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THE CONTENT OF VITAMIN C AND ANTIOXIDANT ACTIVITY IN LESS-KNOWN TYPES OF FRUIT

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ABSTRACT

This study aims to determine the vitamin C content and antioxidant activity in different types of fruit: cornelian cherry (*Cornus mas* L.), service tree (*Sorbus domestica*), medlar (*Mespilus germanica* L.), pawpaw (*Asimina triloba* L.), persimmon (*Diospyros kaki* L.). The aim of the work was to compare these used methods. The fruit's vitamin C content was determined by HPLC system Waters Separations Module 2695 with UV detector 2996. The content of vitamin C reached values from 72.238 µg. g^{-1} (*Diospyros kaki* L.) to 1114.61 µg.g^{-1} (*Sorbus domestica*). The antioxidant activity (DPPH) was determined spectrophotometrically. DPPH radical scavenging activity values were 2.28 (*Mespilus germanica* L.) – 12.34 µmol TE.g^{-1} DW (*Cornus mas* L.). Spearman's test showed a positive relationship between both monitored parameters. The content of vitamin C in the studied species showed a positive relationship with antioxidant activity. Due to the high content of vitamin C and antioxidant activity, future analyses should focus on the determination of other bioactive substances in these fruits.

Keywords: vitamin C, HPLC, antioxidant activity, DPPH, fruit

INTRODUCTION

Fruits and vegetables are the main food sources that provide essential nutrients to sustain life. Their regular consumption reduces the risk of chronic diseases such as cancer and cardiovascular diseases (**Gülçin *et al.*, 2011**). The health-promoting effect is mainly due to bioactive components (**Butt *et al.*, 2015**). The amount of phenolic compounds in the plants or extracts determines their potential antioxidant capacity or the antioxidant activity of their derived products (**Amarowicz & Pegg, 2019**). The ability of natural antioxidants in food to combat the harmful effects of an excess of free radicals and the diseases they generate has recently attracted attention (**Pérez-Torres *et al.*, 2021**).
Cornelian cherry (*Cornus mas* L.), originating from southern Europe and southwest Asia, has been used in ancient times (**Czerwińska & Melzig, 2018**). The fruits of cornelian cherry are a rich source of vitamin C, phenolic acids, flavonoids, anthocyanins and iridoids (**Martinović & Cavoski, 2020**). Cornelian cherry contains significant amounts of phenolic compounds and vitamins with a wide range of biological and pharmacological properties, such as antimicrobial, anti-inflammatory, anti-cancer, anti-diabetic and anti-atherosclerotic effects. It is cultivated not only for ornamental purposes but also for edible fruits, which are used in the production of jam, syrups, yoghurts, liqueurs, wine, soft drinks and cosmetics (**De Biaggi *et al.*, 2018**).
Medlar fruits (*Mespilus germanica* L.) are commonly consumed in Turkey and are gaining importance as a food for human consumption. The pulp or syrup of the fruit has been used as a remedy for enteritis and has many medicinal properties. Medlar also acts as a diuretic and is used to treat kidney and urinary stones (**Gruz *et al.*, 2011; Selcuk & Erkan, 2015**). Medlar is a rich source of bioactive compounds such as phenols, anthocyanins, organic acids, minerals and L-ascorbic acid (**Gülçin *et al.*, 2011; Selcuk & Erkan, 2015**).
Service tree (*Sorbus domestica*) belongs to the twenty most frequently used traditional medicinal plants (**Majić *et al.*, 2015**). Service tree fruits are rich in nutrients and suitable for processing (**Poljak *et al.*, 2021**). They are used as an antioxidant and a means of treating long-term diabetic complications. Fruits are eaten only when they are well-ripe and almost rotten. This fruit is rich in procyanidins, cinnamic acid, quercetin and flavonols. Fruit extract from service tree shows high antioxidant activity (**Lüle & Koyuncu, 2015; Majić *et al.*, 2015**).
Asimina triloba L., commonly known as pawpaw, is the only member of the *Annonaceae* family that grows in the temperate zone. Pawpaw pulp has the potential to be added to various consumer goods, to increase nutritional values or to improve taste (**Lolletti *et al.*, 2021**). Pawpaw fruits have a unique aroma and flavour. From a nutritional point of view, they are a good source of carbohydrates, proteins, lipids, fibre, minerals, and vitamins (mainly vitamins A and C), and are also rich in polyphenols (**Lolletti *et al.*, 2021; Stănică, 2012**).
Persimmon (*Diospyros kaki* L.) is a good source of L-ascorbic acid, carotene, minerals and fibre. The high content of phytochemicals (polyphenols, carotenoids, tocopherols, etc.) in persimmon has a beneficial effect against the formation of free radicals, hypercholesterolemia, diabetes, cancer and hypertension (**Butt *et al.*, 2015; Giordani *et al.*, 2011**).
The beneficial effect of vitamin C (L-ascorbic acid) has been widely recognized since its discovery in the late 20s of 20th century. This popularity is associated with its high abundance of fresh fruits and vegetables (**Arrigoni & Tullio, 2002**). Vitamin C is a strong antioxidant and cofactor of biosynthetic and gene regulatory enzymes. It contributes to the immune defence, supports the function of the epithelial barrier against pathogens, and supports the activity of scavenging oxidants in the skin, thereby protecting cells from environmental oxidative stress. It also treats and prevents scurvy in humans. Vitamin C (L-ascorbic acid) plays an important role in the normal functioning of the immune system, and its use in the prevention or treatment of infections has attracted great interest from doctors and researchers for almost a century. Therefore, we should be interested in species that are a rich source of vitamin C and also exhibit antioxidant activity (**Rucker *et al.*, 2007; Carr & Maggini, 2017**). Fruit is the main source of L-ascorbic acid intake in humans (more than 90%) because the human body does not have the gene encoding L-guluronic acid-1,4-lactone oxidase (**Zheng *et al.*, 2022**). A lack of vitamin C results in weakened immunity and a higher susceptibility to infections (**Carr & Maggini, 2017**). L-ascorbic acid plays an important role in plant resistance to biotic stress, as it scavenges reactive oxygen species, and is also important in resistance to abiotic stress (**Zheng *et al.*, 2022**).
Many plants are popular for their health benefits, but there are still plants that require more attention. These nutritious fruits also include our samples, which show high antioxidant activity, and therefore this research was aimed at determining the vitamin C content and antioxidant activity. An important supply of chemical compounds that can replace chemical medications, insecticides, and additives is found in the fruit components. It can improve our diet by adding beneficial elements including necessary vitamins, minerals, and antioxidants.

MATERIAL AND METHODS

Chemicals

Methanol (99.8%), DPPH (2,2´-diphenyl-1-picrylhydrazyl), Trolox (2,5,7,8-tetramethyl chroman-2-carboxylic acid), metafosforic acid (3%) were purchased from Sigma-Aldrich (Sigma Aldrich Chemie GmbH, Steiheim, Germany).

Fruit samples preparation

Five different types of fruit were used for the analyses, namely: cornelian cherry (*Cornus mas* L.), service tree (*Sorbus domestica*), medlar (*Mespilus germanica* L.), pawpaw (*Asimina triloba* L.) and persimmon (*Diospyros kaki* L.). All species were grown in Slovakia in the Experimental Garden of the Slovak University of Agriculture in Nitra. The fruits were collected at the stage of full maturity. The fruit was cleaned and washed using distilled water (dH_2O). Medlar, pawpaw, and persimmon were peeled. The cornelian cherry and the service tree were cored. An average of 100 g of fruit was used for homogenization. Subsequently, the samples were mixed (Grindomix GM 200, Retsch, Haan, Germany; 30 sec) and homogenized.

For the determination of vitamin C, the extract was prepared as follows: 8 g of the homogenized crop was poured into 25 ml of the extraction agent (3% metaphosphoric acid). This was followed by extraction using ultrasound (UAE – Ultrasonic assisted extraction) for 10 minutes in an ultrasound bath (Bandelin Sonorex Digitec, Sigma Aldrich). Subsequently, the extract was filtered through Whatman filter paper, and then the filtrate was filtered through a 0.45 µm PVDF filter. The filtrate prepared in this way was injected into the column 4 times.

The extract required for DPPH analysis was prepared from 25 g of homogenized sample poured with 50 mL of 80% methanol. The samples thus prepared were extracted for 12 hours on a horizontal shaker (Heidolph Promax 1020, Heidolph Instruments GmbH, Schwabach, Germany). Extracts were filtered through Muktell No 392 paper (Munktell & Filtrac GmbH, Bärenstein, Germany) and stored in sealed 50 mL centrifuge tubes at 4 °C in a refrigerator. The dry matter of the samples was determined on a moisture analyzer (KERN DLB 160 – 3A, KERN & SOHN GmbH, Balingen, Germany).

Determination of vitamin C by HPLC method

The content of L-ascorbic acid (vitamin C) was determined by the method of high-performance liquid chromatography with DAD detection. The external standard method and calibration curves are used for identification and quantification. A Cortecs C_{18} column (dimensions 150 x 4.6 mm, with a particle diameter of 5 µm) with a Security C_{18} pre-column 4 x 3 mm was used for the determination. Mobile phases consisted of methanol and 0.1% H_3PO_4 in ddH_2O (v/v). The isocratic elution was as follows: 0-10 min (20% methanol and 80% acidified water). The mobile phase flow was 0.5 $ml.min^{-1}$ and the sample injection was 20 µl. Column thermostat was set to 30 °C and the samples were kept at 8 °C the sampler manager. The detection and quantification wavelength was set at 254 nm.

Spectrophotometric determination of total antioxidant activity by the DPPH method

We followed the methodology of **Brand-Wiliams *et al.* (1995)** for the determination of the antioxidant activity**.** The DPPH solution was prepared by dissolving 0.025 g of DPPH (2,2-diphenyl-1-picryhydrazyl) in methanol (99.8%) in a 100 mL volumetric flask stored in a dark and cool place. A ten-fold diluted DPPH stock solution was used for analysis. 3.9 mL of DPPH was pipetted into 1 cm wide cuvettes, and then the absorbance value (A0) was measured on a UV-VIS spectrophotometer T92+ (PG Instruments, Leicestershire, United Kingdom) at a wavelength of 515.6 nm. After measuring the initial absorbance, we added 0.1 mL of the extract and mixed it three times using a stirrer. We measured the absorbance (A10) again after ten minutes. Based on the absorbance of the DPPH solution (A0) and the absorbance at time t = 10 minutes (A10) after the addition of the sample, we calculate the percentage of DPPH inhibition using the formula:

$\%DPPH\ inhibition = [(A0 – A10) / A0] \times 100.$

We express the result as trolox equivalent (TE) (µmol TE. g^{-1}) in dry weight (DW) and fresh weight (FW).

Statistical analysis

All analyzes were performed in 4 replicates (n = 4). Results are expressed as arithmetic mean ± standard deviation (SD). First, the dataset was tested for normality. All tested variables were non-parametrically distributed. Non-parametric ANOVA test (Kruskal-Wallis) and Dunn pairwise test with Holm correction was used for comparison between the tested variables. Spearman's correlation coefficient was used to determine the relationship between the investigated parameters (vitamin C and DPPH). Calculations, including graphic presentations, were carried out using the **RStudio (2020)** software package.

RESULTS AND DISCUSSION

Vitamin C content

Most fruits are a source of vitamin C, although the exact content depends on the species, variety and stage of development (**Romero Rodriguez *et al.*, 1992**). The content of L-ascorbic acid in the examined samples ranged from 72.24 µg. g^{-1} (*Diospyros kaki* L.) to 1114.61 µg. g^{-1} (*Sorbus domestica*). Service tree fruits showed several times higher vitamin C content (Table 1). **Egea *et al.* (2010)** measured the content of vitamin C in service tree fruits and found a value of 22.65 $mg.100g^{-1}$, which is approximately 5 times lower than the value measured in the present study. However, there is little published data on the content of L-ascorbic acid in the service tree (**Brindza *et al.* 2009; Egea *et al.* 2010; Mrkonjić *et al.*, 2019**). Furthermore, it is difficult to compare present and previous studies because the same experimental conditions and techniques were not used to determine vitamin C content.

Martinović & Cavoski (2020) investigated vitamin C content in cornelian cherry, and their values ranged from 48 $mg.100g^{-1}$ to 108 $mg.100g^{-1}$ depending on the different genotypes. In another study (**De Biaggi *et al.*, 2018**) the content of L-ascorbic acid was 61.43 $mg.100g^{-1}$ in the fresh matter. These results are higher than our measured results (18.60 $mg.100g^{-1}$), which may be due to different varieties. **Tural & Koca (2008)** report the content of L-ascorbic acid in cornelian cherry in the range of 160 µg. g^{-1} to 880 µg. g^{-1}. **Kostecka *et al.* (2017)** analyzed the vitamin C content using different methods, and the HPLC device results were 63.1 $mg.100g^{-1}$. **Cetkovská *et al.* (2015)** reported values between 199 - 433 µg. g^{-1} in cornelian cherry fruits. Differences between studies may be due to different climatic conditions under which the crops were grown, different degrees of maturity at fruit harvesting, and different sample processing techniques (**Odžaković *et al.*, 2022**).

In medlar, **Gülçin *et al.* (2011)** measured a vitamin C content of 184.6 µg. g^{-1}, which is more than twice as high as our sample, but **Gülçin et al. (2011)** used an aqueous extract of the freeze-dried sample, which may have affected the L-ascorbic acid content. In another study **Rop et al. (2010**) monitored the content of L-ascorbic acid in different stages of medlar maturity, and the values ranged from 17 $mg.100g^{-1}$ (highest maturity) to 59 $mg.100g^{-1}$ (lowest maturity). **Tessa et al. (2021)** observed low amounts of vitamin C (19.47 $mg.100g^{-1}$) because the fruits were collected in the post-ripening period, and the process of fruit development causes a dramatic decrease in the level of L-ascorbic acid. Also, the fruits we examined were in the stage of full maturity (after frost), and therefore the content of L-ascorbic acid is low.

In pawpaw **Nam *et al.* (2018)** determined the amount of vitamin C was only 0.98 $mg.100g^{-1}$ in the fresh matter. **Pande & Akoh (2010)** measured the content of L-ascorbic acid in the value of 9.8 $mg.100g^{-1}$ of fresh matter, although the extraction was carried out in 1M HCl, the result is comparable to our result.

Persimmon cultivars are divided into astringent and nonastringent. This property affects the content of vitamin C in the fruits, so that nonastringent ones have a higher vitamin C content (> 210 $mg.100g^{-1}$ FW) than astringent ones (3.5 $mg.100g^{-1}$ FW), which **Giordani *et al.*** (2011). **Ramin & Tabatabaei (2003)** collected persimmon fruits at two-week intervals in early October, and the highest L-ascorbic acid content was in the fruits after the first collection.

The vitamin C content is influenced by cultivars, as each contains different bioactive compounds (**Martinović & Cavoski, 2020**). The variability of L-ascorbic acid content is also affected by growing conditions, maturity, climate, handling of fresh fruit, processing factors and storage conditions (**Nagy, 1980**). In addition, vitamin C inhibits enzymatic activities, thus preserving the appearance and freshness of foods (**Martinović & Cavoski, 2020**). Aerobic and anaerobic mechanisms are mainly responsible for the decline of vitamin C in fruits (**Nagy, 1980**). L-ascorbic acid has several biological functions, such as scavenging free radicals and thus helps in the prevention of various diseases. Therefore, daily consumption of foods with a high content of L-ascorbic acid contributes to well-being and reduces the risk of chronic diseases (**Mrkonjić *et al.*, 2019**). To maintain adequate stores of vitamin C in the human body, a Recommended Dietary Allowance (RDA) has been proposed over the years. RDAs vary from country to country (**Cerullo *et al.*, 2020**). In the European Union, the EFSA (European Food Safety Authority) set the RDA of vitamin C for women at 95 mg/d and men at 110 mg/d. In contrast, other countries, such as the USA and Canada, considered the potential health effects of vitamin C when deriving the RDA. Their current RDA (90 mg/d for men and 75 mg/d for women) is based on the amount of vitamin C which is believed to provide antioxidant protection (**Carr & Lykkesfeldt, 2021**).

Table 1 Vitamin C content and DPPH antioxidant activity

Fruit	Vitamin C (µg.g^{-1} FW)	Vitamin C (mg.100g^{-1} FW)	AA DPPH (µmol TE.g^{-1} DW)	AA DPPH (µmol TE.g^{-1} FW)	AA DPPH (%)
***Cornus mas* L.**	186.01 ± 2.73	18.60 ± 2.73	12.34 ± 0.01	2.52 ± 0.003	85.80 ± 0.05
Sorbus domestica	1114.61 ± 2.12	111.46 ± 2.12	4.04 ± 0.12	1.35 ± 0.05	56.52 ± 1.72
***Mespilus germanica* L.**	73.40 ± 0.18	7.34 ± 0.18	2.28 ± 0.15	0.70 ± 0.05	26.10 ± 1.52
***Asimina triloba* L.**	125.94 ± 0.66	12.59 ± 0.66	7.82 ± 0.01	2.30 ± 0.003	80.74 ± 0.11
***Diospyros kaki* L.**	72.24 ± 0.85	7.22 ± 0.85	7.38 ± 0.26	1.58 ± 0.06	56.08 ± 1.86

Legend: DW –dry weight, FW- fresh weight, AA – antioxidant activity, DPPH –2,2´-diphenyl-1-picrylhydrazyl, TE –Trolox equivalents, The values are expressed as average ± SD.

Figure 1 shows the relationship between individual types of fruit and vitamin C. There is a significant difference in vitamin C content between types of fruit (p=1.09e^{-03}). Statistical differences were observed between *Cornus mas* L. and *Diospyros kaki* L. ($p_{Holm-adj.}$ = 0.04), *Diospyros kaki* L. and *Sorbus domestica* ($p_{Holm-adj.}$ = 1.31e^{-03}), and between *Sorbus domestica* and *Mespilus germanica* L ($p_{Holm-adj.}$ = 0.04).

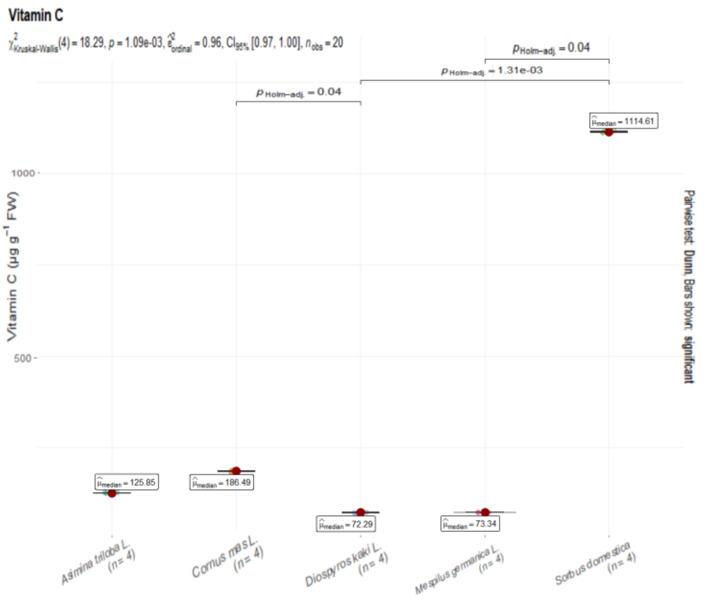

Figure 1 Statistical differences in the content of vitamin C in the investigated types of fruit

Antioxidant activity

By scavenging free radicals, antioxidants act as reducing agents and prevent oxidative damage to biological molecules. They are also widely used as food additives to prevent food degradation and play an important role in the prevention of many lifestyle-related diseases and ageing, being closely related to the generation of reactive oxygen species (ROS) and lipid peroxidation (**Gülçin *et al.*, 2011**). Research on the antioxidant activity of natural products is the basis for the classification of antioxidant plants and the recommendation of the best antioxidant foods for consumption (**Xu *et al.*, 2017**). Antioxidant activity in individual fruit species assessed by DPPH increased in the following order: *Mespilus germanica* < *Sorbus domestica* < *Diospyros kaki* < *Asimina triloba* < *Cornus mas* (Table 1). **Martinović & Cavoski (2020)** report the antioxidant activity of DPPH in values from 6.23 to 19.03 µmol TE. g^{-1} of fresh mass, depending on the different genotypes. They also report in their studies that *Cornus mas* has a higher antioxidant activity compared to strawberries, raspberries and blueberries, which are commonly considered sources of antioxidants in food. Because anthocyanins in the fruit interfere with DPPH and both absorb at the same wavelength, the presence of anthocyanins in the fruit may be the source of the cornelian cherry's strong antioxidant activity (**Martinović & Cavoski, 2020**). The study by **Klymenko et al. (2021)** measured antioxidant activity in the range of 6.75-77.35 µmol TE. g^{-1} FW, and their results could be influenced by the storage of cornelian cherry fruits at -20°C. Regarding the service tree, **Poljak et al. (2021)** found antioxidant activity values from 4.40 to 17.76%, which is several times less antioxidant activity compared to the present results. The difference may be due to the Mediterranean eco-geographical region in which the fruits of service tree (*Sorbus domestica)* ripen or to a difference in the preparation of the extract. **Ognyanov *et al.* (2022)** report that service tree fruits have a good ability to scavenge DPPH radicals. Unfortunately, we could not compare the results, because the authors used not only different extracts to test the antioxidant activity, but also other units of measurement. **Selcuk & Erkan (2015)** observed antioxidant activity in medlar during cold storage. They found that the longer the medlar was in the cold, the higher the antioxidant activity of DPPH. We cannot compare the results of the antioxidant capacity of *Asimina triloba* L. with other studies, because the antioxidant activity is expressed in other units (**Lolletti *et al.*, 2021; Nam *et al.*, 2019; Nam *et al.*, 2017**), as well as the results of *Diospyros kaki* L. (**Giordani *et al.*, 2011; Jang *et al.*, 2010**). The linear reaction range of the DPPH test is narrow, only 2-3 times. Moreover, due to steric inaccessibility, antioxidants that have strong antioxidant activities in the lipid peroxidation system may react slowly or may even be inert to DPPH (**Xu *et al.*, 2017** Sufficient amounts of antioxidants are essential to prevent oxidative stress caused by free radicals. It has been reported that most of the antioxidant capacity of fruits and vegetables might be correlated to the content of total phenolics, anthocyanins and flavonoids (**Gülçin *et al.*, 2011**). Regarding the antioxidant activity, there is a significant difference in DPPH values between the fruit types (p=1.07e^{-03}), which was confirmed by the Kruskal-Wallis test (Figure 2). Statistical differences were observed between *Cornus mas* and *Sorbus domestica*. ($p_{Holm-adj.}$ = 0.04), *Cornus mas*. and *Mespilus germanica* ($p_{Holm-adj.}$ = 1.29e^{-03}), and between *Asimina triloba*. and *Mespilus germanica*. ($p_{Holm-adj.}$ = 0.04).

Spearman's correlation coefficient was used to determine the relationships between monitored parameters vitamin C and antioxidant activity (DPPH). The content of vitamin C in the studied species showed a positive relationship with antioxidant activity. Vitamin C content is positively correlated with DPPH free radical scavenging activity (r = 0.19). **Perna *et al.* (2013)** found a statistically significant correlation between vitamin C content and antioxidant activity, measured by the DPPH assay, which confirmed the antioxidant properties of vitamin C.

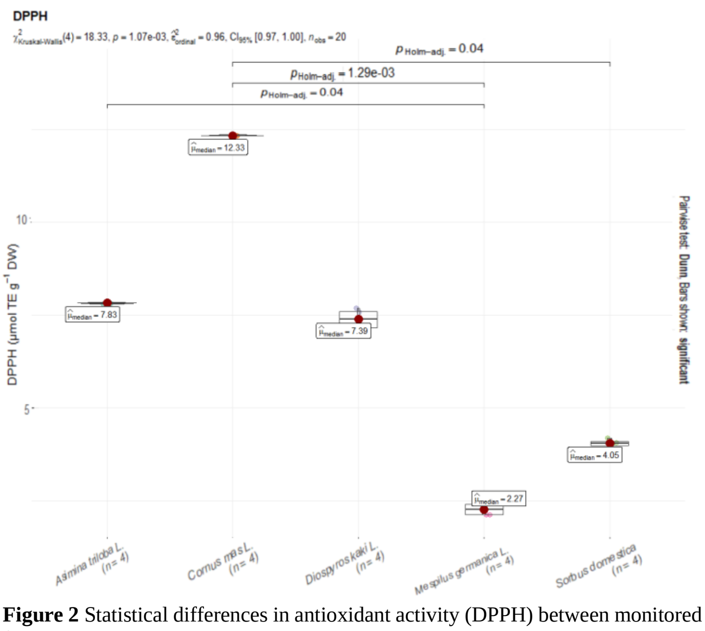

Figure 2 Statistical differences in antioxidant activity (DPPH) between monitored fruit varieties.

CONCLUSION

This study provides information on vitamin C content and DPPH antioxidant activity in underutilized fruits (cornelian cherry, service tree, medlar, pawpaw, persimmon). The study showed that these fruits are rich in vitamin C content and also show high antioxidant activity. The highest content of vitamin C was observed in service tree (*Sorbus domestica*) and the lowest in persimmon (*Diospyros kaki* L.). The antioxidant activity was determined by the DPPH method. Cornelian cherry showed the highest antioxidant activity and medlar showed the lowest antioxidant activity. The study also points to the need to know such types of fruit, because they are a source of bioactive substances and also high-quality nutritional and health-promoting components. In further research, attention should be paid to the content of bioactive substances in these crops, because they are a rich and easily available source of valuable substances. More and more evidence is being placed on the intake of natural vitamins rather than synthetic ones. The statistical evaluation of the results showed a difference between the investigated types of fruit.

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