



**Study on the Efficiency of a Test Strip for Glucose Concentration in Synthetic Urine
Using Crude Papain Extract from *Carica papaya* ‘Kaek Dam’ as a Catalyst in the
Colorimetric Reaction of Tetramethylbenzidine and Hydrogen Peroxide**

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Abstract

Diabetes mellitus is a global health concern that requires regular glucose monitoring for effective disease management. Commercial paper-based glucose test strips commonly employ a glucose oxidase (GOx)-horseradish peroxidase (HRP) enzymatic system to generate a colorimetric response; however, HRP is relatively expensive, requires purification, and is largely imported, limiting the development of affordable point-of-care diagnostic devices. This study investigated the feasibility of using crude papain extract from *Carica papaya* ‘Kaek Dam’ as a natural peroxidase mimic for a low-cost paper-based glucose biosensor. Crude extracts from the peel, flesh, and seeds of immature papaya were analyzed using the Bradford protein assay, and the seed extract, which exhibited the highest protein concentration, was selected for further experiments. Catalytic activity was evaluated using the GOx-TMB-H₂O₂ system by measuring absorbance at 652 nm. A paper-based biosensor was fabricated by immobilizing crude papain extract, TMB, and trehalose onto filter paper and tested with synthetic urine containing glucose concentrations ranging from 0 to 20 mM with GOx. Colorimetric responses were quantified by RGB image analysis. The biosensor produced a concentration-dependent blue color, and both spectrophotometric and RGB analyses showed a positive correlation between glucose concentration and color intensity, confirming the catalytic capability of crude papain extract. These findings demonstrated that crude papain extract was a sustainable and low-cost alternative to HRP for paper-based glucose sensing and had potential for environmentally friendly point-of-care diagnostics in resource-limited settings.

Keywords: Crude papain extract, Peroxidase-like activity, Paper-based biosensor, Glucose detection, Synthetic urine