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Influence of the harvesting seasons on the polyphenol composition and antimicrobial activity of stinging nettle (*Urtica dioica* L.) extracts

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ABSTRACT

The aim of our research was to study the water and ethanol extractable polyphenols of stinging nettle (*Urtica dioica* L.) harvested in different seasons and to determine their antimicrobial activity against certain human pathogenic and food spoiling bacteria and yeasts.

Our results indicate that the spring leaf extracts had higher polyphenol contents than the root one; however, close to the end of the vegetation period these values decreased considerably in both leaves and roots. Detection and quantification of the most abundant phenolic compounds in the spring extracts by HPLC revealed the occurrence of 12 different phenol carboxylic acids and flavonoids. Flavonoid compounds were more abundant than phenol carboxylic acids in the leaves; however, their proportion was equal in the case of the roots. Nettle leaf extracts had remarkable antimicrobial activity, the spring extracts

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were more efficient than the autumn ones. *Escherichia coli* and *Staphylococcus aureus* were sensitive to every leaf extract, while *Listeria monocytogenes* and *Pseudomonas aeruginosa* had reduced but remarkable sensitivity patterns. Among the yeasts, *Candida glabrata* was strongly inhibited by the aqueous leaf extracts. Most of the strains were insensitive to the root extracts, although *Enterococcus faecalis* was inhibited by the root and not the leaf extracts.

KEYWORDS

stinging nettle, phenolic compounds, antimicrobial effects

1. INTRODUCTION

Stinging nettle (*Urtica dioica* L.) is a common herbaceous perennial plant, present in the temperate regions of Europe, Asia, North Africa, and North America, among others in Hungary as well.

Stinging nettle was reported to be used as a folk medicine in the ancient times for curing gangrene, rheumatism, tumours, ulcers, and dog bites (Engels and Brinckmann, 2016). In the Middle Ages nettle was used traditionally for lowering blood pressure and as a diuretic, haemostatic, and anti-inflammatory herb (Upton, 2013).

Current clinical studies, animal experiments, and *in vitro* investigations confirmed the traditional applications or indicated novel pharmacological and clinical effects, such as analgesic, anti-inflammatory, antilipidemic, antiulcer, cardiovascular, diuretic, hepatoprotective, immunomodulatory, and vasoconstrictive effects. Health of the gastrointestinal system and improved metabolism of nutrients were shown to be supported by infusions and extracts of stinging nettle (Gülçin et al., 2004; Chrubasik et al., 2007a; Ghaima et al., 2013; Upton, 2013). Nettle is also used as leafy vegetable, soup, and juice, and the extracts are very popular botanical pesticides in bioorganic farming as the consequence of their insecticidal, fungicidal, and bactericidal efficacy (Bhusal et al., 2022).

Different types of stinging nettle products are available worldwide. Fresh stinging nettle is used for soup preparation, while infusion, decoction, and liquid extracts are made from fresh or dry nettle leaves (Upton, 2013).

The European Medicines Agency (EMA) approved the traditional use of leaf (folium; EMA, 2010) and root (radix; EMA, 2012) of *U. dioica* L. and *Urtica urens* L. for specific therapeutic purposes. The European Pharmacopoeia (2022) provides official quality standards for the dry leaves (Urticae folium) as well as the dry roots (Urticae radix) of the previously mentioned species.

Grauso et al. (2020) published an overview of the chemical composition of *U. dioica* and surveyed the already identified and quantified organic compounds. The list of compounds indicated a wide range of phytochemicals including amino acids, organic acids, carbohydrates, steroids and triterpenoids, fatty acids and ceramides, carotenoids, terpenoids, C13-norisoprenoid, phenolic acids, flavonoids, other phenolic compounds, and volatiles.

Main bioactive phytoconstituents of nettle leaves include flavonoids (glucosides and rutinosides of quercetin, kaempferol, and isorhamnetin), caffeoyl-esters (caffeoylmalic acid,

chlorogenic acid, neochlorogenic acid), caffeic acid, scopoletin (coumarin), and sitosterol (-3-O-glucoside) beside polysaccharides and fatty acids (Chrubasik et al., 2007a). In the nettle root phytosterols, lignans, the lectin UDA (*U. dioica* agglutinin) and ceramides are considered the most important active principles (Chrubasik et al., 2007b).

Several researchers demonstrated the antiviral, antibacterial, and antifungal effects of water and organic solvent extracts of *U. dioica* (Saklani and Chandra, 2012; Ghaima et al., 2013; Joshi et al., 2014). There were attempts to find connection between the pharmacological effects of nettle and the identified and quantified phytochemicals. Saklani and Chandra (2012) presumed the association between the antimicrobial effect and the amount of natural antioxidants present. However, when reports on the antibacterial activity of flavonoids, a significant group of polyphenols, were compared by Cushnie and Lamb (2005, 2006), widely conflicting results were demonstrated. They assumed that discrepancies between reports of antibacterial activity of flavonoids could be attributed to different assays being used and the non-standardised conditions or application of various strains during the assays.

Our aim was to identify and quantify the phenolic compounds of stinging nettle root and leaf extracts and to establish their antimicrobial activity. Nettle extracts were prepared with inorganic and organic liquids, water and ethanol, respectively, because these are considered as the safest and most environmentally friendly solvents. Moreover, these solvents are defined by the EMA for the extraction of bioactive compounds of *U. dioica* and *U. urens* (EMA, 2010).

The European Pharmacopoeia (2022) and the European Medicines Agency (EMA, 2010, 2012) do not indicate any phenological implication, including harvesting periods for leaves and roots of stinging nettle. Upton (2013) made a detailed survey of the considerations for collecting stinging nettle and recommended a harvesting period for dried preparations of between mid-spring and late summer in temperate regions.

Although many researchers investigated the phenolic composition and antimicrobial effect of *U. dioica*, this is the first time that stinging nettle, collected in different seasons in Hungary, has been analysed from all these aspects.

2. MATERIALS AND METHODS

2.1. Plant materials

Stinging nettle (*U. dioica* L.) was collected in Vál (Hungary, Transdanubia region). The plant was harvested in April and September, 2018. The root was washed with water and both plant parts were dried in a loft to constant mass (ca. two weeks) at about 28 °C.

2.2. Preparation of the aqueous and ethanolic extracts from nettle organs

The dry plant material, leaves and roots, were ground into fine powder using Retsch MM 400 ball mill (ProfiLab24 GmbH, Berlin, Germany) and stored in airtight bottles until use. Dry mass of the samples was determined by a moisture analyser (KERN MLS 50-3HA160, Kern GmbH, Grossmaischeid, Germany), which indicated 91.8 ± 0.12 and 91.7 ± 0.09 w/w% for the leaves and roots, respectively.

For the preparation of liquid extracts, 50 g dry mass of leaf and root powders were added to 500 mL water or 96 v/v% ethanol in triplicates. Extraction with water was performed in a water

bath at 80 °C and 100 °C for 3 h, while the ethanolic extract was obtained by shaking the suspension at 200 r.p.m. with an orbital incubator (Gallencamp Manufacture, Cambridge CB4 1TF, UK) at 30 °C. Extracts were cooled at room temperature and centrifuged (Thermo Scientific Sorvall Evolution RC) for 20 min with 8,000 r.p.m. at 4 °C. Supernatant was removed and filtered with 0.45 µm PVDF membrane filter (www.merckmillipore.com).

2.3. Determination of the total polyphenol content (TPC)

The TPC was determined with Folin-Ciocalteu reagent (Singleton and Rossi, 1965).

2.4. HPLC analysis

Analytical HPLC grade standards of different phenolic compounds, phosphoric acid (H_3PO_4), and methanol (MeOH) were purchased from Sigma-Aldrich GmbH (www.sigmaaldrich.com). The standards (0.5 mg mL^{-1}) were dissolved in methanol, and decimal dilutions were used as working standards.

Supernatants of nettle extracts were filtered with 0.22 µm Millipore membrane filter and injected into the HPLC equipment (Waters High Performance Liquid Chromatograph). It was equipped with an absorbance detector (2,487 Dual λ), a binary HPLC pump (1,525), in-line degasser, a column thermostat (set at 40 °C), and an autosampler (717 plus; set at 5 °C) and was controlled using EMPOWERTM2 software. A Kinetex C18 2.6 µm 150 × 4.6 mm column was installed, and the gradient mobile phase was A: H_2O:MeOH:H_3PO_4 = 940:50:1; B: MeOH (0–30 min: A 100%–10%, 30–30.1 min: 10%–100%, 30.1–31 min: A 100%) with a flow rate of 1 cm^3 min^{-1}. The pressure in the column was 4,200 ± 10 psi at a column temperature of 40 °C. The running time was 31 min and each injected volume was 20 µL. The sampling rate was 10 pt s^{-1}, and the following phenolic compounds were monitored at a wavelength of 280 nm as standards: cinnamic acid, chlorogenic acid, dihydroxybenzoic acid, ellagic acid, syringic acid, vanillic acid, pyrocatechol, catechin, epicathechin, rutin, quercetin, and quercitrin. The retention times of the standards were as follows: pyrocatechol 6.8 min, dihydroxybenzoic acid 7.6 min, chlorogenic acid 11.4 min, vanillic acid 11.8 min, syringic acid 12.9 min, catechin 13.9 min, epicathechin 15.8 min, ellagic acid 17.8 min, rutin 17.9 min, quercitrin 19.4 min, cinnamic acid 19.9 min, and quercetin 23.0 min. Two parallel samples were measured in all cases.

2.5. Microbiological analysis

Antimicrobial activity of stinging nettle extracts was tested by the agar well diffusion method against six bacteria and four yeast strains, listed in Table 1.

Aqueous extracts were used in the original concentrations (100 mg dry mass/mL), while the ethanolic extracts were diluted 20-times with double-distilled water, aiming to reduce the antimicrobial effect of ethanol. In the second case, ethanol was added in 5 v/v% to the wells as a control, considering that if it caused inhibition, differences in the diameters of the inhibition zones of the extracts and the control were calculated.

Bacteria were cultivated in TGE (tryptone 5 g L^{-1}, glucose 1 g L^{-1}, yeast extract 2.5 g L^{-1}) and yeasts in YEPD (yeast extract 5 g L^{-1}, peptone 5 g L^{-1}, glucose 10 g L^{-1}) culture media for 24 h. One mL suspensions of each strain, containing 10^7 cells/mL were pipetted onto the surface of YEPD or TGE agar plates, spread evenly, and the excess fluid was removed by pipetting. Wells of 8 mm diameter were cut in the agar plates, and 150 µL extracts were pipetted into the wells.

Table 1. List of microorganisms

Microorganisms	Origin
Bacteria	
Escherichia coli ATCC 8739	American Type Culture Collection
Pseudomonas aeruginosa ATCC 9027	
Enterococcus faecalis NCAIM B 1312	National Collection of Agricultural and Industrial Microorganisms
Staphylococcus aureus NCAIM B 2174	
Listeria monocytogenes CCM 4699	Czech Collection of Microorganisms
Listeria innocua CCM 4030	
Yeasts	
Saccharomyces cerevisiae S288c	American Type Culture Collection
Candida albicans MVH 1	Clinical County Hospital, Tirgu Mures, Romania
Candida glabrata MVH 2	
Candida parapsilosis MVH 3	

Bacteria- and yeast-inoculated plates were incubated at 37 °C for 24 h and at 30 °C for 48 h, respectively. Diameters of the inhibition zones were measured and the inhibition effect was calculated as a mean ± SD of triplicate tests.

3. RESULTS AND DISCUSSION

3.1. HPLC analysis of nettle leaf and root extracts

Phenolic compounds that are present in the aqueous and ethanolic extracts of stinging nettle leaves and roots were separated by HPLC. Two main groups of polyphenols, phenol carboxylic acids and flavonoids (six compounds each, respectively) were identified and quantified. Results are shown in Table 2.

Our results indicate that the sum of the quantified polyphenols in leaf extracts was always higher than that of the root ones, independently, whether the solvent was water or ethanol. Temperature had significant influence on the water-extractable polyphenols of the leaf, while it affected only slightly that of the root. Increasing the temperature of the aqueous extraction from 80 to 100 °C resulted in considerable differences in the sum of the quantified polyphenols, especially in case of the leaf extracts. While temperature increase did not affect the sum of the phenol carboxylic acids, it decreased the total flavonoid content of the leaves in case of the spring extracts. Raising the temperature increased polyphenols nearly tenfold in both groups, extracted from the autumn harvested dry leaves. This difference can be attributed to the fact that the cell walls of the autumn leaves were much more complex and rigid in their structure than those of the spring ones, and maceration at a higher temperature could facilitate extraction of polyphenols. At the same time, the temperature- and time-dependent inactivation of the phenolic compounds, which were rapidly extracted from the spring leaves in comparison to the autumn leaves, may also play a role in the detected differences. Optimisation of the extraction temperature and time would be necessary in order to extract the highest amount of polyphenols from the spring and autumn leaves.

Table 2. HPLC analysis and quantification of polyphenols (μg g^{-1}) in stinging nettle extracts (100 mg dry mass/mL). Total polyphenol content (TPC) was determined with Folin–Ciocalteu reagent

		Spring						Autumn					
		Aqueous extracts 80 °C		Aqueous extracts 100 °C		Ethanolic extracts 30 °C		Aqueous extracts 80 °C		Aqueous extracts 100 °C		Ethanolic extracts 30 °C	
Phenolic compounds		Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root
Phenolcarboxylic acids	Cinnamic acid	582	88	506	101	314	162	173	27	478	52	134	65
	Chlorogenic acid	905	119	545	175	228	85	11	21	116	37	44	n.d.
	Dihydroxy-benzoic acid	302	298	730	146	211	68	21	60	203	103	37	n.d.
	Ellagic acid	130	36	102	n.d.	108	23	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Syringic acid	83	12	69	19	96	9	33	13	181	22	n.d.	n.d.
	Vanillic acid	3,173	548	3,157	736	2,170	146	12	109	1,571	858	185	22
	Subtotal	**5,175**	**1,101**	**5,109**	**1,177**	**3,127**	**493**	**250**	**230**	**2,549**	**1,072**	**400**	**87**
Flavonoids	Pyrocatechin	347	128	154	111	106	36	55	15	476	24	n.d.	n.d.
	Catechin	468	83	384	142	192	61	97	121	244	86	73	n.d.
	Epicatechin	1,208	100	1,165	254	338	31	69	36	495	77	36	68
	Rutin	7,802	231	4,077	85	7,012	204	361	43	3,579	68	304	151
	Quercetin	666	262	467	154	362	36	107	51	539	91	180	48
	Quercitrin	530	53	425	76	210	85	183	28	420	49	27	n.d.
	Subtotal	**11,021**	**857**	**6,672**	**822**	**8,220**	**453**	**872**	**294**	**5,753**	**395**	**620**	**267**
Total		**16,196**	**1,958**	**11,781**	**1,999**	**11,347**	**946**	**1,122**	**524**	**8,302**	**1,467**	**1,020**	**354**
TPC		**18,395**	**2,024**	**19,848**	**3,629**	**8,060**	**517**	**4,495**	**1,155**	**6,917**	**2,453**	**1,732**	**961**

n.d.: not detected.

Evaluation of the spring leaf extracts showed that among the phenol carboxylic acids vanillic acid was the most abundant both in the aqueous and ethanolic extracts (in the range of 3.2 and 2.2 mg g^{-1}, respectively) beside the cinnamic, chlorogenic, dihydroxy-benzoic, ellagic, and syringic acids (in the range of 0.9–0.1 mg g^{-1}). We detected six different flavonoid compounds in both the aqueous and the ethanolic extracts; rutin was the most abundant in both cases (in the range of 7.8 and 7.0 mg g^{-1}, respectively).

Results shown in Table 2 demonstrate that the root was poor in polyphenols; their total concentrations did not exceed 2 mg g^{-1}. In contrast, the sum of the polyphenol compounds extracted from the leaves was 5–10 times higher. These results are consistent with those published by Chrubasik et al. (2007a, 2007b). Total phenolic contents (TPCs) of the extracts determined with Folin–Ciocalteu reagent are close to or slightly different from the sum of the quantified phenols in most cases, indicating that majority of the phenolic compounds were properly quantified.

Plant phenolic compounds are poorly or moderately soluble in water. Organic solvents such as methanol, ethanol, acetone, ethyl acetate, and their various aqueous mixtures generally enhance solubility and the amount of phenolics extracted. Temperature and time of extraction are also crucial in the recovery of plant polyphenols, although solubilisation and analyte degradation could be the conflicting effects (Dai and Mumper, 2010). The solubility characteristics of phenolics and temperature effect on their recovery might account for the identical or lower quantity of the ethanol- than the water-extracted polyphenols at significantly different temperatures (30 and 80 or 100 °C, respectively) in our experiments.

Aqueous solubility of phenol carboxylic acids is outstanding among the other phenolic compounds, although it largely depends on the number and position of their hydroxyl groups. Among the phenol carboxylic acids, concentrations of hydroxybenzoic acids (ellagic, syringic, and vanillic acids) in saturated water solutions are considerably higher than that of the hydroxycinnamic acids (cinnamic and chlorogenic acids) (Furia et al., 2021). These rules are clearly reflected in the results of Table 2.

Flavonoids are known to have low solubility in water, while they are highly soluble in polar solvents such as methanol, ethanol and dimethyl sulfoxide (Ferreira and Pinho, 2012). The total amounts of flavonoids did not differ considerably in the water and ethanol extracted samples. This could be the consequence of the big difference in the temperatures when these two types of solvents were applied.

Orčić et al. (2014) investigated the methanolic extracts of different organs of stinging nettle collected in Serbia. They identified 21 phenolic compounds, with 5-O-caffeoylquinic acid, rutin, and isoquercitrin as the most abundant. HPLC profiles of the leaf and root extracts were only partly identical with those we obtained by HPLC analysis; vanillic and benzoic acids, catechin, rutin, and quercitrin were detected by both Orčić et al. (2014) and us. Garofulić et al. (2021) detected and quantified as much as 27 phenolic compounds of nettle leaves derived from Slovenia by UPLC/ESI-MS analysis, which belonged to benzoic, cinnamic, and other phenol carboxylic acids, flavonols, flavan-3-ols, flavones, and coumarins. Similarly as in case of Orčić et al. (2014), there was only limited overlap with our results regarding the identified phenol carboxylic acids and flavones, although more similarity was observed. These findings confirm that the geographic origin of nettles and harvesting conditions are crucial in the diversity of phenolic compounds present in detectable amount, beside the conditions of extraction and analytical separation techniques (Dai and Mumper, 2010; Otles and Yalcin, 2012; Repajić et al., 2021).

3.2. Antibacterial and antifungal effects of extracts prepared from nettle leaves and roots

Antimicrobial activity of the aqueous and ethanolic extracts of stinging nettle prepared from dry leaves and roots was tested against human pathogenic and food spoiling bacteria and yeasts. Two Gram negative and four Gram positive bacteria as well as four yeast strains, listed in Table 1, were involved in this study. Results are summarised in Table 3.

We found that the inhibition zones were not completely clear in all cases at the end of the incubation period, background growth of the inoculated microbes caused week turbidity. It is supposed that the antimicrobial compounds of the extracts were below the minimal inhibitory concentrations in such cases.

Our results indicate that *Escherichia coli* and *Staphylococcus aureus* strains were sensitive to every leaf extract, but slight differences in the size and clearness of the inhibition zones were found. The food-borne pathogen *Listeria monocytogenes* and its non-pathogenic counterpart, *Listeria innocua* - both living under similar environmental conditions (Mohan et al., 2019) - had similar sensitivity patterns, although spring ethanolic leaf and aqueous root extracts inhibited the *L. innocua* but not *L. monocytogenes* strain. *Pseudomonas aeruginosa* was inhibited by the spring extracts only, nevertheless it was the most sensitive among all tested strains. Interestingly, *Enterococcus faecalis* was insensitive to the aqueous leaf extracts but it was inhibited considerably by every aqueous root extract. Antimicrobial activity of the ethanolic extracts was very limited, only the spring leaf extracts had week antibacterial effect.

The three human opportunistic pathogenic *Candida* strains exhibited two types of sensitivity patterns: *Candida glabrata* was inhibited by every aqueous leaf extract, while growth of *Candida albicans* and *Candida parapsilosis* was not influenced by any of them. *Saccharomyces cerevisiae*, which is an important starter culture in several fermented food and drinks but it can cause food spoiling in sugar containing food (Maráz and Kovács, 2014), was slightly inhibited by the 100 °C aqueous extract of the autumn leaves.

Li et al. (2017) examined the antibacterial effect of the four major flavonoids (luteolin, L-epicatechin, cianidanol, and quercetin) present in the leaf extracts of the medicinal plant *Dracontomelon dao* (Blanco) Merr. et Rolfe, individually or combined in different concentrations. They confirmed the antibacterial activity of these four flavonoids and proved interaction among the pairs. It was shown that luteolin and cianidanol had synergistic effect at high concentrations, while they were antagonistic at low concentrations. These results indicate that the antimicrobial efficacy of plant extracts, that are rich in antimicrobial flavonoids, is influenced by a concentration-dependent interaction of the flavonoids and therefore, prediction of their antimicrobial activity on a dose-dependent manner is practically impossible.

In a recently published review, Kregiel et al. (2018) made an overview of the antimicrobial activity of *U. dioica* extracts and concluded that various nettle fractions have a great variety of antimicrobial capacity as reported by different researchers. Despite the significant differences, stinging nettle exhibits a great potential as the source of antibacterial and antifungal compounds against a wide variety of human pathogenic and food spoiling microorganisms. Microbial spectra of the bacterial and fungal species tested by Gülçin et al. (2004) were partly overlapping with those included in our studies, and the obtained results confirm the conclusions made by Kregiel et al. (2018). All three above mentioned research groups found antibacterial activity of nettle extracts against *S. aureus* and *E. coli*, while the antibacterial and antifungal activity against

Table 3. Antimicrobial effect of nettle extracts (100 mg dry mass/mL). Diameters of the inhibition zones are shown in mm

Microorganisms	Aqueous extracts								Ethanolic extracts			
	80 °C				100 °C				30 °C			
	Spring		Autumn		Spring		Autumn		Spring		Autumn	
	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root
Escherichia coli	13.0 ± 0.5 *t	–	13.0 ± 0.0 t	–	14.5 ± 3.5 t	–	13.0 ± 0.0 t	–	13.0 ± 4.0	–	–	–
Pseudomonas aeruginosa	28.0 ± 0.0	18.0 ± 1.5 t	–	–	23.0 ± 8.0	–	–	–	10.5 ± 0.5	–	–	–
Enterococcus faecalis	–	17.0 ± 2.0	–	17.0 ± 0.5	–	17.0 ± 0.0 t	–	17.0 ± 1.0	14.0 ± 1.0	–	–	–
Listeria monocytogenes	12.0 ± 0.0	–	15.0 ± 0.0 t	–	–	–	15.0 ± 0.0 t	–	–	–	–	–
Listeria innocua	10.0 ± 1.0	–	12.0 ± 0.0 t	–	–	18.0 ± 1.0	12.0 ± 0.0 t	–	11.5 ± 0.5	–	–	–
Staphylococcus aureus	20.0 ± 6.5	–	16.0 ± 0.0 t	–	21.0 ± 4.0	–	15.0 ± 1.0	–	9.5 ± 0.5	–	–	–
Saccharomyces cerevisiae		–	–	–	–	–	12.5 ± 0.0 t	–	–	–	–	–
Candida albicans	–	–	–	–	–	–	–	–	–	–	–	–
Candida glabrata	18.0 ± 2.0	–	17.0 ± 0.5	–	18.0 ± 1.0	–	16.5 ± 0.5	–	–	–	–	–
Candida parapsilosis	–	–	–	–	–	–	–	–	–	–	–	–

*t: turbid inhibition zone.
Diameter of the wells was 8 mm.

P. aeruginosa and *C. albicans*, respectively, were found dissimilar. In these cases, the detected variations may be principally the consequences of the different extraction techniques and evaluation method but the differences in the geographical location, climatic and soil conditions of nettle growth could also attribute to the observed variations (Kregiel et al., 2018).

4. CONCLUSIONS

We confirmed that both the aqueous and the ethanolic extracts of stinging nettle are rich in bioactive phenolic substances. HPLC analysis indicated different chromatographic profiles and concentrations of the phenolic compounds that are present in the aqueous and ethanolic extracts in case of different nettle organs, solvents, and harvesting seasons.

With the exception of ellagic acid, the tested phenolic compounds were present in detectable amounts in both spring and autumn samples, but spring nettle extracts contained them in significantly higher concentrations.

Ethanolic extracts had negligible or zero antimicrobial activity in the applied concentrations, while aqueous extracts were efficient against several pathogenic bacteria and yeast strains. However, if we consider that the ethanolic extracts were diluted 20-times for the microbiological activity tests, while the aqueous extracts were applied in the original concentrations, we might suppose that ethanolic leaf extracts could be more efficient in lower dilutions than the aqueous ones. On the other hand, differences in the inhibition zones might not reflect proportional differences in the activity, because inhibition areas depend not only on the concentration but the diffusion rate of the active compounds, too. It should also be mentioned that if ethanol containing oral herbal medicinal products intended for human (especially paediatric) population, measures to minimise ethanol exposure should be taken (EMA, 2010).

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