ABSTRACT

Xihuangcao is a folk medicinal herb used in southern China with three botanical origins: *Isodon lophanthoides* (IL), *I. lophanthoides* var. *graciliflorus* (ILG) and *I. serra* (IS). They are often used indiscriminately, numerous commercially available herbal products list *Xihuangcao* as an ingredient without listing the source. This situation has led to a growing concern about the differentiation and quality evaluation of *Xihuangcao*. To address this concern, a systematic study was conducted to identify the origin.

The study is divided into five parts, which aimed to establish and apply the authentication methods of the origins. Four *Isodon* species were recorded in research papers as the plant sources. However, a new classification suggested in 2004 and two of the IL varieties were merged. In the ancient herbal documents, ILG was first recorded as the origin plant. IL was the major species in the ancient texts, IS was only listed as an additional sources in recent herbal references. The “yellow juices” which proven to be the exudates of glandular scales was the key identification features recorded.

Macroscopic and microscopic studies provided identification features of the three *Isodon* species. IL and ILG share very similar features, but IS can be easily distinguished. By morphological features, IL and ILG can be distinguished by the shape of leaves, which IL has a broader leaves than ILG; IS can be identified by its very bitter taste and broadly winged petioles. By microscopic features, IL and ILG have a tiny difference in the shape of epidermal cells of leaf, and IS can be recognized by small raphides of calcium oxalate.

In the UPLC-MS fingerprinting and tissue-specific profiling, the chemical profiles the three species were revealed. The chemical profiles of IL and ILG were similar, while IS has its specific chemical profiles. Twenty-seven characteristic peaks were chosen and showed a good distinction of the three species. The tissue-specific profiling of leaves showed the diterpenoids of all the species were accumulated only in the glandular scales.

Lipidomics study on IL, ILG and IS was also conducted. A total of 92 lipids were identified. The variation of lipid profiles of the three *Isodon* species
was further quantified, the results showed that the contents of the lipids in the three *Isodon* species varied. Statistical analyses showed IS has distinctly different lipid profile, while that of IL and ILG are very similar.

Finally, the methods of macroscopic microscopic authentication and UPLC-MS fingerprinting were applied in identifying the source species of commercial *Xihuangcao* products. Twenty-seven batches of *Xihuangcao* decoction pieces were identified, results showed ILG is the major source of the collected samples. The ingredients in eight *Xihuangcao* herbal tea bags were also identified. IS is the major species, and none of the samples match their labels.

The study provided valuable information on the authentication and quality control of folk medicinal herb *Xihuangcao*. The work also provided fundamental information on further studies on the chemical constituents of IL and ILG, also and role of lipids in the production of bioactive diterpenoids in *Isodon* species.
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