



Evaluation of total phenol and flavonoid, antioxidant power and antimicrobial activity of *Perovskia abrotanoides* essential oil for study from Gram-positive and Gram-negative laboratories: a laboratory study

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| ARTICLE INFO | ABSTRACT |
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| <p>Article History: Received: 2024/1/31 Accepted: 2024/4/9</p> | <p>The aim of this study was to determine the total phenol and flavonoid content and investigate the antioxidant and antimicrobial activity of <i>Perovskia abrotanoides</i> essential oil. The total phenol content was determined using the Folin Ciocalteu method, the total flavonoid content using the colorimetric aluminum chloride method, the antioxidant potential using the DPPH and ABTS free radical inhibition methods, and the antimicrobial activity using the disc diffusion agar, well diffusion agar, minimum inhibitory concentration, and minimum bactericidal concentration methods. The total phenol, total flavonoid, DPPH free radical inhibition, and ABTS free radical inhibition of the essential oil were found to be 24.26 mg of gallic acid per gram, 13.15 mg of quercetin per gram, 52.49%, and 56.79%, respectively. The antimicrobial results showed that the antimicrobial effect of the essential oil was higher against Gram-positive bacteria than Gram-negative types, and <i>Staphylococcus aureus</i> and <i>Salmonella typhi</i> were the most sensitive and resistant microbial strains against <i>P. abrotanoides</i> essential oil, respectively. The diameter of the growth inhibition zone in the disc diffusion agar and well diffusion agar tests, as well as the minimum inhibitory and bactericidal concentrations for <i>S. aureus</i>, were 16.50 mm, 17.30 mm, 2 mg/mL, and 128 mg/mL, respectively, and these values for <i>S. typhi</i> were 7.60 mm, 9.20 mm, 16 mg/mL, and 512 mg/mL, respectively. In general, <i>P. abrotanoides</i> essential oil can be used as a natural antioxidant and antimicrobial compound.</p> |
| <p>Keywords:</p> <p><i>Perovskia abrotanoides</i>, Essential oil, Pathogen, Oxidation, Natural preservative.</p> | |
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1-Introduction

The treatment of microbial infections is mostly based on the use of common therapeutic antibiotics. Since common therapeutic antibiotics are widely used, pathogenic microorganisms have mostly become resistant to these chemicals. In addition, common therapeutic antibiotics are usually associated with side effects such as hypersensitivity, immune system suppression, and allergic responses [1-9]. In addition, various forms of active oxygen, also known as reactive oxygen species, include free radicals and non-free radical species. Reactive oxygen species stimulate oxidative stress in the human body and disrupt its antioxidant defense mechanism. Therefore, free radicals attack cellular macromolecules and lead to several physiological disorders. Relative studies have shown that long-term use of chemical preservatives may lead to the outbreak of various cancers [10]. Therefore, the use of chemical preservatives has decreased in recent decades [11, 12]. In fact, the use of medicinal plants as preservatives has recently increased due to their less unwanted side effects [4-6, 8, 13-28].

Essential oils and secondary metabolites of plants have a wide range of applications in medical, food, and health industries. Plant essential oil includes various health properties, including antioxidant and antimicrobial activities [26, 29]. *Perovskia abrotanoides* is a perennial plant that grows wild in Iran, Afghanistan, Pakistan, and Turkmenistan [30]. The essential oil of this plant contains compounds that have antibacterial properties. Also, the diterpenes extracted from it are a type of cellular poison and anti-plasmodium. A poultice made from grinding the root of this plant in water,

sesame oil, and wax is used in the treatment of leishmaniasis. Other features of it can be mentioned as its positive effects on heart function, aldose reductase inhibition, attachment to benzodiazepine receptors, and induction of apoptosis. Traditional medicine studies have shown that this plant has anti-pain and anti-inflammatory effects [31]. It has been reported that the essential oil and extract of *P. abrotanoides*, due to the presence of phenolic, flavonoid, and anthocyanin compounds, have anti-angiogenic, antioxidant, and anti-inflammatory properties [30, 32, 33]. Also, *P. abrotanoides*, due to its high antioxidant effect, reduces lipid peroxidation and antioxidants while reducing blood sugar, inhibit free radicals and reduce the complications of high sugar and fat [31].

In this research, the total phenol, total flavonoid, antioxidant activity, and antimicrobial effect of *P. abrotanoides* essential oil against some pathogenic microorganisms were evaluated under laboratory conditions.

2. Materials and Methods

2.1. Materials

The materials used in this research were of laboratory grade and were purchased from Merck Germany and Sigma USA.

2.2. Essential oil extraction

The dried powder of plant materials (100 g) was subjected to steam distillation using a Clevenger apparatus for 5 h to extract the stem and leaf essential oil. The extracted essential oil was dried with anhydrous Na_2SO_4 , filtered, and stored in a vial at 4 °C [34].

2.3. Total phenolic content

The method of Ahmed et al. [35] was used with some modifications to measure the total phenol content of the essential oil.

For this purpose, 0.2 mL of essential oil or gallic acid (0-0.5 mg/mL) was mixed with 2.5 mL of 10% Folin Ciocalteu reagent. Then, 2 mL of Na₂CO₃ (7.5%) was added and the solution was incubated for 1 h at room temperature. The absorbance of the solution at 756 nm was read and the total phenol content of the essential oil was calculated using the standard curve of gallic acid and expressed as milligrams of gallic acid equivalent per gram of essential oil.

2.4. Total flavonoid content

The total flavonoid content of the essential oil was measured based on the method of Noshad et al. [36]. Briefly, 0.5 mL of the sample was mixed with 300 µL of NaNO₂ solution (1:20 w/v) and the mixture was vortexed for 10 seconds. The resulting mixture was stored for 5 minutes at room temperature and then mixed with 300 µL of AlCl₃ (1:10 w/v), 2 mL of 1 M NaOH, and 1.9 mL of distilled water. The absorbance of the mixture at 510 nm was recorded and the total flavonoid content of the essential oil was expressed as milligrams of quercetin equivalent per gram of essential oil [36].

2.5. Antioxidant Activity

2.5.1. DPPH Radical Scavenging

The antioxidant activity of the essential oil against DPPH free radicals was evaluated according to the method mentioned in the scientific sources with the necessary modifications [37]. In this test, the essential oil was mixed with DPPH solution (1 mL; 90 µM) and then with methanol (95%) to a final volume of 4 mL. After 1 h of incubation at room temperature in the dark, the absorbance of the solution at 515 nm was recorded. The ability of the essential oil to scavenge DPPH free radicals was calculated using the following formula:

$$\text{Antioxidant activity (\%)} = [(A \text{ blank} - A \text{ sample})/A \text{ blank}] \times 100$$

In this formula, A blank and A sample are the absorbance of the control and the sample, respectively.

2.5.2. ABTS Radical Scavenging

In this test, the ABTS and K₂S₂O₈ solutions were initially mixed to produce the cationic radical ABTS solution. Then, 0.1 mL of essential oil or control was mixed with 3.9 mL of the radical ABTS solution and its absorbance at 734 nm was recorded. Then, the antioxidant activity was measured as follows:

$$\text{Antioxidant activity (\%)} = [(A \text{ blank} - A \text{ sample})/A \text{ blank}] \times 100$$

In this formula, A blank and A sample are the absorbance of the control and the sample, respectively [14].

2.6. Antimicrobial Activity

The antimicrobial effect of the essential oil against *Bacillus subtilis*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Shigella dysenteriae*, *Enterobacter aerogenes*, and *Salmonella typhi* was performed according to the following methods.

2.6.1. Disc diffusion agar

A microbial suspension was cultured on petri dishes containing Mueller Hinton agar. Paper disks with a diameter of 6 mm were soaked in 20 µL of essential oil. Then, the disks were placed on the inoculated surface of the culture medium and incubated at 37 °C for 24 h. The diameter of the inhibition zone around the disks was measured in millimeters [38].

2.6.2. Well diffusion agar

In this method, a microbial suspension was spread on Mueller Hinton agar medium in petri dishes. Then, several wells (6 mm in diameter) were created on the surface of the culture medium and filled with 20 µL of essential oil. The petri dishes were kept at a constant temperature of 37 °C for 24 h and the diameter of the inhibition zone around

the wells was measured and expressed in millimeters [38].

2.6.3. Minimum inhibitory and bactericidal concentrations

To determine the minimum inhibitory concentration of the essential oil, the microdilution method in a 96-well plate presented by El-Atki et al. [39] was used with the necessary modifications. Sequential concentrations (1, 2, 4, 8, 16, 32, 64, 128, 256, and 512 mg/mL) of the essential oil were prepared in Mueller Hinton broth and sterilized through 0.22 μm syringe filters. Then, 200 μL of each concentration was added to the wells containing microbial suspensions (20 μL ; equivalent to 0.5 McFarland). The plates were incubated at 37 °C for 24 h, and then 20 μL of 0.5% triphenyltetrazolium chloride solution was added to the wells. After re-incubation at 37 °C for 30 min, the absence of dark red color (as an indicator of microbial growth) in the wells that had been created with the lowest concentration of essential oil was considered as the minimum inhibitory concentration of essential oil.

The minimum bactericidal concentration of the essential oil was performed based on the method of Alghooneh et al. [40]. Based on the results of the minimum inhibitory concentration growth test, 100 μL from the wells without microbial growth was cultured on Mueller Hinton agar medium. In the next step, the medium was incubated under the conditions mentioned above for the antimicrobial minimum inhibitory concentration growth test, and the lowest concentration of essential oil that resulted in bacterial death (i.e., no formation of visible colony) was considered as the minimum bactericidal concentration.

2.7. Statistical analysis

The experiments were performed in three replicates. The data were analyzed using Minitab software (version 16) and one-way analysis of variance. Tukey's test, at the 95% confidence level ($p < 0.05$), was used to determine the difference between the mean data.

3. Results and discussion

Figure 1 shows the total phenol and total flavonoid content of *P. abrotanoides* essential oil. The results showed that the essential oil has a considerable concentration of phenolic and flavonoid compounds; so that the total phenol and total flavonoid content of the essential oil were 24.26 mg of gallic acid per gram and 13.15 mg of quercetin per gram, respectively. Several phenolic acids have been quantified in the methanolic extract of the *P. abrotanoides* plant grown under laboratory conditions and the wild plant by Ghaderi et al. (2019). Rosmarinic acid was the main phenolic acid in the extract of both samples and the total phenol content in the plant grown under laboratory conditions (71 mg of gallic acid per gram) was higher than the wild type plant (55 mg of gallic acid per gram) [41]. Bayat et al. in 2021, investigated the total phenol content of methanolic, ethanolic, hexane, and aqueous extracts in different organs of the *P. abrotanoides* plant (flower, stem, and leaf). The total phenol content in the extracts varied from 0-3 mg of gallic acid per gram and the highest amounts of phenol were obtained in the methanolic extract. Although there was no significant difference between the hexane and aqueous extracts in phenol content, the phenol content in the aqueous extracts (except for the aqueous stem extract) was lower. In addition, the total phenol content in the flower extracts was the highest compared to the leaf and stem extracts [42]. In another

study, the total phenol content of 19-66 mg of gallic acid per gram and total flavonoid content of 2.50 – 4.11 mg of quercetin per

gram in the methanolic extract of *P. abrotanoides* have been reported [32].

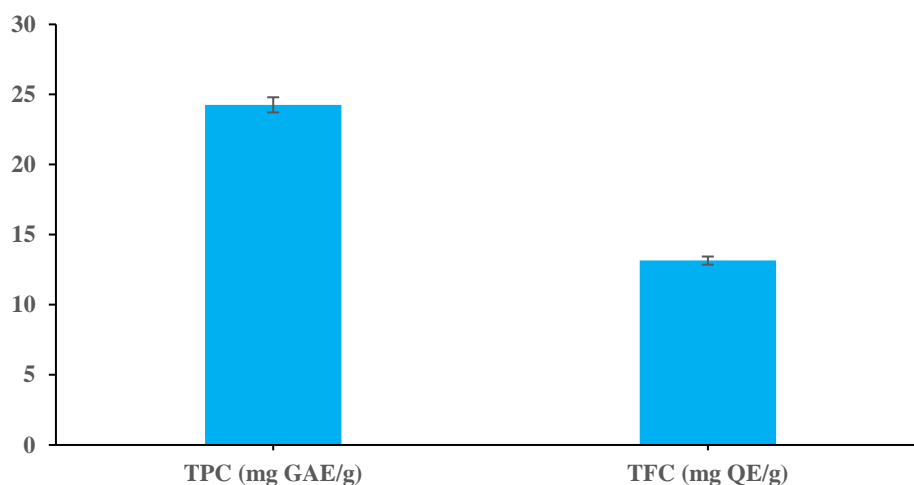


Figure 1. Total phenol content (TPC) and total flavonoid content (TFC) of *P. abrotanoides* essential oil. GAE = Gallic acid equivalent; QE = quercetin equivalent.

Studies have shown that most plants that contain phenolic derivatives also show high antioxidant activity and a positive and significant correlation between the antioxidant activity of plant materials and their phenolic compound contents has been proven [10]. According to the results presented in Figure 2, *P. abrotanoides* essential oil was able to inhibit DPPH (52.49%) and ABTS (56.79%) free radicals. The antioxidant activity of the methanolic extract of *P. abrotanoides* has been reported in the study of Ghaffari et al. in 2018 [32]. In addition, Bayat et al. in 2021 showed that the *P. abrotanoides* plant, especially the flowers, is rich in phenolic

compounds and methanol is the best solvent for the extraction of phenolic and antioxidant compounds [42]. However, the difference in the amount of results presented in this research with the findings of other researchers can be attributed to the change in the composition and quality of the essential oil and extract obtained from plant sources due to the age and diversity of the plant, geographical conditions, drying methods, and extraction methods [2, 13, 15]. According to the results, *P. abrotanoides* essential oil can be used as a natural alternative to synthetic antioxidants to improve the oxidative stability of many food products.

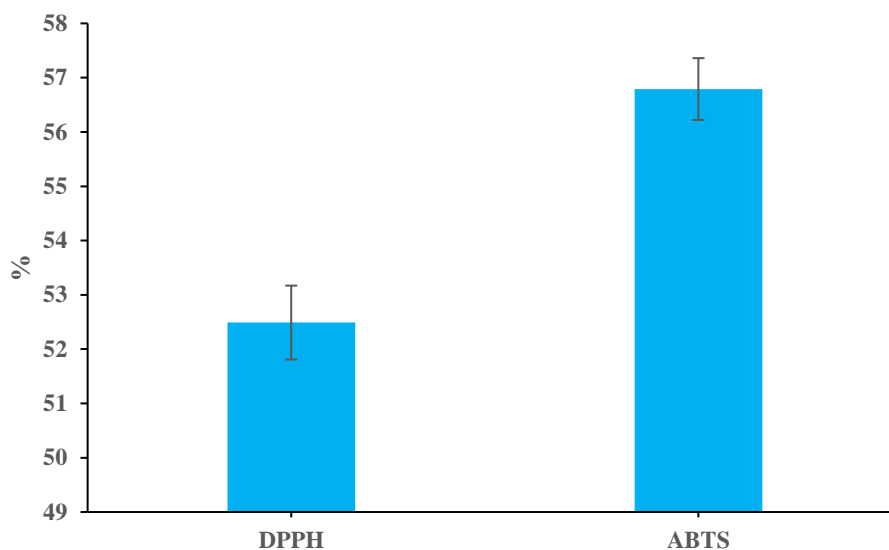


Figure 2. Antioxidant effect of *P. abrotanoides* essential oil based on DPPH and ABTS radical scavenging methods.

The results of the antimicrobial effect of *P. abrotanoides* essential oil against pathogenic bacteria based on the disc diffusion agar method are presented in Figure 3. According to the results, the antimicrobial effect was dependent on the type of bacteria and *S. aureus* and *S. typhi*

were the most sensitive and resistant strains against the essential oil, respectively ($p < 0.05$). The average diameter of the inhibition zones for *B. subtilis*, *S. pyogenes*, *S. aureus*, *Sh. dysenteriae*, *E. aerogenes*, and *S. typhi* were 13.45, 14.80, 16.50, 8.90, 10, and 7.60 mm, respectively.

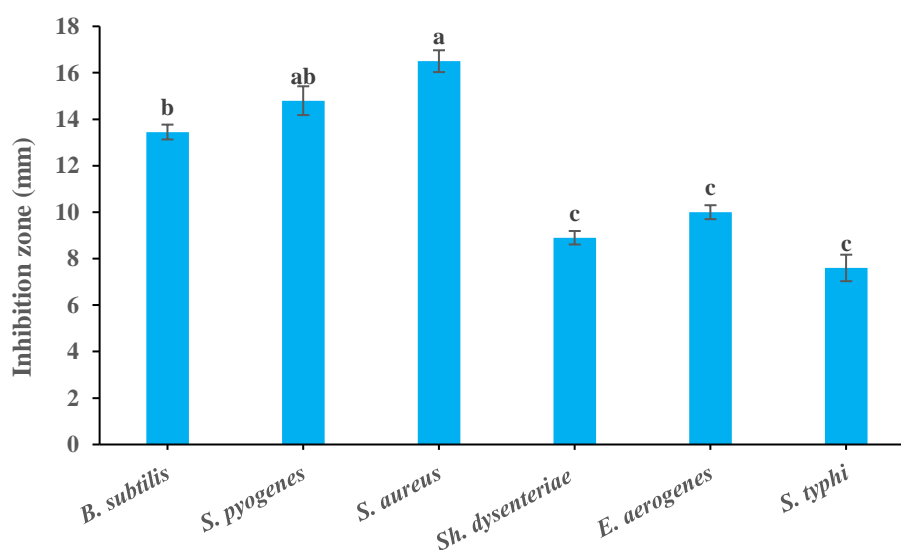


Figure 3. Antibacterial effect of *P. abrotanoides* essential oil based on disc diffusion agar method.

Similar results were observed in the well diffusion agar test (Figure 4), and the

average diameter of the inhibition zones for *B. subtilis*, *S. pyogenes*, *S. aureus*, *Sh.*

dysenteriae, *E. aerogenes*, and *S. typhi* were 14, 16.10, 17.30, 9.60, 10.20, and 9.20 mm, respectively. As observed, the average diameter of the inhibition zone in the well diffusion agar test was larger than the disc diffusion agar method. According to the findings of Alizadeh Behbahani et al., this difference may be due to the fact that

bacterial species in the well diffusion agar method are in direct contact with the essential oil, but the rate of diffusion of the antimicrobial agent from the disk surfaces to the medium determines its inhibitory effect in the disc diffusion agar test [15, 17, 38].

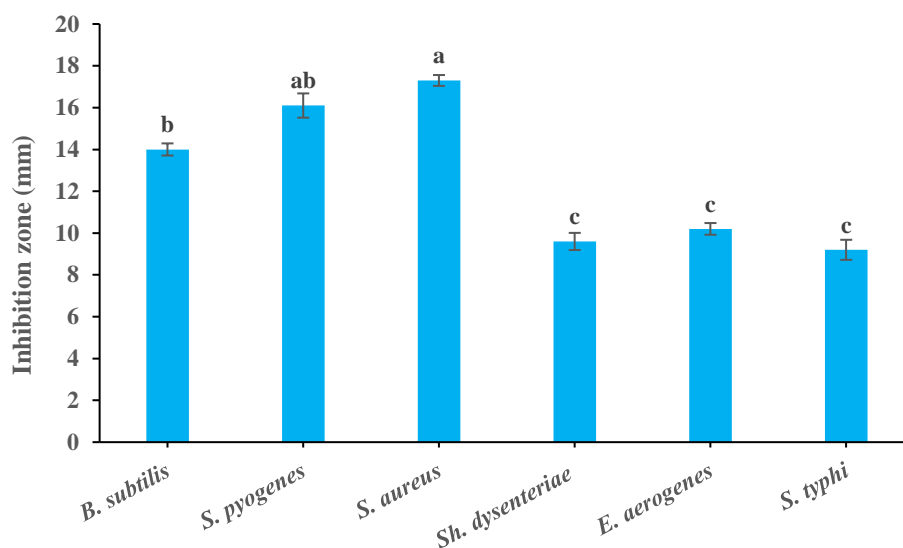


Figure 4. Antibacterial effect of *P. abrotanoides* essential oil based on well diffusion agar method.

The results of the minimum inhibitory and bactericidal concentration of the essential oil against pathogenic bacteria are shown in Figures 5 and 6. The minimum inhibitory concentration for *B. subtilis*, *S. pyogenes*, *S. aureus*, *Sh. dysenteriae*, *E. aerogenes*, and *S. typhi* were 4, 4, 2, 16, 8,

and 16 mg/mL, respectively, and the minimum bactericidal concentration for the above bacteria were 128, 128, 128, 512, 512, and 512 mg/mL, respectively. According to the results, Gram-negative bacteria were more resistant to the essential oil than Gram-positive types.

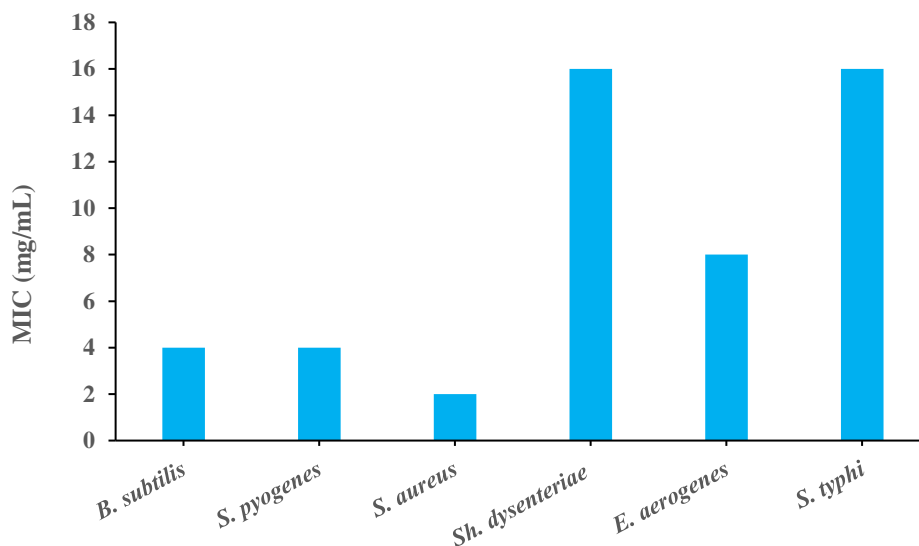


Figure 5. Antibacterial effect of *P. abrotanoides* essential oil based on minimum inhibitory concentration (MIC) method.

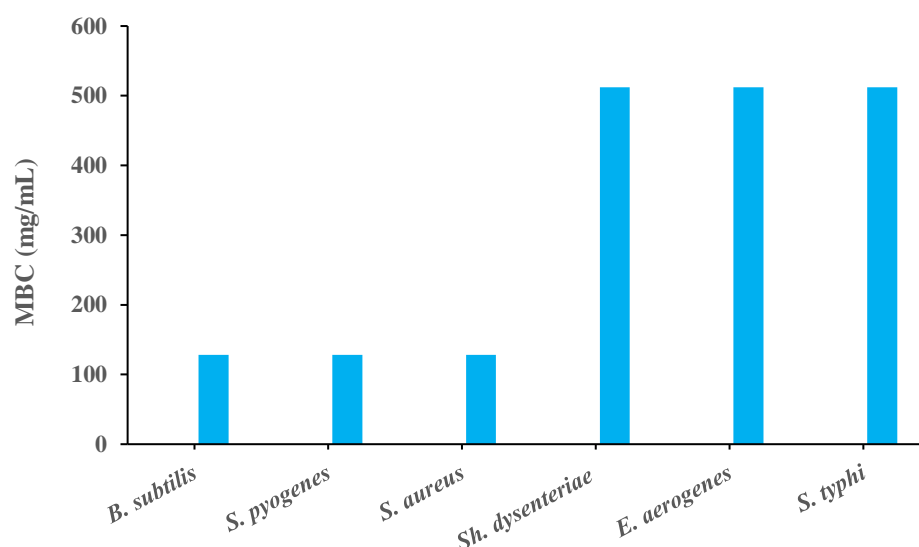


Figure 6. Antibacterial effect of *P. abrotanoides* essential oil based on minimum bactericidal concentration (MBC) method.

In line with the results of this study, Mahboubi and Kazempour in 2009 investigated the antimicrobial activity of *P. abrotanoides* essential oil against microorganisms *S. aureus*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Aspergillus niger*. The results of these researchers showed that *C. albicans* and Gram-positive bacteria, especially *S.*

aureus, were very sensitive to the essential oil, and the antimicrobial effect of the essential oil was attributed to the presence of compounds 1,8-cineole, camphor, and alpha-pinene. In general, Gram-negative bacteria were resistant to *P. abrotanoides* essential oil compared to gram-positive bacteria [30]. The outer layer of Gram-negative bacteria is made up of lipopolysaccharide. This layer forms a hydrophobic permeability barrier that limits

the diffusion of hydrophobic compounds through its lipopolysaccharide coating [38]. Essential oils always represent a complex mixture of different chemical components. The nature and ratio of each component of the essential oil can affect its antimicrobial activity. Mahboubi and Kazempour reported that 1,8-cineole is a lipid-friendly compound and the lipid-friendly property of alpha-pinene is greater than camphor. In general, a lipid-friendly compound has a greater affinity for the cell membrane and shows greater toxicity. Therefore, 1,8-cineole had the best effect on the survival of *S. aureus*, and the effect of alpha-pinene and then camphor was less. After 24 h, the effect of camphor on the survival of bacteria was greater than alpha-pinene and 1,8-cineole. In addition to the lipid-friendly structure of the components, the difference in the antimicrobial activity of the components is attributed to their water solubility. The absorption of monoterpenes is determined by their water solubility and the permeability of the outer coating of microorganisms. The water solubility of camphor is greater than the others and it has more antimicrobial activity than other components against all microorganisms. 1,8-cineole is probably inactive because they were not able to effectively penetrate the outer membrane for a long time. Camphor and alpha-pinene were found in high concentrations in *P. abrotanoides* essential oil and had antimicrobial activity. The high concentration of alpha-pinene and camphor in the essential oil is probably an explanation for its antimicrobial activity against Gram-positive bacteria and yeast. Alpha-pinene destroys the integrity of gram-positive bacterial cells and inhibits respiratory activity in yeast mitochondria and had some antifungal activity, but Gram-negative bacteria were more resistant

to it [30]. The antimicrobial activity of *P. abrotanoides* essential oil against pathogenic microorganisms was also reported in the study of Ashraf and colleagues in 2014 [34].

4. Conclusion

In this study, the essential oil of *P. abrotanoides* was extracted using the Clevenger method and water distillation. The results showed that the essential oil contains significant amounts of phenolic and flavonoid compounds. Also, the essential oil was capable of inhibiting ABTS and DPPH free radicals, indicating the ability of the essential oil to prevent oxidative reactions. In addition, antimicrobial results showed that the essential oil was more capable of preventing growth and also eliminating Gram-positive bacteria compared to Gram-negative strains. The smallest diameter of the inhibition zone of the essential oil was for *S. typhi* bacteria and the largest diameter of the inhibition zone was for *S. aureus* bacteria. Given the acceptable concentration of phenolic and flavonoid compounds in the essential oil, this natural substance can be used to inhibit free radicals and the growth of food pathogens. However, further studies are needed to investigate the potential toxicity of *P. abrotanoides* essential oil for use in in vivo conditions.

5. Acknowledgement

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6. References

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ارزیابی فنل و فلاونوئید کل، قدرت آنتی‌اکسیدانی و فعالیت ضد میکروبی اسانس برازمبل بر تعدادی از باکتری‌های گرم مثبت و گرم منفی: یک مطالعه آزمایشگاهی

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۱- دانشیار، گروه علوم و مهندسی صنایع غذایی، دانشکده علوم دامی و صنایع غذایی، دانشگاه علوم کشاورزی و منابع طبیعی خوزستان، ملاتانی، ایران.

| اطلاعات مقاله | چکیده |
|-----------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| تاریخ های مقاله : تاریخ دریافت: ۱۴۰۲/۱۱/۱۱ تاریخ پذیرش: ۱۴۰۳/۱/۲۱ | هدف از این مطالعه تعیین محتوای فنول و فلاونوئید کل و بررسی فعالیت آنتی‌اکسیدانی و ضد میکروبی اسانس برازمبل (<i>Perovskia abrotanoides</i>) بود. محتوای فنول کل با استفاده از روش فولین سیوکالتو، محتوای فلاونوئید کل با کمک روش رنگ سنجی کلرید آلومینیوم، پتانسیل آنتی‌اکسیدانی با استفاده از روش‌های مهار رادیکال آزاد DPPH و ABTS و فعالیت ضد میکروبی با استفاده از روش‌های دیسک دیفیوژن آگار، چاهک آگار، حداقل غلظت مهارکنندگی و حداقل غلظت باکتری‌کشی ارزیابی گردید. میزان فنول کل، فلاونوئید کل، مهار رادیکال آزاد DPPH و مهار رادیکال آزاد ABTS اسانس به ترتیب برابر با ۲۴/۲۶ میلی‌گرم گالیک اسید در گرم، ۱۳/۱۵ میلی‌گرم کوئرستین در گرم، ۵۲/۴۹ درصد و ۵۶/۷۹ درصد به دست آمد. نتایج ضد میکروبی نشان داد که اثر ضد میکروبی اسانس در برابر باکتری‌های گرم مثبت نسبت به انواع گرم منفی بالاتر بود و <i>استافیلوکوکوس اورئوس</i> و <i>سالمونلا تیفی</i> به ترتیب حساس‌ترین و مقاوم‌ترین سویه‌های میکروبی در برابر اسانس برازمبل بودند. قطر هاله عدم رشد در آزمون دیسک دیفیوژن آگار و چاهک آگار و همچنین حداقل غلظت مهارکنندگی و کشندگی برای باکتری <i>استافیلوکوکوس اورئوس</i> به ترتیب ۱۶/۵۰ میلی‌متر، ۱۷/۳۰ میلی‌متر، ۲ میلی‌گرم در میلی‌لیتر و ۱۲۸ میلی‌گرم در میلی‌لیتر بود و این مقادیر برای باکتری <i>سالمونلا تیفی</i> به ترتیب ۷/۶۰ میلی‌متر، ۹/۲۰ میلی‌متر، ۱۶ میلی‌گرم در میلی‌لیتر و ۵۱۲ میلی‌گرم در میلی‌لیتر بود. بطور کلی، اسانس برازمبل می‌تواند بعنوان یک ترکیب آنتی‌اکسیدان و ضد میکروب طبیعی استفاده شود. |
| کلمات کلیدی: برازمبل، اسانس، پاتوژن، اکسیداسیون، نگهدارنده طبیعی. | |
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