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Development of DNA-based Species-specific Real-time PCR Markers for Discrimination Between *Hemerocallis fulva* and *Veratrum maackii var. japonicum*, and Their Application in Commercial Food Products and Digested Samples by Artificial Gastric Juice

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[Introduction]

Some toxic plants are morphologically similar to edible plants, and poisoning is often caused by accidental ingestion. If edible plants are contaminated by toxic plants when collected, the two plants are assumed to be mixed and eaten together. In fact, due to a similar appearance between the two species, 42 patients have occurred in the past five years. The *Veratrum maackii var. japonicum* is the most common causative plant of poisoning. According to a published paper, *V.maackii* compounds could cause DNA damage in the cerebellum and cerebral cortex of mice. Veratrum alkaloids in *Veratrum maackii* may cause significant gastrointestinal symptoms, bradycardia, hypotension, and arrythmia. Therefore, we needed to develop a discrimination method that distinguishes between *H. fulva*.and *V. maackii.*

[Materials and Methods]

Both crushed leaves of *H. fulva* and *V. maackii* were provided from the Korean Wild Plants Association. A total of 2 commercial food products used in this study were purchased from local markets. 50mg of crushed samples were digested by artificial gastric juice, respectively. Genomic DNAs were extracted from crushed leaves, commercial foods, and digested crushed leaves using CTAB based DNA extraction method. We used chloroplast genes such as *ndhA*, *rpoB*, and *clpP* to developing species-specific primers.

[Results and Discussion]

The efficiency of each primer set was within 90-110%. A linear correlation (R^2>0.99) were obtained between the crossing point values and long DNA concentration. We determined the Ct value of 10pg of the target species as the cut-off line, and the Ct value of all non-target species amplified lather than this cut-off line. Then we evaluated the practicality of the species-specific markers using 2 commercial *H. fulva* food products and digested samples. As a result of the *H. fulva* food products test, all the species-specific markers detected only the target species. In the case of digestion by artificial gastric juice test, we digested crushed samples (0h, 10min, 1h, 2h, 3h, and 4h). All the species-specific markers were able to detect target DNA in digested samples at least within 4h. Considering that most foods are digested within 4h in the human stomach, we thought the results were practical enough. Therefore, we expect that the species-specific markers in this study will be useful tools for distinguish between *H. fulva* and *V. maackii.*

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