Asian Journal of Scientific Research

ISSN 1992-1454

science alert
http://www.scialert.net

ANSInet
an open access publisher
http://ansinet.com

OPEN ACCESS Asian Journal of Scientific Research

ISSN 1992-1454
DOI: 10.3923/ajsr.2019.202.210

Research Article
Phytochemical Screening and Antibacterial Activity of *Eucalyptus camaldulensis*'s Leaves and Bark Extracts

Azzah Ibrahim Alghamdi and Ibtisam Mohammed Ababutain

Department of Biology, College of Science, Imam Abdulrahman Bin Faisal University, P.O. Box 1982-31441, Dammam, Saudi Arabia

Abstract

Background and Objective: Choosing the appropriate antibiotics (bacteriostatic or bactericidal) considered the most important aspect of treatment. This study aimed to determine the type of inhibitory activity of *Eucalyptus camaldulensis*'s leaves and bark extracts whether it is bacteriostatic or bactericidal. **Materials and Methods:** The antibacterial activity of *E. camaldulensis* leaves and bark extracts were assessed against four bacterial isolates (*Pseudomonas aeruginosa* ATCC27853, *Escherichia coli* ATCC25922, *Bacillus subtilis* and *Staphylococcus aureus* ATCC24213). Minimum Inhibitory Concentration (MIC) was estimated using a 2-fold dilution method and Minimum Bactericidal Concentration (MBC) was estimated using a pour plate method. The bactericidal effect was estimated by calculating the ratio between MIC and MBC. Qualitative phytochemical assays were performed using a standard method and GC-MS analysis. **Results:** The results showed that the *E. camaldulensis* extracts have inhibitory activity against all tested bacteria. The results also indicated that the MBC/MIC ratios of *E. camaldulensis* extracts were one to two-fold dilutions which are considered as a bactericidal effect. The result of qualitative phytochemical assays showed the presence of tannin, saponins, flavonoid, carbohydrate and protein compounds in all leaves and bark extracts. The results of the GC-MS analysis showed that *E. camaldulensis* extracts possess a variation in chemical constituents. Some of these compounds have an anti-microbial effect like Cyclononasiloxane, octadecamethyl-, 17-Pentatriacontene, 4H-Pyran-4-one, 3,5-dihydroxy-2-methyl- and Phytol. **Conclusion:** *E. camaldulensis* may be used as a natural antibiotic for its bactericidal effect to treat serious infections caused by pathogenic bacteria.

Key words: Antibacterial activity, bactericidal, bacteriostatic, *Eucalyptus camaldulensis*, natural antibiotic, pathogenic bacteria

Received: July 28, 2018 Accepted: October 16, 2018 Published: March 15, 2019

Citation: Azzah Ibrahim Alghamdi and Ibtisam Mohammed Ababutain, 2019. Phytochemical screening and antibacterial activity of *Eucalyptus camaldulensis's* leaves and bark extracts. Asian J. Sci. Res., 12: 202-210.

Corresponding Author: Ibtisam Mohammed Ababutain, Department of Biology, College of Science, Imam Abdulrahman Bin Faisal University, P.O. Box 1982-31441, Dammam, Saudi Arabia Tel: 00966569270768

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The *Eucalyptus* tree belongs to the Myrtaceae family and it is considered a large aromatic genus that contains about 900 species[1]. For a longtime, attention has been given to the medical characteristics of *Eucalyptus* species; it has been used to treat arthritis, asthma, burns, fever, influenza, sore throat, malaria, wounds and pharyngitis[2]. Also it was used in the soap and cosmetic industries[3]. It has been found that leaves extracts of *Eucalyptus* species have shown many properties and several studies have brought to light the antihyperglycemic, antioxidants, antibacterial activity, ulcer-healing, cytotoxic effect, anti-inflammatory and analgesic effects[4-10]. Some studies have also demonstrated the antimicrobials activity of *Eucalyptus* plants barks[11,12].

Eucalyptus camaldulensis is a fast-growing tree and evergreen that can tolerate drought for long periods and withstand high temperatures[13,14] up to 60°C. Therefore *E. camaldulensis* plants are distributed in eastern region of Saudi Arabia[15].

Drugs that are generated from plants are considered to be relatively safer than chemical drugs[16]. In this regard, traditional medicinal plants are widely used to sustain primary health care needs by more than 80% of the world's population WHO[17]. Moreover, the search of antibiotics (ATBs) is never ending and the study of active substances in plants could enable the modulation of new types of ATBs.

However, ATBs work in two different ways, either inhibiting the growth of the microbe (bacteriostatic) or killing the microbe (bactericidal). This is one of the most important aspects in choosing the appropriate antibiotics for treatment. Where, the bactericidal ATBs are used to treat patients of severe microbial infections and immuno-suppression. While the bacteriostatic ATBs need a healthy immune system to complete the process of eliminating the infection[18,19].

Although the inhibitory activity of the *E. camaldulensis* plant was investigated by few studied, the inhibitory activity was not determined to be bacteriostatic or bactericidal. Therefore this study was aimed to compare between the inhibitory activity for bark and leaves extracts of *E. camaldulensis* and determine the bactericidal or bacteriostatic effect for this plant. Also the phytochemical components of bark and leaves extracts for the *E. camaldulensis* were investigated using standard methods and GC-MS analysis.

MATERIALS AND METHODS

Preparation of plant extracts: The bark and leaves of *E. camaldulensis* plant were collected from a local park in the city of Dammam in Saudi Arabia in June 2015 and were morphologically identified[15]. Plant materials were washed under tap water to remove all dirt and then distributed over filter paper separately to dry at room temperature (20°C±2). The dry plant materials were grinded to powder using an electric mixer. Fifty grams of powdered bark and leaves were transferred individually into 500 mL flasks and 250 mL of different solvents included distilled water, methanol (80%) and ethanol (80%) were added separately to final concentrations of 20% (g mL^{-1}). Flasks were placed in a shaker at 350 rounds per minute (rpm/min) at 15°C for two days. Bacterial filters were used to filter the extracts and 100 mL of each filtered extract was dried using oven at 80°C. Dimethyl Sulfoxide (DMSO) was used to restore the extracts to the final concentration 20%. The crude extracts were kept at 4 °C until used.

Test micro-organisms: Four bacterial species two Gram-negative bacteria; *Pseudomonas aeruginosa* ATCC27853, *Escherichia coli* ATCC25922 and two Gram-positive bacteria; *Bacillus subtilis* and *Staphylococcus aureus* ATCC24213 were kindly provided by King Fahd Hospital, Al Khobar-Saudi Arabia.

Qualitative phytochemical analysis: The presence of biological compounds was tested using standard methods[20,21].

Gas chromatography-mass spectrometry: Gas Chromatography-mass spectrometer QP2010 SE (Shimadzu Made in USA) with 5 Sil MS 5% diphenyl/95% dimethyl polysiloxane capillary column (30 m, 0.25 mmID, 0.25-µm df) was used to identify the bioactive compound of *E. camaldulensis* (leaves and bark). Hundred micro-milliliters of each sample were diluted with 1400 µL of DMSO (100/1400, V/V). For GC-MS detection, electron impact ionization (EI) with ionization energy of 70 eV was used. Carrier gas was helium (99.999%) at a column flow 0.7 mL min^{-1} and injection mode was split. Flow control mode was linear velocity of 29.6 cm sec^{-1} and split ratio 1:10. Injector temperature was 250°C and ion-source temperature 250°C. The oven temperature was programmed from 50-300°C (hold time 3 min) and total run time was 29 min. Compounds were identified by National Institute of Standards and Technology (NIST 08) library match.

Antibacterial susceptibility assay: Seven discs of antibiotics were used Ciprofloxacin (CIP 5 mcg), Piperacillin (PRL 100 mcg), Novobiocin (NV 30 mcg), Ampicillin (AMP

10 mcg), Clindamycin (Cd 10 mcg), Streptomycin (S 10 mcg), Amoxicillin-Clavulanate (AMC 10 mcg). All antibiotic discs were purchased from Bioanalyse Company.

Screening for antibacterial activities: Antibacterial activity of plant extracts was estimated using agar well diffusion technique as described by National Committee for Clinical Laboratory Standards (NCCLS)[22]. Petri plates were inoculated by 1 mL of overnight bacterial broth using inoculum 1-2 $\times 10^8$ CFU mL^{-1} that equivalent to 0.5 McFarland standards and 15 mL of melted nutrient agar medium was poured over the inoculum and mixed well. After cultures were solidified three holes were made and filled with 50 µL of the plant extracts. The plates were then placed in the refrigerator for 1 h for proper diffusion of the plant extracts then plates were placed in incubator at 37°C for 24 h. DMSO was used as a negative control and erythromycin (E5 mcg) disc was used as a positive control. Free zones of bacterial growth appear around the holes were measured in millimeters using ruler. All experiments were conducted in three replicates.

Determination of Minimum Inhibitory Concentration (MIC): The Minimum Inhibitory Concentration (MIC) for plant extracts that showed antibacterial effects were evaluated using 2-fold dilution method[23]. Ninety six well microtiter plates were used and MIC value was calculated after incubation over night at 37°C by using microtiter plate reader at a wavelength of 600 nm. All experiments were conducted in three replicates.

Determination of Minimum Bactericidal Concentration (MBC): From MIC observation, the MBC was carried out. All concentrations of plant extracts which showed no turbidity were transferred in to plates and 12 mL of milted nutrient ager were poured[24]. The MBC was calculated after incubation for 48 h at 37°C. Results were recorded by using binocular microscope and plates with no bacterial colonies were recorded as MBC. All experiments were conducted in three replicates. The ratio between MIC and MBC was calculated.

Statistical analysis: One way ANOVA, SPSS Version[25] 17.0 was used to compare the antibacterial activities of plant extracts and significance was tested at $p<0.01$.

RESULTS

Plant yields: The results showed that the plant yield of leaves extract was higher than the bark extract with values of 40.2, 34.8 and 22.2 g for ethanol, methanol and water extract, respectively. For the bark extract the highest plant yield was with methanol (21.0 g) followed by ethanol (11.6 g) and the lowest was water extract with a value of 8.4 g (Table 1).

Standard methods: The current study showed the presence of saponins, tannins, flavonoids, carbohydrate and protein compounds in all leaves and bark extracts of *E. camaldulensis* (Table 2).

Gas Chromatography-Mass Spectrometry (GC-MS) analysis: GC-MS analysis of the ethanolic, methanolic and water extracts of *E. camaldulensis* leaves and bark had shown a variation in chemical constituents (Table 3). The results showed a total of 12, 10 and 11 compounds identified in the ethanolic, methanolic and water leaves extracts, respectively. The numbers of compounds in the bark extracts were 9, 6 and

Table 1: Plant yields of methanol, ethanol and water extracts of bark and leaves for *Eucalyptus camaldulensis*

Solvent Type	Plant part	Plant weight (g)	Plant extracts weight (g)	Plant yield * (g) (%)
Methanol	Bark	50	10.5	21.0
	Leaves		17.4	34.8
Ethanol	Bark		5.8	11.6
	Leaves		20.1	40.2
Water	Bark		4.2	8.4
	Leaves		11.1	22.2

*Plant extracts weight/Plant weight X100

Table 2: Qualitative phytochemical analysis of leaves and bark of *Eucalyptus camaldulensis* using methanol, ethanol and water as solvents

Solvent type	Plant part	Saponins	Tannins	Flavonoids	Carbohydrate	Protein
Methanol	Bark	++	+	++	++	++
	Leaves	+	+	+	++	+
Ethanol	Bark	+	++	++	++	+
	Leaves	+	++	++	+++	+++
Water	Bark	++	+	+	+++	++
	Leaves	++	+	+	+++	++

+: Exist, ++: Highly exist and +++: Very highly exist

Table 3: GC-MS analysis compounds name, peak area (%) and biological activities of *E. camaldulensis* leaves and bark extracts

	Peak area (%)						
Compound name	EL	ML	WL	EB	MB	WB	Biological activities
Cyclononasiloxane, octadecamethyl-	10.8	3.83	-	-	-	-	Anti- fungal[26]
4,5-Dichloro-1,3-dioxolan-2-one	8.43	10.77	14.09	-	-	-	No activity reported or found
Betulin	7.62	-	-	0.07	-	-	Antiviral and anti-tumour agents[27]
4H-1-Benzopyran-4-one 5,7 -dihydroxy-2-me	4.60	-	-	-	-	-	No activity reported or found
5-Hydroxymethylfurfural	3.45	8.79	6.64	0.44	2.77	0.05	Antiproliferative activity and Antioxidant[28]
Hexadecanoic acid, ethyl ester	3.17	1.53	-	-	-	-	Anti-oxidant, flavor, anti-androgenic, nematicide, hemolytic and hypocholesterolemic[29]
2-Hydroxymethyl-5-(1-hydroxy-1-isopropyl)-	-	7.34	7.22	-	-	-	No activity reported or found
1,2,3-Benzenetriol		6.37	7.36	-	2.01	-	Anti cancer agent[30]
2-Furancarboxaldehyde, 5-methyl-	0.52	-	5.31	0.19	-	-	No activity reported or found
17-Pentatriacontene	-	-	4.33	-	-	-	Antimicrobial activity[31]
D-Allose	-	-	4.26	-	-	-	Anti-oxidative activity[32]
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-	0.50	1.93	3.05	0.05	1.38	-	No activity reported was found
Propane, 3-chloro-1,1,1-trifluoro-	-	-	-	79.62	-	87.01	No activity reported was found
2-Benzimidazolyl methane thiosulfuric acid	-	-	-	6.0	-	-	No activity reported was found
Tungsten, ethyl tris (beta.3-2-propenyl)-	-	-	-	5.55	-	-	No activity reported was found
S-Methyl methanethiosulphonate	-	-	1.71	0.88	9.20	1.33	Anti-mutagenic agent[33, 34]
Catechol	0.15	0.50	2.28	0.10	4.79	0.58	Pesticides, flavors and fragrances[35]
4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-	0.15	-	1.51	-	-	-	Anti-microbial, anti-oxidant and anti-inflammatory[36]
Phytol	1.15	1.06	-	-	-	-	Anti-microbial, anti-cancer and anti-inflammatory[29]
n-Hexadecanoic acid	1.13	0.45	-	-	2.58	0.26	Antioxidant, nematicide, hypochloesterolemi, anti-androgenic and pesticide[30]

EL: Ethanol leaves extract, ML: Methanol leaves extract, WL: Water leaves extract, EB: Ethanol bark extract, MB: Methanol bark extract and WB: Water bark extract

Table 4: Anti-bacterial susceptibility assay, anti-biotic class, bacteriostatic or bactericidal effects and mode of action

				Zone of inhibition (mm)± Standard deviation			
				Gram-negative bacteria		Gram-positive bacteria	
ATBs discs	Antibiotic class	Bacteriostatic or bactericidal effects	Mode of action	*E. coli*	*P. aeruginosa*	*S. aureus*	*B. subtilis*
CIP	Fluoroquinolones	Bactericidal	Targeting DNA gyrase	28.5±0.5	29.0±0.2	24.0±0.4	29.5±0.5
PRL	Beta-lactams	Bactericidal	Inhibit cell wall synthesis	R	7.0±0.5	17±0.5	21.5±0.5
NV	Aminocoumarin	Bactericidal or bacteriostatic	Targeting DNA gyrase	R	8.5±0.5	27.0±0.8	25±0.5
AMP	Beta-lactams	Bactericidal	Inhibit cell wall synthesis	R	R	12.1±0.3	18±0.1
Cd	Lincosamides	Bacteriostatic	Targeting protein synthesis 50S	R	R	23±0.8	18±0.1
S	Aminoglycosides	Bactericidal	Targeting protein synthesis 30S	R	R	15.0±0.8	11±0.1
AMC	Beta-lactams	Bactericidal	Inhibit cell wall synthesis	9.5±0.5	17.0±0.5	R	R

R: Resistance, Ciprofloxacin (CIP 5 mcg), Piperacillin (PRL 100 mcg), Novobiocin (NV 30 mcg), Ampicillin (AMP 10 mcg), Clindamycin (Cd 10 mcg), Streptomycin (S 10 mcg), Amoxicillin-clavulanate (AMC 10 mcg)

5 in the ethanolic, methanolic and water, respectively. All compounds (%) were listed in Table 3.

Antibacterial susceptibility assay: The results showed that six out of seven tested ATBs discs inhibited the growth of Gram-positive bacteria, whereas Amoxicillin-Clavulanate (AMC 10 mcg) failed to inhibit the growth of *S. aureus* and *B. subtilis*. Gram-negative bacterium *P. aeruginosa* was inhibited by four out of seven ATBs discs, whereas *E. coli* was inhibited by only two out of seven ATBs discs (Table 4).

Screening for antibacterial activities: The results showed inhibitory effects of all *E. camaldulensis* extracts on the bacterial strains under study *E. coli*, *P. aeruginosa*, *S. aureus* and *B. subtilis* at varying inhibition zones from 1.7±0.2 to 6.7±0.3 mm (Table 5).

Determination of minimum inhibitory concentration and minimum bactericidal concentration (MIC and MBC): Overall results showed that the extract of *E. camaldulensis* leaves and bark had MIC values ranged between 0.391-25 μg mL^{-1} (Table 6). For bark extracts' MIC ranged between 3.125-25 μg mL^{-1}, while the leaves extracts values were ranged from 0.391-25 μg mL^{-1}. Moreover, the lowest MIC values of ethanol and methanol extracts were 0.391 μg mL^{-1} compared to the lowest MIC values of water was 12.5 μg mL^{-1}.

The values of MBC ranged between 0.781-50 μg mL^{-1}. Bark extracts' MBC ranged between 12.5-50 μg mL^{-1}

Table 5: Antibacterial activity of *Eucalyptus camaldulensis* leaves and bark extracts at concentration of 20% by using agar well diffusion assay

	Zone of inhibition (mm)± Standard deviation							
	Methanol extract		Ethanol extract		Water extract			
No*	Bark	Leaves	Bark	Leaves	Bark	Leaves	DMSO**	E5 mcg***
Gram-negative bacteria								
1	1.7±0.2	3.2±0.3	1.7±0.2	1.8±0.2	3.0±0.3	1.7±0.2	0	9.2±0.3
2	3.0±0.3	3.7±0.7	4.5±0.3	2.5±0.3	5.2±0.2	2.2±0.3	0	R
Gram-positive bacteria								
3	3.2±0.4	4.8±0.2	6.7±0.3	6.0±0.3	5.0±0.3	2.2±0.2	0	15.1±0.2
4	4.7±0.2	5.0±0.3	5.2±0.2	5.8±0.3	4.2±0.4	3.2±0.2	0	25±0.1
Significant $p \leq 0.01$	0.001	0.004	0.000	0.000	0.004	0.008	-	-

*1, *E. coli* ATCC25922. 2, *P. aeruginosa* ATCC27853. 3, *S. aureus* ATCC24213. 4, *B. Subtilis*, **: Dimethyl sulfoxide negative control, ***: Erythromycin (5 mcg) positive control, that target protein synthesis 50S inhibition and R: Resistant

Table 6: Minimal inhibitory concentration, minimal bactericidal concentration, bactericidal effect and MBC/MIC ratios for *Eucalyptus camaldulensis* leaves and bark extract

	Methanol extract				Ethanol extract				Water extract				
	Bark		Leaves		Bark		Leaves		Bark		Leaves		
No.	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	Bactericidal effect
Gram-negative bacteria													
1	25	50	12.5	50	12.5	25	12.5	50	12.5	25	25	50	Yes
MBC/MIC ratio	1		2		1		2		1		1		
2	25	50	6.25	25	6.25	25	6.25	25	12.5	25	12.5	25	Yes
MBC/MIC ratio	1		2		2		2		1		1		
Gram-positive bacteria													
3	3.125	12.5	3.125	12.5	6.25	25	0.391	0.781	25	50	12.5	25	Yes
MBC/MIC ratio	2		2		2		1		1		1		
4	12.5	25	0.391	1.56	6.25	12.5	0.391	0.781	12.5	25	12.5	25	Yes
MBC/MIC ratio	1		2		1		1		1		1		

No. 1: *E. coli* ATCC2592, 2: *P. aeruginosa* ATCC27853, 3: *S. aureus* ATCC24213 and 4: *B. subtilis*

compared to the leaves extracts' were 0.781-50 μg mL^{-1}. In addition, MBC values of ethanol, methanol extract and water were ranging between 0.781-50, 1.56-50 μg mL^{-1} and 25-50 μg mL^{-1}, respectively. The MBC/MIC ratios of *E. camaldulensis* leaves and bark extract was one to two fold dilutions (Table 6).

DISCUSSION

Plant yields result showed that the ethanol and methanol were the best solvents to obtain the highest plant yields this result is in agreement to another study that found ethanol was the best solvent to obtain the highest plant yields[37]. Standard methods result showed that *E. camaldulensis* leaves and bark extracts are rich in bioactive compounds, this result is in line with another study[38] indicated the presence of saponins, tannins, flavonoid and glycosides in extract of *E. camaldulensis*. Moreover this finding agreed with previous phytochemical studies[39,40] showed that *E. camaldulensis* leaves and bark are one of the richest plants in secondary metabolism compounds like essential oils, cineol, cuminal, phellandrene, aromadendral, valeraldehyde, geraniol, cymen, catechol, tannins, terpenes, isoprenoids, phenolics, cardiac glycosides, sterols, saponins and flavonoids.

Results of GC-MS analysis illustrated that the number of the compounds varied according to the solvent types and the difference in the plant part this result is in agreement with several researchers[41,42]. Most of these phytochemical compounds have medical, physiological and antimicrobial activities as previously reported by several investigators as listed in Table 3. However, some of these compounds have no data or report. Thus more investigation is required.

Antibacterial susceptibility assay result showed that Gram-positive bacteria are more sensitive to the antibiotics than Gram-negative bacteria because they lack an outer membrane[43]. Moreover the result indicated that *E. coli* bacterium was resistant to most of ATBs this result was consistent with a previous research[44]. Although most ATBs investigated in this study are similar in their mechanism of action, their inhibitory activity is uneven. The most effective ATB was Ciprofloxacin (CIP 5 mcg), that inhibited both Gram-positive (*S. aureus* and *B. subtilis*) and Gram-negative (*E. coli*

and *P. aeruginosa*). Most tested ATBs used in this study are bactericidal except clindamycin (Cd 10 mcg)[18,45]. Moreover, Novobiocin (NV 30 mcg) works as bactericidal or bacteriostatic depending on its concentration[19].

Screening for antibacterial activities results confirmed that *E. camaldulensis* extracts possesses a broad spectrum against both Gram-positive and Gram-negative bacteria. This may be attributed to the presence of several important secondary metabolism compounds like saponins, tannins and flavonoids. Which, were mentioned in several studies to possess antibacterial properties[46-51]. Overall, inhibitory activity of methanol leaves extract was higher than methanol bark extract this maybe due to the presence of Phytol and 4H-Pyran-4-one, 3,5-dihydroxy-2-methyl- which both have been reported as antimicrobial agents[29,36]. Vice versa, inhibitory activity of water bark extract was higher than water leaves extract despite the presence of 17-Pentatriacontene and 4H-Pyran-4-one, 3,5-dihydroxy-2-methyl- that both have recorded antimicrobial agents[31,36]. This may be attributed to the presence of Propane, 3-chloro-1,1,1-trifluoro- at high concentration (87.01%) and it is also present in high concentration in ethanol bark extract at 79.62%. However, there is no information on the biological activity of this compound. Inhibitory activity of ethanol bark and leaves extracts were high on both Gram-positive bacteria whereas ethanol bark extract was higher on *P. aeruginosa* than ethanol leaves extract. While, they were equal in their inhibitory activity on *E. coli* Table 5). The present study is consistent with other studies[12,14,38,39,52] that proved *E. camaldulensis* extracts possess the ability to inhibit the growth of Gram-positive and Gram-negative bacteria. Moreover, the ethanol extract in both bark and leaves had the highest values of 6.7±0.3 and 6.0±0.3 against *S. aureus*, respectively this is consistent with a prior study[42].

Many species belong to *Eucalyptus* genus exhibited antimicrobial properties. An early study reported that the leaves extracts of *E. viminalis, E. maculata* and *E. globulus* significantly inhibit six Gram-positive bacteria[7]. Moreover, *E. tereticornis* bark and leaves extract inhibit the growth of Gram-positive and Gram-negative bacteria[11].

In general, the current study observed that *E. camaldulensis* extracts inhibit the growth of Gram-negative bacteria, while this bacterial group was not affected by most of the ATBs under study. All the extracts affected the Gram-positive bacteria significantly while they were not affected by Amoxicillin- Clavulanate (AMC 10mcg). This maybe attributed to the presence of phytochemical compounds with antimicrobial properties[52]. Few studies reported the MIC and MBC activity of *E. camaldulensis*. The current study is in agreement with Abubakar[52] who found that water, ethanol and acetone leaves extracts of *E. camaldulensis* had MIC and MBC activity against *E. coli*, *P. mirabilis*, *K. pneumoniae*, *S. typhi* and *S. aureus*.

The current study indicated that the MBC/MIC ratio of *E. camaldulensis* leaves and bark extracts was one to two fold dilution which is considered as a bactericidal[53] (Table 6). There are no reports about the type of effect for *E. camaldulensis* extracts whether bacteriostatic or bactericidal. However, this result is consistent with previous studies that also determined the type of effect using the MBC/MIC ratio for some medicinal plants and found that this ratio does not exceed three to four fold dilutions[54-56].

Overall, the results of the present study showed that *E. camaldulensis* leaves and bark extracts were rich in phytochemical compounds with biological properties. These extracts were able to eliminate the growth of Gram-positive bacteria and Gram-negative bacteria tested in varying degrees, which exceeded the effect of some antibiotics tested. Also, the results showed that all *E. camaldulensis* extracts possessed bactericidal activity against the tested bacteria. Therefore, this plant extracts may be used as a source of antibiotics to treat specific bacterial infections. Moreover, several compounds in high concentration have no report or information mainly Propane, 3-chloro-1,1,1-trifluoro-. Thus further studies are required to investigate their biological properties, if any. This may provide novel compounds, leading to the development of new drugs.

CONCLUSION

This study showed that *E. camaldulensis* leaves and bark extracts have a broad inhibitory effect against both Gram-negative and Gram-positive. Inhibitory activity of this plant maybe attributed to the presence of active substances. Moreover, results demonstrated that the solvent type and plant part had a significant effect on the presence, concentration and inhibitory capacity of plant extract. Also, observed that the all *E. camaldulensis* extracts possessed bactericidal activity against the tested bacteria. Based on these results this plant may be used as natural antibiotic to treat some severe infectious diseases.

SIGNIFICANCE STATEMENT

This study demonstrated the bactericidal activity of leaves and bark extracts of *E. camaldulensis* against Gram-positive and Gram-negative bacteria, which may be beneficial for the manufacturing of effective new antibiotics. This is the first

report of bactericidal activity of alcoholic and aqueous leaves and bark extracts of *E. camaldulensis*. Also, this study demonstrates several compounds in high concentration have no report or information mainly Propane, 3-chloro-1,1,1-trifluoro. Thus further studies are required to investigate their biological properties, if any. This may provide novel compounds, leading to the development of new drugs.

ACKNOWLEDGMENT

The researchers would like to thank the research units at Al Rayyan campus-College of Science-Imam Abdulrahman Bin Faisal University for providing place and devices which were required for this experiment. We also would like to thank Dr. Ahmed Alsayyah, Dr. Reem AlJindan and Msr. Nouf Alromaihi from King Fahd Hospital, Al Khobar-Saudi Arabia, for providing us with the bacterial samples.

REFERENCES

1. Dhakad, A.K., V.V. Pandey, S. Beg, J.M. Rawat and A. Singh, 2018. Biological, medicinal and toxicological significance of Eucalyptus leaf essential oil: A review. J. Sci. Food Agric., 98: 833-848.
2. Elliot, R.W. and D.L. Jones, 1986. Encyclopaedia of Australian Plants Suitable for Cultivation. Vol. 4, Lothian Press, Melbourne.
3. Bajaj, Y.P.S., 1995. Medicinal and Aromatic Plants. In: Biotechnology in Agriculture and Forestry, Bajaj, Y.P.S. (Ed.)., Vol. 8, Springer, Berlin, Heidelberg, New York, pp: 194-196.
4. Gray, A.M. and P.R. Flatt, 1998. Antihyperglycemic actions of *Eucalyptus globules* (Eucalyptus) are associated with pancreatic and extra-pancreatic effects in mice. J. Nutr., 128: 2319-2323.
5. Lee, K.G. and T. Shibamoto, 2001. Inhibition of malonaldehyde formation from blood plasma oxidation by aroma extracts and aroma components isolated from clove and eucalyptus. Food Chem. Toxicol., 39: 1199-1204.
6. Bachir, R.G. and M. Benali, 2012. Antibacterial activity of the essential oils from the leaves of *Eucalyptus globulus*against *Escherichia coli*and *Staphylococcus aureus*. Asian Pac. J. Trop. Biomed., 2: 739-742.
7. Takahashi, T., R. Kokubo and M. Sakaino, 2004. Antimicrobial activities of eucalyptus leaf extracts and flavonoids from *Eucalyptus maculate*. Lett. Applied Microbiol., 39: 60-64.
8. Adeniyi, C.B.A., T.O. Lawal and G.B. Mahady, 2009. *In vitro* susceptibility of *Helicobacter pylori*to extracts of *Eucalyptus camaldulensis* and *Eucalyptus torelliana*. Pharm. Biol., 47: 99-102.
9. Islam, F., J.A. Khanam, M. Khatun, N. Zuberi and L. Khatun *et al*., 2015. A *p* Menth 1 ene 4,7 diol (EC 1) from *Eucalyptus camaldulensis* Dhnh. Triggers apoptosis and cell cycle changes in ehrlich ascites carcinoma cells. Phytother. Res., 29: 573-581.
10. Al-Snafi, A.E., 2017. The pharmacological and therapeutic importance of *Eucalyptus* species grown in Iraq. IOSR J. Pharm., 7: 72-91.
11. Jain, P. S. Nimbrana and G. Kaila, 2010. Antimicrobial activity and phytochemical analysis of *Eucalyptus tereticornis* bark and leaf methanolic extracts. Int. J. Pharmaceutical Sci. Review Res., 4: 126-128.
12. Pandey, B. and S. Singh, 2014. Evaluation of antimicrobial potential of *Eucalyptus camaldulensis* L. J. Pharm. Chem. Biol. Sci., 2: 166-171.
13. Leicach, S.R., A.M. Garau, A.B. Guarnaschelli, M.A. Yaber Grass, N.D. Sztarker and A. Dato, 2010. Changes in *Eucalyptus camaldulensis* essential oil composition as response to drought preconditioning. J. Plant Interact., 5: 205-210.
14. Traore, N., S. Bouare, L. Sidibe, A.A. Somboro and B. Fofana *et al*., 2014. Antimicrobial activity of essential oils of *Eucalyptus camaldulensis* from Mali. Asian J. Plant Sci. Res., 4: 69-73.
15. Anonymous, 2014. Manual of Arriyadh Plants. 2014. High Commsion for the Development of Arriyadh. 1st Edn., King Fahd Natnional Library Cataloging-in-P, Saudi Arabia.
16. Abd EL-Tawab, A.A., A.M.A. Ammar, A.M.A. Hamouda, F.I.A. El-Hofy and A.A.A. Elgamal, 2017. Synergistic antimicrobial activity of black pepper extract with some antibiotics combination on *Escherichia coli* isolated from chickens. Benha Vet. Med. J., 32: 1-6.
17. WHO., 2005. World Health Organization WHO 2002-2005 traditional medicine strategy. World Health Organization, Rome, pp: 1-11.
18. Etebu, E. and I. Arikekpar, 2016. Antibiotics: Classification and mechanisms of action with emphasis on molecular perspectives. Int. J. Applied Microbiol. Biotechnol. Res., 4: 90-101.
19. Davies, J and D. Davies, 2010. Origins and evolution of antibiotic resistance. Microbiol. Mol. Biol. Rev., 74: 417-433.
20. Edeoga, H.O., D.E. Okwu and B.O. Mbaebie, 2005. Phytochemical constituents of some Nigerian medicinal plants. Afr. J. Biotechnol., 4: 685-688.
21. Sofowora, A., 1993. Medicinal Plants and Traditional Medicine in Africa. 2nd Edn., Spectrum Books Ltd., Ibadan, Nigeria, ISBN-13: 9782462195, Pages: 289.
22. NCCLS., 1993. Performance Standards for Antimicrobial Disk Susceptibility Tests: Approved Standard. CCLS Document M2-A5. Vol. 13, NCCLS., Wayne, Pennsylvania, USA.
23. Omura, S., D. van der Pyl, J. Inokoshi, Y. Takahashi and H. Takeshima, 1993. Pepticinnamins, new farnesyl-protein transferase inhibitors produced by an actinomycete. J. Antibiot., 46: 222-228.

24. NCCLS., 1997. Performance standards for antimicrobial disk susceptibility tests; approved standard-sixth edition. NCCLS Document M2-A6, Vol. 17, No. 1, National Committee for Clinical Laboratory Standards, Wayne, PA., USA., January 1997.
25. SPSS., 2007. SPSS for Windows, Base System User's Guide, Release 17.0. University of Sussex, USA., Page: 224.
26. Suriani, N.L., 2016. Identification of the substance bioactive leaf extract *Piper caninum* potential as botanical pesticides. Int. J. Pure Applied Biosci., 4: 26-32.
27. Tolstikov, G.A., O.B. Flekhter, E.E. Shultz, Baltin and A.G. Tolstikov, 2005. Betulin and its derivatives. Chem. Biol. Active., 13: 1-29.
28. Zhao, L., J. Chen, J. Su, L. Li and S. Hu *et al*., 2013. *In vitro* antioxidant and antiproliferative activities of 5-hydroxymethylfurfural. J. Agric. Food Chem., 61: 10604-10611.
29. Tyagi, T. and M. Agarwal, 2017. Phytochemical screening and GC-MS analysis of bioactive constituents in the ethanolic extract of *Pistia stratiotes* L. and *Eichhornia crassipes* (Mart.) solms. J. Pharmacogn. Phytochem., 6: 195-206.
30. Chandran, U., N. Mehendale, S. Patil, R. Chaguturu and B. Patwardhan, 2017. Chapter 5: Network Pharmacology. In: Innovative Approaches in Drug Discovery, Patwardhan, B. and R. Chaguturu (Eds.)., Academic Press, Boston, pp: 127-164.
31. Ingole, S.N., 2017. Phytochemical analysis of *Ficus religiosa* L. (Moraceae) by GC-MS method. Inter. Res. J. N. Applied Sci., 4: 55-63.
32. Ishihara, Y., K. Katayama, M. Sakabe, M. Kitamura, M. Aizawa, M. Takara and K. Itoh, 2011. Antioxidant properties of rare sugar D-allose: Effects on mitochondrial reactive oxygen species production in Neuro2A cells. J. Biosci. Bioeng., 112: 638-642.
33. Nakamura, Y.K., T. Matsuo, K. Shimoi and I. Tomita, 1996. S-methyl methane thiosulfonate, bio-antimutagen, desmutagen, cauliflower (*Brassica oleracea* L. var. botrytis). Biol. Pharm. Bull., 16: 207-209.
34. Sugie, S., K. Okamoto, M. Ohnishi, H. Makita and T. Kawamori *et al*., 1997. Suppressive effects of S methyl methanethiosulfonate on promotion stage of diethylnitrosamine initiated and phenobarbital promoted hepatocarcinogenesis model. Jpn. J. Cancer Res., 88: 5-11.
35. Fiegel, H., H.W. Voges, T. Hamamoto, S. Umemura and T. Iwata *et al*., 2002. Phenol Derivatives in Ullmann's Encyclopedia of Industrial Chemistry. Wiley-VCH., Weinheim, Germany.
36. Jananie, R.K., V. Priya and K. Vijayalakshmi, 2011. Determination of bioactive components of *Cynodon dactylon* by GC-MS analysis. NY Sci. J., 4: 16-20.
37. Do, Q.D., A.E. Angkawijaya, P.L. Tran-Nguyen, L.H. Huynh, F.E. Soetaredjo, S. Ismadji and Y.H. Ju, 2014. Effect of extraction solvent on total phenol content, total flavonoid content and antioxidant activity of *Limnophila aromatica*. J. Food Drug Anal., 22: 296-302.
38. Ibrahim, I.S., M. Ali and A.U. Zage, 2016. Phytochemistry of methanolic and aqueous extracts of *Eucalyptus camaldulensis* leaves, seeds and stem back. Int. J. Adv. Acad. Res., 2: 75-80.
39. Ayepola, O.O. and B.A. Adeniyi, 2008. The antibacterial activity of leaf extracts of *Eucalyptus camaldulensis*(Myrtaceae). J. Applied Sci. Res., 4: 1410-1413.
40. Babayi, H., I. Kolo, J.I. Okogun and U.J.J. Ijah, 2004. The antmicrobial activities of methanolic extracts of *Eucalyptus camadulensis* and *Terminalia catappa* against some pathogenic icroorganisms. Biokemistri, 16: 106-111.
41. Ababutain, I.M., 2015. Impact of solvent type on antibacterial activities of *Lawsonia inermis* leaves. J. Food Agric. Environ., 13: 51-53.
42. Dailey, A. and Q.V. Vuong, 2015. Effect of extraction solvents on recovery of bioactive compounds and antioxidant properties from macadamia (*Macadamia tetraphylla*) skin waste. Cogent Food Agric., Vol. 1. 10.1080/23311932. 2015.1115646
43. Nikaido, H., 1996. Multidrug efflux pumps of gram-negative bacteria. J. Bacteriol., 178: 5853-5859.
44. Arredondo-Garcia, J.L. and C.F. Amabile-Cuevas, 2008. High resistance prevalence towards ampicillin, co-trimoxazole and ciprofloxacin, among uropathogenic *Escherichia coli* isolates in Mexico city. J. Infect. Dev. Count., 2: 350-353.
45. Kahn, C.M., 2005. The Merck Veterinary Manual. 9th Edn., Merck and Co. Inc., USA., pp: 2095.
46. Dzoyem, J.P., H. Hamamoto, B. Ngameni, B.T. Ngadjui and K. Sekimizu, 2013. Antimicrobial action mechanism of flavonoids from *Dorstenia* species. Drug Discov. Ther., 7: 66-72.
47. Lorent, J.H., J. Quetin-Leclercq and M. Mingeot-Leclercq, 2014. The amphiphilic nature of saponins and their effects on artificial and biological membranes and potential consequences for red blood and cancer cells. Org. Biomol. Chem., 12: 8803-8822.
48. Noudeh, G.D., F. Sharififar, M. Khatib, E. Behravan and M.A. Afzadi, 2010. Study of aqueous extract of three medicinal plants on cell membrane-permeabilizing and their surface properties. Afr. J. Biotechnol., 9: 110-116.
49. Maisak, H., S. Jantrakajorn, M. Lukkana and J. Wongtavatchai, 2013. Antibacterial activity of tannin from sweet chestnut wood against *Aeromonas* and *Streptococcal* pathogens of Tilapia (*Oreochromis niloticus*). Thai J. Vet. Med., 43: 105-111.
50. Doss, A., H.M. Mubarack and R. Dhanabalan, 2009. Antibacterial activity of tannins from the leaves of *Solanum trilobatum* Linn. Indian J. Sci. Technol., 2: 41-43.
51. Akiyama, H., K. Fujii, O. Yamasaki, T. Oono and K. Iwatsuki, 2001. Antibacterial action of several tannins against *Staphylococcus aureus*. J. Antimicrob. Chemother., 48: 487-491.

52. Abubakar, E.M.M., 2010. Antibacterial potential of crude leaf extracts of *Eucalyptus camaldulensis* against some pathogenic bacteria. Afr. J. Plant Sci., 4: 202-209.
53. Levison, M.E. and J.H. Levison, 2009. Pharmacokinetics and pharmacodynamics of antibacterial agents. Infect. Dis. Clin., 23: 791-815.
54. Ababutain, I.M., 2017. Anti-probiotic effect of sweet basil (*Ocimum basilicum* L.) leaf extract. Asia Life Sci., 26: 219-228.
55. Ababutain, I.M. and A.I. Alghamdi, 2018. Phytochemical analysis and antibacterial activity of *Vitex agnus-castus* L. leaf extracts against clinical isolates. Asia Life Sci., 27: 11-20.
56. Mbengui, R.D., N.K. Guessennd, G.M. M'boh, J.K. Golly and C.O. Okou *et al*., 2013. Phytochemical screening and study of comparative antibacterial activity of aqueous and alcoholic extracts of the leaves and barks of *Terminalia catappa* on multiresistant strains. J. Applied Biosci., 66: 5040-5048.