PHYTOCHEMICAL COMPARISON AND ANALYSIS OF *BERGENIA CRASSIFOLIA* L. (FRITSCH.) AND *BERGENIA CORDIFOLIA* STERNB.

Renáta Árok,[a] Krisztina Végh, [a]* Ágnes Alberti[a] and Ágnes Kéry[a]

Keywords: *Bergenia crassifolia L.* (Fritsch.); *Bergenia cordifolia Sternb.*; Arbutin; Pigmentary disorders; HPLC-UV

Bergenia crassifolia L. (Fritsch.) and *Bergenia cordifolia* Sternb. species of Saxifragaceae are decorative perennial plants. The most characteristic compound with biological activity is arbutin with skin whitening properties. Arbutin from other sources is used in skin pigmentary disorders. The aim of our work was the identification and qualitative analysis of arbutin using HPLC and UV-spectroscopic methods and quantitative determination of arbutin as well as other pharmaceutically interesting compounds: flavonoids, hydroxycinnamic acids, polyphenols and tannins in domestic cultivars, considering the synergism of phytochemical agents in therapeutic effect in skin disorders. The arbutin content was found to be between 4.8 – 9.8 g/100g in the two *Bergenia* species and significant amount of total polyphenol (4.13 – 9.27 g/100g), tannin (3.70 -6.70 g/100g) and hydroxycinnamic acid (1.80 -2.42 g/100g) was measured.

* Corresponding Authors
Tel: + 36-1-459-1500/55206
Fax: + 36-1-3172979
E-Mail: krisvegh@gmail.com
[a] Department of Pharmacognosy, Semmelweis University, H-1085, Hungary

Introduction

The species belonging to the genus *Bergenia* are herbaceous, perennial plants mainly distributed in the southeastern regions of Central Asia, northern regions of South Asia and Eastern Asia from Siberia and the Altay Mountains in Russia, South to Northern Mongolia.[1,2]

Bergenia species contain various biologically active compounds in their roots, leaves, stems and flowers, which can be mainly classified into three categories, including simple phenols/polyphenols, flavonoids and quinines. The preparations of these plants are used as astringent, anti-inflammatory, bactericidal, haemostatic, immunmodulatory, antiulcer, antihepato-toxic, antifungal, antiarrhytmic preparations. In folk medicine these remedies are used for disinfecting, wound healing, tonic and general strengthening purposes.

The most important compound for the phytochemical researches is arbutin. Arbutin inhibits melanin production and decreases tyrosinase activity.[3] Tyrosinase is a copper-containing enzyme[4], also known as polyphenol oxidase (PPO), which catalyses two initial steps in the formation of pigment melanin: hydroxylation of tyrosine and the oxidation of 3,4-dihydroxyphenylalanine.[5] Overexpression of tyrosinase leads to excessive accumulation of melanin, which causes cutaneous hyperpigmentation including freckles and sentile lentigines, melasma and malignant melanoma. Observations imply that the depigmenting effect of arbutin may be highly effective in both cell culture and human skin models, making arbutin a useful and safe agent for skin whitening. Tyrosinase inhibitors can be applied in the therapy of this skin disorders, especially in malignant melanoma due to their cytotoxicity in melanocytes.[3] *Bergenia* species are cited in literature as being one of the richest in arbutin (15-20%), an important pharmaceutical substance with depigmentation properties. The content of arbutin in vegetative and generative organs of the plant varies in wide limits, being dependent on the method of raw material drying. The plant age is also an important factor affecting arbutin content.

The aim of this study consisted in phytochemical analysis of two *Bergenia* species: *B. crassifolia* and *B. cordifolia*, acclimated in Hungary. The accumulation of flavonoids, hydroxycinnamic acids, polyphenols and tannins in the leaves and flowers were also studied, considering the synergism of phytochemical agents in therapeutic effect in skin disorders.

Experimental

Plant material

Leaves of *Bergenia crassifolia* L. (Fritsch.) and *Bergenia cordifolia* Sternb. were obtained from the Botanical Garden of Corvinus University and Pesthidegkút. One sample of *B. crassifolia* leaves was collected in Finland. Plant samples were harvested in the early spring, dried at room temperature and were authenticated in the Department of Pharmacognosy, Semmelweis University, Budapest, where voucher specimen are deposited.

Qualitative methods

Identification of arbutin by high-performance liquid chromatography

Bergenia samples (3.0 g) were extracted with 50 mL of 80% methanol in ultrasonic bath. After filtration, the extract was evaporated under reduced pressure with a rotary evaporator at 60 °C and was redissolved in super gradient grade methanol (Sigma-Aldrich, Budapest, Hungary). Samples were purified on Supelco SPE

Cartridge LC18 (500 mg / 3 mL) and after evaporation, were redissolved in super gradient grade methanol prior to analysis. Some samples were extracted with 20 mL of 50% methanol in ultrasonic bath. After filtration 0.3 g basic lead acetate was added and filtrated. The extract was evaporated under reduced pressure with a rotary evaporator at 60 ^{o}C and was redissolved in super gradient grade methanol (Sigma-Aldrich, Budapest, Hungary). Samples were purified on Supelco SPE Cartridge LC18 (500 mg / 3 mL) and were redissolved in super gradient grade methanol prior to analysis. One mg/mL arbutin solution was used as a standard. The HPLC analysis was performed with a Jasco system equipped with an ERC-3113 degasser, a Jasco LG-980-02 gradient unit, a PU-980 HPLC pump, a sample injector, and a PU-975 UV–visible detector. Compounds were separated on a 25 cm × 4.6 mm, 5 µm particle, Supelcosil LC-18 column (Sigma–Aldrich). Experiments were performed at 20 °C, the injection volume was 20 µL. Detection was carried out the wavelength of 280 nm and 340 nm. UV-spectra were recorded between 200 and 400 nm.

Quantitative methods

Quantitative determination of arbutin

Herbal drug of 0.40 g was extracted with hot water for 30 min by refluxing on boiling water bath. The extract was filtered into a 250.00 mL volumetric flask and filled up with water. Five mL of the extract was transferred into a separatory funnel and mixed with 45 mL of water, 1 mL of 2% 4-aminoantipyrine, 0.5 mL of diluted NH_4OH and 1 mL of 8% $K_3(Fe/CN/_6)$ solution. After 5 minutes the reaction mixture was extracted with $CHCl_3$. The organic phases were mixed, filtered through Na_2SO_4 sicc. into a 100.00 mL volumetric flask and filled up with $CHCl_3$ to 100.00 mL. The absorption was measured at 455 nm. The blank solution was water. The arbutin content of the crude drug was calculated as arbutin (g/100g).

Total flavonoid, total hydroxycinnamic derivative, total phenoloid and tannin content

Total flavonoid, total hydroxycinnamic derivative, total phenoloid and tannin content were determined according to the valid spectroscopic methods of the European Pharmacopoeia.[6]

Results and discussion

The HPLC method of the European Pharmacopoeia was improved. A gradient of acetic acid in water (1.0%, v/v eluent A) and methanol (eluent B) was applied at a flow rate of 1 mL/min; 0-30 min: A: 11%, B: 89% (linear gradient); 30-31. min.: A: 60%, B: 40% (linear gradient); 31– 35. min: A: 100%, B: 0% (isocratic). Arbutin in the samples was identified by standard addition, using authenticated standard. The standard addition measurement was made with 1:1 mixture of sample and standard.

According to the results the analysed *Bergenia* species contain a high level of arbutin and many flavonol glycosides, presumably quercetin glycosides.

The arbutin content of *B. crassifolia* and *B. cordifolia* leaf extracts was identified by the Rf and UV-spectra of authenticated standard using HPLC method. To improve the quality of chromatograms a purification step was also added to eliminate polyphenols from the sample solutions. Arbutin was detectable with retention time of 3.40 minute. The UV-spectra was measured between 200-600 nm, two maximums of the UV-spectra were observed at 235 nm and 291 nm. The presence of arbutin in the samples was confirmed by using standard addition method. The added arbutin was determined by the characteristic Rf. The HPLC-UV chromatograms of arbutin standard and the samples of *B. crassifolia* and *B. cordifolia* are shown in Figure 1-3.

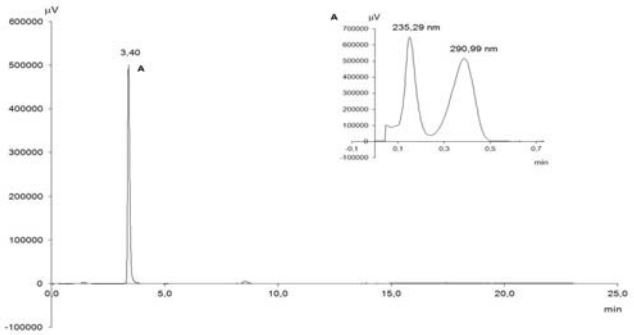

Figure 1. HPLC-UV chromatograms of arbutin standard at 280 nm and UV spectra between 200-600 nm

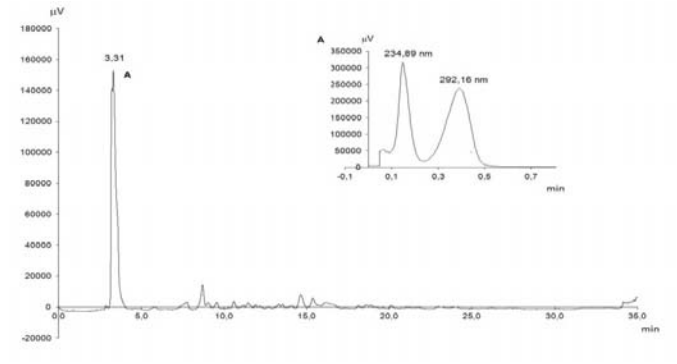

Figure 2. HPLC-UV chromatograms of *Bergenia crassifolia* sample with standard addition

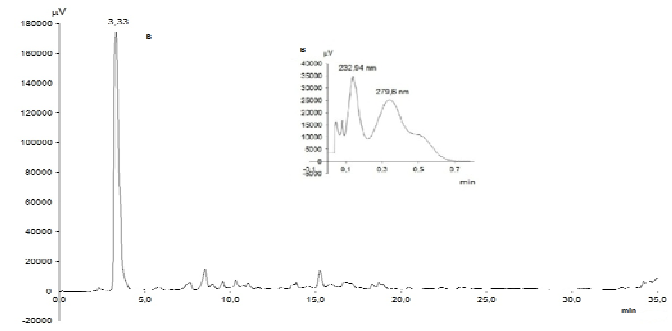

Figure 3. HPLC-UV chromatograms of *Bergenia cordifolia* sample with standard addition

Table 1. Phytochemical characteristics of *Bergenia* samples

Plant sample (*Bergenia*)		Total flavonoid* (g/100g, in hyperoside)	Total polyphenol* (g/100g, in pyrogallol)	Tannin* (g/100g, in pyrogallol)	Hydroxycinnamic acid* (g/100g, in rosmarinic acid)	Arbutin* (g/100g, in arbutin)
B. crassifolia (Finland)	leaf	0.76±0.05	9.27±0.72	5.01±0.34	2.03±0.11	9.83±0.68
B. crassifolia	leaf	0.81±0.04	6.46±0.34	3.70±0.24	2.35±0.23	4.79±0.72
(Corvinus University)	flower	0.88±0.05	4.35±0.57	3.92±0.19	2.42±0.20	2.08±0.13
B. cordifolia	leaf	0.65±0.03	8.20±0.41	3.94±0.15	1.95±0.16	9.48±0.74
(Pesthidegkút)	flower	0.67±0.03	6.31±0.24	4.23±0.31	1.98±0.09	2.51±0.10
B. cordifolia (Corvinus	leaf	0.92±0.06	4.29±0.19	6.50±0.39	1.80±0.12	7.94±0.52
University)	stem	0.95±0.04	4.13±0.25	6.35±0.52	1.95±0.14	7.94±0.61
	flower	0.87±0.07	4.33±0.41	6.70±0.27	1.98±0.30	2.99±0.18

(***** average of 3 determinations)

Total arbutin, flavonoid, polyphenol, tannin and hydroxycinnamic acid content of *Bergenia* samples are summarized in Table 1.

The arbutin content was between 4.8 – 9.8 g/100g. This is lower than the value (17.44 g/100g) was found by Pop et al. and similar than the content (6.96 g/100g) was found by Pozharitskaya et al.[7] Among the investigated parts of the plants the highest amount of arbutin was detected in the leaves. The leaf of species from Finland contained the largest quantities of arbutin. The samples made of *B. crassifolia* and *B. cordifolia* from Corvinus University could be well compared: *B. cordifolia* shows a higher amount of arbutin (7.94 g/100g) than *B. crassifolia* (4.79 g/100g). Arbutin content in the investigated flowers were 2.08 – 2.99 g/100g (Figure 4.).

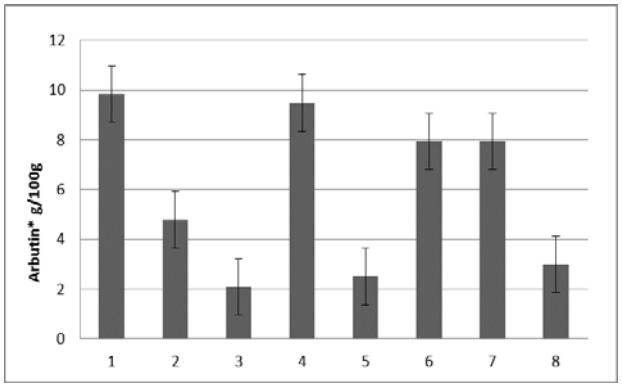

Figure 4.: Total amount of arbutin in *Bergenia* samples (1. *B. crassifolia* (Finland) leaf, 2. *B. crassifolia* (Corvinus University) leaf, 3. *B. crassifolia* (Corvinus University) flower, 4. *B. cordifolia* (Pesthidegkút) leaf, 5. *B. cordifolia* (Pesthidegkút) flower, 6. *B. cordifolia* (Corvinus University) leaf, 7. *B. cordifolia* (Corvinus University) stem, 8. *B. cordifolia* (Corvinus University) flower) (*average of 3 determinations)

The flavonoid, hydroxycinnamic acid, polyphenol and tannin contents are foremost established. The total flavonoid content determined in hyperoside was detectable between 0.65 – 0.95 g/100g. The species from Corvinus University contained the highest amount of flavonoids. The polyphenol and tannin contents were determined in pyrogallol. The content of total polyphenol was 4.13 – 9.27 g/100g, the highest value was 9.27 g/100g in the sample from Finland.

The identified amount of tannin was 3.70 – 6.70 g/100g. In this respect, there was no significant difference between the two species of *Bergenia*. The hydroxycinnamic acid values, determined in rosmarinic acid, were similar, the average content was 2.06 g/100g (Figure 5-6.).

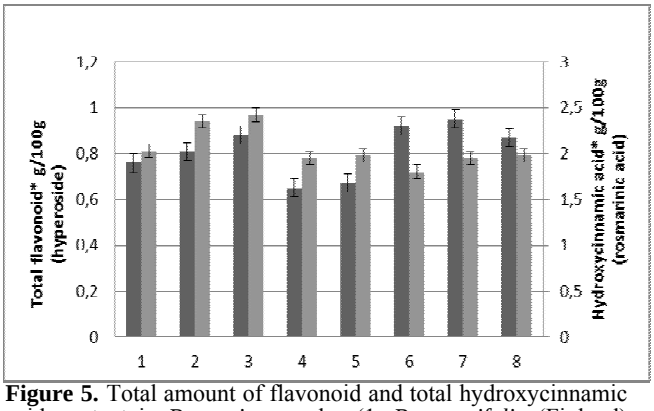

Figure 5. Total amount of flavonoid and total hydroxycinnamic acid content in *Bergenia* samples (1. *B. crassifolia* (Finland) leaf, 2. *B. crassifolia* (Corvinus University) leaf, 3. *B. crassifolia* (Corvinus University) flower, 4. *B. cordifolia* (Pesthidegkút) leaf, 5. *B. cordifolia* (Pesthidegkút) flower, 6. *B. cordifolia* (Corvinus University) leaf, 7. *B. cordifolia* (Corvinus University) stem, 8. *B. cordifolia* (Corvinus University) flower) (*average of 3 determinations)

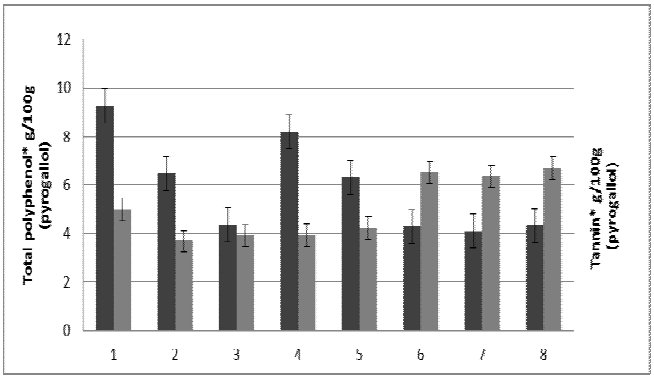

Figure 6. Total amount of polyphenols and tannin in *Bergenia* samples (1. *B. crassifolia* (Finland) leaf, 2. *B. crassifolia* (Corvinus University) leaf, 3. *B. crassifolia* (Corvinus University) flower, 4. *B. cordifolia* (Pesthidegkút) leaf, 5. *B. cordifolia* (Pesthidegkút) flower, 6. *B. cordifolia* (Corvinus University) leaf, 7. *B. cordifolia* (Corvinus University) stem, 8. *B. cordifolia* (Corvinus University) flower) (*average of 3 determinations)

Conclusion

This paper presents a qualitative HPLC method to identify arbutin in two different species of *Bergenia* genus, *Bergenia crassifolia* L. and *Bergenia cordifolia* Sternb. growing in Hungary. In addition content of arbutin and other phenolic compounds (flavonoids, hydroxycinnamic acids, polyphenols and tannins) in the two species was determined and compared for the first time. Due to inhibition of tyrosinase enzyme, which catalyses the rate-limiting step of the melanogenic pathway by arbutin, *Bergenia* species might be suitable for cosmetical and dermatological applications.

Quantitation of other phenolics (flavonoids, hydroxycinnamic acids, polyphenols and tannins) is necessary to complete phytochemical characterization of *Bergenia* samples, since these compounds may also contribute to the pharmacological effect.

References

[1] Pop, C., Vlase, L., Tamas, M. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* **2009,** *37*, 129.

[2] Zhang, Y., Liao, C., Li, J., Liu, X. *J. Med. Plants Res*., **2011,** *5*, 4396.

[3] Chakraborty, A. K., Funasaka, Y., Komoto, M., Ichihashi, M. *Pigment Cell Res.* **1998,** *11*, 206.

[4] Decker H., Tuczek F. *Trends Biochem. Sci.*, **2000**, *25*, 392.

[5] Sánchez-Ferrer, Á., Rodríguez-López, J. N., García-Cánovas, F., García-Carmona, F., *Biochim. Biophys. Acta,* **1995**, *1247*, 1.

[6] *Europian Pharmacopoeia V*., EDQM, **2004,** *2*, 1103 and 2377., **2004**, *1*, 221.

[7] Pozharitskaya, O. N., Ivanova, S. A., Shikov A. N., Makarov, V. G., Galambosi, B. *J. Sep. Sci.* **2007**, *30*, 2447.

Received: 24.05.2012.
Accepted: 31.05.2012.