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In vitro antibacterial proprieties of aqueous extract and essential oil of *Eucalyptus globulus* against multi-resistant *Klebseilla pneumoniae* isolated from hospitalized patients

Marwa Bouras*[1], Naffisa Boutefnouchet Abbaci[1] and Salima Bennadja[2]

[1]*Faculty of Nature and Life Sciences, Department of Biochemistry, Biochemistry and applied Microbiology Laboratory, University Badji Mokhtar of Annaba 23000, Algeria*
[2]*Medicine Faculty, Department of Pharmacy, University Badji Mokhtar of Annaba 23000, Algeria*

ABSTRACT

The present study was conducted to evaluate the antibacterial activity of aqueous extract and essential oil obtained from Eucalyptus globulus leaves. The aqueous extract was obtained by decoction in volume of distilled and essential oil by hydrodistillation method. The antibacterial effect was studied in vitro in five strains: Klebseilla pneumoniae ATCC10031 and four multi-resistant strains of Klebseilla pneumoniae isolated from hospitalized patients. Minimum inhibitory concentration (CMI) and minimum bactericidal concentration (MBC) was determined by agar dilution method. The essential oil has demonstrated a good antibacterial activity against all strains tested with best inhibition zone equal to 18,5±2,12mm for 5µl and 32,50±0,70mm for 10µl. Studied aqueous extract showed a considerable antibacterial effect less than essential oil of the some plant, when the best inhibition zone was 10,0±1,41mm for 5µl and 13,5±0,70mm for10µl. The MIC and MBC of essential oil was ranging respectively from 100 to 400µg/ml and 200µg/ml to 500µg/ml, and for aqueous extract from 300 to 400µg/ml and 400 to 500µg/ml. The results obtained indicated that Eucalyptus globulus essential oil could be used as a potential source of naturel antibiotic for raising problems of infectious diseases caused by multi-resistant Klebseilla pneumoniae after testing the toxic effects on human.

Keywords: Essential oil, aqueous extract, *Eucalyptus globulus*, *Klebseilla pneumoniae*, dics diffusion method, hydrodistillation

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INTRODUCTION

The current problem associated with emerging multi-resistant bacteria presents a serious global medical crisis, requiring constant surveillance, with continuously challenges the scientific community [4].

Traditionally used medicinal plants produce a variety of substances of know therapeutic properties. One of the vital activities possessed by these medicinal plants is antimicrobial. The substances that can either inhibit the growth of bacteria or kill them, with no toxicity or minimum toxicity to host cells are considered candidates for developing new antimicrobial drugs [4].

The *Eucalyptus*, a native genus from Australia belongs to *Myrtaceae* family and comprises about 900 species and subspecies [3, 9, 21]. *Eucalyptus* species are also know to contain bioactive products that display antibacterial, antifungal, analgestic, antioxidative and anti-inflamatory effects [20].

Klebseilla pneumoniae is a Gram negative bacterium, included in enterobacteriaceae. It was recognized as a cause of community acquired pneumonia and is the opportunistic pathogen that can cause pneumonia, urinary tract pathogen

infections, and bacteremia. *K.pneumoniae* is one of the most common nosocomial pathogens, its ability to produce extended spectrum β-lactamases has caused great concern worldwide [10, 11]

In view of this, it was aimed to conduct the study to evaluate the antibacterial activity of aqueous extract and essential oil of *Eucalyptus globulus* leaves against multi-resistant *Klebseilla pneumoniae* strains isolated from hospitalized patients by used disc diffusion method and determination of minimum inhibitory concentration and minimum bactericidal concentration by agar dilution method.

MATERIALS AND METHODS

2-1- 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.

2-2- Microbial strains

The essential oil and aqueous extract of *E.golobulus* were tested against four strains of *Klebseilla pneumoniae* isolated from hospitalized patients. The antibiotic resistance and pathologic sources of strains tested was represented in table 1.

Table 1: Antibiotic resistance and pathologic sources and microorganisms selected

Microorganisms	Age	Sex	Sources	Antibiotic resistance
K.pneumoniae ATCC	-	-	ATCC *10031*	-
K.pneumoniae 01	47	Male	Urine	AMC, AM, TIC, CIP, SXT
K.pneumoniae 02	58	Female	Wound	AMC, AM, TIC, CAZ, CTX, GN, SXT
K.pneumoniae 03	60	Male	Urine	AMC, AM, TIC, CAZ, CTX, GN
K.pneumoniae 04	39	Female	Wound	AMC, AM, TIC, IPM, CAZ, CTX, GN, CIP

AMC: Amoxicilline+clavulanic acid, AM: Ampicillin, TIC: Ticarcillin, CIP: Ciprofloxacin, SXT: Co-Trimethoprim, CAZ: Ceftazidim, GN: Gentamycin, CTX: Cefotaxim

2-3- 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 [22].

2-4- Extraction of essential oil

The hydrodistillation method was using for extraction of *E.globulus* essential oil. The extraction was performed in Clevenger apparatus for 2h. After hydrodistillation, the essential oil obtained was stored at 4°C and protected against light to ovoid alteration in its composition. Yield was calculated according to dry weight of the plant materials by using following formula: [2, 18]

‰Yield= weight of oil/ weight of dried powder of *Eucalyptus globulus* leaves $_x$ 100

2-5- Disc diffusion method

Antibacterial activity of *E.globulus* aqueous extract and essential oil was determinate by agar disc diffusion method. 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 5ųl and 10ųl of essential and two discs by 5ųl and 10ųl of aqueous extract and another disc by 10ųl of dimethylsulfoxid (DMSO) used as a negative control. The Petri dishes were incubated at 37°C for 24h [1, 7, 14, 16].

2-6- Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) by agar dilution method

The MIC and MBC of extracts were performed using agar dilution method reported by Mayachiew and Davahastin. Different concentrations of extracts (1000, 2000, 3000, 4000, 6000, 8000, 10000ųg/ml) were tested; 1ml of each concentrations was 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 10ųl 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 [8, 17, 18].

RESULTS AND DISCUSION

The percentage yield of essential oil extract from *E.globulus* leaves was 2,25‰ for 100g of powder leaves. The yield obtained 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‰) [13, 18]. The difference with these yields could be attributed to some factors such as climate, nature of the sol, age of the tree, time of collection and mode of extraction [12].

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 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 [6].

Table2: Antibacterial effect of *Eucalyptus globulus* essential oil and aqueous extract by used disc diffusion method

Bacterial strains	DMSO (10µl)	Essential oil		Bacterial sensitivity		Aqueous extract		Bacterial sensitivity	
		5µl	10µl	5µl	10µl	5µl	10µl	5µl	10µl
K.pneumoniae ATCC 10031	00,0±0,00	22,5±0,70	34,00±1,41	+++	+++	13,00±1,41	16,00±0,00	+	++
K.pneumoniae01	00,0±0,00	15,00±1,41	31,00±2,82	++	+++	10,00±1,41	13,50±0,70	+	+
K.pneumoniae02	00,0±0,00	16,00±1,41	21,50±2,12	++	+++	09,50±0,70	12,50±0,70	+	+
K.pneumoniae03	00,0±0,00	18,50±2,12	32,50±0,70	++	+++	09,50±0,70	11,50±0,70	+	+
K.pneumoniae04	00,0±0,00	18,00±1,70	29,50±0,70	++	+++	09,00±0,00	10,50±0,70	+	+

DMSO: Dimethylsulfoxid, (-) not sensitive, (+): sensitive, (++) very sensitive, (+++): extremely sensitive

Eucalyptus globulus essential oil showed a potential antibacterial activity, when all strains tested were very sensitive to extremely sensitive. Aqueous extract of the some plant displayed considerable antibacterial effect, but stayed less important than observed with essential oil, when all of the strains tested have been sensitive to aqueous extract; this variability in antibacterial activity could be attributed to the difference of chemical composition between these extracts.

The best inhibition zone of essential oil was observed with *K.pneumoniae ATCC 10031* (22,5±0,70 for 5µl and 34,00±1,41 for 10µl) and for nosocomial strains with *K.pneumoniae03* (18,50±2,12 for 5µl and 32,50±0,70 for 10µl). Aqueous extract was showed best inhibition zone with *K.pneumoniae ATCC 10031* (13,00±1,41 for 5µl and 16,00±0,00 for 10µl) and for multi-resistant strains with *K.pneumoniae01* (10,00±1,41 for 5µl and 13,50±0,70 for 10µl). These results are in agreement with literature which reported that the Gram negative bacterium *K.pneumoniae* was highly sensitive to the essential oils of *E.globulus* [5, 10, 19]

The values of MIC and MBC determined 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 [15].

Table 3: MIC and MBC of extracts 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
K.pneumoniae ATCC 10031	100	200	2,00	300	400	1,33
K.pneumoniae01	200	400	2,50	300	400	1,33
K.pneumoniae02	300	400	1,33	300	500	1,66
K.pneumoniae03	400	500	1,25	400	500	1,25
K.pneumoniae04	400	500	1,25	400	500	1,25

The MIC and MBC of extracts showed varying values against twenty strains tested, it was respectively between 100 to 400µg/ml and 200 to 500µg/ml for essential oil and for aqueous extract ranging from 300 to 400µg/ml and 400 to 500µg/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 suggested antibacterial proprieties of aqueous extract and essential oil extracted from of *Eucalyptus globulus* leaves collected from Algerian east. The essential oil have more effective antibacterial than aqueous extract, that showed potential inhibition against four strains multi-resistant *Klebseilla pneumonia*. These extracts can

be exploited as naturel antibiotic for raising problems of infectious diseases caused by multi-resistant *Klebesiella pneumoniae* which cause a public health problem.

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