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Exploring the medicinal potential of the mangrove plant *Phoenix paludosa*: Insights into bacterial anti-biofilm and anti-quorum sensing activities

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Abstract
Due to arisen of antimicrobial resistance as a global concern, there is an urgent need for innovative therapeutic approaches. The project aims to isolate and characterize pure constituent(s) from mangrove plants *Phoenix paludosa* leaves, for evaluating their bacterial anti-biofilm and anti-QS activities, a novel therapeutic approach for tackling multidrug resistance bacteria. The crude ethanolic extract, ethyl acetate and water fractions showed significant biofilm inhibition of *Pseudomonas aeruginosa* (75.3%, 80.6% & 71.8% inhibition) respectively at the dose of 4mg/ml. In anti-QS activity test, significant percentages of pyocyanin inhibition 77.8%, 70.7% & 69.7% were observed for the above fractions (71.6%, 74.6% and 76.9% inhibition respectively). Pure compound, Trimyristin was isolated from the bioactive ethyl acetate fraction which demonstrated significant biofilm (58.6%), pyocyanin (53.4%), and swarming motility (63.1%) inhibition. This study highlights the potential of *P. paludosa* leaves as a source for developing novel antibacterial based on anti-QS and anti-biofilm mechanism.

Keywords: *Phoenix paludosa*, anti-QS, anti-biofilm, swarming motility, GC-MS, Trimyristin

1. Introduction
Antibiotic resistance is a complex and multifaceted phenomenon that occurs when bacteria and other microorganisms evolve the ability to withstand the effects of antibiotics, rendering these drugs less effective or completely ineffective [1]. The inappropriate use of antibiotics, such as taking antibiotics when they are not needed or not completing a full course of antibiotics, can promote the survival of antibiotic-resistant bacteria. In addition to this, the common misuse in this scenario involves the over prescription of antibiotics for viral infections [2]. Therefore, it is now a growing global health concern where bacteria develop resistance against the antibiotics used to treat infections [3]. This phenomenon can result in a significant threat to public health, as it can lead to the failure of antibiotic treatments, more severe and prolonged infections, and increased healthcare costs [4].

A biofilm is a structured community of microorganisms, primarily bacteria that are embedded in a self-produced matrix of extracellular polymeric substances (EPS). These communities can attach to various surfaces, such as medical devices, pipes, teeth, or living tissues [5]. Within a biofilm, individual bacterial cells work together and are protected by the EPS matrix. This protection can make biofilm-associated bacteria more resistant to antibiotics, the immune system, and other antimicrobial agents [6]. Biofilms can be associated with various problems, including infections (e.g., medical device-related infections, chronic wounds, and dental plaque), biofouling of industrial equipment, and the corrosion of surfaces [7]. Antibiofilm agents aim to disrupt biofilm formation or enhance the susceptibility of biofilm-embedded bacteria to antibiotics. It can also prevent the initial attachment of bacteria to surfaces, interfere with biofilm maturation, or disrupt the biofilm matrix, allowing antibiotics to penetrate and target bacterial cells more effectively [8]. This strategy (antibiofilm) also improves the treatment outcomes for infections, reduced microbial contamination in industrial settings, and a more sustainable approach to managing biofilm-related challenges and thus way contribute to a broader understanding of microbial ecology and the development of innovative solutions for biofilm-associated problems [9]. Quorum sensing (QS) is a communication process used by

bacteria and some other microorganisms to coordinate behavior within a population. It allows bacteria to synchronize their behavior based on the local population density [10]. It is particularly important for pathogenic bacteria in the context of infection, as it enables them to express virulence factors or coordinate attacks on host organisms when their numbers are sufficient for a successful infection. For QS, signaling bacteria secrete different types of pigments are synthesized such as pyocyanin, pyoverdine, green fluorescent protein (GFP) etc. [11]. The synthesis of pyocyanin is primarily controlled by QS process which enhances the bacterial ability to resist antibiotics and becomes capable of surviving adverse conditions. Therefore, inhibition of pyocyanin is identified as a potential anti-microbial strategy [12]. Inhibition of QS, or biofilm formation, is typically a non-violent technique where bacteria are not physically destroyed; instead, their internal communication network that makes them harmful or resistant is disrupted. Nevertheless, these strategies represent innovative avenues for addressing antibiotic resistance and improving the effectiveness of current antibacterial treatments. Bacterial swarming motility is a collective bacterial behavior where groups of bacteria move together in a coordinated manner over solid surfaces. This phenomenon is characterized by the rapid and coordinated movement of bacterial cells across surfaces, creating spreading patterns that are distinguishable from individual bacterial motility [13]. Several bacteria exhibit swarming motility; therefore, specific mechanisms and regulation of swarming motility can vary between bacterial species. Therefore, understanding bacterial swarming motility is important in the study of bacterial ecology, pathogenesis, and biofilm formation and to find out effective treatment without minimal side effects. The Sundarbans, a mangrove forest located in the southern part of Bangladesh, harbors a rich array of plants with diverse ecological adaptive capabilities, largely influenced by its high salinity. This distinctive ecological niche may unveil new avenues for the discovery of novel drug leads [14]. *P. paludosa* Roxb, locally referred to as Hantal, is a thorny palm belonging to the Aracaceae family, commonly found in the Sundarbans with slender stems reaching heights of 6 to 7 meters. Phoenix genus is recognized for its medicinal significance, possessing diuretic, analgesic, ameliorative, antioxidant, and anti-mutagenic properties [15]. In the evaluation of its properties, ethanol extracts from the leaves and stems of this plant were scrutinized for their antioxidant, antidiabetic, and antibacterial activities [16]. In spite of having interesting medicinal values, till date no scientific report is available on this plant about bacterial QS and biofilm inhibitory potential (mechanistic study of antibacterial property). In pursuit of our objectives, we endeavored to carry out this project where we focused on scientifically validating the targeted antimicrobial properties and conducting phytochemical isolation and profiling (via GC-MS) of the above-mentioned plants' extract.

2. Materials and Methods

2.1 Sample collection and preparation of crude extract

After collecting the leaves from the mangrove regions of the Sundarbans in Khulna, specialists at the Bangladesh National Herbarium in Mirpur, Dhaka, identified it as *P. paludosa* where a voucher specimen (specimen number DACB 34176) was then submitted for future reference. After drying (shade), leaves were pulverized into coarse powder. 500 gm of *P. paludosa* powder was placed in clean, flat-bottomed glass containers, and it was soaked in 1500 ml of 96% ethanol. The mixture was kept away for 14 days, with occasional shaking and stirring. Subsequently, the solution was filtered using Whatman filter paper [17]. Following the evaporation of the filtrates, the resulting concentrates, with a yield of 3.84%, were recognized as crude ethanolic extracts derived from the leaves of *P. paludosa*.

2.2 Liquid-liquid partitioning of crude extract

A separating funnel was loaded with 10g of crude ethanolic extract of *P. paludosa*, 400 mL of water, and an equivalent amount of *n*-hexane for solvent-solvent partitioning. After shaking and stirring, the phases were allowed to fully separate. Nonpolar molecules, more soluble in *n*-hexane than water, dissolved in the top layer collected as *n*-hexane, while the bottom layer contained water. The water layer was transferred to a new funnel and the process repeated using dichloromethane and ethyl acetate [18]. The water fraction was dried with a Labocon freeze-dryer (Model: LFD-BT-104), and a rotary evaporator removed organic solvents from each fraction. Yields of dried extracts were recorded as 29% (ethyl acetate), 17% (dichloromethane), 25% (n-hexane), and 19% (water), and these fractions were further used in microbiological research.

2.3 Microorganisms and preparation of inoculum

Microorganisms, obtained from a lyophilized *P. aeruginosa* culture (ATCC 9027, Square Pharmaceuticals Ltd.), were revived in nutrient broth. Following overnight incubation at 37 °C, the bacterial culture produced a clean sample after 15 to 18 hours. Centrifugation at 4,000 rpm for 5 minutes and re-suspension in 20 ml double-distilled water, repeated until the supernatant cleared, preceded spectrophotometric assessment. Serial dilutions were conducted until the optical density reached 0.6 at 600 nm [19].

2.4 Preparation of sample solution (plant extract)

30 mg and 60 mg extracts were dissolved in 13 mL of nutrient broth media using 2 mL of DMSO each, resulting in solutions with concentrations of 2 mg/mL and 4 mg/mL, respectively, in 96-well plate. Additionally, blank (DMSO) was also tested for the microbial assay to assess any potential impact of DMSO alone on bacterial viability.

2.5 Preparation of standard drug solution (Furanone 30)

A standard solution was prepared by dissolving 4 mg of furanone-30 compound in 13 mL of nutrient broth media with the assistance of 2 mL of DMSO. This resulted in a final concentration of 0.2667 mg/ml for the standard solution.

2.6 Assessing of minimum inhibitory concentration (MIC)

The MIC of *P. paludosa* leaf extract was determined following CLSI (Clinical and Laboratory Standards Institute) guidelines (document M26-A) using the broth dilution method [20]. Microorganism density was adjusted to 5×10^5 CFU/mL in Mueller-Hinton Broth (MHB). Cell suspensions (100 µL) were inoculated into 96-well plates with extract concentrations ranging from 0.05 to 25 mg/mL. The essential oil, dissolved in DMSO and serially diluted 2-fold in MHB, served as the test substance. Controls included negative (bacteria + MHB), positive (bacteria + MHB + extract), vehicle (bacteria + MHB + DMSO), and media (MHB). The MIC value was determined after incubation.

2.7 Biofilm inhibition assays: Following the experimental protocol, control, standard, and samples (2 mg/ml and 4

mg/ml) were placed in a 96-well plate with bacterial suspension. The plate was sealed and incubated at 37 ºC for 24 hours, after which the liquid suspension was removed using a Pasteur pipette. Adding a 100 µl solution of 1% w/v crystal violet, the mixture was stained at room temperature for 30 minutes. After dye removal, the wells were washed twice with milli-Q water. Then, 125 µl of 30% acetic acid was added, and the mixture was stained for an additional 30 min. Transferring the solutions to another 96-well plate, absorbance was measured at 570 nm using a microplate reader. The assay was repeated three times, and the averaged absorbance readings for each concentration were calculated. The average of the media control was subtracted, and the result was divided by the mean absorbance of the control, multiplied by 100 [21].

% of inhibition = [(OD in control -OD in treatment)/ OD in control] x 100

2.8 Pyocyanin inhibition assay
Pyocyanin quantification was carried out through the chloroform extraction method [22]. Plant extracts with maximum MIC were applied to *P. aeruginosa* cultures in triplicate, incubated for 3 and 5 days. Falcon tubes containing 4.5 ml sterilized nutrient broth and 0.5 ml plant extract (below MIC) were prepared, along with control tubes. After incubation, the cultures were centrifuged, and the supernatant was mixed with chloroform. The resulting blue layer (chloroform + pyocyanin) was separated, mixed with HCl, and spectrophotometric measurements at 520 nm were taken. Pyocyanin concentration (µg/ml) was calculated by multiplying the OD value at 520 nm by 17.072.

2.9 Swarming motility Test
Agar media was poured into nine petri dishes, and after cooling, 2.5 µl of bacterial suspension was added to the center of each dish. Three dishes were assigned to the standard, control, and test groups, respectively. Nutrient broth, standard solution, and test extracts (2.5 µl each) were added to their corresponding marked dishes. The sealed plates were then incubated at 37 °C, and distances from the agar plate center were measured using slide calipers after 3 and 5 days [23]. Inhibition of swarming motility was calculated by -
% of inhibition = [(DT by control -DT by treatment)/ DT in control] x 100

2.10 Phytochemical tests on fractionated crude extracts
The *n*-hexane, dichloromethane, ethyl acetate, and water fractions of *P. paludosa* leaves were qualitatively tested *in-vitro* to identify major phytoconstituents using standard protocols and reagents [24, 25].

2.11 Isolation of bioactive compound
Ethyl acetate fraction from the ethanolic extract of *P. paludosa* was subjected to chromatographic separation because in the microbiological test (anti-biofilm and anti-QS), this fraction showed more potent activity than the other fractions. 2 gm of bioactive ethyl acetate fraction were dissolved in a minimal amount of methanol. After sonication for lump removal, small amount of silica gel powder was added to the liquid extract. The solvent was evaporated using a rotary evaporator, and the extract was subsequently adsorbed on silica gel. Upon series of attempts and optimizing the mobile phases, a single spot (2.3 mg) was isolated successfully through column chromatography with a R_f value of 0.51 in the mobile phase (dichloromethane: n-hexane =9:1) [26].

2.12 GC-MS analysis
GC-MS analysis was conducted at Jashore University of Science and Technology, Bangladesh, using a T Clarus 690 gas chromatography and Clarus SQ 8C mass spectrometer by PerkinElmer. One microliter of the extract was injected in splitless mode with an inlet temperature of 250 °C. The oven temperature was programmed from 60 °C to 240 °C at 5 °C/min for 4 min, resulting in a total run time of 40 min. Helium gas at a constant flow rate of 1.0 mL/min was used as the carrier gas. The interface transfer line temperature was set at 280 °C, and MS detection was in scan mode with a Quadrupole analyzer temperature of 230 °C and ion source temperature of 150 °C. Electron ionization (EI) mode at 70eV was employed, and the scan time and mass ranges were 1s and 50-600 m/z, respectively. Compound identification was performed by comparing spectral data with the National Institute Standard and Technology Library database [27].

2.13 Statistical analysis
Microsoft Excel and Student's unpaired t-test (GraphPad Prism software, version 5.0; San Diego, CA, USA) were used for the statistical assessment. All experimental results were compared to the blank control group, with significance defined as $p < 0.05$.

3. Results
3.1 Biofilm inhibition
The plant demonstrated notable anti-biofilm activity, particularly in the ethyl acetate fraction showing higher average percentages of biofilm inhibition (80.61%). The ethanolic extract, *n*-hexane, dichloromethane, water fractions, and isolated pure fraction exhibited average biofilm inhibition percentages of 75.3%, 43.3%, 33.2%, 71.8%, and 58.6%, respectively, compared to the standard inhibition (86.7%) at 4 mg/ml. Dose dependent biofilm inhibition was also observed, where higher dose (4 mg/mL) showed better activity than the lower dose (2 mg/mL), presented in Table 1. The isolated pure fraction also displayed significant anti-biofilm activity, highlighting the potential antibacterial benefits of *P. paludosa* leaves extract.

Table 1: Absorbance of the treatments as well as % of biofilm inhibition of *P. paludosa* and standard group (Fu-30) against *P. paludosa*

Treatment groups		**% Biofilm inhibition at different doses and replication**							
Standard (Furanone 30)/ Dose 0.3mg/mL						**87.9**	**86.4**	**85.7**	**86.71±0.02**
Test extracts		**Dose: 2 mg/mL**				**Dose: 4 mg/mL**			
		Rep1	**Rep2**	**Rep3**	**Avg ± SD**	**Rep1**	**Rep2**	**Rep3**	**Avg ± SD**
P. paludosa	EtOH crude extracts	65.84	64.37	63.95	64.72±0.01*	78.52	70.52	75.98	75.34±0.02**
	Fr. *n*-hexane	33.63	36.56	36.65	35.61±0.02	41.29	43.50	45.27	43.35±0.03
	Fr. CH_2Cl_2	24.56	20.23	24.68	23.16±0.03	33.25	33.87	32.73	33.28±0.01
	Fr. EtOAc	72.81	71.76	70.31	71.62±0.03**	81.13	79.55	81.15	80.61±0.04***
	Fr. H_2O	65.45	68.37	63.39	65.73±0.02*	70.47	69.25	75.74	71.82±0.01**
	Pure fraction	45.92	43.54	42.13	43.86±0.05	58.64	55.92	61.37	58.64±0.03*

Values are expressed as Mean ± SD (n =3); *$p < 0.05$, **$p < 0.005$; ***$p < 0.0001$ compared with the control group

3.2 Pyocyanin inhibition

At the highest tested dose (4 mg/mL), both the ethanolic crude extract and ethyl acetate fraction exhibited higher bioactivity, showing statistically significant dose-dependent results in the pyocyanin inhibition test. The ethanolic crude extract and ethyl acetate fraction inhibited pyocyanin production by 77.8%, 70.7%, respectively, while the standard drug furanone 30 achieved an average inhibition of 82.9%. A dose-dependent inhibition of biofilm formation was evident, with the higher concentration (4 mg/mL) exhibiting superior efficacy compared to the lower concentration (2 mg/mL), as illustrated in Table 2.

Table 2: Average pyocyanin inhibition in *P. aeruginosa* culture treated with different test extracts

Treatment groups		**% Pyocyanin inhibition**							
Standard (Furanone 30)/ Dose: 0.3mg/mL						**85.05**	**81.02**	**82.61**	**82.91±0.02**
Test extracts		**Dose: 2 mg/mL**				**Dose: 4 mg/mL**			
		Rep1	**Rep2**	**Rep3**	**Avg ± SD**	**Rep1**	**Rep2**	**Rep3**	**Avg ± SD**
P. paludosa	EtOH crude extracts	34.52	38.91	37.45	36.96±2.24	72.23	83.07	78.33	77.82±0.02***
	Fr. *n*-hexane	17.45	16.54	15.56	16.52±0.95	36.36	33.33	39.13	36.27±0.03
	Fr. CH_2Cl_2	21.56	19.34	17.86	19.59±1.86	45.45	50.33	39.16	44.91±0.03
	Fr. EtOAc	35.42	34.8	33.73	34.65±0.85	72.72	70.84	65.21	70.73±0.02**
	Fr. H_2O	38.12	35.46	37.46	37.01±1.39	71.81	70.17	67.27	69.75±0.03**
	Pure fraction	22.15	20.19	23.58	21.97±1.70	45.46	58.34	56.52	53.44±0.02*

Values are expressed as Mean ± SD (n =3); *p < 0.05, **p <0.005; ***p <0.0001 compared with the control group

3.3 Swarming Motility inhibition

The swarming motility inhibition percentages for the ethanolic extract, ethyl acetate, *n*-hexane, dichloromethane, water fractions, and isolated pure fraction were 71.6%, 74.6%, 51.08%, 35.1%, 76.9%, and 63.1% at 4 mg/mL. The average swarming motility inhibition for the standard was 87.4%. Notably, the ethyl acetate and water fractions exhibited higher inhibition compared to other fractions, with the isolated pure fraction also showing significant inhibition. The inhibition of biofilm formation demonstrated a dose-dependent effect, with the higher concentration (4 mg/mL) exhibiting superior efficacy over the lower concentration (2 mg/mL), as depicted in the Table 3.

Table 3: Swarming motility inhibition of different test extracts on *P. aeruginosa*

Treatment groups		**%Swarming motility inhibition**							
Standard (Furanone 30)/ Dose: 0.3mg/mL						**89.4**	**87.0**	**85.9**	**87.41±1.58**
Test extracts		**Dose: 2 mg/mL**				**Dose: 4 mg/mL**			
		Rep1	**Rep2**	**Rep3**	**Avg ± SD**	**Rep1**	**Rep2**	**Rep3**	**Avg ± SD**
P. paludosa	EtOH crude extracts	30.18	28.90	31.48	30.19±1.29	70.24	74.02	70.62	71.62±0.68**
	Fr. *n*-hexane	25.13	26.54	28.75	26.81±1.82	47.33	54.81	51.12	51.08±0.04
	Fr. CH_2Cl_2	21.86	20.47	18.54	20.29±1.67	44.23	47.43	45.82	35.13±0.02
	Fr. EtOAc	35.84	37.54	39.12	37.50±1.64	72.51	76.25	75.15	74.62±0.05**
	Fr. H_2O	39.68	36.45	37.76	37.96±1.62	76.33	77.75	76.65	76.93±0.04**
	Pure fraction	30.46	32.15	34.51	32.37±2.03	62.57	62.23	64.63	63.15±0.03*

Values are expressed as Mean ± SD (n =3); *p < 0.05, **p <0.005; ***p <0.0001 compared with the control group

3.4 Phytochemical screening on fractionated crude extracts: The ethanolic extract revealed the presence of several important phytoconstituents, including reducing sugar, tannins, flavonoids, saponins, alkaloids, terpenoids, carbohydrates, glycosides and steroids. However, the *n*-hexane fraction lacked tannins, carbohydrates, and glycosides, the chloroform fraction lacked saponins and glycosides, the ethyl acetate fraction lacked saponins and glycosides, and the water fraction notably lacked tannins, saponins, and terpenoids in the experimental results presented in Table 4.

Table 4: Results of preliminary phytochemical tests

Phytochemical groups	***P. paludosa***				
	Ethanolic Extract	**Fr. *n*-hexane**	**Fr. $CHCl_3$**	**Fr. EtOAc**	**Fr. H_2O**
Reducing sugar	+	+	+	+	+
Tannins	+	-	+	+	-
Flavonoids	+	+	+	+	+
Saponins	+	+	-	-	-
Alkaloids	+	+	+	+	+
Terpenoids	+	+	+	+	-
Carbohydrates	+	-	+	+	+
Glycosides	+	-	-	-	+
Steroids	+	+	+	+	+

[‘ + ’ indicates presence and ‘-’ indicates absence]

3.5 Identification of the bioactive compound(s)

The isolated pure fraction underwent GC-MS analysis, and the major peak at a retention time of 38.55 min was identified as Trimyristin, with a calculated peak area of 53.67% shown in Figure 1 and Table 5.

Table 5: Identified compounds through GC-MS analysis from the bioactive ethyl acetate fraction of *P. paludosa*

Serial No.	Retention time (RT)	Name of the compounds	Molecular formula	Molecular weight	% Area
01.	38.56	Trimyristin	C45H86O6	722	53.67
02.	25.36	Neophytadiene	C20H38	278	6.58
03.	26.33	Phytyl Palmitate	C36H70O2	534	2.84
04.	28.21	Tetradecanoic Acid, 10, 13- Dimethyl-, Methyl Ester	C17H34O2	270	3.90
05.	31.76	6-Octadecenoic Acid	C18H34 O2	282	4.64
06.	32.46	2,6,10,14-Tetramethyl-7-(3- Methylpent-4-Enylidene) Pentadecane	C25H48	348	1.97
07.	23.63	Trans-2-Methyl-4-N-Pentylthiane, S,S-Dioxide	C11H22O2S	218	1.63

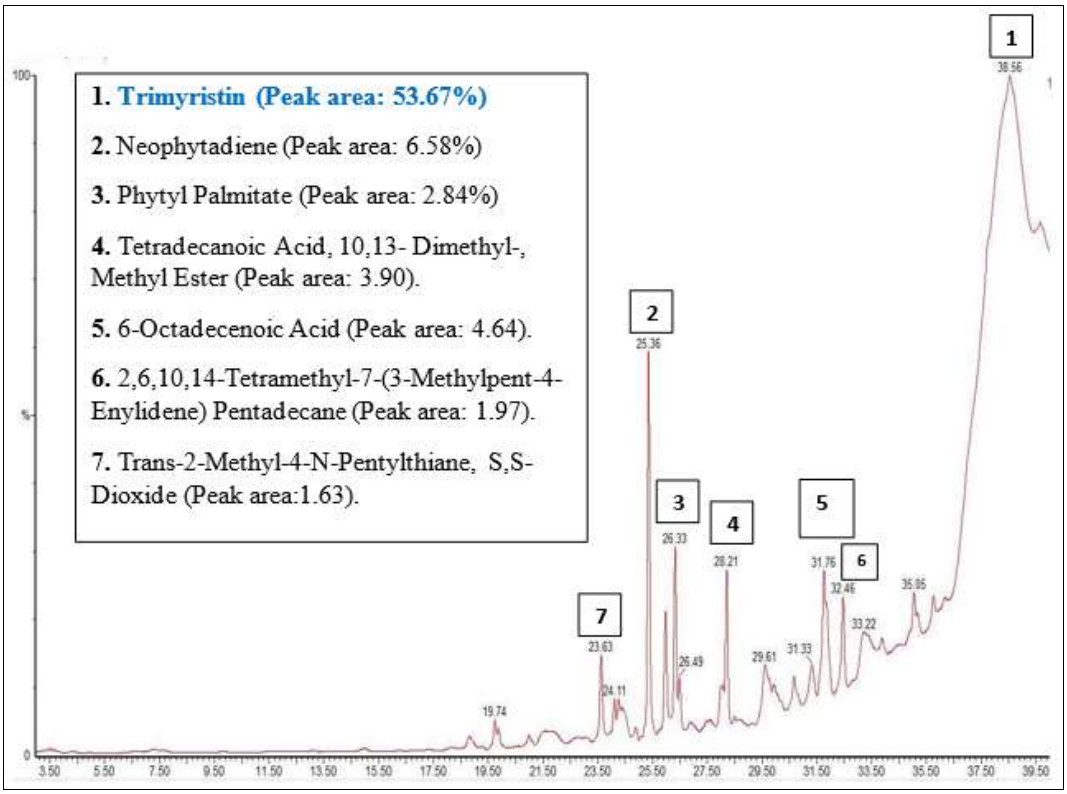

Fig 1: GC-MS Chromatogram of bioactive ethyl acetate fraction of *P. paludosa*

The chemical structure of Trimyristin is illustrated in Figure 2, with an associated R_f value of 0.51 obtained in the mobile phase (dichloromethane: n-hexane = 9:1).

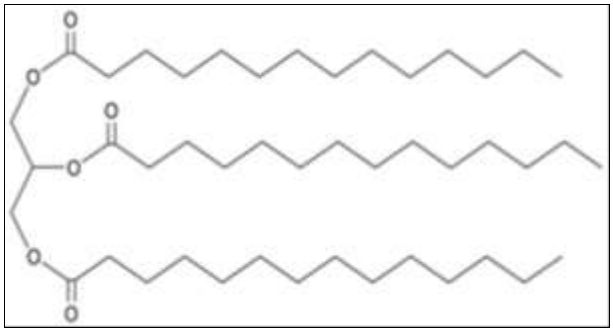

Fig 2: Chemical structure of Trimyristin

Besides this, some other compounds also been identified from this plant as mentioned in Table 5.

4. Discussion

Medicinal plants play a pivotal role in drug discovery, offering a vast and diverse array of bioactive compounds that serve as valuable starting points for pharmaceutical development. Traditional medicine has long relied on the healing properties of plants, providing a wealth of knowledge that guides modern researchers in the search for new drugs [28]. Over the course of centuries, botanical specimens have served as a substantial reservoir of compounds possessing antimicrobial attributes. Numerous traditional medicinal practices have incorporated botanical extracts for the treatment of diverse infections. Although not all compounds derived from plants are viable as autonomous antibiotics, certain entities exhibit notable antimicrobial efficacy. Furthermore, the formulation of standardized extracts or the isolation of specific compounds from these plants holds potential to augment their utility in clinical contexts [29]. The active antibacterial compounds derived from plants encompass a diverse array of bioactive substances. One notable example is allicin, found in garlic (Allium sativum), which has demonstrated antimicrobial efficacy against various bacteria, including *S. aureus* and *E. coli*. Another significant compound is berberine, extracted from plants such as *Berberis* spp. and *C. chinensis*, exhibiting antibacterial properties against Gram-positive and Gram-negative bacteria. Furthermore, tea tree oil, derived from *Melaleuca alternifolia*, contains terpinen-4-ol, which possesses notable antimicrobial activity. These examples underscore the potential of plant-derived compounds in the development of antibacterial agents, contributing to the ongoing exploration of natural sources for therapeutic applications [30-32].

Earlier scientific reports underscored the impact of extraction methods on the quantity and physiological effects of plant extracts, prompting the partitioning of crude extract into

distinct fractions through liquid-liquid extraction [33]. The resulting fractions exhibited varying polarities, with water being the most polar (polarity index 10.2) and hexane the least polar (0.1). Water dissolved the most polar compounds, while ethyl acetate (4.4) and dichloromethane (3.1) fractions accommodated medium polar compounds, and n-hexane housed nonpolar compounds. Given the higher yield percentage in the ethyl acetate fraction (29%) compared to other fractions, it is inferred that the leaves predominantly contain medium polar compounds.

In the anti-biofilm assessment, *P. paludosa* demonstrated varying levels of biofilm inhibition across its ethanolic extract, ethyl acetate, *n*-hexane, dichloromethane, water fractions, and isolated pure fraction, with respective average percentages of 75.34%, 80.61%, 43.35%, 33.28%,71.82%, and 58.64%. Notably, the ethyl acetate fraction exhibited a higher percentage of biofilm inhibition compared to other fractions, and an increase in extract dose corresponded to an elevated biofilm inhibition percentage. The isolated pure fractions also displayed significant anti-biofilm activity, indicating the potential therapeutic impact of individual compounds from the plant leaves in the realm of antibacterial interventions. Previous research has highlighted the antibiofilm activity of natural compounds such as phenolics, essential oils, terpenoids, lectins, alkaloids, polypeptides, and polyacetylenes [34]. Among phenolic compounds, condensed tannins have demonstrated pronounced anti-biofilm action [35], while the pentacyclic triterpenoid ursolic acid has been identified as a biofilm formation inhibitor [36]. Phytochemical screening of the investigated plant leaves revealed the presence of various compounds, including reducing sugar, tannins, flavonoids, alkaloids, saponins, terpenoids, carbohydrates, glycosides, and steroids (Table 4). These constituents, either individually or in combination, may act as anti-QS and anti-biofilm agents, potentially enhancing the efficacy of antibiotics at lower doses against bacterial cells. This strategic approach aims to mitigate resistance concerns and holds promise in addressing multidrug-resistant pathogenic bacteria.

To assess the anti-QS properties of plant extracts, the inhibition of pyocyanin formation and motility inhibition on a soft agar plate were investigated, revealing statistically significant outcomes in a dose-dependent manner. The percentage of pyocyanin inhibition in the ethanolic extract, ethyl acetate, *n*-hexane, dichloromethane, water fractions, and the isolated pure fraction of *P. paludosa* were found to be 77.82%, 70.73%, 36.27%, 44.91%, 69.75%, and 53.44%, respectively, at a concentration of 4 mg/ml after 3 days, with the average percentage of pyocyanin inhibition for the standard being 82.91%. Previous investigations have suggested that tannin chemicals may impede the QS system of bacteria [35]. Flavonoids, which have been explored as QS modulators, were found in this plant extract and have been reported to inhibit pyocyanin production and swarming motility [37]. Similarly, the percentage of swarming motility inhibition in the ethanolic extract, ethyl acetate, *n*-hexane, dichloromethane, water fractions, and the isolated pure fraction of *P. paludosa* at 4 mg/ml after 3 days were 71.62%, 74.62%, 51.08%, 35.13%, 76.93%, and 63.15%, respectively, with the average percentage of swarming motility inhibition for the standard being 87.4%. These findings suggest that the plant extract possesses notable anti-QS effects, with the ethyl acetate and water fractions exhibiting particularly pronounced inhibitory activity.

Trimyristin, a natural triglyceride found abundantly in the seeds of various plants, emerges as a compelling candidate for exploration as a potential antibacterial agent. Recent studies have indicated promising antibacterial activity associated with trimyristin, particularly against a spectrum of bacterial strains [38]. Its potential as an antibacterial agent may be attributed to the presence of myristic acid, one of its constituent fatty acids, which has demonstrated antimicrobial efficacy. Furthermore, the utilization of trimyristin in its natural form or as an isolated compound may offer a sustainable and environmentally friendly alternative in the pursuit of novel antibacterial agents, aligning with the growing interest in natural products for therapeutic applications. However, comprehensive investigations into the mechanisms of action, safety profile, and efficacy against diverse bacterial species are imperative to elucidate and harness the full potential of trimyristin in the development of antibacterial interventions.

5. Conclusion

This study sheds light on the importance of exploring natural sources for innovative solutions to counteract antibiotic resistance, emphasizing the potential of *P. paludosa* in the quest for effective antimicrobial agents. Notably, the ethyl acetate fraction of this plant extract, characterized by medium polarity, exhibited superior activity in both anti-biofilm and anti-QS assays. Furthermore, Trimyristin, a pure compound from this plant, marked the first instance of its identification and demonstrated noteworthy anti-QS and anti-biofilm effects. This pioneering finding underscores the potential of novel compound from the *P. paludosa* as an effective agent against bacterial infections. The ongoing pursuit of bioassay-guided isolation and identification of additional bioactive compounds holds promise for elucidating precise structure-activity relationships, thereby contributing to the development of innovative drugs aimed at combating infectious diseases while mitigating the risk of antibiotic resistance.

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7. Conflict of interest and consent of publication

All authors dedicated their sincere efforts to conducting the experiments. No conflicts of interest are known to be associated with this publication, and the authors have provided their consent for its publication.

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