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Eremurus spectabilis Root Extract: Evaluating Different Extraction Methods and Antimicrobial and Antioxidant Characteristics

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ABSTRACT

The current study aimed to investigate the potential antimicrobial and antioxidant activities of Eremurus spectabilis (*E. spectabilis*) in three extraction techniques. Three methods were selected to extract the aqueous, alcoholic, and hydroalcoholic extracts of *E. spectabilis*. The extraction yield was obtained from 10 g of *E. spectabilis* powder. The carbohydrate test was performed using the phenol sulfuric acid method. The Kjeldahl method was used in two replicates based on the AOAC 2550 standard to determine the protein content. The concentration of phenolic compounds was measured by the Folin-Ciocalteu assay. Based on the results, *E. spectabilis* had 70.33% w/w carbohydrates and 7.1% w/w proteins. The extraction percentages for the aqueous, alcoholic, and hydroalcoholic extracts of *E. spectabilis* were 50%, 10%, and 25%, respectively. The results showed that the aqueous extraction method was the most efficient. The total phenol amount for *E. spectabilis aqueous* extract was 150.04 mg/g. The antioxidant property of the *E. spectabilis* aqueous extract was determined to be 50.71% None of the concentrations of the aqueous extract did not have antimicrobial properties against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. These findings demonstrates the need for further studies on other pathogens and using different concentrations.

Keywords

Antimicrobial, Antioxidant, Eremurus spectabilis, Hydroalcoholic extraction, Alcoholic extraction

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Abbreviations

E. spectabilis: Eremurus spectabilis
E. coli: Escherichia coli

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Introduction

Natural antimicrobial compounds have gained attention due to their compatibility with the body, lower toxicity, and fewer side effects [1, 2]. These compounds, often obtained from plants, animals, or microbial sources, prevent the growth and reproduction of pathogenic microorganisms by various mechanisms [3, 4]. Among these mechanisms, we can mention the destruction of the cell membrane, inhibiting the production of essential proteins, and disrupting the cellular metabolism [5, 6]. Meanwhile, synthetic and chemical antimicrobial compounds, although effective in some cases, are associated with diverse problems, such as increased microbial resistance, high toxicity for human cells, and environmental pollution [7]. The widespread use of these chemical compounds not only reduces the effectiveness of medications over time but also causes irreparable side effects due to their accumulation in the human body and environment [8, 9]. Therefore, natural antimicrobial compounds are valuable and important as a safer and more effective alternative to fight infections, especially when drug resistance has become a serious problem [10, 11].

As a perennial herbaceous plant native to Central Asia and parts of the Middle East, *E. spectabilis* is often referred to as either foxtail lily or desert candle [12]. The plant belongs to the family Asphodelaceae and has been used for medical purposes for a long time. Various bioactive compounds, including glycosides, flavonoids, saponins, and other secondary metabolites, are present in *E. spectabilis* roots [13, 14]. Some of these compounds are highly potent antimicrobial and antioxidant compounds [15], making the plant an ideal subject of study for potential therapeutic applications because of its significant antimicrobial and antioxidant properties [16, 17].

Since the emergence of antibiotic-resistant microorganisms in the past few decades, interest in natural antioxidants and antimicrobials has surged due to growing concerns about the side effects of synthetic compounds and the growing prevalence of side effects caused by synthetic compounds [18-21]. A range of natural products, such as those derived from plants, offer an alternative to traditional medications because they are biocompatible, have a low level of toxicity, and are effective against a wide range of bacteria and viruses [22, 23]. The ethnobotanical and historical relevance of *E. spectabilis* makes it an attractive candidate for further studying its root extracts and investigating its potential medicinal implications [24, 25].

Several extraction methods could be used to extract bioactive compounds from *E. spectabilis* roots; however, it is extremely significant that these methods are compared to determine the most effective one [26]. Some considerations, such as the extraction yield, compound's stability, environmental impact, and operation efficiency are all critical [27]. A systematic evaluation of these methods will greatly help researchers optimize the extraction process, providing root extracts with the most effective antimicrobial and antioxidant properties.

It has been established that the antimicrobial properties of the roots of *E. spectabilis* are particularly important in dealing with the bacteria resistant to antibiotics [28]. According to preliminary studies, these extracts may show a significant activity against various bacterial and fungal pathogens, including those associated with Candida albicans [29, 30]. In addition, the antioxidant properties of the extracts make them suitable for preventing diseases related to oxidative stress [31]. These diseases include cardiovascular diseases, neurodegenerative disorders, and some types of cancer due to their antioxidant properties.

E. spectabilis is a promising source of natural antimicrobial and antioxidant agents [32]. Selecting an extraction method considering the bioactive compounds of a plant is crucial to maximize its potential. Therefore, the current study examined the potential antimicrobial and antioxidant activities of *E. spectabilis* extract.

Results

Chemical properties of E. spectabilis

According to our results, *E. spectabilis* had 70.33% w/w carbohydrates and 7.1% w/w proteins.

Extraction yield

The yield of extraction from *E. spectabilis* root by aqueous, alcoholic, and hydroalcoholic solvents are shown in Figure 1. The extraction yield for the aqueous, alcoholic, and hydroalcoholic extracts of *E. spectabilis* was 50%, 10%, and 25%, respectively (Table 1). The results showed that the aqueous extraction method was the most efficient.

TPC content

As it was shown in Table 1, the TPC content for *E. spectabilis* aqueous extract was 150.04 mg/g.

Antioxidant properties

The RSC% of *E. spectabilis* aqueous extract was equal to 50.71% (Table 1).

Antimicrobial property

None of the concentrations of the aqueous extract had antimicrobial properties against any of the tested

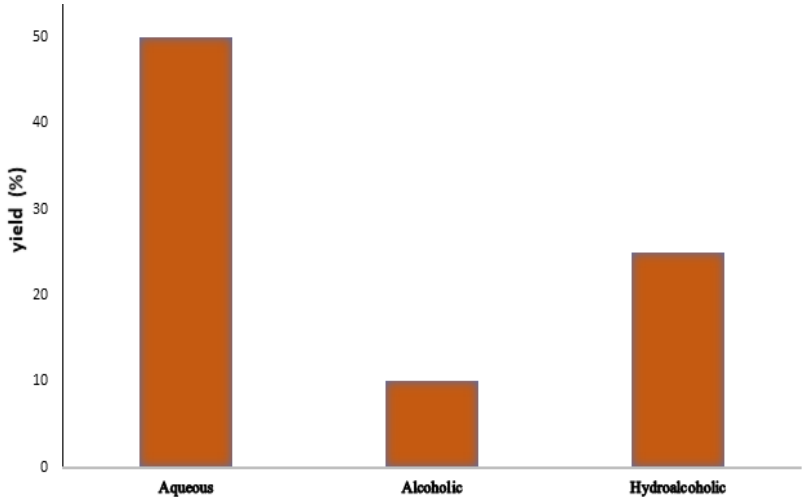

Figure 1.
Extraction yield of E. spectabilis root extracts by different solvents

Table 1.
Chemical properties of E. spectabilis root extracts by different methods.

Extraction method	Extraction yield	(mg/g)TPC	RSC (%)
Aqueous	50	150.04	50.71
Alcoholic	10	-	-
Hydroalcoholic	25	-	-

microorganisms, and no growth inhibition zone was observed. The diameter of the growth inhibition zones of *S. aureus* (ATCC:25923), *E. coli* (NCTC:12900), and *Pseudomonas aeruginosa* (ATCC:27853) for the gentamicin antibiotic disc were 17, 16, and 20 mm, respectively.

Discussion

The results of the present study showed that the main components of *E. spectabilis* were carbohydrate (70.33% w/w) and proteins (7.1% w/w). According to the current study, *E. spectabilis* is a good source of carbohydrates and proteins. The findings of this research correspond with those reported by Salehi *et al.* (2022) regarding the root gum of *E. spectabilis.* The researchers determined that the root gum powder of Eremurus luteus contained an average moisture content of 6.27% (w.b.), 4.17% (d.b.) ash, 6.22% (d.b.) protein, 86.45% (d.b.) carbohydrate, and 8.6% (d.b.) uronic acids [33]. In the present study, the highest extraction percentage was for the aqueous extract (50%), while the lowest was for the alcoholic extract (10%). Phenolic compounds in the aqueous extract of *E. spectabilis* were 150.04 mg/g. A recent study reported that *E. spectabilis* extracted by methanol, ethanol, and aqueous media had a total phenolic content in the range of 31.7-92.15 mg GAE/g and antioxidant activity in the range of 72.01-81.21 mg AAE/g [16].

In addition to flavonoids, phenolic compounds, and saponins, *E. spectabilis* extract has many medicinal properties [12]. A powerful antioxidant is vital for fighting free radicals, leading to preventing cells from being damaged. A high phenol composition gives *E. spectabilis* extracts a strong antioxidant activity [34]. In addition to helping prevent chronic diseases, such as cancer, arthritis, and cardiomyopathies, antioxidant activity may also help treat neurological disorders [23-35].

It is also noteworthy that *E. spectabilis* extract possesses antimicrobial properties that are important for combating various pathogenic microorganisms. Bircan and Kırbağ (2015) reported that zones of inhibition were seen for *S. aureus* (12 mm), *E. coli* (14 mm), and *C. albicans* (9 mm), and *Epidermophyton spp(8mm),* when *E. spectabilis* extract was used [15]. In contrast, Karaman *et al.* (2011) revealed that the 1% concentrations of the methanol, ethanol, and aqueous extracts of *E. spectabilis* showed no inhibitive effect on Yersinia enterocolitica and Pseudomonas aeruginosa [16]. The Eremurus extract has been shown to work as a natural alternative to synthetic antibiotics by exerting inhibitory effects against various bacteria and fungi, suggesting that *E. spectabilis* may be a valuable alternative in the future [18]. Considering that antibiotic resistance is rising, alternative treatments have become more and more necessary, especially in the light of the increasing need for alternative treatments. It has been shown that using the extracts of *E. spectabilis* could be beneficial to animals in a variety of ways, including the prevention of infections and the reduction of oxidative stress-related conditions. Compared to traditional medications, herbal agents are considered safer alternatives with fewer side effects due to the natural origin of their ingredients and their bioactive components [36]. The benefits that will be gained from *E. spectabilis* are on both human and animal sides in terms of enhancing health.

Studies have shown that the main volatile components of the *E. spectabilis* root are carone (terpenoid), carvacrol (monoterpenoid phenolic compound), pentane, 2-methyl (E) caryophyllene (natural bicyclic sesquiterpene), valencene, cadalene, and acetic acid. These volatile components contribute to the antioxidant and antibacterial activity in the *E. spectabilis* root as a defense mechanism against insects, fungi, and

other environmental stresses. On the other hand, glucomannans are water-soluble bioactive polysaccharides in the root of *E. spectabilis* and contribute to the antioxidant activity [37].

Gram-negative organisms are generally believed to be less sensitive to antimicrobial components due to the outer lipopolysaccharide membrane surrounding their cell wall, which provides surface hydrophilicity, preventing access to the antimicrobial components of a predominantly hydrophobic nature. In the current study, the aqueous extract of *E. spectabilis* in the used concentrations did not have antimicrobial properties against any of the tested microorganisms. However, Tuzko *et al.* (2017) concluded that *E. spectabilis* has antimicrobial activity against Gram-negative (E. coli) and Gram-positive (B. subtilis) organisms [17]. Its antimicrobial activity can be attributed to the phenolic compounds, essential oils, and volatile components. It has been reported that n-octane and n-decane, the main components of *E. spectabilis* essential oil, are responsible for the antimicrobial activity due to their hydrophobic nature. Kanani and Mohammadi Sani (2015) showed that the roots of *E. spectabilis* can prevent the growth of Gram-positive and Gram-negative bacteria [38].

Tuzcu *et al.* (2017) examined the antioxidant properties, antimicrobial effects, anticancer properties, as well as the apoptotic and anticancer properties of the aqueous and organic extracts from *E. spectabilis* leaves and roots [17]. In this study, the Folin-Ciocalteu method was used to assess the total content of phenols in these extracts and revealed that the extracts possess significant antioxidant potential. In addition, DPPH radical scavenging assays and lipid peroxidation assays were conducted as further assessments, demonstrating this plant's potent free radical-neutralizing properties. In addition, the antimicrobial efficacy of 500 µg/ml of the extracts of *E. spectabilis* was assessed through disk diffusion and revealed that the extracts were effective against *Listeria monocytogenes*, *Saccharomyces cerevisiae*, *S. aureus*, and *E. coli*. However, in our study, 100 µg/ml concentrations were used. Among the different extracts, the acetone extract of leaves exhibited the highest phenolic and flavonoid content. It also had an antioxidant activity measured at 3703.25 µg ascorbic acid/g dry weight. According to these findings, *E. spectabilis* is a promising natural resource that can be utilized to develop new therapeutic agents for both veterinary and medical uses.

Some limitations were identified in the present study that examined the antimicrobial and antioxidant potential of *E. spectabilis* extract. The extract used in the study may not have had sufficient antimicrobial effects due to its low concentration. The extract may have stronger antimicrobial properties at higher concentrations, which would make it possible to have a greater effect at higher levels. To find out the optimal dosage of the extract for effectively inhibiting microorganisms, further research needs to be conducted using a variety of concentrations of the extract. Furthermore, we tested the extract against a limited range of microorganisms to determine its effectiveness. In order to better understand the extract's antimicrobial properties, it is important to increase the spectrum of microorganisms tested. This would enable us to understand the extract's antimicrobial properties. By extending these tests to a broader range of pathogens, we might be able to identify different types of microbes that are more susceptible to the bioactive compounds of *E. spectabilis*.

Although the current study has some limitations, its results open up a wide range of interesting areas for future research. *E. spectabilis* extract has synergistic effects when combined with other natural antimicrobial agents. In this way, multiple extracts can be used in conjunction to produce more powerful antimicrobial and antioxidant effects resulting from their combined bioactive properties.

The aqueous extract of *E. spectabilis*, despite promising bioactive properties, failed to show any antimicrobial activity against certain bacteria. As a result, it is clear that the extract, in its current form and concentration, is not effective at inhibiting microbial growth. Exploring different extraction methods, higher concentrations, or combining different antimicrobial agents would be beneficial. This will enable us to fully comprehend the potential of *E. spectabilis* in future. Furthermore, a full investigation into the antioxidant properties of the material is required.

Materials & Methods

Materials

The roots of *E. spectabilis* were collected from the heights of the Binaloud mountain range (Razavi Khorasan, Iran) and were washed with water for deflowering and soil removal. Then, they were dried on a cotton cloth in the shade and powdered in a semi-industrial mill. Next, using a sieve with a mesh size of 100 mesh , the powders were sieved and made the same size. The resulting powder was kept in a cool place and away from sunlight until use. DPPH, ethanol, and other chemicals, including culture media, were obtained from Merck. Bacterial strains *S. aureus* (ATCC:25923), *E. coli* (NCTC:12900), and *P. aeruginosa* (ATCC:27853) were obtained from the Food Hygiene Department, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad.

Extraction techniques

Three methods were used to extract *E. spectabilis* gum. In the aqueous extract method, 3% W/V suspension was prepared and homogenized on a magnetic stirrer for one hour. The suspension was heated in a bain-marie at 80°C-95°C for 15 min and smoothed with a linen

cloth to further dissolve. A centrifuge operating at 4500 g (Universal PRP, Iran) was employed at a temperature of 20°C for 10 min to purify the extract. The extract obtained by the extraction process was dried in a fan-assisted oven at a temperature of 40°C. Subsequently, it was ground and filtered through a 100-mesh sieve [39].

An ethanolic extract was prepared by mixing 10 g of *E. spectabilis* root powder with 150 ml of ethanol and stirring in a magnetic stirrer at 150 rpm for 2 h at room temperature (6). The resulting mixture was macerated at room temperature for 24 h and filtered with a 100-mesh sieve. The resulting solution was dried under a low-pressure evaporator at 4°C and kept in the dark.

In the third method, a hydroalcoholic extract was prepared using 10 g of the *E. spectabilis* root powder mixed with 150 ml of the ethanol-water mixture in a ratio of 50:50. The rest of the steps were carried out according to the second method, and the hydroalcoholic extract of E. spectabilis was prepared [17].

Extraction yield

The extraction yield was obtained from 10 g of *E. spectabilis* powder. First, the plate for drying the extract solution was weighed, and the weight difference between the empty plate and the plate containing the dried extract was calculated in all three samples.

Eremurus spectabilis properties

Color

To determine the color, 0.1 g of three aqueous, alcoholic, and hydroalcoholic extracts were dissolved in 6 ml of distilled water, and the absorbance of the sample was measured at 420 nm [40, 41] by a spectrophotometer (UV-VIS single beam spectrophotometer, UNICO, USA).

Total carbohydrates

The carbohydrate test was performed using the phenol sulfuric acid method. A volume of 2 ml of carbohydrate solution was mixed with 1 ml of 5% phenol aqueous solution in a test tube. Afterwards, 5 ml of concentrated sulfuric acid was quickly added to the mixture. The mixture was set for 20 min in a water bath at 30°C. Next, the absorption at 490 nm was recorded by a spectrophotometer. In the end, fructose was used as a standard, and the amount of total sugar was determined based on the absorption standard curve of similar solutions [42].

Protein amount

The Kjeldahl method was used in two replicates based on the AOAC 2550 standard to determine the protein content [43].

Total Phenolic Compounds (TPC)

The concentration of phenolic compounds with potential antioxidant activities can be measured by the Folin-Ciocalteu assay and expressed in gallic acid. A volume of 0.5 ml of the extract (25 ml/250 g) was mixed with 2.5 ml of 10% Folin-Ciocalteu and stirred for 5 min. Next, 2 ml of 5% sodium carbonate solution was added and it was kept for 30 min in a dark place at room temperature. The absorbance of the samples was measured at 760 nm by a spectrophotometer. The TPC content in the extract was measured and reported using a standard curve based on the micrograms of the gallic acid per gram of extract [44].

Antioxidant property

The DPPH radical scavenging method was used to evaluate the antioxidant activity [45]. A volume of 500 µl of the extract was mixed with 4 moles of the methanolic solution of DPPH 0.08 mmol/l and was placed in a bain-marie at 30°C for 30 min. The absorbance of the sample was measured at 517 nm. Radical scavenging capacity was determined using equation 1:

$$RSC\ (\%) = (A_{blank} - A_{sample}/A_{blank}) \times 100 \quad (1)$$

Antimicrobial properties

Mueller Hinton agar culture medium was used to investigate the antibacterial properties of the aqueous extract of E. spectabilis using the disc diffusion method. In this method, from the 24-hour culture of the bacterial strains *S. aureus* (ATCC:25923), *E. coli* (NCTC:12900), and *P. aeruginosa* (ATCC:27853) in Mueller Hinton agar medium, the turbidity liquid equivalent to 0.5 McFarland (1.5×108 CFU/ml) was prepared. After diluting, a suspension with a concentration of 1×104 CFU/ml was obtained and cultured in Mueller Hinton agar and placed at a certain distance from each other and the edge of the plate on the agar medium. A volume of 100 µl of 30%, 40%, and 50% extract concentrations in dimethyl sulfoxide solution was added to the discs and incubated at 37°C. Sterile distilled water was used as a negative control, and gentamicin antibiotic disc was used as a positive control. After 24 h of incubation, the diameter of the zone (Figure 2) was determined [46].

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

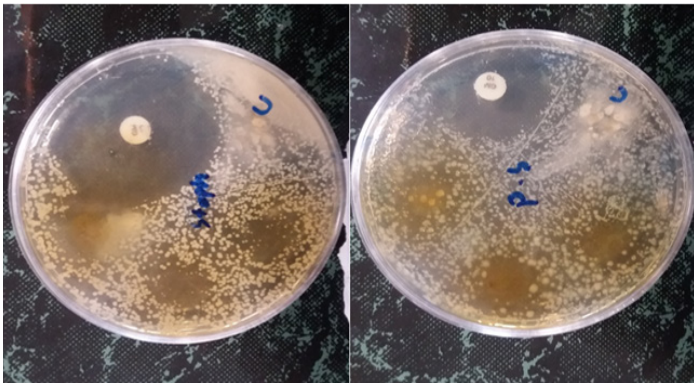

Figure 2.
The measurement of the diameter of the zone of growth inhibition for the microorganisms *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

Authors' Contributions

Conceptualization: Razieh Niazmand; Methodology: Elham Merrikhi Ardebili; Formal analysis and investigation: Elham Merrikhi Ardebili & Razieh Niazmand; Writing - original draft preparation: Elham Merrikhi Ardebili; Writing - review and editing: Elham Merrikhi Ardebili & Razieh Niazmand & Abdollah Jamshidi]; Funding acquisition: Abdollah Jamshidi & Razieh Niazmand; Supervision: Razieh Niazmand & Abdollah Jamshidi. All authors checked and approved the final version of the manuscript for publication in the present journal.

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Conflict of interest

The authors declare that there is no conflict of the interest

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