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Identification of Stemonae Radix based on sequences of four cpDNA regions

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Purpose *Stemona sessilifolia* (Ss), *S. japonica* (Sj) and *S. tuberose* (St) are the sources of Stemonae Radix in the Chinese Pharmacopoeia (CP), which is used for antitussive and insecticidal treatment. We determined the nucleotide sequences of chloroplast *trn*L-F, *trn*H-*psb*A, *pet*B-*pet*D and *trn*K-*rps*16 regions of the 3 CP species and *S. parviflora* (Sp) and further developed a rapid PCR-RFLP method for accurate identification of them.

Materials and methods

37 plant samples (Ss 6, Sj 7, St 22, Sp 2) and 11 crude drug samples collected from various locations in China were examined. By using genomic DNA extracted from dry leaf or root of each sample as template, the 4 cpDNA regions were amplified by polymerase chain reaction and then the nucleotide sequence was directly determined and compared.

Results

The size of determined sequences was 928-961bp in *trn*L-F region, 988-1016bp in *trn*H-*psb*A region, 953-979bp in *pet*B-*pet*D region and 601-605bp in *trn*K-*rps*16 region. *Pet*B-*pet*D and *trn*K-*rps*16 regions, showing relatively high substitution rate, were more informative than *trn*L-F and *trn*H-*psb*A regions. Species-specific sequences enabled the 3 CP species to be clearly distinguished from each other. Intra-species variations were observed in the 3 CP species. Especially, in *S. tuberosa*, 2 types of *pet*B-*pet*D sequences and 4 types each of the other 3 regions resulted in 6 haplotypes. *S. parviflora* was genetically far from the 3 CP species. Moreover, PCR-RFLP methods based on *trn*L-F and *pet*B-*pet*D sequences were developed, enabling a rapid identification of Stemonae Radix derived from each *Stemona* species and from its adulterant, *Asparagus* spp.