CHAPTER V

CONCLUSION

1. The purification of β-glucosidase from *Daldinia eschscholzii* required 4 steps including ammonium sulphate fractionation, SP-sepharose, Phenyl-sepharose, and Superdex-200. The enzyme β-glucosidase was obtained with 50.23 fold purification and 6.28% yield. The specific activity was 77.86 U/mg protein.

2. The enzyme had an apparent molecular weight of 64.2 kDa as analyze by SDS-PAGE, and with a molecular weight of 65,747 Da estimated by MALDI-TOF spectrometry. An isoelectric point, pI, of 8.55, was obtained using isoelectric focusing.

3. The apparent for PNPG as K_m of 1.52 mM, and V_{max}, values of 3.21 mmole/min/mg.

4. Optimal activity with PNPG as the substrate was at pH 5.0 and 50°C. The enzyme was stable at pH 5.0 at temperatures up to 50 °C.

5. This enzyme was competitively inhibited by Glucose and glucono-δ-lactone, with K_i values of 0.79 mM, and 4.72 μM, respectively.

6. The purified β-glucosidase was active against PNPG, cellobiose, sophorose, and gentiobiose, but did not hydrolyze lactose, sucrose, Avicel, and o-nitrophenyl-β-D-galactopyranoside.

7. The activity of β-glucosidase was stimulated by Ca^{2+}, Co^{2+}, Mg^{2+}, Mn^{2+}, glycerol, DMSO, DTT and EDTA and, strongly inhibited by Hg^{2+}.

8. The enzyme was chemically modified by EDC in the presence of glycinamide as nucleophile under various conditions to study role of carboxy groups in the catalytic mechanism of this enzyme.

9. The enzyme was inactivated by the binding of one mol of EDC per mol of the enzyme with a second-order rate constant of 6.73×10^{-2} mM/min. Glucono-δ-lactone as a competive inhibitor, partly protected the active-site carboxy group against chemical modification, with k_d of 70.74×10^{-2} mM.

10. Transglycosylation activity of the enzyme toward alcohol was performed by using PNPG as glucosyl donor substrate. Glucoside products were separated and detected by TLC analysis.

11. The internal amino acid sequence of *D. eschscholzii* β-glucoisdase has similarity to the sequence of the family 3 β-glucosyl hydrolase.