

# Barcoding-HRM analysis for authentication of medicinal plant *Bacopa monnieri* commercial products.

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## Abstract

**Background:** Medicinal plants were consumed as a dietary supplement in expensive price. The authentication of plants was concern in recently years because of the substitutions of plant materials in dietary supplement. *Bacopa monnieri*, herbals for cognitive improvement, were used in Ayurvedic Materia Medica for century. In Thailand, there are three species of plants in the genus *Bacopa* (Plantaginaceae) including *B. monnieri* (L.) Wettst., *B. caroliniana* (Walter) B. L. Rob. and *B. floribunda* (R. Br.) Wettst. Morphological characteristic resemblance of plants in this genus makes it difficult to identify and confound to use. Therefore DNA barcoding, a technique using plant DNA in short regions, provides a powerful tool for solving this problem. High Resolution Melting (HRM) analysis based on nucleotide differences was applied to discriminate *B. monnieri* from other *Bacopa* spp. **Result:** Six candidate barcodes, *matK*, *rbcL*, *psbA-trnH* IGS, *trnL-F* IGS, *ycf1* and *ITS*, of the three taxa were successfully amplified and sequenced with universal primer pairs. The nucleotide polymorphisms of the six regions were found and used to distinguish among the three taxa. HRM analysis of *trnL-F* IGS has been done. The constructed melting curves for *B. monnieri* and other species have significantly different clusters. The assay was effectively applied to commercial herbal products. **Significance:** HRM with specific primers has been developed using DNA barcoding for discrimination the important medicinal species, *B. monnieri*, from other related species. The authentication is also benefit for quality control of medicinal plants for ensuring the safety of the consumers.

## Materials



BM: *Bacopa monnieri*  
(Prom-mi)

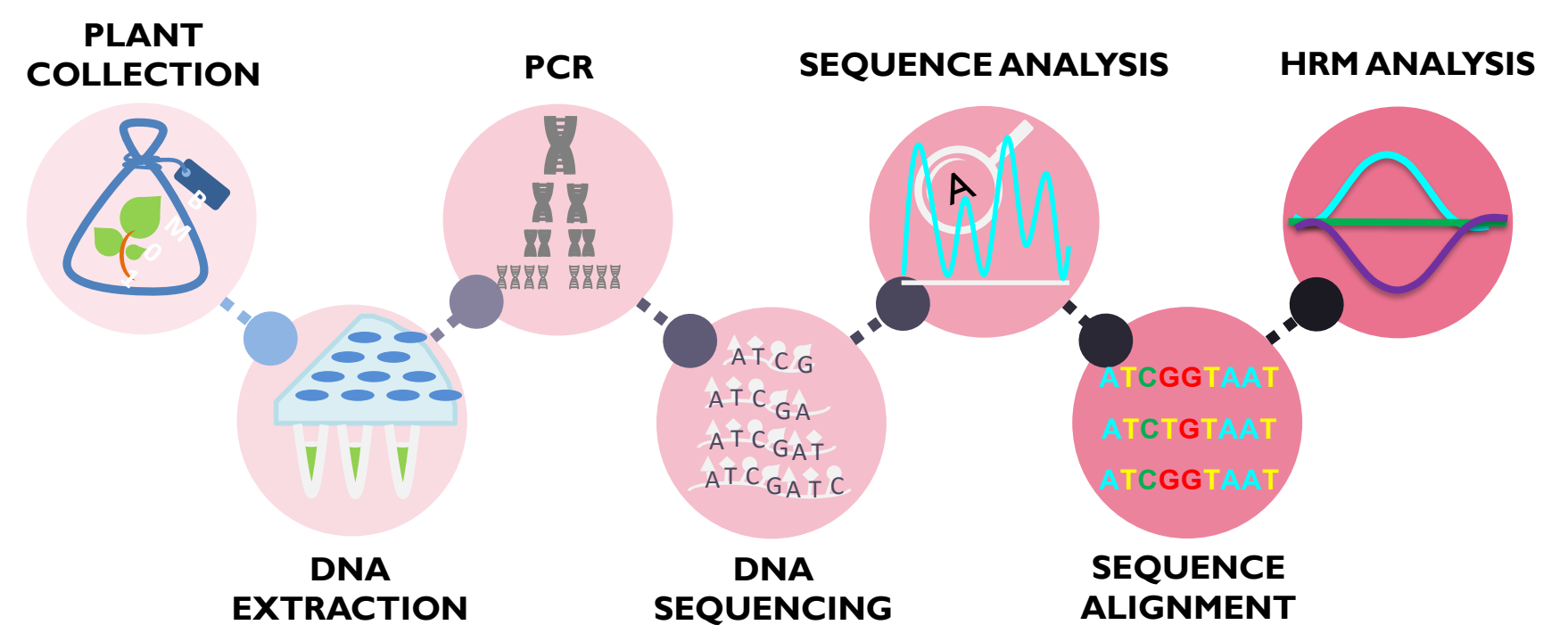


BC: *Bacopa caroliniana*  
(Lan Pailin)



BF: *Bacopa floribunda*  
(Phak Sam Lun)

## Methods



## Results

### *Bacopa* spp. discrimination by *trnL-F* barcoding couple with HRM analysis

*TrnL-F* DNA regions coupled with HRM analysis was evaluated on the three *Bacopa* species. We found that  $T_m$  values of the three species are unique (*B. monnieri*: 74.7°C, *B. caroliniana*: 73.8°C and *B. floribunda*: 75.4°C) (Fig. 1). The difference plot was performed. The results showed differences in the melting curved shapes which typing into three clusters.

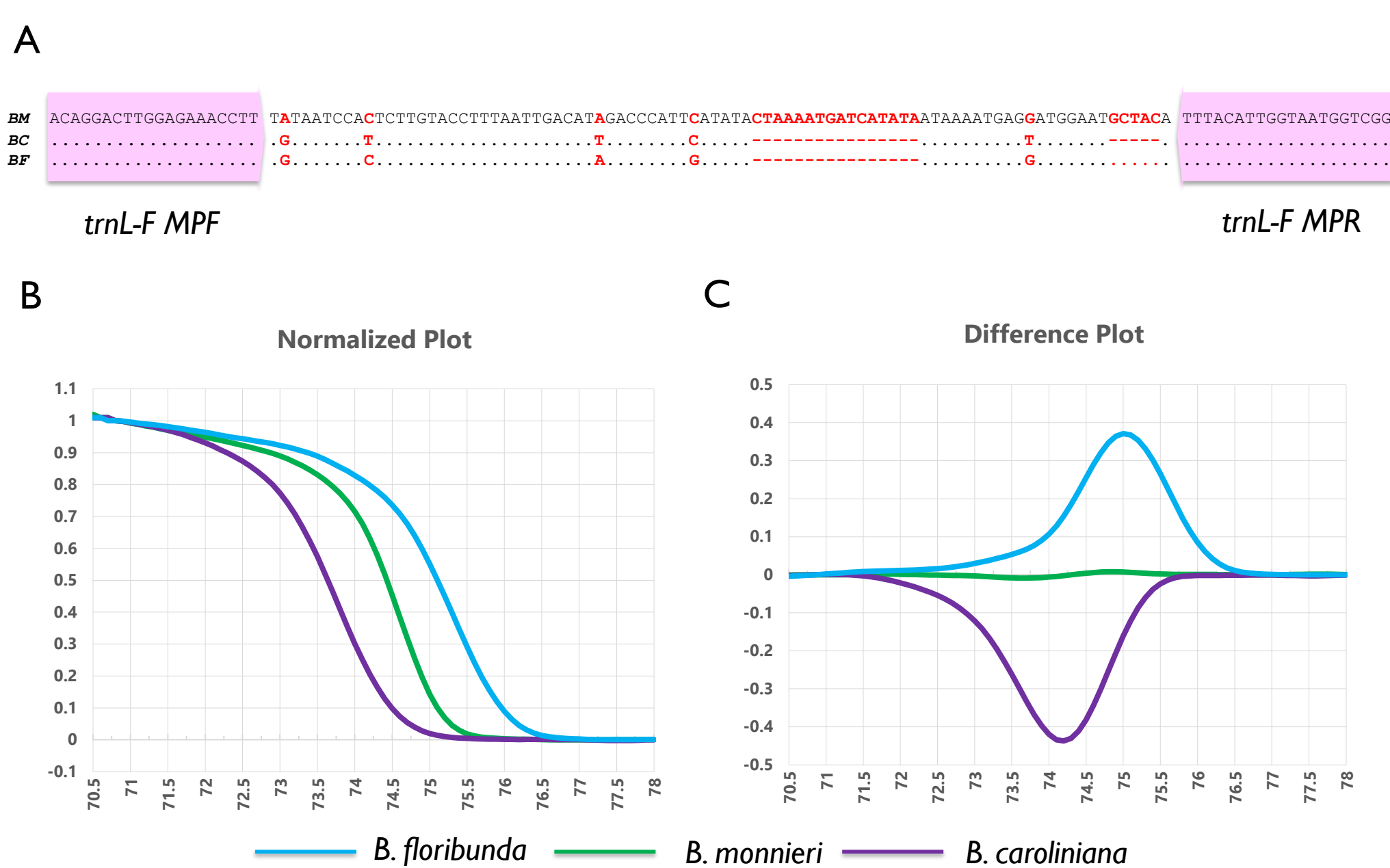


Figure 1

The multiple alignment of DNA sequence amplified using *trnL-F* MP primer set was obtained from three *Bacopa* species; *B. monnieri*, *B. caroliniana* and *B. floribunda* (A). HRM analysis in terms of normalized plot (B) and difference plot (C).

## Discussions

The identity of plant species is essential for medicinal plant manufacturer companies to eliminate the fraudulent of counterfeit raw materials in medical plant product markets. According to the sequence comparison of *B. monnieri*, *B. caroliniana* and *B. floribunda*, 5 polymorphic sites and 21 indels were found. The alteration and the number of nucleotide changes were influenced the shifting of the  $T_m$ . The suitable barcoding regions which chosen to conjugate with HRM analysis should consisted of different GC content among their sequences in order that the significantly different of  $T_m$  for species identification. It was turned out that *trnL-F* region was suitable for plants in the genus *Bacopa*. In previous reports, DNA barcoding based on *trnL-F* regions couple with the HRM analysis were successfully identify the edible plants such as bean crops in family Leguminosae (Madesis et al., 2012), fruit juice (Faria et al., 2013), and *Phyllanthus* species (Buddhachat et al., 2015).

## References

- Russo, A., & Borrelli, F. (2005). *Bacopa monnieri*, a reputed nootropic plant: an overview. *Phytomedicine*, 12(4), 305-317.
- Yadav, A., Ahmad, J., Chaudhary, A. A., & Ahmad, A. (2012). Development of Sequence Characterized Amplified Region (SCAR) marker for the authentication of *Bacopa monnieri* (L.) Wettst. *European journal of medicinal plants*, 2(3), 186.
- Madesis, P., Ganopoulos, I., Anagnostis, A., & Tsafaris, A. (2012). The application of Bar-HRM (Barcode DNA-High Resolution Melting) analysis for authenticity testing and quantitative detection of bean crops (Leguminosae) without prior DNA purification. *Food Control*, 25(2), 576-582.
- Faria, M. A., Magalhães, A., Nunes, M. E., & Oliveira, M. B. P. P. (2013). High resolution melting of *trnL* amplicons in fruit juices authentication. *Food Control*, 33(1), 136-141.
- Buddhachat, K., Osathanunkul, M., Madesis, P., Chomdej, S., & Ongchai, S. (2015). Authenticity analyses of *Phyllanthus amarus* using barcoding coupled with HRM analysis to control its quality for medicinal plant product. *Gene*, 573(1), 84-90.

### Authentication of five commercial products labeled as *B. monnieri*

Five commercial products labeled as *B. monnieri* in dosage forms of powders, capsules and tablets were collected from local markets and drugstores in Thailand. Barcoding-HRM analysis of *trnL* locus developed in this study was applied for identification of the exact plant species. Barcoding-HRM analysis of five commercial products showed that their  $T_m$  was 74.6-74.8°C which agreed with  $T_m$  of *B. monnieri* (Fig. 2). These demonstrated that all commercial products in this study were genuine *B. monnieri*.

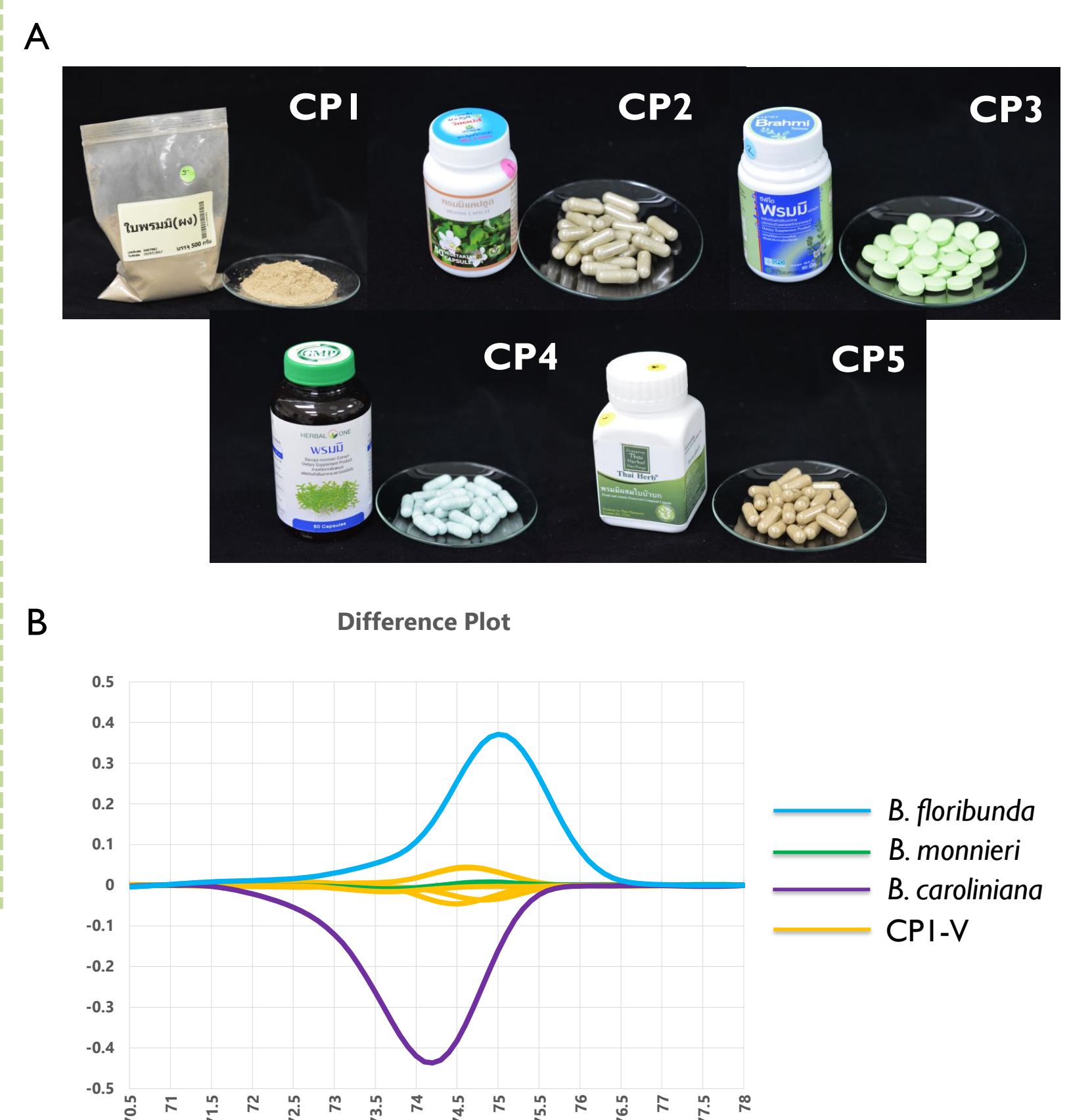


Figure 2

Five commercial products distributed in markets and drugstores in Thailand (A). HRM analysis of *trnL-F* DNA region to detect *B. monnieri* in commercial products (B).

## Conclusions

This is the first study that established the six core DNA barcode of plants in the genus *Bacopa*. We are successfully discriminate *Bacopa* spp. using *trnL-F* loci coupled with HRM analysis. All investigated commercial product was genuine species. Barcoding-HRM analysis should be widespread developed for other medicinal plants for guarantee the identity of the species.

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