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Antioxidant activity in nettle (*Urtica dioica* L.) and garden orache (*Atriplex hortensis* L.) leaves during vegetation period

S. Zeipiņa
Institute of Soil and Plant Sciences, Faculty of Agriculture, Latvia University of Agriculture,
Liela Str. 2, Jelgava, LV-3001, Latvia;
Pure Horticultural Research Centre,
Abavas Str. 2, Pure, Tukuma district, LV-3124, Latvia
E-mail: solvita.zeipina@gmail.com

I. Alsiņa
Institute of Soil and Plant Sciences, Faculty of Agriculture, Latvia University of Agriculture,
Liela Str. 2, Jelgava, LV-3001, Latvia

L. Lepse
Pure Horticultural Research Centre,
Abavas Str. 2, Pure, Tukuma district, LV-3124, Latvia

M. Dūma
Department of Chemistry, Faculty of Food Technology, Latvia University of Agriculture,
Liela Str. 2, Jelgava, LV-3001, Latvia

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Different vegetables are a good source of essential components for human food. Consumers are more and more aware of a healthy and balanced diet. Vegetables usually contain natural antioxidants which neutralize free radicals in the human body. The composition of biologically active compounds in plants is depending on the climate and growing conditions, cultivar properties, plant development stage, harvesting time, and other factors. The aim of the research was to evaluate the fluctuation of antioxidant activity in leaves of four common nettle (*Urtica dioca* L.) clones and three garden orache (*Atriplex hortensis* L.) varieties during vegetation period in Latvian conditions. Experiments were carried out in open field conditions in the Pūre Horticultural Research Centre during the vegetation period of 2014, in two soil fertility variants for nettle and two sowing periods for garden orache. Antiradical activity was determined using the 2.2-diphenyl-1-picrylhydrazyl radical (DPPH*) method. In nettle leaves, antioxidant activity ranged between 17.31–80.77 %, depending on soil fertility, nettle clone and plant age, and in orache leaves between 17.9–54.0 % depending on variety and sowing time. In nettle leaves, the antioxidant activity at the beginning of the vegetation period ranged between 31.0–62.7 %, the highest activity was observed at the stem elongation stage (75.5–78.5 %). A higher antiradical activity was detected in the control variant (without compost treatment) at the 3–5 and 7–9 leaves stage. In other development stages, better results were obtained in the fertilized variant where compost and peat were used as an organic manure. Differences were found also among clones, especially in the 3–5 leave stage. Significant differences for garden orache leaves were found between sowing times. In the first growing period the antiradical activity ranged between 46.1–54.0 %, in the second it was two times less.

Key words: orache, nettle, antioxidant activity

Introduction

Consumers' demand for vegetables with a high nutritional value is increasing. Vitamins (A, C, E), also tocopherols, carotenoids, glutathiones, polyphenols have antioxidant properties [1]. Leafy vegetables are rich in natural antioxidants which can neutralize free radicals in the human body [2, 3].

Across the world, there are known more than 1000 plant species of the nettle family (*Urticaceae*). In Latvia, only two nettle species are found: *Urtica dioica* L., often called common nettle or stinging nettle, and *Urtica urens* L. known as annual nettle. Stinging nettle leaves can be used in early spring as a leafy vegetable in salads and soups [2]. Nettle leaves contain a lot of vitamins and biologically active compounds [4, 5]. Nettles have the antioxidant and antiviral activity. Nettle in medicine is used as a nourishing blood tonic, diuretic, haemostatic, purgative vermifuge, blood purifier, antiarthritic, for seasonal allergies and for the treatment of eczema, rheumatism, haemorrhoids, hyperthyroidism, bronchitis, cancer, and also used for sprains and swellings [6, 7]. Higher doses of nettle can cause blood vessel narrowing. Experiments with animals have shown that stinging nettle has a potential antioxidant effect on ischemic muscle tissues, promoting brain cell survival. R. Upton [8] reports that glutathione, cysteine, and ascorbic acid are natural antioxidants with the antimutagenic effect [8], also phenolic components have antioxidant properties [9].

Over 100 plant species of the *Atriplex* genus are know. The garden orache (*Atriplex hortensis* L.) is grown as a cultivated plant from this genus. It is called also as mountain spinach, sea purslane, saltbush, arroche or gartenmelde [10, 11], it is assumed as one of the oldest cultivated plants, valued mainly for its leaves [12]. For food are used young stems and leaves. Orache is rich in vitamins and biologically active compounds. It has also dietetic and medicinal properties. Used in food, it helps absorb nutrients from food and stimulates digestion [11].

It is referred that stress conditions during plant growth increase the content of antioxidants in plants. Climate and soil, agrotechnological factors, place and date of planting, fertilizing, mulching, salinity are assumed as stress factors influencing the content of antioxidants in plants. Antioxidant content can vary among individual plants of the same species [1].

The aim of the research was to evaluate changes of the antioxidant activity in common nettle (*Urtica dioca* L.) and garden orache (*Atriplex hortensis* L.) leaves during the vegetation period in Latvian conditions.

Materials and methods

The investigations were carried out at the Pure Horticultural Research Centre during the vegetation season of 2014. Four local nettle clones and three garden orache varieties were used for this research. Morphologically different (anthocyanin coloration, leaf colour, plant size) nettle clones were collected in the Pūre village in 2013 autumn and planted in a strongly altered by cultivation soil (ANt) in two variants: control – without any fertilization, and fertilized with peat / manure compost 40 kg·m^{-2}. Nettles were planted in 3 replicates, the total area of each experimental plot was 3 m^2. In the control variant (A), soil contained total N – 0.21 %, P_2O_5 – 352.1 mg·kg^{-1}, K_2O – 133.5 mg·kg^{-1} and organic matter 5.44 %. The fertilized soil variant (B) cantained total N – 0.38 %, P_2O_5 – 417.3 mg·kg^{-1}, K_2O – 223.3 mg·kg^{-1}, and organic matter 8.16 %. Root stolons were planted in double row planting beds, at a 40 cm distance between rows in a bed, 80 cm between centres of planting beds, and 20 cm between plants in a row. Root stolons established very well. First leaves emerged at the beginning of April 2014. First shoots emerged after a couple of weeks in most of the plants. The first plant analyses were performed at this stage. Plants were analysed five times per season at different plant development stages: 3–5 leaves, 7–9 leaves, stem elongation, emergence of inflorescence, and flowering stage.

Garden orache cv.'Red Flash', cv 'Plume Red' and cv 'Plume Green' were grown in four replications, in brown soil with residual carbonates (BRk) (N – 0.10 %, P_2O_5 – 190.4 mg·kg^{-1}, K_2O – 191.8 mg·kg^{-1} and organic matter content 2.97 %), the size of each experimental plot was 3 m^2. For the first growing period, plants were sown on May 20, but for the second – on July 16.

The meteorological parameters used in the investigation (precipitation and average air/soil temperature) were recorded by the automatic meteorological station "Lufft" located at the Pūre HRC (Fig. 1).

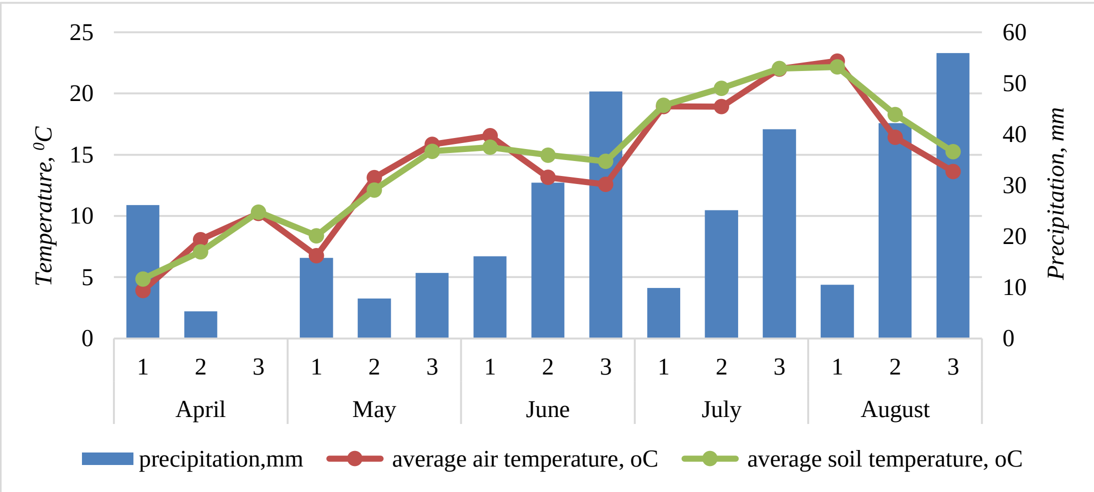

Fig. 1. Meteorological parameters in the vegetation season of 2014, in decades (the Pure HRC automatic station "Lufft" data)

First nettle leaves emerged at the beginning of April when a sufficient precipitation was observed, but in the third decade the precipitation was not observed, and irrigation was done on 24 April when the air temperature was +7.8 °C. Totally, precipitation during the following vegetation period was sufficient, but not regular. The lack of precipitation was observed in the third decade of July and the first decade of August when the average air temperature exceeded +20 °C. In this period, nettles developed flowers.

The length of the first growing period for garden orache was 45 days, but in the second growing period 33 days, which was determined by a higher air temperature enhancing plant growth and development.

Antiradical activity was determined using the 2.2-diphenyl-1-picrylhydrazyl free radical (DPPH*) method.

For determination, 1 ± 0.001 g of nettle and orache mass quantitatively transferred in 20 ml tubes, 10 ml methanol was added. Samples were shaken for 30 minutes, then centrifuged 5 minutes at 5000 rpm. 0.3 ml of an aliquot of the methanolic nettle extract and the garden orache extract was added to a 3 mL DPPH* methanolic solution and spectrophotometrically determined at the 515 nm wavelength. A spectrophotometer UV-1800 (Shimadzu Corporation, Japan) was used. Antiradical activity (%) was calculated by equation (1):

$$\mathrm{ARA} = 100 \times (1 - A_{ss}/A_0), \qquad (1)$$

where A_{ss} – absorbance of the sample solution; A_0 – absorbance of the control DPPH* solution with 0.3 ml of methanol [13].

The results were analysed using ANOVA at the significance level of $p = 0.05$.

Results and discussion

No significant difference was observed for antiradical activity in the nettle leaves among different clones in the control variant without fertilization (A), but it was stated among the development stages ($p = 7.73 \times 10^{19}$). In the variant with fertilized soil (B), significant differences were observed between nettle clones ($p = 0.04$) and development stages ($p = 3.34 \times 10^{-16}$) (Fig. 2).

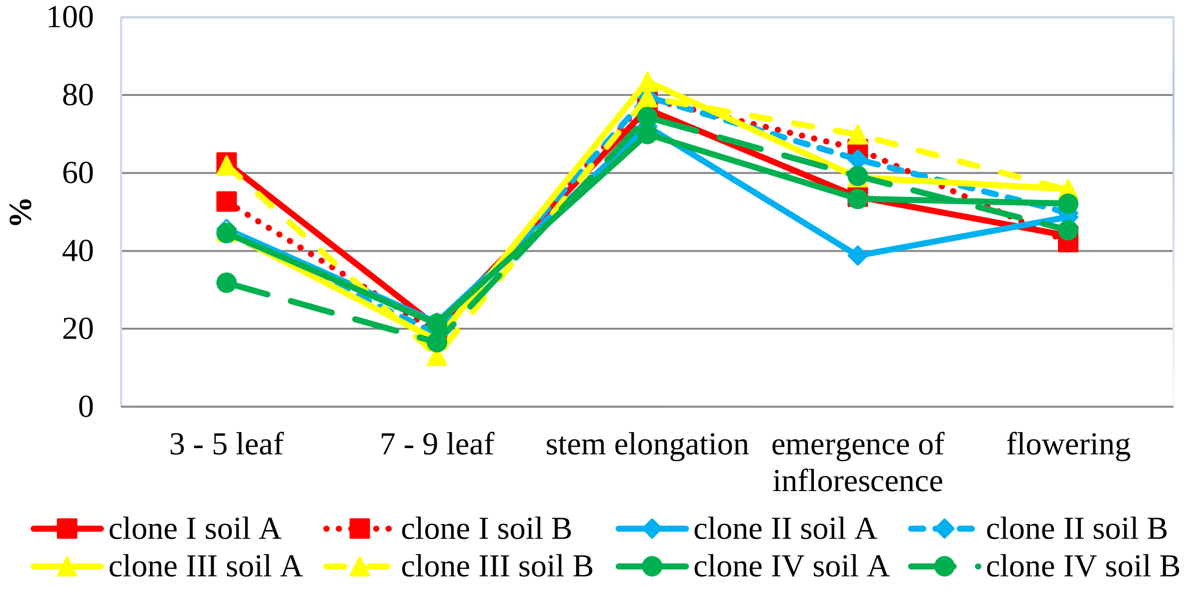

Fig. 2. Antiradical activity in nettle leaves. Control $LSD_{0.05\ (clone)} = 5.29$, $LSD_{0.05(age)} = 5.92$; with fertilization $LSD_{0.05\ (clone)} = 7.37$, $LSD_{0.05(age)} = 8.24$.

The obtained results showed quite clear tendencies of antiradical activity changes in the nettle leaves during the whole vegetation period. On the average, for all samples it was stated between 17.3–80.8 %, depending on fertilization, nettle clone and development stage. There were no clear tendencies in antiradical activity differences between the control and the fertilized variants. At the 3–5 leaf stage, a higher antiradical activity was found for clones I, II and IV in the control variant. The same tendency was observed at the 7–9 leaves stage when all clones showed better results in the control variant. An opposite situation was observed in the next two development stages when a higher antiradical activity was found for all clones in the fertilized variant. In the flowering stage, a higher antiradical activity was found again in the control variant. If to compare the clones, during the whole vegetation period, the highest average antiradical activity was detected for clone III (control variant 52.1 %, fertilized variant 56.0 %) but the lowest for clone II (control variant 45.3 %) and clone IV (fertilized variant 45.5 %). At the 3–5 leaf stage, the antiradical activity ranged between 31.0–62.7 %. Notable differences were observed among clones in the fertilized variant, whereas the majority of clones in the control variant had an almost similar antiradical activity. This leads us to the assumption that in better growing conditions plants can better express their genetically determined properties, and they are more expressed at the beginning of the vegetation period. Also, anthocyanin coloration for two genotypes was clearly visible at the beginning of the vegetation period, and it reduced with plant ageing. At the 7–9 leaves stage, the antiradical activity was only the average values – 16.9–20.2 %. The highest antiradical activity was observed in the stem elongation stage (75.5–78.5 %). The antiradical activity of mature plants was found to be similar to the average – 50–60 %, and there were no clear tendencies for the differences between the genotypes and soil conditions.

No significant differences for antiradical activity in the garden orache leaves were observed among the varieties, but they were observed among the growing periods ($p = 1.45 \times 10^{-6}$) (Fig. 3).

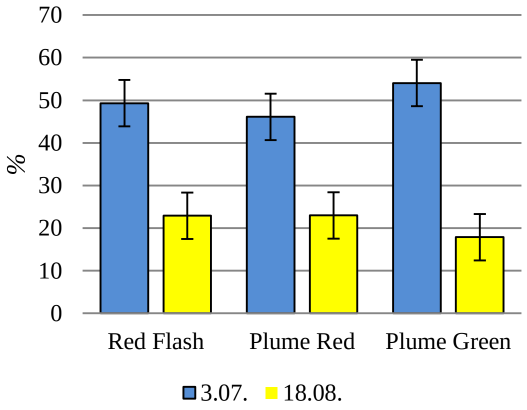

Fig. 3. Antiradical activity in garden orache leaves.
$LSD_{0.05}$ = 10.90; $LSD_{0.05(variety)}$ = 7.71; $LSD_{0.05(sowing\ time)}$ = 6.29.

Results of investigation show quite clear tendencies of antiradical activity differences in the garden orache leaves between both growing periods. In the first growing period antiradical activity ranged between 46.1–54.0 %, and in the second it was on average two times less, 17.9–23.0 %. There were found no differences in antiradical activity between varieties of different leaf coloration – red and green.

Production and consumption of leafy vegetables is more and more interesting growers because their crop can be harvested more than one time per vegetation period and is of high biological value. Results of antioxidant activity in nettle leaves obtained in our trials are higher than it was referred for nettles in Iran, collected in August – 27.1 %, and detected in methanolic extract. In some findings with other extracts the results were quite different: in butanol and ethyl acetate extracts – 62.5 and 62.2 % [9].

It should be stressed that for nettle in all development stages there were no better results in antiradical activity for plants grown in the soil with a higher organic matter content. Probably in the control variant, lower soil organic matter in some development stages was acting as a stress factor. It is in accordance with findings of Oh and colleagues who report that leafy vegetables accumulate more antioxidants, if they are subjected to stress conditions [15].

In Poland, where antioxidant activity was detected for plants of different sowing times (the 2nd and 3rd decades of April and the 1st decade of May), higher antioxidant activity was observed for plants of the last sowing time. In this variant, the antiradical activity was found 31.1 %, but also in both other variants the difference was not so high – respectively 23.6 and 29.1 % [1].

Also in other vegetables differences in antiradical activity have been found for plants of different sowing times. For example, in the USA was compared antiradical activity in lettuce in two different harvesting times (July and September). In July it was higher than in September [14].

In our findings for garden orache, there was observed an indicative tendency of a higher antioxidant activity in plants grown in more severe growing conditions. In the first growing period, regular but not sufficient natural precipitation and not so high air temperature (average day temperature did not exceed +16.5 °C) was observed, contrary to the second growing period when the air temperature was higher and precipitation sufficient. It is in line with the assumption that plants react to stress factors by increasing antiradical activity. These assumptions should be clarified in more detail in further trials.

Conclusions

The results have indicated that the antioxidant activity in nettle leaves is greatly varies among different plant development stages. A higher antiradical activity was observed in the 3–5 leaves stage in the control variant and at the stem elongation stage in the fertilized variant. Antiradical activity in garden orache leaves was absolutely higher in the first growing period in more severe growing conditions. A similar antioxidant activity was found for garden orache varieties with both leave colorations – red and green.

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References

1. **Biesiada A., Tomczak A**. Biotic and abiotic factors affecting the content of the chosen antioxidant compounds in vegetables // Vegetable Crops Research Bulletin. 2012. Vol. 76. N 1. P. 55–78. http://dx.doi.org/10.2478/v10032-012-0004-3
2. **Guil-Guerrero J. L., Rebolloso-Fuentes M. M., Torija Isasa M. E.** Fatty acids and carotenoids from Stinging Nettle (*Urticadioica* L.) // Journal of Food Composition and Analysis. 2003. Vol. 16. N 2. P. 111–119. http://dx.doi.org/10.1016/S0889-1575(02)00172-2
3. **Kim M. H., Kim J. M.,Yoon K. Y.** Effects of blanching on antioxidant activity and total phenolic content according to type of medicinal plants // Food Science Biotechnology. 2013. Vol. 22. N 3. P. 817–823. http://dx.doi.org/10.1007/s10068-013-0150-5
4. **Adamski R**., **Bieganska J**. Studies of chemical substances present in Urtica dioica leaves. Part I, Trace element // Herba Polonica. 1980. Vol. 26. N 3. P. 177–180.
5. **Kukric Z. Z., Topalic-Trivunovic L. N., Kukavica B. M., Matoš S. B., Pavičic S. S., Boroja M. M., Savič A. V.** Characterization of antioxidant and antimicrobial activities of nettle leaves (*Urtica dioica* L.) // Acta Periodica Technologica. 2012. Vol. 43. P. 257–272. http://dx.doi.org/10.2298/APT1243257K
6. **Kavalali G**. The chemical and pharmacological aspects of Urtica. Kavalali G. Urtica: The genus Urtica, Taylor and Francis (CRC Press), Oxford, UK, 2003. P. 25–39.
7. **Upton R., Graff A., Jolliffe G., Langer R., Williamson E.** American Herbal Pharmacopoeia: Botanical Pharmacognosy – Microscopic Characterization of Botanical Medicines, Taylor and Francis (CRC Press), Oxford, UK. 2011, 800 p. http://dx.doi.org/10.1201/b10413

8. **Upton R.** Stinging nettles leaf (*Urtica dioica* L.): Extraordinary vegetable medicine // Journal of Herbal Medicine. 2013. Vol. 3. N 1. P. 9–38. http://dx.doi.org/10.1016/j.hermed.2012.11.001
9. **Chahardehi A. M., Ibrahim D., Sulaiman S. F**. Antioxidant activity and total phenolic content of some medicinal plants in Urticaceae family // Journal of Applied Biological Sciences. 2009. Vol. 3. N 2. P. 25–29.
10. **Carlsson R., Wendy Clarke E. M.** *Atriplex hortensis* L. as a leafy vegetable, and as a leaf protein concentrate plant // Plant Foods for Human Nutrition. 1983. Vol. 33. N 2–3. P. 127–133. http://dx.doi.org/10.1007/BF01091298
11. **Greszczuk M., Jadczak D., Kawecka A**. Effect of sowing date on biological value of garden orache // Acta Scientiarum Polonorum / Hortorum Cultus. 2010. Vol. 9. N 4. P. 163–169.
12. **Wright K. H., Huber K. C., Fairbanks D. J., Huber C. S.** Isolation and characterization of *Atriplex hortensis* and *Sweet Chenopodium quinoa* starches // Cereal Chemistry. 2002. Vol. 79. N 5. P. 715–719. http://dx.doi.org/10.1094/CCHEM.2002.79.5.715
13. **Gülçin I., Küfrevioğlu I., Oktay M., Büyükokoroğlu M. E**. Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (*Urtica dioica* L.) // Journal of Ethnopharmacology. 2004. Vol. 90. N 2–3. P. 205–215. http://dx.doi.org/10.1016/j.jep.2003.09.028
14. **Liu X., Ardo S., Bunning M., Parry J., Zhou K., Stuhnoff C., Stinoker F, Yu L., Kendall P.** Total phenolic content and DPPH radical scavenging activity of lettuce (*Lactuca sativa* L.) grown in Colorado // LWT – Food Science and Technology. 2007. Vol. 40. N 3. P. 552–557. http://dx.doi.org/10.1016/j.lwt.2005.09.007
15. **Oh M. M., Carey E. E., Rajashekar C. B** Antioxidant phytochemicals in lettuce grown in high tunnels and open field // Horticultural, Environment, and Biotechnology. 2011. Vol. 55. N 2. P. 133–139. http://dx.doi.org/10.1007/s13580-011-0200-y

S. Zeipiņa, I. Alsiņa, L. Lepse, M. Dūma

PAPRASTOSIOS DILGELĖS (*URTICA DIOCA* L.) IR SODINĖS BALANDŪNĖS (*ATRIPLEX HORTENSIS* L.) LAPŲ ANTIOKSIDACINIS AKTYVUMAS VEGETACIJOS PERIODU

S a n t r a u k a

Daržovės yra įvairių būtinų komponentų šaltinis. Vartotojai vis daugiau rūpinasi savo sveika ir subalansuota mityba. Daržovės kaupia natūralius antioksidantus, kurie neutralizuoja žmogaus organizme susiformuojančius laisvuosius radikalus. Biologiškai aktyvių junginių sudėtis augaluose priklauso nuo klimato, augimo sąlygų, rūšies, augalo vystymosi, derliaus nuėmimo laiko ir kitų veiksnių. Šio tyrimo tikslas – įvertinti antioksidacinio aktyvumo kitimą paprastosios dilgelės (*Urtica dioca* L.) ir sodinės balandūnės (*Atriplex hortensis* L.), užaugintų Latvijoje vegetacijos periodu, lapuose. Augalai auginti 2014 m. Pūrės sodininkystės tyrimų centre. Antiradikalinis aktyvumas nustatytas taikant 2,2-difenil-1-pikrilhidrazilo radikalo (DPPH$^{\bullet}$) metodą. Didžiausias antioksidacinis aktyvumas nustatytas vegetacijos periodo pradžioje. Paprastosios dilgėlės lapų aktyvumas buvo 17,3–80,8 % atsižvelgiant į dirvožemį, augalo kloną ir amžių; balandūnės lapų aktyvumas buvo 17,9–54,0 % atsižvelgiant į rūšį ir sėjos laiką.