

DOCTORAL THESIS

Comparative study on the chemical constituents and bioactivity between radix astragali and radix hedysari

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Comparative Study on the Chemical Constituents and Bioactivity
between
Radix Astragali and Radix Hedysari

LIU Jing

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for the degree of

Doctor of Philosophy

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ABSTRACT

Radix Astragali (RA), known as “Huangqi” in China, is one of the most popular herbal medicines known worldwide to reinforce “Qi”. RA is traditionally prepared from the dried roots of *Astragalus membranaceus* (MJHQ) and *A. membranaceus* var. *mongholicus* (MGHQ). Radix Hedysari (RH) is named “Hongqi”, which is a similarity of RA. Both herbs have been shown to have cardioprotective, hepatoprotective, hypotensive, immunostimulant, anti-aging, anti-oxidant, anti-diabetic, and anti-inflammatory activities. The bioactive compounds were found to be flavonoids, saponins, polysaccharides, amino acids and some trace elements. In this project, we would focus on the investigation of comparison on chemical constituents and bioactivity between “Huangqi” and “Hongqi” in order to provide the scientific data for the clinical application.

Firstly, comparison study on chemical constituents and immunological effect had been studied. Different constituents in MGHQ, MJHQ and RH were extracted and were analyzed using different methods. Comparison of the immunological effects of extracts was done by using two immunological models. Results showed that flavonoids and saponins present in RA and RH were not only structurally significantly different but also different in their immunological effect. Amino acids extract (AE) in MGHQ shows immunological effect while AE in MJHQ and RH did not. Polysaccharides were the major constituents in MGHQ, MJHQ and RH. The contents of polysaccharides in RH were almost double as those in MGHQ and MJHQ, but were similar in MGHQ and MJHQ. All polysaccharides extracts showed similar levels of immunological effect in both immunological assays.

Secondly, we assessed and compared chemical composition and estrogenic activity of polysaccharides extracted from MGHQ, MJHQ and RH which were proved to be main bioactive compounds. Polysaccharides were isolated by water extraction-alcohol precipitation, hydrolyzed by trifluoroacetic acid into monosaccharides, and then labeled with 1-phenyl-3-methyl-5-pyrazolone (PMP). The labeled monosaccharides derivatives were separated and detected by HPLC. The ratios of arabinose, galacturonic acid, galactose, glucose, mannose and rhamnose in the MGAPS, MJAPS and HPS were, respectively, 2.72: 7.65: 5.88: 206.64: 1: 5.49, 1.54: 9: 6.7: 311.43: 1: 9.21 and 2.01: 6.8:

3.31: 102.6: 1: 3.05. We evaluated the estrogenic potential of the three polysaccharides with estrogen promoter assay in MCF-7 cells by using transient cotransfection. HPS showed higher estrogenic effect than MGAPS and MJAPS while MGAPS and MJAPS had a similar effect.

From our herbal markets, we found that there were many crude drugs of RA, representing both varieties and coming from different geographical regions whereas the source of RH was very limited. Due to complicated sources of RA, quality evaluation and geo-authentic study of RA were carried on. Seeds of 30 RA samples representing the two varieties, from different regions, were cultivated in the geo-authentic producing area under the same conditions. A method, using ultra-performance liquid chromatography coupled with PDA and evaporative light scattering detectors (UPLC-UV-ELSD), was developed to evaluate the quality of these RA samples through a simultaneous determination of four major isoflavonoids and four major saponins. The two RA varieties were successfully distinguished by principal component analysis while samples of the same species with different seeds origins could not be distinguished. A genetic study demonstrated that the internal transcribed spacer sequences of the nuclear ribosomal DNA in MGHQ samples from different geographical regions were highly conservative. These results indicate that the content of the active components in RA depends on the interaction of genotype and environment. At the varietal level, the genetic properties appear to be more important for pharmaceutical quality than environmental factors, while on the intraspecific level environmental factors might be more important than the genetic properties.

In conclusion, this systematic study provides the scientific data for comparison on the chemical constituents and bioactivity between RA and RH. Findings of this work indicate that RH could be used instead of RA in the immunoregulatory function aspect for the clinical application and RH might be more effective in estrogenic activity. Moreover, the geo-authentic study of RA reveals that the local environments such as weather, geographic location, soil conditions, methods of cultivation and processing play a valuable role in the quality of TCMs.

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