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Short Communication

Antimicrobial Effectiveness of Bioactive Phenazine Against Clinical Isolates

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Abstract. The bioactive phenazine extracted from *Pseudomonas aeruginosa* were obtained from the clinical isolates collected from different pathological laboratories of Karachi, Pakistan and its antimicrobial activity was assayed against pathogenic microorganisms. It was found that various gram negative and gram positive bacteria and some species of *Candida* shown to be sensitive against pyocyanin. Extraction was carried out by using chloroform and agar well diffusion protocol was performed for its antimicrobial effectiveness. The maximum average zone of inhibition observed by 20 µL of purified pyocyanin was 16 mm that was against *Proteus* sp. in case of bacteria and 17 mm against *Candida* sp.

Keywords: phenazine, *Pseudomonas aeruginosa*, antimicrobial activity, pyocyanin

Pseudomonas aeruginosa, motile gram-negative bacterium grow in normal atmospheric conditions and hypoxic atmospheric conditions also facilitate its growth (Bettina *et al*., 2012). Different bacterial species produce over 100 phenazine derivatives (Gibson *et al*., 2009) but *P. aeruginosa* is the only non-lactose fermentor that produce pyocyanin (Meyer, 2000; Reyes *et al*., 1981) that may alter the microbial community by inhibiting the growth of sensitive microorganisms (Norman *et al*., 2004). Phenazine is a heterocyclic naturally occuring deep red compound (5-methyl-7-amino-1-carboxy-methylphenazinium betaine) converted to lemon yellow coloured phenazine-1-carboxylic acid (PCA) and finally to the bright blue pyocyacnin 1-hydroxy-5 methylphenazine (Gohain *et al*., 2006).

Pyocyanin regulates the redox mechanism and initiate the production of reactive oxygen species which in turns inhibits cellular respiration, reduction in level of ATP and cAMP thus chloride ion channel could be effected that is controlled by ATP (Winstanley and Fothergill, 2008). The purified form of pyocyanin showed antibacterial effect that varied with the concentration of pyocyanin (Fouly *et al*., 2015; Kanthakumar *et al*., 1993; Baron and Rowe, 1981). Bacterial strains generates increase resistance towards antibiotics, germicides and disinfectants in this regards phenazines have been of great importance from last decades to pharmaceutical and clinical researchers in treating the antibiotic-resistant and susceptible infections (Shouny *et al*., 2011).

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Previous studies revealed that in lung patients the growth of different species of *Candida* sp. has been suppressed by pyocyanin and recurrence of the *Candida* sp. with the suppression of pyocyanin (Kerr,1994), as in this study zone of inhibition against *Candida* sp. by pyocyanin showed some inhibitory action. The available antifungals like azoles and echinocandins are costly and associated with some side effects so there is a need of some other compounds that have better anti-*Candida* effects (Bhattacharyya *et al*., 2013).

Isolation of *P. aeruginosa*. Different strains of *P. aeruginosa* were isolated from clinical samples obtained from pathological laboratories of tertiary care hospital of Karachi, Pakistan and identified by conventional method by performing gram's staining and standard biochemical test (Kathleen and Christopher, 2014).

Pyocyanin extraction and optimization. Soluble pigment of *P. aeruginosa* was extracted by inoculating samples on *Pseudomonas* Cetrimide agar (Fig. 1). They were incubated at 37 °C for 24 h and were observed for colour change. The pigment was extracted using chloroform solvent system as described by Sweedan, (2010). Extraction of pyocyanin was confirmed with

pinkish-red colour production when exposed to 0.2N HCl as shown in Fig. 2-3 (Sudhakar *et al*., 2015).

Antimicrobial assay. Antibacterial activity of pyocyanin was performed with some modifications of the protocol proposed by Sweedan (2010). The tryptic soya broth was inoculated with tests strains which are clinical isolates such as *E. coli, S. aureus, Proteus* sp., *Klebsiella* sp., *Bacillus* sp. and *Candida* sp. for 3 h at 37 °C and the turbidity was checked using 0.5 McFarland standard. Then 100 µL of bacterial suspension was spread by glass rod, 20 µL of purified pyocyanin was added to the prepared wells in the same plate and then incubated at 37 °C for 24 h and the diameter of zone was measured and the results were recorded.

The extracted pyocyanin was tested against different pathogenic microorganisms to identify the inhibitory effect as shown in Table 1. Maximum combatant activity was observed against *Candida* sp. showed 17 mm zone of inhibition (Fig. 4) in terms of diameter followed by *Proteus* sp. which was 16 mm as shown in (Fig. 5). The minimum activity was observed against *E.coli* i.e 10 mm.

In some diseases there is co-infection of *Pseudomonas* in that case our results showed the inhibition of micro-organism by pyocyanin for e.g. in Cystic fibrosis patients

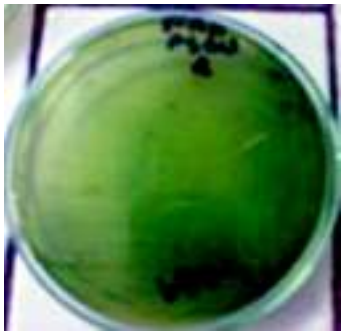

Fig. 1. Growth of *Pseudomonas* on Cetrimide agar.

Table 1. The zone of inhibition (zone diameter) by phenazine against clinical isolates by agar well diffusion

Pyocyanin	Pathogenic microorganism	Zone of inhibitions (mm)
20 µL	*Escherichia coli*	10
	Proteus sp.	16
	Staphylococcus aureus	13.5
	Klebsilla sp.	13
	Bacillus sp.	12.6
	Candida sp.	17

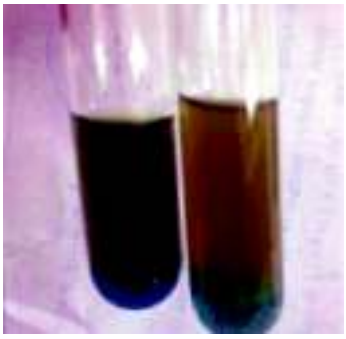

Fig. 2. Extracted Pyocyanin in TSB broth.

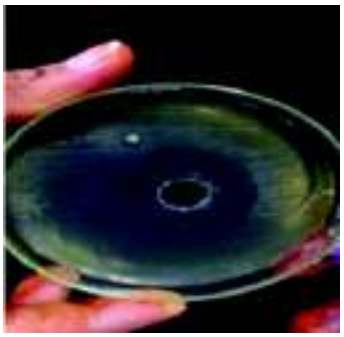

Fig. 4. Antibacterial effect against *Candida* sp.

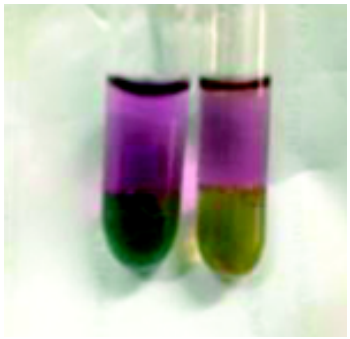

Fig. 3. Pink colour confirms presence of pyocyanin.

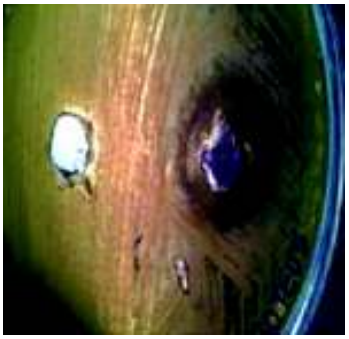

Fig. 5. Antibacterial effect against *Proteus* sp.

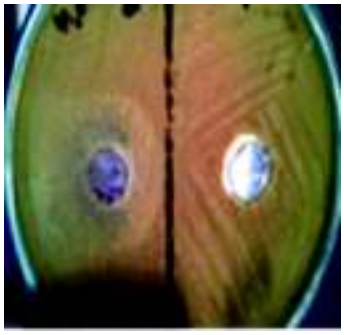

Fig. 6. Antibacterial effect against *S. aureus*.

there is a co-infection with *Staphylococcus aureus* (Abdulrudha, 2011) so the pyocyanin could be the inhibitory molecule for *S. aureus* as in this study zone of inhibition against *S. aureus* was 13.5 mm (Fig. 6). Our study indicates that pathogenic microorganism become susceptible with the action of pyocyanin.

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Conflict of Interest. The authors declare no conflict of interest.

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