STINGING NETTLE – THE SOURCE OF BIOLOGICALLY ACTIVE COMPOUNDS AS SUSTAINABLE DAILY DIET SUPPLEMENT

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Abstract
Environmental conditions and climate change on a global scale affects the overall agriculture and food supply. Consumers demand for vegetables with high nutritional value is increasing. Consumers more and more are thinking about a healthy and balanced diet, but it is not easy to provide year-round fresh vegetables. Nettle (*Urtica)* leaves traditionally are used in early spring as a leafy vegetable in salads and soups. Young leaves before flowering are used for human consumption. Nettle contains a lot of vitamins and biologically active compounds. The research aim was to evaluate different stinging nettle clones, which grow in Pūre village (Tukuma district, Latvia). Samples were collected, when shoots were 10 – 15 cm long. Content of chlorophyll, carotenoids and anthocyanins in five nettle clones leaves were analysed. Biochemical analysis was done in Latvia University of Agriculture, Institute of Soil and Plant Science laboratory. Differences were observed between all clones. Significant difference between genotypes was observed in anthocyanins content, but not in chlorophylls and carotenoids content. Higher anthocyanins content was observed in samples, which grow in places with low nitrogen and phosphorus content. Content of biochemical compounds can influence some metal ions, environmental and other factors.
Key words: nettle, carotenoids, chlorophylls.

Introduction

Nettle (*Urtica)* has a long history as herbal medicine and nourishing food. Nettle species, also known as weed, are widely used as food in early spring. Stinging nettle (*Urtica dioica* L.) young leaves are added to soups or salads as well as dried for winter use (Guil-Guerrero et al., 2003). Lately producers more and more are focusing on nettle as an industrial crop. Also, it is used for textile production.

Nettle production in Europe began in the 19th century. About 500 ha of nettles were cultivated in Germany and Austria and used for textile production. Nettle crop can produce economically significant yields for four years. If grown longer, weed infestations tend to increase and yields reduce. Extensively nettle can be grown for 10 – 15 years, or even without time limit (Vogl and Harti, 2003).

More than 1000 plant species of nettle family (*Urticaceae*) are known across the world. In Latvia, only two nettle species are found:

- *Urtica dioica* L. – often called common nettle or stinging nettle, fibber nettle. It is a perennial herb, 30 – 150 cm in height, lightly green in colour, usually dioecious. Leaves are ovate, rarely lanceolate acuminate. The male and female flower heads are similar in form, branched. The female flowers have purplish stigma.
- *Urtica urens* L. – known as annual nettle, dwarf nettle, small nettle, dog nettle or burning nettle. It is an annual herb, monoecious, 10 – 60 cm in height, clear green in colour. Leaves are ovate and deeply serrate. The male and female flowers are numerous, centrally glabrous or sparsely hispid on the back. It reminds the common nettle in habit but has smaller leaves and short flowers (Kavalali, 2003a).

Common nettle is the only nettle, which can be used for consumption and for medical purposes. Its nutritional value is very high, much higher than other vegetables and herbs usually grown in the gardens. *Urtica* leaves have relatively high level of protein (66%), which is of better quality if compared with the proteins of other leafy vegetables (Hughes et al., 1980). The leaves of nettle are good sources of different significant minerals and vitamins (Adamski and Bieganska, 1980; Kukric et al., 2012). Nettles contain flavonoids, fatty acids, terpenes, protein, vitamins, and minerals. Stinging nettle leaves are rich in vitamin C (20 – 60 mg 100 g^{-1}), B groups vitamins, vitamin K (0.16 – 0.64 mg 100 g^{-1}) and some minerals, such as calcium, iron, magnesium, phosphorus, potassium and sodium (Upton, 2013). Nettle leaves contain nine carotenoids. Lutein and lutein isomers, β-carotene are the basic carotenoids in nettle leaves (Guil-Guerrero et al., 2003). Also important substances are amino acids, glucokinins and chlorophylls (Upton, 2013). Nettle leaves contain significant amount of chlorophylls, 2.5 mg g^{-1} fresh weight (Hojnik et al., 2007). Younger leaves contain more chlorophylls and carotenoids than older ones. Chlorophyll content is very different in leaf vegetables. Lettuce (*Lactuca sativa* L.) total chlorophylls content on average is 0.2 mg g^{-1} (Cruz et al., 2012), dandelion (*Taraxacum officinale* L.) and chicory (*Cichorium intybus* L.) 2.5 mg g^{-1}, rocket (*Eruca sativa* L.) 3 mg g^{-1} (Žnidarčič et al., 2011).

Nettle for medicine is used as a nourishing blood tonic, diuretic, haemostatic, purgative vermifuge, blood purifier, antiarthritic, for seasonal allergiesand for the treatment of eczema, rheumatism, haemorrhoids, hyperthyroidism, bronchitis, cancer and also used for sprains and swellings (Kavalali, 2003b; Upton et al., 2011). *Urtica* species are also used in many phytotherapeutic preparations (Kavalali, 2003b). Different parts of the nettle plant can be used as food, fodder and as material for cosmetics and medicine industry (Vogl and Harti, 2003). Since ancient times, people have used nettle for flailing arthritic or paralytic limbs with fresh stinging nettle to stimulate circulation of blood. Dried herbs used for teas, tablets, capsules and other preparations. Nettle tea is recommended if you have problems with breast, lung, stomach and urinary tract. Preparations from fresh plant material include juice, homeopathic products, liquid extracts (Randall, 2003).

Clinical studies did not show negative effect with nettle product properties. Experiments with animals showed analgesic effect. Nettles have antioxidant and antiviral properties. Higher doses of nettle can cause blood – vessel narrowing (Upton, 2013).

In Latvia, nettles are not cultivated yet. With each year consumers interest on nettle as leafy vegetable and food additive is gradually rising. Therefore, the aim of the research was set: to collect local wild nettle genotypes in the surroundings of Pūre village (Tukuma district) and evaluate the accumulation of biologically active compounds in the clones.

Materials and Methods

Five local nettle (*Urtica dioica* L.) clones were collected in the Pūre village (Tukums district, Latvia 57°2′9″N 22°54′25″E) in the spring of 2013. All clones were grown in places, with soil acidy pH KCl 7±0.2. Growing conditions were different. Content of nitrogen and phosphorus in soil was various. Two clones (clone 1 and clone 5) were collected in places where low phosphorus and nitrogen content was stated. Other two clones (clone 2 and clone 4) were collected in places with medium level content of phosphorus and nitrogen. One clone (clone 3) was growing in the place with relatively higher N, P content in comparison with others. Nitrogen and phosphorus content in soil was determined using express methods. Content of nitrogen and phosphorus was gradation from 1 to 4 (1 – lower lever, 4 – highest level) (Tab.1).

Nitrogen content was determined by using diphenylamine $(C_6H_5)_2NH$) express method, based on the formation of intense blue tint (N,N'-difenil benzidin violet) that results from the reaction between nitrate and diphenylamine ions.

Phosphorus content was determined with express method by using solutions of ammonium molybdate, benzidine and sodium acetate. Soluble silicates and phosphates react with ammonium molybdate in nitric acid solution to form yellow complex molybdates. These complex molybdates bring about the oxidation of benzidine in alkaline solution with the formation of a blue quinoid compound and molvbdenum blue (Магницкий, 1972).

All samples were harvested in one day, when shoots were 10 – 15 cm long. In one sample, there were five shoots. Samples were cooled, packed in a thermo bag and transported ensuring low temperature to the laboratory. On the same day the samples for biochemical analysis were prepared. Analyses were performed in Latvia University of Agriculture, Institute of Soil and Plant Sciences.

The content of chlorophyll, carotenoids and anthocyanins in nettle leaves was analysed spectrophotometrically (Shimadzu Spectrophotometer UV-18000) in two replicates.

Leaf average sample was weighed and placed in a pestle and added a bit of quartz sand, some crystals of $CaCO_3$, and added 1 – 2 mL of 960 g kg^{-1} ethyl alcohol and ground. Samples were placed in a graduated tube, filled up to 10 mL of ET-OH and then centrifuged HermleZ383. The solution optical density at different wavelength (nm) was determined and content of chlorophyll a, chlorophyll b, total chlorophylls and carotenoids were calculated by equations (1) – (4)

$$C_{hla} = 13.7 \times A_{665} - 5.76 \times A_{649} \quad (1)$$

$$C_{hlb} = 25.8 \times A_{649} - 7.60 \times A_{665} \quad (2)$$

$$C_{hl(a+b)} = 6.1 \times A_{665} + 20.04 \times A_{649} \quad (3)$$

Table 1

Nitrogen and phosphorus content in soil, mg kg^{-1}

	1 (no color)	2 (pale blue)	3 (blue)	4 (dark blue)
Nitrogen	<50	50-100	100-150	>150
Phosphorus	<100	100-300	300-600	>600

$$C_c = 4.695 \times A_{440.5} - 0.263 \times C_{(a+b)} \quad (4)$$

where

$C_{hla.} C_{hlb.} C_{hl(a+b)}$ and C_c – concentration of pigments mg dm^{-3};

A – light absorption, at appropriate wavelength;

Calculated content of pigments (mg g^{-1} fresh weight):

$$m = \frac{c \times m_{wei}}{P \times 1000} \quad (5)$$

where

m – content of pigments in plant material, mg g^{-1};

c – concentration of pigments, mg dm^{-3};

m_{vei} – weighed amount of plant material, g;

1000 – Coefficient to recalculate from dm^3 to cm^3 (Гавриленко и Жигалова, 2003).

Leaf average sample was weighed and placed in a pestle and ground. Samples were placed in a graduated tube, 10 mL of 10 g kg^{-1} hydrochloric acid was added, and then centrifuged. The optical density of solution was fixed at 535 nm wavelength and the content of anthocyanins in plant material was calculated (Moor et al., 2005).

The results were analyzed using ANOVA at significance level of $\alpha = 0.05$.

Results and Discussion

Morphological differences of clones were stated. Leaf samples were of different intensity green colour. For some samples purple – violet hue was observed (clone 1 and clone 4). Differences in green pigment - chlorophyll content in analysed clones were also stated. Chlorophyll content in the samples ranged between 1.87 – 2.51 mg g^{-1} fresh weight (Fig. 1).

A result confirms findings of others scientists. Our results were very similar to results obtained in the research performed in Slovenia, where chlorophyll content was on average 2.50 mg g^{-1} fresh weight (Hojnik et al., 2007). A.M. Humphrey (1980) mentions that nettle leaves contain approximately 2.5 mg g^{-1} total chlorophylls. Y.F. Kopytko (2012) refers that nettle leaves contain chlorophyll 10 – 50 mg g^{-1} dry mass, of which 75% are chlorophyll a and 25% chlorophyll b. Also, in our research relation 3:1 to chlorophyll a chlorophyll b was observed. Chlorophyll content in nettle samples was different, although significant differences between clones in chlorophyll content were not observed in the nettle leaves ($p = 0.33$).

The content of carotenoids in nettle leaves was four times lower than chlorophylls content (Fig. 2).

Results show that carotenoids content ranged between 0.52 – 0.63 mg g^{-1} fresh weight. Significant differences between clones are not observed also in carotenoids content in the analysed nettle leaves ($p = 0.28$). In research performed in Serbia leaf samples were collected at different times during vegetation period. It showed differences during carotenoids content, it ranged between 0.22 mg g^{-1} – 0.22 mg g^{-1} fresh weight (Kukric et al., 2012).

Significant differences between clones are observed in anthocyanins content ($p = 0.03$) (Fig. 3).

Anthocyanins content in the analysed samples ranged between 0.22 – 1.99 mg g^{-1} fresh weight. Higher anthocyanins content was observed in clones 1 and 5, which grew in place with phosphorus deficit. It is reported that deficit of nutrients, especially phosphorus and nitrogen, promote the accumulation of anthocyanins was found (Biesiada and Tomczak, 2012). It was stated that in the case when plants have phosphorus deficit, their leaves are dark green-violet colour tone. In this case plants accumulate much more athocyanins as normally. Also, R. Piccaglia with

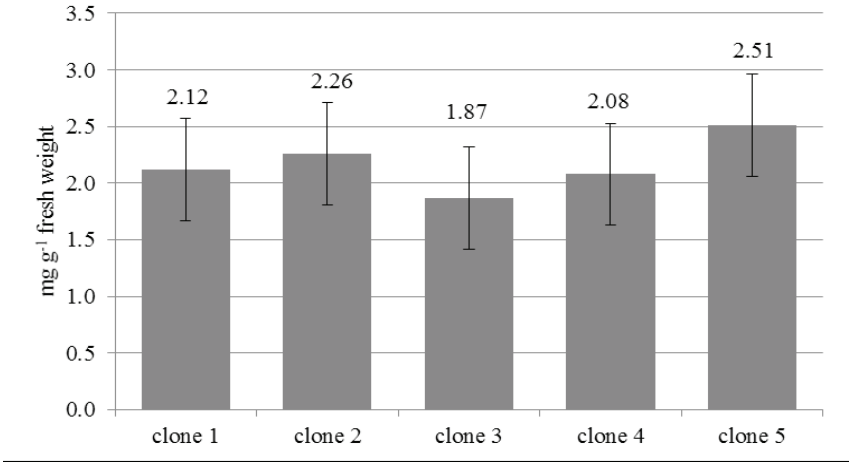

Figure 1. Chlorophylls content in the analysed nettle leaves.

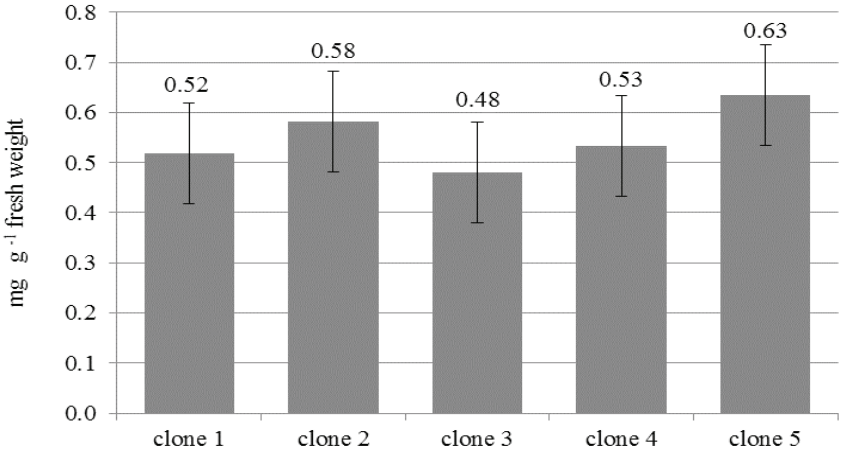

Figure 2. Carotenoids content in nettle leaves.

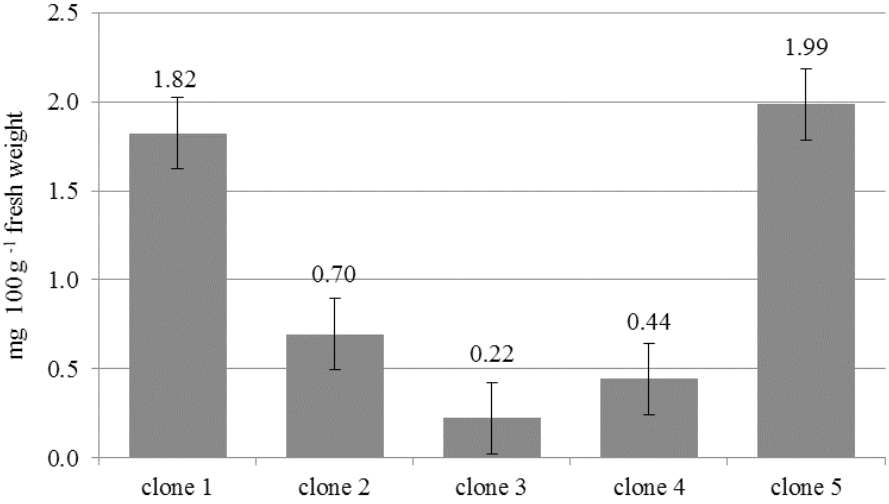

Figure 3. Anthocyanins content in the nettle leaves.

colleagues (Piccaglia et al., 2002) reports that the lower content of anthocyanins was detected if higher doses of phosphorus were added. Our results show that correlation coefficient between nitrogen content in soil and anthocyanins content in leaf sample was $r = -0.95$ ($r > r_{0.05} = 0.878$) and between phosphorus content in soil and anthocyanins content in leaf sample was $r = -0.10$ ($r < r_{0.05} = 0.878$). Higher phosphorus and nitrogen content (clone 3) had negative influence on the anthocyanins accumulation. Clone 3 grew in the place where there was a greenhouse some years ago.

The amount of anthocyanins in leaves is influenced by many factors. One of these factors is presence of some metal ions: aluminum, iron and magnesium in soil. Environmental factors also change leaf tone (Mlodzinska, 2009). Therefore, it is considered essential to plant all the clones in one place where similar agro-ecological conditions are ensured in order to evaluate influence of genotype on the content of biochemically active compounds and exclude the genotype-environment interaction.

Conclusions

High amounts of chlorophyll were detected in the analysed samples. It should be stressed as an important factor that the use of nettle is an important component for biologically functional food. Significant difference between genotypes was observed in anthocyanins content, but insignificant one in chlorophylls and carotenoids content. Stinging nettle leaves have high level of pigments, especially chlorophylls, which provide our daily diet with biologically active compounds.

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