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Biological Effect of Rhamnolipid as Antioxidant and Anticancer Agent.

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Abstract

Forty two isolates were isolated from different sources (water and soil). Vitek 2 system were used for identification the isolates as *P. aeruginosa.*. Twelve biosurfactant producer isolates were selected due to primary screening assay of hemolysis, CTAB, oil dispersion, drop collapse, emulsification index(E24%) methods. Twelve biosurfactant producer isolates were confirmed by 16SrDNA gene identification. Biosurfactants were purified by column chromatography method to obtain honey sticky product. TLC, FTIR, GC-MS, and HPLC were used to characterize purified biosurfactant as rhamnolipid. Rhamnolipid showed anticancer activity against MCF7 and SKG cell lines causing inhibition of viability at IC50 concentration. Acridine orange ethidium bromide staining revealed that rhamnolipid causing necrosis of cancer cells for 72 hrs. treatment with IC50 concentration.

Keywords: *Pseudomonas aeruginosa*, Rhamnolipid, Antioxidant, Anticancer, Cell lines.

Introduction

Pseudomonas aeruginosa is a gram–ve, bacilli–shaped had the potential ability to produce variety of extracellular compounds such as pigments and biosurfactants [1]. Biosurfacant is active surface compounds with hydrophilic and hydrophobic domains, which allow polar and non-polar mediums to have preferential surfaces, for that lowering interface and surface tension [2]. Rhamnolipids are glycolipid compounds made up of Pseudomonas spp., which comprise one or two fatty acid and one or two molecule of rhamnose, hydrophobic group linked via glycocid bound [3]. Rhamnolipids can be used in a variety of industries, including petroleum, bioremediation, agriculture, cosmetics, food, and pharmaceuticals, because of their excellent surface and biological activities [4,5,6]. The present study aimed to evaluate the biological activity of rhamnolipid as antioxidant and anticancer agent.

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 Governorate. One ml soil sample was added to sterile distilled water (10ml) and make dilution, o.1ml on pseudomonas agar base medium was spreaded. 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 [7]. 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

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for 20min at 10000rpm. Rhamnolipid biosurfactant production was investigated in supernatant using various methods (drop collapse, hemolysis test, emulsification index, CTAB method, oil dispersion) [8].

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 [9].

Estimation the rhamnolipid concentration

The L-rhamnose standard curve was used to assess the rhamnolipid content of the supernatant sample [10].

Extraction of rhamnolipid by Chloroform-methanol

After the incubation time, the rhamnolipid biosurfactant was harvested. According to Bharali et al [11] 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 [12].

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 [13].

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.

Rhamnolipids activity as Antioxidants

The free radical scavenging ability of rhamnolipid was determined based on their ability to damage DPPH radical (2,2 -diphenil-1- picrylhydrazyl) was examined by the method defined by [14].

Anti-cancer activity of rhamnolipid

The IRAQ Biotech Cell Bank Unit in Basrah provided human breast cancer (MCF7) and esophageal cancer (SKG) cancer cells, which were maintained in RPMI-1640 added to it 100 units/mL penicillin, 10% Fetal bovine serum, , and 100 µg/mL streptomycin.

Combination cytotoxicity assays

The MTT cell viability assay was performed in microtiter plates to evaluate the cytotoxic influence [15]. The rhamnolipid extracts that gave high cytotoxic effect on MCF7 and SKG cells. Then they were treated with different concentrations of rhamnolipid biosurfactant extracts (50, 100, 250, 450, 650) µg/ml) dissolved with RPMI media and 0.1 % DMSO, then incubated from 72 hrs., at 37 ^{o}C to determine the IC50.

Acridine orang ethidium bromide staining method

MCF7 and SKG are cultured on the slide cover and using a media containing 10% of bovine serum, the plate is covered with its special cover and sealed. With a strip of Parafilm, mark and leave for 24 hours in an

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incubator at 37 ^{0}C and 5% CO2. Then the media is removed, and the cells are exposed to the semi-lethal concentrations (IC50) of the rhamnolipid extracts. After incubation at 37 ^{0}C and CO2 5% cell stained with solution (70µl) of acridine orang and ethidium bromide, then examined with fluorescent microscope and photographed.

Statistical analysis

All data were analyzed statistically by applying uni and multivariate 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 governarate 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 [16,17,8,18,19,20]. Various organic compounds can be break down by *Pseudomonas aeruginosa*, which had the ability to degrade hydrocarbon in contaminated soil [21,22,23,19].

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 *Pseudomonas aeruginosa* by using vitek 2 system [24,25,26,27].

Genetic identification of Pseudomonas spp. by using 16SrDNA gene

The 16SrDNA region of 12 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 12 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 [28,29,30]. Because of some isolates gave 88% identity by using vitek 2 system, the identification was confirmed by 16SrDNA gene.

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 [31,32,33,34,8].

Table 1. Primary Screening Results of Rhamnolipid Producer Isolates.

Isolate no.	CTAB	hemolysis	Oil dispersion	Drop collapse	Emulsification index(E24 %)				
					kerosene	Soybean oil	Olive oil	Diesel oil	Sunflower oil
1	+	+	+	+	73	74	70	55	73
2	+	+	+	+	73	70	68	53	76
8	+	+	+	+	68	78	68	52	63
9	+	+	+	+	80	75	80	55	75
13	+	+	+	+	62	65	65	57	65
14	+	+	+	+	64	72	67	67	55
15	+	+	+	+	73	73	65	52	82
16	+	+	+	+	68	75	73	50	71
22	+	+	+	+	71	77	65	55	74
34	+	+	+	+	76	73	70	56	76
36	+	+	+	+	66	60	60	52	60

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 6, 2021, Pages. 1744-1758
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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 [35,36,37].

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*. For further study 5 isolates were selected.

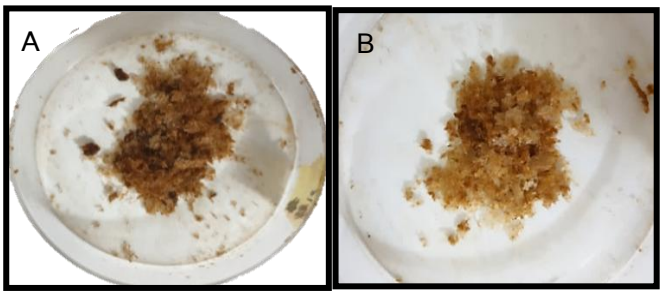
Extraction and purification of rhamnolipid

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, figure (1A).

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* [38] who used solvent extraction of rhamnolipid from *Pseudomonas aeruginosa* UKMP14T. also Kaskatepe *et al* [39] were used chloroform-methanol for extraction rhamnolipid from acidified cell-free supernatant by using equal volume. Another study by Kaya *et al* [40] were used solvent extraction chloroform-methanol for extraction of rhamnolipid from *Pseudomonas spp.* While Salleh *et al* [41] 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, as shown in figure(1B). Tawfeeq and Yesser [42] used the same method and obtained the same honey coloured products. Other study were also used column chromatography to purified rhamnolipid of *P. aeruginosa* [43]. Christova *et al* [44] also used column chromatography to purified RL from *P. aeruginosa*.

Figure 1. crude(A) and purified(B) rhamnolipid from *Pseudomonas aeruginosa* isolates.



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. 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 [8] used rhamnose test to estimate the rhamnolipid concentration in cell- free supernatant from *Pseudomonas aeruginosa*. And Ozdal *et al* [45] were estimated the rhamnolipid concentration in supernatant by phenol-sulfuric acid method from rhamnose standard curve.

Table 2. results of rhamnolipid estimation

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

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 6, 2021, Pages. 1744-1758
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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 rhamnolipid, table (3).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 and image.

sample	Rf1	Rf2	**Image**
P9	0.85	0.94	
P13	0.27	0.85	
P14	0.85	-	
P15	0.705	-	
P42	0.88	-	

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 [46], Lotfabad *et al* [47], Denney [48] , Das *et al* [49], Abdel-Mawgoud *et al* [50], and Oluwaseun *et al* [51].

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 [3].

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 (2).

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 [52] showed that IR spectroscopy gave a good result for analyzing the chemical structure of biosurfactant. In their study, Rikalovic et al [53] 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* [3], and the study of Mehdi et al [54] 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 [38]. All of the above functional groups indicates the glycolipid nature of studied biosurfactant which belong to rhamnolipid [18,9].

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

Wave Number (cm-1)	Assignment

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 6, 2021, Pages. 1744-1758
Received 25 April 2021; Accepted 08 May 2021.

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

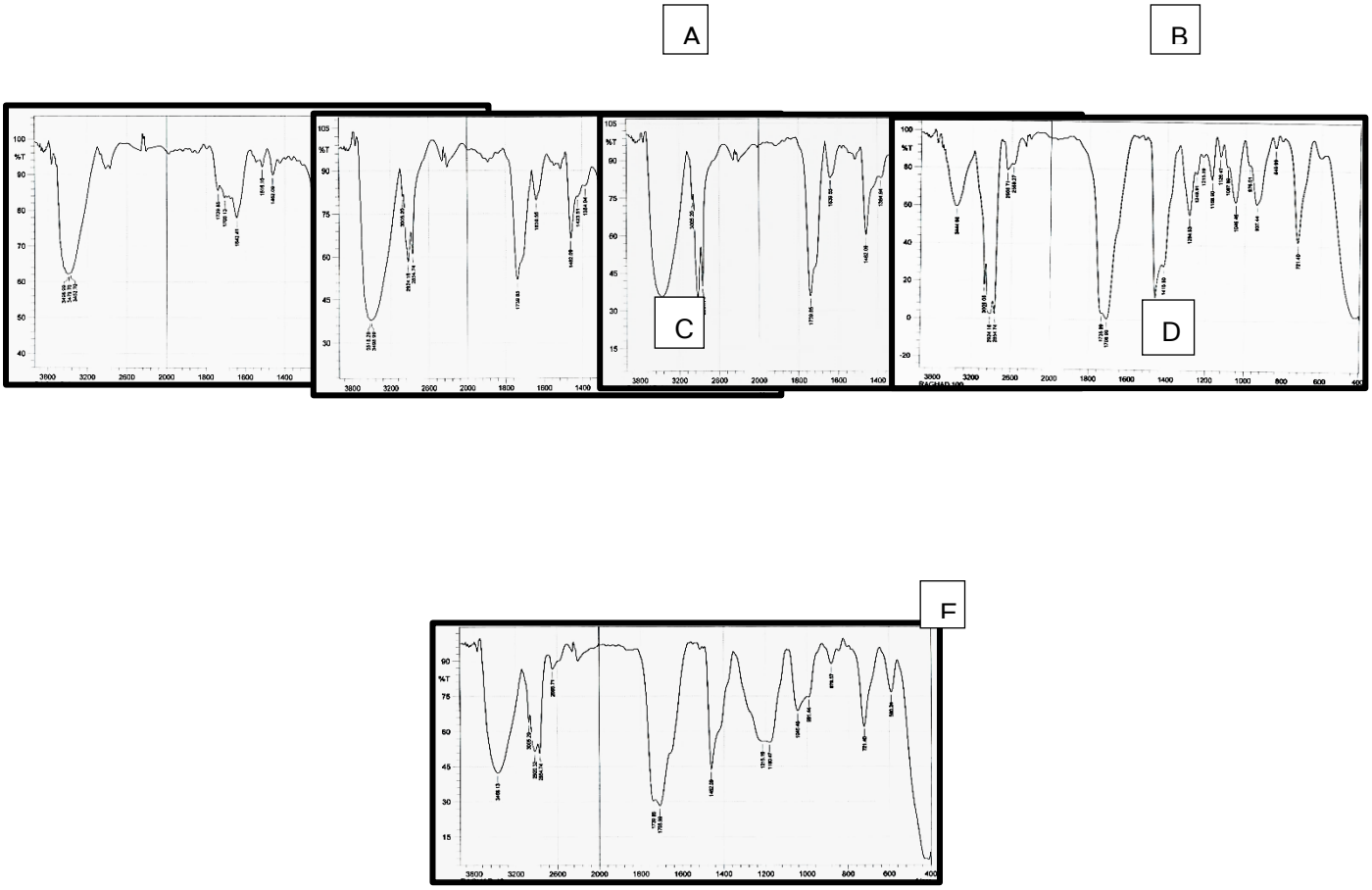

Figure 2. FTIR spectrum of rhamnolipid extracted from P9(A), P13(B), P14(C), P15(D) , P42(E) isolates of *Pseudomonas aeruginosa*.

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 (3)

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 6, 2021, Pages. 1744-1758
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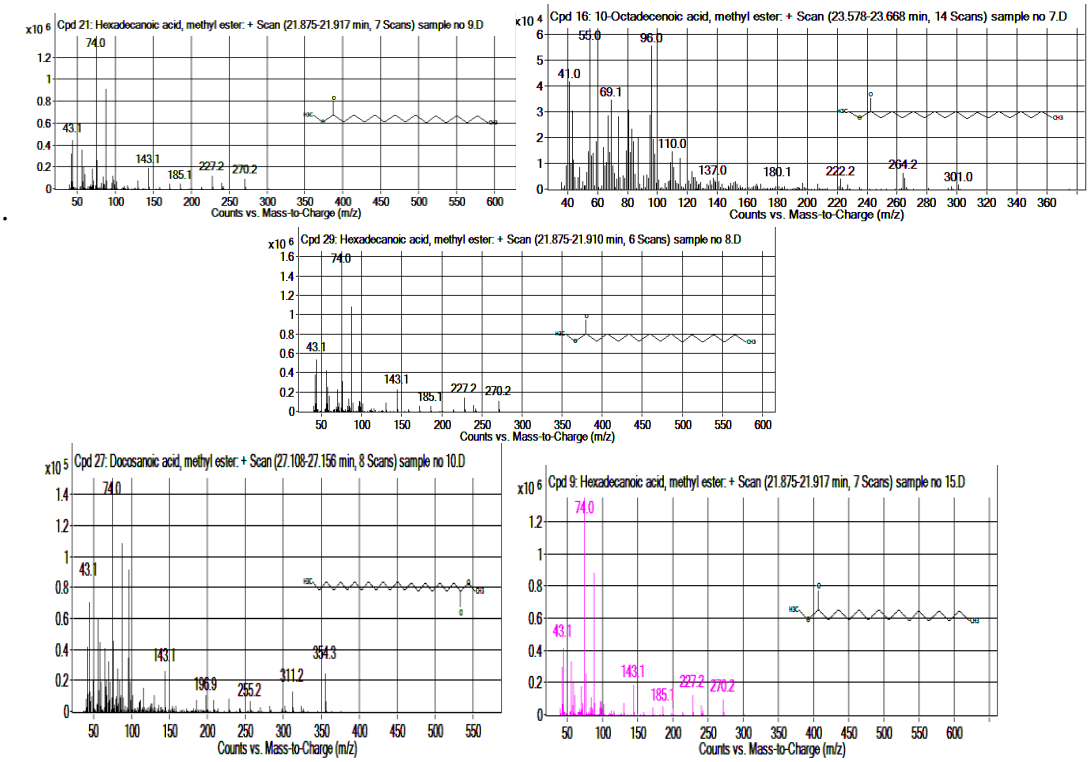

Figure 3. GC-mass spectral analysis of rhamnolipid extracted from P9 ,P13, P14, P15 , P45 isolates of *Pseudomonas aeruginosa*.

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 [18,55,56,57].

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 (4). 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 [58,59,60]. Tawfeeq and Yesser [42] also used HPLC for identification of rhamnolipid produced in sample by *P.aeruginosa.*

Effect of rhamnolipid as antioxidant

In a published paper, the antioxidant activity of rhamnolipid was not thoroughly investigated. The DPPH radical scavenging method was one quick and relatively inexpensive way to evaluate the antioxidant activity of compounds, table (5), and figure (5).

Free radicals are very harmful to human health and can cause several disease like diabetes, cancer etc. various kind of antioxidants mainly from natural sources such as enzymes, carotenoids, ascorbic acid, poly phenols etc. inhibit the cellular damage generally through free radical scavenging property [61]. As an antioxidant, ascorbic acid prevents free radical oxidation by donating one electron to the radical, allowing it to remain stable [62].

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Results of the current research revealed that rhamnolipid exhibited good free radical scavenging activities of DPPH at all concentration, comparing to reference standard ascorbic acid. This results agree with Medoza *et al* [63] who obtained the same results when they tested the ability of rhamnolipid extracts to damage DPPH radical. While Haque *et al* [64] were observed the antioxidant activity of rhamnolipid extracted from *Marinobacter literalis* increase when the rhamnolipid concentration increase the maximum activity at (5mg/ml). While the study of Abdollahi *et al* [65] observed that RL (0.9-7.2mM) extracted from *P. aeruginosa* display low antioxidant activity compare to vitamin E. In another study, Kosaric and Sukan [66] reported the antioxidant activity of mannosyl erythritol lipids (MELs) and Sophrolipids (some types of glycolipid).

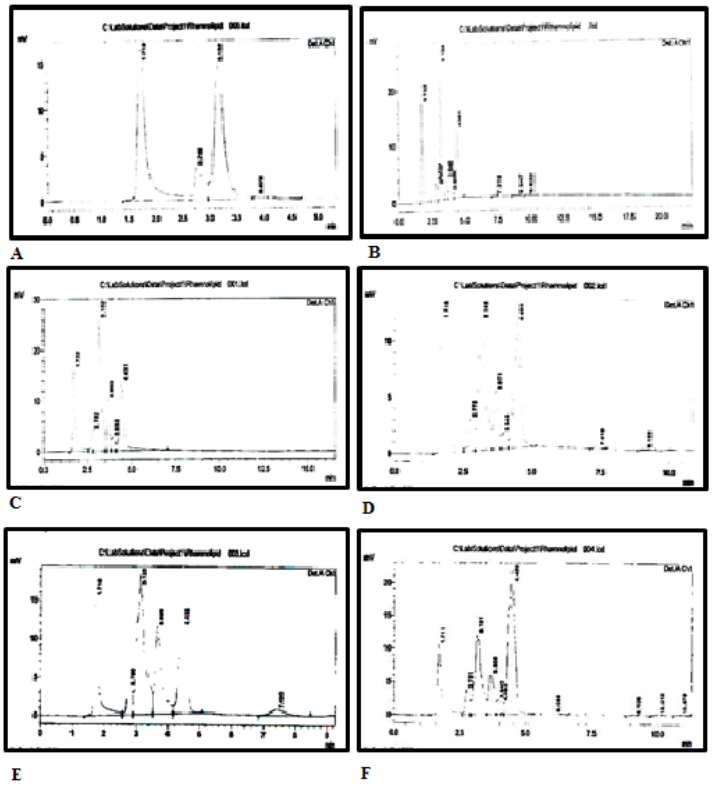

Figure 4. Rrhamnolipid standard curve(A), extracted from P9 (B) ,P13 (C) , P14(D) ,P15 (E) and P42(F) by HPLC analysis.

Table 5. activity of rhamnolipid extracts as antioxidant to DPPH

	Sample	Concentration of rhamnolipid (mg/ml)			
		100	10	1	0.1
% inhibition	Ascorbic acid	95.32	94.30	95.93	93.90
	P9	95.32	94.30	93.49	92.27
	P13	95.73	95.32	94.91	92.07
	P14	95.93	95.73	95.32	91.86
	P15	95.93	95.73	94.71	92.88
	P42	96.13	94.91	93.29	92.07

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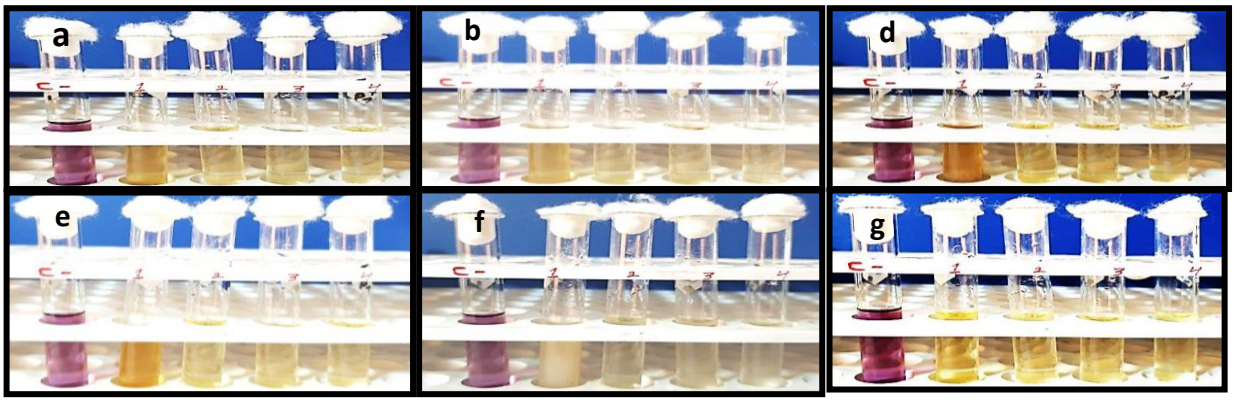


Figure 5. Activity of rhamnolipid as antioxidant agent, a: rhamnolipid extracted from P9 isolate, b: rhamnolipid extracted from P13 isolate, d: rhamnolipid extracted from P14 isolate, e: rhamnolipid extracted from P15 isolate, f: rhamnolipid extracted from P42 isolate, g: ascorbic acid, C-: indicating control negative, (1: 100 mg/ml, 2: 10 mg/ml, 3: 1 mg/ml, 4: 0.1 mg/ml) concentration.

Anticancer activity of rhamnolipid

To evaluate the activity of rhamnolipid extracted from (P9, P13, P14, P15, and P42) isolates of *P. aeruginosa*, two different tumor cell lines (MCF7 and SKG) were exposed first time to one concentration of each rhamnolipid extracts for 27 hrs. incubation period. MTT test was used for determining the number of viable cell with an absorbance at 620 nm. Results showed that rhamnolipid extracts from (P9, P13, P14, P15, and P42) inhibited the propagation of human breast cancer cell line (MCF7), as shown in table (6), while the rhamnolipid extracted from 15 sand 42 had no inhibitory effect on esophagus cancer cell line (SKG) during exposure period, as shown in figure (6).

IC50 value of studied rhamnolipid against MCF7 and SKG cell line was investigated .Results showed that rhamnolipid extracted from (P9) high cytotoxic activity with (IC50=92µ/ml), while (P14) slightly weaker with (IC50=95µ/ml), whereas (P13) less activity with (IC50=354µ/ml) on MCF7 cell line, as shown in figure (7).

The results of rhamnolipid extracted on SKG cell line showed the (P13) high cytotoxic activity with (IC50=177.5µ/ml), while (P9) less cytotoxic activity with (IC50=187µ/ml), as shown in figure (8).

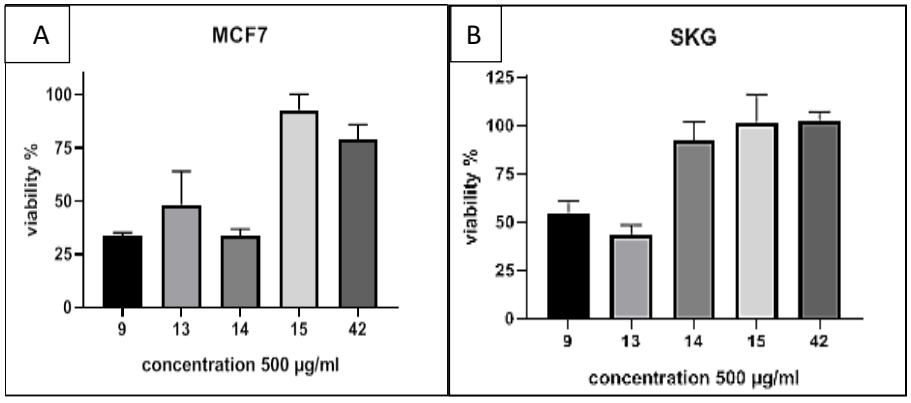
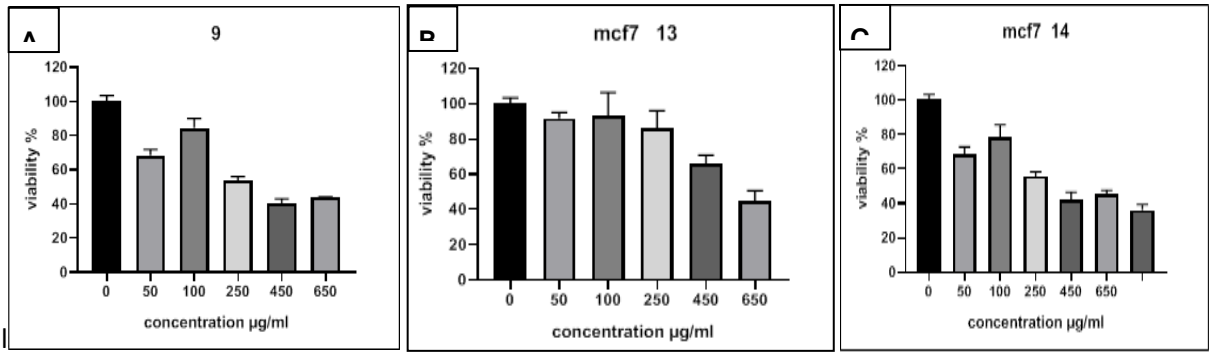


Figure 6. activity of rhamnolipids as anticancer agent against MCF7 (A) and SKG (B) cell lines at 500 µ/ml concentration.



Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 6, 2021, Pages. 1744-1758
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Figure 7. inhibitory effect of different concentrations of rhamnolipid extracted from P9(A), P13(B) and P14(C) isolates against MCF7 cell lines.

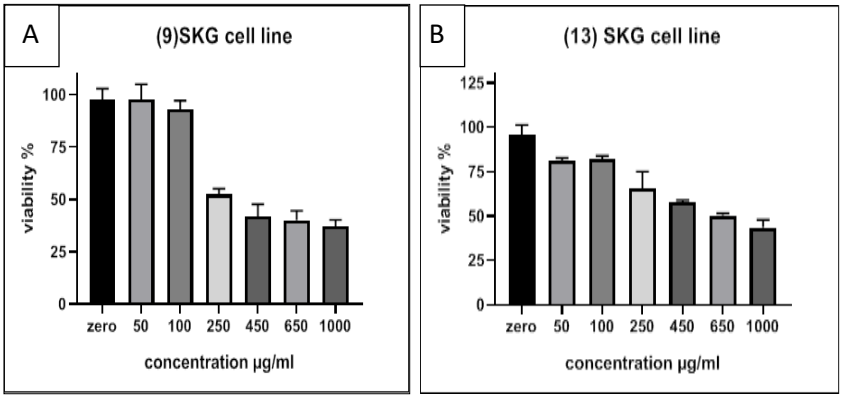

Figure 8. Inhibitory effect of different concentrations of rhamnolipid extracted from P9(A) and P13(B) isolates against SKG cell lines.

One of the most promising discoveries recently revealed that RL has the ability to act as an anticancer agent, slowing the development of some cancers [67]. The results indicate the RL extracts appeared to be effective on inhibition of MCF7 and SKG cell lines. This explain the effect of surface hydrophobicity, and the contact with the cell surface has a greater effect on the survival of the cell. This may be because the cell surface is hydrophobic, and mono-RLs have a more hydrophobic structure than di-RLs, resulting in more efficient cell interaction. This study compatible with Rahimi *et al* [68] reported that Rhamnolipid-1 and Rhamnolipid-2 extracted from *P. aeruginosa* MR01 both inhibited MCF7 cells proliferation. Moreover, RLs extracted from *P. aeruginosa* M14808, which included di-rhamnolipid as major component, inhibited MCF7 cells proliferation [69]. In addition, Allada et al [70] were reported the activity of purified rhamnolipid produced by *Streptomyces coelicoflavus* NBRC (15399) as anticancer agent against propagation of MCF7 cells. Lotfabad et al [47] investigated the rhamnolipid produced by P. aeruginosa MR01 inhibited the propagation of Hela cancer cells when used 5µg/ml concentration because the cytotoxic effect of rhamnolipid. And , Thanomsub et al [71] investigated the biological effects of di-rhamnolipid extracted from *P. aeruginosa* B189 on a human breast cancer cell line (MCF7) during treatment. They discovered that di-Rhamnolipid could effectively stop MCF7 cells from growing.

Acridine Orange-Ethidium Bromide Staining

MCF7 and SKG cell lines exposed to rhamnolipid extracts of P. aeruginosa, as well as control (untreated cells), were stained with acridine orange-ethidium bromide to establish the prevalent form of cell death.

Green live (L) MCF7 cells with normal morphology were observed in control. The cells treated with IC50 (92, 354, 95) µ/ml concentration of rhamnolipid extracted from P9, P13, P14, respectively, for 72 hrs. treatment, were observed necrotic cells (N), as shown in figure (9). SKG cells with normal morphology were observed as green live (L) in control. The cells treated with IC50 (187, 177.5) µ/ml concentration of rhamnolipid extracted from P9 and P13, respectively for 72 hrs. treatment, the necrotic cells (N) were observed in figure (10).

The present study revealed that rhamnolipid extracts of *P. aeruginosa* caused cell necrosis in MCF7 and SKG cells. The majority of the cells were red, but a few had bright green spots, indicating that necrosis had occurred. To demonstrate the cytotoxic effect of glycolipid, Haque et al [71] stained cancer cell lines with acridine-orange-ethidium bromide, which revealed necrotic cells that were red in color. Gupta et al [72] showed the necrotic cells red in colour when stain the prostate cancer cell line (PC3) with acridine orange-ethidium bromide after treated the cells with glycolipid.

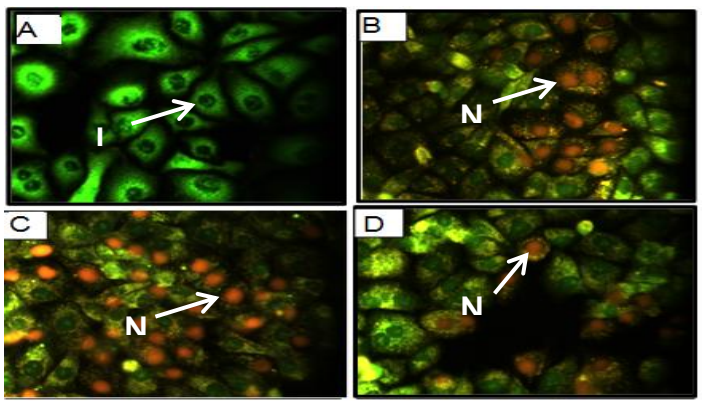

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Figure 9. anti-cancer activity of rhamnolipid extracted from *Pseudomonas aeruginosa* isolate control (A), P9 (B), P13 (C), P14 (D), against MCF7 cell line, L:live, N:necrosis.
Figure 10. anti-cancer activity of rhamnolipid extracted from *Pseudomonas aeruginosa* isolate Control (A), P9 (B), P13 (CS), against SKG cell line L:live, N:necrosis.

Conclusion

Rhamnolipid extracted and purified from *Pseudomonas aeruginosa* showed a good anti- oxidant and anti-cancer activity, against cancerous cell lines (MCF7 and SKG).

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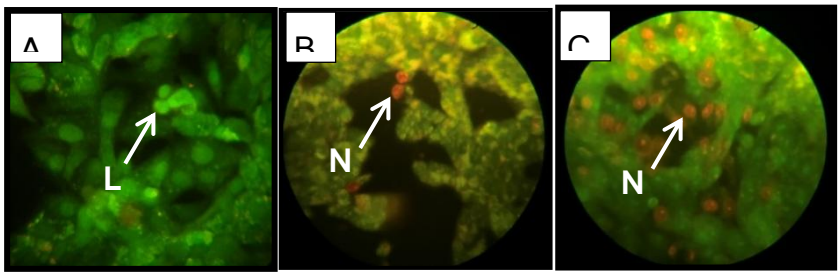

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