Antioxidant and Biochemical Characterisation of Cornelian Cherry
(Cornus mas L.)

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Abstract
Cornelian cherry (Cornus mas L.) is a naturally growing plant especially in Europe and Asia used mainly in traditional medicine in the past. At present, it is beginning to be given the importance again, as it can be an excellent source of bioactive substances with a high antioxidant activity. In this study, antioxidant capacity, total polyphenols, phenolic acids, flavonoids, anthocyanins, basic dry matter components and carbohydrates were investigated. The antioxidant activity was determined by DPPH method, the total polyphenols, phenolic acids, flavonoids, and anthocyanins were determined by spectrophotometric analyses. Analysis of selected bioactive substances showed that cornelian cherry is a rich source of polyphenols, which was reflected in its high antioxidant activity, especially in the stone due to significantly higher values (p < 0.001) of total antioxidant capacity, total polyphenols and total phenolic acid content when compared to the pulp. The stone of cornelian cherry also showed positive values of polyunsaturated fatty acids and carbohydrates content. This study contributes to the analysis of cornelian cherry, which can be used in the preparation of functional foods or in the medicine.

Keywords: antioxidants, Cornelian cherry, polyphenols

1. Introduction
The consumption of plants with a high antioxidant potential plays an important role in the maintenance of health and in disease prevention, such as diabetes, inflammation, cardiovascular disease, cancer, and other metabolic disorders (Terry, Terry & Wolk, 2001). Cornelian cherry (Cornus mas L.) is a plant with a high content of bioactive substances, which are characterized by antioxidant activity. It is a shrub or a small tree that grows mostly in Europe and Asia and its fruits are standing out sour taste (Celik, Bakirci & Şat, 2006). The use of cornelian cherry is abundant, whether in traditional medicine or in food production. In China, cornelian cherry is used in herbal medicine because of its analgesic and diuretic properties. It is also known that cornelian cherry can improve liver and kidney functions and has anti-microbial, anti-histamine and anti-allergic characteristics (Vareed, Reddy, Schutzki & Nair, 2006). Food products from cornelian cherry include for example jam, marmalade, pestil, syrup, paste, sherbet or it can be consumed directly (Celik et al., 2006). In addition to fruit, the use is found also in leaves that are rich in tannins, from the wood and the bark is obtained a dye and from the seed is obtained an oil. The flowers can be used in the treatment of diarrhoea (Demir & Kalyoncu, 2003). Compounds found in fruits such as phenolics, anthocyanins, flavonoids, carotenoids, and vitamins contribute to varying degrees to the antioxidant activity of individual fruits (Pantelidis, Vasilakakis, Manganaris & Diamantidis, 2007). In recent years,
the interest in nutraceuticals and functional foods has led to initiate selection of crops with higher than normal phenolic antioxidant compounds, such as cornelian cherry, blueberries, sea buckthorn, strawberries, plums and peaches (Yilmaz, Ercisli, Zengin, Sengul & Kafkas, 2009). The objective of this study was to analyse biochemical properties, a total antioxidant capacity, total anthocyanins, total polyphenols, flavonoids, and phenolic acids content in cornelian cherry (Cornus mas L.) pulp and stone.

2. Data and Methods

Cornelian cherry was obtained from the Institute of Plant and Environmental Sciences of Slovak University of Agriculture in Nitra, Slovak Republic. Cornelian cherries were washed and separated from stones. Pulps and stones were crushed separately in a mortar with a pestle and used for future analyses. Variability of basic dry matter components and carbohydrate content in evaluated plant parts was determined by chemical analysis in accredited laboratories. An ethanol extract (2 grams of cornelian cherry were weighed and dissolved in 10 mL of 96% acidified ethanol) was formed from the pulp and the stone, which was needed to determine the parameters. A total antioxidant capacity was determined by DPPH method of Sanchez, Larrauri & Saura (1998). Total polyphenols were determined by using the Folin-Ciocalteu reagent method by Singleton & Rossi (1965). Flavonoids were analysed by the procedure of Willet (2002), phenolic acids analysis were followed by protocol of Polskie Towarzystwo Farmaceutyczne (1999). For anthocyanins determination, a weighed sample (2 grams of pulp) was transferred to a beaker, quenched with 10 mL of acidified ethanol, and heated to reflux. The extract was poured after filtration into a 100 mL volumetric flask and the residue was repeatedly extracted with small portions of acidified ethanol (8-9 times). After extraction, the volume of the flask was made up to the mark and the flask was placed into the dark for 15 minutes. After that the absorbance was measured at 535 nm. The results of anthocyanins were calculated based on the formula:

\[
\text{anthocyanins (g/kg)} = \frac{A \times r \times M_v \times V}{e \times n}
\]

Legend: A – absorbance, r – dilution, Mv – molecular weight of the dominant anthocyanin [g/mol], V – volume of the solution [cm³], e – molar absorption coefficient of the dominant anthocyanin [dm³/mol.cm³], n – weight of the sample [g]

All these analyses were performed in triplicate and analysed by spectrophotometer (Jenway 6405 UV/Vis, UK) at different wavelengths and the resulting values were expressed as means ± standard error of the mean (SEM). Statistical software GraphPad Prism 6 was used for statistical analysis. All data were analysed using unpaired parametric t-test to compare the stone and the pulp content values in cornelian cherry and significant differences were set at the level \( p < 0.001 \).

3. Results and Discussion

Cornelian cherry has been recognized as a natural treatment for centuries. Its properties are linked with the secondary metabolites like polyphenols or tannins. These polyphenols are characterized by their antioxidant activity (Szczepaniak, Cisowska, Kusek & Przeor, 2019).
For this reason, the total content of antioxidants in the pulp and the stone was determined and the results were compared with each other. When comparing the stone with the pulp, we obtained a significantly higher antioxidant activity in the stone (Figure 1), which may be due to the presence of a larger amount of total polyphenols present in the stone. The antioxidant activity measured in the stone was $7.84 \pm 0.27$ mg Trolox equivalent antioxidant capacity per gram (mg.TEAC/g) and the antioxidant activity measured in the pulp was $4.19 \pm 0.29$ mg.TEAC/g.

![Figure 1: The total antioxidant capacity](chart1)

**Legend:** S – the stone of cornelian cherry, P – the pulp of cornelian cherry, *** means a significant difference at $p < 0.001$

The comparison of bioactive compounds showed a significantly higher content of total polyphenols (Figure 2) and phenolic acids (Figure 3) in the stone compared to the pulp. Values of the total polyphenols in the stone were $29.61 \pm 0.33$ mg gallic acid equivalents per gram (mg.GAE/g) and the total phenolic acids content in the pulp were $6.66 \pm 0.16$ mg caffeic acid equivalent per gram (mg.CAE/g). Higher amount of polyphenols in the stone may be due to solid character of the stone that is more concentrated compared to the pulp, which contains a larger amount of water (Antolovich, Prenzler, Robards & Ryan, 2000). Polyphenols found in plants are influenced by environmental factors as well as post-harvest processing conditions (Kadir, Sezai, Yasar, Memnune & Ebru, 2009).

![Figure 2: The total polyphenols content](chart2)

**Legend:** S – the stone of cornelian cherry, P – the pulp of cornelian cherry, *** means a significant difference at $p < 0.001$
Figure 3: The total phenolic acids content
Legend: S – the stone of cornelian cherry, P – the pulp of cornelian cherry, *** means a significant difference at $p < 0.001$

When comparing the content of flavonoids (Figure 4), higher levels were measured in the pulp, but no significant difference between the groups was recorded. Our data of total flavonoids content were expressed in mg quercetin equivalent per gram (mg.QE/g). The role of flavonoids is protecting plants against various biotic and abiotic stresses and they play an important role in the interaction between the plant and their environment (Samanta, Das & Das, 2011).

Figure 4: The total flavonoids content
Legend: S – the stone of cornelian cherry, P – the pulp of cornelian cherry

Of the total weight of 2 grams of cornelian cherry pulp, the value of anthocyanins was $0.54 \pm 0.02$ g/kg (Figure 5). In the study of Hamid, Yousef, Jafar & Mohammad (2011), the genotype can influence content of anthocyanins found in cornelian cherry, however they did not find any statistically significant correlation between antioxidant activity and content of anthocyanins. David, Danciu, Moldovan & Filip (2019) identified three main anthocyanins in cornelian cherry, which are cyanidin-3-O-galactoside, pelargonidin-3-O-glucoside and pelargonidin-3-O-rutinoside, responsible for antioxidant activity in this fruit.
Results from basic dry matter components are stated in Table 1 and carbohydrate content in evaluated plant parts is stated in Table 2. It is known that saturated fatty acids can increase low-density lipoprotein (LDL), which is a strong risk factor for cardiovascular disease (Briggs, Petersen & Etherton, 2017). Our results showed that Cornelian cherry stone has less content of saturated fatty acids when compared to the pulp and also the stone showed higher values of polyunsaturated fatty acids, which have positive effects on health. Determination of carbohydrate content showed that pulp has higher amount of fructose. At present, researchers are developing foods that have lower sugar content and become alternative sources of sweeteners, which would reduce dietary sugar intake and reduce the risk of diseases such as diabetes, high cholesterol, obesity, and cardiovascular disease (Edwards, Rossi, Corpe, Butterworth & Ellis, 2016). Results of our analyses indicate that cornelian cherry stone has much more potential in preparation of healthy foods due to its better properties than the pulp.

### Table 1: Basic dry matter components

| Component                | SI | Stone  | Pulp  |
|--------------------------|----|--------|-------|
| Dry matter               | %  | 90.73  | 85.59 |
| Proteins                 | %  | 2.41   | 4.03  |
| Ash                      | %  | 1.07   | 2.75  |
| Lipids                   | %  | 4.42   | 1.68  |
| Saturated fatty acids    | g/100 g fat | 12.20 | 39.70 |
| Monounsaturated fatty acids | g/100 g fat | 21.70 | 12.50 |
| Polyunsaturated fatty acids | g/100 g fat | 64.10 | 33.00 |

### Table 2: Carbohydrate content

| Carbohydrates | SI | Stone  | Pulp  |
|---------------|----|--------|-------|
| Fructose      | g/kg | 2.6   | 69.5  |
| Maltose       | g/kg | <0.5  | <0.5  |
| Sucrose       | g/kg | 2.5   | <0.5  |
| Lactose       | g/kg | <0.5  | <0.5  |
4. Conclusion

As a conclusion, this study clearly shows the potential value of cornelian cherry. Cornelian cherry could be considered a valuable source of natural antioxidants. Particularly high values of bioactive substances, polyunsaturated fatty acids, carbohydrates, and antioxidant capacity were shown by the stone of cornelian cherry in comparison with the pulp. The use of cornelian cherry can be in a functional food production, a nutraceutical supplementation and possible in a medicine or a pharmacy.

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