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Analysis of Anthocyanins in cornelian cherry (*Cornus mas L.*)

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Abstract. This study aimed to determine and analyze the total anthocyanin content in extracts from cornelian cherry (*Cornus mas L.*). The fresh fruits of cornelian cherry (*Cornus mas L.*) were extracted with the solvent of EtOH 96% /0.1% HC 1 (1:1, ratio) at room temperature for 4 h in the dark. The extraction process was an important step in the isolation and identification of anthocyanins. For this purpose, the total anthocyanin contents in cornelian cherry (*Cornus mas L.*) fruits was performed with a variety of pH and analyzed by UV-Vis Spectroscopy. The results showed that the total anthocyanin content (expressed as cyanidin-3-glucoside) in the extract was 146.12 to 328.2 mg/100g total anthocyanin of dry material. All statistical analysis was performed using the MS Excel program and SPSS 22.0 statistics software.

Keywords: Anthocyanins, Extract, cornelian cherry (*Cornus mas L.*), UV-Vis Spectroscopy

Introduction

Fruits and vegetables are a good source of natural antioxidants, containing many different antioxidant components which provide protection against harmful free radicals and have been associated with lower incidence and mortality rates of cancer and heart diseases in addition to a number of other health benefits [1]. Anthocyanins are red colorants belonging to the flavonoids and widely appearing in fruits, vegetables, and flowers. Also, they have antioxidants and inflammatory effects [2],[3].

Cornelian cherry (*Cornus mas L.*) is a species from the Cornaceae family. It can be found naturally in Europe and Asia. In Kosovo it is part of the spontaneous flora and is found in the forests spread on the plains and hills. Commonly, the most frequent use of cornelian cherry fruits is to produce different drinks, syrups, gels, jams, and other fruit-based products. *Cornus mas L.* fruits have been used for centuries as traditional cuisine and folk medicine in various countries of Europe and Asia [4].

Cornus mas L. is famous for being a rich source of vitamin C and polyphenols. Significant amounts of flavonoids, anthocyanins, and iridoids were identified in the fruits of the Cornelian cherry.

Cornelian cherry (*Cornus mas* L.) that has been traditionally used as a food and medicine, due to its unique flavors, nutritional properties, and health benefits. These fruits are well-known for their nutrition and health-promoting values as a source of vitamin C, phenolic acids, flavonoids, anthocyanins, and iridoids. Despite their health benefits, *Cornus mas* L. fruits are still being underutilized as food or ingredients. Recent research has highlighted the potential of *Cornus mas* L. fruits to be used in novel food developments as functional foods [5].

Cornelian cherry fruits, describing both sensory and qualitative attributes and pro-health qualities. Flavonoids, like anthocyanins, possess the biological activity and affect fruit color. They have, among others, the ability to modulate the activity of several enzymes, thus preventing enzymatic browning reactions [6].

Biologically active compounds are linked with intense radical scavenging potential and antitumor properties. The fruits of *Cornus mas* L. are not only consumed fresh but also used to produce many food productions such: liquors, jams, yogurt, different desserts, and other fruit-based products [7]. Recently, research into the potential application of *Cornus mas* L. has been conducted and expanded by numerous examples of novel functional food applications based on the Cornelian cherry's fruits extracts. Foods including extracts from the Cornelian cherry featured a higher antioxidative potential than they were also accepted by consumers [8],[9]. There have been very limited studies related to assessments of bioactive compounds of cornelian cherry in Kosovo. In the present study, we aimed to determine and analyzed the content of bioactive compounds such as anthocyanins content in different cornelian cherry (*Cornus mas* L.) fruits and possible beneficial health effects. Many further studies need the assessment to quantify and isolate the phytochemicals as anthocyanins from *Cornus mas* L. fruits grown in the Kosovo region which might serve as cheap natural antioxidants in the food and drug industry.

Materials and methods

Sampling Preparation

The preparation of samples has been previously described [10]. The extraction process was an important step in the isolation and identification of anthocyanins. The fresh cornelian cherry (*Cornus mas* L.) fruits were extracted and acid hydrolyses using an acidified aqueous solvent of EtOH 96% /0.1% HCl (1:1, ratio) at room temperature for 4 h in the dark. Following hydrolysis, samples were cooled to room temperature and 1 mL was subjected to the total anthocyanin analysis.

Total anthocyanin content

Total anthocyanin content was measured with the pH differential absorbance method with some modifications [6]. The absorbance of the extract was measured at 510 and 700 nm in buffers at pH 1.0 (hydrochloric acid–potassium chloride, 0.2 M) and 4.5 (acetate acid–sodium acetate, 1 M). The absorbance of each dilution was measured at

520 and 700 nm using a distilled water as a blank. Absorbance (A) was calculated as follows:

$$A = (A_{520} - A_{700})_{\text{pH}1.0} - (A_{520} - A_{700})_{\text{pH}4.5}$$

The anthocyanin concentration (mg/L) was calculated using the following formula:

$$\text{Anthocyanin content} = (A \times \text{MW} \times \text{DF} \times 1000) / (\epsilon \times l)$$

Where MW is the molecular weight of cyanidin-3-glucoside (449.2 gmol⁻¹), DF is the dilution factor, and ϵ is the molar extinction coefficient of cyanidin-3-glucoside ($\epsilon = 26\,900 \text{ L cm}^{-1} \text{ mol}^{-1}$). Total anthocyanin was calculated in the sample as mg per 100 g of extracts and dry material (FW).

For this purpose, anthocyanin contents in *Cornus mas* L. fruits were determined with UV-Vis Spectroscopy (Genesys10S UV-Visible). Cyanidin-3-glucoside is commonly used to create the standard curve. The results showed that the total anthocyanin content was expressed as cyanidin-3-glucoside.

Statistical analysis

All data were expressed as the mean \pm standard deviation of triplicate experiments. All statistical analyses performed using the MS Excel program and SPSS 22.0 statistics software. Differences were tested for significance using the ANOVA procedure, with a significance level of $p < 0.05$.

Results and Discussion

Results for total anthocyanins content on cornelian cherry (*Cornus mas* L.) fruits are summarized in Table 1. and Figure 1.

Table 1. Total anthocyanin content of *Cornus mas* L. fruit extracts and dry material (mg/100 g dry material)

| Sample | TAc (CyGE mg/100 g) |
|---|-------------------------------|
| Extract of <i>Cornus mas</i> L. fruits | 328.2 \pm 1.02 ^b |
| Dry material of <i>Cornus mas</i> L. fruits | 146.12 \pm 0.6 ^a |

^{a,b}: means \pm SD in the same column differ significantly at level $p < 0.05$; TAc-total anthocyanin contents; CyGE—cyanidin-3-glucoside equivalents. Values represent means SD of three measurements (n = 3).

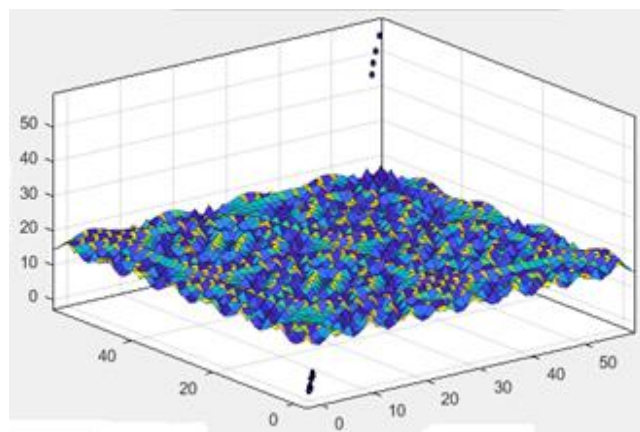


Figure 1. The 3D data of total anthocyanin content of *Cornus mas* L. extracts and dry material (mg/100 g dry material)

The results showed that the total anthocyanin content (expressed as cyanidin-3-glucoside) in the extract of the *Cornus mas* L. fruits was 328.2 mg/L, which is equivalent to 146.12 mg/100 g total anthocyanin of dry material.

This is the first study to characterize the anthocyanin content of the *Cornus mas* L. fruits in Kosovo. Similar results for anthocyanin content of the *Cornus mas* L. fruits have been reported by others researchers. Therefore, anthocyanins are regarded as important antioxidants in berry fruits. However, in humans, the bioavailability of dietary anthocyanins is low [10].

Our results were similar by **Kucharska et al.** [2] in their work report total anthocyanin content of the *Cornus mas* L. fruits was observed 134.57 to 341.18 mg/100g dry material.

Kazimierski et al. [5] reported total anthocyanin content of the *Cornus mas* L. fruits was observed 27.52 to 160.51mg/100g dry material, these values are much lowest from our findings.

Until now, no detailed information was available for the biologically active compounds of cornelian cherry (*Cornus mas* L.) fruit grown in the Kosovo region. This is the first research of this type in Kosovo and it should give us a novel result of the cornelian cherry (*Cornus mas* L.) as the cheapest source of biologically active compounds such as anthocyanins.

Conclusion

Cornelian cherry (*Cornus mas* L.) is a valuable fruit crop, highly evaluated for its nutritional and sensory value. In our work, we evaluated the content of anthocyanins in selected varieties of cornelian cherry (*Cornus mas* L.) as mentioned, this is the first

study to characterize anthocyanins content of the cornelian cherry (*Cornus mas* L.) in Kosovo. In addition, the total anthocyanin value of cornelian cherry (*Cornus mas* L.) sampled helps to give a realistic picture of what values might be expected from growing available cornelian cherry (*Cornus mas* L.) cultivars as well as from new ones developed through breeding in the near future. These data will help provide a baseline for researchers studying the health effects of phytochemicals in cornelian cherry (*Cornus mas* L.) as well as product developers for nutraceutical, natural colorant, and other industries interested in these properties and health benefits from the health properties of anthocyanins.

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