Jundishapur J Nat Pharm Prod. In Press(In Press):e63640. doi: 10.5812/jjnpp.63640.

Published online 2019 June 26. **Research Article**

Phytochemical Properties and Antibacterial Effects of *Salvia multicaulis* Vahl., *Euphorbia microsciadia* Boiss., and *Reseda lutea* on *Staphylococcus aureus* and *Acinetobacter baumanii*

Majid Asadi-Samani [1, 2], Mansoor Khaledi [3], Fatemeh Khaledi [3], Saeed Samarghandian [4] and Abolfazl Gholipour [5, *]

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

[*]*Corresponding author*: Medical Plants Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Rahmatiyeh, Shahrekord, Iran. Email: gholipour_abolfazl@yahoo.com

Received 2017 November 04; **Revised** 2019 March 05; **Accepted** 2018 April 30.

Abstract

Background: Plants have long served as a rich source of drugs. Given some microorganisms' acquisition of resistance to the current antibiotics, there is a need for discovering new drugs.
Objectives: The aim of the present study was to investigate the phytochemical properties and antibacterial effects of *Salvia multicaulis* Vahl., *Euphorbia microsciadia* Boiss., and *Reseda lutea* against *Acinetobacter baumanii* and *Staphylococcus aureus*.
Methods: In this experimental study, hydroalcoholic (ethanol 70%) plant extracts were prepared by maceration. Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) were determined by CLSI broth microdilution and Müller-Hinton agar assay for each sample, respectively. Total phenolic content was measured by Folin-Ciocalteu colorimetric assay and expressed in terms of gallic acid equivalent and total flavonoid content by aluminum chloride colorimetric method and in terms of rutin equivalent.
Results: Findings showed that 1, 4, and 1 mg/mL were derived as MICs and 4, 16, and 8 mg/mL as MBCs for *S. multicaulis* Vahl., *E. microsciadia* Boiss., and *R. lutea*, respectively, against *S. aureus*; 2, 8, and 2 mg/mL were derived as MICs and 16, 32, and 16 mg/mL as MBCs for *S. multicaulis* Vahl. *R. lutea*, and *E. microsciadia* Boiss., respectively, against *A. baumanii*. In addition, *E. microsciadia* Boiss. and *S. multicaulis* Vahl. were found to contain the highest total phenolic and flavonoid content, respectively.
Conclusions: The studied plants that were collected from Chaharmahal and Bakhtiari Province can be used to produce antibiotics due to their phenols and flavonoids and exert antibacterial effects on the studied bacteria.

Keywords: Medicinal Plants, Drug Resistance, Minimum Inhibitory Concentration, Minimum Bactericidal Concentration, Phytochemistry

1. Background

Evidence indicates that the antibiotic resistance of bacteria has increased (1). *Acinetobacter baumanii* and *Staphylococcus aureus* are two particularly ubiquitous bacteria. *A. baumanii* is a Gram-negative and non-glucose fermenting coccobacillus that is one of the main common causes of nosocomial infections because of high levels of drug resistance (2). In Chile, Carbapenem resistance of *A. baumanii* in the ICU was reported to be up to 70% (3). A study in 2012 showed that the resistance of *A. baumanii* to Imipenem and Meropenem in the ICU-admitted patients was 76% and 80.2%, respectively (4). A study in Ohio, USA showed that the mortality was 70% because of resistant *A. baumanii* infection and the mortality only 25% because of non-resistant *A. baumanii* infection (5). Moreover, *Staphylococcus aureus* is one of the life-threatening pathogens for the human that can cause a wide spectrum of diseases, ranging from skin infections to respiratory and urinary tract infections. This bacterium can cause certain life-threatening diseases such as endocarditis, toxic shock syndrome toxin, and osteomyelitis (6). Asia is addressed as one of the regions with the highest prevalence of methicillin-resistant *S. aureus* (MRSA) worldwide, and vancomycin-resistant *S.*

aureus strains have been reported in certain Asian countries as well (7). The latest findings have shown that the prevalence of MRSA is 73% of the clinical samples collected from the hospitals in Taiwan (8).

Given the reported extensive drug resistance, there is a need for developing and using new antibacterial drugs that do not lead to drug resistance and also have efficient therapeutic effects. In this regard, the use of medicinal plants is an approach to discover new drugs (9-11). In addition, the medicinal plants that are collected from the regions with different climates may have different amounts of active compounds and exert biological activities of different degrees (12,13). In this regard, the aim of this study was to investigate the phytochemical properties and antibacterial effects of *Salvia multicaulis* Vahl., *Euphorbia microsciadia* Boiss., and *Reseda lutea*, collected from Chaharmahal and Bakhtiari province, against *A. baumanii* and *S. aureus*.

To date, 1000 plant species from the Salvia genus of the subfamily Nepetoideae and the family Lamiaceae have been identified, of which 56 are native to Iran (14). *Salvia multicaulis*Vahl. grows mainly in Central and East Asia (15). The main compounds of this plant include 1,8-cineole, α-pinene, and camphor (16). The pharmaceutical effects of this plant include anti-inflammatory, antimicrobial, and analgesic effects (15). *Euphorbia microsciadia* Boiss is from the family of Euphorbiacea that is one of the largest families of flowering plants, including over 300 genera and 5000 species (17). In relevant textbooks, *E. microsciadia* has been reported to have anti-anxiety, analgesic, antipyretic, and antimicrobial effects (18). *Reseda lutea* L., also known as *Reseda vulgaris*, is a member of the family Resedaceae (19). *R. lutea* has been reported to have cytotoxic, antitumor, anti-HIV, antibacterial, and anti-inflammatory effects (19, 20). This plant contains benzyl isothiocyanate and 2-(α-L-rhamnopyranosyloxy) benzyl isothiocyanate that has cytotoxic effects (19).

2. Methods

2.1. Collecting Plants

The plants (*E. microsciadia*, *S. multicaulis* Vahl. and *R. lutea*) were collected from different regions of Chaharmahal and Bakhtiari Province (in Iran) such as Saman, Shahrekord, and Teshniz between late March 2016 and late September 2016 and then identified as the plants of interest by a botanist (Dr. Shirmardi) at the Research Center of the Agricultural Jihad Organization of Chaharmahal and Bakhtiari province.

2.2. Extraction

Extraction was conducted by maceration of the aerial parts of *E. microsciadia* and *S. multicaulis* Vahl. and the seeds of *R. lutea* in triplicate (each time for 72 hours). In this method, water and butyric acid-free bitter ethanol at 30/70 ratio were used (ethanol 70%). The resulting extract was filtered using a filter paper and evaporated under next-to-vacuum pressure and 40°C by a rotary evaporator to concentrate. The resulting solution was stored at -20°C until later use.

2.3. Preparing Different Dilutions of Extract

Ninety-six mg of each extract was weighed using a digital scale and stock solutions of the extracts prepared using dimethyl sulfoxide (DMSO; Sigma, USA) and distilled water. Then different dilutions (0.25, 0.5, 1, 2, 4, 8, and 16 mg/mL) of the extracts were prepared using Mueller-Hinton agar. The maximum concentration of DMSO was 0.2% in the final concentration (21, 22).

2.4. Preparing Standard Bacterial Strains

S. aureus strain (ATCC 12923) and *A. baumanii* strain (PTCC 1855) were purchased as lyophilized from Iranian Research Organization for Science and Technology.

2.5. Preparing Microbial Suspension

To prepare a microbial suspension equivalent to 0.5 McFarland standard (1.5×10^8 CFU/mL), a 24-hour culture of the bacteria was performed on blood agar, and then a suspension with 0.5 McFarland turbidity prepared in normal saline.

2.6. Determining Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs)

The antimicrobial effects of the extracts were determined by broth microdilution in a sterile 96-well plate (SPL life science; Korea) with reference to 0.5 McFarland standard (1.5×10^8 CFU/mL). In this method, the first well was considered negative control (culture medium + the extract) and the second well positive control (culture medium + the bacterium). After adding 95 μL culture medium and 100 μL extracts to microplate wells and diluting them, we incubated the samples at 37°C for 24 hours. The concentration of the last (most diluted) well without turbidity was considered MIC (23). To determine MBC, we subsequently performed a culture of the samples of each well at 10 μL on Mueller-Hinton agar and incubated them at 37°C for 24 hours. The lowest concentrations of the extract in which the bacteria could not grow were considered MBCs. The tests to determine the MICs and MBCs were conducted in triplicate (24).

2.7. Determining Total Phenolic and Flavonoid Content

Total phenolic content was measured by Folin-Ciocalteu colorimetric assay and expressed in terms of gallic acid equivalent and total flavonoid content by aluminum chloride colorimetric method and in terms of rutin equivalent (25-27).

For total phenolic content, 0.1 mL of extract was transferred to a test tube, and 0.5 mL of Folin-Ciocalteu reagent was added and mixed gently. After 5 minutes incubation, 0.4 mL of 7.5% (w/v) sodium carbonate was added to the mixture. The mixture was allowed to stand at room temperature for 30 minutes. UV-vis absorption measurements were carried out at 765 nm using a spectrophotometer (UNICO 2100: USA). The standard calibration curve was plotted using gallic acid in methanol. The TPC was expressed as gallic acid equivalents (mg GAE/g dry weight of the extract).

For flavonoid content, 0.5 mL of the extract was transferred to a test tube and mixed with 1.5 mL of methanol. Then 0.5 mL of 2% aluminum chloride (AlCl3) and 3 mL of 5% potassium acetate were added to the extract. After 40 minutes incubation, UV-vis absorption measurements were carried out at 415 nm using a spectrophotometer (UNICO 2100: USA). The standard calibration curve was plotted using rutin in methanol.

4. Results

The *S. aureus* was susceptible to *S. multicaulis* and inhibited at 1 mg/mL, but was resistant to 0.5 mg/mL of this extract. Therefore, the most acceptable MIC of this plant against *S. aureus* was determined 1 mg/mL. On the other hand, this bacterium was completely eliminated by *S. multicaulis* and could not grow at 4 mg/mL concentration. Therefore, the MBC of this plant against *S. aureus* was determined 4 mg/mL. The results on the antimicrobial effect of hydroalcoholic *S. multicaulis* extract on *A. baumanii* showed that this extract at 2 mg/mL could inhibit this bacterium, with 16 mg/mL determined as its MBC for *A. baumanii*. Furthermore, the MIC and MBC of *E. microsciadia* for *S. aureus* were determined 4 mg/mL and 16 mg/mL, respectively, and the MIC and MBC of this plant for *A. baumanii* 8 mg/mL and 32 mg/mL, respectively; the MIC and MBC of *R. lutea* for *S. aureus* were determined 1 mg/mL and 2 mg/mL, respectively, and the MIC and MBC of this plant for *A. baumanii* 8 mg/mL and 32 mg/mL, respectively (Tables 1 and 2).

According to Table 3, all three plants contained phenols and flavonoids. *E. microsciadia* and *S. multicaulis* contained the highest total phenolic and flavonoid contents, respectively (Table 3).

5. Discussion

This study was conducted to investigate the phytochemical properties and antibacterial effects of S.multicaulis, *E. microsciadia*, and *R. lutea* against *A. baumanii* and *S. aureus*, showing that these plants can be considered alternatives for developing new antibiotics against some microorganisms that are resistant to routine drugs, owing to phenols and flavonoids and exerting antibacterial effects.

Our results showed that *S. multicaulis* could exert inhibitory and bactericidal effects on both *S. aureus* and *A. baumanii* but these effects on *S. aureus* were more potent. The flavonoid content of this plant was also higher than the other two plants. The study of Akin et al. to investigate the antimicrobial effects of *Salvia cryptantha* and *Salvia heldreichiana*, revealed that these two species did not exert any inhibitory effect on *S. aureus* (28). Inconsistently, our study with *S. multicaulis* showed that this plant exerted a potent inhibitory effect on this bacterium. The study of Alim et al. indicated that methanol *Salvia cedronella* extract had inhibitory effects on *S. aureus* and *B. cereus*, with an MIC of 31.25 μg/mL, which is in agreement with our study. Alim et al. attributed their observations about the inhibitory properties to the high concentrations of phenols and flavonoids in *S. cedronella* (29). Fazly Bazzaz et al. reported that Salvia chloroleuca exerted a partial inhibitory effect on *S. aureus*, while *Salvia ceratophylla*, *Salvia lourifolia*, *Salvia limbata*, and *Salvia macrosiphone* had no inhibitory effect on this bacterium (30).

Our results were promising regarding the antimicrobial effects of *E. microsciadia* to inhibit *S. aureus* and *A. baumanii* thus its MIC and MBC for *S. aureus* were determined 4 mg/mL and 16 mg/mL and for *A. baumanii* 8 mg/mL and 32 mg/mL, respectively. Also, *E. microsciadia* contained comparatively higher concentrations of phenols and flavonoids. The study of Eshraghi et al. investigated the effects of 10 plant species on the Nocardia genus, showed that *Euphorbia denticulate* could inhibit the growth of *Nocardia asteroides* and *Nocardia brasiliensis* (31). Kaveh et al. observed that the species of *Euphorbia* were rich sources of flavonoids, and *E. microsciadia* contained abundant phenols such as kaempferol (32).

Moreover, *R. lutea* was found to exert antimicrobial effects on *S. aureus* and *A. baumanii*. This plant was also found to contain phenols and flavonoids. The study of Boroumand et al. showed that *R. lutea*-assisted synthesis of silver nanoparticles led to the inhibition of *Escherichia coli* in vitro (33). Benmerache et al. reported that chloroform *Reseda phyteuma* L. extract had inhibitory effects on *S. aureus*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* so that its MIC for all three bacteria was 80 μg/mL and the

Table 1. The Minimum Inhibitory Concentrations and Minimum Bactericidal Concentrations of the Hydroalcoholic Extracts of Studied Plants for *Staphylococcus aureus*

Plants, Effect	Concentration (mg/mL)[a]							
	0.25	0.5	1	2	4	8	16	32
***Salvia multicaulis* Vahl**[b]								
MIC	-	-	+	+	+	+	+	+
MBC	-	-	-	-	+	+	+	+
***Euphorbia microsciadia* Boiss.**[b]								
MIC	-	-	-	-	+	+	+	+
MBC	-	-	-	-	-	-	+	+
Reseda lutea[c]								
MIC	-	-	+	+	+	+	+	+
MBC	-	-	-	-	-	+	+	+

[a](+), Lack of microorganism growth in culture medium and the antimicrobial activity of the hydroalcoholic (ethanol 70%) extracts of plants; (-), Microorganism growth in culture medium and lack of antimicrobial activity of the hydroalcoholic extracts of plants.
[b]The extraction of aerial parts.
[c]The extraction of seeds.

Table 2. The Minimum Inhibitory Concentrations and Minimum Bactericidal Concentrations of the Hydroalcoholic Extracts of Studied Plants for *Acinetobacter baumanii*

Plants, Effect	Concentration (mg/mL)[a]							
	0.25	0.5	1	2	4	8	16	32
***Salvia multicaulis* Vahl**[b]								
MIC	-	-	-	+	+	+	+	+
MBC	-	-	-	-	+	-	+	+
***Euphorbia microsciadia* Boiss.**[b]								
MIC	-	-	-	-	+	+	+	+
MBC	-	-	-	-	-	-	-	+
Reseda lutea[c]								
MIC	-	-	-	+	+	+	+	+
MBC	-	-	-	-	-	-	+	+

[a](+), Lack of microorganism growth in culture medium and the antimicrobial activity of the hydroalcoholic (ethanol 70%) extracts of plants; (-), Microorganism growth in culture medium and lack of antimicrobial activity of the hydroalcoholic extracts of plants.
[b]The extraction of aerial parts.
[c]The extraction of seeds.

Table 3. Total Phenolic and Flavonoid Content of the Studied Plants[a]

Plants	Used Organ	Total Phenolic Content mg GAE/g DW	Flavonoid Content mg RU/g DW
***Euphorbia microsciadia* Boiss**	Shoot	69.56 ± 6.75	24.39 ± 1.38
***Salvia multicaulis*Vahl.**	Shoot	63.96 ± 5.11	26.74 ± 2.24
***Reseda lutea* L.**	Seeds	65.32 ± 3.72	21.93 ± 2.67

[a]Values are expressed as mean ± SD.

inhibition zone diameters for *P. mirabilis*, *S. aureus*, and P. aeruginosa were 13 mm, 12 mm, and 11 mm, respectively (34), while the study of Yildirim et al. in Turkey showed that *R. lutea* showed no antimicrobial effect on *S. aureus*. That study also demonstrated that this plant could not exert any antimicrobial effect on *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Serratia marcescens*, *Salmonella typhimurium*, *P. aeruginosa*, *Proteus vulgaris*, and *E. coli*, meanwhile had a partial inhibitory effect on *Klebsiella pneumoniae* (35).

The concentrations of bioactive compounds in a medicinal plant may vary and, therefore, exhibit various biological effects depending on the location and time of its collection as well as its part of use (12,13). The inconsistency in the findings of our study and others' can be attributed to different concentrations of the bioactive compounds due to the difference in the occurrence locations of the studied plants as well as their parts of use. The plants that were investigated in the current study and collected from Chaharmahal and Bakhtiari province could pave

the way for producing antibiotics due to their phenols and flavonoids and exerting antibacterial effects on the studied bacteria.

Acknowledgments

This article is a product of a research project approved by the Shahrekord University of Medical Sciences. This project is registered in the department of research and technology of the Shahrekord University of Medical Sciences under the code 2402-77-01-1393. We thank everybody who helped us in this research project.

Footnotes

Conflict of Interests: It Was not declared by the authors.

Funding/Support: Shahrekord University of Medical Sciences.

References

1. Bagheri N, Azadegan-Dehkordi F, Rafieian-Kopaei M, Rahimian G, Asadi-Samani M, Shirzad H. Clinical relevance of Helicobacter pylori virulence factors in Iranian patients with gastrointestinal diseases. *Microb Pathog*. 2016;**100**:154–62. doi: 10.1016/j.micpath.2016.09.016. [PubMed: 27666510].
2. Zarrilli R, Pournaras S, Giannouli M, Tsakris A. Global evolution of multidrug-resistant Acinetobacter baumannii clonal lineages. *Int J Antimicrob Agents*. 2013;**41**(1):11–9. doi: 10.1016/j.ijantimicag.2012.09.008. [PubMed: 23127486].
3. Rivera G, Bulnes J, Castillo C, Ajenjo MC, Garcia P, Labarca J. Extensively drug-resistant Acinetobacter baumannii isolated in a university hospital: Role of inter-hospital transmission. *J Infect Dev Ctries*. 2016;**10**(1):96–9. doi: 10.3855/jidc.6713. [PubMed: 26829543].
4. Labarca JA, Salles MJ, Seas C, Guzman-Blanco M. Carbapenem resistance in Pseudomonas aeruginosa and Acinetobacter baumannii in the nosocomial setting in Latin America. *Crit Rev Microbiol*. 2016;**42**(2):276–92. doi: 10.3109/1040841X.2014.940494. [PubMed: 25159043].
5. Spellberg B, Bonomo RA. The deadly impact of extreme drug resistance in Acinetobacter baumannii. *Crit Care Med*. 2014;**42**(5):1289–91. doi: 10.1097/CCM.0000000000000181. [PubMed: 24736340]. [PubMed Central: PMC4184139].
6. Otto M. Staphylococcus aureus toxins. *Curr Opin Microbiol*. 2014;**17**:32–7. doi: 10.1016/j.mib.2013.11.004. [PubMed: 24581690]. [PubMed Central: PMC3942668].
7. Chen CJ, Huang YC. New epidemiology of Staphylococcus aureus infection in Asia. *Clin Microbiol Infect*. 2014;**20**(7):605–23. doi: 10.1111/1469-0691.12705. [PubMed: 24888414].
8. Lai CC, Lin SH, Sheng WH, Hsueh PR. Decrease in the incidence of meticillin-resistant Staphylococcus aureus nosocomial bloodstream infections in Taiwan. *Int J Antimicrob Agents*. 2013;**41**(6):591–2. doi: 10.1016/j.ijantimicag.2013.02.002. [PubMed: 23507412].
9. Kalantari H, Larki A, Latifi SM. The genotoxicity study of garlic and pasipy herbal drops by peripheral blood micronucleus test. *Acta Physiol Hung*. 2007;**94**(3):261–66. doi: 10.1556/APhysiol.94.2007.3.10. [PubMed: 17853777].
10. Asadi-Samani M, Moradi MT, Mahmoodnia L, Alaei S, Asadi-Samani F, Luther T. Traditional uses of medicinal plants to prevent and treat diabetes; an updated review of ethnobotanical studies in Iran. *J Nephropathol*. 2017;**6**(3):118–25. doi: 10.15171/jnp.2017.20. [PubMed: 28975089]. [PubMed Central: PMC5607970].
11. Hosseini Z, Lorigooini Z, Rafieian-Kopaei M, Shirmardi HA, Solati K. A review of botany and pharmacological effect and chemical composition of Echinophora species growing in Iran. *Pharmacognosy Res*. 2017;**9**(4):305–12. doi: 10.4103/pr.pr_22_17. [PubMed: 29263622]. [PubMed Central: PMC5717781].
12. Asadi-Samani M, Rafieian-Kopaei M, Lorigooini Z, Shirzad H. A screening to determine total phenol and flavonoid content of some iran's medicinal plants grown in chaharmahal va Bakhtyari province. *Indian J Nat Prod Resour*. 2018;**9**(4):296–302.
13. Asadi-Samani M, Rafieian-Kopaei M, Lorigooini Z, Shirzad H. A screening of anti-breast cancer effects and antioxidant activity of twenty medicinal plants gathered from Chaharmahal va Bakhtyari province, Iran. *J Pharm Pharmacogn Res*. 2019;**7**(3):213–22.
14. Kharazian N. Chemotaxonomy and flavonoid diversity of Salvia L.(Lamiaceae) in Iran. *Acta Bot Bras*. 2014;**28**(2):281–92. doi: 10.1590/s0102-33062014000200015.
15. Salimikia I, Aryanpour M, Bahramsoltani R, Abdollahi M, Abdolghaffari AH, Samadi N, et al. [Phytochemical and wound healing effects of methanolic extract of Salvia multicaulis vahl. In rat]. *J Med Plant*. 2016;**1**(57):38–46. Persian.
16. Mohammadhosseini M, Pazoki A, Akhlaghi H. Chemical composition of the essential oils from flowers, stems, and roots of Salvia multicaulis growing wild in Iran. *Chem Nat Comp*. 2008;**44**(1):127–8. doi: 10.1007/s10600-008-0039-3.
17. ELhassan GOM, Adhikari A, Abdalla OM, Shukrulla A, Khalid A, Choudhary MI, et al. Chemical constituents of euphorbia polyacantha Boiss. and their immunomodulatory properties. *Record Nat Prod*. 2015;**9**(1):146.
18. Nasiri Semnani S, Rahnema M, Alizadeh H, Ghasempour H. Evaluation of antimicrobial effects of euphorbia cyparissias extracts on intra macrophages Salmonella typhi. *J Biol Active Prod Nature*. 2013;**3**(1):64–71. doi: 10.1080/22311866.2013.782751.
19. Radulovic NS, Zlatkovic DB, Ilic-Tomic T, Senerovic L, Nikodinovic-Runic J. Cytotoxic effect of Reseda lutea L.: A case of forgotten remedy. *J Ethnopharmacol*. 2014;**153**(1):125–32. doi: 10.1016/j.jep.2014.01.034. [PubMed: 24509155].
20. Bedoya LM, Sanchez-Palomino S, Abad MJ, Bermejo P, Alcami J. Anti-HIV activity of medicinal plant extracts. *J Ethnopharmacol*. 2001;**77**(1):113–6. [PubMed: 11483387].
21. Moradi MT, Karimi A, Alidadi S, Hashemi L. Anti-adenovirus activity, antioxidant potential, and phenolic content of dried flower buds of Syzygium aromaticum extract in HEp2 cell line. *Marmara Pharmaceut J*. 2017;**21**(4):852–9. doi: 10.12991/mpj.2017.4.
22. Moradi MT, Karimi A, Fotouhi F, Kheiri S, Torabi A. In vitro and in vivo effects of Peganum harmala L. seeds extract against influenza A virus. *Avicenna J Phytomed*. 2017;**7**(6):519–30. [PubMed: 29299435]. [PubMed Central: PMC5745536].
23. Khaledi M, HeidariSureshjani R, Gholipour A, Mardanpour Shahrekordi E, RoohiBroojeni H. [Study of the antimicrobial effects of the hydroalcoholic extract of Teucriumchamaedryson the bacteria Streptococcus mutansinvitro]. *J Shahrekord Univ Med Sci*. 2016;**17**(6):61–7. Persian.
24. National Committee for Clinical Laboratory Standards. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved Standards. NCCLS Document M7-A5*. Wayne: National Committee for Clinical Laboratory Standards; 2001.
25. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol*. 1999;**299**:152–78. doi: 10.1016/s0076-6879(99)99017-1.

26. Dulf FV, Vodnar DC, Socaciu C. Effects of solid-state fermentation with two filamentous fungi on the total phenolic contents, flavonoids, antioxidant activities and lipid fractions of plum fruit (Prunus domestica L.) by-products. *Food Chem*. 2016;**209**:27–36. doi: 10.1016/j.foodchem.2016.04.016. [PubMed: 27173530].
27. Karimi A, Moradi MT. Total phenolic compounds and in vitro antioxidant potential of crude methanol extract and the correspond fractions of Quercus brantii L. acorn. *J Herb Med Pharmacol*. 2015;**4**(1):35–9.
28. Akin M, Demirci B, Bagci Y, Baser KHC. Antibacterial activity and composition of the essential oils of two endemic Salvia sp. from Turkey. *Afr J Biotechnol*. 2010;**9**(15):2322–7.
29. Alim A, Goze I, Goze HM, Tepe B, Serkedjieva J. In vitro antimicrobial and antiviral activities of the essential oil and various extracts of Salvia cedronella Boiss. *J Med Plant Res*. 2009;**3**(5):413–9.
30. Bazzaz BS, Haririzadeh G. Screening of Iranian plants for antimicrobial activity. *Pharmaceut Biol*. 2003;**41**(8):573–83. doi: 10.1080/13880200390501488.
31. Eshraghi S, Amin GH, Othari A. Evaluation of antibacterial properties and review of 10 medicinal herbs on preventing the growth of pathogenic Nocardia species. *J Med Plant*. 2009;**4**(32):60–78.
32. Kaveh M, Noori M. Fruit flavonoids of some species of subgenera esula and chamaesyce (Euphorbia) in Iran. *Int J Mod Bot*. 2014;**4**(1):1–7. doi: 10.5923/j.ijmb.20140401.01.
33. Nasiri Boroumand M, Montazer M, Dutschk V. Biosynthesis of silver nanoparticles using Reseda Luteola L. and their antimicrobial activity. *Ind Text*. 2013;**64**(3):123–78.
34. Benmerache A, Berrehal D, Khalfallah A, Kabouche A, Semra Z, Kabouche Z. Antioxidant, antibacterial activities and flavonoids of Reseda phyteuma L. *Pharm. Lett*. 2012;**4**:1878–82.
35. Yildirim AB, Karakas FP, Turker AU. In vitro antibacterial and antitumor activities of some medicinal plant extracts, growing in Turkey. *Asian Pac J Trop Med*. 2013;**6**(8):616–24. doi: 10.1016/S1995-7645(13)60106-6. [PubMed: 23790332].