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Crude aqueous *Proteus mirabilis* extract with quorum sensing inhibitory activity can increase the susceptibility of multidrug resistant *Pseudomonas aeruginosa* to antimicrobials

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Abstract

Background: Suppression of quorum sensing (QS) that regulates many virulence factors, including antimicrobial resistance, in bacteria may subject the pathogenic microbes to the harmful consequences of the antibiotics, increasing their susceptibility to such drugs.

Aim: The current study aimed to make an aqueous crude extract from the soil *Proteus mirabilis* isolate with the use of the gas chromatography-mass spectrometry (GC-MS) technique for its analysis, and then, study the impact of the extract on clinical isolates of *Pseudomonas aeruginosa.*

Methods: Preparation of crude extracts from *P. mirabilis* (both organic and aqueous), which were then analyzed by GC-MS to detect the bioactive ingredients. Furthermore, the extract's capability to interfere with both the expression of the QS of *P. aeruginosa* and its antibacterial resistance was examined.

Results: The highest GC-MS peak (37.11%.) appeared for 1,3-benzodioxole, 4-methoxy-6-(2-propenyl), along with the presence of other components of antibacterial activities. When the aqueous extract was added to the culture of two multi-drug resistant (MDR) *P. aeruginosa,* a significant reduction in the expression of the QS regulatory gene *LasI* occurred*,* indicating its interference with QS. Moreover, upon adding the extract to the culture of *P. aeruginosa* (MDR) and then subjecting it to Amikacin and Colistin, already not effective on the bacteria, the isolates became more susceptible to these antibiotics showing zones of inhibition of 25 and 17 mm, respectively.

Conclusion: The crude aqueous extract of the soil *P. mirabilis* isolate might be a potential producer of QS inhibitors with antibacterial activities that render the MDR *P. aeruginosa* more susceptible to antibiotics to whom they already exerted resistance.

Keywords: Antibacterial activity, GC-MS analysis, *Pseudomonas aeruginosa, Proteus mirabilis* extract, Quorum sensing inhibition.

Introduction

The majority of *Pseudomonas aeruginosa* strains are motile, and they are rod-shaped, encapsulated, and non-spore-forming bacteria (Razook *et al.,* 2020). This is a multi-drug resistant (MDR) opportunistic pathogen that can cause acute or long-term infection in immunocompromised patients suffering from sepsis, burns, trauma, cancer, chronic obstructive pulmonary disease, and ventilator-associated pneumonia (del Mar Cendra and Torrents, 2021; Jurado-Martín *et al.,* 2021; Khudair and Mahmood, 2021; Rossi *et al.,* 2021). Because *P. aeruginosa* mutates quickly and adapts to become resistant to medicines, treating *P. aeruginosa* infections is quite challenging (Blomquist and Nix, 2021).

Additionally, due to its propensity to flourish on moist surfaces, *P. aeruginosa* is among the top-ranked microorganisms responsible for hospital-acquired infections, which are frequently seen in medical devices (ventilation) (Jangra *et al.,* 2022). The pathogen *P. aeruginosa* belongs to the MDR-ESKAPE group. *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *P. aeruginosa*, and *Enterobacter* are together referred to by the abbreviation ESKAPE. According to the World Health Organization, the bacterium *P. aeruginosa* is categorized as a "critical" pathogen since it is resistant to the antibiotic carbapenem. This means that there is a crucial demand for new medicines to cure infections caused by this bacterium in clinical settings (Daikos *et al.,* 2021). Epidemiological studies have demonstrated an annual mortality rate of around 700,000 individuals due to bacterial infections that show antibiotic resistance (Botelho *et al.,* 2019). Resistance

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to established antibiotics remains a persistent issue in hospital-acquired infections attributed to *P. aeruginosa* (Haque *et al.,* 2018; Al-Khazraji, 2023). This versatility in resistance mechanisms poses significant challenges in treating infections caused by *P. aeruginosa*, necessitating judicious antibiotic use and the improvement of novel therapeutic policies to combat its resistance (Breidenstein *et al.,* 2011).

Quorum sensing (QS) is a regulatory system that has a significant function in bacterial physiological processes. It regulates various characteristics, including the production of biofilms, resistance to antibiotics, and the virulence factors expression (Sethupathy *et al.,* 2016; Mohammed and Zgair, 2022). The QS system and its corresponding inhibitors offer pioneering approaches for the treatment of bacterial illnesses (Dong and Zhang, 2005; Kociolek, 2009). A wide range of QS inhibitors have been discovered in plants, fungi, actinomycetes, and macroalgae. These substances (QS inhibitors) involve halogenated furanone (C30) and extracts derived from garlic (Kociolek, 2009). Because they are easy to isolate and cultivate and have no detrimental effects on the environment or agricultural productivity, microbes are also an excellent source for the extraction of QS inhibitors (John, 2010). Furthermore, QS inhibitors have the potential to decrease the synthesis of bacterial virulence factors, thereby lowering the possibility of septic shock in the event of an infection (Dong and Zhang, 2005).

Metabolites from natural sources have been verified as effective agents of great importance against various microbes and their biofilms (Francis and Namasivayam, 2024), protecting against economically important insect pests (Sowmya and Namasivayam, 2024), along with preventing hypercholesterolemia (Kokila *et al.,* 2024). Bioactive compounds from natural sources have also antioxidant and antimicrobial activities in food applications and the food industry (Lavanya *et al.,* 2024). Furthermore, natural extracts showed inhibition of QS-mediated virulence factors produced by *P. aeruginosa* (Namasivayam *et al.,* 2020). In one study, the environmental isolate *Proteus mirabilis* was found to be a possible producer of QS inhibitory molecules (Yu *et al.,* 2018). However, the bioactive components of that extract were not analyzed in that investigation. For the purpose of analysis, the technique of gas chromatography-mass spectrometry (GC-MS) is well established and has been used routinely since the early 1980s (Biermann and McGinnis, 1988). It uses a tiny amount of plant extracts and is one of the greatest, fastest, and most accurate methods for identifying a wide range of substances, including alcohols, alkaloids, nitro compounds, long-chain hydrocarbons, organic acids, steroids, esters, and amino acids (Razack *et al.,* 2015; Shah and Abdul-Jalil, 2022). Additionally, it is utilized for analyzing the acquired extracts and can serve as a valuable instrument for quantifying the levels of specific active compounds found in the environment among other sources (Uma *et al.,* 2009). It is a method that integrates two distinct analytical techniques in order to analyze mixtures of chemical compounds. Gas chromatography is used to separate different components of a mixture, whereas mass spectroscopy is used to evaluate each of these components individually (Qinghua *et al.,* 2005). Thus, the current investigation aimed at analyzing the aqueous crude extract of *P. mirabilis* by GC-MS technique to detect the active ingredients of the extract that might harbor the antimicrobial effect, along with studying the molecular effects of the extract on suppressing the expression of the QS regulatory gene and its phenotypic alteration to the antibacterial resistance.

Materials and Methods

Bacterial isolates

Proteus mirabilis

An isolate of *P. mirabilis* was obtained from the study of Abdul Hussain and Lafta (2024). The isolate was confirmed by the Vitek-2 system.

Pseudomonas aeruginosa

In total, 8 isolates of *P. aeruginosa* obtained from Baqubah General Hospital, Baqubah, Iraq, were used in this study. Among the isolates, four were grown from cystic fibrosis patients; the other four were from burn wound infections. These bacteria were cultivated on different agar media, and many biochemical tests were used (data not shown). The assay of polymerase chain reaction (PCR) was applied for the identification of the clinical isolates of *P. aeruginosa* based on partial amplification of the *recA* gene. Specific primers were designed in this study using Prim3Plus software; their sequences are stated in Table 1. The oligonucleotides were provided in a lyophilized form by Macrogen Company (Korea).

The genomic DNA of *P. aeruginosa* isolates was extracted by the use of the gSYNC™ DNA Extraction Kit (Geneaid Biotech Ltd., Taiwan) following the procedure specified by the manufacturer. Totally, the volume of the PCR reaction was 25 µl, which included: 12.5 µl of the PCR Master mix (5×), 1 µl of each forward and reverse primer (10 µM), 5 µl of DNA samples, along with 5.5 µl of nuclease-free water. The components of the PCR reaction were then placed in the thermal cycler (Simpliamp, Singapore). First, optimization was done to choose the most appropriate annealing temperature for the primers. Then, the PCR program was set as follows: initial denaturation for one cycle of 1 minute at 95°C, and then 30 cycles of (denaturation at 95°C for 30 seconds, annealing for 30 seconds at 57°C, and extension for 30 seconds at 72°C), and one cycle of 7 minutes for the final extension at 72°C. Eventually, the PCR amplicons were analyzed on a 1% agarose gel stained with Ethidium bromide. No template control was also included in the last lane. Electrophoresis was done for 50 minutes at 65 V, and

Table 1. The nucleotide sequences of the primers specific to the recA gene.

Primer	Primer sequence (5′ to 3′)	Tm	Product size (bp)
Forward	ACTGCCTGGTCATCTTCATC	57.6	104
Reverse	CGAGGCGTAGAACTTCAGTG	58.7	

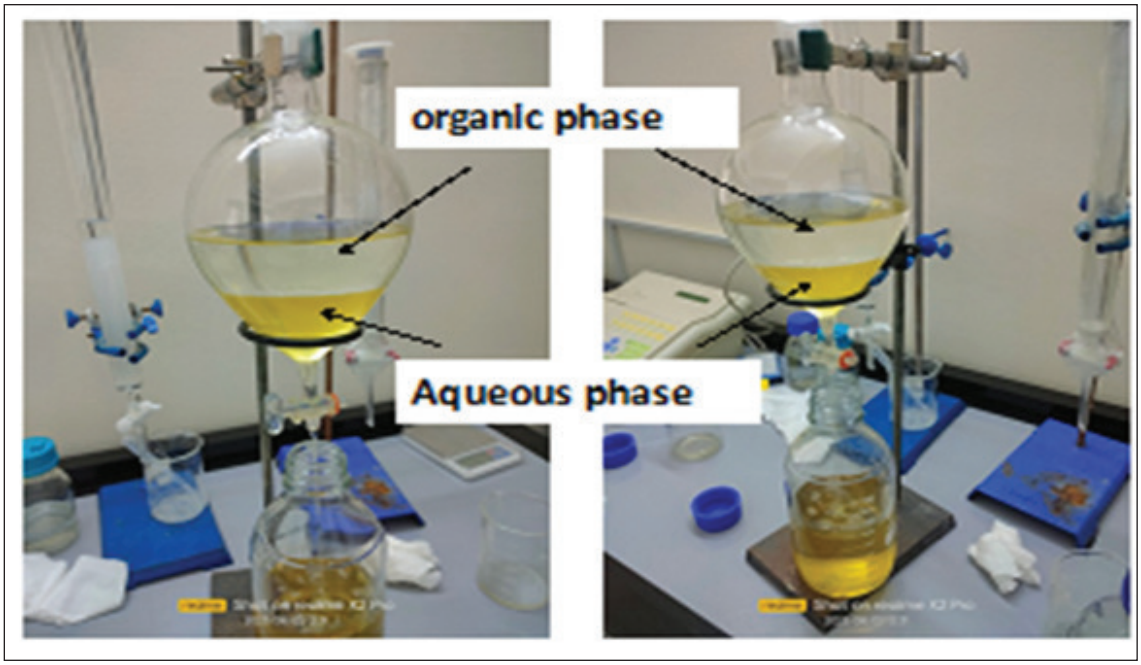

Fig. 1. Preparation of *P. mirabilis* extracts, including organic and aqueous phases.

the gel images were captured by a UV transilluminator connected to the camera (Clraver, UK).

Antibiotic susceptibility test

The Vitek-2 compact system using the AST-N222 card was applied, based on the instructions of the manufacturer, to check the susceptibility of the 8 clinical isolates of *P. aeruginosa* toward the antibiotics tested. The card contained 14 antibacterial agents, including: amikacin, gentamicin, tobramycin, ciprofloxacin, ticarcillin, colistin, ceftazidime, cefepime, imipenem, piperacillin, +mezlocillin, meropenem, piperacillin/tazobactam, and ticarcillin/clavulanic acid.

Preparation of *P. mirabilis* extracts

Organic and aqueous extracts were made from a culture of *P. mirabilis* according to the method of Dong and Zhang (2005). Briefly, a single colony from an overnight culture of *P. mirabilis* was inoculated into 1 l of Luria Bertani (LB) broth (Liofilchem, Italy) and subjected to incubation at a temperature of 37°C on a shaker incubator (Pars Azma, Iran) for a duration of 2 days. The culture was spun at 4°C for 15 minutes at a speed of 12,000 rpm. Then, after collecting the supernatant, it was filtered via a 0.22 µm membrane. Using an equivalent volume of ethyl acetate (Alpha Chemika, India), the resulting filtrate was extracted twice. The aqueous crude extract was assigned to the aqueous fraction subsequent to the extraction process, whereas the organic crude extract was assigned to the organic phase. Finally, the extracts were subjected to concentration by the use of a rotary evaporator (Buchi-R100, Switzerland) for the aqueous crude extract or by incubation at 40°C for two days in the case of the organic crude extract. Figure 1 shows the organic and aqueous phases of *P. mirabilis* extracts.

GC-MS analysis

Analysis of the aqueous *P. mirabilis* extract by GC-MS was performed by the Central Laboratory, Tehran University, Tehran, Iran. The analysis was conducted via the use of a 6,890 Gas Chromatograph (Agilent, US) connected to Mass Spectrometer model 5973N (Agilent, US). For this analysis, 30 m length, 0.25 mm inner diameter, and 0.25 µm resident layer thickness of an HP-5MS capillary column with a static phase of 5% methylphenyl siloxane were used. The GC Inlet Line's temperature was adjusted to 250°C, and a scan range of 20–600 m/z was applied. The ionization energy was 70 eV, and the carrier gas employed was helium (1.0 ml/min). Based on each component's retention index, identification was accomplished, and the supplier's MS

Table 2. The nucleotide sequences of the oligomers used in qRT-PCR.

Gene	Oligonucleotide sequence (5′ to 3′)	Tm (°C)	Amplicon size (bp)
LasI	F: 5′-CCGTAGGGGTGGAGAAGAT-3′	59	110
	R: 5′-ATTGAGTTCGATGCGCAAG-3′	60	
recA*	F: 5′- ACTGCCTGGTCATCTTCATC-3′	57.6	104
	R: 5′-CGAGGCGTAGAACTTCAGTG-3′	58.7	

*Means the same primer set was used in the conventional PCR for bacterial identification.

solution software assisted in controlling the system and data acquisition.

Impacts of the P. mirabilis aqueous extract on the QS regulatory gene of P. aeruginosa

RNA extraction and cDNA synthesis

Before the RNA extraction, two *P. aeruginosa* isolates (number 1 and 8) were grown separately in nutrient broth (Hi-Media, India), and each was treated with the aqueous *P. mirabilis* extract at sub-MIC at a concentration of 16 µg/ml. Then, the bacterial cells were harvested in a microcentrifuge tube by spinning at 13,000 rpm for 1 minute. This step was repeated to obtain enough amount of the cell pellet. The whole cell RNA was extracted from the bacterial cell pellets, either treated or not with the aqueous extract, by using the TRIzol™ RNA extraction kit (Thermo Fisher, USA), and the company's instructions were followed. The extracted RNA was stored in the freezer until use. For the cDNA synthesis, the extracted whole-cell RNA was treated with the cDNA ready-to-use kit (Bioneer, Korea). The synthesized cDNA was immediately employed as a template to study gene expression by quantitative real-time PCR (qPCR).

qPCR

The qPCR assay was used in this study to examine the potential impact of the aqueous *P. mirabilis* extract on the expression of the *P. aeruginosa lasI* gene, which is a QS regulator gene. For this reason, the qPCR reaction mixture was prepared from the following: 12.5 µl of 2×qPCR Master mix (Tinzyme, China), 2 µl of the cDNA samples with or without treatment with the aqueous extract, 1 µl (10 µM) of each forward and reverse primers (Table 2), as well as DNase-free distilled water up to 25 µl. Then, these components were mixed well, put in the qPCR equipment known as Exicycler™96 (Bioneer, Korea), and the qPCR reaction was performed. The setting of the qPCR program was as follows: initial denaturation (95°C, 10 minutes) for 1 cycle, then 40 cycles of denaturation (95°C, 20 seconds), annealing (60°C, 60 seconds), and extension (72°C, 60 seconds). Afterward, the qPCR reaction was pursued by analyzing the melt curve to verify the specificity of the amplified genes and also to exclude the presence of primer dimers or amplified gDNA contaminants.

To calculate the data of the qPCR assay, first, the Ct values of the target *lasI* gene and the reference or housekeeping *recA* gene were copied to the Excel sheet of Microsoft software. Then, the gene expression fold change was estimated based on the following equations: $\Delta Ct_{treatment} = Ct_{target} - Ct_{reference}$, $\Delta Ct_{control} = Ct_{target} - Ct_{reference}$, $\Delta\Delta Ct = \Delta Ct_{treatment} - \Delta Ct_{control}$, and Fold change in gene expression = $2^{-\Delta\Delta CT}$ (Schmittgen and Livak, 2008).

Effects of the *P. mirabilis* aqueous extract on the antibacterial response of *P. aeruginosa*

Two *P. aeruginosa* isolates (isolate no. 1 from cystic fibrosis and isolate no. 8 from burn wound) were cultivated either with or without the aqueous extract of *P. mirabilis* on Mueller-Hinton agar medium (Hi-Media, India). The bacterial suspension was prepared by mixing a few colonies of *P. aeruginosa* from a fresh culture with 5 ml of sterile normal saline to yield a suspension equivalent to McFarland tube no. 0.5 (1.5×10^8 CFU/ml). Simultaneously, 2 ml of the aqueous crude extract of *P. mirabilis* (32 µg/ml) was mixed with the same volume of bacterial suspension in a sterile tube. By using a sterile cotton swab, the bacterial suspension (with or without *P. mirabilis* extract) was spread in different directions on Mueller-Hinton agar and left for 10 minutes. Subsequently, the antimicrobial discs, including Colistin (CL, 10 µg), Ciprofloxacin (CIP, 5 µg), and Amikacin (AK, 10 µg), were picked by sterile forceps and pushed firmly at suitable distances on the agar surface to confirm their contact with the medium. After that, the plates were inverted and incubated at 37ºC for 24 hours. The next day, zones of inhibition were measured in millimeters by a ruler, and compared with the readings stated in CLSI (2020).

Statistical analysis

The data were initially subjected to one-way ANOVA to assess the presence of statistically significant differences among the group means. Tukey's HSD test was subsequently utilized to compare all possible pairs of means and identify significant differences among the groups at a significance level of $p < 0.05$.

Results

Identification of P. aeruginosa

All four cystic fibrosis isolates and the four burn wound bacteria were confirmed at the molecular level as *P. aeruginosa*, in which a band of approximately 104 bp as the expected size delineated by the designed primers appeared on the agarose gel (Fig. 2).

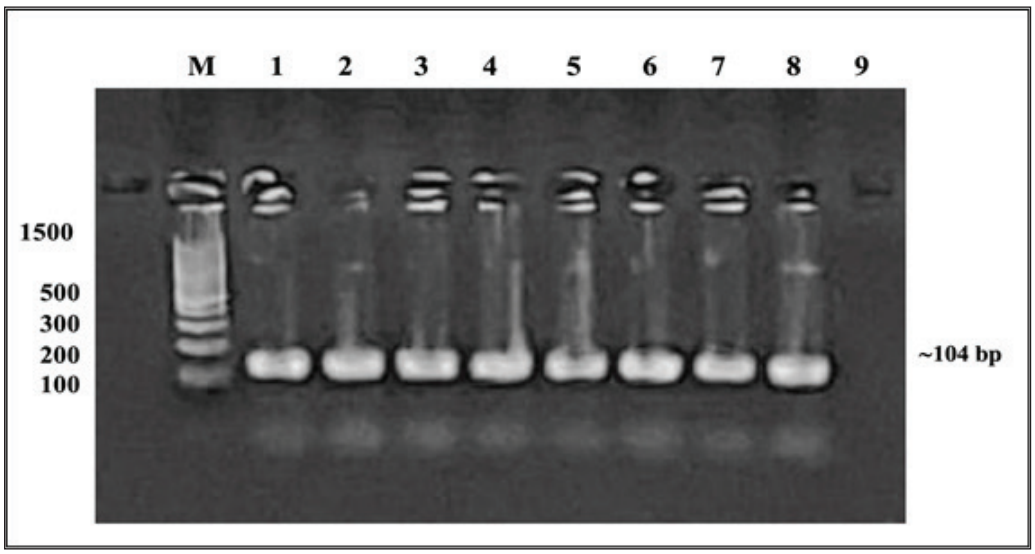

Fig. 2. Agarose gel (1%) shows DNA bands of roughly 104 bp of the partially amplified recA gene of *P. aeruginosa*. M: 100 bp DNA molecular size marker; lanes 1–4: DNA of cystic fibrosis isolates; lanes 5–8: DNA from burn wound isolates; lane 9: the negative control (without the PCR product). Electrophoresis was done at 65 V for 50 minutes.

Table 3. The antibacterial susceptibility of *P. aeruginosa* clinical isolates.

No.	Antimicrobial	*P. aeruginosa*							
		1	2	3	4	5	6	7	8
		MIC/In	MIC/In	MIC/In	MIC/In	MIC/In	MIC/In	MIC/In	MIC/In
1	Ticarcillin	32/S	32/S	32/S	32/S	32/S	32/S	≥128/R	≥128/R
2	Ticarcillin/ clavulanic acid	≤8/S	16/S	32/S	16/S	16/S	≤8/S	64/S	64/S
3	+ Mezlocillin	R	S	R	R	R	R	R	R
4	Piperacillin	8/R	≤4/S	16/R	8/R	8/R	16/R	16/R	8/R
5	Piperacillin/ Tazobactam	≤4/S	≤4/S	/	/	/	≤4/S	≤4/S	≤4/S
6	Ceftazidime	≥64/R	2/S	≥64/R	≥64/R	≥64/R	≥64/R	≥64/R	≥64/R
7	Cefepime	8/I	≤1/S	8/I	8/I	8/I	≥ 64/R	32/R	32/R
8	Imipenem	0.5/S	1/S	2/S	0.5/S	0.5/S	≥ 16/R	2/S	8/R
9	Meropenem	≤0.25/S	≤0.25/S	≤0.25/S	0.5/S	≤0.25/S	≤0.25/S	≤0.25/S	≤0.25/S
10	Amikacin	16/R	≤2/S	16/R	16/R	16/R	16/R	16/R	16/R
11	Gentamicin	4/S	≤1/S	4/S	4/S	4/S	4/S	4/S	4/S
12	Tobramycin	≥16/R	≤1/S	≥16/R	≥16/R	8/R	≥16/R	≥16/R	≥16/R
13	Ciprofloxacin	2/R	≤0.25/S	2/R	2/R X	1/R	2/R	2/R	1/R
14	Colistin	≥16/R	1/S	≥16/R	≥16/R	≥16/R	≥16/R	≥16/R	≥16/R

MIC: Minimum Inhibitory Concentration; In: Interpretation; R: Resistant; S: Susceptible. All the numbers above refer to µg/ml MIC values.

Antibiotic susceptibility of the isolates

The antibiotic susceptibility of eight isolates of *P. aeruginosa* is demonstrated in Table 3 below. Most of the isolates were MDR. For instance, isolate no. 8 showed resistance to 10 out of 14 antimicrobial agents, followed by isolates no. 6 and 7, which were resistant to 9 out of 14 antibacterial agents. While isolates no. 1, 3, 4, and 5 showed resistance to seven

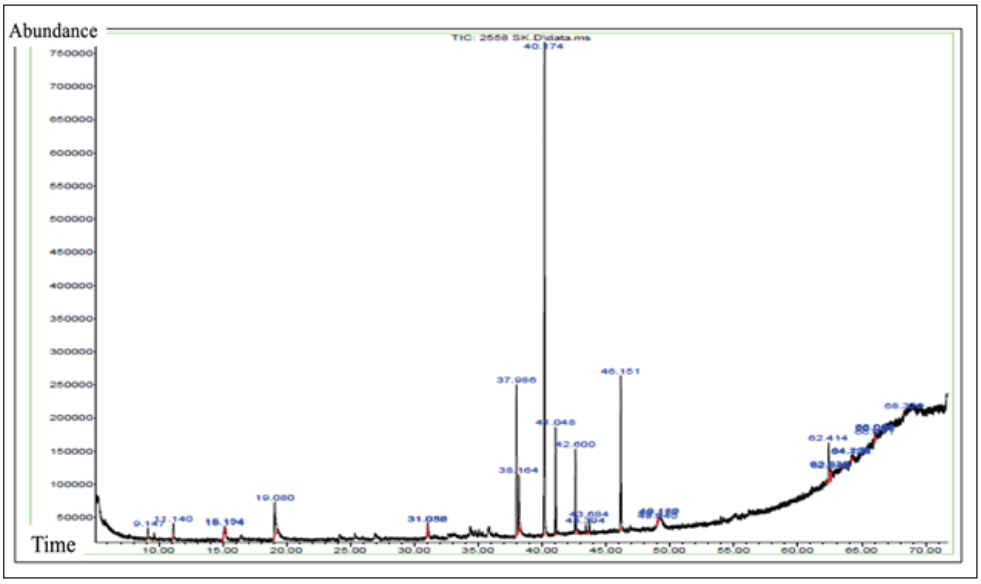

Fig. 3. GC-MS chromatogram of the aqueous *P. mirabilis* extract. *X* axis represents time; *Y* axis is the abundance.

drugs. Nevertheless, isolate no. 2 was susceptible to all of the tested antibiotics. The isolates, regardless of whether they were obtained from burned or cystic fibrosis patients, shared a common resistance profile. In particular, these isolates revealed resistance to each of +mezlocillin, piperacillin, ceftazidime, amikacin, tobramycin, ciprofloxacin, and colistin (Table 3).

Extracts of P. mirabilis

Organic and aqueous extracts were made successfully from a culture of *P. mirabilis*. The aqueous extract was tuned into a powder form with a net weight of 320 mg, while the organic phase was made as a pasty texture in a Petri dish and weighed roughly 1.3 g.

The GC–MS analysis

The GC-MS chromatogram of the aqueous *P. mirabilis* extract yielded 27 peaks, each of which corresponded to a particular bioactive compound (Fig. 3). The active components with their molecular weights (MWs), retention time (RT), concentration (peak area %), and their nature are presented in Table 4. The main compound present in the extract was 1,3-benzodioxole, 4-methoxy-6-(2-propenyl), whose peak area was 37.11%. Additionally, apiol and 1,3-benzodioxole, 4,7-dimethoxy-5-(2-propenyl) also appeared in another area of 12.96%. A third large peak in an area of 10.54% was occupied by dodecane, 1-chloro- and 2-propenamide, and 2-methyl-N-phenyl. Another peak area of 7.98% belonged to benzene, 1,2,3-trimethoxy-5-(2-propenyl). Further compounds were present but in smaller amounts, such as benzene, 1,2,3,4-tetramethoxy-5-(-propenyl), which appeared in the area of 6.56%. Cyclotetradecane, 1-tetradecanol, and 1-dodecanol occurred at a peak area of 5.52%. At the peak area of 4.76%, there were octadecanoic acid and 1H-imidazole-2-methanol, 1-decyl. The compounds succinic acid, 4-methoxy-2-methylbutyl pentadecyl ester, and succinic acid, heptyl pentyl ester, occurred at a peak area of 4.65%. Moreover, other compounds in smaller areas of 1% or less occurred too. Furthermore, numerous substances with antibacterial activities also occurred in the aqueous extract in very small amounts, such as amino acid derivatives (1.09% of N-methyl-dl-leucine), flavonoids (0.64% of 2H-1-benzopyran, 2,2-diphenyl-), quinolines (0.22% of benzo[h]quinoline, 2,4-dimethyl-), and another small peak area of 0.07% of benzo[h]quinoline, 2,4-dimethyl (quinoline).

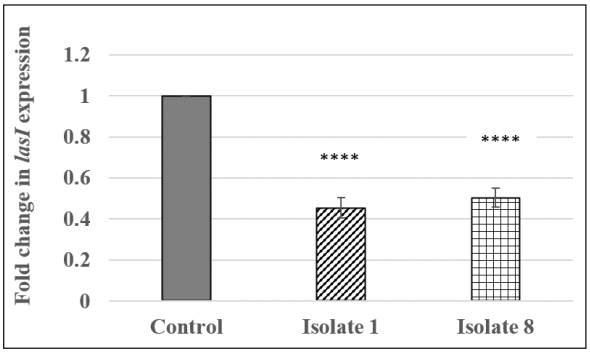

Fig. 4. Fold change in the gene expression of *LasI* of *P. aeruginosa* (isolates 1 and 8) following treatment with the aqueous extract of *P. mirabilis* compared to the negative control (without treatment). Statistical significance was analyzed by one-way ANOVA, and a significance of $p < 0001$ is indicated by ****.

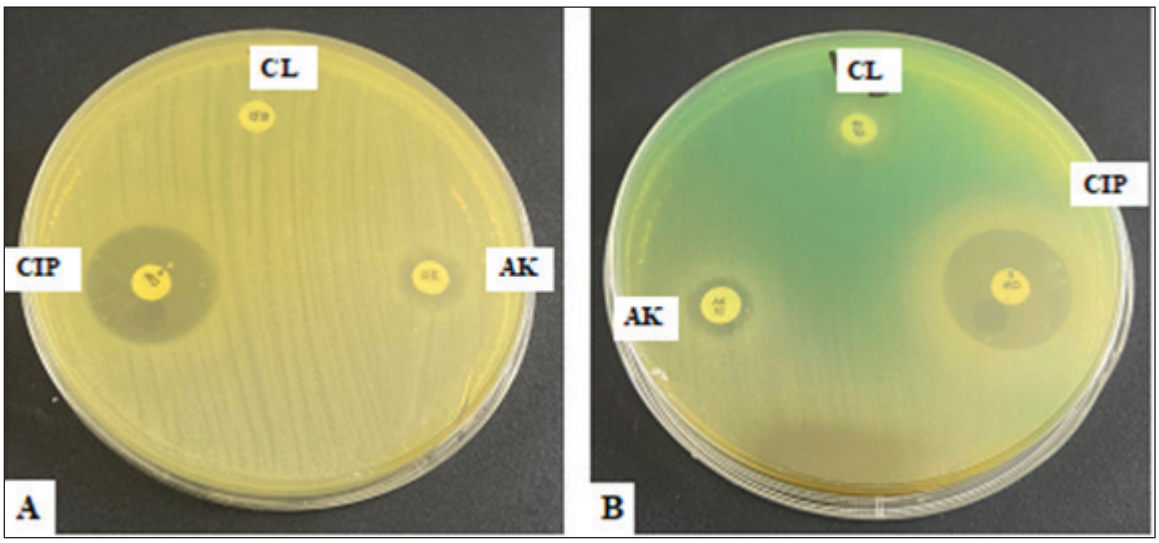

Fig. 5. Antibacterial susceptibility test of *P. aeruginosa*, without treatment with the aqueous extract of *P. mirabilis*, toward Colistin (CL, 10 µg), Ciprofloxacin (CIP, 5 µg), and Amikacin (AK, 10 µg). (A) *P. aeruginosa* isolate no. 1 from cystic fibrosis. (B) *P. aeruginosa* isolate no. 8 from a burn wound.

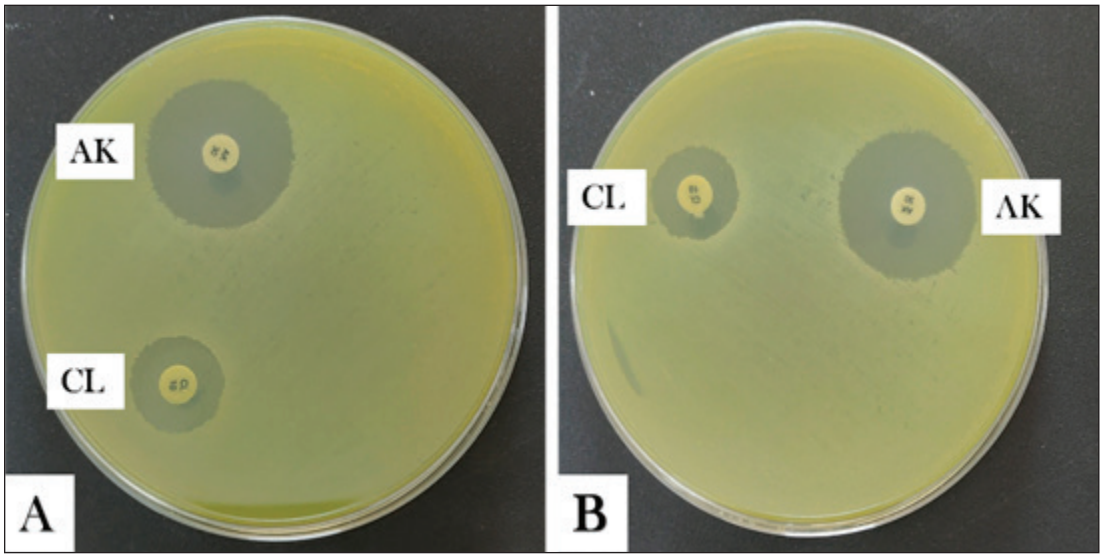

Fig. 6. Antibacterial susceptibility test of *P. aeruginosa*, treated with the aqueous extract of *P. mirabilis*, toward Colistin (CL, 10 µg) and Amikacin (AK, 10 µg). (A) *P. aeruginosa* isolate no. 1 from cystic fibrosis. (B) *P. aeruginosa* isolate no. 8 from a burn wound.

Impacts of the P. mirabilis extract on the expression of the QS regulatory gene

The qPCR technique analyzed the gene expression of *recA* and *lasI* of *P. aeruginosa* with or without treatment with the aqueous extract of *P. mirabilis*. Treatment with the aqueous extract of *P. mirabilis* had the capacity to reduce the expression of the QS gene *LasI* in isolates no. 1 and 8 of *P. aeruginosa* compared to the negative control (Fig. 4).

The one-way ANOVA indicated statistically significant differences ($p < 0.0001$) in gene expression among the three groups (i.e., the control, isolate 1, and isolate 8). Tukey's HSD test demonstrated significant differences ($p < 0.0001$) in gene expression between the control group and both isolate 1 and isolate 8 for both comparisons. However, there was no significant difference ($p = 0.2877$) between isolate 1 and isolate 8.

Table 4. Compounds identified in the aqueous extract of *P. mirabilis* by GC-MS.

Peak no.	The compound's name	MW (g/mol)	RT	Peak area %	The compound's nature
1	(2E)-2-Methyl-2-Pentenal	98.14	9.145	1.16	Aldehyde
	2-Pentenal, 2-methyl-				
2	1,2-Ethanediamine, N, N-bis(1-methylethyl)-	186.34	11.140	1.14	Diamines
	Thiazole, 2-amino-5-methyl-	114.17			Heterocyclic compounds
	2(1H)-Pyridinone-1,3-d2, 4-(hydroxy-d)-	97.12			Pyridinones
3	2,4,6-Trimethylboroxin	125.5	15.152	0.59	Boron compound
	2-ethyl-4-methylthiophene	126.22			Thiophenes
	Ethanone, 1-(2-thienyl)-	126.18			Ketones
4	2-Ethyl-5-Methylthiophene	126.22	15.175	0.13	Thiophenes
5	Succinic acid, 4-methoxy-2-methylbutyl pentadecyl ester	428.6	19.079	4.65	Ester compound
	Succinic acid, heptyl pentyl ester	286.41			
6	N-Methyl-dl-leucine	145.20	31.035	1.09	Amino acid derivative
	(E)-2-Methyl-2-penten-1-ol	100.16			Alcohol
	1H-Indole-3-carboxylic acid, 6-bromo-5-methoxy-1-methyl-2-(4-morpholinylmethyl)-, 2-(dimethylamino)ethyl ester	575.9			Ester derivative
7	Isobutyramide, N-isobutyl-	129.20	31.058	0.74	Amide compound
	Propargyl ethyl sulfide	100.18			Alkyl sulfides
	2-Propanamine, N-methyl-N-(1-methyl ethyl)-	115.21			Amines
8	Dodecane, 1-chloro-	204.78	37.985	10.54	Chloroalkane
	2-Propenamide, 2-methyl-N-phenyl-	281.4			Amide
9	Cyclotetradecane	196.37	38.162	5.52	Cycloalkane
	1-Tetradecanol	214.39			Alcohols
	1-Dodecanol	186.33			Fatty alcohol
10	1,3-Benzodioxole, 4-methoxy-6-(2-propenyl)-	192.21	40.174	37.11	Heterocyclic aromatic compounds
11	Benzene, 1,2,3-trimethoxy-5-(2-propenyl)-	208.25	41.048	7.98	Phenylpropanes
12	Benzene, 1,2,3,4-tetramethoxy-5-(-propenyl)-	238.27	42.603	6.56	Phenylpropanes
13	2-Methyl-1-dodecene	182.35	43.391	0.76	Alkenes
	1-Tridecene				
	6-Tridecene				
14	Carotol	222.37	43.683	1.11	Sesquiterpenoids
	3a(1H)-Azulenol, 2,3,4,5,8,8a-hexahydro-6,8a-dimethyl-3-(1-methylethyl)-, [3R (3.alpha.,3a.alpha.,8a.a				
	(+)-Calarene Or (+)-.Beta.-Gurjunene				
15	Apiol	222.24	46.152	12.96	Phenylpropanoids
	1,3-Benzodioxole, 4,7-dimethoxy-5-(2-propenyl)-				

Continued....

Peak no.	The compound's name	MW (g/mol)	RT	Peak area %	The compound's nature
16	2-Oxo-4-nitrosomethyl-6-trifluoro-methyl-1,2-dihydropyrimidine	221.13	49.038	0.15	Pyrimidines
	1,2,3,4-Tetrahydro-1,7-dimethyl-6H-[1,2,4] triazino[4,3-b][1,2,4,5]te trazin-6-one	193.24			Heterocyclic compounds
	2-(1-Piperidino)-5-nitropyridine	217.18			
17	N-(1,1-Dimethyl-3-oxobutyl)-2-methylazetidine	169.22	49.118	0.20	Amides
	Fumaric acid, 2-ethylhexyl 8-chlorooctyl ester	374.94			Esters
	4a(2H)-Naphthalenol, octahydro-a-dimethyl-, [4S-(4.alpha.,4a.alph a.,8a.beta.)]-	182.3025			Terpenoids
18	Palmitic Acid, N-Octyl Ester	368.6	49.181	0.13	Fatty acid esters
	4a(2H)-Naphthalenol, octahydro-a-dimethyl-, [4S-(4.alpha.,4a.alph a.,8a.beta.)]-	182.3025			Terpenoids
	1,4'-Bipiperidine	212.29			Piperidines
19	Octadecanoic acid	284.5	62.412	4.76	Fatty acids
	1H-imidazole-2-methanol, 1-decyl-	222.37			Heterocyclic compounds
20	2H-1-Benzopyran, 2,2-diphenyl-	284.3	62.532	0.64	Flavonoids
	Acetic acid, [4-(1,1-dimethylethyl)phenoxy]-, methyl ester	188.34			Esters
21	Adamantane-1-(3,3-dichloropropyn-yl)	128.98	62.589	0.72	Substituted adamantanes
	1-(3,3-Dichloro-1-Propynyl)Adamantane	243.17			
	Ethanone, 2-(2-benzothiazolylthio)-1-(3,5-dimethylpyrazolyl)-	303.4			Ketones
22	Pyrido[2,3-d]pyrimidine, 4-phenyl-	207.23	64.195	0.24	Heterocyclic compounds
	2-(4-tert-Butylbenzyl)-1,3-propanediol	224.77			Alcohols
23	2',4'-Dimethyloxanilic Acid N' Veratrylidenehydrazide	144.21	64.224	0.04	Hydrazone
	Indolizine, 2-(4methylphenyl)-	207.27			Heterocyclic compounds
	1H-Indole, 1-methyl-2-phenyl-	235.28			Indoles
24	1,3-dimethyl-4 azaphenanthrene	207.27	66.013	0.22	Polycyclic aromatic compound
	Benzo[h]quinoline, 2,4-dimethyl-	207.2704			Quinolines
	1-methyl-4-phenyl-5-thioxo-1,2,4-triazolidin-3-one	207.25			Thiazolidinones
25	Pyrido[2,3-d]pyrimidine, 4-phenyl-	207.23	66.053	0.50	Heterocyclic compounds
	Thymol, TBDMS derivative	222.3987			Terpenoids
26	2',4'-Dimethyloxanilic Acid N' Veratry lidenehydrazide	144.21	66.093	0.30	Hydrazides
	6-(4-Chloro-Phenyl)-5-Nitro-Piperidin-2-One	445.9			Amides
	Acetic acid, [4-(1,1-dimethylethyl)phenoxy]-, methyl ester	188.34			Esters
27	2',4'-Dimethyloxanilic Acid N' Veratry lidenehydrazide	144.21	68.327	0.07	Hydrazides
	1H-Indole, 1-methyl-2-phenyl-	235.28			Indoles
	Benzo[h]quinoline, 2,4-dimethyl-	207.2704			Quinolines

RT = Rtention time.

Impact of the P. mirabilis aqueous extract on the antibiotic susceptibility

Prior to the aqueous extract treatment, *P. aeruginosa* was resistant to amikacin and colistin (Fig. 5). However, *P. aeruginosa* became more susceptible to these antibiotics upon mixing these isolates with the extract of *P. mirabilis*. A zone of inhibition of 25 mm was seen against Amikacin and 17 mm in the case of Colistin (Fig. 6).

Discussion

In the current study, eight *P. aeruginosa* isolates (four from cystic fibrosis and the others from burn wound patients) were included and confirmed by the molecular technique (PCR) based on the partial amplification of the *recA* gene. This gene codes for the RecA protein, which is involved in repairing DNA damage, particularly double-strand breaks, by catalyzing the exchange of DNA strands between homologous sequences (Del Val *et al*., 2019). When studying the antimicrobial susceptibility of the clinical isolates, this research showed that most of the isolates were multidrug resistant (MDR). This finding agrees with many researchers, who concluded that *P. aeruginosa* has multi-resistance toward a wide range of antibiotics, especially beta-lactam antibiotics (Mohanty *et al*., 2021; Ahmed *et al*., 2022). Gaining resistance is common and occurs in bacterial cells either via mutational changes or the acquirement of resistance genes through horizontal gene transfer, and the acquired resistance is highly contributing to the development of MDR strains. This, in turn, augments the complexity of eliminating this microorganism and results in increasing cases of permanent infections (Munita and Arias, 2016).

Among the isolates included in this study, isolates no. 1 and 8, in particular, were chosen for later experiments as they showed resistance to 7 and 10 antibiotics, respectively. Then, an aqueous crude extract of the soil bacterium *P. mirabilis* was made in this study, and its bioactive compounds were identified by the GC-MS analysis. The GC-MS analysis uncovered the occurrence of different groups of composites, including esters, alcohols, alkanes, amides, ketones, aldehydes, terpenoids, heterocyclic compounds, and other derivatives, in the soil bacterial extract. The foremost compound found in the bacterial extract is 1,3-benzodioxole, 4-methoxy-6-(2-propenyl) whose peak area is 37.11%. This compound is also known as myristicin, which is broadly dispersed in the plant kingdom and belongs to the apiole group of compounds, which are known for their synergistic activity. The compound was separated and identified from the hexane portion of Piper mullesua inflorescence alcoholic extract. It demonstrated effective larvicidal efficacy toward fourth-instar larvae of Spilarctia obliqua within 24 hours when applied topically (Srivastava *et al*., 2001). According to Tisserand and Balacs (1994), it was utilized as an antioxidant, anti-inflammatory, and antidepressant. It has also been observed that the chemical myristicin is larvicidal against the Aedes aegypti mosquito *(*Marston *et al*., 1995*)*.

The second major component present in the extract of the present study is phenylpropanoids, including apiol, as well as 1,3-benzodioxole and 4,7-dimethoxy-5-(2-propenyl) (12.96%). Other phenylpropanes are also present in the bacterial extract; these are represented by benzene, 1,2,3,4-tetramethoxy-5-(-propenyl) (6.56%), and 1,2,3-trimethoxy-5-(2-propenyl) (7.98%). Phenylpropanoids are specific compounds that are often found to have a role in plants' defense against both living and non-living threats. They can act as a chemical or physical barrier to stop invasions, take part in plant communication that triggers defense responses, or act as a direct toxic weapon against microorganisms or insects (Ramaroson *et al*., 2022). Their mechanism of action on microbes and insects likely stems from disrupting crucial cellular machinery and structures (Ramaroson *et al*., 2022). Importantly, some chemical compounds found in the bacterial extract of the current study, especially myristicin and apiol, are also present in the essential oil of parsley [*Petroselinum crispum* (Mill) Fuss] (Linde *et al*., 2016). In addition to its bactericidal action against all tested bacteria, primarily *S. aureus*, parsley essential oil had bacteriostatic activity against all tested bacteria, primarily *S. aureus*, *Listeria monocytogenes*, and *Salmonella enterica*. Moreover, this essential oil demonstrated fungicidal and fungistatic efficacy against every tested fungus (Linde *et al*., 2016). The third chief compound occurring in the aqueous extract of *P. mirabilis* in this study is dodecane, 1-chloro-, which is chloroalkane, and it also belongs to alkyl halides (Wiraswati *et al*., 2023). The compound 1-chloro-dodecane was found in Spongia officinalis var ceylonensis extract and demonstrated anti-proliferative properties on three various cancer cell lines (Ka and Tr, 2021). Moreover, the plant *Breynia cernua* contains many compounds, including dodecane and 1-chloro-. This medicinal plant shows promise as a possible source of novel plant-derived medications for the treatment of various ailments (smallpox, cervical cancer, breast cancer, and wounds) (Wiraswati *et al*., 2023). Dodecane, 1-chloro-has also been isolated from *Streptomyces* spp. and exerted antibacterial and bioactive action (Al-Rrubaye *et al*., 2020).

Importantly, the aqueous extract of the soil bacterium used in the present research contained quinolines (e.g., benzo[h]quinoline, 2,4-dimethyl-) and flavonoids (e.g., 2H-1-benzopyran, 2,2-diphenyl-). Concerning quinolones, they are among the earliest types of artificial antibacterial substances, possessing a wide range of effectiveness against many types of bacteria (Moussaoui *et al*., 2020). Quinolones have the ability to hinder the activity of two specific enzymes, topoisomerase IV and DNA gyrase, which ultimately prevent bacterial DNA replication (Hooper, 1999).

These enzymes can be observed in Gram-positive and Gram-negative bacteria. Based on the Quinolone used for the medication as well as the host, either DNA gyrase or topoisomerase IV can be chosen as the main target in the treatment (Pham *et al*., 2019). Regarding flavonoids, they are generally present in flowers, seeds, vegetables, fruits, nuts, stems, tea, wine, propolis, and honey (Cushnie and Lamb, 2005). For ages, preparations comprising these chemicals as primary physiologically active ingredients have been utilized for curing human ailments (Cushnie and Lamb, 2005). Flavonoids with antifungal, antiviral, and antibacterial properties have garnered attention in anti-infective research, leading to their isolation and structural identification by several research groups. Numerous excellent studies have looked into the relationship between flavonoids' structural make up and possible antibacterial capabilities (Cushnie and Lamb, 2005).

In addition, succinic acid, 4-methoxy-2-methylbutyl pentadecyl ester, and succinic acid, heptyl pentyl ester were detected in the bacterial extract of this investigation at 4.65%. Both compounds are esters derived from succinic acid, which might possess biological activity, including antimicrobial properties, among others (Huang *et al*., 2022). Succinic acid might act by damaging the structure of bacterial cell membrane as well as disrupting the intracellular structure leading to increased leakage of the cellular components and reducing the cell size. The findings of the aforementioned authors imply that succinic acid can be exploited in the food industry due to its ability to regulate *S. aureus* and *Pseudomonas fluorescens* contamination (Huang *et al*., 2022). Other minor elements present in the *P. mirabilis* extract, ranging from 1.16% to 0.04%, are worth mentioning for their known antimicrobial activity. Among the minimal components found in the *P. mirabilis* extract is carotol. Carotol has been reported to exhibit antimicrobial effects versus various fungi and bacteria, and its mechanism of action may involve disruption of microbial membranes and interference with microbial metabolism (Mahboubi *et al*., 2019). Furthermore, thymol, one of the terpenoids, is also present in the extract of this study. It has been demonstrated to damage microbial cell membranes and reduce the activity of microbial enzymes, and it has been extensively investigated for its antimicrobial properties, particularly against bacteria and fungi (Nagoor Meeran and Prince, 2012).

In the current research, *P. aeruginosa* (the MDR isolates 1 and 8) treated with the *P. mirabilis* crude aqueous extract at sub-MIC showed decreased expression of the QS regulatory gene, *LasI,* using the sensitive technique qPCR. This means that the extract successfully interfered with the expression of the QS-regulated gene at the molecular level. These results are in harmony with those observed in the study of Yu and his colleagues (Yu *et al*., 2018), in which the filtrate of *P. mirabilis* isolated from soil significantly inhibited QS production by *P. aeruginosa*. This finding was further confirmed phenotypically in the current study when the pyocyanin producer isolate of *P. aeruginosa,* particularly isolate no. 8 from a burned patient, was treated with the sub-MIC of the *P. mirabilis* aqueous extract, complete disappearance of the greenish dye of *P. aeruginosa* occurred compared with the same isolate not treated with the extract. Importantly, when the same MDR isolates of *P. aeruginosa* were treated with the extract and then subjected to Amikacin and Colistin, they became susceptible to these antibiotics with zones of inhibition of 25 mm against Amikacin and 17 mm in the case of Colistin. These findings agree with what is stated in CLSI (2020), where *P. aeruginosa* showing inhibition zones of 11–17 mm are considered susceptible to Colistin 10 µg. Pertaining to Amikacin 30 µg, zones of 18–26 mm are considered susceptible according to CLSI, (2020). However, in this investigation, a disk of 10 µg Amikacin, which is three times lower than the amount tested in CLSI, was used and exerted a zone of 25 mm. Compared with another study, the aqueous extract of *P. mirabilis* has been shown to exert antagonistic effects on QS-regulated phenotypes, such as the resistance to antibiotics, mainly Kanamycin (Yu *et al*., 2018). Overall, the soil bacterium extract applied in this investigation has a powerful effect against pathogenic clinical isolates of *P. aeruginosa.*

Conclusion

Pseudomonas aeruginosa, an opportunistic pathogen, is notorious as MDR, producing several acute and long-term infections in those with impaired immune systems. It is well-known that the treatment against *P. aeruginosa* infections is very difficult owing to its quick mutations and the rise of resistance to antibiotics. Therefore, studies are needed to attempt new therapeutic targets or antibiotic adjunct agents that can prevent or decrease bacterial resistance to the current drugs. Thus, this study was successful in rendering the MDR clinical *P. aeruginosa* isolates to be more susceptible to the tested medications (amikacin and colistin). This occurred due to mixing *P. aeruginosa* isolates (MDR) with the crude aqueous extract of the soil *P. mirabilis* isolate. This study is the first to analyze the extract of the *P. mirabilis* soil isolate by GC-MS, which showed the existence of many components with antibacterial activity. Even the compounds found at low percentages in the extract had antimicrobial activities. This means that the crude extract of the soil bacterium might be much more effective than using purified extracts. The data here support the role of the extract as a QS inhibitory agent that had the capacity to decrease the *LasI* gene expression (the QS regulatory gene) at the molecular level. However, one limitation of the study is examining the effect of the extract on two isolates only of *P. aeruginosa*, but not the eight isolates used in this study. Additionally,

the effect of the extract on various virulence factors and other QS genes regulating the pathogenicity of *P. aeruginosa* was not covered. Further future studies are needed to study more characteristics of the extract, such as its interference with other virulence factors genotypically and phenotypically and its impact on QS of other bacteria not only *P. aeruginosa*. Moreover, it is necessary to extend the work on the extract by doing *in vivo* evaluation in laboratory and field animals.

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Conflict of interest

The researchers declare no conflict of interest is present.

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Authors' contributions

IJL put the concept and design of the study and wrote the manuscript. SRA collected the required data and performed the experiments. All authors revised and approved the final manuscript.

Data availability

All data are included in this research article.

References

Abdul Hussain, S.R. and Lafta, I.J. 2024. Quorum sensing inhibitors as potential mitigators interfering with *Pseudomonas aeruginosa* pathogenicity. Ph.D. Dissertation, Department of Microbiology/College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq.

Ahmed, N.M., Baraka, A., Kabbashi, A.S., Mohammed, S.E., Arbab, M. and Mubara, A.K. 2022. Antimicrobial activity of Lepidium Sativum against multi-drug resistant and sensitive *Pseudomonas aeruginosa*: a microbiological study from Khartoum State, Sudan. Al-Kindy Col. Med. J. 18(2), 96–100.

Al-Khazraji, S.F.R. 2023. Study on tssC1 gene mediating biofilm antibiotics resistance of *Pseudomonas aeruginosa*. Iraqi J. Sci. 64(9), 4356–4365.

Al-Rrubaye, T.S., Risan, M.H. and Al-Rubaye, D. 2020. Gas chromatography-mass-spectroscopy analysis of bioactive compounds from Streptomyces spp. isolated from Tigris river sediments in Baghdad city. J. Biotechnol. Res. Center. 14(1), 63–71.

Biermann, C.J. and McGinnis, G.D. 1988. Analysis of carbohydrates by GLC and MS. Boston, MA: CRC press.

Blomquist, K.C. and Nix, D.E. 2021. A critical evaluation of newer β-lactam antibiotics for treatment of *Pseudomonas aeruginosa* infections. Ann. Pharmacother. 55(8), 1010–1024.

Botelho, J., Grosso, F. and Peixe, L. 2019. Antibiotic resistance in *Pseudomonas aeruginosa*–mechanisms, epidemiology and evolution. Drug Resist Updates. 44, 100640.

Breidenstein, E.B.M., de la Fuente-Núñez, C. and Hancock, R.E.W. 2011. *Pseudomonas aeruginosa*: all roads lead to resistance. Trends Microbiol. 19(8), 419–426.

CLSI. Clinical and Laboratory Standards Institute. 2020. Performance standards for antimicrobial susceptibility testing. 30th ed. Wayne, PA: CLSI Supplement M100.

Cushnie, T.P.T. and Lamb, A.J. 2005. Antimicrobial activity of flavonoids. Int. J. Antimicrob. Agents. 26(5), 343–356.

Daikos, G.L., da Cunha, C.A., Rossolini, G.M., Stone, G.G., Baillon-Plot, N., Tawadrous, M. and Irani, P. 2021. Review of ceftazidime-avibactam for the treatment of infections caused by *Pseudomonas aeruginosa*. Antibiot. 10(9), 1126.

del Mar Cendra, M. and Torrents, E. 2021. *Pseudomonas aeruginosa* biofilms and their partners in crime. Biotechnol. Adv. 49, 107734.

Del Val, E., Nasser, W., Abaibou, H. and Reverchon, S. 2019. RecA and DNA recombination: a review of molecular mechanisms. Biochem. Soc. Trans. 47(5), 1511–1531.

Dong, Y.H. and Zhang, L.H. 2005. Quorum sensing and quorum-quenching enzymes. J. Microbiol. 43(spc1), 101–109.

Francis, A.L. and Namasivayam S.K.R. 2024. Combatting microbial biofouling network associated with Chembarambakkam lake water using Acalypha indica derived metabolites. Environ. Qual. Manag. 34(2), e22270.

Haque, M., Sartelli, M., McKimm, J. and Bakar, M.A. 2018. Health care-associated infections–an overview. Infect. Drug Resist. 11, 2321–2333.

Hooper, D.C. 1999. Mode of action of fluoroquinolones. Drugs 58, 6–10.

Huang, S., Chen, X., Yan, R., Huang, M. and Chen, D. 2022. Isolation, identification and antibacterial mechanism of the main antibacterial component from pickled and dried mustard (Brassica juncea Coss. Var. foliosa Bailey). Mol. 27(8), 2418.

Jangra, V., Sharma, N. and Chhillar, A.K. 2022. Therapeutic approaches for combating *Pseudomonas aeruginosa* infections. Microbes Infect. 24(4), 104950.

John, J.E. 2010. Natural products as lead-structures: a role for biotechnology. Drug Discov. Today. 15(11–12), 409–410.

Jurado-Martín, I., Sainz-Mejías, M. and McClean, S. 2021. *Pseudomonas aeruginosa*: an audacious pathogen with an adaptable arsenal of virulence factors. Int. J. Mol. Sci. 22(6), 3128.

Ka, A.K. and Tr, K. 2021. Cultivable bacterial symbionts from the marine sponge with biological activity. Biomed. J. Sci. Tech. Res. 36(3), 28635–28646.

Khudair, A.N. and Mahmood, S.S. (2021). Detection of the antiseptic resistance gene among *Pseudomonas aeruginosa* isolates. Iraqi J. Sci. 62(1), 75–82.

Kociolek, M.G. 2009. Quorum-sensing inhibitors and biofilms. Antiinfect. Agents Med. Chem. 8(4), 315–326.

Kokila, V., Namasivayam, S.K.R. and Amutha, K. 2024. Hypocholesterolemic potential of *Bacillus amyloliquefaciens* KAVK1 modulates lipid accumulation on 3T3-L1 adipose cells and high fat diet-induced obese rat model. World J. Microbiol. Biotechnol. 40, 206.

Lavanya, M., Namasivayam, S.K.R. and John, A. 2024. Developmental formulation principles of food preservatives by nanoencapsulation—fundamentals, application, and challenges. Appl. Biochem. Biotechnol. In Press, doi: 10.1007/s12010-024-04943-1.

Linde, G.A., Gazim, Z.C., Cardoso, B.K., Jorge, L.F., Tešević, V., Glamočlija, J., Soković, M. and Colauto, N.B. 2016. Antifungal and antibacterial activities of *Petroselinum crispum* essential oil. Genet. Mol. Res. 15(3), 15038538.

Mahboubi, M., Mahdizadeh, E. and Tabar, R.H. 2019. Chemical composition and antimicrobial activity of *Fraxinus excelsior* L. seeds essential oil. Jundishapur J. Nat. Pharm. Prod. 14(1), :e61105>

Marston, A., Hostettmann, K. and Msonthi, J.O. 1995. Isolation of antifungal and larvicidal constituents of *Diplolophium buchanani* by centrifugal partition chromatography. J. Nat. Prod. 58, 128–130.

Mohammed, H.A. and Zgair, A.K. 2022. Detection of quorum sensing genes of *Pseudomonas aeruginosa* isolated from different areas in Iraq. Iraqi J. Sci. 63(11), 4665–4673.

Mohanty, S., Baliyarsingh, B. and Nayak, S.K. 2021. Antimicrobial resistance in *Pseudomonas aeruginosa*: a concise. Antimicrob. Resist. One Health Perspect. 49, 177–192.

Moussaoui, O., Bhadane, R., Sghyar, R., El Hadrami, E.M., El Amrani, S., Ben Tama, A., Rodi, Y.K., Chakroune, S. and Salo-Ahen, O.M.H. 2020. Novel amino acid derivatives of quinolines as potential antibacterial and fluorophore agents. Sci. Pharm. 88, 57.

Munita, J.M. and Arias, C.A. 2016. Mechanisms of antibiotic resistance. Microbiol. Spectr. 4(2), 481–511.

Nagoor Meeran, M.F. and Prince, S.M.P. 2012. Protective effects of thymol on altered plasma lipid peroxidation and nonenzymic antioxidants in isoproterenol-induced myocardial infarcted rats. J. Biochem. Mol. Toxicol. 26(9), 368–373.

Namasivayam, S.K.R., Jerry Angel, J., Arvind Bharani, R.S. and Valli Nachiyar, C. 2020. Terminalia chebula and Ficus racemosa principles mediated repression of novel drug target Las R—the transcriptional regulator and its controlled virulence factors produced by multiple drug resistant *Pseudomonas aeruginosa*—biocompatible formulation against drug resistant bacteria. Microb. Pathog. 148, 104412.

Pham, T.D., Ziora, Z.M. and Blaskovich, M.A. 2019. Quinolone antibiotics. Medchemcomm, 10, 1719–1739.

Qinghua, S., Yangmin, M. and Huqiang, Q. 2005. Preliminary study on chemical composition and insecticidal activity of root bark from Periploca sepium. Acta Agric. Bor. Sin. 14(6), 141–144.

Ramaroson, M.L., Koutouan, C., Helesbeux, J.J., Le Clerc, V., Hamama, L., Geoffriau, E. and Briard, M. 2022. Role of phenylpropanoids and flavonoids in plant resistance to pests and diseases. Mol. 27, 8371.

Razack, S., Hemanth Kumar, K., Nallamuthu, I., Naika, M. and Khanum, F. 2015. Antioxidant, biomolecule oxidation protective activities of *Nardostachys jatamansi* DC and its phytochemical analysis by RP-HPLC and GC-MS. Antioxid. 4(1), 185–203.

Razook, B.R.F., Al-ani, A.N. and Mahmood, M.M. 2020. Hematological picture of rabbits immunized with *Pseudomonas aeruginosa*. Iraqi J. Vet. Med. 44(E0), 64–68.

Rossi, E., La Rosa, R., Bartell, J.A., Marvig, R.L., Haagensen, J.A.J., Sommer, L.M., Molin, S. and Johansen, H.K. 2021. *Pseudomonas aeruginosa* adaptation and evolution in patients with cystic fibrosis. Nat. Rev. Microbiol. 19(5), 331–342.

Schmittgen, T.D. and Livak, K.J. 2008. Analyzing real-time PCR data by the comparative CT method. Nat. Protoc. 3(6), 1101–1108.

Sethupathy, S., Prasath, K.G., Ananthi, S., Mahalingam, S., Balan, S.Y. and Pandian, S.K. 2016. Proteomic analysis reveals modulation of iron homeostasis and oxidative stress response in *Pseudomonas aeruginosa* PAO1 by curcumin inhibiting quorum sensing regulated virulence factors and biofilm production. J. Proteom. 145, 112–126.

Shah, S.M. and Abdul-Jalil, T.Z. 2022. Qualitative and quantitative estimation or chemical constituents from leaves and roots of Iraqi Agave Attenuata by GC-MS and RP-HPLC (Conference Paper). Iraqi J. Pharm. Sci. 31(Suppl.), 75–85.

Sowmya, R. and Namasivayam S.K.R. 2024. Pesticidal activity of extra cellular metabolites extracted from the green muscardian fungi Metarhizium anisopliae. Environ. Qual. Manag. 34(1), e22235.

Srivastava, S., Gupta, M.M., Prajapati, V., Tripathi, A.K. and Sushil Kumar. 2001. Insecticidal activity of Myristicin from *Piper mullesua*. Pharm. Biol. 39(3) 226–229.

Tisserand, R. and Balacs, T. 1994. Nutmeg: A review of the toxicology and pharmacology of nutmeg and its essential oil. Int. J. Aromather. 6, 28–38.

Uma, B., Prabhakar, K., Rajendran, S. and Lakshmi Sarayu, Y. 2009. Studies on GC/MS spectroscopic analysis of some bioactive antimicrobial compounds from Cinnamomum zeylanicum. J. Med. Plants. 8(31), 125–131.

Wiraswati, H.L., Fauziah, N., Pradini, G.W., Kurnia, D., Kodir, R.A., Berbudi, A., Arimdayu, A.R., Laelalugina, A., Supandi and Ma'ruf, I.F. 2023. *Breynia cernua*: chemical profiling of volatile compounds in the stem extract and its antioxidant, antibacterial, antiplasmodial and anticancer activity in vitro and in silico. Metabolites 13(2), 281.

Yu, S., Zhu, X., Zhou, J. and Cai, Z. 2018. Biofilm inhibition and pathogenicity attenuation in bacteria by *Proteus mirabilis*. R. Soc. Open Sci. 5(4), 170702.