Anthocyanin-based gel from *Clitoria ternatea*: Antimicrobial efficacy against *Streptococcus pneumoniae* and zebrafish toxicity assessment

S Sivasankara Narayani¹, S Mahesh Kannan¹, P Lokesh², K Kulasekarapandian³, J Ravindran⁴

¹Department of Microbiology, Ayya Nadar Janaki Ammal College, Sivakasi, India, ²School of Biosciences and Technology, Vellore Institute of Technology, Vellore, India, ³Department of Physics, Ayya Nadar Janaki Ammal College, Sivakasi, India, ⁴Preclinical Department, Universiti Kuala Lumpur Royal College of Medicine Perak, Malaysia.

ABSTRACT

Introduction: Anthocyanins exhibit antibacterial activity against a broad spectrum of microorganisms by destabilizing plasma membrane and damaging cell structures. They inhibit both Gram-negative and Gram-positive bacteria. This study aims to evaluate the antimicrobial activity of anthocyanins extracted from Clitoria ternatea flowers against microorganisms isolated from the eye. The study involves the formulation of a gel using the extracted pigment and an analysis of its toxicity. Materials and Method: Anthocyanin was extracted from Clitoria ternatea (ACT) flowers by a pH differential method. The organism isolated were identified through biochemical tests and 16s rRNA sequencing. Antimicrobial activity, MIC, and DNA fragmentation assays were performed. The ACT extract was formulated into a gel, with toxicity analysed using zebrafish. Results: The highest yield from dry flowers in acidified methanol (DCM) of $15.1129 \pm 0.05 \,\mu\text{g/g}$. The swab samples yielded seven and nine colonies, respectively. A single colony was selected for analysis, showing Gram-positive cocci in chains. Biochemical tests were positive for methyl red and negative for Voges-Proskauer, indole, citrate, triple sugar iron, and starch hydrolysis. 16s rRNA sequencing confirmed Streptococcus pneumoniae. DCM extract showed a 7 mm zone of inhibition against Streptococcus pneumoniae, outperforming other extracts. The MIC was 57.6 µg/ml, and the DNA fragmentation assay showed smudges on the agarose gel, indicating DNA destruction. ACT gel was prepared using Carbopol. The spreadability of the gel was 32 seconds, and it spread uniformly with no solid particles. Since the fish movement, gills, and mouths appeared normal, the gel was considered nontoxic. Conclusion: This study concludes that ACT - DCM exhibits potent antimicrobial activity against Streptococcus pneumoniae and can be used in eco-friendly gel formulations.