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Anti-biofilm Activity of Rhamnolipid Extracted From *Pseudomonas aeruginosa*

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Abstract

P. aeruginosa isolated from water and soil samples and identified by Vitek 2 system. Based on primary screening assays such as (hemolysis, CTAB, oil dispersion, drop collapse, and emulsification index (E24 %) methods, 5 isolates were chosen for their ability to produce biosurfactants. The identification of 5 isolates were confirmed by 16SrDNA gene. Crud biosurfactant purified by column chromatography and obtained pure rhamnolipids honey colour product. TLC, FTIR, GC-MS, and HPLC were used to characterize the biosurfactants. The biosurfactant was found to be rhamnolipid. Rhamnolipid prevent the adhesion of *P. aeruginosa, E. cloacae, S. aureus, P. mirabilis* cells and biofilm formation of these bacteria. Rhamnolipid also disrupted the pre-formed biofilms of these bacteria.

Keywords: Rhamnolipids, *Pseudomonas aeruginosa*, antibacterial, anti-biofilm.

Introduction

Biosurfactants are a diverse group of microbially produced active surface compounds. Because of their high surface tension and emulsifying properties, rhamnolipids have been extensively studied and used[1,2]. Rhamnolipids have the greatest merits over synthetic surfactants in terms of being less toxic and producing them from renewable waste substances [3,2]. *Pseudomonas spp.* is found to produce the most rhamnolipids [4]. Rhamnolipid is a glycolipid made up of one or two β-hydroxy fatty acids and one or two rhamnose sugar molecules with a glycosidic link to a hydrophobic group. Microbial fermentation produces a variety of congeners with different fatty-acid chain lengths [5]. Rhamnolipids have been studied extensively for their potential applications in bioremediation, increased oil recovery, therapeutics, pharmaceuticals, agriculture, detergents, cosmetics, and cleaners [6,7,8,9]. Rhamnolipid is investigated to be able to break up biofilms formed by microorganisms that form biofilms [10]. The present research aimed to evaluate the biological activity of identified rhamnolipid as antibacterial, anti-biofilm against pathogenic bacteria isolated from diabetic foot infection.

Materials and Methods

Sample collection and Bacterial identification

Fifty samples of soil and water were collected during December 2018– May 2019 from different places in Basrah city. One ml soil sample was added to sterile distilled water (10ml) and make dilution, o.1ml on pseudomonas agar base medium was spraded. Colonies were gram stained, oxidase, and catalase tested. Positive catalase, oxidase and negative gram staining bacilli were identified by using Vitek 2 system analysis. Isolates were further identified using 16SrDNA gene which were sequenced (Macrogen company) and analyzed by using searching tool BLAST.

Primary screening the rhamnolipid biosurfactant producer

50ml of mineral salt medium [11] with soybean oil as carbon source, was despised in 250 ml Erlenmeyer flasks to determine the ability of isolates to produce rhamnolipid biosurfactant. 1% of bacterial growth (18hrs.) inoculated in flasks, at 30^{o}C for 7days with shaking (120rpm) were incubated. centrifuge the cultures for 20min at 10000rpm. Rhamnolipid biosurfactant production was investigated in supernatant using various methods (drop collapse, hemolysis test, emulsification index, CTAB method, oil dispersion) [12].

Production of Rhamnolipid biosurfactant

Inoculating 50 ml of liquid mineral salts medium with soybean oil into (250 ml) flasks with pre-selected bacterial isolates, the procedure used according to Abbasi et al [13].

Estimation the rhamnolipid concentration

The L-rhamnose standard curve was used to assess the rhamnolipid content of the supernatant sample [14].

Extraction of rhamnolipid by Chloroform-methanol

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After the incubation time, the rhamnolipid biosurfactant was harvested. According to Bharali et al [15] extraction was carried out.

Purification of Rhamnolipid biosurfactants

The stationary phase or packing material used in column chromatography was silica gel 60, chloroform-methanol (3:2) was used as the mobile phase the procedure done according to Pathaka and Pranav [16].

Chemical characterization of rhamnolipid

Thin layer chromatography (TLC)

The presence of glycolipid (rhamnolipid) was detected using thin layer chromatography. The extracted rhamnolipid was dissolved in (9:1) mixture of chloroform and methanol. The plate of 60 silica gel as stationary phase, solution of chloroform:methanol:acetic acid (65:15:2) was used as mobile phase [17].

FTIR – Spectroscopy analysis

The IR spectra of the partially purified rhamnolipid were recorded in KBr pellets using FTIR Spectrophotometer, at the range (400-3800) cm-1.

Gas chromatography mass spectrophotometer (GC-MS)

A shimadzu Qp2010 quadrupole gas chromatography Mass spectrophotometer (GC-MS) instrument fitted with a carbox tool (30m x 0.25 mmID; 0.25 im film thickness) capillary column (intercut DB5Ms-Japan) was used for rhamnolipid analysis in Nahran Omar laboratories/Basrah oil company. In the capillary column, one liter of sample was injected. The helium gas used as carrier.

High performance liquid chromatography (HPLC) method

Rhamnolipid purity were tested by high performance liquid chromatography (Research Center of Veterinary Medicines / Authority of Industrial Research and Development / Iraq) with C 18 column (4.6 x 5mm) and rhamnolipid was detected at 262nm wavelength, 1.4ml/min the rate of flow and mobile phase used 0.2% of (A) formic acid in ions-free water and (B) methyl cyanide.

Determination of Minimum Inhibitory Concentration (MIC) of RL

The antimicrobial activity of the rhamnolipid was tested using the macro broth dilution method with 12 sterile test tubes against a test culture of pathogenic bacteria isolated from a diabetic foot infection. Optical density values at 625 nm are calculated using a Spectrophotometer. In comparison to the positive control, the MIC was the lowest rhamnolipid concentration that visually inhibited microbial development [18].

Anti-biofilm activity of Rhamnolipids by Tissue culture plate method

Different concentration of rhamnolipid (100, 50, 25, 12.5, 6.250, 3.12, 1.6, 0.8, 0.4,and 0.2) mg/ml was prepared in Tryptic Soy broth (TSB) with 1% glucose. 180 µl of different concentration of rhamnolipid and 20 µl of the tested bacteria was put in 96-well flat bottom microtiter plate and culture without rhamnolipid considered as positive control while, control negative TSB only. The wells of the microtiter plate were sucked out and washed with 200µl of phosphate buffer saline (PBS) after 24 hours of incubation at 37°C. After that, 200µl of methanol was applied to the microtiter plate wells for 15 minutes of fixation. The wells were stained by using 200µl of crystal violate(1%) for 20 minutes. The stain was aspirated from wells and wash with distilled water (D.W.). The wells were then filled with 33 percent acetic acid, and the optical density (OD) of each well was measured at 630 nm using a microtiter plate reader [19]. Optical density for detection biofilm formation recorded as (<0.120:weak/non, 0.120-0.240:moderate, >0.240:strong/high).

Statistical analysis

All data were analyzed statistically by applying uni and multivariants ANOVA test using SPSS (version 24) program.

Results and Discussion

Sample collection

Fifty samples (soil and water) were collected from various harbours and fields of oil in governorate of Basrah. On selective medium forty two isolates of *Pseudomonas spp.* were obtained from samples and numbered serially P1-P42. *P. aeruginosa* have different habitats and isolated from variety ecosystem [20,21,12,22,23,24]. Various organic compounds can be break down by *Pseudomonas aeruginosa*, which had the ability to degrade hydrocarbon in contaminated soil [25,26,27,23].

Identification of *Pseudomonas ssp.*by Vitek 2 system

The forty two isolates that gave gram negative rod, catalase and oxidase positive subjected to identification by Vitek 2 system analysis. The results of identification revealed that all bacterial isolates were belong to *Pseudomonas aeruginosa* with probability ranging (88%-99%). Many studies were identified

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Pseudomonas aeruginosa by using vitek 2 system [28,29,30,31]. Because of some isolates gave 88% identity by using vitek 2 system, the identification was confirmed by 16SrDNA gene.

Genetic identification of *Pseudomonas spp.* by using 16SrDNA gene

The 16SrDNA region of 5 isolates that gave positive results for all primary screening tests of rhamnolipid biosurfactants were sequenced and analyzed by using searching tool Blasts. The identity of 5 isolates were confirmed as *P. aeruginosa*. Biosurfactant producing bacteria were identified by genetic analysis using 16SrDNA gene, because of this gene is highly conserved than others. It has more variable domain, which could be used for examining more closely organisms [32,33,34].

Primary screening of biosurfactant producer isolate

From the results of the (CTAB, hemolytic activity, oil dispersion, drop collapse assay, and emulsification index E_{24}%) tests that used for primary screening of rhamnolipid biosurfactant producer isolates, showed that twelve isolates out of forty two isolates gave the positive results for all tests used, as shown in table (1). And these isolates were selected for further study. More than one method should be used to examine the ability of bacteria to produce biosurfactant, and this finding is with agreement of the studies of [35,36,37,38,12].

Table 1. Results of primary screening of rhamnolipid producer isolates.

Isolate	CTAB	hemolysis	Oil dispersion	Drop collapse	Emulsification index(E24 %)				
					kerosene	Soybean oil	Olive oil	Diesel oil	Sunflower oil
9	+	+	+	+	80	75	80	55	75
13	+	+	+	+	62	65	65	57	65
14	+	+	+	+	64	72	67	67	55
15	+	+	+	+	73	73	65	52	82
42	+	+	+	+	66	73	70	53	73

Vegetable oils, Glycerol, and molasses are all considered as good carbon and energy sources for producing rhamnolipid, and the genus Pseudomonas is capable of utilizing them [39,40,41]. In this research, carbon and energy source as soybean oil in mineral salt medium were used to enhancement the production of rhamnolipid biosurfactant. The addition of calcium, magnesium, potassium, sodium salt and trace elements to media to improve rhamnolipid production in *Pseudomonas aeruginosa*.

Extraction and purification of rhamnolipid

Chloroform-methanol (2:1) was used to extract rhamnolipid from isolate supernatant. The crud rhamnolipid biosurfactant was yellowish-brown in colour and sticky at room temperature.

The used solvent extraction method by chloroform: methanol (2:1) was a good method for extraction rhamnolipid biosurfactant from acidified cell-free supernatant.This study consistent with Sabturani *et al* [42] who used solvent extraction of rhamnolipid from *Pseudomonas aeruginosa* UKMP14T. also Kaskatepe *et al* [17] were used chloroform-methanol for extraction rhamnolipid from acidified cell-free supernatant by using equal volume. Another study by Kaya *et al* [43] were used solvent extraction chloroform-methanol for extraction of rhamnolipid from *Pseudomonas spp.* While Salleh *et al* [44] used the mixture of the solvent to give it large quantities. Column chromatography method was used for purification of rhamnolipid biosurfactant extracted from these isolates .The purified rhamnolipid extracts appeared as honey colour. Tawfeeq and Yesser [45] used the same method and obtained the same honey coloured products. Other study were also used column chromatography to purified rhamnolipid of *P. aeruginosa* [46]. Christova *et al* [47] also used column chromatography to purified RL from *P. aeruginosa*.

Estimation of rhamnolipid

Using the phenol-sulphuric acid process, the presence group of rhamnose in the rhamnolipid biosurfactant molecule was determined. The concentration of rhamnose estimated from Dubois standard curve of L-rhamnose, figure (1). The results in table (2) showed that rhamnolipid concentration in each sample equal to the rhamnose concentration in (P9, P13, P14, P15, and P42).

Sumathi and Yogananth [12] used rhamnose test to estimate the rhamnolipid concentration in cell- free supernatant from *Pseudomonas aeruginosa*. And Ozdal *et al* [48] were estimated the rhamnolipid concentration in supernatant by phenol-sulfuric acid method from rhamnose standard curve.

Table 2. Results of rhamnolipid estimation.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 6, 2021, Pages. 1731-1743
Received 25 April 2021; Accepted 08 May 2021.

Sample No.	OD at wave length (490nm)	Rhamnolipid concentration (µg/ml)
P9	0.078	40
P13	0.051	40
P14	0.0445	10
P15	0.026	50
P42	0.0365	30

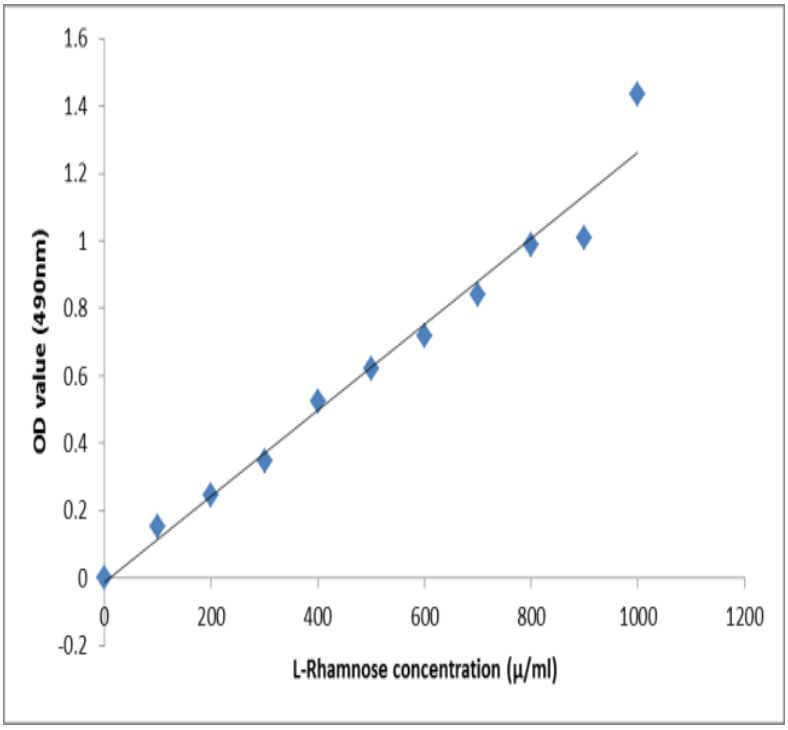

Figure 1. Standard curve of L-Rhamnose by Dubois et al. method for L-Rhamnose estimation

Chemical Characterization of rhamnolipid biosurfactant

Thin layer chromatography (TLC)

Plates of silica gel-60, and chloroform:methanol:acetic acid, (TLC) was used for indication of rhmnolipid table (3) and figure (2).The presence of two spots at different R_f values indicating the presence of two compounds of rhamnolipid differs in their molecular weight.

Table 3. Thin layer Chromatography (TLC) of rhamnolipid extracts with their R_f values.

sample	R_{f1}	R_{f2}
P9	0.85	0.94
P13	0.27	0.85
P14	0.85	-
P15	0.705	-
P42	0.88	-

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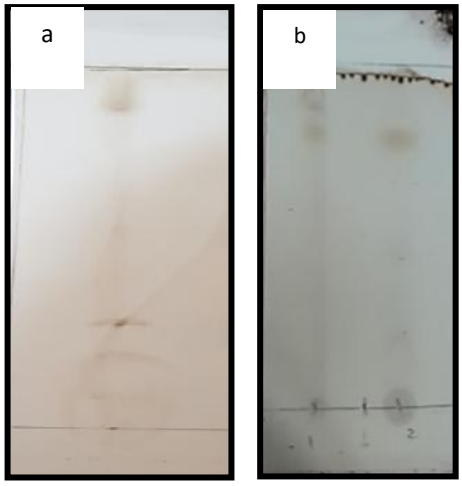

Figure 2. Thin layer chromatographs (TLC) of the extracted rhamnolipid of *Pseudomonas aeruginosa* isolates, a: two spots with different R_f value, b: one spot.

Glycolipid (rhamnolipid) consist of sugar (rhamnose) one or two and fatty acid one or two, and had series of structures. The results of current research are in consistent with other reported studies of George and Jayachandran [49], Lotfabad *et al*[50], Denney [18], Das *et al* [51], Abdel-Mawgoud *et al* [52] and Oluwaseun *et al* [53].

Previous TLC results of rhamnolipid mixture revealed the identification of various spots on TLC plates of rhamnolipid extracts formed by various isolates of *P. aeruginosa*. The variation in rhamnolipid value may be due to the fact that different strains generate rhamnolipid with different compositions [5].

FTIR analysis

Rhamnolipid biosurfactant extracted from (P9, P13, P14, P15, and P42) isolates have been characterized by Infra-Red spectroscopy. Wave length in cm^{-1} of bands observed in infrared spectrophotometer of these rhamnolipid biosurfactants in the (400-3800 nm) region, table (4) and figure (3).

The Fourier transform infrared spectroscopy technique is used to determine the feature groups of various compounds based on their characteristic absorption bands. Result of FTIR analysis of rhamnolipids extracted from different isolates of *P. aeruginosa* showed that all compounds displayed the fingerprinting region of functional groups of rhamnolipid, the band between 3500 and 3400 cm^{-1} related to the vibrating stretching of O-H group. Also IR analysis indicated that all samples contain C-H stretching which belong to CH3 and CH2 alkyle group; while C=O stretching at 1735 which belong to ester groups.

Kiefer et al [54] showed that IR spectroscopy gave a good result for analyzing the chemical structure of biosurfactant. In their study, Rikalovic et al [55] showed identical results with the present study regarding of the functional groups spectra of rhamnolipid. Also the result in this situation are in consistent with study of Abdel-Mawgoud *et al* [5] and the study of Mehdi et al [56] from FTIR spectrometry, the OH stretching vibration related to hydroxyl group, CH and CH2 and carbonyl group C=O belong to the lipid moiety of rhamnolipid [42]. All of the above functional groups indicates the glycolipid nature of studied biosurfactant which belong to rhamnolipid [22,13].

Table 4. FTIR analysis and wave numbers of bands of rhamnolipid extracts from *P. aeruginosa*.

Wave Number (cm-1)					Assignment
P9	P13	P14	P15	P42	
3479	3498	3468	3494	3468	Symmetric OH stretching
2989	2924	2924	2924	2920	Asymmetric stretching of Alkane
1739	1739	1739	1735	1739	C=O Alkyle stretching
1643	1639	1639	1458	1462	Carboxylic stretching
1049	1003	1003	1045	1045	C-O-C stretching

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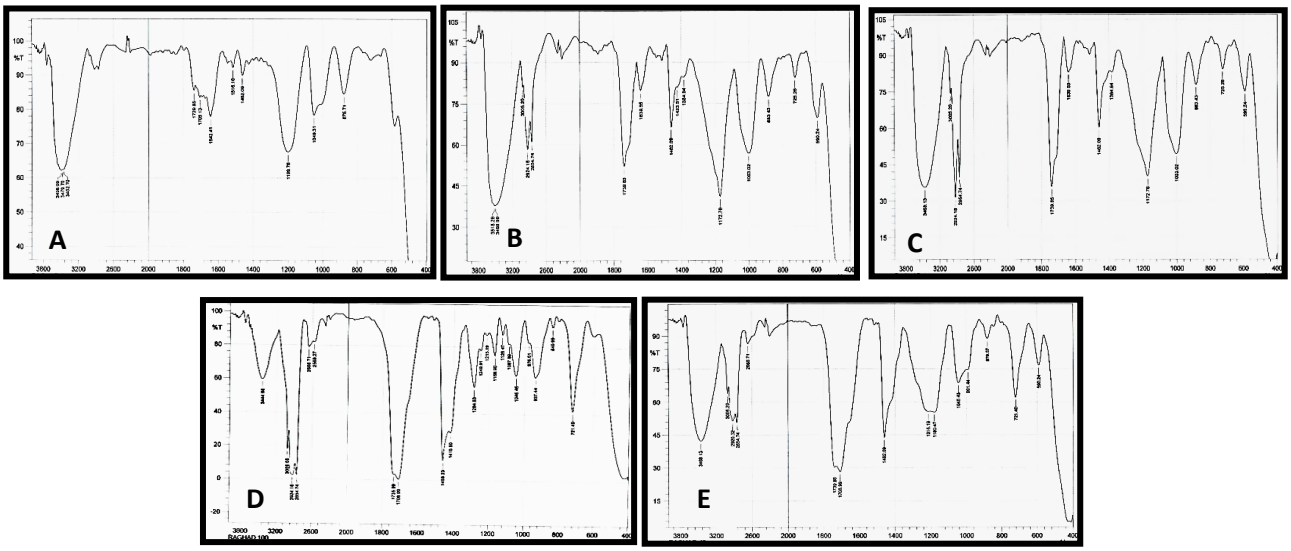
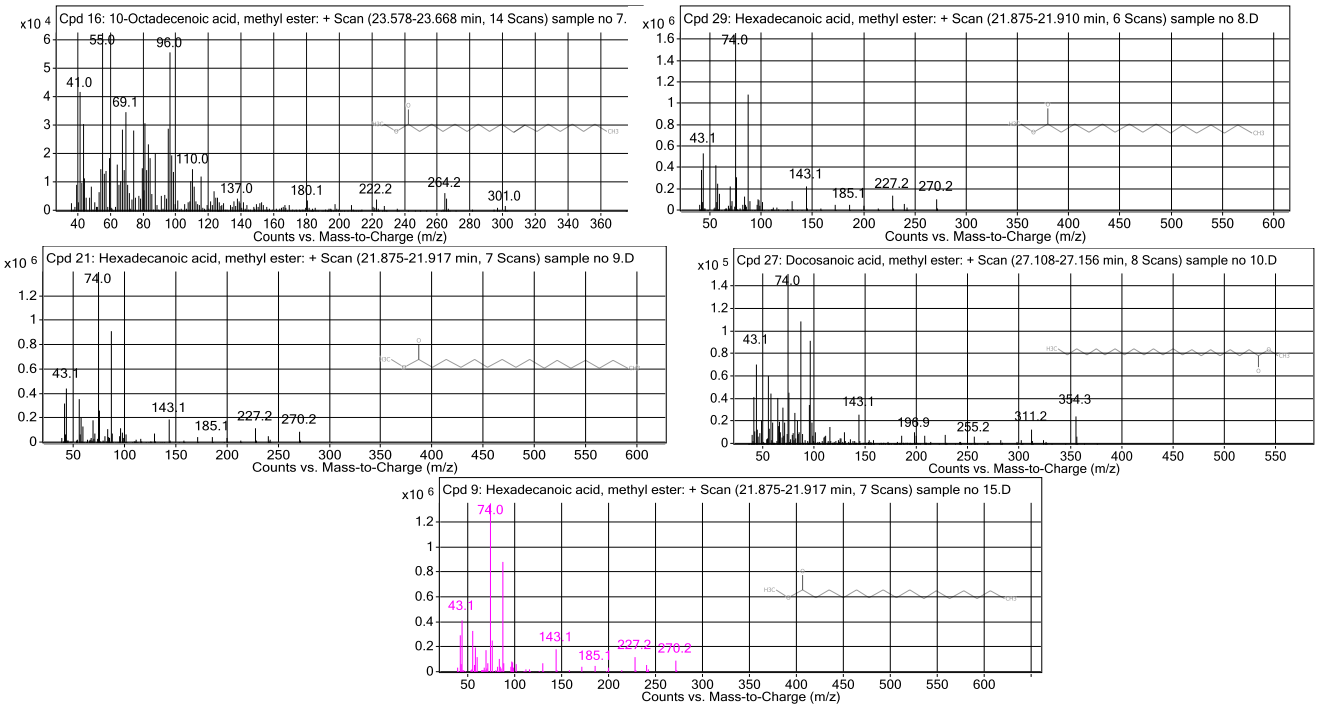


Figure 3. FTIR spectrum of rhamnolipid extracted from P9(A) ,P13(B), P14(C), P15(D) , P42(E) isolates of *Pseudomonas aeruginosa*.

Gas chromatography–mass spectrometry (GC-MS) analysis of rhamnolipid

GC-MS chromatogram of rhamnolipid extracts from (P9, P13, P14, P15, and P42) of *Pseudomonas aeruginosa* was performed showing sharp peaks at acquisition time (23.599, 21.896, 21.896, 27.129, and 21.896) minutes, respectively on the basis of gas chromatography analysis, mass spectrum analysis which identified as (octadecenoic acid, hexadecanoic acid, hexadecanoic acid, docosanoic acid, and hexadecanoic acid), respectively, that gave intense molecular ion peak at (301.0, 270.2, 270.2, 354.3, and 270.2) m/z, respectively, with its structure, as shown in the figure (4).

Therefore, the study indicates the biosurfactant extracts were rhamnolipid according to other studies that GC-MS examination of a rhamnolipid compound derived from *P. aeruginosa* indicates the presence of fatty acid [22,57,58,59].



Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 6, 2021, Pages. 1731-1743
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Figure 4. GC-mass spectral analysis of rhamnolipid extracted from P9, P13, P14, P15, P42 isolates of *Pseudomonas aeruginosa*.

High performance liquid chromatography (HPLC) analysis

Rhamnolipid extracts in this study analyzed by HPLC, showed that each of them exhibit three peaks, were observed in retention time of (1.732, 2.774, 3.154) for (P9), (1.722, 2.782, 3.152) for (P13), (1.716, 2.775, 3.146) for (P14), (1.713, 2.760, 3.123) for (P15), (1.711, 2.751, 3.131) for (P42) as in standard curve of rhamnolipid and appeared fourth peak in retention time of (4.381, 4.431, 4.453, 4.458, 4.459) for (P9, P13, P14, P15,P 42), respectively, in rhamnolipid extracts which not found in standard curve, as shown in figure (5). HPLC is an analytical technique for detecting a compound in a sample. In the present study the results of HPLC analysis revealed that rhamnolipid extracts from different isolates of *P. aeruginosa* gave four compound having different retention time which provided evidence to presence different compounds of rhamnolipid biosurfactant in samples compared to standard rhamnolipid.

These results consistent with studies obtained from previous works who used HPLC for identification the presence of RL in samples [60,61,62]. Tawfeeq and Yesser [45] also used HPLC for identification of rhamnolipid produced in sample by *P.aeruginosa.*

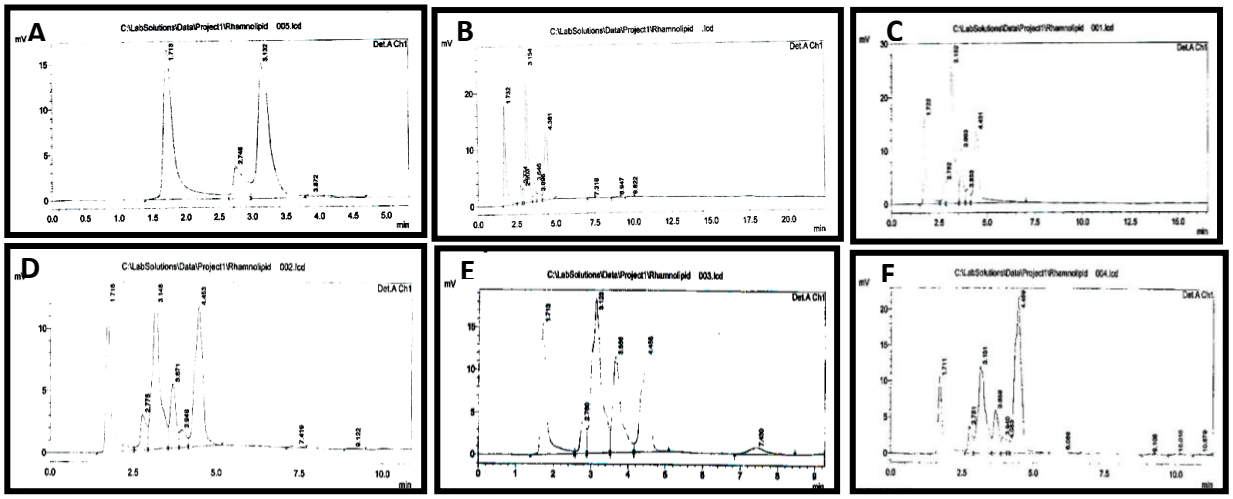

Figure 5. Rhamnolipid standard curve(A), extracted from P9 (B) ,P13 (C) , P14(D) ,P15 (E) and P42(F) by HPLC analysis

Minimum inhibitory concentration (MIC) of rhamnolipids

The results of rhamnolipid extracted from (P9, P13, P14, P15, and P42) showed the MIC values range (3.12 and 6.250) µ/ml against the tested biofilm forming bacteria as shown in table (5).

Table 5. Results of MIC of rhamnolipid extracts from *P. aeruginosa* against pathogenic bacteria.

Sample No.	Concentration (mg /ml)			
	P. aeruginosa	E. cloacae	S. aureus	P. mirabilis
	MIC	MIC	MIC	MIC
P9	3.12	6.250	3.12	3.12
P13	6.250	3.12	3.12	3.12
P14	6.250	6.250	6.250	3.12
P15	3.12	3.12	3.12	6.250
P42	3.12	6.250	3.12	3.12

Antibacterial activity of various concentrations of bacterial biosurfactant against pathogenic G+ve and G-ve bacterial strains has previously been published [63,64]. Due to differences in their chemical structure and bacterial cell composition, RL biosurfactant from different bacterial strains was stated to have varying antibacterial effects [65,66,67]. Gaur *et al* [68] who reported that RL extracted from *Planococcus sp* exhibit bactericidal properties against G+ve and G-ve pathogenic bacteria. The disparity in their values may be due to

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differences in the form of RL produced and the bacteria that generate it. Because of the differences in structural structure, the successful antibacterial dose of biosurfactant differed [65].

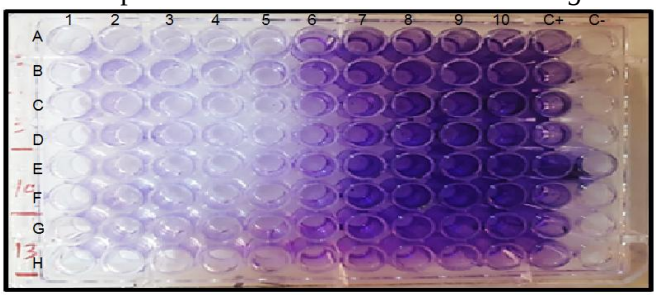
Anti-biofilm activity of rhamnolipids by TCP method

The results of rhamnolipid extracted from (P9, P13, P14, P15, and P42) isolates showed anti-biofilm activity against pathogenic bacteria started at a concentration same to MIC value (3.12 and 6.250)mg/ml, when incubated with different concentration of rhamnolipid extracts (0.2-100) mg/ml and the effect increase when increase the concentration, when compared with control positive, as illustrated in table (6) and figure (6).

Table 6. Anti-biofilm activity of rhamnolipid extracts of P. aeruginosa isolates by tissue culture plate method measured of OD at wave length 630 nm.

Sample	Bacteria	Concentration of Rhamnolipid (mg/ml)										Control	
		100	50	25	12.5	6.250	3.12	1.6	0.8	0.4	0.2	C+	C-
P9	P. aeruginosa	0.013	0.091	0.011	0.014	0.087	0.145	0.162	0.254	0.631	0.621	0.612	0.032
	E. cloacae	0.034	0.052	0.055	0.054	0.074	0.123	0.131	0.242	0.391	0.385	0.393	0.034
	S. aureus	0.035	0.091	0.085	0.107	0.118	0.168	0.251	0.253	0.332	0.332	0.486	0.033
	P. mirabilis	0.037	0.092	0.096	0.091	0.099	0.122	0.253	0.261	0.302	0.303	0.303	0.035
P13	P. aeruginosa	0.036	0.071	0.085	0.085	0.084	0.188	0.09	0.211	0.234	0.242	0.361	0.032
	E. cloacae	0.096	0.086	0.098	0.09	0.078	0.085	0.178	0.21	0.211	0.254	0.26	0.035
	S. aureus	0.035	0.078	0.097	0.102	0.114	0.142	0.136	0.27	0.277	0.272	0.276	0.033
	P. mirabilis	0.037	0.067	0.062	0.074	0.075	0.062	0.186	0.277	0.276	0.264	0.266	0.033
P14	P. aeruginosa	0.038	0.079	0.077	0.073	0.066	0.182	0.25	0.256	0.305	0.374	0.374	0.036
	E. cloacae	0.067	0.063	0.075	0.073	0.063	0.062	0.128	0.245	0.257	0.381	0.385	0.034
	S. aureus	0.062	0.065	0.093	0.092	0.102	0.135	0.294	0.274	0.267	0.236	0.357	0.033
	P. mirabilis	0.067	0.086	0.08	0.077	0.062	0.088	0.11	0.264	0.278	0.396	0.404	0.033
P15	P. aeruginosa	0.047	0.064	0.055	0.066	0.069	0.174	0.166	0.203	0.187	0.208	0.305	0.036
	E. cloacae	0.084	0.092	0.097	0.076	0.061	0.087	0.136	0.166	0.167	0.232	0.239	0.193
	S. aureus	0.071	0.058	0.076	0.052	0.068	0.121	0.134	0.143	0.158	0.232	0.274	0.033
	P. mirabilis	0.043	0.042	0.055	0.053	0.058	0.082	0.113	0.151	0.212	0.364	0.376	0.033
P42	P. aeruginosa	0.095	0.085	0.062	0.064	0.081	0.133	0.165	0.171	0.205	0.262	0.263	0.033
	E. cloacae	0.052	0.082	0.099	0.064	0.066	0.073	0.102	0.19	0.218	0.233	0.244	0.033
	S. aureus	0.073	0.067	0.076	0.075	0.085	0.085	0.167	0.218	0.214	0.254	0.315	0.033
	P. mirabilis	0.072	0.075	0.062	0.066	0.076	0.097	0.411	0.215	0.278	0.347	0.345	0.033

Figure 6. Anti-biofilm activity of rhamnolipid extracted from *Pseudomonas aeruginosa* isolates by using



tissue culture plate method (1-10) different concentration of rhamnolipid (100-0.2 mg/ml), c+: control positive, c-: control negative, *P. aeruginosa* (A,B), *E. cloacae* (C,D), *S. aureus* (E,F), *P. mirabilis* (G,H).

This results in massive economic losses and puts pressure on the medical community to find alternative methods of treating diseases related to biofilms. As a result, attempts are being made to find effective antimicrobial molecules that are not susceptible to bacterial resistance mechanisms, such as those found in biofilms [69].

The TCP method was used to detect the anti-biofilm behavior of RLs in this current research. The present study TCP method was revealed that RL extracts from (P9, P13, P14, P15 and P42) isolates of *P. aeruginosa* were showed a highly significance activity as anti-biofilm against all tested pathogenic bacteria the effects started from MIC and increase the effect with increased the concentration of RL extracts. This finding is in line with another study that found that incubating P. aeruginosa with 0.2 mM of RL prevented adhesion and biofilm formation [70]. *P. aeruginosa* RL dispersed the biofilms of *Disulfovibirio vulgaris, E. coli*, and *S. aureus*, according to Wood et al [71]. Yamasaki et al[72] also found that RL inhibited the growth and biofilm formation of all oral bacteria tested. Furthermore, Silva et al [73] found that by using RL to eliminate *S. aureus* biofilms at 25°C and 1% concentration, they were able to remove about 35 percent of biofilm biomass (using various RL concentrations).

Biosurfactants, such as RLs, impact the initial attachment on different surfaces, preventing the formation of biofilms [74,75]. The effect of RL on biofilm formation may be linked to the destruction of the cell wall, changes in cell wall morphology, or ultimately disconnected biofilm architecture that forms individual cells [76]. RLs were influenced on chemistry of surface and change it charge and hydrophobicity and prevent adhesions and biofilm formation [77].

Conclusion

Study concluded that rhamnolipid exhibited antibacterial and anti-biofilm activities against pathogenic bacteria isolated from diabetic foot infection.

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