

## **INFLUENCE OF PRESERVATIVES ON QUALITY ELDERFLOWER SYRUP (*Sambucus nigra* L.)**

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*Original scientific paper*

### **Summary**

Elder plant lat. *Sambucus nigra* L. (*Adoxaceae*) is a shrub height up to 7 m (rarely to 10 m) with numerous vertical shoots that grow next to each other from the base. This plant has a great use in traditional medicine because it has a wide range of activities such as anti-inflammatory, especially for respiratory tract infections, antiviral, antibacterial, laxative, diuretic, antioxidant and many others. For research purposes elderflowers were collected from five different locations (Tuzla, Travnik, Tuzla, Zenica and Zivinice) and the syrup was prepared according to traditional recipes. The first analysis of samples was performed after immersing the flowers in water and left them at room temperature for 24 hours while the second analysis was performed after the addition of sugar and citric acid under the same conditions. The analysis included chemical and microbiological methods. From a microbiological point of view it has been shown that the addition of preservatives reduced the number of bacteria, and that such syrup is safe for consumption. Chemical analysis showed the syrup called after treatment reduces their significant properties but is still suitable for use.

**Keywords:** antioxidant activity, anthocyanins, CFU / ml, *Escherichia coli*, coagulase-positive staphylococci, sulphite reducing clostridia

### **Introduction**

Elder plant lat. *Sambucus nigra* L. (*Adoxaceae*) is significant medicinal plant and natural resources of this area and has always been known in folk medicine. It is a shrub height up to 7 m (rarely to 10 m) with numerous vertical shoots that grow next to each other from the base (Atkinson and Atkinson, 2002). The leaves are opposite, odd pinnate composed, saw tooth serrated, bright green collared with unpleasant odour, up to 30 cm in length (Grlić, 2005). The flowers are yellowish-white, small, form a large thyroid inflorescence with strong and pleasant odour. Flowering time is in May and June, and the fruits ripen in September. The fruits are dark purple berries rounded juicy stone berries 3-5 mm in diameter grouped in large bunches. It grows in sunny places or shade, on the edges of the wet forest, near villages and neglected places. It favours the wetter and deeper sandy and clayey soil Godet (2000) i Grlić (2005). It is widespread in the area of Europe, Asia, North America and North America. Use of Elder plant in folk medicine and cooking has a long tradition. It is also used in horticulture, pharmacy and is a significant bio indicator. Parts of plants that are in use are the leaves, flowers, ripe fruits and bark. Medicinal properties of Elder plant has a wide range of activities such as anti-inflammatory, especially for respiratory tract infections, antiviral, antibacterial, laxative, diuretic, antioxidant and many other (Barak

et al., 2002; Gorchakova et al., 2007; Zakary-Rones et al., 2004; Pool-Zobel et al. 1999). Besides juice and concentrates, elderberry fruits may be used for the industrial production of jam, jelly, desserts, wine, cakes, candies and colouring of mixed juices. Elderberry juice also contains many primary metabolites including various sugars and organic acids. Fruit extracts, containing phenolic compounds, are characterized by higher antioxidant activity than many isolated pure phenolic compounds or vitamins.

### **Materials and methods**

#### *Sample collection and preparation of syrup*

Elder flowers (*Sambucus nigra* L.) were collected from 5 different locations and altitudes in the area of Bosnia and Herzegovina (Table 1). Samples were collected at the same amount and at the same time to ensure confidential, accurate results and stored at 4 °C for 24 hours. Elderflower syrup is prepared according to traditional recipes:

- 15-20 of flowers was immersed in 1.5 l of water and stored for 24 hours at room temperature after which time samples were taken for the first analysis.
- The rest of the syrup was treated with 500 g of sugar and 5 g citric acids and again stored at room temperature for 24 hours and the samples were taken for analysis second time.

**Table 1.** Sampling localities

No.	LOCALITY	Altitude (m)
1.	Bugojno (Karadže)	550
2.	Travnik (Vlašić)	1900
3.	Tuzla (Moluška rijeka)	250
4.	Zenica (Kopilo)	320
5.	Živinice (Litve)	300

### Chemical methods of analysis

Chemical analysis are generally included parameters such as pH value, which was determined potentiometrically, turbidity was determined directly in the turbidimeter and expressed in Nephelometric Turbidity Unit (NTU units), electrical conductivity was measured on the conductivity meter and the density was determined using a pycnometer. In addition to general parameters were determined and anthocyanins (total and monomeric) with pH differential method where a certain volume of the sample was transferred to a volumetric flask of 10 cm<sup>3</sup>, which are then dilute to the mark with 0.025 mol/l buffer of potassium chloride and 0.4 mol/l buffer of sodium-acetate. After 15 minutes the absorbance was measured at 520 nm and 700 nm and the calculated value of anthocyanin expressed in mg/mL. The antioxidant activity of the samples was determined using H<sub>2</sub>O<sub>2</sub> test (level of neutralizing H<sub>2</sub>O<sub>2</sub> - "H<sub>2</sub>O<sub>2</sub> scavenging" depending on the concentration of extracts) (Ruch et al., 1989). The solution of H<sub>2</sub>O<sub>2</sub> (0.6 cm<sup>3</sup>) with concentration of 40 mmol/l was added to extracts, after incubation for 10 minutes at a dark place absorbance was measured at 230 nm and further calculation is derived value for neutralization capacity H<sub>2</sub>O<sub>2</sub>.

### Microbiological methods of analysis

Microbiological analysis was performed before and after the addition of sugar and citric acids who served as preservatives according to traditional recipe. The analysis was performed on the following types of bacteria:

1. *Escherichia coli*,
2. Coagulase positive *Staphylococcus aureus*,
3. Sulfite-reducing *Clostridia*,
4. The total number of bacteria.

Samples for analysis were prepared under sterile conditions in a laminar with air flow. In sterile

Erlenmeyer flask with 180 ml of sterile physiological solution we added 20 ml of an untreated juice, and left it on the magnetic mixer for 15 minutes to homogenize the sample. After sample preparation, under sterile conditions, with a Bunsen burner we have prepared a decimal dilution of the sample, and a nutrient medium that we used for the isolation of different types of bacteria. *Escherichia coli* was analyzed from the dilution sample by transferring 1 ml of the sample in 15 ml of sterile nutrient medium Brilliant Green Bile 2% Broth (Hi Media Laboratories), and incubated at 44 °C for 24 hours. Coagulase positive *Staphylococcus aureus* and sulfite-reducing *Clostridium* are also analyzed from the 10<sup>-1</sup> dilution, and seeded in salt broth and iron sulphite agar, and allowed to incubate for 24 hours at 37 °C. The total number of bacteria was analyzed in 1 ml of sample dilution on basic agar. The procedure was repeated in the same manner and under identical conditions for all samples.

## Results and discussion

### Results of chemical analysis

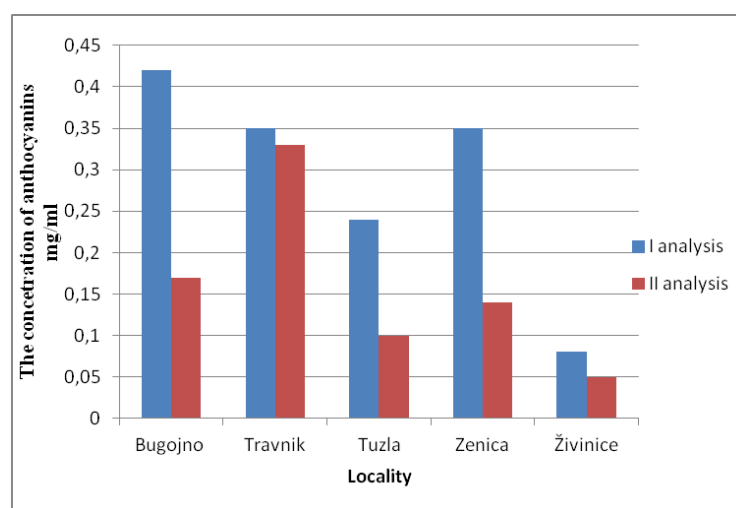
The general parameters (Table 2) chemical analysis is mainly decreasing after treatment with a preservative such a pH value before treating showed mild acidic medium while after treatment decreased considerably and shows a very acidic medium. The turbidity and electrical conductivity of samples were also decreased after treatment with sugar and citric acid. The only general parameter that was increased was the density of samples. These changes were expected due to the addition of sugar and citric acids. The highest density was measured in a sample from the Travnik locality. We assume that the reason for that is the high altitude at which the sample was pattern is picked and low pollution in the sampling area.

**Table 2.** General technological parameters of analyzed samples before and after the treatment with sugar and citric acids

No.	Locality	pH		Electroconductivity ( $\mu\text{S}$ )		Turbidity (NTU)		Density ( $\text{g/cm}^3$ )	
		Before treatment with sugar and citric acids	After the addition of sugar and citric acids	Before treatment with sugar and citric acids	After the addition of sugar and citric acids	Before treatment with sugar and citric acids	After the addition of sugar and citric acids	Before treatment with sugar and citric acids	After the addition of sugar and citric acids
1.	Bugojno	6.62	3.76	3180	1019	73.5	59	1.0200	1.1868
2.	Travnik	5.89	2.92	3620	914	81	77.4	1.0135	1.1938
3.	Tuzla	5.95	3.43	5210	1646	153	150	1.0212	1.1630
4.	Zenica	5.53	3.17	3690	560	78.9	58.5	1.1830	1.1830
5.	Živinice	6.19	3.20	4140	1042	95.6	90.5	1.0200	1.1850

The amount of anthocyanin was increased after the treatment (Fig. 1). The highest amount of anthocyanins before treatment shows a sample from Bugojno while after the treatment pattern of Travnik. According to previous research, higher amounts of anthocyanins include elderberry (Galić, 2007). An important application is their antioxidant activity, which plays a key role in the prevention of neurological and cardiovascular diseases, cancers and

diabetes (Ćujić et al., 2013). Anthocyanins can act an antioxidant, anti-inflammatory, anticancer, antidiabetic, oedematous, have the effect of capillary permeability, acting hepatoprotective, antibacterial, radioprotective, immunomodulatory, inhibit herpes virus, influenza virus, the protective function of endothelial cells in comparison to the various stress agents (Pascual and Sanches, 2008; Croizer and Cliffprd, 2010).

**Fig. 1.** Comparative display of anthocyanin content before and after the treatment of syrup with sugar and citric acids

Antioxidants as important parameters are the name of the syrup is decreased after the treatment, which

indicates that a greater amount of antioxidants in the syrup have no sugar and citric acids (Fig. 2).

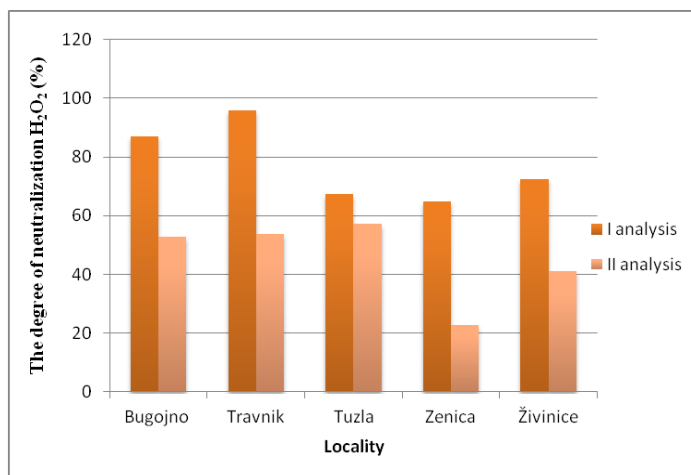


Fig. 2. Impact of sugar/citric acid treatment on antioxidant activity of analyzed syrups

Results of microbiological analysis

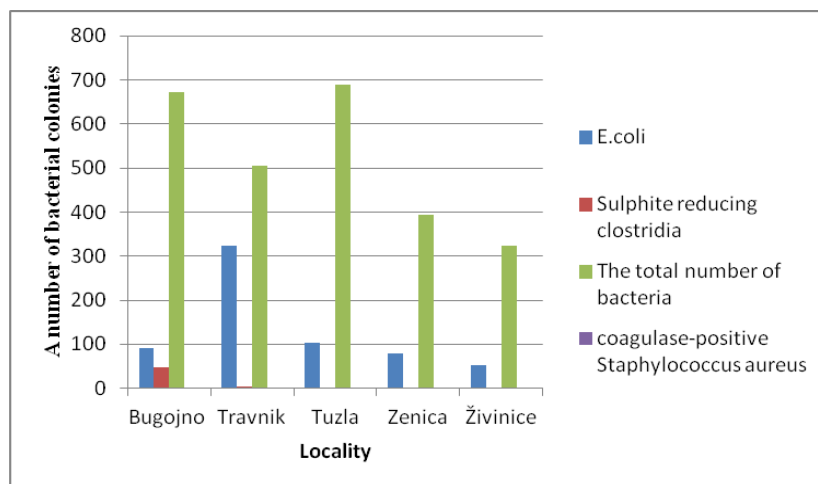
The results of microbiological analysis of *Sambucus nigra* L. flowers before and after addition of sugars and citric acids in water are shown in Table 3. Based on what we can see that the acidification of the environment with a citric acids and added sugar

caused the lysis of bacterial cells, which resulted that in the samples completely left out the growth and development of microorganisms.

From Table 3 we can clearly see that all the samples before treatment with sugar and citric acids were a suitable medium for the growth and development of all tested species of bacteria.

Table 3. Comparison show CFU / ml (Colony Forming Units) of the tested bacterial strains in syrup *Sambucus nigra* syrup

No.	Locality	<i>E.coli</i>		<i>Sulphite reducing clostridia</i>		<i>The total number of bacteria</i>		<i>Coagulase-positive Staphylococcus aureus</i>	
		<i>Determined of CFU/ml</i>							
		<i>Before treatment with sugar and citric acids</i>	<i>After the addition of sugar and citric acids</i>	<i>Before treatment with sugar and citric acids</i>	<i>After the addition of sugar and citric acids</i>	<i>Before treatment with sugar and citric acids</i>	<i>After the addition of sugar and citric acids</i>	<i>Before treatment with sugar and citric acids</i>	<i>After the addition of sugar and citric acids</i>
1.	Bugojno	91	0	48	0	672	0	0	0
2.	Travnik	323	0	3	0	504	0	0	0
3.	Tuzla	103	0	0	0	689	0	0	0
4.	Zenica	78	0	0	0	394	0	0	0
5.	Živinice	52	0	0	0	323	0	0	0



**Fig. 3.** Number of bacterial colonies from the syrup before treatment with sugar and citric acids, determined on selective nutrient media

From Fig. 3 we can clearly observe that samples from the Travnik locality have the largest number of *E. coli* colonies grown on VRBG media. In samples from the area of Tuzla were established the total number of bacterial colonies in most, even 689 CFU / ml. With increasing colonies of *E. coli* that have a characteristic purple colour on VRBG medium, colonies are piqued under sterile conditions and biochemical characteristics were analyzed. In Klinger's double sugar piqued colonies of *E. coli* were sown by pricking and zigzag movements on the slant agar. Simmons citrate, and peptone for the reaction with an indole were also inoculated with the isolated bacterial colonies and left to incubate for 24 hours at 37 °C.

In the analyzed samples were not detected increase of *Staphylococcus aureus* colonies. T test has proven the greatest statistically significant difference ( $p$ ) in the total number of bacteria in the amount of 0.004659973 when we compared the number of bacterial colonies which grew after incubating in the different nutritive media, from samples taken before treatment with the sugar and citric acids and after treatment. It has been found that there is a significant difference at the level of 0.05 in the number of bacterial species grown before and after the treatment of juice and pH value established after the first and the second analysis ( $p = 0.000308725$ ).

In recent years, more and more we talk about natural production, healthy food, local cuisine and the preservation of traditional recipes. Particularly noteworthy is the consumption of natural juices and syrup prepared from fresh plant parts without adding any chemicals and preservatives in order to maintain the quality and medicinal properties. Originally it

was thought that the natural acidity of certain juices enough to prevent the possible harmful effects of the consumption of these juices (Mihajlović et al., 2013). But studies have shown that for example *Salmonella* can survive in the juice of the orange and up to 3 weeks (Hodgson, 2001). The fact is that microorganisms are everywhere around us, and so are the plants we use in nutrition source of potentially harmful microbes that are the natural or adopted. *E. coli*, which is pathogenic to man is a natural inhabitant of the intestine through which faeces animals are transferred to the fruit which is why it is important to take care of hygiene, sterilization equipment and methods of keeping the product when creating natural remedies. Our analysis confirmed that untreated juice is not the safest for consumption. Some of the traditional methods of preservation are adding sugar, citric acid or pasteurization (Smith & Stratton, 2007). Microbiological analysis of samples after the addition of sugar and citric acids gave negative results showing the efficient preservation of the desired product and its safe use.

## Conclusions

Results of chemical analyzes have shown that the general parameters decreased after treatment as well as antioxidant activity while the amount of anthocyanin increase. If we take into account all the parameters, syrup that is prepared from a flowers picked in Travnik (Vlašić) showed the best properties. It is assumed that the reason for this is higher altitude and less contamination of the plant itself. Based on the results of microbiological analysis, a statistically significant difference was

found in the number of bacterial species grown before and after treatment. Microbiological analysis proved that the syrup after the addition of sugar and citric acids is safe for consumption. From the aspect of the chemical analysis of the syrup after treatment reduces its significant properties but is still suitable for use.

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