

THE MORPHOLOGICAL AND ANTIOXIDANT CHARACTERISTICS OF INFLORESCENCES WITHIN WILD-GROWING GENOTYPES OF ELDERBERRY (*SAMBUCUS NIGRA* L.)

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ABSTRACT

The aim of this study was to determine the basic morphological characteristics (weight, length) and antioxidant activity (using DPPH method) of elderberry (*Sambucus nigra* L.) inflorescences as well as some elderberry-derived food products prepared from fresh (honey, alcoholic extract, tea infusions) and dried inflorescences (syrup). For the study of problematic, it was used 113 wild-growing genotypes of elderberry from 56 locations in Slovakia growing at an altitude of 98.15 – 712.32 m. The weight of fresh inflorescences ranged from 0.45 to 57.59 g (75.65% coefficient of variation value), the total length of inflorescences ranged from 19.0 to 282.0 mm (22.42%), the length of inflorescence's stems from 9.0 to 197.0 mm (31.51%), a number of petals predominated pentanumerous petals. Variability in primary and secondary branching reported a low to high degree of variability among as well as within the genotypes. Results showed significant differences in the shape of inflorescences and the colour of flowers among each genotype. Antioxidant activity by DPPH method in elderberry inflorescence water extract was between 85.12 and 89.29%. Activation of tea infusions and beverages was ensured using a mechanism Kalyxx based on galvanic effect. In beverages made from 10% diluted honey prepared from fresh inflorescences in the carbohydrate-based extract, anti-radical activity was determined in the range of 16.81 – 24.16%. In an alcoholic extract from fresh inflorescences, anti-radical activity was between 90.99 and 93.16%. In beverages acquired from the syrup of flowers, we identified antioxidant activity ranging from 37.92 (10%) to 62.82% (40%). Results indicated that elderberry inflorescences and elderberry-derived food products can be attractive to consumers and in future can increase the assortments of healthy products.

Keywords: elderberry-derived products; tea infusions; syrup; honey; beverages; DPPH radical

INTRODUCTION

Elderberry (*Sambucus nigra* L.) grows freely throughout Europe. All parts of this plant i.e. bark, roots, leaves, flowers and fruits have medicinal properties, and therefore they are economically used primarily in food, pharmaceutical and cosmetic industry (Hejný, 2001). In traditional culinary practice, it used fresh and dried inflorescences to prepare tea infusions, syrups, alcohol extracts and other products (Grieve, 1971; Miraj, 2016; Tarko et al., 2015). The inflorescences are commonly used as tea infusions, giving a very common refreshing drink in Northern Europe and the Balkans. In Europe, these plant parts are made into a syrup, which is diluted with water before drinking (Jørgensen et al., 2000; Miraj, 2016) or is possible by fermentation process prepare beverage similarly like wine. In south-western Sweden, it is traditional to make a snaps liqueur flavoured with elderflower. Inflorescences are also used in a mildly

alcoholic sparkling elderflower 'champagne' (Miraj, 2016).

In Slovakia we can find some elderberry-derived food products particularly in health-food stores (elderberry wines, mixtures of dried flowers and berries for tea infusions, syrups, beverages, sweets, jams and jellies).

The inflorescences consist of aromatic white flowers, which are clustered in umbrella cymes (Jančovičová, 2011). In elderberry upgrows rich inflorescences with flowers almost in one line at the end of twigs (Paganová, 2001; Atkinson and Atkinson, 2002). Mratinić and Fotirić (2007) observed five selected clones from the natural populations of elderberry in Serbia and determined that average length of inflorescences ranged from 106.7 to 143.2 mm, the width from 72.8 mm to 158.8 mm. Kabuce (2006) also determined the average length of inflorescences 10 – 20 cm. Stems (Pedicels) of inflorescences are generally short, tomentose with two

bracteoles (Atkinson and Atkinson, 2002). Watson and Dallwitz (2007) reported small, hermaphrodite, unfrequently diclinous flowers, radial symmetric with 3 – 5 sepals, 3 – 5 gamopetalous petals 3 – 5 mm in length, 3 – 5 free stamens and yellow anthers with 3 coupled styles, which form 3 seeds in the fruit. These authors also documented that small, white or yellow-and-white flowers collected in large, rich, multifloral and flat umbels are characterized by a specific and intense aroma. According to Grieve (1971), fresh flowers have a slightly bitter taste and an unpleasant odour, but by maceration of flowers in distilled water gradually obtained a pleasant aroma. It was also reported that elderberry inflorescences are rich in biologically active compounds.

Elderflowers contain about 3% flavonoids; dominants are the following: rutin, quercetin, isoquercetin, kaempferol, astragalin (WHO, 2002; EMEA/HMPC, 2007; Ivanišová et al., 2015; Tomašková et al., 2017), nicotiflorin (Hänsel et al. 1994; Willer 1997), hyperoside (Willer, 1997; Fleming, 2000; WHO, 2002); from phenolic acids chlorogenic acid and caffeic acid derivatives are dominant (3%) (Fleming, 2000; Wu et al., 2004; Thole et al., 2006). Volatile oil (0.03 – 0.14%), high share (65%) of free fatty acids, including among others palmitic acid (share 38%) (Fleming, 2000) and linoleic acid, 7% alkanes (Barnes et al., 2002) amines (choline, etylamine, isobutylamine, isoamylamine), organic acids (e.g. valeric acid, ascorbic acid, citric acid, malic acid, tartaric acid, fumaric acid, shikimic acid) (Mikulic-Petkovsek et al., 2016), vitamins, mineral compounds (8%) and saccharides were also determined in elderflowers. Numerous other constituent types have been identified, including ethers and oxides, ketones, aldehydes, alcohols and esters (Toulemonde and Richard, 1983).

The consumption of elderberry helps in the prevention and therapy for a number of diseases, such as diabetes (Gray et al., 2000; Netzel et al., 2005; Fowler, 2010; Bhattacharya et al., 2013; Folmer et al., 2014; Song et al., 2014), obesity (Chrubasik et al., 2008; Christensen et al., 2010; Petruț et al., 2017), elderberry also exhibited antibacterial, antifungal (Kong, 2009; Hearst et al., 2010; Krawitz et al., 2011; Kinoshita et al., 2012), and antitumour activity (Thole et al., 2006; Pehlivan Karakas et al., 2012), immune system stimulation (Ciocoiu et al., 2010; Groza et al., 2010; Frøkiær et al., 2012), protection against UV radiation (Chen et al., 2012; Jarzycka et al., 2013), diuretic and laxative activity (Beaux et al., 1999; Picon et al., 2010).

The aim of this study was to determine the basic morphological characteristics (weight, length) and antioxidant activity (using DPPH method) of elderberry inflorescences as well as some elderberry-derived food products prepared from fresh (honey, alcoholic extract, tea infusions) and dried inflorescences (syrup).

Scientific hypothesis

There are significant differences between various genotypes in morphometrics parameters and antioxidant activity of some products from elderberry. Inflorescences are rich in biologically active compounds with strong antioxidant activity and for this reason, can be used for preparing various kinds of products for human nutrition.

MATERIAL AND METHODOLOGY

Biological material

Sambucus nigra inflorescences were collected in 2008 – 2010 from bushes and trees growing within Slovakia localized at an altitude of 98.15 – 712.32 m above the sea level. The inflorescences were picked from bushes with stems (pedicels); the flowers were evaluated for uniformity of the colour and then transported to the laboratory for analysis. The plants were botanically identified in the Institute of Biodiversity Conservation and Biosafety of the Slovak University of Agriculture in Nitra. Samples were marked as SN (*Sambucus nigra*) in morphometric analyses, HSN (elderflower honey), TSN (tea infusion), AE (alcohol extract) in antioxidant analyses and appropriate number.

Morphometrical analysis of elderberry inflorescences

The following properties were measured by morphometrical analysis:

- Inflorescence weight in g, n = 10 was detected for flower clusters with blossoming flowers by measuring of weight with analytical scales (Kern ADB-A01S05, Germany).
- Inflorescence totally length in mm, n = 10 measurement was performed from the base to the apical part of the flower clusters and was measured using a ruler.
- Inflorescence's pedicel length in mm, n = 5 measurement was performed from the base to the apical part of the flower clusters and was measured using a ruler.
- Number of petals, n = 5.
- Number of primary and secondary branches in flower clusters, n = 10.
- Shape of inflorescence.
- Compactness of inflorescences and time of entry into the flowering phase.

Antioxidant analysis of elderberry-derived products

Chemicals

All the chemicals used were of analytical grade and were purchased from Sigma-Aldrich (St. Louis, MO, USA) and CentralChem (Slovakia).

Preparation of elderberry inflorescences products

The tested fresh inflorescences and elderberry-derived products were made by a traditional procedure using the processing methods of native inhabitants within Slovakia.

- Tea infusions were obtained by dried inflorescences and activation using a mechanism Kalyxx based on galvanic effect. From tea infusions, 14 variants with 4 controls were tested.
- Honey was obtained from fresh inflorescences boiled for one hour in 1 L water, together with one lemon sliced using sucrose with ratio 1 : 1. Next day infusion strained through a gauze and boiled again. Finally, tea infusion was put into the glass and hermetically sealed. Totally 10 variants of honey with 4 controls were tested.

c) Alcoholic extract was prepared from fresh inflorescences poured 40% alcohol and put in dark place. Totally 10 variants of alcohol extracts with 4 controls were tested.

Syrup was produced from fresh inflorescences, lemon slices, 6 pieces mint's stem, lemon balm and 2 L water mixed, allowed to stand 24 hours with the addition of sucrose with a ratio 1:1 and boiled for 30 minutes. Totally 4 samples of syrup in 4 concentrations were tested.

Activation of tea and beverages

Activation of tea and beverages was ensured using a mechanism Kalyxx – based galvanic effect. Fluid activation can be achieved by simply pouring through the mechanism. In the experiment, it was also provided multiple activations. Double activation was ensured by flushing the fluid through the mechanism device 2-fold, three times 3-fold. Activation is provided by increasing the biopoly of the fluids themselves.

Free radical scavenging activity

Free radical scavenging activity was measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to **Brand-Williams et al. (1995)**. An amount of 0.1 mL of sample of juice, wine, syrup, liqueur; sample of fruits conserved by honey, jam, compote and jelly was homogenized in a mortar and 20 g of the blended mass was extracted for 24 hours in 200 mL of distilled water, filtrated (Whatman No. 1) and after filtration the extract was used for measuring. For detection of free radical scavenging activity, the extract was mixed with 3.9 mL of DPPH radical (0.025 g was soluble in 96% ethanol and diluted as needed). Absorbance was registered at 515 nm at regular time intervals until the reaction equilibrium was reached (10 minutes) by using spectrophotometer (Genesys 20 UV-VIS, USA). The DPPH scavenging activity (% inhibition) was calculated to fresh matter (FM) by using of the following equation:

$$\% Inh = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0 – absorbance of control reaction and A_1 – absorbance in presence of the sample.

Statistical analysis

It was evaluated the variability of the test files in each character using descriptive statistics. For the characteristics of the files, it was used the basic descriptors of variability: average, minimum measured value, maximum measured value, the coefficient of variation (%). Data were analysed with ANOVA test and differences

between means compared through the Tukey-Kramer test ($\alpha = 0.05$). The degree of variability was determined by the coefficient of variation values. The given parameter is independent of the unit of the evaluated character. Theoretically, they can acquire different values (**Stehlíková, 1998**). Cluster dendrogram were performed in the free software for scientific data analysis PAST 2.10.

RESULTS AND DISCUSSION

Morphological analysis of elderberry inflorescences

The weight, compactness and uniformity of flowering (simultaneous flowering) of elderflower-clusters belong among considerable characteristics for practical uses in preparing of various teas. In elderberry upgrows rich inflorescences with flowers located on the top of branches and collected in corymbs (**Paganová, 2001; Atkinson and Atkinson, 2002**).

In the collection of 113 wild-growing elderberry morphological specifics of each genotypes was confirmed and the fresh weight of inflorescences was determined: it was in the range from 0.45 (SN-77) to 57.59 g (SN-34). Genotypes with high inflorescences weight also provide their high-quality for practical uses. The average coefficient of variation was 75.65%, which shows a very high degree of variability (Table 1).

In addition to the harvesting and drying of the flowers, the length of inflorescences is also important. In the evaluated collections, it was determined the length of the flower clusters ranged from 19.0 (SN-13) to 282.0 mm (SN-51). The average coefficient of variation was 22.42%, which shows a high degree of variability.

Mratinić and Fotirić (2007) in their study of five selected clones from the natural populations of elderberry in Serbia, determined the average length of inflorescences in the range of 106.7 – 143.2 mm, the width from 72.8 to 158.8 mm. **Kabuce (2006)** determined average length of inflorescences in the range of 100 – 200 mm. Thus, the Slovak populations are more variable than the Serbian and Lithuanian ones, on this characteristic.

Flowers aggregated in inflorescences – cymes, corymb and umbels in panicles. Inflorescences are terminal, large, repeatedly branched, compound, flat-topped (**Watson and Dallwitz, 2007**). The pedicels of inflorescences are generally short, tomentose, with two bracteoles (**Atkinson and Atkinson, 2002; Watson and Dallwitz, 2007**).

For the evaluation the length of a pedicel, it was determined the range from 9.0 (SN-04) to 197.0 mm (SN-55). The average coefficient of variation was 31.50%, which shows a very high degree of variability.

Watson and Dallwitz (2007) reported small,

Table 1 The variability of some morphometric parameters of inflorescences *Sambucus nigra* L.

Characteristics	Unit	n	min	max	mean	V%
Inflorescences weight	g	113	0.45	57.59	9.54	75.65
Inflorescences length	mm	113	19.0	282.0	171.66	22.42
Pedicel inflorescences length	mm	113	9.0	197.0	75.11	31.51
Number of petals	pcs	113	4.0	8.0	5.07	10.36
Primary branching	pcs	64	2.0	8.0	4.95	9.58
Secondary branching	pcs	64	1.0	14.0	6.10	87.0

Note: n – the number of measurements; min, max – minimal and maximal measured values; mean – arithmetic mean; V% – coefficient of variation.

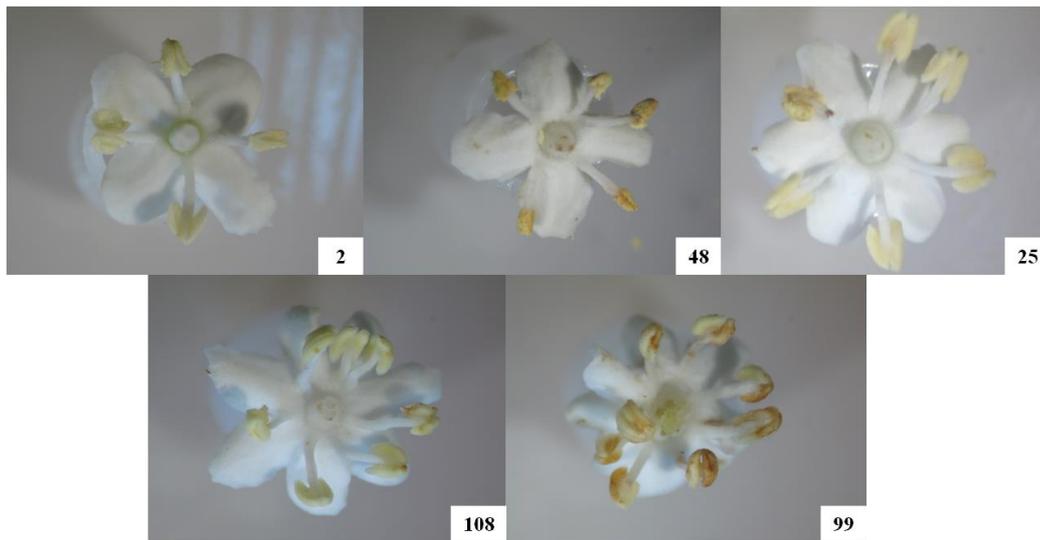


Figure 1 The variability of petal's number in collection of *Sambucus nigra* genotypes from wild-growing populations in Slovakia.

hermaphrodite, unfrequently diclinous flowers, radial symmetric with 3 – 5 sepals, 3 – 5 gamopetalous petals 3 – 5 mm in length, 3 – 5 free stamens and yellow anthers with 3 coupled styles, which form 3 seeds in the fruit (Paganová, 2001; Atkinson and Atkinson, 2002). A small part of flowers is also tetramerous (Skalická, 2001; Paganová, 2001).

Also, the observation on collection of genotypes identified other aspects of investigation these plants. The average number of petals for flowers of different

primary branching and, conversely very high degree of variability (87.0%) in secondary ones.

The analysis of coefficient of variation showed the difference of variability of morphometrical characters between *Sambucus nigra* genotypes (Figure 2). Data showed that the most variable parameters were the following: inflorescences weight – from 11.56 to 77.32%, panicle of inflorescences length – from 7.02 to 71.96%, secondary branching – from 0.0 to 61.43%, inflorescences length – from 2.31 to 49.55%. These results indicate the

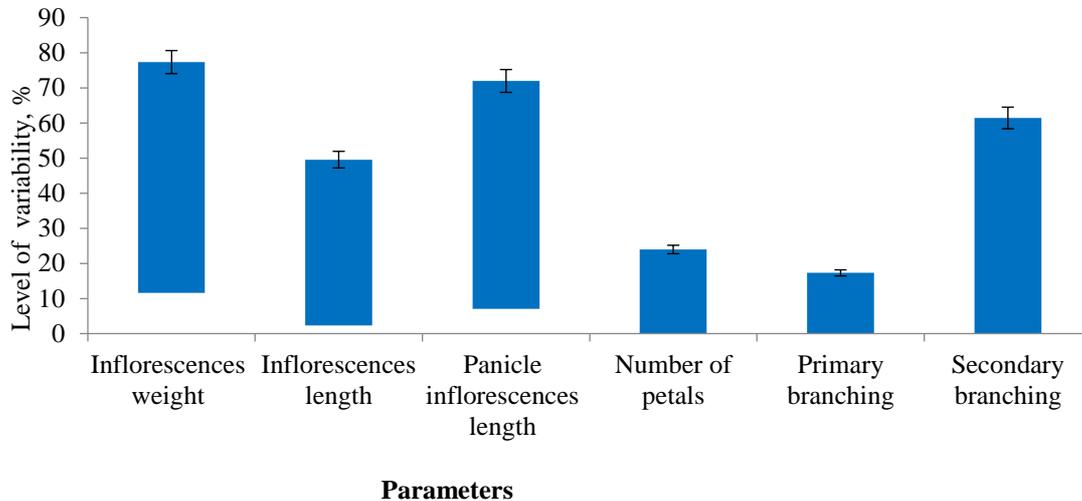


Figure 2 Level of the variability of morphometric parameters of *Sambucus nigra* L. (%).

genotypes ranged from 4 to 8. The average coefficient of variation was 10.36%, which shows a medium degree of variability. For the great majority of flowers there were determined pentamerous flowers, less frequently hexamerous and tetramerous ones (Figure 1).

In the collection of 64 wild-growing elderberry genotypes it was evaluated primary and secondary branching. It was identified in the range from 2 to 8 branches in primary branching and from 1 to 14 branches in secondary one. It was not identified variability in the primary branching of inflorescences in 44% of genotypes. Low degree of variability (9.58%) was determined in

promise of breeding in this way of investigations. The more stable signs are the following: primary branching – CV from 0.0 to 17.29% and a number of petals – CV from 0.0 to 23.96%.

The 6 characteristics of the 113 genotypes *Sambucus nigra* were used in clustering, and the resulting clusters are presented in Figure 3. The *Sambucus nigra* genotypes were divided into four clusters. Cluster III and cluster IV contained the largest number of genotypes. Cluster I and cluster II contained the 4 and 3 genotypes, which differ from other genotypes of collection by all parameters.

Significant differences between genotypes have been identified in the shape, size, colour of the inflorescences

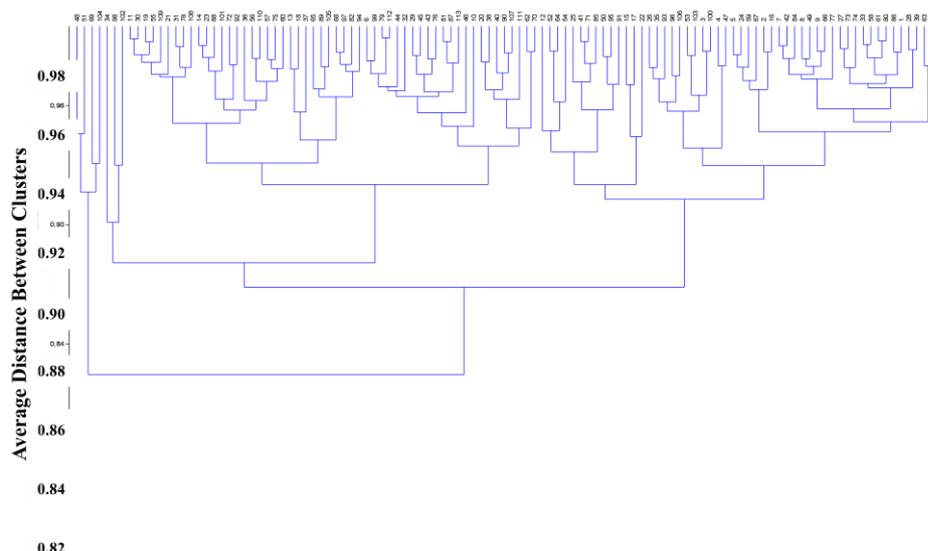


Figure 3 Cluster dendrogram based on morphometrics parameters of *Sambucus nigra* L.

and flowers. This is illustrated in Figure 4. Inflorescences are terminal, large, repeatedly branched, compound, flat-topped, umbelliform corymbs and cymes (Watson and Dallwitz, 2007). They consist of aromatic white flowers, which are grouped in umbrella cymes (Jančovičová, 2011). For the great majority of inflorescences there are determined creamy white flowers. The inflorescences were characterized by a more compact as well as a reduced arrangement of flowers. Our results were in accordance to Pišťanková (2002), which also found medium-sized, more compact clusters with creamy white medium-sized flowers in the variety Bohatka. Accordingly, in the variety Dana, the inflorescences are characterized as big, with smaller creamy white flowers, flower clusters are semi-loose. For this reason, the cultivation of elderberry in the monoculture (Wažbińska and Puczel, 2002) and in the landscaping (Wažbińska, 2000) began to grow in many

European countries.

Antioxidant activity

Antioxidant activity of tea infusions

Sambucus nigra is one of the main sources of biologically active substances for various practical uses. Elderflowers are particularly useful for the preparation of syrups and other traditional and innovative beverages. Measurement of the antioxidant activity of activated tea infusions from dried inflorescences was determined on 14 samples received from variants of dried inflorescences by pouring boiling water.

Variant TSN-01 was a control sample without activation by Kalyxx (Figure 3). Other samples were successively activated from 1 to 13 times. When assessing tea infusion samples it was determined antioxidant activity in the range

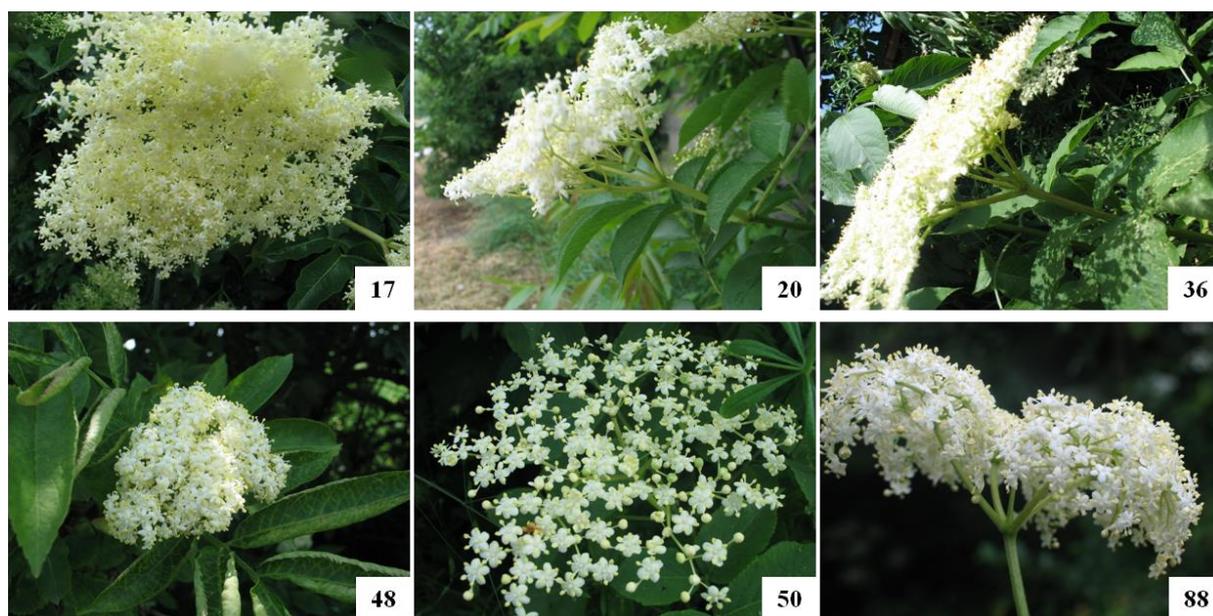


Figure 4 The comparison of selected genotypes of elderberry (*Sambucus nigra* L.) in the shape and size of inflorescences (*Sambucus nigra* L.) from wild-growing populations in Slovakia.

from 85.12 (TSN-10) to 89.29% (TSN-04). Compared results with the control variant were screened the presence or slightly reduced of anti-radical activity. It wasn't detected statistically significant differences among variants ($P < 0.05$). Tea infusion samples retained a high degree of anti-radical activity even after multiple activations (Figure 5).

Socaci et al. (2015) determined antioxidant activity from samples of fresh and dried inflorescences using DPPH radical. The capacity ranged from 28.4% (dried inflorescences) to 52.54% (fresh inflorescences) by DPPH inhibition.

Autors Mlynarczyk and Walkowiak-Tomczak (2017) determined the antioxidant activity of fresh and dried inflorescences by spectrophotometric method using ABTS radical. The fresh inflorescences were characterized by stronger bioactive properties than dried inflorescences.

Diankov and Parlapanska (2013) reported results extraction from dried inflorescences at ambient temperature 50 % ethanol-in-water solution. The best antioxidant capacity ($IC_{50} = 6.58$) was measured for the 90 min. extract by DPPH method.

Socaci et al. (2015) determined antioxidant capacity of elder samples of fresh and dried inflorescences using

DPPH method. The antioxidant capacity ranged from 28.4% (dried inflorescences) to 52.54% (fresh inflorescences) of DPPH inhibition.

Antioxidant activity of beverages prepared from elderflower honey

In the other experiment in our study it was determined antioxidant activity of activated beverages of dilute/aqueous honey prepared from fresh inflorescences in the saccharide extract. In beverages prepared from 10% honey diluted in 150 mL of water it was determined antioxidant activity in the range from 16.81% (HSN-02) to 24.16% (HSN-10). It was observed an increasing of antioxidant activity in many variants compared to the control variant HSN-K (16.18) (Figure 6). The enlarging of activation positively correlates with an increasing in antioxidant activity. That means that traditionally prepared products from inflorescences are a significant source of antioxidants.

The antioxidant activity of diluted beverages from alcohol extracts

Very high antioxidant activity was determined in the test of the multiple activated alcohol extracts of fresh

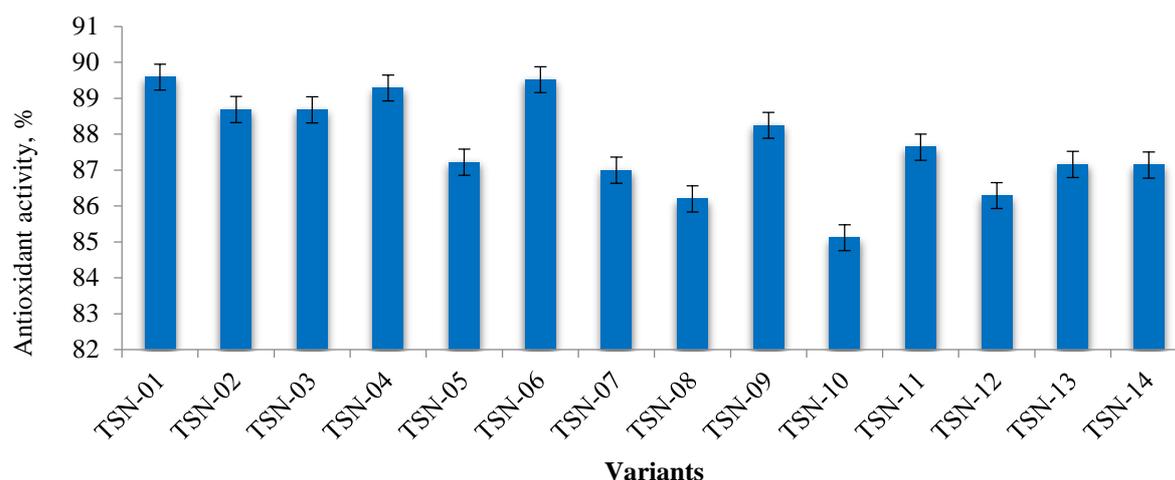


Figure 5 Antioxidant activity of tea infusions prepared from dried inflorescences of elderberry.

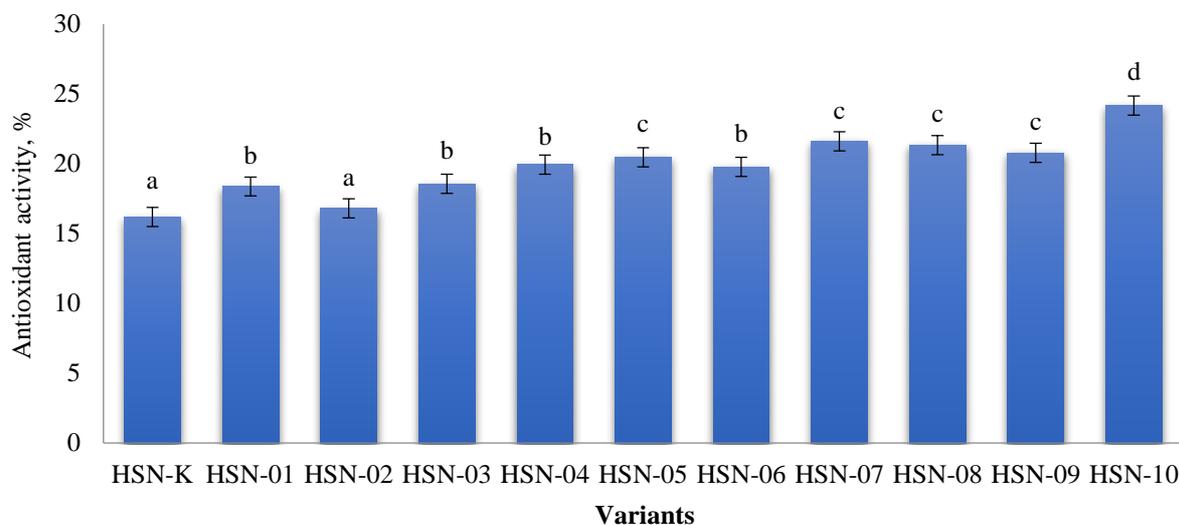


Figure 6 Antioxidant activity of the multiple activated beverages prepared from aqueous „elderflower honey“ (means in columns followed by different letters are different at $p = 0.05$. Each value represents the mean of three independent experiments ($\pm SD$)).

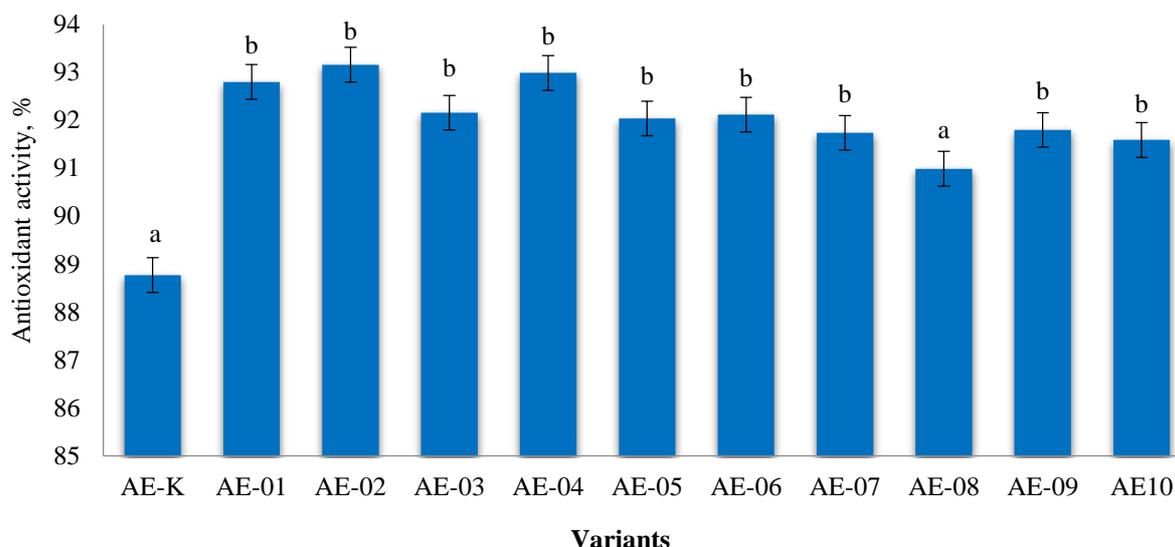


Figure 7 The variability provided antioxidant activity of the multiple diluted beverages prepared from alcohol extract (AE) of fresh inflorescences of elderberry (means in columns followed by different letters are different at $p = 0.05$. Each value represents the mean of three independent experiments (\pm SD)).

Table 2 Statistical characterization of variability of antioxidant activity of beverages prepared by increasing the concentration of syrup from inflorescences of elderberry in aqueous medium (SS).

Concentration %	n	mean	V%
10	4	37.920a	5.851
20	4	55.193b	1.911
30	4	62.643c	1.681
40	4	62.823c	6.745

Note: n – number of evaluated inflorescences; mean – average of file; V% – coefficient of variation.

inflorescences in alcohol (Figure 7). In the control variant of the alcohol extract without activation it was determined the 88.77% antioxidant activity. The antioxidant activity was increased in the range of 91.59 (AE-10) to 93.16% (AE-02) by activation, in addition to the 8-fold activated variant.

The presented results expressly demonstrate that inflorescences of elderberry are important sources of antioxidants that are clearly apparent in both aqueous and alcohol environment, in both cold and cooked beverages.

The antioxidant activity of elderflower syrup

In our study it was also determined the antioxidant activity of beverages prepared from the syrup of elderflower inflorescences. In accordance with the methodology there were prepared 4 variants of beverages from prepared syrup by extracting fresh inflorescences in the saccharide solution by increasing the syrup concentration in 100 mL of drinking water at room temperature +23°C. This method was used for preparing beverages in a concentration of 10 to 40% (Table 2). The results showed that average antioxidant activity was found in the range from 37.92 (10%) to 62.82% (40%). Simultaneously the results documented that increasing the syrup concentration in beverages also increased antioxidant activity.

Halvorsen et al. (2002) determined an antioxidant capacity of 4.31 mmolL.100g⁻¹ using the ferric reducing antioxidant potential (FRAP) method. In another study it

was detected scavenger activity of inflorescences extract on the two radicals in concern: DPPH and OH•, characterized the extract as a more potent scavenger with respect to OH• radicals – the IC50 value for OH• was determined to be 0.0122 µg.mL⁻¹ compared to IC50 value of the 0.152 µg.mL⁻¹ for DPPH (Stoilova et al., 2007).

The results obtained in our study confirmed that both fresh and dried elderberry flowers recorded significant antioxidant activity and they can serve as a good source of bioactive compounds in human diet or as functional ingredients in different foods. Antioxidant activity of extracts from dried elderberry flowers is comparable to those obtained for other medicinal plants, reported in the literature (Karcheva et al., 2010).

CONCLUSION

Based on the survey data and the evaluation of 113 selected genotypes from the wild-growing populations of *Sambucus nigra* within Slovakia there was determined significant variability in all evaluated morphometrical characteristics of inflorescences. The weight of inflorescences was in the range from 0.45 to 57.59 g, the length of the flower clusters ranged from 19.0 to 282.0 mm, the length of a pedicel was determined the range from 9.0 to 197.0 mm. The analysis of coefficient of variation showed the difference of variability of morphometrical characters between *Sambucus nigra* genotypes. Data showed that the most variable parameters were the

following: inflorescences weight – from 11.56 to 77.32%, panicle of inflorescences length – from 7.02 to 71.96%, secondary branching – from 0.0 to 61.43%, inflorescences length – from 2.31 to 49.55%.

These findings document that in natural populations of elderberry in Slovakia it is possible to detect genotypes with required economic characters for practical use. The findings have confirmed that both fresh and dried inflorescences used in elderberry-derived food products have high antioxidant activity and they can be used as a significant natural source of bioactive components in human diet or as functional components of various foods or nutritional supplements. Inflorescences of elderberry are also widely used for therapeutic purposes, which can be obtained by simple and traditionally proven methods and recipes in the form of natural extracts, essential oils, syrups, concentrates and other forms.

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