+	++	13,00±0,00	15,50±0,70	+	++
S. aureus49	00,0±0,00	08,50±0,70	08,50±0,70	12,00±0,00	+	+	15,50±0,70	19,50±0,70	++	++
S. aureus04	00,0±0,00	16,0±0,00	09,50±0,00	12,00±1,41	+	+	17,00±0,00	19,00±0,00	++	++
S. aureus52	00,0±0,00	12,00±0,00	10,50±0,70	16,00±0,00	+	++	14,00±0,00	16,00±0,00	++	++
S. aureus28	00,0±0,00	11,00±0,00	09,00±0,00	14,50±0,70	+	++	25,50±0,70	31,00±0,70	+++	+++
S. aureus27	00,0±0,00	11,00±0,00	10,00±0,00	14,00±0,00	+	+	18,00±0,00	20,00±0,00	++	+++
MRSA45	00,0±0,00	00,0±0,00	08,00±0,00	10,00±0,00	-	+	15,00±0,00	20,00±0,00	++	+++
MRSA58	00,0±0,00	00,0±0,00	10,00±1,41	12,50±2,12	+	+	16,50±0,70	20,50±0,70	++	+++
MRSA47	00,0±0,00	00,0±0,00	00,00±0,00	09,50±2,12	-	+	13,50±0,70	15,00±0,00	++	++
MRSA40	00,0±0,00	00,0±0,00	00,00±0,00	08,50±0,70	-	+	16,00±0,00	19,50±0,70	++	++
MRSA05	00,0±0,00	00,0±0,00	09,00±0,00	14,50±0,70	+	++	16,00±0,00	19,50±0,70	++	++
MRSA 41	00,0±0,00	00,0±0,00	08,00±0,00	14,50±0,70	-	++	15,50±0,70	20,00±0,00	++	+++
MRSA59	00,0±0,00	00,0±0,00	12,00±0,00	15,50±0,70	+	++	15,00±0,00	17,00±0,00	++	++
MRSA23	00,0±0,00	00,0±0,00	10,00±1,41	15,00±1,41	+	+	16,50±0,70	20,00±0,00	++	+++

appreciated as follows: not sensitive (-) for diameter equal to 8mm or below; sensitive (+) for diameter between 8 and 14mm; very sensitive (++) for diameter 14 to 20mm and extremely sensitive (+++) for diameter equal or larger than 20mm[1]. DMSO: Dimethylsulfoxid, (-) not sensitive, (+): sensitive, (++) very sensitive, (+++): extremely sensitive. The values of MIC and MBC determineted by agar dilution method were shown in the table 3. According to the values of MIC and MBC; the report CMB/CMI was calculated to determine bacteriostatic or bactericidal effect of extracts study. When this report is superior to 4, extract have a bacteriostatic effect, and bactericidal effect when it is less than or equal 4[5].

DISCUSSION

The yield of essential oils was higher (2.25‰) than that obtained by Selvakumar P et al (0.72 to 0.8‰) and Manika N et al (1.7 to 2.1‰)[14,18]. Difference with these yields compared to those previously reported in the literature for some aromatic plants such as *Eucalyptus* species collected in the other geographic areas in the world, could be attributed to some factors such as climate, nature of the sol, age of the tree, time of collection, mode of extraction[7]. The antibacterial activity of essential oil displayed considerable against different strains tested, but stayed lower than that of aqueous extract which showed highest antibacterial

Table 3: The values of MIC and MBC determined by agar dilution method.

Bacterial strains	Essential oil			Aqueous extract		
	MIC (µg/ml)	MBC(µg/ml)	MBC/MIC	MIC (µg/ml)	MBC (µg/ml)	MBC/MIC
S. aureus ATTC29223	200	400	2,00	400	500	1,25
S. aureus12	200	500	2,50	300	400	1,33
S. aureus34	300	400	1,33	300	400	1,33
S. aureus01	400	400	1,00	400	500	1,25
S. aureus60	400	500	1,25	400	500	1,25
S. aureus25	300	400	1,33	400	500	1,25
S. aureus37	300	400	1,33	400	500	1,25
S. aureus49	200	300	1,50	300	400	1,33
S. aureus04	200	400	2,00	400	400	1,00
S. aureus52	300	500	1,60	400	500	1,25
S. aureus28	500	500	1,00	400	500	1,25
S. aureus27	200	300	1,50	400	400	1,00
MRSA45	500	500	1,00	400	500	1,25
MRSA58	400	500	1,00	400	500	1,25
MRSA47	500	500	1,00	400	400	1,25
MRSA40	500	500	1,00	400	500	1,25
MRSA05	500	500	1,00	400	500	1,25
MRSA 41	400	500	1,25	500	500	1,00
MRSA59	500	500	1,00	400	400	1,00
MRSA23	400	500	1,25	400	500	1,25

activity; this variability in antibacterial effect could be attributed to the chemical composition of these extracts.

The best inhibition of essential oil was observed with *S. aureus 37* (13,50±0.50 for 5ql and 16,00±1,41 for 10ql) and for aqueous extract with *S. aureus 28* (25,50±0,70 for 5ql and 31,00±0,70 for 10ql), but it stayed less important than with methicillin resistance *S. aureus* strains; where the highest activity of essential oil was observed against MRSA59 (12,00±0,00 for 5ql and 15,50±0,70 for 10ql) and with MRSA58 (16,50±0,70 for 5ql and 20,50±0,70 for 10ql) for aqueous extract. In general, the essential oil and aqueous extract of *E. globulus* had a superior inhibitory activity against twenty strains tested, compared with the best antibiotic used for treatment of infectious diseases caused by *Staphylococcus aureus:* oxacillin. These results are in agreement with literature which reported that the Gram positive bacterium *S. aureus* was highly sensitive to the essential oils of *E. globulus* then Gram positive bacteria[2,15,21]. The MIC and MBC of extracts showed varying values against twenty strains tested, the best MIC and MBC of essential oil and aqueous extract was respectively 200mg/ml and 300mg/ml. All reports MBC/MIC of two extracts were less than four which determine bactericidal effect of essential oil and aqueous extract of *Eucalyptus globules* leaves.

CONCLUSION

Our results confirmed antibacterial proprieties of *Eucalyptus globulus* essential oil and aqueous extract. The aqueous extract has more effective antibacterial than essential oil extracted from the leaves of some plant, that showed potentienl inhibition against eleven strains of methicillin sensitive *Staphylococcus aureus* and eight strains methicillin resistance *Staphylococcus aureus*. These extracts may be suggested as naturel antibiotic for the treatement of infectious diseases caused by *Staphylococcus aureus* after testing the toxic effects on human.

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