

EFFICACY OF BARBERRY (*Berberis vulgaris* L.) FRUIT EXTRACTS AGAINST ANTIBIOTIC-RESISTANT PATHOGENIC *Pseudomonas aeruginosa*.

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ABSTRACT

The rise of antibiotic-resistant bacteria makes treating bacterial infections difficult. Thus, new antibiotics and antimicrobials are urgently needed. Natural therapies like medicinal plant and herb extracts are becoming increasingly popular for health issues. Based on biofilm development as a virulence factor, this study examined the antibacterial efficacy of alcoholic and aqueous Barberry (*Berberis vulgaris* L) fruit extracts against antibiotic-resistant clinical *Pseudomonas aeruginosa* isolates. At dosages evaluated, both water- and alcohol-based Barberry fruit extracts affect clinical strains of antibiotic-resistant *P. aeruginosa*. Alcoholic Barberry extract outperforms water-based Barberry extract against bacteria and biofilm. According to GC-Mass results, showed the presence of 21 chemical compounds in the alcoholic extract of barberry fruits and the most important chemical compounds in the alcoholic extract of barberry fruit were Oxalic acid (3.07%), Acrylic acid, 2,3 epoxy propyl ester (1.24%), Vinyl methyl ketone (6.49%), Carbamimidic acid, methyl ester (62.03%), and Bis (2,3-epoxypropyl) ether (5.89%).

Keywords: Antibacterial efficacy, Anti-biofilm efficacy, Antibiotic resistance, Barberry, *Berberis vulgaris* L, *Pseudomonas aeruginosa*.

فعالية مستخلصات ثمار نبات الزرشك (*Berberis vulgaris* L.) ضد بكتيريا الزائفة الزنجارية الممرضة المقاومة للمضادات الحيوية

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الخلاصة

إن مقاومة المضادات الحيوية تشكل حالياً أخطر تهديد عالمي لفعالية علاج الالتهابات البكتيرية، ويظل البحث عن مضادات حيوية جديدة ومضادات ميكروبية أخرى حاجة ملحة في معركة البشرية ضد الالتهابات البكتيرية المقاومة للمضادات الحيوية. وقد كان هناك اهتمام متزايد بالنباتات والأعشاب الطبية ومستخلصاتها لإيجاد بدائل علاجية طبيعية جديدة. لذلك، هدفت الدراسة الحالية إلى معرفة النشاط المضاد للبكتيريا للمستخلصات المائية والكحولية لثمار نبات الزرشك (*Berberis vulgaris* L.) ضد العزلات السريرية للزائفة الزنجارية *Pseudomonas aeruginosa* المقاومة للمضادات الحيوية وتأثير هذه المستخلصات على إنتاج الأغشية الحيوية كعامل ضراوة مهم. أثبتت الدراسة الحالية أن المستخلص المائي والكحولي لثمار نبات الزرشك فعال كمضاد بكتيري ومضاد لتكوين الأغشية الحيوية ضد *P. aeruginosa* عند التركيزات المختبرة مع اختلافات كبيرة. كما أظهرت الدراسة أن المستخلص الكحولي كان أكثر فعالية كمضاد للبكتيريا ومضاد للأغشية الحيوية من المستخلص المائي للزرشك، وأظهرت نتائج كروماتوغرافيا الغاز- مطياف الكتلة (GC-MS) وجود 21 مركباً كيميائياً في المستخلص الكحولي لثمار الزرشك وكان المركب المهم هو حمض الأساليك Oxalic acid (3.07%) وحمض الأكريليك Acrylic acid, 2,3 epoxy وإستر إيبوكسي بروبيل Epoxy



propyl ester (1.24%) وفينيل ميثيل كيتون Vinyl methyl ketone (6.49%) وحمض الكارباميديك إستر الميثيل Bis(2,3-إيثر (2,3-إيبوكسي بروبيل) إيثر (62.03%) وبيس (89.5%) epoxycyclopentyl)ether.
الكلمات المفتاحية: الفعالية، مقاومة المضادات الحيوية، الزرثك، الزانفة الزنجارية.

INTRODUCTION

The WHO produced a list of 12 drug-resistant microorganisms that pose a severe health risk, including *Pseudomonas aeruginosa*. *P. aeruginosa*, a common gram-negative opportunistic bacteria, causes pneumonia, bacteremia, and UTIs (Urinary Tract Infections) (Shehab *et al.*, 2020). Overuse of antibiotics has led to several resistance mechanisms in *P. aeruginosa*. This causes multidrug-resistant bacteria, which plague conventional therapy and treatment. This has fostered the development of more effective and new antimicrobial medicines and more efficient antibiotic administration and targeting (Alnuaimi *et al.*, 2023). Natural therapies like medicinal plant and herb extracts are becoming increasingly popular for health issues. Because many plants include flavonoids, tannins, saponins, alkaloids, terpenes, and heavy metals, scientists have developed antibacterial, antifungal, antioxidant, and anticancer drugs from them (Al-Hayanni *et al.*, 2022).

Barberry plants (*Berberis vulgaris* L.) grow across Europe and Asia. Dewick (2002) & Damaschin (2006) describe *B. vulgaris* as a shrub that grows to 1-3 m and has lanceolate leaves, prickly, yellow wood, and yellow flowers with oblong red berries. Roots, bark, leaves, and fruit are medicinal plant constituents. Traditional Asian medicine, notably in Iran, utilizes Barberry components for their antibacterial, fever-reducing, and anti-pruritic properties (Balázová *et al.*, 2024). Palmatine, berberine, and berberine are the main alkaloids in this plant. Primary components have several medical benefits (Fatehi *et al.*, 2005; Javadzadeh & Fallah, 2012; Imanshahidi & HJ, 2008). It is effective against numerous *Candida* and *Staphylococcus aureus* species (Freile *et al.*, 2003). Berberine works against *Helicobacter pylori*, *Trichomonas vaginalis*, *Giardia lamblia*, *Leishmania donovani*, and *Entamoeba histolytica* in vitro. Barberry fruit extracts are antibacterial and anti-biofilm, therefore this study tested them against antibiotic-resistant *P. aeruginosa*.

MATERIAL AND METHODS

Bacterial Isolates

Six *P. aeruginosa* isolates diagnosed by the Vitek2-system according to manufacturing company (Biomerieux) were used in the current study isolated from Iraqi patients, Three of them were metallo-beta-lactamase (MBL) and three were extended-spectrum lactamase (ESBL). The ability of these isolates to produce biofilms was tested according to Shinde *et al.* (2021), and they were vigorous biofilm producers.

- Preparation of Barberry Fruit Extracts

The Department of Biology/College of Science in Baghdad purchased *B. vulgaris* fruit from the local markets and recognized it as a remedy for women. Al-Hayaani & El-Shora (2021) provided the protocol for preparing ethanolic and water-based extracts from dried Barberry fruits. The mixture was macerated for 24 hours at 37°C in a shaker incubator. The



remaining solvent was evaporated and kept at 4°C. until they were needed, the unprocessed extracts were stored in sterile containers at 4°C.

-Antibacterial Activity of Barberry Fruit Extracts

The MICs (minimum inhibitory concentrations) of the Barberry extracts against the selected six bacterial isolates were determined using the broth microdilution technique in accordance with the procedure outlined by **Strompfová et al. (2024)** on a 96-well microtiter plate. From the highest concentration (500 µg/ml), serial dilutions were made in the following steps: (250, 125, 62.5, 31.25, 15.6, 7.8, 3.9, 1.95, 0.97, and 0.48) µg/ml. The MIC data were deciphered by observing the transition from blue to pink. the MIC value was defined as the lowest concentration at which color change was observed. The sub-MIC well follows the MIC well.

Antibiofilm Activity of Barberry Fruit Extracts

A microtiter plate with 199 µL of Mueller-Hinton broth supplemented with 1% glucose was used to study the anti-biofilm effect of Barberry fruit extracts, with a few modifications made in accordance with **Abd. Alaameri et al. (2023)**. The microplates were incubated at 37°C for 24 hours after inoculation with 100 µL of suspended bacterium and 100 µL of each concentration of sub-MIC. The wells were dried at 37°C for less than an hour after rinsing the adhesive cells twice with PBS (Phosphate Buffer Saline). After that, the specimen was incubated for 15 minutes after being stained with crystal violet. Twice, PBS was washed from the microplate wells that had been stained with crystal violet. Once the microplate wells have been air-dried, the biofilm color may be re-solubilized using 150 µL of 95% ethanol. After 5-10 minutes, the microplate reader was analyzed using a spectrophotometer with an optical density of 580 nm.

-Statistics Analysis

For this data analysis, we consulted SPSS V.16, the Statistical Package for the Social Sciences. According to **Smitha et al. (2021)**, the statisticians used analysis of variance and linear regression to determine the mean, standard deviation, and significance levels.

RESULTS AND DISCUSSION

Antibacterial Activity of Barberry Fruit Extracts

The antibacterial activity of alcoholic and aqueous Barberries extract against the six biofilm-producing *P. aeruginosa* isolates selected for the study: P1, P2, P3, P4, P5, and P6. was investigated. Table (1), and Figure (1) indicate that the MIC activity of alcoholic extract began at 125 µg/mL for 2 isolates (P3, P6) and 62.5 µg/mL for 4 isolates (P1, P2, P4, P5).

Table (1): The results of MICs of alcoholic Barberry extract against *Pseudomonas aeruginosa* isolates.

No. of <i>P. aeruginosa</i> isolate	250 µg/mL	125 µg/mL	62.5 µg/mL	31.2 µg/ml
P1	-	-	MIC	sub-MIC
P2	-	-	MIC	sub-MIC
P3	-	MIC	sub-MIC	
P4	-	-	MIC	sub-MIC
P5	-	-	MIC	sub-MIC
P6	-	MIC	sub-MIC	

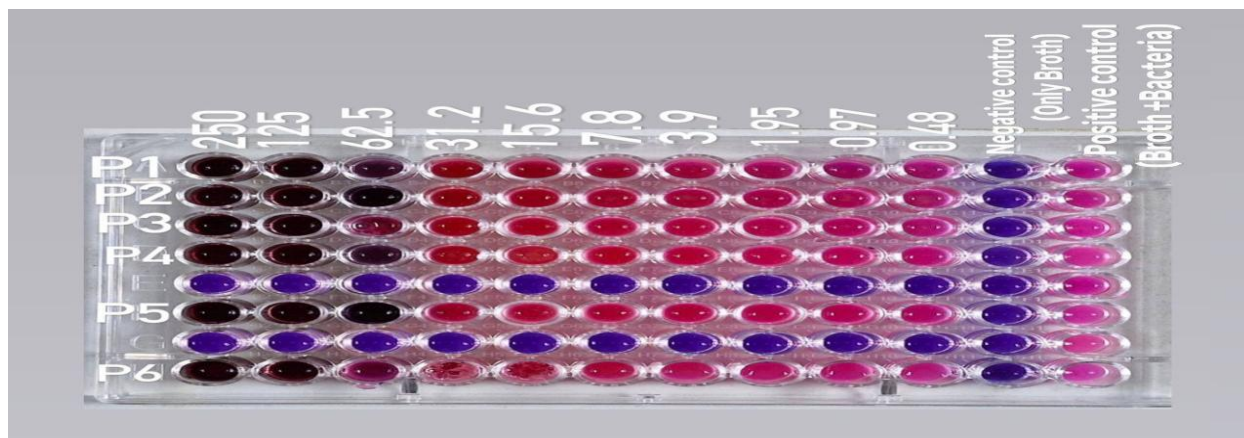


Figure 1: Antibacterial activity of alcoholic Barberry extract against 6 *P. aeruginosa* isolates by microtiter plate method.

The aqueous extract only 1 isolate (P3) shows MIC at a concentration of 250 µg/mL ,as shown in Table (2) , and Figure (2).

Table (2): The results of MIC of aqueous Barberry extract against *Pseudomonas aeruginosa* isolates.

No. of <i>P. aeruginosa</i> isolate	250 µg/mL	125 µg/mL	62.5 µg/mL	31.2 µg/mL
P1	-	-	-	-
P2	-	-	-	-
P3	MIC	Sub MIC		
P4	-	-	-	-
P5	-	-	-	-
P6	-	-	-	-

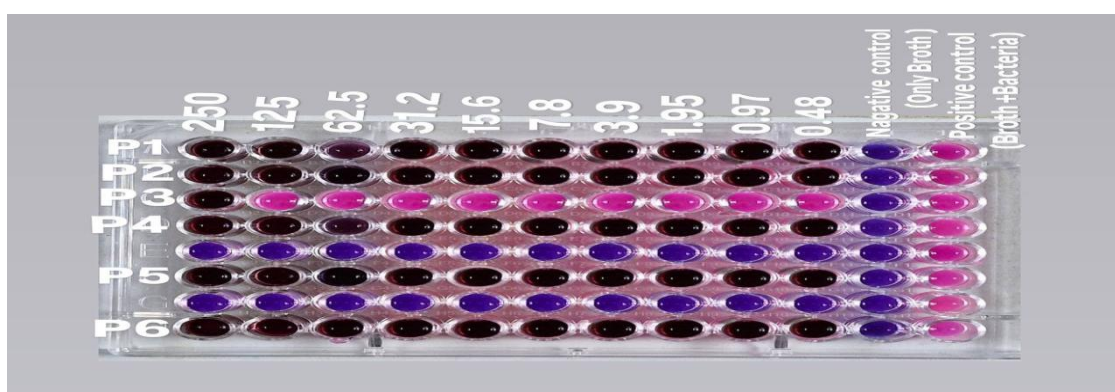


Figure (2): Antibacterial activity of aqueous Barberry extract against 6 *P. aeruginosa* isolates by microtiter plate method.



In the disc assay study by (Al-Zahar *et al.*, 2022), the extracts were evaluated for bactericidal efficacy against pathogenic Gram-positive and Gram-negative *Staphylococcus aureus* and *E. coli* strains. *Berberis vulgaris* effectively inhibited infections in vitro, with the fruit extract having a substantially lower MIC value (64 µg/mL) against *S. mutans*.

The antimicrobial activities of *B. vulgaris* have been of interest due to the plant's rich phytochemical content, particularly berberine, an alkaloid with well-documented antimicrobial properties. *B. vulgaris* has been traditionally used in various cultures for its medicinal properties, and scientific studies have provided evidence supporting its antimicrobial efficacy.

Among the several actions shown by *Berberis vulgaris* is an antimicrobial one (Gul *et al.*, 2022). Its long history of therapeutic use is attributable to its relatively low cytotoxicity and mutagenicity against human cells. There are several proposed processes by which berberine exerts its antibacterial effects on bacteria, including DNA and RNA binding, enzyme inhibition, and surface morphological modification. There is substantial evidence that berberine may inhibit the growth of some harmful microbes, including *Helicobacter pylori* (Mahady *et al.*, 2003), *S. aureus*, and *S. epidermidis* (Aksoy *et al.*, 2020). The antibacterial properties of barberry are particularly potent when applied to *Candida spp.* (Freile *et al.*, 2003).

Antibiofilm Activity of Alcoholic Barberry Fruit Extract

Using the sub-MIC concentration determined from the antibacterial impact investigation of the extract, the inhibitory effect of the alcoholic Barbary extract was evaluated against six biofilm-forming *P. aeruginosa* isolates. Figure 3 shows that the application of Barbary extracts significantly reduced the virulence factor of biofilms compared to all isolates except P2, and the findings demonstrated inhibitory effectiveness when comparing the treated bacterial isolates with the control. The active ingredients in antibacterial treatments decrease bacterial cell colonization on tooth surfaces and epithelial mucosa, which is why these agents may kill or hinder biofilm development. According to Roy *et al.* (2018), plant extracts primarily work by interfering with the electrostatic response, van der Waal, and Brownian forces that mediate the adherence and deposition of bacteria to surfaces. This inhibits the early stage of biofilm development in SRB (Sulphate Reducing Bacteria) isolates. Research suggests that plant extracts may reduce bacterial cell adhesion and proliferation by blocking the availability of nutrients. Another research found that Aloe vera extract exhibited satisfactory antibiofilm action against a variety of bacteria that are resistant to drugs (Cardosa *et al.*, 2021; Cherian *et al.*, 2019; Galleh *et al.*, 2021) The Result show in Table (3).

Table (3): Antibiofilm activity of *Berberis vulgaris* fruits extract

<i>P. aeruginosa</i> isolates No.	Control	Barbary extract
P1	0.776±0.040 ^a	0.676±0.046 ^b
P2	0.368±0.007 ^a	0.288±0.023 ^a
P3	0.457±0.015 ^a	0.368±0.100 ^b
P4	0.811±0.033 ^a	0.573±0.009 ^b
P5	0.622±0.045 ^a	0.407±0.011 ^b
P6	0.534±0.021 ^a	0.350±0.032 ^b

Below $p > 0.05$, similar lowercase letters in a row indicate no coefficient differences. A row of lowercase letters indicates significant coefficient differences below $p \leq 0.05$.

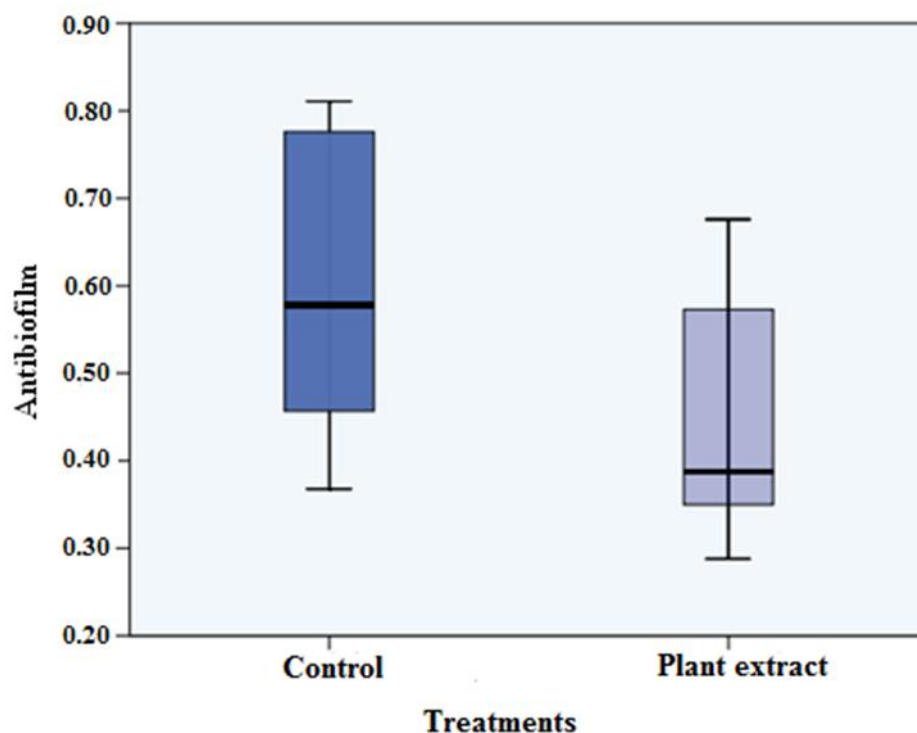


Figure (3): Anti-biofilm activity of alcoholic Barberry fruits extract.

Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

GC–Mass was measured for the alcoholic extract of the fruits of Barberry, and this examination is necessary to identify the active compounds inside the plant fruits that are responsible for the biosynthesis processes of nanoparticles. The results in **Figure (4)** and **Table (4)** showed 21 chemical compounds in the alcoholic extract of Berberis fruits and the important compound were *Oxalic acid* (3.07%), *Acrylic acid, 2,3 epoxy propyl ester* (1.24%) *Vinyl methyl ketone* (6.49%), *Carbamimide acid, methyl ester* (62.03%), *Bis (2,3-epoxypropyl) ether* (5.89%). According to **Behravan et al., (2019)**, the green production of silver nanoparticles relies heavily on the active phytochemicals found in Barberry extract, including terpenoids, resins, flavonoids, and more. The functional groups, such hydroxyls and aldehydes, of flavonoids and terpenoids convert silver ions (Ag^+) into silver nanoparticles (Ag^0) by acting as reducing agents. To further guarantee the stability of the nanoparticles and avoid aggregation, these chemicals and resins also act as stabilizing agents by creating a protective coating around them. Because of their dual function, nanoparticles may be synthesized more efficiently, and their bioactivity and homogeneity are both improved, making them useful in biological and antibacterial contexts (**Macak et al., 2024**).

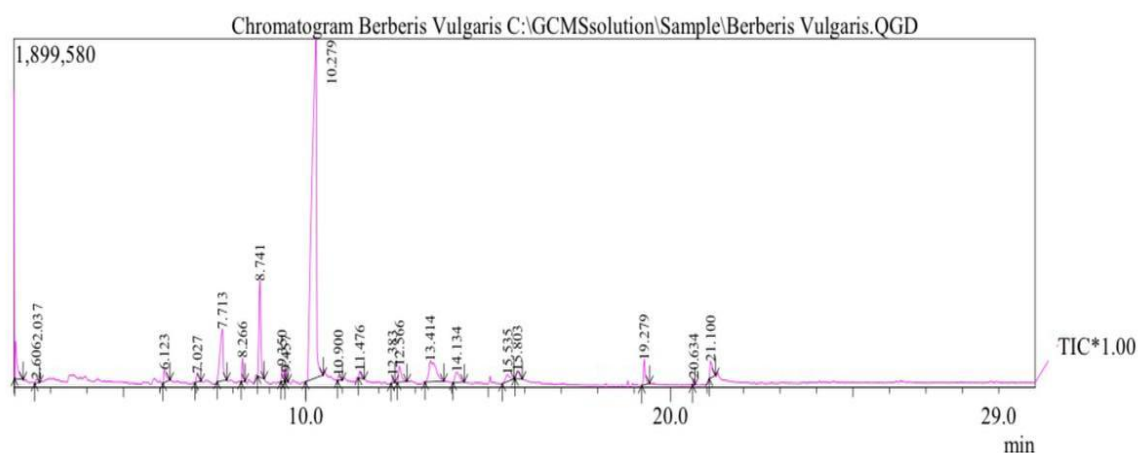


Figure (3): The chemical compounds in the alcoholic extract of *Berberis vulgaris* fruits.

Table (4): GC-MS analysis results of *Berberis vulgaris*.

Compound	Active grope	R. Time	Area%	Activity
Oxalic acid	Flavonoid	2.037	3.07	Anti-oxidative Anti-inflammatory Anti-mutagenic Anti-carcinogen
Acrylic acid,2,3 epoxy propyl ester	Terpenoids	6.623	1.24	Antiplasmodial Antibacterial
Vinyl methyl ketone	Resin	7.713	6.49	Antibacterial Wound healing
Carbamimidic acid, methyl ester	Terpenoids	10.279	62.03	Antibacterial Antiplasmodial
Bis(2,3-epoxypropyl) ether	Terpenoids	13.414	5.89	Antibacterial Antiplasmodial

CONCLUSIONS

The results showed that the aqueous and alcoholic extract of Barberry fruit might be a potent agent for treating biofilm-producing pathogenic bacteria like *Pseudomonas aeruginosa*, which heal states like nosocomial infections and the alcoholic extract was more effective than the aqueous extract of the Barberry. The Barberry fruit alcoholic extract's antimicrobial effectiveness may be ascribed to the bioactive chemical components present in this plant, including flavonoids, and terpenoids.



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