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RESEARCH ARTICLE

Characterization and Antibacterial Activity of the Natural Biopolymer Extracted from *Pseudomonas aeruginosa*

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ABSTRACT

Objective: This study aimed to extract, purify, characterize, and assess the antibacterial efficacy of the biopolymer extracted from *Pseudomonas aeruginosa*.

Methods: The ice-cold 96% ethanol and isopropanol were used for biopolymer extraction. For protein precipitation, 60% ammonium sulfate was utilized. The solubility, chemical color reactions, fourier transform infrared spectroscopy (FTIR), ultraviolet (UV)-visible spectroscopy and high-performance liquid chromatography (HPLC) techniques were used for the characterization of the biopolymer. The antibacterial efficacy of the biopolymer against the pathogens isolated from bacterial vaginosis was evaluated using the broth microdilution method.

Results: The extraction of biopolymer produced by *P. aeruginosa* with ice-cold 96% ethanol and isopropanol yielded 9.22 and 0.2 g/L, respectively, and could be successfully purified using 60% ammonium sulfate. The purified biopolymer exhibited solubility in distilled water. The color chemical reagents methods indicated the presence of monosaccharides, disaccharides, polysaccharides, and amino acids in the composition of the biopolymer. The carbohydrate average concentration of sugars in the samples was 1083.33 µg/mL. The UV-vis spectra of the produced biopolymer showed an absorbance peak at 285 nm. FTIR spectra revealed the main functional groups of the examined biopolymer. The FTIR spectra of the biopolymer exhibited main absorption bands at 3257.48, 2924.42, 2858.63, 1743.60, 1616.00, 1417.07, and 817.00 cm^{-1}. HPLC findings showed one clear peak indicating the purity of the produced biopolymer, with a peak absorbance of 145.930 mAU. Biopolymer showed the highest percentage of inhibition (98.3%) recorded against *Bacillus cereus,* followed by *Staphylococcus haemolyticus* (96.8%) at a concentration of 250 µg /mL.

Conclusions: This work concluded that *P. aeruginosa* is a potential biopolymer-accumulating bacteria and could be used for low-cost biopolymer production to combat bacteria that have developed antibiotic resistance.

INTRODUCTION

Biopolymers are biomolecule polymers derived from natural sources, either chemically or from biological material.[1,2] These molecules have properties that are suitable for a wide range of medicinal applications and serve various biological functions.[3,4] Based on the structure of the repeating unit,

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biopolymers can be categorized into three groups: proteins, polysaccharides, and nucleic acids.[5] Bacteria are regarded as the primary cell factories because they can produce a variety of extracellular and intracellular biopolymers, such as polyamides, polysaccharides, polyphosphates, polyesters, proteinaceous substances, and extracellular DNA, from nitrogen and carbon sources.[6,7] Maji8 states polysaccharides are biopolymers consisting of 20 and 60,000 monosaccharides linked through O-glycosidic bonds in a linear or branched manner to form polysaccharides. Based on cellular location, microbial polysaccharides are either Intracellular or extracellular.[9] Some microbes, including *Pseudomonas aeruginosa*, retain these compounds at high concentrations, enabling them to withstand unfavorable circumstances .[10] Scientists have been exhaustively trying to understand the processes involved in biopolymer production by microbial cell factories.[11] Exopolysaccharides (EPS) are produced by diverse microorganisms, including yeasts, fungi, and bacteria, using various raw materials.[12] The composition and generation of EPS directly relate to the supply of carbon.[13] With a range of biochemical structures, biopolymers produced by microbes vary greatly, leading to a wide range of chemical and physical properties; while some are polycationic, others are polyanionic.[14] The non-toxicity and biodegradability of biopolymers contribute to preserving a safe and secure environment. [15] Microbial biopolymers are promising targets for producing newly discovered antibacterial medications.[16, 17] EPSs may contain many functional groups, including carbonyl, phosphate, and hydroxyl groups, that are assumed to be essential for EPS's antibacterial and antioxidant functions.[18] These polymers may inhibit Gram-positive and Gram-negative microbes either in vitro or in vivo.[19] Antibiotic resistance (ABR) is increasingly recognized as a key health issue and is a developing global public health concern.[21,22] Bacterial vaginosis (BV) is the most prevalent cause of vaginal discharge on a worldwide scale, with prevalence rates varying between 23 and 29% in different areas of the world.[20] The antibiotics' effectiveness is diminished With the development and spread of bacterial resistance. Antibiotic resistance creates a challenge for developing new alternatives to overcome infections.[23] According to O'Neill,[24] if we don't come up with proactive solutions to this issue by 2050, 10 million lives per year and a total of 100 trillion USD in economic output will be in danger due to the increase of drug-resistant illnesses. Therefore, this study aimed to extract, purify, and characterize biopolymer produced by *Pseudomonas aeruginosa* strains and study its medical application as an antimicrobial agent.

MATERIALS AND METHODS

Bacterial isolates and Screening for biopolymer Production

The present study used environmental *P. aeruginosa* isolates (P1, P2, and P3) obtained from the Laboratory of Applied Microbiology, Department of Biology, College of Sciences, Basrah University, for biopolymer production. These strains were activated on nutrient agar medium (Oxoid, U.K), re-identified by conventional bacteriological methods, and then stored on glycerol agar at -20°C until use. To screen for biopolymer production activity, 5 mL of overnight broth culture of each isolate was added to 250 mL Erlenmeyer flask containing 100 mL of sterilized biopolymer-producing media as described earlier[25] with some modifications. Then, the culture media was supplemented with 50 mL date juice, 0.5 g NaNO3, 1.0 g yeast extract, 0.5 g KH2PO4, 0.25 g MgSO4.7H2O, pH 7.0. Further, 1-mL stock solution: $FeCl_3$.6H2O (0.08 g/L), $CuSO_4$.5H2O (0.075 g/L), H_3BO_3 (0.15 g/L) were added to the media and incubated at 35°C on a shaker at 180 rpm for 72 h. The cultures were centrifuged at 6,000 rpm for 30 minutes, and the cell-free supernatant was used to extract biopolymer.

Detection of biopolymers accumulation by Sudan black test

P. aeruginosa isolates were qualitatively analyzed for biopolymer production using slide,[26] plate.[27, 28] Black Stain methods. In the slide black stain method, the observation of blue-black droplets with pink color cytoplasm of the examined bacterial strain indicates accumulation of biopolymer granules, while in the plate method, the formation of black colony indicates biopolymer production. The positive isolates were subjected to biopolymer extraction and purification.

Extraction and partial purification of bacterial biopolymers by organic solvents

The produced bacterial biopolymers were extracted using two organic solvents, ice-cold 96% ethanol,[29] and isopropanol.[30] The bacterial cells were collected from the broth cultures by centrifugation at 6000 rpm for 20 min. The ice-cold ethanol 96% was added at a ratio of (1:3) v/v, while isopropanol was added at a ratio of (1:2) v/v to the supernatant and kept at 4˚C for 24 h to precipitate the biopolymer. The precipitates were centrifuged at 6000 rpm for 20 minutes at 4˚C, then collected, dried, and weighed. The purification was performed according to an author[31] with some modifications; the biopolymer was extracted from the supernatant after centrifugation of the isolate culture at 6000 rpm for 20 minutes, and then precipitated with ammonium sulfate salt at a concentration of 60% w/v (saturation ratios). Salt is added gradually, with constant stirring, and after complete dissolution, the solution is left for one hour at 4°C.

Characterization of biopolymer

Solubility test

The solubility of the extracted biopolymers was examined in water, acetone, ethanol, and dimethyl sulfoxide (DMSO). The findings were reported as soluble, partially soluble, and insoluble.

Color chemical reagents

To determine the chemical composition of the created biopolymer, various compounds were utilized,[32] including the Molisch test, to explore the presence of carbohydrates in the

obtained bacterial biopolymers, 1-mL of α- naphthol solution was added to 1-mL of the prepared bacterial biopolymer then the mixture was shaken well, then drops of concentrated sulfuric acid were added. The appearance of a violet ring indicates a positive reaction. In the iodine test, 1-mL of iodine reagent and 1-mL of biopolymer were mixed and thoroughly shaken for the detection of polysaccharides. The appearance of violet, dark blue, or red color indicates a positive reaction. To detect the reducing sugar, Benedict's test, 1-mL of Benedict's reagent was added to 1-mL of the prepared bacterial biopolymer, shaken well, and heated for 5 minutes in a water bath at 100°C. The formation of an orange precipitate indicates a positive reaction. Barfoed's test, for demonstration of mono-reducing sugar, 1-mL of Barfoed's reagent was mixed with 1-mL of the prepared biopolymer, then shook well and heated for 10 minutes in a water bath at 100 \°C; the formation of a red precipitate indicates a positive reaction. Ninhydrin test, for detection of amino acids, 1-mL of ninhydrin reagent was mixed with 1-mL of the prepared biopolymer, shaken well, and heated for 10 min in a water bath at 100°C. The appearance of blue or violet color indicates a positive reaction. Biuret test, test was used to discover the presence of proteins by adding 1-mL of NaOH (10%), $CuSO_4$ (1%) mixture to 1-mL of the prepared biopolymer and then shaking well. The appearance of a violet color indicates a positive reaction.

Estimation of the Carbohydrate Concentration

The carbohydrate concentration of the biopolymer was measured using the phenol–sulfuric acid method by preparation of a glucose standard curve.[33]

Ultraviolet-visible spectrum

This test was conducted at the University of Tehran, Iran. UV-vis spectra in the 200–800 nm range were used to determine the optical characteristics of the bacterial biopolymer. Distilled water was used as a blank reagent.[34]

Fourier transforms infrared Spectra

FTIR was conducted at the University of Tehran, Iran, to identify the functional groups of the purified *P. aeruginosa* biopolymer. FTIR spectra were collected at a spatial resolution of 4 cm^{-1} in the transmission mode, in the range of 400 to 3900 cm^{-1}.[35]

High-performance liquid chromatography

HPLC analysis was conducted using the HPLC device at the College of Pharmacy, Clinical Laboratory Sciences Branch, University of Basrah, Iraq. The solution was prepared from biopolymer-produced juice dates by dissolving 5 mg of each sample in 350 µL of distilled water, the sample distance traveled by the solvent 500 µL of acetonitrile and 20 µL of DMSO, and the metaphase consisted of 25:75 v/v of distilled water and acetonitrile respectively.[36]

The Bacterial isolates

The bacterial strains were isolated from Iraqi females who suffered from vaginosis. The isolates were genetically identified in a previous study by the authors.[37] The following bacterial strains were identified in samples (Table 1).

Table1: Pathogenic bacteria isolated from vaginal samples.

Bacterial species	*Isolation code*	*Isolates Number*
Staphylococcus haemolyticus	M1,M3,M11,M37,M12	5
Bacillus cereus	M5	1
Staphylococcus hominis	M6,M16	2
Escherichia coli	M15,M27,M33,M34,M22	5
Staphylococcus epidermidis	M17,M18	2
Staphylococcus aureus	M24	1
Klebsiella pneumonia	M26,M35,M36,M23	4
Enterococcus faecalis	M13,M28	2
Macrococcus caseolyticus	M31	1
Streptococcus agalactiae	M39	1

The antibacterial efficacy of the bacterial biopolymer

Before experiments, bacterial isolates from the stocks were cultured on blood agar media. The susceptibility of these bacteria to biopolymer was examined by broth microdilution method in 96-well microtiter plates.[38] Two-fold serial dilution of the examined biopolymer at concentrations 250, 125, 62.5, and 31.25 µg/mL was done using a nutrient broth medium. From each bacterial isolate, 100 µL of inoculum at a concentration of 1.5×10^6 CFU/mL (matched to 0.5 McFarland turbidity standards) was inoculated to each well. The positive control was inoculated with bacterial suspension only, while the negative well was left blank without inoculation. The plates were sealed using a perforated plate seal and incubated at 37°C for 24 hours.

RESULTS AND DISCUSSION

Screening of *P. aeruginosa* isolates for biopolymer production.

The increased demand for natural biopolymers for diverse industrial applications in the last few years has led to a renewed interest in EPS production by microorganisms.[39] Sudan black slide and plate stain methods were used to demonstrate the biopolymer production by *P. aeruginosa* isolates (P1, P2, and P3). All isolates showed the ability to store the biopolymer intracellular (Figures 1a and b). The obtained results agreed with those reported earlier.[40, 41] Aljuraifani *et al.*,[42] used the Sudan Black B (SBB) dye method for staining *P. aeruginosa* to detect PHA granules production. Abdelrhman *et al.*,[43] used the same stain for screening the biopolymer produced by some marine bacterial species. They reported that Screening of *P. aeruginosa* isolates for biopolymer production showed the ability to store the biopolymer intracellular.

Extraction and purification of biopolymer

The biopolymer produced by *P. aeruginosa* (P3) was extracted with ice-cold 96% ethanol and isopropanol yielded 9.22 and 0.2 g/L, respectively (Figure 2 a). The extraction with

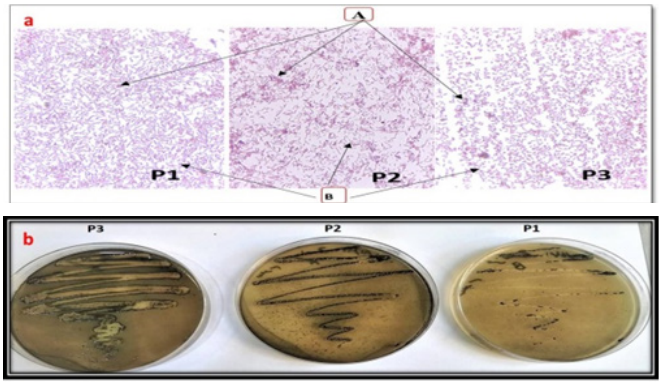

Figure 1(a): Sudan black slide stain method, *P. aeruginosa* isolates (P1, P2, and P3). (A). Dark-stained granules, (B). Bacterial cell stained red.
(b): Sudan black slide plate stain method *P. aeruginosa* (P1, P2, and P3) colony stained black.

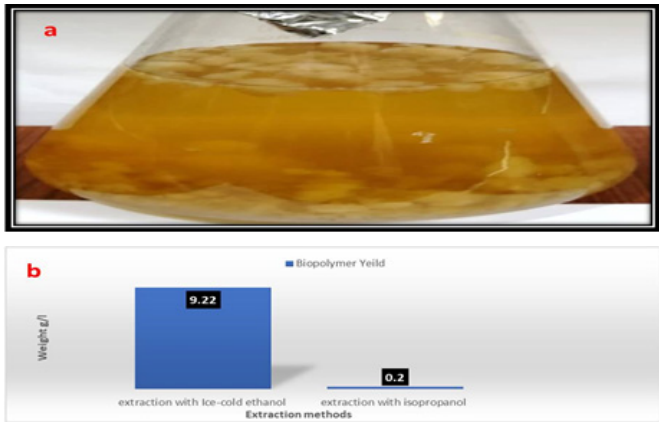

Figure 2(a): Extraction of biopolymer with through the ice-cold 96% ethanol.
(b): The yield obtained by the two extraction methods (ice-cold 96% ethanol and isopropanol) of biopolymer produced by *P. aeruginosa*)

ice-cold ethanol exhibited a better biopolymer yield (Figure 2b). Sivakumar *et al.*,[44] used ice-cold ethanol to extract *Pseudomonas* biopolymer and found that the maximum biopolymer produced among isolates was 3.17g/L. Also, the same technique was carried out in previous studies for extraction of biopolymer from *Bacillus licheniformis* strain CC91, *Bacillus thuringiensis* strain LU3, and *Bacillus paramycoides* SKA2.2 biopolymers.[45] Optimization of the separation processes is critical to the economics of biopolymer synthesis.[46] For protein precipitation, 60% ammonium sulfate was used. Baker *et al.*,[47] purified protein polymers using ammonium sulfate precipitation; they noticed that protein-polymer conjugates may find several new uses when protein solubility is increased in salt solutions through polymer conjugation. Ammonium sulfate is one of the best salts for precipitating proteins without producing denaturation.[48]

Characterization of biopolymer

The purified biopolymer was characterized by the determination of solubility, chemical color reaction, FTIR, UV-visible spectroscopy, and HPLC technique. The solubility test proved that the partially purified biopolymer was soluble in distilled water, and insoluble in dimethyl sulfoxide (DMSO), acetone, and 96% ethanol. The physical nature and the biopolymer's chemical structures determined the biopolymer's solubility in solvents and *vice-versa*. Binma *et al.*,[49] characterized the biopolymers isolated from palm oil; the obtained biopolymers were soluble in water and had two sizes (large and small) of molecular weight. Biopolymers with enough electronegative atoms and/or functional groups that can form hydrogen bonds with water tend to dissolve in water and are called water-soluble polymers and have a wide range of medical and industrial applications.[50] Using the color chemical reagents methods, the Molisch test showed the appearance of a purple ring, indicating the presence of carbohydrates in the composition of the polymer (Figure 3a). The Molisch test is easy to perform, and each biopolymer can be distinguished based on its color findings because it produces unique reaction products.[51,52] The iodine test proved that *P. aeruginosa* (P3) biopolymer contains oligosaccharides (Figure 3b). Parwani *et al.*,[53] recorded a similar finding who they characterized *Moringa oleifera* seed biopolymer-PVA composite hydrogel. Benedict's test exhibited an orange precipitate, indicating the presence of reducing disaccharides (Figure 3c). The same results were shown for reducing sugars like maltose, fructose, and lactose based on the qualitative method of Benedict.[54] Barfoed's test confirmed the presence of reducing monosaccharides (Figure 3d). The Biuret test indicated the absence of proteins in the composition of the tested product (Figure 3e), as it is a colorimetric method designed specifically for the detection of proteins and peptides is the Biuret method.[55] The ninhydrin test proved the presence of amino acids in the product's composition (Figure 3f). This result agreed with those of[56] who applied the ninhydrin reaction to analyze amino acids, peptides, and proteins in agricultural and biomedical sciences.

Determination of the carbohydrate concentration

The Dubies standard curve showed that the carbohydrate content of the biopolymer average concentrations of sugars in the samples were 1083.33 µg/mL^{-1}. Quero-Jiménez *et al.*,[57] evaluated the concentration of carbohydrates in the product of microbial origin. They found that the average concentrations of sugars in the samples were 10732.26 µg/mL^{-1}. The standard method for quick determination of the total carbohydrate content of bacterial and plant polysaccharides is still the phenol-sulfuric acid method.[58, 59]

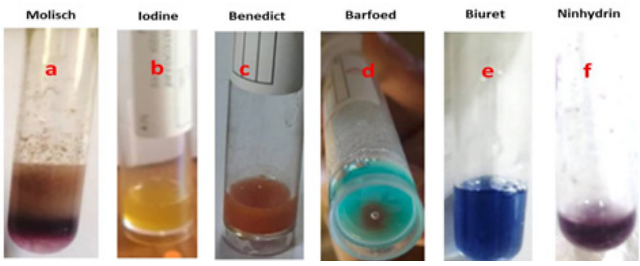

Figure 3: Color of chemical reagents for detection of the biopolymer, (a) Molisch test +ve (purple ring), (b) Iodine text +ve (yellow color), (c) Benedict test +ve (orange precipitate), (d) Barfoed test +ve (red precipitate), (e) Biuret test -ve (blue color), (f) (Ninhydrin test +ve (dark purple).

UV-visible spectrophotometer analysis of biopolymer

The UV-vis spectra of the produced biopolymer by *P. aeruginosa* showed an absorbance peak centered at around 285 nm (Figure 4). This result was consistent with those reported by[60] who analyzed PCL/PMMA biopolymer blend films using a UV-visible spectrophotometer. They indicated that an absorption band was centered around 280 nm. Abdelrazek *et al*.,[34] studied the properties of the PCL/PMMA biopolymer blend using a UV visible spectrophotometer. They proved that no absorption peaks at wavelength >280 nm.

Fourier Transform-infrared analysis of the extracted biopolymer

The chemical characterization of the isolated biopolymer was carried out using FTIR. The FTIR spectra revealed the main functional groups of the examined biopolymer, Table 2 and Figure 5. Similar findings were reported.[61] The FTIR spectra of the *P. aeruginosa* (P3) biopolymer exhibited main absorption bands at 3257.48, 2924.42, 2858.63, 1743.60, 1616.00, 1417.07, and 817.00 cm^{-1}. The broad bands observed at 3257.48 cm^{-1} in the spectra correspond to the N-H stretching of amines. The two bands detected at 2924.42 and 2858.63cm^{-1} represent the stretching of the C-H alkane compound. Absorption bands revealed the presence of strong ketones C-O stretch groups at 1743.60 cm^{-1}. The bands that appeared at 1616.00, 1417.07, and 817.00 cm^{-1} correspond to the C=C stretch, C-H bend, and C-H bend vibration, suggesting the presence of aromatic compounds. Previous studies have reported absorption bands at 2986.44, 2858.50, and 1637.56 cm^{-1} for biopolymers.[42] FTIR spectra of the pure PHA of *P. aeruginosa* and standard PHB showed two intense absorption bands at 1,740 and 1220 cm^{-1} that correspond with C-O stretching groups and ester carbonyl C=O group, respectively. The peaks recorded at 2,922 and 3,340 cm^{-1} represent bands of C-H stretching and O-H bonding, respectively.[62] Mahgoub *et al*.,[63] reported the same functional groups.

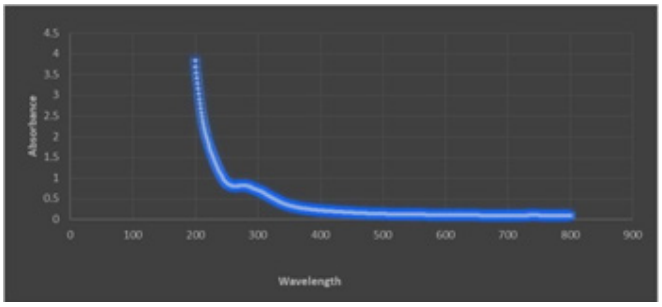

Figure 4: The spectrum of UV-vis absorption of biopolymer, absorbance peak centered at around 285 nm

Table 2: Functional groups detected in the biopolymer produced by *Pseudomonas aeruginosa* (P3) by FTIR spectrum analysis

Functional class	*Functional group*	*Appearance*	*Transmission (cm^{-1})*
Amines	N-H stretch	Board, Medium	3257.48
Alkane	C-H stretch	Medium, strong	2924.42
Alkane	C-H stretch	Medium, strong	2858.63
Ketones	C-O stretch	Strong	1743.60
Alkenes	C=C stretch	Conjugated	1616.00
Aromatic Compounds	C=C stretch	Medium, weak	1417.07
Aromatic Compounds	C-H bend	Strong	817.00
Aromatic Compounds	C-H bend	Strong	774.31

High-performance liquid chromatography analysis of the biopolymer

The HPLC analysis showed one clear peak, indicating the purity of the produced biopolymer, and the detention time for the apparent peak was 2.130 minutes, with a peak absorbance of 145.930 mAU, Figure 6. These results were nearly similar to those obtained by an author.[64] Owlia *et al*.,[65] applied a novel HPLC technique for determining alginate in *P. aeruginosa*, and they concluded that the proposed approach was a simple and valid method for bacterial alginate testing. HPLC for biopolymers represents a significant step forward in the bioanalytical properties, such as the materials' nature (monomer versus aggregate).[66] The HPLC assay method is simple, specific, exact, accurate, robust, and stability-indicating, and it may be successfully employed for routine sodium alginate analysis in bulk medication and pharmaceutical dosage form.[67, 68]

The antibacterial activity of biopolymer

The antibacterial activity of the biopolymer against 24 isolates was tested by the broth microdilution method. The results showed that antibacterial efficacy increased with

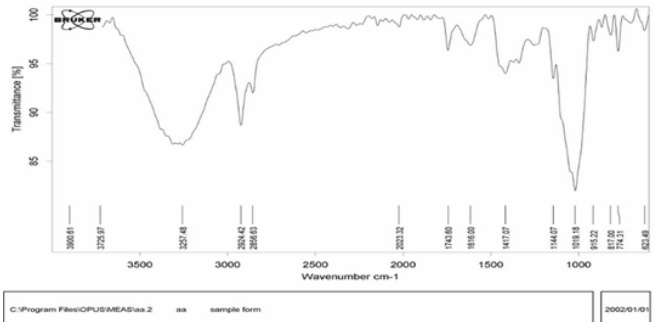

Figure 5: FTIR spectra of the purified biopolymer extracted from *P. aeruginosa*

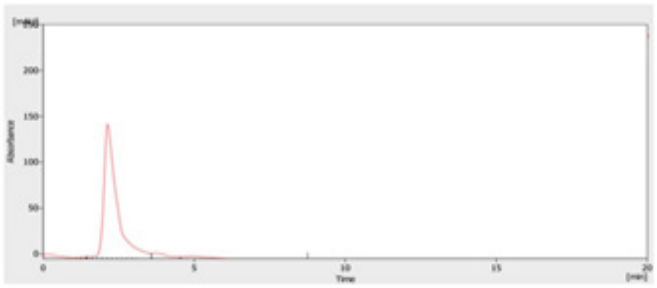

Figure 6: HPLC analysis of biopolymer extracted from *P. aeruginosa*

Table 3: Antibacterial activity of biopolymer against pathogens isolated from bacterial vaginosis.

NO. of isolates	*Bacterial species*	*Biopolymer concentration (µg/mL)*				
		250	*125*	*63*	*32*	*Control*
		Percentage of inhibition (%)				
1	*S. haemolyticus* yasmun69	95.9	47.7	45.9	25.7	0.414
2	*S. haemolyticus* Hakim 1980	68.7	35.6	32.9	51.6	0.559
3	*S. haemolyticus* yasmun69	52.7	38.9	-	7.88	0.450
4	*S. aureus* S21	59.3	-	19	-	0.415
5	*S. haemolyticus* EE103-B1	96.8	59.3	71.3	51.8	0.599
6	*S. hominis* R14	89.9	15.8	1.04	-	0.276
7	*E. coli* EC87E	84.3	58.4	50.7	30	0.559
8	*E. coli* 01P2R2D2E5	73.6	38.2	38.7	23.8	0.405
9	*E. coli* IAUK 8735	54.4	54.6	40.5	21.8	0.559
10	*E. coli* 152-a blue	78	64.5	48.2	31.3	0.509
11	*S. hominis* subsp. novobiosepticus	61.3	3.46	-	33.5	0.375
12	*Macrococcus caseolyticus* ZY02	38.8	-	-	-	0.049
13	*K. pneumoniae* NK 2.bp-1	86.9	61.9	21.4	19.5	0.611
14	*E. faecalis* ABC3	80.8	50.7	-	-	0.661
15	*K. pneumoniae* M1	90.6	51.6	31.1	20.1	0.438
16	*S. agalactiae* 149	34.8	35.7	-	-	0.115
17	*B. cereus* H6	98.3	-	-	-	0.300
18	*S. epidermidis* Y19	57	55.1	-	-	0.414
19	*S. epidermidis* BP11	-	-	-	-	0.319
20	*S. haemolyticus* OB058	55.6	43.1	8.88	-	0.383
21	*E. faecalis* UFVCC1180	20.3	-	13.6	10.2	0.177
22	*E. faecalis* ABC3	7.39	-	-	-	0.203
23	*E. coli* EC87E	63.7	55.4	44.3	-	0.942
24	*E. coli* 06P2R2D2E3	70.6	61.7	44	39.8	0.698

increased biopolymer concentration against gram-positive and gram-negative bacteria, Table 3. The highest percentage of inhibition (98.3%) was recorded against *Bacillus cereus* strain Gvt-Sh-12 at a concentration of 250 µg/mL, followed by *Staphylococcus haemolyticus* strain EE103-B1 (96.8%). The minimum percentage of inhibition (7.3%) was recorded towards *Enterococcus faecalis* strain ABC3 at the same concentration, while there was no effect toward *S. epidermidis* at all concentrations. On the other hand, some isolates showed a non-gradient inhibition according to the concentrations. The present study's findings revealed the difference in the inhibition rates among the isolates. These variances may be due to EPS interactions with various cell walls of gram-positive and gram-negative bacteria, which may cause variances in susceptibility. Additionally, *S. epidermidis* did not experience any effects, these indicate that the biopolymer produced in the present study had a mode of action that may involve more complicated processes than merely interacting with bacterial cell walls. This was consistent with.[69] *Lacto bacillus* EPSs contain a variety of functional groups, such as carbonyl, phosphate, and hydroxyl groups, which are thought to be crucial to the antibacterial action,[18, 70] where functional groups in the structure of EPS probably interact with bacterial cell walls in some way to produce antimicrobial effects.[71] Salachna *et al*.,[72] reported that EPS might promote the accumulation of secondary metabolites in the growth media, which may harm gram-positive and Gram-negative bacteria. A few studies demonstrated the potent antimicrobial action of EPS from microbes against several pathogens in vitro. They identified several potential antibacterial mechanisms of EPS, which include preventing cell division, rupturing the cell wall and cytoplasmic membrane, and degrading DNA.[73, 74] According to research[74], EPS produced by *Bifidobacterium longum* inhibited the growth of *Vibrio parahaemolyticus*, *Salmonella typhimurium*, *S. aureus*, and *B. cereus*. Many studies evaluated the antimicrobial activities of bacterial biopolymers against different pathogens and recorded the different antimicrobial activity of the biopolymers against different bacterial species.[75-77] Elmi, *et al*.,[78] evaluated the antibacterial activities of EPS from Enterococcus strains and noted that the most susceptible microbes were *S. aureus* and *E. faecalis*.

CONCLUSION

This work proved that *P. aeruginosa* is a potential biopolymer accumulating bacteria and could be used for low-cost biopolymer production to combat bacteria that have developed resistance to antibiotics. Up to date, the main obstacle facing biopolymer production from pathogenic microorganisms is how to obtain the final safe product.

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