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Natural Resources Containing Arbutin. Determination of Arbutin in the Leaves of *Bergenia crassifolia* (L.) Fritsch. acclimated in Romania

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Abstract

Bergenia crassifolia (L.) Fritsch. is cited in literature as being one of the richest in arbutin (15-20%), an important pharmaceutical substance with disinfecting properties (in genitourinary diseases) and also depigmentation properties (skin whitening agent). The aim of this study consisted in determination of arbutin content in leaves of *Bergenia crassifolia* acclimated in Romania. The optimum parameters for the extraction of arbutin and the dynamics of the accumulation of arbutin in *Bergenia crassifolia* leaves during the four seasons were also studied. The content of arbutin varied between 17.44% and 22.59% dry weight, values which are similar to those found in literature

Keywords: arbutin, *Bergenia crassifolia*

Introduction

Hydroquinone O-β-D-glucoside, also called arbutin, is spread in more superior plants such as: *Arctostaphylos uva-ursi* (L) Spreng., *Vaccinium vitis-idea* L., *Pyrus communis* L., *Lathyrus* sp. From the pharmacologic point of view arbutin is of interest due to two therapeutic applications. The antibacterial properties recommend the use of the leaves of *Arctostaphylos uva-ursi* in the form of infusions for the treatment of the urogenital tract infections (cystitis, nephritis, gonorrhoea, endometriosis, etc.). It also shows the property of suppressing melanin biosynthesis in the human skin. The pharmacological active compound is hydroquinone which originates from arbutin by in vivo glucoside cleavage.

In Romania, the best source for obtaining arbutin by extraction is represented by the leaves of *Arctostaphylos uva-ursi (L) Spreng.* (bearberry). However, this plant is protected by the law, being considered a nature's monument since it only vegetates in two small areas (The Apuseni Mountains, Alba County and Rachitis Hill, Suceava County). In this context, for the first time in Romania, the percentage of arbutin was determined in the species *Bergenia crassifolia* (L.) Fritsch. *(Saxifragaceae).*

Bergenia crassifolia (Siberian tea, Elephant's ears, Leather bergenia) (Fig. 1) is a perennial plant, native from Central and Eastern Asia, from Siberia and the Altay Mountains in Russia, South to Northern Mongolia and Xinjiang in China. The name *Bergenia* commemorates Karl August of Bergen (1704-1759), a German physician and botanist (Anisko, 2008).

Bergenia crassifolia contains a complex of biologically active phenolic compounds such as simple phenols, flavonoids, phenolcarboxylic acids, coumarins and tannins. This plant is cited in literature as an important source of arbutin (15-20%).

Although in Romania *Bergenia crassifolia* is not considered to be a medicinal plant, in Russian Federation preparations of this plant are used as astringent, anti-inflammatory (probably due to bergenan, a pectin from its structure), bactericidal and haemostatic remedies. In the folk medicine is used for wound healing, disinfecting, tonic and general strengthening purposes (Shilova *et al.*, 2006). It has been demonstrated that bergenan from leaves of *Bergenia crassifolia*, which belong to galacturonans, shows the immunostimulating activity *in vivo* and the phagocytic activity *in vitro* (Golovchenko *et al.*, 2006). Khazanov *et al.* (2000) demonstrated an important cerebroprotective activity of leaves extract in hypoxia.

The content of arbutin in vegetative and generative organs of the plant varies in wide limits, from 27.9% in roots to 19.92% and 7.5% in leaves being dependent on the method of raw material drying. The plant age is also an important factor affecting arbutin content: the first-year leaves contain 8% arbutin and old leaves contain only 3.3% arbutin (Lubsandorzhieva *et al.*, 1999).

The aim of this study consisted in determination of arbutin content in species *Bergenia crassifolia* acclimated in Romania (Botanical Garden of Cluj-Napoca). The optimum parameters for the extraction of arbutin and the dynamics of the accumulation of arbutin in *Bergenia crassifolia* leaves during the four seasons were also studied.

Materials and methods

The leaves and petioles of *Bergenia crassifolia L.* were collected in April, August, October 2005 and February 2006, from the Botanical Garden of Cluj Napoca, Roma-

Fig.1. *Bergenia crassifolia* (L.) Fritsch.

nia. The plant leaves were dried at room temperature and powdered. Voucher specimens were deposited in the Herbarium of this institution.

In order to establish the complete extraction of arbutin, five simple methods were used. These methods differed one from another by the solvent used and the extraction way. For this purpose, only the leaves collected in October 2005 were used. The results obtained were interpreted using t Test for unpaired values.

Method 1

50 mg dried powdered material was sonicated with 20 ml of solvent (methanol:water = 5:95) at 25°C for 30 min, after a preliminary humectation with 1 ml methanol for 24 hours.

Method 2

50 mg dried powdered material was sonicated with 20 ml of solvent (methanol:water = 5:95) at 25°C for 30 min.

Method 3

50 mg dried powder material was extracted with 20 ml of solvent (methanol:water = 50:50) and heat under a reflux condenser on a water bath for 15 min.

Method 4

50 mg dried powder material was extracted with 20 ml of solvent (methanol:water = 5:95) heat under a reflux condenser on a water bath for 15 min.

Method 5

50 mg dried powder material was extracted with 20 ml distilled water heat under a reflux condenser on a water bath for 30 min.

Five assays were performed for each method. After sonication, the volume of all samples was adjusted to 25 ml with distilled water, in measuring flask. After homogenisation, an aliquot of 1 ml solution was centrifuged at 10000 rpm for 1 min. From the supernatant, an aliquot of 0.1 ml was diluted successively, such as in the final step all the samples were diluted 1:100.

For the quantitative determination of arbutin, a new selective and sensitive HPLC-MS method was elaborated.

The experiment was carried out using an Agilent 1100 Series HPLC system (Agilent USA) consisting of a G1322A degasser, G1311A quaternary gradient pump and a G1313A autosampler. The chromatographic separation was achieved using a reversed-phase analytical column (Zorbax SB-C18 100mmx3.0mm i.d., 3.5µm particle) maintained at 45°C. The mobile phase consisted of 100% water containing 50µM sodium acetate. The mobile phase was delivered with a flow rate of 1mL/min and the injection volume was 5µL. All solvents were filtered through 0.5µm (Sartorius) filters and degassed through ultrasonication.

The detection of arbutin was in single ion monitoring (SIM) mode using an ion trap mass spectrometer with electrospray positive ionization. In these chromatographic conditions, the retention time for arbutin was 1.6 min (Fig. 2).

Nitrogen was used as nebulising and drying gas. The instrument was set to the following tune parameters: nebulising gas pressure of 60psi, drying gas flow of 12L/min, drying gas temperature 300°C, capillary voltage +4000V. The chromatographic data were processed using Chemstation and Data Analysis software from Agilent, USA.

Results and disscusion

For the interpretation of the results obtained, all the method proposed were compared to method 3 (considered as a reference) employing water:methanol (50:50) and heat under a reflux condenser on a water bath. This procedure was used by Stahl and Schild (1981) for the sample preparation for the quantitative determination of arbutin by spectrophotometry.

Method 1 can be considered as a variant of method 2 because the solvent use for extraction is the same and the extraction way utilises the ultrasounds. Whether the humectation of vegetal powder with methanol (as an inert solvent) increase the extraction efficiency was also tested.

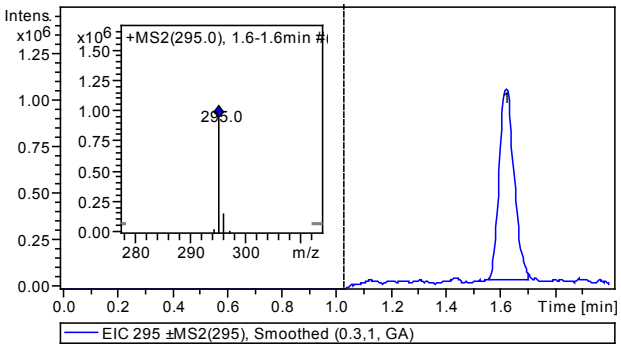

Fig. 2. Typical chromatogram of arbutin from *Bergenia crassifolia* extract and its ESI-mass spectra (fig. insert)

Tab. 1. Different parameters used in the establishment of the extraction process

Method	Solvent used	Extraction way	Observation
1	methanol:water = 5:95	Ultrasounds (25°C for 30 min)	Humectation with methanol
2	methanol:water = 5:95	Ultrasounds (25°C for 30 min)	Parejo *et al.* (2001)
3	methanol:water = 50:50	Heat under a reflux condenser for 15 min.	Stahl and Schild (1981)
4	methanol:water = 5:95	Heat under a reflux condenser for 15 min.	-
5	distilled water	Heat under a reflux condenser for 30 min.	4th European Pharmacopeia (2002)

Method 2 employing methanol:water (5:95) and an ultrasonic bath for 30 min. was proposed by Parejo *et al.* (2001). The authors used this method for the extraction of arbutin from *Arcostaphyllos uva-ursi* leaves. The quantitative analysis was performed by HPLC.

According to the results obtained it was demonstrated that the use of mentioned solvent and of ultrasounds determined a total extraction of arbutin from vegetal samples at 25°C. At higher temperature (45°C) a decreasing of arbutin concentration was observed, probably due to its degradation.

The 4th extraction method combine the solvent utilised for methods 1 and 2 with the boiling of mixture (solvent and vegetal powder).

Method 5 employing distilled water as solvent and reflux heating on a water bath for 30 min. is officinal in 4th European Pharmacopeia (2002) for the extraction of arbutin from *Arctostaphyllos uva-ursi* leaves.

For the statistic interpretation of the results, t Test for unpaired values was used. Method 3 (considered as a reference) has been compared to each one of the other four employed methods.

According to the results presented, statistically significant differences in extraction efficiency were observed only between methods 4-3 ($p < 0.05$). Consequently, the optimum extraction method combined a mixture of methanol:water (5:95) as a solvent and heat under a reflux condenser on a water bath for 15 min.

The humectation of vegetal powder with methanol for 24 hours did not increase the extraction efficiency significantly. Although the usage of ultrasound extraction diminishes the time needed for the preparation of samples, the results have pointed out that heating induces superior extraction rated capacities.

In order to establish the dynamics of the accumulation of arbutin in *Bergenia crassifolia* leaves during the four seasons, the proposed method was used to determine the content of arbutin in different samples of raw material.

The content of arbutin varied between 17.44% and 22.59% dry weight, values which are similar to those found in literature (Lubsandorzhieva *et al.*, 1999) (Fig. 2). In spring (April 2005) the content of arbutin was the smallest found (17.44%), but in summer the concentration of arbutin was considerably increased (21.56%).

On the basis of the obtained results it's obvious that the higher arbutin content in *Bergenia crassifolia* leaves acclimated in Romania is during the autumn (October 2005, 22.59%). Parejo *et al.* (2001) were observed the same dynamics of the accumulation of arbutin in *Arctostaphyllos uvae-ursi* leaves.

This dynamics of accumulation seems to be entirely justifiable, if we consider the protective role that arbutin may play in plants.

The high level of arbutin found in summer can be considered a response to climatic conditions, especially to drought. In autumn and in winter, arbutin content is higher due to environmental stress conditions such as low temperature.

Arbutin is found in diverse species and many of the plants in which it occurs in high levels are adapted to stress conditions, for example arctic low temperatures (*Vaccinium* spp., *Arctostaphyllos* spp.) or drought stress (*Myrothamnus flabelifolia*).

Maybe due to its high content of arbutin, *Bergenia crassifolia* is able to resist in environmental stress conditions characteristic to Siberia, being a species with evergreen foliage.

Tab. 2. Values of standard deviations, coefficients of variations and mean values (mg arbutin/ml) for each method tested

Extraction method	Mean values (mg arbutin/ml)	Standard deviation	Coeff. of variation (%)
1	245.85	13.66	5.56
2	242.25	13.49	5.57
3	254.11	16.83	6.63
4	276.38	11.61	4.20
5	269.91	18.99	7.04

Tab. 3. „p" values for all four comparative tests

Methods compared	„p" values
1-3	0.420
2-3	0.256
4-3	0.045*
5-3	0.202

* statistically significant when $p < 0.05$

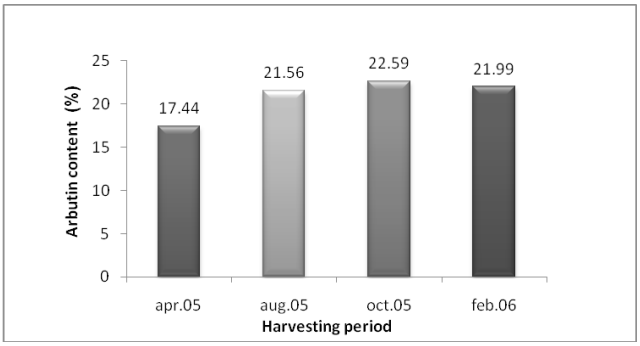

Fig. 2. The Dynamics of the accumulation of arbutin in *Bergenia crassifolia* leaves during four seasons

The role of arbutin in plants is still unknown, but as a source of hydroquinone it might reduce the rate of peroxidation of unsaturated plants membrane lipids, one of the events commonly considered to play a role in the deterioration of plant cells during aging or exposure to stress (Suau *et al*., 1991).

Conclusions

For the first time in Romania the content of arbutin (4-hydroxyphenyl-β-D-glucopyranoside) a glycosilated hydroquinone with disinfecting properties (in genitourinary diseases) and also depigmentation properties (skin whitening agent) was determined in the species *Bergenia crassifolia (L.)*. Arbutin has been found until now in high concentrations in the leaves of several plants species such as *Vaccinium vitis-idea L.* and *Arctostahyllos uva-ursi L.* (8-10%) but *Bergenia crassifolia (L.)*, proved to be one of the richest in arbutin (15-20%).

To establish the complete extraction of arbutin, out of the five simple methods we used, the optimum extraction was obtained with a mixture of methanol:water (5:95) and a reflux heating for 15 min.

The content of arbutin varied between 17.44% and 22.59% dry weight, values which are similar to those found in literature, the dynamics of the accumulation of the active principle being established during the four seasons. The highest content of arbutin was observed in autumn (October 2005).

Maybe due to its high level of arbutin, *Bergenia crassifolia* is able to resist in environmental stress conditions characteristic to Siberia.

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