



Antimicrobial Effects of Ethanol Extract of *Eremurus persicus* Leaves on *Staphylococcus Aureus* under Laboratory Conditions

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ABSTRACT

Background: The aim of this study was to investigate the antimicrobial effects of ethanol extract of *Eremurus persicus* leaves on *Staphylococcus aureus* under laboratory condition.

Methods: The ethanol extract of paste leaves were collected using a rotary machine. 12 strain of *Staphylococcus aureus* were collected from urinary tract infection of Zabol city (Iran).

Results: The results showed that MIC and also MBC of *Eremurus persicus* ethanol extract against *Staphylococcus aureus* were 5 and 2.55 ppm, respectively.

Conclusion: The results of this study showed that ethanol extract of *Eremurus persicus* leaves has a significant antibacterial effect and can be used to deal with specific pathogenic bacteria.

Key words: Antimicrobial activity, Ethanol extract, Iranian paste, *Staphylococcus aureus*

1. Introduction

Man has realized the benefits of medicinal herbs since old-time and has used various plants to treat common diseases [1]. The consumption of medicinal herbs has decreased gradually because of growth in urban life and the population, so synthetic drugs in many cases have been replaced [2]. However, the consumption of these drugs have caused problems such as increasing resistance of microorganisms and reducing its impact as a result of the creation of continued application [3, 4]. On the other hand, due to increase in the number of different bacterial strains and resistance to

antibiotics, many attempts have been made to use the plant's potential antimicrobial activities [5, 6].

Iranian paste plant (*Eremurus persicus*, Joub. & Spach) is grown in central areas of Iran. This plant grows in alluvial lands of mountainous heights with a natural habitat [7]. It is a grassy perennial plant with long narrow leaves, 30 to 70 cm wide and is complex at the base. The flowers flourish white to pinkish in the flower's clusters flowery on the stems of flowering. The flowers are seen with perianth segments slightly curved and the leaves and flower stalks are covered with soft and fine wool

and also with rather inflated leaves with thick and fleshy and drawn lumps [8]. *Eremurus persicus* has been used to treat liver diseases, gastritis, constipation, and diabetes in traditional medicine. The anti-glycation activities of extract of *Eremurus persicus* plant was studied and 5, 6, 7-methoxy-coumarin of *Eremurus persicus* with anti-glycation properties was reported [9].

Staphylococcus aureus is an opportunistic pathogen crime that causes various infections in humans and animals [10]. *Staphylococcus aureus* is one of the most important pathogens among the bacteria which causes food poisoning and infects hundreds of thousands of people every year [11]. *Staphylococcus aureus* are found in the skin's mucous membrane of the mammals,

various foods, and surroundings. And after a viral infection, it causes pneumonia, bovine mastitis, inflammation of the veins, meningitis, urinary tract infection, local inflammation of the bones, and skin lesions, etc. [12]. The aim of this study was to investigate the effects of antimicrobial activity of *Eremurus persicus* plant ethanol extracts against *Staphylococcus aureus* pathogenic.

2. Material and Methods

2.1. Preparation of samples

Mature leaves of *Eremurus persicus* (figure 1) were collected from mountainous areas of Zagros (elevation: 2850m). Leaves were dried in shade under suitable conditions.



Figure 1. The characteristic of leaves and flowers of *Eremurus persicus* (photo: www.nargil.ir)

2.2. Extraction

Mature Leaves of *Eremurus persicus* were collected in Zagros region in spring and dried in the shade. To prepare the ethanolic extract, 10 g of dry plant powder was placed in half-liter Erlenmeyer flasks containing 100 ml of 96% ethanol. The contents of the Erlenmeyer flasks were mixed at room temperature for 24 hours

by a shaker (Pars Azma-Iran) at 130 rpm, and then filtered through Whatman 2 paper. The isolation of the solvent from the extract was performed by a rotary apparatus (Heidolph-Germany) using a vacuum pump (vacuum distillation). The obtained weighted extract was then dissolved in DMSO solvent. The obtained extract was stored in the refrigerator at 4 °

C until it was used in antimicrobial tests [13].

2.3. Strains of bacteria

12 strains of *Staphylococcus aureus* were isolated from urine samples of patients admitted to Amir Al-Moemenin hospital in Zabol city; then cultured on Blad agar and Mannitol salt agar (MSA) special culture media and were identified by the use of catalase biochemical test and growth in differential mannitol salt agar medium and angulated on the lam as *Staphylococcus aureus* species.

2.4. Half McFarland suspension preparation

To prepare the microbial suspension, the bacterial was inoculated to the inclined agar nutrient medium (Merck, Germany) from saving culture. After the growth of bacteria colonies, the medium is washed with normal saline and concentrated bacterial suspension was obtained. Then some of the bacterial suspension poured into tube containing normal saline. Its turbidity was measured with a spectrophotometer at 630 nm. Until the turbidity of the solution with a solution of half McFarland, diluent saline and bacterial suspension with a concentration of 1×10^8 cfu/ml was prepared.

2.5. Antimicrobial test of extract

Bacterial isolates sensitive to the plant extracts were checked by dilution in the wells. 100 ml nutrient broth Mueller Hinton (MHB) was added to seven wells of the Microtiter plates. 100 ml of the diluted solution of extract was added to first well. After mixing, 100 ml was removed from the first wells and added to the second well. This process was repeated till we reached the last well. Then 100 ml medium was removed from the last well and 100 ml of microbial suspension containing 10^7

units per ml, equivalent to 5.0 McFarland, was added. It was then incubated at 37°C for 24 hours. The first well that stopped the growth of the bacteria, after being incubated, was considered as MIC.

2.6. Determination of MIC and MBC of medicinal plant extracts

To determine the minimum inhibitory concentration (MIC) of the plant extract, 100 microliters of Müller Hinton broth (Merk-German Company) was added to each well of microtiter plate. In the first well, 100 μL of the 20 mg/ml extract was added and after mixing, 100 μL was removed from the first well and added to the second well and continued to the last well. Afterward, 10 microliter of each bacterial suspension (cfu = $1/510$ per ml, half McFarland) added to wells contents. DMSO was added (without extract) to the negative control well, then the microtiter plate was incubated for 24 h at 37°C . The MIC was defined as the lowest concentration needed to stop the growth of bacteria at the end of 22 h of incubation [14, 15]. To determine the minimum bactericidal concentration (MBC), at the end of 24 h of incubation, 10 microliter of wells contents were cultured on a Nutrient agar medium (Merk-German Company) and the plates were examined for bacterial growth after 24 hours of incubation. The lowest concentration of extract which inhibited 99.9% of bacterial growth was considered as MBC. All antimicrobial tests were repeated at least 3 times [2, 16].

3. Results

The MIC of ethanolic extract of *Eremurus persicus* leaves on *Staphylococcus aureus* varied between 5 to 10 ppm and also MBC on *Staphylococcus aureus* varied between 2.5 to 20 ppm (Table 1).

Table 1. MIC and MBC of ethanolic extract of *Eremurus persicus* leaves against *Staphylococcus aureus*

bacteria strain	MIC (PPm)	MBC (PPm)
1	5	10
2	10	20
3	10	20
4	10	20
5	5	10
6	10	20
7	10	20
8	5	10
9	10	20
10	10	20
11	5	10
12	5	2.5

4. Discussion

This study investigated the *in vitro* antifungal activity of *Phlomis lanceolata*, *Rhynchospora elephas*, *Otostegia persica*, and *Eremurus persicus* on the clinical isolates of the pathogenic fungi *Aspergillus fumigatus*, *A. flavus*, *Trichophyton mentagrophytes*, *T. rubrum*, *T. verrucosum*, *Microsporum canis*, *M. gypseum*, *Epidermophyton floccosum* and the yeast *Candida albicans*. Among the investigated species, *P. lanceolata* sub-fractions were found to have fungicidal activity. The MIC and MFC were found to be considerable in petroleum ether, chloroform and ethyl acetate fractions (100 and 200 mg/mL) against the studied fungi and the yeast *Candida albican* [17].

It should be noted that the flowering aerial parts of *Eremurus persicus* were collected from Golpayegan (Isfahan, Iran) in May 2010. The extract was tested for its antibacterial activity against 4 Gram-positive bacteria strains, *i.e.* *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Bacillus cereus*, *Streptococcus pyogenes* and 5 Gram-negative bacterial strains, *i.e.* *Escherichia coli*, *Salmonella typhi*, *Shigella dysantriae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*. Its cytotoxic activity was also investigated using the MTT assay.

The antibacterial activity of *E. persicus* against *S. aureus* (MIC = 125 mg/ml), *B. cereus* (MIC = 15.62 mg/ml), *E. coli* (MIC = 125 mg/ml), *S. typhi* (MIC = 31.25 mg/ml), *S. dysantriae* (MIC = 0.48 mg/ml) was reported for the first time [18].

In another research, it was found that the chemical composition of essential oils of aerial parts of *Sonchus arvensis* and roots of *Eremurus spectabilis* were determined by GC-MS technique. Also, antibacterial activity of methanolic extracts of these plants was evaluated on some gram-positive and gram-negative bacteria by disk-diffusion and micro-dilution method to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The bacterial strains included: *Bacillus cereus* PTCC1015, *Staphylococcus aureus* PTCC1431, *Salmonella enterica* PTCC1709, and *Escherichia coli* PTCC1399. The results showed thirty-five compounds in the hydro-distilled essential oil from aerial parts of *S.arvensis*, representing 99.82 % of the total oil. N-Octane (29.82 %) and n-Decane (11.09 %) were the main components. The MIC and MBC varied respectively between 12.5-100 and 100 to >400 mg ml⁻¹ for all gram-positive and gram-negative bacteria. The lowest MIC and MBC observed against *S.aureus*, 12.5

and 100 mg ml⁻¹, respectively. The methanolic extracts of *S.arvensis* and *E.spectabilis* also could inhibit the growth of gram-positive and gram-negative bacteria [19].

5. Conclusion

The results of this study showed that ethanol extract of paste leaves has a significant antibacterial effect and also can be used to deal with specific pathogenic bacteria.

Abbreviation

Not applicable

Conflict of interest

All authors declare no conflict of interest exists.

Consent for publications

All authors read and approved the final manuscript for publication.

Availability of data and material

All the data is embedded in the manuscript.

Authors' contributions

All authors designed the research and wrote the paper. All authors read and approved the final manuscript.

Ethics approval and consent to participate

No human or animals were used in the present research.

6. References

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