



DETERMINATION OF ANTIOXIDANT CAPACITY AND POLYPHENOLS CONTENTS IN FRUITS OF GENOTYPES OF *CHAENOMELES JAPONICA* (THUNB.) LINDL.

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In traditional Chinese medicine, the fruits of various species of *Chaenomeles* Lindl. have been used for thousands of years in the treatment of rheumatoid arthritis, hepatitis, asthma, and the common cold and also have reported anti-inflammatory, antinociceptive, antimicrobial, antioxidant, immunoregulatory, antitumor, hepatoprotective, antiparkinsonian, and antioxidant activities. Fruits contain many bioactive components, including polyphenols, triterpenes, and organic acids. Cold-pressed *Chaenomeles japonica* (Thunb.) Lindl. seed oil is one of the richest sources of natural micro constituents such as fatty acids, carotenoids, squalene, polyphenols, phytosterols, tocopherols and other compounds with high biological activity. The aim of this work was to evaluate the biological activity of fruits of *Chaenomeles japonica* genotypes, as potential species for cultivation and use in Ukraine. The objects of the research were 13 genotypes of *Chaenomeles japonica* selection in M.M. Gryshko National Botanical Garden (Kyiv, Ukraine). The raw materials were collected in the season of full ripeness (October). 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, FRAP – ferric-reducing antioxidant power). Total polyphenol content (TPC) was evaluated using the Folin-Ciocalteu reagent assay. The results for AOA ($\mu\text{mol Trolox/g}$) determined by the DPPH method varied from 10.08 to 21.42, those obtained by the ABTS method varied from 31.13 to 70.87, and those obtained by the FRAP method varied from 14.97 to 42.51. The results for TPC varied from 162.46 to 408.09 mg/100 g. The results showed that all fruit extracts exhibited strong antioxidant activities, which generally correlated positively with the total phenol contents. Due to the content of biologically active substances, the fruits are valuable raw materials for various types of processing in the confectionery, distillery, pharmaceutical industries, production of natural low-calorie products for children, diet and treatment and prophylactic nutrition. This means that genotypes of *Chaenomeles japonica* may be used as a source of new health resources when improving the nutritional properties of the world's less traditional fruit species.

Keywords: *Chaenomeles japonica*, genotypes, fruits, antioxidant activity, phenols contents

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Introduction

The genus *Chaenomeles* Lindl. belongs to the Rosaceae Juss. family and comprises four species: *C. cathayensis* (Hemsl.) C.K.Schneid., *C. japonica* (Thunb.) Lindl., *C. speciosa* (Sweet) Nak. and *C. thibetica* T.T.Yu (Phipps et al., 1990). *Japanese quince* (*Chaenomeles japonica*), a shrub naturally occurs in central and southern Japan (Phipps et al., 1990). Compared to the other three species *Japanese quince* best suited to the climate of northern Europe, where it was introduced in 1869 (Weber, 1964).

In traditional Chinese medicine, the fruits of various species of *Chaenomeles* have been used for thousands of years in the treatment of rheumatoid arthritis, hepatitis, asthma, and the common cold (Zhang et al., 2010, 2014) and also have reported anti-inflammatory, antinociceptive, antimicrobial, antioxidant, immunoregulatory, antitumor, hepatoprotective, antiparkinsonian, and antioxidant activities (Xie et al., 2007; Zhao et al., 2008; Zhang et al., 2010; Zhang et al., 2014).

Fruits by contains many bioactive components, including polyphenols, triterpenes, and organic acids (Lesińska, 1987; Lesińska et al., 1988; Hellín et al., 2003; Ros et al., 2004; Strek et al., 2007; Hallmann et al., 2011; Du et al., 2013; Baranowska-Bosiacka et al., 2017; Klymenko et al., 2017; Watychowicz et al., 2017). Cold-pressed *Chaenomeles japonica* seed oil is one of the richest sources of natural micro constituents such as fatty acids, carotenoids, squalene, polyphenols, phytosterols, tocopherols and other compounds with high biological activity (Górnaś et al., 2014).

The aim of this work was to evaluate the biological activity of fruits of *Chaenomeles japonica* genotypes, as potential species for cultivation and use in Ukraine.

Materials and methodology

Biological material

The objects of the research were 13 genotypes (Ch-01 – Ch-13) of *Chaenomeles japonica* selection in M.M. Gryshko National Botanical Garden of NAS of Ukraine (Kyiv). The raw materials were collected in the season of full ripeness (October).

Chemicals and spectral measurements

1.1-diphenyl-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), 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

The amount of about 2 g of fruits was homogenized and extracted with 80% aqueous methanol to a final volume of 20 mL at room temperature. The extraction was performed in the ultrasonic

bath (Polsonic, Poland) for 15 minutes. All extracts were filtered through pickling mixture paper filters (Whatman filter No.1), and then they were subjected to analyses.

Determination of total polyphenol content (TPC)

Total phenolic contents of fruits were determined by 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 H₂O, and after 3 min 1 mL 20% sodium carbonate. Total polyphenols were determined after 1 h of incubation at room temperature in the dark. The absorbance of the resulting blue colour was measured at 765 nm with a Shimadzu UV-VIS spectrophotometer. The standard curve was prepared using different concentrations of gallic acid. The results were calculated as mg of gallic acid equivalent (GAE)/1 g. All the determinations were performed in triplicates.

Determination of Reducing power, (FRAP) 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 FeCl₃ 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 minutes 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 the determinations were performed in triplicates.

Determination of (DPPH) radical scavenging activity

The DPPH free radical scavenging activity of fruits extracts was measured from bleaching of the purple colour of (2,2-diphenyl-1-picryl hydrazyl) out as described by Yen and Chen (1995). Exactly 0.5 ml solution of different concentrations of extracts 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 the determinations were performed in triplicates.

Determination of ABTS radical scavenging activity

ABTS (2,2'-azino-bis (3-ethyl benzothiazoline-6-sulfonic acid) assay was based on the method of Re et al. (1999). Briefly, ABTS radical cation is 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 with an absorbance of 0.700 ± 0.040 at 734 nm. The reagent blank reading was taken (A₀). After the addition of 3.0 ml of diluted ABTS⁺ solution to 30 μl of plant extracts, the absorbance reading was taken exactly 6 min after the 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 the determinations were performed in triplicates.

Statistical analysis

Basic statistical analyses were performed using PAST 2.17. The variability of all these parameters was evaluated using descriptive statistics. Correlation coefficients were calculated by CORR analysis.

Results and discussion

Last years in the gardening of our country, as well as other countries, new fruit and berry plants till recently meeting only in nature, are entered. The non-traditional fruits plants differ by high maintenance of valuable biologically active substances and have an important economic value. Works on the introduction and selection of new for a culture in Ukraine species are conducted the M.M. Gryshko National Botanical Garden of Ukraine (NBG) in Kyiv. By basis for the creation of cultivars sewed rich genetic fund of fruits plants, collected in collections of NBG, which presented more than by 120 species and varieties of growing wild and 2000 cultivars of different species (Klymenko and Grygorieva, 2013; Klymenko et al., 2014, 2017). The Department of Fruit Plants Acclimatisation of NBG explores biochemical composition and biological activity different from species of non-traditional plants namely *Cornus mas* L. (Klimenko, 2004; Kucharska et al., 2015), *Chaenomeles* spp. (Klymenko and Mezhenkyj, 2013), *Pseudocodynia sinensis* Schneid. (Monka et al., 2014; Grygorieva et al., 2019), *Asimina triloba* (L.) Dunal (Klymenko and Grygorieva, 2016; Brindza et al., 2019), *Morus nigra* L. (Kucelova et al., 2016), *Ziziphus jujuba* Mill. (Ivanišová et al., 2017), *Castanea sativa* Mill. (Grygorieva et al., 2018e), *Diospyros virginiana* L. (Grygorieva et al., 2018c, d), *Sambucus nigra* L. (Horčinová Sedláčková et al., 2018), *Mespilus germanica* L. (Grygorieva et al., 2018b), *Elaeagnus multiflora* Thunb. (Grygorieva et al., 2018a), *Aronia michurinii* A. Skvorts. et Yu. Maitullina. (Vinogradova et al., 2018).

Chaenomeles spp. collection fund of NBG a great diversity (Figure 1). The collection of *Chaenomeles* in the department is represented by several species, in particular, *Ch. japonica* (Thunb.) Lindl., *Ch. speciosa* (Sweet) Nakai, *Ch. × superba* (Frahm) Rehder, *Ch. cathayensis* (Hemsl.) Rehd. and numerous hybrid varieties – fruit and decorative. It is a representative genetic bank of species for the reproduction and introduction of *Chaenomeles* as valuable fruits, medicinal and decorative plants in farmer and amateur plants. Selection work with *Chaenomeles* in the NBG has been conducted for more than 50 years, and four varieties of *Chaenomeles japonica* are incorporated in the Register of Plant Varieties of Ukraine. There are 17 more promising genotypes (Klymenko and Mezhenkyj, 2013).

Phenolic compounds can act as low molecular weight antioxidants, protect cells from the effect of oxidative stress developing in conditions of hypothermia. Having high reaction activity due to the presence in the structure of aromatic rings and free hydroxyl groups, they can easily engage in free-radical reactions and connect the active forms of oxygen and peroxy radicals produced in the cells under stress conditions. Polyphenols are secondary metabolites of plants and are generally involved in defense against ultraviolet radiative or aggression by pathogens. They are formed in all organs of plants from sugars and participate in the process of cellular respiration, transferring hydrogen from oxidative molecules. Phenolic compounds exhibit a strong effect on the growth of plants, inhibiting germination of seeds, growth of

stems and roots. They have strong anti-bacterial properties and provide plant immunity to fungal and especially to bacterial infection.



Figure 1 Fruits of different cultivars of *Chaenomeles japonica* (Thunb.) Lindl. selection of M.M. Gryshko National Botanical Garden (Kyiv, Ukraine)

The amount of total polyphenols contents varied with the genotypes (Figure 2). Total polyphenols contents were ranged from 162.46 ± 2.41 to 408.09 ± 8.67 mg/100 g.

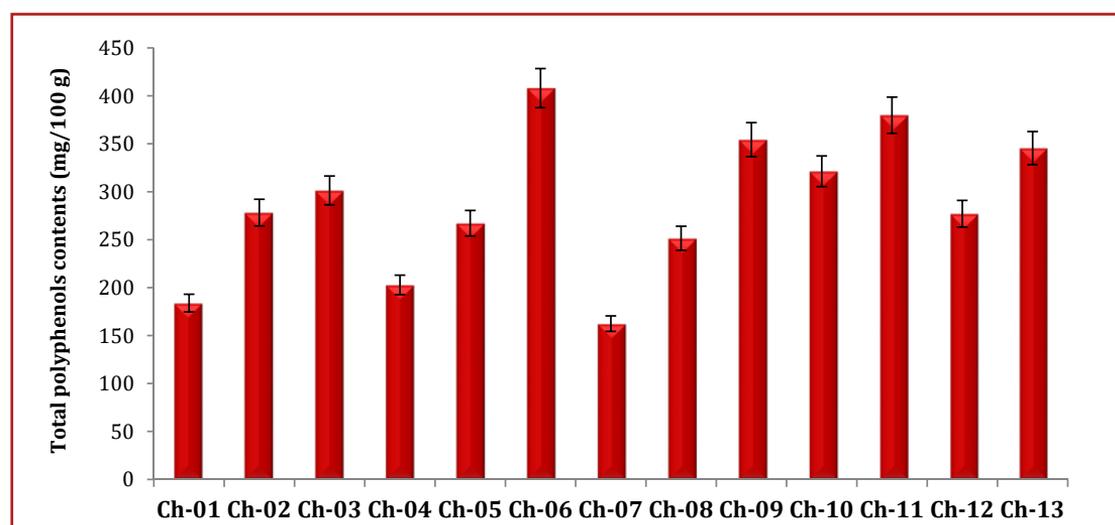


Figure 2 Total polyphenols contents of genotypes of *Chaenomeles japonica* (Thunb.) Lindl. fruits extracts based on gallic acid equivalents (GAE, Line) (mg/100 g)

Our results are consistent with data of researches investigated the polyphenols of *C. sinensis* and *C. speciosa* (Miao et al., 2016). Miao et al. (2016) report that great in *C. sinensis* total polyphenol content fruits from 345 to 590 mg GAE/g DW and in *C. speciosa* from 356 to 404 mg GAE/g DW. Du et al. (2013) in fresh weight identified total polyphenol content 19.35 ± 0.59 mg GAE/g FW.

Antioxidants are substances that can prevent or slow damage to cells caused by free radicals, unstable molecules that radicals, that the body produces as a reaction to environmental and other pressures. 'Antioxidant' is not really the name of a substance, but rather it describes what a rang of substances can do. Flavonoids, flavones, catechins, polyphenols, and phytoestrogens are all types of antioxidants and phytonutrients, and they are all found in plants – based foods. The best sources of antioxidants are plant – based foods, especially fruits and vegetables. Food that is particularly high in antioxidants are often referred to as a 'super food' or 'functional food'.

The DPPH radical scavenging activity of each *Chaenomeles japonica* genotypes extracts is shown in Figure 3. Antioxidant activity of fruits genotypes ranged from 10.08 (Ch-07) to 21.42 (Ch-06) $\mu\text{Mol Trolox/g}$.

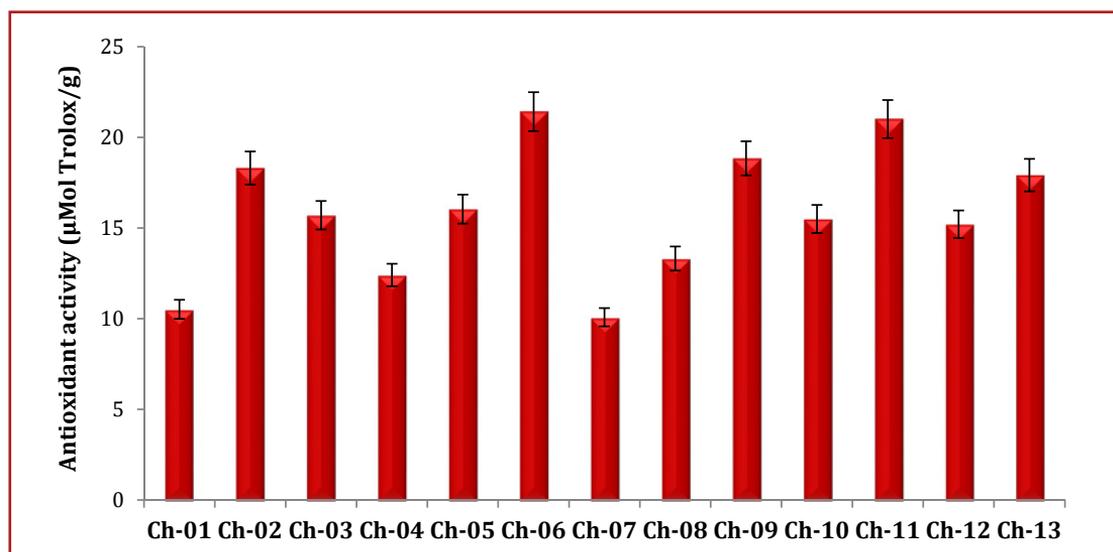


Figure 3 Antioxidant activity of *Chaenomeles japonica* (Thunb.) Lindl. extracts determined by DPPH method ($\mu\text{Mol Trolox/g}$)

Other stable free radical cation, ABTS^+ , was used to evaluate the antioxidant activity of the *Chaenomeles japonica* extracts.

As shown in Figure 4 antioxidant activity ranged from 31.13 (Ch-01) and 31.54 (Ch-07) to 70.29 (Ch-09) and 70.87 (Ch-06) $\mu\text{Mol Trolox/g}$ and had the most results comparing with other methods.

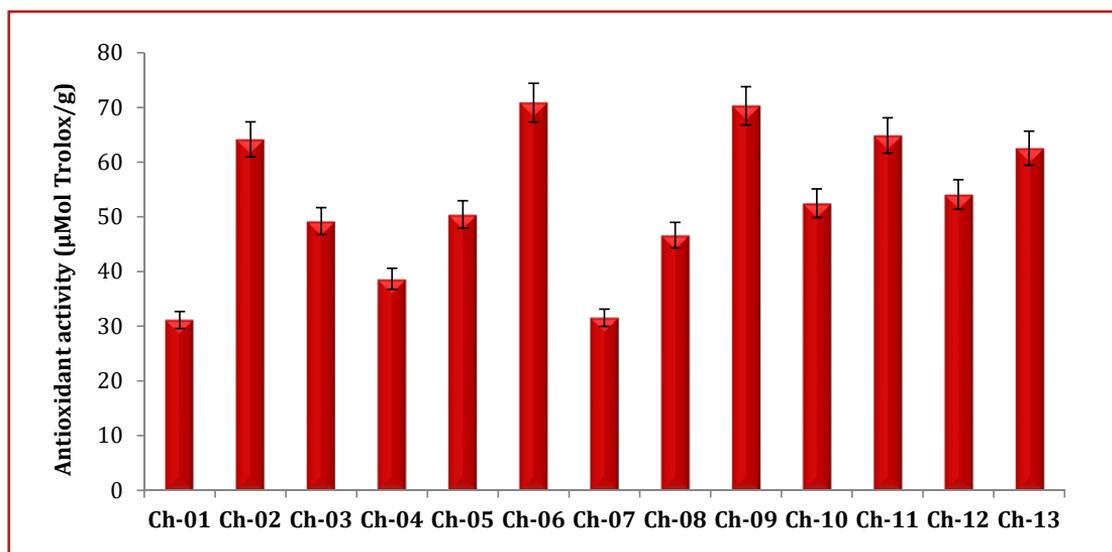


Figure 4 Antioxidant activity of *Chaenomeles japonica* (Thunb.) Lindl. extracts determined by ABTS method (μMol Trolox/g)

Antioxidant activity of *Chaenomeles japonica* genotypes evaluated by FRAP method (Figure 5) ranged from 14.97 (Ch-07) and 15.64 (Ch-01) to 40.22 (Ch-11) and 42.51 (Ch-06) μMol Trolox/g.

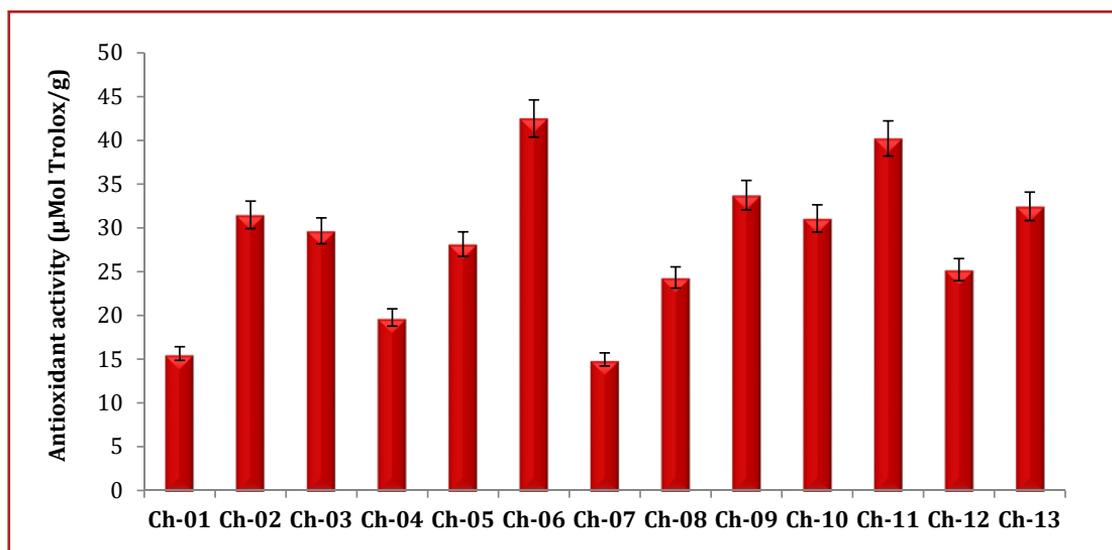


Figure 5 Antioxidant activity of *Chaenomeles japonica* (Thunb.) Lindl. extracts determined by FRAP method (μMol Trolox/g)

Our results compared favourably with previous studies on *C. japonica* (Nawirska et al., 2007; Tarko et al., 2010; Du et al., 2013), and showed equivalent antioxidant activity. Nawirska et al. (2007) investigated the antioxidant activity of *C. japonica* by ABTS, DPPH and FRAP methods.

Results were ranged 13.97 ± 0.06 , 18.21 ± 0.34 and 6.12 ± 0.01 $\mu\text{Mol Trolox/g DW}$, respectively. Du et al. (2013) investigated the antioxidant activity of *C. japonica* by ABTS (118.15 ± 4.10 $\mu\text{Mol Trolox/g FW}$) and FRAP (19.10 ± 0.76 $\mu\text{Mol Trolox/g FW}$) methods.

The Pearson correlation coefficients between antioxidant activities and TPC were depicted in Figure 6.

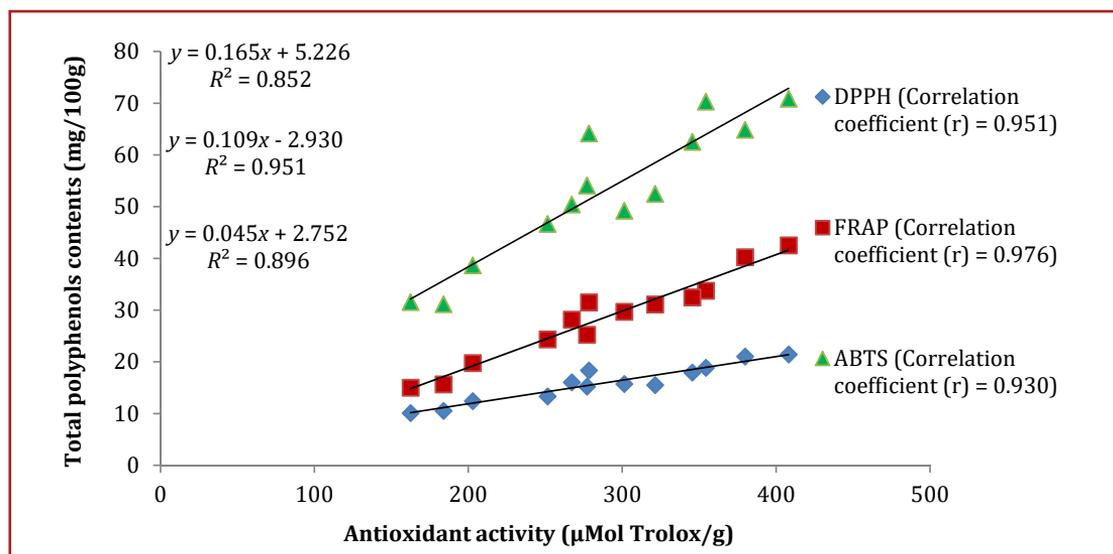


Figure 6 Correlation between antioxidant activity and TPC of *Chaenomeles japonica* (Thunb.) Lindl. fruits extract

The findings of this study indicate that total polyphenols present dramatically high and positive correlations with ABTS scavenging capacity, DPPH and FRAP ($r = 0.930$, $p < 0.05$; $r = 0.951$, $p < 0.05$ and $r = 0.976$, $p < 0.05$, respectively), which confirmed that fruits had strong antioxidant activities as previously reported (Rasmussen et al., 2005; Du et al., 2013).

Conclusions

This study demonstrates the potential of fruits *Chaenomeles japonica* in Ukraine, as a prospective source of valuable polyphenol contents with high antioxidant activities and health beneficial 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 contribute to the strong antioxidant activity. Due to the content of biologically active substances, the fruits are valuable raw materials for various types of processing in the confectionery, distillery, pharmaceutical industries, production of natural low-calorie products for children, diet and treatment and prophylactic nutrition. Our results point to the fact that observed plants of *Chaenomeles* are suitable natural sourced have high biological efficiency – antioxidant activity. This means that some *Chaenomeles* cultivars be used as a source of new health sources when improving the nutritional properties of the world's less traditional fruit species.

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