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Evaluation of the antioxidant, antibacterial, phenolic, and flavonoid potential of the total essential oil of *Artemisia sieberi* in laboratory conditions

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ABSTRACT

Artemisia sieberi plant (*Artemisia sieberi*), one of the Artemisia species with wide distribution in Iran and the Middle East, has received attention due to its various medicinal properties, including antimicrobial effects. The aim of this study was to determine the total phenolic and flavonoid content, antioxidant activity (DPPH and ABTS radical scavenging), and antimicrobial activity (agar disk and well diffusion, minimum inhibitory concentration, and minimum bactericidal concentration) of the essential oil of *Artemisia sieberi*. The total phenolic and flavonoid contents were determined to be 44.62 ± 0.53 mg gallic acid equivalent (GAE)/g and 18.20 ± 0.29 mg quercetin equivalent (QE)/g, respectively. Antioxidant activity, expressed as the percentage of DPPH and ABTS radical scavenging, was found to be 58.26 ± 1.57% and 63.74 ± 1.42%, respectively. The results of the antimicrobial activity evaluation, using both disk diffusion and well diffusion methods, demonstrated that the Gram-positive bacteria *Streptococcus pyogenes* and *Listeria monocytogenes* were the most susceptible strains to *Artemisia sieberi* essential oil. The minimum inhibitory concentration of this essential oil against the Gram-positive bacteria *Streptococcus pyogenes*, *Listeria monocytogenes*, and *Bacillus cereus* was determined to be 8 mg/mL, 128 mg/mL, and 128 mg/mL, respectively. Furthermore, this essential oil was effective against Gram-negative bacteria such as *Shigella dysenteriae* and *Salmonella typhimurium* at a concentration of 512 mg/mL, except for Klebsiella pneumoniae, which showed sensitivity at a concentration of 256 mg/mL. Therefore, Siberian wormwood essential oil can be used as an effective natural antimicrobial agent against bacterial infections.

1.Introduction

The genus Artemisia, comprising over 500 herbaceous and shrubby plant species, is considered one of the most numerous and diverse genera within the Asteraceae family. This genus is distributed worldwide, spanning from northern temperate to subtropical and tropical regions, with its species found in a wide range of habitats including pastures, deserts, forests, and agricultural lands [1]. *Artemisia sieberi* is a perennial evergreen shrub that grows to a height of 50 to 150 cm in open fields, roadsides, valleys, and deserts, featuring alternate, palmate, and hairy leaves. This medicinal plant is widely used throughout the Middle East as an antiparasitic, antimalarial, and for treating gangrenous and infected wounds [2]. *Artemisia sieberi* has a long history of use in the traditional medicine of various communities and is recognized as a rich source of secondary compounds with numerous medicinal properties [3]. Scientific studies have demonstrated that extracts and essential oils of *Artemisia sieberi* possess potent antimicrobial activity against a broad spectrum of bacteria, fungi, and parasites. These properties are attributed to the presence of sesquiterpene lactones and other secondary metabolites found in the plant. Furthermore, these compounds also act as antioxidants, protecting cells from damage caused by free radicals [3, 4].

Antioxidant components such as total phenol, total tannin, and total flavonoid, along with their derivatives like gallic acid, tannic acid, and quercetin, are considered important nutrients due to their significant health benefits. A growing body of epidemiological and laboratory studies provides strong evidence that certain edible plants and their secondary compounds possess antioxidant activities, which can offer substantial protective effects against carcinogenesis in humans [5]. *Artemisia* species also exhibit antioxidant properties that may reduce the risk of complex diseases by counteracting oxidative stress [6].

Essential oils are volatile and concentrated extracts derived from various parts of plants, recognized for their unique aromas and flavors [7]. These oils hold significant potential in the field of biomedicine due to their effectiveness in treating a wide array of bacterial, fungal, and viral disorders [8]. Essential oils are effective against various diseases because they contain different aldehydes, phenolics, terpenes, and other antibacterial components. Previous studies have indicated that *Artemisia* essential oils possess antimicrobial properties, suggesting their potential for diverse medicinal applications [9]. Furthermore, the essential oils of certain *Artemisia* species have also been found to exhibit antifungal activity against *Fusarium* species such as *F. moniliforme*, *F. solani*, and *F. sporotrichioides* [10].

Numerous studies have highlighted the significant potential of this plant for its antioxidant, antibacterial, and antifungal properties [11, 12, 13]. These characteristics position the plant as a natural alternative to synthetic antioxidants and antibiotics. Taheri et al. (2018) investigated the inhibitory effect of aqueous and alcoholic extracts of *Artemisia sieberi* on the growth of *Candida albicans*. The ethanolic extract showed the strongest antifungal effect, inhibiting fungal growth at lower concentrations compared to aqueous and methanolic extracts. However, they reported that *Candida albicans* showed resistance to the hydroalcoholic extracts [14].

Mahboubi et al. (2014) examined the chemical properties of *Artemisia sieberi* essential oil (from Iran and France), identifying beta-thujone, camphor, cineole, and alpha-thujone as its main components. They also reported similar antimicrobial activity for the *Artemisia* essential oils, observing the greatest antimicrobial effect against *Staphylococcus aureus* and *Candida albicans* [15]. Additionally, Hashemi et al. (2014) reported on the

antioxidant effect of *Artemisia sieberi* essential oil on the oxidative stability of frying oil [16].

The objective of this study was to extract *Artemisia sieberi* essential oil and evaluate its total phenolic, total flavonoid content, antioxidant activity, and antimicrobial activity against several bacteria responsible for infection and food poisoning. This was done to explore its potential applications in the food and pharmaceutical industries.

2- Materials and Methods

2.1- Chemicals and Microbial Strains

This study utilized DPPH free radical, ABTS free radical, sodium carbonate, aluminum trichloride, Folin-Ciocalteu reagent, quercetin, triphenyl tetrazolium chloride, and Mueller-Hinton Broth and Agar culture media. All chemicals were procured from Merck (Germany) and Sigma-Aldrich (USA). The microbial strains used were *Bacillus cereus*, *Streptococcus pyogenes*, *Listeria monocytogenes*, *Shigella dysenteriae*, *Klebsiella aerogenes*, and *Salmonella typhimurium*. These strains were obtained from the microbial collection of the Department of Food Science and Engineering, Khuzestan University of Agricultural Sciences and Natural Resources.

2.2- Essential Oil Preparation

Artemisia sieberi were obtained from an accredited medicinal plant supplier in Ahvaz. After botanical identification of the plant's scientific name was confirmed by a botanist at the Faculty of Agricultural Sciences and Natural Resources, Khuzestan, the dried leaves were ground and then subjected to essential oil extraction using a Clevenger apparatus. For each extraction, 100 grams of the crushed plant material were combined with one liter of water in the apparatus, and the extraction process was carried out for three hours. The extracted essential oil was stored at 4∘C in dark glass bottles [17].

2.3- Total Phenol and Flavonoid Content

Total phenol content was measured using the Folin-Ciocalteu colorimetric method, expressed as gallic acid equivalents. For this, 500µL of essential oil diluted in methanol was mixed with 2.5 mL of 10% Folin-Ciocalteu reagent. Subsequently, 4 mL of 7% sodium carbonate was added, and the mixture was incubated in a water bath at 45∘C for 15 minutes to facilitate the aqueous phase development. The absorbance was then read at 765 nm using a spectrophotometer [18]. The total flavonoid content of *Artemisia sieberi* essential oil was determined by the aluminum chloride colorimetric method, following the procedure by Heydari et al. (2020). Briefly, 500µL of essential oil diluted with methanol (1:10 dilution ratio) was mixed with 100µL of 10% aluminum chloride solution and 100µL of 1 M potassium acetate solution. The volume was then adjusted to 5 mL with distilled water. The mixture was allowed to stand at room temperature for 30 minutes, and its absorbance was measured at 415 nm using a spectrophotometer. Total flavonoid content was calculated based on a quercetin standard calibration curve and expressed as milligrams of quercetin equivalent per gram (mg QE/g) [19].

2.4- Evaluation of Essential Oil Antioxidant Activity

The antioxidant activity of Iranian Artemisia essential oil was assessed using two methods: DPPH free radical scavenging and ABTS free radical scavenging assays.

2.4.1- DPPH Free Radical Scavenging Activity

The DPPH free radical scavenging activity was determined according to the method described by Alizadeh-Behbahani et al. (2019). In this method, 1 mL of essential oil was mixed with 1 mL of 0.2 mM DPPH solution. After 30 minutes, the absorbance was measured at 517 nm. The scavenging power of the essential oil against DPPH free radicals was calculated using the following formula [20]:

Inhibition %=([AC-AS]/AC) ×100

Where AC represents the absorbance of the control sample (DPPH methanolic solution without essential oil), and AS represents the absorbance of the test sample (DPPH methanolic solution with essential oil).

2.4.2- ABTS Free Radical Scavenging Activity

For this method, ABTS and potassium persulfate solutions were first mixed to generate ABTS free radicals. Then, 3 mL of the essential oil was added to 3 mL of the ABTS radical solution. The absorbance of the sample was subsequently read at 734 nm. The ABTS free radical scavenging activity of the essential oil was calculated using the following formula [21]:

Inhibition %=([AC-AS]/AC) ×100

Here, AC represents the absorbance of the control sample (ABTS radical cation solution without essential oil), and AS represents the absorbance of the test sample (ABTS radical cation solution with essential oil).

2.5- Antimicrobial Activity

The present study employed various methods to evaluate the antimicrobial effect of Artemisia sieberi essential oil. These methods included disc diffusion, agar well diffusion, determination of Minimum Inhibitory Concentration (MIC), and Minimum Bactericidal Concentration (MBC).

2.5.1- Disc Diffusion Agar

This assay was performed in sterile Petri dishes (80 mm diameter) containing Mueller-Hinton Agar medium. Briefly, 0.1 mL of the test microorganism's suspension was spread over the culture medium. Subsequently, sterile paper discs (6 mm diameter) impregnated with 30µL of essential oil were placed on the surface of the medium previously inoculated with the microorganism. To prevent potential interference from other essential oils, only one disc was placed per Petri dish. The plates were then incubated for 24 hours at 37∘C. After incubation, the diameter of the zone of bacterial growth inhibition around each disc was measured [22].

2.5.2- Well Diffusion Agar

In this assay, 100µL of bacterial suspension with an approximate concentration of 1.5×10^8 colony forming unit (CFU)/mL was inoculated onto Mueller-Hinton Agar medium. Wells, 6 mm in diameter, were then created in the agar. Subsequently, 50µL of the essential oil was pipetted into each well. The plates were incubated for 18 to 24 hours at 37◦C. Antimicrobial activity was evaluated by measuring the diameter of the zone of bacterial growth inhibition around the wells [23].

2.5.3- Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined using the broth microdilution method [23, 24]. The essential oil was dissolved in Mueller-Hinton Broth containing Tween 20 (at a final concentration of 10% v/v). Serial two-fold dilutions of the essential oil were prepared in a 96-well plate. Subsequently, all wells were inoculated with bacteria, and the plates were incubated for 24 hours at 37∘C. Bacterial growth was visualized by adding 20µL of a 0.05% aqueous solution of 2,3,5-triphenyl tetrazolium chloride (TTC). The MIC was defined as the lowest concentration of the essential oil that prevented visible bacterial growth (indicated by the absence of a red precipitate at the bottom of the wells after TTC addition). The Minimum Bactericidal Concentration (MBC) was defined as the lowest concentration that resulted in the death of 99.9% of bacterial cells. To determine the MBC, aliquots were taken from each well where no visible bacterial growth was observed and sub-cultured onto Mueller-Hinton Agar medium. These plates were then incubated for 24 hours at 37◦C. All experiments were performed in triplicate [26].

2.6- Statistical Analysis

Statistical analysis of the data was performed using SPSS software version 18. To assess the significance of differences between means, one-way ANOVA and Duncan's post-hoc test were used at a 95% confidence level (p<0.05). Graphs were plotted using Excel software version 9.

3- Results and Discussion

3.1- Total Phenol and Flavonoid Content

Medicinal plants have gained a special standing in both traditional and modern medicine due to their long history of treating various ailments. Among the active compounds present in medicinal plants, phenolic and flavonoid compounds have received particular attention from researchers because of their significant role in human health [11]. The results obtained from measuring total phenolic compounds by the Folin-Ciocalteu method and flavonoids by the aluminum chloride colorimetric method are presented in Figure 1. The total phenolic content was 44.62±0.53 mg gallic acid equivalent per gram, and the total flavonoid content was 18.20±0.29 mg quercetin equivalent per gram. Our findings align with several other studies that have reported the presence of these compounds in the same plant species but from different regions worldwide [11]. One study investigated the bioactive compounds in methanolic extracts of *Artemisia* species, reporting that *A. sieberi* contained 194.30 mg gallic acid equivalent per gram of total phenols and 18.22 mg quercetin equivalent per gram of total flavonoids, followed by *A. judaica* and *A. monosperma* [27]. Furthermore, Aryanfar et al. (2018) examined the phytochemical characteristics of two *Artemisia* species (*A. aucheri* and *A. sieberi*), and their results indicated that *A. sieberi* had higher total phenol and flavonoid content compared to *A. aucheri* [28].

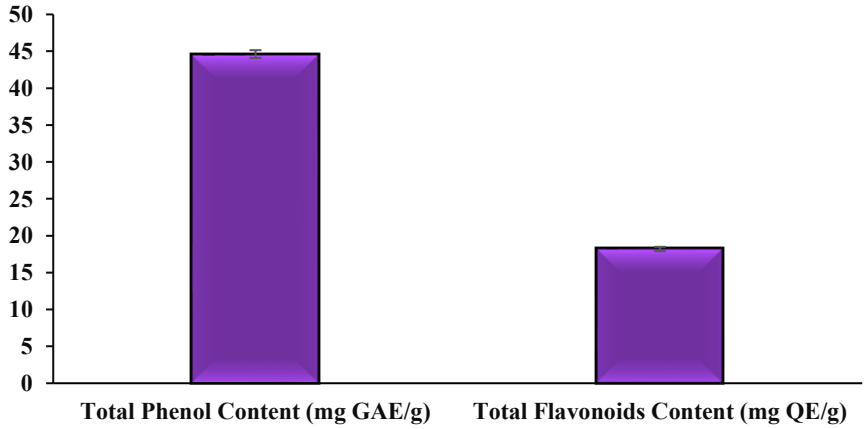

Fig. 1– Total phenolic and flavonoid content of *Artemisia sieberi* essential oil

Numerous studies have attributed antioxidant capacity to the presence of phenolic and flavonoid compounds. These compounds possess a high ability to neutralize non-physiological free radicals such as DPPH and ABTS [11]. The scavenging percentages for DPPH and ABTS free radicals were 58.26±1.57% and 63.74±1.42%, respectively (Figure 2). Previous studies by Nasr et al. (2020) demonstrated that this *Artemisia species* exhibits antioxidant activity (DPPH free radical scavenging of 61.3±0.29% and ABTS free radical scavenging of 71.3±3.2%) [6], which aligns with our findings. Mahboubi et al. (2015), in their investigation of *Artemisia sieberi* essential oil, showed that this essential oil possesses high antioxidant activity, attributing it to compounds such as 1,8-cineole, camphor, α-thujone, β-thujone, borneol, and santolina alcohol [29]. Research has consistently shown that *Artemisia sieberi* possesses high antioxidant potential [30, 31]. Ghasemi et al. (2021) examined the antioxidant activity of the essential oil and extract of *Artemisia sieberi* Besser. Their findings indicated that the essential oil and

extract of this species exhibited 84.04% and 89.33% antioxidant activity (DPPH), respectively [32]. Furthermore, variations in antioxidant capacity exist among different *Artemisia species* [33]. These differences can be attributed to the quantity of phenolic compounds, including flavonoids, as well as the climatic conditions and soil characteristics of their growing regions.

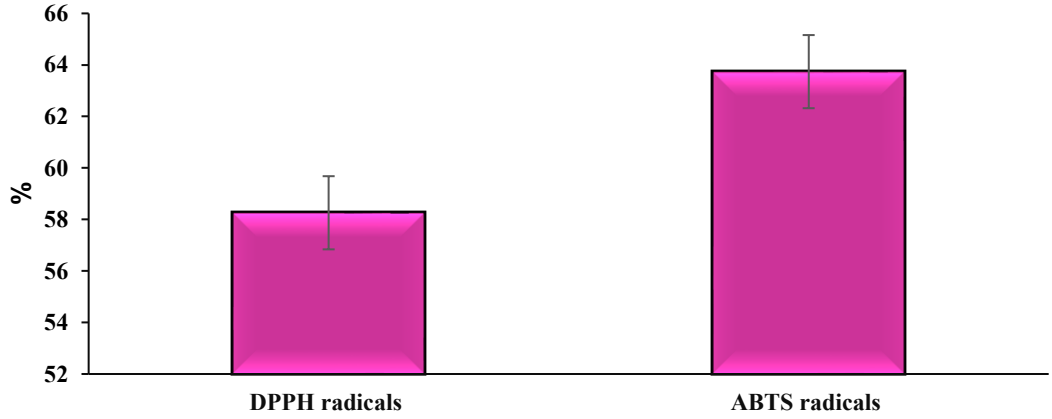

Fig. 2- Antioxidant activity of *Artemisia sieberi* essential oil.

3.2- Disc Diffusion and Well Diffusion Agar

The antimicrobial effects of *Artemisia sieberi* essential oil against pathogenic bacterial strains, as determined by the agar disc diffusion method, are presented in Figure 3. The results showed that Gram-positive bacteria—*Bacillus cereus* (12.40±0.62 mm), *Streptococcus pyogenes* (16.60±0.52 mm), and *Listeria monocytogenes* (15.30±0.43 mm)—exhibited significantly larger inhibition zones compared to Gram-negative bacteria—*Shigella dysenteriae* (10.80±0.28 mm), *Klebsiella aerogenes* (12.00±0.46 mm), and *Salmonella typhimurium* (11.60±0.48 mm) ($p<0.05$).

The results obtained from the agar well diffusion method were similar to those from the disc diffusion method. In this study, *Streptococcus pyogenes* was identified as the most sensitive strain, while *Salmonella typhimurium* was the most resistant strain to *Artemisia sieberi* essential oil (Figure 4). Notably, the diameter of the inhibition zones in the agar well diffusion method was larger for all tested microorganisms compared to the disc diffusion method. This can be attributed to the direct contact of the extract with the bacteria in the agar well method, whereas in the disc diffusion method, the inhibitory effect of the extract relies on the diffusion of its antimicrobial compounds from the disc surface into the bacterial medium [34, 35]. Therefore, the agar well method more accurately reflects the sensitivity of bacterial strains due to this direct contact.

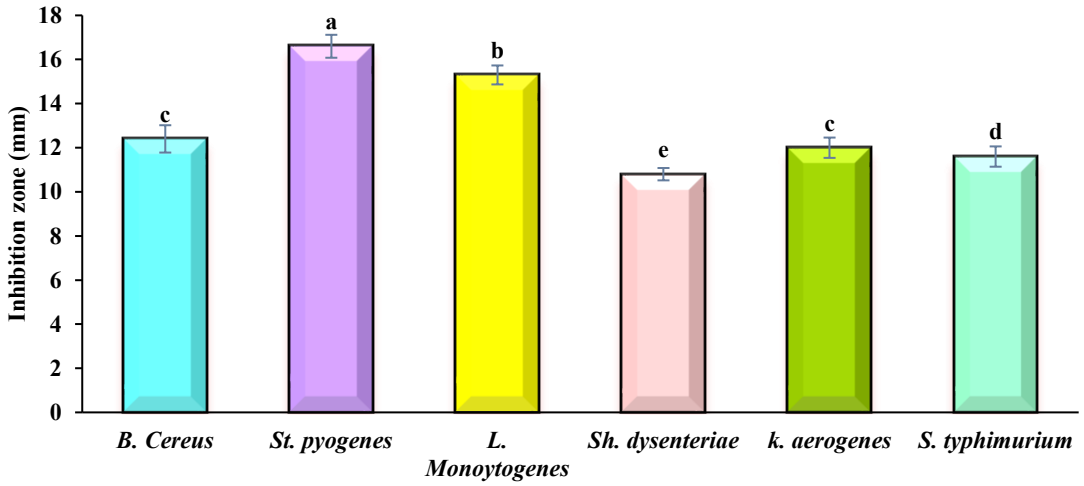

Fig. 3- Antimicrobial activity of *Artemisia sieberi* essential oil based on disk diffusion agar assay.

Mahboubi et al. (2015) investigated the antimicrobial effect of *Artemisia sieberi* essential oil against bacterial strains (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*) using the disc diffusion method. Their research indicated that *Pseudomonas aeruginosa* was the most sensitive bacterium, while *Escherichia coli* was the most resistant to the A. sieberi essential oil [29]. The findings of these researchers are comparable to the results of our study. Another study also demonstrated the antimicrobial effect of *Artemisia sieberi* essential oil. This study showed that A. sieberi essential oil, containing alpha-thujone, beta-thujone, and camphor as its main compounds, exhibited significant antimicrobial activity against a broad range of pathogenic microorganisms, including bacteria and fungi. Specifically, Gram-positive bacteria and fungi were more sensitive (showing larger inhibition zone diameters) than Gram-negative bacteria. Among Gram-positive bacilli, *Listeria monocytogenes* and *Bacillus cereus*, and among Gram-positive cocci, *Streptococcus mutans* showed greater sensitivity than other tested strains [36]. Furthermore, the antibacterial and antifungal activities of this essential oil were also reported by Mohammadi et al. (2017) [37].

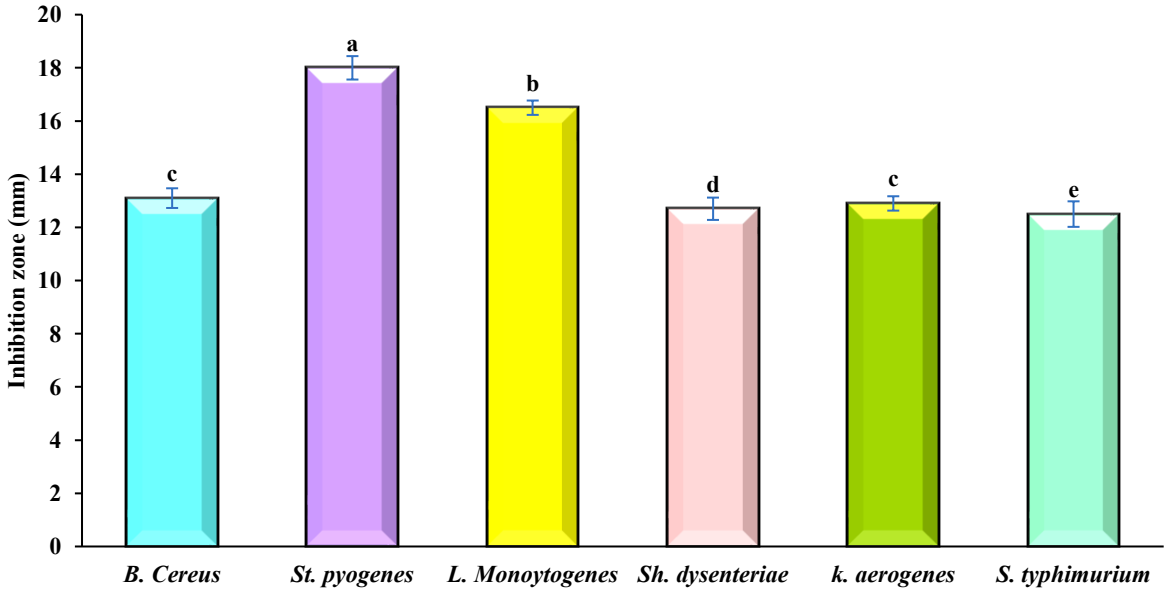

Fig. 4- Antimicrobial activity of *Artemisia sieberi* essential oil based on well diffusion agar assay.

3.3- Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The antimicrobial activity results of *Artemisia sieberi* essential oil, based on the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) methods, are presented in Figures 5 and 6.

Streptococcus pyogenes and *Listeria monocytogenes* were the most sensitive bacteria to *A. sieberi* essential oil, with an MIC and MBC of 8 mg/mL. In contrast, *Shigella dysenteriae* and *Salmonella typhimurium* were the most resistant bacteria, showing an MIC and MBC of 128 mg/mL ($p<0.05$). These findings were consistent with the results obtained from both the agar disc diffusion and agar well diffusion methods. In a study by Mohammadi et al. (2017), results indicated that this essential oil exhibited strong antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus*, with MICs of 50 and 25µg/mL, respectively. Its activity against Gram-negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa*, was slightly weaker, with an MIC of 100µg/mL for both bacteria [37].

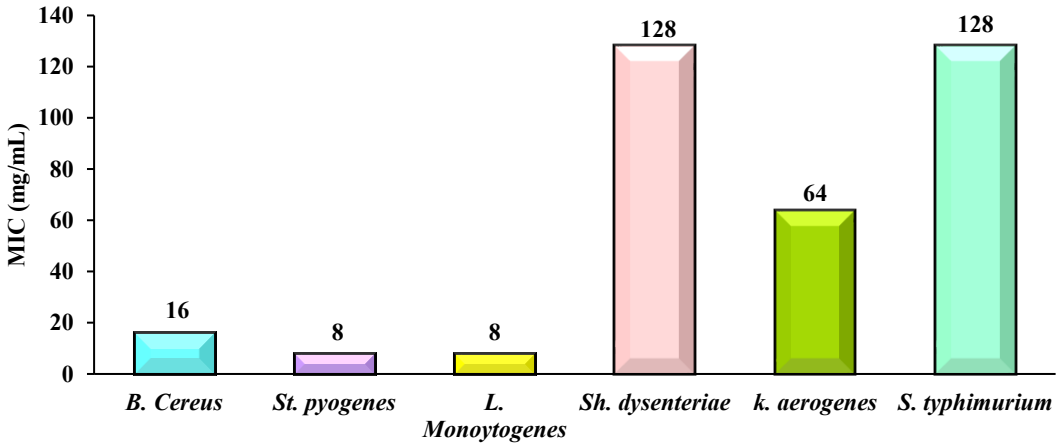

Figure 5. Antimicrobial activity of essential oil of *Artemisia sieberi* based on minimum inhibitory concentration method.

In a study by Ghasemi et al. (2021), the antifungal effects of *Artemisia sieberi* essential oil on the fungus Botrytis cinerea were investigated. The results indicated that this essential oil exhibited potent antifungal activity against the fungus, completely inhibiting mycelial growth at concentrations of 1000 and 1500µL/L. This effect was attributed to the monoterpenes, which are the primary contributors to the strong antifungal activity of *Artemisia sieberi* essential oil [32]. Monoterpenes are a class of volatile organic compounds naturally found in many plants, known for their strong antimicrobial and antifungal properties [38].

The antimicrobial activity of *Artemisia sieberi* essential oil has been confirmed by numerous studies. Nasr et al. (2021) and Singh et al. (2023) reported the antibacterial and antifungal effects of *Artemisia sieberi* essential oil using Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) methods [30, 33]. Jamal et al. (2023) tested the antimicrobial activity of *Artemisia sieberi* essential oil against four different bacterial species (Bacillus subtilis, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*), as well as one fungal species (*Candida tropicalis*). The findings of this study underscore the significant potential of *Artemisia sieberi* essential oil as a natural antimicrobial agent against a broad range of pathogenic microorganisms. Specifically, its potent antifungal and antibacterial activity against some important human pathogens,

including *Bacillus subtilis* and *Staphylococcus aureus*, is noteworthy [2].

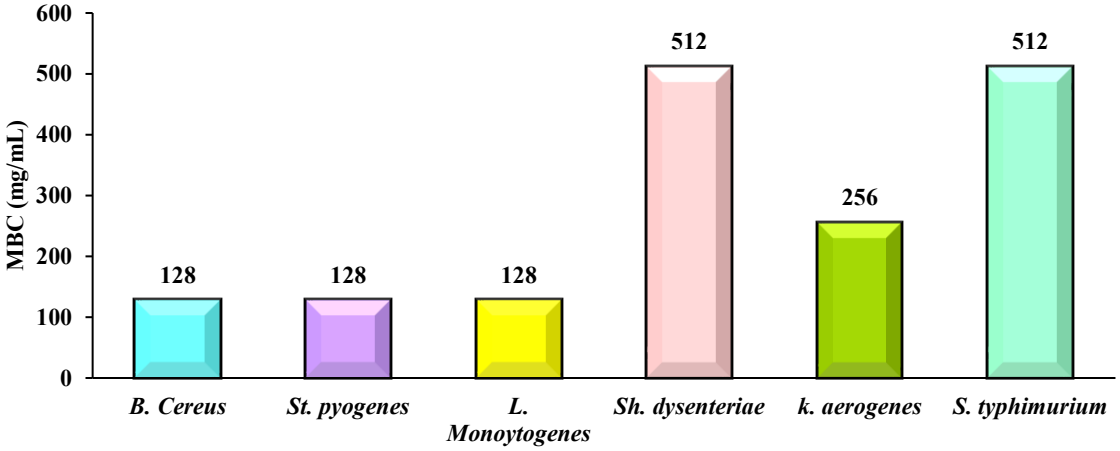

Figure 6. Antimicrobial activity of essential oil of *Artemisia sieberi* based on minimum bactericidal concentration method.

4- Overall Conclusion

Our results revealed a strong positive correlation between the antioxidant activity of the essential oil and its phenolic and flavonoid compounds. These findings indicate that phenolic and flavonoid compounds play a primary role in conferring the antioxidant activity of the essential oil. Based on the antibacterial activity assessment, *Artemisia sieberi* essential oil demonstrated broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria, with the most pronounced effects observed on *Streptococcus pyogenes* and *Listeria monocytogenes*.

Therefore, due to its bioactive compounds possessing antioxidant and antibacterial properties, *Artemisia sieberi* essential oil holds significant potential for application in the pharmaceutical and food industries as a natural source of bioactive compounds. However, for its practical use as a natural preservative in the food industry, more extensive studies are necessary regarding its safety, compatibility with other food additives, and stability under various processing conditions.

5- Acknowledgements

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ارزیابی پتانسیل آنتی‌اکسیدانی، ضدباکتریایی، فنولی و فلاونوئید کل اسانس درمنه سیبری در شرایط آزمایشگاهی

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چکیده

گیاه درمنه سیبری (*Artemisia sieberi*)، یکی از گونه‌های جنس درمنه (Artemisia) با پراکنش گسترده در ایران و خاورمیانه، به دلیل خواص دارویی متنوع از جمله اثرات ضد-میکروبی مورد توجه قرار گرفته است. هدف از این پژوهش، تعیین محتوای فنول و فلاونوئید کل، فعالیت آنتی‌اکسیدانی (مهار رادیکال آزاد DPPH و ABTS) و فعالیت ضد-میکروبی (دیسک و چاهک آگار، حداقل غلظت مهارکنندگی و کشندگی) اسانس درمنه سیبری بود. مقدار فنول و فلاونوئید کل به ترتیب برابر با ۰/۵۳±۴۴/۶۲ میلی‌گرم گالیک اسید در گرم و ۰/۲۹±۱۸/۲۰ میلی‌گرم کوئرستین در گرم تعیین شد. فعالیت آنتی‌اکسیدانی نیز براساس درصد مهارکنندگی رادیکال آزاد DPPH و ABTS به ترتیب برابر با ۱/۱±۲۶/۵۷ و ۱/۴۲±۶۳/۷۴ به دست آمد. نتایج حاصل از ارزیابی فعالیت ضدمیکروبی به دو روش دیسک و چاهک آگار نشان داد که باکتری‌های گرم مثبت *استرپتوکوکوس پیوژنز* و *لیستریا مونوسیتوژنز* حساسترین سویه‌ها به اسانس درمنه سیبری بودند. حداقل غلظت کشندگی این اسانس روی باکتری‌های گرم مثبت؛ *استرپتوکوکوس پیوژنز*، *لیستریا مونوسیتوژنز* و *باسیلوس سرئوس* به ترتیب ۸ میلی‌گرم بر میلی‌لیتر و ۱۲۸ میلی‌گرم بر میلی‌لیتر تعیین شد. همچنین، این اسانس روی باکتری‌های گرم منفی مانند *شیگلا دیسانتری* و *سالمونلا تیفی موریوم* با غلظت ۵۱۲ میلی‌گرم بر میلی‌لیتر موثر بود، به جز *کلبسیلا ائروژنز* که در غلظت ۲۵۶ میلی‌گرم بر میلی‌لیتر حساسیت نشان داد. بنابراین، از اسانس درمنه سیبری می‌تواند به عنوان یک ضدمیکرب طبیعی موثر در برابر عفونت‌های باکتریایی مورد استفاده قرار گیرد.