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Extracted of Pyocyanin from Pseudomonas Aeruginosa and Determination Their Activity as Anti MDR Bacteria

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ABSTRACT

Objective: Using the Vitek-2 technology, bacteria were identified and pyocyanin was recovered from environmental Pseudomonas aeruginosa that was isolated from different soil in Hilla City. Various pigment concentrations were tested for their antibacterial properties against pathogenic bacteria that cause burns and wound infections. The findings demonstrated that pigment had a positive impact on all bacteria.

Methods: After Pseudomonas aeruginosa from burns and wounds was isolated and identified, isolates that could generate pyocyanin to inhibit the development of certain pathogenic isolated bacteria from clinical infections as MDR bacteria were chosen for the investigation.

Results: Following the collection of 40 burn and wound samples, 15 Pseudomonas aeruginosa isolates were obtained. Pyocyanin was extracted from one isolate in order to assess its efficacy against certain harmful bacteria. The results of this investigation demonstrated that all of the bacteria were multidrug resistant and that pyocyanin had a potent antibacterial impact.

Conclusion: The potential of pyocyanin, which was obtained from P. aeruginosa, as an anti-MDR bacterial and alternative anti-bacterial agent was demonstrated by this study.

KEYWORDS: Pyocyanin; Pseudomonas aeruginosa; MDR; Antimicrobial.

INTRODUCTION

The rod-shaped, asporogenous, monoflagellated bacteria Pseudomonas aeroginosa is Gram-negative. It is around 0.5-1.0 μm broad and 1-5 μm length. Aerobic respiration is the ideal metabolism of P. aeroginosa, an obligatory bacterium. But it can also breathe anaerobically using other electron acceptors, such nitrate. As a result, P. aeroginosa is a very invasive microbe that has been discovered in sewage, soil, water, people, animals, plants, and hospitals [1]. Degrading aromatic hydrocarbons like methybenzenes, which are byproducts of the petroleum industry and utilised as solvents for paints and enamels as well as in the manufacturing of medications and chemicals, is one way that P. aeroginosa is employed in biotechnology. Methylbenzenes are thought to be pervasive environmental pollutants found in soil, groundwater, and the atmosphere [2]. Additionally, P. aeroginosa can break down toluene, the most basic form of methylbenzene, by oxidising the existing methyl group to the aldehyde, alcohol, and acid, then converting it to the catechol. As a result, it may be applied to pollution operations control [3]. The redox-action of pigments that these bacteria make are called phenazines. Both virulence factors and iron acquisition are impacted by these pigments [4]. Pseudomonas aeroginosa active cultures generate the blue-green phenazine and water-soluble pigment known as pyocyanin. The pyocyanin has antimicrobial properties against several bacteria [5,6].

Because they produce a vast array of bioactive substances considered as one of the secondary metabolites, including poisons, alkaloids, pigments, and antibiotics, microbes have recently drawn attention as biological agents that can effectively address a variety of health-related issues [7, 8]. Natural and environmentally (eco-friendly) bio-pigments have also attracted a lot of attention because of its excellent safety record, growing capacity to lessen health-related issues and consumer acceptance [9]. Bacterial pigments are the most desired and attractive target among all natural sources, surpassing those of other plants or animals. This is ascribed to their quick growth, long-term availability, and ease of management of microbial cell factories for large rates of output.

Furthermore, their biological actions have been diversified, including antibacterial, anticancer, and anticoagulant properties [10]. However, because P. aeruginosa secretes a variety of colours, including redox-active phenazine chemicals, it is one of the most advantageous bacteria in terms of biotechnology application.

Yet, because it secretes a variety of colours, P. aeruginosa is among the most advantageous microorganisms in terms of technological application, including redox-active phenazine compounds" Phenazines are not only virulence factors but also secondary metabolites with advantageous biological activity and uses. Phenazines comprise the most important component in this family, pyocyanin (blue-green), pyorubrin (red-brown), pyoverdine (yellow and fluorescent), and pyomelanin (light-brown) [11, 12].

Therefore, in order to assess the antibacterial efficacy against certain MDR bacteria, various burn and wound samples were gathered, followed by the isolation and identification of Pseudomonas aeruginosa and the extraction of pyocyanin.

MATERIALS AND PROCEDURES

Bacterial isolates' (isolation and identification)

For 24 to 48 hours, 40 burn and wound samples were grown on (MRS agar) and (MRS broth) in an anaerobic environment at 37 °C. If a colony did emerge, it was identified by its isolate morphology (shape ,colony form, colour, borders, pigment type, textures and elevations), and it was examined under a light microscope after Gram's staining. 20% glycerol was used to preserve and store P. aeruginosa isolates (at -80 °C). [13].

The bacterial inoculum's preparation

Proliferation was controlled at 0.5 McFarland after bacteria were activated and isolated for 18 hours at 37°C using pseudomonas chromogenic agar.

Test for antibiotic susceptibility

The Clinical Laboratory Standard Institute (CLSI) recommendations 2021 used the Kirby-Bauer disc diffusion procedures to evaluate antibiotic resistance. This trial included five antibiotics. The susceptibility of some isolates was evaluated using several antibiotic discs, including Cephalothin (KF-30 µg), Novobiocin (NV-5 µg), Methicillin (ME-5 µg), Clarithromycin (CLR-15 µg), and Doxycycline (DO-30 µg), and which were acquired from Bioanalyse, Turkey [15].

Extraction of pyocyanin

Using Pseudomonas aeruginosa bacteria and a few biochemical reagents, the technique of [14] extracts pyocyanin pigments for use in subsequent testing.

Pyocyanin's antibacterial action on pathogenic microorganisms

Using the disc diffusion technique, the antibacterial activity of P. aeruginosa pyocyanin isolated against a few harmful bacteria was assessed [16]. Proteus mirabilis, Streptococcus pnumoniae, Enterobacter sp., and Staphylococcus aureus were among the pathogenic isolates that were injected and kept in nutritional broth. Different concentrations of pyocyanin were evaluated as anti-bacteria, and the microbial concentration was 0.5 MacFarland on plates following an overnight incubation at 37 °C.

RESULTS AND DISCUSSION

As shown in Table 1, 15 isolates of Gram-positive P. aeruginosa bacteria from burn and wound were selected from 40 samples and identified by biochemical testing. These isolates had a variety of morphological, physiological, and biochemical characteristics.

The 15 isolates under investigation were all determined to be Gram-negative, spherical, smooth, and pale yellow on MacConkey agar because they were unable to ferment lactose; some of them were green and smelled like grapes. The capacity of the bacteria to break down blood on the enriched medium known as blood agar was assessed, and the majority of the isolates seemed to be beta hemolytic. On this medium, several sizable, mucoid colonies were seen.

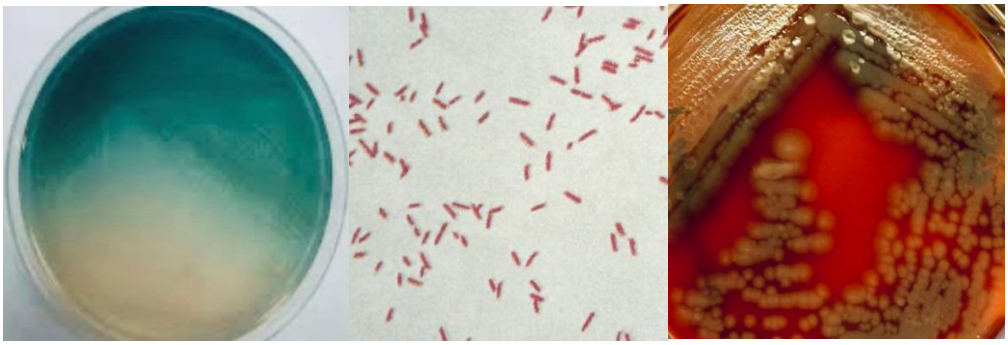

Figure 1: A/ pyocyanin P. aeruginosa pigment, B/ Gram negative P. aeruginosa, C/ Beta haemolysis of P. aeruginosa

Table: Biochemical test of P. aeruginosa

Test	Result
Gram stain	-
Catalase	+
Oxidase	+
KOH	+
Methyl red	-
Indole	+
Citrate utilization	+
Growth at (42°C)	+
Voges Proskauer	_
Hemolysis	+
Motility	+
Urease	-

According to Table, every isolate had positive test results for urease, methyl red, Gramme stain, voges proskauer, and oxidase, but negative findings for catalase, oxidase, KOH, indole, citrate utilisation, growth at 42°C, hemolysis, and motility [15].

Susceptibility profiles of pathogenic bacteria isolates

Several harmful bacteria obtained from various parts of the human body were utilized to demonstrate the effects of various antibiotics. In this study, five antibiotics were used: cephalothin (KF-30 µg), Novobiocin (NV-5 µg), methicillin (ME-5 µg), clarithromycin (CLR-15 µg) and doxycycline (DO-30 µg), Figure 1-4 shows the antibacterial action of four concentrations of pyocyanin (5000, 4000, 3000, and 2000 µg/ml).

It was demonstrated by the current study's results that every bacterial isolate is resistant to the antibiotics being studied. Cephalothin, methicillin, ampicillin, and Novobiocin are all 100% resistant to the isolates, while doxycycline and clarithromycin are 75%. This indicates that all of the bacterial isolates under the study, appeared resistant to every antibiotic disc are used in our test (MDR), and that the chosen antibiotic disc was ineffective against every isolated bacterium. [16]

Antibacterial action of pyocyanin isolated from P. aeruginosa against pathogenic bacteria:

Proteus mirabilis, Streptococcus pnumoniae, Enterobacter sp., and Staphylococcus aureus were among the harmful microorganisms against which the antibacterial efficacy of pyocyanin was assessed. Figure (2-5) illustrate the various sizes at which the inhibition zone is obtained.

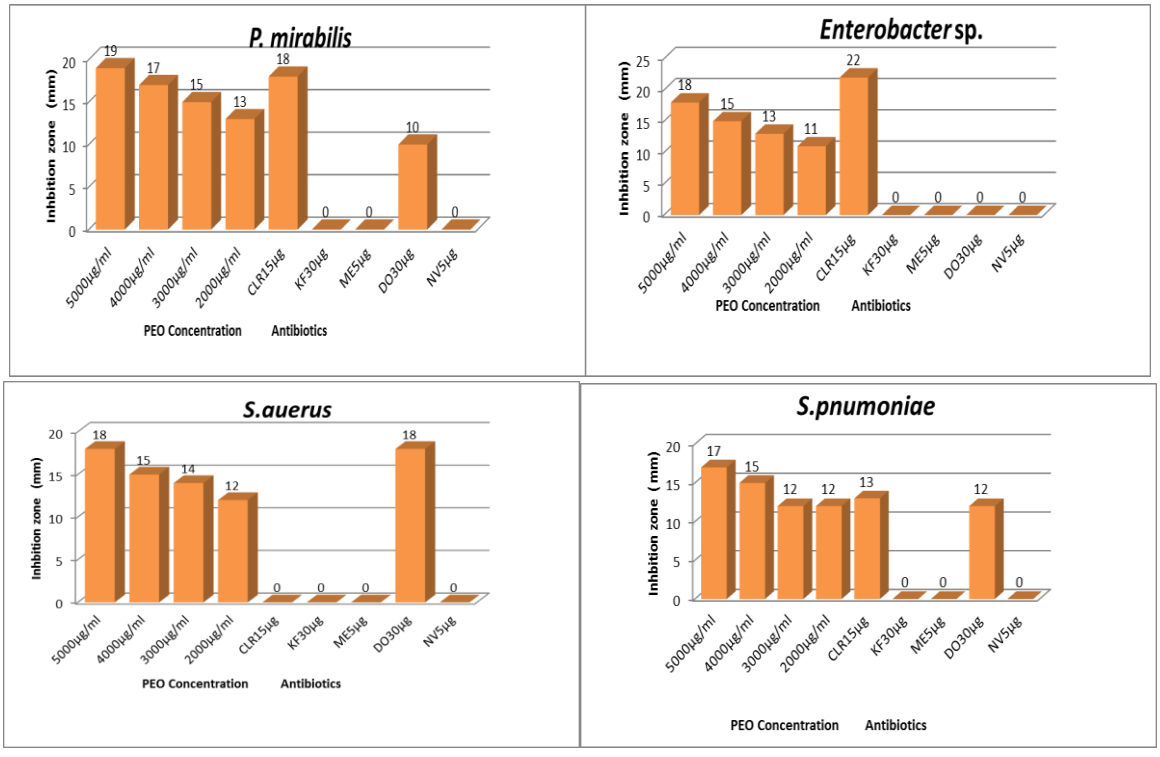

Figure (2-5): Antibacterial action of pyocyanin and some antibiotics against some bacteria.

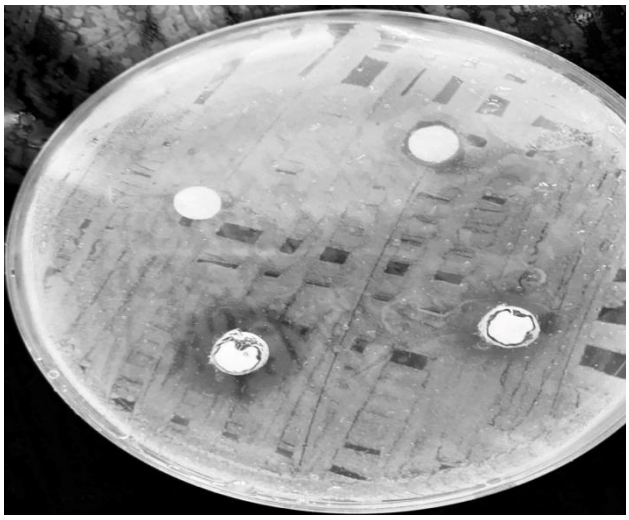

Figure (6): Action of pyocyanin as antibacterial in Mueller agar with different conc.

pyocyanin showed diameter of inhibition zone as clearly directly propor-tional with the decreased of pyocyanin concentrations that exceeded the effectiveness of several medicines. 5000 µg/ml concentration of pyocyanin gaves highest inhibition zone area against the bacterial pathogenic isolates, 19 mm appeared maximu zone of pyocyanin inhibition against P. mirabilis (Fig. 2-5) and the least isolate was affected in the sensitive in comparison with the selected antibiotic discs followed by S. pnumoniae . S. aureus and Enterobacter sp. are the second bacterial isolates that are susceptible to pyocyanin. Pyocyanin contributes to the degradation of biomolecules by rapidly compromising the integrity of the bacterial cell membrane and generating reactive oxygen species (ROS), including superoxide species [17]. The term "minimum residual illness" refers to the acquisition of non-susceptibility to at least one antibacterial antibiotic or category out of three or more kinds. The researcher said that pyocyanin has the capacity to suppress multi-drug-resistant bacteria (MDR) based on all the results that were in agreement with other studies [18].

How P. aeruginosa regulate the colonization of other bacterial isolate species in the different culture and if probiotics are employed in combination with other appropriate pathogenetic or aetiological therapies will determine the outcome [19].

CONCLUSION

Pyocyanin-like compounds were found to be present in Pseudomonas aeruginosa isolated and identified from burn and wounds when screened for antimicrobial chemicals. These substances were shown to be effective against both Gram-positive and Gram-negative bacteria. The fact that Pseudomonas aeruginosa pyocyanins have a limited antibacterial range and are typically ineffective against Gram-negative bacteria makes this study significant. The pyocyanin-like compounds that P. aeruginosa produces are thoroughly characterised, and their antibacterial efficacy in various applications is assessed.

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