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Original scientific paper

Determination of protein and mineral contents in stinging nettle

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Abstract: Content of proteins and minerals (Mg, Ca, Cu, Zn, Mn and Co) in leaves, stem and root of stinging nettle collected from different localities from the Republic of Macedonia were determined. Generally, the higher content of proteins and minerals were determined in leaves, followed by the content in stem and root. In the quantity of proteins significant differences were determined depending to the organ of stinging nettle. The highest determined values for the protein content in leaves expressed to the dry mass were 26.89% in leaves, 14.54% in stem and 10.89% in root. The values for the calcium content in stinging nettle were higher in comparison to the magnesium values. Zink content in the leaves was two and five times higher than the content of copper and manganese, respectively. In leaves, stem and root non significant differences in cobalt content were determined.

Introduction

Stinging nettle (*Urtica dioica* L., *Urticaceae*) as a weed plant widespread in the world, predominantly in wasteland areas with characteristically unpleasant stinging hairs on the stems and leaves is characterized by economical important potentials [Kavalali, 2003] [Bisht, Bhandari, & Bisht, 2012].

The presence of valuable biologically important compounds such as proteins, vitamins, phenolic components, macro and microelements, tannins, flavonoids, sterols, fatty acids, carotenoids and chlorophylls [Guil-Guerrero, Rebolloso-Fuentes, & Torija-Isasa, 2003] [Nica, et al, 2012] [Vega-Maray, Fernández-González, & Valencia-Barrera, 2006] [Grevsen, & Fretté, 2008] [Pinelli, Ieri, & Vignolini, 2008] [Kopyt'ko, Lapinskaya, & Sokol'skaya, 2012] [Kukrić, et al, 2012], contributes to the utilization of stinging nettle in different ways. The high nutritive values caused stinging nettle leaves to be included in the human consumption, as a tonic for strengthening the body, in the preparation of soups and various dishes and as a natural source of food flavouring [Wetherilt, 2003] [Council of Europe, 2008]. As an animal food, stinging nettle leaves are important for increase and improve of body weight and meat quality [Hanczakowska, Wiytkiewicz, & Szewczyk, 2007] [Khosravi, et al, 2008] [Kwiecien, & Mieczan, 2009]. Stinging nettle leaf extract is used in the manufacture of personal care products and pharmaceutical products, like shampoos, toothpaste and creams with certain functionality [Rayburn, et al, 2009] [Patil, Sapkale, & Surwase, 2010]. The potential of the stinging nettle fibres in the production of natural textiles is investigated, also [Vogl, & Hartl, 2003] [Fischer, Werwein, & Graupner, 2012]. Traditionally, in herbal medicine stinging nettle is used as a diuretic agent and for the treatment of rheumatism and arthritis. Nowadays, in form of leaves and roots extracts, stinging nettle is used as supportive therapy to help relieve rheumatic complaints and seasonal allergy symptoms [Roschek, et al, 2009], in reducing difficulties in urination associated with early stages of benign prostatic hyperplasia [Safarinejad, 2005] [Nahata, & Dixit, 2012] [Schulze-Tanzil, et al, 2002] and in control of glucose level in diabetes [Mehri, et al, 2011] [Sarkhail, 2011] [Namazi, Tarighat, & Bahrami, 2012]. Antimicrobial and antioxidant activities [Kukrić, et al, 2012] [Gülçin, et al, 2004] [Modarresi-Chahardehi, et al, 2012], the possibilities for decreasing of cardiovascular risks [Alisi, et al, 2008] and investigations of chemo preventive properties [Güler, 2013] of stinging nettle extracts in breast cancer cells are still researched.

Alterations in chemical composition and quantity of components of stinging nettle is related to environmental growth factors such as temperature, moisture, light, soil type and nutrients. Among others, development stage, harvest term, form and type of organs, as well as the conditions of storage and drying are important [Grevsen, & Fretté, 2008] [Biesiada, et al, 2009] [Krystofova, et al, 2010] [Kowol, et al, 2011] [Biesiada, et al, 2010].

Determination of proteins and mineral materials content in the leaves, stem and root of stinging nettle collected from different regions of the Republic of Macedonia was set as an objective of this research.

Materials and methods

Plant Materials. The stinging nettle was collected in the first week of June in 2012 from different regions of the Republic of Macedonia. Table 1 presents the localities where the samples of stinging nettle were collected. The collected leaves, stems and roots were with satisfactory organoleptic characteristics without presence of sand and mud. The samples were air dried and then grounded using Retsch ZM1 mill (Germany) and sieved (0.1 mm particle size). The samples placed in dark glass bottles were stored at 4°C in a refrigerator.

Table 1. Altitude and designation of the locality where stinging nettle was collected

Locality	Latitude	Longitude	Altitude (m)
Andon Dukov, Ohrid region	41°08'31'' N	20°45'23''E	695
Markov monastery, Skopje region	41°53'56'' N	21°24'29'' E	380
Village Nikolic, Gevgelija region	41°15'46'' N	22°44'47'E'	180
Popova Sapka, Tetovo region	41°00'47'' N	20°53'03'' E	1694
Lesnica, Tetovo region	41°55'41'' N	20°49'25 E	1450
Gevgelija town	41°08'21'' N	22°30'09'' E	53

Determination of dry matter and proteins content. The dry matter content was determined by drying at 105 °C till constant mass (AOAC, 925.10), the proteins content was determined from the nitrogen content by Kjeldahl method (AOAC, 978.04) using factor 6.25, and calculated as N x 6.25 [AOAC, 1995].

Sample preparation for determination of macro and microelements. 5 g sample (0.0001 g accurately weighted) was heated very carefully with 50 cm^3 6M HCL until the volume decreased to 5 cm^3. Afterwards, 10 cm^3 of hot water was added and again heated for 10 min, filtrated and filled with distillated water in 50 cm^3 volumetric flask.

Atomic absorption spectrophotometry: Content of the macro and microelements in the samples of the stinging nettle were measured with double-beam atomic absorption spectrophotometer (AA M5000, Perkin Elmer, USA). The main source used for AA was the hollow cathode lamp (HCL). The dual option burner system was used with the flow spoiler in a spray chamber. The common oxidant/fuel combination used in AA was air-acetylene. The best wavelengths were selected for our analysis based on the concentration range of sample. The slit width (0.2 nm) was the optimum for the elements which are determined. The following wavelengths (nm) were chosen for metal analysis: Mg (285.2), Ca (422.7), Cu (324.8), Mn (279.5), Zn (213.9) and Co (240.7). The concentration of the elements of interest was determined by using the standard conditions. The standards were prepared by suitable dilution of the stock standard solution to bring the concentration of the elements into a suitable range for analysis (linear range). The analyte concentration of all the samples to be analyzed falls within the linear range and one calibration standard is used. The top of the linear range for determined elements was between 0.25 and 0.30 A.U. The method of standard additions was used to avoid the matrix interferences. The method was based upon the linear relation between the absorbance (AU) and concentration ($\mu g\cdot cm^3$) of the determined element. Samples were made in triplicate. The obtained results are expressed in % and $mg\cdot kg^{-1}$ on a dry mass weight in sample.

Results and discussion

The mean values for the content of proteins determined in leaves, stems and roots of stinging nettles collected from the six regions from the Republic of Macedonia are presented in Table 2. Generally, the highest quantities of proteins expressed in relation to the corresponding dry mass were determined in the leaves in comparison with the stem and the root. The content of proteins in leaves ranged from 16.08 ± 0.38% to 26.89 ± 0.39% depending on the locality where the sample was collected. The highest determined proteins content in the stem and root was 14.54 ± 0.27% and 10.89 ± 0.11%, respectively. The results of protein content of the stinging nettle leaves correspond with the results reported by Adamski & Bieganska [Adamski, & Bieganska, 1984] [Kukrić, et al, 2012].

Table 2. Dry mass and protein contents in leaves, stem and root of stinging nettle

	Sample material	Andon Duhov Ohrid region	Markov monastery Skopje region	Village Nikolic Gevgelija region	Popova Sapka Tetovo region	Lesnica Tetovo region	Gevgelija town
Dry mass (% ± SD[1])	*Leaves*	91.28 ± 0.12	92.50 ± 0.22	90.40 ± 0.23	91.28 ± 0.12	91.49 ± 0.17	91.07 ± 0.13
	Stem	91.85 ± 0.25	91.63 ± 0.27	92.24 ± 0.23	92.31 ± 0.25	91.55 ± 0.24	93.21 ± 0.20
	Root	89.27 ± 0.24	93.91 ± 0.22	93.04 ± 0.23	93.63 ± 0.17	93.91 ± 0.27	93.00 ± 0.13
Proteins (% *± SD[2])	*Leaves*	18.91 ± 0.36	26.89 ± 0.39	16.08 ± 0.38	18.33 ± 0.34	19.03 ± 0.34	21.73 ± 0.37
	Stem	7.67 ± 0.22	14.54 ± 0.27	8.25 ± 0.17	8.07 ± 0.32	8.92 ± 0.21	11.25 ± 0.18
	Root	3.31 ± 0.23	10.89 ± 0.11	7.92 ± 0.26	7.39 ± 0.14	6.95 ± 0.11	3.98 ± 0.25

1 - Mean value ± S.D. (standard deviation) for number of replication n=3
2 - Mean value ± S.D. (standard deviation) for number of replication n=9
** Calculated to the corresponding dry mass weight*

In Table 3, the content of macro (Mg and Ca) and microelements (Cu, Zn, Mn and Co) determined in the stinging nettle is given. The stinging nettle organs, leaves, stem and root, contained higher amount of calcium than magnesium. The content of magnesium and calcium in the leaves was almost three times higher than in stems and roots where none significant differences were confirmed. The calcium content expressed in relation to the dry mass ranged from 2.63 ± 0.05% to 5.09 ± 0.04% in leaves, 0.76 ± 0.03% to 1.42 ± 0.05% in stem and 0.61 ± 0.12% to 0.92 ± 0.06% in root. In the leaves, zinc was found with the highest content (27.44 ± 0.23 mg kg^{-1} dry mass), followed by the content of copper (17.47 ± 0.09 mg kg^{-1} dry mass), manganese (17.17 ± 0.06 mg kg^{-1} dry mass) and cobalt (0.21 ± 0.06 mg kg^{-1} dry mass). The mean values for cobalt content showed insignificantly higher quantities in leaves, than in stem and root. The ranges of the cobalt content expressed to the corresponding dry mass were in the following boundaries: 0.11 - 0.21 mg kg^{-1}, 0.10 - 0.18 mg kg^{-1} and 0.08 - 0.16 mg kg^{-1} for leaves, stem and root, respectively. The obtained results for the metal profiles in the stinging nettle leaves were confirmed with the reported literature data [Grevsen, & Fretté, 2008] [Biesiada, et al, 2009] [Krystofova, et al, 2010] [Kowol, et al, 2011] [Biesiada, et al, 2010]. The quantities of elements found in the stinging nettle leaves, stem and root do not exceed the limits of health hazards and toxicological limits, also [Liu, Goyer, & Waalkes, 2008].

Table 3. Content of macro and microelements in leaves, stem and root of stinging nettle

Content	Sample material	Andon Duhov Ohrid region	Markov monastery Skopje region	Village Nikolic Gevgelija region	Popova Sapka Tetovo region	Lesnica Tetovo region	Gevgelija town
Mg (% *± SD[1])	*Leaves*	2.51 ± 0.15	2.53 ± 0.13	3.56 ± 0.07	3.36 ± 0.14	2.63 ± 0.14	3.08 ± 0.14
	Stem	1.28 ± 0.07	1.67 ± 0.15	1.15 ± 0.07	0.94 ± 0.06	0.79 ± 0.09	1.00 ± 0.06
	Root	0.94 ± 0.12	0.88 ± 0.06	1.03 ± 0.07	0.94 ± 0.15	0.69 ± 0.17	1.15 ± 0.21
Ca (%*± SD[1])	*Leaves*	2.63 ± 0.05	2.94 ± 0.02	5.09 ± 0.04	3.43 ± 0.11	2.83 ± 0.15	3.38 ± 0.05
	Stem	1.06 ± 0.06	1.42 ± 0.05	0.89 ± 0.02	0.76 ± 0.03	1.11 ± 0.03	1.07 ± 0.02
	Root	0.89 ± 0.06	0.76 ± 0.03	0.92 ± 0.06	0.68 ± 0.02	0.61 ± 0.12	0.89 ± 0.07
Cu (mg kg^{-1}*± SD[1])	*Leaves*	12.26 ± 0.31	12.27 ± 0.24	9.85 ± 0.13	17.47 ± 0.09	12.00 ± 0.11	11.07 ± 0.09
	Stem	10.88 ± 0.05	11.05 ± 0.18	7.92 ± 0.11	15.31 ± 0.21	11.05 ± 0.15	10.55 ± 0.12
	Root	10.34 ± 0.11	9.58 ± 0.06	6.57 ± 0.20	14.42 ± 0.26	10.01 ± 0.14	10.55 ± 0.12
Zn (mg kg^{-1}*± SD[1])	*Leaves*	27.44 ± 0.23	21.44 ± 0.18	27.43 ± 0.06	16.97 ± 0.09	26.58 ± 0.16	19.35 ± 0.06
	Stem	25.22 ± 0.15	20.01 ± 0.19	25.92 ± 0.08	15.35 ± 0.14	25.22 ± 0.13	18.19 ± 0.28
	Root	25.18 ± 0.03	19.55 ± 0.17	24.31 ± 0.20	13.91 ± 0.09	24.05 ± 0.13	16.53 ± 0.18
Mn (mg kg^{-1}*± SD[1])	*Leaves*	4.03 ± 0.08	11.96 ± 0.14	17.17 ± 0.06	12.99 ± 0.15	14.07 ± 0.25	20.87 ± 0.31
	Stem	3.29 ± 0.11	10.15 ± 0.26	15.85 ± 0.06	11.65 ± 0.12	13.02 ± 0.16	19.07 ± 0.24
	Root	2.78 ± 0.17	9.94 ± 0.21	14.02 ± 0.15	10.43 ± 0.11	12.34 ± 0.12	17.99 ± 0.05
Co (mg kg^{-1}*± SD[1])	*Leaves*	0.14 ± 0.02	0.17 ± 0.03	0.21 ± 0.06	0.13± 0.07	0.11 ± 0.02	0.18 ± 0.05
	Stem	0.11 ± 0.04	0.14 ± 0.01	0.18 ± 0.02	0.11± 0.09	0.10 ± 0.01	0.16 ± 0.06
	Root	0.10 ± 0.01	0.13 ± 0.02	0.16 ± 0.03	0.09± 0.01	0.08 ± 0.02	0.14 ± 0.03

[1] - Mean value ± S.D. (standard deviation) for number of replication n=3
** Calculated to the corresponding dry mass weight*

Conclusion

The results of this study show that the stinging nettle collected from the Republic of Macedonia can be considered as a source of valuable components. High content of proteins and minerals determined in leaves, stem and root is of great importance for the introduction of the stinging nettle in nourishment, as well as from medicinal and phytotherapeutic point of view. Considering that the data given in the literature is in regard to the components in leaves and root, the determined values for the quantities of proteins and minerals in the stem of the stinging nettle are of significance, with indications of new directions for its utilization.

References

Adamski, R., Bieganska, J. (1984). Investigation of substances present in *Urtica dioica* L. Leaves. Part II. Analyses for proteins, aminoacids and nitrogen non-proteins substances. Herba Pol. 30(1), 17-26.

Alisi, C.S., Emejulu, A.A., Alisi, P.N.C. et al. (2008). Decreased cardiovascular risk and resistance to hyperlipemia-induced hepatic damage in rats by aqueous extract of *Urtica dioica*. Afr. J. Biochem. Res. 2(4), 102-106.

AOAC. (1995). Official Methods of Analysis. 16th ed. Association of Official Analytical Chemists, Washington, USA, pp. 780.

Biesiada, A., Kucharska, A., Sokół-Łętowska, A. et al. (2010). Effect of the age of plantation and harvest term on chemical composition and antioxidant activity of stinging nettle (*Urtica dioica* L.). Ecol. Chem. Eng. A 17(9), 1061-1068.

Biesiada, A., Wołoszczak, E., Sokół-Łętowska, A. et al. (2009). The effect of nitrogen form and dose on yield, chemical composition and antioxidant activity of stinging nettle (*Urtica dioica* L.). Herba Pol. 55(3), 84-93.

Bisht, S., Bhandari, S. Bisht, N.S. (2012). *Urtica dioica* (L): an undervalued, economically important plant. Agric. Sci. Res. J. 2(5), 250-252.

Council of Europe. (2008). Committee of Experts on Flavouring Substances. Natural Sources of Flavourings. Report No. 3. Belgium, Council of Europe Publishing, pp. 208.

Fischer, H., Werwein, E., Graupner, N. (2012). Nettle fibre (*Urtica dioica* L.) reinforced poly(lactic acid): A first approach, J. Compos. Mater. 46, 3077-3087.

Grevsen, K., Fretté, X.C., (2008). Christensen, L. P. Concentration and composition of flavonol glycosides and phenolic acids in aerial parts of stinging nettle (*Urtica dioica* L.) are affected by nitrogen fertilization and by harvest time. Europ. J. Hort. Sci. 73(1), 20-27.

Guil-Guerrero, J.L., Rebolloso-Fuentes, M.M., Torija-Isasa, M.E. (2003). Fatty acids and carotenoids from stinging Nettle (*Urtica dioica* L.). J. Food Comp. Anal. 16, 111-119.

Gülçin, I., Küfrevioğlu, I. Ö., Oktay, M. et al. (2004). Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (*Urtica dioica* L.). J. Ethnopharmacol. 90, 205-215.

Güler, R.E. (2013). Investigation of chemopreventive properties of *Urtica Dioica* L., in MCF-7 and MDA 231 breast cancer cell lines. New J. Med. 30(1), 50-53.

Hanczakowska, E., Wiytkiewicz, M., Szewczyk, A. (2007). Effect of dietary nettle extract on pig meat quality. Med. Wet. 63, 525-527.

Kavalali, G. (2003). An Introduction to *Urtica* Botanical Aspects. In: *Urtica*, edited by Kavalali, G. Taylor & Francis, London and New York, pp. 1-11.

Khosravi, A., Boldaji, F., Dastar, B. et al. (2008). The use of some feed additives as growth promoter in broilers nutrition. Poultry Sci. 7, 1095-1099.

Kopyt'ko, F.Y., Lapinskaya, E.S., Sokol'skaya, T.A. (2012). Application, chemical composition and standardization of nettle raw material and related drugs: A rewiev Pharm. Chem. J. 45(10), 622-631.

Kowol, J., Kwapuliński, J., Brodiak-Dopierala, B. et al. (2011). Influence of a transboundary emission on bioavailability of metals of stinging nettle from soil. Polish J. Environ. Stud. 20(1), 115-124.

Krystofova, O., Adam, V., Babula, P. et al. (2010). Effects of various doses of selenite on stinging nettle (*Urtica dioica* L.). Int. J. Environ. Res. Public Health 7, 3804-3815.

Kukrić, Z.Z., Topalić-Trivunović, N.Lj., Kukavica, M.B. et al. (2012). Characterization of antioxidant and antimicrobial activities of nettle leaves (*Urtica dioica* L.). APTEFF 2012, 43, 259-272.

Kwiecien, M., Mieczan, W. (2009). Effect of addition of herbs on body weight and assessment antibiotic of physical and chemical alterations in the tiba bones of broiler chickens. J. Elem. 14, 705-715.

Liu, J., Goyer, A.R., Waalkes, P.M. (2008). Toxic Effects of Metals. In: Toxicology, edited by Klaassen, D. C. The McGraw-Hill Companies, pp. 931-981.

Mehri, A., Ranjbar-Hasani, S., Larijani, B. et al. (2011). systematic review of efficacy and safety of *Urtica dioica* in treatment of diabetes. Int. J. Pharmacol. 7(2), 161-170.

Modarresi-Chahardehi, A., Ibrahim, D., Sulaiman, F.S. et al. (2012). Screening antimicrobial activity of various extracts of *Urtica dioica*. Rev. Biol. Trop. 60 (4), 1567-1576.

Nahata, A., Dixit, V.K. (2012). Ameliorative effects of stinging nettle (*Urtica dioica*) on testosterone-induced prostatic hyperplasia in rats. Andrologia 44(1), 396-409.

Namazi, N., Tarighat, A., Bahrami, A. (2012). The effect of hydro alcoholic nettle (*Urtica dioica*) extract on oxidative stress in patients with type 2 diabetes: A randomized double-blind clinical trial. Pak. J. Biol. Sci. 15(2), 98-102.

Nica, D., Bordean, D.M., Gergen, I. et al. (2012). Comparative assessment of trace metal accumulation in celery and nettle leaves. JHFB. 16(2), 217-220.

Patil, S.M., Sapkale, G.N., Surwase, U.S., (2010). Bhombe, B.T. Herbal medicines as an effective therapy in hair loss: A review. Res. J. Pharm. Biol. Chem. Sci. 1(2), 773-781.

Pinelli, P., Ieri, F., Vignolini, P. (2008). Extraction and HPLC analysis of phenolic compounds in leaves, stalks, and textile fibers of *Urtica dioica* L. *J. Agric. Food Chem.* 56(19), 9127-9132.

Rayburn, K., Fleischbein, E., Song, J. et al. (2009). Stinging nettle cream for osteoarthritis. Altern. Ther. Health Med. 15(4), 60-66.

Roschek, B., Fink, R.C., McMichael, M. et al. (2009). Nettle extract (*Urtica dioica*) affects key receptors and enzymes associated with allergic rhinitis. Phytother. Res. 23(7), 920-926.

Safarinejad, M.R. (2005). *Urtica dioica* for treatment of benign prostatic hyperplasia: A prospective, randomized, double-blind, placebo-controlled, crossover study. J. Herbal Pharmacother. 5(4), 1-11.

Sarkhail, P. (2011). *Urtica dioica* improves glucose control in diabetes on the basis of animal studies. Asian J. Anim. Vet. Adv. 6, 865-867.

Schulze-Tanzil, G., Souza, P., Behnke, B. et al. (2002). Effects of the antirheumatic remedy Hox alpha - a new stinging nettle leaf extract - on matrix metalloproteinases in human chondrocytes *in vitro*. Histol. Histopathol. 17, 477-485.

Vega-Maray, A.M., Fernández-González, D., Valencia-Barrera, R. (2006). Allergenic proteins in *Urtica dioica*, a member of the *Urticaceae* allergenic family. Ann. Allergy Asthma Immunol. 2006, 97, 343-349.

Vogl, C.R., Hartl, A., (2003). Production and processing of organically grown fiber nettle (*Urtica dioica* L.) and its potential use in the natural textile industry: A review. Am. J. Alternative Agric. 18(3), 119-128.

Wetherilt, H. (2003). Nutritional Evaluation of *Urtica* Species. In: *Urtica*, edited by Kavalali, G. Taylor & Francis, London and New York, pp. 84-92.

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