

Pyocyanin (5-methylphenazin-1-one) Inhibits the Growth of Bacterial Species Colonized on Chronic Diabetic Wounds

SDAK Wijesinghe¹, WMID Nakkawita², AD De Silva^{2,3}, US Gunwardana³, and HS Jayasinghearachchi^{4#}

¹School of Health Science, British College of Applied Studies, Sri Lanka

²Faculty of Medicine, General Sir John Kotelawala Defence University, Sri Lanka

³Biomedical Laboratory, Faculty of Medicine, General Sir John Kotelawala Defence University, Sri Lanka

⁴Institute for Combinatorial Advance Research and Education, General Sir John Kotelawala Defence University, Sri Lanka

#jayasinghearachchihs@kdu.ac.lk

Pyocyanin (5-methylphenazin-1-one), a blue-green pigment synthesized by *Pseudomonas aeruginosa*, inhibits bacterial and fungal species, but its antimicrobial effect on microbial species colonized on chronic diabetic wounds is limited. In this study, microorganisms isolated from chronic diabetic wounds at the microbiology laboratory at the University Hospital of KDU were grown on Luria Agar plates. Microbial pellet harvested from LA-grown sub-cultures were subjected to Next Generation Sequencing (NGS) with 16S metagenomics sequencing analysis at the Biomedical Laboratory, KDU. Microbial lawns prepared on Muller Hinton Agar (MHA) medium were used for all antimicrobial assays of pyocyanin against wound colonizers. Three concentrations of pyocyanin (23, 45, and 54 µg/ml) were selected. One plate was kept without pyocyanin discs. Microbial lawns were well-grown in plates that did not receive pyocyanin discs. Clear zones with diameters 20.8 mm, 22.0 mm and 24.3 mm were observed surrounding the disks impregnated with 54 µg/ml indicated the complete inhibition of the growth of the microbial colonizers, whereas microbial growth was not completely inhibited by lower concentrations tested (23, 45 µg/ml), and therefore completely clear zones were absent indicating growth of resistant colonizers when used lower concentrations (23, 45 µg/ml). Metagenome analysis showed the highest abundance of microbial colonizers belongs to genera *Acinetobacter*, *Klebsiella*, *Brenneria*, *Photobacterium*, *Pseudomonas*, *Serratia*, *Citrobacter*, *Enterobacter*, and *Cronobacter*. Pyocyanin found to be a good antimicrobial agent and, further investigations should be carried out to identify minimum inhibitory concentration (MIC) for eliminating the harmful microorganisms while retaining beneficial microbial colonizers that support for wound healing process.

Keywords: *pyocyanin, chronic diabetic wound colonizers, 16S NGS metagenome analysis, antimicrobial activity*