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Multifunctional roles of hemolymph serum in the innate immunity of freshwater crab *Barytelphusa cunicularis* (Westwood in Sykes, 1836)

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Understanding innate immunity in crustaceans is essential due to their ecological and aquaculture importance, yet the functional roles of hemolymph serum in freshwater crabs remain poorly characterized. In this study, the immune properties of serum from the freshwater crab *Barytelphusa cunicularis* were investigated with emphasis on its biochemical composition and functional activity. Biochemical profiling revealed a predominance of proteins over carbohydrates and lipids, indicating a serum rich in effector molecules. Phenoloxidase (PO) activity, a key enzyme in invertebrate immunity, exhibited optimal activity at 25 °C in Tris–HCl buffer (pH 7.5) using DL-DOPA as substrate, and was significantly influenced by immune-related activators and inhibitors such as trypsin, SDS, laminarin, and PTU ($P < 0.05$). Hemagglutination assays revealed lectin-like activity, with the serum strongly agglutinating vertebrate erythrocytes, while carbohydrate inhibition confirmed galactose-specific binding. Additionally, the serum exhibited pronounced lysozyme and antimicrobial activities against five bacterial and three fungal pathogens. Microbial agglutination titers (32–512) further demonstrated its capacity for broad pathogen recognition and binding. These findings highlight the coordinated immune strategy of *Barytelphusa cunicularis* serum involving pathogen recognition, enzyme activation, and antimicrobial defense, providing new insights into the multifunctional roles of hemolymph serum in crustacean innate immunity.

Keywords: Phenoloxidase, Hemagglutination, Lysozyme, Microbial agglutination, Antimicrobial defense