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Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 14, 2020, p. 501-509
https://doi.org/10.5219/1314
Received: 8 February 2020. Accepted: 19 April 2020.
Available online: 28 July 2020 at www.potravinarstvo.com

ISSN 1337-0960 (online)

ESTIMATION OF PHENOLIC COMPOUNDS CONTENT AND ANTIOXIDANT ACTIVITY OF LEAVES EXTRACTS OF SOME SELECTED NON-TRADITIONAL PLANTS

Olga Grygorieva, Olena Vergun, Svitlana Klymenko, Mykhailo Zhurba, Vladimíra Horčinová Sedláčková, Eva Ivanišova, Ján Brindza

ABSTRACT

The aim of the research is the determination of the total antioxidant activity and the content of phenolic compounds of the leaves of 12 species of non-traditional plants, namely, *Amelanchier alnifolia* (Nutt.) Nutt. ex M. Roem., *Aronia mitschurinii* A.K. Skvortsov & Maitul., *Castanea sativa* Mill., *Chaenomeles japonica* (Thunb.) Lindl., *Cornus mas* L., *Diospyros kaki* L., *Diospyros lotus* L., *Diospyros virginiana* L., *Lycium barbarum* L., *Lycium chinense* Mill., *Pseudocydonia sinensis* (Thouin) C.K. Schneid., *Ziziphus jujuba* Mill. Total phenolic content was evaluated using the Folin-Ciocalteu reagent assay. Antioxidant activity was measured using two different methods (DPPH – 2,2-diphenyl-1-picrylhydrazyl, MRAP – molybdenum reducing antioxidant power). Significant variability was observed in phenolic compounds content and total antioxidant activity. Total polyphenol content ranged from 38.02 (*Z. jujuba*) to 80.58 (*C. sativa*) mg GAE.g^{-1} DM, total flavonoid content from 22.47 (*P. sinensis*) to 54.61 (*L. barbarum*) mg QE.g^{-1} DM and phenolic acids content from 3.51 (*A. mitschurinii*) to 24.67 (*Ch. japonica*) mg CAE.g^{-1} DM. All tested samples exhibited DPPH• radical scavenging activities with values from 6.92 (*A. mitschurinii*) to 9.0 (*C. mas*) mg TEAC.g^{-1} DM. Antioxidant activity by molybdenum reducing antioxidant power method ranged from 109.43 (*A. mitschurinii*) to 322.95 (*C. mas*) mg TEAC.g^{-1} DM. Differences between the species of non-traditional plants were significant in all observed parameters. Obtained results of phytochemical composition demonstrated the possibility of leaves' use of non-traditional plants as sources of valuable bioactive compounds with health-promoting and disease-preventing properties.

Keywords: non-traditional plants; leaves; phenolic compounds; antioxidant activity

INTRODUCTION

Fruits constitute is a large group of functional food, whose consumption delivers several health benefits, but very limited studies about the leaves of plants especially lesser-known and non-traditional plants, among which fruit plants. Biological activity in plants present in leaves has attracted much attention to their beneficial health effects **(Yildirim, Oktay and Bülaloúlu, 2001; Olszewska, 2011; Amjad and Shafighi, 2012; Nam, Jang and Rhee, 2017; Yılmaz and Seyhan, 2017; Bhatt et al., 2018)**. Leaves of non-traditional plants are one of the promising sources of antioxidants **(Ipatova et al., 2003; Calliste et al., 2005; Sakanaka, Tachibana and Okada, 2005; Priya and Nethaji, 2015; Ferlemi and Lamari, 2016; Klymenko, Grygorieva and Brindza, 2017; Urbanaviciute et al., 2019)**. They can use in the tea production and may have potential health benefits as a therapeutic aid in many illnesses which could be attributed to their antifungal, anti-inflammatory, antimicrobial and antioxidant activities. For example, tea made up from leaves of the plant *Camellia sinensis* (L.) Kuntze, is the second most consumed beverage in the world **(Costa, Gouveia and Nobrega, 2002; Rietveld and Wiseman, 2003)**.

The leaves *Ziziphus jujuba* have been used for herbal tea as a folk medicine for hemorrhaging, diarrhea **(Mahajan and Chobda, 2009)** and also been used to improve sleep, nourish the heart and soothe the nerves **(Zhang et al., 2014)**. The effects of *Diospyros virginiana* leaf and bark were comparable to that of standard drug, Silymarin. The ethanolic extract *Diospyros virginiana* leaf and bark is not only an effective hepatoprotective agent but also possesses significant antioxidant activity **(Priya and Nethaji, 2015)**.

The leaves of *Diospyros kaki* are most widely used in countries in eastern Asia, including China, Korea, and Japan **(Ahn et al., 2017)**. They contain abundant bioactive chemicals, such as flavonoids, polyphenols, organic acids, and vitamins, which could contribute to their pharmacological characteristics, such as their potent radical-scavenging and antioxidant properties **(Kim et al., 2006; Lee et al., 2006)**. The leaves of *Diospyros kaki* show medicinal effects against hemostasis, diuresis, constipation, and hypertension, have beneficial effects on

eye diseases in humans **(Ryu et al., 2015; Xie et al., 2015; Ahn et al., 2017)**.

Aronia melanocarpa leaf extract effectively reduced lipid and protein peroxidation in brain homogenates obtained from rats subjected to immobilization-induced oxidative stress **(Cuvorova et al., 2005)**.

Scientific hypothesis

The results of this study will provide new knowledge and useful information about the content of phenolic compounds in some selected non-traditional plants leaves and the antioxidant activity of their extracts, which will give a wide range of possibilities to employing these plants as the sources of phenolic compounds.

MATERIAL AND METHODOLOGY

Selection of plants

Objects of this study were leaves of 12 species of non-traditional plants, namely, *Amelanchier alnifolia* (Nutt.) Nutt. ex M. Roem., *Aronia mitschurinii* A.K. Skvortsov & Maitul., *Castanea sativa* Mill., *Chaenomeles japonica* (Thunb.) Lindl., *Cornus mas* L., *Diospyros kaki* L., *Diospyros lotus* L., *Diospyros virginiana* L., *Lycium barbarum* L., *Lycium chinense* Mill., *Pseudocydonia sinensis* (Thouin) C.K. Schneid., *Ziziphus jujuba* Mill. (Figure 1). The raw materials were collected in the 2018 August on the experimental collection of the Institute of Biological Conservation and Biosafety, the Slovak University of Agricultural in Nitra.

The collected samples were packed in cotton bags and air-dried for several days. The air-dried samples were finely powdered using laboratory blender and kept in zip-locked bags until further analysis. Duplicate specimens were collected for the herbarium preparation.

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

The dry non-traditional plants leaves were used for the detection of total phenolic content and total flavonoid content. An amount of 0.25 g of each sample was extracted with 20 mL of 80% ethanol for 24 h. Then, the sample in 80% ethanol was centrifuged at 4605 RCF (Rotofix 32 A, Hettich, Germany) for 10 min and the supernatant was used for measurement with the DPPH and molybdenum reducing antioxidant power methods.

Total polyphenol, flavonoid, and phenolic acid content

The total polyphenol content (TPC) was measured by the method of **Singleton and Rossi (1965)** using the Folin-Ciocalteu reagent. A quantity of 0.1 mL of each sample was mixed with 0.1 mL of the 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 the spectrophotometer Jenway (6405 UV/Vis, England). Gallic acid (25 – 300 $mg.L^{-1}$; R^2 = 0.998) was used as the standard. The results were expressed in $mg.g^{-1}$ DM gallic acid equivalent.

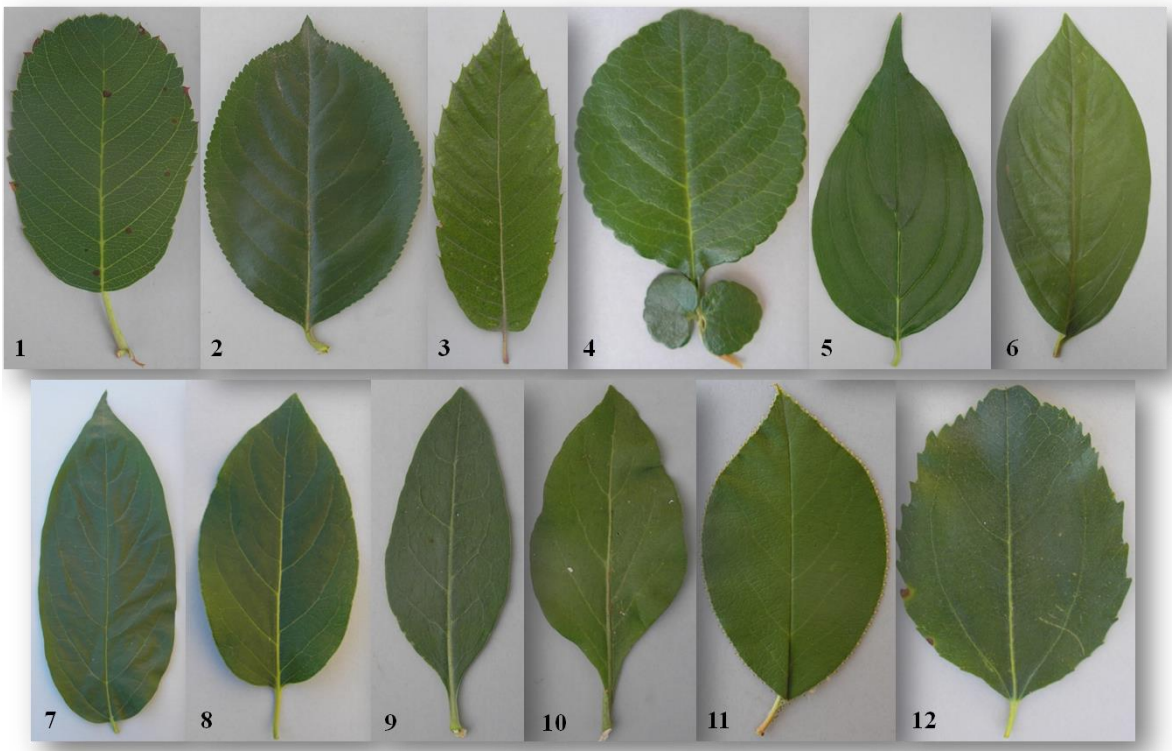

Figure 1 Leaves of non-traditional plants. Note: 1 – *Amelanchier alnifolia* (Nutt.) Nutt. ex M. Roem.; 2 – *Aronia mitschurinii* A.K. Skvortsov & Maitul.; 3 – *Castanea sativa* Mill.; 4 – *Chaenomeles japonica* (Thunb.) Lindl.; 5 – *Cornus mas* L.; 6 – *Diospyros kaki* L.; 7 – *Diospyros lotus* L.; 8 – *Diospyros virginiana* L.; 9 – *Lycium barbarum* L.; 10 – *Lycium chinense* Mill.; 11 – *Pseudocydonia sinensis* (Thouin) C.K. Schneid.; 12 – *Ziziphus jujuba* Mill.

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 0.1mL of 10% (w/v) ethanolic solution of aluminum chloride, 0.1 mL of 1 M potassium acetate and 4.3 mL of distilled water. After 30 min in darkness, the absorbance at 415 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Quercetin (1 – 400 mg/L; R2 = 0.9977) was used as the standard. The results were expressed in $mg.g^{-1}$ DM quercetin equivalent.

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

Determination of antioxidant activity

Free radical scavenging activity

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

Molybdenum reducing antioxidant power

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

Statistical analysis

Basic statistical analyses were performed using PAST 2.17. Data were analyzed with ANOVA test and differences between means compared through the Tukey-Kramer test (α = 0.05). The variability of all these parameters was evaluated using descriptive statistics. Correlation coefficients were calculated by CORR analysis.

RESULTS AND DISCUSSION

The main classes of natural antioxidant compounds such as flavonoids and phenolic acids often show a high correlation with antioxidant activity and have been identified in following non-traditional plants species: *Aronia melanocarpa* **(Tolić et al., 2015)**, *Cydonia oblonga* **(Bystrická et al., 2017)**, *Ziziphus jujuba* **(Ivanišová et al., 2017)**, *Diospyros virginiana* **(Grygorieva et al., 2018)**, *Sambucus nigra* **(Horčinová Sedláčková et al., 2018)**, *Asimina triloba* **(Brindza et al., 2019)**, *Cornus mas* **(Klymenko et al., 2019b)**, *Chaenomeles japonica* **(Klymenko et al., 2019a)** and other.

Total phenolic acid (TPA), flavonoids (TFC) and polyphenols (TPC) contents

Phenolic compounds have been proven to have a particularly strong antioxidant effect **(Scalbert et al., 2005; Pandey and Rizvi, 2009)**, which is closely related to the anti-inflammatory **(Pastore et al., 2009)**, strong antimicrobial **(Cushnie and Lamb, 2005)**, antiviral **(Chávez et al., 2006)**, and anticancer **(Kandaswami et al., 2005)** effect. Based on a large amount of scientific data proving the beneficial effect of phenolic compounds in humans, it is appropriate to perform estimation of these compounds content of leaves extracts of some selected non-traditional plants.

The amount of total phenolic acid content varied with the plant species (Figure 2). Total phenolic acids were ranged from 3.51 (*Aronia mitschurinii*) to 24.67 (*Chaenomeles japonica*) mg $CAE.g^{-1}$ DM.

Comparing the works of literature, **Barreira et al. (2010)** reported a total phenol content of 228.37 – 522.98 $mg.g^{-1}$ of *Castanea sativa*. According to **Lavola, Karjalainen and Julkunen-Tiitto (2012)**, total phenolic acid content for *Amelanchier alnifolia* cultivars was from 22.78 to 26.75 $mg.g^{-1}$ DW. In this case, chlorogenic acids had maximal values (17.55 – 20.16 $mg.g^{-1}$). Also, this study showed that that among investigated organs of *A. alnifolia* cultivars leaves had the most content phenolic acids as well as other phenolic compounds. In our study leaves, extracts of this species showed less content of phenolic acids. The total phenolic contents of water extracts of *Cornus mas* was 341.09 $mg.g^{-1}$ **(Stankovic et al., 2014)**. Methanol extracts of *Ziziphus jujuba* had total phenolic content 68.10 $mg.g^{-1}$ **(Al-Saeedi et al., 2016)**. The total amount of phenolic compounds in the methanol extracts of *Chaenomeles japonica* leaves varied from 12.94 to 64.79 mg $GAE.100g^{-1}$ **(Urbanaviciute et al., 2019)**. The total phenolic contents have been investigated in other plant leaf extracts, including *Mangifera indica* L. (65 $mg.g^{-1}$), *Anacardium occidentale* L. (58.57 $mg.g^{-1}$), *Cymbopogon citratus* (DC.) Stapf (28.30 $mg.g^{-1}$), *Carica papaya* L. (21.80 $mg.g^{-1}$) **(Iyawe and Azih, 2011)**, *Euphorbia* spp. (19.10 – 20.30 $mg.g^{-1}$) **(Gapuz and Besagas, 2018)**, and *Azadirachta indica* A. Juss. (14.43 $mg.g^{-1}$) **(Iyawe and Azih, 2011)**.

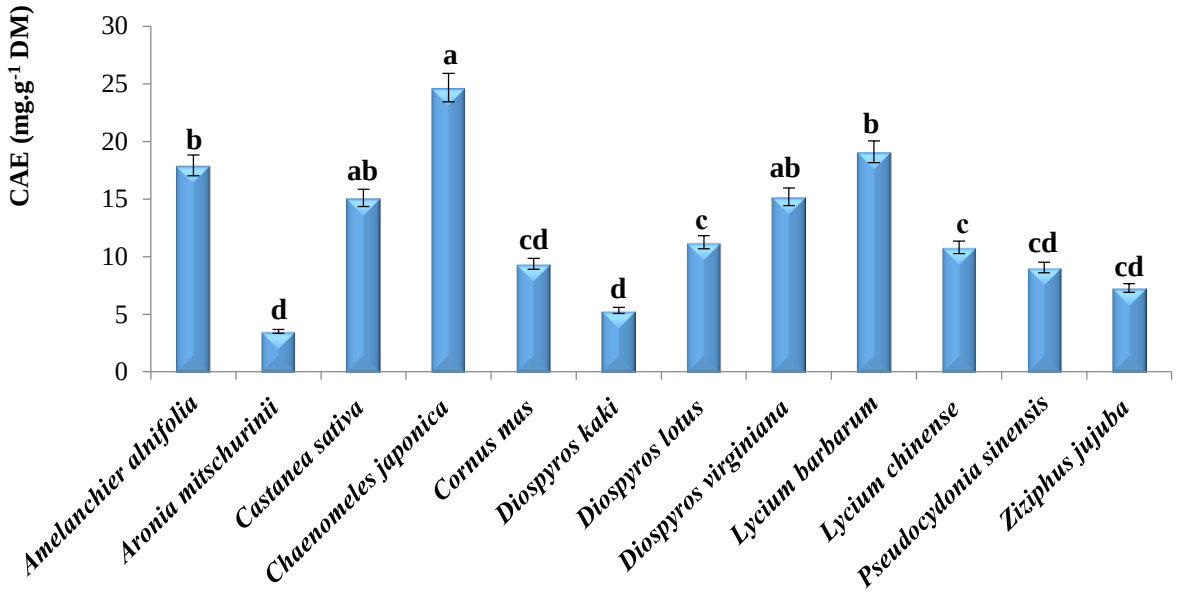

Figure 2 Total phenolic acid content in leaves of non-traditional plants.
Note: different superscripts in each column indicate the significant differences in the mean at p <0.05; CAE – caffeic acid equivalent.

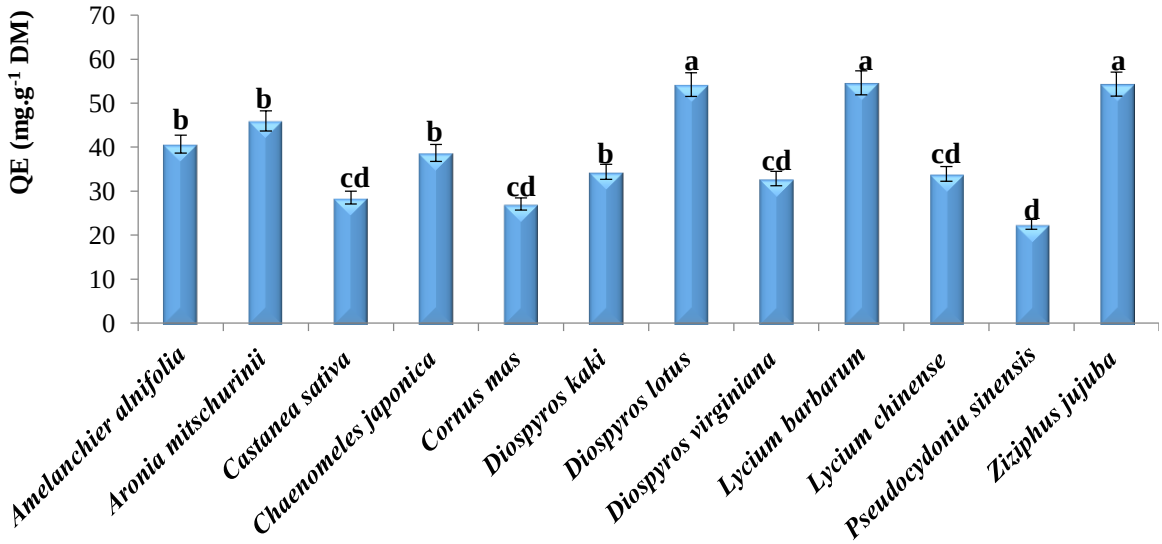

Figure 3 Total flavonoid content in leaves of non-traditional plants.
Note: different superscripts in each column indicate the significant differences in the mean at p <0.05); QE – quercetin equivalent.

The biological effects of many plant species depend on flavonoids; therefore, studies on the variation in their content are important and relevant **(Liaudanskas et al., 2014)**.

In the present work, *Lycium barbarum*, *Ziziphus jujuba* and *Diospyros lotus* leaf extract showed the highest amount of flavonoids (54, 61, 54.35 and 54.21 mg QE.g^{-1} DM, respectively) (Figure 3). The least value of flavonoids determined in extracts of *Pseudocydonia sinensis* (22.47 mg QE.g^{-1} DM).

According to **Barreira et al. (2010)**, total flavonoid contents *Castanea sativa* 73.31-90.39 mg.g^{-1}. Water extracts of *Cornus mas* leaves had total flavonoid contents 22.18 mg.g^{-1} **(Stankovic et al., 2014)**. *Aronia mitschurinii* leaves extracts in another study showed the content of flavonoids 103.6 – 163.7 mg CE.g^{-1} DW **(Thi and Hwang, 2014)**. Also, **Shahin et al. (2019)** found that the total content of flavonoids in dried leaves of *A. melanocarpa* was 96.16 mg.mL^{-1}. It should be noted that most studies about antioxidant parameters of *Aronia* species concerning of berries. The value of this parameter for *Ziziphus jujuba* methanol extracts was 90.28 mg.g^{-1} **(Al-Saeedi et al., 2016)** but ethanol extract of this species in our study was less. A study by **Aryal et al. (2019)** showed the flavonoid content in methanol leaves extracts eight selected wild vegetables from Nepal ranged from 37.86 to 66.1 mg QE.g^{-1}.

Natural plant antioxidants include main classes such as phenolic compounds, vitamins, carotenoids, etc. Phenolic compounds are a large group of antioxidants that can have structures from simple molecules to polyphenols (like flavonoids). Moreover, polyphenol compounds

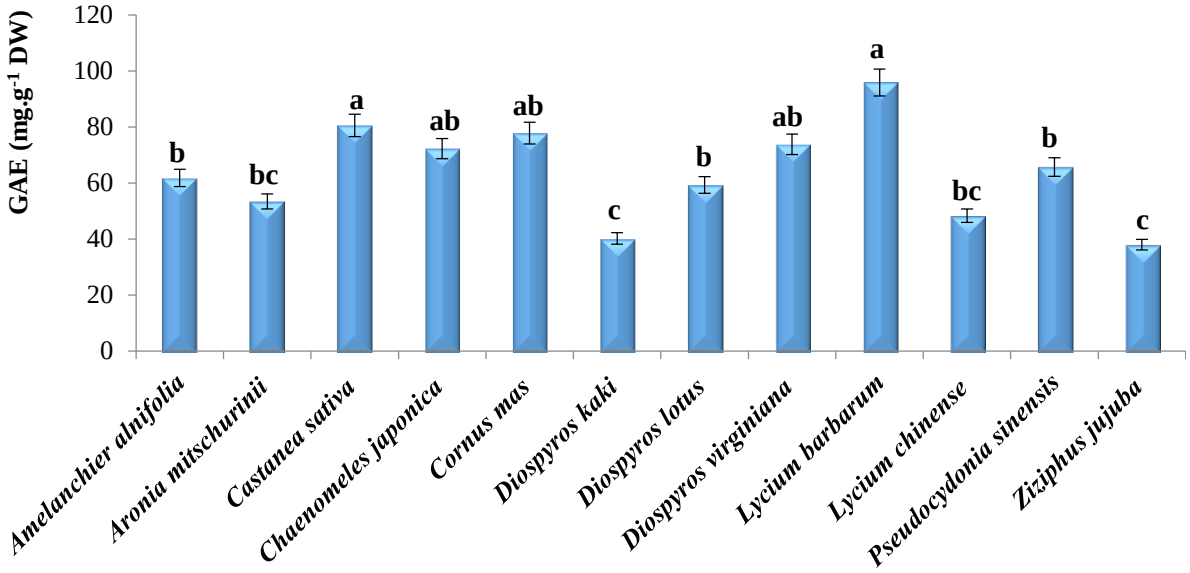

Figure 4 Total polyphenol content in leaves of non-traditional plants.
Note: different superscripts in each column indicate the significant differences in the mean at p <0.05; GAE – gallic acid equivalent.

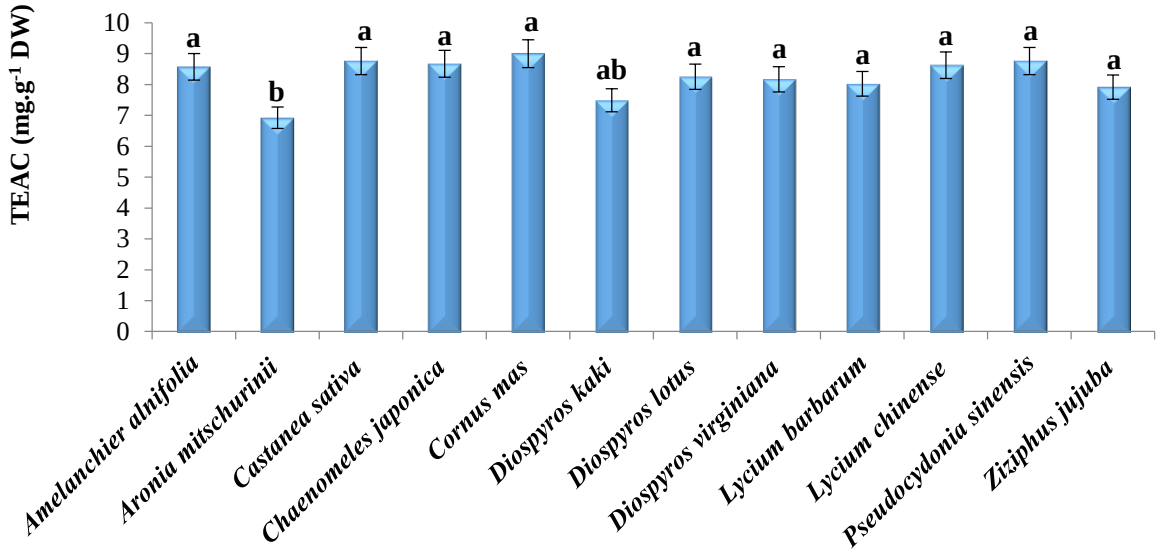

Figure 5 Antioxidant activity in leaves of non-traditional plants evaluated by the DPPH method.
Note: different superscripts in each column indicate the significant differences in the mean at p <0.05; TEAC – Trolox equivalent antioxidant capacity.

characterized by numerous activities that can be useful in human life such as antimicrobial, antifungal, anti-inflammatory, etc. **(Lourenço, Moldão-Martins and Alves, 2019)**.

The concentrations of total polyphenols content in non-traditional plant leaves are presented in Figure 4.

The highest total polyphenol content was obtained from *Lycium barbarum* and *Castanea sativa* (95.84 and 80.58 mg GAE.g^{-1} DW, respectively). In contrast, the lowest polyphenol content (40.24 and 38.02 mg GAE.g^{-1} DW, respectively) was obtained from *Diospyros kaki* and *Ziziphus jujuba* leaves.

Thi and Hwang (2014) found that *Aronia mitschurinii* total polyphenol content varied from 139.3 to 250.8 mg GAE.g^{-1} DW showed higher results than in the present study. The result of another study represented by **Shahin et al. (2019)** related to *Aronia melanocarpa* demonstrated that the total polyphenol content of dried leaves was 765.63 mg GAE.g^{-1}. As reported **Męczarska et al. (2017)**, leaves of *Amelanchier alnifolia* demonstrated the total content of polyphenols 185.23 mg GAE.g^{-1} DW that was 3 times less comparing with our result.

The antioxidative ability of leaves extracts

After studying the total phenolic compounds content it is important to examine and assess the antioxidant activity in the extracts of non-traditional plant species leaves. The results obtained during studies will be useful for the assessment and standardization of the quality of plant raw materials and will allow predicting an antioxidant effect of leaves non-traditional plants species.

The DPPH radical scavenging activities of non-traditional plant leaves at different stages of growth are shown in Figure 5. All tested samples exhibited DPPH•

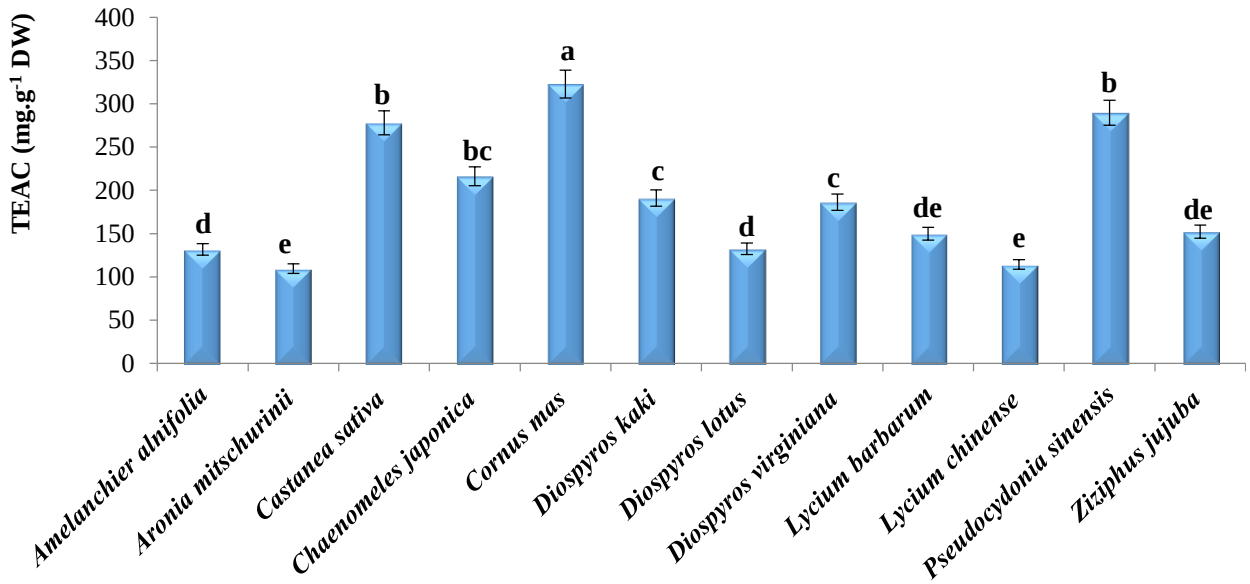

Figure 6 Antioxidant activity in leaves of non-traditional plants evaluated by the molybdenum reducing antioxidant power (different superscripts in each column indicate the significant differences in the mean at p <0.05); TEAC – Trolox equivalent antioxidant capacity.

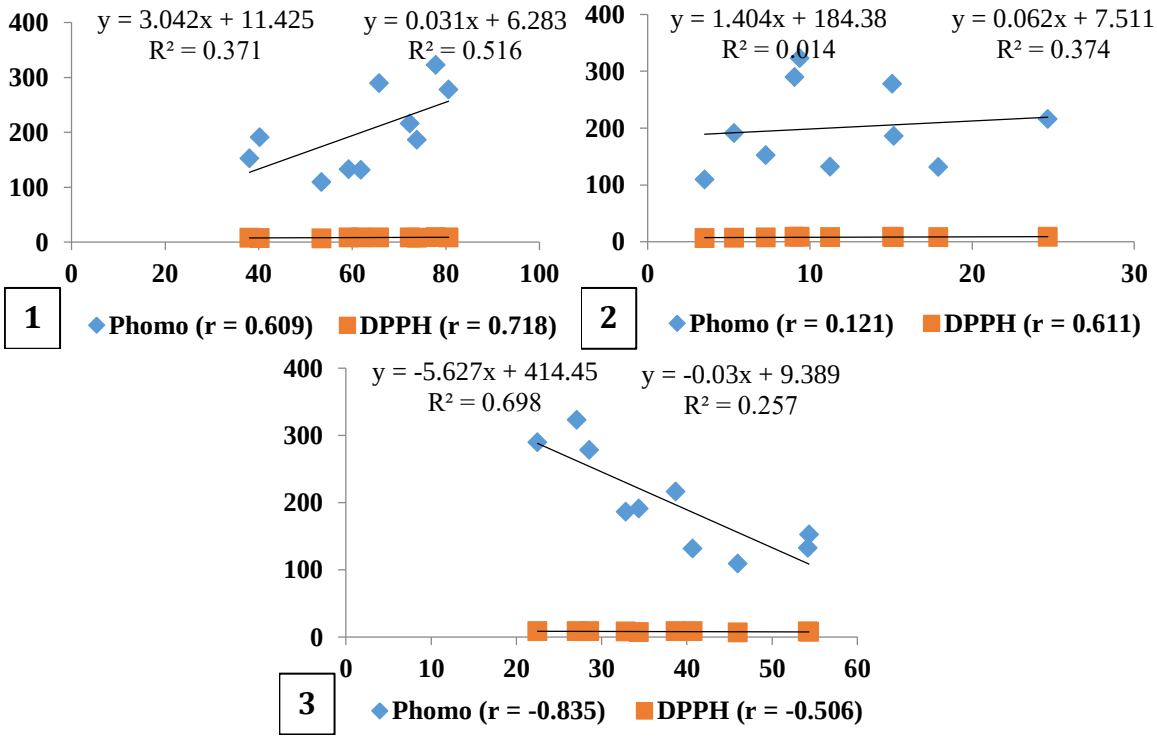

Figure 7 Correlation between antioxidant activity and TPC (1), TPA (2), and TFC (3) of non-traditional plants leaves extract.

radical scavenging activities with values from 6.92 (*Aronia mitschurinii*) to 9.0 (*Cornus mas*) mg TEAC.g^{-1} DW.

Serteser et al. (2009) reported that the methanolic extracts of the *Cornus mas* fruits showed EC50 (mg/ml) (DPPH reduction) values as 0.71.

The DPPH radical scavenging activity of the distilled water and 80% ethanol extracts of samples *Aronia mitschurinii* increased in a concentration-dependent manner (12.5~100 µg.mL^{-1}) **(Thi and Hwang, 2014)**. *Ziziphus spina-christi* (L.) Desf. ethanolic extracts exhibited good radical scavenging activity, with IC50 (the extract concentration providing 50% of inhibition) values of 54.3 µg.mL^{-1}, respectively (Khaleel et al., 2016). In the leaves of wild-growing plants of Nepal, the DPPH radical scavenging potency with a minimum IC50 value was recorded from 9.89 to 45.68 mg.mL^{-1} **(Aryal et al., 2019)**.

Antioxidant activity by molybdenum reducing antioxidant power method ranged from 109.43 (*Aronia mitschurinii*) to 322.95 (*Cornus mas*) mg TEAC.g^{-1} DM (Figure 6).

Comparison analyses of obtained data with results of other reviews often difficult to use in a similar study because of using different methods of determination of

antioxidant parameters, especially it is concerning standards that used and units.

The Pearson correlation coefficients between antioxidant activities and TPC, TPA, and TFC were depicted in Figure 7.

The result revealed the existence of a positive and negative correlation between tested antioxidant assays. TPC was significantly correlated with DPPH and phosphomolybdate assays (r = 0.609 – 0.718, p <0.05). The considerable correlation was noted in TPA and DPPH assay (r = 0.611).

CONCLUSION

In conclusion, the results of this study will provide new knowledge and useful information about the content of phenolic compounds in some selected non-traditional plants leaves and the antioxidant activity of their extracts, which will give a wide range of possibilities to employing these plants as the source of phenolic compounds. The highest total amounts of polyphenols and flavonoids were determined in the *Lycium barbarum* leaves (95.84 mg GAE.g^{-1} DW and 54.61 mg QE.g^{-1} DW, respectively). The preliminary experiments examining the antioxidant activity of leaf extracts of some selected non-traditional plants by the DPPH and phosphomolybdenum assays have shown that these extracts possess a strong antioxidant activity, which positively correlated with the total polyphenols content (r = 0.609-0.718, p <0.05). The ethanol extracts obtained from the apple leaves of the *Cornus mas* showed the highest TE values: 9.0 mg.g^{-1} DW by the DPPH method, 322.95 mg.g^{-1} DW by the molybdenum reducing antioxidant power. The findings of this study support the fact that leaves of some selected non-traditional plants are promising sources of potent antioxidants that can confirm the potential of investigated plants as a raw material in medical practice as well as the development and production of dietary supplements and cosmetic preparations rich in biologically active compounds.

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Acknowledgments:

The publication was prepared with the active participation of researchers in International Network AgroBio*Net* within the project ITMS 25110320104 ″Innovation of Test Methods and Procedures for the Detection of Sources of Bioactive Substances for the Improvement of Health and Quality of Life″. The authors is grateful Polish National Commission for UNESCO, Bilateral Scholarship of the Ministry of Education, Science, Research and Sport (Slovak Republic) and Visegrad Fund. Experimental activities were realized in laboratories of Excellent Center for the Conservation and use of Agrobiodiversity at the Faculty of Agrobiology and Food Resources, and Research Centre Agrobiotech (Slovak Agricultural University in Nitra).

Contact address:

*Mgr. Olga Grygorieva, PhD., M. M. Gryshko National Botanical Garden of the NAS of Ukraine, Department of Fruit Plants Acclimatisation, Timiryazevska 1, 04014, Kyiv, Ukraine, Tel.: +380671988082,
E-mail: olgrygorieva@gmail.com
ORCID: https://orcid.org/0000-0003-1161-0018

Mgr. Olena Vergun, PhD., M. M. Gryshko National Botanical Garden of the NAS of Ukraine, Cultural Flora Department, Timiryazevska 1, 04014, Kyiv, Ukraine, Tel.: +380975398541,
E-mail: olenavergun8003@gmail.com
ORCID: https://orcid.org/0000-0003-2924-1580

Prof. Svitlana Klymenko, M. M. Gryshko National Botanical Garden of the NAS of Ukraine, Department of Fruit Plants Acclimatisation, Timiryazevska 1, 04014, Kyiv, Ukraine, Tel.: +380976732625,
E-mail: cornusklymenko@gmail.com
ORCID: https://orcid.org/0000-0002-9110-0466

Mgr. Mykhailo Zhurba, PhD., M. M. Gryshko National Botanical Garden of the NAS of Ukraine, Department of Fruit Plants Acclimatisation, Timiryazevska 1, 04014, Kyiv, Ukraine, Tel.: +380673676542,
E-mail: zhurbamikhail@gmail.com
ORCID: https://orcid.org/0000-0001-5318-3961

Ing. Vladimíra Horčinová Sedláčková, PhD., Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources, Institute of Biodiversity Conservation and Biosafety, Trieda Andreja Hlinku 2, 949 76 Nitra, Slovakia, Tel.: +421376414779,
E-mail: vladimira.sedlackova@uniag.sk
ORCID: https://orcid.org/0000-0002-5844-8938

Ing. Eva Ivanišová, PhD, Slovak University of Agricultural in Nitra, Faculty of Biotechnology and Food Resources, Department of Plant Storage and Processing, Trieda Andreja Hlinku 2, 949 76 Nitra, Slovakia, Tel: +421376414421,
E-mail: eva.ivanisova@uniag.sk
ORCID: https://orcid.org/0000-0001-5193-2957

doc. Ing. Ján Brindza, PhD, Slovak University of Agricultural in Nitra, Faculty of Agrobiology and Food Resources, Institute of Biological Conservation and Biosafety, Trieda Andreja Hlinku 2, 949 76 Nitra, Slovakia, Tel: +421376414787,
E-mail: Jan.Brindza@uniag.sk
ORCID: https://orcid.org/0000-0001-8388-8233

Corresponding author: *