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# Optimization and Characterization of Glycolipid Produced from *Pseudomonas aeruginosa*

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**Abstract**

Rhamnolipids (RLs) are surface-active chemicals primarily generated by *Pseudomonas aeruginosa* and classified as glycolipid biosurfactants. They have attracted interest in several fields because of their unique properties. Therefore, this study aimed to extract, optimize and characterize the glycolipid from a clinical isolate of *P. aeruginosa*. One hundred-twenty specimens were gathered. Hemolysis test (primary screening) was performed to estimate the isolates that have the ability to produce biosurfactant (glycolipid). Only nine isolates of *Pseudomonas* spp. showed the highest hemolysis zones ranged between (26-40 mm), respectively. These isolates were subjected to secondary screening and emulsification index ($E_{24}$%) to choose the best biosurfactant (glycolipid) produced isolate, whereas *Pseudomonas* sp. P30 isolate showed the highest $E_{24}$% value, which was 60.6%, and was later subjected to the VITEK system, which confirmed this isolate was *P. aeruginosa* with a probability of 86%. Optimization of culture conditions was performed, and the results revealed that the best condition was when the isolate was cultured in mineral salt medium (MSM) containing olive oil and a mixture of $NH_4Cl$ and peptone as carbon and nitrogen sources at pH 7 and 72 hrs. of incubation. Four solvents were utilized to select the best solvent for glycolipid extraction, and the best solvent mixture was methanol: chloroform (1:2). Glycolipid was partially purified using silica gel column chromatography. In addition, Fourier transforms infrared (FTIR) spectrum and Fatty acid analysis (GC-Mass) used to characterize glycolipid. The study indicated that the biosurfactant extract was rhamnolipid.

**Keywords:** Biosurfactant, Olive oil, Emulsification Index, Chromatography, Glycolipid.

# تحديد الظروف المثلى وتوصيف الدهن السكري المنتج من الزائفة الزنجارية

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**الخلاصة**

الرامنوليبيدات (RLs) هي مواد كيميائية نشطة سطحيًا تُنتَج بشكل أساسي بواسطة الزائفة الزنجارية، وتصنّف ضمن المستحلبات الحيوية من نوع الدهون السكرية. وقد جذبت هذه المركبات اهتمامًا واسعًا في العديد من المجالات نظرًا لخصائصها الفريدة. لذلك، هدفت هذه الدراسة إلى استخلاص، تحسين، وتوصيف الدهن السكري من عزلة سريرية لبكتيريا الزائفة الزنجارية. تم جمع 120 عينة، وأُجري اختبار التحلل الدموي

(كمرحلة فحص أولية) لتحديد العزلات القادرة على إنتاج المستحلبات الحيوية (الدهون السكرية). أظهرت تسع عزلات فقط أعلى مناطق تحلل دموي، تراوحت بين (26–40 ملم). خضعت هذه العزلات إلى مرحلة الفحص الثانوي واختبار مؤشر الاستحلاب (E24%) لاختيار العزلة الأكثر كفاءة في إنتاج المستحلب الحيوي. أظهرت عزلة الزائفة P30 أعلى قيمة لمؤشر E24% بلغت 60.6%. تم تأكيد هوية هذه العزلة باستخدام نظام (VITEK)، حيث تبين أنها عزلة زائفة زنجارية بنسبة احتمالية بلغت 86%. تم تحسين ظروف النمو، وأظهرت النتائج أن أفضل الظروف كانت عند زراعة العزلة في وسط أملاح معدنية (MSM) يحتوي على زيت الزيتون ومزيج من $NH_4Cl$ والبيبتون كمصادر للكربون والنيتروجين، عند درجة حموضة pH 7 وفترة حضانة بلغت 72 ساعة. تم استخدام أربع مذيبات لاختيار أفضل مذيب لاستخلاص الدهن السكري، وأظهرت النتائج أن أفضل مزيج من المذيبات كان الميثانول: الكلوروفورم (1:2). تمت تنقية الدهن السكري جزئيًا باستخدام كروماتوغرافيا عمود السيليكا جل. بالإضافة إلى ذلك، تم استخدام مطياف الأشعة تحت الحمراء بتحويل فوريه (FTIR) وتحليل الأحماض الدهنية بواسطة كروماتوغرافيا الغاز –مطياف الكتلة (GC–Mass) لتوصيف الدهن السكري. أشارت نتائج الدراسة إلى أن المستحلب الحيوي المستخلص هو الرامنوليبيد.

## 1. Introduction

Glycolipids are amphiphilic molecules, whereas hydrophilic molecules may be amino acid, cyclic peptide, phosphate, carboxylic acid, alcohol, or glucose, while hydrophobic molecules are either hydroxyl fatty acid, a-alkyl-b-hydroxy fatty acid or long-chain fatty acid. These compounds that microorganisms excrete extracellularly and occasionally cell-to-cell during development on water-immiscible substrates [1,2]. Because biosurfactants (BS) are non-toxic and have antimicrobial, antifungal, and antiviral properties, they may find application as therapeutic agents. They trigger an increase in membrane permeability, which in turn leads to cell lysis and metabolite loss due to leaky membranes. Partitioning at the interfaces allows these chemicals to modify microbial adhesion properties [3,4]. The industrial, agricultural, food, cosmetic, and pharmaceutical sectors all use surfactants substantially. Most of these surfactants are synthetically created by chemical processes and could be harmful to ecosystems [5,6]. Secondary metabolites, such as the glycolipid and glycoprotein produced by bacteria, are crucial to the organisms' existence because they aid in nutrient transport, host-microbe interactions, and biocide production [7]. Its production from renewable feed-stocks is made possible by a rich microbial diversity, including *Pseudomonas aeruginosa* [8], which is a widespread bacterium [9,10].

*P. aeruginosa* produces a number of glycolipids, although rhamnolipid is among the best recognized [11]. Rhamnolipids are biosurfactants that have been extensively studied due to their potential industrial and biomedical applications. One or two rhamnose moieties connected to one or two fatty acid chains make up these molecules. They exhibit surface-active properties, which make them useful in various biotechnological processes such as bioremediation, improved recoveries of oils, and pharmaceutical formulations [12,13].

The biosynthesis of glycolipids in *P. aeruginosa* is a complex process involving multiple enzymes and metabolic pathways. Understanding the regulation of glycolipid production in this bacterium is crucial for developing strategies to control its pathogenicity and exploring the biotechnological potential of these compounds [14-16].
Overall, the production of glycolipids by *P. aeruginosa* represents an intriguing aspect of its biology with implications for both basic research and practical applications. Therefore, this work aimed to optimize and characterize the glycolipid produced by *P. aeruginosa*.

## 2. Materials and method

*Collection of samples and isolation of P. aeruginosa*

One hundred-twenty specimens were collected from clinical sources (wound, burn and Middle ear infections), kept in a transport medium tube and transported into a laboratory during the period of four months (from October 2022 to January 2023). The experiments were done in the environment laboratory in the Department of Biotechnology at the College of Science, University of Baghdad. All specimens were subjected to various examinations, including microscopic (gram staining), cultural characteristics (colonies on MacConkey and Cetrimide agar plates) and biochemical test (oxidase test), in addition to confirmative test (VITEK 2 system). Subsequently, the acquired isolates were preserved at 4°C in a 20% glycerol solution. A total of 120 specimens were collected from clinical sources (wound, burn and middle ear infections) and distrusted as follows: 51.67% (n=62/120) from the wound, 30% (n=36/120) from burn and 18.3% (n=22/120) middle ear sources.

*Screening program for glycolipid production*

The screening program for *Pseudomonas* spp. involved both primary and secondary screening to assess its ability to produce glycolipid and choose the best isolate for production, as described below:

*Primary screening (qualitative/hemolysis test)*

The obtained *Pseudomonas* spp. isolates were evaluated for the production of glycolipid on blood agar plates [17]. For the selection of the highest glycolipid-produced isolates, previously activated bacterial culturing inoculum of each isolate (1 x $10^6$ CFU/ml) was placed into wells formed on blood agar medium using cork borer (5 mm in diameter). Plates were incubated for 24 hrs. at 37°C. The results were represented as diameters of clear zones (mm) formed around wells, which were estimated using an electronic ruler [5].

*Biosurfactant production using secondary (quantitative) screening*

*Preparation of inoculum*

To prepare the inoculum of bacterial isolates, the previously activated bacterial culture in brain heart infusion (BHI) broth was made, and the cells were counted using a spectrophotometer to obtain 1x$10^8$ cell/ml (O.D 0.5), which was obtained via dilution of cells using same broth.

*Biosurfactant production*

A mineral salt medium (MSM) was used to produce the extracellular biosurfactant. The MSM was autoclaved and then inoculated with 2% (1x $10^8$ CFU/ml) of activated *Pseudomonas* sp. inoculum and incubated in a shaker incubator at 37˚C for 96 hours [18]. After incubating for 96 hours, the extracellular biosurfactant was extracted using centrifugation at 10,000 rpm for 15 minutes. Centrifuging 10 milliliters of culture allowed us to assess the dry biomass. Furthermore, the use of the cell-free supernatant for testing emulsification activity was also conducted.

*Emulsification Index (E24%)*

Two ml of toluene was added into cell-free supernatant, then mixed for 2 min. and subsequently left for 24 hrs. The emulsifier layer height was determined at room temperature. According to the following equation, the emulsification index was estimated as described by Patil *et al.* [19].

$$Emulsification\ Index\ (E24) = \frac{Emulsion\ layer\ height\ of\ (mm)}{Broth\ total\ height\ (mm)} \times 100\%$$

*Estimation of dry weight (biomass)*

Ten milliliters of culture was centrifuged and filtered through 0.2 mm filter paper to estimate biomass. The following is an estimate of the paper's dry weight after 24 hours of drying at 80°C [20]:

The dry weight (mg)= the weight of paper (with biomass) – the weight of paper (without biomass)

*Glycolipid Extraction*

After incubating for 96 hours, the extracellular biosurfactant was extracted using centrifugation at 10,000 rpm for 15 minutes. In order to determine the optimal solvent for extraction, the biosurfactant-containing supernatant was moved to a separating funnel and subjected to a series of solvent extractions using different ratios of v/v methanol and chloroform (1:2), di ethyl ether (1:1), ethyl acetate (1:1), and petroleum ether (1:1). After removing the watery layer from the bottom of the separating funnel, the emulsion layer was transferred to a glass Petri dish and dried at temperatures ranging from 40 to 45 °C until it became powder. After weighing, the final product was placed in a clean vial and kept at 5°C until needed again [21].

*Optimal conditions of glycolipid production*

Different parameters, including pH value, nitrogen source, carbon source, and time of incubation, were tested to determine the optimal conditional situations for glycolipids (biosurfactants) production from the local isolate of *Pseudomonas* sp.. The experiments were described as follows:

*Optimum carbon source*

Different carbon sources, including soybean, glucose, glycerol, glycerol + glucose, olive oil, and coconut oil, were examined. A 1% (OD = 0.5; $1x10^8$ CFU/ml) of a previously activated *Pseudomonas* sp. was suspended in 50 ml of sterile MSM containing 2% of each tested carbon source at pH 7 and placed in a shaker incubator at 37°C for 72 hours. Then the emulsification index (E24%) was measured.

*Optimum nitrogen source*

Different nitrogen sources, including organic (protease-peptone, yeast, and urea) and non-organic ($NH_4Cl$, $NaNO_3$, and $NH_4NO_3$) sources, were examined. The MSM was prepared as follows: For estimation of organic source, MSM contains $NH_4Cl$ with 5 g/L of either protease - peptone, yeast, or urea. For estimation of inorganic source, MSM contains protease-peptone with 1 g/L of either $NH_4Cl$, $NaNO_3$, or $NH_4NO_3$. Control: MSM contains protease- peptone and $NH_4Cl$ as nitrogen sources.

A previously activated *Pseudomonas* sp. (1%; OD = 0.5; $1x10^8$ CFU/ml) was suspended in 50 ml of sterile MSM containing 2% of the best carbon source and examined nitrogen source at pH 7 and placed in a shaker incubator at 37°C for 72 hours. Then the emulsification index (E24%) was measured.

*Optimal pH value*

Different pH values, including pH 5, 6, 7, 8 , and 9, were examined. A previously activated *Pseudomonas* sp. (1%; OD = 0.5; $1x10^8$ CFU/ml) was suspended in 50 ml of sterile MSM containing 2% of each tested carbon source and the best selected nitrogen source at each different pH value and placed in a shaker incubator at 37°C 72 hours. The emulsification index (E24%) was measured.

*Optimum incubation time*

Different incubation periods, including 24, 48, 72, 96 and 120 hours, were estimated. A previously activated *Pseudomonas* sp. (1%; OD = 0.5; $1x10^8$ CFU/ml) was suspended in 50 ml of sterile MSM containing 2% of each tested carbon source and the best selected nitrogen source at the optimal pH value and placed in a shaker incubator at 37°C for different incubation times. The emulsification index (E24%) and biomass were measured.

*Partial purification of glycolipid*

The glycolipid was partially purified using a dialysis tube and thin-layer chromatography as follows:

*Dialysis*

A dialysis tube with a molecular weight cutoff 8,000- 10,000 Daltons was used to concentrate the biosurfactant solution (2.5 g of glycolipid and 10 ml of phosphate buffer saline) was put in the dialysis tube, and the tube closed on both sides then immerse in sucrose for 1hr in cold condition until biosurfactant concentrated.

*Silica gel chromatography*

Following this process, glycolipid was partly isolated from the crude mixture: A chloroform-dissolved 60 slurry of heat-activated silica gel was transferred to a 3.5*30 cm glass chromatography column. The column was supplemented with 10 g of crudely extracted glycolipid, and diluted in 4 ml of chloroform. Separation of mono-glycolipid required two steps: first, the column was washed with chloroform at a flow rate of 60 ml/h to completely elute the natural lipids, and second, 250 and 200 ml of a chloroform/methanol in 50:3 and 50:5 ratios were added, respectively. The last step in eluting glycolipid was using a 50:50 mixture of the aforementioned solvents with 100 ml of methanol alone. Then, layer chromatography was used to verify the fractions [22].

*Characterization of glycolipid*

*Fourier transforms infrared (FTIR) spectrum*

After the substance was mixed with KBr, FTIR spectra of the biosurfactant were analysed using data collected from wavelengths ranging from 500 to 4000 wave numbers per centimeter. Spectra were obtained using a UV spectrophotometer manufactured by Shimadzu-I, Raffinity-1. Another way the spectra have been shown is as intensity vs wave number [23].

*Gas chromatography- Mass spectroscopy (GC-Mass)*

Gas chromatography (GC) was used to analyse biosurfactants and find out what percentage of fatty acids they contained. The following method was used to analyse the fatty acid structure. The procedure included dissolving 10 mg of partly purified biosurfactant in 1 ml of sulphuric acid-methanol (1%) at 90 ◦C for 15 hours, followed by adding 1 ml of hexane while stirring, and finally, extracting the hexane layer after the sulphuric acid had evaporated. 1 millilitre of dilute white vinegar was added to the hexane layer and stirred until completely mixed. After hexane was utilised for recovery, the fatty acid methyl ester was subjected to gas chromatography (GC) using helium as the carrier gas. The GC was fitted with a fused silica capillary column (30 m x 0.25 mm, 0.25 m film thickness) by Shimadzu [21].

## 3. Results and Discussion

*Isolation of P. aeruginosa*

Only 35% of isolates (n=42/120) were identified as *Pseudomonas* spp., which were grown on MacConkey agar as flat, colorless colonies, while their colonies appeared as green colonies on Cetrimide agar. These isolates were Gram-negative rod bacterial cells under a light microscope, in addition to their positive result for oxidase. *P. aeruginosa* is a leading cause of illness and death in hospitalized patients owing to its high level of antibiotic

resistance [24]. The findings of a study conducted by Shatti *et al.* [25] detected that forty (35.1%) out of 114 patient samples were confirmed to be *P. aeruginosa*.

*Screening program for biosurfactant (glycolipid) production*

*Primary screening*

A hemolysis test was performed to estimate the isolates that have the ability to produce biosurfactants (glycolipid). All forty-two isolates of *Pseudomonas* spp. produced β-hemolysis with different diameters of lysis zones on blood agar, as shown in Figure 1. The results are illustrated in Table 1. Only nine isolates of *Pseudomonas* spp. (P2, P11, P12, P17, P26, P29, P30, P33 and P38) showed the highest hemolysis zones ranged between (26-40 mm) and were selected as biosurfactant-producers for further experiments.

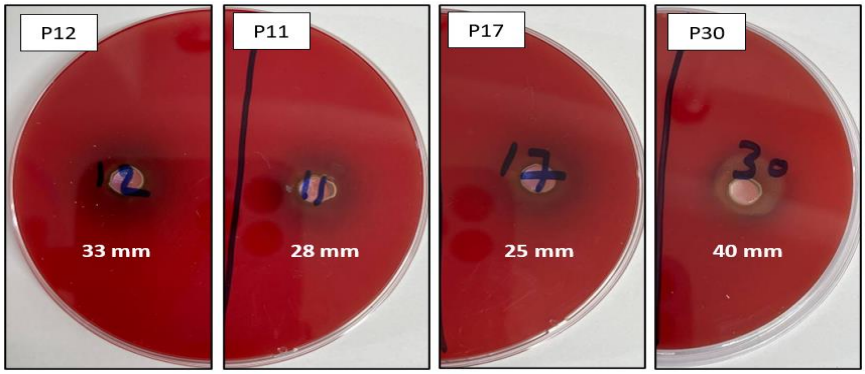


**Figure 1:** β-hemolysis produced by *Pseudomonas* spp. isolates on blood agar plate.

**Table 1:** Results of hemolysis test.

| No. | Hemolysis zone (mm) | No. | Hemolysis zone (mm) | No. | Hemolysis zone (mm) | No. | Hemolysis zone (mm) |
|---|---|---|---|---|---|---|---|
| **P1** | 13 | **P12** | 28 | **P23** | 10 | **P33** | 35 |
| **P2** | 27 | **P13** | 11 | **P24** | 11 | **P34** | 15 |
| **P3** | 11 | **P14** | 16 | **P25** | 10 | **P35** | 25 |
| **P4** | 10 | **P15** | 21 | **P26** | 26 | **P36** | 10 |
| **P5** | 6 | **P16** | 10 | **P27** | 25 | **P37** | 20 |
| **P6** | 7 | **P17** | 26 | **P28** | 17 | **P38** | 30 |
| **P7** | 14 | **P18** | 14 | **P29** | 30 | **P39** | 11 |
| **P8** | 12 | **P19** | 20 | **P30** | 40 | **P40** | 12 |
| **P9** | 11 | **P20** | 15 | **P31** | 25 | **P41** | 10 |
| **P10** | 10 | **P21** | 16 | **P32** | 9 | **P42** | 11 |
| **P11** | 33 | **P22** | 14 | | | | |

Glycolipid, like rhamnolipids, are a type of lipid molecule that, in the right concentration, can exhibit hemolytic activity, meaning they can break up red blood cells by lysing the cell membrane and destroying the cell wall [26]. There is a detergent-like process that, when activated, may enhance the permeabilization of human erythrocytes. This process causes the red blood cells to swell out of their typical biconcave disc form and lyse [27].

*Secondary screening*

Nine isolates of *Pseudomonas* spp. (P2, P11, P12, P17, P26, P29, P30, P33 and P38) were subjected to secondary screening, and the emulsification index ($E_{24}$%) was calculated to choose the best biosurfactant (glycolipid) produced isolate. The results in Table 3 indicated

that *Pseudomonas* sp. P30 isolate showed the highest $E_{24}$% value, which was 60.6%, as shown in Figure 2. Since *Pseudomonas* sp. P30 is more productive in producing biosurfactants, so it was chosen for further experiments.

**Table 3:** Secondary screening of biosurfactant (glycolipid) produced isolates of *Pseudomonas* spp. using emulsification index ($E_{24}$%)

| No. of isolate | $E_{24}$% | No. of isolate | $E_{24}$ % | No. of isolate | $E_{24}$ % |
|---|---|---|---|---|---|
| **P2** | 37.8 | **P17** | 10 | **P30** | **60.6** |
| **P11** | 49.6 | **P26** | 32.5 | **P33** | 52 |
| **P12** | 36 | **P29** | 46.2 | **P38** | 47.3 |

*Confirmative test using VITEK 2 system*

This test was utilized to confirm the identification of *Pseudomonas* sp. P30. Using Gram-positive (GP) card which is composed of 43 biochemical tests. Based on the results, this isolate was *P. aeruginosa,* with a probability of 86%**.**

*Optimization of culture conditions for the production of glycolipid*

*Best carbon source*

As shown in Figure 2, the findings showed that olive oil was the best carbon source, with an E24% of 61.58%.

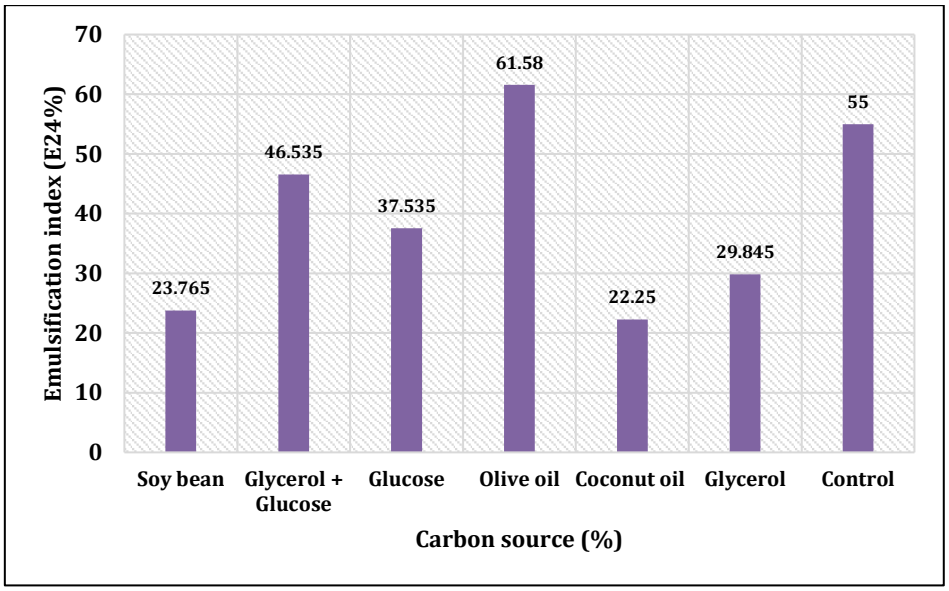


**Figure 2:** Influence of carbon sources on the production of biosurfactant from a local isolate of *Pseudomonas aeruginosa* P30 in MSM medium at pH 7 and 37 ºC for 72 hours.

*Best nitrogen source*

The results indicated that control ($NH_4Cl$ and peptone) was the best nitrogen source, with E24% equal to 65.2%, as shown in Figure (3).

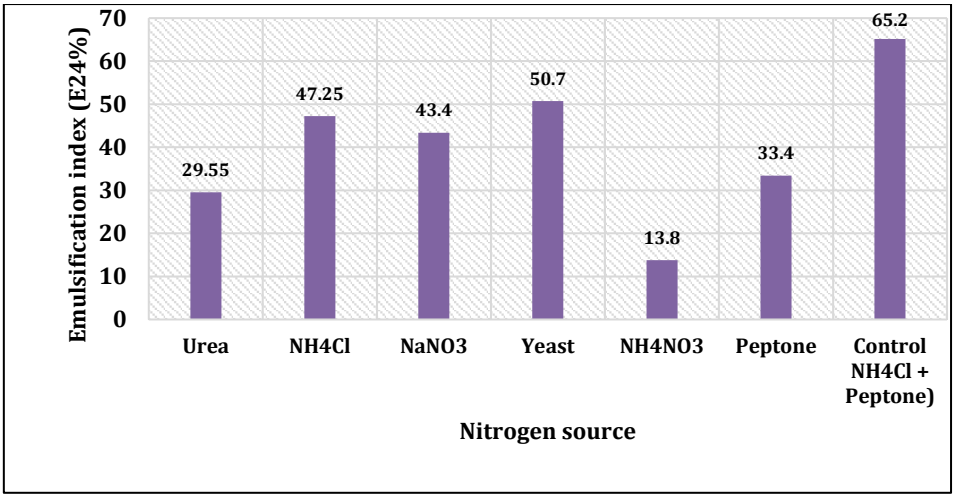


**Figure 3.** Influence of nitrogen sources on the production of biosurfactant from a local isolate of *Pseudomonas aeruginosa* P30 in MSM medium at pH 7 and 37 ºC for 72 hours.

*Optimal pH value*

The results indicated that pH 7 was the optimal pH value, with E24% at 66.5%, as shown in Figure 4.

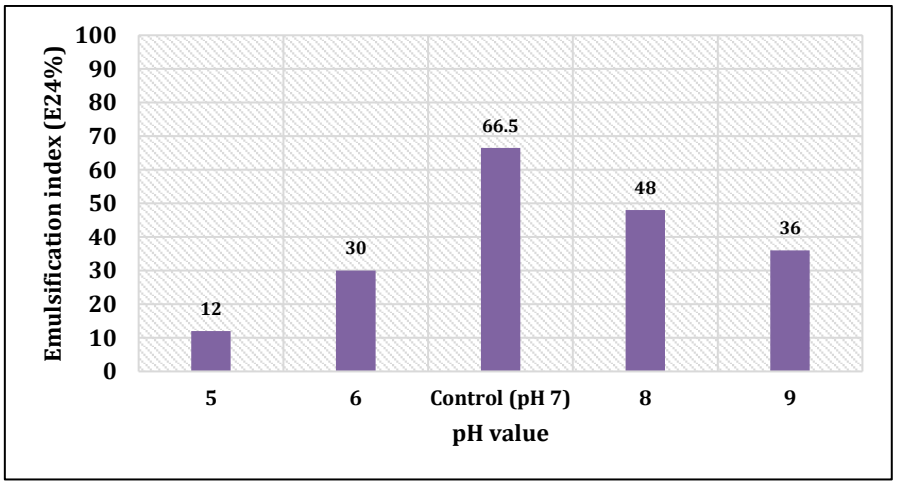


**Figure 4:** Influence of different pH values on the production of biosurfactant from a local isolate of *P. aeruginosa* P30 in MSM medium at 37 ºC for 72 hours.

*The effect of incubation time*

Different incubation periods, including 24, 48, 72, 96 and 120 hours, were examined to estimate the best incubation time for glycolipid and biomass production. The results indicated that the highest E24% of glycolipid was 67.8% after 72 hours of incubation, then this value was decreased to 25.4% after 148 hours of incubation, and no result was estimated after 120 hours, as shown in Figure 5, while the biomass was reached to 5.15 g/L of weight after 120 hours of incubation.

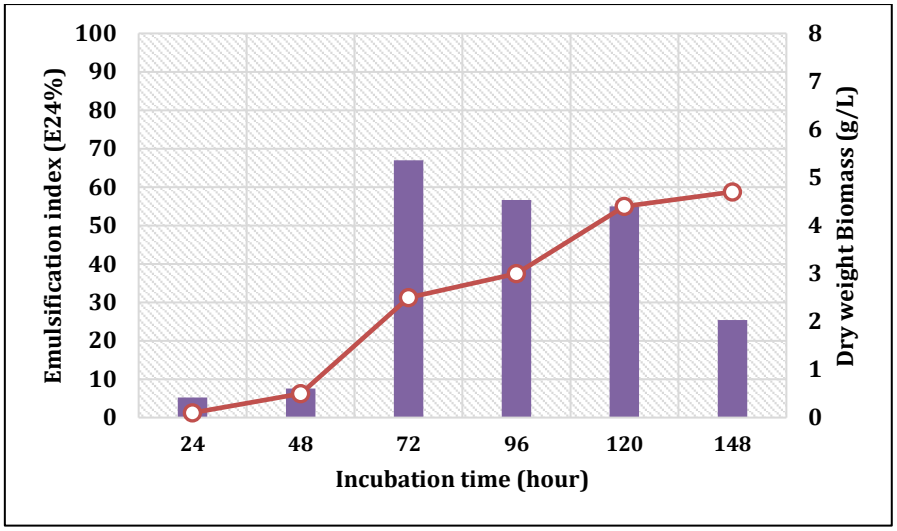


**Figure 5:** Influence of different periods of incubation on the production of glycolipid and biomass formation from a local isolate of *Pseudomonas aeruginosa* P30 in MSM medium at pH 7 and 37 ºC.

*Extraction of biosurfactant*

Four solvents were utilized to select the best solvent for extraction of glycolipid produced by *P. aeruginosa* P30. The results showed that the methanol: chloroform solvent mixture was the best choice for biosurfactant extraction, with the highest obtained weight of 5.1 g (Table 4). Methanol and chloroform are frequently utilized as solvents for biosurfactant extraction because of their capability to dissolve an extensive array of organic compounds, such as proteins and lipids [28]. The rhamnolipid biosurfactant was successfully extracted from the acidified cell-free supernatant using a solvent extraction procedure including chloroform: methanol (2:1).

**Table 4:** Determination of the best solution system for extraction of biosurfactant.

| Solvent | Ratio | The obtained weight of biosurfactant (g) |
|---|---|---|
| **Methanol: Chloroform** | **1:2** | **5.1** |
| **Di ethyl ether** | 1:1 | 4.15 |
| **Ethyl acetate** | 1:1 | 4.169 |
| **Petroleum ether** | 1:1 | 4.115 |

*Purification of biosurfactant*

The refined biosurfactant was obtained using silica gel column chromatography (3.5 × 30 cm). The column was loaded with 5.1 g of crude biosurfactant dissolved in chloroform. The emulsification activity of each fraction was assessed after collection. The findings of the correlation between fraction number and emulsification activity are shown in Figure 6. There were two glycolipid peaks identified in the data; the first peak occurred in elution 2 with a fraction number between 62 and 67 in the chloroform: methanol (50:5) mixture, and the second peak occurred in elution 3 with a fraction number between 71 and 76 in the same mixture. The results also showed that the emulsification activity was lowest at the first peak (E24%= 70) and highest at the second peak (E24%= 90). Column chromatography was used in several studies to purify biosurfactant compounds to purify biosurfactant produced by *Pseudomonas* sp. [29], and other microorganisms, including *L. plantarum* [1]. In a study by Alshaikh *et al.* [30], the fractions that were collected after the crude RL was purified using column chromatography were analysed using thin-layer chromatography (TLC). Rhamnose

domains, which correspond to mono- and di-rhamnolipids, were indicated by the spots found on the TLC plates (Rf values of 0.41 and 0.17, respectively).

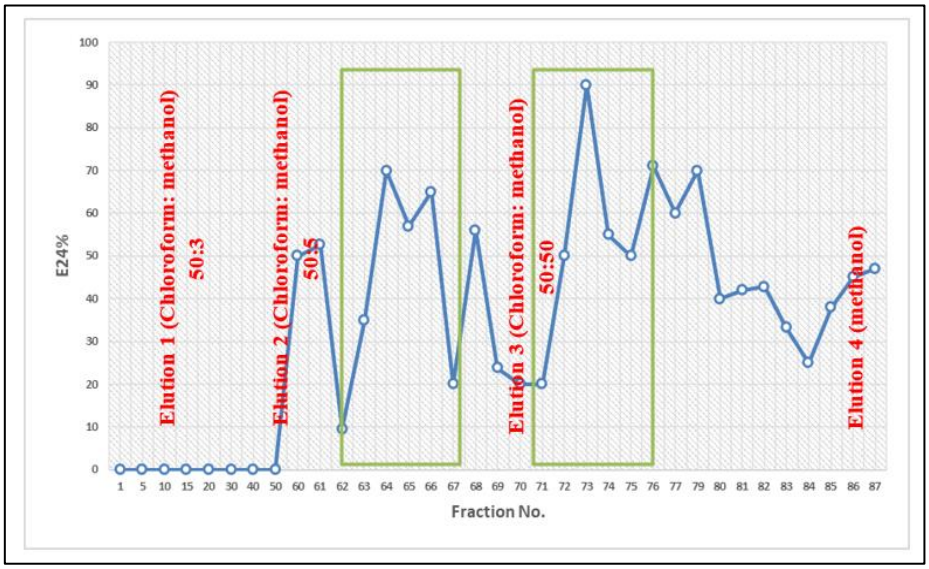


**Figure 6:** This layer chromatography for biosurfactant purification from *Pseudomonas aeruginosa* P30 using a silica column (3.5 × 30 cm) equilibrated and eluted with solvents, in flow rate 60 ml/hr.

*Characterization of glycolipid*

*Fourier transforms infrared (FTIR) spectrum*

The FTIR analysis validated the biosurfactant's glycolipid origin by revealing the presence of aliphatic hydrocarbon chains (lipids) and a polysaccharide component. Based on Figure 7, the band between 3839.77 and 3772.82 $cm^{-1}$ indicated the presence of free –OH groups due to –OH stretching of carboxylic acid groups and H-bonding of polysaccharides, respectively. Two bands at 3192.45 and 2431.70 $cm^{-1}$ may be due to the presence of the aldehyde, methyl and C-CH groups in the polysaccharide. Also, C=O, C=C, C-N, S=O and C-CH stretching were appeared at (1775.46 - 1601.63), (1538.83 - 1493.32), (1452.32-1410.79), (1311.89), (1053.70) and (973.08-620.43) $cm^{-1}$, respectively. The absorption peak around 1051 $cm^{-1}$ is assigned to C–O–C in the rhamnose molecule in glycolipid. The C-O-C bond in the glycolipid rhamnose molecule is responsible for the absorption peak at around 1051 cm-1.

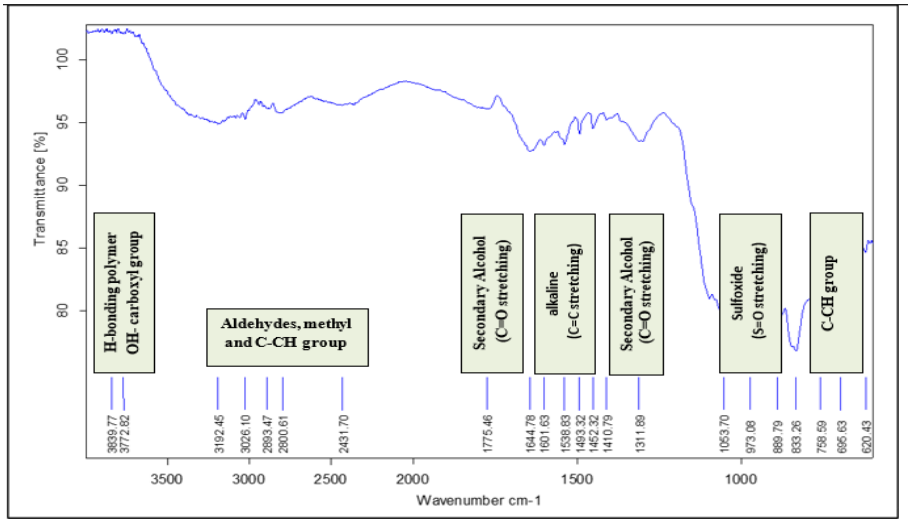


**Figure 7:** FTIR spectrum analysis glycolipid produced by *Pseudomonas aeruginosa* P3.

*Fatty acid analysis (GC-Mass):*

Figure 8 shows that many components are made up of pure biosurfactants. According to previous research that found fatty acids in GC-MS analyses of rhamnolipid compounds produced by *P. aeruginosa*, this study concluded that the biosurfactant extracts were really rhamnolipids [31]. The results also indicated that the major compound of biosurfactant was hexadecanoic acid with 100% relative abundance. The results were in agreement with Rantan and Kumar [32], where they found that Rhamnolipids contained hexodecanoic acids in addition to other compounds.

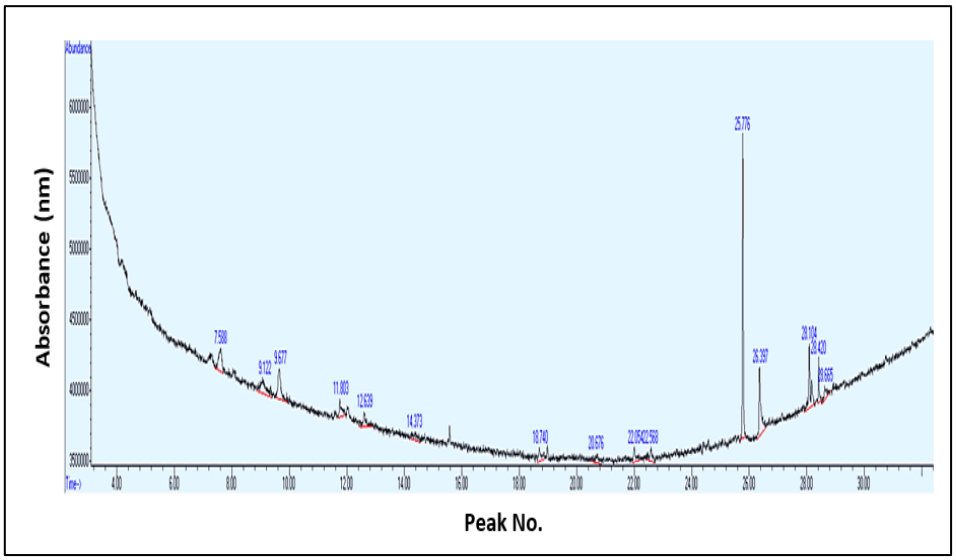


**Figure 8:** The GC mass analysis of partially purified biosurfactant produced by *Pseudomonas aeruginosa* P3.

## 4. Conclusion

In conclusion, *Pseudomonas aeruginosa*'s ability to produce glycolipids, especially rhamnolipids, is important for understanding its pathogenicity and physiology, and it also has great promise for medicinal and industrial applications. Rhamnolipids have biocompatibility and special surface-active features that make them promising candidates for use in bioremediation, innovative therapeutics, and environmentally friendly detergents. They have the potential to become an invaluable asset in various sectors due to forthcoming research and technical advancements that expand their usefulness even further.

## 4. Ethical Clearance

The experiments mentioned in this investigation were approved by the local committee of the Biotechnology Department (CSEC/0123/0010). The University of Baghdad staff conducted the investigation.

## 5. Conflict of Interest

There are no conflicts of interest between authors.

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