Antimicrobial activity of the leaves essential oil of *Eucalyptus camaldulensis* Dehnh.

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Abstract: *Essential oil from the* leaves of *Eucalyptus camaldulensis* Dehnh. belonging to the family Myrtaceae was tested for its antimicrobial activity against six standard bacteria, two Gram positive bacteria (*Bacillus subtilis, Staphylococcus aureus*), four Gram negative (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris, Pseudomonas aeruginosa*) and two standard fungi (*Aspergillus niger, Candida albicans*) using the cup plate agar diffusion method. The essential oil of *E. camaldulensis* showed high activity (22mm) against *S.aureus,* (20mm) *against E.coli* and *C. albicans*. It also possessed moderate activity (17mm) against *B.subtilis, Kl. Pneumoniae and Pr. vulgaris* ,(18mm) against *Ps. aeruginosa* and (16mm) against *A.niger*. The minimum inhibitory concentrations (MICs) of *E. camaldulensis* essential oil against standard bacteria were determined using the agar plate dilution method and the MICs were12.5 µg/ml. The essential oil of *E. camaldulensis* was further tested against fifty clinical isolates, collected randomly from patients of Khartoum Teaching Hospital laboratory and Soba Hospital. The antibacterial activity of two reference drugs and the antifungal activity of two reference drugs were determined against six bacteria and two fungi and their activities were compared with the activity of essential oil of *E. camaldulensis* leaves. The essential oil of *E. camaldulensis* leaves can be of potential use as antimicrobial agents.

Keywords: Antimicrobial activity, Essential oil, *Eucalyptus camaldulensis*, Leaves, Minimum Inhibitory Concentrations.

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1. Introduction

Eucalyptus camaldulensis Dehnh., local names (ban, kafur), belongs to the family Myrtaceae (subfamily Myrtoideae) and is also known as river red gum or Murray red gum. It is a tree of the genus *Eucalyptus,* a native to Australia (Quattrocchi 2000) and is probably the most widespread *Eucalyptus* species in Australia (Hillis, 1966). Eucalyptus is one of the diverse genus of flowering plants in the world and comprises about 800 species (Gil et al. 2010). Eucalyptus has been used in folk medicine throughout the world as anti-inflammatory, analgesic and antipyretic remedies for the symptoms of respiratory infections, such as cold, flu, and sinus congestion (Rahimi et al. 2012, Shahwar et al. 2012 and Hmamouchi, 1997). Essential oils from Eucalyptus species have been approved as food additives: 1,8-cineole is the main constituent of eucalyptus oil (Samate et al. 1998). In addition, the oil possesses a wide spectrum of biological activity including anti-microbial, fungicidal, insecticidal/insect repellent, herbicidal, acaricidal and nematicidal (Singla et al. 2014). The main uses of the leaves of some species are the production of essential oil (Shahwar et al. 2012).

The eucalyptus oil is a complex mixture of a variety of monoterpenes and sesquiterpenes, aromatic phenols, oxides, ethers, alcohols, esters, aldehydes and ketones (Brooker and Kleinig 2006) and the extracts are also widely used in modern pharmaceutical and cosmetic industries (Arfao et al.2013) and have a therapeutic application in the treatment of pulmonary infections by inhalation (Low et al. 1974) and (Hasegawa et al. 2008). Previous studies on the essential oil of the flowers of *E.camaldulensis* revealed the presence of 1,8-cineole, β -pinene and spathulenol as the most abundant constituents (Giamakis et al. 2001). The essential oil of the leaves was found to contain *p*-cymene, γ-terpinene, α-pinene, 1, 8-cineole, terpinen-4-ol, α-terpineol, carvacrol and thymol as the major components (Siramon and Ohtani 2001). The major components of the essential oil of the fruits were aromadendrene, α-pinene, drimenol, and cubenol (El-Ghorab et al.2007).

A pentacyclic triterpenoid, named camaldulin along with ursolic acid lactone acetate and ursolic acid lactone were isolated from *E. camaldulensis*. All exhibited spasmolytic action (Begum et al. 2000). Later the same authors isolated another triterpenoid acid named eucalyptanoic acid which

also exhibited aspasmolytic action (Begum et al. 2000). Some flavonoid glycosides were isolated from the leaves of *E. camaldulensis* (Abd-Alla et al. 1980).

The purpose of the study is to evaluate the antimicrobial activity of *Eucalyptus camaldulensis* against standard bacteria and fungi, to isolate pathogenic bacteria and to determine the minimum inhibitory concentrations.

2. Materials and methods

2.1 Plant material

The Leaves of *Eucalyptus camaldulensis* were collected from Medicinal and Aromatic Plants and Traditional Medicinal Research Institute in October 2013 and they were identified and authenticated by the taxonomist Dr. Haider Abd alGader (Medicinal and Aromatic Plants and Traditional Medicine Research Institute, Khartoum, Sudan). A voucher specimen was deposited in the Herbarium of the institute.

2.2 Method of extraction

The oil of the tested *Eucalyptus camaldulensis* leaves was obtained by hydrodistillation technique using Clevenger's apparatus. Hundred grams from plant materials were placed in a two liters round bottom flask and distilled water was added and mixed thoroughly. The contents of the flask were boiled gently for four hours until the volatile oil has been distilled. The crude volatile oil of plant was transferred by means of a pipette into a separate brown glass bottle. Anhydrous sodium sulphate was added; agitated gently to absorb the water and the clear oil was decanted into brown glass bottle and kept in the refrigerator until needed for analysis.

The oil was dissolved in methanol (1:10) and was then tested against the standard organisms.

2.3 Test microorganisms

The oily solution of *E.camaldulensis* was tested against two Gram positive bacteria *Bacillus subtilis* (NCTC 8236) and *Staphylococcus aureus* (ATCC 25923). Four Gram negative organsims, *Escherichia coli* (ATCC 25922), *Klebieslla pneumonia* (ATCC 53657), *Proteus*

vulgaris (ATCC 6380), *Pseudomonas aeruginosa* (ATCC 27853) and two standard fungi, *Aspergillus niger* (ATCC 9763) and *Candida albicans* (ATCC7596). The tested organsims were obtained from the Department of Microbiology, MAPTMRI and National Health Labroatory, Khartoum, Sudan.

Sixty clinical isolates of *Bacillus subtilis, Staphylococcus aureus*, *Escherichia coli, Klebieslla pneumonia, Proteus vulgaris* and *Pseudomonas aeruginosa* were collected from Khartoum and Soba Hospital. The bacterial cultures were maintained on nutrient agar and the fungal cultures were maintained on Dextrose agar and inculated at 37 °C for 18 hours.

2.4 *In vitro* testing the oil *Eucalyptus camaldulensis* oil for antimicrobial activity

The cup-plate agar diffusion method (Kavanagh, 1972) was adopted with some minor modifications to assess the antibacterial of the prepared extracts. One ml of the standardized bacterial stock suspension 10^8 –10^9 C.F.U/ ml were thoroughly mixed with 100ml of molten sterile nutrient agar which was maintained at 45 ºC. 20ml aliquots of the inoculated nutrient agar were distributed into sterile Petri-dishes. The agar was left to set and in each of these plates 4 cups (10 mm in diameter) was cut using a sterile cork borer (No. 4) and agar discs were removed. Alternate cups were filled with 0.1 ml sample of the oil using automatic microliter pipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37 ºC for 18 hours. Two replicates were carried out for the oil against each of the tested organisms. After incubation the diameters of the resultant growth inhibition zones were measured, averaged and the mean values were tabulated. Positive control involving the addition of the solvent (methanol) instead of the oil was carried out.

For antifungal activity Sabouraud dextrose agar was used instead of nutrient agar. The inoculated medium was incubated at 25°C for three days for the *A. niger* and two days for *C. albicans.*

3. Results

Table 1. Antimicrobial activity of the methanol oily solution of *E. camaldulensis* leaves

Tested bacteria and fungi used (Mean diameter of Inhibition Zone in mm)									
		B. subtilis	***S. aureus***	***E. coli***	***K. pnumoniae***	***Pr. vulgaris***	***Ps. aeruginosa***	***A.niger***	***C.albicans***
		17	22	20	17	17	18	16	20
		Mean diameter of Inhibition Zone in mm							
Drugs	**(µg/ml)**	***B.s***	***S.a***	***E.c***	***K.pn***	***Pr.v***	***Ps.a***		
Ampicillin	40	15	25	-	35	-	16		
	20	14	20	-	26	-	13		
	10	13	18	-	25	-	12		
	5	12	15	-	21	-	-		
Gentamicin	40	29	35	32	26	25	23		
	20	22	33	30	24	24	22		
	10	20	30	17	21	23	21		
	5	17	28	-	20	22	19		
	Mean diameter of Inhibition Zone in mm								
			A. n		***C. a***				
Clotrimazole	40		30		42				
	20		22		40				
	10		19		33				
	5		16		30				
Nystatin	50		28		17				
	25		26		14				
	12.5		23		-				

Concentration of oil dissolved in methanol (1:10) at 0.1ml/cup. ; Mean diameter of Inhibition, > 18 Sensitive. , 14 -18: Moderate, < 14: Resistant. Methanol as positive control (-).

Table 2. Minimum inhibitory concentrations of the essential oil *E. camaldulensis* leaves**.**

Microorganisms	**Concentration used(mg/ml)**			
	100	**50**	**25**	**12.5**
B.subtilis	17	16	15	14
S.aureus	22	19	18	17
E.coli	20	19	18	17
K.pnumoniae	17	16	15	14
Pr.vulgaris	17	16	15	14
Ps.aeruginosa	18	17	16	15
A.niger	16	15	14	13
C.albicans	20	18	17	15

Table 3. The activity of *E. camaldulensis* leaves oil against different clinical isolates

Microorganisms	Number of isolates	No. of clinical isolates		
		Sensitive	Moderate	Resistant
B. subtilis	10	8	2	-
S.aureus	10	-	9	1
E.coli	10	3	6	1
K.pnumoniae	10	1	8	1
Pr.vulgaris	10	5	5	-
Ps.aeruginosa	10	-	4	6

4. Discussions

The antimicrobial activity of the crude oil of *Eucalyptus camaldulensis* leaves family (Myrtaceae) was evaluated by the agar plate diffusion method against six standard bacteria. According to the result presented in Table 1, *E.camaldulensis* exhibited high activity (22mm) against *S.aureus*, (20mm) against *E.coli* and *C.albicans*. It also showed moderate activity (18mm) against *Ps.aeruginosa*, (17mm) against *B.subtilis*, *K.pneumoniae*, *Pr.vulgaris* and (16mm) against *A.niger* . This result showed that the oil of *E.camaldulensis* exhibited variable activity against the organisms tested and this was similar to the results of Akin et al. (2007) and Farouk et al. (2015) from Egypt. Unlike Babayi et al. (2004) found that methanol extract of *E.camaldulensis* inhibited the growth of *B.subtilis, S.aureus* but had no inhibitory effects on *Ps.aeruginosa*, *E.coli* and *C. albicans*. Also Mohammad and Naimch (2010) found that the methanol extract of *E.camaldulensis* inhibited the growth of *B.subtilis, S.aureus* but no inhibitory effect on *E.coli*. This means that the oil of *E.camaldulensis* has no effect on *E.coli.*

E.camaldulensis exhibited higher activity against *S.aureus* and *E.coli* than the other organisms tested. This result was different from that reported by Bachir and Benali (2008 and 2014), who found that the effects on the Gram negative bacteria *E.coli* was more than the Gram positive *S.aureus.* Similar to our result, Lima et al. (2013) found that the essential oil of *E.camaldulensis* is more effective against *S.aureus* than *E.coli.*

Akin *et* al. (2007) from Northern Cyprus and Panahi et al. (2011) from South West Iran found that *E.camaldulensis* showed different result that the oil was more active against *S.aureus* but no

inhibition of the growth of *E.coli* was seen. The oil showed high activity against *E.coli,* moderate activity against *Pr.vulgaris* and *Ps.aeruginosa*. On the contrary Mehani and Ladjet (2011) found that the essential oil of the plant *E.camaldulensis* have moderate activity against *Ps.aeruginosa.*

These results would indicate the potential usefulness of *E.coli* as microbiostatic, antiseptic and as disinfected agent.

The result of minimum inhibition concentrations from Table 2 showed that 12.5µg/ml was the lowest concentration to inhibit the growth of all organisms tested.

Comparison of observation given in Tables 1 and 4 showed that the leaves oil of *E. camaldulensis* dissolved in methanol inhibited *B. subtilis*, *Pr.vulgaris* and *Ps.aeruginosa* more than 40µg/ml Ampicillin. It also inhibited *Staphylococcus* more than 20µg/ml Ampicillin. It inhibited *E.coli* more than 10 µg/ml Gentamicin and inhibited *K.pneumoniae* less than 5µg/ml Ampicillin. The oil of *E.camaldulensis* inhibited *A.niger* similar to 5 µg/ml Clotrimazole and inhibited *C.albicans* more than 50 µg/ml Nystatin.

From Table 3, it was clearly seen that the leaves oil of *E.camaldulensis* showed high activity against the clinical isolates of *B.subtilis*, *Pr.vulgaris* moderate activity against *S. aureus, E.coli, K.pnumoniae* and low activity against *Ps.aeruginosa.*

5. Conclusions

The essential oil of *E. camaldulensis* showed various degree of inhibitory activity against the microorganisms tested. The obtained result may justify the use of the Sudanese *E. camaldulensis* as antimicrobial therapy in folkloric medicine in Sudan and the neighboring countries.

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