Original Research Article

Phytochemical Screening and Antimicrobial Activity of Leaves Extracts of *Eucalyptus Camaldulensis* and *Eucalyptus Microtheca*

ABSTRACT

Aims: The aim of this study is to investigate the phytochemical constituents and antimicrobial activity of the leaves extracts of *Eucalyptus camaldulensis* and *Eucalyptus microtheca.*

Study design: Extraction of *E. camaldulensis* and *E. microtheca* leaves using five solvents off different polarities, and scanning their phytochemical constituents and antimicrobial activity against five microorganisms.

Place and Duration of Study: Industrial chemistry and microbiology Departments, International University of Africa, Khartoum – Sudan, January 2017.

Methodology: The leaves of *E. camaldulensis* and *E. microtheca* were extracted using water, ethanol, chloroform, ethyl acetate and petroleum ether; the extracts were used for phytochemical screening. Five concentration (100, 50, 25, 12.5 and 6.25 mg/mL) of each extracts prepared and tested against five organisms, namely; *Staphylococcas aureus*, *Klebsiella pneumonia*, *Eschericchia coli*, *Pseudomonas aeruginosa* and *Candida albicans* (fungi). Muller Hilton agar was used for growth of microorganism strains. The inhibition activity was evaluated using cup plate method with slight modification.

Results: The results of the phytochemical screening revealed the presence of tannins, steroids, saponins, flavonoids and phenolic compounds in all tested extracts of both plants. In addition the leaf extracts from *E. camaldulensis* and *E. microtheca* were found to be effective against *S. aureus*, *P. aeruginosa*, *E. coli*, *K. pneumonia* and *C. albicans*. The aqueous and ethanolic extracts of both plants displayed highest antimicrobial activity compare to other extracts. These two extracts were significantly inhibited the growth of the five pathogenic microorganisms particularly at high concentrations (100 and 50 mg/mL); while there was no inhibition effect detected at low concentrations (25 and 12.5 mg/mL). The diameter of inhibition zones at concentrations of 100 and 50 mg/mL were found to be between 11 and 35 mm.

Conclusion: These results indicate that *E. species* might be exploited as natural antibiotic for the treatment of several infectious diseases caused by these five microorganisms.

Keywords: Eucalyptus camaldulensis, Eucalyptus microtheca, phytochemical screening, antimicrobial activity, inhibition zone

1. INTRODUCTION

Infectious diseases remain a serious public health problem; thus there is an increasing need for new compounds that can act as a direct antimicrobial or as an indirect effect increasing resistance mechanism of microorganisms. Some chemical substances or group of compounds that produce a definite physiological action in the human body can be obtained from medicinal plants. These chemical substances are called secondary metabolites. The most important of these bioactive groups are alkaloids, terpenoids, steroids, flavonoids, tannins and phenolic compounds [1]. Medicinal plants traditionally used against many infectious diseases, represent a high potential for discovering such compounds. *Eucalyptus* is native to Australia and Tasmania, and the genus *Eucalyptus* contains about 600 species. The Aborigines (native Australians) have traditionally used *Eucalyptus* leaves to heal wounds and fungal infections [2].The *Eucalyptus* tree is a large, fast-growing evergreen. The tree can grow to 375 - 480 feet (125 - 160 meters). *Eucalyptus* trees belong to the *myrtaceae* family. Their name originates from the Greek word "*Eucalyptol*" which means "well covered". *Eucalyptus* trees

thrive in environments that maintain average temperatures of about 60 °C [3]. Leave extracts of *Eucalyptus* have been approved as food additives [4]. Recently, attention has been focused on the medicinal properties of these extracts. Research data has demonstrated that the extracts exhibited various biological effects, such as antiseptics, against infections of the upper respiratory tract [5, 6], antibacterial, antihyperglycemic [7], insecticidal activities [8, 9], antioxidant and natural cytotoxic [10]. Essential oils of various species of *Eucalyptus* have been used in the pharmaceutical industry [11]. *Eucalyptus* oil has numerous traditional uses; for example it has been used as a traditional non-ingestive treatment for coughs and colds, a topically applied medication for relief of muscular pain and as a solvent/sealer in root canal dentistry. It has uses as a fragrance in soaps, detergents, perfumes and as a flavoring in food. Essence of *Eucalyptus* and its major component is widely used in manufacturing of softeners, pomades, antitussive syrups, toothpastes and also as flavor in many medicines. Also this component is used as fragrance in soaps, powders and other washing materials [12]. Phytochemicals screening of crude extracts of various parts of both *E. camaldulensis* and *E. microtheca* showed the presence of tannins, saponins, glycosides, steroids, anthraquinones, alkaloids and flavonoids [13, 14]. The leaf extracts of *E. camaldulensis* and *E. microtheca* have been reported to significantly inhibit growth of *P. aeruginosa, E. coli* and *S. aureus* [15, 16]. Herein, the phytochemical constituents and antimicrobial activity of the *Eucalyptus camaldulensis* and *Eucalyptus microtheca* leaf extracts was studied using *Staphylococcas aureus*, *Klebsiella pneumonia*, *Eschericchia coli*, *Pseudomonas aeruginosa* and *Candida albicans* (fungi) as a target microorganisms.

2. MATERIALS AND METHODS

2.1 Plant collection and preparation

The leaves of *E. camaldulensis* and *E. microtheca* were collected from the College of Forestry, Sudan University of Science and Technology, Khartoum, Sudan. The taxonomy identification of the plant was done by a Botanist of the same college. The collected leaves were washed, dried at room temperature, grinded to proper size and stored in polyethylene bags for further work.

2.1.1 Preparation of extracts

Dried grinded leaves of *E. camaldulensis* and *E. microtheca* (10 g) were accurately weighed into five different conical flasks. A 100 mL of distilled water, ethanol, petroleum ether, chloroform and ethyl acetate were added to each flask separately, with occasional shaking; then left for 72 hours. The crude extracts of the five flasks were filtered, the solvents were evaporated and then the extracts were kept for further analysis.

2.1.1.1 Preparation of graded concentration of the leaves extracts

A stock solutions of leaf extracts were prepared by dissolving one gram of the extract into 5 mL of the extraction solvent for proper dissolution (200 mg/mL), then a series of diluted solutions (100, 50, 25,12.5 and 6.25 mg/mL) were prepared.

2.2 Phytochemical screening

Qualitative phytochemical screening of both plants extracts were performed using the methods of Harborne (1984) [18], for identification of the following secondary metabolites: alkaloids with Mayer Wagner and Dragendoff's reagents; tannin and phenolic compounds with (0.1% $FeCl_3$); saponins with (foaming test); flavonoids using (10% $FeCl_3$), lead acetate, potassium hydroxide; triterpenes and sterols (Salkowski's test).

2.3 Antimicrobial activity

2.3.1 Microorganisms

The microorganisms used for the antimicrobial assay in this study were as follows: *S. aureus*, *K. pneumonia*, *E. coli*, *P. aeruginosa* and *C. albicans*. The organisms were obtained from Microbiology Laboratory, department of Microbiology, International University of Africa, Khartoum, Sudan.

2.3.1.1 Preparation of microbial suspensions

One mL aliquots of a 24 hours broth culture of the test organisms were aseptically distributed onto Mueller-Hinton agar and incubated at 37 °C for 24 hours. The microbial growth was harvested and washed off with 100 mL sterile normal saline, to produce a suspension containing about 108 -109 C.F.U./mL. The suspension was stored in a refrigerator at 4 °C till used.

2.3.2 Antimicrobial assay

The antimicrobial activity of the crude extracts was determined by means of the agar well diffusion method. One mL of the standardized bacterial stock suspension 108 - 109 C.F.U./mL were thoroughly mixed with 100 mL of sterile molten nutrient agar which was maintained at 45 °C. A 20 mL aliquots of the inoculated nutrient agar were distributed into sterile petri-dishes. The agar were left to dry and in each of these plates 4 cups (10 mm in diameter) were cut using a sterile cork borer (No. 4) and agar discs were removed. Alternate cups were filled with 0.1 mL sample of each extracts using automatic micro-pipette, and allowed to diffuse at room temperature for 2 hours. The plates were then incubated in the upright position.

3. RESULTS AND DISCUSSION

3.1 Phytochemical screening

Phytochemical screening results of the crude extracts of *E. camaldulensis* and *E. microtheca* leaves shown in Table1 below, confirmed the presence of phenolic compounds, flavonoids, steroids, tannins and saponins in the two plants. These results are disagreed with Mahmoud (2012) [18]; which performed phytochemical analysis of alcoholic and aqueous extracts of *E. microtheca;* his results indicated that only alcoholic extract of *E. microtheca* contained alkaloids. Also disagreed with Sani *et al.* (2014), where revealed the present of alkaloids in ethanolic extracts of *E. camaldulensis* leaves. While Chuku *et al*. (2016); were studied the methanol, ethanol and petroleum ether extracts of *E. camaldulensis*. Their results showed the presence of alkaloids in ethanol and petroleum extracts [16]. The variation on the phytochemical constitutes of the extracts of *E. camaldulensis* and *E. microtheca* under our study with the other studies perhaps due to climate condition and the environment where the plant growth.

Table1. Phytochemical screening of leaves extracts of *E. camaldulensis* and *E. microtheca*

Phytochemical	*E. camaldulensis*					*E. microtheca*				
	Water	Chloroform	Ethanol	Ethyl acetate	Petroleum ether	Water	Chloroform	Ethanol	Ethyl acetate	Petroleum ether
Phenols	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	+
Steroids	+	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+	+	+
Alkaloids	-	-	-	-	-	-	-	-	-	-

** Keys: (+): present, (−): absent.*

3.2 Antimicrobial activity

The antimicrobial activity of the extracts was quantitatively assessed by measuring the diameter of the IZ. The results of the antimicrobial activity of the crude extracts of *E. camaldulensis* and *E. microtheca* were presented in Table 2; only high concentrations (100 and 50 mg/mL) were showed inhibition

activity against tested microorganisms; while no antimicrobial activity observed using low concentrations (25, 12.5 and 6.25 mg/mL). The antimicrobial activity of the aqueous extracts of *E. microtheca* showed highest inhibition effects against all tested microorganisms particularly in high concentrations 100 and 50 mg/mL except in *K. pneumonia* where the inhibition was low at 50 mg/mL. At high concentrations (100 and 50 mg/mL), the aqueous extracts of *E. camaldulensis* exhibited the highest inhibition effect on all the pathogenic microorganisms; except in *P. aeruginosa*, *K. pneumonia* and *C. albicans* where a low inhibition activities were observed using 50 mg/L of the leafs extract. As shown on table 2, as the concentration increases the inhibition zone increases and vice versa. The ethanolic extract of *E. microtheca* leaves performed highest inhibition effects against *E. coli*, *P. aeruginosa*, *S. auruas*, *C. albicans* and *K. pneumonia* in high concentrations 100 and 50 mg/mL, except the 50 mg/mL *extract of K. pneumonia*, which showed no inhibition effects. For *E. camaldulensis* the ethanolic extracts showed highest inhibition effects against all tested microorganisms in 100 mg/mL, while it showed low effects against these microorganisms in 50 mg/mL of the extract. There were no significant differences in the activities of the ethanol and aqueous extracts at concentration of 100 mg/mL. Also a decreasing of the inhibition effect was noted as the ethanolic extracts concentrations decrease. Seyyed *et al.* (2014), the results of his study on the ethanolic extracts of *E. microtheca*; revealed that it had a potential application in infection control, especially against *E. coli* and *P. aeruginosa* [19]. Moreover, the ethyl acetate extracts of *E. microtheca* at concentration of 100 mg/mL inhibited the growth of all tested microorganisms. *E. coli* is only the affected organism by ethyl acetate extracts of *E. microtheca* at concentration of 50 mg/mL. On the other hand ethyl acetate extracts of *E. camaldulensis* at 100 mg/mL had low inhibition effect; while 50 mg/mL of the extract exhibited no inhibition effect against *P. aeruginosa.* Chloroform extract of *E. microtheca* displayed inhibition on *C. albicans, P. aeruginosa, E. coli* and *S. auruas* at concentration of 100 mg/mL while there was no any inhibition on *K. pneumonia*; for the concentration of 50 mg/mL only inhibition at *C. albicans* was detected. Chloroform extract of *E. camaldulensis* inhibited all microorganisms except *P. aeruginosa* at concentration of 100 mg/L; but at 50 mg/mL no inhibition activity was observed. Petroleum ether extracts of *E. microtheca* inhibited only *S. auruas* at concentration 100 mg/mL, and no inhibition effects for all tested microorganisms at concentration 50 mg/mL. Petroleum ether extract of *E. camaldulensis* inhibited only *S. auruas* and *C. albicans* at concentration of 100 mg/mL, but at concentration of 50 mg/mL, no inhibition effects for all microorganisms. Petroleum ether extracts revealed a less inhibition effect on the tested microorganisms compared to the other four extracts used in this study. According to what mentioned above, the antimicrobial activities of the extracts of both plants against five different microorganisms varied considerably depending on the kind of solvent and concentration of the extract used. The above mentioned phytochemical constitutes present in leaves extracts of *E. camaldulensis* and *E. microtheca* possibly the reason for the antimicrobial activity of the extracts on the tested microorganisms. The occurrence of tannins in *E. camaldulensis* and *E. microtheca* increases the industrial value of the plant, because tannins are used in a number of industries such as leather tanning, pharmaceutical and food industries [20, 21].

Table 2: Antimicrobial activity of leaves extracts of *E. camaldulensis* and *E. microtheca*

Solvent	Conc. mg/mL	*E. camaldulensis*					*E. microtheca*				
		Inhibition zone diameter in mm					Inhibition zone diameter in mm				
		E. coli	*P. aeruginosa*	*S. auruas*	*K. pneumonia*	*C. albicans*	*E. coli*	*P. aeruginosa*	*S. auruas*	*K. pneumonia*	*C. albicans*
Distilled water	100	35	30	34	18	35	33	35	35	22	35
	50	15	12	20	11	13	23	20	23	13	15
Ethanol	100	30	33	22	20	34	30	23	35	21	28
	50	12	15	12	12	13	16	15	20	0	17
Petroleum ether	100	13	13	18	14	16	10	0	15	0	11
	50	0	0	0	11	13	0	0	0	0	0
Ethyl acetate	100	15	15	12	14	15	20	16	22	12	17
	50	13	0	10	11	4	16	0	0	0	0
Chloroform	100	16	11	17	17	15	16	16	20	0	30

	50	0	0	0	10	11	0	0	11	0	15

4. CONCLUSION

Herein, Phytochemical screening of crude extracts of *E. camaldulensis* and *E. microtheca* leaves had confirmed the presence of phenolic compounds, flavonoids, sterols, tannins, saponins and terpenes. These compounds could be potential control of clinical pathogenic bacteria and fungi. The extracts of the plant proved to be active against *S. aureus*, *P. aeruginosa*, and *E. coli*, *Klebcella* and *C. albicans* at high concentrations. Ethanol and distilled water extracts showed highest inhibition at high concentrations; while ethyl acetate and chloroform extracts has moderate inhibition activity. Only petroleum ether extract was an active against some pathogenic micro-organisms even at high concentrations. These results indicate that the *E. microtheca* might be exploited as natural antibiotic for the treatment of several infectious diseases caused by these five germs.

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