AcademicPres

Available online at www.notulaebotanicae.ro

Print ISSN 0255-965X; Electronic 1842-4309

Not. Bot. Hort. Agrobot. Cluj 38 (1) 2010, 57-60

Notulae Botanicae Horti Agrobotanici Cluj-Napoca

Phytochemical Analysis of Isoflavons from some *Fabaceae* Species Extracts

Daniela HANGANU[1)], Laurian VLASE[2)], Neli OLAH[3)]

[1)] *"Iuliu Hatieganu" University of Medicine and Pharmacy, Faculty of Pharmacy, Department of Pharmacognosy-Phytotherapy, 12 I. Creanga Str., Cluj-Napoca, Romania; handa_1964@yahoo.com*

[2)] *"Iuliu Hatieganu" University of Medicine and Pharmacy, Faculty of Pharmacy, Department of Pharmaceutical Technology and Biopharmaceutics, 12 I. Creanga Str., Cluj-Napoca, Romania*

[3)] *"Vasile Goldis" West University, Faculty of Pharmacy, Department of Drug Industry and Pharmaceutical Biotechnology, Arad and SC Plant Extrakt SRL, Radaia, Cluj, Romania*

Abstract

Phytoestrogens are natural compounds synthesized almost exclusively by plants of the *Fabaceae* family. To find new sources of phytoestrogens, we analyzed the isoflavons from different extracts obtained from plants of the Romanian spontaneous flora belonging to the *Fabaceae* family: *Genistella sagittalis*, *Genista tinctoria*, *Cytisus albus*, *Coronilla varia*, *Lotus cornyculatus* and *Dorycnium herbaceum*. The qualitative and quantitative analysis were performed by liquid chromatography coupled with mass spectrometry. The hydroalcoholic extracts were obtained by sonication for 10 minutes at 60°C. They were analyzed before and after hydrolysis. Some of the studied extracts presented a decreased level of isoflavons: *Cytisus albus, Coronilla varia, Lotus cornyculatus* and *Dorycnium herbaceum*. Other extracts were found to be rich in isoflavons. The high quantities of heterosides (daidzin, genistin, ononin) and aglycons (daidzein, genistein and formononetin) were found in *Genistella sagittalis* and *Genista tinctoria*. The results obtained for hydrolyzed extracts are inconclusive.

Keywords: isoflavons, isoflavon heterosides, LC/MS analysis

Introduction

Isoflavons are a flavanic subgroup found almost exclusively in plants of the *Fabaceae* family. They have exceptionally interesing, multidirectional therapeutic properties. In addition to antiinflammatory, antimycotic and radical scavenging properties, they also exhibit both estrogenic and antiestrogenic effects, being phytoestrogenes. Daidzein, genistein, formononetin and their 7-O-glucosides, daidzin, genistin, ononin are natural agonists or antagonists of estrogenic receptors E_2 and they have been associated with a decreased risk of hormone dependent cancers, arthritis and neurodegenerative diseases. They also diminish the risk of diseases that appear due to the lack of hormones from menopause such as osteoporosis and cardiovascular troubles. Isoflavons may be important antioxidant agents, since they exhibit hydroxyl groups in rings A and/or B and are thus, capable of donating hydrogen to free radicals (Franke and Custer, 1994; Setchell, 1998; Bruneton, 1999; Dragomirescu *et al*., 2003; Luczkiewicz and Glod, 2003).

In the Romanian spontaneous flora there is a series of fabacee which could become significant sources of isoflavons, some of them being worthy to be transferred into culture, due to their increased isoflavon content. As a result some species of this family were included in the study: *Genistella sagittalis, Genista tinctoria, Cytisus albus, Coronilla varia, Lotus cornyculatus* and *Dorycnium herbaceum*. These were analysed for the first time in this work, except for *Genista tinctoria*.

The purpose of this study was the identification and quantitative determination of the isoflavons from these species by LC/MS in order to find new isoflavon sources.

Materials and methods

Vegetal material represented by the aerial parts (*herba*) obtained from *Genistella sagittalis, Genista tinctoria, Cytisus albus, Coronilla varia, Lotus cornyculatus* and *Dorycnium herbaceum* harvested during the blooming stage from the Cluj county (valea Draganului, Romania) spontaneous flora, in June-July 2008 were analyzed. After the natural drying process performed in the shadow, the blooming aerial parts of the above mentioned plants were powdered.

Each extract was obtained from 1 g plant material in 20 ml of 50% vol. ethanol. Extractions were carried out in an Elmasonic S15H high-intensity ultrasound probe system having 35 W effective power and 37 KHz ultrasonic frequency (Mauricio *et al*., 2003).

In order to study the isoflavon aglycons, a hydrolysis with HCl 2 N, at 70°C for 60 minutes was performed on each extract (Bucur *et al*., 2006; Deheleanu *et al*., 2006; Fodorea *et al*., 2005; Peev *et al*., 2007; Tero-Vescan *et al*., 2009; Vlase *et al*., 2005).

The HPLC analysis was carried out using an Agilent 1100 HPLC system equipped with a degasser, binary

pump, autosampler and column thermostat. For the separation of compounds, a reversed-phase Zorbax SB-C18 analytical column (100 x 3.0 mm i.d., 3.5 μm particles) was used. The column was operated at 48°C. The mobile phase used for the separation of isoflavons was a mixture of acetic acid 0.1% (V/V) in water (A) and methanol (B), in linear gradient mode, as follows: until 2 min, 20% B, at 10 min 40% B, at 10.5 min 40% B, at 11.5 min 45% B and hold 45% B until 17 min. Methanol of HPLC analytical-grade and acetic acid of analytical-grade from Merck (Germany) were both used. The flow rate was 1 ml/min. For detection and quantification, the HPLC system was coupled with an Agilent 1100 Ion Trap SL mass spectrometer, operated with an electrospray (ESI) ion source in negative ion mode. The vaporization gas used by mass spectrometer was nitrogen, at 65 psi; the dry gas was also nitrogen at a flow rate of 12 l/min and heated at 360°C. The capillary potential was set at +2500 V. The analysis mode of isoflavons was either in single ion monitoring mode (SIM) - for aglycons or in single reaction monitoring mode (SRM) - for glycosides (Tab. 1). The following standards were used: daidzein, glycitein, genistein, formononetin, daizin, genistin, ononin from Merck (Germany). Methanolic stock solutions (1 mg/ml) of the above standards were prepared and stored at 4°C, protected from daylight. They were appropriately diluted with double distilled water before being used as working solutions. The calibration curves for all isoflavons were built in range of 40-4000 ng/ml. In order to fit the calibration curves, a quadratic model and 1/y weighting scheme were used. The accuracy of the calibration points, for each compound, was no more than ±15%.

Tab. 1. The retention time of isoflavons and their mass spectrometry detection parameters

Compound	Retention time (min)	Detection mode	Parent m/z ion [M-H]-	Quantified m/z ion
Daidzin	3.7	SRM	415	253
Genistin	5.5	SRM	431	268, 269
Ononin	8.9	SRM	429	267
Daidzein	9.2	SIM	253	253
Glycitein	10.2	SIM	283	283
Genistein	11.0	SIM	269	269
Formononetin	14.4	SIM	267	267

Results and discussion

The retention time, detection mode, parent ion's and quantified ion's *m/z* for identified isoflavon heterosides and isoflavon aglycons are shown in Tab. 1.

A typical chromatogram of a standard mixture of isoflavons is presented in Fig. 1, while the chromatogram of *Genistella sagittalis* extract is shown in Fig. 2.

The results of the un-hydrolyzed extracts are shown in Tab. 2.

The studied un-hydrolyzed extracts contain isoflavons as heterosides (7-O-glucosides) but also as aglycons. In the *Genista tinctoria* we found a high concentration of hetero-

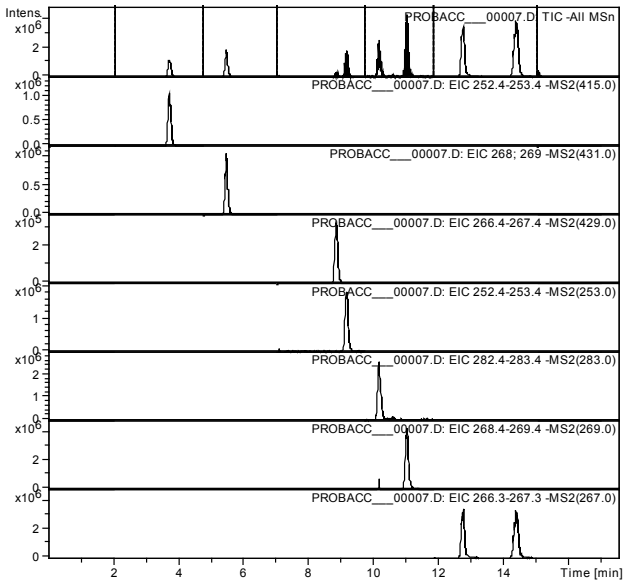

Fig. 1. The chromatogram of a standard mixture, MS detection - chromatogram 1: TIC-all standards, chromatogram 2: daidzin (3.7 min) , chromatogram 3: genistin (5.5 min), chromatogram 4: ononin (8.9 min), chromatogram 5: daidzein (9.2 min), chromatogram 6: glycitein (10.2 min), chromatogram 7: genistein (11.0 min), chromatogram 8: formononetin (14.4 min)

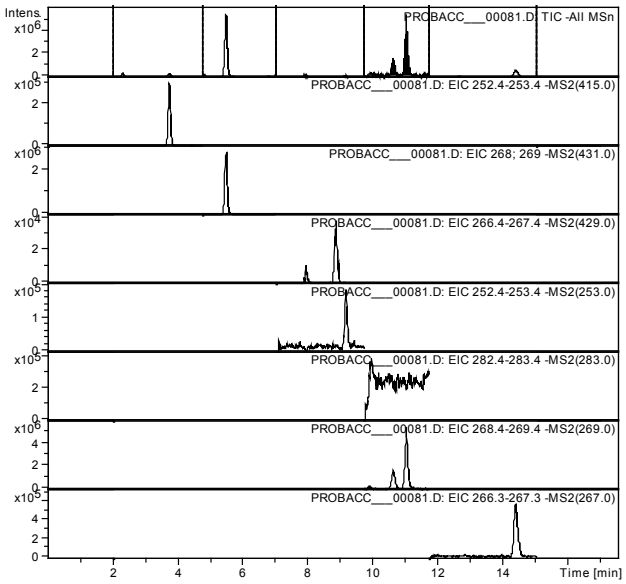

Fig. 2. The chromatogram of *Genistella sagittalis* extract (unhydrolyzed), MS detection-1: TIC, chromatogram 2: daidzin (3.7 min), chromatogram 3: genistin (5.5 min), chromatogram 4: ononin (8.9 min), chromatogram 5: daidzein (9.2 min), chromatogram 6: glycitein (10.2 min), chromatogram 7: genistein (11.0 min), chromatogram 8: formononetin (14.4 min)

sides: daidzin (22.32 µg/ml), genistin (130.17 µg/ml) and ononin (242.70 ng/ml) respectively of aglycons: daidzein (8,81 µg/ml), genistein (73.77 µg/ml) and formononetin (675.70 ng/ml). *Genistella sagittalis* contains heterosides: daidzin (2.89 µg/ml), genistin (51.60 µg/ml)) and ononin (890.20 ng/ml) respectively aglycons: daidzein (0.55 µg/ml), genistein (9.02 µg/ml) and formononetin (839.50 ng/ml). *Coronilla vari* and *Lotus cornyculatus* contain small amounts of genistin (1.10 µg/ml respectively 0.52 µg/ml) and genistein (0.34 µg/ml respectively 0.26 µg/ml) and *Dorycnium herbaceum* contains only genistin (0.52 µg/ml). *Cytisus albus* contains heterosides: daidzin (0.47 µg/ml), genistin (6.65 µg/ml)), ononin (192.60 ng/ml) respectively aglycons: genistein (1.61 µg/ml).

Of all the analysed isoflavons, the most important estrogenic activity was that of the genistein and daidzein, both being considered un-glycosilated pharmacologically active forms of their glycosilated precursors: genistin, daidzin. Daidzein can be synthetised under the action of the intestinal microbial flora, and also from the formononetin by demethylation (Bruneton, 1999; Setchell, 1998). As a result of all the analysed extracts, the ones obtained from *Genistella sagittalis* and *Genista tinctoria* proved to be the most valuable, *Genista tinctoria* having an increased level of genistein and daidzein along with ononin and formononetin. *Genistella sagittalis* is remarked due to a higher content of ononin and formononetin. Supplementary aglycon quantities within the obtained extracts result from genistin and daidzin enzymatic hydrolise (Bruneton, 1999; Setchell, 1998).

Genista tinctoria and *Genistella sagittalis* are the most significant natural sources of phytoestrogene isoflavons from the analysed *Fabaceae* species of the spontaneous flora. These species, valuable from the poin of view of their isoflavon content, widespread in the Romanian spontaneous flora are worthy to be introduced in culture. The comparison between the two species shows that *Genista tinctoria* is richer in daidzin and genistin, respectively in daidzein and genistein. *Genistella sagittalis* is richer in ononin and formononetin.

The higher quantities of isoflavons found in *Genista tinctoria* (in comparison to other studies) may be due to the extraction method. This study shows that sonication is a more efficient extraction method than the classical extraction ones and thus we can extract a higher amount of isoflavons, both heterosides and aglycons (Mauricio *et al*., 2003; Tero-Vescan *et al*., 2009).

In the hydrolyzed extracts both heterosides and free aglycons have been identified (Tab. 3).

The obtained results reflect some incongruities that derived from the hydrolysis conditions which are classic for polyphenols, that led either to an incomlete hydrolyze of heterosides or to the degradation of some structures (dimers, oligomers, addition products of isoflavones with cinnamic acid) and also to the formation of new quantities from the structures already existent in the plants (Bruneton, 1999). Sometimes these conditions can destroy the structures of the aglycon type and as a result, these were found in smaller quantities than in the un-hydrolyzed extracts.

Tab. 2. The content of isoflavon compounds from the un-hydrolyzed extracts

Sample	Daidzin µg/ml	Genistin µg/ml	Ononin ng/ml	Daidzein µg/ml	Genistein µg/ml	Formononetin ng/ml
Genistella sagittalis	2.89	51.60	890.20	0.55	9.02	839.50
Genista tinctoria	22.32	130.17	242.70	8.81	73.77	675.70
Cytisus albus	0.47	6.65	192.60	0.00	1.61	0.00
Coronilla varia	0.00	1.10	0.00	0.00	0.34	0.00
Lotus cornyculatus	0.00	0.75	0.00	0.00	0.26	0.00
Dorycnium herbaceum	0.00	0.52	0.00	0.00	0.00	0.00

Tab. 3. The content of isoflavon compounds from the hydrolyzed extracts

Sample	Daidzin µg/ml	Genistin µg/ml	Ononin µg/ml	Daidzein µg/ml	Genistein µg/ml	Formononetin ng/ml
Genistella sagittalis	2.49	34.40	1.02	0.35	5.25	202.40
Genista tinctoria	23.82	134.25	0.64	5.09	50.23	0.00
Cytisus albus	0.52	7.98	0.00	0.00	0.74	0.00
Coronilla varia	0.00	1.11	0.00	0.00	0.00	0.00
Lotus cornyculatus	0.12	2.13	0.00	0.00	0.46	0.00
Dorycnium herbaceum	0.00	0.49	0.00	0.00	0.00	0.00

Conclusions

By meas of the LC/MS analysis we qualitatively and quantitatively determined isoflavons under the form of heterosides and aglycons from the following *Fabaceae* species belonging to the Romanian spontaneous flora: *Genistella sagittalis, Genista tinctoria, Cytisus albus, Coronilla varia, Lotus cornyculatus* and *Dorycnium herbaceum*.

The results present a great variation of the isoflavon content and composition within the analysed species, some proving to have a decreased isoflavon contend *Cytisus albus*, *Coronilla varia*, *Lotus cornyculatus* and *Dorycnium herbaceum*.

On the other hand, *Genista tinctoria* and *Genistella sagittalis* are an important source of isoflavon. These species, valuable in terms of their isoflavon content, widespread in Romanian spontaneous flora, are worthy to be introduced into culture.

The study indicates that the used extraction method, sonication, is an efficient one.

The following studies can demonstrate their significant therapeutic use.

In the hydrolyzed extracts the obtained results reflect some incongruities that derived from the hydrolysis conditions.

References

Bruneton, J. (1999). Pharmacognosie. Phytochimie. Plantes medicinales, 3eedition, Edition TEC@DOC, Paris pp.347-351.

Bucur, L., L. Vlase, C. Fodorea and V. Istudor (2006). HPLC analysis of the polyphenolic compounds from the extracts obtained from flowers and young branches of *Elaeagnus angustifolia* L. Revista de Chimie 57(2):189-192 (in Romanian).

Dehelean, C. A., L. Vlase, C. Peev, D. Antal and C. Soica (2006). Comparative analysis of polyphenols content from birch tree foliar buds and leaves. Revista de Chimie 57(8):862-865.

Dragomirescu, A., D. Antal and C. Deheleanu (2003). Phytoestrogens. The role of isoflavons. Farmacia 51(6):15-24 (in Romanian).

Fodorea, C. S., L. Vlase, S. E. Leucuta and M. Tămaş (2005). Phytochemical Study on Some Polyphenols of *Geranium pyrenaicum*. Chem. Nat. Compd. 41(4):400-403.

Franke, A. and L. Custer (1994). Quantitation of Phytoestrogens in Legumes by HPLC. J. Food Chem. 42:1905-1913.

Luczkiewicz, M. and D. Glod (2003). Callus cultures of *Genista* plants-in vitro material producing high amounts of isoflavones of phytoestrogenic activity. Plant Science 165:1101-1108.

Mauricio, A. R., M. Palma and C. G. Barroso (2003). Ultrasound-assisted extraction of soy isoflavones. J. Chromatogr. A. 1012:119-125.

Peev, C. I., L. Vlase, D. S. Antal, C.A. Dehelean and Z. Szabadai (2007). Determination of some polyphenolic compounds in buds of *Alnus* and *Corylus* species by HPLC. Chem. Nat. Compnd. 43(3):259-262.

Setchell, K. D. R. (1998). Phytoestrogens: the biochemistry, physiology and implications for human health of soy isoflavones. Am. J. Clin. Nutr. 68:1333-46.

Tero-Vescan A., S. Imre, C. E. Vari, A. Osan, M. Dogaru and C. Csedo (2009). Determination of some isoflavonoids and flavonoids from *Genista tinctoria* L. by HPLC- UV. Farmacia 57(1):120-127.

Vlase, L., L. Radu, C. Fodorea, S. Leucuta and S. Gocan (2005). Determination of Phenolic Compounds from Geranium sanguineum by HPLC. J. Liq. Chromatogr. Relat. Technol. 28(19):3109-3117.