CHAPTER VI
CONCLUSIONS

Determination of pseudojujubogenin glycosides using immunological assays including immunochromatographic strip, eastern blotting and immunoaffinity column are based on antigen-antibody reaction. Because of *B. monnieri* herbal products acted as memory enhancer was increasingly produced to support the consumer demand, the necessary to confirmation of the quality of herbal products were also rising.

The compound acted as memory enhancer, pseudojujubogenin glycosides from *B. monnieri*, were focused because the ease of growing in Thailand, the time of planting also short that proper to up scaling for industry level. To screening of the pseudojujubogenin glycosides in herb and herbal product, immunochromatographic strip was developed. This immunochromatographic strip is based on competitive antigen-antibody reaction resulting that the sample gave two red spot both capture and control zone when the sample have no pseudojujubogenin glycosides or lower than the detection limit. In opposite, the sample that have pseudojujubogenin glycosides equal or higher than the detection limit gave only one red spot at control zone. Due to the rapid of the assay and small volume of sample indicated that immunochromatographic strip is proper for screening of pseudojujubogenin glycosides and the detection limit is 125 $ng.ml^{-1}$.

Only ten-to-fifteen min of analyzing, the strip could give the result that is the advantage of this method more than other immunological assays; however the sensitivity of this method is lower than other immunoassay. In our study, the strip could be used for analyzing around six month when stored in refrigerator. This assay provides a simple step and effective cost-performance in the experiment.

Moreover, the sample that gave a positive result on the immunochromatographic strip could be continued to determine the total pseudojujubogenin glycosides and analyzed of each pseudojujubogenin glycosides by eastern blotting. Eastern blotting using PES membrane can be detected the amount of each pseudojujubogenin glycosides. The detection limit of eastern blotting for

detection of pseudojujubogenin glycosides is 3.125 ng level. The eastern blotting established in our study could be applied to determine pseudojujubogenin glycosides content from plant sample. Various parts of *B. monnieri* were selected to analyze of each pseudojujubogenin glycosides. Furthermore, the extract of *B. monnieri* from *in vitro* culture were also analyzed, these result suggested the proper *in vitro* culture condition for *B. monnieri* to gave a high content of pseudojujubogenin glycosides. The total pseudojujubogenin glycosides content in plant samples using eastern blotting technique have a good correlation with ELISA method (r^2 = 0.9721). These results suggested that eastern blotting could be used for determination of both total and each amount of pseudojujubogenin glycosides.

The specific binding of antigen-antibody could be worked for isolation of pseudojujubogenin glycosides from the sample. At the first of isolation, the sample solution was allowed through the immunoaffinity column that containing of anti-bacopaside I PAb which specific bound to pseudojujubogenin glycosides. Then, non specific compound was eliminated from the column. Finally, the eluant was collected and determined by ELISA. Isolation of pseudojujubogenin glycosides using immunoaffinity column that contained anti-bacopaside I PAb in this study could be worked but gave the low column capacity that was not appropriate for industry level. Therefore the immunoaffinity column should be developed the column capacity. In general, ELISA is more sensitive method compared to HPLC as described (Phrompittayarat et al., 2007a,b). When detecting very low concentration of these compounds is required, the combination of ELISA and immunoaffinity column for concentration of active compound may be useful techniques of the samples from natural resource and animal plasma.

The immunological assays using anti-bacopaside I antibody including immunochromatohraphic strip, eastern blotting, ELISA and immunoaffinity column are based on antigen-antibody reaction that have specific binding resulting in specificity for detection and separation of pseudojujubogenin glycosides. Beside the plant, the herbal product that launched in the market also could be analyzed by these methods. Moreover, that methods gave the idea for develop immunological assay for analyzed other glycoside.