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Antibiofilm Activity of *Conocarpus erectus* Leaves Extract and Assessment Its Effect on *pelA* and *algD* Genes on Multi-drug Resistant *Pseudomonas aeruginosa*

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ABSTRACT

Background: Due to its various resistance mechanisms, *Pseudomonas aeruginosa* is the most prevalent opportunistic infection that kills hospitalized patients. Thus, therapeutic options become limited.

Objective: The study aimed to estimate the antibiofilm effectiveness of Conocarpus erectus leaf extracts against MDR *P. aeruginosa* isolates and examines *pelA* and *algD* gene expression.

Subjects and Methods: One hundred-fifty clinical samples were collected from five Baghdad hospitals between September 2021 and January 2022. Samples were grown on different mediums. Despite cetrimide agar's ability to detect *P. aeruginosa*, only 83 isolates developed at 42°C. VITEK 2 compact system identification followed. This study examined 83 of *P. aeruginosa* isolates for resistance vs 10 medications. Disk diffusion was used for this investigation.

Results: The results showed that *P. aeruginosa* isolates were most resistant to Ceftriaxone, Amoxicillin-Clavulanic acid, and Trimethoprim. Microtiter plate biofilm detection is done with 10 multi-drug-resistant isolates. All isolates produced significant biofilm. Maceration and Soxhlet equipment produced methanolic and aqueous extracts. The phytochemical screening of Conocarpus erectus revealed flavonoids, phenols, alkaloids, tannins, glycosides, and saponins in methanolic and aqueous extracts. 32 mg/ml Conocarpus erectus methanolic leaf extract prevented clinical *P. aeruginosa* biofilm development. After treatment with the sub-MIC of the methanolic extract, *P. aeruginosa*'s biofilm-forming genes pelA and algD had minimal expression. **Conclusion:** This study demonstrated that Conocarpus erectus methanolic extracts contain significant phytochemical content, making them therapeutic. The antibiofilm compound in Conocarpus erectus extract downregulates the algD and pelA genes in *P. aeruginosa* despite antibiotic resistance.

Keywords: *Pseudomonas aeruginosa*, *Conocarpus erectus*, antibacterial, antibiofilm, MIC, *pelA* and *algD* genes.

INTRODUCTION

Clinically and epidemiologically, *Pseudomonas aeruginosa* ranks high prevalence of MDR. Non-fermenting Gram-negative bacilli cause most nosocomial and opportunistic infections in immunocompromised patients [(1)]. Its virulence factors trick the host's immune system. Biofilm, lipopolysaccharide, flagellum, type IV pili, type III secretion system, exotoxin A, proteases, alginate, quorum sensing, biofilm formation, type VI secretion systems, and airspace oxidant production are virulence factors [(2)].

When bacteria stick to a surface and build a polysaccharide matrix, they produce biofilms. They do this to protect their microorganisms from outside threats. Biofilms can cover biotic and abiotic surfaces (EPS). Stress allows *P. aeruginosa*'s biofilm form to avoid the host immune response and have better antimicrobial resistance than its planktonic form [(3)].

In therapeutic plants, one can discover a great deal of material that possesses antibacterial properties. Because of the potential antibacterial activity that they possess, the extracts of numerous medicinal plants are utilized in the treatment of a wide variety of ailments. The screening and subsequent market trading of particular bioactive components is essential to the success of a large number of herbal firms [(4)].

The existence of active compounds in plants is what causes them to have antimicrobial effects. These chemicals include quinones, phenols, alkaloids, flavonoids, terpenoids, essential oil, tannins, lignans, glucosinolates, and others. Some secondary metabolites are also involved [(5)]. *Conocarpus* wood might have been in the past utilized for fire, furniture, and charcoal, as it is very hard and strong wood [(6)].

Because of their low rate of combustion, the wood and bark of this tree are often used in the smoking process, particularly for red meat and fish. Orchitis, prickly heat, headache, anemia, bleeding, catarrh, diabetes, diarrhea, conjunctivitis, tumors, gonorrhea, and syphilis are just some of the many conditions that have traditionally been treated with this species. It has also been used as an antipyretic and anti-inflammatory in the treatment of fever (Decoction of leaves) and swellings [(7)].

AIM OF THE STUDY

The study aims to estimate the antibiofilm effectiveness of Conocarpus erectus leaf extracts against MDR *P. aeruginosa* isolates and examines *pelA* and *algD* gene expression.

SUBJECTS AND METHODS

Collection of plant:

The expert from the Department of Biology in the College of Science at the University of Baghdad identified the plant leaves seen in Iraqi marketplaces as belonging to the species Conocarpus erectus L. After being washed and dried at room temperature, the leaves are then ground and placed in an airtight container to be

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kept at 4°C until further analysis.

Preparation of aqueous extract:

The aqueous extract was created by ***N'Guessan et al.*** it is briefly, 200g laraurs nobilis leaves macerated in 1400ml distilled water for 72 hours before filtering through Whatman No.1 paper. At 50°C, a rotary evaporator extracts water from filtrate under vacuum. The extract was kept at 4°C in amber glass vials until it was analyzed [(8)].

Preparation of methanolic extract:

The methanolic extract was produced in accordance with the Soxhlet apparatus, as stated above (AACC, 1984). For six hours at temperatures between 40 and 60°C, 100grams of laraus nobilis leaves were steeped in 700 milliliters of methanol. To remove the methanol from the solution, it was first filtered through Whitman No. 1 filter paper and then evaporated in a rotary evaporator at 40 °C while under a vacuum. The extract remained in amber glass vials at a temperature of 4 °C until it was examined.

Phytochemical tests of *Conocarpus erectus* extracts:

According to the results of the phytochemical screening, both the aqueous and the methanolic leaf extracts were shown to exhibit significant levels of activity [(9)].

High-Performance Liquid Chromatography (HPLC):

Isolated Conocarpus erectus in methanol and water (HPLC). Calculate the plant's chemical concentration using this equation:

$$C.\ sam = \frac{Cst * Asam}{Ast} * \frac{D\ .\ F}{Wt}$$

C. sam= concentration of sample, ***Cst***= concentration of standard, ***Asam***= area of sample, ***Ast***= area of standard, ***D.F***= dilution factor, ***Wt***= weight of sample [(10)].

Isolation of bacteria:

A total of 120 clinical specimens were collected from patients who were referred to many hospitals in Baghdad (Burns Hospital / Medical City, Al-Karama Teaching Hospital, Al-Kindi Teaching Hospital, Al-Yarmouk Teaching Hospital, and Al-Mahmoudia Teaching Hospital). Nutrient agar was used for the majority of the collected specimens' streaks growth. The plates were kept in an aerobic 37°C incubator for 24 hours.

Antibiotic susceptibility test:

Susceptibility to 14 different antibiotics was tested using the Kirby-Bauer method [(11)]. Bacterial olonies were suspended in 4 ml of normal saline in a test tube. CFU/ml = 1.5 x 10^8. A little amount of the bacterial suspension was smeared gently and evenly with a sterile cotton swab over Mueller-Hinton agar medium and then incubated at 37°C for 10 minutes. Next, the antimicrobial discs were placed firmly on the agar with sterile forceps to guarantee that they would come into contact with the media. After 18 to 24 hours of incubation at 37°C, the plates were switched. Inhibition zones around discs were measured in millimeters (mm) in accordance with CLSI protocols [(12)].

Study the antibiofilm activity of *Conocarpus erectus* extract:

The 96-well microtiter plates examined Conocarpus erectus methanolic extract antibiofilm activity. Plant extracts were 400 mg/ml in broth, and two-fold dilutions on the plate yielded 200-1.562 mg/ml methanolic extracts. First row (A) wells received 200 µl of methanolic extract. B-H had broth alone. Columns had two micropipette dilutions (from rows A-H). 100 µl from the starting concentrations in row A was transferred to the next row with the broth, correctly mixed, and continued until row (H), when the last 100µl was discarded. All wells except the negative control received 100µl of 1×10^6 CFU/ml bacterial inoculum [(13)].

Molecular Detection of *algD* and *pelA* gene:

This stage involved adding 12.5 µl of OneTaq (NEB®) mastermix, 3 µl of DNA sample, 1 µl of each primer at 10 pmol/µl, and 7.5 µl of free-nuclease water to the appropriate PCR conditions for the gene [(14)].

Gene expression Analysis Using qRT PCR Technique:

The gene expression of *pelA* and *algD*, which are involved in biofilm formation, was measured in resistant isolates before and after methanolic extract treatment. The sub-MIC methanolic extract allowed bacterial growth.

RNA was extracted by using TRIzol™ Reagent according to the the protocol described by the manufacturer. In order to assess the gene expression of pelA and algD gene, primers utilized in this Study include ***PelA*** FF: CCTTCAGCCATCCGTTCTTCT, RR: TCGCGTACGAAGTCGACCTT (118 bp) [(15)], ***algD*** FF: GAGGAATACCAGCTGATCCGG, RR: CACCGAGTTCAAGGACCTGAA (190 bp) (Newly Designed) and House Keeping gene FF: GTGCTATACCGCTGGGATCAA, RR: GGTTCTATTTGCTGTGAATCC (238bp)(Newly Designed). The reaction mixture were Luna Universal qPCR Master Mix: 10 µl, Forward primer (10µM): 1 µl, Reverse primer (10µM):1 µl, Template DNA: 5 µl, Nuclease-free Water: 3 µl. Moreover, after several trials, the thermo cycler protocol was optimized and the protocol included were initial denaturation was 95 °C for 60 seconds (1cycle), denaturation was 95 °C for 15 seconds (40 cycle), Annealing was 60 °C for 30 seconds (40 cycle),and Melt Curve 60-95 °C for 40 minutes (1 cycle).

Ethical considerations:

Institutional Review Boards at all participants' hospitals (Burns Hospital/Medical City, Al-Karama Teaching Hospital, Al-Kindi Teaching Hospital, Al-Yarmouk Teaching Hospital, and Al-Mahmoudia Teaching Hospital) gave their stamp of approval to the study according to agreement 86105.

RESULTS

Phytochemical tests of *Conocarpus erectus*:

Conocarpus erectus extract phytochemical characterizations in methanolic and aqueous form are displayed in (Table **1**). Flavonoids, phenols, alkaloids, tannins, glycosides, and saponins were all present in the methanolic extract. Aqueous extract, unlike methanolic extract, contains every component excluding the alkaloids.

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Table (1): Phytochemical screening of conocarpus erectus extracts

Phytochemical compound		Aqueous extract	Methanolic extract	Result
Flavonoids		+	+	yellow color
Phenols	**ferric chloride**	+	+	bluish green color
	lead acetate	+	+	reddish brown precipitate
Alkaloids	**Wagner's test**	-	+	reddish brown precipitate
	Meyer s test	-	+	White precipitate
Tannins		+	+	white gelatin
Glycosides		+	+	violet ring
Saponins		+	+	thick foam

(+) Positive, (-) negative

High-performance liquid chromatography (HPLC)

According to HPLC analysis was carried out in order to determine the one-of-a-kind phenolic profiles possessed by Conocarpus erectus.

In comparison to the reference chemicals, the phenolic compounds catechin, gallic acid, quercetin, kaempferol, and taxifolin acid were shown to have statistical significance in the methanolic and aqueous extracts (Figures**1** and **2**, respectively).

A quantitative investigation of Conocarpus erectus extracts came to the conclusion that the percentages of total volume in the aqueous and methanolic forms of the extract are quite different from one another (Table **2**).

The phytochemical screening and HPLC analysis of extracts of Conocarpus erectus found that the methanolic extract contained a greater number of ingredients than the aqueous extract did. The methanolic extract will be used as the testing medium for the antibiofilm agent.

Table (2): Quantitative analysis of Conocarpus erectus extracts

Active constituents	Methanolic extract (ppm)	Aqueous extract (ppm)
Catechin	20.8	12.5
Gallic acid	12.8	48.9
Quercetin	22.2	13.6
Kaempferol	12.4	18.9
Taxifolin	40.8	34.7
Rutin	12.8	24.7

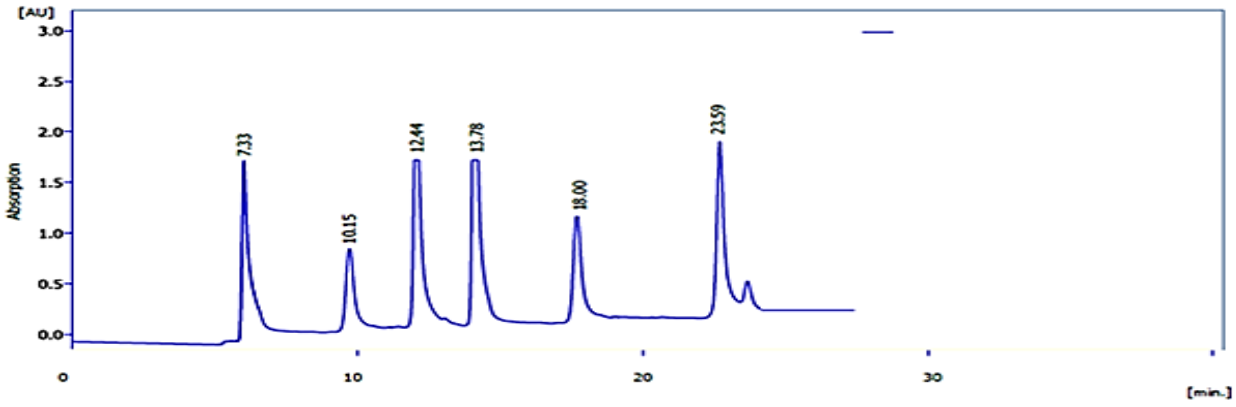

Figure (1): HPLC chromatogram of phenolic compounds in *Conocarpus erectus* methanolic leaves extract.

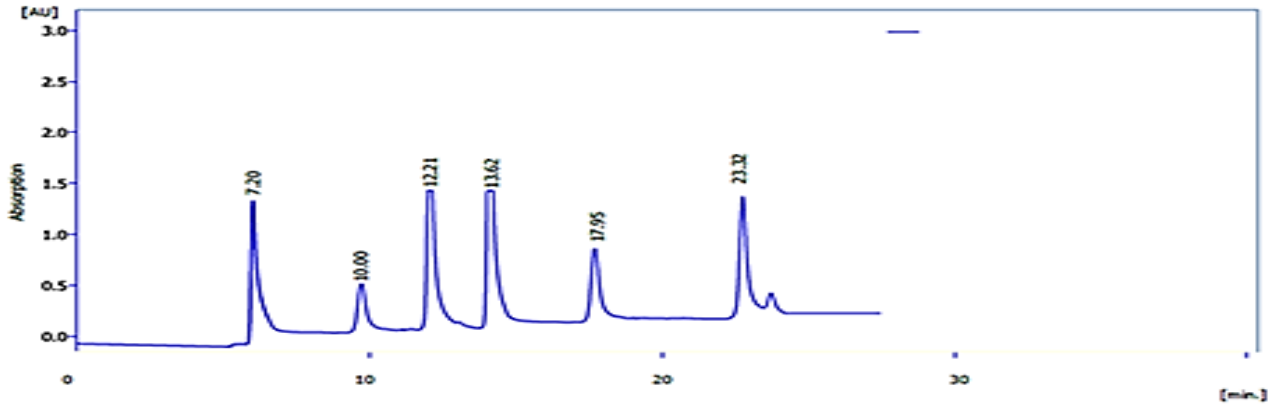

Figure (2): HPLC chromatogram of phenolic compounds in *Conocarpus erectus* aqueous leaves extract.

Antibiotic Susceptibility test:

In this study, the susceptibility of ten medications to 83 *P. aeruginosa* isolates was examined. The *P. aeruginosa* isolates with the highest rates of resistance were CRO and AUG, with 92.7% and 89.2%, respectively, and TMP coming in at 79.5% (Table **3**). Ten *P*. aeruginosa isolates were chosen for this research because they had shown drug resistance to several different substances (Table **4**).

Table (3): Antibiotic susceptibility test of eighty-three *P. aeruginosa*

Antibiotic	Sensitive No. / (%)	Resistance No. / (%)
IMI	42 (50.6%)	41 (49.3%)
CEP	39 (46.9%)	44 (53.0%)
TMP	17 (20.4%)	66 (79.5%)
TOB	50 (60.2%)	33 (39.7%)
AK	30 (36.1%)	53 (63.8%)
CIP	52 (62.6%)	31 (37.3%)
TC	47 (56.6%)	36 (43.3%)
CS	37 (44.5%)	46 (55.4%)
AUG	9 (10.8%)	74 (89.2%)
CRO	6 (7.2%)	77 (92.7%)

(*P*): *P. aeruginosa,* (**IM1):** Imipenem**, (CFP):** Cefepime**,** (**TMP):** Trimethoprim**,** (**TOB):** Tobramycin**,** (**AK):** Amikacin**,** (**CIP):** Ciprofloxacin**,** (**TC):** Ticarcillin**,** (**CS):** Colistin**, (AUG):** Amoxicillin-Clavulanic acid**,** (**CRO):** Ceftriaxone.

Table (4): Antibiotic susceptibility test of ten *P. aeruginosa*

No.	Number of Isolate	IMI	CFP	TMP	TOB	AK	CIP	TC	CS	AUG	CRO	Percentage of Resistance
P1	P_7	R	R	R	R	R	S	R	R	R	R	**90%**
P2	P_{10}	R	R	R	R	R	S	R	R	R	R	**90%**
P3	P_{20}	R	R	R	R	R	R	R	R	R	R	**100%**
P4	P_{32}	R	R	R	R	R	R	R	R	R	R	**100%**
P5	P_{37}	R	R	R	R	R	S	R	S	R	R	**80%**
P6	P_{48}	R	R	R	R	R	S	R	S	R	R	**80%**
P7	P_{56}	R	R	R	R	R	R	R	R	R	R	**100%**
P8	P_{58}	R	R	R	R	R	S	R	R	R	R	**90%**
P9	P_{65}	R	R	R	R	R	S	R	R	R	R	**90%**
P10	P_{82}	R	R	R	R	R	R	R	R	R	R	**100%**

Antibiofilm activity of *Conocarpus erectus* methanolic extract:

The results of this study showed the *Conocarpus erectus* methanolic extract reduced the biofilm formation of *P. aeruginosa* at 6.25 mg/ml, and inhibited 80% and 100% of the biofilm formation at 25 and 50 mg/ml respectively, as shown in (**Table 5**).In this study, *P. aeruginosa* showed multidrug-resistant to common antibiotics used.

Table (5): Biofilm formation of *P. aeruginosa* clinical isolates before and after treatment with *Conocarpus erectus* methanolic extract

Isolates	Before treatment (Control)	After treatment concentrations (mg/ml)							
		1.562	3.125	6.25	12.5	25	50	100	200
P_1	Strong	Strong	Moderate	Weak	Weak	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	Strong	Strong	Moderate	Weak	No Biofilm	No Biofilm	No Biofilm	No Biofilm
P_4	Strong	Strong	Strong	Moderate	Weak	No Biofilm	No Biofilm	No Biofilm	No Biofilm
P_5	Strong	Moderate	Weak	No Biofilm	No Biofilm	No Biofilm	No Biofilm	No Biofilm	No Biofilm
P_6	Strong	Moderate	Weak	No Biofilm	No Biofilm	No Biofilm	No Biofilm	No Biofilm	No Biofilm
P_7	Strong	Strong	Strong	Moderate	Weak	Weak	No Biofilm	No Biofilm	No Biofilm
P_8	Strong	Strong	Moderate	Weak	Weak	No Biofilm	No Biofilm	No Biofilm	No Biofilm
P_9	Strong	Strong	Moderate	Weak	No Biofilm	No Biofilm	No Biofilm	No Biofilm	No Biofilm
P_{10}	Strong	Strong	Strong	Moderate	Weak	Weak	No Biofilm	No Biofilm	No Biofilm

Detection of *pelA* and *algD* Genes:

In this study, the PCR technique was used to detect the *pelA* and *algD* genes that are responsible for the biofilm formation in *P. aeruginosa*. The results showed that all isolates of *P. aeruginosa* contain *pelA* and *algD* genes, as shown in (Figures **3** and **4**).

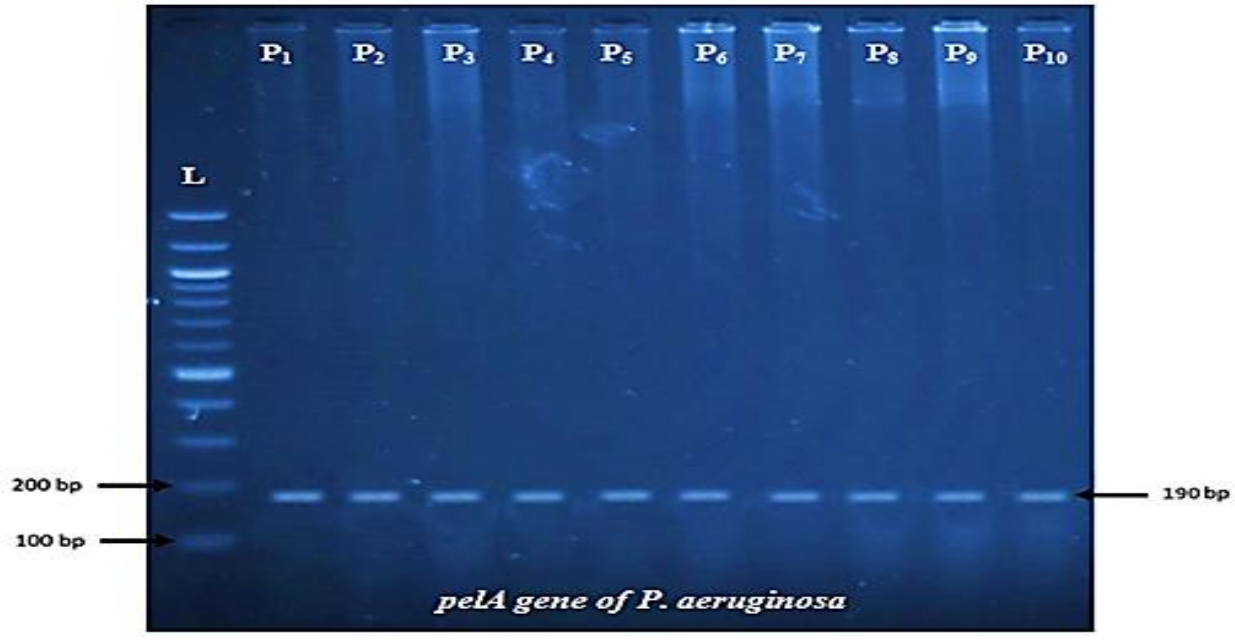

Figure (3): Gel electrophoresis of amplified *pelA* (118 bp), from *P. aeruginosa* using conventional PCR. Agarose 2% stained with Ethidium bromide dye DNA ladder 100-1500 bp and visualized on a UV transilluminator.

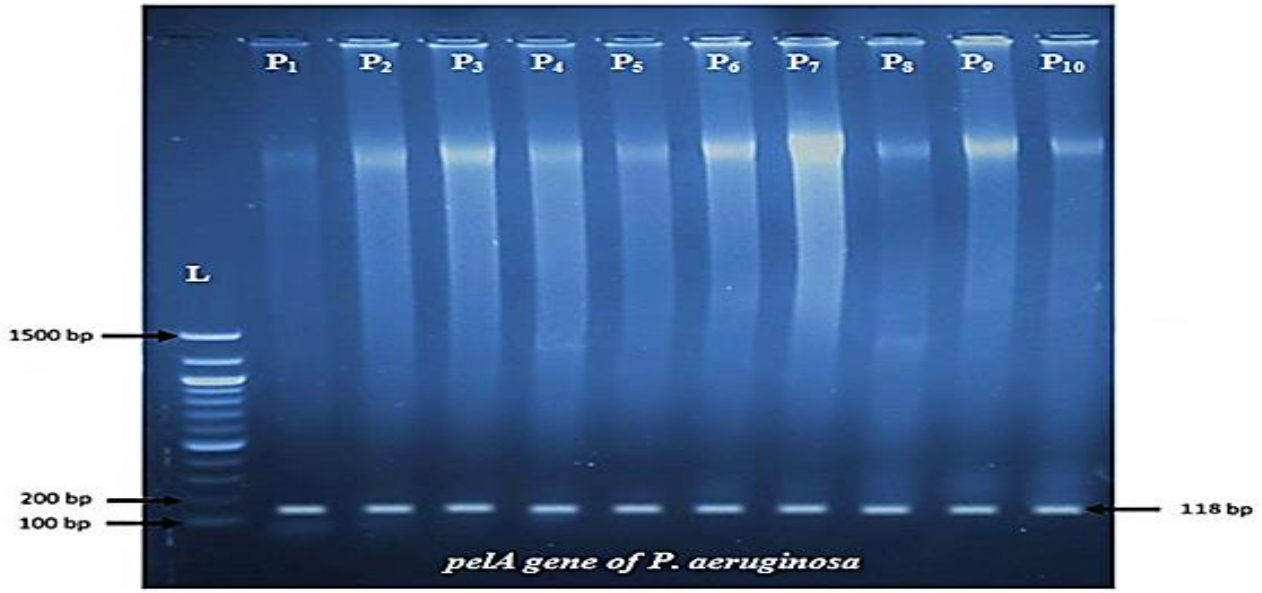

Figure (4): Gel electrophoresis of amplified *algD* (190 bp), from *P. aeruginosa* using conventional PCR. Agarose 2% stained with Ethidium bromide dye DNA ladder 100-1500 bp and visualized on a UV transilluminator.

Gene expression of *pelA* and *algD* genes:

The present study performed a quantitative RT-PCR test to evaluate mRNA expression of the pelA and algD genes in treated isolates by comparing them to untreated isolates and those treated with sub MIC doses of methanolic Laurus nobilis leaves extract. Results showed that expression of *pel*A and *algD* genes dropped (Tables **6** and **7**).

Table (6): Gene expression results for *pelA* before and after treatment with methanolic *Conocarpus erectus* methanolic extract

Groups	Samples	Ct gene 16s RNA	Ct target gene	Delta Ct	Delta Delta Ct	Folding
After treated	P_1	33.67	16.66	-17.01	3.9	0.0669
	P_2	31.76	26.25	-5.51	-3.62	0.0813
	P_3	36.36	22.61	-13.75	-6.71	0.00955
	P_4	31.66	37.32	5.66	7.77	0.004458
	P_5	21.02	39.93	18.91	14.74	0.00004
	P_6	29.71	32.94	3.23	11.55	0.000333
	P_7	20.73	18.22	-2.51	-1.43	0.3711
	P_8	34.99	32.98	-2.01	3.95	0.0647
	P_9	31.99	26.01	-5.98	-4.22	0.0536
	P_{10}	28.83	19.98	-8.85	1.14	0.453
Before treated (Control)	C_1	37.20	14.23	-22.97	0.00	1.00
	C_2	31.72	29.82	-1.89	0.00	1.00
	C_3	40.02	32.98	-7.04	0.00	1.00
	C_4	31.76	29.65	-2.11	0.00	1.00
	C_5	28.57	32.74	4.17	0.00	1.00
	C_6	38.62	30.29	-8.32	0.00	1.00
	C_7	34.09	33.01	-1.08	0.00	1.00
	C_8	44.05	38.09	-5.96	0.00	1.00
	C_9	36.77	35.01	-1.76	0.00	1.00
	C_{10}	44.98	34.99	-9.99	0.00	1.00

Table (7): Gene expression results for *algD* before and after treatment with *Conocarpus erectus* methanolic extract

Groups	Samples	Ct gene 16s RNA	Ct target gene	Delta Ct	Delta Delta Ct	Folding
After treated	P_1	30.60	33.64	3.04	0.48	0.71
	P_2	29.32	30.52	1.2	6.66	0.0098
	P_3	35.43	36.33	0.9	8.1	0.00364
	P_4	23.0	20.43	-2.57	-4.49	0.0445
	P_5	32.20	33.38	1.18	-6.62	0.1016
	P_6	32.33	30.15	-2.18	-3.32	0.1001
	P_7	22.33	21.03	-1.3	-0.21	0.6241
	P_8	39.29	40.7	1.41	-0.34	0.7900
	P_9	29.99	30.56	0.57	5.19	0.0273
	P_{10}	37.72	28.04	-9.68	-10.35	0.00076
Before treated (Control)	C_1	29.12	31.68	2.56	0.00	1.00
	C_2	38.46	33.00	-5.46	0.00	1.00
	C_3	42.62	35.42	-7.2	0.00	1.00
	C_4	21.01	2331	1.92	0.00	1.00
	C_5	20.42	28.22	7.8	0.00	1.00
	C_6	23.06	24.20	1.14	0.00	1.00
	C_7	26.6	25.51	-1.09	0.00	1.00
	C_8	32.39	34.14	1.75	0.00	1.00
	C_9	40.45	35.83	-4.62	0.00	1.00
	C_{10}	35.74	36.41	0.67	0.00	1.00

DISCUSSION

A procedure known as phytochemical screening can be used to extract bioactive molecules from plants, including flavonoids, alkaloids, carotenoids, tannins, and phenolic compounds [16,17]. This finding was in agreement with Findings that the aqueous extracts of Conocarpus erectus included active chemicals such as flavonoids, phenols, tannins, glycosides, and saponins but no alkaloids [18]. This outcome was consistent with finding that the fruit extract of Conocarpus erectus contained comparatively greater levels of phenolic components such gallic acid and quercetin [19]. Moreover, **Saadullah *et al.*** [20] reported flavonoid content of *Conocarpus lancifolius* methanol extracts from leaves and roots (Quercetin, Gallic acid, Chlorogenic acid, Caffeic acid, Hydroxybenzoic acid, Coumaric acid and Ferulic acid). The aqueous extract of *Conocarpus erectus* leaves was analyzed by Ultra-performance liquid chromatography, and it was found to contain 10 different major compounds. These compounds were as follows: caffeic acid, quercetin, chlorogenic acid, gallocatechin, pauciflorol A, myricetin, myricetin 3-glucoronide, apigenin-7-O-glucoside, fertaric acid, and discretine [21]. Having this skill is either innate or something they've picked up through mutations or lateral gene transfer [22]. *P. aeruginosa* is an opportunistic pathogen causing infections, especially in immune-compromised patients. It is the leading cause of nosocomial infections such as urinary tract infections, surgical site infections, pneumonia, bacteremia and septicaemia [23]. Drug resistance bacteria are responsible for increased cost, length of hospital stay and mortality [24]. Biofilm formation is a never-ending cycle in which bacterial colonies cling to a surface via extracellular polymeric substances (EPS) matrix [25]. Microorganisms create a close-knit community on a surface, either alive or nonliving, and release polymers to enclose themselves in a biofilm. Infections produced by biofilm-forming bacteria are notoriously difficult to treat due to the development of multidrug resistance [26]. The ability of *Pseudomonas aeruginosa* to produce biofilms gives it a major advantage over its planktonic cousin in its ability to tolerate the impacts of environmental stressors [27].

The antibiofilm efficacy of Conocarpus erectus leaf extracts appears to be related to the concentrations of phenolic compounds present in the extracts (Catechin, Gallic acid, Quercetin, Kaempferol, Taxifolin acid, and Rutin). For example, phenolic compounds can act as hydrogen ion carriers, any reducing agents that bind with adenosine triphosphate, which represents an oxidizing agent, and then stop the oxidation; or they can bind in active sites with the enzymes inside the cell and work to close them, making them unable to bind to the basic materials, thereby metabolically inhibiting the enzymes and nullifying their work [28]. There are matrix proteins, lipid vesicles, exopolysaccharides, and extracellular DNA all in the extracellular matrix that surrounds the

bacteria that live there (eDNA). There are three exopolysaccharides in the *P. aeruginosa* biofilm matrix (alginate, Pel and Psl) [(29)] .For *P. aeruginosa* to be fit, form a biofilm, infect the host, and become pathogenic, it needs to be able to release extracellular chemicals [(35)]. The results of the current study agreed with the result of **Elmaraghy *et al.*** [(30)] who detected *algD* and *pelA* genes in 42 isolates (89.4%). Moreover, revealed that all isolates had the *algD* gene and showed a high capacity of alginate biofilm formation which interfered with the response of the *P. aeruginosa* isolates to antibiotics [(25)]. Many researchers used plant extracts to study their effect on gene expression, **Pangastuti *et al.*** [(31)] used the ethyl acetate extract of *Curcuma aeruginosa rhizomes* and proved that the plant extract could decrease the expression of *P. aeruginosa* virulence factors that controlled by quorum sensing. Moreover, **Al-Bayati** [(32)] demonstrated that remarkable down regulation effect of *Ficus carica* extract on the *lasA* and *lasB* genes for 80% of *P. aeruginosa* isolates. Furthermore, the total flavonoids of *Potentilla Kleiniana* inhibited *P. aeruginosa* by damaging its cell membrane, reduced bacterial motility and consequently initial adhesion, and reduced the extracellular polymeric substances production and consequently inhibited biofilm maturation [(33)]. However, a number of studies have shown that using a variety of diverse methods as physical forces therapy against pathogenic bacteria, such as Nanoparticles[(34)] ,and audible sounds and magnetic fields, can assist to diminish the resistance of some pathogenic bacteria that produced a deadly infection [(35)].

CONCLUSION

Conocarpus erectus methanolic extracts had rich phytochemical contents, so, the presence of these phytochemicals contributes to medicinal as well as physiological properties in the treatment of different ailments. Therefore, the extract from this plant could be seen as a good source of valuable drugs. Furthermore, the *Conocarpus erectus* extract displays an antibiofilm agent on *P. aeruginosa*, which can down-regulation of the *algD* and *pelA* genes despite the bacterial isolates' resistance to antibiotics.

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Author contribution: Authors contributed equally in the study.

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