



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

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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 baumannii* 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. baumannii*. 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 baumannii* and *Staphylococcus aureus* are two particularly ubiquitous bacteria. *A. baumannii* 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. baumannii* in the ICU was reported to be up to 70% (3). A study in 2012 showed that the resistance of *A. baumannii* 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. baumannii* infection and the mortality only 25% because of non-resistant *A. baumannii* 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. baumannii* 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 Euphorbiaceae 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. baumannii* 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 μ L culture medium and 100 μ L 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 μ L 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. baumannii* showed that this extract at 2 mg/mL could inhibit this bacterium, with 16 mg/mL determined as its MBC for *A. baumannii*. 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. baumannii* 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. baumannii* 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. baumannii* 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. baumannii* 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 hel-dreichiana*, 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. baumannii* thus its MIC and MBC for *S. aureus* were determined 4 mg/mL and 16 mg/mL and for *A. baumannii* 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. baumannii*. 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
<i>Salvia multicaulis</i> Vahl^b								
MIC	-	-	+	+	+	+	+	+
MBC	-	-	-	-	+	+	+	+
<i>Euphorbia microsciadia</i> Boiss.^b								
MIC	-	-	-	-	+	+	+	+
MBC	-	-	-	-	-	-	+	+
<i>Reseda lutea</i>^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.

^bThe extraction of aerial parts.

^cThe extraction of seeds.

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

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

^bThe extraction of aerial parts.

^cThe 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
<i>Euphorbia microsciadia</i> Boiss	Shoot	69.56 ± 6.75	24.39 ± 1.38
<i>Salvia multicaulis</i> Vahl.	Shoot	63.96 ± 5.11	26.74 ± 2.24
<i>Reseda lutea</i> L.	Seeds	65.32 ± 3.72	21.93 ± 2.67

^aValues 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.

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Footnotes

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

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