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Original Article

Investigation of the Antimicrobial Activity of Nine Medicinal Plants on Standard Bacteria

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ABSTRACT

Introduction: Medicinal plants have important roles in the treatment of infections. This study aimed to investigate the relationship among the amount of phenol, flavonoid, and antioxidant properties, as well as the effect of antimicrobial properties of methanolic extracts of nine medicinal plants against standard bacteria.
Materials and Methods: Nine plants were collected from Zabol, located in the south-eastern of Iran and identified in the botanical laboratory of the University of Zabol, Iran. The soaking process prepared extracts including *Althaea officinalis*, *Calotropis procera*, *Eryngium caucasicum*, *Malva Sylvestris*, *Nerium oleander*, *Saponaria officinali*, *Satureja hortensis*, *Sinapis alba*, and *Urtica dioica*, and total phenol and flavonoid content were measured by folin-ciocaltio reagent and aluminum chloride by colorimetric methods, antioxidant activity by 2, 2-diphenyl-1-picrylhydrazyl method, and antibacterial activity of extracts against standard bacteria (*Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Proteus mirabilis*, *Escherichia coli*, *Bacillus cereus*, *Streptococcus mutans*, *Hafnia elevi*, *Enterococcus fecalis*) were evaluated.
Results: The results showed that the methanol extract of *N. oleander* with an average of 3.36 mg/g and *C. procera* with an average of 0.48 mg/g of dry weight have the highest and lowest amounts of phenolic compounds, respectively. *C. procera* extract (ith an average of 85.54 mg/ml was the most effective and *M. sylvestris* extract with an average of 21.80 mg/ml had the least role in inhibiting free radicals. The results of the antimicrobial activity of different extracts showed that the largest non-growth zone diameter in bacteria *P. mirabilis*, *E. coli*, and *H. alevi* is related to the extract of *N. oleander*.
Conclusion: The results of this study showed the differences in the number of effective compounds of the studied plants and their antioxidant properties. Also, after carefully examining the effects of these extracts *in vitro* and *in vivo*, it is suggested that these extracts be studied as a substitute for chemical drugs to treat infections.

1. Introduction

One of the current problems of bacterial infection treatment is their resistance increase to antibiotics. Antibiotic-resistant bacteria cause significant mortality, compared to non-resistant bacteria[1-4]. Among the antibiotic-resistant Gram-negative bacteria that cause hospital infections, one can mention *Enterobacteriaceae*, *Pseudomonas*, and *Acinetobacter* species, and among the Gram-positive bacteria, one can mention *Staphylococcus*, *Streptococcus*, and *Enterococcus* species[5-7]. The increasing resistances of pathogenic bacteria and the constant change in the sensitivity patterns of microorganisms to antibiotics have made the treatment of infectious diseases difficult and expensive; moreover, the worrying issue is the death of many people due to infections resistant to antibiotic treatment[8-10]. In addition to these problems, some of these antibiotics have adverse effects, including severe sensitivity, suppression of the immune system, and allergic reactions in the host. Therefore, there is a need to develop natural and alternative antimicrobial drugs for the treatment of infectious diseases. In recent decades, plant

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materials, especially medicinal plants, have been considered a natural source for the treatment of infectious diseases worldwide [11].

Medicinal plants are among the potential resources that have been receiving attention for their therapeutic and medicinal properties for a long time; these plants are a rich source of raw materials for many drugs, which are mainly considered plant secondary metabolites. Although these materials are made by guiding genetic processes, their construction is significantly influenced by environmental factors[12]. Determination of the number of effective compounds and antioxidant value in medicinal plants is of great importance due to their wide use in various industries, such as food, medicine, cosmetics, as well as sanitary and industrial goods[13].

Antioxidants are polyphenol compounds that are found in all plants, as well as all their parts, such as leaves, stems, fruits, roots, and seeds[15]. Antioxidants can remove free radicals, superoxide, and hydroxyl radicals through the transfer of single electrons and prevent their destructive effect on cell membranes, proteins, and nucleic acids[14]. In other words, antioxidants in low concentration significantly delay or prevent the oxidation of lipids, and their antioxidant activity depends on the total amount of their phenolic and flavonoid compounds[15].

One of the plants' largest groups of secondary metabolites is phenolic compounds (phenols and flavonoids). Phenolic compounds are made of aromatic nuclei or several OH groups. Due to having a hydroxyl group as a hydrogen or electron donor, they can neutralize free radicals and produce stable phenoxyl compounds[16]. These compounds have high antioxidant properties and are usually found in fruits, vegetables, leaves, seeds, roots, and other plants. They include compounds, such as flavonoids, flavonols, anthocyanins, anthraquinone, stilbenoid, and their derivatives[17]. The antioxidant properties of these compounds depend on their ability to donate electrons to trap free radicals by forming stable phenoxyl compounds[18]. Flavonoids are among the phenolic compounds that directly inhibit the active molecules of superoxide, hydrogen peroxide, and hydroxyl radicals. Phenolic acids, stilbenes, tannins, lignans, and lignins are usually found in leaves and wood parts (e.g., stems and branches)[13] and their antioxidant properties are due to the presence of phenolic hydroxyl groups in their structure. Trapping and removing free radicals is one of the critical roles of the antioxidant activity of these compounds[19].

Due to the increasing resistance of microorganisms to antibiotics, the use of antimicrobial compounds in plants as natural agents that have lethal and inhibitory effects on pathogenic agents has received more attention. Moreover, owing to the diversity of climate and a result of the very diverse plant flora in Iran, it is possible to identify effective plant substances in different native plants of the country and extract them industrially to produce these substances in large quantities. Therefore, the introduction of valuable species or ecotypes is of great importance in terms of having highly effective substances, and on the other hand, providing antioxidant reserves from natural sources is considered important to reduce the effects of oxidative damage caused by free radicals[20].

2. Materials and Methods

2.1. Study procedure

Medicinal plant parts were collected from Zabol, located in the south-eastern in Iran and then they were identified in the botanical laboratory of University of Zabol (Table 1). Plant materials were dried in the shade and then ground into powder to prepare methanolic extract (96%). Extraction was done by the soaking method[1]. For this purpose, initially, first 5 grams of all plant samples were weighed using a digital scale and poured into the desired solvent in a ratio of 1:10. After 48 hours at room temperature on the shaker, filtration was performed using Whatman No. 1 filter paper and Buchner funnel. The extracted extract was allowed to be separated from the solvent based on the density difference with the used solvents before smoothing it with a clean paper. Most of the solvents were removed using a rotary evaporator. The concentrated extract was spread on the surface of the glass plates in the form of a thin sheet and then transferred to the oven to remove the remaining solvent and the dried sediment of each extract was obtained. After drying, the extracts were scraped from the surface of glass plates using a metal blade, and the resulting powder was stored in dark-colored glass containers in the refrigerator at 4ºC until the next tests.

2.2. Extraction for total phenol, total flavonoid, and antioxidant measurements

The methanolic extract was prepared by cold maceration method with a ratio of 1:20 dry plant material and 80% methanol solvent[2].

2.3. Measurement of total phenol

The amounts of phenolic compounds in methanolic plant extracts were measured[5,21].

Table 1. The names, family and the parts of the plants used in this study

Row	Scientific name row	Family	The part used
1	*Althaea officinalis*	Malvaceae	Leaves, roots, and flowers
2	*Calotropis procera*	Apocynaceae	Sap, root, stem, leaf, and flower
3	*Eryngium caucasicum*	Apiaceae	All parts of the plant
4	*Malva sylvestris*	Malvacea	All parts of the plant
5	*Nerium oleander*	Apocynaceae	Flowering branches
6	*Saponaria officinalis*	Caryophyllaceae	Leaves and roots
7	*Satureja hortensis*	Lamiaceae	Leaf
8	*Sinapis alba*	Brassicaceae	Seed
9	*Urtica dioica*	Urticaceae	Leaves, roots, flowers, and seeds

2.4. Total flavonoid assay

The flavonoid content of these extracts was measured by the aluminum chloride colorimetric method[22,23].

2.5. Measurement of antioxidant activity

DPPH free radical inhibition activity was measured according to the method of Barros et al[23].

2.6. Bacterial strains and culture conditions

Bacterial strains were obtained from the standard laboratory of the Department of Veterinary Medicine, University of Zabol, Iran. Bacterial strains included *S. pyogenes* ATCC® 19615™, *S. saprophyticus* ATCC® 15305, *S. pneumoniae* ATCC 49619, *Hafnia alvei* ATCC 51873, *S. aureus* ATCC® 25923, *Serratia marcescens* ATCC 274, *Enterococcus faecalis* ATCC 29212, *P. mirabilis* ATCC 35659, and *Acinetobacter baumannii* ATCC 19606 propagated on nutrient agar culture medium and kept in a refrigerator at 4°C until use.

To prepare bacterial suspension from fresh and young bacterial cultures, several colonies were transferred to the Mueller Hinton Broth culture medium. To equalize the turbidity of the microbial suspension prepared according to McFarland standard tube number 0.5 (turbidity equal to 1.5×10^8 bacteria per milliliter), the light absorption at the wavelength of 630 nm was set in the range of 0.08 to 0.1. To reach a concentration of 1.5 ×107 bacteria per milliliter, the bacterial suspension with a McFarland turbidity of 0.5 was diluted to 0.1. The antimicrobial effects of the extracts with a concentration of 250 mg/ml were investigated by the diffusion method in agar. With the help of a sterile swab of turbidity equal to 1.5×10^7 bacteria per milliliter, it was cultured uniformly on Mueller Hinton agar culture medium. Then, several wells with a diameter of 6 mm and a depth of 5 mm were created at appropriate intervals. Afterward, 100 microliters of the extracts were poured into the corresponding well. The antibiotic ciprofloxacin was used as a positive control. After 24 hours of incubation at 37°C, the diameter of the non-growth of bacterial samples was measured in millimeters. To confirm the results of the test for each of the extracts and each bacterial sample, it was repeated three times.

2.7. Statistical analysis

The statistical analysis of the data was performed using SPSS software (Version 20) in the form of a completely random design in three repetitions; moreover, Duncan's statistical test was used to compare the statistical means of averages in a data group and it was considered statistically significant when the p value was < 0.05.

3. Results

The results of variance analysis of the data showed that the effect of different plant extracts on phenolic compounds, flavonoids, and inhibition of free radicals was significant at the level of 1% (Table 2).

Based on the average data comparison results, it was found that among the studied plants, *N. oleander* extract with an average of 3.36 and *C. procera* with an average of 0.484 mg/g dry weight of the extract had the highest and lowest amount of phenol, respectively (Figure 1). Also, *N. oleander* extract showed the highest amount of flavonoids with an average of 399.65 and *U. dioica* with an average of 160.53 mg/g dry weight of the extract (Figure 2).

Table 2. The amounts of phenol, flavonoid, and antioxidant activity in different plant extracts

Source of variation (sov)	Degrees of freedom(df)	Phenol	Flavonoid	Antioxidant activity
Plant	8	2.42**	48273.9**	1748.92**
Error	21	0.091	64.5	24.06
Coefficient of variation		28.03	2.27	8.1

*, and **: no significant difference, as well as the significant difference at 0.5% and 1%, respectively

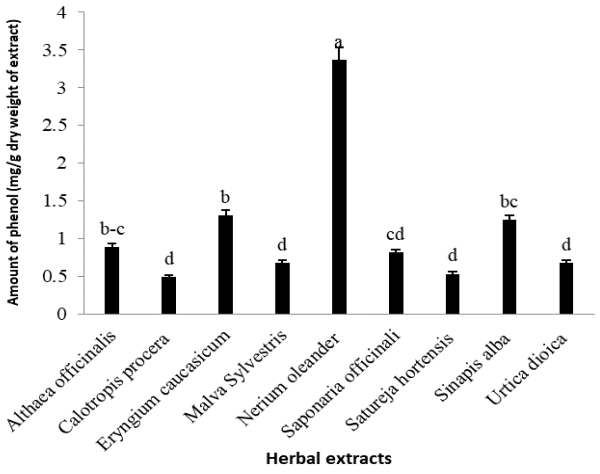

Figure 1. The effect of different plant extracts on the amount of phenolic compounds

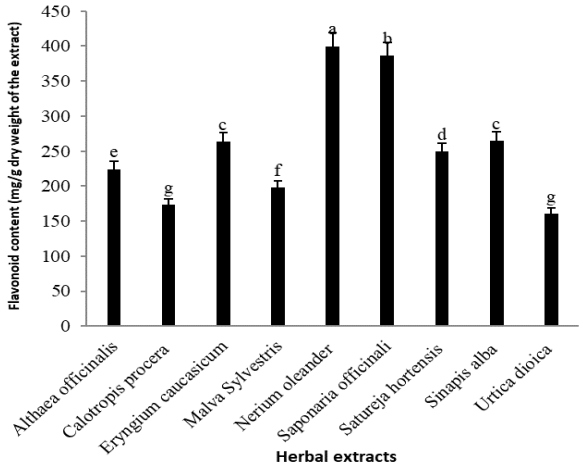

Figure 2. The effect of different plant extracts on the amount of flavonoid compounds

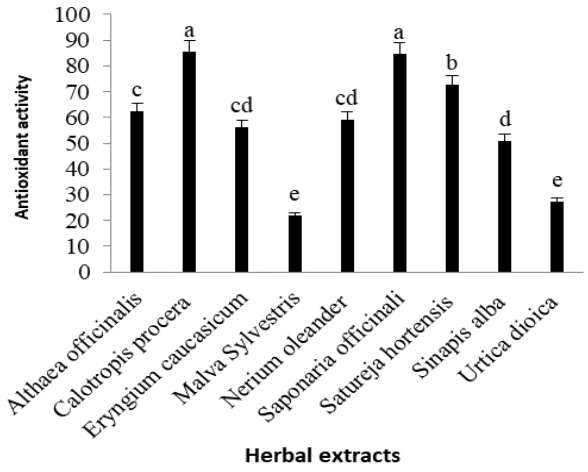

Figure 3. The effect of different plant extracts on the amount of antioxidant activity

Additionally, the results showed that *C. Procera* extract (average 85.54 mg/ml) was the most effective among different plant extracts, and *M. Sylvestris* extract (intermediate 21.80 mg/ml) had a minor role in inhibiting free radicals (Figure 3).

The results obtained from the variance analysis of the data in Table 3 showed that the non-growth zone diameter of the investigated bacteria was affected by different plant extracts, and the difference was statistically significant at the level of 1%.

Based on the average comparison results, it was found that the methanolic extract of *S. officinalis* with growth zone diameter was the most effective extract on *S. aureus* and *S. pyogenes*; in addition, the methanolic extract of *A. officinalis* had the least effect on *S. aureus*. In *S. pyogenes* bacteria, the smallest non-growth zone diameter was related to *C. procera*, *E. caucasicum, S. alba*, and *U. dioica* extracts (Figures 4, 5).

Table 3. The non-growth zone diameter of some pathogens in different plant extracts

Source of variation (sov)	Degrees of freedom (df)	*S. aureus*	*S. pyogenes*	*P. aeruginosa*	*S. pneumoniae*	*H. elevi*	*E. fecalis*	*p. mirabilis*	*E. coli*	*S. mutans*	*B. cereus*
Herbal extract	9	383**	0.315**	0.92**	0.79**	0.57**	0.35**	0.38**	0.69**	0.69**	0.58**
Error	120	0.006	0.064	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006
Coefficient of variation		13.35	13.93	12.82	12.89	12.69	13.26	12.57	12.89	12.46	13.86

* and ** are significant at 1% and 5% probability level, respectively, and ns is not significant at the 5% level.

The results showed that among the studied medicinal plants, the methanolic extract of the plants used had an antimicrobial effect on the studied strains. Thus, the largest non-growth zone diameter of *H. elevi*, *P. mirabilis*, and *E. coli* bacteria was related to the methanolic extract of *N. oleander* plant. However, in *P. mirabilis*, there was no significant difference in terms of the diameter of the absence zone diameter between *N. oleander* and *U. dioica* extracts (Figures 6, 7, 8). On the other hand, based on the average comparison results in Figure 9, it was found that the highest and lowest non-growth zone diameter in *E. phicalis* bacteria were related to the methanolic extracts of medicinal plants, namely *U. dioica*, and *C. procera*.

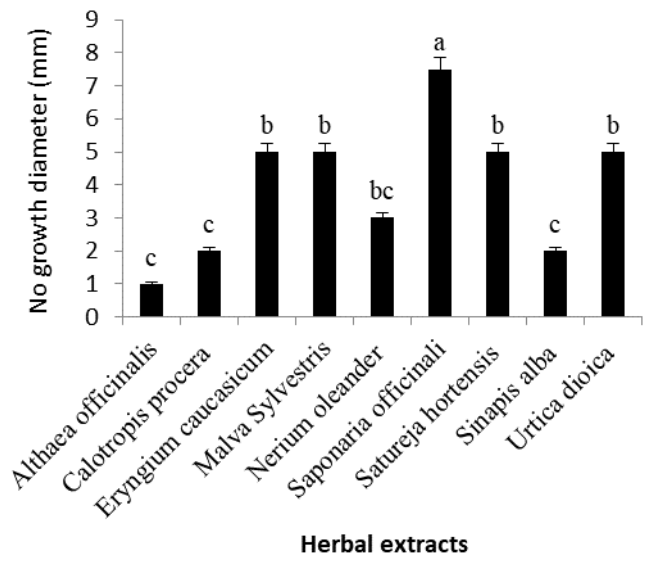

Figure 4. Comparison of the average zone diameter of methanol extract of different plants on *S. aureus* bacteria

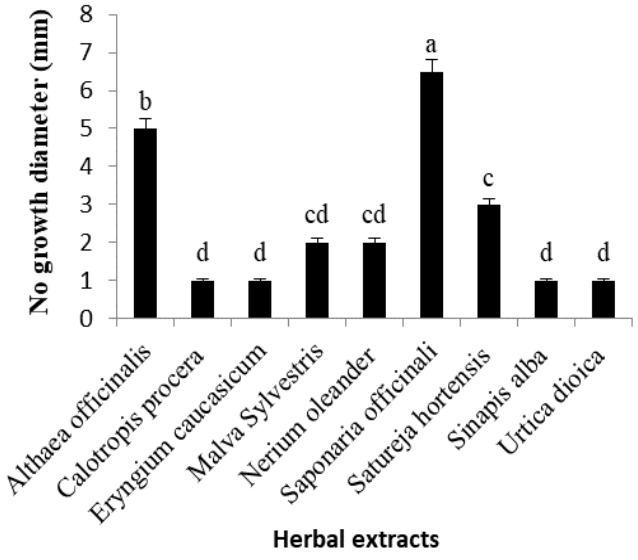

Figure 5. Comparison of the average non-growth zone diameter of methanol extract of different plants on *S. pyogenes*

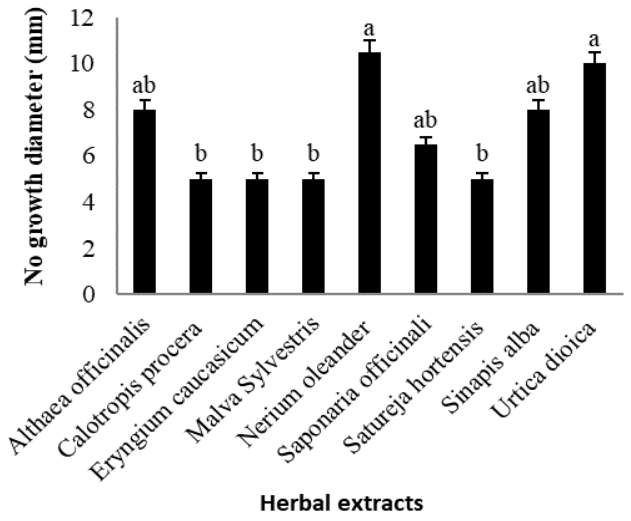

Figure 6. Comparison of the average non-growth zone diameter of methanol extract of different plants on *P. mirabilis*

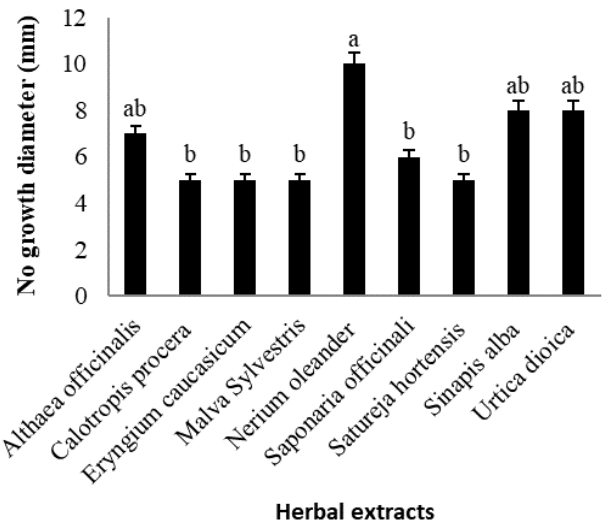

Figure 7. Comparison of the average non-growth zone diameter of methanolic extracts of different plants on *H. elevi*

Based on the average comparison results, it was found that the methanolic extract of *S. hortensis* with the largest growth zone diameter was the most effective extract on *P. aeruginosa*, Streptococcus pneumonia, and Streptococcus mutants (Figures 10, 11 and 12). In *B. cereus* bacteria, the highest non-growth zone diameter was associated with *S. alba* extract and the lowest diameter was associated with *C. procera* and *M. Sylvestris* extracts (Figure 13).

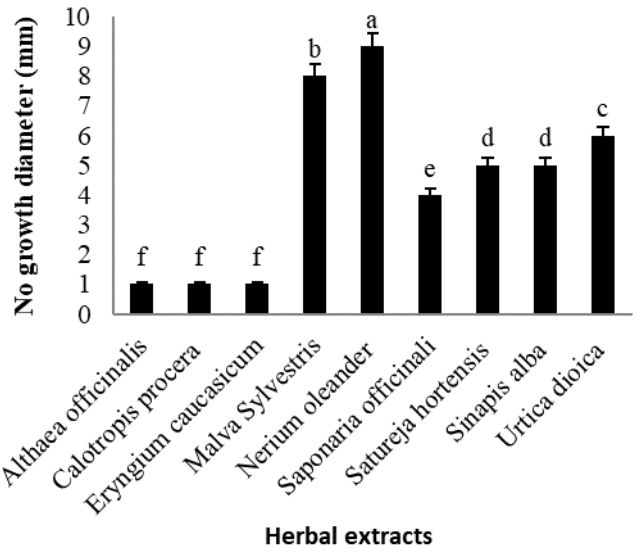

Figure 8. Comparison of the average non-growth zone diameter of methanol extract of different plants on *E. coli*

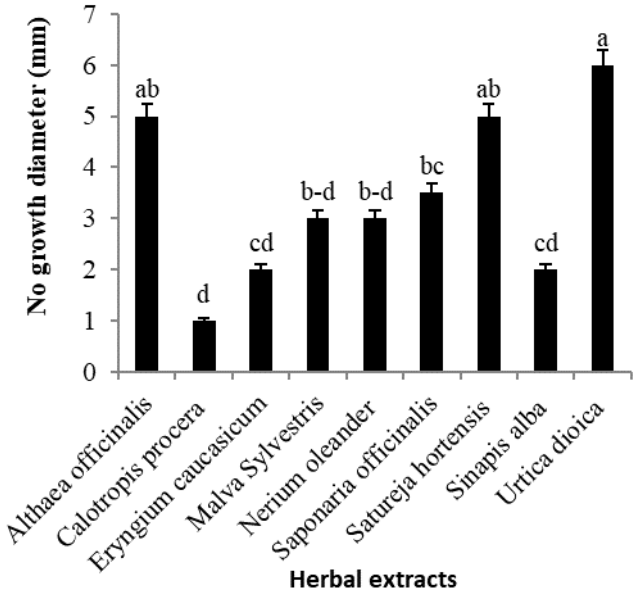

Figure 9. Comparison of the average non-growth zone diameter of methanolic extracts of different plants on *E. phicalis*

4. Discussion

Based on the results of this study, it was found that there is a significant difference among the methanolic extracts of different studied plants in terms of the amount of phenolic, flavonoid, and antioxidant activity. Among the investigated plants, the number of phenolic compounds varied between 0.484 and 3.36, and flavonoid compounds were between 160.53 and 399.65 mg/g dry weight of the extract. The highest amount of phenolic compounds (3.36 mg/g dry weight of the extract) and flavonoid compounds (399.65 mg/g dry weight of the extract) belonged to the medicinal plant *N. oleander*. On the other hand, the lowest amount of the above compounds was related to the methanolic extracts of *C. procera* (0.484 mg/g dry weight of the extract) and *U. dioica* (160.53 mg/g dry weight of the extract), respectively. This difference between the examined plant extracts in terms of the number of compounds may be influenced by genetic differences, as well as climatic and geographical differences, such as altitude in different regions. This shows the effect of these factors on the amount of phenolic and flavonoid

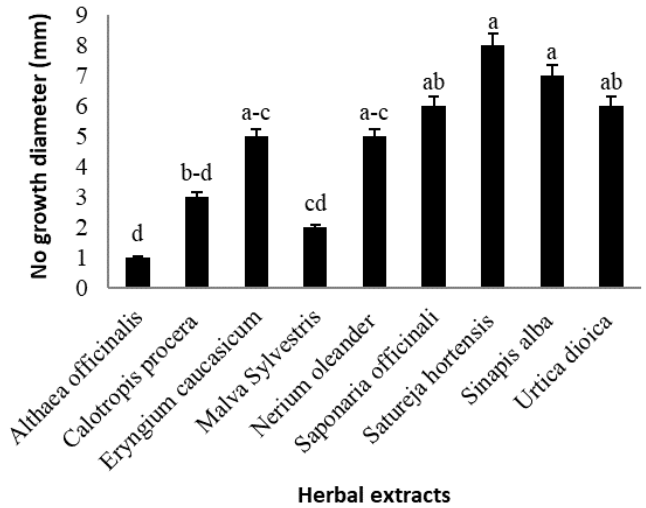

Figure 10. Comparison of the average non-growth zone diameter of methanol extract of different plants on *S. pneumoniae*

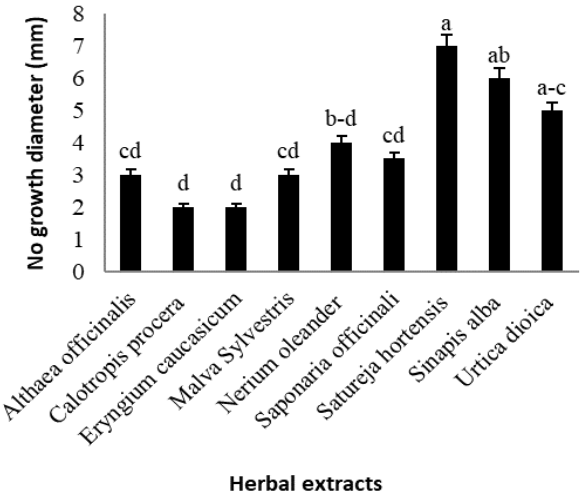

Figure 11. Comparison of the average non-growth zone diameter of methanol extract of different plants on *P. aeruginosa*

compounds in all medicinal plants. These results are consistent with the results of a study by Terronen and Hakkinen[24], who stated that the amount of phenolic and flavonoid compounds of plants in different regions is influenced by factors, such as genetic differences, as well as climatic and geographical differences in different regions.

One of the methods of evaluating the antioxidant activity of plants is the use of 2,2-diphenyl-1-pyrylhydrazyl (DPPH)[17]. In this method, the compounds that change the DPPH free radical color from purple to yellow by taking hydrogen or electron are compounds with antioxidant ability. Therefore, on this basis, DPPH stable radical scavenging model is used to evaluate the free radical scavenging ability in various samples[25,26]. Based on the results of the DPPH test, it was observed that there is a wide range of antioxidant properties among the investigated plants; some of these plants had strong antioxidant activity and others had less activity. The highest and lowest levels of free radical scavenging activity were related to the extracts of *C. procera* (85.54 mg/ml) and *M. Sylvestris* (21.8 mg/ml), respectively. One of the methods of evaluating the antioxidant activity of plants is using the DPPH method.

The results of various pieces of research have shown that the ability of plant extracts to inhibit free radicals depends on the phenolic compounds since the antioxidant property increases with the increase of phenolic compounds[15,26]. However, based on the results of this study, it was found that the ability of the extracts of the examined species to inhibit free radicals is not so dependent on phenolic compounds. In some cases, despite the higher or lower phenolic compounds, the antioxidant properties are not proportional to these compounds, which indicates other influencing factors that exist in these plants and affect their antioxidant properties during reactions[17].

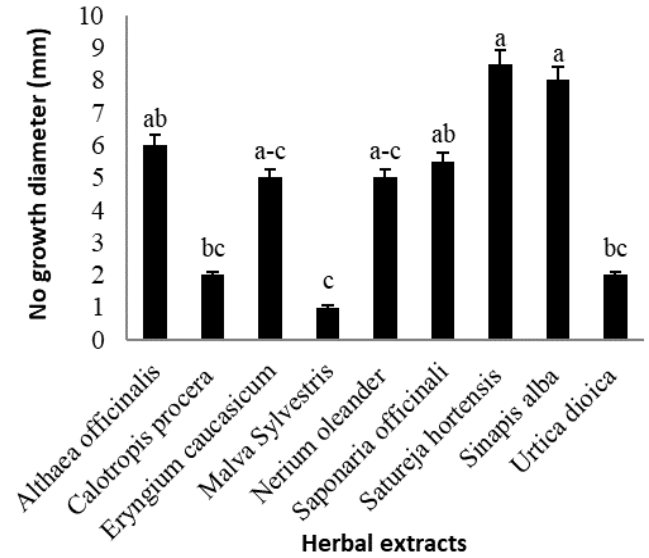

Figure 12. Comparison of the average non-growth zone diameter of methanol extract of different plants on *S. mutans*

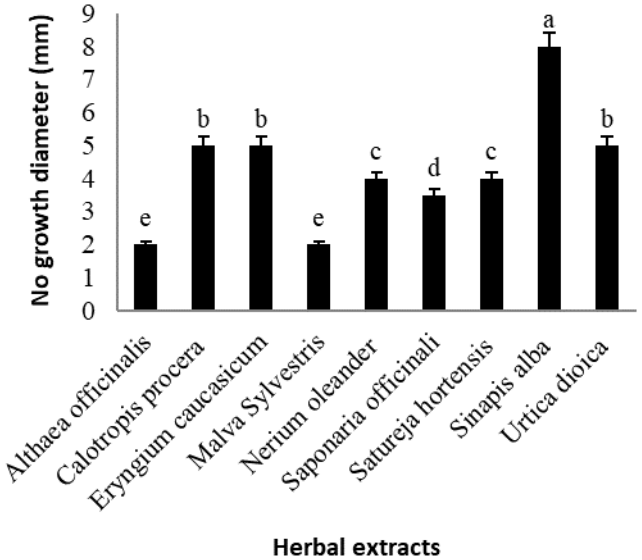

Figure 13. Comparison of the average non-growth zone diameter of methanol extract of different plants on *B. cereus*

It has been reported that antioxidant activity has a positive correlation with the antimicrobial properties of the extracts[27]. Based on the results of the antimicrobial activity of the extracts, it was found that among all the extracts studied against the investigated strains, the methanolic extracts of *N. oleander*, *S. officinali*, *U. dioica*, *S. alba*, and *S. hortensis* had the largest growth zone diameter. A study by Sabzali et al.[28] investigated and reported the effect of methanol extract from the oleander plant on 9 Gram-negative and positive strains (*E. coli*, *S. aureus*, *Staphylococcus epidermidis*, *Enterobacter aerogenes*, *Klebsiella pneumonia*, *Morganella morganii*, *B. cereus*, *Salmonella typhi*, and *P. aeruginosa*). The hydroalcoholic extract of *N. oleander* has a significant bactericidal effect on the bacteria under study, which confirms the results of the above study. In another study, Malik et al.[29], found the antibacterial effect of *N. oleander* extract has been investigated on *S. aureus*, *E. coli*, and *P. aeruginosa* bacteria, and the results stated that the above extract has a significant effect on Gram-negative bacteria.

In the same vein, a study by Golpasand et al.[30] investigated the effect of *S. hortensis* extract and essential oil on three pathogenic strains of Streptococcus, including S. mutant strain, and stated that the aqueous extract tested had no antibacterial effect on the three evaluated microorganisms but its essential oil had a strong antibacterial effect on *S. mutans*, *S. Salivarius*, and *S. Sanguis*, which confirms the results of the above study. In a report, the antibacterial effect of three types of *S. hortensis* medicinal plant extracts on nine pathogenic strains, including *S. aureus*, *E. coli*, *E. faecalis*, and *P. aeruginosa* was investigated. The results showed that all three types of extracts have a significant antibacterial effect on the investigated strains, which is in line with the findings of this study stating that the methanol extract of *S. hortensis* has a significant effect on the said strain[31]. In another report, the effect of different concentrations of *S. hortensis* extract on the biofilm formation of five human pathogens, including *S. aureus*, *S. pyogenes*, *S. pneumoniae*, *Staphylococcus saprophyticus*, and *p. mirabilis* was investigated. The results showed that *S. hortensis* plant extract at a concentration of 100 ppm completely prevented the formation of *S. pneumoniae* and *S. saprophyticus* bacteria biofilm. Also, at concentrations of 50 ppm and 100 ppm, the biofilm formation of *S. aureus* and *P. mirabilis* bacteria was zero. In this case, the amount of biofilm formation of *S. pyogenes* bacteria is gradually reduced with increasing concentration, but not completely inhibited, which is consistent with the findings of this research[32].

In another study Mahboubi and Kazempour[33] studied the effect of *S. hortensis* extract was evaluated on three pathogenic microorganisms, including *S. aureus*, *P. aeruginosa*, *E. coli*, and *S. typhi*murium by broth microdilution method. Their results showed that *P. aeruginosa* was more resistant to *S. hortensis* extract, while *S. aureus* and *E. coli* were more sensitive to it. So that the MIC level for *S. aureus* was 2 ml/ml, that of the *E. coli* and *S. typhi*murium was 1 ml/ml, and that of the *P. aeruginosa* was 8 ml/ml. Contrary to the findings of these researchers, the use of *S. hortensis* extract during the present study completely inhibited the growth of *P. aeruginosa* bacteria. Thus, the largest diameter of non-growth zone diameter during the application of *S. hortensis* extract was related to the above bacteria.

Countless studies have been performed on the *U. dioica* plant in various fields of medicine and medicinal plants; however, little information has been reported about its antimicrobial properties. In a study Gülçin et al.[34] found that the aqueous extract of *U. dioica* has antimicrobial properties on *E. coli*, *E. aerogenes*, *S. epidermidis*, and *Candida albicans*, while this extract had no effect on Gram-negative resistant bacterium *P. aeruginosa*, and contrary to the results of these researchers, the methanolic extract of *U. dioica* had a significant inhibitory effect on all the investigated strains.

In a study by Modarresi-Chahardehi et al.[35], the antimicrobial properties of alcoholic extracts of *U. dioica* were investigated on several Gram-negative and positive bacteria, including *B. cereus*, *E. coli*, and *S. aureus* using the plate diffusion method. The results showed that *U. dioica* extract has an inhibitory effect on the investigated strains; however, it had the highest inhibitory effect on Gram-positive bacteria. The best results were for Gram-positive bacteria of the genus Bacillus, which is in line with the findings of this research that the methanolic extract of *U. dioica* affects all Gram-negative and positive strains.

There are reports on the antimicrobial effects of *A. officinalis* extract[36-38]. The results of this study show that *A. officinalis* extract is effective against Gram-negative and positive bacteria. In another study Valiei et al.[39] found the effect of hexane extract of the flower and root of *A. officinalis* on some Gram-negative and positive bacteria, such as *P. aeruginosa*, *E. coli*, *Bacillus subtilis*, *E. faecalis*, *S. aureus*, and *S. epidermidis* were investigated. The results showed that the hexane extract of flowers and roots has an inhibitory effect on all investigated strains. Zareii et al.[40] researched, the effect of different concentrations of *A. officinalis* extract was investigated on *E. coli*, *K. pneumoniae*, *S. aureus*, and *Streptococcus agalactiae* bacteria using the agar diffusion method and tube dilution. The results showed that the alcoholic extract of *A. officinalis* in all dilutions and a dose-dependent manner caused a significant increase in the non-growth zone diameter in all four bacterial strains so that the maximum non-growth zone diameter of all four bacteria was observed at the concentration of 800 mg/ml. Furthermore, the comparison between Gram-positive and Gram-negative bacteria showed that *A. officinalis* extract has better antimicrobial effects on Gram-positive bacteria.

The results of this research showed that the methanolic extract of *E. caucasicum* inhibited the growth of all Gram-negative and positive strains, so it had the highest inhibition diameter on Gram-positive bacteria and the least effect on Gram-negative bacteria. These results were consistent with the findings of previous pieces of research, which investigated and expressed[41-43] the effect of *E. caucasicum* root and stem extract on Gram-negative (*E. coli*

and *P. aeruginosa*) and positive (*S. aureus* and *B. subtilis*) bacteria using the minimum growth inhibitory concentration method. *E. caucasicum* root and stem extract inhibited the growth of all four investigated bacteria; however, the effect and inhibitory power of the extracts on Gram-positive bacteria was more than that on Gram-negative bacteria.

So far, many different studies have been conducted regarding the evaluation of the antimicrobial properties of *M. Sylvestris* species in different parts of the world and Iran. In a study by Hassanpour et al.[44], it was found that the non-polar extract of the aerial parts of *M. Sylvestris* plant in Arsbaran region has significant growth inhibitory effects on *S. aureus*, *B. cereus*, *E. coli*, and *P. aeruginosa*. Also, the inhibitory effects on Gram-positive bacteria are more than that on Gram-negative bacteria, although the results of this research showed that the methanolic extract of *M. Sylvestris* has a significant effect on Gram-negative bacteria in addition to Gram-positive bacteria. Moreover, it had the greatest effect on the Gram-negative bacteria *E. coli*, and the smallest non-growth zone diameter was related to the Gram-positive bacteria *S. mutans*. In addition, these results are in line with the findings of a study by Mehran et al., who stated that *M. Sylvestris* plant extract has an antimicrobial effect on *S. mutans* bacteria. It is worth mentioning that the diameter of the aura of its lack of growth was not as large as the results of this research. In a report by Eghbal et al.[45], the antimicrobial effect of the medicinal plant *M. Sylvestris* on some Gram-negative and positive bacteria was investigated by the disk diffusion method. The results showed that this plant has significant inhibitory effects on all types of Gram-negative and positive bacteria, which confirms the results of the above study.

So far, many different studies have been conducted regarding the evaluation of the antimicrobial property of different organs of the *C. procera* plant in different parts of the world. In all the research, the antimicrobial effect of this valuable plant has been proven on the investigated strains[46-48]. Based on the results of this research, it has been determined that the methanolic extract of *C. procera* has a great inhibitory effect on Gram-negative and positive bacteria, some Gram-positive and negative bacteria had a greater effect and others had a lesser effect. However, it has an inhibitory effect. This positive effect of *C. procera* extract on Gram-negative and positive strains is consistent with the findings of previous pieces of research[49,50]. In separate reports, these researchers investigated the antimicrobial effect of *C. procera* plant extracts on *E. coli*, *S. aureus*, Staphylococcus albus, *S. pyogenes*, *S. pneumoniae*, *B. subtilis*, and *P. aeruginosa* bacteria. The results showed that the above extracts had different inhibitory effects; however, they inhibited the growth of Gram-negative and positive strains. The other study, Bilal et al.[51] has investigated the antimicrobial effect of the methanolic extract of *C. procera* on *P. aeruginosa*, *E. coli*, *p. mirabilis*, *E. faecalis*, *B. cereus*, *S. typhi*, and *K. pneumonia* by disc diffusion method. The results showed that the methanolic leaf extract had the highest inhibitory effect on *P. mirabilis*, *B. cereus*, and *P. aeruginosa* bacteria, but it had no inhibitory effect on *E. coli*, *E. faecalis*, *S. typhi*, and *K. pneumonia* bacteria. Based on the results of this research, the methanolic extract of *C. procera* leaves had a significant inhibitory effect on all Gram-negative and positive strains.

The largest non-growth zone diameter was related to *S. aureus* bacteria and the smallest was related to *B. cereus*, *E. fecalis*, and *S. pyogenes*. However, it had a significant inhibitory effect on all the investigated bacteria. The effectiveness of *S. officinali* extract on Gram-negative and positive strains is consistent with the findings of the study of previous research[52]. These researchers investigated the effect of methanolic, acetone, and ethanolic extracts on some Gram-negative and positive strains, including *S. aureus*, *E. faecalis*, and *E. coli*. Their results showed that all three extracts used have a significant inhibitory effect against all three studied strains. In another study by Sengul et al.[53], the effect of aqueous and methanolic extracts of *S. officinali* was investigated on 27 pathogenic strains, including *S. pyogenes*, *S. aureus*, *E. coli*, *P. mirabilis*, *P. aeruginosa*, and *B. cereus*. The results showed that the methanolic extract had a better performance in inhibiting bacteria than the aqueous extract. Among the studied strains, it had the highest inhibitory effect on *S. aureus*, *E. coli*, *P. mirabilis*, and *P. aeruginosa* bacteria, but it did not affect *S. pyogenes* and *B. cereus*. Contrary to the findings of these researchers, the methanolic extract of *S. officinali* had an inhibitory effect on all the investigated strains.

Based on the results of this research, it was found that the methanolic extract of *S. alba* had a positive effect on all investigated bacteria, so it had a greater effect on some Gram-positive and negative bacteria and a lesser effect on others. However, it has a significant inhibitory effect on the investigated strains. This positive effect of the above extract on the investigated strains is consistent with the results of a previous study by Sujatha et al.[54]. These researchers investigated the effect of different concentrations of hexane extract of *S. alba* on three pathogenic strains of *S. pneumoniae*, *S. Typhi*, and *K. Pneumoniae*. The results showed that the inhibitory power of the above extract was dependent on the concentration, and the growth inhibition of the studied strains was obtained only at the concentrations of 100 and 150.

5. Conclusion

The results of this study showed the differences in the number of effective compounds of the studied plants and their antioxidant properties. This may be due to the influence of various factors, such as the type of species, climate of the region, soil environment, altitude above sea level, and geographical location. Each of these factors can have a significant effect on the quantity and quality of the mentioned compounds. The results of the microbial test showed that all the medicinal extracts mentioned in this study had antimicrobial properties; however, they had different effects on the growth of Gram-negative and positive bacteria. The reason for this may be related to

various reasons, including the structural differences between the walls of these two groups of bacteria. More in-depth analysis is required to isolate and identify the bioactive secondary compounds in this plants in order to develop novel alternative antibiotics.

Declarations

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

This study was prepared and done by team member with specific contribution of each author. All the authors read and approved the final version of the manuscript.

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Availability of data and materials

The data used in this study can be made available from the corresponding author upon reasonable request.

Ethical considerations

The authors declare that this manuscript is original and is not being considered elsewhere for publication. Other ethical issues, including consent to publish, misconduct, fabrication of data, and redundancy, have been checked by the authors.

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