DNA barcoding of plants in Thai Herbal Pharmacopoeias as a reference for quality control of plant origins and herbal products

Suchada Sukrong, Natapol Pornputtapon, Thathree Padungscharoen, and Jirayut Jaipaew
Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 11220, Thailand.

Abstract
Background: The use of herbs as medicines and dietary supplements in Thailand was increased dramatically in the past few decades. The increasing demand of raw materials might lead to the intentionally or unintentionally substitution with other plant species in herbal drug regimens. There were 62 monographs (herbs and extracts) in Thai Herbal Pharmacopoeias (THP) Volume I-V and more than 200 listed medicinal plants that officially promulgated by the Ministry of Public Health. We aim to develop a herbal DNA reference library for Thai herbal species using DNA barcode regions (matK and rbcl) and used for authentication of herbal products. Results: Forty-three plants listed in Thai Herbal Pharmacopoeias and 154 of Thai medicinal plants were barcoded for matK and rbcl and used for construction of phylogenetic tree. The information of DNA sequences of these loci provides the species-specific barcode for identification of the botanical origins of different plant species. Herbal products were subjected to be tested using the DNA barcoding method and they were successfully authenticated. Significance: This is the first time to compile a DNA reference library of Thai medicinal plants. Regulatory agencies (FDA) may propose DNA barcoding for manufacturers and merchants to ensure identity of raw materials and processed herbal drugs.

Materials
Table 1. Sources and number of medicinal plants used for DNA library.

<table>
<thead>
<tr>
<th>No. of Monographs in Thai Herbal Pharmacopoeia (Volume)</th>
<th>No. of Monographs in The Monographs of Selected Thai Materia Medica</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>11</td>
<td>10</td>
</tr>
</tbody>
</table>

Methods
- DNA extraction, PCR amplification, and direct sequencing of barcoding loci (matK & rbcl)
- Maximum likelihood analysis
- Authentication of herbal products by comparing sequences against DNA barcoding reference library

Results and Discussions
matK and rbcl regions were successfully amplified and sequenced from all plants. The barcoding dataset was established as a reference for frequently used medicinal plants in Thailand. The phylogenetic relationship of plants in THP and Thai Materia Medica was created. Constructed trees from matK and rbcl are similar. Here, phylogenetic tree based on plastid rbcl sequence is selected for presentation (Fig. 1). Ten single herbal products supplied by Thai FDA were tested against our generated DNA barcode dataset (Fig. 2). DNA was successfully recovered from all herbal preparations including tablets, capsules, and gels. Successful PCR amplification of rbcl and matK from herbal products was 100% and 80%, respectively. However, we found that type of Taq polymerase is play an important role to obtain clear PCR products. All tested products were authenticated by comparing sequences of recovered amplicons. Herbal products contained corrected plant species as labeled on the packages. However, eight herbal products (A, B, D-G, and J) out of 10 were strongly confirmed for the present of the plants as listed on the packages since the fully information from both of barcoding loci were recovered.

Conclusion
The Thai herbal DNA barcoding reference library was initiated. DNA barcoding is useful for the authentication of herbal products as we were able to authenticate many herbal products from Thai FDA. The next step is the development of the DNA herbal library such as vouchers and SOPs for testing single herbal products and mixture.

References

Acknowledgement
We thanks The Research Unit of DNA Barcoding of Thai Medicinal Plants, Chulalongkorn University, Food Drug Administration of Thailand (FDA) and The Department of Thai Traditional and Alternative Medicine, Ministry of Public Health, Thailand.

Fig 1. Maximum likelihood analysis of rbcl region of Thai medicinal plants. The tree is un-rooted tree representing taxonomic classification of plants. Green clades represent monophyletic clade based on plant’s families, red clades are otherwise. The bootstrap values are given on the nodes.

Fig 2. Ten single herbal products provided by Thai FDA that were subjected to test by DNA barcodes. A: Curcuma longa tablets, B: Centella asiatica capsules, C: Kaempferia parviflora capsules, D: Centella asiatica tablets, E: Curcuma longa capsules, F: Kaempferia parviflora capsules, G: Centella asiatica cream, H: Centella asiatica powder, I: Curcuma longa powder, J: Zingiber montanum powder.