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Evaluation of the efficiency of Pyocyanin purified from pseudomonas aeruginosa as anticancer agent toward human breast cancer cell line CAL-51

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ABSTRACT

Pseudomonas aeruginosa was isolated from Tigris River. These isolates could produce two types of pigments on the cetrimide agar plates. The quantity of productivity for Pyocyanin pigment ranged between 5.9 and 13.7 µg/ml and increased with increasing the incubation period to 72 hours. The Pyocyanin was purified by silica gel chromatography. The cytotoxic activity of the Pyocyanin on tumor cell line CAL-51 and normal cell line REF using a range of Pyocyanin concentrations caused a reduction in the cell viability in CAL-51 and REF cell lines in a dose-dependent manner. The doses of 50, 100, 150 ng/ml, 200 ng/ml, and 400 ng/ml were non-significant in the inhibition ratios of 14%, 20%, 24%, 27% and 37 %, respectively, in the REF cell line, on the other hand, showed the best inhibition ratio with 33%, 45%, 52%, 64%, and 71%.

Key words: *P. aeruginosa*, pyocyanin, beast cancer

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INTRODUCTION

Pseudomonas aeruginosa is a commercially important opportunistic bacteria renowned for its capacity to secrete phenazine chemicals. It is in charge of a wide range of functions, including dye decoloration, food colorants, antibacterial, antifungal, nematocidal, and pesticide degradation [1, 2].

Among gram-negative bacteria, *Pseudomonas aeruginosa*, a frequent opportunistic and nosocomial infection, produces a wide range of colors as secondary metabolites [3]. These pigments are required for bacterial pathogenicity and biological control, but not for bacterial growth and proliferation [4].

Pseudomonas aeruginosa produces pyrocyanin and other members of the phenazine family, which have been linked to pathophysiological processes [5]. Pyocyanin is a water-soluble blue-green phenazine pigment produced in enormous amounts by active cultures of Pseudomonas aeruginosa, with potential in medicine, agriculture, and environmental protection [6, 7]. The broad range of features of this secondary metabolite have piqued the interest of researchers. Pyocyanin has a wide range of biotechnological applications, including anticancer activity and the capacity to stop pathogenic bacteria and fungus from growing [8]. Biofilm management has also been achieved by using bacteria-produced pigments. Because pyocyanin is produced by no other Gram-negative bacteria, its presence aids in organism identification. Pyocyanin has antimicrobial properties that are similar to antibiotics. As a result, the purpose of this study was to detect pyocyanin in P. aeruginosa and test its anticancer effectiveness against the CAL-51 and REF cell lines.

MATERIALS AND METHODS

Isolation and identification of *P. aeruginosa* producers for Pyocyanin

Fifteen water samples were collected from the Tigris River. The samples were serially diluted in phosphate buffer saline up to 106 times, then plated on cetrimide agar and incubated for 24 hours-48 hours at 35°C. *P. aeruginosa* colonies with the distinctive blue-green appearance were separated and re-streaked for purification and isolation on nutrient agar. The purified isolated colony of the *P. aeruginosa* bacterial strain was then processed for morphological characterizations such as colony shape, pigment color, gram staining, and cell shape, as well as biochemical and physiological identification tests such as indole production, citrate utilization, catalase, oxidase, methyl red, and vogus proskuaer reactive compounds tests .

Production and extraction of Pyocyanin

Color formation in the broth was detected when P. aeruginosa was grown in nutritional broth and incubated at 35°C for 24 hours, 48 hours, and 72 hours. To produce cell-free supernatant, the soup was centrifuged for 15 minutes after incubation. The pyocyanin-containing supernatant was thoroughly mixed with an equal volume of chloroform, and a blue solvent layer was formed after vortexing. After collecting the blue layer, a 0.1N HCl solution (20% of the volume of the blue layer) was added and vortexed, resulting in an acidified pink upper layer. The pink layer was then neutralized with Tris-Base before being treated with chloroform once more. To ensure purity, the technique was performed multiple times. Using a UV-visible spectrophotometer, the pigment concentration (µg/ml) in extracted solution was estimated by measuring the optical density (absorbance) at 520 nm wavelength and multiplying the optical density value (OD520) by 17.072 [9].

Purification of Pyocyanin

The extracted pigment was purified using column chromatography (column size 45 cm × 3.5 cm) with silica gel as the stationary phase and a 1:1 methanol-chloroform solution as the mobile phase. The presence of pyocyanin and its concentration were determined for each fraction after elution [10].

Maintain CAL-51 and REF cell lines

The CAL-51 (human breast cancer cell line) and REF cell lines were grown in RPMI-1640 with 10% fetal bovine serum, 100 units/mL penicillin, and 100 g/mL streptomycin. Cells were passaged twice a week with Trypsin-EDTA, reseeded at 80% confluence, and incubated at 37°C.

Treatment of cell lines with Pyocyanin

The cytotoxic effect of varying quantities of pure pyocyanin was investigated in this study. In 96 flat well micro-titer plates, the cells (1 × 104-1 × 106/ml) were grown. The total culture media of each well is 200 µl. Covered with sterilized parafilm and gently shacked. Plates were incubated for 24 hours at 37°C with 5% CO_2. Purified pyocyanin was introduced to wells in CAL-51 and REF cell lines at 50 ng/ml, 100 ng/ml, 150 ng/ml, 200 ng/ml, and 400 ng/ml after incubation. Each concentration and control were done in triplicate (treated cells with a medium free from serum). A plate was incubated at 37°C with 5% CO_2 during the exposure period (24 hours). After exposure, fill each well with 10 l MTT solution. For 4 hours, plates were incubated at 37°C with 5% CO_2. After removing the MTT solution, the crystals in the wells are solubilized by shaking for 15 minutes at 37°C with 130 L of DMSO (Dimethyl Sulphoxide). The absorbance at 492 nm was measured using a microplate reader, and the test was performed three times [11]. The following formula is used to calculate the percentage of cytotoxicity (cell growth inhibition rate): A-B/A *100 = Viability Inhibition (percentage).

RESULTS AND DISCUSSION

Isolation and identification of *P. aeruginosa* producers for Pyocyanin

After culturing water samples on cetrimide agar plates, 4 Pseudomonas aeruginosa isolates produced pigmented blue-green circular colonies with smooth margins as

well as morphological distinct properties such as rod cell shape of gram-negative *P. aeruginosa* with positive citrate utilization, catalase, and oxidase while negative for indole production, methyl red and vogus proskuaer reactive compound tests. Two isolates produced yellow-green pigment and 2 did not Figure 1.

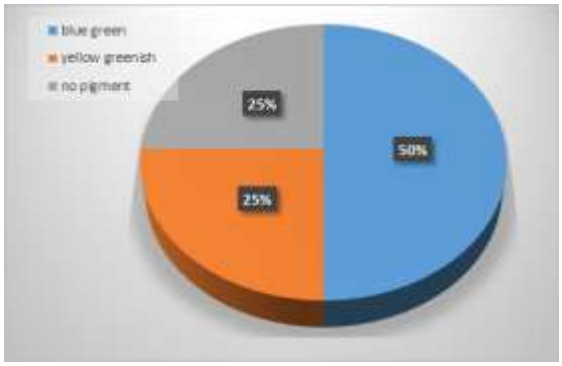

Fig. 1. Types of pigments produced by *P. aeruginosa*

Color change in the solid media suggested the synthesis of soluble pigments, specifically Pyocyanin. Pyocyanin production was seen in liquid media in various colors of green [12]. *P. aeruginosa* colonies producing blue-green Pyocyanin isolated from three aquatic habitats in Pakistan (river, swimming pool, and drainage water). *P. aeruginosa* isolates from three different environments showed extremely comparable growth performance, phenotypic, and metabolic profile [13]. *P. seudomonas* aeruginosa produces a variety of pigments, including Pyocyanin (blue-green), Pyomelanin (light brown), Pyoverdin (yellow, green, and fluorescent), and Pyorubrin (red), all of which are responsible for pseudomonads' color change [14].

Production and extraction of Pyocyanin

The quantity of productivity for this pigment ranged between 5.9 µg/ml and 13.7 µg/ml after growing Pseudomonas aeruginosa isolates that produced Pyocyanin pigment in nutrient broth, as shown in Figure 2. With Pseudomonas aeruginosa 3, the amount of production increased with the lengthening of the incubation period, reaching 24.6 µg/ml after 72 hours and a shift in color from light to dark green.

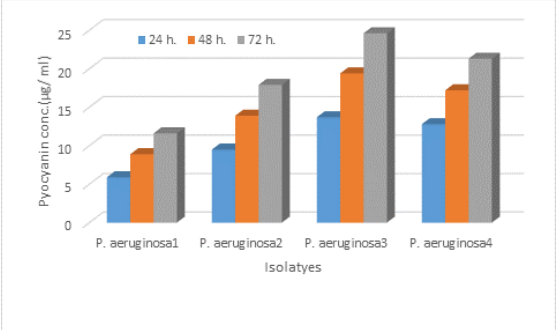

Fig. 2. The quantity of productivity for pyocyanin by *P. aeruginosa* isolates

The emergence of Pyocyanin pigment from inoculated *P. aeruginosa* strain started in nutrient broth culture media within 10 hours of incubation, according to this study. However, from 24 hours to 72 hours, there was a progressive increase in Pyocyanin concentration, which coincided with increased cell proliferation and, eventually, bacterial density Also (3) studied the effect of incubation time on Pyocyanin production and found that the greatest Pyocyanin production occurs at 72 hours and diminishes as incubation time increases.

Purification of Pyocyanin

The extracted Pyocyanin was run on column chromatography using a silica gel as the stationary phase, and after elution, two peaks formed, one of which contained Pyocyanin pigment, as shown in Figure 3, with a final concentration of 34.9 µg/ml.

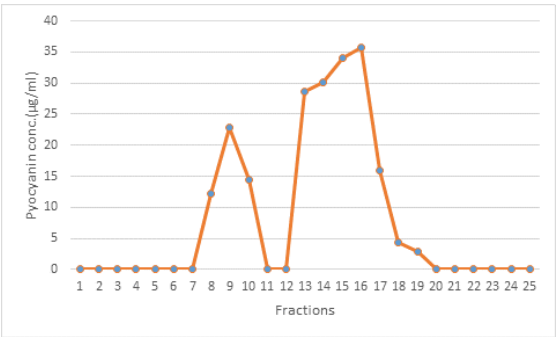

Fig. 3. Column chromatography using a silica gel for purification of *P. aeruginosa*

Pyocyanin's effect on CAL-51 and REF cell lines

The MTT assay was used to test the pyocyanin's cytotoxic activity on the tumor cell line CAL-51 and the normal cell line REF. This assay was used on normal and tumor cell lines to detect cell viability using

a range of pyocyanin concentrations. The vitality of the CAL-51 breast cancer cell line was determined using a variety of pyocyanin concentrations (50, 100, 150, 200, and 400 ng/ml). The results in Figure 4. demonstrated that pyocyanin reduced cell viability in the CAL-51 and REF cell lines in a dose-dependent manner. The doses of 50, 100, 150, 200, and 400 ng/ml were non-significant ($P<0.05$) in the inhibition ratios of 14, 20, 24, and 27 and 37 %, respectively, in the REF cell line, on the other hand, showed the best inhibition ratio ($p<0.05$) with 33, 45, 52, 64, and 71 %.

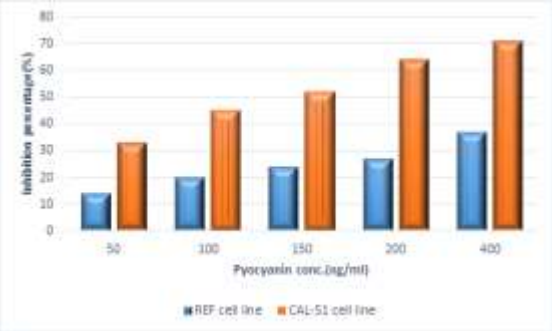

Fig. 4. Cytotoxic effect of purified pyocyanin on REF and CAL-51 cell lines

In comparison to the control, a nine-day treatment with pyocyanin resulted in a 67 %| reduction in HepG2 cell counts. The researchers discovered that pyocyanin-induced cell death is caused by oxidative stress caused by increased ROS, DNA damage, caspase-3 activation, and the acceleration of senescence and apoptosis [15]. When pyocyanin enters a cell, it interacts directly with cellular NADH and NADPH, causing an increase in reactive oxygen species (ROS). The produced ROS can cause oxidative stress and cell damage in the host cells [16]. Pyocyanin, a blue-green pigment generated by Pseudomonas aeruginosa, disrupts host redox cycles, causing reactive oxygen species to be formed and cellular oxidative damage to occur [17-19].

CONCLUSION

Pyocyanin may be a promising anti-neoplastic agent for the treatment of increasing of breast cancer. Thus, Pyocyanin may be a promising anti-neoplastic agent for the treatment of increasing cases of breast cancer.

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