Flavonoids and Phenol Carboxylic Acids in the Oriental Medicinal Plant *Astragalus membranaceus* Acclimated in Poland

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Astragalus membranaceus (Fisch.) Bunge has been was successfully acclimated in Central Europe. We report the content of isoflavones and some other polyphenolic compounds in roots and aerial parts that have been analyzed by means of TLC and HPLC.

The total amount of isoflavones in leaves, was 0.55 mg g^{-1} dry weight, and of the flavonols – up to 3.54 mg g^{-1}. In the roots isoflavonoid content was extremely variable, but reached 3.04 mg g^{-1}, whereas flavonols content was 0.49 mg g^{-1}.

Key words: Astragalus membranaceus, Flavonoids

Introduction

Astragalus membranaceus Bunge, a perennial belonging to the Fabaceae family delivers an important herbal drug in Traditional Chinese Medicine. The root (Astragali Radix – Huang Qi), has been applied in a variety of diseases and is recommended for general health improvement. It becomes increasingly popular in modern phytotherapy as well as in alternative medicine. Additionally, the growing interest in herbal products in environmentally oriented populations has led to increasing consumption of active plant ingredients in form of functional foods. Astragali Radix has been shown to have following activities: immunostimulation of respiratory system, hence the effective prevention of cold, stimulation of phagocytosis in leukocytes, hepatoprotective, hypotensive and vasodilatating (Zhang *et al.*, 1990). The extract of this root has also insecticidal properties with potential application in forestry (Park *et al.*, 1997). Among constituents that contribute to its biological activity are steroid saponin glycosides (astragalosides), isoflavonoids as well as other polyphenolic compounds (flavonols, phenolic acids), essential oils and polysaccharides (Cheshuina, 1990; He and Findlay, 1991; Ganzera *et al.*, 2000; Ma *et al.*, 2002). Isoflavonoids, detected in *A. membranaceus* possess antioxidant and antimutagenic properties (Wong *et al.*, 1992; Hong *et al.*, 1994; Shirataki *et al.*, 1998).

Native in Northern and Central China, Huang Qi has been successfully acclimated and introduced to herbal horticulture in moderate regions in Europe like Germany and Poland. The quality evaluation of the crude drug harvested from acclimated plants is therefore of great importance. No data are available on potential use of the aerial part of the plant as a source of isoflavonoids.

The aim of presented study was:

1. to assess the quality of the Huang Qi roots harvested in Poland with respect to their isoflavonoid content and composition;
2. to test the feasibility of routine chromatographic methods for determination of isoflavonoids in the drug;
3. to compare the flavonoid fraction from roots and epigeous part of this species.

Instrumentation and Experimental Procedures

Thin layer chromatography (TLC)

Methanol extracts from air-dried and ground roots and herbs (100 g of each) were obtained by overnight extraction in 80 °C with 500 ml of analytical grade Me-OH. The extracts were concentrated in vacuo, redissolved in methanol and separated with ethyl acetate (EA). EA fraction was further separated by column chromatography on silica gel. The fractions obtained were analyzed by TLC performed in Chromdes D-9, saturated horizontal chambers (Chromdes, Lublin, Poland) on Merck Silica Gel 60 glass plates using 8 different eluents. The chromatograms were observed in UV/VIS before and after processing with 5% $AlCl_3$ spray or incubation in NH_3 vapor. The flavonoids and phenolic acids were identified by comparison to co-chromatographed standards and available literature data (Mabry *et al.*, 1970).

High performance liquid chromatography (HPLC)

Plant material was homogenized with 80% methanol in 80 °C, sonicated for 30 min, and centrifuged at 14,000 rpm followed by filtration. The reversed-phase HPLC was performed by Merck LiChroCart 125–3 with Purospher RP-18 column (5 mm) using Ll-7100 pump controlled by Multisolvent Delivery System F-7000 HSM and Merck-Hitachi Diode Array Detector L-7455 (Merck KGaA, Darmstadt, Germany). Gradient elution was carried out with A – acetonitrile 80%, water 15.5%, formic acid 4.5% and B – water 95.5%, formic acid 4.5%. Flow rate was 1 ml min^{-1}. Detection wavelength was either 260 nm or 360 nm.

Results and Discussion

Roots

Following polyphenolic compounds have been detected in Astragali Radix by means of TLC:

Formononetin (an isoflavone), quercetin, rutin, phenolic acids: chlorogenic, caffeic, ferulic, *p*-coumaric;

and by HPLC, isoflavonoids: calycosin glucosides (7-O-β-D-glucoside (**1**), and 7-O-β-D-glucoside 6″-O-malonate (**2**), ononin (**3**), 3-hydroxy-9,10-dimethoxypterocarpan-3-O-β-D-glucoside (**4**), calycon (**5**), 7,2′-dihydroxy-3′,4′-dimethoxyisoflavan-7-O-β-D-glucoside (**6**), formononetin-7-O-β-D-glucoside-6″-O-malonate (**7**), formononetin (**8**); and flavonols: quercetin and kaempferol.

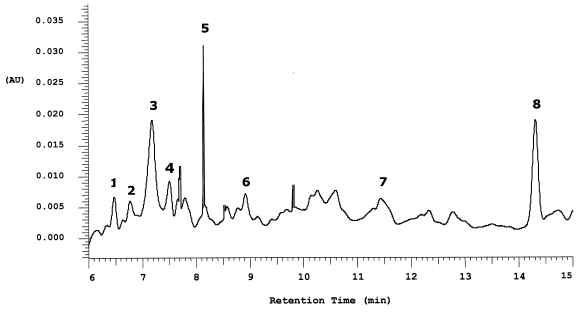

Fig. 1. The HPLC chromatogram of Astragali radix extract. The detection wavelength was 260 nm. The peaks were identified as: calycosin glucosides (7-O-β-D-glucoside (**1**), and 7-O-β-D-glucoside 6″-O-malonate (**2**), ononin (**3**), 3-hydroxy-9,10-dimethoxypterocarpan-3-O-β-D-glucoside (**4**), calycon (**5**), 7,2′-dihydroxy-3′,4′-dimethoxyisoflavan-7-O-β-D-glucoside (**6**), formononetin-7-O-β-D-glucoside-6″-O-malonate (**7**), formononetin (**8**).

Aerial parts

Following compounds have been identified in Astragali Herba by TLC and HPLC:

formononetin, other flavonoids (flavonols and glycosides): quercetin, rutin, quercitrin, isorhamnetin, kaempferol, luteolin; phenolic acids: chlorogenic, caffeic, ferulic, *p*-coumaric.

The maximal amount of isoflavonoid constituents determined by HPLC in the plant was: 3.04 mg g^{-1} and 0.55 mg g^{-1}, for roots and herb respectively. The actual abundance of individual compounds was very variable. In aerial part, the flavonols were the predominating group reaching 3.54 mg g^{-1} dry weight, whereas in roots they reached only 0.49 mg g^{-1}. These results are consistent with our preliminary study (Matkowski *et al.*, 2002).

Dependence on imported material is disadvantageous due to often poor quality of imported herbs. A contamination with heavy metals has been reported in Chinese herbal drugs (Gertner *et al.*, 1995; Ko, 1998) and the botanical uniformity of plant material has been unsatisfactory (Cheng-Kur *et al.*, 2000). A support for domestic plantations of oriental medicinal herbs shall therefore be recommended. However, the prerequisite for this is that the quality of drugs from cultivated plants will meet all requirements with respect to the composition of bioactive substances. The isoflavonoid compounds contribute to the active principles of this drug, and have been chosen as "marker compounds" for standarization of Astragali radix (Lin *et al.*, 2000).

For determination of phytochemicals in Huang Qi, chromatographic methods have been applied including silica gel column chromatography (Toda and Shirataki, 1998), advanced HPLC (Xiao *et al.*, 2001) and coupled systems as HPLC-EIMS (Lin *et al.*, 2000) Isoflavonoid compounds that have been previously identified in roots of *A. membranaceus* (Lin *et al.*, 2000; Ma *et al.*, 2002) include formononetin, other aglycone calycosin, and several glycosides, with typical for this genus malonate glucosides, afromormosin, odoratin. These latter two were not identified in our study, probably due to the relative simplicity and insufficient sensitivity of applied techniques so that some non-abundant compounds might have been omitted resulting in underestimation of total isoflavonoid content as well. Nevertheless, the procedures de-

scribed in this paper, have been also applied for isoflavonoid analysis in another leguminous Chinese medicinal plant *Pueraria lobata* (Matkowski *et al.*, 2003). The phenolic acids in roots may also contribute to the therapeutic activity.

Aerial parts of *A. membranaceus* have sporadically been subjected to phytochemical analysis. One report indicates the presence of flavonol aglycones, quercetin, isorhamnetin and kaempferol in Astragali Herba (Cheshuina, 1990). This was confirmed by the results of present research, where the flavonols were the prevailing group of flavonoids. Other flavonols and phenolic acids have been also found that had not been previously reported.

Concluding, while the green aerial part of the *A. membranaceus* plant contains a detectable amount of isoflavones, the root remains the only reasonable source of these active compounds, not to be substituted by the leaves. Even though for detailed phytochemical examination and complete determination of every isoflavonoid contained in Astragali radix, more advanced techniques ought to be applied, the present results have confirmed an adequate suitability of applied here chromatographic methods for quality evaluation of this crude drug and suggest that plants cultivated in European climate can be used for phytotherapeutic purposes.

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