Acta fytotechnica et zootechnica 2
Nitra, Slovaca Universitas Agriculturae Nitriae, 2012, s. 38 – 41

ANTIOXIDANT AND α-GLUCOSIDASE INHIBITION ACTIVITIES AND POLYPHENOL CONTENT OF FIVE SPECIES OF *AGRIMONIA* GENUS

ANTIOXIDAČNÁ AKTIVITA, INHIBÍCIA α-GLUKOZIDÁZY A CELKOVÝ OBSAH POLYFENOLOV V PIATICH DRUHOCH RODU *AGRIMONIA*

Renata KUBÍNOVÁ, Dagmar JANKOVSKÁ, Veronika BAUEROVÁ

University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic

Phytochemical analysis and a comparative study of antioxidant activity, inhibition of α-glucosidase and content of total polyphenols and flavonoids were established in five species of *Agrimonia* genus. Plants were collected in Medicinal Herbs Centre of Masaryk university Brno, and these were *Agrimonia eupatoria* L., *Agrimonia procera* Wallr., *Agrimonia leucantha* Kunze, *Agrimonia japonica* (Miq.) Koidz and *Agrimonia coreana* Nakai. HPLC profile of *Agrimonia* herbs shows high content of quercetin and apigenin glycosides. The content of total polyphenols in aqueous extracts correlates with antioxidant activity (r = 0.92), the aqueous extract of *Agrimonia procera* works as an excellent antioxidant (86.7 % of DPPH reduction). The highest content of flavonoids was observed in methanolic extract of *Agrimonia eupatoria* which has the highest inhibition of α-glucosidase.

Keywords: *Agrimonia* species, antioxidant activity, polyphenol content, flavonoids, HPLC

The *Agrimonia* species have been reported to possess several biological activities, such as antioxidant, anti-inflammatory, antiviral, antibacterial, anti-diabetic, diuretic (Copland et al., 2003; Kwon et al., 2003; Shin et al., 2010). Decoction can be used in traditional medicine for a treatment of diabetes mellitus or coronary diseases (Gray and Flatt, 1998; Correia et al., 2006). Inhibitors of α-glucosidase are promising drugs in the treatment of diabetes mellitus, and antioxidants from natural sources can prevent the oxidative stress associated with diabetes mellitus. The individual with diabetes has a 2- to 6-fold increased risk of cardiovascular disease (Wolff, 1993) and *Agrimonia* herbs could exert a beneficial effect in the diabetic environment. *A. eupatoria* is the most common *Agrimonia* species in Europe and the aerial parts are used in form of infusion, decoction or tincture. The scientific investigations are focused on research of the most common *A. eupatoria* and *A. pilosa* (Jung et al., 2010; Kato et al., 2010; Lee et al., 2010), there are no information about species growing in eastern Asia – *A. japonica* and *A. coreana*. In this work, the phytochemical profile of five *Agrimonia* species was investigated and its correlation with antioxidant activity and inhibition of α-glucosidase was verified.

Material and methods

Plant material

Agrimonia species were collected in Medicinal Herbs Centre of Masaryk University in July 2008. *Agrimonia coreana* Nakai, *Agrimonia japonica* (Miq.) Koidz, *Agrimonia procera* Wallr., *Agrimonia eupatoria* L., *Agrimonia leucantha* Kunze were identified by the chief scientific researcher of Medicinal Herbs Centre, Ing. Pavel Musil. Voucher specimens are deposited in the herbarium of Department of Natural Drugs, Faculty of Pharmacy, Brno.

Extraction

Aqueous and methanolic extracts from flowering aerial parts of *Agrimonia* were prepared by microwave extraction (10 min, 500 W, 150 °C): 0.1 g of dried aerial parts/6 ml of water or methanol (Jain et al., 2009). After filtration, 5 ml of extract was used. Polyphenol and flavonoid content, phytochemical analysis, antioxidant activity (%) and inhibition of α-glucosidase (%) were determined.

Qualitative analysis of constituents of extracts

For HPLC (high performance liquid chromatography), qualitative analysis Agilent Technologies 1100 with diode array detector was used (column Supelcosil ABZ + Plus 150 × 4.6 mm, parts size 3 µm and method of gradient elution – in 0. min 10 % of acetonitril and 90 % of 40 mM HCOOH, till 30. min to 100 % of acetonitril).

Determination of antioxidant activity

For determination of antioxidant activity, method of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical was used (Blois, 1958). The DPPH radical scavenging activity was determined routinely using 60 µM DPPH. The reaction mixture contained: 1.8 ml solution of DPPH and 0.2 ml of aqueous (methanolic) extract. The absorbance was measured at 517 nm after 5 min on spectrophotometer Unicam Helios. Scavenging effect (%) was calculated according to the formula: $(1 - (A_{sample} / A_{control})) \times 100$.

Inhibition of α-glucosidase

The inhibition of α-glucosidase was assayed on a microplate reader according to the standard method (Valentová et al., 2010), with slight modification: 170 µl of 0.1M phosphate buffer (pH 7.0), 20 µl of enzyme solution (0.2 U/mL α-glucosidase) and 20 µl of extracts were mixed. After an incubation time (15 min, 37 °C), the reaction was initiated by adding 20 µl of substrate solution (2.5mM p-nitrophenyl-α-D-glucopyranoside). After an additional 15 min at 37 °C, the reaction was stopped by adding 0.2M Na_2CO_3 (80 µl). The absorbance was measured at 405 nm. One set of mixtures prepared with an equivalent volume of MeOH instead of tested samples was used as a control. Another set of mixtures prepared with an equivalent volume of phosphate buffer instead of enzyme was used as a blank.

The inhibitory rates (%) were calculated according to the formula $(1 - (A_{sample} - A_{blank}) / A_{control}) \times 100$.

Determination of total polyphenols

Total polyphenol content was measured using the Folin-Ciocalteu reagent (Singleton and Rossi, 1965). The reaction mixture contained: 10 µl of aqueous extract, 10 µl of Folin-Ciocalteu reagent and 310 µl of sodium carbonate (7.5%). The absorbance was measured at 760 nm after 10 min on microplate reader (BioTech Synergy HT). The amount of total polyphenols was calculated as a gallic acid equivalent from the calibration curve of gallic acid (GA) standard solutions and expressed as mg gallic acid/1 g dry plant material.

Determination of flavonoids

Total flavonoid content was measured using aluminium chloride (Correia et al., 2006). The reaction mixture contained: 1 ml of methanolic extract, 9 ml of methanol and 200 µl of aluminium chloride (10%). The absorbance was measured at 425 nm after 30 min on spectrophotometer Unicam Helios. The amount of total flavonoids was calculated as a quercetin equivalent (QE) from the calibration curve of quercetin standard solutions and expressed as mg quercetin/1 g dry plant material.

Statistical analysis

All measurements were done in triplicate and are presented as means ± standard deviation. Polyphenol content values were plotted against antioxidant activity and the correlations were analyzed by calculating the r correlation coefficient. Statistical significance of the differences among the study species was performed by post-hoc analysis using Tukey test (ANOVA). Differences with $p < 0.05$ were considered significant.

Results and discussion

A comparative study of antioxidant activity and content of total polyphenols and flavonoids were established in five species of *Agrimonia* genus. The highest levels of flavonoids were recorded in the methanolic extract of *A. eupatoria*, and flavonoid content values correspond with high DPPH scavenging activity of *A. eupatoria* (Tab. 1).

The HPLC profile recorded at 280 nm of methanolic extract of *A. eupatoria* (Fig. 1) shows more of quercetin-glycosides than methanolic extract of *A. procera*. These compounds were identified as hyperoside, isoquercitrin and rutin, retention characteristics were compared to standards. Also apigenin 7-O-glucoside and apigenin 6-C-glucoside (isovitexin) were identified by co HPLC/DAD with standards in all extracts of *Agrimonia*. These compounds have been reported previously in *A. eupatoria* (Bilia, et al., 1993; Correia, et al., 2006) or in *A. pilosa* (Zhu et al., 2009). The HPLC analysis of methanolic extracts of *A. procera* and *A. leucantha* showed two peaks with retention time of 18.7 and 32.5 min. According to their UV spectra, these compounds were associated with flavanone structure. Spectral and chromatographic characteristics correspond to flavanones with hydroxyl group (one or two) like pinocembrin or pinostrobin. These flavanones can contribute to antioxidant potential of methanolic extracts of *A. procera* and *A. leucantha*. Their influence on antioxidant potential is not significant enough to influence quercetin-glycosides. The antioxidant activity of methanolic extracts of *A. coreana*

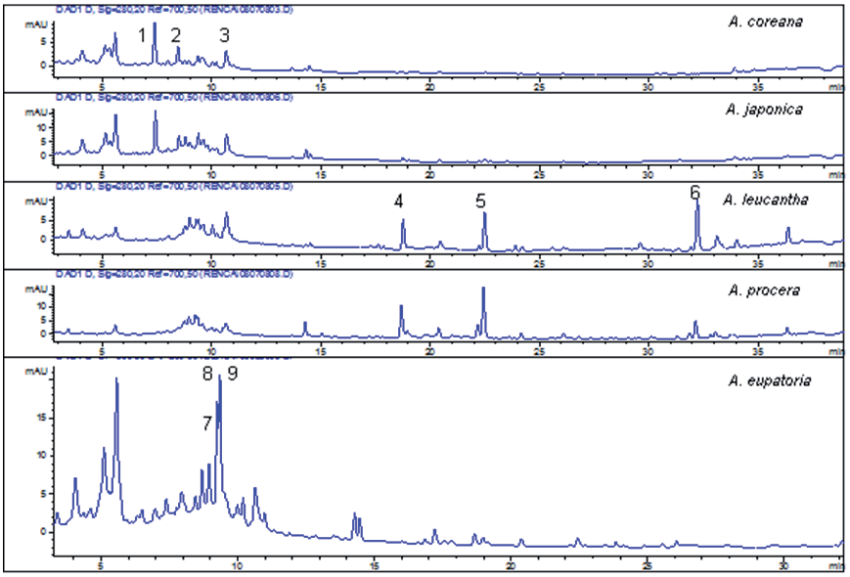

Figure 1 HPLC analysis of methanolic extracts of five *Agrimonia species*
(1) polar flavanone, (2) isovitexin, (3) apigenin-7-O-glucoside, (4, 5, 6) non-polar flavanones, (7) rutin, (8) hyperoside, (9) isoquercitrin

Obrázok 1 HPLC analýza metanolických extraktov druhov rodu *Agrimonia*
(1) polárne flavanóny, (2) isovitexin, (3) apigenín-7-O-glukozid, (4, 5, 6) nepolárne flavanóny, (7) rutín, (8) hyperosid, (9) isokvercitrin

Table 1 Antioxidant activity, inhibition of α-glucosidase and flavonoid content of methanolic extracts

Plant (1)	% DPPH reduction (2 mg dry plant/ml) (2)	% inhibition of α-glucosidase (0.1 mg dry plant/ml) (3)	Flavonoid content (mg QE/g of dry plant) (4)
A. coreana	27.7 ± 3.2***	88.8 ± 10.9	1.1 ± 0.1
A. japonica	48.7 ± 5.9*	93.8 ± 0.8	1.3 ± 0.1
A. procera	48.5 ± 1.0*	87.2 ± 4.8	1.4 ± 0.2
A. eupatoria	63.9 ± 0.9	94.2 ± 1.7	3.5 ± 0.3
A. leucantha	44.5 ± 2.4**	90.2 ± 6.8	0.7 ± 0.1

significant difference: *p <0.05; **p <0.01; ***p <0.0001 vs. *A. eupatoria*
signifikantný rozdiel: *p <0,05; **p <0,01; ***p <0.0001 oproti *A. eupatoria*

Tabuľka 1 Antioxidačná aktivita, inhibícia α-glukozidázy a obsah flavonoidov v metanolickom extrakte
(1) rastlina, (2) % redukcie DPPH (2 mg sušenej rastliny/ml), (3) % inhibície α-glukozidázy (0,1 mg sušenej rastliny/ml), (4) obsah flavonoidov (mg QE/g sušenej rastliny)

Table 2 Antioxidant activity, inhibition of α-glucosidase and polyphenol content of aqueous extracts

Plant (1)	% DPPH reduction (1 mg dry plant/ml) (2)	% inhibition of α-glucosidase (0.1 mg dry plant/ml) (3)	Polyphenol content (mg GA/g of dry plant) (4)
A. coreana	70.4 ± 0.8***	82.2 ± 11.2	68.9 ± 6.3
A. japonica	81.9 ± 1.9	88.3 ± 3.0	88.6 ± 2.3
A. procera	86.7 ± 0.5	77.6 ± 6.1*	104.8 ± 0.5
A. eupatoria	78.0 ± 3.3**	98.9 ± 0.2	72.4 ± 3.8
A. leucantha	82.0 ± 0.8	88.4 ± 6.9	90.1 ± 6.3

significant difference: *p <0.05; **p <0.01; ***p <0.0001 vs. *A. procera* (DPPH reduction) and vs. *A. eupatoria* (inhibition of α-glucosidase)
signifikantný rozdiel: *p <0,05; **p <0,01; ***p <0,0001 oproti *A. procera* (redukcia DPPH) a oproti *A. eupatoria* (inhibícia α-glukozidázy)

Tabuľka 2 Antioxidačná aktivita, inhibícia α-glukozidázy a obsah polyfenolov vo vodnom extrakte
(1) rastlina, (2) % redukcie DPPH (1 mg sušenej rastliny/ml), (3) % inhibície α-glukozidázy (0,1 mg sušenej rastliny/ml), (4) obsah polyfenolov (mg GA/g sušenej rastliny)

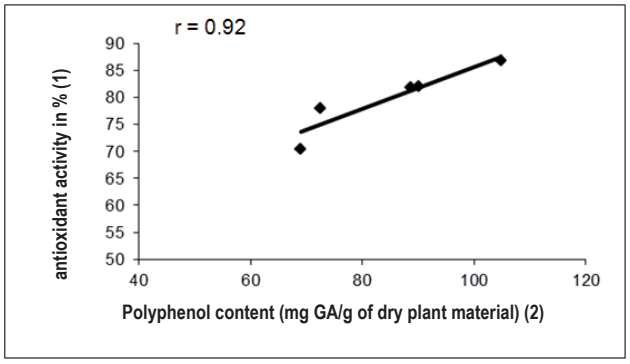

Figure 2 Correlation between antioxidant activity and polyphenol content of aqueous extracts

Obrázok 2 Korelácia medzi antioxidačnou aktivitou a celkovým obsahom polyfenolov
(1) antioxidačná aktivita, (2) obsah polyfenolov (mg GA/g sušenej rastliny)

and *A. japonica* can be affected by flavanone with retention time of 7.4 min. This flavanone is a dominant peak in HPLC profile of methanolic extracts of *A. coreana* and *A. japonica* and it is polyphenol flavanone like taxifolin. *A. coreana* has low content of quercetin glycosides and this flavanone is an important compound in its methanolic and aqueous extracts. Methanolic extracts of all *Agrimonia* herbs contain polar compounds with retention time from 2 min to 6 min and these compounds support antioxidant activity of extracts.

HPLC profile of the aqueous extracts of all *Agrimonia* herbs is similar to HPLC profile of the methanolic extracts. As the major compounds of the aqueous extracts were determined apigenin-glucoside, quercetin-glycosides, polar flavanone in *A. coreana* and *A. japonica* and non identified polar compounds with retention time from 2 min to 6 min. As polar compounds, proanthocyanidins and catechins have been identified previously in *A. eupatoria*, compounds with relevant antioxidant activity (Correia et al., 2006).

The aqueous extracts from *Agrimonia* herbs exhibited higher antioxidant activity than the methanolic extracts. The aerial parts of *A. eupatoria* are used as infusions or decoctions in folk medicine for haemostatic, diuretic and anti-inflammatory properties. In the Czech Republic, *A. procera* is used less than *A. eupatoria*, but its polyphenol content is higher than in aqueous extract of *A. eupatoria*, and *A. procera* is an excellent antioxidant (Tab. 2). The aqueous extract of *A. leucantha* also demonstrated strong antioxidant activity. Polyphenol content values correlate with DPPH scavenging activity (Fig. 2).

In previous study, an aqueous extract from *A. pilosa* and its fractions containing especially rutin, hyperosid and quercitrin, exerted also strong antioxidant activities (Zhu et al., 2009). An acetone-hexan extract of *A. procera* demonstrated better DPPH scavenging activity than an acetone-hexan extract of *A. eupatoria* (Venskutonis et al., 2007). HPLC profile of the extract of *A. procera* indicated content of polar compounds that could contribute to antioxidant potential of *A. procera*.

In the α-glucosidase bioassay, hyperoside demonstrated stronger activity than standard acarbose, compound used to treat type II. diabetes by the mechanism of α-glucosidase inhibition (Fan et al., 2010). In our study, *A. eupatoria* with the highest content of flavonoids is an excellent inhibitor of α-glucosidase (Tab. 1, 2).

Conclusions

On the basis of our results, the highest antioxidant activity is possessed by aqueous extract of *A. procera*. This species of genus *Agrimonia* is a strong antioxidant (mainly the aqueous extract) and the methanolic extract is also an interesting source of non-polar flavanones. We can suggest that *A. procera* has better antioxidant properties than commonly used *A. eupatoria* but *A. eupatoria* is richer in flavonoid glycosides that can contribute to inhibiton of α-glucosidase. Polyphenol profile of *Agrimonia* herbs, antioxidant activity and inhibition of α-glucosidase support the traditional use of these plants as an anti-diabetic and anti-inflammatory drug.

Súhrn

Cieľom práce bola fytochemická analýza vodných a metanolických extraktov, stanovenie antioxidačnej aktivity a inhibície enzýmu α-glukozidázy v piatich druhoch rodu *Agrimonia*. Testované druhy boli *Agrimonia eupatoria* L., *Agrimonia procera* Wallr., *Agrimonia leucantha* Kunze, *Agrimonia japonica* (Miq.) Koidz a *Agrimonia coreana* Nakai. Rastliny boli získané z Centra liečivých rastlín Masarykovej univerzity. Dominantnými obsahovými látkami všetkých extraktov sú kvercetínové glykozidy, ďalej isovitexin a apigenín-7-O-glukozid. Najvyšší obsah flavonoidov bol stanovený v metanolickom extrakte druhu *Agrimonia eupatoria*, najvyšší obsah celkových polyfenolov vo vodnom extrakte druhu *Agrimonia procera*. Tieto extrakty vykazovali najvyššiu antioxidačnú aktivitu. Extrakty z druhu *Agrimonia eupatoria* vykazujú najvyššiu schopnosť inhibície α-glukozidázy. Výsledky naznačujú, že druhy rodu *Agrimonia* rastúce na Slovensku je možné využiť ako fytoterapeutiká nielen pri prevencii chorôb súvisiacich s oxidačným stresom, ale taktiež ako doplnkovú terapiu *diabetes mellitus*.

Kľúčové slová: druh *Agrimonia*, antioxidačná aktivita, obsah polyfenolov, flavonoidy, HPLC

References

BILIA, A.R. – PALME, A. – MARSILI, A. – PISTELLI, L. – MORELLI, I. 1993. A flavonol glycoside from *Agrimonia eupatoria*. In: Phytochemistry, vol. 32, 1993, p. 1078 – 1079.

BLOIS, M.S. 1958. Antioxidant determination by the use of a stable free radical. In: Nature, vol. 181, 1958, p. 1199 – 1200.

COPLAND, A. – NAHAR, L. – TOMLINSON, C.T.M. – HAMILTON, V. – MIDDLETON, M. – KUMARASAMY, Y. – SARKER, S.D. 2003. Antibacterial and free radical scavenging activity of the seeds of *Agrimonia eupatoria*. In: Fitoterapia, vol. 74, 2003, p. 133 – 135.

CORREIA, H. – GONZÁLEZ-PARAMAS, A. – AMARAL, M.T. – SANTOS-BUELGA, C. – BATISTA, M.T. 2006. Polyphenolic profile characterization of *Agrimonia eupatoria* L. by HPLC with different detection device. In: Biomed. Chromatogr., vol. 20, 2006, p. 88 – 94.

FAN, P. – TERRIERA, L. – HAY, A.E. – MARSTON, A. – HOSTETTMANN, K. 2010. Antioxidant and enzyme inhibition activities and chemical profiles of *Polygonum sachalinensis* F.Schmidt ex Maxim (*Polygonaceae*). In: Fitoterapia, vol. 81, 2010, p. 124 – 131.

GRAY, A. – FLATT, P.R. 1998. Actions of the traditional anti-diabetic plant, *Agrimonia eupatoria* (agrimony): effects on hyperglycaemia, cellular glucose metabolism and insulin secretion. In: Brit. J. Nutrition, vol. 80, 1998, p. 109 – 114.

JAIN, T. – JAIN, V. – PANDEY, R. – VYAS, A. – SHUKLA, S.S. 2009. Microwave assisted extraction for phytoconstituents – An overview. In: Asian J. Research Chem., vol. 2, 2009, p. 19 – 25.

JUNG, C.H. – KIM, J.H. – PARK, S. – KWEON, D.H. – KIM, S.H. – KO, S.G. 2010. Inhibitory effect of *Agrimonia pilosa* Ledeb. In inflammation by suppression of iNOS and ROS production. In: Immunol. Invest., vol. 39, 2010, p. 159 – 170.

KATO, H. – LI, W. – KOIKE, M. – WANG, Y. – KOIKE, K. 2010. Phenolic glycosides from *Agrimonia pilosa*. In: Phytochemistry, vol. 71, 2010, p. 1925 – 1929.

KWON, D.H. – KWON, H.Y. – KIM, H.J. – CHANG, E.J. – KIM, M.B. – YOON, S.K. – SONG, E.Y. – YOON, D.Y. – LEE Y.H. – CHOI, I.S. – CHOI, Y.K. 2005. Inhibition of hepatitis B virus by an aqueous extract of *Agrimonia eupatoria* L. In: Phytother. Res., vol. 19, 2005, p. 355-358.

LEE, K.Y. – HWANG, L. – JEONG, E.J. – KIM, S.H. – KIM, Y.C. – SUNG, S.H. 2010. Effect of neuroprotective flavonoids of *Agrimonia eupatoria* on glutamate-induced oxidative injury to HT22 hippocampal cells. In: Biosci. Biotechnol. Biochem., vol. 74, 2010, p. 1704 – 1706.

SHIN, W.J. – LEE, K.H. – PARK, M.H. – SEONG, B.L. 2010. Broad-spectrum antiviral effect of *Agrimonia pilosa* extract on influenza viruses. In: Microbiol. Immunol., vol. 54, 2010, p. 11 – 19.

SINGLETON, V. – ROSSI, J. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. In: Am. J. Enol. Vitic., vol. 16, 1965, p. 144 – 158.

VALENTOVÁ, M. – MAREK, R. – ŠVAJDLENKA, E. – KUBÍNOVÁ, R. – SUCHÝ, V. 2011. A new isoflavanone from Iresine herbstii. In: Fitoterapia, vol. 82, 2011, p. 272 – 275.

VENSKUTONIS, P.R. – SKEMAITE, M. – RAGAZINSKIENE, O. 2007. Radical scavenging capacity of *Agrimonia eupatoria* and *Agrimonia procera*. In: Fitoterapia, vol. 78, 2007, p. 166 – 168.

WOLFF, S.P. 1993. Diabetes mellitus and free radicals. In: Brit. Med. Bull., vol. 49, 1993, p. 642 – 652.

ZHU, L. – TAN, J. – WANG, B. – HE, R. – LIU, Y. – ZHENG, CH. 2009. Antioxidant activities of aqueous extract from *Agrimonia pilosa* LEDEB and its fractions. In: Chem. Biodivers., vol. 6, 2009, p. 1716 – 1726.

Contact address:
PharmDr. Renata Kubínová, Ph.D., Ústav přírodních léčiv, Farmaceutická fakulta VFU, Palackého 1-3, 612 42 Brno, phone: (+420) 541 56 28 34, e-mail: kubinovar@vfu.cz