Theses of doctoral (Ph.D.) dissertation

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Investigation of the extracts and bioactive components of stinging nettle (*Urtica dioica* L.)

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Antecedents of the work, set goals

The role of food in developed countries has considerably changed in recent decades. Consumers not only look for tasty nutrients, but also pay more and more attention to the health protection and disease prevention effects of foods. Foods often enriched with active ingredients having beneficial effect on health are called functional foods.

My research work aimed at finding valuable, biologically active components in the stinging nettle's leaf and root (*Urtica dioica* L.), and preparing an extract that, thanks to its antimicrobial and reducing effects, could be suitable in the form of tincture (perhaps of powder) - even in terms of food safety - for using as functional food, food ingredient, or natural additive to products undergone various technological/processing treatments, and could also play an important role in microbiological protection in food processing.

I intended to investigate which solvent among the four known ones would be the most suitable for extracting the stinging nettle's components having antioxidant and antimicrobial effect. For how long and at what temperature should this extraction be performed? At what time of the year should the plant's leaves be harvested, or is it worth making an extract from nettle root?

During my experiments, I intended to answer several questions in this connection:

- How can I reach maximum amount of extract with the help of supercritical, Soxhlet, and conventional extraction methods, adjustable operation parameters (pressure, temperature, extraction time) and solvents related to various methods (supercritical CO_2, ethanol, hexane and water)?
- To what extent is a given solvent favourable for extracting polyphenols and how much antioxidant/reducing capacity can I detect in the leaf or root with the help of it?
- I intended to investigate which phenological phase in the plant's vegetation period was the most suitable for harvesting leaves and roots so that the total polyphenol content (TPC) and antioxidant/reducing capacity (FRAP, DPPH) would be the highest.
- During microbiological experiments, I investigated which method, solvent, setting of operation parameters and which concentration of extracts would be suitable for detecting antimicrobial activity against microorganisms that are important in respect of food safety and medicine. In case of a positive result, I intended to find a connection between the antimicrobial effect of extracts and the components most likely responsible for this effect.

Materials and methods

Materials

Plant materials

I used the stinging nettle's (*Urtica Dioica* L.) roots and leaves as raw material, the botanical identification of which was determined with the help of Dr. Péter Radics, senior lecturer at Szent István University, Faculty of Horticultural Science, Department of Medicinal and Aromatic Plants. I harvested the plant samples in 4 phenological phases in the robinia forests in Vál, Central Hungary.

Extraction solvents

Distilled water, ethanol, n-hexane, supercritical CO_2

Standards used for HPLC

pyrocatechin, quercetin, quercitrin, rutin, catechin, epicatechin, chlorogenic acid, cinnamic acid, dihydrobenzoic acid, syringic acid, vanillin acid, ellagic acid

Microorganisms used during the investigation of antimicrobial effect

Candida albicans, *Candida glabrata*, *Candida parapsilosis*, *Saccharomyces cerevisiae*, *Enterococcus faecalis*, *Escherichia coli*, *Listeria innocua*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*

Operations and investigations I used during my research work

Operations: drying, chopping, sieving, vacuum filtration, extraction (conventional, Soxhlet, supercritical), distillation - evaporation

Investigations: determination of dry matter content, particle size, extraction yield. TPC, FRAP, DPPH, measurement of polyphenol profile with the help of HPLC method and investigation of antimicrobial effect.

Preparing samples

Drying:

The plant's fresh parts - in the case of root after thorough washing – were dried in a well-ventilated place protected from light at about 30°C for two to three weeks.

The extracts were vaporized in a rotary vacuum evaporator, and in a laboratory drying oven at 80°C for 24 hours to constant mass.

Chopping

First I chopped roughly the fresh plant parts with pruning scissors, then continued the cutting with a grinder (BOSCH TSM6A013B). I used a knife mill (Retsch GM 200 Grindomix) and a mixer mill (MM 400) to chop the dried plant parts.

Extractions

- I performed conventional extraction to determine extraction yields and for analytical and microbiological measurements. I used duplicator with mixer and Memmert water bath as extraction apparatus.
- I performed Soxhlet extraction with laboratory Soxhlet extractor.
- I measured supercritical extraction (SFE) with pilot plant equipment. The high-pressure supercritical carbon dioxide passed through the chopped, dried drug dissolved the substances and then by releasing the pressure in the separator, the carbon dioxide released from the extract.

Determination of dry matter content

The dry matter content in the samples was determined in two ways: in the laboratory drying oven at 105 °C for 24 hours to constant mass before supercritical and Soxhlet extraction, and with rapid moisture analyser KERN MLS 50-3HA160 before conventional single extratcion.

Determination of particle size

The particle size distribution in the chopped samples was determined in two ways: on the one hand with laboratory sieve shaker, when I carried out the evaluation with RRSB (Rosin-Rammler-Sperling-Bennet) method, and determined the characteristic particle size, uniformity coefficient and specific surface of the bulk of chopped plants. On the other hand, I determined the particle size distribution and measured the above detailed data with laser diffraction equipment.

Determination of yields

In order to determine extraction yields, I vaporized the obtained extracts in each case, then I weighed it. I determined the result in mass percentage with respect to the dry matter content in the measured material.

Methods used during my analytical measurements

- Determination of total polyphenol content (TPC)
- Determination of antioxidant/reducing capacity (with the help of FRAP method)
- Determination of antioxidant/reducing capacity (with the help of DPPH method)
- Determination of the quantity of rutin and quercetin with the help of HPLC measurement

Methods used during my microbiological investigations

- determination of antimicrobial effect with agar well diffusion test
- determination of antimicrobial effect with disc diffusion test

Results

Determination of dry matter content in leaf and root in percentage of the measured amount of drug

	Leaf	Root
Fresh	18.25±0.84	22.90±0.90
Dried	90.80±0.02	91.07±0.08

Determination of particle size with sieve analysis

Based on the results of sieve analysis, the particle distribution in the chopped drug was modeled using the particle distribution function developed by Rosin-Rammler-Sperling-Bennet (RRSB):

$$R(x) = 100 \exp[-(x/x_o)^n]$$

where R(x) - amount retained on sieve, sum (%)
x - particle size (mm)
x_o - characteristic particle size (mm)
n - uniformity coefficient (-)

Based on the graphs, I determined the chopped bulk's average particle size x_o (mm), uniformity coefficient n (-) and the multiplication of its specific surface with x_o $F*x_o$ $(m^2/kg)*(mm)$. My data is presented in the following table:

	x_0 (mm)	n	Surface (m^2/kg)
Leaf	0.45	1.45±0.01	33.33±0.01
Root	0.50	1.41±0.10	32.72±4.80

Determination of particle size with laser particle meter

After having chopped first with laboratory mixer, then with mixer mill, I evaluated the drugs' characteristic particle size (x_o) with this equipment, the bulk's uniformity coefficient (n) and specific surface (F) based on the measured data using a computer program (FRITSCH, Program "analysette 22").

The following table contains my results:

	x_0 (mm)	n	Surface (m^2/kg)
Leaf	0.150±0.001	1.18±0.02	119.46±0.27
Root	0.042±0.001	3.05±0.16	359.37±3.42

Results of extraction yields

I investigated the effect of extraction solvents (water, ethanol, n-hexane, supercritical CO_2), temperature (simple-single: 60-80-100 ^oC) and pressure (supercritical extraction: 100 to 450 bar) on the yield in the case of three extraction methods (conventional extraction, Soxhlet and supercritical extraction). Based on the results of supercritical extraction completed on nettle roots, it was found that the amount of yield can be higher by increasing the pressure from 100 bar to 450 bar.

The following table contains the comparisons of extraction yields.

Solvent	Drug		
	Yield weight %		
	Dried root	**Dried leaf**	**Fresh leaf**
supercritical CO_2 (100 - 450 bar)	0.32 - 0.61	1.11 - 1.52	-
Soxhlet n-hexane (autumn)	0.67 ± 0.12	-	-
Soxhlet n-hexane (spring)	0.77 ± 0.04	1.94 ± 0.07	-
Soxhlet ethanol 96% (autumn)	7.78 ± 0.47	21.73 ± 1.47	-
Soxhlet ethanol 96% (spring)	8.00 ± 0.48	18.13 ± 1.57	-
ethanol 20% conventional simple	15.49 ± 0.31	20.45 ± 0.77	33.55 ± 2.28
ethanol 70% conventional simple	12.87 ± 0.17	21.16 ± 0.27	32.63 ± 0.64
water scald conventional simple	14.18 ± 0.87	26.69 ± 0.83	42.25 ± 1.19
water 60°C 3 hour conventional simple	17.04 ± 1.74	33.02 ± 0.30	-
water 80°C 3 hour conventional simple	19.41 ± 1.35	29.85 ± 0.13	-
water 100°C 3 hour conventional simple	21.23 ± 0.29	31.13 ± 0.49	48.40 ± 3.20
ethanol 50% conventional multiple	-	25.46 ± 0.74	-
water 80°C 3 hour conventional multiple	20.45 ± 0.24	-	-

The yield in dry matter content and expressed in weight percentage may have varied more orders of magnitude, depending mainly on the solvent, and to a lesser extent, on the time, temperature and pressure of the extraction.

Results of microbiological experiments

The following three tables contain the results of my microbiological experiments. The first two tables show the antimicrobial activity of the extracts of a spring and autumn nettle leaf. The third table describes the antimicrobal properties of the nettle root extracts.

The antimicrobial effect of the mixtures of spring nettle leaves of 100 mg/ml dry matter concentraion, based on the diameter (mm) and quality of the self-pruning zone. The diameter of agar well was 8 mm.

Microorganism		80 °C aqueous extract	100 °C aqueous extract	5% ethanol extract
Candida albicans	zone mm			
	inhibition	no	no	no
Candida glabrata	zone mm	**18.0 ± 2.0**	**18.0 ± 1.0**	
	inhibition	**total**	**total**	no
Candida parapsilosis	zone mm			
	inhibition	no	no	no
Saccharomyces cerevisiae	zone mm			
	inhibition	no	no	no
Enterococcus faecalis	zone mm			**14.0 ± 1.0**
	inhibition	no	no	**total**
Escherichia coli	zone mm	**13.0 ± 1.5**	**14.5 ± 3.5**	**13.0 ± 4.0**
	inhibition	**partial**	**partial**	**total**
Listeria innocua	zone mm	**10.0 ± 1.0**		**11.5 ± 0.5**
	inhibition	**total**	no	**total**
Listeria monocytogenes	zone mm	**12.0 ± 0.0**		
	inhibition	**total**	no	no
Pseudomonas aeruginosa	zone mm	**28.0 ± 0.0**	**23.0 ± 8.0**	**10.5 ± 0.5**
	inhibition	**total**	**total**	**total**
Staphylococcus aureus	zone mm	**20.0 ± 6.5**	**21.0 ± 4.0**	**9.5 ± 0.5**
	inhibition	**total**	**total**	**total**

The antimicrobial effect of the mixtures of autumn nettle leaves of 100 mg/ml dry matter concentraion, based on the diameter (mm) and quality of the self-pruning zone. The diameter of agar well was 8 mm.

Microorganism		80 °C aqueous extract	100 °C aqueous extract	5% ethanol extract
Candida albicans	zone mm			
	inhibition	no	no	no
Candida glabrata	zone mm	**17.0 ± 0.5**	**16.5 ± 0.5**	
	inhibition	**total**	**total**	no
Candida parapsilosis	zone mm			
	inhibition	no	no	no
Saccharomyces cerevisiae	zone mm	no	**12.5 ± 0.0**	
	inhibition	no	**partial**	no
Enterococcus faecalis	zone mm			
	inhibition	no	no	no
Escherichia coli	zone mm	**13.0 ± 0.0**	**13.0 ± 0.0**	
	inhibition	**partial**	**partial**	no
Listeria innocua	zone mm	**12.0 ± 0.0**	**12.0 ± 0.0**	
	inhibition	**partial**	**partial**	no
Listeria monocytogenes	zone mm	**15.0 ± 0.0**	**15.0 ± 0.0**	
	inhibition	**partial**	**partial**	no
Pseudomonas aeruginosa	zone mm			
	inhibition	no	no	no
Staphylococcus aureus	zone mm	**16.0 ± 0.0**	**15.0 ± 1.0**	
	inhibition	**partial**	**total**	no

Antimicrobial effect of spring leaf samples and quercetin, based on the diameter (mm) and quality of self-pruning zone, in mixture of 100 mg/ml dry matter concentration. The diameter of agar well was 8 mm.

Root				
Microorganism		**80 °C aqueous extract**	**100 °C aqueous extract**	**5% ethanol extract**
Candida albicans	zone mm			
	inhibition	no	no	no
Candida glabrata	zone mm			
	inhibition	no	no	no
Candida parapsilosis	zone mm			
	inhibition	no	no	no
Saccharomyces cerevisiae	zone mm			
	inhibition	no	no	no
Enterococcus faecalis	zone mm	**17.0**	**17.0**	
	inhibition	**total**	**partial**	no
Escherichia coli	zone mm			
	inhibition	no	no	no
Listeria innocua	zone mm		**18.0**	
	inhibition	no	**total**	no
Listeria monocytogenes	zone mm			
	inhibition	no	no	no
Pseudomonas aeruginosa	zone mm	**18.0**		
	inhibition	**partial**	no	no
Staphylococcus aureus	zone mm			
	inhibition	no	no	no
Quercetin				
Mikroorganizmus		aqueous solution 80 °C (20 µg/ml)		ethanol solution (10 µg/ml)
Pseudomonas aeruginosa	zone mm	**13.0 ± 1.0**		**11.0 ± 0.1**
	inhibition	total		total

Antimicrobial effect of aqueous extracts was more pronounced against bacteria, than yeasts. Growth of Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Listeria monocytogenes was inhibited, while antifungal effect was detected only in case of Candida glabrata.

In our microbiological experiments we have found that the antimicrobial effect of the nettle leaf is much more significant than in the nettle root.

New scientific results - Theses

1. My experiments proved that as a result of the extraction of common nettle's leaves and roots, performed with different methods, the yield in dry matter content and expressed in weight percentage may have varied in its range depending mainly on the solvent, and to a lesser extent, on the time, temperature and pressure of the extraction. The following table contains the yield values.

	Supercritical	**Soxhlet**	**Soxhlet**	**Conventional simple**	**Conventional simple**
	CO_2	**n-hexan**	**ethanol**	**ethanol**	**water**
Leaf	1.1 – 1.5	1.8 – 1.9	18.1 – 21.7	20.5 – 21.2	26.7 – 31.1
Root	0.3 – 0.6	0.7 – 0.8	7.8 – 8.0	12.9 – 15.5	14.2 – 21.2
Comment	100 bar - 450 bar	95%	96%	70% - 20%	(tea) 60-80-100 °C

2. I found that the extraction of polyphenols and the resulting antioxidant/reducing capacity depended on the solvent, the best of which was water, the second-best is ethanol, then hexane and supercritical CO_2 the efficiency of which was close to that of hexane. In the case of conventional extractions, I expressed the values of total polyphenol content in mMGSE/g dry matter unit. I demonstrated the difference in efficiency of water and ethanol in case of leaf and root samples resulting from conventional extractions in the following two tables.

Leaf	**Conventional extraction**	**Conventional extraction**
	ethanol	**water**
	30.1 – 19.1	71.0 – 30.0
Comment	april-oct, 2018. 70%	april-okt, 2018. 100 °C

Root	**Conventional extraction**	**Conventional extraction**
	ethanol	**water**
	23.9 – 15.4	25.3 – 23.4
Comment	april-okt, 2018. 70%	april-okt, 2018. 100 °C

I demonstrated in the following table the difference in total polyphenol content of extracts prepared with ethanol, n-hexane and supercritical CO_2 obtained from root samples, expressed in mMpirogallol equivalent/g dry matter unit, resulting from Soxhlet and supercritical extractions.

Root	Supercritical	Soxhlet	Soxhlet
	CO_2	**n-hexan**	**ethanol**
	0.7	0.7	1.6
Comment	300 bar	95%	96%

3. In the series of experiments I performed throughout the life cycle of the nettle – from April to October –, I proved that the total polyphenol content in term of mMGSE/g dry matter dimension was the highest in the early spring plant. The polyphenol content of the leaves gradually decreased by the end of the vegetation period. This change was less perceptible in the root, and in some cases, even a slight increase could be observed during the vegetation period, which is presented in the following table.

	April				May				June				October			
	tea	60 °C	80 °C	100 °C	tea	60 °C	80 °C	100 °C	tea	60 °C	80 °C	100 °C	tea	60 °C	80 °C	100 °C
Leaf	59.0	65.0	68.0	71.0	52.0	50.0	57.9	60.3	46.1	48.1	51.4	52.6	40.0	39.0	33.8	30.0
Root	20.0	19.8	22.3	24.2	20.0	20.7	24.5	25.3	20.0	22.0	22.2	24.3	20.0	21.0	22.0	23.4

Among the extraction conditions, increasing the temperature and duration of the extraction resulted in an increase in the total polyphenol content and the related antioxidant capacity. The amount of polyphenolic compounds in the leaves was significantly higher than in the roots. The antioxidant capacity was also higher in the leaves than in the roots, but it decreased in both cases by the end of the vegetation period.

Therefore, in terms of health protection, spring leaves are best to harvest, use and consume.

4. My microbiological experiments proved that the 80°C and 100°C aqueous extracts of the common nettle's leaves equally had antimicrobial effect on the propagation of the following microbial strains: *Candida glabrata, Escherichia coli, Listeria monocytogenes, Listeria innocua, Pseudomonas aeruginosa* and *Staphylococcus aureus.* In order to reach

positive results, before preparing the extracts, it is necessary to chop the dried drug to less than 0.15 mm particle size.

I proved that the time of harvesting the plant (April to October), depending on the size and quality of the self-pruning zone, significantly influenced the development of the antimicrobial effect. Based on the size of the self-pruning zone, I found an average antimicrobial activity 20-30% higher in spring leaf samples than in autumn samples.

Compared to the root, the aqueous extracts of the leaves had stronger inhibitory effect, both in spring and in autumn.

In the case of ethanol extracts, only spring leaf samples showed inhibition.

Based on literature data, rutin and quercetin are highly responsible for antimicrobial activity. Examining the antimicrobial activity of the two substances, I found that no inhibitory effect could be measured in case of rutin, while in case of quercetin, only in aqueous extracts of 80°C showed such effect, because – according to my measurements – heat treatment of 100°C probably inactivated the compound.

Conclusions, suggestions

My research work aimed at making a product from the stinging nettle's leaf and root (*Urtica dioica* L.) - with different extraction methods, solvents and setting of parameters - that, thanks to its antimicrobial and reducing effects, could be suitable in the form of tincture (perhaps of powder) - even in terms of food safety - for using as functional food, food ingredient, or natural additive to products undergone various technological/processing treatments, and could also play an important role in microbiological protection in food processing.

Based on my results it was found that water was the best solvent in respect of either making nettle extracts or environmental protection, out of which valuable bioactive ingredients/ polyphenolic compounds should be obtained by extraction of at least 60 °C and during for at least 3 hours, since higher temperatures are favourable for the extraction of these compounds. Therefore, if the aim is not making a vitamin-rich fresh drink, but binding free radicals harmful to the body during a detox diet, the extraction from the drug should be made as described above.

My experiments also proved that it is worth harvesting and then drying the plant at the beginning of vegetation period (April).

I found that aqueous extractions performed for 3 hours and at high temperature (80 °C and 100 °C) were also beneficial for antimicrobial compounds, since antimicrobial activity can be related to the presence of polyphenols. During my experiments, I managed to develop a method available for anyone using a household kettle to make a detoxing/free radical scavenging drink at home.

Nettle extracts can also be vaporized and used as food additive in a solid powder form in food technology processes or in packaging materials having preservative effect. Based on best practices in Mediterranean countries, I recommend using the fresh leaves of the plant in salads or using the dried leaves in spice mixtures.

During my experiments, I also investigated the antimicrobial activity of rutin and quercetin, and the presence of the same components in my extracts. I found that quercetin contributed to the inhibitory effect of the solutions, but rutin had no effect on the tested microorganisms. The question therefore arises as to which polyphenolic compound or compounds, and in what composition, are responsible for the antimicrobial activity at a given phenological phase of the plant. It would also be interesting to see how the amount of each component changes throughout the entire phenological phase of the plant.

Related publications

Journal articles with impact factor

1. Kornélia Kőszegi, Gyula Vatai, Erika Békássy-Molnár: Comparison the Soxhlet and supercritical fluid extraction of nettle root (*Urtica dioica* L.). *Periodica Polytechnica Chemical Engineering*, 59(3), 168-173 (2015). IF: 0,84
2. Arijit Nath, Máté András Molnár, Attila Csighy, Kornélia Kőszegi, Ildikó Galambos, Klára Pásztorné Huszár, András Koris and Gyula Vatai: Biological activities of lactose-based prebiotics and symbiosis with probiotics on controlling osteoporosis, blood-lipid and glucose levels Review, *Medicina* 54(6), 98 (2018). DOI: 10.3390/medicina5406009 IF: 1,467
3. Kornélia Kőszegi, Erika Békássy-Molnár, Noémi Koczka, Tímea Kerner, Éva Stefanovits-Bányai: Changes in total polyphenol content and antioxidant capacity of stinging nettle (*Urtica dioica* L.) from spring to autumn. *Periodica Polytechnica Chemical Engineering* DOI: 10.3311/PPch.14338 (2019). IF: 1,248

Journal articles without impact factor, foreign-language

Kornelia Koszegi, Joseph Michael Kocsis, Gyula Vatai, Erika Bekassy-Molnar: Antimicrobial effects of the stinging nettle (*Urtica Dioica* L.). *Review Analecta Technica Szegedinensia*, 11(2), 10-15 (2017)

Book detail in Hungarian language

Békássyné Dr.Molnár Erika, Dr.Friedrich László, Galicz István, Dr.Hitka Géza, Kőszegi Lászlóné, Nágl Péter, Pásztorné Dr.Huszár Klára, Stégerné Dr.Máté Mónika, Török Zita *Módszertani Füzet* VII. Szemelvények az élelmiszeripari technológiák oktatásának módszertanáról. Budapesti Corvinus Egyetem (2015) 2,1 ív

Conference publication in Hungarian language (total)

Terék O., Papp I., Honfi P., Jámborné Benczúr E., Kőszegi L.né., Máthé Á.: Előzetes eredmények a 'Bacardi' krizantémfajta fotoszintézissel kapcsolatos poszthárveszt folyamatairól különböző tartósító oldatok esetén., „Hagyomány és haladás a növénynemesítésben" XV. Növénynemesítési Tudományos Napok 2009. március 17. MTA Agártudományok Osztályának Növénynemesítési Bizottsága. Conference publication 482-486 (2009)

Conference publication in Hungarian language (summary)

1. Kőszegi Lászlóné, Fogarassy Eszter, Békássyné Molnár Erika, Vatai Gyula: A must sűrítése során alkalmazott membránszűrési eljárások optimális megvalósításának tervezése „Műszaki Kémiai Napok'2011. Veszprém." 2011. április 27-29. Poszter. Konferenciakidvány 264

2. Kőszegi K., Galambos I., Békássy-Molnár E., Vatai Gy.: Komplex eljárás ivóvíz előállítására nagy arzén- és huminsav-tartalmú kútvízből (Kísérleti terv, első eredmények). „Műszaki Kémiai Napok'2012. Veszprém." 2012. április 24-26. Conference presentation. Conference publication 22.

International conference publication (total)

1. K. Koszegi, G. Gurin, I. Galambos, Gy. Vatai, E. Bekassy-Molnar: Industrial application of GEH package for coexistent removal of arsenic and humic acid. PERMEA 15-19 September 2013 Warsaw, Poland. Angol nyelvű konferencia előadás. Konferenciakiadvány E6-E14

2. Kőszegi K, Simándi B, Vatai Gy, Békassy-Molnár E. Extraction of the active substances of the stinging nettle (*Urtica Dioica* L.) by Soxhlet- and supercritical extraction. Élelmiszertudományi Konferencia 2013. november 7-8. Budapest, Magyarország, BCE 1118 Budapest, Villányi út 35-43. Conference presentation in Hungarian, Conference publication 84-87

3. Koszegi K., Simandi B., Vatai Gy., Bekassy-Molnar E.: Comparison the Soxhlet- and supercritical fluid extraction of nettle root (*Urtica dioica* L.) „Műszaki Kémiai Napok" 2014. Veszprém 2014. május 14-16. Conference presentation in English, Conference publication 82-88

4. Koszegi Kornelia, Kocsis Joseph Michael, Vatai Gyula, Bekassy-Molnar Erika: Antimicrobial effects of the stinging nettle (*Urtica dioica* L.). Proceedings of 1st International Conference on Biosystems and Food Engineering, Budapest (2016), ISBN 978-963-269-598-3, PDF E125. 7

International conference publication (summary)

1. Fogarassy E., Koszegi K., Bekassy S., Bekassy Molnar E., Vatai Gy.: Optimization of grape juice concentration by using multistep membrane technique. Training Nanostructured materials and membranes for Energy. Lilleström, Norvégia (2009). Poster (in English).

2. Koszegi K., Simandi B., Vatai Gy., Bekassy-Molnar E.: Examining the effectiveness of different extractions using nettle leaf. The Scientific board of the 8th International Congress

of Food Technologists, Biotechnologists and Nutritionists 21 to 24 October 2014 in Opatija, Croatia. Poster (in English).

3. Kőszegi K., Békássy-Molnár E., Stefanovits-Bányai É., Szabó P., Végvári Gy., Maráz A.: Chemical composition and antimicrobial effect of aqueous and ethanolic extracts of stinging nettle (*Urtica dioica* L.). 2nd International Conference on Biosystems and Food Engineering Budapest, 2018. június 8. Conference proceedings in English. PDF E231, 1

4. Kornélia Kőszegi, Éva Stefanovits-Bányai, Tímea Kerner, Erika Békássy-Molnár: Stinging nettle extract health benefits -A comparative analysis Young Researchers'International Conference on Chemistry and Chemical Engineering (YRICCCE II) Budapest, 2018. május 3-5. Conference proceedinngs in English 66

5. Kőszegi K, Végvári Gy, Békássy-Molnár E, Maráz A.: Characterization of phenolic compounds of stinging nettle (*Urtica dioica* L.) extracts by HPLC analysis and determination of their antimicrobial activity. 3rd International Conference on Biosystems and Food Engineering, Budapest on 4th of December, 2019. Conference proceedings in English PDF E231, 1