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Studying the Diversity of Antibacterial and Biochemical Properties of Khiarak (*Ixiolirion tataricum* L.) Organs

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ABSTRACT

Ixiolirion tataricum L. with the Persian name of "Khiarak" belongs to the Amaryllidaceae family and is regarded as one of the most important plant sources of antioxidant and antimicrobial compounds. The aim of this research is to investigate the effect of aerial organ treatment on the biochemical and antibacterial properties of this plant. Samples were fully collected from its habitat in Asadabad County, located in the western part of Hamadan Province and Some biochemical traits, including dry matter percentage, protein, total phenol, the ability to inhibit the free radical diphenyl picrylhydrazyl, and antibacterial activities in the plant organs such as bulb, leaf, stem, and inflorescence, were measured. The results showed that the type of plant organ significantly affected the percentage of protein, total phenol, and the ability to inhibit the free radical diphenyl picrylhydrazyl. Among the organs, the highest levels of protein, total phenol, and antioxidant properties were found in the inflorescence. The assessment of the antimicrobial properties from the extract of all four organs indicated that the highest antibacterial effect was present in the stem of Ixiolirion, while the effects of the extract from other organs on two bacteria (Staphylococcus and Escherichia coli) were not significant. Additionally, as the concentration of the extract increased, its effect on inhibiting bacterial growth intensified, and the diameter of the inhibition zone increased. The inflorescence organ has more applications in traditional medicine and pharmacy regarding protein, total phenol, and antioxidant properties, while the stem organ has more significance concerning antibacterial properties.

Keywords: Antimicrobial, Ixiolirion plant, Organ, Phytochemical compounds

INTRODUCTION

The Amaryllidaceae family is a large family of flowering monocotyledonous and bulbous plants, comprising about 1,000 species in 79 genera. The genus Ixiolirion from the Amaryllidaceae family contains three species, with Ixiolirion tataricum (Pall.) Schult. & Schult.f is the only species observed in Iran according to the flora of Iran [1]. Identifying native plants in pastures is of great importance as useful and valuable germplasm. Among these, medicinal plants hold special significance due to their wide applications [2]. Ixiolirion is one of the most important species of the Amaryllidaceae family, with limited studies conducted on it. An image of the plant is shown in Figure 1.

Fig. 1 The photograph of the *Ixiolirion tataricum* L. plant in nature

Research indicates that the quantity and quality of chemical compounds produced in different plant organs vary and in addition to that they are influenced by environmental and ecological factors, harvest time, and genetics [3]. Studies by Keneshloo *et al*. [4] also demonstrated that, in comparing the yield and chemical compositions of different organs (leaf, inflorescence, stem, and flowering branch) of the medicinal plant *Matricaria chamomilla*, the yield of essential oil from the inflorescence, leaf, and flowering branch varied based on the dry weight of the plant. Generally, the production of active substances in plants can be considered a result of plant growth, which includes metabolic, morphological changes, and differentiation in various plant organs [5]. Mehrpoor *et al*. [6] also indicated in a study on medicinal plants that considering the characteristics of the growth location, geographical position, and the impact of ecological factors on the plant in nature, especially on its organs, are major factors that can significantly affect the levels of active substances in the plant.

Various plant essential oils and extracts exhibit high antioxidant and antimicrobial activities. Due to their significant biological activities, including antioxidant, antifungal, and antibacterial properties, plant extracts are notably used in food products [7]. Antibiotic-resistant bacteria can lead to higher mortality rates compared to non-resistant bacteria. Antibiotic-resistant gram-negative bacteria responsible for nosocomial infections comprise species such as Escherichia coli, Enterobacteriaceae, Pseudomonas, and Acinetobacter. Gram-positive bacteria include species like Staphylococcus, Streptococcus, and Enterococcus [8]. Given the escalating bacterial resistance to antibiotics, global efforts are underway to develop new alternative treatments [9]. The emergence of drug-resistant strains necessitates the search for new antimicrobial agents. Plants and their compounds, including essential oils and extracts, have the potential to replace chemical drugs, especially since the side effects of these compounds are lower compared to chemical drugs [10]. Secondary metabolites derived from plants, such as phenolic compounds, have strong potential to scavenge free radicals, existing in all different parts of the plant including leaves, fruits, seeds, roots, and bark [11]. Falleh *et al*. [12] reported that phenolic compounds are present in almost all parts of the plant and play roles in most physiological processes such as cell growth, seed germination, and fruit ripening. One of the most important characteristics of this group is their antioxidant properties, which allow them to lose hydrogen and trap free radicals [13]. The results of the study by Fazeli-nasab *et al*. [14] indicated that plants with high levels of phenols and flavonoids exhibited relatively high antioxidant and antimicrobial properties; however, since antioxidant properties are related to specific components of phenolic and flavonoid compounds, it cannot be concluded that plants with high levels of phenolic and flavonoid substances will necessarily have high antioxidant properties [15].

In the pursuit of eliminating or reducing chemical and synthetic compounds in food products, extensive research has been conducted to find natural antioxidants from plant sources as substitutes for chemical agents [16]. One of the chemical compounds in Ixiolirion is an antioxidant. Antioxidants can prevent the activity of free radicals, protect body cells from the harmful effects of radicals, and minimize their impact [17]. The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method is a common approach to investigate antioxidant effects. This molecule has the capacity to initiate chain reactions of peroxidation through direct reactions with antioxidants; thus, antioxidants can play an inhibitory role by trapping it and reducing this molecule, resulting in the formation of a yellow compound that indicates radical inhibition, with an absorption spectrum at a wavelength of 516 nm signaling the occurrence of this inhibitory reaction [18]. The antioxidant activity of phenolic compounds in plants is primarily due to their redox properties and chemical structures, which play a significant role in neutralizing free radicals [19].

Studies show that plants, as rich sources of antioxidant compounds, also exhibit high antimicrobial effects. Furthermore, the use of natural substances compared to chemical ones is of great importance, and undoubtedly, the use of essential oils and plant extracts are considered suitable alternatives [20]. A significant issue in antibiotic treatments is the increasing resistance of bacterial infections to antibiotics. Additionally, the occurrence of side effects from the use of chemical drugs as antibiotics in treating infectious diseases has been on the rise. Antimicrobial compounds obtained from plants eliminate bacteria through mechanisms different from those of antibiotics, which is crucial in treating infections caused by resistant microbial strains. With the increasing resistance of bacteria to antibiotics, efforts are underway to replace treatments, particularly by using medicinal plants that have high compatibility with the human body [21]. The antimicrobial mechanism of plant extracts is attributed to their hydrophobic properties, which facilitate the entry of these substances into the phospholipids of bacterial cell membranes, leading to disruption of their structure and increased permeability. This results in the leakage of ions and other cellular contents, ultimately leading to cell death [22]. Plants from the Amaryllidaceae family are known for producing unique structural alkaloids with a wide range of physiological effects, including antitumor, antiviral, cytotoxic, acetylcholinesterase inhibitory, immunostimulatory, anti-inflammatory, analgesic, and DNA-binding activities and some of them are also used in the treatment of allergy diseases [23]. Nowadays, due to the adverse effects of synthetic drugs, the use of plants and their natural compounds as medicines, functional foods, and as antimicrobial agents with inhibitory and lethal effects on pathogens has gained more attention in Iran and European countries [24,25]. In recent years, numerous studies have been conducted on the antimicrobial properties of various plants against certain pathogenic bacteria, including Staphylococcus aureus and Escherichia coli, for example Toolabi *et al*. [26] investigated the antibacterial effects of Ziziphus fruit extract on foodborne Escherichia coli and their results showed that the methanolic extract of Ziziphus exhibited very favorable antibacterial effects against Escherichia coli. The narcissus flower, being one of the ornamentals and highly fragrant plants in the Amaryllidaceae family, has had its extracts from various parts, including inflorescence, stem, and bulb, studied. Alkaloids are the main secondary metabolites in narcissus, possessing antimicrobial, antioxidant, and anti-inflammatory properties, and have therapeutic applications [27]. The extract of the narcissus plant, due to its various alkaloids, has numerous antioxidants, anti-inflammatory, and antimicrobial activities [28]. Zamanifar *et al*. [29] also investigated the antimicrobial effects of the medicinal plants Harmala and Lavender against the pathogenic microorganism Staphylococcus aureus in laboratory conditions, reporting the diameter of the growth inhibition zone resulting from the effect of the methanolic extract of Harmala.

Considering the necessity of optimally utilizing plant organs that contain effective compounds, the aim of the present study is to investigate the chemical compounds in different organs of the plant, qualitatively assess the presence of active substances, and evaluate the antimicrobial potential of the wild Ixiolirion species from the Amaryllidaceae family in the mountainous region of Asadabad County, Hamadan Province.

MATERIALS AND METHODS

This research was conducted in 2025 to examine the phytochemical compounds, including dry matter percentage, protein, total phenol, and the antioxidant and antimicrobial properties in various organs of the medicinal plant Ixiolirion.

Plant samples were collected from the highlands of Asadabad County, located in the west of Hamedan Province, and the herbarium numbers for all three selected specimens are 521-1, 521-2, and 521-3, respectively. The geographical conditions and some soil characteristics of the test site presented in Tables 1 and 2.

Table 1 Geographical characteristics of the location of the *Ixiolirion tataricum* L. samples collection

Province	County	Climate	Latitude	Longitude	Height above sea level (m)
Hamedan	Asadabad	Cold – Semi-dry	34° 46´ N	48° 07´ E	1591

Table 2 Some physical and chemical properties of soil

Soil depth	Sand (%)	Clay (%)	Silt (%)	pH	EC (dS/m)	Organic matter (%)	Nitrogen (%)	Absorbable phosphorus (mg/kg)	Absorbable potassium (mg/kg)
0-30	60.6	11.5	27.9	7.3	0.64	0.3	0.02	12.8	198

After collecting the samples, the bulb, stem, inflorescence, and leaves were separately dried at room temperature and in the shade with proper ventilation. After transferring the dried parts of the plant to the laboratory, they were ground, and the dry matter percentage was measured using a scale with an accuracy of 0.01 grams.

Measurement of Total Protein

To measure total protein, the Bradford method [30] was used. A quantity of 0.2 grams from each organ was homogenized in a cold porcelain mortar with two millilitres of a 0.1 M phosphate buffer at a pH of 6.8 and then centrifuged. The resulting supernatant was used to measure the amount of soluble protein, which was read using a spectrophotometer (HITACHI 340 model, Japan) at a wavelength of 595 nm.

Measurement of Total Phenol

For measuring total phenol, methanolic extracts (70% methanol) were used, and the evaluation was performed using the Folin-Ciocalteu reagent through colorimetry. The phosphotungstic acid present in the reagent acts as an oxidizing agent that quickly reduces the oxidized phenolic hydroxyl groups, resulting in the formation of a blue color, with maximum absorbance at a wavelength of 760 nm. For this purpose, 500 microliters of the prepared extract were mixed with 4500 microliters of distilled water and 100 microliters of the Folin solution. After 2 minutes, 1 milliliter of 20% sodium carbonate (Na_2CO_3) was added. The samples were kept in the dark for 2 hours, and then the absorbance of the samples was measured at 765 nm, expressed as grams of gallic acid per kilogram of wet weight of the sample [31,32] (Fig. 2).

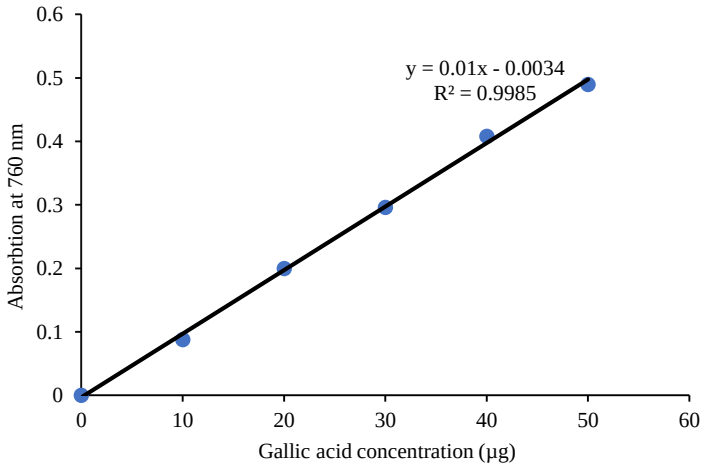

Fig. 2 Gallic acid standard curve for the total phenolic content determination

Evaluation of Antioxidant Activity

To assess the antioxidant activity of various extracts, a factor known as IC50 is used, which refers to the concentration of the extract at which 50% of the DPPH radicals in the reaction medium are inhibited. A lower concentration indicates a higher antioxidant activity of the extract [33]. The antioxidant activity of the extract was evaluated using the radical scavenging capacity measurement with 2, 2-diphenyl-1-picrylhydrazyl (DPPH) [34]. In this method, 300 milligrams of the dried sample were powdered and transferred into a Falcon tube along with 9 milliliters of pure methanol. The Falcon tubes were kept in the dark at 4 degrees Celsius for 24 hours. Then, 0.1 milliliters of the plant extract were added to 3.9 milliliters of the prepared DPPH stock solution (0.004 grams of DPPH in 100 milliliters of methanol) and left in the dark for 30 minutes. The absorbance was read at a wavelength of 517 nm using a spectrophotometer. Finally, the results were calculated as IC50 using the equation 1, where A_{blank} and A_{sample} represent the absorbance of the control and the sample, respectively.

$$C_{50} = \left[(A_{blank} - A_{sample})/A_{blank}\right] \quad \text{(Eq.1)}$$

Evaluation of Antibacterial Activity

In this study, lyophilized bacteria Staphylococcus aureus (Gram-positive) (ATCC25923) and Escherichia coli (Gram-negative) (ATCC25922) obtained from the Faculty of Veterinary Medicine at Bu-Ali Sina University were used and the antibacterial property of the methanolic extract of Ixiolirion was assessed using the disk diffusion method. In this method, bacteria were initially cultured in nutrient agar. After 24 hours, a colony from each bacterium was picked using a sterile loop and cultured in an Erlenmeyer flask containing nutrient broth (liquid medium). After another 24 hours, the bacteria were cultured lawn-style on Mueller-Hinton agar. The methanolic extract was prepared in three concentrations: 100, 300, and 500 mg/kg, and 50 microliters of the extract were injected onto blank disks. After 24 hours of bacterial culture on Mueller-Hinton agar, the disks coated with the extract were placed on the plates and ciprofloxacin antibiotic disks were used as controls. The plates were then incubated at 37 degrees Celsius for 24 hours and the diameter of the inhibition zone, if present, was measured in millimeters [35] (Fig. 3).

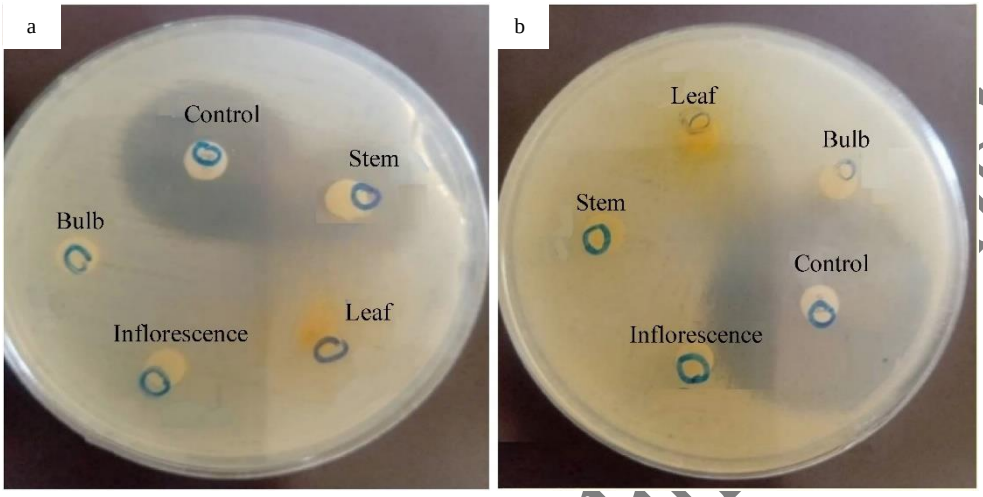

Fig. 3 Measurement of the inhibition zone diameter to determine antibacterial properties using the disk diffusion method, (a) Staphylococcus bacteria, and (b) Escherichia coli bacteria

This experiment was conducted in a completely randomized block design with three replications using SPSS version 19 statistical software, and mean comparisons were performed using Duncan's multiple range test at a 5% significance level.

RESULTS

The results of the analysis of variance for each of the traits examined in Table 3 showed that, unlike the percentage of dry matter, there were significant differences at the 1% probability level (p≤1%) among the different organs of Ixiolirion regarding traits such as protein percentage, total phenols, and DPPH radical scavenging rate, which indicates the antioxidant capacity of the plant.

Table 3 Analysis of variance on the effect of organ type on some phytochemical properties of the *Ixiolirion tataricum* L. plant

		Mean Squares			
Variation source	Df	Dry matter percentage	Protein	Total polyphenols	DPPH inhibition rate
Block	2	0.00^{ns}	0.13^{ns}	0.05^{ns}	5.57^{*}
Treatment	3	4.05^{ns}	111.84^{**}	9.81^{**}	249.43^{**}
Error	6	0.05	1.03	1.01	7.30

ns, *, and **: not significant and significant at levels of 5% and 1%, respectively

Protein Percentage

The analysis of variance indicated that the effect of the organ treatment on the protein percentage was significant at the 1% level (Table 3). The protein content in the inflorescence of Ixiolirion was 18%, which was higher than that in other plant organs. The lowest protein content was observed at 6% in the bulb of the plant (Table 4).

Table 4 Mean comparison of the effect of organ type on some phytochemical properties of the *Ixiolirion tataricum* L. plant

Organ	Protein (%)	Total polyphenols	DPPH inhibition rate
Bulb	6.00 c	0.07 b	81.00 a
Inflorescence	18.00 a	0.11 a	64.00 c
Leaf	15.44 b	0.03 c	64.00 c
Stem	6.62 c	0.02 c	78.37 b

Percentage of Total Phenol

The results of the analysis of variance indicated that the effect of the organ treatment on the total phenol content was significant at the 1% level (Table 3). The results obtained from the mean comparison table showed that the highest total phenol content, 0.11%, was found in the inflorescence of the plant, which represented an increase of approximately 450% compared to the stem (Table 4).

Percentage of DPPH Inhibition

According to the results of the analysis of variance, the effect of the organ treatment on DPPH inhibition was significant at the 1% level (Table 3). The mean comparison table indicated that the lowest DPPH inhibition was observed in the inflorescence and leaves of Ixiolirion. Demonstrating their highest antioxidant capacity (Table 4).

Antibacterial Activity

The examination of the diameters of the inhibition zones revealed that the methanolic extract from the stem of Ixiolirion at concentrations of 100, 300, and 500 mg/mL inhibited the growth of Staphylococcus aureus and Escherichia coli, with the highest growth inhibition observed at a concentration of 500 mg/ml. The diameter of the inhibition zone for Staphylococcus aureus at 500 mg/mL was 21±0.002 mm, which was approximately three times the diameter of the inhibition zone for Escherichia coli (Table 5). The average inhibition zone diameter for the antibiotic ciprofloxacin (as a control) was 28 mm for Staphylococcus aureus and 39 mm for Escherichia coli. The growth inhibition of bacteria by methanolic extract from other plant organs was not significant.

Table 5 Diameter of inhibition zones (mean ± SD) (mm) in different concentration of studied plants extract.

Organ	Bacteria	Diameter of the growth inhibition zone at different concentrations of methanol extract		
		100 mg/ml	300 mg/ml	500 mg/ml
Stem	*S. aureus*	14 ± 0.24	18 ± 1.03	21 ± 0.002
	E. coli	4 ± 0.42	6 ± 1.37	7 ± 0.04
Inflorescence	*S. aureus*	0	0	0
	E. coli	0	0	0
Leaf	*S. aureus*	0	0	0
	E. coli	0	0	0
Bulb	*S. aureus*	0	0	0
	E. coli	0	0	0

DISCUSSION

Based on the results obtained from the mean comparison table, the effect of the type of organ treatment on the protein content of the plant showed that the highest percentage of protein was found in the inflorescence. Makari *et al*. [36] also reported significant differences in protein percentages among different organs and their mixtures in a study on various parts of rhubarb and the percentage of protein in inflorescence and leaves was significantly higher than in stem organs and a mixture of organs.

The results of the mean comparison regarding the type of plant organ on the storage of total phenols indicated that the amount of this biochemical compound in the inflorescence was nearly twice that of the stem. A study on the thyme plant revealed that the methanol extract of the plant contains a substantial amount of phenols and exhibits potent antibacterial activity against Gram-positive bacteria. The research indicated a strong correlation between the total phenol content and the antibacterial efficacy of the extract under study, suggesting that the plant could serve as a promising source of natural bioactive compounds [37]. The effect of the type of organ on the content of secondary metabolites, including essential oil components, phenols, flavonoids, and biological properties such as antioxidant activity, has been confirmed in many previous studies [38]. Klanitalaei *et al*. [39] reported that different parts of marshmallow (flower, fruit, seed, root, and leaf) have various medicinal uses attributed to their high mucilage content, phenolic compounds, and antioxidant properties. Marshmallow plants also contain different amounts of flavonoid compounds, oleic acid, riboflavin, anthocyanin, sitosterol, and other phytochemical compounds in their leaves and flowers [40]. Numerous studies have demonstrated the antioxidant activity of phenolic compounds. Hazrati *et al*. [41] demonstrated in a study on the medicinal plant *Stachys schtschegleevi* that the highest quality of effective compounds was observed during the flowering stage and in the flower organs and significant differences in total phenol and flavonoid content between vegetative and reproductive organs, which aligns with the current research. Reigosa Roger *et al*. [42] reported that the type and production rate of phenolic compounds produced depend on the species, plant organ, and possible stress intensity. In a study investigating the total phenol content and antioxidant properties of various parts of marshmallow, it was discovered that the flower part exhibited the highest total phenol content, measuring 3.38 mg of gallic acid. Furthermore, this experiment reported that the flower part of the plant possessed superior antioxidant properties compared to the root [43]. Azadeh *et al*. [44] demonstrated that in their examination of several species within the Althaea genus, the flowers contained higher levels of total phenols, flavonoids, and anthocyanins than the vegetative parts of the plant. It seems that the amount of phenolic compounds is related not only to geographical and environmental conditions but also to the growth stage of the plants, with higher amounts found in organs that appear later. Naghiloo *et al*. [45] also indicated in their research on the medicinal plant *Astragalus compactus* L. that the amounts of phenolic compounds in the extract of this plant depended on the growth stage, with the highest amounts observed during the reproductive stage. Makari *et al*. [36] showed that the accumulation of phenolic compounds is a type of defensive response to biotic and abiotic stresses. The presence of phenolic compounds in plant extracts is one of the main reasons for their antimicrobial effects. Therefore, considering the high levels of phenolic compounds during the flowering phenological stage, these compounds may be used as natural antioxidants and plant free radical scavengers [36].

In this study, the lowest DPPH radical scavenging was observed in the inflorescence and leaves of Ixiolirion, indicating their highest antioxidant capacity. The differences in antioxidant activity among various plant organs have been demonstrated in numerous studies. The observations of this research, along with the results of other researchers, indicate that the type of organ used can have a considerable impact on the levels of secondary metabolites. Research conducted by Mehrpoor *et al*. [6] on the medicinal plant Ferula also showed that

the type of plant organs is one of the important factors influencing secondary metabolites, with different levels of secondary metabolites in various organs and the study revealed significant differences in the amounts of phenolic, flavonoid compounds, and antioxidant capacity across the three plant organs: root, stem, and leaf. According to the results obtained from the mean comparison table, it is noted that the highest percentage of total phenols and the highest antioxidant capacity were observed in the inflorescence. Therefore, it is expected that there is a reciprocal relationship between these two factors. Khalili & Ebrahimzadeh [46] also attributed the antioxidant activity of plants to the presence of phenolic compounds in them. The results of the study by Fazelinasab *et al*. [14] also indicated that plants with high levels of phenols and flavonoids had relatively higher antioxidant and antimicrobial properties.

The DPPH test is one of the oldest indirect methods for assessing antioxidant properties, based on the reaction of the stable free radical DPPH with hydrogen-donating compounds such as phenols [6]. The antioxidant activity derived from phenolic compounds extracted from various tissues (*Artemisia annua* L.) was examined by Song *et al*. [47] and the results showed that the concentration required to reduce the activity of a biological or biochemical process by half with DPPH free radical reduction method differed among various organs with the amounts reported from highest to lowest in the inflorescence, leaves, stem, and root, which did not contradict the findings of this research. In another study on the levels of secondary metabolites and antioxidant activity of aerial parts (stem, leaf, flower, and flower axis) of the plant Satureja, it was concluded that the type of organ used and ecological factors play an undeniable role in the levels of secondary metabolites in medicinal plants [48]. Hemmati Hassan Gavyar and Armand's [49] results also showed that leave had higher antioxidant power compared to fruit and stem, with the antioxidant power of stem being lower than the others. Sadeghi and Zareie [50] also reported that in all tests, the hexane extract of the inflorescence of the medicinal plant *Descurainia Sophia* and the leave of the medicinal plant *Fumaria vaillantii* had higher DPPH inhibition activity and high antioxidant properties due to their high phenolic and flavonoid percentages compared to other organs. Salmaneian *et al*. [51] reported that the levels of phenolic and flavonoid compounds in the methanolic extract of the medicinal plant *Eryngium caucasicum* were higher, and this extract also exhibited higher antioxidant activity. Jamshidi *et al*. [52] evaluated the methanolic extracts of several native plants of Mazandaran in terms of flavonoid and phenolic compounds. In this study, they demonstrated a suitable relationship between antioxidant activity and the phenolic compounds of the plants. Javanmardi *et al*. [53] also reported a positive linear relationship between antioxidant activity and the total phenolic content of 23 local basil and local *Ocimum accessions* varieties in Iran, which is consistent with the results of the present study.

In this research the investigation of antibacterial properties in different plant organs showed that only the methanolic extract from the stem inhibited the growth of Staphylococcus aureus and Escherichia coli and both bacteria exhibited less resistance with increasing extract concentration, leading to larger inhibition zones. However, extract from other organs did not have a significant effect on bacterial inhibition, with inhibition zones measuring zero. Additionally, as the concentration of the extract increased, the diameter of the inhibition zone also grew, which is consistent with the findings of Rouhi *et al*. [54]. According to the results, the antibacterial effect of the ethanolic extract of *Johernia aromatica* intensified with increasing concentration, such that at 300 mg/ml, the inhibition zone diameter for all microorganisms was greater than at other concentrations [55]. The examination of antibacterial properties indicated that Staphylococcus aureus showed less resistance with increasing extract concentration, resulting in larger inhibition zone [21]. In a study, it was discovered that the methanol extract of the marigold plant, at concentrations of approximately 30 mg/ml, inhibits the growth of the tested Gram-positive bacteria, requiring higher concentrations to affect Gram-negative bacteria. Additionally, the essential oil of marigold exhibits a notable inhibitory effect on Staphylococcus aureus, Bacillus cereus, and Escherichia coli [56]. Entezari *et al*. [57] also reported that the methanolic extract of *Echinophora platyloba* exhibited antibacterial effects against Staphylococcus aureus. The larger inhibition zone diameter for Staphylococcus aureus may be attributed to the higher sensitivity of this Gram-positive bacterium to antimicrobial agents. Tajbakhsh [58] noted the effectiveness of the extract from the Narcissus plant on Staphylococcus aureus and the greater sensitivity of Gram-positive bacteria compared to Gram-negative bacteria to antibacterial agents, which aligns with the current study. Jafarnezhad *et al*. [59] demonstrated in a study on two species from the Apiaceae family that the methanolic extracts of *Bupleurum rutundifolium* and *Jurinea sintenisii* had a significant antimicrobial effect on Gram-positive bacteria. The results of the study on the antibacterial effects of the methanolic extract of *Carum copticum* L. against Gram-positive bacteria were also greater than those against Gram-negative bacteria, with the largest inhibition zone observed for the strain *S. aureus* [60]. The results indicate that the methanol extract's effectiveness against gram-negative bacteria is considerably weaker compared to Gram-positive strains. For instance, at a concentration of 400 mg/ml, the methanolic extract from the leaf exhibits a modest inhibitory effect against Escherichia coli, while showing no inhibitory effect on Pseudomonas aeruginosa at any of the tested concentrations. This discrepancy could be attributed to the presence of cell wall lipopolysaccharides, which potentially hinder the essential oil and extract's active compounds from reaching the cytoplasmic membrane of Gram-negative bacteria. Generally, plant products induce cytoplasm granulation, cytoplasmic membrane rupture, inactivation or inhibition of intracellular and extracellular enzyme activity, and cell wall disintegration. As a result, most plant extracts demonstrate inhibitory effects on Gram-positive bacteria but exhibit less impact on Gram-negative bacteria [56]. The greater sensitivity of Gram-positive bacteria appears to be related to the thicker mucopeptide of their cell walls, while the cell walls of Gram-negative bacteria are significantly thinner. Kim *et al*. [61], in a study examining antibacterial properties, showed that Escherichia coli exhibited resistance to plants extracts due to its Gram-negative nature, meaning that the presence of lipids in its cell membrane conferred resistance to the extracts. In contrast, in a research results showed that the extracts affected three bacteria: Bacillus subtilis, Staphylococcus aureus, and Micrococcus luteus, which is due to their gram-positive nature (lacking lipids in their cell membranes) [62].

Given the consumer preference, especially among patients, for using medicinal plants to treat infections due to fewer side effects compared to chemical drugs, this approach could serve as a low-side-effect method to combat infection-causing bacteria, particularly Staphylococcus aureus [63].

CONCLUSION

Based on the results obtained from this research, among the studied organs of *Ixiolirion tataricum* L., the highest levels of protein, total phenols, and antioxidant properties were found in the inflorescence, while the highest antimicrobial activity was observed in the stem.

Therefore, the plant has been more successful in accumulating and storing beneficial biochemical compounds in its aerial parts. The assessment of total phenol percentage and DPPH radical scavenging capacity indicated a positive relationship between the amount of phytochemical compounds in Ixiolirion organs and its antioxidant capacity. Overall, the results demonstrated that the highest antimicrobial property of the Ixiolirion plant was derived from the methanolic extract of its stem, which had the most significant inhibitory effect on the Gram-positive bacterium Staphylococcus aureus and this effect increased with increasing extract concentration, leading to a larger diameter of the growth inhibition zone. The findings of this research highlight the antibacterial and antioxidant effects of the Ixiolirion plant, which could reduce the side effects and undesirable impacts of chemical antibiotics and synthetic antioxidants in the pharmaceutical industry.

CONFLICT OF INTEREST

The authors declare no conflict of interest in this study.

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