Pharmacological screening of Polygonaceae species and isolation of biologically active compounds from *Rumex aquaticus* L. and *Rumex thyrsiflorus* Fingerh.

Summary of PhD Thesis

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University of Szeged

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INTRODUCTION

According to WHO, of the 56.4 million deaths worldwide in 2015, more than half (54%) were due to 10 most common causes. Ischaemic heart disease and stroke have remained the leading causes of death globally in the last 15 years. The third most common cause is lower respiratory infections while the others are chronic obstructive pulmonary disease, lung cancer (along with trachea and bronchus cancers), diabetes, dementias, diarrhoeal diseases, tuberculosis and road injuries.

Despite the wide spectra of antibacterial pharmaceutics, even more people are dying in consequence of bacterial infections. The uncontrolled usage of antibiotics may increase the selection pressure of resistant strains. The hospital-acquired infections are still one of the major problems of modern medicine and often caused by methicillin-resistant Staphylococcus aureus (MRSA). It can cause wound, lower respiratory and urinary infections or septicaemia. Severe infections are more common in intensive care units and in older population, which can elongate their hospital stays and increase the therapeutic costs. Besides MRSA, several bacterial strains, including Staphylococcus epidermidis, Moraxella catarrhalis, Streptococcus pyogenes, Pseudomonas aeruginosa, Escherichia coli and Klebsiella pneumoniae, can cause nosocomial infections.

Xanthine oxidase (XO) is an enzyme present in significant concentrations in the gastrointestinal tract and liver. It is responsible for the metabolism of hypoxanthine and xanthine to uric acid in the purine catabolic pathway, yielding superoxide radicals. XO is an important source of $O_2^-$ and has been reported in various pathological processes, e.g. in various forms of ischaemic tissue and vascular injuries, inflammatory diseases, stroke, diabetes mellitus, rheumatic disease, liver disorders, renal failure and chronic heart failure. Moreover, excessive levels of uric acid in the blood can cause gout. XO inhibitors are able to hinder the synthesis of uric acid in the organism and, as anti-inflammatory agents, can alleviate the symptoms of inflammation-associated diseases.

Globally, stroke is the second leading cause of death above the age of 60, and millions are left permanently disabled. Stroke therapy has been diminutive at best, with recombinant tissue plasminogen activator, but only a subset of the population qualifies for this therapy, and it also suffers from the crippling limitation of a three hour therapeutic window starting from the onset of stroke symptoms. It has an urgent need to develop
effective neuroprotective agents that would prevent the occurrence and/or aid recovery from stroke, thereby tremendously reducing the societal and economic costs associated with it.

It has been recognized since ancient times that nature is a potential source of pharmacologically important drugs. This has resulted in the use of a large number of medicinal plants to treat various diseases, and some medicaments in Western medicine (e.g. atropine, morphine, quinine and digitoxin) are based on the traditional use of such drugs.

Plants belonging in the family Polygonaceae are known to produce a large number of biologically important secondary metabolites, e.g. anthraquinones, naphthalenes, stilbenoids, and flavonoids. Different plant parts are used in traditional medicine for the treatment of several disorders (e.g. infections, inflammation, diarrhoea, constipation, mild diabetes and jaundice). The genus *Rumex* has attracted the attention of many investigators because of its phytoconstituents and medicinal properties. The extracts of these plants, and compounds isolated from them, have been demonstrated to possess various pharmacological activities, including anti-inflammatory, antioxidant, antitumor, antibacterial, antiviral and antifungal properties *in vitro* and *in vivo*.

**AIMS OF THE STUDY**

A few years ago, the research group of the Department of Pharmacognosy at the University of Szeged started a screening programme to investigate the pharmacological activities of species belonging to the family Polygonaceae and to identify the bioactive compounds of the selected plants. In the course of the work, different pharmacological screenings were performed with plants of the Polygonaceae family, especially members of the genus *Rumex*.

In order to achieve these aims, the main tasks were:

- A review of the literature on the genus *Rumex*, from aspect of the chemistry and pharmacological properties of the plants.
- Extraction of plant materials of *Rumex* species with various solvents for the screening, and investigation of the xanthine oxidase inhibitory and antibacterial activities of the extracts.
• Identification of the bioactive secondary metabolites of *Rumex aquaticus*: isolation, structure elucidation and *in vitro* evaluation of antibacterial, XO-inhibitory and neuroprotective potentials of the extracts and/or isolated compounds.

• Phytochemical and pharmacological analysis of *Rumex thyrsiflorus*: isolation, structure determination of the compounds and *in vitro* antibacterial evaluation of the extracts and compounds.

**MATERIALS AND METHODS**

Plants were collected between June and September 2010 (*R. aquaticus* was collected in July 2012), in several regions of the Carpathian Basin (Hungary and Romania).

For the antimicrobial and the XO inhibitory screening assays, extracts were prepared from air-dried plant materials (roots, herb, leaves and flowering parts) with MeOH, and then the solutions were evaporated to dryness under vacuum. The residues were dissolved in 50% aqueous MeOH and then solvent–solvent partitions were performed between *n*-hexane (extracts A) and CHCl₃ (extracts B), and the residues gave extracts C. After the extraction with MeOH, the residual plant materials were dried and extracted with boiling H₂O. The filtered extracts were freeze-dried, affording extracts D.

The plants for the preparative phytochemical work were processed in a same way. The grinded plant material was percolated with MeOH. Then the extract was concentrated and diluted with H₂O. For the solvent–solvent partition, different solvents with different polarity were used, in order to separate the nonpolar and the polar compounds of the extract. Then the obtained extracts were exposed to further separation methods in order to isolate their potential pharmacologically active secondary metabolites.

The compounds were isolated by multistep chromatographic methods, including open-column chromatography (CC), vacuum-liquid chromatography (VLC), centrifugal partition chromatography (CPC), rotation planar chromatography (RPC), medium-pressure liquid chromatography (MPLC), preparative layer chromatography (PrepTLC), gel filtration (GF) and high-performance liquid chromatography (HPLC). Normal- or reversed-phase SiO₂ or Sephadex LH-20 were applied as stationary phases.

The isolated compounds were characterized and their structures were elucidated by means of different spectroscopic methods (NMR, MS).
Antimicrobial activity of the plant extracts was tested against 11 standard bacterial strains (S. aureus, methicillin-resistant S. aureus, S. epidermidis, Bacillus subtilis, M. catarrhalis, S. pyogenes, Streptococcus pneumoniae, Streptococcus agalactiae, P. aeruginosa, E. coli and K. pneumoniae). The antibacterial screening assay was performed by disc-diffusion method. The active extracts and compounds were further subjected to determine their minimal inhibitory concentrations (MICs) by microdilution method.

The XO inhibitory assay’s method is based on a modified protocol of Sigma, a continuous spectrophotometric rate determination: the absorbance of XO induced uric acid production from xanthine was measured at 290 nm for 3 min on 37 °C in a 96-well plate, using the plate reader FluoSTAR Optima (BMG LABTECH). The XO inhibitory effect was determined via the decreased production of uric acid. Allopurinol served as positive control.

Oxygen Glucose Deprivation (OGD) model – one of the most reliable and frequently used models to induce ischemia – was applied to test the effects of drugs in ischemia. OGD mimics the interruption of blood supply, by depriving the cells of both oxygen and glucose, and is also amenable to study reperfusion injury, which is a common occurrence in clinical settings.

The effects of isolated compounds on cell viability and neurite outgrowth were evaluated under OGD conditions, using rat pheochromocytoma (PC12) cell line as it is a well-established model cell line to study neuroprotection as well as amenable to differentiation, which is beneficial for the neurite outgrowth studies.

Protection of PC12 cells against OGD induced cell death can be used to measure the neuroprotective activity of test agents. For the cell viability assay, PC12 cells were seeded onto 24-well plates. Following a 24 h incubation period for acclimatization, RPMI was replaced with HBSS which contained all standard components except glucose. The cells were then treated with the test compounds (10 µM) and exposed to OGD conditions in an anaerobic glove box for 1 h. Compounds were dissolved in MeOH and appropriate solvent controls were used. A separate 24-well plate with cells in RPMI and no drug treatment served as control. Cell viability assay was performed using MTT cell proliferation assay kit.
For the neurite-outgrowth assay, PC12 cells were differentiated with nerve growth factor (NGF). The differentiated PC12 cells were then subjected to the same OGD treatment schedule outlined in the previous assay and treated with compounds isolated from *R. aquaticus* (1 µM and 10 µM). Following OGD, the HBSS medium was replaced with RPMI and allowed to incubate at 5% CO₂ at 37 °C for 24 h to simulate reperfusion conditions. At the end of the incubation period, cells were washed with 1 × PBS buffer, fixed with freshly prepared 4% para-formaldehyde, and permeabilized using 0.3% Triton X-100 in 1 × PBS. The cells were then incubated with 1% bovine serum albumin fraction V in 1 × PBS at room temperature for 1h. After being washed, the cells were incubated overnight at 4 °C with primary antibody, rabbit anti-synaptophysin, followed by goat anti-rabbit secondary antibody. The cells were then washed with 1 × PBS followed by incubation with phalloidin for 30 min. Phalloidin stains F-actin and serves as a neuronal marker. Following another wash, the coverslips were transferred to slides, mounted with DAPI, and sealed. Fluorescent images of slides were captured using Nikon Eclipse Ti microscope. The images were then analyzed by the automated program NeuriteTracer to assess the neurite length.

**RESULTS AND DISCUSSION**

**Antibacterial properties of *Rumex* species**

Plants belonging to the genus *Rumex* are used worldwide in the traditional medicine for the treatment of various diseases, including bacteria-related dermatologic conditions, bacterial and fungal infections, e.g. dysentery or enteritis.

Our study aimed to screen the antibacterial activity of *Rumex* species, collected in the Carpathian Basin, against standard bacterial strains. The further objective of this work was the isolation of the pharmacologically active components of two active species, *R. aquaticus* and *R. thyrsiflorus*.

The antibacterial effects of *n*-hexane, CHCl₃, aqueous MeOH and H₂O extracts, prepared from different parts of 14 *Rumex* species, were investigated against *S. epidermidis*, *S. aureus*, MRSA, *B. subtilis*, *M. catarrhalis*, *S. pyogenes*, *S. pneumoniae*, *S. agalactiae*, *P. aeruginosa*, *E. coli* and *K. pneumoniae* using the disc diffusion method. From the investigated species, only *R. crispus* and *R. hydrolapathum* were tested
previously for antibacterial activity. Mainly the n-hexane and CHCl₃ extracts, prepared from the roots of the plants, displayed high antibacterial activity (inhibition zones >15 mm) against one or more bacterial strains at 50 mg/mL concentration. From the active fractions, three n-hexane extracts (R. alpinus roots, R. aquaticus roots and R. patientia roots against S. aureus and R. alpinus roots on MRSA); four CHCl₃-soluble fractions (R. acetosa roots on S. epidermidis and S. aureus; R. conglomeratus herbs on M. catarrhalis; R. crispus roots against S. pneumoniae; R. pulcher whole plant on B. subtilis) and two aqueous MeOH extracts (R. crispus herb and R. patientia flowers against S. epidermidis) exerted strong antibacterial activity. None of the H₂O extracts have shown any activity on the investigated microbial strains.

The results of our antibacterial screening have provided important data for selection of Rumex species and their different extracts with potential inhibitory properties against bacteria for detailed pharmacological and chemical experiments.

**Xanthine oxidase inhibitory properties of Rumex species**

In recent years, a number of research groups have commenced explorations of potential XO inhibitors from a wide variety of traditional folk medicines. Numerous studies have dealt with investigations of the XO inhibitory activities of plant extracts used in certain countries for the treatment of hyperuricaemia, and especially gout.

The XO inhibitory activity of H₂O and organic extracts of 14 selected species belonging in Rumex genus occurring in the Carpathian Basin were tested in vitro. A total of 73 extracts prepared with n-hexane, CHCl₃, 50% MeOH or H₂O from different plant parts (aerial parts, leaves, flowers, fruits and roots) were investigated. It was found that the CHCl₃ extracts of R. acetosa, R. acetosella, R. alpinus, R. obtusifolius subsp. subalpinus and R. patientia demonstrated the highest XO inhibitory activity (>85% inhibition) at 400 µg/mL. The IC₅₀ values of the active extracts were also determined. Especially the CHCl₃ extracts (B) of the whole plant of R. acetosella (IC₅₀ = 19.3 ± 3.1 µg/mL), the CHCl₃ extract (B) prepared from the flowers and fruits of R. alpinus (IC₅₀ = 23.4 ± 3.0 µg/mL), the herb extract (B) of R. conglomeratus (IC₅₀ = 23.4 ± 4.0 µg/mL), the root extract (C) of R. hydrolapathum (IC₅₀ = 25.4± 2.2 µg/mL), the flowers extracts (B) and (C) of R. patientia (IC₅₀ = 27.6± 3.3 µg/mL and
18.9 ± 1.2 μg/mL), and the flowers and fruits extract (C) of *R. stenophyllus* (IC$_{50}$ = 27.4 ± 0.4 μg/mL) exhibited high activity against XO.

A comparison of the measured activities with the ethnomedicinal uses of the plants led to the conclusion that our screening results for several *Rumex* species are in accordance with the traditional uses of the plants against gout, inflammatory diseases and chronic heart failure.

Earlier publications on the highly active *R. acetosella* indicated the presence of phenolic compounds and its antioxidant capacity, but its XO inhibitory activity has not been investigated previously. However, the ability of inhibiting XO is strongly connected with the antioxidant capacity, since reactive oxygen species are produced during the formation of uric acid in the presence of XO.

**Isolation of biologically active secondary metabolites of *R. aquaticus* and *R. thyrsiflorus***

With the combination of different chromatographic methods (e.g. RP-VLC, prepTLC and HPLC), 23 compounds were isolated from *R. aquaticus* roots and aerial parts and *R. thyrsiflorus* roots (Figures 1–3).

![Diagram](image)

**Figure 1.** Isolation of compounds from the aerial parts of *R. aquaticus*
The isolated compounds are naphtalenes [musizin (1), musizin-8-\(\text{O}\)-glucoside (2) and torachrysone-8-\(\text{O}\)-glucoside (3)], a naphtoquinone [\(2\)-\text{methoxystypandrone} (4)], anthraquinones [emodin (5), chrysophanol (6), phycion (7), citreorosein (8), emodin-8-
O-glucoside (9) and chrysophanol-8-O-glucoside (10); stilbenes [resveratrol (11) and piceid (12)]; flavonoids [quercetin (13), quercetin-3,3′-dimethylether (14), isokaempferide (15), quercetin-3-O-arabinoside (16), quercetin-3-O-galactoside (17), catechin (18) and epicatechin (22)]; a proanthocyanidin [procyanidin B5 (23)]; monoacylglycerols [1-stearoylglycerol (19) and 1-palmitoylglycerol (20)]; and β-sitosterol (21) (Figure 4). All compounds were isolated for the first time from the roots of *R. thyrsiflorus*. Apart from musizin-8-O-glucoside, all compounds were isolated for the first time from *R. aquaticus*.

![Chemical structures](image)

<table>
<thead>
<tr>
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<th>R₁</th>
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<th>R₃</th>
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</tr>
<tr>
<td>2</td>
<td>H</td>
<td>glu</td>
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<td>3</td>
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<td>OH</td>
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<tr>
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<tr>
<td>17</td>
<td>gal</td>
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<th>Compound</th>
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Antimicrobial properties of the isolated compounds

The highly active extracts of the aerial parts and roots of *R. aquaticus* and the roots of *R. thyrsiflorus* were subjected to a bioassay-guided, multistep separation procedure and 23 compounds were isolated. The antibacterial activities of the isolated compounds were tested, and it was observed that different types of secondary metabolites are responsible for the antibacterial effects of the plants; especially naphthalenes [musizin (1), musizin-8-O-glucoside (2) and 2-methoxystypandrone (4)], isolated from *R. aquaticus*, exerted remarkable antibacterial effects against several bacterial strains showing that these compounds can be at least partly responsible for the antibacterial activity of the plant (Table 1).

**Table 1.** Antibacterial properties of the isolated compounds (1, 2 and 4)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Antibacterial activity - MIC (µg/mL)</th>
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<tbody>
<tr>
<td></td>
<td><em>S. epidermidis</em></td>
</tr>
<tr>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
</tr>
<tr>
<td>vancomycin</td>
<td>2</td>
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</table>

Previously, the antimicrobial effect of musizin (1), 2-methoxystypandrone (4) and torachrysone was tested by Nishina et al. and 2-methoxystypandrone (4) proved to be the most active against *S. aureus*, *S. lutea* and *S. cerevisiae*.
Xanthine oxidase inhibitory properties of the isolated compounds

The XO inhibitory potency of the isolated compounds from *R. aquaticus* were evaluated. It was observed that mostly the flavonoid-type compounds exerted activity against XO. The most potent compound was quercetin (13) (IC$_{50}$: 0.85 ± 0.03 µM), followed by quercetin-3,3’-dimethylether (14) and isokaempferide (15). Quercetin-glycosides (16 and 17) and catechin (18) did not possess any activity. Besides flavonoids, some of the naphthalene-derivatives possessed activity. Emodin (5) showed weak activity, but citreorosein (8) had remarkable effect (IC$_{50}$: 7.88 ± 0.77 µM). (Table 2.)

Structure-activity relationship studies have been performed previously on the inhibitory potency of flavonoids on XO, and it was concluded that flavanones, dihydroflavonols and flavanols were not capable of inhibiting XO, and the presence of hydroxy groups at C-5 and C-7, and the double bond between C-2 and C-3 are important in terms of the efficacy. The results also correlated with the observations of Lin et al., that glycosylation of flavonoids causes a decrease in the affinity for XO, probably because of the nonplanar structure, the steric hindrance or the hydrophilicity of these compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>XO inhibition 20 µg/mL (%± SD)</th>
<th>IC$_{50}$ (µM ± SD)</th>
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<tr>
<td>1</td>
<td>15.41 ± 6.17</td>
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<tr>
<td>2</td>
<td>28.39 ± 1.83</td>
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<td>3</td>
<td>25.26 ± 2.37</td>
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</tr>
<tr>
<td>5</td>
<td>10.53 ± 1.21</td>
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<tr>
<td>8</td>
<td><strong>83.52 ± 2.90</strong></td>
<td>7.88 ± 0.77</td>
</tr>
<tr>
<td>11</td>
<td>27.62 ± 5.25</td>
<td></td>
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<tr>
<td>13</td>
<td><strong>94.19 ± 1.34</strong></td>
<td><strong>0.85 ± 0.03</strong></td>
</tr>
<tr>
<td>14</td>
<td>48.72 ± 1.68</td>
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<tr>
<td>15</td>
<td>43.37 ± 2.90</td>
<td></td>
</tr>
<tr>
<td>allopurinol</td>
<td></td>
<td>7.49 ± 0.29</td>
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</table>

*Only compounds showed activity (> 10% inhibition) are listed.*
Neuroprotective properties of two isolated compounds of *R. aquaticus*

In the field of stroke recovery, there is an urgent need for agents that would prevent the debilitating effects of the disorder, thereby tremendously reducing the societal and economic costs associated with it. In our study, the neuroprotective effects of two flavonoids [quercetin-3-O-arabinoside (16) and quercetin-3-O-galactoside (17)], isolated from *R. aquaticus*, were proved in the OGD model of *in vitro* ischemia using rat PC12 cell line (Figure 5). This model is a robust and validated model for preliminary screening of neuroprotective agents. Plant-derived flavonoids belong to the broader class of polyphenolic compounds, which are purported to have salutary effects in various disease states. What makes flavonoids a fascinating class of molecules is that, in addition to their chemical diversity and abundance in natural sources; their pharmacological effects are also multi-faceted. Apart from their well-known antioxidant properties, they are also known to have profound anti-inflammatory, anti-apoptotic and neurotrophic effects as evidenced in various models of ischemia.

![Figure 5](image-url)  
*Figure 5*. Neuroprotective effect of compounds 16 and 17 in an OGD model of ischemia. PC12 cells exposed to OGD conditions for 1 h showed significantly reduced cell viability. Cells exposed to the drugs (16 and 17 at a concentration of 10 µM) during OGD, exhibited a 100% increase in viability. Data expressed as percentage of control, #: vs control, *: vs OGD treatment, P < 0.05, n = 3
Oxidative stress is one of the primarily implicated mediators of ischemic pathogenesis. The brain is extremely sensitive to the consequences of oxidative stress, as it is one of the prime consumers of oxygen and has a relatively low antioxidant defence. It is thought to be intimately involved in mediating other ensuing cell death mechanisms like excitotoxicity, mitochondrial dysfunction and inflammation, thus forming a vicious circle culminating in large-scale cell death. The observed neuroprotective effects of compounds 16 and 17 could be attributed to their antioxidant properties, although effects on inflammatory and other apoptotic pathways cannot be ignored. Moreover, the polypharmacology exhibited by flavonoids could be particularly useful in the complex, rapidly changing microenvironment manifested in ischemic pathology, and needs to be thoroughly investigated.

Another highlight of our study is that, in addition to preventing cell death under simulated ischemic conditions, the drugs were also able to induce neurite outgrowth in the surviving cells, thereby suggesting a role in restoration of the neuronal network. Rapid restoration of neurological function following injury is paramount to the prevention of debilitating consequences of ischemic stroke. Re-establishment of brain plasticity following stroke is key to recovery. It aids the regeneration and functional integration of severed neuronal networks. Our investigation of the cellular mechanism for the observed restorative effects revealed that quercetin-3-O-galactoside (17) (10 μM) enhanced the expression of synaptophysin – a marker of synaptic plasticity. This can be a good indicator of recovery because re-formation of synapsis is crucial to the functional integration of the restored neurons. This study revealed a notable difference in the neurite outgrowth-inducing potencies between the two flavonoids tested, with quercetin-3-O-arabinoside (16) exhibiting higher potency. In addition, we did not observe any considerable change in synaptophysin expression with compound 16. This could mean that the subtle structural differences between 16 and 17 are probably driving them to occupy different receptors, thereby initiating different cellular cascades that ultimately affect neurorestoration and survival (Figure 6).
Figure 6. Neurite outgrowth inducing effect of compounds 16 and 17 in dPC12 cells under OGD conditions. dPC12 cells were subjected to OGD with/without compounds 16 and 17 (1 μM and 10 μM) and reperfused for 24 h after which their neurites were measured using immunocytochemical analysis. Cells subjected to OGD show a drastic reduction in neurite length. Both the drug treated groups exhibited significantly higher neurite lengths (16: 1 μM and 10 μM; 17: 10 μM). Data expressed as pixels, not absolute length as it is a comparative study. #: vs control, *: vs OGD treatment, P < 0.05

Our results reveal that secondary metabolites of Rumex species can be regarded as promising starting materials in the search for new pharmaceutical discoveries, in consequence of their pharmacological potential, and in particular their noteworthy XO inhibitory and neuroprotective effects.
ACKNOWLEDGEMENTS

I express my deepest gratitude to my supervisors, Prof. Judit Hohmann (director of Department of Pharmacognosy), and Dr. Andrea Vasas, for the management of my work. I am greatly obliged to them for their never-failing professional guidance, humanity and encouragement, which have continually inspired me during my work.

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I would like to extend my special thanks to my dear husband, for his love, support, inspiration and understanding attitude during these years. I will forever be grateful to my family, whose love and support has enabled me to complete this work.

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THE THESIS IS BASED ON THE FOLLOWING PUBLICATIONS:

   If: 2.628

   If: 2.694

   If: 3.055

   If: 2.698*

   If: 0.506*

(*The impact factor for the year 2016 is given.)*

OTHER PUBLICATIONS:

   If: 1.160

   If: 2.660

   If: -

   If: 1.440
Investigation of Antimicrobial, Antioxidant, and Xanthine Oxidase−Inhibitory Activities of Phellinus (Agaricomycetes) Mushroom Species Native to Central Europe
If: 1.104

Isolation of Chemical Constituents of *Centaurea virgata* Lam. and Xanthine Oxidase Inhibitory Activity of the Plant Extract and Compounds
If: 2.331

**PRESENTATIONS HELD IN THE THEME OF THE THESIS:**

Flavonoid-glycosides from *Rumex aquaticus* with neuroprotective activity
*Planta Medica: Natural Products and Medicinal Plant Research* 2014, 80: P1L110.

Neuroprotektív hatású flavonoidok izolálása a *Rumex aquaticus*ból
*Gyógyszerészet* 2014, 58:(Suppl. I.) p. 87.

Neuroprotektív hatású vegyületek izolálása a *Rumex aquaticus*ból

Antibacterial activity of *Rumex aquaticus* and *R. thyrsiflorus* extracts and isolation of the biologically active compounds