Iraqi Journal of Science, 2026, Vol. 67, No. 3, pp: 1379-1390
DOI: 10.24996/ijs.2026.67.3.9

ISSN: 0067-2904

Assessment of *Populus euphratica* Activity on *Staphylococcus aureus* (MRSA) Biofilm Formation

Rana B.H. ALDalwy*, Lamiaa A. Gharb
Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.

Received: 11/9/2024 Accepted: 17/3/2025 Published: 30/3/2026

Abstract

The rise in resistant infections, which are primarily brought on using antibiotics carelessly, is a serious issue for the community. This study aimed to examine the antibiofilm and antibacterial activity of *Populus euphratica* ethanolic extract against the resistance of *Methicillin-resistant Staphylococcus aureus* (MRSA) isolates. Leaves of *Populus euphratica* were collected and extracted using the Soxhlet apparatus. Active chemical compounds were detected by some reagents and screened using Gas chromatographic–Mass spectral (GC-Mass) analysis. Fifteen clinical MRSA isolates from several different clinical samples were detected for biofilm formation, and an antibiotic susceptibility test was performed to investigate the sensitivity pattern. Minimal Inhibitory Concentration (MIC and Sub MIC) for most sensitive antibiotic and *Populus euphratica* extract were investigated. The results showed that this plant has a greater percentage of glycosides and polyphenolic compounds than other compounds. In GC/MS analysis, twenty-six compounds have been detected; 8-Octadecenoic acid methyl ester(E) was the most abundant compound (13.98 %), followed by Eicosane (10.77%). On the other hand, the ethanolic extract of *Populus euphratica* has a good antibacterial effect against MRSA isolates in a concentration-dependent manner. The study also revealed that *Populus euphratica* was highly effective against biofilms in comparison with the most sensitive antibiotic (Ciprofloxacin). In conclusion, the studied plant can be used as an alternative and natural defense material against chronic infections caused by bacterial biofilms.

Keywords: Euphrates poplar, Biofilms, Antibacterial Agents, Ciprofloxacin

تقيم فعالية نبات الحور الفراتي على البكتريا المكورات العنقودية الذهبية المقاومة للمثيسلين في تكوين الأغشية الحيوية

رنا الدلوي* ، لمياء عبد الزهرة غريب

قسم علوم الحياة، كلية العلوم،جامعة بغداد، بغداد، العراق

الخلاصه :

يعد ارتفاع حالات العدوى المقاومة، التي تحدث في المقام الأول عند أستخدام المضادات الحيوية بلا مبالاة ، قضية خطيرة للمجتمع. تهدف هذه الدراسة الى فحص النشاط المضاد للأغشية الحيوية والمضاد للبكتريا للمستخلص الأيثانولي لنبات الحورالفراتي ضد مقاومة عزلات المكورات العنقودية الذهبية المقاومة

*Email: Rana.Hamdi2302@sc.uobaghdad.edu.iq

للمشيلين MRSA. تم جمع أوراق الحور الفراتي واستخلاصها بأستخدام جهاز السوكسليت .تم الكشف عن المركبات الكيميائية الفعالة بواسطة بعض الكواشف وفحصها باستخدام تحليل مطياف الكتلة الكروماتوغرافي للغاز (GC–Mass) . تم الكشف عن خمسة عشرعزلة سريرية من المكورات العنقودية الذهبية (MRSA) من العديد من العينات السريرية المختلفة لتشكيل الأغشية الحيوية، وتم اجراء اختبار حساسية المضادات الحيوية للتحقيق في نمط الحساسية. تم الكشف عن الحد الأدنى من التركيز المثبط (MIC, SUB MIC) للمضاد الحيوي الأكثر حساسية ومستخلص الحور الفراتي. أظهرت النتائج ان هذا النبات يحتوي على نسبة اكبر من الجليكوسيدات والمركبات البوليفونول من المركبات الأخرى. في تحليل جهاز مطياف الكتلة الكروماتوغرافي، تم اكتشاف ستة وعشرون مركباً، وكان المركب استر ميثيل 8–اوكتاديسينويك (E) هو المركب الأكثر وفرة (١٣،٩٨٪)، يليه المركب ايكوسان (١٠،٧٧٪).ومن ناحية أخرى، كان المستخلص الايثانولي للحورالفراتي له تأثير جيد ضد العزلات المكورات العنقودية الذهبية المقاومة للميثسيلين بطريقة تعتمد على التركيز. كشفت الدراسة ايضاً ان نبات الحورالفراتي كان فعالاً للغاية ضد الأغشية الحيوية مقارنة بالمضاد الحيوي الأكثر حساسية (سيبروفلوكساسين). ويستنتج من هذا انه بالأمكان استخدام النبات المدروس كمادة دفاعية بديلة وطبيعية ضد الالتهابات المزمنة التي تسببها الأغشية الحيوية البكتيرية.

Introduction

Herbal medicine is based on the premise that plants contain natural substances that can promote health or alleviate disease. A variety of herbs and herbal extracts contain different phytochemicals with biological activity that can reduce high cholesterol concentrations in the blood, provide some protection against cancer, and stimulate immunity [1]. The effectiveness is due to the nature of the substance's presence in plants, such as alkaloids, phenols, tannins, flavonoids, and saponins, which are considered antibacterial substances. Alkaloids are characterized by their ability to penetrate the bacterial cell and interfere with DNA. Tannins also work to inhibit transporter enzymes present in the cell membrane, while phenols are characterized by their ability to form a complex with extracellular proteins and a complex with the cell wall, which leads to the disruption of the bacterial cell membrane [2]. Numerous types of bacteria, such as (*Pseudomonas aeruginosa, Escherichia coli , Mycobacterium tuberculosis, Staphylococcus aureus, Methicillin-resistant Staphylococcus aureus* (MRSA) and *Klebsiella pneumoniae*) are resistant to antibiotics. It has been demonstrated that the presence of antibiotic-resistant bacteria lowers the efficacy of currently prescribed drugs and raises yearly death rates from treatment failure. For the treatment of infectious illnesses, plant natural products (higher plant extracts and pure compounds) provide a substantial alternative to traditional methods [3]. One of the indirect mechanisms by which bacteria develop resistance to medicinal drugs is biofilm development. The physical barrier formed by enclosed exopolymer materials could be the reason for biofilms increased resistance to antibiotics [4]. Poplar is a common name for *P. euphratica* that belongs to the Salicaceae [5], and is sometimes referred to as the Euphrates poplar, or desert poplar [6]. The species can be found natively in a large area, spanning from western China to North Africa, the Middle East, and Central Asia, and many countries of the world, including Algeria, China, Egypt, India, Iran, and Iraq [7]. The scientists discovered many chemical components in this plant, such as triterpenoids, flavonoids, phenolics, steroids, organic acids and their glycosides, polysaccharides, amino acids, and essential elements. [8]. The chemicals derived from *P. euphratica* could be potential sources for novel therapeutic agents and might be used in medicine [9]. The goal of this study was to examine the antibiofilm and antibacterial effect of *P. euphratica* extract against MRSA isolates.

Materials and Methods

Plant extract preparation:

Fresh leaves of *P. euphratica* were collected, cleaned with tap water, and spread in an electric oven with good ventilation to make sure the plants were completely dry. Dried leaves were grinded by using an electrical blender. Ethanolic extract was prepared by placing 25 g of powdered leaves in a Soxhlet device and using 500 ml of ethanol alcohol for eight hours [10]. Then the extracts were filtered using filter paper with a porosity of 0.45 microliters for the purpose of sterilizing the extracts. The extract was concentrated in a rotary evaporator and then preserved in sterilized containers in the refrigerator until use.

Detection of the active compounds in leaves extract of P. euphratica:

Dragendroff's reagent was used to detect alkaloids according to the Mondal method [11], while the detection of flavonoids was done according to Harborne [12]. On the other hand, lead acetate was used to detect tannins according to the method of Evans [13]. As well as Saponins were also detected, according to Shinata [14], and glycosides were detected using a Benedict reagent [12].

Gas Chromatography –mass spectrometry (GC-MS) analysis

GC/MSD Chem instrument and AcqMethod QC3 were used to identify the active chemical compounds in this study. The carrier gas was helium with a mobile phase flow rate set at 1.21 mL min -1. The temperature of the instrument's oven was raised from 100 °C to 260 °C at a rate of 10 °C min -1, and the volume per injection was set at 2 Pl. In GC-MS, an electron ionization energy system was used with 70 eV [15]. The start and end times were 10 min and 70 min, respectively.

Bacterial Isolates

Bacterial isolates were obtained from the Laboratories of the Biology Department/ College of Sciences/ University of Baghdad. In this study, 15 samples were collected from several different clinical samples of people infected with methicillin-resistant *S. aureus* (MRSA) bacteria.

Biofilm formation

Detection of biofilm formation was performed essentially for MRSA bacterial isolates. In brief, each isolate was propagated in tryptic soy broth containing 1% glucose at 37°C for 24h; thereafter, bacterial culture was adjusted to McFarland standard no. 0.5. A volume (180 of medium and 20 of bacterial culture) was added to three wells of sterile 96-well polystyrene microplates. All plates were covered with their lids to avoid evaporation and incubated under aerobic conditions at 37°C for 24h. Three wells filled with bacteria-free tryptic soy broth were considered as a negative control. After incubation, the growth medium was removed from the biofilm plate and washed thrice with phosphate-buffered saline, followed by fixation at 60°C for 1 hr. An aliquot (200 µl) from crystal violet was added to the wells at room temperature for 15 min. Afterwards, the plates were washed thrice and covered with 0.1% phosphate-buffered saline at room temperature for 10 min. Then, 200 microliters of glacial acetic acid was added at a concentration of 33% to each well. The absorbance was read with an ELISA reader at a wavelength of 630nm to estimate the ability of the isolates to form a biofilm [16].

The classification of biofilm intensity is summarized in Table 1.

Table 1: classification of biofilm intensity

OD630	Biofilm
OD ≤ ODc* 2 ODc≥OD>ODc 4 ODc≥OD>2ODc OD >4ODc	Non-producer Weak Moderate Strong

Antibiotics sensitivity test

The disc diffusion method was used to test the isolates for their antibiotic susceptibilities in accordance with the standards of the Clinical and Laboratory Standards Institute (CLSI 2023). The subsequent antibiotics were utilized: Gentamicin (10 µg), Azithromycin (15 µg), Ciprofloxacin (5 µg), Clindamycin (2 µg), Rifampin (5 µg), Chloramphenicol (30 µg), Tetracycline (30 µg), Cefoxitin (30 µg). The source of all the antibiotic discs was from Hi-media / India.

Antibacterial activity P. euphratica against MRSA using the agar diffusion method

The inhibitory effectiveness of the prepared crude plant extract was tested against isolated bacterial species at concentrations that ranged from (100, 80, 40, and 20 mg/ml); the extract was prepared using DMSO as a diluent. A sterile cotton swab was used to spread the inoculum of bacteria uniformly on the surface of the Mueller Hinton plate. 100 µl for each dilution was added to the four wells, and wells (5 mm in diameter) were cut in the agar. DMSO was used as a control. Finally, the plates were incubated overnight at 37°C. The inhibition zones were measured with a ruler after the completion of the incubation period [17].

Determination of Minimal Inhibitory Concentration (MIC) for most sensitive antibiotics and P. euphratica extract

The inhibitory activity of three types of antibiotics, including Gentamicin (10µg), Ciprofloxacin (5 µg), and Rifampin (5 µg) against the isolated bacterial species was detected. Then, the MIC value of the most sensitive antibiotics was determined using dilution methods.One gram from each antibiotic (Gentamicin and Ciprofloxacin) was diluted with 10 ml of D.W while rifampin was diluted by using (9 ml methanol:1ml D.W) [18]. The same method for the inhibitory effectiveness of the prepared extract was tested against isolated bacterial species at a concentration of 20 mg/ml, according to Ngaffo *et.al.* [19]. The bacterial isolates MRSA were activated by the brain heart infusion BHI at 37°C for 24 hours. Then, 100 microliters of the bacterial suspension were transferred to the Eppendorf containing BHI and glucose, and the turbidity was compared with a 0.5 McFarland tube. The wells of the plate were filled with three replicates for each isolate with a volume of 200 microliters of the (bacterial suspension 100 microliters and 100 of plant extract); the same applies to the most sensitive antibiotics using a micropipette and incubated at 37°C for 24 hours. The contents of the plate were emptied by washing them three times with buffer phosphate saline and leaving it to dry. After that, Resazurin sodium salt (0.015) was prepared with 100 D.W and mixed with a vortex device, then 20 microliters of Resazurin sodium salt was added to each well by using a micropipette and incubated at 37°C for 2 hours, then the plate was washed three times with buffer phosphate and left to dry at room temperature.

Antibiofilm activity of P. euphratica extract and sensitive antibiotic on the biofilm at MIC and sub -MIC concentration

The MIC and SUB MIC method was done was for each of the *P. euphratica* extract and the most sensitive antibiotics (Ciprofloxacin) according to Hemati *et al.,* [20]. And evaluate their effect on the biofilm formation of the bacterial isolates depending on the same protocol as the biofilm formation in the previously mentioned method [16]. The biofilm inhibition rate was measured as in the equation, biofilm inhibition (%) = (Control OD- Test OD / Control OD) × 100.

Statistical Analysis

The Statistical Analysis System- SAS (2018) program was used to detect the effect of different groups on study parameters. The least significant difference was used to significantly compare between means. The chi-square test was used to significantly compare the percentages of 0.05 and 0.01 probability in this study**.**

Results and Discussion

Detection of the active compounds in P. euphratica leaves extract

The active compound contents of the plant leaves are summarized in Table 2 . The results showed that six groups of mainly active compounds gave positive results in the ethanolic alcoholic extract of the plant studied, with a quantitative change in the presence rates observed through the amount of precipitate or foam formed after the test. These totals include (alkaloids, flavonoids, polyphenolic compounds, tannins, saponins, and polysaccharides). It was noted that the extract of *P. euphratica* contained a greater percentage of glycosides and polyphenolic compounds, and the other components had medium percentages.

Table 2: Active compounds in the ethanolic extract of *P. euphratica* leaves

Test Name	Reagent	Result of ethanolic extract	Indication
Alkaloids	Dragan Groff	+	Orange, brown precipitate
Flavonoids	7.5%$FeCl_3$ solution	+	Dark color
Polyphenolic Compounds	ferric chloride 5%solution ferric chloride 1%solution	+++ +++	a-Brown color b-Dark color
Tannins	Lead Acetate 1%solution	+	creamy precipitate
Saponins	Foam formation	++	Foam
polysaccharides, Carbohydrates and glycosides	Benedict reagent	+++	Reddish-brown precipitate

(+++ strong, ++ medium, + weak)

The *P. euphratica* tree is rich in phenols and their glycosides, including populin and salicin. *P. euphratica* extract has long been used by some people in some parts of Iraq to treat eczema and other skin disorders because previous investigations on the plant have revealed the identification of several phenolic compounds and volatile oils [21]. Traditionally, this plant is used to treat painful musculoskeletal joint pain conditions, inflammation, fever, antifungal, anticancer, and antioxidant [22].

GC/MS analysis of P. euphratica leaves extract

GC/MS analysis of *P. euphratica* leaf extract detected fifty-eight compounds. The main defined compounds were twenty-six. These compounds, with their retention times and percentage of composition, were listed in Table 3. The major constituents were 8-octadecenoic acid, methyl ester(E)-(13.98 %), followed by Eicosane (10.77%). Eicosane

could be as antioxidant, anti-inflammatory and antibacterial [23]. As well as the compound 8-Octadecenoic acid, methyl ester could be used as an antioxidant and antibacterial [24].

Table 3: Main components in *P. euphratica* leaves extract by GC/MS

No	RT (min)	Components	Area%
1	13.70	3-Hexanol	1.86
2	14.12	2-Pentene, 3-methyl-, (E)-	1.13
3	17.24	Proline, 3,4-didehydro-	2.03
4	28.56	p-Cymene	0.54
5	26.09	Decanal	2.19
6	31.98	6-hydroxy-2(1h)-pyridinone	5.88
7	51.82	Neophytadiene	0.98
8	55.25	Bis(2-ethylhexyl) phthalate	7.64
9	56.42	n-Hexadecanoic acid	6.26
10	57.30	Tridecanol	1.60
11	57.39	trans-Sesquisabinene hydrate	1.70
12	60.02	2,2-Dimethylpropanoicacid,2,6-di methylnon-1-en-3-yn-5-yl ester	1.72
13	60.78	phytol isomer	2.64
14	61.78	9,12,15-Octadecatrien-1-ol	8.18
15	61.89	[1S,4aS,8aS] - 1,2,3,4,4a,7,8,8a - octahydro 1,4a,5,6 - tetramethy l - 1 - naphthalene – methanol	4.27
16	62.06	Ethyl Oleate	1.48
17	64.17	1-octadecanol	4.12
18	64.66	Allyl Cyclohexyl carbonate	1.78
19	66.13	8-Octadecenoic acid, methyl ester(E)-	13.98
20	66.69	Nonanoic acid, pentadecyl ester	1.35
21	67.91	Cyclohexanol, 4-[(trimethylsilyl)o xy]-, cis-	1.66
22	69.37	Heptadecene-(8)-carbonic acid-(1)	1.41
23	69.48	2,6,10,14,18,22-Tetracosahexaene	3.55
24	69.57	Tricyclo [4.2.1.1(2,5)] deca-3,7-die ne-9,10-diol, 9-methyl-, stereoisomer	1.04
25	70.17	Cyclohexane, 1-(cyclohexylmethyl)-2-ethyl-, cis-	1.18
26	67.24	Eicosane	10.77

Antibiotics sensitivity test:

The results in Table 4 showed that most of the MRSA bacterial isolates were resistant to the antibiotics, Cefoxitin (100%), Azithromycin (93.33%), Clindamycin (86.67%), Chloramphenicol (93.33%), and Tetracycline (86.67%) However, it was sensitive to Ciprofloxacin (80.0%), Rifampicin (93.33%), and Gentamycin (86.67%). The result presented highly significant ($P<0.01$) variability in Antibiotics. MRSA always exhibits resistance to multiple antimicrobial agents, including Penicillin, Methicillin, Oxacillin, Cefoxitin, Amoxicillin-clavulanic acid, Amoxicillin-sulbactam, Quinolones, Macrolides, Cephalosporins, Tetracycline, and Chloramphenicol [25]. One of the reasons for the resistance of bacterial isolates to beta-lactam antibiotics, is the production of lactamase enzymes that work to break down the beta-lactam ring, which leads to inhibiting the action of antibiotics that belong to the penicillin group [26]. Antibiotic resistance is becoming an

increasingly global issue. Bacterial infections cause greater health and financial consequences for MRSA [27].

Table 4: Antibiotic susceptibility testing against isolates of MRSA

Antibiotics	Resistance	Sensitive	P-value
Cefoxitin	15 (100%)	0 (0.00%)	0.0001 **
Gentamycin	2 (13.33%)	13 (86.67%)	0.0045 **
Azithromycin	14 (93.33%)	1 (6.67%)	0.0008 **
Ciprofloxacin	3 (20.00%)	12 (80.00%)	0.0201 *
Clindamycin	13 (86.67%)	2 (13.33%)	0.0045 **
Rifampicin	1 (6.67%)	14 (93.33%)	0.0008 **
Chloramphenicol	14 (93.33%)	1 (6.67%)	0.0008 **
Tetracycline	13 (86.67%)	2 (13.33%)	0.0045 **
P-value	0.0001 **	0.0001 **	---
* (P≤0.05), ** (P≤0.01).			

Investigation of the susceptibility of isolates to biofilm formation

The current results showed that all the bacterial isolates produced biofilm, and the intensity of the biofilm varied among them; about 86,67 % of MRSA bacterial isolates created strong biofilms, whereas only 13.33% had moderate biofilm, as shown in the Table (5).

Table 5: Biofilm formation by MRSA bacterial isolates.

Biofilm formation	No.	Percentage (%)
Strong	13	86.67
Moderate	2	13.33
Weak	0	0.00
Total	15	100
Chi-Square (χ^2)	---	9.836 **
P-value	---	0.0002
** (P≤0.01).		

By forming the biofilms, MRSA can produce strains of these pathogens that either become extremely resistant to the same antimicrobials or become susceptible to certain antimicrobials in standard laboratory testing. Treatment for infectious disorders involving biofilms may become challenging as a result [28]. It is now necessary to examine the antibacterial qualities of novel compounds considering the bacterial resistance issue. The bacterial cells immersed in their own extracellular matrix define the growth mode or biofilm. Bacterial biofilms are linked to a variety of illnesses, including chronic tissue infections such as cystic fibrosis and infections of catheters or prosthetic joints [29].The pathophysiology of MRSA infections involves biofilm. Biofilm gene expression is induced as a stress response in microorganisms exposed to stress circumstances. Bacteria can thrive in stressful environments due to the slime-like glycocalyx known as biofilm. [30]. They can also attach to and colonies on biotic or abiotic surfaces, such as prosthetic surfaces, which can serve as a substrate for microbial adhesion, and spread throughout the entire body [31].

Antibacterial activity P. euphratica against MRSA using the agar diffusion method

The current study showed that the leaves extract of *P. euphratica* has good antibacterial efficacy against the bacterial isolates in a concentration-dependent manner. The plant extract revealed inhibitory properties against all isolates. The inhibition zones were 17± 13, 15 ± 11,13 ± 0, and 11 ± 0 mm at 100, 80, 40, and 20 mg/ml, respectively.

Determination of MIC and (Sub-MIC) for most sensitive antibiotics and P. euphratica extract:

The antibacterial effect of *P. euphratica* extract and Ciprofloxacin were examined by using MIC assays. The results showed that the MIC values were10 mg/ml and 0.25 µg /ml for each the plant extract and the most sensitive antibiotic (Ciprofloxacin) respectively for all MRSA isolates . On the other, the Sub- MIC value was 5 mg/ml for plant extract while the Sub- MIC value for the most sensitive antibiotic (Ciprofloxacin) was 0.125 µg /mL for all MRSA isolates.

Antibiofilm activity of P. euphratica extract and sensitive antibiotic at MIC concentration

The results clarified that the MIC level of ethanolic extract of *P. euphratica* was more effective than the antibiotic (Ciprofloxacin) against most bacterial isolates. The lower antibiofilm activity of plant extract appeared in isolate no.5 with a percentage (54%), while in isolate no. 4 it showed the highest antibiofilm activity with inhibition (89%). The effectiveness differs from one isolate to another, with highly significant (P≤0.01) variability in the formation of biofilms, as shown in Table 6.

Table 6: The activity of MIC for *P. euphratica* extract and antibiotic on biofilm

Bacterial isolates numbers	*P. euphratica* extract in 10 mg/ml concentration	Antibiotic Ciprofloxacin at 0.25 µg /mL concentration	P-value
1	61%	43%	0.0008 **
2	55%	41%	0.0001 **
3	63%	40%	0.0006 **
4	89%	55%	0.0001 **
5	54%	53%	0.802 NS
6	65%	65%	1.00 NS
7	72%	61%	0.0398 *
8	77%	51%	0.0041 **
9	73%	75%	0.159 NS
10	55%	63%	0.0084 **
11	59%	51%	0.072 NS
12	81%	52%	0.0001 **
13	77%	60%	0.0023 **
P-value	0.0001 **	0.0001 **	---
* (P≤0.05), ** (P≤0.01).			

The antibiofilm activity of *P. euphratica* may be acquired from different concentrations of phenolic glycosides and other secondary compounds [32]. Phenols also increase the activation of enzymes responsible for basic metabolic reactions through their specialized interference with proteins, which leads to their denaturation and, thus, the inability of the bacteria to continue growing [33].

Antibiofilm activity of P. euphratica extract and sensitive antibiotic at sub -MIC concentration

The results clarified that the sub- MIC level of ethanolic extract of *P. euphratica* was more effective than the antibiotic Ciprofloxacin against most isolates. The effectiveness differs from one isolate to another, with highly significant (P≤0.01) variability in the formation of biofilms, as shown in Table 7.

Table 7: Effect of Sub MIC of *P. euphratica* extract and antibiotic on biofilm

Bacterial isolates numbers	*P. euphratica* extract at 5 mg/ml concentration	Antibiotic Ciprofloxacin at 0.125 ug/mL concentration	P-value
1	40%	20%	0.0074 **
2	43%	39%	0.0001 **
3	43%	18%	0.0001 **
4	53%	36%	0.0091 **
5	34%	43%	0.074 NS
6	71%	67%	0.502 NS
7	65%	38%	0.0036 **
8	75%	48%	0.0057 **
9	70%	75%	0.648 NS
10	51%	60%	0.0081 **
11	18%	5%	0.0094 **
12	78%	49%	0.0002 **
13	74%	58%	0.0007 **
P-value	0.0001 **	0.0001 **	---
** (P≤0.01).			

The biofilm is responsible for providing additional protection for bacteria against antibiotics as well as enhancing their colonization of host cells. Thus, it is an important virulence factor for the survival of bacteria [34]. It is known that excessive doses and long-term use of antibiotics can lead to microbial resistance to the antibiotic, which is a serious health problem. To overcome the virulence of bacteria, including the formation of biofilms, there has been a move to use natural herbal plant extracts as alternatives to antibiotics with potential effects against antibiotic-resistant bacteria [35]. There may be variation in the effectiveness of crude extract due to the synergistic effects of the crude extract components of bioactive compounds [36], as well as the selectivity of bioactive compounds against clinical isolates. In addition, these bioactive compounds are responsible for the ethanolic extracts' antibiofilm activity. The herbal extracts also contain the glycoside hydrolase enzyme, which helps break down the glycosidic linkages in the biofilm's polysaccharide chain into smaller subunits or monomers, thereby inhibiting the biofilm [37].On the other hand, the indirect activity of plant extracts on bacterial growth may lead to an indirect effect on the DNA (chromosome or plasmid) of cells [38].

Conclusion:

Ethanolic extract from leaves of *P. euphratica* could be considered a good source of active chemical compounds, and its antibacterial and antibiofilm activities make it a candidate as a plant extract that can be used as a natural antibacterial agent.

Conflict-of-interest statement

The authors have no conflicts of interest to declare.

References:

[1] S. Vitale, S. Colanero, M. Placidi, G. Di Emidio, C. Tatone, F. Amicarelli and A.M. D'Alessandro, "Phytochemistry and biological activity of medicinal plants in wound healing," *Molecules,* vol. 27, no.11, pp. 35-66, 2022.

[2] K. Shrinet, R.K. Singh, A.K. Chaurasia, A. Tripathi and A. Kumar, "Bioactive compounds and their future therapeutic applications. In Natural Bioactive Compounds ," *Academic Press,* vol. 174, pp. 337-362, 2021.

[3] T. R. Prashith Kekuda, K.S. Vinayaka and S.K. Praveen Kumar , "Antimicrobial activity of Salix tetrasperma Roxb. (Salicaceae)," *International Journal of Herbal Medicine,* vol. 5, no.5, pp. 192-195, 2017.

[4] S. A. Al-Wendawi and L. A.Gharb, "Antioxidant, antibacterial and antibiofilm potentials of anise (Pimpinella anisum) seeds extracted essential oils," *Iraqi Journal of Agricultural Sciences,* vol. 52, no.2, pp. 348-358, 2021.

[5] T. D. Hong, S. Linington and R. H. Ellis, *Seed storage behavior: a compendium*, Italy: International Plant Genetic Resources Institute, 1996.

[6] J. T. Zhai, Y. L. Li, Z. J. Han, Z. J. Li, "Morphological, structural and physiological differences in heteromorphic leaves of Euphrates poplar during development stages and at crown scales," *Plant biology*, Vol.22, no.3, pp.366-375, 2020.

[7] J. G. Isebrands and J. Richardson, *Poplars and willows: trees for society and the environmentnt,*U.K. : CAB International, 2014.

[8] I. Guleria, A. Kumari, M. A. Lacaille-Dubois, Nishant, V. Kumar, A. K. Saini, J. Dhatwalia and S. Lal, , "A review on the genus Populus: A potential source of biologically active compounds," *Phytochemistry Reviews,* vol. 21, no.4, pp.1-60, 2021.

[9] A. Movahedi, A. A, Z. Yaghuti, H. Wei, P. Rutland, W. Sun, M. Mousavi, D. Li, and Q. Zhuge, "Plant secondary metabolites with an overview of Populus," *International journal of molecular sciences,* Vol. 22,no.13, pp.6890, 2021.

[10] J. B. Harborne, *Textbook of Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis*, London : Chapman and Hall Ltd, 1998.

[11] H. Mondal, H. Hossain, K. Awang, S. Sanjib, M. Sheikh and A. S. Jamil, "Anthelmintic activity of ellagic acid, a major constituent of Alternanthera sessilis against Haemonchus contortus," *Pakistan Veterinary Journal,* vol.35, no.1, pp.58-62, 2015.

[12] J. B. Harborne, *Phytochemical Methods,* New Delhi: Springer, 2005, p. 17.

[13] W. C. Evans, , *Trease and Evans. Pharmacognosy*, 15th Edition, Edinburgh: Saunders Elsevier, 2002, p. 553-557.

[14] A.E.T.A. Shihata and E.M. Mark, "The fate of yeast in the digestive tract of Drosophila.," *The American Naturalist,* vol. 85, no.825, pp. 381-383, 1951.

[15] A. Qadir , M. Aqil , A. Ali, F.J. Ahmad, S. Ahmad , M. Arif and N. Khan, "GC-MS analysis of the methanolic extracts of Smilax China and Salix alba and their antioxidant activity.," *Turkish Journal of Chemistry,* vol.44, no.2,pp.352-363, 2020.

[16] H.M Kemung , L.T.H. Tan, K.Y. Khaw, Y.S. Ong, C.K. Chan, D.Y.S. Low, S.Y. Tang and B.H Goh, "An optimized anti-adherence and anti-biofilm assay: Case study of zinc oxide nanoparticles versus MRSA biofilm," *Progress In Microbes & Molecular Biology,* vol. 3, no.1, pp. a0000091, 2020.

[17] A.M. Hamad and Q.M. Atiyea, "In vitro study of the effect of zinc oxide nanoparticles on Streptococcus mutans isolated from human dental caries.," *In Journal of Physics: Conference Series,* Vols. Vol. 1879, no.2, pp. 022041, 2021.

[18] B. Kowalska-Krochmal and R. Dudek-Wicher, "The minimum inhibitory concentration of

antibiotics: Methods, interpretation, clinical relevance.," *Pathogens,* vol.10, no.2, pp.165, 2021.

[19] C.M. Ngaffo, S.B.Tankeo, M.G.F. Guefack, B.E.Wamba, P. Nayim, I.N.Bonsou, V. Kuete and A.T. Mbaveng , "In vitro antibacterial and antibiotic-potentiation activities of five edible plant extracts and mode of action against several MDR Gram-negative phenotypes," *Investigaional Medicinal Chemistry and Pharmacology,* vol.4, no.1, pp.00049, 2021.

[20] S. Hemati , E. Kouhsari, N. Sadeghifard, A. Maleki, N. Omidi , Z. Mahdavi and I. Pakzad, "Sub-minimum inhibitory concentrations of biocides induced biofilm formation in Pseudomonas aeruginosa," *New Microbes and New Infection,* vol. 38, pp.100794, 2020.

[21] M.A. Ezghayer and E.J. Kadhim, "UPLC-ESI-MS/MS and Various Chromatographic Technique for Identification of Phytochemicals in Populus euphratica Oliv. Leaves Extract.," *Iraqi Journal of Pharmaceutical Sciences,* vol. 29, no.1, pp.94-114, 2020.

[22] N. Tawfeek, M.F Mahmoud, D.I. Hamdan, M. Sobeh, N. Farrag, M.Wink and A.M. El-Shazly, "Phytochemistry, pharmacology and medicinal uses of plants of the genus Salix," *Frontiers in pharmacology,,* vol.12, pp.593856, 2021.

[23] Z.K.Wu., H.Y. Li, Y.L Zhu, M.Q. Xiong and J.X. Zhong, "Neuroprotective and anti-inflammatory effects of eicosane on glutamate and NMDA-induced retinal ganglion cell injury.," *International Journal of Ophthalmology,* vol.17, no.4, pp. 638, 2024.

[24] S.R.Farhan, A.H.AL-Azawi, W.Y.Salih and A.A. Abdulhassan, "The antibacterial and antioxidant activity of Moringa oleifera seed oil extract against some foodborne pathogens.," *Indian Journal of Forensic Medicine & Toxicology,* vol.15, no.4, pp.2529-253, 2021.

[25] F. M Alshabrmi and E.A. Alatawi, "Unraveling the mechanisms of Cefoxitin resistance in methicillin-resistant Staphylococcus aureus (MRSA): structural and molecular simulation-based insights.," *Journal of Biomolecular Structure and Dynamics,* pp. 1-11, 2023.

[26] R.Cherazard, , M. Epstein, T.L. Doan, T. Salim, S. Bharti and M.A.Smith, "Antimicrobial resistant Streptococcus pneumoniae: prevalence, mechanisms, and clinical implications.," *American journal of therapeutics ,* vol.24, no.3, pp.e361-e369., 2017.

[27] R.R.Gurung, P.Maharjan and G.G.Chhetri , "Antibiotic resistance pattern of Staphylococcus aureus with reference to MRSA isolates from pediatric patients.," *Future science OA,,* vol. 6, no.4, pp. FSO464, 2020.

[28] Y.Y.Yong, G.A.Dykes, and W.S.Choo, "Biofilm formation by staphylococci in health-related environments and recent reports on their control using natural compounds.," *Critical reviews in microbiology,* vol. 45, no.2, pp. 201-222, 2019.

[29] R.M. Donlan and J.W. Costerton, "Biofilms: survival mechanisms of clinically relevant microorganisms," *Clinical Microbiology Reviews*, vol. 15, no. 2, pp. 167-193, 2002.

[30] D.Dhanasekaran and N.Thajuddin, eds., Microbial Biofilms: Importance and Applications. BoD–Books on Demand, 2016.

[31] S. Enany and L.C.Alexander, eds., The rise of virulence and antibiotic resistance in Staphylococcus aureus. BoD–Books on Demand, 2017.

[32] Raj, S.P.; Solomon, P.R.; Thangaraj, B. (Eds.) Salicaceae BT—Biodiesel from Flowering Plants; Springer: Singapore, pp. 491–494, 2022;

[33] J.M.Badi, H.M.Resen and A.M.Majeed, "Antimicrobial Activity of Populous Euphratica Leaves Extract on Growth of Some Gram-Negative Bacteria.," *Engineering and Technology Journal,* Vols. 34, no.5B, pp.169-175., 2016.

[34] S. A. Jasim, Z. A.Hatem and Abd Z. Mohammed, "Virulence Factors and Clinical Features of Streptococcus pyogene," *Annals of the Romanian Society for Cell Biology,* pp. 603-614, 2021.

[35] S.S.Fajriani, H.Handayani and D.Putri, "The role of green tea extract on inhibiting Porphyromonas gingivalis as a major periodontitis pathogen:in vitro study.," *Systematic Reviews in Pharmacy,* vol. 11, no.8, pp.152-155., 2020.

[36] M.L.Bittencourt, P.R.Ribeiro, R.L.Franco, Hilhorst, H.W., R.D.de Castro and L.G.Fernandez, Metabolite profiling, antioxidant an antibacterial activity of Brazilian propolis: Use of correlation and multivariate analyses to identify pote, "Metabolite profiling, antioxidant an antibacterial

activity of Brazilian propolis: Use of correlation and multivariate analyses to identify potential bioactive compounds," *Food Research International,* vol. 76, pp. 449-457, 2015.

[37] N.T.M. Jaddoa, and L.A.Gharb, "The antibiofilm activity of Hibiscus sabdriffa L. against methicillin-resistant Staphylococcus aureus.," *Iraqi Journal of Agricultural Sciences,* vol. 52, no.3, pp. 626-631, 2021.

[38] F.J. Álvarez-Martínez, E. Barrajón-Catalán, M. Herranz-López and V. Micol, "Antibacterial plant compounds, extracts and essential oils: An updated review on their effects and putative mechanisms of action," *Phytomedicine*, vol. 90, p. 153626, 2021.