

Antimicrobial Activity of Pyocyanin for Inhibition of *Pseudomonas aeruginosa* Urinary Tract Pathogens

Abstract:

This study aim to detect the antimicrobial Activity of Pyocyanin for Inhibition of *Pseudomonas aeruginosa* Urinary Tract Pathogens. Five different isolates of *Pseudomonas aeruginosa* were isolated from wound infection and eye drops. The isolates were tested for the production of pyocyanin by growing in nutrient agar after incubation at 37°C for 18 hour. Considerable amount of blue pigment pyocyanin were produced by *Pseudomonas aeruginosa* after grown in lauryl broth medium and extracted by chloroform extraction method. Maximal pigment achieved after 72 hours and used in sensitivity test against isolated microorganisms " *S.aureus* , *S.epidermis*, *S.saprophyticus*, *E.coli*, *K.pneumoniae*, *P.aeruginosa*, *C.frundii* and *C.albicans* ". The antimicrobial activity of the pigment was evaluated using Mueller Hinton Agar medium. The isolated microbes from urinary tract infection have different percentages of microorganisms " *S.aureus*15%, *S.epidermis*5%, *S.saprophyticus*10%, *E.coli*40%, *K.pneumoniae*10%, *P.aeruginosa*5%, *C.frundii* 5% and *C.albicans*10% ". The pigment have properties that make it an important bioactive compound which has the ability to arrest the electron transport chain of bacteria and exhibit antibacterial activity towards gram positive and un capsulated gram negative by different zone of inhibition at different concentrations. The inhibition zone determined by using 25%,50%,75% and 100%. The *S.aureus* inhibition zone at 25% was 16mm, 50% was 17.5mm, 75% was 20mm and 21mm at 100% concentration, *S.epidermis* at 25% was 17mm, 50% 20mm, 75% was 21mm and 31mm at 100%, *S.saprophyticus* at 25% was 19mm, 50% was 23mm, 75% was 25mm and 33mm at 100%. *E.coli* at 25% was 13mm, 50% was 15mm, 75% was 25mm and 22mm at 100%. *C.frundii* was resistant to 25% and 50% concentration and given inhibition zone 11,13mm at 75,100% concentration. Also *K.pneumoniae*, *P.aeruginosa*, and *C.albicans* were resistant to pyocyanin. This study concluded that pyocyanin possess antibacterial activity against U T pathogens.

Key words: *Pseudomonas aeruginosa*, pyocyanin, Urinary tract pathogen.

Introduction

Urinary tract has become a major public health problem, It can be cause by different bacteria gram positive and gram negative bacteria; such as *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Staphylococcus epidermidis*, *Enterococci*, *Haemolytic streptococci*, *Escherichia coli*, *Proteus species*, *Klebsilla strain* and *Pseudomonas aeruginosa*, (1). *Pseudomonas aeruginosa* is belonging to the family pseudomonadaceae. It is wide spread in the environment where in majority of which are responsible for nosocomial infections. In addition, they are also found associated with chronic infections in cystic fibrosis patients and wound infections especially of burns (9), it is gram-negative rod, motile, an obligate aerobic, the bacterium is ubiquitous in soil and water, and on surfaces in contact with soil or water, (2) its metabolism is respiratory and never fermentative, but it will grow in the absence of O₂ if NO₃ as terminal electron acceptor is available as a respiratory electron acceptor, *Pseudomonas aeruginosa* is oxidase positive (2), it produces a green fluorescent pigment (fluorescein) and a blue pigment (pyocyanin), which gives colonies and infected wound dressings a greenish-blue coloration, *Pseudomonas aeruginosa* grows well on simple

bacteriological media, and most strains elaborate the blue phenazine pigment pyocyanin which together impart the characteristic blue-green coloration to agar cultures (3), *Pseudomonas aeruginosa* is an opportunistic pathogen to human (1) and (8) meaning that it exploits some break in the host defenses to initiate an infection, it cause urinary tract infection, skin infection, respiratory infection, external ear infection, eye infection, and septicaemia (1), it also produces a sweet grape-like scent, so wound dressings and agar plates are often sniffed for organism identification, *Pseudomonas aeruginosa* has weak invasive ability, healthy people just don't get infections with this guy, However, once inside a weakened patient, the story changes. It elaborates numerous exotoxins including exotoxin A, which has the same mechanism of action as diphtheria toxin (stops protein synthesis) but is not antigenically identical. Some strains also possess a capsule that is antiphagocytic and aids in adhesion to target cells for example in the lungs. (3). Pyocyanin is phenazine antibiotic pigment produced by *P.aeruginosa*, the antibiotic action of pyocyanin pigment against different bacteria and yeast was investigated , *P.aeruginosa* is the only known organism of the *pseudomonads* and other glucose–nonfermenting gram-negative bacilli capable of producing the

very distinctive water-soluble pigment pyocyanin (4), it is considered as a product of secondary metabolites, pyocyanin has phenazine nucleus that means it belongs to the phenazine family (electron acceptor), which stimulates redox cycling in bacteria, and human epithelial and liver cells, Pyocyanin has a variety of pharmacological effects on eukaryotic and prokaryotic cells and has bactericidal activity against many bacteria such as, *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Enterococci*, *Haemolytic streptococci*, *Escherichia coli*, *Proteus species*, *Klebsilla* strain which may allow *P.aeruginosa* an advantage over competing bacteria occupying the same niche (5), the inhibitory action of pyocyanin is the result of its unique redox potential, it was also proposed that, during respiration, pyocyanin becomes reduced and univalently reduces oxygen to the toxic superoxide radical, the resistance of various bacteria to pyocyanin would therefore be dependent upon the levels of superoxide dismutase and catalase possessed by the organism and on the presence of oxygen (6), pyocyanin is a redox- phenazine compound that kills mammalian and bacterial cells through the generation of reactive oxygen intermediates, *P aeruginosa* resists pyocyanin because of the limited redox cycling of this compound and that under

conditions favoring pyocyanin production; catalase and superoxide dismutase activities are increased (7). The biofilm formation on various antiseptics and disinfectants and the mechanism involving drug resistance toward various antibiotics has been one of the strategies in the emergence and the release of the resistant strain in the environment (10). However, Pseudomonads are known to produce pigments resolved to survive under oxidative stress imposed by environmental hazards and exhibits antagonism. This pigment is known Phenazines are heterocyclic compounds that are produced naturally and substituted at different points around their rings by different bacterial species (11). Pyocyanin is a water-soluble blue-green phenazine pigment produced in large quantities by active cultures of *Pseudomonas aeruginosa*. Pyocyanin (*N*-methyl-1-hydroxyphenazine) has antibiotic activity against a wide variety of microorganisms (12-16). The ability of opportunistic human pathogen; *P.aeruginosa* to acquire resistance to a broad range of antibiotics has made effective therapy more difficult. Several recent investigations have dealt with the problem of antibiotic resistance in *P.aeruginosa* (17) and (18). A multidrug resistant *P. aeruginosa* strain has caused an outbreak in a neurosurgery ward (19).The increase in bacterial strains

resistance, both to antibiotics and other disinfectants and germicides, led researchers to investigate other options, in treating both antibiotic-resistant and susceptible infections. In this concern,

Materials and Methods:

This study was conducted in Khartoum state, forty samples of urine were collected from Khartoum Educational Hospital. Collection and transport of sample According to (1) aseptically patients were given a urine container to collect midstream of urine. Instruction was given to the patient to get midstream urine, the sample was labeled very well. After the collection, 1% boric acid was added to the sample as the preservative until transported to laboratory.

Solid media “Nutrient, Blood, CLED, Sabouraud Dextrose, Mannitol Salt, Eosin Methylene Blue, Mueller Hinton and DNase Agar” was cultured by streaking tested organism with sterile loop in the surface of the media, semi solid media “Kligler Iron Agar” was cultured by inserted sterile straight wire containing tested organism in tube and stabbed in media, Broth media “Peptone water, MR – VP and Citrate medium” was cultured by

phenazines have been of great interest to pharmaceutical and clinical research groups for the last 50 years (16), (20) and (21).

inserted sterile loop containing tested organism in tube. Results of cultural characteristics, primary and secondary tests for bacterial identification were recorded, also *C.albicans* was isolated and confirmed by Germ Tube Test.

Pyocyanin was prepared by growing *P.aeruginosa* in LB media, and then sub cultured on tryptic soy broth, and incubated at 37°C for 18 hrs, centrifuged 3000 rpm for 30min, and supernatant was taken. An aliquot of 5ml from culture then extracted into 1ml of chloroform, and then reextracted into 1ml of 0.2 N HCl. The absorbance of this solution was measured at 520 nm (22). The different pathogenic bacteria were cultured on brain-heart broth 24 hr, and the bacteria was cultured on a nutrient agar by spreader and left to dry for 15min after that wells made by cork borer, and filled with 0.1µl of pyocyanin extraction and incubated for 24 hrs, and the results were recorded.

Result

Staphylococcus aureus , *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Escherichia coli* ,*Citrobacter freundii* , *Klebsilla pneumoniae* ,*Pseudomonas. aeruginosa* ,*Candida albicans* were isolated from urine, Incidence of UTI by *E.coli* is more high than incidence by other microorganisms .*S.epidermidis* and *P.aeruginosa* have low incidence and

Table (1): Percentage of microorganism.

Isolated Microorganism	Percentage %
<i>S.aureus</i>	15%
<i>S.saprophyticus</i>	10%
<i>S.epidermidis</i>	5%
<i>E.coli</i>	40%
<i>P.aeruginosa</i>	5%
<i>K.pneumoniae</i>	10%
<i>C.freundii</i>	5%
<i>C.albicans</i>	10%

associated with catheter as shown in table (1).

Pyocyanin inhibited the growth of *S.aureus*, *S.saprophyticus*, *S.epidermidis*, *E.coli* and *C.freundii* indicated by zone of inhibition, and did not affect the growth of *K.pneumoniae* *P.aeruginosa* and *C.albicans* as shown in table (2), Figure (1 – 4).

Table (2): Effect of pyocyanin against isolated pathogens

Microorganism	Diameter of zone by mm			
	Concentration of pyocyanin			
	25%	50%	75%	100 %
<i>S.aureus</i>	16	17.5	20	21
<i>S.epidermis</i>	17	2	21	31
<i>S.saprophyticus</i>	19	23	15	33
<i>E.coli</i>	13	15	21	22
<i>K.pneumoniae</i>	0	0	0	0
<i>C.freundii</i>	0	0	11	13
<i>P.aeruginosa</i>	0	0	0	0
<i>C.albicans</i>	0	0	0	0

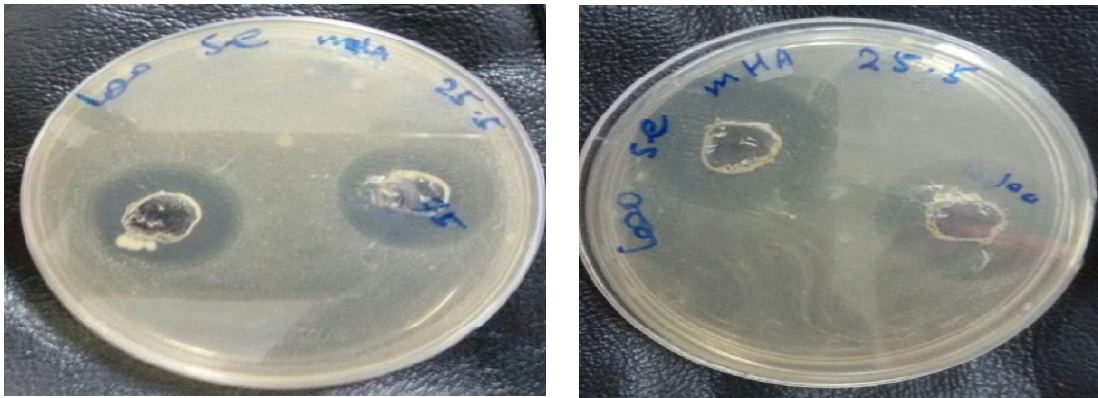


Figure (1): Left and right show inhibition zone of pyocyanin (75 and 100) % concentration against *S.epidermidis* .



Figure (2): Left, right and middle show inhibition zone of pyocyanin (25, 50, 75 and 100)% concentration against *S.aureus*.

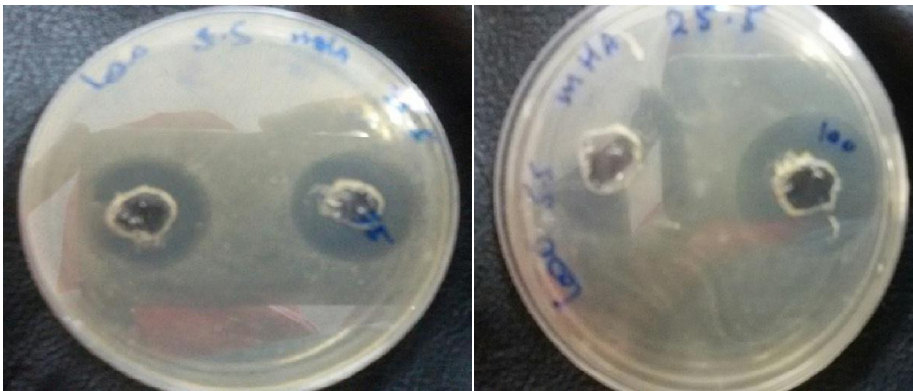


Figure (3): Left and right show inhibition zone of pyocyanin (75 and 100) % concentration against *S.saprophyticus*.



Figure (4): show inhibition zone of pyocyanin 100% concentration against *E.coli*.

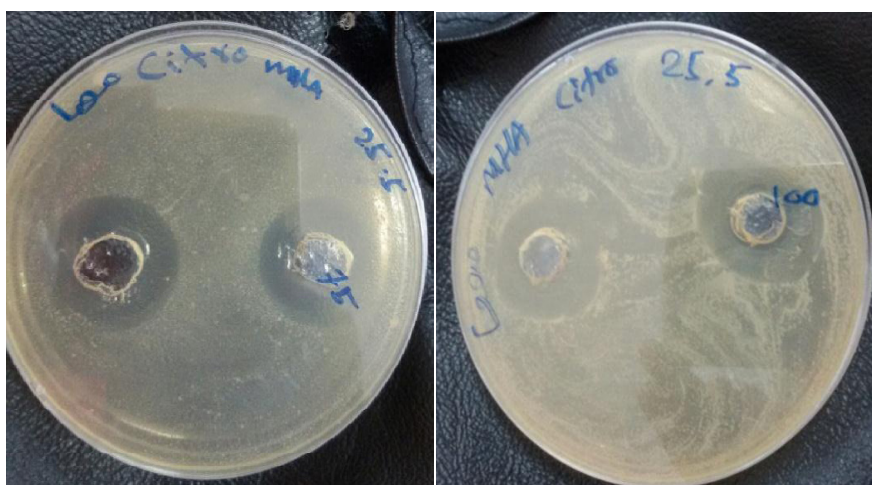


Figure (5): Left and right show inhibition zone of pyocyanin (75 and 100) % concentration against *C.freundii*.

Discussion

According to the result the pyocyanin added at different concentrations (25,50,75, and 100) %, best results were showed by using 75%,100% concentration of pyocyanin that inhibited the growth of gram positive and uncapsulated gram negative bacteria by appearance of large zones around wells. This result confirm the finding of (23). This findings are agreed with (24) they found that

pyocyanin has antibacterial activity, and can use as antibiotic against pathgenic bacteria.

conclusion

This study concluded that pyocyanin possess antibiotic activity against gram positive and uncapsulated gram negative isolated pathogens of UTIs and more efficiency with high concentration than at low concentration. pyocyanin has antibacterial activity Emphasis with the commitment by good personal hygein to reduce urinary tract

infection. More research is needed for antimicrobial activity of pyocyanin against other microorganisms. Further investigation under controlled circumstances will be needed to discover other procedures for pyocyanin production as an antibiotic. Experimental research finding in vivo to study physiological changes of pyocyanin as antibiotic.

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