Int J Enteric Pathog. 2014 May; 2(2): e16515. DOI: 10.17795/ijep16515

Published online 2014 May 10. **Research Article**

Antibacterial Effect of *Eucalyptus microtheca*

Seyyed Mansour Seyyednejad [1,*]; Hossein Motamedi [1]; Fariba Dehghani Najvani [1]; Zahra Hassannejad [1]

[1]Department of Biology, Faculty of Science, Shahid Chamran University, Ahvaz, IR Iran

*Corresponding author: Seyyed Mansour Seyyednejad, Department of Biology, Faculty of Science, Shahid Chamran University, Daneshgah Sq., Golestan Blvd., Ahvaz, Khouzestan, Iran. Tel: +98-6113331045; +98-9163130836, Fax: +98-6113331045, E-mail: sm.seyyednejad@gmail.com

Received: November 30, 2013; **Revised:** February 9, 2014; **Accepted:** February 17, 2014

Background: Medicinal plants have now attracted more attention due to their antibacterial activity and also increasing antibiotic resistance among bacteria. Native plants of each region are potential resources for this purpose.

Objectives: The aim of the present study was to detect the antibacterial effect of *Eucalyptus microtheca* (Myrtaceae family) which is currently used as an antibacterial fumigation medicine.

Materials and Methods: Using standard disk diffusion method, the antibacterial activity, MIC, and MBC indexes of alcoholic extracts from this plant were tested on some pathogenic bacteria. The structural changes following the exposure to these extracts were also investigated in test bacteria.

Results: Significant antibacterial activity was found against gram-positive and gram-negative bacteria, which among them, *Escherichia. coli* and *Pseudomonas. aeruginosa* showed the most sensitivity and *Staphylococcus. aureus* the least. The value of MIC and MBC for both extracts was 8 mg/mL for *E. coli,* while they were 8 mg/mL and 16 mg/mL for *Bacillus cereus*, respectively. Both MIC and MBC values of methanolic and ethanolic extracts against *P. aeruginosa* were 8 and 16 mg/mL respectively. SEM revealed structural changes in the affected bacteria that suggest the cell wall was the main target site of active constituents.

Conclusions: It can be concluded that this plant has potential application in infection control, especially against *E. coli* and *P. aeruginosa* and regarding their recent reported epidemic, this plant can be a good choice for antibiotic discovery.

Keywords:Eucalyptus; Plants, Medicinal; Anti-Bacterial Agents

1. Background

Since the ancient time aromatic plants have been used for their pharmaceutical properties (1-3). Many active constituents exist in these plants that show antibacterial, antifungal, antiviral and antioxidant effects (4). The history of medicinal plants application for treatment of infectious disease dates back to ancient times, and people all over the world have experienced this tradition (5).

Humans are always vulnerable to infectious disease caused by bacteria, fungi and viruses, thus finding effective and alternative treatments are invariably necessary (6). One of the suitable choices for this purpose is medicinal aromatic plants like Eucalyptus, which is an important source of chemical substances with potential therapeutic effects (1).

Eucalyptus is a diverse genus of evergreen aromatic flowering trees in Myrtaceae family, which contains over 600 species (1, 7). *Eucalyptus* species is famous for its rapid growth; some members of this species attain gigantic sizes and are among the tallest trees in the world with 20 to 50 m high (7). It is indigenous in Australia, Tasmania, New Guinea and its neighboring islands (7). The members of this species are cultivated, particularly in sub-tropical and warm temperate climates (1, 7).

Anticancer, anti-inflammatory, antioxidant, antifungal and antiviral effects have been attributed to the leaf extracts of this plant (4). In Some studies, it has been reported that phytochemicals such as essential oils, sterols, alkaloids, glycosides, flavonoids, tannins and phenols are effective substances present in *Eucalyptus* (8, 9). In recent years with regard to the emergence of multidrug-resistant pathogenic bacteria, searching new antibacterial substances from natural sources such as plants has gained more attention (10). This problem is of great importance, especially in developing countries because infectious diseases can be life threatening (11).

Khuzestan is a tropical region in south-west of Iran that due to its border with Iraq, hot climate and persistence of infectious agents, especially enteric pathogens, the incidence of infectious diseases is relatively higher than other regions. Therefore, the need for effective treatment

Implication for health policy/practice/research/medical education:
Antibacterial activity of ethanolic and methanolic extracts of *Eucalyptus microtheca* was studied based on disk diffusion method, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) indexes. The structural changes of the affected bacteria were also investigated using scanning electron microscopy (SEM) to find the main target of active constituents of the extracts. The antibacterial property of this plant has not been previously studied in Khuzestan, Iran. These data can be used for discovering new antibacterial agents against resistant bacteria.

of such infections is obvious, and due to the frequent use of antibiotics, emergence of drug-resistant strains is inevitable. In this regard, finding medicinal plants that can be cultivated in this climate and simultaneously have considerable antibacterial properties is important.

2. Objectives

The present study has been focused on determining and comparing the antibacterial activity of methanolic and ethanolic extracts of *Eucalyptus microtheca*, the major *Eucalyptus* species cultivated in south of Iran, Khuzestan. These extracts can be used alone or in combination with other antibacterial compounds.

3. Materials and Methods

3.1. Plant Collection and Identification

Plant samples were collected from a farmland in Ahvaz, Khuzestan province. The plant identification was done by the services provided by the herbarium of the department of Biology, Faculty of Science, Shahid Chamran University, Ahvaz, IR Iran.

3.2. Plant Extract Preparation

Plant leaves were dried in the shade at room temperature for ten days and then powdered using electronic blender. Ethanolic and methanolic extracts were prepared from mixing 1 g of finely powdered plant with 10 mL of 80% ethanol or methanol (ethanol- or methanol-distilled water, 8:2 w/v). Then these samples were vortexed for one minute and then centrifuged (3000 rpm) for 15 minutes. Finally, the supernatant was harvested. This process was repeated three times, and then the solvents were evaporated (5, 11).

3.3. Bacterial Strains

The target cultures used in this study were prepared from the bacterial culture collection of Biology Department; Shahid Chamran University and consisted of *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia,* and *Proteus mirabilis*. They were originally isolated from clinical specimens.

3.4. Antibacterial Assessment

Antibacterial activity of the ethanolic and metanolic extracts was investigated using paper disk diffusion method (kirby-bauer) against test bacteria. All tests were repeated 3 times. Stock culture of test bacteria were grown in nutrient broth medium at 37˚C for 22 hours. Final cell concentration were adjusted to 0.5 McFarland turbidity which is approximately equal to 105 CFU/mL and then a lawn culture was prepared on Muller-Hinton agar (MHA, Merck) using sterile cotton swab (11).

Four extracts with different concentrations (100, 200, 400 and 600 mg/mL) were prepared. The sterile filter paper disks (6 mm diameter) were saturated through adding 40 µL from each extract so that the final concentrations of effective dose in these disks were 4, 8, 16 and 24 mg (5).

The prepared disks were placed on lawn cultures of the bacteria. The plates were left at room temperature for one hour to allow the diffusion of extract into the medium, and then were incubated at 37˚C for 24 hours.

The inhibition zone diameter around each disk was measured (mm). Standard antibiotic disks were also used besides these prepared disks. In order to determine the possible inhibitory effect of solvents on test bacteria, disks containing 80% ethanol and methanol were used simultaneously (12).

3.5. Investigating of Minimum Inhibitory Concentration

To determine the minimum inhibitory concentration (MIC) against most sensitive bacterial species, a twofold serial dilution of each extract (0.5, 1.0, 2.0, 4.0, 8.0, 16.0 and 32 mg/mL) was prepared in tubes containing 1 mL Muller Hinton broth and 30 µL of bacterial suspension with 0.5 McFarland turbidity. These tubes were incubated at 37˚C for 24 hours. The least concentration of crude extract in the mentioned serial dilution that had inhibited the growth of the test microorganism was considered as MIC (5, 11-13).

3.6. Minimum Bactericidal Concentration Determination

To determine the minimum bactericidal concentration (MBC), a loopful of broth from those tubes which did not exhibit any visible growth in the MIC assay was cultured on freshly prepared sterile Muller-Hinton agar and then incubated at 37˚C for 18-24 hours. After incubation, the highest dilution (least concentration) that inhibited colony formation on this solid medium was considered as MBC (11).

3.7. SEM Analysis

For finding the structural changes induced as a result of bacterial exposure to extracts, a sample from those species inhibited by the extract were prepared for scanning electron microscopy (SEM). These samples were coated by carbon and studied by SEM of central laboratory of Shahid Chamran University.

4. Results

Table1 presents the results of antibacterial activity for ethanolic and methanolic extracts of *E. microtheca*. These results show that different concentrations of ethanolic

and methanolic extracts of this plant have high antibacterial activity against gram-positive (*B. cereus* and *S. aureus*) and gram-negative (*P. mirabilis*, *E. coli* and *P. aeruginosa*) bacteria, but they didn't have any activity against *K. pneumoniae* and *S. typhi* even at their highest concentration.

The maximum effect was observed against *E. coli* for both ethanolic and methanolic extracts and *P. aeruginosa* for methanolic extract while the least effect was seen against S. *aureus* in the case of ethanolic extract even at 24 mg effective dose. There was no significant difference between antibacterial activities of ethanolic and methanolic on tested microorganisms except in the case of *P. aeruginosa* that methanolic extract was more effective than the ethanolic extract.

Generally, the antibacterial effects of both extracts were developed along with increasing their concentration, but this increase was not proportional. The results of antibacterial activity for standard antibiotics are presented in Table 1. As we can see from these results, all the tested bacteria were resistant to nafcillin. *E. coli* was sensitive to novobiocin and doxycycline and *P. aeruginosa* to novabiocin and colistin, but they were resistant to the other tested antibiotics. However, they were sensitive to alcoholic extracts and this result is noticeable. Disks containing 80% ethanol and methanol did not have any zone of inhibition; Therefore they could not interfere with the test results. MIC and MBC values of ethanolic and methanolic extracts against *E. coli* and *B. cereus* were shown in Table 2. MIC and MBC values of both extracts for *E. coli* were the same (8 mg/mL), while the MBC of *B. cereus* was twofold greater than MIC (MIC = 8 mg/mL, MBC = 16 mg/mL). MIC and MBC values of methanolic extract against *P. aeruginosa* were equal (8 mg/mL) and for the ethanolic extract was 16 mg/mL.

In SEM analysis as it can be found in Figure 1 that the rod-shaped bacteria have been deformed. The length of bacteria has been shortened, and they have changed to some extent, into coccus, ovoid or irregular shapes. These structural changes can lead to loss of cell wall integrity and consequently its lysis.

Table 1. Inhibition Zone (mm) of Alcoholic Extracts From *Eucalyptus microtheca* at Various Concentrations and Standard Antibiotics on Tested Bacteria [a]

	Amount of Extract, mg								Antibiotic Disks				
	Ethanolic				Methanolic								
	4	8	16	24	4	8	16	24	NF, 1 mcg	CB, 100 mcg	NB, 30 mcg	DX, 30 mcg	CL, 10 mcg
S. aureus	12	15	16	18	14	15	15	19	R	13	30	15	R
B. cereus	13	15	16	22	11	14	16	22	R	7	18	18	R
S. typhi	R	R	R	R	R	R	R	R	R	23	30	26	R
E. coli	16	17	22	23	16	17	22	23	R	R	17	11	R
P. aeruginosa	15	17	18	19	15	17	20	23	R	R	12	R	15
K. pneumoniae	R	R	R	R	R	R	R	R	R	R	15	R	11
P. mirabilis	15	16	18	19	15	17	18	19	R	15	17	R	R

[a] Abbreviations: CB, carbenicillin; CL, colistin; DX, doxycycline; NB, novobiocin; NF, nafcillin; R, resistant.

Table 2. MIC and MBC Values Against Most Sensitive Bacterial Species [a]

Bacterial Spp.	Ethanolic Extract, mg/mL		Methanolic Extract, mg/mL	
	MIC	MBC	MIC	MBC
E. coli	8	8	8	8
B. cereus	8	16	8	16
P. aeruginosa	16	16	8	8

[a] Abbreviations: MBC, minimum bactericidal concentration; MIC, minimum inhibitory concentration.

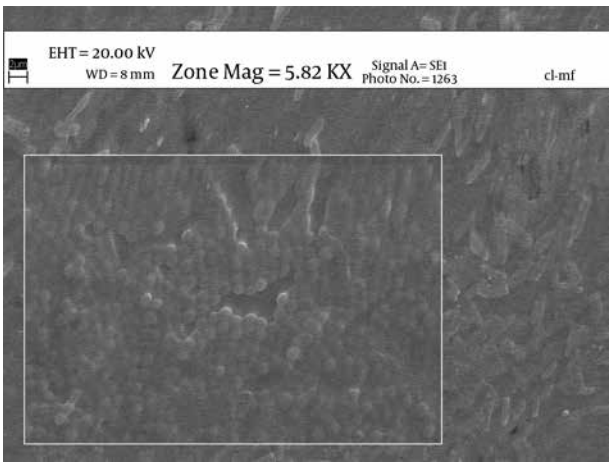

Figure 1. SEM analysis of a sensitive bacterium to alcoholic extract of Eucalyptus microtheca.

5. Discussion

Medical plants have been always a significant source for treatment of infectious disease and with respect to the ecophysiological differences between plants that have been grown in different geographical regions, continued and widespread studies are necessary to find pharmaceutical effects of these plants (1). Appearance of newly resistant bacteria highlights investigating antimicrobial agents more (14). The antibacterial activity of the leaf extracts of *E. microtheca* was studied in our research, and results showed noticeable antibacterial activity.

These results can be attributed to phytochemical compounds of this plant that some of these chemicals were reported by Ayepola and Adeniyi, which include saponins, saponin glycosides, steroid, cardiac glycosides, tannins, volatile oils, phenols and balsam. In some other reports polyphenolic compounds and essential oils of the plant were considered responsible for anti-microbial activity (4, 8, 9, 15, 16).

The major component in essential oil of *Eucalyptus* species is 1, 8-cineoled (4). Cineole is a monoterpenoid cyclic ether that can affect cytoplasmic membrane of targeted bacteria (17). This chemical is in ethanolic fraction and as a result the prepared extract in this study contains this potent antibacterial compound (18).

According to the results, leaf extracts of native *E. microtheca* in Khuzestan have significant antibacterial activity against both gram-positive and gram-negative pathogenic bacteria. All the tested bacteria were sensitive to ethanolic and methanolic extracts except *S. typhi* and *K. pneumoniae*. The maximum effect was observed on *E. coli* by both ethanolic and methanolic extracts and *P. aeruginosa* by methanolic extract, while the lowest effect was seen on *S. aureus*.

The antibacterial effects were increased in higher concentrations, but this increase was not significant. The inhibitory effect against *S. aureus* is noticeable because resistant strains of this species appear each year, and their treatment can be a major problem in near future, especially in case of hospital-acquired infections that are resistant to methicillin and to some extent, to vancomycin (11). The antipseudomonal activity is also of great importance, particularly in case of methanolic extract. This species can also cause nosocomial infections, and different strains have considerable antibiotic resistance (13).

In a similar study, the methalonic extract of *Eucalyptus* leaves have shown antibacterial activity against *S. aureus*, *E. coli* and *P. aeruginosa*; interestingly high antibacterial activity against *P. aeruginosa* was also found in our research (7). The anti-bacterial activity of ethanolic extract of this plant has been reported by Abubakar (8), but in our research ethanolic extract didn't have any antibacterial activity against *K. pneumoniae* and *S. typhi* even at highest concentration. Presence of the polysaccharide capsule in these two bacteria can act as a barrier to the entrance of active antibacterial compounds (13).

It could also be due to the differences among chemical components of plants that have been cultivated in divergent ecological regions. Ahvaz, a town in south of Iran, has very dry and warm climate and the weather condition is very decisive in producing officinal substances (5). In other study, Jahan et al. by comparing the antibacterial activity of aqueous and hydro-alcoholic extracts of this plant reported that the least activity was obtained by aqueous extract while the maximum effect was recognized by the alcoholic extract against *E. coli* and *Bacillus subtilis*. By Phytochemical screening of this plant, presence of tannins, saponins, cardiac glycosides and steroids have been proved (1). Presence of phenolic compounds and tannins could be the main reason for antibacterial activity of *E. microtheca* leaves extracts, and it could be related to their ability to bind to proteins and inhibiting protein synthesis (14).

In the study of structural changes induced by these extracts, it was obvious that the shape and integrity of the cell walls had been affected. These can be due to the active constituents on penicillin binding proteins (PBPs). These proteins are involved in the cell wall synthesis, shape of the bacterium and its division, so it can be suggested that the possible target of alcoholic extracts of *E. microtheca* would be these proteins. Localization of PBPs in cell wall of rod shape bacteria is in the central and lateral regions of cell wall and in cocci is in the central region. The central and lateral PBPs localization in cocci and bacilli are responsible for the cell growth. In bacilli this localization also directs the cell shape and elongation. Deformity of bacilli that was found in this study can be a clue that the extracts of the studied plant would act on the cell wall. As a rule, those antibacterial agents that affect cell wall have no side effects for eukaryotic cells, so *E. microtheca* can be regarded as a source for antibiotic discovery that has the minimum side effects for humans and animals. Further detailed studies are needed for this purpose.

With regard to these results and increase in antibiotic resistance in pathogenic bacteria, it can be concluded that leaf extracts of *E. microtheca* have potential application in treatment of infections, especially *E. coli* and *P. aeruginosa* that are common infectious agents in the community and hospitals. Further studies on antibacterial properties of this plant are required in order to find its active antibacterial components. It can be purified and fractioned and the most effective part be used as an antibacterial agent. These studies can be promising for overcoming resistant and sometimes overwhelming infections.

Acknowledgements

There is no acknowledgment.

Authors' Contribution

All authors participated equally in the present study.

Financial Disclosure

There is no disclosure.

Funding/Support

The research was granted by the vice chancellor for research of Shahid Chamran University (54314777).

References

1. Jahan M, Warsi MK, Khatoon F. Studies on Antibacterial Property of Eucalyptus-The Aromatic Plant. *Int J Pharm Sci Rev Res.* 2011;**7**(2):86–8.
2. Karakas FP, Yildirim A, Turker A. Biological screening of various medicinal plant extracts for antibacterial and antitumor activities. *Turk J Biol.* 2012;**36**(6):641–52.
3. Vaghasiya Y, Chanda SV. Screening of Methanol and Acetone Extracts of Fourteen Indian Medicinal Plants for Antimicrobial Activity. *Turk J Biol.* 2007;**31**(4):243–8.
4. Sadlon AE, Lamson DW. Immune-modifying and antimicrobial effects of Eucalyptus oil and simple inhalation devices. *Altern Med Rev.* 2010;**15**(1):33–47.
5. Seyyednejad SM, Koochak H, Darabpour E, Motamedi H. A survey on Hibiscus rosa—sinensis, Alcea rosea L. and Malva neglecta Wallr as antibacterial agents. *Asian Pac J Trop Biomed.* 2010;**3**(5):351–5.
6. Matasyoh JC, Maiyo ZC, Ngure RM, Chepkorir R. Chemical composition and antimicrobial activity of the essential oil of Coriandrum sativum. *Food Chem.* 2009;**113**(2):526–9.
7. Nagpal N, Shah G, Arora NM, Shri R, Arya Y. Phytochemical and Pharmacological aspects of eucalyptus genus. *Int J Pharm Sci Rev Res.* 2010;**1**(12):28–36.
8. Abubakar EMM. Antibacterial potential of crude leaf extracts of Eucalyptus camaldulensis against some pathogenic bacteria. *Afr J Plant Sci.* 2010;**4**(6):202–9.
9. Patra A, Jha S, Sahu AN. Antidiabetic activity of aqueous extract of Eucalyptus citriodorahook. in alloxan induced diabetic rats. *Pharmacogn Mag.* 2009;**5**(19):51.
10. Doughary JH, Pukuma MS, De N. Antibacterial effects of Balanites Aegyptaca L. Drel. and Moringa oleifera Lam on Salmonella typhi. *Afr J Biotechnol.* 2007;**6**:2212–5.
11. Harley J. *Laboratory Exercises in Microbiology.* 9th edNew York, United States: McGraw-Hill Science; 2013.
12. Cockerill F. *Performance Standards for Antimicrobial Susceptibility Testing: Twenty-second Informational Supplement.*Englewood, Colorado: Clinical and Laboratory Standards Institute; 2012.
13. Ghasemi Pirbalouti A, Bahmani M, Avijgan M. Anti-Candida activity of some of the Iranian medicinal plants. *Electron J Biol.* 2009;**5**(4):85–8.
14. Ayepola OO, Adeniyi BA. The antibacterial activity of leaf extracts of Eucalyptus camaldulensis (Myrtaceae). *J Appl Sci Res.* 2008;**4**(11):1410–3.
15. Ebadollahi A, Safalizadenh MH, Pourmirza AA, Nouri-Ganbalani G. Comparison of Fumifant Toxicity of Eucalyptus Globolus Labill and Lavandula Stoechas L. Oils Against Different Stages of Tribolium Castaneum Herbst. *Indian J Agricu Res.* 2010;**44**(1):26–31.
16. Safaei-Ghomi J, Ahd AA. Antimicrobial and antifungal properties of the essential oil and methanol extracts of Eucalyptus largiflorens and Eucalyptus intertexta. *Pharmacogn Mag.* 2010;**6**(23):172–5.
17. Boland DJ, Brophy JJ, House APN. *Eucalyptus leaf oils: use, chemistry, distillation and marketing.*Melbourne: Inkata Press; 1991.
18. Mulyaningsih S, Sporer F, Reichling J, Wink M. Antibacterial activity of essential oils from Eucalyptus and of selected components against multidrug-resistant bacterial pathogens. *Pharm Biol.* 2011;**49**(9):893–9.