Antioxidant Flavonoid Glycosides in *Viola Tricolor* L.

Theses of the Dissertation

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Abstract

In this thesis the chemical composition of heartsease (*Viola tricolor* L.) was studied to support the evidence-based determination of its biological activities.

Henceforth, a proper sample preparation method was developed, yielding a fraction rich on polar constituents (characteristic of the traditionally applied aqueous extract), which was further separated by conventional Sephadex LH-20 column chromatography. HPLC-UV analysis of the fractions suggested that the two main flavonoid components had been successfully isolated. They were definitly identified by LC-MS^n and NMR studies as violanthin (6-*C*-glucosyl-8-*C*-rhamnosyl apigenin) and rutin (3-*O*-rhamnoglucosyl quercetin) (1). Furthermore, sixteen of the minor flavonoid components (*C*-glycosides, *O*-glycosides and *C*,*O*-glycosides) were tentatively identified by nanoLC-MS^n (2). Although violanthin could not be quantified in the lack of a commercially available reference molecule, rutin was quantitatively determined by HPLC with UV detection ((0.42$\pm$ 0.01) %). The antioxidant capacities (electron-donor and hydrogen donor activities) of the fractions were determined by the TEAC and DPPH assays, respectively. In respect to their antioxidant properties, the highest electron-donor capacity was measured with rutin, and a fraction enriched on flavonoids exhibited the highest hydrogen donor activity (1).

Garden pansies (*V. x wittrockiana* Gams.) are plants of complex hybrid origin. In this thesis, beside a comparative HPLC study, the anthocyanidin and flavonoid contents as well as the antioxidant capacities of garden pansies of different petal color and heartsease were compared.The anthocyanidin and flavonoid contents of the samples were quantified by spectroscopic methods registered in the European Pharmacopoeia 5.0. While the highest anthocyanidin content was measured in the violet flower sample, the white and yellow pansy samples showed the highest flavonoid content. The antioxidant capacity of the samples was determined by the TEAC assay. The heartsease and pansy samples were observed to be as good antioxidants as the well-known ginkgo leaf. In addition, significant correlation was found between the flavonoid content and the antioxidant capacity of the samples (3).

(1) V. Vukics, A. Kery, G. K. Bonn, and A. Guttman. Major flavonoid components of heartsease (*Viola tricolor* L.) and their antioxidant activities. *Anal. Bioanal. Chem.*, 390:1917–1925, 2008. (2) V. Vukics, T. Ringer, A. Kery, G. K. Bonn, and A. Guttman. Analysis of heartsease (*Viola tricolor* L.) flavonoid glycosides by micro-liquid chromatography coupled to multistage mass spectrometry. *J. Chromatogr. A*, 1206:11–20, 2008. (3) V. Vukics, A. Kery, and A. Guttman. Analysis of polar antioxidants in heartsease (*Viola tricolor* L.) and garden pansy (*Viola x wittrockiana* Gams.). *J. Chrom. Sci.*, 46:823–827, 2008.

1 Preliminaries, aims

Application of plant products in therapy has contributed several important results to the development of modern medicine. In this process it is important to reevaluate such traditional herbs, that have long been featured in pharmacopeiae, that is, to map their chemical constitution, to prove their known effects on a scientific basis and to look for possible new fields of application. The most significant results of today's herbal research are: the quicker and more precise identification of herbal bioactive components, the application of molecular biology test systems to explore the full spectrum of action of plant extracts and their synergic interactions, and the possibility of more precise clinical studies.

The herb of heartsease -also known as wild pansy- (*Violae tricoloris herba*)has a long history in phytomedicine, and is official in the 5th European and the VII. Hungarian Pharmacopeiae. It has been utilized to treat various skin disorders, upper-respiratory problems, and also used as a diuretic. Although the use of heartsease as herbal medicine goes back centuries, the biological activity of its main secondary metabolites has hardly been studied. Reports deal only with the cytotoxicity of its cyclic peptides and its antimicrobial activity. Some of the newest results report on its positive effect on mitochondria.

Our knowledge in regards to the chemical structures of its main components is also very limited. Papers from as early as the 1970s report on carotenoids, anthocyanidins, and flavonoids being present in heartsease. Obsolate chromatographical and spectroscopical methods were used to identify flavonoid aglycones (quercetin, luteolin), O- (rutin, lu- teolin-7-glycoside) and C-glycosides (scoparin, saponarin, saponaretin, violanthin, orientin, and isoorientin, vicenin-2 as well as vitexin).

In our work we had the possibility to join a complex biological-effect screening study with our wild pansy extracts. The main point of the study was the exploration of the effect on mitochondrial mechanisms, the expression and activity of constitutive nitrogene-monoxide synthase (cNOS) enzymes and the expression of the closely related inducible heat-shock proteins (HSP). Since the nitrogen-monoxide (NO) produced by the cNOS is a multi-functional transmitter, it is not surprising that the insufficient cNOS functionality is partly responsible for illnesses such as diabetes, cardiovascular-, Parkinson-, and Alzheimer-diseases. Hence we have noticed the strong biochemical responses of *Viola tricolor* water extracts: the clear growth of mitochondrium number, the 50-120% induction of the expression of mitochondrial enzyme (NOS and COX-4) and heat-shock protein (HSP 25, 60, and 90) expression. We have measured a prominent effect in

glucose tolerance test (decrease of insulin resistance) as well as beneficial vascular and intestinal effects in diabetic animals.

Further biochemical and pharmacological investigations required the phytochemical reevaluation of *Viola tricolor*. Hence my thesis is related to the above body of work by the study of potential hydrofil bioactive components of the plant. Accordingly, our aim was to

1. develop a proper extraction method to replace the water extract of heartsease, and obtain an extract, which was microbiologically more stable and easier to work with.

2. develop a suitable fractionation protocol for the separation of this polar extract by conventional column chromatography.

3. analyze the fraction's chemical composition by chromatographic and spectroscopic methodologies, such as HPLC, LC-MS, and NMR.

4. characterize the antioxidant properties of the fractions in different *in vitro* test systems.

5. compare the chemical composition and antioxidant activity of heartsease with other *Viola* species.

2 Material and methods

2.1 Plant materials

For the scouting analyses heartsease herb (*Viola tricolor* L., Violaceae) was obtained from three different sources: (i) collected and identified in Transilvania by Prof. Kalman Csedo (Babes-Bolyai University), (ii) Fitopharma Ltd. (Budapest, Hungary) (SN=28-56-05-VI/24), (iii) MDR 2000 Ltd. (Godollo, Hungary). In the further analyses, the sample from Fitopharma Ltd. was used.

Garden pansies (*Viola* x *wittrockiana* Gams., Violaceae) were cultivated in Nagyrecse (Hungary). They were selected by petal color: violet, violet-white, white and yellow, and collected as herbs, flowers and leaves.

2.2 Sample preparation

Solvent extraction 5.0 g dried and freshly powdered heartsease herb was sonicated two times with 50 mL chloroform for 25 minutes in an ultrasonic

bath at 30 °C. The chloroform extract was evaporated to dryness under reduced pressure at 30 °C. The plant residue was dried at room temperature and re-extracted two times with 40 mL methanol for 15 minutes in an ultrasonic bath at 30 °C.

Conventional open column chromatography The stationary phase bed was prepared by equilibrating Sephadex LH-20 beads for at least 24 hours in 50% methanol. After transferring the slurry to the column (glass, homemade, 35 x 1.5 cm) the bed (final geometry: 28 cm times 1.5 cm) was allowed to settle. 0.2 g methanolic extract was dissolved in 2 mL 50% methanol and introduced to the column. Elution was carried out at a flow rate of 1.0 mL/min with 21 mL 50% methanol then 10 mL 70% methanol and 100% methanol until no more component were detectable.

2.3 Qualitative analyses

TLC Sample solutions were spotted on 0,2 mm Kieselgel 60 F_{254} fluorescent silica-gel plates. Plates were read attended and/or unattended in visible and/or UV (λ = 254 and 365 nm) light. Developing mixture: EtAc(100)/cc. CH_3COOH(11)/H_2O(26)/HCOOH(11); color reagent: Naturstoff reagent

HPLC-UV The ABLE-E & Jasco HPLC apparatus consisted of an ERC-3113 degasser, an LG-980-02 solvent mixer, a PU-980 pump and a 20 μL Rheodyne 7725 injector. The instrument was equipped with a type UV-975 UV-VIS detector. UV spectra were recorded during the HPLC separation by manually setting the recording time. For the separation, gradient elution from 13% to 18% ACN in 20 minutes (A = 0.5% H_3PO_4) was performed at a flow rate of 1.5 mL/min on a Hypersil ODS (250 x 4.6 mm, 5μm column. Before injection, each sample was filtered on an Acrodisc PVDF 0.20 μm membrane Sartorius syringe filter. The eluate was monitored at 340 nm.

LC-MS^n All analyses were performed on a μLC system coupled to linear ion trap mass spectrometer (LTQ, Thermo Fisher). The LC device consisted of the Ultimate μ HPLC pump, the Switchos μ column-switching device and the FAMOS μ-Autosampler. A monolithic capillary column was used for separation (260 x 0.2memm). μ-HPLC of flavonoids was carried out employing reversed phase conditions using 0.1% formic acid

in water and 0.1% formic acid in acetonitril as solvents A and B, respectively, at a flow rate of 1 μL/min and 35 °C. Separation was performed with a linear gradient (in 50 min from 0% B to 10% B). Hyphenation to the mass spectrometer was carried out by a nanoflow electrospray ionization source from Proxeon with Pico Tips (FS360-20-10) from New Objective. Mass spectrometric data were obtained on the linear ion trap LTQ from Thermo Fisher. Measurements in the positive ion mode were performed as follows: source voltage 1.7 kV, capillary temperature 220 °C, capillary voltage 41 V, tube lens 115 V; in the negative mode: source voltage 1.3 kV, capillary temperature 220 °C, capillary voltage -50 V, tube lens -152 V.

NMR 18 mg *Fraction E* was dissolved in 1 ml DMSO-d6 and characterized on a two-channel Varian Inova 600 MHz NMR spectrometer equipped with a waveform generator, a pulsed field gradient (PFG) unit and a dual inverse broad-band probe-head. In addition to registering the ^{1}H-NMR and ^{13}C-NMR spectra standard 2D experiments (COSY, TOCSY, NOESY, HSQC, HMBC) and selective 1D TOCSY-TOCSY and TOCSY-NOESY experiments were applied for assignment.

2.4 Quantitative analyses

Anthocyanidin and flavonoid content The antocyanidin and flavonoid content of the dried samples were determined by applying the methods of the European Pharmacopoeia 5.0, paragraph '*Myrtilli fructus recens*' and '*Solidaginis herba*' [**?**].

2.5 In vitro antioxidant assays

TEAC assay ABTS was dissolved in water in 7 mM concentration. Its radical monocation was produced by reacting the ABTS solution with 2.45 mM (final concentration) potassium persulfate and letting the mixture stand in dark at room temperature for at least 12 h before use. The $ABTS^{\bullet+}$ stock solution was diluted with spectroscopic grade ethanol to 0.9 absorbance unit at 734 nm. At least four different volumes of the diluted sample were added to 2.5 ml ABTS solution resulting in different final concentrations and producing inhibition of the blank solvent between 20% - 80%. Absorbance values were measured at 734 nm after 0, 0.5; 0.66; 0.83; 1; 1.5; 2; 2.5; 3; 4; 5 and 6 minutes. For the determination of the so-called inhibition percentage, we extrapolated the final absorbance by numerically

solving the simplest possible reaction kinetics model. The inhibition percentage produced by a given sample concentration was calculated as (100-A_t/A_0)*100 (A_t: the extrapolated final absorbance, A_0: the absorbance of the blank solvent). The antioxidant activity was characterized by plotting the inhibition percentage of the samples as a function of sample concentration followed by linear regression (data not shown). The concentration resulting in 50% inhibition is referred to as IC_{50} value.

DPPH assay DPPH$^\bullet$was dissolved in HPLC grade methanol in 0.25 g/L concentration. The DPPH$^\bullet$stock solution was diluted with HPLC grade methanol to 0.9 absorbance unit at 515 nm. At least four different volumes of the diluted sample were added to 2.5 ml DPPH$^\bullet$solution resulting in different final concentrations and producing inhibition of the blank solvent between 20% - 80%. Absorbance values were measured at 515 nm after 0, 0.5, 0.66, 0.83, 1, 1.5, 2, 2.5, 3, 4, 5, 6 minutes. The inhibition percentage was calculated as for the TEAC assay.

3 New scientific results

Although heartsease (*Viola tricolor* L.) has been extensively used in the traditional medicine for centuries, its biological activities and secondary metabolite composition have hardly been investigated. The chemical composition of heartsease was studied as first in the literature to support the evidence-based determination of its biological activities. Our examinations covered the qualitative and quantitative analyses of heartsease's hydrophyl –potentially bioactive– constituents, the determination of their antioxidant capacities, as well as the comparison of heartsease with an other *Viola* species, namely garden pansy (*V. x wittrockiana* Gams.).

1. As the traditional internal administration of heartsease herb is as a tea, we primarily aimed at the thorough analyses of components, which are supposed to be present in this aqueous extract. Aqueous solutions, however, are microbiologically instable and difficult to handle (e.g. to evaporate). Henceforth, a methanol-applying extraction method was developed, yielding a fraction rich on polar constituents. The samness of heartsease methanolic and aqueous extracts' compositions was verified by TLC and HPLC analyses.

2. A fractionation protocol was developed for the separation of the potentially bioactive hydrophyl components. The methanolic extract enriched on polar components was separated by conventional

Sephadex LH-20 or polyamide column chromatography. HPLC-UV analyses of the fractions suggested that the two main flavonoid components had been successfully isolated. In addition, purified flavonoid fractions containing only two-three components were obtained, suitable for the scouting LC-MS analyses.

3. The main flavonoid component –contrary to former literature data– was identified by LC-MS^n and NMR studies as violanthin (6-*C*-glucosyl-8-*C*-rhamnosyl apigenin).

4. By comparing retention times, UV spectra, molecular masses, and fragmentation patterns with reference standard molecules, the second main flavonoid component was suggested to be rutin (3-*O*-rhamnoglucosyl quercetin).

5. In addition, another fourteen flavonoid glycosides were tentatively identified by LC-MS^n. 3 flavonoid *O*-, 7 *C*- and 3 *C,O*-glycosides with kaempferol, quercetin, isorhamnetin, crysoeriol, apigenin and luteolin aglycones were reported for the first time in heartsease. Besides, our results confirmed the presence of rutin, violanthin, isoorientin and vicenin-2.

6. Although violanthin could not be quantified in the lack of a commercially available reference molecule, rutin – the second main flavonoid component – was quantitatively determined by HPLC with UV detection. The examined heartsease herb samples contained 0.42 ± 0.01 % rutin.

7. The antioxidant activity of heartsease herb was characterized as first in the literature. The antioxidant capacities (electron-donor and hydrogen donor activities) of the above described fractions were determined by the trolox equivalent antioxidant capacity (TEAC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays, respectively. In respect to their antioxidant properties, the highest electron-donor capacity was measured with isolated rutin, and a fraction enriched on flavonoid glycosides exhibited the highest hydrogen donor activity.

8. Garden pansies (*Viola x wittrockiana* Gams.) were characterized from a phytochemical point of view as first in the literature. Besides a comparative HPLC study, the anthocyanidin and flavonoid contents as well as the antioxidant capacities of garden pansies of different

Table 1: Flavonoid glycoside components in heartsease methanol extract characterized by LC-MS^n

	MW (Da)	Rt	Structure
O-glycosides			
10	594	42.7	kaempferol-3-*O*-deoxyhexosyl(1→6)hexoside
11	610	36.5	quercetin-3-*O*-deoxyhexosyl(1→6)hexoside
12	624	46.7	isorhamnetin-3-*O*-deoxyhexosyl(1→6)hexoside
13	756	34.0	quercetin-3-*O*-deoxyhexosylhexoside-7-*O*-deoxyhexoside
C-glycosides			
14	448	33.0	luteolin-6-*C*-hexoside
15	462	39.5	chrysoeriol-6-*C*-hexoside
16	564	33.0	apigenin-6-*C*-pentoside-8-*C*-hexoside
17	564	35.5	apigenin-6-*C*-hexoside-8-*C*-pentoside
18	578	35.0	apigenin-6-*C*-deoxyhexoside-8-*C*-hexoside
19	578	37.5	apigenin-6-*C*-hexoside-8-*C*-deoxyhexoside
20	594	31.6	apigenin-6,8-di-*C*-hexoside
21	594	33.0	luteolin-6-*C*-deoxyhexoside-8-*C*-hexoside
22	594	35.0	luteolin-6-*C*-hexoside-8-*C*-deoxyhexoside
C,*O*-glycosides			
23	740	30.6	apigenin-X-*O*-hexoside-Y-*C*-deoxyhexoside-Z-*C*-hexoside
24	740	34.3	apigenin-6-*C*-deoxyhexoside-(6″-*O*-hexosyl-8-*C*-hexoside)
25	740	39.7	apigenin-(6″-*O*-hexosyl-6-*C*-hexoside)- 8-*C*-deoxyhexoside

petal color and heartsease were compared. Apparently, similar major components were found in herb, leaf and flower samples, however, differences were observed in their component ratios. The main component of the flower samples was proposed to be rutin, as the main component of the herb and leaf samples was suggested as violanthin.

9. The anthocyanidin and flavonoid contents of the samples were quantified by spectroscopic methods registered in the European Pharmacopoeia 5.0. While the highest anthocyanidin content was measured in the violet flower sample, the white and yellow pansy samples showed the highest flavonoid content.

10. The antioxidant capacity of the samples was determined by the TEAC assay. Our data revealed that in this test system the heartsease and pansy samples were as good antioxidants as the well-known antioxidant ginkgo leaf.

11. In addition, significant correlation was found between the flavonoid content and the antioxidant capacity of the samples supporting the assumption that the antioxidant activity of heartsease and of garden pansies are mainly due to their flavonoid coponents. On the other hand, no correlation was observed between the antioxidant capacity and the anthocyanidin content.

12. According to our study garden pansies provide a more valuable flavonoid source than heartsease. This fact –supported by further examinations– may bear also therapeutical significance.

Table 2: The antocyanidin and flavonoid contents, as well as the antioxidant capacity of heartsease and garden pansies of different petal color.

		Anthocyanidin content (g cyanidin-3-glucoside/ 100 g sample)	Flavonoid content (g rutin/ 100 g sample)	IC_{50} value (g/ml)
Violet pansy	flower	1.52 ± 0.06	1.21 ± 0.07	(1.57 ± 0.05)x 10^{-5}
	leaf	0.05 ± 0.002	0.10 ± 0.006	(3.86 ± 0.18)x10^{-5}
	herb	0.31 ± 0.01	0.38 ± 0.02	(2.92 ± 0.04)x10^{-5}
Violet-white pansy	flower	0.19 ± 0.01	2.58 ± 0.15	(8.59 ± 0.25)x10^{-6}
	leaf	0.04 ± 0.002	0.03 ± 0.002	(3.18 ± 0.09)x10^{-5}
	herb	0.05 ± 0.004	0.62 ± 0.03	(1.99 ± 0.10)x10^{-5}
White pansy	flower	0.09 ± 0.004	2.01 ± 0.12	(6.35 ± 0.08)x10^{-6}
	leaf	0.06 ± 0.002	0.16 ± 0.01	(5.32 ± 0.12)x10^{-5}
	herb	0.08 ± 0.003	0.59 ± 0.03	(1.42 ± 0.06)x10^{-5}
Yellow pansy	flower	0.31 ± 0.01	2.93 ± 0.18	(6.98 ± 0.28)x10^{-6}
	leaf	0.11 ± 0.004	0.11 ± 0.007	(4.00 ± 0.09)x10^{-5}
	herb	0.11 ± 0.006	0.62 ± 0.04	(1.87 ± 0.05)x10^{-5}
Heartsease	herb	0.02 ± 0.0008	0.50 ± 0.03	(4.17 ± 0.10)x10^{-5}
Rutin				(0.12 ± 0.01)x10^{-5}

4 List of publications

4.1 Publications related to the dissertation

1. **Viktoria Vukics**, Barbara Hevesi Toth, Thomas Ringer, Krisztina Ludanyi, Agnes Kery, Guenther K. Bonn, and Andras Guttman. Quantitative and qualitative investigation of the main flavonoids in heartsease (*Viola tricolor* L.). *J. Chrom. Sci.*, 46:97–101, 2008.

2. **Viktoria Vukics**, Agnes Kery, Gunther K. Bonn, and Andras Guttman. Major flavonoid components of heartsease (*Viola tricolor* L.) and their antioxidant activities. *Anal. Bioanal. Chem.*, 390:1917–1925, 2008.

3. **Viktoria Vukics**, Thomas Ringer, Agnes Kery, Guenther K. Bonn, and Andras Guttman. Analysis of heartsease (*Viola tricolor* L.) flavonoid glycosides by micro-liquid chromatography coupled to multistage mass spectrometry. *J. Chromatogr. A*, 1206:11–20, 2008.

4. **Viktoria Vukics**, Agnes Kery, and Andras Guttman. Analysis of polar antioxidants in heartsease (*Viola tricolor* L.) and garden pansy (*Viola x wittrockiana* Gams.). *J. Chrom. Sci.*, 46:823–827, 2008.

5. **Viktoria Vukics**, and Andras Guttman. Structural characterization of flavonoid glycosides by multi-stage mass spectrometry. *Mass Spectrom. Rev.*, Published Online: Dec 30 2008. DOI:10.1002/mas.20212

4.2 Other publications

1. **Vukics Viktória** és Kéry Ágnes. Metodikai gondok és lehetőségek flavonoid hatóanyagú kivonatok standardizálásánál. *Acta. Pharm. Hung.*, 75:133–139, 2005.

2. **Viktoria Vukics**, Barbara Hevesi Toth, Adam Fukasz, and Agnes Kery. Impact of flavonoid composition of medicinal plants: Difficulties in selecting an LC method. *Chromatographia Suppl.*, 63:93–100, 2006.

3. Barbara Hevesi Toth, Andrea Balazs, **Viktoria Vukics**, Eva Szoke, and Agnes Kery. Identification of *Epilobium* species and willowherbs (Onagraceæ) by HPLC analysis of flavonoids as chemotaxonomic markers. *Chromatographia Suppl.*, 63:119–123, 2006.

4.3 Talks

1. **Vukics Viktória**, Fukász Ádam, Blázovics Anna, Kéry Ágnes. XI. Magyar Gyógynövény Konferencia, Dobogókő, 2005. okt. 13-15: A vadárvácska és izolált fenoloidjainak antioxiáns hatása

2. **Viktoria Vukics** and Agnes Kery. II. PhD. Joint Meeting on Biomedical Sciences, Budapest, 6-7 November 2005: Antioxidant activity of wild pansy (*Viola tricolor*) L. and its phenolic constituents

4.4 Posters

1. Agnes Kery, Pal Apati, Andrea Balazs, Ildiko Papp, Eva Nagy, **Viktoria Andrasek**, Eva Szoke, and Anna Blazovics. 3. World Congress on Medicinal and Aromatic Plants, Thailand, Chiong Mai, 3-7 February 2003: Antioxidant Activity of Medicinal Plants in Different Systems. PP04-11

2. **Andrasek Viktória**, Apáti Pál, Balázs Andrea, Blázovics Anna, Papp Ildikó és Kéry Ágnes. Congressus Pharmaceuticus Hungaricus XII., Budapest, 2003. máj. 8-10. : Flavonoid tartalom, összetétel és antioxidáns hatás *Viola* fajokban. P-2

3. Hevesi T. Barbara, Balázs Andrea, **Vukics Viktória**, Szőke Éva és Kéry Ágnes. Semmelweis Egyetem PhD. Tudományos napok, Budapest, 2005. ápr. 14-15. : A kisvirágú füzike flavonoid összetételének és antioxidáns hatásának vizsgálata. PII/2

4. **Viktoria Vukics**, Anna Blazovics, Adam Fukasz, Agnes Kery. 1st BBBB Conference on Pharmaceutical Sciences, Siofok, 25-28 September 2005: The antioxidant activity of *Viola tricolor* L. measured in different *in vitro* antioxidant systems. P-58

5. Barbara Hevesi Toth, Andrea Balazs, **Viktoria Vukics**, Eva Szoke, and Agnes Kery. 1st BBBB Conference on Pharmaceutical Sciences, Siofok, 25-28 September 2005: Flavonoid composition and antioxidant capacity of willow-herb. P-18

6. Agnes Kery, Bela Simandi, Ildiko Papp, Aniko Gava, **Viktoria Vukics**, Eva Lemberkovics, and Eva Szoke. 1st BBBB Conference on Pharmaceutical Sciences, Siofok, 25-28 September 2005: Quality of medicinal plant products prepared by supercritical fluid extraction. P-23

7. **Vukics Viktória**, Blázovics Anna, Fukász Ádam, Hevesi T. Barbara, Kéry Ágnes. A Magyar Szabadgyök-kutató Társaság III. Konferenciája, Debrecen, 2005. okt. 13-15. : A vadárvácska *Viola tricolor* L. antioxidáns hatásásnak vizsgálata.

8. **Viktoria Vukics** and Agnes Kery. Lippay János – Ormos Imre – Vas Károly Tudományos Ülésszak, Budapest, 2005. okt. 19-21. : A vadárvácska *Viola tricolor* L. és néhány kertészeti árvácska faj összehasonlító vizsgálata. page 142

9. **Vukics Viktória**, Tory Kálmán, Kolonics Attila és Kéry Ágnes. Congressus Pharmaceuticus Hungaricus XII., Budapest, 2006. máj. 25-27. : Vadárvácska frakciók összetételének és biológiai hatásának vizsgálata. P-101

10. Alberti Ágnes, **Vukics Viktória**, Hevesi T. Barbara és Kéry Ágnes. Congressus Pharmaceuticus Hungaricus XII., Budapest, 2006. máj. 25-27. : Áfonya fajok fenoloidjainak ósszehasonlító vizsgálata. P-87

11. Gáva Anikó, Simándi Béla, Szarka Szabolcs, **Vukics Viktória**, Szőke Éva és Kéry Ágnes. Congressus Pharmaceuticus Hungaricus XII., Budapest, 2006. máj. 25-27. : Betulin és betulinsav vizsgálata *Alnus*, *Betula* és *Platanus* fajokban. P-89

12. **Viktoria Vukics**, Thomas Ringer, Agnes Kery, Gunther Bonn, and Andras Guttman. 8th Horvath Medal Award Symposium, Innsbruck, Austria,14-15. April 2008. : LC-MS^n analysis of flavonoid glycosides in heartsease (*Viola tricolor* L.) 37B