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ANTIOXIDANT ACTIVITIES AND PHENOLIC COMPOUNDS IN FRUITS OF VARIOUS GENOTYPES OF AMERICAN PERSIMMON (*DIOSPYROS VIRGINIANA* L.)

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ABSTRACT

Background. American persimmons (*Diospyros virginiana* L.) are known as a widespread cultivar which were traditionally used by Native Americans as a food source, and since ancient times have been used in folk medicine. The objective of this study was to evaluate the antioxidant activity and phenolic content of *Diospyros virginiana* genotypes.

Material and methods. The content of the total antioxidant activity and phenolic compounds from the fruits of the American persimmon (*Diospyros virginiana* L.) of six genotypes were compared. Antioxidant activity (AOA) was measured using three different photometric methods – DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) and FRAP (ferric-reducing antioxidant power). Total phenolic content (TPC) was evaluated using Folin-Ciocalteu reagent assay.

Results. The results for AOA (μMol Trolox/g) determined by the DPPH method varied from 51.68 (DV-05) to 100.87 (DV-03), those obtained by the ABTS method varied from 65.40 (DV-05) to 142.26 (DV-03), and those obtained by the FRAP method varied from 45.06 (DV-05) to 109.30 (DV-03). The results for TPC varied from 590.75 ±27.98 mg/100 g (DV-05) to 1325.12 ±77.30 mg/100 g (DV-03). The highest results for AOA and TPC were achieved for the fruits of genotypes DV-03 and DV-05. A positive linear correlation was found between antioxidant activity and total phenolic content in the examined plant material.

Conclusion. The results showed that all fruit extracts exhibited strong antioxidant activities, which generally correlated positively with the total phenolic content. This study demonstrates the potential of the fruits of *Diospyros virginiana* grown in Ukraine as a possible source of valuable polyphenol content, with high antioxidant activities and health-promoting properties. The high contents of phenolic compounds and significant linear correlation between the values of the concentration of phenolic compounds and antioxidant activity indicated that these compounds contributed to the strong antioxidant activity.

Keywords: American persimmon, fruit, antioxidant activity, phenolic compounds

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INTRODUCTION

Only in recent years has the search for new sources of antioxidants and polyphenols in fruits of less popular orchard plants been widely reported: *Vaccinium corymbosum* L. (Ścibisz and Mitek, 2007), *Cornus mas* L., *Chaenomeles japonica* (Thunb.) Lindl. ex Spach (Nawirska-Olszańska et al., 2011), *Prunus spinosa* L. (Sikora et al., 2013), *Actinidia arguta* (Siebold & Zucc.) Planch. ex Miq., *Crataegus monogyna* Jacq., *Gaultheria procumbens* L., *Schisandra chinensis* (Turcz.) Baill. (Pliszka et al., 2016), *Morus nigra* L. (Kucelova et al., 2016), *Ziziphus jujuba* Mill. (Ivanišová et al., 2017).

American persimmons (*Diospyros virginiana* L.) a widespread fruit used traditionally by Native Americans as a source of food, and since ancient times have also been used in folk medicine (Briand, 2005; Foster and Duke, 1999; Hamel and Chiltoskey, 1975; Mallavadhani et al., 1998; Ross et al., 2014).

The fruits of the persimmon are an excellent dietary product. They are consumed fresh and pastes, jams, syrups, and marinades can be made from them. The fruits have also been used to make wine, brandy, white wine vinegar and beer (Bartram, 1772; Briand, 2005). Additionally, the by-products from processing persimmon fruits or leaves can be used in animal nutrition as a source of bioactive compounds (Gálik et al., 2016; Herkeľ et al., 2016) and thereby improve the performance of farm animals.

Leaves, fruits, calyx and seeds contain ($g \cdot kg^{-1}$) protein – 123.6, 33.2, 218.4, 128.1, and cellulose – 138.8, 37.9, 158.4, 182.0. Lipids – 9.10 $g \cdot kg^{-1}$, fructose – 9.56%, glucose – 10.67% (Grygorieva et al., 2009), ascorbic acid – 30.11–49.7 mg% (Grygorieva et al., 2012) have also been detected in the fruits, alongside 106 volatile compounds, of which 83 have been identified. These include alcohols, saturated and unsaturated aldehydes, ketones, fatty acids, esters and terpenoids (Grygorieva et al., 2017).

The leaves accumulate lupeol, betulin, betulinic acid (Shukla et al., 1989), catechins, leucoanthocyanins, anthocyanins, saponins and other compounds (Chetverikova et al., 1959). The leaves of *Diospyros virginiana*, by biochemical composition, have the highest content of ascorbic acid and mineral compounds compared to other species (Grygorieva et al., 2012; Richter, 2001). Some investigations reported the presence of the betulinaldehyde and ursulic acid in the roots of these plants (Wang et al., 2011).

The fruits demonstrate a positive effect on the central nervous system; the complex of biologically active compounds stimulates the activity of endocrine glands, promotes better iron absorption, improved hematopoiesis, prevents the formation of carcinogens and cholesterol stones in the gallbladder, influences the intracellular hepatic circulation of bile acids, and stimulates bactericidal action on colon bacillus (*Escherichia coli*), and aurococcus (*Staphylococcus aureus*) (Mallavadhani et al., 1998). The fruits and leaves have antifungal (Wang et al., 2011), antimicrobial (Charley et al., 1999; Isfahani et al., 2014) and antitumor (Shukla et al., 1989) effects. The bark has antiseptic (Briand, 2005), hepatoprotective and antipyretic (Priya and Nethaji, 2014; 2015) effects. Teas made from American persimmon leaves are a caffeine-free healthy alternative to black or green tea (Kobayashi et al., 2017).

The objective of this study was to evaluate the antioxidant activity and phenolic content of six *Diospyros virginiana* genotypes. The obtained data shows that the investigated plants have potent antioxidant activity, that can be used for the further investigation and utilization of *Diospyros virginiana*.

MATERIAL AND METHODS

Biological material

The fruits (Fig. 1) of 6 genotypes of *Diospyros virginiana* (DV-1–DV-6) collected in the M. M. Gryshko

Fig. 1. Fruits of *Diospyros virginiana* L.

National Botanical Garden of NAS of Ukraine (NBG) were the subject of these investigations. The raw materials were collected in the season of full ripeness (October).

Chemicals and spectral measurements

1,1-di-phenyl-2-picrylhydrazyl (DPPH) ferrous chloride, tripyridyltriazine (TPTZ), kaliumperoxodisulfat, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid; ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman--2-carboxylic acid (Trolox) and gallic acid were obtained from Sigma Chemical Co. (Sigma-Aldrich, Poland, Poznań). Methanol was obtained from POCh Poland. All chemicals and solvents were of analytical grade. All UV-V measurements were recorded on a Shimadzu UV-2401PC (Kyoto, Japan).

Preparation of extracts for analysis of total polyphenols and antioxidant activity

Around 2 g of fruits were homogenized and extracted with 80% aqueous methanol to a final volume of 20 mL at a room temperature. Extraction was performed in an ultrasonic bath (Polsonic, Poland) for 15 minutes. All extracts were filtered through pickling mixture paper filters (Whatman filter no. 1), and subjected to analyses.

Determination of total polyphenol content (TPC)

Total phenolic content (TPC) of the fruits was determined using the Folin-Ciocalteu reagent method according to Gao et al. (2000). Plant extracts (0.1 mL) were mixed with 0.2 mL of Folin-Ciocalteu reagent and 2 mL of H_2O, and, after 3 min, 1 mL 20% sodium carbonate. Total polyphenols were determined after 1 h of incubation at room temperature in a dark room. The absorbance of the resulting blue color was measured at 765 nm with a Shimadzu UV-VIS spectrophotometer. The standard cure was prepared using different concentrations of gallic acid. The results were calculated as mg of gallic acid equivalent (GAE/1 g). All determinations were performed in triplicate.

Determination of reducing power (FRAP) and radical scavenging activity

Ferric reducing antioxidant power (FRAP) was measured using Benzie and Strain (1996). An aliquot (1.0 ml) of the diluted extract was added to 3 ml of FRAP solution (acetate buffer (300 μM, pH 3.6), a solution of 10 μM TPTZ in 40 μM HCl, and 20 μM $FeCl_3$ at 10:1:1 (*v/v/v*) ratio). The mixture was shaken and left at room temperature for 10 min. The absorbance was read at 593 nm after 10 min using a Shimadzu UV2401PC spectrophotometer. The standard curve was prepared using different concentrations of Trolox. The results of the assay were expressed in μM Trolox per 1 g. All determinations were performed in triplicate.

Determination of DPPH radical scavenging activity

The DPPH free radical scavenging activity of the fruit extracts was measured from bleaching of the purple color of (2,2-diphenyl-1-picrylhydrazyl), as described by Yen and Chen (1995). Exactly 0.5 ml solution, at different concentrations of extract, was added to 2 ml of DPPH. The mixture was shaken and left at room temperature for 10 min. The absorbance was measured at 517 nm, using a Shimadzu UV2401PC spectrophotometer. The standard curve was prepared using different concentrations of Trolox. The results of the assay were expressed in μM Trolox per 1 g. All determinations were performed in triplicate.

Determination of ABTS radical scavenging activity

ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay was based on the method of Re et al. (1999). Briefly, ABTS radical cation was generated by reacting 7 mM ABTS and 2.45 mM potassium persulfate via incubation at room temperature (23°C) in the dark for 12−16 h. The ABTS solution was diluted to an absorbance of 0.700 ±0.040 at 734 nm. The reagent blank reading was taken (A_0). After the addition of 3.0 ml of diluted $ABTS^+$ solution to 30 μl of plant extract, the absorbance reading was taken exactly 6 min after initial mixing (A_t). The standard curve was prepared using different concentrations of Trolox. Results of antioxidant activity were expressed in μMol Trolox equivalents (TE/g). All determinations were performed in triplicate.

Statistical analysis

Analysis of variance was performed by ANOVA procedures. Statistical analysis was performed using

Statictica 8.0. Correlations between the data were obtained using a correlation coefficient (*r*).

RESULTS AND DISCUSSION

In the department of the fruit plants of the M. M. Gryshko National Botanical Garden of NAS of Ukraine, the gene fond the collection of American persimmon was collected, including 15 American cultivars (John Rick, Meader, weber, Prok, Yates, Szukis, supersweet, Pieper, NC 10, Hess, Geneva Long, Garretson, Evelyn, Dickie, Early Golden) and 4 Ukrainian cultivars. The different plants were assessed according to their ecological and biological properties and economic value. As result of analytical selection, the most promising forms with different characteristics (size of fruit, productivity, winter resistance, term of maturity, etc.) were chosen (Grygorieva et al., 2009; 2011; 2017).

Antioxidant activity of *Diospyros virginiana*

The scavenging of DPPH$^{\bullet}$ free radical is a common method to evaluate the antioxidant activity of antioxidants. DPPH$^{\bullet}$ is a relatively stable free organic radical due to the delocalization of the spare electron, which reduces its capacity to be absorbed by antioxidants. Thus, DPPH$^{\bullet}$ does not dimerize, as happens with most free radicals (Pisoschi and Negulescu, 2011). The DPPH radical scavenging activity of each *Diospyros virginiana* genotype extract is shown in Figure 2.

The antioxidant activity of *Diospyros virginiana* genotypes ranged from 51.68 (DV-05) to 100.87 (DV--03) µMol Trolox/g. The scavenging effect of extracts (by DPPH method) of the studied *Diospyros virginiana* genotypes decreased in the following order: DV-03 > DV-02 > DV-06 > DV-01 > DV-04 > DV-05. The best activity was detected in the sample of DV-03.

Another stable free radical cation, ABTS^{+}, was used to evaluate the antioxidant activity of the American persimmon extracts. The ABTS radical scavenging effect of extracts from *Diospyros virginiana* genotypes showed similar trends to that of the DPPH radical scavenging activity (Fig. 2).

As shown in Figure 2, the antioxidant activity of extracts of *Diospyros virginiana* genotypes measured by ABTS^{+} ranged from 65.40 (DV-05) to 142.26

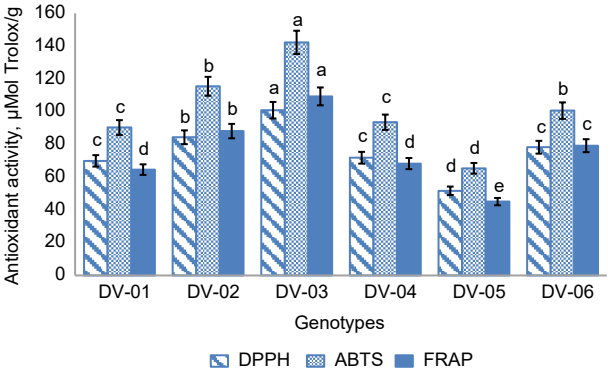

Fig. 2. Antioxidant activity of *Diospyros virginiana* L. extracts determined by different methods, µMol. Means in columns followed by different letters are different at $p = 0.05$. Each value represents the mean of three independent experiments (±SD)

(DV-03) µMol Trolox/g, and had the highest results compared with other methods.

These results indicate that the American persimmon extracts have different radical scavenging activity, depending on genotype. The scavenging capacity for ABTS radical by the extracts was relatively high compared to that for DPPH radical for all genotypes. In this case the scavenging effect of the extracts (by ABTS method) of the studied *Diospyros virginiana* genotypes decreased in the following order: DV-03 > DV-02 > DV-06 > DV-04 > DV-01 > DV-05.

The antioxidant activity of *Diospyros virginiana* genotypes evaluated by FRAP method (Fig. 2) ranged from 45.06 (DV-05) to 109.30 (DV-03) µMol Trolox/g. Ferric reducing antioxidant power assay uses antioxidants as reductants in a redox-linked colorimetric method, employing an easily reduced oxidant system. It should be noted that the results of antioxidant activity obtained in the first experiment (by DPPH method) was fairly similar to the FRAP method results. And in this case the scavenging effect of the extracts was in the same order as with the results obtained by DPPH method.

Our results compared favorably with previous studies on *Diospyros kaki* L. (Chen et al., 2008; Ercisli et al., 2008; Heras et al., 2017; Oksuz et al., 2015; Pu et al., 2013), and showed similar antioxidant activity. Chen et al. (2008) reported that the antioxidant

activity of *Diospyros kaki* was higher than grape, apple and tomato. Pu et al. (2013) investigated the antioxidant activity of *Diospyros kaki* cultivars by ABTS, DPPH and FRAP methods. Results ranged from 47.86 to 3,716.28, from 190.83 to 2,223.11 and from 90.10 to 957.74 μMol/100 g respectively. Oksuz et al. (2015) reported that antioxidant activity by ABTS and DPPH was 364.85 and 217.60 mg TEAC/100 g respectively.

Assessment of total polyphenol content

Polyphenols are secondary metabolites of plants and are generally involved in defense against ultraviolet radiation or aggression by pathogens. In food, polyphenols may contribute to bitterness, astringency, color, flavor, odor and oxidative stability. Fruits like grapes, apples, pears, cherries and berries contain up to 200–300 mg polyphenols per 100 grams fresh weight (Pandey and Rizvi, 2009). Polyphenols are of interest because of their potential use as prophylactic and therapeutic agents in the treatment of many diseases, and much work has been presented by the scientific community which focuses on their antioxidant effects. Plant polyphenols have been studied with the intention of finding compounds which protect against a number of diseases related to oxidative stress and free radical-induced damage, such as cardiovascular and neurodegenerative diseases, cancer, diabetes, autoimmune disorders and some inflammatory diseases (Vladimir-Knežević et al., 2012).

The results for TPC determined by the Folin-Ciocalteu method ranged from 590.75 mg/100 g (DV-05) to 1325.12 mg/100 g (DV-03) (Fig. 3). Total phenolic content of the studied genotypes of *Diospyros virginiana* fruit extracts expressed as mg GAE/100 g of matter decreased in following order: DV-03 > DV-02 > DV-06 > DV-04 > DV-01 > DV-5.

Our results are consistent with data from research which investigated the polyphenols of *Diospyros* spp. such as *Diospyros kaki* (Achiva et al., 1997; Denev and Yordanov, 2013; Ercisli et al., 2008; Oksuz et al., 2015; Toyoda et al., 2008), *Diospyros lotus* L. (Ayaz et al., 1997; Gao et al., 2014). Ercisli et al. (2008) reported that great variation of total phenolic content (from 15.7 to 42.3 mg GAE/g) was observed among genotypes *Diospyros kaki*. Also, according to this

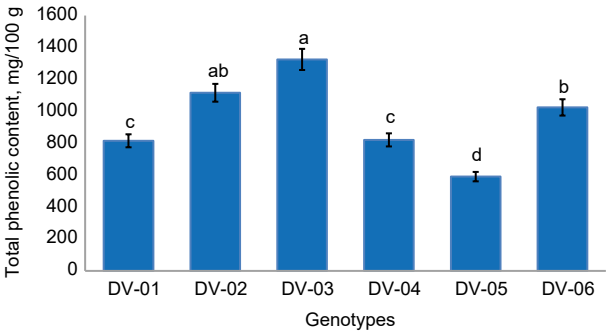

Fig. 3. Total phenolic content of genotypes of *Diospyros virginiana* L. fruit extracts based on gallic acid equivalents (GAE, Line; mg/100 g). Means in columns followed by different letters are different at $p = 0.05$. Each value represents the mean of three independent experiments (±SD)

report, the polyphenol content of *Diospyros lotus* acetone extract was 1.6, methanol extract – 3.3, water extract – 14.5 mg GAE/g (Gao et al., 2014). The obtained results regarding antioxidant activity (Fig. 2) showed a high correlation with the results for the total phenol content.

Correlation analysis was used to explore the relationships between individual phenolic compounds and antioxidant capacities (ABTS, DPPH, and FRAP), measured for all fruit extracts from six *Diospyros virginiana* genotypes (Fig. 4).

The findings of this study indicate that the phenolic content presents high positive correlations with ABTS scavenging capacity, DPPH and FRAP ($r = 0.982$, $p < 0.05$; $r = 0.985$, $p < 0.05$ and $r = 0.994$, $p < 0.05$, respectively). There were no significant differences between the different methods of antioxidant activity determination or total phenolic content ($P > 0.05$).

Ercisli et al. (2008) found there was a poor correlation ($r = 0.711$) between total phenolic content and antioxidant activity in the *Diospyros kaki* samples. Zou et al. (2017) identified a slightly stronger correlation ($r = 0.823$) between total phenolic content and antioxidant activity in the *Diospyros kaki* samples. Several studies have reported on the relationship between total polyphenol content and antioxidant activity due to the strong correlation between these two factors in fruits (Chen et al., 2008; Fu et al., 2010; Li et al., 2011; Pu et al., 2013; Velioglu et al., 1998).

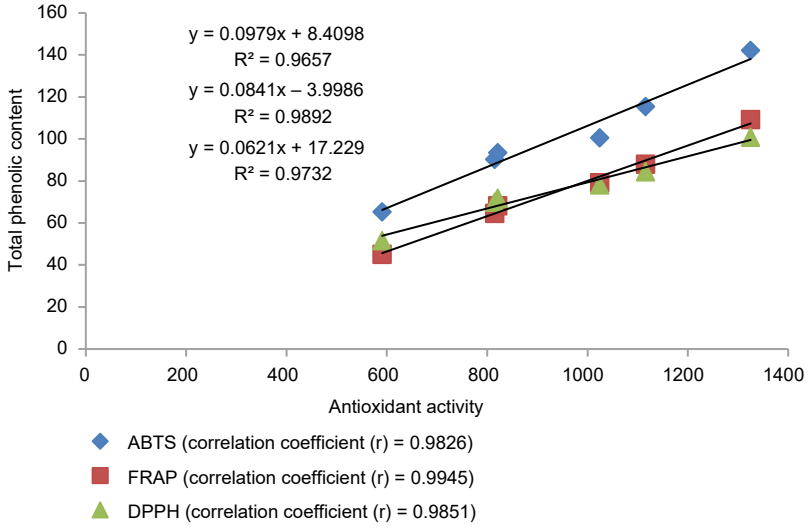

Fig. 4. Linear correlation between total phenolic content and antioxidant activity (TEAC)

CONCLUSION

The antioxidant activities and total phenolic contents of fruit extracts from six *Diospyros virginiana* genotypes were studied. The results showed that all fruit extracts exhibited strong antioxidant activities, which correlated positively with the total phenol contents. This study demonstrates the potential of fruits of *Diospyros virginiana*, grown in Ukraine, as possible source of valuable polyphenol content with high antioxidant activities and health-promoting properties. The obtained data showed that, of the three methods of determining antioxidant activity in extracts of *Diospyros virginiana* fruits, the strongest results were found by the ABTS method. The high contents of phenolic compounds and significant linear correlation between concentration of phenolic compounds and antioxidant activity indicated that these compounds contribute strongly to antioxidant activity.

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