Composition and content of antioxidants in nodules and leaves of stachis (Stachys sieboldii Mig) of Bochonok and Rakushka varieties

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Abstract. Providing the world population with high-quality and functional food products is one of the main tasks of modern scientists. The introduction and subsequent cultivation of new and rare crops makes it possible to expand the diet. Stachys sieboldii Mig, or stakhys, is a vegetable plant with medicinal properties. Of particular value is the underground part of the plant, its nodules. But even the leaves accumulate microelements, antioxidants and phenolic compounds.

1. Introduction
The vegetable plant Stachys sieboldii Mig belongs to the deadnettle family, whose nodules have medicinal properties, while the aerial organs of this plant have been little studied [1]. Stachys plants planted in the Moscow region in the spring-autumn period form a branched, well-leafy stem that looks like mint. A mass of bright green leaves is formed on the main and lateral shoots, which is a reproducible raw material rich in photosynthetic pigments to produce a food-grade green dye.

The aim of the work was to study the composition and content of biologically active substances (including antioxidants) in nodules and aerial parts of plants grown in open and protected ground.

2. Methods and materials
The content of chlorophylls and carotenoids in ethanol 95% was determined by the equations proposed by Lichtenthaler [2]. The content of the reduced form of ascorbic acid (AA) was determined by the iodometric method based on titration of ascorbic acid in colored extracts with potassium iodate in an acidic medium in the presence of potassium iodide and starch, the fractional composition and content of phenolic compounds as described in the procedure [3]. The elemental composition was determined by the method of emission spectral analysis, the result was expressed in terms of ash. The total antioxidant capacity (TAC) was determined by amperometric method, the result was expressed in GAE/g [4–5].
3. Results

The content of plant phenols in nodules was studied. These include aromatic organic compounds that contain one or more hydroxyl groups linked to carbon atoms of an aromatic benzene ring. In stachys nodules, there were no significant differences between varieties in terms of the content of phenolic compounds (PS) (Table 1)

Table 1. Fractional composition and content of phenolic compounds (% of absolutely dry mass)

| Sample                        | Bochonok       | Rakushka       |
|-------------------------------|----------------|----------------|
| total PSs                     | 3.87±0.11      | 3.78±0.11      |
| simple PSs and hydroxybenzoic | 0.55±0.05      | 0.47±0.05      |
| acids                         |                |                |
| hydroxycinnamic acids         | 0.11±0.01      | 0.10±0.01      |
| flavonoids                    | 0.44±0.04      | 0.51±0.04      |
| condensed and polymer PSs     | 2.77±0.11      | 2.70±0.11      |

Stachys nodules contain stachiosis tetrasaccharide with an inulin-like action. Due to the increased content of stachyose in nodules, this culture is of pharmacological value in the prevention and treatment of diabetes [6]. It was shown that when using stachys powder, the growth of beneficial intestinal flora is stimulated.

Flavonoids and phenolcarboxylic acids, which accumulate in larger quantities in stachys nodules of the Rakushka and Bochonok varieties, are not only highly biological, but also exhibit an antihistamine effect, reducing capillary permeability and are used as a vasodilator [7]. At the same time, flavonoids containing 3,4-dioxigroup (quercetin, myricetin, luteolin and their glycosides) have the highest P-vitamin activity. In addition to biological activity, they exhibit antioxidant activity. The amount of phenolic compounds in Bochonok stachys nodules is from 5.97 to 6.12 mg/g in dry weight.

The dynamics of changes in the TAC may indicate the degree of resistance of plants to cold stress [5, 8]. A higher content of antioxidants was found in the autumn before nodules were planted in the soil in the winter compared to those dug in the spring (Table 2).

Table 2. Content of antioxidants in Bochonok stachys nodules

| Sample         | TAC [mg GAE/g] | AA [mg%] |
|----------------|---------------|---------|
|                | water extract | ethanol extract |       |
| autumn nodules | 1.56±0.05     | 2.91±0.05 | 15.1±0.8 |
| spring nodules | 1.39±0.05     | 2.50±0.05 | 13.3±0.8 |

Moreover, stachys samples with different mass of nodules differed in the unequal total content of hydrophilic antioxidants. Table 3 presents the results of the determination of TAC in nodules before planting in the winter. A decrease in the mass of stachys nodules led to a decrease in the total antioxidant capacity.

In the spring of May, young plants appeared in the protected ground, which reached a height of 7–9 cm, the leaves were located on the main shoot forming 6–7 tiers, the length of which ranged from 3 to 6 cm. The root system reached up to 7 cm and the length of the nodules was up to 3 cm.

Table 3. TAC in Bochonok stahis nodules before planting in ground

| Nodule mass [g] | TAC [mg GAE/g] |
|-----------------|---------------|
| 2.10±0.05       | 1.38±0.06     |
| 1.90±0.05       | 1.13±0.05     |
| 1.50±0.05       | 0.85±0.03     |
| 1.40±0.05       | 0.75±0.03     |
| 0.80±0.05       | 0.64±0.03     |

It should be noted that after the winter dormant period, in the protected ground, as well as after wintering in the open ground, part of the nodules acquired a brown color, although white seedlings formed from the nodule. As can be seen from Table 4, brown nodule retained antioxidant activity, and stachys seedling had a 1.7 times higher total antioxidant content. At the same time, the total
antioxidant capacity in the overwintered white nodule of stachys was higher in comparison with the seedling.

### Table 4. TAC in Bochonok stahis nodules after winter

| Sample                  | TAC [mg GAE/g] |
|-------------------------|----------------|
| brown nodule seedling   | 0.58±0.03      |
| brown nodule            | 0.34±0.03      |
| white nodule seedling   | 0.79±0.05      |
| white nodule            | 1.23±0.06      |

In May 2019, young stachys plants grown in protected ground formed 6–7 tiers of leaves on a tetrahedral green stem. Leaves located on different tiers differed in the total antioxidant content (Table 5). The smallest antioxidants were found in the juvenile leaves of the lateral shoots (0.27 mg GAE/g), and the maximum amount of antioxidants accumulated in young leaves of the fifth tier with an incompletely formed leaf blade (0.70 mg GAE/g).

### Table 5. TAC in Bochonok stahys plants grown in protected ground

| Sample                              | TAC [mg GAE/g] |
|-------------------------------------|----------------|
| first pair of leaves                 | 0.50±0.03      |
| second pair of leaves                | 0.41±0.02      |
| third pair of leaves                 | 0.31±0.02      |
| fourth pair of leaves                | 0.50±0.03      |
| fifth pair (young) of leaves         | 0.70±0.04      |
| side leaves                          | 0.27±0.01      |
| petioles                             | 0.20±0.01      |
| stem                                 | 0.16±0.01      |
| stolons                              | 0.18±0.01      |
| roots                                | 0.57±0.03      |
| nodule                               | 0.33±0.02      |

Apparently, leaves of 2–4 tiers with a lower content of antioxidants can act as donors of antioxidants for juvenile developing leaves, petioles, and underground organs – stolons and nodules, in which significantly less antioxidants were found. (From 0.16 to 0.33 mg GAE/g) with the exception of the root system (0.57 mg GAE/g).

It is interesting to note that in May 2018, the total antioxidant content in stachys nodules was higher (0.87–1.33 mg GAE/g) than in the leaves of these samples (0.72–0.77 mg GAE/g). At the same time, in May 2019, in the protected ground, the maximum content of antioxidants in young leaves was higher than in nodules (Table 5).

Photosynthetic pigments are biologically active compounds. Therefore, in the leaves of different tiers, as well as in the stems and petioles, the content of chlorophylls and carotenoids was studied (Table 6). A minimal amount of chlorophyll was present in the stems and petioles. Whereas young leaves, as well as leaves of the fourth and second tiers, accumulated the maximum amount of photosynthetic pigments.

Recently emerged new technologies in physiological and medical research confirm the important role of microelements at the level of metabolic reactions and submolecular processes, the activity of which depends on the presence of certain macro- and microelements contained in our daily diet.

The features of the accumulation of minerals in nodules of Bochonok stachys were studied (Table 7). The main share of ash elements in nodules is potassium. A significant contribution is made by phosphorus and molybdenum. The remaining macronutrients are many times less. Moreover, sulfur is a biogenic element in the composition of proteins and glutathione, has an antioxidant effect [9]. Microelements form range from silicon to manganese. Manganese (Mn) is a cofactor and activator of
many enzymes (pyruvate kinase, decarboxylase, superoxide dismutase), is involved in the synthesis of glycoproteins and proteoglycans, and has an antioxidant effect.

Table 6. Content of photosynthetic pigments in Bochonok stahys plants

| Sample      | Xια | Xιδ | Xια+δ | Kap |
|-------------|-----|-----|-------|-----|
| stalks      | 0.09±0.01 | 0.04±0.01 | 0.13±0.01 | 0.04±0.01 |
| petioles    | 0.15±0.01 | 0.05±0.01 | 0.21±0.01 | 0.08±0.01 |
| leaves      |       |     |       |     |
| lower       | 0.73±0.03 | 0.21±0.01 | 0.94±0.04 | 0.33±0.02 |
| second      | 1.06±0.05 | 0.36±0.02 | 1.41±0.07 | 0.47±0.02 |
| third       | 0.94±0.05 | 0.30±0.02 | 1.24±0.07 | 0.43±0.02 |
| fourth      | 1.07±0.05 | 0.30±0.02 | 1.26±0.07 | 0.49±0.03 |
| fifth       | 1.04±0.05 | 0.32±0.02 | 1.36±0.07 | 0.48±0.03 |
| juvenile    | 1.04±0.05 | 0.31±0.02 | 1.35±0.07 | 0.48±0.02 |
| side        | 0.67±0.03 | 0.21±0.01 | 0.88±0.04 | 0.32±0.02 |

Table 7. Elementary composition of Bochonok stuhys, wt %

| Macroelements | Microelements |
|---------------|---------------|
| K             | Si            | 0.57 % |
| P             | Cu            | 0.20 % |
| Mo            | Zn            | 0.19 % |
| Mg            | Fe            | 0.10 % |
| Cl            | Co            | 0.11 % |
| S             | Al            | 0.17 % |
| Ca            | Mn            | 0.05 % |

4. Conclusion
A direct correlation was found between the mass of Bochonok stachys nodule and the total antioxidant capacity.

The maximum total antioxidant capacity was found in young leaves (fifth tier) of the Bochonok stachys plants, which exceeded the amount of antioxidants in the leaves of the first tier by 1.75 times, and in the lateral leaves by 2.5 times.

A minimal amount of antioxidants was found in the stalk and petioles of the aerial organs of the stachys plant, which was 3.5 times less than in young leaves.

References
[1] Tundis R, Peruzzi L and Menichini F 2014 Phytochemical and biological studies of Stachys species in relation to chemotaxonomy: a review Phytochem. 102 7–39
[2] Lichtenhalter H K 1987 Chlorophylls and carotenoids – pigments of photosynthetic biomembranes Methods in Enzymol. 148 350–82
[3] Gins M S, Gins V K, Kolesnikov M P et al 2010 Method for the analysis of phenolic compounds in vegetable crops (Moskaw: Ministry of Agriculture of the Russian Federation)
[4] Cosio M S, Buratti S, Mannino S and Benedetti S 2006 Use of an electrochemical method to evaluate the antioxidant activity of herb extracts from the Labiatae family Food Chem. 97 725–31
[5] Levko G D, Gins M S, Zdolnikova E A et al 2016 Influence of the total content of water-soluble antioxidants in rhizomes on winter hardiness of garden iris varieties (Iris hybrida L.) Russian vegetables 1(30) 76–81
[6] Yin J, Yang G, Wang S and Chen Y 2006 Purification and determination of stachyose in Chinese artichoke (Stachys sieboldii Miq.) by high-performance liquid chromatography with evaporative light scattering detection Talanta 70(1) 208–12
[7] Lichota A, Gwozdzinski L and Gwozdzinski K 2019 Therapeutic potential of natural compounds in inflammation and chronic venous insufficiency *Europ. J. of Med. Chem.* **176** 68–91

[8] Huang M and Guo Z 2005 Responses of antioxidative system to chilling stress in two rice cultivars differing in sensitivity *Biol. Plantarum* **49**(1) 81–4

[9] Feroci G and Fini A 1998 Study of the antioxidant effect of several selenium and sulphur compounds *J. Trace Elem. Med. Biol.* **12** 96–100