The Antibacterial and Antioxidant Characteristics of *Berberis vulgaris* Fruit Extracts

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ABSTRACT

Background: Foodborne illnesses, which result from contaminated foods, are considered a worldwide public health threat. Some wild fruits may have antioxidant and antibacterial effects. These wild fruits can be used as a food additive, which is used on food as a preservative. Berberis possesses nutritional, health supplements, and antimicrobial properties. **Aims of the study**: This study aimed to investigate the antioxidant capacities, free radical scavenging activities, and antibacterial activities against some bacterial foodborne illnesses. **Methodology**: For testing antimicrobial properties of the *Berberis vulgaris* fruits, the water extracts of the fruits were tested against important foodborne pathogenic bacteria using the agar well diffusion method. The 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging assay was used to determine the antioxidant activity of *Berberis* fruit extracts and compared with that of butylated hydroxytoluene (BHT) through the decrease in absorbance at 517 nm measurement. **Results:** *Berberis vulgaris* fruit extract had the highest antibacterial activity against Methicillin-resistant *Staphylococcus aureus*, with an inhibition zone of 25 mm. While on Gram,-negative bacteria, a small zone of 8 mm for *Salmonella typhimurium*, and no zone for *Escherichia coli* and *Pseudomonas aeruginosa*. The fruit extract of *B. vulgaris* showed a high free radical scavenging activity in distilled water (84.79%). **Conclusion**: This study revealed that wild-type *B. vulgaris* can be used as an effective natural antimicrobial and antioxidant in the food industry. Barberry extract showed the highest activity against MRSA, while small zones of reduced growth or no zones against the tested Gram-negative bacteria.

Keywords: *Berberis vulgaris*, antibacterial, antioxidant, foodborne pathogen.

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INTRODUCTION

Various herbal products, like oils and extracts, have been studied for their anti-inflammatory, antibacterial, antioxidant, and radical-scavenging properties. This increased interest is due to consumers being more informed about the side effects of synthetic chemical compounds. As a result, there is growing attention to substituting synthetic chemicals with natural compounds in food preservation. Nowadays, different natural antioxidants extracted from plants and fruits can serve this purpose rather than other substances (1,2). The misuse of antimicrobial agents has resulted in an increasing resistance to antibiotics among many types of bacteria. As a result of this growing resistance, researchers have conducted more studies to create and develop new antimicrobial agents. Herbs are considered ideal for this purpose due to their properties, including lower complications, ease of access, and cost-effectiveness compared to synthetic alternatives (3-5).

The use of an antibacterial combination has a significant impact by preventing the rise of new mutated strains and works on a broader spectrum of bacteria. Additionally, when different antimicrobial agents are used together,

their combined effects may allow for using fewer drugs and preservatives as well as at lower doses. Phytochemicals and various micronutrients are known for their benefits as they work as antioxidants, scavenging free radicals that cause damage to the cellular lipids, proteins, and DNA, leading to the onset of numerous degenerative conditions. Phytochemicals encompass over a dozen categories of biologically active compounds and have been recognized for their benefits to replace synthetic chemicals (6,7).

Plants, including their leaves, roots, and fruits, contain substances that have high antioxidant properties. These antioxidants help combat the free radicals, which decrease the process of fibrosis. Antioxidants work as a protective agent that protects the human body from oxidative stress and free radicals. Many fruits naturally contain antioxidants that can counteract the action of free radicals by providing electrons to render them into harmless molecules (8,9). The role of wild and semi-wild edible fruits has been proven due to their abundance of antioxidants, like flavonoids, anthocyanins, organic acids, and various other compounds (10,11). Certain flavonoids have anticancer properties, leading to a growing interest in fruits rich in anthocyanidin and anthocyanin. Anthocyanins are phenolic complexes that have a role in determining the color of fruits and vegetables. These phenolic complexes are significant in the juice and wine industry (12,13). The antioxidant abilities of phenolics resulted from their characteristics to function as reducing hydrogen donors and singlet oxygen quenching agents (14).

The fruits of Barberry are rich in compounds that possess antioxidant properties, helping to combat the effects of free radicals and lower the risk of chronic diseases and cancer (15). Barberry fruit is rich in pigments that are packed with components like phenolic compounds, flavonoids, carotenoids, and anthocyanins. These natural chemicals serve as anti-inflammatory, anti-mutagenic, antioxidant, and prevent hepatitis, inhibit specific enzymes, and safeguard food in the stomach from free radicals that can cause chronic illnesses (16,17). This study aimed to examine antibacterial and antioxidant activities of the B. vulgaris fruit extracts that are used widely as general health supplements, also as food preservation for inhibition of foodborne pathogens, to increase their shelf life, in addition to treatment of infectious diseases.

Methodology

Fruit Extracts Preparation.

To evaluate the antioxidant properties of *Berberis* fruits, we followed a method based on Meng's extraction process, with some adjustments (18). Initially, the fruit was dried at 55°C for three days. After drying, the fruits were gently crushed in a mortar, and 3g of the crushed fruits were weighed. To extract the antioxidants, we combined 3g of fruits with 30ml of water, allowing the solution to shake in a shaker for 15 hours (200 rpm) at room temperature. Following this, the samples were transferred to centrifuge tubes, and then centrifuged at 5000 rpm for 15 minutes at 4°C. Finally, the aqueous extracts were filtered using Whatman No. 1 filter paper (18).

Antibacterial Activity of Water Extracts of Wild Fruit Extracts.

Agar well diffusion method: the antimicrobial activity of Barberry extract on pathogenic bacteria was examined by the agar well diffusion method.

Pathogenic strains that are resistant to Methicillin, including *Staphylococcus aureus* (MRSA), *Escherichia coli*, *Salmonella typhimurium*, and *Pseudomonas aeruginosa*, were used for testing the antibacterial activity. The bacteria were cultured on Brain Heart Infusion broth for 18 hours at 37°C. Compared with the 0.5 MacFarland Standards (5x10-8 CFU/mL). The pathogenic strains were then spread through swapping Mueller-Hinton Agar plates, and 20µl of Berberis fruit water extracts were applied to these plates. The wells (6 mm in diameter) were made with a sterile bore, and 50 µL of prepared extract was delivered into each well. The plates were then placed in an incubator at 37°C for 24 hours. After the incubation period, the inhibition zones that formed because of the water extracts were recorded and measured.

Determination of Antioxidant Characteristics of Berberis Fruits.

The DPPH (1,1-Diphenyl-2-picryl-hydrazyl) radical scavenging activity test (RSA) was conducted following the method outlined by G¨ulçin. The (1e) absorbance was measured at 517nm. The reduction in absorbance of (1e) indicates the remaining DPPH solution or the effectiveness of the free radical scavenging (19). For ABTS analysis, a

slightly modified version of the procedure detailed by Huang and his colleagues was used (20). To summarize, 0.008g of ABTS was dissolved in DW, then mixed with 13.2mg of potassium persulfate to produce a dark blue solution after 16 hours of incubation. Later, the solution was diluted with ethanol to achieve an OD 734 reading of 0.7 before mixing 100µl of it with 2.4 ml ABTS solution and incubating for 6 min, at room temperature, before recording the absorbance. In both the DPPH and ABTS tests, each extract (6.67 mg/mL) was diluted in a 1:1 (v/v) ethanol/water solution. The percentage inhibition values were determined using the following equation:

Inhibition% = [(ADBBH or ABTS - A extract)] / ADBBH or ABTS X 100

Statistical Analysis

Statistical analysis carried out by GraphPad Prism version 9. The Kruskal–Wallis test is used for comparing the results of inhibition zones between the tested microorganisms. *P*-value less than 0.05, considered statistically significant.

RESULTS

Results of Antibacterial Activity

The antibacterial properties were measured by observing whether an inhibition zone was formed around the well containing the extract or not. The means of the triple inhibition zones were measured. *B. vulgaris* extract (0.3 g/mL) showed significant variation among the tested bacterial species (Kruskal–Wallis H = 9.65, df = 3, p = 0.022). The extract exhibited the strongest inhibition against MRSA (25.0 ± 1.0 mm), which was significantly higher (p=0.02) than the inhibition zones observed for Gram-negative bacteria, including *S. typhimurium* (8.0 ± 1.0 mm), *E. coli* (6.3 ± 0.6 mm), and *P. aeruginosa* (6.3 ± 0.6 mm), as shown in Table 1 and figure.1 below.

Table (1): Inhibition zones (mm) of *Berberis vulgaris* on the tested bacteria

Bacterial species	Inhibition zones (mm)			Mean ± SD (mm)	B. vulgaris 0.3 gm/ml on the tested bacteria
	Plate (1)	Plate (2)	Plate (3)		
Gram-positive bacteria MRSA	25	24	26	25.0 ± 1.0	25mm
Gram-negative bacteria *S. typhimurium*	9	8	7	8.0 ± 1.0	8mm
Escherichia coli	6	6	7	6.3 ± 0.58	R*
Pseudomonas aeruginosa	7	6	6	6.3 ± 0.58	R*

R: RESISTANT, *(6MM) DIAMETER WELL.

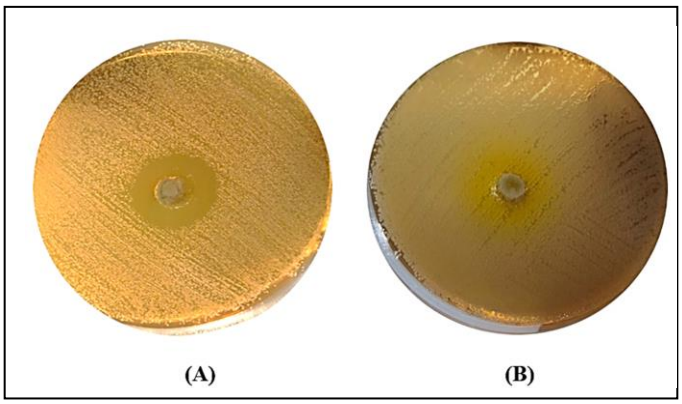

Figure 1. A- Inhibition zone of *B. vulgaris* on MRSA (25 mm). B- Inhibition zone of *B. vulgaris* on *E. coli* (zero zone).

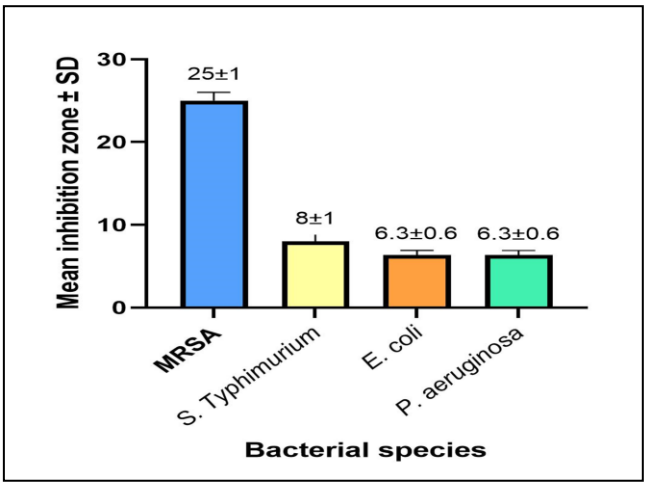

Figure 2. The results of the statistical analysis revealed different microorganisms, p = 0.02.

Antioxidant activity:

The antioxidant activity of fruits was 84.79% in the water extract. The DPPH radical scavenging activity test was used to determine antioxidant activity.

DISSCUSION

This study investigated the antibacterial activities of the *B. vulgaris* fruit extracts. The fruit extract was effective against Gram-positive bacteria, including MRSA, and Gram-negative bacteria such as *S. typhimurium*. While other Gram-negative bacteria, *E. coli* and *P. aeruginosa*, showed a resistance response. Antioxidant activity of *Berberis* fruits was high (84.79%) in water extract. Results of the present study for having antimicrobial effects on these bacteria were proved by other studies, and according to the studies of Salehi and Eroğlu, the extracts of *B. vulgaris* and *Berberis Cartagena* had antimicrobial activity for *Bacillus cereus*, *S. typhimurium*, *Yersinia enterocolitica*, and *S. aureus*. The antimicrobial effect of this plant is attributed to its high content of phenolic compounds (21, 22).

The present study indicated that the fruit extract has no such effect on Gram-negative bacteria. Such a result, approved by other studies as well, Wojtyczka and his colleagues in 2014, indicated that the fruit extracts have a weak effect on this group of bacteria (23). These slight differences may result from the differences in the concentration of the extract or due to bacterial strain differences. The antimicrobial effect of any plant or even synthetic drug is affected by several factors, among them the strain of the bacteria, because the geographical distribution shows differences by having different strains of the same species with varying responses to a specific antimicrobial agent (24).

Regarding the antioxidant activity of *B. vulgaris* fruit extract, the present study indicated a considerable percentage (84.79%) in the water extract. According to a study carried out by Motalleb and his colleagues in 2005, they found that the *B. vulgaris* extracts exhibited DPPH radical scavenging activity of 82.52 ± 0.64% and 73.62 ± 1.87%, for water and ethanol extracts, respectively, which proves our results (25). Antioxidants of fruits and vegetables include carotenoids, ascorbic acid, and phenolic compounds. Phenolic compounds, such as flavonoids, are recognized as sources of antioxidants (26, 27).

The antioxidant properties of the fruits came from their ability to capture the free radicals and chelate the heavy metals (28). *Berberis* fruits, like other fruits, possess an antioxidant ability, and their high antioxidant content enables barberry fruits to aid in preventing oxidative stress, which may potentially reduce the risk of chronic illnesses (29). Because the flavonoids are the main contributors to the total reducing power in different fruit species, it's likely that the antioxidant capabilities of *Berberis* fruit species were affected by weather conditions during the growing season (30, 31). These findings align with research by Koncić *et al*. in 2010, regarding the relationship between antioxidant capacity and phenolic complexes, in *B. vulgaris* and *B. croatia* fruits (32).

The results of the present study, like previous studies, highlight the potential of using extracts from Berberis fruits as antimicrobials for pharmaceutical and therapeutic applications. Anthocyanins serve as color pigments, giving fruits and vegetables their dark brown to red color. The color of these fruits and the presence of anthocyanins are closely linked during the ripening process. With hydroxyl groups in three carbon rings, they exhibit chelating properties that make them antioxidants. As fruits ripen, transitioning from green to red, anthocyanins play a role in enhancing the fruits' antioxidant capabilities (33, 34).

Differences between the results of the present study and other previous studies could be related to the concentration of the phenolic compounds and flavonoids that are used in the present study compared to those in their studies. According to the studies of Yildiz and his colleagues in 2014 and Ersoy and his colleagues in 2018, in Turkey, their concentrations were higher than our concentration (35, 36). The concentration and amount of the phenol and flavonoid content differ among different plant species and even within the same species in different geographical locations. This results in genetic variations among different plants and other environmental factors like the amount of light, temperature, components of the soil, humidity, use of fertilizers, time of harvesting, and even storage conditions (37). This will explain the differences between the results of the different studies regarding the antimicrobial activity of *Berberis* extracts on different types of bacterial species.

CONCLUSION

Most types of plants contain antioxidants with different concentrations. Based on their chemical components, different plants can be used for pharmacological and therapeutic purposes. *B. vulgaris* contains a high amount of antioxidant and antibacterial compounds. Different pathogenic bacteria show different responses to the antimicrobial properties of this plant. Further studies are needed to enhance the use of this plant as an antimicrobial agent to replace synthetic chemical drugs and to benefit from its antioxidant properties for medical uses.

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الخصائص المضادة للبكتريا ومضادات الأكسدة لمستخلص ثمرة *Berberis vulgaris*

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الخلاصة

خلفية عن الموضوع: تعتبر الأمراض المنقولة بالغذاء والتي تنتج عن الأطعمة الملوثة تهديدا للصحة العامة في جميع أنحاء العالم، قد يكون لبعض الفواكه البرية تأثير مضاد للأكسدة ومضاد للبكتريا. يمكن استخدام هذه الثمار البرية كأضافات غذائية، حيث يتم استخدامها في الأطعمة كمادة حافظة، يمتلك البرباريس مكملات غذائية وصحية وخصائص مضادة للميكروبات. **الهدف من الدراسة:** أستهدفت هذه الدراسة إلى دراسة القدرات المضادة للأكسدة وأنشطة الكسح الجذري والأنشطة المضادة للبكتريا ضد بعض الأمراض البكتيرية المنقولة بالغذاء. **المواد وطرق العمل:** لاختبار الخصائص المضادة للميكروبات لثمار *Berberis vulgaris* ، تم اختبار مستخلصاتها المائية ضد البكتيريا المسببة للأمراض الهامة المنقولة بالغذاء باستخدام طريقة نشر الآكار. تم استخدام اختبار (DPPH) لتحديد نشاط مضادات الأكسدة في مستخلصات ثمار البرباريس ومقارنته مع نشاط (BHT) من خلال انخفاض الامتصاصية عند قياس 517 نانومتر. **النتائج:** كان لمستخلص ثمار *Berberis vulgaris* أعلى فعالية مضادة للبكتريا ضد المكورات العنقودية الذهبية المقاومة للميثيسيلين مع منطقة تثبيط 25 ملم. بينما على البكتريا سالبة الكرام اظهرت فعالية قليلة (8ملم) على *S. Typhimurium* ولم تظهر اية تأثير على النوعين الاخرين. أظهر مستخلص ثمار نبات *Berberis vulgaris* فعالية عالية في التخلص من الجذور الحرة في الماء المقطر بنسبة (84.79%). **الاستنتاج:** كشفت هذه الدراسة أن النوع البري *Berberis vulgaris* يمكن استخدامه كمضاد طبيعي فعال للميكروبات ومضاد للأكسدة في صناعة الأغذية. أظهر مستخلص البرباريس أعلى نشاط ضد MRSA، في حين أن المناطق الصغيرة ذات النمو المنخفض ضد البكتريا سالبة الكرام التي تم اختبارها.

الكلمات المفتاحية: *Berberis vulgaris* ، مضادات البكتريا، مضادات الأكسدة ، مسببات الأمراض المنقولة بالغذاء.