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The effect of extracted exopigments of *pseudomonas aeroginosa* on different microorganisms : an invetro study

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ABSTRACT

Pseudomonas aeruginosa is gram-negative Bacteria are among the most important pathogenic agents in both human and veterinary medicine, responsible for a wide range of diseases affecting humans and animals. These include wound and burn infections, eye and skin infections, urinary tract infections, otitis media, septicemia, and infections of bones and joints. Otitis media, in particular, is a frequently encountered condition in domestic animals such as cats and dogs. In order to isolate and identify *Pseudomonas aeruginosa* strains capable of producing pyocyanin and to assess their antibiotic resistance, a total of 120 ear swabs were obtained from domestic cats clinically diagnosed with otitis media. These samples were collected from cats presented to private veterinary clinics in Tikrit City from December 2024 to march 2025, using sterile cotton swabs.The isolated *P. aeruginosa* strains showed a high capability to produce the extracellular pigment pyocyanin, and they exhibited resistance to a wide range of antibiotics compared to *Staphylococcus aureus, Enterococcus faecalis, and Escherichia coli*, which were found to be sensitive to antibiotics. In contrast, the isolated *P. aeruginosa* strains were resistant to antifungal agents, unlike *Candida albicans*, which was sensitive to the antifungal drug.

Introduction

Pseudomonas aeroginosa is Gram-negative, rod-shaped bacterium that is non-spore-forming and equipped with a single polar flagellum for motility [8,14]. It is primarily an obligate aerobe, utilizing oxygen as its preferred electron acceptor for respiration. Nevertheless, it is also capable of anaerobic growth by using nitrate or other alternative electron acceptors. This metabolic versatility contributes to its high invasiveness and adaptability, allowing it to thrive in diverse habitats, including soil, water, plants, animals, humans, sewage systems, and healthcare environments [9]. Pseudomonas caused many diseases in human and animals, such as wound infection, burn infection, eye infection, skin infection, urinary tract infections, otitis media, bacteremia and bone joint infections[5].the important infection caused disease in pets animals such as dogs and cats otitis media[2]. A distinctive feature of *Pseudomonas aeruginosa* is its ability to produce a blue-green pigment known as pyocyanin (1-hydroxy-5-methylphenazine), which is soluble in chloroform. The pathogenic effects of *P. aeruginosa* are largely linked to a variety of virulence factors secreted by this microorganism. [6]. One of the applications of *P. aeroginosa* in biotechnology is its ability to degrade aromatic hydrocarbon such as methybenzenes, which are the by-products of petroleum industries and used as solvents for enamels and paints as well as in production of drugs and chemicals[13]. Methylbenzenes are considered as environmental contaminants that ubieties atmosphere, soil, groundwater, and surface waters [4]. Additionally, pyocyanin exhibits antimicrobial activity against a variety of microorganisms [3].

Material and methods :

Samples collection:

in December 2024 to march 2025, a total of forty Samples from the ears of domestic cats clinically diagnosed with otitis media were collected. The cats had been brought to private veterinary clinics in Tikrit City, and the infected ear sites were sampled using sterile cotton swabs, which were immediately labeled with relevant case information. The swabs were transported under refrigerated conditions in a cool box to the Microbiology Laboratory, Department of Microbiology, College of Veterinary Medicine, for bacterial isolation and identification.

Culture Media Preparation

The following culture media were prepared and used for the study:

a) Blood agar
b) Mueller–Hinton agar
c) Brain Heart Infusion broth supplemented with glycerol
d) King's Medium A Base

Isolation and Identification of Pseudomonas aeruginosa

The isolation process involved a combination of cultural, morphological, and biochemical examinations:

Cultural characteristics — Observation of colony morphology on selective and differential Morphological characteristics — Microscopic examination of Gram-stained smears to confirm Gram-negative rod morphology.

Biochemical Tests

The isolates were subjected to the following biochemical assays:

a) Catalase test
b) Oxidase test
c) Indole production test
d) Methyl red test
e) Voges–Proskauer test
f) Citrate utilization testmedia.

Antibiotic Susceptibility Testing

The antibiotic resistance profiles of the isolates were determined using the well diffusion method on Mueller–Hinton agar. A panel of commonly used antibiotics was tested to assess sensitivity and resistance patterns.

Pigment Production

The ability of the isolates to produce the extracellular pigment pyocyanin was evaluated using King's Medium A Base, following standard protocols for pigment extraction and identification.

Results :

Bacterial isolation :

The results of bacterial isolation revealed the presence of *Pseudomonas aeruginosa* in the form of pure colonies. On Mueller-Hinton agar, the colonies were medium-sized, mucoid, and emitted a grape-like odor, in addition to producing pigment. On blood agar, the colonies ranged from medium to large in size, were gray in color, and exhibited beta (β) hemolysis

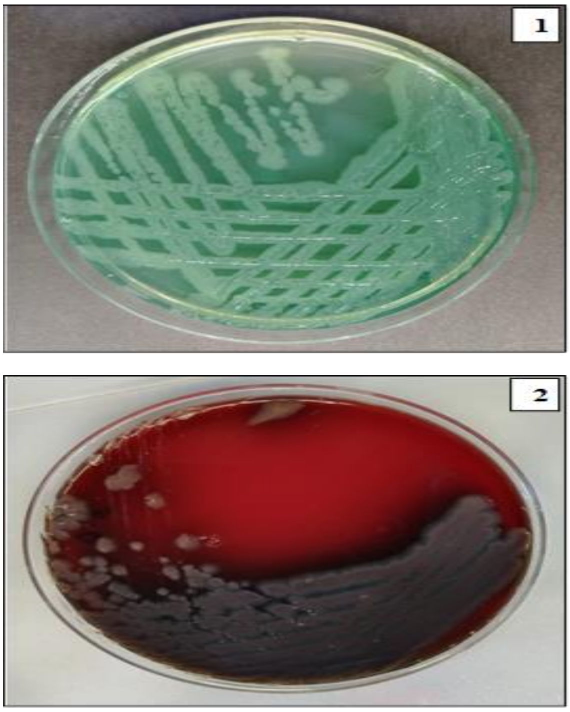


Figure 1 growth of Pseudomonas aeruginosa on culture media (1) growth On Mueller-Hinton agar with producing pigment (2) growth On blood agar complete blood hemolysis beta (β) hemolysis.

Table 1 Biochemical tests for identification Pseudomonas aeruginosa:

	Test name	Result
1	Catalase test	+ve
2	Oxidase test	+ve
3	Indole test	-ve
4	Methyl Red test	-ve
5	Voges Proskauer test	-ve
6	Citrate protaction test	+ve
7	Nitrate Reduction test	+ve
8	Urease protaction test	-ve

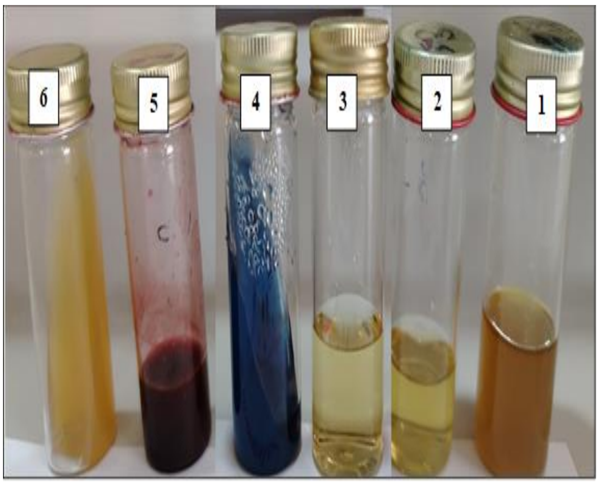


Figure 2 Biochemical tests for Pseudomonas aeruginosa (1) Indole test (2) Methyl Red test (3) Voges Proskauer test (4) Citrate protaction test (5) Nitrate Reduction test (6) Urease protaction test.

Antibiotic sensitivity test:

The results of antibiotic susceptibility testing showed that the *Pseudomonas aeruginosa* isolate, which was later used in the production of the extracellular pigment, exhibited a multidrug-resistant (MDR) phenotype to commonly used antibiotics that are typically employed to treat various types of bacterial infections, as illustrated in Table 2.

Table 2 Antibiotic sensitivity test for Pseudomonas aeruginosa:

	Disk	antibiotics	Result
1	LEV5	Levofloxacin	R
2	CN10	Gentamycin	R
3	CIP10	Ciprofloxacin	R
4	CTX10	Ceftriaxone	R
5	AMC30	Amoxicillin+Clavulinic acid	R
6	CAZ30	Ceftazidime	R
7	MEM10	Meropenem	R

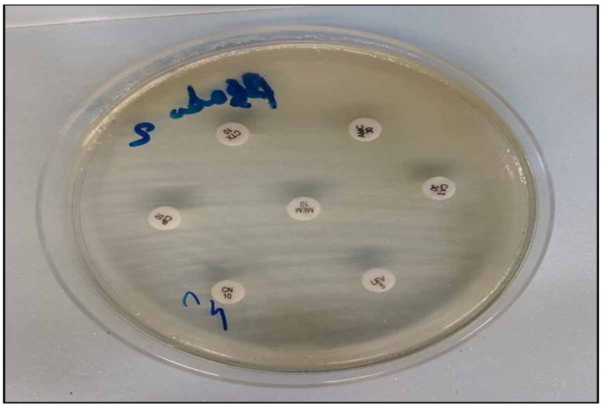


Figure 3 Antibiotic sensitivity test on Mueller-Hinton agar

Production of exopigmentation:

The results demonstrated the ability of the *Pseudomonas aeruginosa* isolate to produce extracellular pigment in large quantities using King's Medium A Base, as shown in Figure 4.

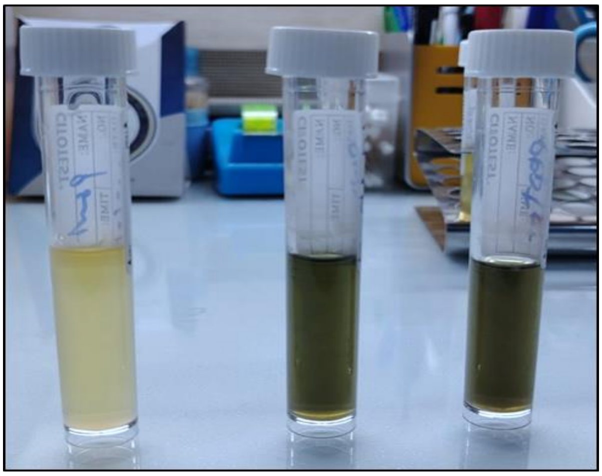


Figure 4 Production of exopigmentation from Pseudomonas aeruginosa that used in the study

Well diffusion method sensitivity test:

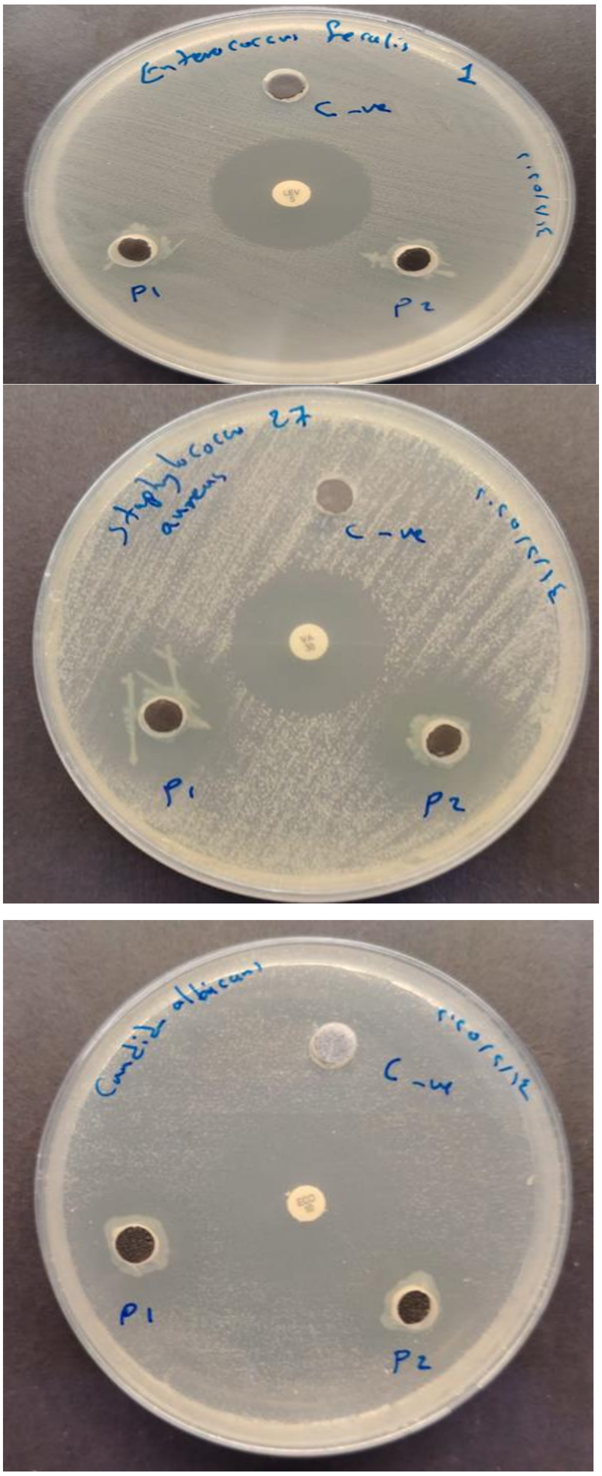
Antibiotic susceptibility testing demonstrated that *Staphylococcus aureus* was sensitive to Vancomycin (VA30). Additionally, both *Enterococcus faecalis* and *Escherichia coli* exhibited sensitivity to the tested antibiotics. Furthermore, the yeast *Candida albicans* showed susceptibility to Levofloxacin (LEVS) and Econazole (ECO10), indicating the potential effectiveness of these agents against both bacterial and fungal pathogens.

The results of antibiotic susceptibility testing using the well diffusion method indicated that the *Pseudomonas aeruginosa* isolate, which was subsequently used for extracellular pigment production, exhibited a multidrug-resistant (MDR) profile against a broad range of commonly used antibiotics for various bacterial infections, as shown in Table (3) and Figure (5).

Table 3 Well diffusion method sensitivity test on Mueller-Hinton agar by uses of pathogenic bacterial and fungal:

	Type	Well	Result
1	*Staphylococcus aureus*	Control -ve	R
2		P1	S
3		P2	S
4		Disc VA30	S
5	*Enterococcus fecalis*	Control -ve	R
6		P1	R
7		P2	R
8		Disc LEV5	S
9	*Escherichia coli*	Control -ve	R
10		P1	R
11		P2	R
12		Disc LEV5	R
13	*Candida albicans*	Control -ve	R
14		P1	S
15		P2	S
16		Disc ECO10	S

P= pyocyanin pigment, VA30=Vancomycin 30 µg, LEV5=Levofloxacin 5 µg, ECO10=Econazole 10 µg.



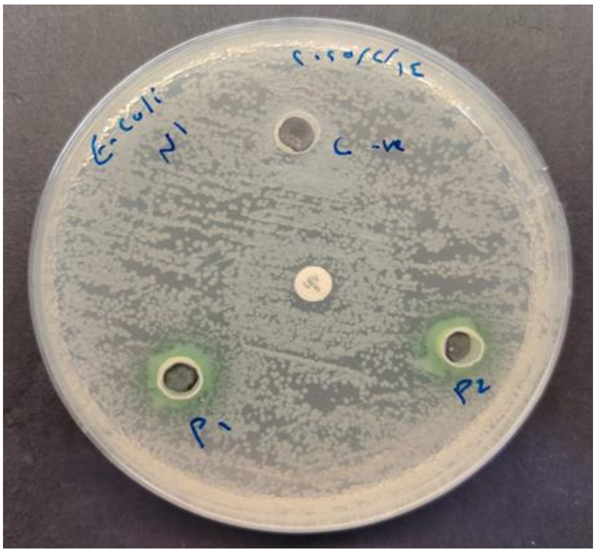


Figure 5 Well diffusion method sensitivity test on Mueller-Hinton agar that show activity of exopigmentation antimicrobial and antifungal .

Discussion :

The results of this study demonstrated the antimicrobial efficacy of the Pyocyanin compound, extracted from *Pseudomonas aeruginosa*, against various strains of Gram-positive bacteria and its antifungal activity against certain types of fungi. However, its antimicrobial activity was weak against different strains of Gram-negative bacteria such as *E. coli* and *Enterococcus faecalis*. This variation is consistent with what was mentioned by[15],that Pyocyanin possesses selective activity depending on the type of bacterial cell wall. A previous study by [12]indicated that Pyocyanin functions by stimulating the production of reactive oxygen species (ROS), which cause damage to bacterial proteins and nucleic acids—particularly in bacteria that lack antioxidant defenses. This concept was supported by an earlier study conducted by[4], which clarified the mechanism of toxicity through the depletion of intracellular glutathione and catalase levels. Another study from 2021 demonstrated Pyocyanin's antifungal activity and its potential use as a natural antifungal agent effective against *Candida albicans*, *Aspergillus*, and *Fusarium*[7]. A previous study also confirmed the prevalence of otitis media in pets, especially cats and dogs, caused by *Pseudomonas aeruginosa*, and highlighted the clear link between Pyocyanin production and antibiotic resistance[6], supporting our study and emphasizing the potential therapeutic significance of Pyocyanin. Two different studies conducted in 2021 found that Pyocyanin is not only effective against bacteria but also has beneficial effects on the immune system, such as stimulating the innate immune response and activating phagocytic cells[11,16]. A previous study also demonstrated that Pyocyanin has the ability to disrupt or prevent the formation of biofilms, which increases its efficacy against chronic and resistant isolates. Our findings are consistent with two independent reports that addressed the challenges of treating otitis media in pets due to increased antibiotic resistance, encouraging the use of alternative solutions such as Pyocyanin[1,2]. Other studies have shown that *Pseudomonas aeruginosa* can survive in hospital and clinic environments, increasing its potential to develop antibiotic resistance. However, the availability of products like Pyocyanin and their conversion into therapeutic agents could drastically transform the treatment landscape [8,14].Other studies have also suggested the possibility of incorporating Pyocyanin into antioxidant-based antimicrobial and antifungal topical applications, representing a promising and practical direction[10].

Conflict of Interest

The authors declare that there is no conflict of interest.

Acknowledgment

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تأثير الصبغات المستخلصة من الزوائف الزنجارية على الاحياء المجهرية المختلفة : دراسة مختبرية

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الملخص

الزوائف الزنجارية من الجراثيم الممرضة المهمة في المجالين الطبي و البيطري حيث تسبب العديد من الامراض للانسان والحيوان ، منها اخماج الجروح والحروق واخماج العين واخماج الجلد والمجاري البولية والاذن الوسطى وتجرثم الدم واخماج العظام والمفاصل من بين هذه الأمراض، يبرز التهابات الأذن الوسطى التي تُلاحظ بشكل متكرر في الحيوانات الأليفة مثل الكلاب والقطط. ولغرض عزل وتشخيص بكتيريا الزوائف الزنجارية المنتجة للخضاب الخارجي (البايوسيانين) ومدى مقاومتها للمضادات الحيوية جمعت 40 مسحات من اذان القطط الأليفة الواردة إلى العيادات البيطرية الخاصة في مدينة تكريت والتي شخصت على انها حالات التهاب الاذن وذلك خلال شهر كانون الأول 2024. حيث جمعت مسحات الأذن من القطط المصابة باستخدام مسحات قطنية معقمة و تم عزل وتشخيص الزوائف الزنجارية والتي اظهرت قابليتها على انتاج الخضاب الخارجي وبكميات كبيرة وكانت ذات صفة مقاومة للعديد من المضادات الحيوية مقارنة بالمكورات العنقودية الذهبية والمكورات المعوية البرازية والايشيريكيا القولونية حيث كان لهم حساسة للمضاد الحيوي ومقاوم للمضادات الفطرية مقارنة بخمائر المبيضات البيضاء فقد كانت حساسة للمضاد الفطري.

الكلمات المفتاحية: الزائفة الزنجارية ، الصبغات البكتيرية، بيوسيّانين.