

Genome-based authentication of black cohosh (*Cimicifuga racemosa*; Ranunculaceae) supplements available in the Japanese markets

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Abstract

Black cohosh is one of the most popular herbal medicines which is produced from the roots and rhizomes of *Cimicifuga racemosa* (Ranunculaceae). Investigations so far have found that some of the black cohosh products were adulterated with other related *Cimicifuga* species, thus the accurate and convenient technique for the identification of the botanical sources is required. In the present study, we have developed a DNA analytical method to discriminate *C. racemosa* from six related species by an Amplification Refractory Mutation System (ARMS) analysis. Two kinds of species-specific sense primers were designed on the basis of the nucleotide substitution at position 61 on the *trnL* gene among the seven species, and the presence of 284 bp fragments was detected upon PCR amplification. The resultant fragments were species-specific when this method was applied for the referential plant samples. Commercial black cohosh products were then tested in the same way and the result indicated that three of the eight products were not derived from *C. racemosa*. Moreover, TLC and HPLC analyses were performed for marker compounds in sixteen commercial products to determine the reliability of the ARMS analysis. These metabolic analyses completely followed the results of the ARMS analysis and strongly suggested its usefulness.

Keywords : black cohosh, *Cimicifuga racemosa*, species identification, polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) analysis, amplification refractory mutation system (ARMS) analysis

I Introduction

Recently, herbal medicinal products available as non-prescription drugs and/or dietary supplements have gained a significant importance in the role in self-medication all over the world. Besides the increased demand of herbal medicinal products, substitution or adulteration is not rare occurrences, and unfortunately, can have tragic consequences^{1, 2)}. Therefore, unequivocal identification of the botanical sources of herbal products is an elementary and critical step for their assurance of quality, efficacy and safety of herbal products, and effective methods are needed³⁻⁵⁾. We have investigated the main constituents and botanical sources of the crude

drugs and medicinal products available in the Japanese market to establish effective methods for authentication of their origin⁶⁻⁹⁾. Most recently, the first of the herbal medicines which are available in Europe has been approved as a direct over the counter (OTC) drug by the Ministry of Health, Labour and Welfare of Japan (MHLW), and more attention has focused on the verification method for Western herbal medicines.

Black cohosh (*Cimicifuga racemosa* (L.) Nutt.) is a medicinal plant and its roots and rhizomes have been traditionally used for the treatment of menopausal symptoms and menstrual dysfunction in Europe, and the manufactured drugs and supplements of black cohosh are recently one of the biggest-

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