Asian Journal of Medicine and Health

4(4): 1-9, 2017; Article no.AJMAH.33379

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

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. They managed the analyses of the study and managed the literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJMAH/2017/33379
Editor(s):
(1) Triveni Krishnan, Division of Virology, National Institute of Cholera and Enteric Diseases, Kolkata, India.
Reviewers:
(1) Masaaki Minami, Nagoya City University, Japan.
(2) Guadalupe García-Elorriaga, Mexican Social Security Institute, Mexico.
(3) Ronald Bartzatt, University of Nebraska at Omaha, USA.
Complete Peer review History: http://www.sciencedomain.org/review-history/19312

Original Research Article

Received 12th April 2017
Accepted 20th May 2017
Published 2nd June 2017

ABSTRACT

This study aims 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 hours. Considerable amount of blue pigment pyocyanin were produced by *Pseudomonas aeruginosa* after growing 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. freundii* 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. epidermidis* 5%, *S. saprophyticus 10%, E. coli* 40%, *K. pneumoniae 10%*, *P. aeruginosa* 5%, *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 uncapsulated gram negative by different zone of inhibition at

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different concentrations. The inhibition zone was determined by using 25%, 50%, 75% and 100%. The *S. aureus* inhibition zone at 25% was 16 mm, 50% was17.5 mm, 75% was 20 mm and 21 mm at 100% concentration, *S. epidermidis* at 25% was 17 mm, 50% 20 mm, 75% was 21 mm and 31 mm at 100%, *S. saprophyticus* at 25% was 19 mm, 50% was 23 mm, 75% was 25 mm and 33 mm at 100%. *E. coli* at 25% was 13 mm, 50% was 15 mm, 75% was 25 mm and 22 mm at 100%. was resistant to 25% and 50% concentration and given inhibition zone 11,13 mm 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.

Keywords: Pseudomonas aeruginosa; pyocyanin; urinary tract pathogen.

1. INTRODUCTION

Urinary tract infection has become a major public health problem, It can be caused by different gram positive and gram negative bacteria; such as *Staphylococcus aureus, Staphylococcus saprophyticus, Staphylococcus epidermidis, Enterococci, Haemolytic streptococci, Escherichia coli, Proteus* species*, Klebsiella strain* and *Pseudomonas aeruginosa,* [1] *Pseudomonas aeruginosa* belongs to the family *Pseudomonadaceae*. It is widely spread in the environment where majority 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 [2], it is gram-negative rod, motile, an obligate aerobe, the bacterium is ubiquitous in soil and water, and on surfaces in contact with soil or water, [3] its metabolism is respiratory and never fermentative, but it will grow in the absence of O_2 if NO_3 as terminal electron acceptor is available as a respiratory electron acceptor, *Pseudomonas aeruginosa* is oxidase positive [3], 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 [4], *Pseudomonas aeruginosa* is an opportunistic pathogen to human [1] and [5] meaning that is exploits some break in the host defenses to initiate an infection, it causes 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 organism, 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. [4]. 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 non-fermenting gram-negative bacilli capable of producing the very distinctive water-soluble pigment pyocyanin [6], 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, Klebsiella* strain which may allow *P. aeruginosa* an advantage over competing bacteria occupying the same niche [7], 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 [8], 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 [9]. 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 as Phenazines and 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* [17,18]. 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, phenazines have been of great interest to pharmaceutical and clinical research groups for the last 50 years [16,20,21].

2. 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 was done 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, Eiosin Methyline 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 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.

Pyocaynin 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 at 3000 rpm for 30 min, and supernatant was taken. An aliquot of 5 ml from culture was then extracted into 1 ml of chloroform, and then re-extracted into 1 ml of 0.2 N HCl. The absorbance of this solution was measured at 520 nm [22]. The different pathogenic bacteria were cultured in brain-heart broth for 24 hrs, and the bacteria was cultured on nutrient agar by spreader and left to dry for 15 min after that wells were made by cork borer, and filled with 0.1 µl of pyocyanin extract and incubated for 24 hrs, and the results were recorded.

3. RESULTS

Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus, Escherichia coli, Citrobacter freundii, Klebsiella 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 is associated with catheter as shown in Table 1.

Pyocyanin inhibited the growth of *S. aureus, S. saprophyticus*, *S. epidermidis, E. coli and C. frundii* indicated by zone of inhibition, and did not affect the growth of *K. pneumoniae P. aeruginosa* and *C. albicans* as shown in Table 3, Figs. (1-4). And explained in the statistical analysis.

Table 1. Percentage of microorganism

Isolated microorganism	Percentage %
S. aureus	15%
S. saprophyticus	10%
S. epidermidis	5%
E. coli	40%
P. aeruginosa	5%
K. pneumoniae	10%
C. fruendii	5%
C. albicans	10%

Table 2. Effect of pyocyanin against isolated pathogens

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

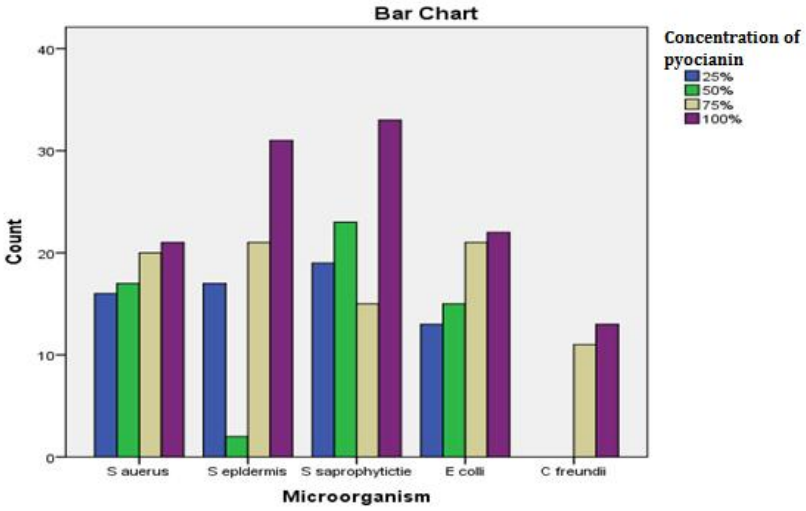
Case processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Microorganism * Concentration of pyocyanin	330	100.0%	0	0.0%	330	100.0%

Chi-Square tests

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	36.122[a]	12	.000
Likelihood Ratio	48.710	12	.000
Linear-by-Linear Association	5.249	1	.022
N of Valid Cases	330		

a. 2 cells (10.0%) have expected count less than 5. The minimum expected count is 4.15.



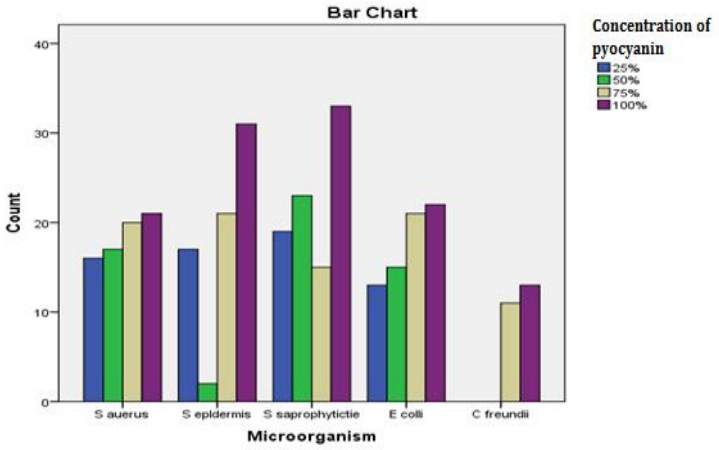
Microorganism * Concentration of pyocyanin cross tabulation

			Concentrations of pyocyanin				Total
			25%	50%	75%	100%	
Microorganism	*S. auerus*	Count	16_a	17_a	20_a	21_a	74
		% within Microorganism	21.6%	23.0%	27.0%	28.4%	100.0%
	S. epidermidis	Count	17_a	2_b	21_a	31_a	71
		% within Microorganism	23.9%	2.8%	29.6%	43.7%	100.0%
	S. saprophyticus	Count	$19_{a, b}$	23_b	15_a	$33_{a, b}$	90

Microorganism * Concentration of pyocyanin cross tabulation							
			Concentrations of pyocyanin				Total
			25%	50%	75%	100%	
		% within Microorganism	21.1%	25.6%	16.7%	36.7%	100.0%
	E. colli	Count	13_{a}	15_{a}	21_{a}	22_{a}	71
		% within Microorganism	18.3%	21.1%	29.6%	31.0%	100.0%
	C. freundii	Count	0_{a}	$0_{a, b}$	11_{c}	$13_{b, c}$	24
		% within Microorganism	0.0%	0.0%	45.8%	54.2%	100.0%
Total		Count	65	57	88	120	
		% within Microorganism	19.7%	17.3%	26.7%	36.4%	

Chi-square tests			
	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	36.122[a]	12	.000
Likelihood ratio	48.710	12	.000
Linear-by-linear association	5.249	1	.022
N of valid cases	330		

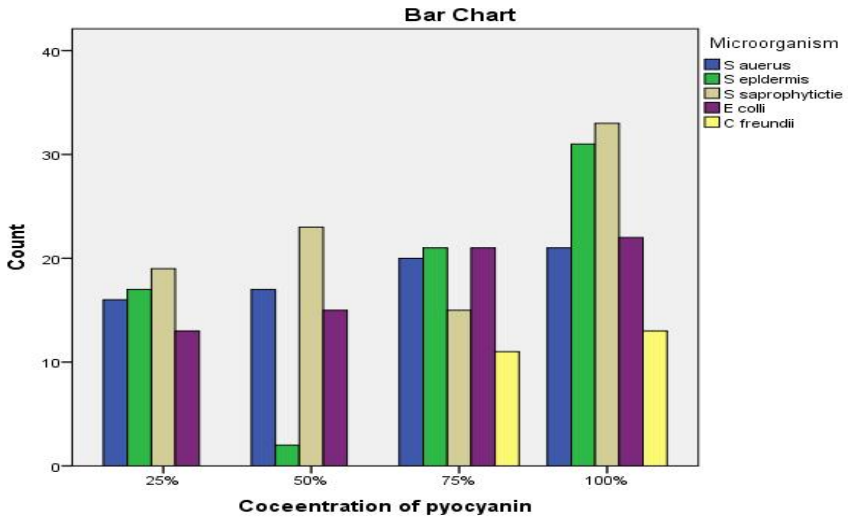
a. 2 cells (10.0%) have expected count less than 5. The minimum expected count is 4.15



Concentrations of pyocyanin * Microorganism Cross tabulation					
			Microorganism		
			S. auerus	*S. epidermidis*	*S. saprophyticus*
Concentrations of pyocyanin	25%	Count	16_{a}	17_{a}	19_{a}
		% within Concentration of pyocyanin	24.6%	26.2%	29.2%
	50%	Count	17_{a}	2_{b}	23_{a}
		% within Concentration of pyocyanin	29.8%	3.5%	40.4%
	75%	Count	$20_{a, b}$	$21_{a, b}$	15_{b}
		% within Concentration of pyocyanin	22.7%	23.9%	17.0%
	100%	Count	21_{a}	31_{a}	33_{a}
		% within Concentration of pyocyanin	17.5%	25.8%	27.5%
Total		Count	74	71	90
		% within Concentration of pyocyanin	22.4%	21.5%	27.3%

Concentrations of pyocyanin * Microorganism cross tabulation					
			Microorganism		Total
			E. coli	*C. freundii*	
Concentration of pyocyanin	25%	Count	13_a	0_a	65
		% within Concentration of pyocyanin	20.0%	0.0%	100.0%
	50%	Count	15_a	$0_{a, b}$	57
		% within Concentration of pyocyanin	26.3%	0.0%	100.0%
	75%	Count	$21_{a, b}$	11_a	88
		% within Concentration of pyocyanin	23.9%	12.5%	100.0%
	100%	Count	22_a	13_a	120
		% within Concentration of pyocyanin	18.3%	10.8%	100.0%
Total		Count	71	24	330
		% within Concentration of pyocyanin	21.5%	7.3%	100.0%

Each subscript letter denotes a subset of Microorganism categories whose column proportions do not differ significantly from each other at the .05 level



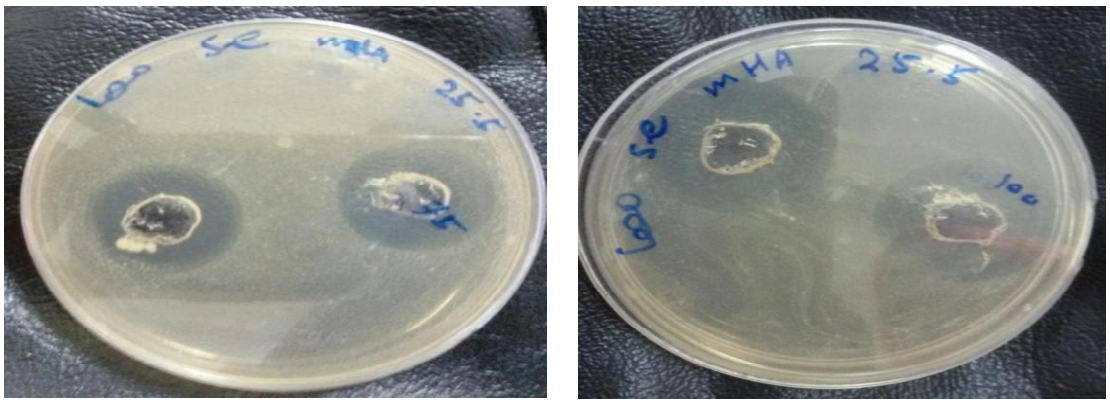


Fig. 1. Left and right show inhibition zone of pyocyanin (75 and 100) % concentration against *S. epidermidis*

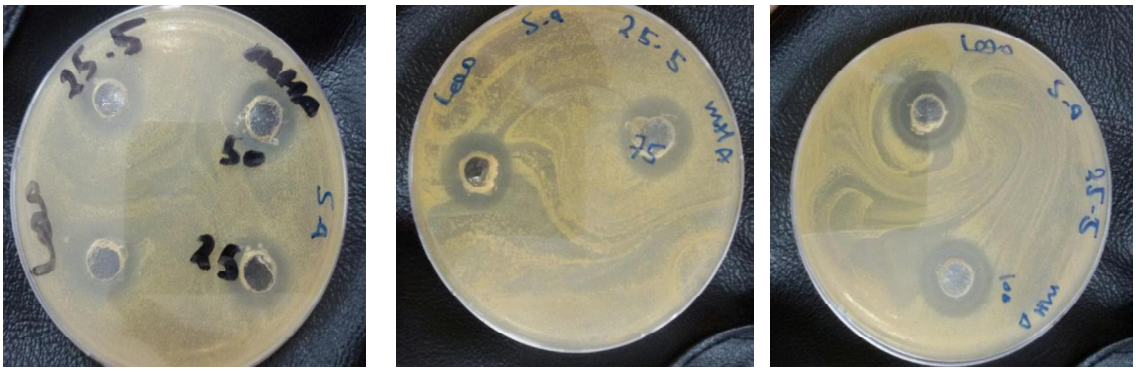


Fig. 2. Left, right and middle show inhibition zone of pyocyanin (25, 50, 75 and 100)% concentration against *S. aureus*

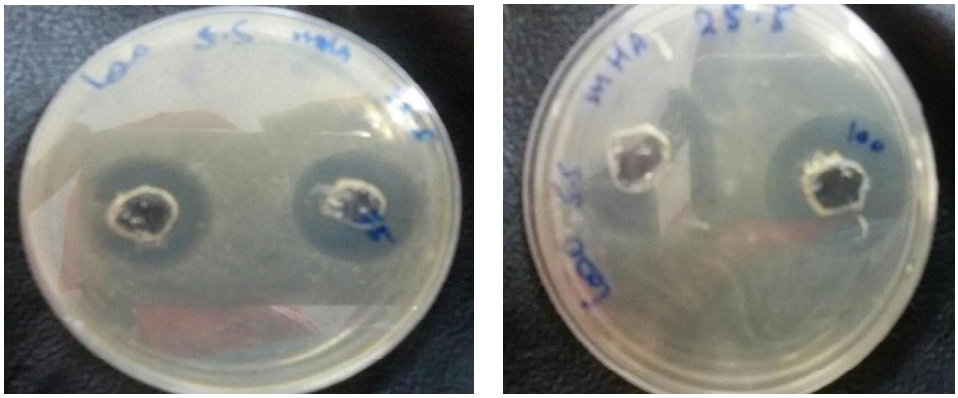


Fig. 3. Left and right show inhibition zone of pyocyanin (75 and 100) % concentration against *S. saprophyticus*

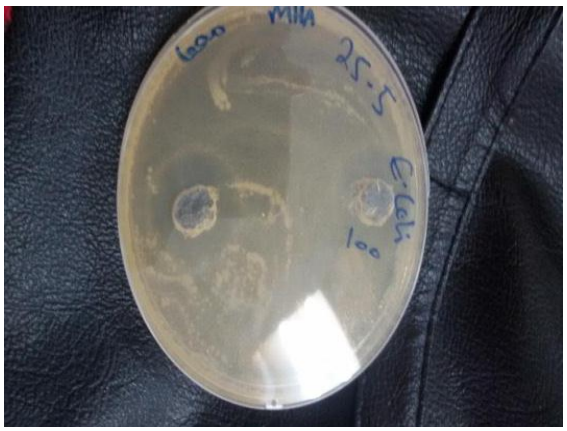


Fig. 4. Show inhibition zone of pyocyanin 100% concentration against *E. coli*

4. 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 noncapsulated gram negative bacteria by appearance of large zones

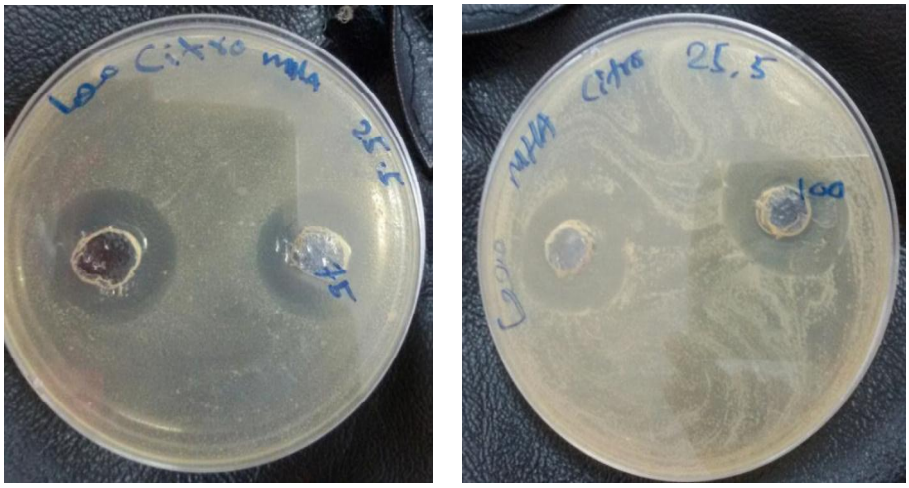


Fig. 5. Left and right show inhibition zone of pyocyanin (75 and 100) % concentration against *C. freundii*

around wells. These results confirm the finding of El-Shouny et al. [23]. These findings agreed with Enass et al. [24]; they had found that pyocyanin has antibacterial activity, and can be used as antibiotic against pathogenic bacteria.

5. CONCLUSION

This study concluded that pyocyanin possess antibiotic activity against gram positive and noncapsulated gram negative bacteria isolated as pathogens of UTIs and had more efficiency with high concentration than at low concentration. Pyocyanin has antibacterial activity but commitment by maintaining good personal hygiene to reduce urinary tract infection needs to be emphasised. More research is needed for antimicrobial activity of pyocyanin against other microorganisms. Further investigation under controlled conditions will be needed to discover other procedures for pyocyanin production as an antibiotic. There is urgent need for experimental research to find in vivo applications and also to study physiological changes of pyocyanin as an antibiotic.

CONSENT

As per international standard or university standard written patient consent has been collected and preserved by the authors.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/19312