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The antibacterial effects of wild and cultivated *Allium hirtifolium* Boiss on *Pseudomonas aeruginosa* and *Enterococcus faecalis* and antibiotic resistance patterns of the strains using disk diffusion

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ABSTRACT

The widespread use of antibiotics has recently led to increased drug resistance in most bacteria. The studies on antimicrobial properties of plant species, especially endemic plants, can help to use nature-based drugs, with greater efficacy, to control and treat bacterial infections.This study was conducted to investigate and compare the antimicrobial effects of wild and cultivated *Allium hirtifolium* Boiss on *Pseudomonas aeruginosa* and *Enterococcus faecalis*, and to determine the antibiotic patterns of the strains. Extraction was done by maceration and minimum inhibitory concentration (MIC) tested by Broth Microdilution.To determine minimum inhibitory concentration, the wells without opacity were cultured separately on Mueller-Hinton agar. As well, the antibiotic susceptibility of the isolated strains was investigated by Kirby-Bauer disk diffusion susceptibility test with reference to amikacin, gentamicin, vancomycin, and penicillin. The findings demonstrated that the lowest MIC and the highest MBC were obtained for cultivated *A. hirtifolium* leaf extract on *E. faecalis* and *P. aeruginosa*, respectively. Moreover, *E. faecalis* was found to have the highest antibiotic resistance to penicillin with a 12-mm inhibition zone diameter.

KEY WORDS: *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Allium hirtifolium* Boiss, Antibiotic resistance pattern.

1. INTRODUCTION

In the recent years, the excessive use of antimicrobial drugs has led to increased antibiotic resistance in most bacteria (Taghvaee, 2013). Resistance to common antibiotics is an old issue. Antibiotic resistance occurs usually as a mutation, and because chromosomal mutations are much more frequently seen in bacteria than other organisms, microbes are constantly changing (Tabatabaei Yazdi, 2014). Nowadays, nosocomial infections are a well known medical issue in developing and developed countries. *Pseudomonas aeruginosa* and *Enterococcus faecalis* are considered two common causes of nosocomial infections, especially in wound wards (Adabi, 2015). *P. aeruginosa* is widely distributed in the nature and considered an opportunisic pathogen for human beings, which can cause a broad range of diseases including urinary tract infections, wound-induced infections, and respiratory infections. As well, *P. aeruginosa* is a primary cause of ventilator-associated pneumonia in ICUs, which may lead to irreparable consequences in the people who need to have longer stays in hospital(Lanini, 2011; Qiu, 2007). This bacterium has various mechanisms of antibiotic resistance including drug non-enzymatic deactivation, change in the drug target, and antibiotics exogenous systems (Hauser, 2005). Furthermore, *P. aeruginosa* is intrinsically resistant to many antimicrobial and disinfectant substances such as ammonium, hexachlorophene, and iodized solutions, such that the emergence of the strains with multidrug resistance is considered a main problem facing treatments for *P. aeruginosa*. Enterococci are gram-positive and catalase-negative cocci that are able to grow in the presence of 6% salt and 40% bile salts. These bacteria are considered natural intestinal microflora. *E. faecalis* (85-90%) and *Enterococcus faecium* (5-10%) are the most common Enterococci involved in human infections. These two bacteria can cause urinary tract infections, wound infections, and even endocarditis (Cetinkaya, 2000). Yet, the greater importance attached to these microorganisms is related to their intrinsic resistance to many widely used antibiotics (oxacilin and cephalosporins) or having genes with acquired resistance (to aminoglycosides and vancomycin). Therefore, the Enterococci that are a constituent of the natural microbial flora of the patients treated with broad-spectrum antibiotics and those with malignancies can proliferate and cause disease in the body of these patients (Murray, 1995). Antimicrobial plant-based compounds have many therapeutic properties, and not only are effective in treating infectious diseases but also can reduce many of the side effects due to antimicrobial agents (Rafieian-kopaei, 2013). In the recent years, many studies have been conducted on the inhibitory effects of nature-based substances on microorganisms, and experimental works have demonstrated that the extracts of some plants can exert strong bactericidal effects on microorganisms (Ghasemi-pirbalouti, 2015). In this regard, the compounds that are non toxic to human beings and cause no side effects seem necessary. Since a wide variety of plants can grow in Iran because of its climatic conditions, investigating antimicrobial properties of different species of plants, especially endemic, can help to use nature-based drugs, with greater efficacy, to control and treat bacterial infections. Regarding that most plants from

family Alliaceae, such as *Allium cepa*, *Allium sativum*, Allium jesdianum and Allium ampeloprasum have antimicrobial properties, the aim of this study is to investigate the antimicrobial effects of *Allium hirtifolium* Boiss, a plant from family Alliaceae, to contribute to the pharmacological data on the antimicrobial properties of this plant. *A. hirtifolium* is a perennial plant from family Alliaceae and native to Iran, and spontaneously occurs in the uplands and mountainous regions.The diameter of *A. hirtifolium* is 2.5-4 cm. It has grey, thin, and ridged peels and a bare stem of 80-120 cm in height (Mozaffarian, 2011). *A. hirtifolium* is an appetizer and is effective in strengthening gastrointestinal tract. Linolenic acid, linoleic acid, palmitic acid, palmitoleic acid, stearic acid, and oleic acid are the main fatty acids of *A. hirtifolium*. *A. hirtifolium* has a special taste, and the slices of its dried bulb are used as additives to yoghurt and pickles (Salunkhe, 1998; Ebrahimi, 2008). Given the side effects due to chemical drugs and increased multidrug resistance, this study is conducted to investigate the antimicrobial effects of wild and cultivated *A. hirtifolium* on *P. aeruginosa* and *E. faecalis* and to determine the antibiotic resistance patterns of these strains.

2. MATERLAL and METHODS

First, necessary investigations were conducted on reliable references to select the region of the study. We selected to gather the study samples from Kouhrang region, Chaharmahal va Bakhtiari in mid-spring, 2015. The samples were transferred to the Medicinal Plants Research Laboratory of the Islamic Azad University of Shahrekord Branch and *A. hirtifolium* samples were confirmed and assigned the herbarium no. 1265 according to the botanical keys and the flora of Iran and also with reference to the herbarium samples of Chaharmahal va Bakhtiari Research Center for Agriculture and Natural Resources.The cultivated plants were harvested before flowering. Then, the aerial organs and the bulbs of the cultivated and wild *A. hirtifolium* were kept separately in shadow at 25-30°C for two weeks to dry. The dried plants were pulverized with a laboratory mill. Extraction was done using ethanol 70% and the extracts were concentrated with a rotary at 40°C for eight hours. Then, the concentrated extracts of *A. hirtifolium* leaf and bulb were incubated at 37°C until they were dried. To prepare different dilutions, 4.04 g of each extract, weighed by a digital weight, was dissolved with 1 mL of dimethyl sulfoxide (5%). Then, 16, 32, 64, 128, 256, 512, 1024, and 2045 µg/mL dilutions of the extracts were prepared with Mueller-Hinton Broth medium. *P. aeruginosa* and *E. faecalis* were provided from the Iranian Research Organization for Science and Technology and cultured according to the manufacturer instructions.In the next step, bacterial suspension was prepared by McFarland 0.5 standard (10^5 CFU/mL). To investigate the antimicrobial effects, Broth Microdilution method in 96 sterile wells was adopted. According to this technique, the first well is considered negative control and the second well positive control. After Mueller-Hinton Broth medium and the extract were introduced, the bacteria were introduced into microplate wells, and the samples were incubated at 37°C for 24 hours after dilution. The concentration of the final (most diluted) well with no opacity was considered representative of minimum inhibitory concentration (MIC).To determine minimum bactericidal concentration (MBC), all the wells without opacity were cultured separately on blood agar and incubated at 37°C for 24 hours and the lowest concentration of the extract in which the bacteria could not grow was considered representative of the MBC. To investigate the antibiotic resistance of the strains, Kirby-Bauer disk diffusion test was used. For this, an amount of bacterial colony was taken out and dissolved with sterile physiology serum. After a homogeneous solution was prepared and mixed using a sterile swab, it was transferred to the Mueller-Hinton agar medium. After culture, the antibiogram disks, gentamicin (10 µ/g), amikacin (30 µ/g), vancomycin (39 µ/g), and penicillin (10 µ/g) (PadTan Teb Co., Tehran, Iran) that were taken out of the refrigerator half an hour before test, were transferred on the culture medium with reference to the isolated bacterium, and incubated at 37°C for 24 hours. Then, the diameters of the inhibition zones were measured with a ruler and the results were compared with CLSI.

3. RESULTS and DISCUSSION

Table.1.Results of antimicrobial test of wild *Allium hirtifolium* leaf extract

Standard strains	Gram	MIC µg/mL	MBC µg/mL
Enterococcus faecalis	+	256	128
Pseudomonas aeruginosa	-	1024	512

Wild *A. hirtifolium* leaf extract had the highest MBC on *E. faecalis*, and *P. aeruginosa* was mostly resistant to *A. hirtifolium* leaf extract.

Table.2. Results of antimicrobial test of wild *Allium hirtifolium* bulb extract

Standard strains	Gram	MIC µg/mL	MBC µg/mL
Enterococcus faecalis	+	256	128
Pseudomonas aeruginosa	-	256	1024

Table.3. Results of antimicrobial test of cultivated *Allium hirtifolium* leaf

Standard strains	Gram	MIC µg/mL	MBC µg/mL
Enterococcus faecalis	+	128	64
Pseudomonas aeruginosa	-	512	2048

As Table 2 shows, wild *A. hirtifolium* bulb exerted inhibitory and bactericidal effects on both gram-positive and gram-negative bacteria. *P. aeruginosa* was partly resistant to *A. hirtifolium* bulb extract. *E. faecalis* was highly resistant to cultivated *A. hirtifolium* leaf extract. The highest inhibitory effect was exerted on *E. faecalis* growth. Cultivated *A. hirtifolium* leaf extract exerted the highest bactericidal effect on *P. aeruginosa* (Table 3). In addition, cultivated *A. hirtifolium* bulb extract exerted the highest inhibitory effect on *E. faecalis* growth and the highest bactericidal effect on *P. aeruginosa* (Table 4).

Table.4. Results of antimicrobial test of cultivated *Allium hirtifolium* bulb extract

Standard strains	Gram	MIC µg/mL	MBC µg/mL
Enterococcus faecalis	+	128	64
Pseudomonas aeruginosa	-	512	2048

The inhibitory and bactericidal effects of the extracts of cultivated and wild *A. hirtifolium* leaf and bulb were different, such that wild *A. hirtifolium* leaf and cultivated *A. hirtifolium* bulb extracts exerted the greatest inhibitory effects on *E. faecalis*.Furthermore, the highest inhibitory effect on *E. faecalis* growth was exerted by cultivated *A. hirtifolium* leaf extract. The difference in the MIC and the MBC can be related to the differences in the bacterial strains, the types of culture media, the extraction techniques, the solvents, weather conditions, soil composition, growth conditions, and the seasons of harvesting (Tajkarim, 2010). Therefore, a medicinal plant extract may exert significant effects on an organism but smaller or no effects on other microorganisms. Moreover, some works have demonstrated that gram-negative bacteria are less resistant to plant-based extracts than gram-positive bacteria. This may be due to the difference in the cell wells of gram-positive and gram-negative bacteria (Walter, 2011). A work indicated that *A. hirtifolium* extract had strong antimicrobial effects because of containing phenolic, flavonoid, and antioxidant compounds (Leelarungrayub, 2006). An important explanation of inhibiting bacterial growth by plant-based extracts is the presence of phenolic compounds in these extracts. Phenolic compounds enable the extracts and the essential oils to contribute significantly to decomposing cell and mitochondrial membrane lipids and changing membrane permeability and therefore bacterial cell death. Besides that, the quercetin in plants and their derivatives has been found to cause bacterial growth inhibition through inhibiting DNA gyrase (Tavassoli, 2011). Because quercetin is an important compound of *A. hirtifolium*, we can argue that quercetin is a main cause of inhibiting bacterial growth by *A. hirtifolium* extract. Antibiotics are vitally required to treat many human diseases. However, certain reasons such as the excessive use of antibiotics can lead to antibiotic resistance in bacteria, which has resulted in increasing development of new antibiotics (Dehghan, 2014). In this study, the effect of antibiotic resistance of *E. faecalis* and *P. aeruginosa* was investigated by antibiogram test using disk diffusion. Tables 5 and 6 show the results.

Table.5. Results of *Pseudomonas aeruginosa* resistance patterns to antibiotics

Inhibition zone diameter (mm)	Disk content (mg)	Antibiotics
Pseudomonas aeruginosa ATCC10145		
26 Suceptible	30	Amikacin
24 Suceptible	10	Gentamicin

Table.6. Results of *Enterococcus faecalis* resistance patterns to antibiotics

Inhibition zone diameter (mm)	Disk content (mg)	Antibiotics
Enterococcus faecalis ATCC29212		
18 Suceptible	30	Vancomycin
12 Resistant	10	Penicillin

According to Table 5, the antibiotic resistance pattern obtained in this study represents *P. aeruginosa* susceptibility to two antibiotics used for treatment, amikacin and gentamicin. According to Table 6, *E. faecalis* is resistant to penicillin with the greatest susceptibility to vancomycin. Overall, the studied strains had different resistance patterns to different antibiotics.

These resistance patterns are seen frequently due to the genes localized on mobile genetic elements such as transpozons and integrons and are easily spread among the bacteria (Bou, 2000). Therefore, penicillin cannot be effective in treating infection due to *E. faecalis* . Moreover, the present study demonstrated that *P. aeruginosa* was not resistant to amikacin and vancomycin. An important explanation of this finding can be the less frequent prescription of amikacin and vancomycin by physicians, no constant use and use of these two antibiotics only in life-threatening conditions.

4. CONCLUSION

Overall, this study demonstrated the inhibitory effects of ethanolic wild and cultivated *A. hirtifolium* extracts on the studied bacteria. Interestingly, many internal and external (environmental) factors can contribute to secondary metabolites content, the types of their compounds, and the rates of their production in plants, such that even the phase of the growth of the plant, climatic conditions of the regions where the plant is harvested, and the used tissue or organ of the plant may influence its antimicrobial properties.Moreover, we found that *E. faecalis* was resistant to penicillin and therefore penicillin cannot be used to treat the infection due to *E. faecalis*. Given the positive effects of wild and cultivated *A. hirtifolium*, leaves and bulbs of this plant can be suitable alternatives to synthetic drugs and antibiotics to reduce the side effects of these treatments and drug resistance.

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REFERENCES

Adabi M, Talebi Taher M, Arbabi L, Afshar M, Fathizadeh, Minaeian , Moghadam-Marageh , Majidpour, Determination of Antibiotic Resistance Pattern of *Pseudomonas aeruginosa* Strains Isolated from Patients with Burn Wounds, Journal of Ardabil University of Medical Sciences, 15(1), 2015, 66- 74.

Bou G, Oliver A, Martinez-Beltran J, OXA-24, a novel class D beta-lactamase with carbapenemase activity in an Acinetobacter baumannii clinical strain. Antimicrob Agents Chemother, 44(6), 2000, 1556-61.

Cetinkaya Y, Falk P, Mayhall G, Vancomycin resistance Enterococci. Clin Microbial ReV, 13, 2000, 686-707.

Dehghan Gh, Zarini Gh, Hajizadeh M, Phytochemical investigation and antimicrobial, antifungal and synergistic activities of chloroform fractions of the root of *Ferula szovitsiana*. J Shahrekord Univ Med Sci,15(6), 2014, 10-17.

Ebrahimi R, Zamani Z, Kashi A, Genetic diversity evaluationof wild Persian shallot (*Allium hirtifolium* Boiss.) using morphological and RAPD markers, Scientia Horticulturae 119, 2008, 345–351.

Ghasemi-pirbalouti A , Ahmadzadeh Y, Malekpoor F, Vayiation in antioxidant, and antibacterial activities and total Phenolic content of the bulbs of mooseer (*Allium hirtifolium* Boiss) 2015.

Hauser AR, Sriram P, Severe *Pseudomonas aeruginosa* infections. Tackling the conundrum of drug resistance. Postgrad. Med, 117, 2005, 41–48.

Lanini S, D'Arezzo S, Puro V, Martini L, Imperi F, Piselli P, Molecular epidemiology of a *Pseudomonas aeruginosa* hospital outbreak driven by a contaminated disinfectant-soap dispenser. PLoS One, 16, 6(2), 2011, 17064.

Leelarungrayub N, Rattanapanone V, Chanarat N, Gebicki JM, Quantitativeevaluation of the antioxidant properties of garlic and shallot preparations . nutrition .22(3), 2006, 74-266.

Mozaffarian V, Medicinal and Aromatic Plants recognize Iran. Publications of farhang moaser.Tehran, 2011.

Murray PR, Baron EJ, Pfaller MA, (editors). Manual of clinical microbiology. ASM, Washington D.C, 1995.

Qiu D, Eisinger VM, Rowen DW, Yu HD, Regulated proteolysis controls mucoid conversion in *Pseudomonas aeruginosa*. Proc Natl Acad Sci USA, 104(19), 2007, 8107-8112.

Rafieian-kopaei M, Keshvari M , Asgary S, Salimi M, Heidarian E. Potential role of a nutraceutical spice (Allium hirtifolium) in reduction of atherosclerotic plaques. J HerbMed Pharmacol, 2(2), 2013, 23-28.

Salunkhe D.K and Kadam S.S, Handbook of Vegetable science and Technology. Marcel Dekker,Inc, 1998, 72.

Tabatabaei Yazdi F, Alizade Behbahani B, Heidari Sureshjani M, The comparison of antimicrobial effects of Chevil (Ferulago angulata) extract with a variety of common therapeutic antibiotics in vitro, Arak Medical University Journal (AMUJ) Original Article, 17(84), 2014, 35-46.

Taghvaee R, Shojapour M, Sadeghi A, Pourbabay AA, The Study of Antibiotic Resistance Pattern and the Frequency of Extended-Spectrum Beta-Lactamases (ESBL) in *Pseudomonas aeruginosa* Strains Isolated from Medical Centers in City, Iran. Qom Univ Med Sci J, 7 (4), 2013, 36-41.

Tajkarim MM, Ibrahim SA, Cliver DOAntimicrobial herb and spice compounds in food, Food Control, 21, 2010, 1199-18.

Tavassoli S, Mousavi S M, Emam-Djomeh Z, Comparative Study of the Antimicrobial Activity of Rosmarinus officinalis L. Essential Oil and Methanolic Extract. Middle-East Journal of Scientific Research, 9(4), 2011, 467-71.

Walter C, Shinwari Z, Afzali I, Malik R, Antibacterial activity in herbal products used in Pakistan. Pak J Bot, 43, 2011, 155-62.