



Antimicrobial & Antioxidant Activity of a Novel Exopolysaccharide Production by *Pseudomonas Savastanoi* pv. *Savastanoi* Bacterium Isolated from Olive Knot

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Abstract

The present study aimed Isolation, characterization of EPS from *Pseudomonas savastanoi* isolated from olive tree (*olea europea*) from different sites of Mosul city, and selected the isolation to produce EPS, Seven isolates of *P. savastanoi* Pss1 , Pss2, Pss3 , Pss4 , Pss5 , Pss6, Pss7 were selected as the best isolates producing EPS based on the appearance of colonies and their growth with a viscous mucous. The results of the secondary screening showed that the best isolate was *P. savastanoi* (Pss3), gave best yield of EPS, produced on liquid King B Medium reached 3 g/l after three days of incubation. The EPS composition Extracted from bacteria was characterized by using TLC and HPLC technique, the results showed that the main units that make up the product such as glucose, fructose and mannose, The results of the study revealed that the inhibitory that EPS towards the growth of some pathological microorganisms and the Antioxidant Activity by effect of EPS on DPPH scavenging ability.

Keywords: *P.savastanoi*, EPS, HPLC, Antimicrobial, Antioxidant.

الفعالية المضادة للميكروبات والأوكسدة للسكر المتعدد الخارجي الجديد المنتج بواسطة *Pseudomonas savastanoi* pv. *Savastanoi* المعزولة من عقدة الزيتون

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الخلاصة

هدفت الدراسة الحالية إلى عزل وتوصيف EPS من بكتريا *Pseudomonas savastanoi*. عزل المعزولة من عقد الزيتون (*olea europaea*) لمواقع مختلفة من مدينة الموصل، واختيار العزلة الاكفا لإنتاج عديد السكريات الخارجي (EPS)، تم انتخاب سبع عزلات من *Pseudomonas savastanoi* وهي Pss1 و Pss2 و Pss3 و Pss4 و Pss5 و Pss6 و Pss7 كأفضل عزلات منتجة للسكريات الخارجية بناءً على المظهر الخارجي للمستعمرات ونموها اللزج على الوسط الصلب. أظهرت نتائج الفرز الثانوي أن أفضل عزلة من بين العزلات السبعة كانت *Pseudomonas savastanoi* (Pss3) حيث أعطت أفضل ناتج للسكر المتعدد الخارجي والذي بلغ 3 غم / لتر على وسط King M السائل بعد ثلاثة أيام من التلقيح، وتم الكشف عن تركيب السكر المنتج EPS والمستخلص من البكتيريا باستخدام تقنية TLC و HPLC، وأظهرت النتائج أن الوحدات الرئيسية التي يتكون منها المنتج هي الجلوكوز والفركتوز والمانوز، وهذا ما تؤكدته النتائج التشخيصية بتقنية HPLC. وأظهرت نتائج التأثير التثبيطي للـ EPS المعزول من عزلة *Pseudomonas savastanoi* على بعض البكتيريا المسببة للأمراض، وأظهرت النتائج تثبيط EPS تجاه نمو بعض الكائنات الدقيقة المرضية ونشاط مضادات الأوكسدة من خلال تأثير وقدره EPS على كبح الجذور الحرة.



1. Introduction

The genus *Pseudomonas* includes a variety of Gram-negative bacteria that affect both animals and plants. Pathogenic plant species, *Pseudomonas* are responsible for many agricultural important crops diseases. Symptoms are an overgrowth of tumor that occurs in young branches and include lesions of buds, twigs, leaves and kernels, ulcers, dying, fruits leaf spots, and fine rot [1,2]. There symptoms It is an overgrowth and tumor that appears on leaves and fruits as well as immature branches and stems [2].

There are pathogens of plants Oleaceae family [3]. *pseudomonas* has a unique ability to induce gallbladder or tumor formation on their host. *Pseudomonas savastanoi* PV. Savastanoy (Pss) is specified for olive trees [3]. This disease is common in olive of Mediterranean countries and it was discovered in Australia [4]. (Pss) can survive on the surface of the aerial parts of olive trees and is an opportunistic wound pathogen. Wounds from natural leaf scarring, frost and hail, and Agricultural practices such as pruning and harvesting greatly contribute to spread of the pathogen [5]. Olive knot is the source of pollination contains a large concentration of Psv cells and can be long lasting. The spread of the pathogen enhanced during periods of precipitation upon perfusion. The polysaccharide produced by microbes can be classified to intracellular, polysaccharide (glycogen), capsular, polysaccharide, which is very few Associated cell surface (for example, K300-Antigen) and bacterial extracellular polysaccharides (such as, xanthan, sphingane, alginate, cellulose, etc) biofilm formation and pathological [6, 7].

There are different species of bacteria and yeasts that produce microbial exopolysaccharides (EPSs). many large-scale uses in the food, pharmaceutical, petroleum, and medical domains acceptable biotechnology products [8, 9] many chemical structures, including sugar and non-sugar components, capability and range Monosaccharide formulations, with non-carbohydrates Diverse alternatives and connection types producedction of EPS [10]. Excellent agent and bacterial diversity appears finding new EPSs that are appropriate for specialized uses [11].

The objective of this research paper is Isolation, characterization of EPS from *Pseudomonas savastanoi* PV. Savastanoy and study of its antimicrobial and antioxidant efficacy.

2. Materials and Methods

2.1 Bacterial Isolates

Seven isolates were obtained from *Pseudomonas savastanoi* bacteria isolated from olive nodes and isolated on King B Medium selection medium. Olive nodes were excised from the stems of olive trees (*Olea europaea*) from different locations in the city of Mosul/Iraq, collected in sterile bags, and surface sterilized by immersing them twice in 96% ethyl alcohol for 1-2 minutes each time, then washed (3-4 times with sterile distilled water and transferred to flasks containing (3%) sodium hypochlorate NaOCl and left in it for (10-15) minutes, then washed with distilled water several successive washes, then preserved by placing them between two sterile filter papers. The sterilized pieces were distributed superficially by placing them on a surface (20) ml of Nutrient Agar medium was stored at a temperature of (28±2) °C (48) hours to ensure the efficiency of its surface sterilization, Then the uncontaminated nodes were taken, cut with a sterilized scalpel into small pieces, then crushed in (10) ml of saline solution. Physiology using a glass rod sterilized with alcohol flame. and prepared decimal dilutions, took



(0.1) ml from the last dilution and spread it on the surface of the Nutrient Agar medium to obtain single colonies. They were incubated for (48-72) hours at a temperature of (28 ± 2) °C and then transferred. Each of these colonies was transferred to the surface of solid King Medium B, The isolates were subjected to primary and secondary screening processes to study their ability to produce EPS [12].

2.2 Preparation of *P. savastanoi* inoculum

P. savastanoi bacteria By transferring the loop drive of an inoculum the developing culture bacteria on King B Medium solid medium of all Isolates into a conical flask containing 20 mL of sterile King B liquid medium.

2.3 Preliminary screening to detect ability of bacteria to produce an exopolysaccharides:

The purified isolates were subjected to a preliminary screening process to reveal the ability of *Pseudomonas savastanoi* isolates to produce exopolysaccharides (EPS) according to what was stated in [13].

2.4 Production and extraction of exopolysaccharides (EPS):

The sterile liquid King B Medium was used for the development of *Pseudomonas savastanoi* bacteria and the production of Exopolysaccharides.

2.5 Biomass determination

After the culture medium was inoculated, the flasks were placed in the incubator and the required incubation period had expired, the flasks were withdrawn from the incubator then for 20 minutes, the culture liquid was centrifuged at 6000 rpm/min. and the supernatants was left aside for the determination of the polysaccharide. The precipitate was collected in clean glass Petri dishes of known weight, thus the dried precipitate was estimated by weight difference using a Germany / Sartorius scale [14].

2.6 Secondary screening of *P. savastanoi* isolates on exopolysaccharide production

To create a complete selection database for isolates that are most efficient in their ability to produce external polysaccharides and to characterize them in the future in experiments on cultivation, purification and various applications, a sterile liquid King B Medium was prepared for the development of isolates of *Pseudomonas savastanoi* and the production of exopolysaccharides. This medium was distributed in conical flasks. Which it contains 50 ml media per beaker, with 3 Replicates for each treatment, was inoculated with *Pseudomonas savastanoi* bacteria inoculant (Pss1, Pss2 and Pss3) of 48 hours at a rate of 2%. The decanters were placed in the New Brunswick Scientific vibratory incubator at a shaking rate of 150 rpm at 28 ± 2 °C and for incubation periods (3) days, to follow the most efficient isolation in production.

2.7 Exopolysaccharide isolation and precipitation

The method was adopted [15] to isolate EPS from these bacteria with a modification procedure including adding (1%) of Na₂EDTA to the growing culture, then it was returned to the vibrating incubator and left them for (30 minutes), then the culture was discarded Bacterial centrifuge with a speed of (6000 rpm / min) type Germany / Hettich / D 72000. After that, EPS was a precipitation by acetone and according to what was stated in [16].

2.8 Molecular purification of exopolysaccharides by Dialysis

The polysaccharide precipitate was dissolved with distilled water and Savage reagent (5:1 ratio of chloroform to n-butanol) were mixed of 4:1 volume / volume, with the mixture being stirred about five cycles using a magnetic stirrer device, a centrifugation process was carried



out to remove the organic layer glucose and protein content was estimated based on the standard curve for glucose sugar and the standard curve for protein respectively [17]. The membrane screening process was accomplished by placing the EPS solution prepared in the above paragraph in Dialysis sac cellophane of the type 8000 ~ 14,400 da, [18].

2.9 Characterization of the exopolysaccharide of *P. savastanoi* measurement of the EPS's total carbohydrate content of the EPS in supernatants

The whole thing carbohydrate the content of exopolysaccharide was determined according to [19].

2.10 Measurement of the EPS's total protein concentration in supernatants

The amount of total protein in the bacterial filtrate was estimated by relying on the Fulin method [20] modified by [21].

2.11 Determining the type of sugar as a component of an EPS

The quality of the sugar units forming the exopolysaccharide was determined using TLC technique, by placing 50 mg of freeze-dried sugar in clean test tube and then adding to it 5 cm³ of sulfuric acid 1N H₂SO₄, the tubes were tightly closed and then placed at 100 °C for 24 hours, after which the neutralization of sulfuric acid was achieved and gradually adding barium hydroxide until the pH reached 7, the precipitate was removed by filtration and the clear part was taken and a process of centrifugation was completed [22], after which the plate of TLC was prepared using the impregnation solution that was formed from n-butanol: ethanol: water 4: 5: 5 (volume / volume) respectively, then the plate was heated in the oven at a degree of 100 °C until the sugars spots appeared clearly and the sugar units of exopolysaccharide of *P. savastanoi* were determined by conformity of the sample spots under study with the spots of standard sugars used in the experiment. Law of velocity of flow [19].

2.12 Starch detection test

This test was performed to detect the starch content in the exopolysaccharide, using iodine according to [23].

2.13 The composition of EPS monomers was determined using high-performance liquid chromatography.

Following the acid decomposition procedure outlined in paragraph (2.9.3), the EPS diagnostic process was carried out in the laboratories of the Ministry of Science and Technology/Department of Environment and Water, using a high performance liquid chromatography system (SYKAMN–Germany), for the purpose of diagnosing the basic units of exopolysaccharide according to what was stated in [24].

2.14 The biological activity of an exopolysaccharide against pathogenic bacteria

- Bacterial isolates used in the experiment
- Filter paper discs diffusion

The disc diffusion method was utilized to assess exopolysaccharide's biological activity against pathogenic bacteria, as reported by [25].

2.15 EPS's Antioxidant Activity using DPPH Free Radical Scavenging

Actively remove DPPH free radicals for EPS Determined according to the method of [26] 0.04 g DPPH was dissolved in 100 mL of methanol Note that the concentration of (DPPH) is (400ug/ml) to prepare the standard solution (vitamin C) and sample the concentration of the standard solution was (500 ppm) and using the dilution law, the other concentrations were prepared 30, 60, 120, 250 ppm from vitamin C and sample [27] Using a UV-VIS Shimadzu spectrophotometer, absorbance was measured at 517 nm. [28]. Higher free radical activity was



shown by the reaction mixture's increased absorbance [29]. The following method was used to calculate the percent DPPH scavenging effect: DPPH scavenging effect (%) or Percent inhibition = $(A_0 - A_1) / A_0 \times 100$.

3. Results

3.1 Ability of bacteria to produce EPS by Preliminary screening

The current study was able to reveal the susceptibility of different isolates of the bacterium *Pseudomonas savastanoi*. The results of the initial detection tests showed six isolates have the ability of the bacterial isolates to produce the EPS, and the initial detection of their ability to produce these compounds was through color statements that gave a positive test by forming a violet ring in the liquid growth media. After adding Mulch's reagent to the liquid medium The superations was colored red in these media the Congo Red stain was added Fig. 1-A-2, also characteristic mucous of its colonies developing in the solid medium Fig. 1-B-2. As well as by capturing the developing colonies. In solid media and bacterial superations discoloration in media Liquid is red for Congo Red Table (1) indicate the bacteria's production of these carbohydrates, As well as the characteristic mucous of its colonies growing in the solid medium as shown in Fig. 1-B-2 and its appearance in a red color of varying intensity as a result of picking up the color of the Congo Red has been added to the growing medium. Fig. 1-B-1.

Table 1- Preliminary detection test for the ability of isolates of *Pseudomonas savastanoi* to capture the Congo Red.

Sampling site	Symbol of isolation	Congo red test
Al fadhiah	Pss1	+++
Errachidia	Pss2	++
Nineveh gardening	Pss3	++++
Bahshika	Pss4	+++
Kibbeh	Pss5	++
Church Container	Pss6	++

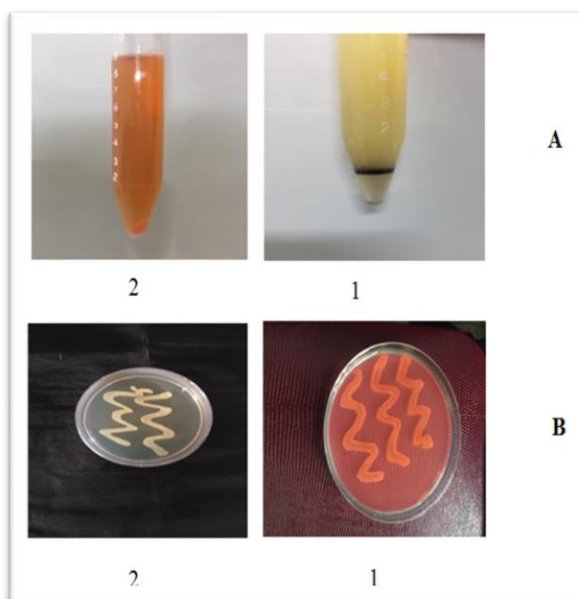


Figure -1 This Testing the ability of bacteria to produce

A-1: The violet ring forms in liquid media

A-2: The bacterial supernatants are red in liquid media

B-1: The appearance of colonies in red in solid media containing Congo Red stain

B-2-: mucous of colonies in solid media.

3.2 Secondary screening of selective bacterial isolates and their ability to produce EPS

After conducting the initial detection tests on the ability of bacterial isolates to produce the EPS by adding the Congo Red stains to solid and liquid media of king B and noting the contrast of isolates in their capture of the stains as well as the characteristic mucous of their colonies developing in the solid medium,. In selecting the most efficient isolation and describing it in the future in the experiments of purification, characterization, and the use of extracted EPS in various applications, The three isolates (Pss1, Pss3, and Pss4) were selected, and gave a clear indication of its production of *EPS*, and this is proven by the results indicated in this field, as isolation was distinguished (Pss3) in recording the highest rate of EPS production, which reached (3.33) g / l, followed by isolation (Pss1) which produced (1.91) g / l, and its live mass reached (1.30, 0.91) respectively table (2). isolation (Pss3) was the highest rate of EPS production for this selected to complete the research path in the purification and characterization of exopolysaccharide and its subsequent use in some applications.

Table 2- Secondary screening of selected bacterial isolated and their ability to produce EPS in liquid medium.



Symbol of isolation	Exopolysaccharide (G / l)	Biomass (G / l)	Final pH
Pss1	1.91	0.91	6.72
Pss3	3.33	1.30	6.44
Pss4	1.72	0.81	6.23

2.3 Characterization of the exopolysaccharide of *P. Savastanoi* (Pss3) bacteria

2.3. 1. The exopolysaccharide's total carbohydrate content

The exopolysaccharide's total carbohydrate content produced by the bacterium *Pseudomonas savastanoi* was estimated in this study, and in four purification stages that included: The bacterial suspension (before the centrifugation process), The bacterial supernatants (after the centrifugation of the bacterial suspension), And the precipitant compound. With acetone and Na₂EDTA, obtained after the dialysis process. The results are revealed and after dropping the carbohydrate content values of these compounds on the standard curve for glucose, A gradual decrease in the carbohydrate content after each purification stage of the previously produced exopolysaccharides compounds, as the total carbohydrate content estimation of the bacterial suspension showed that it contains 64% carbohydrates, 41% of the bacterial supernatants and 27% of the compound precipitated with acetone, with a decrease in the carbohydrate content of the resulting sugar after the purification by dialysis process, to 21%. As observed in Fig 2.

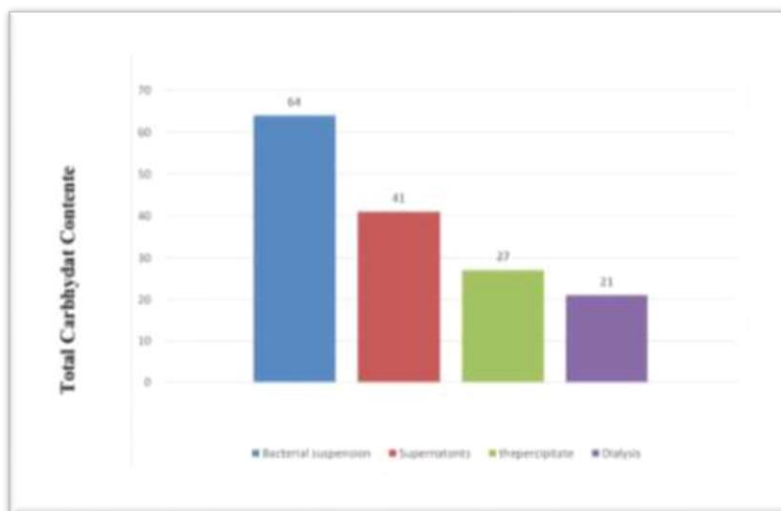


Figure -2 Total carbohydrate content of exopolysaccharides in bacteria *P. Savastanoi*

3.3. 2. determination of the EPS total protein content

The total protein content of the EPS obtained from bacteria was estimated, and after dropping the protein content values for these compounds on the standard protein curve, It was found that there was a gradual decrease in its quantity after each purification stage, which included: The bacterial suspension, the bacterial supernatants, the precipitant compound and obtained after the dialysis process as the estimation of the total protein content of the bacterial suspension showed that it contains 71.6%, 56% of the bacterial supernatants and 38% of the compound



precipitated with acetone, noting that the protein content of sugar resulting after purification by dialysis process decreased to 18% as observed in Fig 3.

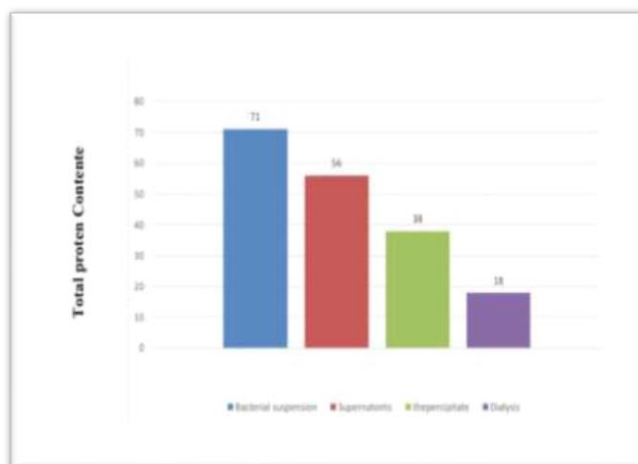


Figure -3 Total protein content of exopolysaccharides in bacteria *P. Savastanoi*

3.3. 3. Iodine detection

The iodine test for the EPS solution showed a negative as no color appeared when adding iodine solution to the sugar product solution which indicated the absence of starch.

3.3. 4. Specific determination of EPS units

After carrying out the acidic hydrolysis of exopolysaccharide in isolation (Pss3) from *P. savastanoi* bacteria and purified by membrane sorting using dialysis process and the results showed TLC technique, That the EPS components of the exopolysaccharide under study were (glucose, fructose and mannose sugar) and these sugar spots were very apparent in the samples, and the relative distance of migration on the TLC layer is equal to the relative distance of the standard sugars Fig 4.

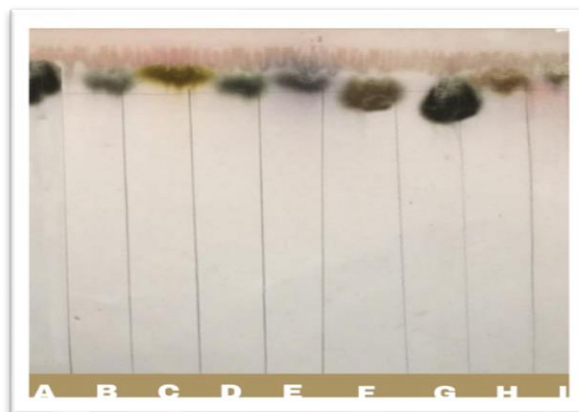
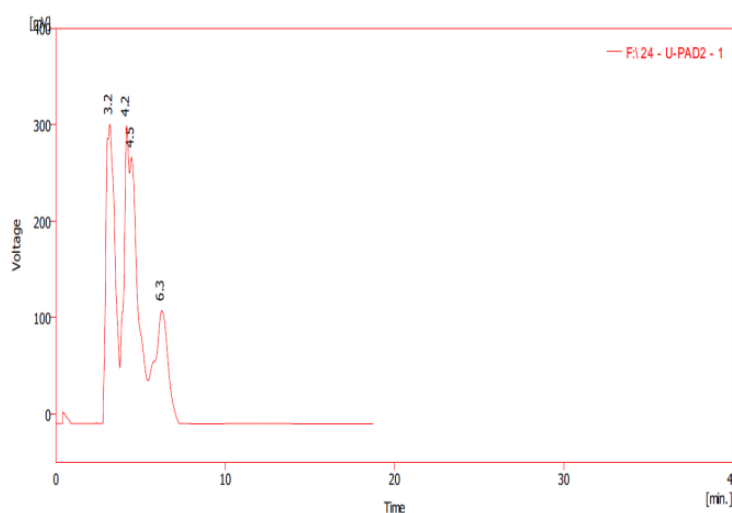


Figure -4 The locations of the main units forming the EPS on the TLC: A. The sugar sample extracted in *P. Savastanoi* bacteria, B. glucose standard sample, C. rhamnose standard sample, D. Fructose standard sample, E. xylose Standard sample, F. galactose Standard sample, G. sucrose Standard sample, H. mannose standard sample, I. lactose standard sample.



3.3. 5 HPLC Analysis

The identities and quantities of the various monosaccharides in the carbohydrates and glycoproteins are determined by monosaccharide composition. The data utilized for studying the structure of carbohydrates, which has importance in quantification. High-performance analysis was used to determine the monosaccharide composition of EPS. Germany's SyKAM liquid chromatography (HPLC) model. Column is C18- NH with dimensions (250 mm x 4.6mm, 5 μ n). The Mobile phase composition was Mobile phase = D.W: MeOH (98: 2), samples at a flow rate of 0.7 ml/min were identified. The HPLC analysis of the Bacterial extracts revealed the presence of many natural products which may be responsible for the antimicrobial activities they elicit. EPS from *P. Savastanoi* was hydrolyzed by sulfuric acid shown in Fig 5, three kinds of monosaccharide were found, glucose, fructose and manous as compared with standard monosaccharides glucose, fructose and manous.



Result Table (Uncal - F:\24 - U-PAD2 - 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	3.188	9596.057	285.676	52.0	39.9	0.57	
2	4.192	2631.658	193.332	14.3	27.0	0.24	
3	4.472	3953.163	168.606	21.4	23.5	0.38	
4	6.252	2277.574	68.767	12.3	9.6	0.52	
	Total	18458.452	716.381	100.0	100.0		



Calibration Summary Table (ESTD - F:\sugers - Signal 1)

Compound Name	Reten. Time	Left Window	Right Window	Peak Type	Peak Color	LOD	LOQ	RB	Resp. Factor
glucose	3.200	0.200 min	0.200 min	Ordnr		0.020	0.000	A	0.0000
fructose	4.200	0.200 min	0.200 min	Ordnr		0.000	0.000	A	0.0000
manous	6.300	0.200 min	0.200 min	Ordnr		0.000	0.000	A	0.0000

Figure -5 The HPLC data and result tables of compounds detected EPS in *P. Savastanoi*: (A) HPLC chromatogram showing the detection of sample EPS, (B) calibration summary table of Reten Time for standard compound.

3.3.4. Antimicrobial Activities of EPS

A few studies have shown that EPS from microorganisms has substantial antibacterial behavior, particularly against human pathogenic bacteria, and the current study underlines this aspect, the results showed that EPS of *Staphylococcus lentus* has the most antibacterial effects at 1 mg/mL., followed by *K. pneumonia* and *S. aureus*, where the zone of inhibition diameter was 26, 24, and 23 mm respectively. Table (3) and Fig 6.

Table 3- Antibacterial activity of EPS of *P. Savastanoi* Zone of inhibition.

No	Microorganisms	Distilled	Erthromycin	EPS
		water	50mg/ml	1mg/ml
		(-ve control)	(+ve control)	
1	<i>Escherichia coli</i>	-	9	13
2	<i>Proteus mirabilis</i>	-	14	22
3	<i>Klebsiella pneumonia</i>	-	10	24
4	<i>Staphylococcus aureus</i>	-	10	23
5	<i>Staphylococcus lentus</i>	-	15	26

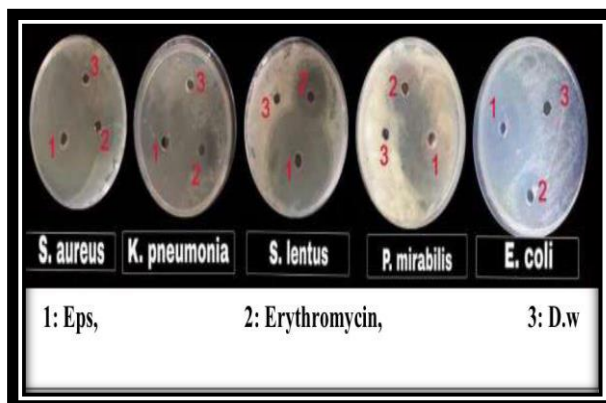


Figure -6 Diameter of the inhibition zones (mm) of the purified exopolysaccharide from *P. savastanoi* isolate towards the tested pathogen bacteria

3.3.5. Scavenging of DPPH Radicals Provides Antioxidant Activity

The DPPH radical has been using to study the antioxidative activity. in the present study, The impact of *P. savastanoi*'s EPS about DPPH scavenging ability is shown in fig 7 The activity of scavenging of 30 ppm of EPS was (21.9%) which similar to that of 30 ppm ascorbic acid (22.5%) in addition, scavengings activity increased with EPS concentration, and the concentration (120, 250) ppm of ascorbic acid (41.8, 55.9)% respectively have activity similar to that 120ppm EPS (37.9%) and 250ppm EPS(49.8%) respectively.

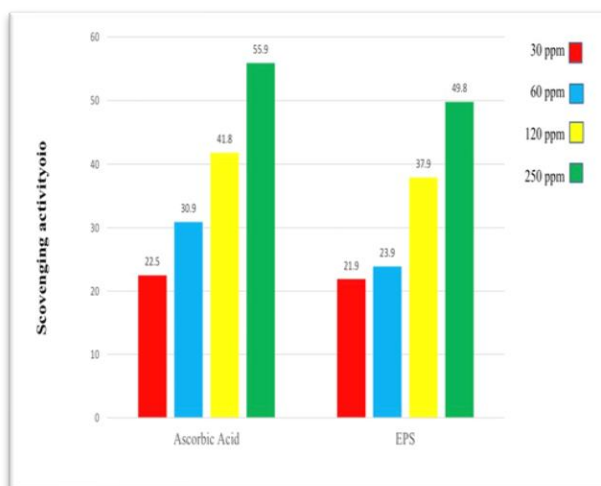


Figure -7 Scavenging effect of EPS with the impact of ascorbic acid on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radicals.



4. Discussion

The study of the ability of microorganisms to produce different compounds has been the focus of many scientific researches, as well as studying their importance to the organism producing them, such as fungi, algae or bacteria [30]. The initial detection of their ability to produce these compounds was through colorimetric tests, which gave a positive test by forming a violet ring in the liquid growth medium as a result of the reaction of Purphole compound (the product of the reaction of sugars with sulfuric acid) with the α -naphthol colorimetric reagent[31], as well as by capturing the developing colonies in the solid medium and discoloration of the bacterial superatoints in the liquid media that added Congo Red, the liquid is red in Congo Red, which is probably pigment to the ability of the diphenyldiazo-bis- α -naphthyla-minesulfonate compound in the pigment to bind with the polysaccharide that is secreted outside the bacterial cell growing in solid media [32], or present within The bacterial superatoints in liquid media, and bacterial capture of this stain was approved as a color detector to indicate the bacteria's production of these carbohydrates [33] In *Pseudomonas aeruginosa*[34]. As an indication of its production from exopolysaccharides to select more efficient in producing EPS. As a result of growth of bacterial colonies with the appearance of sticky mucous on solid medium Appropriate[35], while explaining the phenomenon of contrast color Bacterial colonies in the intensity of their capture of the red color, to the contrast of the ability of each of EPS compounds to bind to the amyloid substance responsible for giving the red color [36], in addition to the overlap of the color of this pigment with the color of pyocyanin in bacteria of the genus *Pseudomonas*, which resulted in giving a dark red color when grown on the king B medium [37].

The isolation of bacteria from different sources and the identification of the most efficient isolate in its production of EPS and a study has been internationally recorded, The results indicated as isolation was distinguished (Pss3) in recording the highest rate of EPS production after four days of incubation, the results obtained are consistent with results of one of the research that found a difference in the ability of isolates to produce exopolysaccharide[23], This study also dealt with measuring of the carbohydrate and protein content of the EPS, and in the various stages of its purification, as the results showed their decrease osition of microbial EPS that is producedby a certain species [38]. Indicated the difference between sugars produced accafter each stage of purification, and this was confirmed by the results of the study that it conducted [39].

As for the sugars that make up the EPS, the results of TLC showed that they are generally composed of glucose, fructose and mannose, and this is what many researchers have indicated about the containment of these compounds produced by genus *Pseudomonas* bacteria, For example, the study conducted by[40] the sugar isolated from *P. putida* bacteria which contains glucose and mannose units as basic units for the sugar produced from them. depending on a variety of factors, such as the type of bacterial strain, the components of the medium, the carbon offer, nitrogen source, and carbon/nitrogen ratio in the fermentation medium all influence the type of sugar generated by bacteria [38], These data are consistent with the results of high-



performance EPS analysis determined the monosaccharide content . Liquid chromatography (HPLC) As compared with standard monosaccharides glucose,fructose and manous, the culture conditions strain, influence the amount and the compording to the different bacterial strain and the components of the media, as *P. putida* and *Pseudomonas fluorescens* produced an EPS composed up of glucose, galactose, and pyruvate. [41, 24] It was found that the EPS produced by *P. fluorescens* Pathovar II contained galactose, mannose, rhamnose, glucose, ribose, arabinose, and xylose.. The effect of exo polysaccharides (EPS) isolated from *Pseudomonas savastanoi* on some species of pathogenic bacteria, the results in this field showed the clear effect of EPS's role in controlling pathogenic microbial growth isolates under test and that is through the inhibitory effect of EPS the agar well diffusion method was used to test several human pathogenic microorganisms [42] . The result is consistent in somehow with [43,44] Several possible antibacterials are suggested EPS mechanisms, such as inhibition of cell division, Disruption of the cell wall, cytoplasmic membrane, and DNA degradation [45] . As for the use of EPS as an antioxidant, they conclude from the results of the experiment that the mechanical EPS is an antioxidant by inhibiting free radicals that can be exploited and utilized in food applications, and this agrees with what was mentioned[46,47] about the EPS obtained from the isolate of *Lactobacillus plantarum* C88, which indicated that the EPS is subject to the theory that the electron-donating groups increase the efficiency of free radical scavenging and that the electron acceptor groups reduce the scavenging efficiency of free radicals, These results indicate the strong antioxidative effect of this EPS on DPPH radical scavenging and consistent in somehow with[44] .

5. Conclusion

The current study revealed that EPS isolated from *P. savastanoi* isolate in this study had an effective alternative sources of antimicrobial drugs, antioxidant activity vital effectiveness against other microorganisms.

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7. Disclosure and conflict of interest

The authors declare that they have no conflicts of interest.



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