Chapter 5

CONCLUSIONS

1. Rubber peroxidase (RBP) was purified from *Hevea brasiliensis* leaves by $(NH_4)_2SO_4$ precipitation, DEAE ion exchange chromatography, and Sephadex G-75 size exclusion chromatography, respectively.
2. The purified RBP was used to prepared the RBP-antibody conjugates by using glutaraldehyde, sodium periodate and sulfo-SMCC as cross-linkers.
3. Among the three different cross-linkers tested, periodate was the best cross-linker. The RBP-anti-rabbit IgG conjugate and the RBP-anti-human IgG conjugate, which retained 68% and 76% peroxidase activity, respectively, were obtained.
4. The prepared RBP-antibody conjugates, once can be kept at -20 ^{o}C for eight months without losing peroxidase and immunological activity.
5. The RBP-anti-rabbit IgG conjugate was used as a secondary antibody to detect vitellogenin in mullet plasma and HMG-CoA synthase in C-serum of rubber latex equally well as the commercial horseradish peroxidase (HRP) -anti-rabbit IgG conjugate by the Western blot technique.
6. The RBP-anti-human IgG conjugate was used as a secondary antibody to determine anti-leptospira antibody in human serum by the indirect ELISA technique.

7. Purified RBP was also used as a coupled enzyme in the cholesterol esterase/oxidase reaction to determine human serum cholesterol.
8. The RBP from a Sephadex G-75 column was further purified in a Con-A Agarose column. Three protein peaks - RBPA, RBPB and RBPC were obtained, corresponding to their ND-PAGE analysis.
9. Amino acid sequence analysis of RBP1, RBP2 and RBP3 fractions from the Con-A agarose column was performed. The peptides RBPA_K24, RBPA_K25, RBPA_K28, RBPB_K32, RBPB_K35, and RBPA_CN19 showed high similarity to known plant peroxidase sequences, whereas RBPB_CN20, RBPB_K17, RBPB_K30 and RBPB_K34 were related to Concanavalin A and RBPC_K24 to β-glycosidase, respectively.
10. Degenerate primers for the first PCR were designed from the conserved region GAHTF, which was found in peptide RBPA_K28 and HFHDCF found in alignment of other plant peroxidases.
11. The cDNA encoding RBP was cloned by performing 3́, 5́ Rapid Amplification of cDNA Ends (3́, 5́ RACE), using gene-specific primer designed from the first PCR nucleotide sequence and RNA from *Hevea* leaves as template.
12. Two cDNA sequences, one containing a complete cDNA, *rbp1* and a 3′cDNA fragment of *rbp2* were obtained.