## ABSTRACT

Protease is an enzyme that catalyzes the hydrolysis of peptide bonds in proteins, cleaving amino acid chains into shorter peptides or individual amino acids widely applied in the food, detergent, and pharmaceutical industries. Microbial proteases are particularly desirable due to lower cost, ease of scale-up, and ethical advantages compared to animal- or plant-derived enzymes. The endophytic bacterium Delftia lacustris Z8, isolated from the bark of waru (*Hibiscus tiliaceus*), is known to produce bioactive secondary metabolites, making it a promising candidate for enzyme studies. This isolate was selected to: purify its protease using ammonium sulfate fractionation and dialysis; characterize the effects of temperature and pH; and determine enzyme kinetics in terms of Km and Vmax. The study methods included reviving strain Z8 on Zobell medium, confirming its phenotype through macroscopic and microscopic identification, adapting it to casein-based medium, generating growth curves, and producing the enzyme. Enzyme activity and specific activity were measured before and after purification. The protease was further characterized under varied temperature and pH, with kinetic parameters derived from Lineweaver–Burk and Michaelis–Menten plots. Results showed the highest specific activity of 1192.693 U/mg in fraction 5 post-purification, significantly higher than the 335.426 U/mg measured before purification. Optimal enzyme activity occurred at 37.5 °C and pH 6.5. Lineweaver–Burk analysis yielded Km is 0.050 M and Vmax is 2500 M/min, while Michaelis–Menten analysis produced Km is 0.033 M and Vmax is 2311.605 M/min.



**Keywords**: *Delftia lacustris* Z8, Enzyme Kinetics, Enzyme Purification, pH, Protease, Temperature