CHAPTER V

CONCLUSION

Medicinal plant authentication is a quality assurance process. The proper authentication of herbal raw materials is critically important to the safety and efficacy of herbal medicines. Hence, the purpose of this study was to perform pharmacognostic evaluation and DNA fingerprinting of *L. speciosa*.

To reach the objective, an investigation was conducted in two steps. The first step, pharmacognostic specifications were performed. Macroscopical and microscopical characteristics, physicochemical properties, preliminary phytochemical screening, and TLC fingerprinting profiles were examined. The results indicated that the macroscopic and microscopic characteristics of leaves, and thin layer chromatographic patterns can be effectively used together as important tools in authentication of crude drugs sold in the crude drug market. The physicochemical evaluation of crude drug gives important parameters in detecting adulteration or improper handling of drugs. From this investigation, the requirements of the specification should be established (in integer). The loss on drying, moisture content, total ash and acid insoluble ash should not be more than 10%, 9%, 9% and 2% w/w respectively. These physicochemical parameters were useful for detecting low-grade products as well as the extractive values. The ethanol-, water- and dichloromethane-extractive values were determined to be not less than 8%, 10% and 2%, respectively. These specifications can also be used as quality assurance of the crude drug. *L. speciosa* leaves obtained from various sources of Thailand which contained corosolic acid ranging from 0.01-0.75 % base on dry weight. The difference in contents in the crude drugs may be due to the environment of the plant cultivation. Moreover, collected season and storage condition may also lead to fluctuation in corosolic acid content.

The second step, the DNA fingerprint of *L. speciosa* and closely related species, *L. macrocarpa*; *L. loudonii* and *L. floribunda*, was investigated using AFLP method. DNA based polymorphism assay may offer an alternative method to identify herbal medicines. This investigation found three candidates species-specific of *L.*

ER3AAG/MS3CTG, and 190 bp fragment from ER3AAC/MS3CAT. It should be further developed to SCAR marker for identification of banaba. SCAR method proved to be a rapid and easy-to-perform analytical tool to achieve species authentication of crude drugs. In addition, this study was successful in constructing the dendrogram based on AFLP bands. Genetic relationship analyzed by Jaccard's similarity matrix and UPGMA method showed that the two species, *L. speciosa* and *L. macrocarpa*, formed in the same cluster. *L. loudonii* and *L. floribunda* were placed in other groups as an individual branch.

Based on the result from this study, it is reasonable to say that the pharmacognostic specifications and its DNA fingerprinting could serve as a basis for proper identification, collection and investigation of *L. speciosa* leaves. It will provide useful information for its correct identity and may enable those who handle this plant to maintain its quality control. Thus, these techniques developed here have been successfully proved as a powerful tool for the identification of *L. speciosa* and authentication of *L. speciosa* crude drugs.