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ANTIBACTERIAL AND ANTIBIOFILM ACTIVITY OF *ALBIZIA LEBBECK* LEAVES EXTRACTS IN *PSEUDOMONAS AERUGINOSA* ISOLATED FROM URINARY TRACT INFECTION

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Microbial infections have become one of the most pressing public health issues worldwide as a result of the emergence of resistance to current antibiotics. This has prompted scientists to examine the antibacterial characteristics of medicinal plants.

***The aim of the study**: Extract secondary metabolites from Albizia lebbeck and analyze how they affect Pseudomonas aeruginosa.*

***Materials and methods**: From the labs of the University of Baghdad's Genetic Eng. and Biotech. Institute, 10 isolates of P. aeruginosa were obtained. The identification was confirmed by cultivating the isolates on cetrimide agar and using the VITEK2 technology.*

***Results**: The chemical analysis of the methanolic and aqueous extracts revealed the presence of secondary metabolite molecules such as alkaloids, flavonoids, glycosides, phenols, saponins, and tannins. The total phenols content of the methanolic and aqueous extracts was 71.11 mg/g and 45.15 mg/g, respectively. Furthermore, the results indicated that at a concentration of 50 mg/ml, the methanolic extract outperformed the aqueous extract in free radical scavenging by 78.65% to 91.20%. Using the disk diffusion method, the methanolic extract also demonstrated higher antibacterial activity than the aqueous extract of Albizia lebbeck leaves, and its potency increased with increasing concentration. The methanolic extract's lowest inhibitory concentration against P. aeruginosa isolates was determined to be 16 mg/ml, whereas the aqueous extract's was 32 mg/ml. According to the biofilm formation experiment, the methanol extract inhibits the formation of biofilms at a concentration of 200 mg/ml, whereas the aqueous extract does so at a concentration of 400 mg/ml.*

***Conclusion**: This work demonstrated that the secondary metabolites extracted from Albizia lebbeck leaves have a considerable antibacterial and antibiofilm action on P. aeruginosa, even though the bacterial isolates create a strong biofilm. This research has shown that Albizia lebbeck leaves can be used in traditional therapy for bacterial infections and diseases caused by oxidative stress since they contain therapeutic phytochemicals with strong antibacterial and antioxidant effects*

***Keywords**: Albizia lebbeck, Antibiofilm activity, Antibacterial activity, Antioxidant activity, Total phenolic content, Pseudomonas aeruginosa*

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1. Introduction

Bacterial and fungal resistance to antibiotics is one of the issues in the biological and medical sciences; some of these microbes are more than 90% resistant to chemical treatments [1]. Since the 1950s, several dangerous bacteria have shown evidence of antibiotic resistance, which has been growing along with the creation of new chemicals and drugs. Furthermore, the negative effects of these medications started to manifest slowly [2]. The misuse of antibiotics has resulted in the majority of bacteria today displaying increased drug resistance to a variety of medications [3]. One of the factors behind the growing use of herbs as low-risk, affordable, and natural substitutes to synthetic antibiotics in the treatment of bacterial infections has been this. The use of natural ingredients in the manufacturing of drugs is growing. In addition to being utilized directly as therapeutic medicines [4, 5], additionally, bioactive substances can be used as building blocks or templates to create new physiologically active compounds. Studies on the use of herbal medicines as a substitute for medications and antibiotics were consequently conducted.

The *Albizia lebbeck* can be found throughout India, as well as in Australia and South Africa. The bark is used for toothaches, hemorrhoids, diarrhea, and gum issues. Certain allergic disorders, like bronchial asthma, can also be prevented by the methanolic extract of the leaves and bark. The chemical makeup of the *Albizia lebbeck* plant was examined, and it was discovered that it contains flavonoids, tannins, saponins, and macrocyclic alkaloids, as well as compounds such as NbenzoylLphenylalaninol and 3',5dihydroxy4',7dimethoxyflavones. Additionally, it has been discovered that the leaves of the plant contain a novel hexaglycosylated saponin called albiziahexoside, which has

antibacterial, antifungal, antituberculous, and anticancer effects [6].

2. Planning (methodology) of research

The study protocol describing the stages of the work is represented below:

1. Description the antibiotic susceptibility test of *pseudomonas aeruginosa* bacteria used in this research.

2. Discussion of problems connected of the bacteria such as formation of biofilm and finding the solutions of this problem.

3. Usage the secondary metabolites of *Albizia lebbeck* due to their antibacterial, antibiofilm and antioxidants properties.

4. Assessment of the Minimum Inhibitory Concentration (MIC) of *Albizia lebbeck* extracts.

5. Analysis of obtained results and their relationships with other scientific works.

6. Conclusions highlighting the importance of secondary metabolites extracted from *Albizia lebbeck* leaves as antibacterial and antibiofilm action on *P. aeruginosa.*

3. Materials and methods

Pseudomonas aeruginosa isolates.

From Institute of Genetic Engineering and Biotechnology at the University of Baghdad collected ten ready-made clinical isolates of *P. aeruginosa* that had previously been identified using multiple chemical and molecular diagnostic techniques. Their diagnosis was confirmed by growing them on cetrimide agar medium and incubating them at a temperature of 37°C for 24 hours, in addition to using the VITEK2 system to diagnose the isolates, which were previously obtained from different hospitals in the city of Baghdad. The isolates were re-cultured on nutrient agar media to activate them, and they were cultivated for 24 hours at 37°C in an aerobic environment.

Antibiotic susceptibility test.

The World Health Organization endorsed the Kirby-Bauer technique, which was used to assess the susceptibility of 14 different antibiotics [7]. To create a moderately turbid bacterial suspension, the standard turbidity solution of a 0.5 McFarland preparation containing 1.5×10^8 CFU/ml was compared to one or two bacterial colonies that had been isolated fill a test tube with one milliliter of regular saline solution, from the original culture. Using a sterile cotton swab, evenly and gently spread a quantity of the bacterial solution over Mueller Hinton agar media, and then let it for 10 minutes. The antimicrobial discs were then securely positioned on the surface to guarantee that they made contact with the agar. After that, the plates were turned over and let to sit at 37°C for hours. The Clinical Laboratories Standards Institute (CLSI) [8], states that the inhibition zones that formed around the discs was measured using a metric ruler.

Assessment of biofilm formation.

The quantitative evaluation of biofilm formation with specific modifications was done using the colorimetric microtiter plate method, which previously published in [9]. Overnight, the turbidity of the *P. aeruginosa* culture was increased to a McFarland value of 0.5. The suspensions were placed 1% glucose diluted 1:100 in 200 µl of sterile, flat-bottomed polystyrene microtiter plates. The wells were washed three times with sterile phosphate buffered saline (PBS, pH 7.3) following a 24-hour incubation period at 37°C. After fixing the adhering biofilms with 99% methanol for 15 minutes, the plate was allowed to air dry. 200 µl of 0.1% crystal violet was used to stain biofilms at room temperature for five minutes, after which they were rinsed with water and allowed to dry. The biofilm in each well was eliminated by treating each well with 200 µl of 95% ethanol for 30 minutes. The optical density (OD) at 630 nm was measured using a microtiter plate reader (ELISA). Each trial was replicated three times across three distinct iterations of each experiment. Additionally, a cutoff value (ODc) was determined. ODc is calculated as follows: ODc = average OD of negative control + (3 × SD of negative control). ODc is defined as three standard deviations (SD) over the average OD of the negative control. The isolates were classified into four groups based on their OD: weak biofilm producers ($ODc < OD < 2 \times ODc$), moderate biofilm producers ($2 \times ODc < OD < 4 \times ODc$), strong biofilm producers ($4 \times ODc < OD$), and nonbiofilm producers ($OD < ODc$) [10].

Collection of Albizia lebbeck L.

The plant *Albizia lebbeck L.*, purchased from a Baghdad City plantation, was recognized by a specialist from the Dep. of Biology, Faculty of Sci., University of Baghdad. The leaves were pulverized with a grinder and kept at 4°C for additional examination after being washed well with water and allowed to air dry.

Preparation of Albizia lebbeck extracts.

Four hundred grams of powdered *Albizia lebbeck* leaves were first macerated in two liters of petroleum ether solvent in order to extract the fat from the leaves. After being collected, the residue was separated into two halves and left to air dry. Following the methods of AACC [11] and N'Guessan *et al.* [12], respectively, the two batches were extracted separately using methanol and water to yield a methanolic extract and an aqueous extract.

Examination of the chemical compounds of Albizia lebbeck leaves extracts.

The chemical makeup of aqueous and methanolic *Albizia lebbeck* leaf extracts was determined by method Suleiman and Ateeg [13].

Detection of total phenolic content of Albizia lebbeck leaf extracts.

To ascertain the total phenolic content (TPC) in the methanolic and aqueous extract of *Albizia lebbeck*, the N-benzoyl-L-phenylalaninol 3', 5-dihydroxy-4', 7-dimethoxyflavones (Folin-Ciocalteu reagent) was utilized. Every methanolic and aqueous extract sample contained 0.4 ml of each extract 1.6 ml of a 7.5% sodium carbonate solution, and 2.0 ml of a ten-fold diluted Folin-Ciocalteu reagent, resulting in a total volume of 5 ml. After 30 minutes at room temperature, the mixture was placed in a spectrophotometer and the absorbance was measured at 760 nm. The test tubes holding the mixture were sealed with para-film. The total phenolic content was ultimately calculated using the gallic acid standard curve, and the data were presented as milligrams of gallic acid equivalent per gram of dry weight (Fig. 1) [14].

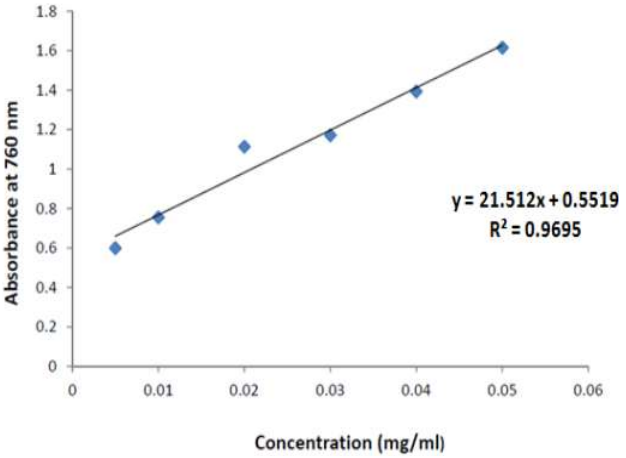


Fig. 1. Standard curve of Gallic acid [15]

Antioxidant activity assessment (*DPPH test*).

To assess using the method outlined by [16]. The antioxidant properties of the aqueous and methanolic extracts of *Albizia lebbeck* were assessed by measuring the radical scavenging ability of the samples. The mixture was allowed to sit for 30 minutes after adding 100 ul of methanolic and aqueous extract at concentrations of 0.625, 1.25, 2.5, 5, 10, and 20 mg/ml to 5 ml of newly prepared 0.004% 2, 2diphenyl1picrylhydrazyl (DPPH) in methanol. The absorbance was determined at 517 nm. Vitamin C, a natural antioxidant, and butylated hydroxytoluene (BHT), an artificial antioxidant, were among the positive controls. Each test was performed three times. The decline in DPPH expressed as a percentage was calculated using the following formula:

$$\%\text{Reduction} = \frac{\text{A DPPH} \times \text{Abs Dil.}}{\text{A DPPH}} \times 100,$$

accordingly, A DPPH – average DPPH solution absorption; A Dil. – average absorption between each dilution's three absorption measurements.

A graphic of the collected data was produced using Microsoft Excel. The EC_{50} of each extract the effective concentration of the extract or chemical at which DPPH is reduced by 50% was calculated using the graphic.

Examine the antibacterial properties of Albizia lebbeck extracts.

To assess the antibacterial activity reported by [17], the disc diffusion method was used. The bacterial culture that had been adjusted to the 0.5 McFarland standards was evenly injected onto Muller Hinton agar plates. The experiment was carried out after the dishes had been dried for fifteen minutes. A concentration of 100 and 200 mg/ml was produced by mixing 0.2 g and 0.4 g of methanolic extract and aqueous extract with 1 ml of distilled water. The 20 µl of each dilution were then infused into sterile blank discs with a diameter of 6 mm. Distilled water and DMSO discs served as negative controls. All discs were thoroughly dried before being placed on the Mueller Hinton agar surface. The plates were maintained at a temperature of 37°C for 24 hours. The size of the inhibitory zone around the discs was measured after incubating the plates to assess the aqueous and methanolic extracts' antibacterial action. The test was performed three times in order to verify the outcomes.

Assessment of the minimum inhibitory concentration (*MIC*) *of Albizia lebbeck extracts.*

A 96-well microtiter plate and the broth microdilution technique were used to calculate the *Albizia lebbeck* extracts' minimum inhibitory concentration (MIC). The working solution was made up of plant extracts in broth at a concentration of 128 mg/ml. The methanolic and aqueous extracts were sequentially diluted in twofold dilutions directly on the plate to achieve concentrations of 1, 2, 4, 8, 16, 32, 64, and 128 mg/ml., the first well of row (A) received 200 µl of the methanolic and aqueous extracts of *Albizia lebbeck* that were produced. In each column, only rows B through H had 100 microlitres of broth. Using a micropipette, double serial dilutions were produced in steps down the columns, beginning with rows A through H. Before discarding the final 100 µl, the process was repeated up to row (H). With the exception of the column containing 200 µl of negative control broth medium, the total volume of each test well containing the extracts was decreased to 100 µl after 100 µl of the initial concentrations in row A were removed and the subsequent row was treated with the appropriate amount of broth. Each well of the negative control received only 100 µl of the bacterial inoculum, which has a concentration of 1.5×10^8 CFU/ml. Microtiter plates were incubated at 37°C for 18 to 20 hours. Resazurin dye, in a volume of 20 µl, was subsequently put into each well, and any colour changes were observed after 30 minutes of sitting. In the resazurin broth test, the Minimum Inhibitory Concentrations were determined to be the lowest extract concentrations at which the color of the broth did not shift from blue to pink, as seen in broth microdilution [18].

Examine the anti-biofilm abilities of Albizia lebbeck extracts.

Using a 96-well microtiter plate, the methanolic and aqueous extracts of *Albizia lebbeck* were tested for their antibiofilm activity. A concentration of 400 mg/ml was the operational solution for the aqueous and methanolic *Albizia lebbeck* extracts, which resulted in concentrations of 3.125, 6.25, 12.5, 25, 50, 100, 200, and 400 mg/ml. While the wells in rows B through H only contained 100 µl of broth, the wells in row A included 100 µl of each sample. Using a micropipette, two-fold serial dilutions were carefully started with rows A–H and preceded along the columns. Following a thorough mixing of the 100 µl of broth, 100 µl of the initial concentrations in row A were taken out and moved to the following row, and so on, until the last row (H), when the final 100 µl were thrown away. 100 µl of the bacterial inoculum at a concentration of 1.5×10^8 CFU/ml was added to each well, with the exception of the negative control. The same procedure was utilized (assessment of biofilm formation), as detailed in the paragraph.

Statistical analysis.

The (SAS) program, which performs statistical analysis, was used to assess the influence of several variables on research parameters. Utilizing the least significant difference (LSD) test, this research made a notable comparison between means.

3. Results

Antibiotic susceptibility test.

All ten isolates of *Pseudomonas aeruginosa* were tested against the following 14 antibiotics: Meropenem, Azetreonam, Imipenem, Tobramycin, Cefotaxime, Ceftriaxone, Carbenicillin, Gentamicin, Piperacillin, Cephalothin, Ceftazidime, Cefepime, Amikacin, and Ciprofloxacin. The findings showed that *P. aeruginosa* isolates frequently showed extremely high levels of resistance to the drugs used in this study, as shown in Table 1.

Detection of biofilm formation.

Biofilm development is assessed using the microtiter plate technique and the ELISA reader measures absorbance at 630 nm. As demonstrated in Table 2, all isolates produced biofilms that were 100% strong, according to the findings.

Phytochemical screening of Albizia lebbeck leaves extracts.

The solvents used in the serial exhaustive extraction for this study, methanol and water, have varying polarity indexes. As a result, extracts of *Albizia lebbeck* leaves were shown to have a variety of secondary metabolites, including alkaloids, flavonoids, glycosides, phenols, saponins, and tannins, as shown in Table 3.

Total phenolic content of Albizia lebbeck leaves extracts.

The total phenolic contents of the aqueous and methanolic extracts were assessed using the Folin-Ciocalteu reagent. The overall phenolic content (TPC) of the *Albizia lebbeck* extracts increased consistently with increasing concentration, albeit with significant variability ($P \leq 0.01$). The maximum values in methanolic and aqueous extracts were 71.11 and 45.15 mg/g at 50 mg/ml, respectively, as seen in Table 4.

Antioxidant activity of Albizia lebbeck leaves extracts.

With notable variations across concentrations ($P \leq 0.01$), *Albizia lebbeck* extracts displayed concentration-dependent free radical scavenging effects. The scavenging activity increased steadily with increasing extract concentrations. When compared to the synthetic antioxidant BHT and the natural antioxidant vitamin C, whose respective amounts were (97.41 and 92.41), the methanolic extract showed a greater free radical scavenging activity of (91.20) at a concentration of 20 mg/ml, which was higher than the aqueous extract's (78.65) at the same concentration, as shown in Table 5.

Table 1

Antibiotic resistance in *P. aeruginosa* isolates

Antibiotic / Isolate	MER	ATM	IMI	TOB	CTX	CRO	CAR	GN	PRL	KF	CAZ	CFM	AK	CTP	Percentage of resistance
P_1	R	R	S	R	R	R	R	R	R	R	R	R	R	S	85.71%
P_2	R	R	S	R	R	R	R	R	R	R	R	R	R	S	85.71%
P_3	R	R	S	R	R	R	R	R	R	R	R	R	R	S	85.71%
P_4	R	R	S	R	R	R	R	R	R	R	R	R	R	S	85.71%
P_5	R	R	S	R	R	R	R	R	R	R	R	R	R	S	85.71%
P_6	R	R	S	R	R	R	R	R	R	R	R	R	R	S	85.71%
P_7	R	R	S	R	R	R	R	R	R	R	R	R	R	R	92.85%
P_8	R	R	S	R	R	R	R	R	R	R	R	R	R	R	92.85%
P_9	R	R	S	R	R	R	R	R	R	R	R	R	R	R	92.85%
P_{10}	R	R	R	R	R	R	R	R	R	R	R	R	R	R	100%

Note: P – P. aeruginosa; S – Sensitive,® Resistant; MER – Meropenem; ATM – Azetreonam; IMI – Imipenem; TOB – Tobramycin; CTX – Cefotaxime; CRO – Ceftriaxone; CAR – Carbenicillin; GN – Gentamicin; PRL – Piperacillin; KF – Cephalothin; CAZ – Ceftazidime; CFM – Cefepime; AK – Amikacin; CIP – Ciprofloxacin.

Table 2

Biofilm forming of *P. aeruginosa* isolates

P. aeruginosa isolates	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	P_{10}
Biofilm forming	Strong	Strong	Strong	Strong	Strong	Strong	Strong	Strong	Strong	Strong

Note: P – P. aeruginosa isolates; Control negative (cut off) = 0.11.

Table 3

Phytochemical screening of *Albizia lebbeck* leaves extracts

Phytochemical compound		Aqueous extract	Methanolic extract
Alkaloids	Meyer's test	+	+
	Wagner's reagent	+	+
Tannins	Lead acetate	+	+
	Ferric chloride	+	+
Phenols	Lead acetate	+	+
	Ferric chloride	+	+
Saponins		+	+
Flavonoids		+	+
Glycosides		+	+

As demonstrated in Fig. 2, the radical scavenging capacity (EC_{50}) of the aqueous, methanolic extract, BHT, and V. C was (14.9, 9.5, 1.1 and 0.5) mg/ml, respectively.

Antibacterial activity of Albizia lebbeck extracts. Disk diffusion method.

The antibacterial activity of *Albizia lebbeck* leaf extracts against isolates of *P. aeruginosa* was assessed using the disc diffusion technique. Tables 6 and 7 demonstrate that at dosages of 200 and 400 mg/ml, the methanolic extract outperformed the aqueous extract, resulting in the *P. aeruginosa* isolates exhibiting the largest inhibitory zone of 20.00 and 28.33 mm, respectively. The aqueous extract, however, generated inhibitory zones of 13.00 and 17.00 mm, respectively. This study suggests that the antibacterial activity is brought about by the high phenolic component content in the methanolic and aqueous extracts of *Albizia lebbeck.*

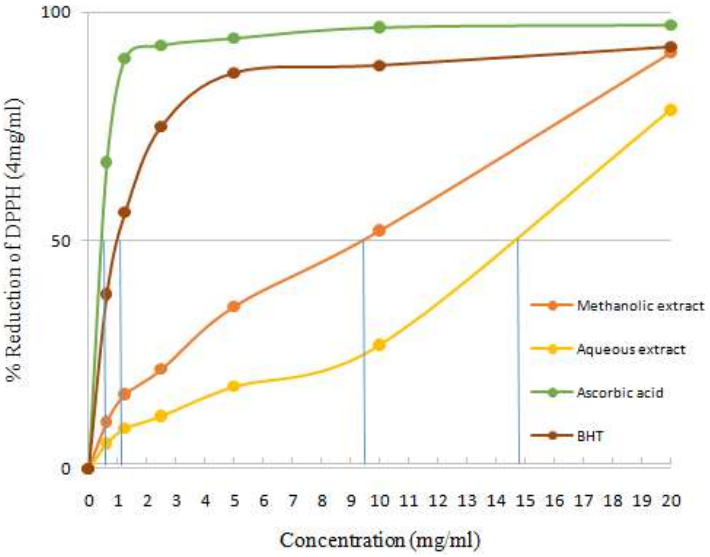


Fig. 2. EC_{50} of *Albizia lebbeck* leaves extract

Table 4

Total phenolic content of *Albizia lebbeck* extracts

Concentration (mg\ml)	Aqueous extract (mg\g)	Methanolic extract (mg\g)	LSD value
12.5	23.48 ± 0.01	34.67 ± 0.20	0.563**
25	37.01 ± 0.02	56.99 ± 0.04	0.154**
50	45.15 ± 0.02	71.11 ± 0.02	0.079**
LSD value	0.070**	0.418**	–

*Note: ** – $P \leq 0.01$.*

Table 5

Radical scavenging activity of *Albizia lebbeck* extracts

Concentration mg/ml	Aqueous extract	Methanolic extract	BHT	Vit. C	LSD value
0.625	5.47 ± 0.21	10.22 ± 0.12	37.24 ± 0.13	67.29 ± 0.18	0.556 **
1.25	8.78 ± 0.03	16.12 ± 0.07	56.53 ± 0.05	89.16 ± 0.10	0.239 **
2.5	11.68 ± 0.12	21.92 ± 0.11	74.07 ± 0.08	92.73 ± 0.19	0.436 **
5	17.93 ± 0.08	34.52 ± 0.28	85.73 ± 0.36	94.13 ± 0.22	0.840 **
10	26.62 ± 0.23	56.47 ± 0.29	88.28 ± 0.25	96.62 ± 0.25	0.846 **
20	78.65 ± 0.20	91.20 ± 0.13	92.48 ± 0.08	97.41 ± 0.09	0.448 **
LSD value	0.514 **	0.588 **	0.600 **	0.571 **	–

*Note: ** – $P < 0.01$.*

Table 6

Antibacterial activity of *Albizia lebbeck* methanolic extract in *P. aeruginosa*

No. of isolate	Methanolic extract		LSD value
	100 mg/ml	200 mg/ml	
P_1	16.33 ± 0.58	28.33 ± 0.58	2.170**
P_2	16.00 ± 0.00	21.33 ± 0.58	1.535**
P_3	15.67 ± 0.58	22.00 ± 1.00	3.069**
P_4	14.33 ± 0.58	21.33 ± 0.58	2.170**
P_5	20.00 ± 0.00	25.33 ± 0.58	1.535**
P_6	16.33 ± 0.58	22.33 ± 0.58	2.170**
P_7	12.33 ± 0.58	18.33 ± 0.58	2.170**
P_8	14.67 ± 0.58	21.33 ± 0.58	2.170**
P_9	17.33 ± 0.58	25.33 ± 0.58	2.170**
P_{10}	16.00 ± 1.00	23.00 ± 0.00	2.658**
LSD value	1.341**	1.407**	–

*Note: means with different big letters in the same column and small letters in the same row are significantly different; ** – $P \leq 0.01$; P – P. aeruginosa.*

Table 7

Antibacterial activity of *Albizia lebbeck* aqueous extracts extract in *P. aeruginosa*

No. of Isolate	Aqueous extract		LSD value
	100 mg/ml	200 mg/ml	
P_1	9.33 ± 0.58	15.33 ± 0.58	2.170**
P_2	9.33 ± 0.58	15.33 ± 0.58	2.170**
P_3	8.00 ± 1.00	14.00 ± 0.00	2.658**
P_4	8.67 ± 0.58	15.00 ± 1.00	3.069**
P_5	10.00 ± 0.00	15.67 ± 0.58	1.535**
P_6	11.33 ± 0.58	17.00 ± 0.00	1.535**
P_7	6.67 ± 0.58	10.00 ± 0.00	1.535**
P_8	10.33 ± 0.58	15.33 ± 0.58	2.170**
P_9	13.00 ± 0.00	16.33 ± 0.58	1.535**
P_{10}	10.33 ± 0.58	15.33 ± 0.58	2.170**
LSD value	1.341**	1.272**	------

*Note: means with different big letters in the same column and small letters in the same row are significantly different; ** – $P \leq 0.01$; P: P. aeruginosa.*

Calculation of the (*MIC*) *of the extracts from Albizia lebbeck.*

According to the data, the minimum inhibitory concentration (MIC) of the methanolic extract was lower than that of the water-based extract. As indicated in Table 8 and Fig. 3, 4, the methanolic extract MIC for all *P. aeruginosa* isolates was 16 mg/ml, except for isolate No. 7, which exhibited a MIC of 32 mg/ml. With the exception of isolate No. 7, which exhibited a MIC of 64 mg/ml, all *P. aeruginosa* isolates had a MIC of 32 mg/ml for the aqueous extract.

Anti-biofilm activity of Albizia lebbeck Extracts.

The methanolic leaf extract from *Albizia lebbeck* entirely prevented *P. aeruginosa* from creating a biofilm at a concentration of 200 mg/ml and 80% at a concentration of 100 mg/ml, as can be seen in Table 9. However, as demonstrated in Table 10, at a 400 mg/ml dosage, the aqueous extract totally stopped the *P. aeruginosa* isolates' ability to produce biofilms.

Table 8

MIC of *Albizia lebbeck* methanolic and aqueous extracts on *P. aeruginosa*

Isolate	Methanolic extract	Aqueous extract
	MIC (mg/ml)	MIC (mg/ml)
P_1	16	32
P_2	16	32
P_3	16	32
P_4	16	32
P_5	16	32
P_6	16	32
P_7	32	64
P_8	16	32
P_9	16	32
P_{10}	16	32

Note: P – P. aeruginosa.

Table 9

Biofilm formation of *P. aeruginosa* before and after treatment with *Albizia lebbeck* methanolic leaves extract

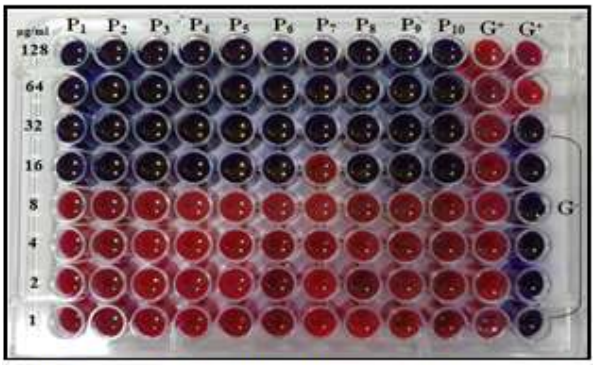
Isolates	Before treatment (control)	After treatment concentration mg/ml							
		Concentration (mg/ml)							
		3.125	6.25	12.5	25	50	100	200	400
P_1	Strong	Strong	Moderate	Moderate	Moderate	Weak	Weak	Weak	No Biofilm
P_2	Strong	Strong	Strong	Moderate	Moderate	Moderate	Weak	Weak	No Biofilm
P_3	Strong	Strong	Moderate	Moderate	Moderate	Moderate	Weak	Weak	No Biofilm
P_4	Strong	Strong	Moderate	Moderate	Moderate	Weak	Weak	No Biofilm	No Biofilm
P_5	Strong	Strong	Strong	Moderate	Moderate	Weak	Weak	No Biofilm	No Biofilm
P_6	Strong	Strong	Strong	Moderate	Moderate	Moderate	Moderate	Weak	No Biofilm
P_7	Strong	Strong	Moderate	Moderate	Moderate	Weak	Weak	No Biofilm	No Biofilm
P_8	Strong	Strong	Moderate	Moderate	Moderate	Weak	Weak	No Biofilm	No Biofilm
P_9	Strong	Strong	Moderate	Moderate	Moderate	Weak	Weak	No Biofilm	No Biofilm
P_{10}	Strong	Strong	Moderate	Moderate	Weak	Weak	Weak	Weak	No Biofilm

Note: P – P. aeruginosa.

Table 10

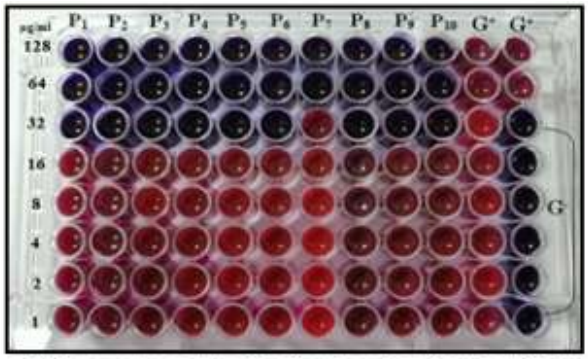
Biofilm formation of *P. aeruginosa* before and after treatment with *Albizia lebbeck* aqueous leaves extract

Isolates	Before treatment (control)	After treatment							
		Concentration (mg/ml)							
		3.125	6.25	12.5	25	50	100	200	400
P_1	Strong	Strong	Moderate	Moderate	Moderate	No Biofilm	No Biofilm	No Biofilm	No Biofilm
P_2	Strong	Strong	Moderate	Weak	Weak	No Biofilm	No Biofilm	No Biofilm	No Biofilm
P_3	Strong	Moderate	Moderate	Weak	Weak	No Biofilm	No Biofilm	No Biofilm	No Biofilm
P_4	Strong	Moderate	Moderate	Weak	Weak	No Biofilm	No Biofilm	No Biofilm	No Biofilm
P_5	Strong	Strong	Strong	Moderate	Moderate	Weak	Weak	No Biofilm	No Biofilm
P_6	Strong	Moderate	Moderate	Moderate	Weak	Weak	No Biofilm	No Biofilm	No Biofilm
P_7	Strong	Strong	Moderate	Moderate	Weak	Weak	No Biofilm	No Biofilm	No Biofilm
P_8	Strong	Strong	Strong	Moderate	Weak	Weak	No Biofilm	No Biofilm	No Biofilm
P_9	Strong	Strong	Moderate	Moderate	Weak	Weak	No Biofilm	No Biofilm	No Biofilm
P_{10}	Strong	Moderate	Moderate	Moderate	Weak	Weak	Weak	No Biofilm	No Biofilm



(P): *P. aeruginosa* isolate, (C^+): Control positive (Bacteria + Media), (C^-): Control negative (Media only)

Fig. 3. MIC of *Albizia lebbeck* methanolic extract on *P. aeruginosa*



(P): *P. aeruginosa* isolate, (C^+): Control positive (Bacteria + Media), (C^-): Control negative (Media only)

Fig. 4. MIC of *Albizia lebbeck* aqueous extract on *P. aeruginosa*

4. Discussion

Six of *P. aeruginosa* were 85.71% antibiotic resistant, three isolates were 92.85% resistant, and the isolate number 10 was 100% resistant to the antibiotics utilized in this study. This resistance is either inherited or gained through mutations in genetic material or horizontal gene transfer, according to [19]. Because of the improper usage of antibiotics, resistance has developed in *P. aeruginosa* bacteria, resulting in the development of multidrug-resistant (MDR) forms of *P. aeruginosa* bacteria [20].

The capacity of *P. aeruginosa* to produce biofilm is a key factor in its pathogenicity since it enables the bacteria to flourish in a variety of environments, including burn wounds, and eventually results in chronic illnesses [21]. Prior research has established a correlation between the multidrug resistant phenotype of *P. aeruginosa* and its ability to produce biofilms [22]. [23] brought attention to the importance of biofilm and how it contributes to the development of high levels of antibiotic resistance in a variety of bacterial species. [24] discovered that every isolate could form biofilms, which is in line with the results of the current study.

As it is commonly known, repeated extraction with solvents of varying polarity is a method for enhancing the solubility of phytochemicals in plant matrices [25]. Many variables, such as time, temperature, solvent concentration, solvent polarity, and changes in the structure of phytochemicals (functional groups), affect how well they dissolve in organic or polar solvents or solvents with differing polarities [26]. The need for their in-depth phytochemical investigation and/or pharmacological assessment is established by performing a qualitative phytochemical screening of medicinal plants [27].

On another hand, the health advantages of phenolic compounds and their possible biological effects have been investigated using total phenolic content [28]. According to this study, the methanolic extract of *Albizia lebbeck* had a greater TPC content than the aqueous extracts. Additionally, it has been demonstrated that water can absorb proteins and carbohydrates from the plant matrix that may affect the TPC and some biological functions of plant extracts [25].

To determine a substance's ability to act as a hydrogen donor or free radical scavenger, the relatively stable DPPH radical has been widely used. This ability was employed to determine antioxidant activity [29]. In addition, the antioxidant activity is represented using an Effective concentration (EC50). The half maximal Effec-

tive Concentration (EC_{50}), which is frequently used as a measure of a drug's potency, is the amount of a drug, toxin, or antibody that, after a certain exposure period, produces a response that is halfway between the baseline and maximum [30].

Polyphenols, which are found in plants, are known to mediate antioxidant and free radical scavenging capabilities [31, 32]. Due to their reductionoxidation characteristics, phytochemical compounds have antioxidant action because they can function as metal chelators, singlet oxygen quenchers, or hydrogen donors (reducing agents). The DPPH test used in this study evaluates a plant extract's capacity to introduce hydrogen atoms or electrons into the stable radical DPPH produced in solution. The mechanisms by which antioxidants in natural products interact with DPPH are likely to be HAT, multistep reactions (single electron transfer and sequential proton loss electron transfer), or a synergistic combination of these activities. Single electron transfer and sequential proton loss electron transfer mechanisms are more common in nonaqueous media because organic solvents are more chemically reactive and may create hydrogen bonds with antioxidants [33].

Numerous phenolic compounds can be utilised to treat human disorders and have antibacterial qualities against plant diseases. Furthermore, a number of produced phenolic chemicals have antibacterial activities that use processes that are different from those used by conventional drugs, indicating that they may have a key role in enhancing antibacterial treatment [34].

As well as, the methanol extracts of *Albizia lebbeck* leaves include secondary metabolites that may be responsible for their antibacterial properties. For example, alkaloids, tannins, saponins, and polyphenols (such as phenols and flavonoids) have all been shown to have antibacterial action [35]. They have a direct effect on microorganisms and reduce the virulence factors in germs [36]. By targeting numerous nucleic acid enzymes, alkaloids intercalate microbial DNA and penetrate cells, inflicting long-term damage to microbial cells [37]. By precipitating microbial proteins and depriving the microbes of these vital nutrients, tannins and saponins prevent bacterial development [38]. Additionally, tannins have the potential to damage enzymes, transport proteins, adhesions, and the cell walls of bacteria. Gallotannins exhibit antibacterial action by binding to iron in microbial cell membranes, which renders membrane-bound proteins inactive [39]. The observed antibacterial action is also correlated to the concentration of the extracts' secondary metabolites. For example, methanolic extracts have higher concentrations of phenols and saponins than aqueous extracts.

The minimum inhibitory concentration (MIC) of antibacterial medications against *P. aeruginosa* has been determined using the technique that uses the redox colorimetric indicator resazurin. Resazurin can be seen clearly with the naked eye, and the MIC can be identified without the need of a spectrophotometer. Living cells reduce resazurin, which is blue when oxidized, to a pink hue [40]. The MIC of the plant extracts was ascertained using the broth microdilution method and a 96-well microtiter plate. The method that employs the redox colorimetric indicator resazurin has been used to measure the minimum inhibitory concentration (MIC) of antibacterial drugs against *P. aeruginosa*. The flavonoid compounds found by [41] and [42] have been shown to have potent antibacterial effects against a number of pathogenic microorganisms in vitro.

Through the inhibiting or reducing biofilm formation using a concentration-dependent approach, phenolic compounds were shown to be able to inhibit the growth of biofilms. Among the antibacterial mechanisms discovered [36] are decreased membrane fluidity, interactions between bacterial proteins and cell walls, damage to the cytoplasmic membrane, and inhibition of energy metabolism, nucleic acid synthesis, or cell wall formation. On the other hand, plant phenolic antibiofilm activity has demonstrated that, despite not affecting bacterial growth, it has effects that lessen biofilms by disrupting bacterial regulatory mechanisms like quorum sensing or other global regulator systems. This is in addition to the harm they cause to bacteria [43, 44]. Phenolic chemicals produced from *B. ciliata* may be used to treat infectious diseases caused by *P. aeruginosa* PAO1, particularly biofilms, as stated by [45]. Furthermore, [46] found that even though the bacterial isolates of *P. aeruginosa* formed significant biofilms before treatment, the phenolic compounds isolated from *C. sinensis* leaves demonstrated high *P. aeruginosa* antibiofilm activity that can downregulate the pelA gene.

Practical relevance. One of the factors behind the growing use of herbs as low-risk, affordable, and natural substitutes to synthetic antibiotics in the treatment of bacterial infections has been this. The use of natural ingredients in the manufacturing of drugs is growing. In addition to being utilized directly as therapeutic medicines. So Bacterial and fungal resistance to antibiotics is one of the issues in the biological and medical sciences, as several dangerous bacteria have shown evidence of antibiotic resistance, which has been growing along with the creation of new chemicals and drugs.

Research limitations. The "cytotoxicity or potential side effects" of the extracts were not evaluated. Without toxicity profiling on mammalian cells, the safety of using these extracts as antimicrobial agents cannot be confirmed, especially when used *in vivo* study.

Further research prospects. Bioactive substances can be used as building blocks or templates to create new physiologically active compounds. Studies on the use of herbal medicines as a substitute for medications and antibiotics were consequently conducted.

5. Conclusion

This work demonstrated that the secondary metabolites extracted from *Albizia lebbeck* leaves have a considerable antibacterial and antibiofilm action on *P. aeruginosa*, even though the bacterial isolates create a strong biofilm. This research has shown that *Albizia lebbeck*

leaves can be used in traditional therapy for bacterial infections and diseases caused by oxidative stress since they contain therapeutic phytochemicals with strong antibacterial and antioxidant effects.

Conflict of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this article.

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Data availability

Manuscript has no associated data.

Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies.

Authors' contributions

Inas Humam Abdulaal: Drafting of the manuscript, Administrative technical or material support; **Ahmed H. AL-Azawi:** Supervision, Conception and design, Analysis and interpretation of data; **Diana Ghanem Yahya:** Statistical analysis.

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