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A study on the antimicrobial, antioxidant, and phytochemical compounds of the essential oil of *Artemisia persica*

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ABSTRACT

Essential oils are volatile, aromatic organic compounds produced as secondary metabolites in plants. They often exhibit significant biological activities, including antimicrobial, anti-inflammatory, and antioxidant properties. In this study, the antimicrobial, antioxidant activities and phytochemical compounds of the essential oil of Iranian Artemisia were investigated. The antimicrobial activity of the essential oil was evaluated based on agar disk diffusion, agar well diffusion, minimum inhibitory concentration and minimum bactericidal concentration methods. Furthermore, to investigate the phytochemical compounds, including total phenolic and flavonoid contents, Folin-Ciocalteu and aluminum chloride colorimetric methods were used, respectively. Antioxidant activity was evaluated based on DPPH and ABTS radical scavenging activity. Antimicrobial tests revealed that the essential oil under investigation exhibited significant inhibitory effects against both Gram-positive and Gram-negative bacteria, including *Streptococcus pyogenes*, *Shigella dysenteriae*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Klebsiella aerogenes*. The examined essential oil contained acceptable amounts of phenolic compounds (31.2 ± 1.27 mg GAE/g) and flavonoid compounds (17.49 ± 1.19 mg QE/g) and had a significant ability to inhibit DPPH free radicals. (52.43±1.41) and ABTS (57.49±1.37). The present study showed that the investigated essential oil has a significant potential as a therapeutic agent for diseases caused by oxidative stress and microbial infections.

1.Introduction

In the current era, with the escalating phenomenon of drug resistance, discovering novel antimicrobial compounds has become one of the most critical research priorities in global health. Scientists worldwide have initiated extensive efforts to identify and develop new antimicrobial drugs from various sources, including plants, animals, and microorganisms [1]. Pathogenic microorganisms, through gradual evolution and genetic mutations, have acquired the ability to overcome existing antimicrobial drugs. Evidence suggests that this phenomenon is expanding, with the number of resistant microbes increasing daily. Developing countries face an even more severe challenge in this regard due to lower awareness regarding the rational use of antibiotics. The indiscriminate and self-prescribed use of these drugs, without proper medical supervision or necessary oversight, creates conditions conducive to the proliferation and spread of resistant microbes [2, 3].

The genus *Artemisia*, belonging to the Asteraceae family, is an aromatic plant of significant economic importance in traditional medicine. This genus comprises approximately 500 species and is widely distributed across the Northern Hemisphere, ranging from arid deserts to high mountain ranges. In Iran, 34 species, and in Turkey, 23 species of this genus have been identified [4]. *Artemisia persica* (Iranian wormwood) is a perennial herbaceous plant, growing to an approximate height of 60 cm, characterized by narrow, feather-like leaves and yellow capitulum inflorescences [5, 6].

Artemisia species are used to treat certain bacterial infections, such as abscesses, acne, and diphtheria. These plants are also employed as febrifuges, anthelmintics, antidiarrheals, antispasmodics, and anti-inflammatories. Furthermore, they've been used to treat malaria, nervous and respiratory system disorders, inflammation, and abdominal pain [7]. These properties can be attributed to the presence of various bioactive compounds. The bitter and strong aroma and flavor of many *Artemisia* species stem from terpenoids and sesquiterpene lactones. Phenols and flavonoids are also notable components of this genus, recognized for their significant antioxidant, anti-inflammatory, and antimicrobial properties [8].

Essential oils are volatile, aromatic compounds extracted from various plant parts, including flowers, leaves, stems, and roots. Due to their high concentration of bioactive compounds, they possess numerous therapeutic properties and health benefits [9, 10]. The use of essential oils and plant extracts as natural alternatives to chemical preservatives is an emerging approach in the food industry. These compounds help maintain food safety and extend shelf life by inhibiting the growth and controlling pathogenic microorganisms. However, using these essential oils at high doses can negatively affect the taste, aroma, and color of food products. Therefore, finding the optimal dose that maintains antimicrobial properties while minimizing impact on sensory characteristics is crucial [11]. A 2014 study by Nikbakht et al. on the essential oil compounds of *Artemisia persica* revealed that this plant contains a wide array of volatile organic compounds. Among the 28

identified compounds, β-thujone was identified as the main component of this essential oil, with a substantial concentration of 75.23% [6]. This finding indicates that Iranian *Artemisia* essential oil is rich in monoterpene compounds, particularly β-thujone, a compound of research interest due to its diverse medicinal properties, including antimicrobial and anti-inflammatory activities [12, 13].

This research aimed to investigate the total phenolic and flavonoid content and the antioxidant potential of *Artemisia persica* essential oil. Furthermore, the antimicrobial activity of *Artemisia persica* essential oil against pathogenic microorganisms—*Streptococcus pyogenes*, *Shigella dysenteriae*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Klebsiella aerogenes*—was evaluated under laboratory conditions.

2. Materials and Methods

2.1. Chemicals

In this study, aluminum trichloride, methanol, sodium hydroxide, sodium persulfate, Folin-Ciocalteu reagent, quercetin, DPPH and ABTS free radicals, Tween 80, triphenyltetrazolium chloride, and Mueller Hinton Agar and Broth media from Merck and Sigma brands were used.

2.2. Preparation of *Artemisia persica* Essential Oil

Essential oil was extracted from the *Artemisia persica* plant using a Clevenger apparatus. For this, the plant was first dried in the shade and then powdered using a laboratory mill. One hundred grams of the powdered plant material were placed into a 2-liter distillation flask, and distilled water was added. By controlling the temperature and the flow rate of cold water through the condenser, the distillation process was carried out for 3 hours, and the plant's essential oil was extracted [14].

2.3. Preparation of 0.5 McFarland Standard and Microbial Suspension

To activate microbial strains for evaluating the antimicrobial activity of *Artemisia persica* essential oil, the 0.5 McFarland standard method was used. First, all strains were cultured on Mueller Hinton Agar for 24 hours at 37°C. Subsequently, a bacterial suspension with turbidity equivalent to 0.5 McFarland (approximately 1.5×10^8 colony forming unit (CFU)/mL) was prepared for each strain [15].

2.4. Total Phenolic Content

First, 0.5 mL of *Artemisia persica* essential oil was combined with 2.5 mL of Folin-Ciocalteu reagent (10% v/v). After standing for 10 minutes at room temperature, 2 mL of sodium carbonate (7.5% w/v) was added. After incubating for 30 minutes in the dark, its absorbance was recorded at 765 nm. The total phenolic content of *Artemisia persica* essential oil was reported as milligrams of gallic acid equivalents per gram of essential oil (mg GAE/g) [16].

2.5. Total Flavonoid Content

In this study, the aluminum chloride colorimetric method was employed to determine the total flavonoid content of *Artemisia persica* essential oil. Briefly, 0.5 mL of the essential oil was mixed with 75 µL of 5% (w/v) sodium nitrite and left at room temperature for 5 minutes. Subsequently, 150 µL of 10% (w/v) aluminum trichloride was added, and the mixture was again held at room temperature for 5 minutes. Finally, 1 mL of 1 M sodium hydroxide was added to the solution, and its absorbance was immediately recorded at 510 nm. The total flavonoid content was reported as milligrams of quercetin equivalents per gram of essential oil (mg QE/g) [17].

2.6. Evaluation of Antioxidant Activity

2.6.1. DPPH Free Radical Scavenging Method

To measure the DPPH free radical scavenging activity, 0.1 mL of the essential oil was combined with 3.9 mL of a 0.12 mM methanolic DPPH solution. The solution was then stirred and left in the dark for 30 minutes. Subsequently, the absorbance of the sample was measured at 517 nm using a UV-Vis spectrophotometer. For the control sample, a similar procedure was followed, except methanol was used instead of the essential oil. The antioxidant capacity of the essential oil against the DPPH free radical was determined using the following formula [18]:

Inhibition activity (%)= (Absorbance of Control−Absorbance of Sample)/Absorbance of Control×100

2.6.2. ABTS Free Radical Scavenging Method

First, equal volumes of potassium persulfate solution (2.45 mM) and ABTS solution (7 mM) were mixed together. This mixture was then left in the dark at room temperature for 16 hours to generate the ABTS radicals. Subsequently, methanol was added until the absorbance of the ABTS radical solution reached 0.7 at 734 nm. Following this, 0.1 mL of the essential oil was mixed with 3.9 mL of the ABTS radical solution and allowed to stand at room temperature for 6 minutes. Finally, the absorbance of the solution was measured at 734 nm. The antioxidant capacity of the essential oil against the ABTS free radical was determined using the following equation [19]:

Inhibition activity (%)= (Absorbance of Control−Absorbance of Sample)/Absorbance of Control×100

2.7. Evaluation of Essential Oil Antimicrobial Activity

2.7.1. Well Diffusion Agar

First, 6 mm diameter wells were created in plates containing Mueller Hinton Agar medium. Then, 100 µL of bacterial suspensions, prepared according to the 0.5 McFarland standard, were spread evenly on the surface of the medium using an L-shaped spreader. Next, 60 µL of *Artemisia persica* essential oil was added to each well, and the plates were incubated for 24 hours at 37°C. After incubation, the diameter of the zones of inhibition around the wells was measured and recorded in millimeters [20].

2.7.2. Minimum Inhibitory Concentration (MIC)

The antimicrobial activity of extracts and essential oils against microorganisms was evaluated using the micro-dilution method to determine their minimum inhibitory concentration (MIC). To do this, serial dilutions of the essential oil (4, 8, 16, 32, 64, 128, 256, and 512 mg/mL) were prepared. Then, 75 µL of each dilution and 10 µL of bacterial suspension (0.5 McFarland turbidity) were added to each well of a 96-well microplate. The microplate was then incubated for 24 hours at 37°C. After incubation, 10 µL of 5% triphenyltetrazolium chloride was added to each well, and the plate was incubated again for 30 minutes. Finally, the first well that showed no color change was recorded as the minimum inhibitory concentration [21].

2.7.3. Minimum Bactericidal Concentration (MBC)

For this, 5 µL from the wells where bacterial growth was inhibited were cultured onto Mueller Hinton Agar plates. These plates were then incubated at 37°C for 24 hours. The concentration that prevented the growth of microorganisms

was determined as the minimum bactericidal concentration (MBC) [22].

2.8. Statistical Analysis

The results of this study were analyzed using One-Way Analysis of Variance (ANOVA) with SPSS version 18 software. Mean comparisons were performed using Duncan's test at a significance level of 95% (p>0.05). It should be noted that all chemical and antimicrobial experiments were conducted in triplicate.

3. Results and Discussion

Over 600 secondary metabolites have been identified in *Artemisia* plant species, showcasing a wide array of biological and medicinal effects. Some of the notable compounds in these plants include caffeic acid derivatives, flavonoids, coumarins, monoterpenes, sesquiterpenes, sesquiterpene lactones, sterols, polyacetylenes, and artemisinin [23]. Figure 1 illustrates the total phenolic and flavonoid content of the essential oil extracted from *Artemisia persica*. The essential oil contained 31.2±1.27 mg GAE/g total phenols and 17.49±1.19 mg QE/g total flavonoids. In a study by Hooshmand et al. (2023), the phenolic and flavonoid content in ethanolic, methanolic, and ethyl acetate extracts of *Artemisia* plants from different regions of Iran (Semnan, Golestan, Gilan) were investigated. Their findings indicated that the total phenolic content across various solvents and regions ranged from 17.51–42.06 mg GAE/g, and the total flavonoid content ranged from 3.72–7.94 mg QE/g [23]. In another study, Mazaraei et al. (2017) reported the highest phenolic and flavonoid content for *Artemisia* in the methanolic extract. According to their findings, the total phenolic content was 35.2 mg GAE/g, and the total flavonoid content was 15.9 mg QE/g [24]. A study by Younesi et al. (2016) aimed to determine the chemical composition (total phenols, flavonoids, flavonols, and flavanols) of *Artemisia herba-alba* essential oil. The results showed that the total phenolic content was 17.65 mg GAE/g and the total flavonoid content was 13.96 mg QE/g, respectively [25]. Therefore, it can be stated that the levels of these compounds can vary depending on the plant's ecology, as well as the solvent and extraction methods employed.

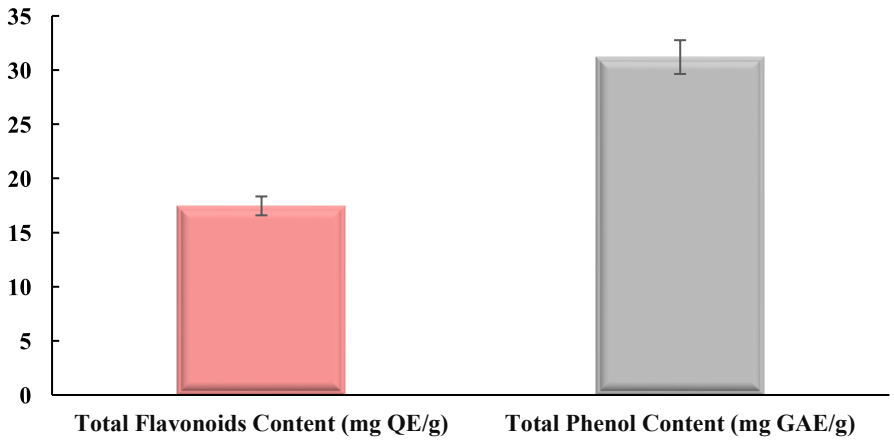

Figure 1. The total phenol content (TPC) and total flavonoid content (TFC) of *Artemisia persica* essential oil.

Due to the complex nature of plant extracts, which contain a mixture of chemical compounds with various functions, two complementary assay methods were used to

evaluate their antioxidant activity: the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay. The DPPH method is widely used to measure antioxidant capacity because, in the presence of an antioxidant, the stable DPPH radical is reduced to its colorless form. ABTS is an alternative method for assessing antioxidant activity, based on the ability of antioxidants to neutralize ABTS radical cations [8]. The results for the antioxidant activity of *Artemisia persica* essential oil are reported in Figure 2. According to the findings, the essential oil's antioxidant activity in scavenging DPPH free radicals was 52.43±1.41%, and for ABTS free radicals, it was 57.49±1.37%. Numerous studies have shown a direct correlation between an increase in the total phenolic and flavonoid content in plant extracts and an increase in their DPPH activity [14]. This correlation is likely due to the ability of phenols and flavonoids to neutralize DPPH free radicals by donating hydrogen or electrons.

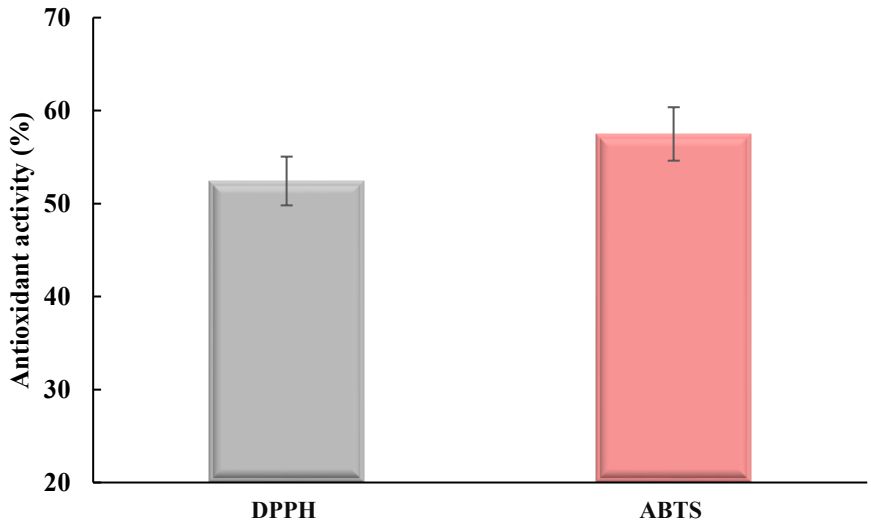

Figure 2. The antioxidant activity of *Artemisia persica* essential oil based on DPPH and ABTS radical scavenging methods.

Figure 3 presents the results of the antimicrobial activity of *Artemisia persica* essential oil against pathogenic bacteria, evaluated by the agar well diffusion method. As the figure illustrates, Gram-positive bacteria exhibited significantly larger inhibition zone diameters compared to Gram-negative bacteria when exposed to the essential oil. These findings align with the structural and functional differences between the cell walls of Gram-positive and Gram-negative bacteria. Gram-positive bacteria consistently produced considerably larger zones of inhibition against the tested antimicrobial agent than Gram-negative bacteria. The diameter of the inhibition zones varied among the Gram-positive bacteria: *Streptococcus pyogenes* showed the largest zone at 13.80±0.36 mm, followed by *Staphylococcus aureus* at 13.10±0.47 mm, and *Bacillus subtilis* at 12.20±0.48 mm. Among the Gram-negative bacteria tested, *Escherichia coli* displayed the highest sensitivity to the *Artemisia* essential oil, with an inhibition zone diameter of 11.20±0.25 mm. *Klebsiella aerogenes* and *Shigella dysenteriae* showed the next highest sensitivities, with zones of

10.50±0.23 mm and 9.50±0.40 mm, respectively ($p<0.05$).

In a study by Nahid et al. (2017), the inhibition zone diameters for the methanolic extract of *Artemisia indica* (at 100 mg/mL) were reported as 15 mm against Escherichia coli, 18 mm against Staphylococcus aureus, 18 mm against Pseudomonas putida, 17 mm against *Salmonella enterica*, and 15 mm against *Streptococcus pyogenes*. They also noted that *Artemisia indica* exhibited high antibacterial activity against *Staphylococcus aureus*. Furthermore, they reported that the plant extract possessed the ability to inhibit the growth of both Gram-positive bacteria (*Streptococcus pyogenes* and *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*, *Salmonella enterica*, and *Pseudomonas putida*) [26]. Mahboubi and Ghazian Bidgoli (2009) investigated the antibacterial and antifungal effects of *Artemisia khorassanica* essential oil. Their findings indicated that the antifungal activity of *Artemisia khorassanica* essential oil was stronger than its antibacterial activity. Gram-positive bacteria also showed greater resistance to *Artemisia khorassanica* essential oil compared to Gram-negative bacteria. The mean diameter of the inhibition zone created by *Artemisia khorassanica* essential oil was larger for Gram-positive bacteria and fungi. They attributed the differences in inhibition zone diameters among these Gram-negative bacteria to various factors, including cell wall structure and composition, enzymatic activity, and mechanisms of resistance to the antimicrobial agent [27].

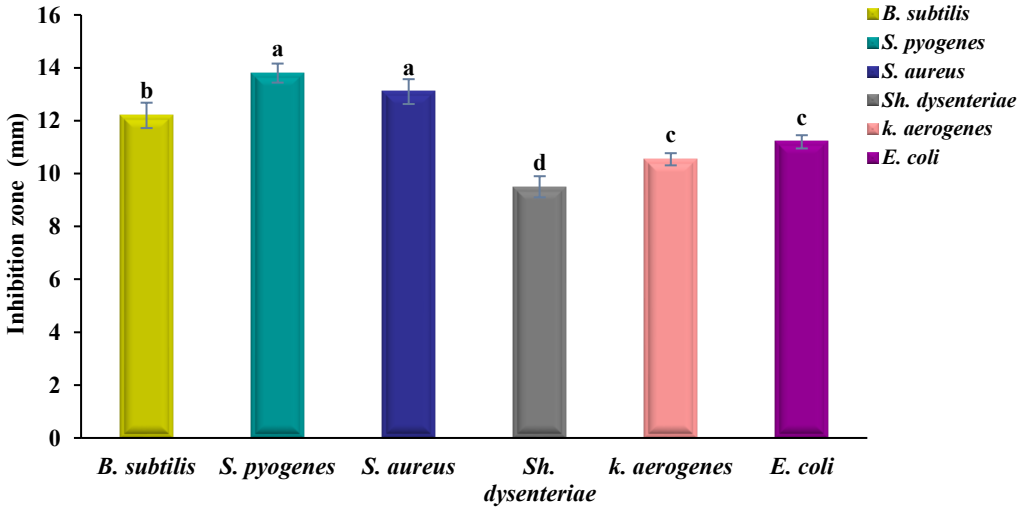

Figure 3. Antimicrobial activity of ethanolic extract of *Artemisia persica* based on well diffusion agar method.

In assessing the antimicrobial efficacy of plant extracts, two crucial criteria are the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC). The MIC represents the lowest concentration of an extract that can inhibit the growth of 90% of bacteria, whereas the MBC is the lowest concentration that kills 99.9% of bacteria. Figure 4 illustrates the antimicrobial results of *Artemisia persica* essential oil based on its minimum inhibitory concentration against pathogenic bacteria. The lowest MIC (16 mg/mL) was observed for *Streptococcus pyogenes*, while the highest MIC (256 mg/mL) was found for *Shigella dysenteriae*.

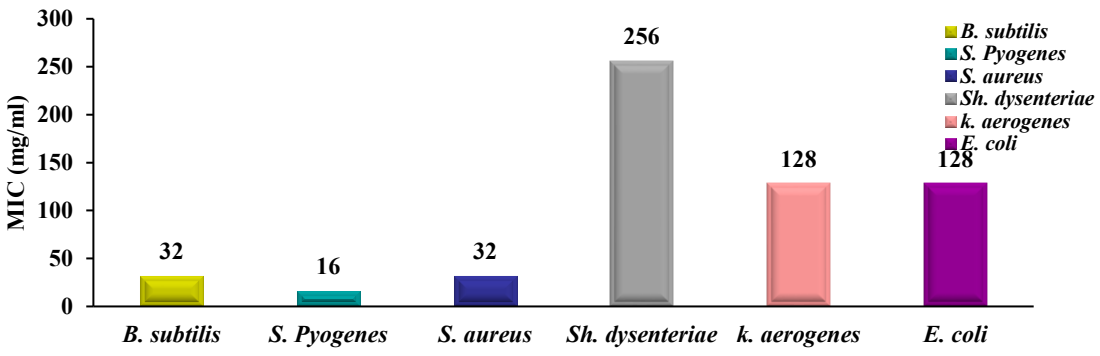

Figure 4. Antimicrobial activity of essential oil of *Artemisia persica* based on minimum inhibitory concentration method.

Figure 5 illustrates the antimicrobial results of *Artemisia persica* essential oil based on its Minimum Bactericidal Concentration (MBC) against pathogenic bacteria. The MBC for *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli* was 256 mg/mL. For *Shigella dysenteriae* and *Klebsiella aerogenes*, the MBC was 512 mg/mL, and for *Streptococcus pyogenes*, it was 128 mg/mL.

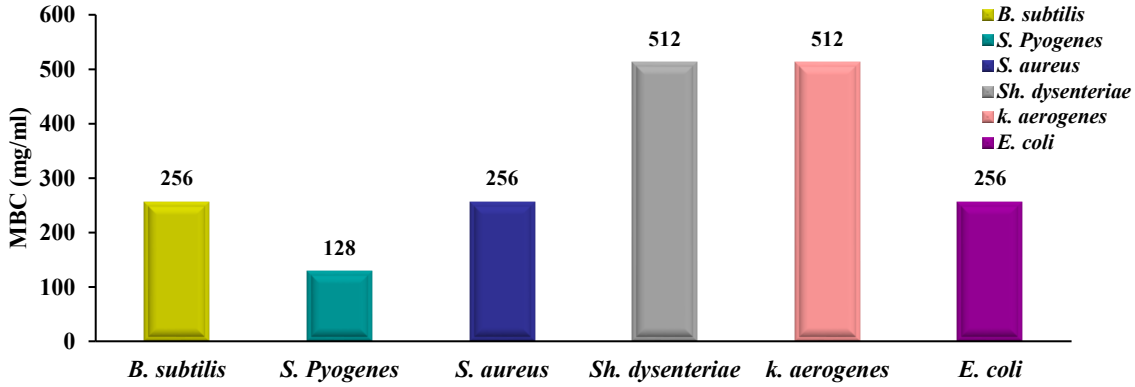

Figure 5. Antimicrobial activity of essential oil of *Artemisia persica* based on minimum bactericidal concentration method.

The antimicrobial effect of the methanolic extract of *Artemisia persica* on two Gram-positive bacteria was investigated by Niakan et al. (2017). They reported that the minimum inhibitory concentration (MIC) of the extract for both bacteria was 100 µg/mL. The minimum bactericidal concentration (MBC) of the extract was 200 µg/mL for *Staphylococcus aureus* and 400 µg/mL for *Bacillus subtilis* [28]. In another study, the antibacterial activity and the MIC and MBC of *Artemisia* essential oil were examined against a broad spectrum of bacteria. It was found that the essential oil had a stronger inhibitory effect on Gram-positive bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Bacillus subtilis*) compared to Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus vulgaris*) [4]. Khezri et al. (2021) evaluated the antibacterial effect of *Artemisia persica* essential oil at concentrations of 75 and 150 ppm, combined with *Lactobacillus paracasei*, to improve the shelf life and microbial and sensory quality of *doogh* (a traditional Iranian yogurt drink). The results of their study indicated that the combination of *Artemisia persica* essential oil at 150 ppm and the probiotic

Lactobacillus paracasei had a synergistic effect in inhibiting the growth of pathogenic bacteria *Listeria monocytogenes* and *Escherichia coli* in *doogh* [29].

4. Conclusion

In this study, Iranian *Artemisia* essential oil was evaluated for its phytochemical composition, including total phenols and flavonoids, as well as its antioxidant and antimicrobial activities. The results demonstrated that the essential oil contains significant amounts of phenolic and flavonoid compounds. The presence of these compounds, acting as antioxidants, likely underlies the observed antimicrobial and antioxidant activities of this essential oil. Furthermore, the findings suggest that due to its antioxidant and antimicrobial properties, the essential oil possesses high potential for application in treating diseases stemming from oxidative stress and microbial infections. However, to broaden the applications of this essential oil in the food and pharmaceutical industries, more extensive studies are necessary to precisely identify the molecular mechanisms behind its antioxidant and antimicrobial activities.

5. Acknowledgements

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مطالعه‌ای بر فعالیت‌های ضدمیکروبی، آنتی‌اکسیدانی و ترکیبات فیتوشیمیایی اسانس درمنه ایرانی

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چکیده

اسانس‌ها ترکیبات آلی فرار و معطری هستند که به عنوان متابولیت‌های ثانویه در گیاهان تولید می‌شوند. که اغلب دارای فعالیت‌های بیولوژیکی قابل توجهی همچون خواص ضد-میکروبی، ضدالتهابی و آنتی‌اکسیدانی هستند. در این مطالعه، فعالیت‌های ضدمیکروبی، آنتی‌اکسیدانی و ترکیبات فیتوشیمیایی اسانس گیاه درمنه ایرانی بررسی شد. فعالیت ضد-میکروبی اسانس براساس روش‌های دیسک دیفیوژن آگار، چاهک آگار، حداقل غلظت بازدارندگی و کشندگی بررسی شد. همچنین، به منظور بررسی ترکیبات فیتوشیمیایی شامل ترکیبات فنولی و فلاونوئیدی کل از روش‌های رنگ‌سنجی فولین-سیوکالتو و کلرید آلومنیوم و فعالیت آنتی‌اکسیدانی بر اساس مهار رادیکال آزاد DPPH و ADTS استفاده شد. نتایج آزمون‌های ضدمیکروبی نشان داد که اسانس مورد مطالعه، اثر مهاری قابل ملاحظه‌ای بر باکتری‌های گرم مثبت و گرم منفی از جمله *استرپتوکوکوس پیوژنز*، *شیگلا دیسانتری*، *اشرشیا کلی*، *استافیلوکوکوس اورئوس*، *باسیلوس سوبتلیس* و *کلبسیلا ائروژنز* از خود نشان داد. اسانس مورد بررسی حاوی مقادیر قابل قبولی از ترکیبات فنلی (mg GAE/g ۳۱/۲±۱/۲۷) و فلاونوئیدی (mg QE/g ۱۷/۴۹ ±۱/۱۹) بود و توانایی قابل توجهی در مهار رادیکال‌های آزاد DPPH (۵۲/۴۳ ±۱/۴۱) و ABTS (۵۷/۴۹ ±۱/۳۷) داشت. مطالعه حاضر نشان داد که اسانس مورد بررسی، پتانسیل قابل توجهی به عنوان یک عامل درمانی برای بیماری‌های ناشی از استرس اکسیداتیو و عفونت‌های میکروبی دارد.