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EVALUATION OF THE ANTIOXIDANT ACTIVITY AND PHENOLIC CONTENT OF CHINESE QUINCE (*PSEUDOCYDONIA SINENSIS* SCHNEID.) FRUIT

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ABSTRACT

Background. Neglected and underutilized plant species could serve as a valuable source of natural bioactive compounds. The objective of this study was to evaluate the biological activity of Chinese quince (*Pseudocydonia sinensis* Schneid) genotypes of Ukrainian and Slovak origin.
Materials and methods. The content of the total antioxidant activity (DPPH method and molybdenum reducing antioxidant power), total polyphenol, flavonoid and phenolic acid compounds in the pulp and peel of Chinese quince were compared across five genotypes from Slovakia and three from Ukraine.
Results. All tested samples exhibited DPPH$^{\cdot}$ radical scavenging activities with values from 6.17 to 9.56 mg TEAC (Trolox equivalent antioxidant capacity) per gram of dry matter (DM). Antioxidant activity, measured using the molybdenum reducing antioxidant power method, ranged from 69.82 to 225.04 mg TEAC per gram of DM. Total polyphenol content was from 34.73 to 82.02 mg GAE (gallic acid equivalent), while total flavonoid content was from 0.50 to 26.72 mg QE (quercetin equivalent) per gram DM. Phenolic acid content varied from 1.12 to 8.39 mg CAE (caffeic acid equivalent) per gram DM. The peel extracts contained the highest content of bioactive compounds when compared with the pulp extract (from 15.30 to 32.60%). All observed parameters differed significantly between the genotypes. Strong positive correlations ($p \leq 0.05$) were observed between the content of phenolic acids and flavonoids in the peel in plants from Slovakia ($r = 0.951$, $r = 0.928$, respectively); between the phenolic acid and antioxidant capacities detected using the MRP method – $r = 0.950$ and $r = 0.955$ for peel and pulp, respectively; between the determination of antioxidant activity by the DPPH and MRP methods in the peel and pulp in plants from Ukraine ($r = 0.986$, $r = 0.998$, respectively). Significantly positive correlations were found between all the parameters in the samples of Ukrainian origin.
Conclusion. The results showed that all fruit extracts exhibited strong antioxidant activities, which generally correlated positively with the total phenolic content. This study demonstrates that Chinese quince fruit grown in Ukraine and Slovakia is a perspective source of valuable polyphenol content with high antioxidant activity and is a valuable fruit for use in the agriculture and food industries.

Keywords: *Pseudocydonia sinensis*, fruits, antioxidant activity, phenolic compounds

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INTRODUCTION

Growing interest about neglected and underutilized plant species which could serve as a valuable source of natural bioactive compounds has been emerging worldwide. The biological properties of plants like *Cornus mas* L., *Chaenomeles* spp., *Diospyros virginiana* L., *Lonicera* spp., *Morus nigra* L., *Ziziphus jujuba* Mill., *Vaccinium* spp., *Sambucus nigra* L. have been discussed recently (Hamauzu et al., 2005; Kazimierski et al., 2019; Scibisz and Mitek, 2007; Xue et al., 2009).

Pseudocydonia sinensis Schneid. (Chinese quince) of the family *Rosaceae* Juss. is a Chinese and East Asian native plant represented by one species from the genus *Pseudocydonia* C.K. Schneid. It is closely related to the East Asian genus, *Chaenomeles* Lindl., and to the European genus, *Cydonia* Mill. (Suzuki, 1994). Fruit of the *Pseudocydonia sinensis* are very fragrant, yellow edible pomes with an elliptical (var. *ellipsoidea*) or ovoid (var. *ovoidea*) shape (Choi et al., 2018; Klymenko et al., 2017; Mihara et al., 1987). Their fruit are very big with a height of 98.06–124.48 mm, an average diameter of 62.33–88.64 mm, and an average weight in the range of 197.85–466.38 g (Monka et al., 2014). The main volatile compounds in Chinese quince peel are expected to be (E,E)-α--farnesene, isobutyl octanoate, ethyl octanoate, isobutyl 7-octanoate, and hexyl hexanoate (Mihara et al., 1987). In the peel, ethyl 2-methylpropanoate, ethyl (E)-2-butenoate, ethyl 2-methyl butanoate, methional, (Z)-3-hexenyl acetate, β-ionone, ethyl nonanoate, and γ-decalactone were found as the potent aroma-active compounds (Choi et al., 2018). The fresh fruit of *Pseudocydonia sinensis* are sour and hard, and consumed after processing into spreads, marmalades, jams, fruit jellies, candied pulp, sweetened syrups and juices, wines, liqueurs, and candies (Hamauzu et al., 2006; Klymenko et al., 2017; Monka et al., 2014).

The fruit of the Chinese quince were widely used in traditional Chinese medicine as antitussives of central or peripheral action to suppress the cough reflex. The fruit are applied for the treatment of asthma, colds, sore throat, mastitis, rheumatoid arthritis, and tuberculosis (Chung et al., 1988a; 1988b; Hamauzu et al., 2005; 2014; Mihara et al., 1987). Active ingredients of the fruit include organic acids, flavonoids rutin and quercetin, procyanidins, and volatile compounds (Hamauzu et al., 2005; 2014). Recent pharmacological studies have shown the antibacterial, antihaemolytic (Osawa et al., 1997), anti-inflammatory (Osawa et al., 1999), antipruritic (Oku et al., 2003), antioxidant and antiviral (Hamauzu et al., 2005; 2007; Sawai et al., 2008; Sawai-Kuroda et al., 2013), anti-ulcerative (Hamauzu et al., 2006), gastroprotective (Hamauzu et al., 2018), antitumor (Chun et al., 2012), and antimicrobial (Essuman et al., 2017; Kabir et al., 2015) properties of Chinese quince fruit.

The objective of this study was to evaluate the antioxidant activity, total polyphenol, flavonoid, and phenolic acids content in the *Pseudocydonia sinensis* fruit of genotypes from different growing conditions (Slovakia and Ukraine).

MATERIALS AND METHODS

Biological material

Pseudocydonia sinensis (Fig. 1) were collected in the period of full ripeness (November 2018) from trees growing in an arboretum Mlyňany (Vieska nad Žitavou, Slovakia; 160–210 m a.s.l.) and M.M. Gryshko National Botanical Garden (Kyiv, Ukraine; 197 m a.s.l.). The plants were botanically identified at the Institute of Biodiversity Conservation and Biosafety of the Slovak University of Agriculture in Nitra. Samples from Slovakia were identified as PS and appropriate number (PSe-01 – PSe-05 for peel and PSu-01 – PSu-05 for pulp). Samples from Ukraine were identified as PU and appropriate number (PUe--01 – PUe-03 for peel and PUu-01 – PUu-03 for pulp). The peel and pulp were dried in the oven (Binder 115, Germany) at 38°C for 48 h before analysis.

Chemicals

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

Preparation of sample extracts

An amount of 0.25 g of each sample was extracted with 20 mL of 80% ethanol for 2 h in a laboratory shaker GFL 3005 (GFL, Burgwedel, Germany). Then, the samples were centrifuged at 4605 RCF (Rotofix 32 A, Hettich, Germany) for 10 min and the supernatant

Fig. 1. Morphology of fruit of *Pseudocydonia sinensis* Schneid.: A – shape of fruit, B – vertical and horizontal cross-section of fruit

was used for measurement of the antioxidant activity (DPPH and phosphomolybdenum method) and detection of the total polyphenol, total flavonoid and phenolic acid contents.

Free radical scavenging activity – DPPH method

The free radical scavenging activity of the samples was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH; Sanches-Moreno et al., 1998). An amount of 0.4 mL of sample was mixed with 3.6 mL of DPPH solution (0.025 g DPPH in 100 mL ethanol). The absorbance of the reaction mixture was determined with a spectrophotometer Jenway (6405 UV/Vis, England) at 515 nm. Trolox (6-hydroxy-2,5,7,8-tetramethyl--chroman-2-carboxylic acid; 10–100 mg/L; $R^2 = 0.989$) was used as the standard and the results were expressed in mg/g DM Trolox equivalents.

Molybdenum reducing antioxidant power

The molybdenum reducing (MRP) antioxidant power of the samples was determined using the method of Prieto et al. (1999) with slight modifications. A mixture of sample (1 mL), monopotassium phosphate (2.8 mL, 0.1M), sulfuric acid (6 mL, 1M), ammonium heptamolybdate (0.4 mL, 0.1M) and distilled water (0.8 mL) was incubated at 90°C for 120 min, then cooled to room temperature. The absorbance at 700 nm was detected with a spectrophotometer Jenway (6405 UV/Vis, England). Trolox (10–1000 mg/L; $R^2 = 0.998$) was used as the standard and the results were expressed in mg/g DM Trolox equivalent.

Total polyphenol content

The total polyphenol content (TPC) was measured using the method of Singleton and Rossi (1965) using a Folin-Ciocalteu reagent. A quantity of 0.1 mL of each sample was mixed with 0.1 mL of Folin-Ciocalteu reagent, 1 mL of 20% (w/v) sodium carbonate and 8.8 mL of distilled water. After 30 min in darkness, the absorbance at 700 nm was measured with a spectrophotometer Jenway (6405 UV/Vis, England). Gallic acid (25–300 mg/L; $R^2 = 0.998$) was used as the standard. The results were expressed in mg/g DM gallic acid equivalent.

Total flavonoid content

The total flavonoid content (TFC) was determined by the modified method described by Shafii et al. (2017). An aliquot of 0.5 mL of the sample was mixed with 0.1 mL of 10% (w/v) ethanolic solution of aluminium chloride, 0.1 mL of 1M potassium acetate and 4.3 mL of distilled water. After 30 min in darkness, the absorbance at 415 nm was measured using a spectrophotometer Jenway (6405 UV/Vis, England). Quercetin (1–400 mg/L; $R^2 = 0.9977$) was used as the standard. The results were expressed in mg/g DM quercetin equivalent.

Total phenolic acid content

Total phenolic acid (TPA) content was determined with the method of Farmakopea Polska (1999). A 0.5 mL of the sample was mixed with 0.5 mL of 0.5M hydrochloric acid, 0.5 mL Arnov's reagent (10% $NaNO_2$ + 10% Na_2MoO_4), 0.5 mL of 1M sodium hydroxide (w/v) and 0.5 mL of water. Absorbance at 490 nm was measured using a spectrophotometer Jenway (6405 UV/Vis, England). Caffeic acid (1–200 mg/L, $R^2 = 0.999$) was used as the standard and the results were expressed in mg/g DM caffeic acid equivalents.

Statistical analysis

Statistical analyses were performed using PAST 2.17. Data were analyzed with the ANOVA test and the Tukey-Kramer test ($p = 0.05$) and the results were used to calculate the differences between the means. Correlation coefficients were calculated using the CORR analysis.

RESULTS AND DISCUSSION

Antioxidant activity

The antioxidant activity of fruit is mainly based on the presence of biologically active compounds – polyphenols, flavonoids, phenolic acids, anthocyanins, catechins, ascorbic acid and β-carotene. The content of antioxidants in fruit is of importance for reducing the risk of cardiovascular and neurological disease and could lower the risk of cancer (Karadeniz et al., 2005). In recent years, numerous methods have been widely applied for the analysis and evaluation of the antioxidant capacity of fruit (Kucelova et al., 2016; Kucharska et al., 2017; Luximon-Ramma et al., 2003; Netzel et al., 2006; Pliszka, 2017).

In our study, the antioxidant activity of *Pseudocydonia sinensis* peel ranged from 9.19 (PSe-05) to 9.56 (PSe-02) and from 9.31 (PUe-02) to 10.23 (PUe-01) mg TEAC/g DM, for genotypes from Slovakia and Ukraine, respectively (Fig. 2).

The antioxidant activity of the pulp varied from 6.17 (PSu-02) to 8.55 (PSu-01) and from 5.39 (PUu-02) to 6.85 (PUu-03) mg TEAC/g DM for genotypes from Slovakia and Ukraine, respectively. The variation coefficient showed a low to medium variability of the antioxidant activity of the peel and pulp of genotypes from Slovakia and Ukraine with 2.38, 5.39%, and 12.57, 11.96%, respectively. In a study by Monka et al. (2014), the antiradical activity of aqueous and methanolic extracts of dry peel was 91.87–93.25%, and of dry pulp 80.39–84.11%, which showed the high antioxidant potential of different extracts. In another study, the antioxidant activity of *Cydonia oblonga* Mill. extracts evaluated by the DPPH assay showed a higher activity in pulp than in jam (Baroni et al., 2018). Comparable analysis of the antioxidant activity of *Cydonia oblonga* Mill. pulp, peel, seed and jam extracts identified that methanolic peel extracts demonstrated the strongest activity, followed by pulp and

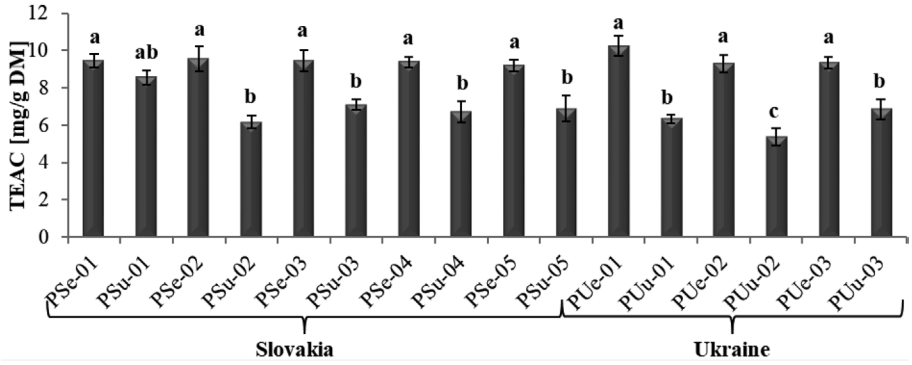

Fig. 2. Antioxidant activity of the peel and pulp of the fruit of *Pseudocydonia sinensis* Schneid. genotypes from Slovakia and Ukraine evaluated by the DPPH method (different superscripts in each column indicate the significant differences in the mean at $P < 0.05$): TEAC – Trolox equivalent antioxidant capacity

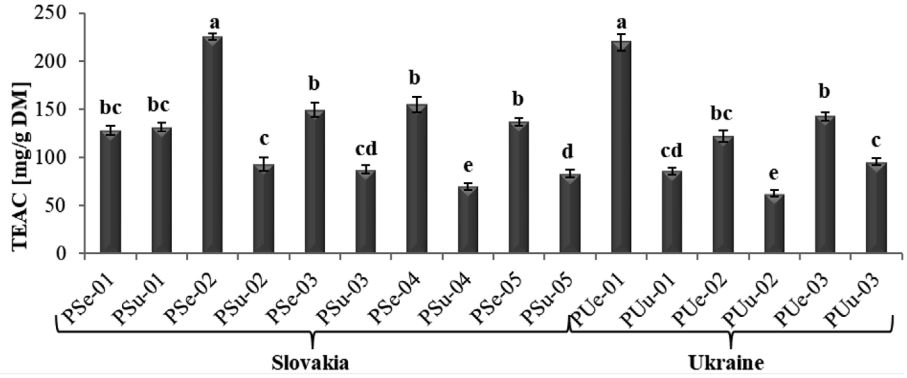

Fig. 3. Antioxidant activity of *Pseudocydonia sinensis* Schneid. peel and pulp fruit genotypes from Slovakia and Ukraine evaluated by the molybdenum reducing antioxidant power (different superscripts in each column indicate the significant differences in the mean at $P < 0.05$): TEAC – Trolox equivalent antioxidant capacity

seed extracts. Also, different study determined that the phenolic fraction of seed extracts had a stronger antioxidant activity than the peel and pulp extracts (Silva et al., 2004). According to Manzoor et al. (2012), the antioxidant activity using DPPH method of methanol extracts of *Malus domestica* Borkh. cultivars was higher in peel extracts (71.7–84.9%) than in pulp ones (43.9–52.8%).

The reducing power is one of the methods in the study of antioxidant capacity, which is generally associated with the presence of reductants, when Trolox is used as the antioxidant standard. A study by Issa et al. (2016) showed that the reducing power of *Malus domestica* Borkh. extracts increased with an increase in the ascorbic acid concentration. The alcoholic extract of apple fruit was the most potent reducing agent (Issa et al., 2016).

The antioxidant activity of peel extract measured by molybdenum reducing antioxidant power was from 127.93 (PSe-01) to 222.0 (PSe-02) and from 122.31 (PUe-02) to 219.31 (PUe-01) mg TEAC/g DM for genotypes of Slovak and Ukrainian origin, respectively (Fig. 3).

The antioxidant activities of pulp extracts were from 82.92 (PSu-05) to 131.13 (PSu-01) and from 62.19 (PUu-02) to 95.44 (PUu-03) mg TEAC/g DM for genotypes from Slovakia and Ukraine, respectively. The high variability of the antioxidant activity of peel and pulp from the fruit of genotypes from Slovakia and Ukraine was confirmed by the variation coefficient (24.24%, 31.78% and 24.82%, 21.07%, respectively). Our results were in agreement with previous studies confirming the higher antioxidant activity of the peel of *Pseudocydonia sinensis* in comparison with the pulp (Monka et al., 2014). The higher antioxidant activity of the peel of other fruit than of the pulp has been widely reported (Ajila et al., 2007; Liu et al., 2018; Xue et al., 2009).

Total polyphenol, flavonoid and phenolic acid content

Current research supports the role of polyphenols as effective antioxidants in the prevention of cancers, cardiovascular and neurodegenerative diseases, diabetes mellitus, autoimmune disorders and some inflammatory processes (Duthie and Brown, 1994; Dong et al., 2009; Milner, 1994; Vladimir-Knežević et al., 2012; Williams et al., 2004). In food, the polyphenols may contribute the bitterness, astringency, color, flavor, odor and oxidative stability. Fruits like grapes, apples, pears, cherries, and berries contain up to 200–300 mg of polyphenols per 100 g of fresh fruits (Pandey and Rizvi, 2009). Määttä-Riihinen et al. (2004) reported the high content of chlorogenic and neochlorogenic acids in chokeberry and sweet rowanberry, of the *Rosaceae* family. The polyphenols of *Pseudocydonia sinensis* are considered to be the most important bioactive ingredients because of their various

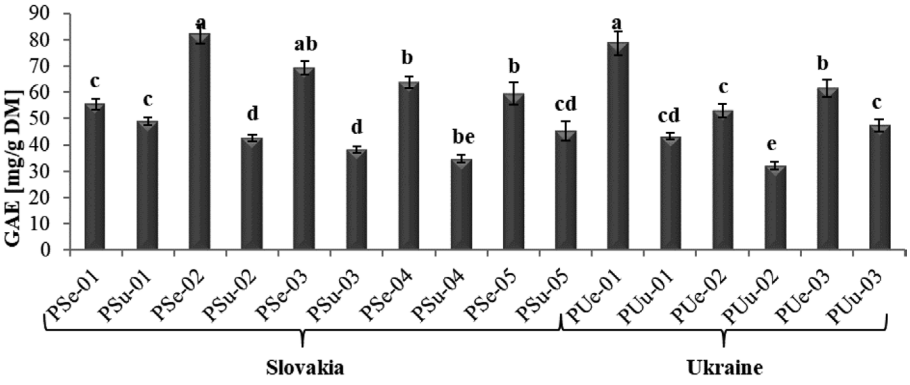

Fig. 4. Total polyphenol content in the peel and pulp of fruit of *Pseudocydonia sinensis* Schneid. genotypes from Slovakia and Ukraine (different superscripts in each column indicate significant differences in the mean at $P < 0.05$): GAE – gallic acid equivalent

pharmacological actions and high content (Oku et al., 2003; Osawa et al., 1999).

The content of total polyphenols (Fig. 4) in the peel was from 55.61 (PSe-01) to 82.02 (PSe-02) and from 53.17 (PUe-02) to 78.67 (PUe-01) mg GAE/g DM in the genotypes of Slovakia and Ukraine, respectively. The content of total polyphenols in the pulp ranged from 34.73 (PSu-04) to 48.99 (PSu-01) and from 32.31 (PUu-02) to 47.41 (PUu-03) mg GAE/g DM in fruit from Slovakia and Ukraine, respectively. The variability of the total polyphenols in the peel and pulp of fruit of genotypes from Slovakia and Ukraine (15.57, 20.16% and 13.39, 19.02%, respectively) was confirmed with the variation coefficient. Differences in the content of polyphenols have previously been identified, and Al-Snafi (2016) highlighted the highest content of phenolic compounds in the peel of *Cydonia oblonga*. As reported by Manzoor et al. (2012), the total content of polyphenols in methanol extracts of the peel and pulp of *Malus domestica* Borkh. cultivars was 1907.5–2587.9 mg GAE/100 g DW and 1185.2–1475.5 mg GAE/100 g DW, respectively.

Flavonoids are considered to be an indispensable source of the components of nutraceutical, pharmaceutical, medicinal and cosmetic applications and studies on the content of flavonoids in different parts of non-traditional or neglected fruit plants are important (Kumar and Pandey, 2013). This is one of the most important groups of phenolic compounds with health-promoting effects widely found in fruit and vegetables. Flavonoids are synthesized in particular sites and are responsible for attracting pollinators (Panche et al., 2015).

The total flavonoid content in the peel (Fig. 5) was from 11.0 (PSe-01) to 26.72 (PSe-02) and from 9.15 (PUe-03) to 26.18 (PUe-01) mg QE/g DM for genotypes from Slovakia and Ukraine, respectively. The total flavonoid content in the pulp varied from 0.59 (PSu-05) to 1.07 (PSu-02) and from 0.55 (PUu-02) to 0.87 (PUu-03) mg QE/g DM for fruit from Slovakia and Ukraine, respectively. The high variability of flavonoid content in the peel and pulp in the fruit of genotypes from Slovakia and Ukraine was confirmed with the variation coefficient – 36.36, 61.78% and 22.82, 22.50% respectively. Amirahmadi et al. (2017) identified that the similar genotypes of *Cydonia oblonga* shared the total content of flavonoids in fruit of 6.2 mg QE·g^{-1}.

Phenolic acids are secondary metabolites that are present in plant products, mostly in bound form. The well-known phenolic acids are chlorogenic, caffeic, *p*-coumaric, ferulic and vanillic. Alongside the other polyphenolic compounds, phenolic acids are powerful antioxidants with antibacterial, antiviral, anticarcinogenic, anti-inflammatory and vasodilatory activities (Mattila and Hellström, 2007).

The total phenolic acid content was found to vary significantly among the various *Pseudocydonia sinensis* genotypes and parts of the fruit (Fig. 6). The content of phenolic acids in the peel was from 4.40 (PSe-04) to 8.39 (PSe-02) and from 3.56 (PUe-03) to 7.45

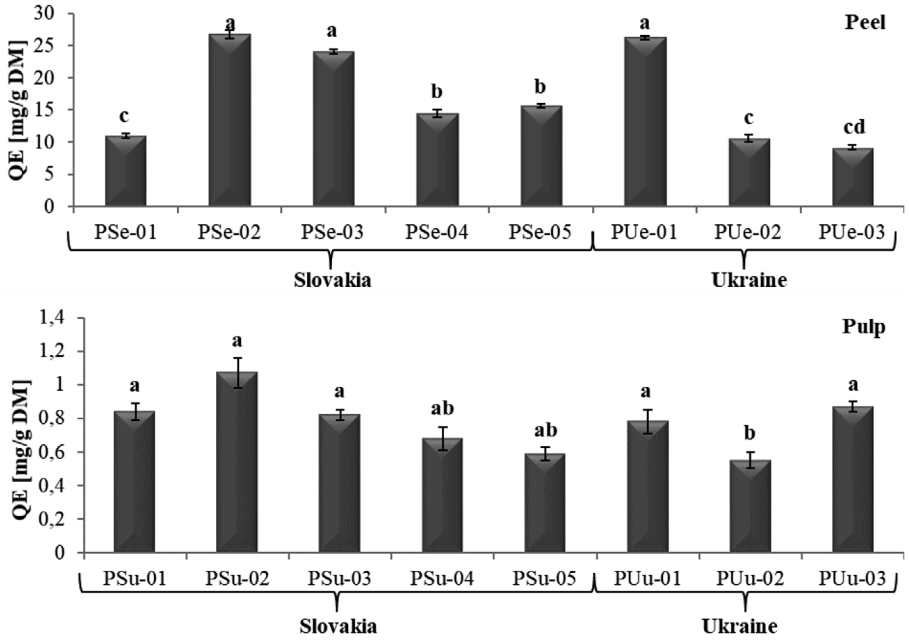

Fig. 5. Total flavonoid content in the peel and pulp of fruit of *Pseudocydonia sinensis* Schneid. genotypes from Slovakia and Ukraine (different superscripts in each column indicate significant differences in the mean at $P < 0.05$): QE – quercetin equivalent

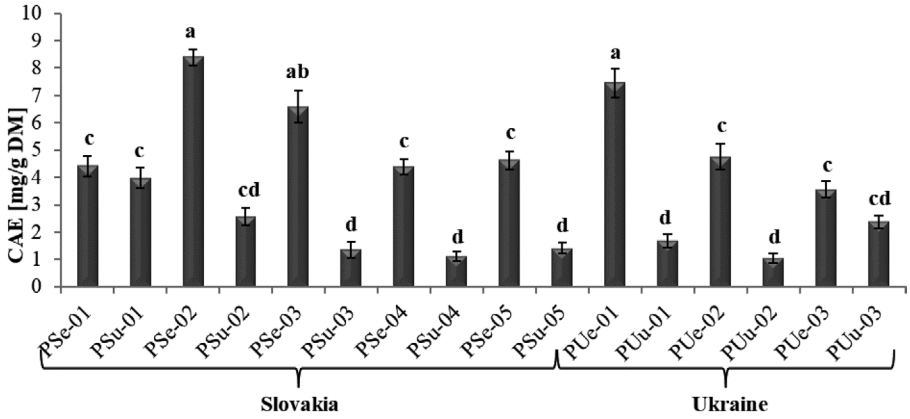

Fig. 6. Total phenolic acid content in *Pseudocydonia sinensis* Schneid. genotypes (different superscripts in each column indicate the significant differences in the mean at $P < 0.05$): CAE – caffeic acid equivalent

(PUe-01) mg CAE/g DM for genotypes from Slovakia and Ukraine, respectively. The content of these compounds in the pulp varied from 1.12 (PSu-04) to 3.97 (PSu-01) and from 1.04 (PUu-02) to 2.38 (PUu-03) mg CAE/g DM for genotypes of Slovak and Ukrainian origin, respectively. The high variability of the phenolic acid content was confirmed with the variation coefficient of 31.13, 37.89% and 57.36, 39.51%

for the peel and pulp for fruit of Slovak and Ukrainian origin, respectively.

The content of phenolic compounds in the fruit of *Pseudocydonia sinensis* was in agreement with previous research (Hamauzu et al., 2006). Hamauzu et al. (2005) found a total phenolic content of 1280 mg/100 g of FW in the fruit of *Pseudocydonia sinensis* that was four and 20 times higher than in *Cydonia oblonga* Mill. and in *Malus domestica* Borkh., respectively. Hamauzu et al. (2006) showed that the phenolic compounds of *Pseudocydonia sinensis* fruit were effective protectors against ethanol-induced gastric ulcers. The phenolic compounds of *Pseudocydonia sinensis* fruit could enhance the antioxidant capacity of blood after oral administration (Hamauzu et al., 2006). In a report by Mattila et al. (2006), the highest content of phenolic acids was described in the chokeberry, of 96 mg per 100 g FW, and sweet rowanberry, of 75 mg per 100 g FW. The content of phenolic compounds in other fruit, for example, in dark plum, cherry, citrus fruit, and some apple varieties, was less than 30 mg per 100 g FW, which was significantly lower. The content of phenolic acids in apples was shown to be dependent on the cultivar. Traditionally known fruit, such as pear, peach and nectarine, contained low concentrations of phenolic acids (less than 10 mg per 100 g FW).

Differences between present and previously conducted studies in the chemical composition of fruit of *Pseudocydonia sinensis* could be attributed to the geographical plant origin and different methods of extraction.

Correlation analysis was used to explore the relationships between the phenolic compounds and antioxidant capacities with the DPPH and MRP methods for peel and pulp extracts from five *Pseudocydonia sinensis* genotypes (Table 1).

Our findings indicated strong positive correlations for the content of phenolic acid and flavonoids in the peel in plants from Slovakia ($r = 0.951$, $r = 0.928$, respectively). A strong positive correlation was identified between the phenolic acid and antioxidant capacities, detected using the MRP method – $r = 0.950$ and $r = 0.955$ for the peel and pulp, respectively. In addition, a strong positive correlation was identified between determinations of the antioxidant activity using the DPPH and MRP methods in the peel and pulp in

Table 1. Pearson's correlation coefficients (r) between the antioxidant capacity and total phenolic compounds measured in the peel and pulp of *Pseudocydonia sinensis* Schneid. fruit

Parameter	Total polyphenols	Phenolic acids	Flavonoids	MRP method	Total polyphenols	Phenolic acids	Flavonoids	MRP method
	peel				pulp			
Slovakia								
Phenolic acids	0.951*				0.780			
Flavonoids	0.928*	0.950*			0.440*	0.511		
MRP method	0.947	0.873*	0.776*		0.813*	0.955*	0.774*	
DPPH method	0.643*	0.676	0.520*	0.621	0.571*	0.659	0.458	0.807*
Ukraine								
Phenolic acids	0.805				0.959			
Flavonoids	0.920*	0.973			0.694	0.960*		
MRP method	0.992*	0.873*	0.961*		0.939*	0.964*	0.957*	
DPPH method	0.958	0.941*	0.993	0.986*	0.796*	0.878*	0.797*	0.998

MRP – molybdenic reducing power.
*Significant according to the t-test ($p \leq 0.05$).

plants from Ukraine ($r = 0.986$, $r = 0.998$, respectively). Significantly positive correlations were found between all the parameters in the samples of Ukrainian origin. A strong correlation between the antioxidant activity and the content of phenolic acids in the fruit of *Malus domestica*, *Vitis vinifera*, *Cydonia vulgaris*, *Pyrus communis*, *Punica granatum* using the DPPH method was demonstrated by Karadeniz et al. (2005).

CONCLUSION

The antioxidant activity, total polyphenol, flavonoid and phenolic acid content of fruit (peel and pulp) extracts of *Pseudocydonia sinensis* genotypes from two different agroecological conditions (Slovakia and Ukraine) were studied. The results revealed a higher content of bioactive compounds in the peel compared with the pulp. All extracts exhibited strong antioxidant activities, which correlated positively with the content of polyphenols, flavonoids, and phenolic acids. This study demonstrates the potential application of the fruit of *Pseudocydonia sinensis* as a possible source of valuable polyphenols with high antioxidant activities and health-promoting properties.

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