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ORIGINAL PAPER

QUINCE SEEDS AS A POTENTIAL SOURCE OF MINERAL AND BIOLOGICAL ACTIVE COMPOUNDS*

Anna Krzepiłko[1], Roman Prażak[2]

[1] Department of Biotechnology, Microbiology and Human Nutrition
[2] Institute of Plant Genetics, Breeding and Biotechnology
University of Life Sciences in Lublin, Poland

Abstract

Seeds of fruit shrubs are herbal material of relatively low popularity and a narrow range of applications. The aim of the study was to assess selected quality characteristics of the seeds of two species of flowering quince – *Chaenomeles japonica* cv. Nicoline and *C.* × *superba* cv. Fascinacion. Determination of the content of selected minerals showed that the seeds of *C. japonica* contained more potassium, iron, manganese and copper and less calcium than *C.* × *superba*. The antioxidant and antimicrobial properties of the seeds were tested in ethanol and ethanol-water extracts. For both species, the total phenolic content in the ethanol extract was statistically significantly higher than in the ethanol-water extract. This was not true of the content of antioxidants measured by the ABTS and DPPH methods. The total phenolic and antioxidant content in the seeds was much higher in *C.* × *superba* than in *C. japonica*. The highest phenolic content in the seeds was 51.49 mg GAE 100 g^{-1} DW for *C.* × *superba* and 8.63 mg GAE 100 g^{-1} DW for *C. japonica*. The total antioxidant content measured by the ABTS method in the seeds of *C. japonica* was 1.69 and 3.38 mmol TE 100 g^{-1} DW in the E and EW extracts, respectively, while in the seeds of *Ch.* × *superba* it was 18.22 and 18.78 mmol TE 100 g^{-1} DW. Measured by the DPPH method, it ranged from 1.76 to 11.34 mmol TE 100 g^{-1} DW, depending on the species. The antibacterial properties of the seeds, i.e. the susceptibility of bacterial strains to the seed extracts, were assessed by the disc diffusion method. The ability of extracts of *C.* × *superba* seeds to inhibit bacterial growth proved much greater than that of extracts of *C. japonica* seeds. In the case of both *Chaenomeles* species, the most susceptible bacteria were *Enterobacter aerogenes* and *Enterococcus faecalis*, whereas *Salmonella enterica*, *Serratia marcescens* and *Escherichia coli* were not susceptible to extracts of *C. japonica* seeds. Higher antibacterial activity against *E. aerogenes* and *E. faecalis* was observed for the ethanol extract, in which it surpassed the activity in ethanol-water extract. The study showed that the seeds of both *Chaenomeles* species contain valuable minerals and phytocompounds with antioxidant and antimicrobial properties.

Keywords: antibacterial activity, antioxidant capacity, *Chaenomeles* ssp., minerals, seed extracts, total phenolic content

Roman Prażak, PhD., DSc., Institute of Plant Genetics, Breeding and Biotechnology, University of Life Sciences in Lublin, St. Akademicka 15, 20-950 Lublin, Poland, e-mail: roman.prazak@up.lublin.pl, ORCID ID: 0000-0002-6920-1017

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INTRODUCTION

Shrubs of the genus *Chaenomeles* (quince), belonging to the family *Rosaceae* (subfamily *Maloideae*), are native to China, Japan and Korea. Quinces are easily crossed within the genus. *Chaenomeles japonica* often forms hybrids with *C. speciosa*, resulting in *C.* × *superba* Fran. Owing to their modest requirements, ease of interspecific crossing, and wide tolerance to climate conditions, cultivation of these shrubs has spread through Europe and North America (Seneta et al. 2021).

Quinces are planted mainly for the ornamental attributes of their beautifully flowering shrubs and the exceptional health-promoting properties of the fruit. In the food industry, the fruits of these shrubs are used to produce juice, liqueurs, syrups, preserves, and candied snacks. The quince fruits have been shown to possess various pharmacological properties, including antibacterial, anti-inflammatory, antiproliferative, antidiabetic, antihyperlipidaemic, antihyperglycaemic, immunomodulatory, and antioxidant activity (Zhang et al. 2014). The flowering quinces produce fruit rich in minerals, especially iron, molybdenum, magnesium, sodium, copper, zinc and phosphorus (Lesińska 1985). Fourteen phenolic acids have been identified in the pericarp and seeds of quince. The dominant compounds are caffeic acid, protocatechuic acid, gallic acid, p-hydroxybenzoic acid, p-coumaric acid, syringic acid, and vanillic acid (Sokołowska-Woźniak et al. 2002). Strong antioxidant properties resulting from the high content of phenolic acids, isoflavonoids, catechins, anthocyanidins, phytoalexins and tannins have been reported in extracts from seeds of soybeans, chickpeas, lentils, peas, beans, broad beans and faba bean (Troszyńska et al. 1997, Mazur et al. 1998). Cereal grains and seeds of coffee, cocoa, chestnut, peanuts, vines and peaches also contain high amounts of phenolic compounds (phenolic acids, flavones, isoflavones, anthocyanins, lignins, stilbenes), vitamin E, trace elements such as selenium, copper, zinc and manganese, which are components of antioxidant enzymes (Kähkönen et al. 1999, Fugetta 2004, Grajek 2004, Szajdek 2004, Glucin 2006, Dykes, Rooney 2007, Kumar 2014).

The quince seeds, which are usually a by-product of the process of obtaining pulp, contain unconventional proteins with a valuable amino acid composition. Quince seeds have been used as material for pressing oil, whose composition is similar to that of other vegetable oils (Radziejewska-Kubzdela, Górnaś 2020, Mišina et al. 2022). There is currently an increasing tendency to look for new, innovative means of managing waste from the fruit and vegetable industry, apart from their traditional use as compost or animal feed. Numerous studies confirm that apple, avocado, plum, and citrus seeds are rich in nutrients, bioactive compounds, and dietary fibre (Fidelis et al. 2019), and thus can be an effective source of extracts for the production of nutraceuticals and enriched food. Extracts of quince seeds might be used to reduce pathogens in food, which seems a promising solution in light of the growing tolerance of microbes to common preservatives as well as growing consumer awareness of their negative effects on human health (Kikowska et al. 2019).

In the present study, the content of selected minerals was determined in the seeds of *C. japonica* cv. Nicoline and *C.* × *superba* cv. Fascinacion, cultivated in Poland, and the content of biologically active substances – polyphenolic compounds and antioxidants – was determined in extracts from the seeds. Total phenolic compounds (TPC) as well as total antioxidant capacity (TAC) by the ABTS and DPPH methods were determined in the extracts. In addition, the antibacterial effect of the extracts to selected gram-negative and gram-positive bacteria was determined.

MATERIAL AND METHODS

Plant material

The seeds of two flowering quince species, *Chaenomeles japonica* (Thunb.) Lindl. ex Spach cv. Nicoline and *C.* × *superba* (Frafm) Rehder. cv. Fascinacion were the research material. The two quince species differing in the size of fruits and seeds (Figure 1) were grown in a field trial established in 2015 at the Experimental Station, University of Life Sciences in Lublin (51°13′59″ φN, 22°34′0″ λE, elevation: 225.48 m). Three plants of each species were grown on medium silty soil of loess origin, with the granular composition of clay dust, classified as good wheat complex. Basic agronomical procedures were performed during the growing season. Each year, fertilizer doses were based on soil analysis. In the research year

Fig. 1. Quince pericarps and seeds (from the left): *Chaenomeles* × *superba*, *Chaenomeles japonica*

of 2021, fertilization was as follows: N – 120 kg ha^{-1}, P – 60 kg ha^{-1}, K – 20 kg ha^{-1}.

Quince fruits were harvested at the end of October, during the full maturity stage. The bushes of these species reached a height of up to 1.0 m (*C.* × *japonica*) or 1.5 m (*C.* × *superba*), and the weight of fruit in the range of 30-45 g in the case of the *C. japonica* and 40-80 g in the case of the *C.* × *superba.* In the analysed fruits, number of *C.* × *japonica* seeds ranged from 42 (weighing 2.16 g) to 76 (weighing 3.65 g) and number of *C.* × *superba* seeds ranged from 26 (weighing 2.18 g) to 43 (weighing 3.26 g).

Ten fruits were taken from each quince bush and 15 seeds were obtained from each fruit (primary sample). Then, by pooling the primary samples, a laboratory sample was obtained (n=150 seeds).

The seeds were washed with distilled water, dried at 105°C to a constant weight, and grounded in the laboratory to obtain homogenous material.

Determination of mineral composition

The content of K, Mg, Ca, Mn, Fe, Zn, and Cu was determined in the ground seeds (after wet mineralization in extra pure HNO_3) by atomic adsorption spectroscopy according to PN-EN ISO 6869:2002. Elements were analysed in three laboratory samples prepared from seeds from the test plants. The results were expressed in mg per 100 g dry weight (DW) of seeds.

Preparation of extracts

Extracts of the seeds were obtained: ethanol (E), i.e. ethanol 96% (v/v), and ethanol-water (EW), i.e. ethanol 40% in water (v/v). Ground seeds were mixed with extraction solution in a 1:10 weight ratio and then homogenised for 5 min while the samples were cooled in an ice bath. The samples were mixed (24 h, 60 rpm) and then centrifuged for 5 min at 5,000 rpm. The supernatant was frozen and subjected to biochemical and microbiological analysis.

Determination of total phenolic content

The Folin-Ciocalteu spectrophotometric method is used to determine the total content of polyphenols by exploiting their ability to produce coloured products of a reaction with the components of Folin reagent (phosphomolybdic and phosphotungstic acid salts). The absorbance of the solutions is proportional to the total content of phenolic compounds in the sample. Absorbance was measured after 1 h at 765 nm (Singleton et al. 1999). The results were expressed in gallic acid equivalent (GAE) per 100 g dry weight (DW) of seeds.

Determination of total antioxidant capacity

Total antioxidant capacity (TAC) was determined in the extracts by the ABTS or DPPH method. The ABTS method involves spectrophotometric measurement of the colour reduction reaction between the $ABTS^{+}$ radical cation and the antioxidants in the sample. Coloured $ABTS^{+}$ radicals are generated from ABTS (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) during chemical reactions with $K_2S_2O_8$. $ABTS^{\cdot +}$ is soluble in both water and organic solvents, which makes it possible to determine the antioxidant capacity of hydrophilic and lipophilic compounds. When antioxidants reduce radical cations ($ABTS^{\cdot +}$), the solution loses colour, and the decrease in the intensity of the colour is proportional to the concentration of antioxidants. Spectrophotometric measurements were performed at 414 nm, 30 min after the reagents were mixed (Re et al. 1999).

The DPPH method uses the DPPH• (2,2-diphenyl-1-picrylhydrazil) radical, which takes on a dark violet colour in a solution. In the reaction between DPPH and an antioxidant, the radical is reduced, and the ethanol solution loses its violet colour. The decrease in absorbance is proportional to the antioxidant properties of the sample (Molyneux 2004). The absorbance of the samples was measured at 517 nm, 30 min after initiation of the reaction (Addai et al. 2013).

In both the ABTS and DPPH methods, TAC was expressed as trolox equivalent (TE) per 100 g dry weight of seeds (Re et al. 1999, Molyneux 2004).

Determination of the antimicrobial activity of the extracts

The antimicrobial activity of the extracts against selected gram-negative and gram-positive bacteria was tested by the disc-diffusion method in accordance with EUCAST methodology (CSLI 2012). Reference strains of bacteria were obtained from the Polish Microorganism Collection: gram-negative bacteria *Enterobacter aerogenes* PCM 1836, *Serratia marcescens* PCM 549, and *Salmonella enterica* Enteritidis PCM 930; gram-positive bacteria *Staphylococcus aureus* PCM 2267 and *Enterococcus faecalis* PCM 2786. The strains were stored in a deep freezer. The antimicrobial properties of the seed extracts were tested using 24 h cultures of bacteria in nutrient broth (Sigma-Aldrich, Germany), which were diluted in 0.9% NaCl to obtain a density of 0.5 McFarland. Standardized suspensions of bacteria were plated on Petri dishes with Mueller–Hinton agar. Next, sterile filter paper discs were placed on the media, and 10 µl of the antimicrobial agent was applied to each of them. Extracts from the seeds were previously filtered through a 0.45 µm membrane filter. Control samples were prepared with the solutions used to prepare the extracts – a 40% aqueous solution of ethanol or 96% ethanol (10 µL/disc), but bacterial inhibition was not observed in either case. The antibiotic kanamycin (30 µg/disc) was used as a positive control. In the disc-diffusion method, the size of the growth inhibition zone around the discs indicates the antimicrobial activity of the agent tested.

Statistical analysis

All tests were performed in a minimum of three replicates. The results were subjected to one-way analysis of variance. The average polyphenol content and antioxidant capacity obtained for the stored extracts are shown in Figures 1-3. The significance of differences was determined using the Tukey test. Results were considered statistically significant at α=0.05. Values marked with the same letters indicate a lack of statistically significant differences between groups. The correlation between variables was examined using the Pearson (r) linear correlation coefficient.

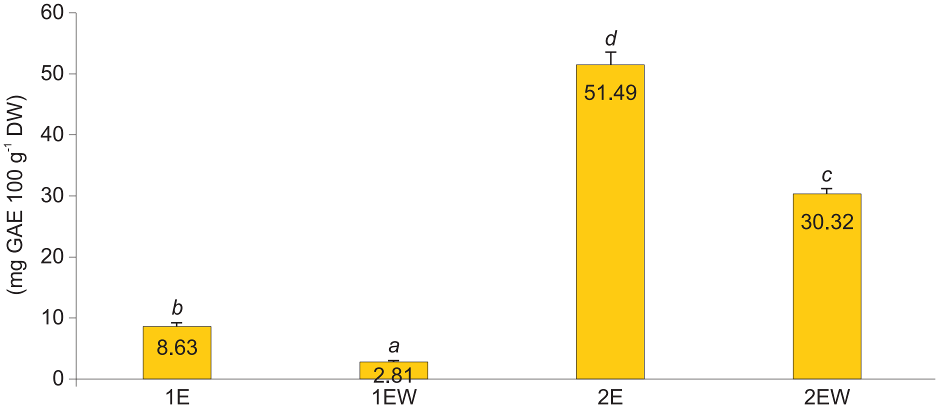

Fig. 2. Total phenolic content in seeds extracts. Species: 1 – *Chaenomeles japonica*, 2 – *Chaenomeles* × *superba*. Extracts: E – ethanol, EW – ethanol-water. Error bars represents ± SD. Different lowercase letters indicate significant differences at α=0.05

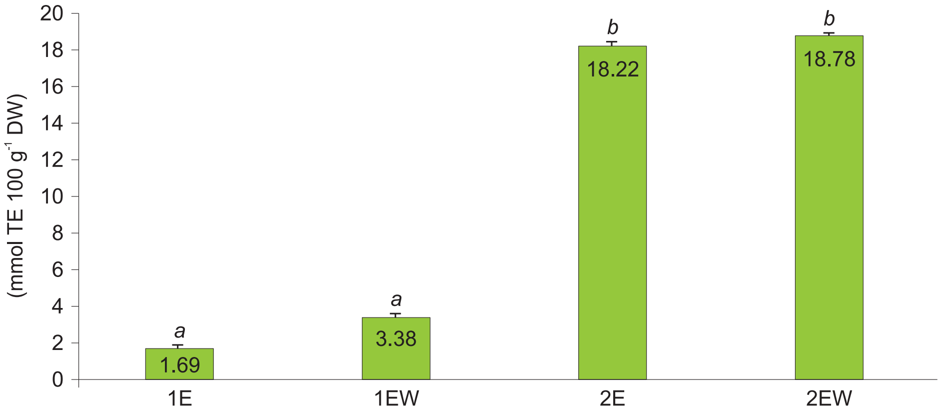

Fig. 3. Total antioxidant capacity seeds extracts determined by ABTS method. Species: 1 – *Chaenomeles japonica*, 2 – *Chaenomeles* × *superba*. Extracts: E – ethanol, EW – ethanol-water. Error bars represents ± SD. Different lowercase letters indicate significant differences at α=0.05

RESULTS AND DISCUSSION

C. japonica cv. Nicoline and *C.* × *superba* cv. Fascinacion differed in the concentrations of minerals, i.e. K, Ca, and Mg (macroelements) and Fe, Zn, Mn, and Cu (microelements). Content of potassium, magnesium, iron, manganese and copper was higher in the seeds of *C. japonica*, while the concentrations of calcium and zinc were higher in the seeds of *C.* × *superba* (Table 1).

Potassium content was higher in the seeds of *C. japonica* (887.19 mg 100 g^{-1} DW) than in the seeds of *C.* × *superba* (781.02 mg 100 g^{-1} DW). The results indicate that the quince seeds are rich in potassium. According to Turkiewicz et al. (2021), the content of potassium was 543.4 mg 100 g^{-1} DW in the seeds of *C. japonica* and 368.9 mg 100 g^{-1} DW in the seeds of *C.* × *superba*.

The potassium content in the seeds of *C. japonica* was on average 106.17 mg 100 g^{-1} DW higher than in the seeds of *C.* × *superba*, content of magnesium was higher by 2.33 mg 100 g^{-1} DW, iron by 0.64 mg 100 g^{-1} DW, manganese by 0.95 mg 100 g^{-1} DW, and copper by 0.59 mg 100 g^{-1} DW. However, the calcium content was higher in the seeds of *C.* × *superba* than in the seeds of *C. japonica*, on average by 10.54 mg 100 g^{-1} DW, and the zinc content was higher by 0.54 mg 100 g^{-1} DW.

The calcium content in the quince seeds, reached 20.13 mg 100 g^{-1} DW or 30.67 mg 100 g^{-1} DW. On the other hand, the magnesium content in the quince seeds was much lower than in hazelnuts, a good source of this nutrient, containing on average 280 mg Mg 100 g^{-1} DW (Cosmulescu et al. 2004). The content of calcium and magnesium in the quince seeds in the present study was within the range reported by Turkiewicz et al. (2021) for the seeds of various species and varieties of *Chaenomeles* (calcium from 13.9 to 49.7 mg 100 g^{-1} DW, magnesium from 21.7 to 94.9 mg 100 g^{-1} DW).

The quince seeds contained iron at concentrations of 1.92-2.56, similar to walnut seeds (2.06 mg 100 g^{-1} DW) and more than peanuts, Brazil nuts, pecans, and pistachios (1.11-1.80 mg 100 g^{-1} DW) – Malinowska, Szefer (2007). Other authors have reported iron concentrations in the quince seeds from 2.58 to 7.22 mg 100 g^{-1} DW (Turkiewicz et al. 2021), i.e. somewhat higher than in the present study. The contents of zinc, manganese and copper obtained in the present study in *C. japonica* cv. Nicoline and *C.* × *superba* cv. Fascinacion were similar to the results reported by Turkiewicz et al. (2021). The content of manganese and copper in the quince seeds was similar to their levels in Brazil nuts (1.06 mg Mn 100 g^{-1} DW and 1.39 mg Cu 100 g^{-1} DW) – Malinowska, Szefer (2007). Since the human daily requirement for manganese is 1.80 mg, it can be fully satisfied by 100 g of *C.* × *superba* seeds. The adult person's requirement for copper is 700 μg/day (Gaetke et al. 2014), and in our study the copper concentration in the quince seeds was 1.37 or 1.96 mg 100 g^{-1} DW, so they may be a good source of this mineral.

Table 1

Mineral contents in seeds of *Chaenomeles* sp.

Species	Mineral content (mg 100 g^{-1} DW)						
	K	Ca	Mg	Fe	Zn	Mn	Cu
Chaenomeles japonica	887.19±13.31*a*	20.13±15.71*a*	36.83±27.10*a*	2.56±2.35*a*	4.56±0.45*a*	1.81±0.21*a*	1.96±0.22*a*
Chaenomeles × *superba*	781.02±30,64*b*	30.67±25.04*b*	34.50±21.78*a*	1.92±1.42*b*	5.10±0.23*a*	0.86±0.19*b*	1.37±0,18*b*

Means sharing the same letter within a column are not significantly different at $p<0.05$.

The content of bioactive compounds was analysed using 96% ethanol and 40% ethanol-water extracts. Extraction is often used to study the content of bioactive compounds and the microbiological properties of plants. This method relies on the action of the solvent or solvent mixture on plant material. Ethanol has many advantages as a solvent for extraction. It has low toxicity, evaporates easily at low temperatures, has a preservative effect, and facilitates rapid absorption of the extract in the human body. As ethanol is safe for human consumption, ethanol plant extracts can be directly ingested or applied to the skin (Gurjar et al. 2012). According to Cruz et al. (2017), ethanol and ethanol-water extracts from various plants have high TPC and TAC values, similar to those obtained using other solvents, such as acetone, n-hexane or methanol. In the study of Urbanavičiūtė et al. (2020), extracts prepared using 50% v/v and 70% v/v ethanol from quince fruits had higher TPC than the corresponding methanol extracts.

Quince seeds, treated mainly as waste material, contain valuable bioactive substances – phenolic compounds (Sokołowska-Woźniak et al. 2002). Total phenolic content (TPC) in the seed extracts of the quince species fell within a fairly wide range, from 2.81 mg GAE 100 g^{-1} DW to 51.49 mg 100 g^{-1} DW (Figure 2).

This parameter also varied depending on the solvent used. Higher content of phenolic compounds was noted in the 96% ethanol extracts than in the 40% extracts in both genotypes. The content of phenolic compounds was higher in the *C.* × *superba* seed extracts – 30.32 and 51.49 mg GAE 100 g^{-1} DW for 40% and 96% ethanol, respectively (Figure 2).

According to Soong and Barlow (2004) the seeds of *C.* × *superba* cv. Fascinacion had higher TPC than the seeds of breadfruit (27.7 mg GAE g^{-1} DW), but lower than the seeds of longan, avocado or tamarind (62.6, 88.2 and 94.5 mg GAE g^{-1} DW, respectively). Other authors have shown highly varied phenolic content in quince seeds (Turkiewicz et al. 2021). The content of phenolic acids was the lowest in seeds of *C. speciosa* cv. Simonii (1.15 mg GAE g^{-1} DW) and the highest in the seeds of *C.* × *superba* cv. Crimson and Gold (3.56 mg GAE g^{-1} DW). The average TPC in quince seeds (45.3 mg GAE g^{-1} DW) was higher than in three cultivars of *C. japonica* investigated by Urbanavičiūtė et al. (2020).

The total phenolic content in our study, measured in seed extracts, was significantly lower than in the quince pericarp extracts (Urbanavičiūtė et al. 2020). Urbanavičiūtė et al. (2020) found that TPC in *C. japonica* fruit extracts ranged from 3906 mg GAE 100 g^{-1} DW (Rondo variety) to 4,550 mg GAE 100 g^{-1} DW (Darius). According to Tarko et al. (2010), the fruit of *C. japonica* had a high concentration of phenolic compounds (645 mg GAE 100 g^{-1} FW) and 32% higher antioxidant potential than Cornelian cherry.

The quince seeds are an unusual source of antioxidants, but in our study we noted their high content of these substances. The total content of all antioxidants can be measured in biological samples using methods based on reduction of synthetic radicals, such as DPPH and $ABTS^{+\cdot}$, and expressed as synthetic Trolox equivalent (Cybul, Nowak 2008). The total antioxidant capacity of the extracts from the seeds determined by the ABTS method was higher in *C.* × *superba* than in *C. japonica* (Figure 3).

In the case of *C.* × *superba* seeds, similar total antioxidant capacity was noted for the 96% ethanol (E) and 40% ethanol-water (EW) extracts (18.22 and 18.78 mmol TE 100 g^{-1} DW, respectively). In the case of *C. japonica*, antioxidant capacity measured by the ABTS method was higher in the 40% ethanol extract (Figure 3).

The TAC of the seeds determined by the DPPH method also varied between the two genotypes, with higher values obtained in the E and EW extracts of *C.* × *superba* (11.27 and 11.34 mmol TE 100 g^{-1} DW, respectively). The TAC of *C. japonica* was similar for the E and EW extracts (1.91 and 1.76 mmol TE 100 g^{-1} DW, respectively) and lower than in case of *C.* × *superba* (Figure 4).

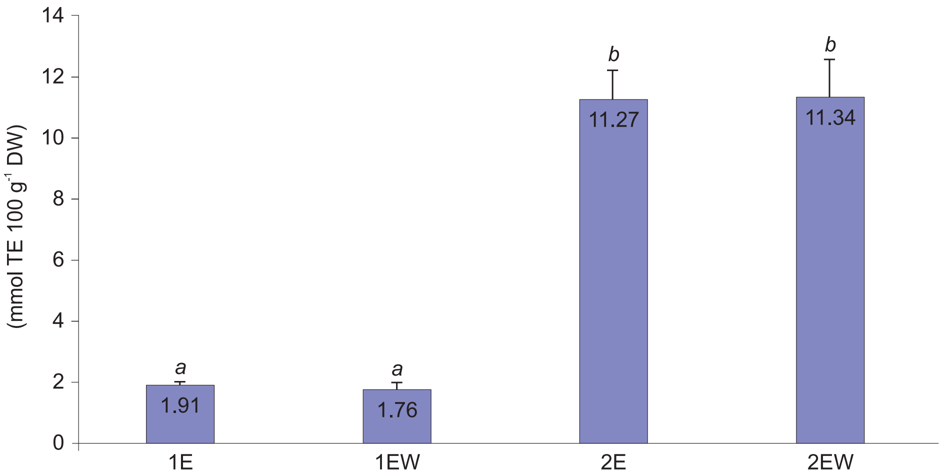

Fig. 4. Total antioxidant capacity in extracts seeds determined by DPPH method. Species: 1 – *Chaenomeles japonica*, 2 – *Chaenomeles* × *superba*. Extracts: E – ethanol, EW – ethanol-water. Error bars represents ± SD. Different lowercase letters indicate significant differences at α=0.05

Quince seeds are less known as a source of antioxidants than the fruits of this plant. The quince fruit was used for medicinal purposes in traditional Chinese medicine, due to the presence of bioactive compounds with antioxidant properties (Antoniewska et al. 2017). The antioxidant properties of the fruit of *C. japonica* are associated with the presence of large amounts of polyphenolic compounds and vitamin C (Ros et al. 2004). The free-radical scavenging activity of the fruit extracts of three *C. japonica* varieties, determined by the DPPH and ABTS methods, ranged from 99.1 μmol TE 100 g^{-1} DW (Rondo) to 115.9 μmol TE 100 g^{-1} DW (Darius) and from 372 μmol TE 100 g^{-1} DW (Rondo) to 682 μmol TE 100 g^{-1} DW (Rasa), respectively (Urbanavičiūtė et al. 2020). Zujko and Witkowska (2009) reported antioxidant potential of 8.44 mmol TE 100 g^{-1} DW in apples and 7.13 mmol TE 100 g^{-1} DW in pears. The antioxidant activity of the seeds of both quince species in our study was similar or significantly higher than the values for these fruits.

The antioxidant activity of seed extracts of *Chaenomeles* does not depend solely on the presence of polyphenols, so determinations by other methods (ABTS and DPPH) are fully justified. The reaction mechanism of antioxidants with ABTS is hydrogen atom transfer (HAT), while the DPPH reagent is based on a combination of HAT mechanism and single electron transfer (SET – single electron transfer). Many authors, when measuring the antioxidant properties of plant extracts, use several methods simultaneously, including the ABTS and DPPH methods. These methods complement each other, because DPPH allows for the determination of hydrophobic antioxidants, while ABTS captures the presence of both hydrophobic and hydrophilic antioxidants (Re et al. 1999, Molyneux 2004).

There was a high positive correlation between TPC and TAC determined by the ABTS method (r=0.89) and the DPPH method (r=0.91) in the seed extracts. The ABTS and DPPH methods of determining antioxidant capacity were also highly correlated (r=0.99) – Table 2.

Table 2

Significant values of correlation coefficients at α<0.05 between total phenolic content (TPC) and antioxidant capacity (TAC method of analysis – ABTS, DPPH)

Method of analysis	TPC	ABTS	DPPH
TPC	1.00		
ABTS	0.89	1.00	
DPPH	0.91	0.99	1.00

In contrast to the total phenolic content, the solvent used for extraction did not affect the total antioxidant activity of the extracts in either the ABTS or DPPH method. No correlation was found between TPC and TAC and the content of macro- and microelements.

Plant extracts can be used to treat infectious diseases caused by microbes. The antibacterial activity of the quince seed extracts against selected opportunistic bacteria for humans was determined by the disc diffusion method. No growth inhibition zones were found for *Staphylococcus aureus*, *Salmonella enterica*, or *Serratia marcescens* in the case of *C. japonica* (Table 3). Zones of inhibition were noted for *C.* × *superba*, ranging from 4.83 to 6.01 mm. The E and EW extracts were effective against *Enterobacter aerogenes* and *Enterococcus faecalis* (inhibition zones 3.50-7.58 mm; Table 3). Antimicrobial activity against *E. aerogenes* was higher for the EW and E extracts from the seeds of *C. japonica* (inhibition zones 6.38-7.58 mm, respectively). Inhibition zones for this bacterial species were smaller in the case

Table 3

The amount of bioactive substances introduced with the extract and the biostatic effect of the extracts on selected strains of bacteria

Extract*	The amount of antioxidant substances put on the disc calculated for the methods			Microorganism/zone of inhibition (mm)				
	TPC (µg GAE)	ABTS (µmol TE)	DPPH (µmol TE)	EA	EF	SA	SE	SM
1E	0.009	0.019	0.017	7.58±1.55	3.67±0.68	0.00	0.00	0.00
1EW	0.003	0.018	0.034	6.38±0.77	3.50± 0.90	0.00	0.00	0.00
2E	0.040	0.112	0.360	5.90±0.90	5.33±0.80	5.73±0.67	6.10±1.96	5.90±1.58
2EW	0.032	0.114	0.380	5.83±0.70	3.95±0.57	5.03±0.81	4.83±1.55	5.83±1,41
Negative control – ethanol				0.00	0.00	0.00	0.00	0.00
Kanamycin				23.00	26.00	26.8	27.00	26.00
Significant values of correlation coefficients at $\alpha<0.05$ between the amount of phenolic compounds introduced and the growth inhibition zone				ns	0.70	ns	ns	ns
Significant values of correlation coefficients at $\alpha<0.05$ between the amount of introduced antioxidant compounds ABTS and the zone of growth inhibition				-0.51	0.53	ns	ns	ns
Significant values of correlation coefficients at $\alpha<0.05$ between the amount of introduced antioxidant compounds DPPH and the zone of growth inhibition				ns	0.54	ns	ns	ns

* Extracts: E – ethanol, EW – ethanol-water, from the seeds of 1 – *Chaenomeles japonica*, 2 – *Chaenomeles* × *superba*. Microorganism: EA – *Enterobacter aerogenes*, EF – *Enterococcus faecalis*, SA – *Staphylococcus aureus*, SE – *Salmonella enterica*, SM – *Serratia marcescens*, ns – not significant values of correlation coefficients at $\alpha<0.05$

of EW and E extracts from the seeds of *C.* × *superba* (EW 5.83 and E 5.90). The strongest antibacterial activity was exhibited by the ethanol extract of *C.* × *superba* seeds, which caused the largest inhibition zones in all bacteria. The E extracts exhibited stronger antimicrobial activity against *E. aerogenes*, *E. faecalis*, *S. aureus*, *S. enterica*, and *S. marcescens* than the EW extract. There was a high, positive, statistically significant correlation at α<0.05 between the amount of phenolic compounds (*r*=0.70) and antioxidant substances (*r*= 0.53, *r*=0.54) applied to the disc with the extract and the size of the growth inhibition zones for *E. faecalis* (Table 4). No correlations were found for other bacteria. Only the size of the inhibition zone of *E. aerogenes* was shown to be negatively correlated (*r*=-0.51) with TAC determined by the ABTS method.

There are no literature reports on the antibacterial effect of extracts of quince seeds. In the present study, extracts of *C.* × *superba* seeds had an antibacterial effect against all tested species of bacteria, while the extracts of the seeds of *C. japonica* were effective against only two species. The highest antibacterial activity against *E. aerogenes* and *E. faecalis* was observed for the 96% ethanol extract, which surpassed the 40% ethanol-water extract in inhibiting bacterial growth. For *E. faecalis*, the content of antioxidants and phenolic compounds in the extract were shown to be positively correlated with their antimicrobial activity. Phenolic compounds are regarded as promising biological compounds that could serve as a new type of antimicrobial agent (Krzepiłko et al. 2021). The quince seeds are a good raw material for processing and isolation of therapeutic antimicrobial agents. They remain an undervalued source of bioactive compounds with antimicrobial activity. Among the seeds of 21 plant species tested by Kumarasamya et al. (2002), all of them showed varied antibacterial activity. *Centaurium erythraea* and *Prunus padus* were especially promising, as their extracts killed methicillin-resistant *Staphylococcus aureus*. Extracts from pumpkin or pistachio seeds inhibited the growth of bacteria such as *S. aureus*, and sunflower seed extracts exhibited activity against *Proteus vulgaris* (Kırbaşlar et al. 2012). Ethanol extracts of the seeds and skin of pomegranate inhibited clinical isolates of *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Nozohour et al. 2018). Cruz et al. (2017) showed moderate or strong antibacterial activity of ethanol and ethanol-water extracts from the seeds of palm *Butia catarinensis* against *Bacillus cereus* and *Escherichia coli*. Antimicrobial activity was also shown for extracts from red raspberry seeds against *Escherichia coli*, *Salmonella typhimurium*, *Salmonella enteritidis*, and *Listeria monocytogenes* (Bauza-Kaszewska et al. 2021).

Extracts from quince seeds may have a variety of applications, including in the cosmetics and food industries, as a rich source of bioactive compounds and natural antibacterial agents.

CONCLUSION

Differences between *Chaenomeles* species were noted for the content of minerals and total phenolic content in the seeds and the total antioxidant capacity of seed extracts. In *C.* × *superba* the total phenolic and antioxidant content in the seeds was much higher than in *C. japonica*. Both ethanol and ethanol-water solutions allowed the extraction of phenolic compounds and antioxidants from the tested quince seeds (Figures 2-4). Extracts from the seeds of *C.* × *superba* inhibited the development of all bacterial species tested, which confirms that they can be used as natural antimicrobial agents. We obtained a higher concentration of phenolic compounds and a higher antimicrobial activity in the ethanol extracts. The results of our experiments suggest that further purification and identification of compounds contained in ethanol extracts may improve their antimicrobial properties.

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