Phytochemical and *in vitro* biological evaluation of potentially active compounds in *Epilobium* species

Thesis of doctoral (Ph.D.) dissertation

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SUMMARY

*Epilobium* species (willow-herbs) are common members of the Hungarian flora. Their blooming, perennial shoots are used as traditional medicine in the therapy of prostatic diseases, especially in benign prostatic hyperplasia (BPH). Inspite of good therapeutic experiences, none of the species are registered neither in the Hungarian (Ph.Hg. VIII.) nor in the European (Ph. Eur. 6.) Pharmacopoeia. Due to the lack of adequate amount of *in vitro* and *in vivo* studies regarding the mechanism of action, *Epilobium* species may be less popular among other medicinal herbs used in the same indication. On the other hand, macromorphology of *Epilobium* species is very similar and mistaken identity is recurrent, what increases the uncertainty of their use even more.

Aim of our study was to improve the knowledge of *Epilobium* species, through comparative phytochemical analysis and *in vitro* studies, modeling the pathological process, on mechanism of action.

We have investigated five *Epilobium* species, commonly occurring in Hungary: *E. parviflorum* Schreb., *E. roseum* Schreb., *E. tetragonum* L., *E. montanum* L., *E. angustifolium* L. Both the collected and cultivated plant material was examined and identified by cautious macro- and micromorphological methods. During phytochemical analysis, we have mainly focused on revelation of the possibly potent substances: phenoloids and sterols. After thin-layer chromatographic (TLC) examination, we have elaborated a new, high performance liquid chromatographic (HPLC-UV, HPLC-MS/MS) method for the analysis of willow-herb phenoloids. Due to „HPLC fingerprints“ -which revealed similarities and differences in phenoloid composition of species- 16 components, among them 11 flavonol-glycosides, have been identified. A macrocyclic tannin, oenothein B, considered as active compound, has been observed in all samples. Explored fragmentation patterns of oenothein B resulted in completely new data on its mass-spectrometric analysis. Quantitative data have been published on total polyphenol (22.3-34.8 g/100g herb), tannin (18.7-25.8g/100g herb) and flavonoid (0.69-0.83g/100g herb) content of *Epilobium* species [1, 2].

Sterol composition of species has been studied by TLC and gas chromatographic (GC-MS) methods. β-sitosterol, a pharmacologically important compound, was the sole identifiable sterol component in n-haxane extract of samples, whose quantitative content in *E. parviflorum* (0.13±0.02g/100g herb) has been published first.

We have compared the *in vitro* antioxidant capacity of the polar extract of species by two different spectrophotometric methods (ABTS, DPPH), and a significant activity of *E. parviflorum* has been established. Elements of BPH pathomechanism have been *in vitro* simulated in order to reveal the mechanism of action of *E. parviflorum*.
Our team was the first to prove the lipid-peroxidation inhibitory (IC$_{50}$: 2.37mg/ml) effect of *E. parviflorum* in TBA assay and also its antioxidant cell-protective effect, comparable to that of catalase enzyme on fibroblast cells. The anti-inflammatory effect of *E. parviflorum* has been confirmed by verifying its COX enzyme inhibitory action (IC$_{50}$: 1.4μg/ml) [3]. We have investigated the aromatase enzyme inhibitory effect of the extract (0.32-3.2μg/ml) on choriokarcinomic placenta cells, however the study applied has not provided significant results to verify this activity.

We have investigated the *E. parviflorum* extracts agonistic and antagonistic action on steroid receptors, and we were the first to state that the willow-herb extracts has not been acted on steroid receptors in the concentration range examined (0.01-1000μg/ml) [4].

Since there is no evidence on the components which are undoubtedly responsible for beneficial effect, it is not possible to draw conclusion from the revealed variances occurring in phytochemical composition of *Epilobium* species to the possible differences in biological effect or the replace ability. Although, it can be established that simultaneous application of botanical and phytochemical examination is essential. Investigation of „HPLC fingerprint” of flavonoids is particularly important, especially in case of *E. angustifolium*.

*E. parviflorum* has the highest antioxidant capacity among the species examined, however further comparative investigations are necessary to prove other selective activities of the species.

Based on our *in vitro* studies *E. parviflorum* possess multi factorial antioxidant and anti-inflammatory (COX inhibitory) effects, but does not influence the steroid homeostasis on receptorial level.

1. Introduction

Herbal medicines are used the most in the therapy of urological diseases. One of the best examples of modern phytotherapy is the treatment of benign prostatic hyperplasia (BPH). Proper herbal extracts are often first-chosen remedies in initial state of the disease and later they could beneficially complement the medical treatment.

Blooming, aerial shoots of *Epilobium* (willow-herb) species (*Onagraceae*), common members of the Hungarian flora, have been used for very long time in Central Europe. Their modern application does not differ from their traditional usage: *Epilobium* infusion (tea) is recommended in case of prevention and complementary treatment of prostatic disorders, especially BPH. Inspite of good therapeutic experiences, none of the species is registered neither in the Hungarian (Ph.Hg. VIII.) nor in the European (Ph. Eur. 6.) Pharmacopoeia. Due to the lack of adequate amount of *in vitro* and *in vivo* studies regarding the mechanism of action, *Epilobium* species may be less popular among other medicinal herbs used in the same indication. On the other hand, macromorphology of *Epilobium* species is very similar and mistaken identity is recurrent, what increases the uncertainty of their use even more.

2. Objectives

Main object of our study was to investigate the most common *Epilobium* species in Hungary from the point of phytochemical composition and their mechanism of biological action. We would like to contribute to the extension of knowledge in Hungarian *Epilobium* species and promote the evidence based application of the proper quality willow-herb drugs.

First of all, we aimed to supply reliable plant material for the study, by collecting and harvesting, and to identify them by macro- and micromorphological examination.

Regarding the potential beneficial effect of the phenoloids and fitosterols, we aimed to investigate mainly these groups of compounds by modern phytoanalitical methods (TLC, HPLC-MS/MS, GC-MS).
Knowing the phytochemical similarities and differences of *Epilobium* species, we aimed to compare willow-herbs with respect to their antioxidant capacity, to select the most potent sample and to subject the chosen species to further *in vitro* studies.

To reveal the mechanism of biological action of *Epilobium* extracts, our purpose was to apply *in vitro* studies suitable to simulate some parts of the BPH pathomechanism. Among our intention was investigation of antioxidant capacity, anti-inflammatory (COX inhibitory) action and the steroid hormone receptor agonistic- and antagonistic activity of *Epilobium* extracts.

### 3. Materials and methods

#### 3.1. Plant material


#### 3.2. Botanical examination

Due to the similar external habit of *Epilobium* species, their distinction is rather difficult. The reliable identification requires detailed macro- and micromorphological examination. Morphological studies were performed on fresh plant material, right after gathering.

#### 3.3. Preparation of extracts

The most common application form of *Epilobium* is the herbal tea, i.e. water infusion, that was prepared by 20 min long steeping. Filtered extracts were left to cool down, protected from light, afterwards they were frozen and freeze-dried as soon as possible.

Acetonic (80% v/v) extracts suitable for examination of polar compounds of *Epilobium* were prepared by extraction in ultrasonic bath, and afterwards evaporated to dryness under reduced pressure.
Hexane (n-hexane) extracts suitable for examination of apolar compounds of *Epilobium* were prepared by Soxhlet extractor and afterwards evaporated to dryness under reduced pressure.

All samples employed in phytochemical and *in vitro* studies were prepared from the dried extracts.

3.4. Phytochemical analysis

3.4.1. Chromatographic methods

- **Thin layer chromatographic (TLC) examination of flavonoid composition** in acetonic extracts: Layer: Kieselgel 60F$_{254}$, 100x200mm; Eluent mixture: etil-acetate : formic acid : acetic acid : water (100:11:11:26); Evaluation: after spraying with Naturstoff (1% methanolic diphenylboric acid β-ethylamine ester) and poly-ethylene-glycol (PEG) reagents.

- **Comparative high performance liquid chromatographic (HPLC) examination of water and acetonic extracts**: HPLC-DAD apparatus: Jasco UV-970 HPLC system; Column: 5μm, Supelcosil LC-18 (250x4.6mm); Eluents: acetonitrile (ACN) – 2.5% (v/v) acetic acid.

- **HPLC-MS/MS examination of *Epilobium* polyphenols** in acetonic extracts: HPLC-DAD-MS/MS apparatus: Agilent 6410, triple quadrupole MS analyzer coupled Agilent 1100 HPLC system; Column: 5μm, Supelcosil LC-18 (250x4.6mm); Eluents: acetonitrile (ACN) – 2.5% (v/v) acetic acid; Electrospray ionization (ESI) settings: negative ion mode; Mass domain recorded (m/z): 50-1600 (m/z).

- **TLC study of β-sitosterol content**: Layer: Kieselgel 60F$_{254}$, 100x100mm; Eluent mixture: n-hexane: ethyl-acetate (3:1); Evaluation: after spraying with cerium-sulphate reagent.

- **Gas chromatographic (GC-MS) examination of *Epilobium* phytosterols** in hexane extracts: GC apparatus: Agilent 6890N; MS detector: Agilent 5973N; Column: Agilent HP-5MS capillary ((5%-phenyl)-methyl-polysiloxane, 30m x 250μm x 0.25μm); Electro-impact ionization: 70eV; Mass domain recorded (m/z): 40-600 (m/z); Collision energy: 10-60kV.
3.4.2. Quantitative studies

- **Flavonoid content** was determined according to the method described in the „Solidaginis herba” item of the Hungarian Pharmacopoeia (Ph. Hg. VIII.), equivalent to the European Pharmacopoeia (Ph. Eur. 6.).

- **Total polyphenol and tannin content** was determined according to the method described in the Hungarian Pharmacopoeia (Ph. Hg. VIII.), equivalent to the European Pharmacopoeia (Ph. Eur. 6.).

- **β-sitosterol content of *Epilobium parviflorum***: GC apparatus: Agilent 6890N; FID detector (330°C, suitable for quantitative analysis); Column: Agilent DB-5MS (divder liquid: (5%-phenyl)-methylpolysiloxane, 25m x 200μm x 0.33μm); Internal standard: 5α-cholestan-3on (12.65 μg/ml).

3.5. *In vitro* studies of biological action

Elements of the complex pathomechanism of BPH have been *in vitro* simulated in order to reveal the way of action of *Epilobium* extracts. The sample investigated in expedient *in vitro* studies was selected based on the results of the comparative antioxidant capacity assay.

- **Antioxidant capacity assay** was performed by help of ABTS (2,2’-azinobis-(3-ethyl-benzothiazoline-6-sulphonic acid)) and DPPH (2,2-diphenyl-1-pikrylhydrazil) „stable” free radical compounds, in two different spectrophotometric methods.

- **Lipid peroxidation inhibitory** effect of *Epilobium parviflorum* was tested in thiobarbituric acid (TBA) assay by measuring the malondialdehyde quantity released during lipid peroxidation. Membrane lipid liposomes were prepared from calf brain extracts.

- **Antioxidant cellprotective effect** of *E. parviflorum* was investigated on fibroblast cells (143RB) after a pro-oxidant treatment, with hydrogen-peroxide, and a pre- and simultaneous treatment with *Epilobium* extracts. Positive control: catalase enzyme; Evaluation: after Neutral Red cell dyeing.

- **COX inhibitory action** of *E. parviflorum* was examined on macrophage cells (RAW 264.7) by measuring the released amount of PGE₂. Quantification of PGE₂ was performed based on an immunological competitive binding technique, offered by PGE₂ assay kit (R&D Systems).
- **Oestrogen-/androgen receptor binding activity** of *E. parviflorum* was studied on genetically modified yeast cells (*Saccharomyces cerevisiae*). The receptorial binding was traceable by spectrophotometrical observation of the β-galactozidase released.

- **Anti-oestrogen/ anti-androgen activity** of *E. parviflorum* was investigated with the same method used in steroid receptor binding assay, but in constant presence of steroid receptors ligands (17β-oestradiol, dihydro-testosterone).

- **Aromatase enzyme inhibitory assay** was performed on choriocarcinomic placenta cells (JEG3). The aromatase activity was examined by measuring the $^3$H release in media after conversion of radiolabeled androstendione. We could conclude to aromatase activity from the radioactive intensity of the medium.

### 4. Results

#### 4.1. Botanical examination

Due to cautious macro- and micromorphological examination, we have reliably identified the plant material employed and contributed to the extension of the image database of *Epilobium* species.

#### 4.2. Phytochemical analysis

##### 4.2.1. TLC examination of flavonoids

Based on the results of TLC study, all of the *Epilobium* species examined are rich in flavonoid components. One definite variation was observed in case of *Epilobium angustifolium* which main flavonoid compound, in contrast with other examined species, was not myricitrin, but a quercetin-glycoside. Flavonoid composition of further species did not show remarkable diversity based on TLC, except some individual difference in the ratio of components.

##### 4.2.2. Comparative high performance liquid chromatographic (HPLC) examination of water and acetonic extracts

The traditional and most common application form of *Epilobium* is still the herbal tea. For this reason we have found it necessary to compare the HPLC fingerprint of water
extracts and that of acetonic extracts, which are easier to handle in laboratory work. Based on our results, composition of acetonic extracts does not differ from the water extracts’ in respect to the main compounds, besides acetonic extracts were found to be richer in some minor components.

4.2.3. HPLC-MS/MS examination of *Epilobium* polyphenols

The HPLC–MS/MS examination of *Epilobium* species resulted in detection of 20 diverse and distinctive components; thus far 16 of them have been identified. *Epilobium* species contain these components in various combinations and ratios. Oenothein B, the potentially active compound, was present in all species examined. Explored fragmentation patterns of this macrocyclic tannin component resulted in completely new data on its mass-spectrometric analysis. Oenothein B was detected with two different molecular ion forms: a double charged ((m/z)-783Da ([M-2H]^2-)) and a single charged ((m/z)-1567Da)) form, which fragmentation patterns have been first published.

Table 1.: Components detected in *Epilobium* samples.

<table>
<thead>
<tr>
<th>Component</th>
<th>Rt (min)</th>
<th>[M-H]^(-)</th>
<th>Product ions (m/z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Oenothein B</td>
<td>3.0 - 7.3</td>
<td>1567 / 783</td>
<td>915, 765, 450, 301</td>
</tr>
<tr>
<td>2. Ellag- and gallotannin derivatives</td>
<td>3.0 - 7.6</td>
<td>1473, 1208, 1065, 923</td>
<td>-</td>
</tr>
<tr>
<td>3. Caffeic acid – pentose ester</td>
<td>5.9</td>
<td>311</td>
<td>179, 149, 135</td>
</tr>
<tr>
<td>4. Chlorogenic acid</td>
<td>7.2</td>
<td>353</td>
<td>191, 135</td>
</tr>
<tr>
<td>5. Not identified</td>
<td>9.2</td>
<td>381</td>
<td>300, 283, 229, 185</td>
</tr>
<tr>
<td>6. Myricetin-3-O-hexose-gallate</td>
<td>11.8</td>
<td>631</td>
<td>479, 316, 151</td>
</tr>
<tr>
<td>7. Myricetin-3-O-hexoside</td>
<td>14.05</td>
<td>479</td>
<td>316, 287, 271, 179, 151</td>
</tr>
<tr>
<td>8. Not identified</td>
<td>15.5</td>
<td>615</td>
<td>300, 169</td>
</tr>
<tr>
<td>9. Quercetin-3-O-hexose-gallate</td>
<td>16.4</td>
<td>615</td>
<td>463, 300, 151</td>
</tr>
<tr>
<td>10. Ellagic acid-pentoside</td>
<td>16.5</td>
<td>433</td>
<td>300, 271, 228</td>
</tr>
<tr>
<td>11. Myricetin-3-O-pentoside</td>
<td>16.9</td>
<td>449</td>
<td>316, 287, 151</td>
</tr>
<tr>
<td>12. Myricetin-3-O-rhamnoside</td>
<td>17.3</td>
<td>463</td>
<td>316, 271, 179, 151</td>
</tr>
</tbody>
</table>
High ratio of macrocyclic ellag- and gallotannins was characteristic in all *Epilobium* species examined. Myricetin, quercetin, kaempferol and their various glycosides were dominant in samples, but their combination and ratio were distinctive in all cases. The most remarkable difference was observed in case of *E. angustifolium* which, in contrast with other species, exclusively contained flavonol-glucuronide components, and its main flavonoid compound was identified as quercetin-glucuronide.

### 4.2.4. Quantitative determination of the flavonoid-, total polyphenol- and tannin content

Based on our results, there was no significant difference between the flavonoid content of *Epilobium* species examined (0.73-0.83g/100g herb), but decided variances were measured in the total polyphenol and tannin content of samples (total polyphenol: 22.3-34.8g/100g dried herb, tannin: 18.7-25.8g/100g dried herb) (Table 2.).
Table 2.:  
Flavonoid-, total polyphenol- and tannin content *Epilobium* samples examined

<table>
<thead>
<tr>
<th>Sample</th>
<th>Flavonoid content (hyperoside)</th>
<th>Total polyphenol content (g/100g dried herb)</th>
<th>Tannin content (pyrogallol)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. parviflorum</em></td>
<td>0.83 ± 0.03</td>
<td>34.8 ± 0.7</td>
<td>25.8 ± 0.9</td>
</tr>
<tr>
<td><em>E. angustifolium</em></td>
<td>0.69 ± 0.02</td>
<td>22.3 ± 0.9</td>
<td>18.7 ± 0.8</td>
</tr>
<tr>
<td><em>E. roseum</em></td>
<td>0.82 ± 0.03</td>
<td>28.6 ± 1.0</td>
<td>21.4 ± 0.8</td>
</tr>
<tr>
<td><em>E. montanum</em></td>
<td>0.80 ± 0.04</td>
<td>30.2 ± 0.9</td>
<td>25.2 ± 0.9</td>
</tr>
<tr>
<td><em>E. tetragonum</em></td>
<td>0.73 ± 0.04</td>
<td>25.8 ± 1.0</td>
<td>18.7 ± 0.9</td>
</tr>
</tbody>
</table>

4.2.5. Gas chromatographic (GC-MS) examination of *Epilobium* phytosterols

The literature refers to the phytosterol content of *Epilobium* species but modern phytochemical studies have not been performed thus far. Remarkable variances could not been observed between the hexane extracts of the species examined. Long-chained fatty acids and alcohols were dominant in samples and the only detectable phytosterol compound was β-sitosterol.

4.2.5. β-sitosterol content of *Epilobium parviflorum*

As in almost all the herbal remedies used in the treatment of BPH, *Epilobium* contains β-sitosterol, but quantitative particulars have been unknown so far. Due to the two-step extraction method (first with n-hexane, than with ethanol) we were able to determine the free and the glycoside- or ester-bounded β-sitosterol content of *E. parviflorum* sample. Distinction of the free and the bounded forms may be important because of their different pharmacokinetic properties.

Based on our results, the β-sitosterol content of *E. parviflorum* was 0.13 ± 0.02 (g/100g dried herb), and more than 15% of this was present in glycoside- or ester-bounded form. On the standard deviation, the repeatability and accuracy measurements, the gas chromatographic method applied is reliable and accurate.
4.3. In vitro studies of biological action

4.3.1. Antioxidant capacity

Two spectrophotometric assays (ABTS, DPPH) were employed to determine the antioxidant capacity of Epilobium samples. Results obtained from the two methods correlated well. Interesting observation, that roughly twice as much sample concentration was necessary in all cases to eliminate the DPPH free radicals than in case of ABTS. On the results of the antioxidant capacity assay Epilobium extracts possess significantly high radical scavenger activity (EC$_{50}$ value was between 1.71μg/ml and 3.00μg/ml). All samples examined were good antioxidants, comparable to Trolox or ascorbic acid (Trolox EC$_{50}$: 7.96 ± 0.238μM; ascorbic acid EC$_{50}$: 14.29 ± 0.43μM). Among species, Epilobium parviflorum showed the highest antioxidant capacity (1.71 ± 0.05μg/ml), therefore this sample was selected for further expedient in vitro studies.

4.3.2. Lipid peroxidation inhibitory effect

Lipid peroxidation inhibitory effect of E. parviflorum extracts have been first examined in TBA assay. Based on our results, the extracts showed concentration-dependent inhibition of lipid peroxidation at doses over 0.20mg/mL (IC$_{50}$ acetone: 2.37 ± 0.12mg/mL; IC$_{50}$ water: 8.11±0.23mg/ml) but at lower concentrations, both extracts seemed to be pro-oxidant. The positive control, propyl-gallate (10$^{-4}$M) showed 50% inhibition under the same conditions.

4.3.3. Antioxidant cellprotective effect

The viability of cultured, human fibroblast cells was measured after pro-oxidant treatment, with hydrogen-peroxide, in the absence and presence of E. parviflorum extracts. In the concentration range examined the extracts exerted protective effect against the oxidative damage (IC$_{50}$ water: 3.3μg/ml, IC$_{50}$ acetone: 5.2μg/ml). The effect was steady and concentration-dependent and the protective action was comparable to that of catalase enzyme (250IU/ml) which protected 64% of the cells under the same conditions. Pretreatment seemed to be less efficient than simultaneous treatment, with extract and peroxide, and than pre- and simultaneous treatment together. Destruction of cells caused by pro-oxidant impact and the protective effect of Epilobium extracts could also be observed visually.
4.3.4. COX enzyme inhibitory effect

COX enzyme inhibitory effect of *E. parviflorum* extracts was investigated on macrophage cells. In the study applied, *Epilobium* extracts decreased the PGE$_2$ release of cells, thus showing concentration-dependent COX-enzyme inhibitory action (IC$_{50}$ acetone: 1.4±0.1µg/mL IC$_{50}$ water: 5.5±0.2µg/mL). Our results have affirmed the anti-inflammatory effect of *E. parviflorum* extracts. Under similar experimental conditions, the positive control indomethacin suppressed the LPS-stimulated PGE$_2$ release on macrophages with an IC$_{50}$ value of 21±3µM.

4.3.5. Oestrogen-/androgen receptor binding activity

Direct steroid receptor binding ability of *E. parviflorum* extracts was first investigated. The study applied is straightforward and offers prompt results in the oestrogen- or androgen receptor binding activity. According to our results, none of the extracts (water, acetone, n-hexane) showed steroid receptor binding activity in the concentration range examined (0.01-1000µg/ml).

4.3.6. Anti-oestrogen/ anti-androgen activity

Based on our results *E. parviflorum* extracts, in the concentration range examined (0.01-1000µg/ml), did not antagonized the receptor binding of steroid ligands, therefore did not show anti-oestrogen or anti-androgen effect.

4.3.7. Aromatase inhibitory assay

Aromatase inhibitory effect of *E. parviflorum* extracts (0.32-3.2µg/ml) was first investigated. Letrozol, a known aromatase inhibitor, was used as positive control. The assay applied, did not furnish significant data to verify the activity. Standard deviation values of the results obtained might indicate a methodical error, therefore additional revision of the measurement is necessary.
Conclusion

Since there is no evidence on the sure components responsible for beneficial effect, it is not possible to draw conclusion from the revealed variances occurring in phytochemical composition of *Epilobium* species to the possible differences in biological effect or the replace ability. Although, it can be established that simultaneous application of botanical and phytochemical examination is essential. Investigation of „HPLC fingerprint” of flavonoids is particularly important, especially in case of *E. angustifolium*.

*E. parviflorum* has the highest antioxidant capacity among the species examined, however further comparative investigations are necessary to prove other selective activities of the species.

Based on our *in vitro* studies *E. parviflorum* possess multifactorial antioxidant and anti-inflammatory (COX inhibitory) effects, but does not influences the steroid homeostasis on receptorial level.
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Article:

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Antioxidant and antiinflammatory effect of Epilobium parviflorum Schreb. Phytotherapy Research, 23: 719-724;

Identification of Epilobium Species and Willow-herbs (Onagraceae) by HPLC Analysis of Flavonoids as Chemotaxonomic markers. Chromatographia, 63: 119-123;

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Az Epilobium parviflorum kivonat hatásmechanizmusának in vitro vizsgálata. Acta Pharmaceutica Hungarica, 79: 3-9;

A kisvirágú füzike (Epilobium parviflorum Schreb.) és néhány Magyarországon gyakran előforduló füzike faj botanikai és fitokémiai összehasonlítása. Kertgazdaság különkiadás: A fajtaválaszték fejlesztése a kertészetben, 229-235;

Oral presentation:

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Az Epilobii herba fitoterápiás értékelése. Semmelweis Egyetem PhD Tudományos Napok, Budapest;
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A kisvirágú füzike (*Epilobium parviflorum* Schreb.) és néhány Magyarországon gyakran előforduló füzike faj botanikai és fitokémiai összehasonlítása – Lippay János – Ormos Imre – Vas Károly Tudományos Ülésszak, Budapest;

Antioxidáns flavonoidok vizsgálata kisvirágú füzike mintákban. A MTA Flavonoidkémiai Munkabizottságának tudományos előadóülése, Budapest.

*Poster presentation:*

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Poliphenol Composition and Antioxidant Capacity of Some *Epilobium* Species - 19th International Symposium on Pharmaceutical and Biomedical Analysis, 2008, Gdansk, Lengyelország;


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