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Original article

Synergistic Activity of Three Iranian Medicinal Plants in Combination with Ceftazidime and Neomycin against Bacterial Strains Causing Nosocomial Infections

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Abstract

Background and objectives: This study aimed to investigate the antimicrobial properties of *Salvia limbata, Centella asiatica,* and *Bacopa Monnieri* extracts against *Staphylococcus aureus, Pseudomonas aeruginosa, Acinetobacter baumannii* and methicillin-resistant *Staphylococcus aureus* (MRSA). We also examined the synergistic effect of these extracts with ceftazidime and neomycin. The antimicrobial effects of these plants had been reported before but synergistic effect with broad spectrum antibiotics such as ceftazidime and neomycin was an important issue that we tried to determine. **Methods:** Methanol extracts were prepared by percolation method and phenolics content was determined by Folin–Ciocalteu method. The minimum inhibitory concentrations (MIC) of the extracts were determined by the Clinical and Laboratory Standards Institute (CLSI) broth microdilution method. Checkerboard assay was used to evaluate the synergistic effect of the extracts with ceftazidime and neomycin. **Results:** *Salvia limbata* methanolic extract with MIC of 25, 100, and 150 mg/mL could inhibit the growth of *S. aureus, P. aeruginosa,* and MRSA, respectively. *Bacopa monnieri* with MIC of 50 mg/mL and 100 mg/mL inhibited the growth of *S. aureus* and *P. aeruginosa*, respectively. *Centella asiatica* did not affect the studied strains. The fractional inhibitory concentration (FIC) results showed partial synergistic activity between *S. limbata* with ceftazidime and neomycin against *P. aeruginosa* and MRSA. The MIC of ceftazidime and neomycin in combination with *S. limbata* was reduced fourfold for each antibiotic. **Conclusion:** *Salvia limbata* is a potentially rich source of bioactive compounds with antimicrobial properties that can be used with ceftazidime and neomycin to provide a synergistic effect.

Keywords: ceftazidime; neomycin; *Pseudomonas aeruginosa*; *Salvia limbata; Staphylococcus aureus*

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Introduction

To date, bacterial infections have been a serious threat to human life. The discovery and prevalence of antibiotics have led to a decrease in the importance of infection. Nonetheless, the phenomenon of antibiotic resistance has increased the importance of bacterial infections once again. Microbial infections resistant to antibiotics are now of the most important and

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prominent challenges threatening public health [1,2].

Microbial infections cause millions of deaths worldwide each year. According to the World Health Organization's report, about 9.2 million people died from infections in 2013, about 17% of all deaths worldwide. However, human overuse of antibiotics and the evolution of microbial agents have made existing antibiotics less effective or even ineffective [3,4]. A study presented to the United Nations in 2019 suggested that infections caused by antimicrobial resistance will cause 10 million annual deaths by 2050, and the economic crisis resulting will be as bad as the financial downturn between 2008 and 2009 [5].

The COVID-19 outbreak at the end of 2019 led to new antibiotic resistance challenges [6]. An article published in the Lancet has noted the possibility of intensifying antibiotic resistance following the outbreak of COVID-19 [7].

Antibiotic resistance is the process by which the bacterium adapts to the action of antibiotics. For example, it produces enzymes that lead to the removal or alteration of antibiotics. The genes encoding these enzymes are developed in the environment by genetic exchanges between bacteria. The most common of these genes are found in hospitals, nursing homes, and animal husbandry centers. The widespread bacterial strains such as *Staphylococcus aureus*, and methicillin resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in the hospital environment is a serious problem [8-12]. Thus, immediate solutions and new effective drugs to deal with and prevent future crises are among the essential and strategic research priorities.

Staphylococcus aureus is a Gram-positive, immobile, voluntary anaerobic, catalase and coagulase-positive, and a coconut-shaped bacterium that causes many diseases such as severe food poisoning, dangerous bacteremia, endocarditis, meningitis, and osteomyelitis [13,14]. In recent years, *S. aureus* has gain high adaptability to antibiotics. For instance, MRSA is resistant to beta-lactam antibiotics [15].

Pseudomonas aeruginosa is a Gram-negative, absolute aerobic, motile, and oxidase-positive bacterium. It is one of the most important causes of nosocomial infections that can cause various infections in immunocompromised patients. Resistant isolates of this bacterium cause severe skin and systemic diseases in people with diabetes, burns, and bedsores [16,17].

Acinetobacter baumannii is an opportunistic Gram-negative bacterium that causes nosocomial infections, especially in patients admitted to the intensive care unit. It causes septicemia, pneumonia, and urinary tract infections following hospitalization. In the last decade, *A. baumannii* strains with multiple drug resistance have been reported, which may result from the over-use of antimicrobial agents [18,19].

Salvia limbata from the Lamiaceae family is one of the most valuable types of medicinal herb with important and broad therapeutic properties. In Iranian traditional medicine, the sage decoction was used as a blood sugar reducer, sedative and anticonvulsant, improver of general weakness and fatigue, as poultice for burns, wounds, and insect bites, and as mouthwash against mouth and throat ducts inflammation [20,21].

Bacopa monnieri belongs to the Scrophulariaceae family. This family comprises more than 100 species of aquatic plants scattered in warm and temperate regions. This plant has been used to improve memory, treat mental disorders, anticholinesterase, and as a rejuvenating substance. Many studies have been done on this plant and its components in recent decades, especially saponins. The plant's analgesic and anti-ulcer properties are due to the activities of superoxide dismutase, catalase, and glutathione peroxidase. The antioxidant properties of the plant protect against oxidative damage in cardiovascular diseases and some instances of cancer [22].

Centella asiatica from the Umbelliferae family is a semi-aquatic plant that grows on the water's edge. The plant effectively improves and repairs elastic connective tissue, reduces fibrosis, improves rapid wound healing, and treats hypertrophic keloids and scars. It has an effect similar to corticosteroids in patients, attributed to the composition of asiaticoside [23].

In recent decades, traditional medicine, one of the oldest treatment methods, has become popular due to the high prevalence of nosocomial infections and bacterial resistance in most parts of the world. Today, developing new therapeutics against infections is essential more than ever. In this regard, the interest in plant products to fight infectious diseases has increased. Researchers have tested a variety of plants to combat bacterial resistance to antibiotics, which are mainly used to

treat most infections.
Thus, the present study aimed to investigate the antimicrobial properties of methanol extract of *S. limbata*, *C. asiatica*, and *B. monnieri* that were recommended for antimicrobial effects against the standard strains of *S. aureus*, *P. aeruginosa*, *A. baumannii* and MRSA. The study ultimately examined the synergistic effects of these extracts with ceftazidime and neomycin antibiotics.

Material and Methods

Ethical considerations

All procedures were approved by the Ethic Committee of Shahid Sadoughi University of Medical Sciences, Iran (approval number: IR.SSU.MEDICINE.REC.1398.127).

Chemicals

Methanol, dimethyl sulfoxide (DMSO), gallic acid and Folin-Ciocalteu reagent, were provided from Merck Chemical Company (Germany). MTT was purchased from Sigma-Aldrich (St. USA). Ceftazidime and neomycin were supplied by Jaber Ebne Hayyan Pharmaceutical Company (Iran).

Plant material

The aerial parts of the studied plants were collected in the summer of 2021. A botanist confirmed the taxonomic identification of plant species, and a voucher specimen was deposited at the Herbarium of Medicinal Plants Research Center of Shahid Sadoughi University of Medical Sciences in Yazd, Iran. *Centella asiatica* (L.) Urban (SSU0084) was collected from Anzali wetland region, Guilan Province, *Bacopa monnieri* (L.) Pennell (SSU0085) was collected from springs in Sang Rud region of Guilan Province, and *Salvia limbata* C. A. Mey (SSU0083) was collected from Dorodzan region of Fars Province.

Bacterial strains

Staphylococcus aureus strain (ATCC 6538p), MRSA (ATCC*3359*), *P. aeruginosa* (ATCC 9027) and *A. baumannii* (ATCC BAA-747) were purchased from the Persian Type Culture Collection (Tehran, Iran).

Preparation of plant extracts

The plants were dried and powdered by an electric mill; the methanol extracts were prepared by the percolation method [24]. The plant powder (100 g) was soaked in 80% methanol, then it was added to percolator where 150 ml of 80% methanol was added to it. The solution was percolated each day for seven days. Finally, the extract was dried under lab condition.

Total phenolics content

In order to determine the total phenolics content of the Plants, the Folin-Ciocalteu method was performed with some modifications [25].
The basis of this method is the reduction of folate reagent by phenolic compounds in alkaline environment and the formation of a blue complex that has a maximum light absorption at wavelength of 725 nm. Gallic acid, which is a type of phenolic acid was used as the standard at concentrations of 25, 50, 100, 125, 150 µg/mL in order to construct standard calibration curve as mg of gallic acid equivalents per g dried plant powder.
In the case of extract, 400 µL of the extract solution was mixed with 3 mL of distilled water in a test tube and 3 mL of sodium bicarbonate 6% (w/v) was added to the contents of the tube as a blank. In the sample tube, 1.5 mL of Folin-Ciocâlteu (diluted at a ratio of 1:10 with distilled water) and 200 µL of the extract solution were poured and placed at 25 °C for 5 min, 1.5 mL of sodium bicarbonate 6% (w/v) was added. Then the sample tubes were incubated at 25 °C for 90 min.
The absorption was measured by spectrophotometer against blank at the wavelength of 725 nm. This operation was performed on three different days and at the end, the average of the results was calculated.

Preparation of bacterial inoculation

In order to evaluate the antimicrobial activity, the microbial stock culture was separately inoculated for 24 h before the experiments to activate in Trypticase Soy Broth (TSB) culture medium and placed in an incubator at 37 °C. Afterward, it was cultured in Muller-Hinton Agar (MHA) at 37 °C. After incubation and formation of separate colonies for each microbe, 4 to 5 colonies were selected and transferred to a tube containing normal saline. Then, the turbidity was compared with the 0.5 McFarland standard.

Determining minimum inhibitory concentrations (MIC)

First, three concentrations (100, 200, 300 mg/mL) of each three available extracts were prepared using 4% DMSO as solvent. The bacterial

inoculation was matched to the 0.5 McFarland standard tube in terms of turbidity. Following the CLSI protocol, inoculation was diluted at a ratio of 1:20 [26]. Hundred µL Mueller–Hinton Broth (MHB) culture medium was added to all wells. Then, serial dilution was prepared on a 96-well plate. Ten µL of the diluted bacterial suspension was added to each well. One house was used as a positive control (culture medium + bacteria) and another as a negative control (culture medium + extract). A well was used to control the environment regarding turbidity and non-pollution of the culture medium. It was placed in an incubator at 37°C for 24 h. After the incubation period, the last well that was clear and had no turbidity was considered as MIC. It was difficult to detect the minimum inhibitory concentration of bacteria in the extracts due to their turbidity. Therefore, five µL of tetrazolium salt with the concentration of 5 mg/mL was added to each well and placed in an incubator at 37 °C for 2 h to solve this problem. No color change was observed if the germ did not grow [27].

Synergy measurement by checkerboard analysis

The checkerboard method in broth was used to investigate the interaction between antibiotics and extracts against bacterial strains [28].

First, a new culture was prepared from standard and resistant strains, then the MIC of antibiotics and extracts was determined on standard and resistant strains. Various concentrations were determined according to the MIC of the strains, and four concentrations lower than the MIC (1/2, 1/4, … MIC) were considered for antibiotics and extracts. In an entirely sterile condition, 25 µL of MHB was added to each well of the 96-well plate. Antibiotic concentrations were poured horizontally, and extracts were poured vertically into the wells. Twenty-five µL of each antibiotic and extract concentration was poured into the wells, so the volume of each well containing the antibiotic and extract reached 50 µL. In column 1 and row 8, where the extract and antibiotic were present separately, 25 µL of MHB medium was added to increase the volume to 50 µL, similar to the rest of the wells. Column 12 was considered as the spositive control, and the last well of the first column was considered as the control to confirm the lack of environmental pollution. Turbidity of 0.5 McFarland was prepared from the pure culture of each strain and diluted 100 times in bacterial suspension in tubes containing MHB medium. Then, 10 µL of MHB medium containing bacteria and 40 µL of MHB medium were spread into all microplate wells. The final volume of each well reached 100 µL. The microplates were incubated for 24 h at 37°C. After one day, the microplates were examined for bacterial growth. The turbidity was considered an indicator of bacterial growth.

Like the study of the synergistic effect using the MIC test, the FIC index was calculated for the first well in which no growth was observed.

FIC for the extract =
MIC of plant extract in combination/MIC of plant extract alone

FIC for antibiotics =
MIC of antibiotic in combination/MIC of antibiotics alone

FIC extract + FIC antibiotic =
FIC (fractional inhibitory concentration) index

The interaction between antibiotics and extracts is interpreted based on the number obtained from the calculation of the FIC fraction index. The FIC index is interpreted as follows: FIC index of <0.5 as the synergistic effect, 0.5 < FIC < 0.75 as partial synergism activity, 0.75 < FIC < 1 as additive effect, 1 < FIC < 4 indifferent and FICI > 4 known as antagonism [28].

Results and Discussion

The standard curve of gallic acid was constructed to determine the amount of total phenolic content. The related equation was y = 0.0049x -0.0016 and total phenolic content of *S. limbata*, *B. monnieri* and *C. asiatica* methanol extracts equivalent to gallic acid were 6.3, 5.5 and 8.2 (mg/g of plant dry weight), respectively (Table 1). According to the results shown in Table 2, neomycin MIC was 4, 8, and 128 µg/mL, respectively against *S. aureus*, *P. aeruginosa*, and MRSA. MIC of ceftazidime for *P. aeruginosa* was 8 µg/mL.

As shown in Table 3, *S. limbata* methanol extract with MIC of 25, 100, and 150 mg/mL could inhibit the growth of strains of *S. aureus*, *P.aeruginosa*, and MRSA, respectively.

Table 1. Total phenolics of *Salvia limbata, Bacopa monnieri* and *Centella asiatica* methanol extracts

Extracts	Phenolic content (mg/g of plant dry weight)
Salvia limbata	6.3
Bacopa monnieri	5.5
Centella asiatica	8.2

Table 2. Minimum inhibitory concentration in µg/mL (MIC) of neomycin and ceftazidime

Antibiotics	Bacterial Strains		
	Pseudomonas aeruginosa	*Staphylococcus aureus*	*MRSA*[*]
Neomycin	8	4	128
Ceftazidime	8	-	-

[*] methicillin-resistant *Staphylococcus aureus*

Bacopa monnieri with 50 mg/mL and 100 mg/mL MIC inhibited the growth of *S. aureus* and *P. aeruginosa*, respectively. *Centella asiatica* did not affect the studied strains. Also, all extracts were ineffective gainst *A. baumannii*.

The checkerboard method was used in broth to investigate the interaction between antibiotics and extract of studied plants. FIC index was calculated and interpreted after combining antibiotics and extracts in selected concentrations (Table 4).

The results of fractional inhibitory concentration (Table 4) showed that the combination of 32 µg/mL of neomycin and 75mg/mL of methanol extract of *S. limbata* showed partial synergism activity against MRSA strain. Also, the combination of neomycin and methanol extract of *S. limbata* and *B. monnieri* was ineffective against the standard strain of *S. aureus* and *P. aeruginosa* in all sub MIC concentrations.

As shown in Table 4, the combination of ceftazidime in concentrations of 2 and 4 µg/mL with 50 and 25 mg/mL of *S. limbata* methanol extract against *P. aeruginosa* strain had partial synergism activity. Also, the combination of ceftazidime and *B. monnieri* methanol extract was ineffective against the standard strain of *P. aeruginosa* in all sub MIC concentrations.

Medicinal plants have long been used in traditional medicine [29]. The study of the antibacterial properties of native plants has many benefits for the production of new drugs and the control of pathogens. It has been shown that the *Salvia* genus has shown antibacterial activity against bacteria such as *S. aureus, Escherichia coli*, *Salmonella typhi*, *P. aeruginosa*, and even some fungi such as *Candida albicans* [30,31].

The present study aimed to investigate the antimicrobial properties of methanol extracts of *Salvia limbata, Centella asiatica, and Bacopa Monnieri* against standard strains of *S. aureus*, *P. aeruginosa*, *A. baumannii*, and MRSA. The synergistic effect of these extracts with ceftazidime and neomycin antibiotics was also investigated.

Table 3. Minimum inhibitory concentration in mg/mL (MIC) *Salvia limbata*, *Centella asiatica*, and *Bacopa Monnieri* methanol extracts

Plants	Bacterial Strains			
	Acinetobacter baumannii	***Pseudomonas aeruginosa***	***Staphylococcus aureus***	**MRSA**[**]
S. limbata	Nd[*]	100	25	150
B. monnieri	nd	100	50	nd
C. asiatica	nd	nd	nd	nd

[*] nd: not determined; methicillin-resistant *Staphylococcus aureus*

Table 4. Interaction of neomycin and ceftazidime with the methanol extract of *Salvia limbata* against *Pseudomonas aeruginosa* and MRSA strain

Microbial strains	Methanol extract of *Salvia limbata* (mg/mL)		Neomycin (µg/mL)		Ceftazidime (µg/mL)		FIC index	Inference
	MIC_1	MIC_2	MIC_1	MIC_2	MIC_1	MIC_2		
MRSA *strain*	150	75	128	32	-	-	0.75	Partial synergism
Pseudomonas	100	50	-	-	8	2	0.75	Partial synergism
aeruginosa	100	25			8	4	0.75	Partial synergism

MIC_1: Minimum inhibitory concentration of the extract or antibiotic alone. MIC_2: Minimum inhibitory concentration of the combination of the extract and antibiotic; FIC index: fractional inhibitory concentration; FIC index <0.5 as the synergistic effect, 0.5 < FIC < 0.75 as partial synergism activity, 0.75 < FIC < 1 as additive effect, 1 < FIC < 4 indifferent and FICI > 4 known as antagonism; MRSA: methicillin-resistant *Staphylococcus aureus*

According to the results, the most effective extracts on *S. aureus* and MRSA and *P. aeruginosa* was *S. limbata*. *Centella asiatica* extract did not affect the studied strains.

The fractional inhibitory concentration (FIC) results showed partial synergistic activity between the methanol extract of *S. limbata* and both ceftazidime and neomycin against *P. aeruginosa* and MRSA. The MIC of ceftazidime and neomycin combined with methanol extract of *S. limbata* was reduced fourfold for each antibiotic.

Application of neomycin alone on MRSA with MIC 128 µg/mL and in combination with methanol extract of *S. limbata* with MIC 32 µg/mL showeda an inhibitory effect. Also, the application of ceftazidime alone on *P. aeruginosa* with MIC 8 µg/mL and in combination with methanol extract of *S. limbata* with MIC 2 µg/mL demonstrated an inhibitory effect.

The fact that *B. monnieri* had no synergistic effect with ceftazidime and neomycin may be related to the chemical components of the antibiotics being studied, which require further investigation.

Interestingly, *S. Limbata* had partial synergistic effects with ceftazidime (inhibitor of cell wall synthesis) and neomycin (inhibitor of protein synthesis) and reduced the minimum inhibitory concentration for both extracts and antibiotics. According to the results of this study, *S. Limbata* can be used as a complementary drug combined with ceftazidime and neomycin.

In 2012, Olgicad et al. studied the synergistic antibacterial activity of *Salvia officinalis* and chicory extracts combined with amoxicillin and chloramphenicol. The results showed that different ethyl acetate, and acetone extracts of *S. officinalis* ere effective on Gram-positive and Gram-negative bacteria. Acetone and ethyl acetate extracts of *S. officinalis* and *Cichorium intybus* and the mentioned antibiotics, inhibited the growth of tested bacteria at lower concentrations compared to the individual drugs separately. The *S. officinalis* acetone extract and amoxicillin compound showed the strongest effect [32].

The synergism of *salvia* and *rosemary* on resistant *Escherichia coli* was consistent with our results. *Salvia limbata* has shown a synergistic property with ceftazidime and ceftriaxone antibiotics due to inhibition of cell wall synthesis [33].

In 2020, Leilabandian et al. investigated the antimicrobial properties of ethanol extracts of *Zataria multiflora*, *Salvia verticillata*, and *Froriepia subpinnata* on *Pactobacterium carotovorum* and *P. aeruginosa*, with minimum inhibitory concentrations of 3.12 to 6.25 mg/mL for the alcoholic extract of *Zataria multiflora* and MIC values of 12.3 to 25 mg/mL for *Salvia verticillata* extract; also, MIC values of 12.5 to 25 mg/mL for *Froriepia subpinnata*. After examining the synergistic properties of these plants, it was found that *Z. multiflora* and *S. verticillata* had synergic activity for *P. aeruginosa* but showed an antagonistic effect for *P. carotovorum* [34]. A study was conducted by Fazlul et al. in 2019 to evaluate the antibacterial and antifungal properties of aqueous, ethanol, diethyl ether, or ethyl acetate extracts of *B. monnieri*; effective results were obtained in confirming this claim [35]. According to their study, ethanol extract of *B. monnieri* had a potent antifungal effect on *Candida albicans* and *Aspergillus flavus*. Diethyl ether extract of *B. monnieri* had a potent Antimicrobial effect on Gram-positive bacteria (*S. aureus*) and Gram-negative bacteria (*E. coli*). Ethyl acetate extract of *B. monnieri* had less inhibitory potency. Also, the aqueous extract did not affect the studied strains. The finding of our study showed that the methanol extract of *B. monnieri* had an antimicrobial effect on Gram-positive bacteria (*S. aureus*) and Gram-negative bacteria (*P. aeruginosa*).

Conclusion

Antibiotic combinations with plant extracts can be a significant basis for a new approach to combat microbial resistance and for the introduction of new drugs. Many plant extracts have antimicrobial properties; however, they can also be combined with chemical antibiotics to potentiate and provide synergistic effects. The present study showed that neomycin and ceftazidime antibiotics in combination with sage (*Salvia limbata*) extract could be used in lower concentrations. It is worth mentioning that further studies, especially in vivo studies, are required as this study was done only in vitro. Our findings indicate that *S. limbata* extract is a rich and potential source of bioactive compounds with antimicrobial properties that can be combined with ceftazidime and neomycin to provide a synergistic effect.

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None.

Author contribution
Fatemeh Tavakoli designed the research and prepared the manuscript; Azadeh Emami performed the experimental studies and helped in manuscript preparation; Mohammad Beyk collected the plants and performed extraction; Ali Mohammad Ranjbar contributed in design of the study and manuscript preparation.

Declaration of interest
The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

References
[1] Blair JMA, Webber MA, Baylay AJ, Ogbolu DO, Piddock LJV. Molecular mechanisms of antibiotic resistance. *Nat Rev Microbiol*. 2015; 13(1): 42–51.

[2] Giedraitiene A, Vitkauskiene A, Naginiene R, Pavilonis A. Antibiotic resistance mechanisms of clinically important bacteria. *Medicina (Kaunas).* 2011; 47(3): 137–146.

[3] World Health Organization. WHO methods and data sources for global burden of disease estimates 2000-2011. [Accessed 2022]. Available from: https://www.who.int/healthinfo/statistics/GlobalDALYmethods_2000_2011.pdf.

[4] World Health Organization. Antimicrobial resistance: global report on surveillance; 2014. [Accessed 2022]. Available from: https://apps.who.int/iris/handle/10665/112642.

[5] Carr C, Smith A, Marturano M, Hakki L, Friedman J, Guidry C, McGrew P, McGinness C, Juan Duchesne J, Schroll R . Ventilator associated pneumonia: how do the different criteria for diagnosis match up? *Am Surg.* 2019; 85(9): 992–997.

[6] Pelfrene E, Botgros R, Cavaleri M. Antimicrobial multidrug resistance in the era of COVID-19: a forgotten plight? *Antimicrob Resist Infect Control*. 2021; 10(1): 1–6.

[7] Strathdee SA, Davies SC, Marcelin JR. Confronting antimicrobial resistance beyond the COVID-19 pandemic and the 2020 US election. *Lancet*. 2020; 396(10257): 1050–1053.

[8] Malmir S, Bahreinian M, Zahiri Yeganeh S, Mirnejad R, Moosazadeh Moghaddam M, Saberi F. Molecular mechanisms of resistance to conventional antibiotics in bacteria. *Int J Med Rev*. 2018; 5(3): 118–129.

[9] Khameneh B, Iranshahy M, Soheili V, Bazzaz BSF. Review on plant antimicrobials: a mechanistic viewpoint. *Antimicrob Resist Infect Control.* 2019; 8(1): 118–145.

[10] Keyes K, Lee MD, Maurer JJ. Antibiotics: mode of action, mechanisms of resistance, and transfer. In: Torrence ME, Isaacson RE, Eds. Microbial food safety in animal agriculture: current topics. Iowa: Iowa State Press, 2003.

[11] Alekshun MN, Levy SB. Molecular mechanism of antibacterial maltidrug resistance. *Cell*. 2007; 128(6): 1037–1050.

[12] Hosseini SMJ, Saberi M, Hosseini Doust SR, Yaribeigi H. Evaluation of antibiotic resistance in bacteria isolated from patients hospitalized in Imam Khomeini and burn hospitals in Ahvaz City, Iran. *Qom Univ Med Sci J*. 2013; 7(6): 21–26.

[13] Carroll KC, Hobden JA, Miller S, Morse SA, Mietzner TA, Detrick B, Mitchell TG, Mc Kerrow JH, Sakanari JA, Eds. Jawetz, Melnick & Adelberg's medical microbiology. New York: McGraw Hill, 2019.

[14] Llamazares C, Del Olmo NS, Soliveri J, Javier de la Mata F, Copa-Patiño JL, García-Gallego S. Insight on the structure-to-activity of carbosilane metallodendrimers in the fight against *Staphylococcus aureus* biofilms. *Antibiotics*. 2021; 10(5): 2–12.

[15] Chew YL, Mahadi AM, Wong KM, Kheng Goh J. Anti-methicillin-resistance *Staphylococcus aureus* (MRSA) compounds from *Bauhinia kockiana* Korth and their mechanism of antibacterial activity. *BMC Complement Altern Med*. 2018; 18(1): 70–78.

[16] Hawkey PM, Warren RE, Livermore DM, McNulty CAM, Enoch DA, Otter JA, Wilson APR. Treatment of infections caused by multidrug-resistant Gram-negative bacteria: report of the British society for antimicrobial chemotherapy/healthcare infection society/British infection association joint working party. *J Antimicrob Chemother*. 2018; 73(3): 2–78.

[17] Wanger VE, Iglewski BH. *P. aeruginosa* biofilms in CF infection. *Clin Rev Allergy Immunol*. 2008; 35(3): 124–134.

[18] Sun D, Crowell SA, Harding CM, Silva

PMD, Harrison A, Fernando DM, Mason KM, Santana E, Loewen PC, Kumar A, Liu Y. KatG and KatE Confer *Acinetobacter* resistance to hydrogen peroxide but sensitize bacteria to killing by phagocytic respiratory burst. *Life Sci*. 2016; 148: 31–40.

[19] Protic D, Pejovic A, Andjelkovic D, Djukanovic N, Savic D, Piperac P, Markovic Denic L, Zdravkovic M, Todorovic Z. Nosocomial infections caused by *Acinetobacter baumannii*: are we losing the battle? *Surg Infect*. 2016; 17(2): 236–242.

[20] Rajabi Z, Ebrahimi M, Farajpour M, Mirza M, Ramshini H. Compositions and yield variation of essential oils among and within nine *Salvia* species from various areas of Iran. *Ind Crop Prod.* 2014; 61: 233–239.

[21] Iravani M, Mahinpour R, Zahraei Z, Toluei Z. In vitro evaluation of the antimicrobial properties of essential oils and methanolic extracts of four species of *Salvia* L. *Res Med*. 2019; 43(3): 157–163.

[22] Vijay R, Shukla J, Saxena R. Propagation of *Bacopa monnieri* (BRAHMI): important medicinal plant. *CIBTech J Biotechnol.* 2016; 5(3): 17–23.

[23] Wong JX, Ramli S. Antimicrobial activity of different types of *Centella asiatica* extracts against foodborne pathogens and food spoilage microorganisms. *LWT*. 2021; 142: 1–6.

[24] Shafizadeh gerdkohi H, Ramezani V, Eslahi E, Emami A, Ranjbar AM, Tavakoli F. Effect of hydroalcoholic extract of *Oliveria decumbens* Vent on diarrhea and symptoms of morphine withdrawal syndrome in mice. *Sci J Kurdistan Uni Med Sci.* 2021; 26(3): 1–12.

[25] Slinkard K, Singleton VL. Total phenol analysis: automation and comparison with manual methods. *Am J Enol Vitic.* 1977; 28(1): 49–55.

[26] Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: a review. *J Pharm Anal*. 2016; 6(2): 71–79.

[27] Moussa SH, Tayel AA, Al-Hassan AA, Farouk A. Tetrazolium/formazan test as an efficient method to determine fungal chitosan antimicrobial activity. *J Mycology*. 2013; Article ID 753692.

[28] Vazirian M, Hamidian KH, Noorollah M, Manayi A, Samadi N. Enhancement of antibiotic activity and reversal of resistance in clinically isolated methicillin-resistant *Staphylococcus aureus* by *Trachyspermum ammi* essential oil. *Res J Pharmacogn*. 2019; 6(1): 1–10.

[29] Bhatia P, Sharma A, George AJ, Anvitha D, Kumar P, Dwivedi VP, Chandra NS. Antibacterial activity of medicinal plants against ESKAPE: an update. *Heliyon*. 2021; 7(2): 1–12.

[30] Alam MZ, Ahmad Khan MS. Phytomedicine from Middle Eastern countries: an alternative remedy to modern medicine against *Candida* spp. infection. *Evid Based Complement Altern Med.* 2021; Article ID 6694876.

[31] Jafari B, Jafari Sales A, Khaneshpour H, Fatemi S, Pashazadeh M, Al-Snafi AE, Shariat A. Ntibacterial effects of *Thymus vulgaris*, *Mentha pulegium*, *Crocus sativus* and *Salvia officinalis* on pathogenic bacteria: a brief review study based on Gram- positive and Gram-negative bacteria. *Jorjani Biomed J*. 2020; 8(3): 58–74.

[32] Stefanović OD, Stanojević DD, Comić LR. Synergistic antibacterial activity of *Salvia officinalis* and *Cichorium intybus* extracts and antibiotics. *Acta Pol Pharm*. 2012; 69(3): 457–463.

[33] Amirian F, Kazemi Pour N, Khoshroo SMR, Sayadi A, Karmostaji A, Mousavi SM. Synergistic effect and antibacterial activities of extracts of *Salvia* and *Rosemary officinalis* against *Esherichia coli* isolated from clinical urinary tract infection. *Ann Mil Health Sci Res*. 2017; 15(4): 1–7.

[34] Bandian L, Moghaddam M, Bahraini M. Investigate the antimicrobial activity and synergistic effects of *Zataria multiflora*, *Salvia verticillata* and *Froriepia subpinnata* ethanolic extracts on bacterial vegetables decay. *J Food Microbiol.* 2021; 8(1): 45–57.

[35] Fazlul MKK, Deepthi SP, Mohammed I, Farzana Y, Munira B, Nazmulmhm MHM. Antibacterial and antifungal activity of various extract *Bacopa monnieri*. *Int J Pharm Res*. 2019; 11(1): 2–25.

Abbreviations

MRSA: methicillin-resistant *Staphylococcus aureus*; MIC: minimum inhibitory concentrations; CLSI: Clinical and

Laboratory Standards Institute; FIC: fractional inhibitory concentration; DMSO: dimethyl sulfoxide; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; ATCC: American Type Culture Collection; TSB: trypticase soy broth; MHA: Mueller-Hinton agar; MHB: Muller-Hinton broth