Department of Pharmaceutical Biology and Botany[1], Wrocław University of Medicine, Dept. Fruit, Vegetable & Herbs Processing[2], University of Agriculture in Wrocław, Poland

Flavonoids of *Puerariae lobata*: chromatographic analysis of leaves and roots of cultivated plants

A. Matkowski[1] , D. Woźniak [1], J. Oszmiański[2], E. Lamer-Zarawska[1]

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Dr. Adam Matkowski, Department of Pharmaceutical Biology and Botany, Al. Jana Kochanowskiego 10, PL-51-601 Wrocław, Poland
bbsekret@biol.am.wroc.pl

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Pueraria lobata (Wild.) Ohwi (Fabaceae), – kudzu, has great potential in phytomedicine. *Puerariae radix* (kudzu root) has been used for centuries in Japanese and Chinese medicines. A remarkable feature is its ability to reduce alcohol intake in addicted individuals [1, 2]. Isoflavonoids, daidzein and its glucoside daidzin are the putative anti-alcoholic agents, whereas puerarin and other C-glycosides act in cardiovascular protection. Also the phytoestrogenic activity can be attributed to isoflavones [3]. Flowers of kudzu have been used in Chinese medicine [4], but not other epigeous parts. However, isoflavones can be induced in stems of *P. lobata* [5] and in tissue culture [4, 6]. Kudzu is a vigorous vine, capable to colonize quickly vast areas of land. It has become an aggressive pest weed in the Southwest of North America [2]. Hence, further introduction in warm climate areas should be restrained. Yet, there is growing demand on drugs from this species but availability and sometimes the quality of Chinese drugs can be poor [7]. This led us to attempt the introduction of this species to moderate climate in Central Europe (Germany and Poland).

The aim of present study was to assess the quality of the kudzu roots harvested in Poland with respect to their isoflavonoids content and composition, to compare the flavonoids from roots and leaves of this species and finally to test the feasibility of simple TLC methods for the quality monitoring and standardization of the crude drug as compared to the HPLC.

By means of TLC, we detected the presence of flavonoids and isoflavonoids in both investigated crude drugs (*Puerariae radix* and *Puerariae folium*). 12 spots have been observed after processing with ammonia fumes and 5 isoflavonoids have been identified as formononetin (R_F in eluent 2. – 0.90), genistein (0.80), daidzein (0.77), daidzin (0.40) and puerarin (0.48). HPLC analysis also revealed these five compounds. In the micropropagated plantlet, the considerable amount of 3′-methoxypuerarin was detected, but no formononetin. Other compounds of potential therapeutic activity were also present such as flavonoid aglycones and glycosides: apigenin, luteolin, quercetin, rutin, hyperoside and quercitrin, as well as phenolic acids: caffeic, chlorogenic, *p*-coumaric, ferulic acid. In roots, the total amount of isoflavones was about 30-fold higher than in the leaves, where the flavonols were a prevailing group.

Cultivated plants should become a major source of crude drugs for domestic markets, but the ecological threats related to introduction of invasive species, have to be considered. Lack of quality control in the country of origin may result in unreliable condition of imported material [7]. A support for domestic plantations of oriental medicinal herbs shall therefore be recommended. The prerequisite is that the quality of drugs from acclimated plants will meet all requirements with respect to the composition of bioactive substances. In recent studies on chemistry of *P. lobata* and related species, the HPLC analysis was usually employed, with spectroscopic identification techniques [3–5, 8–10] or Capillary Electrophoresis with electrochemical detection [11]. CC and TLC had been applied for isolation of isoflavonoids by Kubo and Fujita [12]. The methods used in this study, had been also applied for analysis of tissue cultures of kudzu and for flavonoid analysis of another Chinese herb – Huang Qi (*Astragalus membranaceus*) [6].

Our results indicate that TLC can be adequate for overall screening of the quality of crude drugs harvested from cultivated plant. It may offer an affordable alternative to routine advanced methods, if the extreme accuracy is not required. The presence of 12 putative isoflavonoid spots is consistent with HPLC study and with literature data, where about 15 isoflavonoid compounds were identified [3, 11, 13]. Present results suggest the good quality of the crude drug from acclimated plant, but require further research to identify remaining compounds. Nonetheless, the total amount of isoflavones while confirming the excellent value of *Puerariae radix*, also implies that the leaves can only be an additional but not an alternative source.

Table: Quantitative analysis by HPLC of flavonoid compounds in different plant material from *Pueraria lobata*

Compounds	Organs	mg/100 g freshweight
Total isoflavonoids	Roots	927.90
	Leaves	3.28
	In vitro plantlet	47.66
Puerarin	Roots	522.30
	In vitro plantlet	9.11
Daidzin	Roots	178.00
	In vitro plantlet	15.06
3′-Methoxypuerarin	*In vitro* plantlet	23.49
Total flavonols	Leaves	204.70
	In vitro plantlet	13.03

Experimental

Roots and leaves were harvested from *P. lobata* grown in Medicinal Plant Garden at the Medical University in Wrocław, Poland. The *in vitro* propagated plantlets (gift from Dr. B. Thiem, Med. University, Poznań, Poland) were used without separating roots and leaves.

Thin Layer Chromatography: Methanol extracts obtained by extraction of material dried at 80 °C were concentrated *in vacuo*, redissolved in methanol and separated with ethyl acetate (EA). EA fraction was used for further analysis. For establishing optimal elution and separation parameters the extracts were chromatographed on Merck Silica Gel 60 glass plates in horizontal chambers (Chromdes Lublin, Poland). The solvents tested were: 1. $CHCl_3$: methanol – 9 : 1, 2. EA : formic acid : water – 8 : 1 : 1, 3. n-hexane : EA – 2 : 1. The analysis for the presence of isoflavonoids was performed using Merck HP TLC Si60 F254s and HP TLC RP-18 WF254s plates. The optimal solvent gradient system was n-hexane: acetone – 10 : 1 through 2 : 10, and system 2. for isocratic elution. The spots were identified by comparison of retention factors (R_F) to authentic standards and observation in UV/VIS before and after processing with $AlCl_3$ spray or ammonia fumes, referring to literature data [13].

Analysis by HPLC was performed in Merck LiChroCart 125-3 with Purospher RP-18 column (5 mm), diode array detector Merck-Hitachi L-7455 was set to 260 nm for isoflavones or 360 nm for flavonols. Gradient elu-

tion 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}.

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