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Antibacterial Effect of The Hydroalcoholic Extracts of Four Iranian Medicinal Plants on *Staphylococcus aureus* and *Acinetobacter baumanii*

Mansoor Khaledi[1], Majid Asadi-Samani[2], Ahmad Mahmoodi-Kouhi[3], Abolfazl Gholipour[4*]

[1]Students Research Committee, Shahrekord University of Medical Sciences, Shahrekord, Iran
[2]Young Researchers and Elite Club, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran
[3]Student Research Committee, Dezful University of Medical Sciences, Dezful, Iran
[4]Medical Plants Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran

Corresponding Author: Dr. Abolfazl Gholipour
Mailing Address: Shahrekord University of Medical Sciences, Rahmatiyeh, Shahrekord, Iran
e-mail ✉ gholipour_abolfazl@yahoo.com
Relevant Conflicts of Interest/Financial Disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 12 April 2017; **Revised:** 26 April 2017; **Accepted:** 27 April 2017; **Published:** 30 April 2017

ABSTRACT

Acquiring infectious diseases due to resistant infectious agents leads to serious problems such as taking higher doses of antibiotics, additional treatments, lengthened hospital stay, and imposing additional costs. The aim of the current study is to study antibacterial effects of the hydroalcoholic extracts of four Iranian medicinal plants, occurring in Chaharmahal va Bakhtiari, on *Staphylococcus aureus* and *Acinetobacter baumanii*. In this experimental study, the hydroalcoholic extracts of the plants were prepared by maceration. To investigate the antibacterial effects, microdilution and determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were used. DMSO and distilled water were used as solvent. The MIC and MBC of *Dianthus orientalis*, *Ziziphora clinopodioides*, *Euphorbia* sp., and *Acanthophyllum glandulosum* Bunge ex Boiss. for *S. aureus* were derived 4, 0.5, 2, and 2 mg/ml and 16, 8, 8, and 16 mg/ml, respectively. Also the MIC and MBC of plants for *A. baumanii* were derived 4, 1, 0.5, and 2 mg/ml and 16, 8, 8, and 32 mg/ml, respectively. The greatest antibacterial effect was displayed by *Z. clinopodioides* on *S. aureus.* The greatest bactericidal effect on *A. baumanii* was exerted by the recently identified species, *Euphorbia* sp. These plants can serve as suitable choices to produce antibiotics to fight treatment-resistant bacteria.

Key Words: Minimum inhibitory concentration, Minimum bactericidal concentration, Medicinal plants, Drug resistance.

DOI: 10.24896/eijppr.2017722

INTRODUCTION

Different resistant rates have been reported for *Acenitobacter baumanii* and *Staphylococcus aureus* worldwide. *S. aureus* resistance to penicillin was reported for the first time in 1942. *S. aureus* can acquire resistance to other antibiotics such as erythromycin, tetracycline, streptomycin, and methicillin [1]. Besides that, *A. baumanii* has recently been reported to develop multidrug resistance (MDR). While carbapenem was previously used to treat *A. baumanii* infection, the emergence of carbapenem-resistant strains has increased concerns worldwide [2]. *A. baumanii* is a gram-negative cocobacillus and an important bacterium of a group of bacteria consisting of *Enterococcus faecium*, *S. aureus*, *Klebsiella pneumoniae*, *A. baumanii*, *Pseudomonas aeruginosa*, and *Enterobacter spp.*, collectively referred to as ESKAPE. *A. baumanii* is an opportunistic bacterium an important cause of nosocomial infections that lead to a high rate of mortality according to Infectious Diseases Society of America [3].

A. baumanii is responsible for 2-10% of nosocomial infections due to gram-negative bacteria. *A. baumanii* is an important cause of pneumonia, bacteremia, meningitis, and urinary tract infections [4].

S. aureus is an important human pathogen. This gram-positive bacterium is a main cause of bacteremia, endocarditis, and respiratory tract infections [5]. The prevalence of bacteremia due to *S. aureus* has been reported 5015 people per 1000-individual population in hemodialysis patients in the USA [6]. A 34% increase in infection with *S. aureus* in Europe caused much concern [7].

Indeed, acquisition of infectious diseases due to resistant infectious agents can lead to serious problems such as taking higher doses of antibiotics, additional treatments, lengthened hospital stay, and imposing additional costs [8]. These issues alongside ineffectiveness of chemical drugs to fight these pathogens have necessitated development of new antibiotics. The use of medicinal plants and their derivatives is an approach to fight such various pathogens [9-11]. Also medicinal plants' effects have been investigated and confirmed on several diseases [12-18]. In this regard, researchers are seeking to identify medicinal plants and their derivatives to develop effective antibacterial drugs. The current study was conducted to study antibacterial effects of the hydroalcoholic extracts of four Iranian medicinal plants occurring in Chaharmahal va Bakhtiari province, *Dianthus orientalis*, *Ziziphora clinopodioides*, *Euphorbia* sp., and *Acanthophyllum glandulosum* Bunge ex Boiss., on *S. aureus* and *A. baumanii* in vitro. These plants or some of their species have been reported, in traditional medicine, to display antibacterial effects.

MATERIALS AND METHODS

The collection of the plants

The plant samples were collected from different regions of Chaharmahal va Bakhtiari province including Saman county between March 2016 and September 2016 and then confirmed by the botanist of Research Center of the Construction Crusade of the province (Dr. Shirmardi). *D. orientalis*, *A. glandulosum* Bunge ex Boiss., *Z. clinopodioides*, and *Euphorbia sp.* were studied in this study.

Extraction

To prepare hydro alcoholic extracts, maceration was used with duplicate, 72-hour extraction. In this method, aqueous and bitter ethanolic solvents without butyric acid (30:70) were used. Then, the resulting extract was evaporated to concentrate using filter paper under pressure approximate to vacuum under 40°C with a rotary evaporator. The extracts were stored at -20°C till later use.

Preparing standard bacterial strain

S. aureus and *A. baumanii* with standard numbers ATCC 12923 and PTCC 1855, respectively, were purchased from the Iranian Research Organization for Science and Technology and used.

Preparing microbial suspension

To prepare microbial suspension equal to 0.5 McFarland standard (10^5 CFU/ml), 24-h culture was conducted on blood agar and then a suspension with 0.5 McFarland turbidity in normal saline was prepared.

Investigating antimicrobial activity using well microplate method

After conducting bacterial culture and preparing microbial suspension, broth microdilution in a sterile 96-well plate according to 0.5 McFarland standard (10^5 CFU/ml) was used to determine the antimicrobial effects of the extracts.

Table 1. The bactericidal effects of the studied plants

Extracts	MBC (mg/ml)		MIC (mg/ml)	
	A. bumani	*S. aureus*	*A. bumani*	*S. aureus*
Dianthus orientalis	16	16	4	4
Ziziphora clinopodioides	8	8	1	0.5
Euphorbia sp.	8	8	0.5	2
Acanthophyllum glandulosum Bung. ex Boiss	32	16	2	2

Acenitobacter baumanii: A. bumani; Staphylococcus aureus: S. aureus.

In this method, the first well was considered negative control (containing the culture medium and the extract) and the second well considered to be positive control (containing the culture medium and the bacterium). After the culture medium (95 µl) and the extracts (100 µl) were introduced into the microplate wells, 5 µl bacterium was added, and after dilution, the resulting samples incubated at 37°C for 24 h. The concentration of the final (most diluted) well in which no turbidity developed was considered to represent minimum inhibitory concentration (MIC).

To determine minimum bactericidal concentration (MBC), all wells without turbidity were separately cultured on the blood agar medium and incubated at 37°C for 24 hours. The minimum concentrations of the extract in which the bacteria were not able to grow were considered to represent MBC. The tests to determine the MIC and MBC were conducted in triplicate [19].

RESULTS AND DISCUSSION

4 mg/ml *D. orientalis* extract could inhibit *S. aureus* and *A. baumanii*, and 16 mg/ml of this extract could destroy these bacteria.

Z. clinopodioides displayed more potent bactericidal activity against *S. aureus* and *A. baumanii* than *D. orientalis*. 0.5 and 1 mg/ml *Z. clinopodioides* inhibited and eliminated *S. aureus*, respectively. 2 and 0.5 mg/ml *Euphorbia sp.* was found to inhibit *S. aureus* and *A. baumanii*, respectively. In addition, 8 mg/ml *Euphorbia sp.* was able to eliminate these two bacteria. 2 mg/ml *A. glandulosum* Bung. ex Boiss. could inhibit *S. aureus* and *A. baumanii*. Sixteen and 32 mg/ml *A. glandulosum* could eliminate *S. aureus* and *A. baumanii*, respectively.

The most potent antibacterial effect was displayed by *Z. clinopodioides* on *S. aureus.* The most potent bactericidal effect on *A. baumanii* was exerted by the recently identified species *Euphorbia* sp. The least potent bactericidal effect among the studied plants was displayed by *D. orientalis* (Table 1).

This study that was conducted to study antibacterial effects of the hydro alcoholic extracts of four Iranian plant species on *S. aureus* and *A. baumanii* in vitro, demonstrated that the studied plants can serve as suitable alternatives to develop antibiotics to fight treatment-resistant bacteria. The findings showed that *D. orientalis* displayed the same inhibitory effect on *S. aureus* and *A. baumanii* at 4 mg/ml for both bacteria. In addition, the MBCs of the two bacteria were similar. Muthanna *et al*. study on the antibacterial effects of *D. caryophyllus* demonstrated that this plant exerted inhibitory effect on *S. aureus* [20]. Casigliaa *et al*. argued that the antimicrobial effect of *D. caryophyllus* could be due to the presence high amounts of thymol and its derivatives in this plant [21]. In addition, although *D. orientalis* is from family Caryophyllaceae and a rich source of flavonoids, Naghibi *et al*. reported that this plant had no cytotoxic and anticancer effect [22]. However, Chandra *et al*. study found that this plant was effective in treating colon cancer [23]. This finding deserves further research.

Our findings on another plant from family Caryophyllaceae namely *A. glandulosum* demonstrated that this plant exerted antimicrobial effect such that the MIC of this plant for both *S. aureus* and *A. baumanii* was 2 mg/ml, and its MBC for *S. aureus* and *A. baumanii* was 16 and 32 mg/ml, respectively. Egamberdieva *et al*. reported that the people of Uzbekistan have long used *A. glandulosum* as a disinfectant substance [24]. Juan *et al*. study also confirmed the antimicrobial effect of this plant because of its large amounts of saponin [25]. Karamian *et al*. study demonstrated that *Proteus vulgaris* and *Citrobacter amalonaticus* displayed the greatest susceptibility to saponin compounds [26]. Chandra *et al*. argued that plants from family Caryophyllaceae can generally exert more potent antibacterial effects on gram-negative bacteria due to the presence of eugenol and thymol in these plants [23].

Our findings on *Z. clinopodioides* demonstrated that this plant exerted more potent antibacterial effect on *S. aureus* and *A. baumanii* compared to the other three plants. Soltani *et al*. study demonstrated that *Z. clinopodioides* exerted pleasant effects on the in vitro growth of *Listeria monocytogenes*[27]. Besides that, Shahbazi study showed that the inhibitory effects of this plant on *S. aureus* and *Salmonella typhimurium* were optimal [28]. A study also demonstrated that *Z. clinopodioides*, combined with niacin, exerted bactericidal effect on *Escherichia coli* [29]. A study to investigate the antibacterial effects of *Z. clinopodioides* on *S. aureus*, *Bacillus cereus*, *Bacillus subtilis*, *L. monocytogenes*, *S. typhimurium*, and *E. coli* showed optimal antibacterial effects of this plant on the studied bacteria. Overall, the MIC and MBC were reported to be 2-2.5 µl/ml, which were lower compared to the current study. That study also reported that *Z. clinopodioides* contains carvacrol (64.2%) and thymol (19.2%) and that the antibacterial effects of this plant could be attributed to the phenolic compounds [30].

In the current study, *Euphorbia sp*. was found to exert more potent antibacterial effect on *A. baumanii* than that on *S. aureus*. Jayalakshmi *et al*. study demonstrated that methanolic *Euphorbia cotinifolia* extract exerted inhibitory effects on *E. coli*, *K. pneumoniae*, *B. subtilis*, *B. cereus*, *Salmonella typhi*, *Enterobacter aerogenes*, and *S. aureus* such that the MIC was reported 0.312-1.25 mg/ml [31], which is in agreement with our study. de Araújo *et al*. study indicated that *Euphorbia sp*. exerted inhibitory effects on *S. aureus* and *Staphylococcus epidermidis*. de Araújo *et al*. argued that this effect is mainly due to the presence of large amounts of ferulic acid and phenolic compounds in this plant [32], which is completely consistent with our study. It is obieous that medicinal plants and their drivates can prevent a lot of diseases via their antioxidant and anti-inflammatory effects [33-46].

CONCLUSION

Because we currently observe high resistance of the bacteria to common antibiotics, the medicinal plants studied in the current study can be suitable alternatives to develop antibiotics to fight treatment-resistant bacteria because of their phenolic compounds and antibacterial effects. However, further research should be conducted to extract the active compounds of these plants and study their effects on pathogenic bacteria.

CONFLICT OF INTEREST

The authors declared no competing interests.

ACKNOWLEDGMENT

This project has been registered by Medical Plants Research Center of Shahrekord University of Medical Sciences (No: 3370-77-08-1396); We thank Deputy of Research and Technology of the Shahrekord University of Medical Sciences for supporting this research.

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HOW TO CITE THIS ARTICLE: Khaledi, M., Asadi-Samani, M., Mahmoodi-Kouhi, A. and Gholipour, A., 2017. Antibacterial Effect of The Hydroalcoholic Extracts of Four Iranian Medicinal Plants on Staphylococcus Aureus and Acinetobacter Baumanii. *International Journal of Pharmaceutical and Phytopharmacological Research, 7*(2), pp.10-14. **DOI: 10.24896/eijppr.2017722**