Chapter

Biochemical Composition of Japanese Quince (*Chaenomeles japonica*) and Its Promising Value for Food, Cosmetic, and Pharmaceutical Industries

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Abstract

Japanese quince (*Chaenomeles japonica*) is one of the most underutilized plant species that have high nutrient value and a positive impact on human health. Due to the high content of bio-compounds, such as phenols, vitamin C, triterpenes, fibers, essential amino acids, and microelements, the fruits, leaves, and seeds are excellent raw materials for functional food production. In addition, their biochemical composition and anti-inflammatory, anticancer, and antibacterial properties expanded their uses in the pharmaceutical field. Moreover, it was demonstrated that quince waste after industrial processing is still valuable and suitable for remanufacturing and developing innovative high value-added products, which can provide economic and ecological benefits. This chapter presents the biochemical composition and possible application of *C. japonica* cultivars Rasa, Darius, and Rondo. The optimization of processing and extraction parameters was evaluated to increase the extraction efficiency of biologically active compounds and to reduce the extraction time and cost of electricity and environmentally harmful solvents. Moreover, the detailed nutritional and pharmacological value of Japanese quince can help for more selective plant organs application. Our study revealed that cultivars Rasa, Darius, and Rondo are very valuable with many new options for utilization, including food, cosmetic, and pharmaceutical industries.

Keywords: Japanese quince, fruit, leafs, seeds, phenols, triterpenes, by-products, oil, antioxidant activity, anticancer, antibacterial

1. Introduction

The *Chaenomeles* genus is one of the oldest cultivated plants from the *Rosaceae* family, a subgenus of *Maloideae*, phylogenetically very close to the genera *Cydonia*, *Pyrus*, and *Malus* [1–3].) *Chaenomeles japonica* named Japanese quince (JQ) is a dwarf shrub from East Asia (**Figure 1**), was used in Chinese medicine around 3000 years

Figure 1.
Chaenomeles japonica *cultivar rondo growing in the garden of Babtai, Lithuania, on the left – in may, and on the right – in September. Photo I. Urbanavičiūtė.*

ago [3]. Among several species of the *Chaenomeles* genus, JQ is the most suitable to the North European climate. JQ propagation by seeds is undesirable and causes high genetic variation in morphological and quality characteristics. JQ breeding program funded by the European Research Project "Japanese quince (*C. japonica*)*–a new European fruit crop for the production of juice, flavor, and fiber* (FAIR5-CT97-3894, 1998-2001)," resulted in new improved JQ cultivars. Three needleless cultivars have been released and registered in the European Union after passing the DUS (distinctness, uniformity, and stability) tests. Cultivars Darius, Rondo, and Rasa were released in the Baltic region with the quality and desirable characteristics of fruits and products [4, 5]. JQ is a cross-pollinated crop, and a minimum of three different cultivars should be planted in the same garden to ensure a highly successful fruit set and yield [6]. Nowadays, cultivation and scientific research on JQ are mostly concentrated in the North part of Europe, in Poland, Finland, Sweden, Latvia, and Lithuania.

Properties of JQ fruit such as firmness, and a high amount of juice and fibers characterized them as a potential crop in horticulture [7–9]. The knowledge about the biochemical characteristics of the JQ fruit recently increased, and their bioactive compound quantity and composition highlighted them as promising raw materials with a positive influence on human health [3, 10, 11]. The abundance of compounds with strong antioxidant properties, such as phenols, triterpenes, and vitamin C, can contribute to chronic disease prevention [2, 12–14]. Phenols as natural antioxidants are well-known with a positive effect on human health as anti-inflammatory [15], neuroprotective [16], anticancer [17], antiviral [18], improving cardiovascular activity [19], antidiabetic [20], and blood cholesterol and triglyceride-lowering [21, 22].

JQ fruits are also rich in fibers, as well organic acids, mainly malic and citric acids at levels of 10 times that of apple fruits [8, 23]. However, due to their characteristics, such as firmness, sourness, and astringency they are not suitable for fresh consumption, so mostly used for the production of juices, jams, syrups, alcoholic and non-alcoholic beverages, etc. [7, 24–27]. If JQ fruits are not immediately processed or to be frozen, and appropriate storage should be considered to ensure minimum losses in quality parameters and biochemical composition. Several studies have shown that JQ fruits could be stored without significant losses in quality under a controlled atmosphere system or at 1°C and 85% relative humidity [9, 28]. Moreover, an unavoidable big amount of waste is generated during processing, mostly consisting of seeds [7, 29]. Even so, recent studies showed that seeds left after industrial manufacturing can be used for oil recovery, mucilage extraction, and as a source of organic acids, polyphenols, triterpenes, and microelements [29–32]. Another residual, such as pomace, also is suitable for secondary uses, for example, pectin and fiber extraction [33, 34].

Significant amounts of biologically active compounds, including phenols and triterpenes, were detected also in JQ leaves [11, 35, 36]. Since the branches of the wild JQ plant are mostly prickly, collecting their leaves is a hard and complicated process.

The new cultivars Darius, Rondo, and Rasa are needleless, which can enrich the use-value of the whole plant. In order to expand the utilization of these new cultivars, it was necessary to determine their biochemical value. Moreover, the optimization of process parameters will help increase the extraction efficiency, as high temperature, oxidation, and other decomposition processes have negative effects on sensitive bio-compounds. Adjusting extraction parameters such as polarity of the solvent and its ratio to water, time, temperature, additional energy source (ultrasound, microwave, etc.), have a crucial impact on the efficiency of phenols extraction [37–40]. This chapter aims to determine the impact of genotype and extraction methods on quince *C. japonica* biochemical composition and to evaluate possible applications of three cultivars Rasa, Darius, and Rondo cultivated in Lithuania.

2. Materials and methods

2.1 Chemicals

All the solvents, reagents, and standards were used of analytical grade. The following substances were used in the study: Ethanol 96% (*v*/*v*) (AB Strumbras, Kaunas, Lithuania), procyanidin C1, procyanidin B2, quercetin, hyperoside, avicularin, quercitrin, kaempferol 3-O-glucoside, luteolin 7-O-glucoside, phloridzin, formic acid, acetonitrile, (+)-catechin, (−)-epicatechin, rutin, isoquercitrin, chlorogenic acid, p-coumaric acid, caffeic acid, hydrochloric acid (Sigma-Aldrich, Steinheim, Germany). Where used purified de-ionized water was prepared with the Milli–Q® (Millipore, Bedford, MA, USA) water purification system.

2.2 Plant material

Fruits, leaves of the Japanese quince cultivars, Darius, Rondo, and Rasa were collected from test gardens belonging to the Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry, Babtai (55° 60´ N, 23° 48′ E). The fruits were harvested from late August to mid-September, depending on the technical maturity, for each cultivar randomly from five shrubs in September. Leaves were collected for each cultivar randomly at different seasons, spring (May), summer (August), and autumn (October). Fruits and leaves were frozen (at −40°C) in a freezer with air circulation, and then lyophilized with a sublimator Zirbus 3 × 4 × 5 (ZIRBUS technology GmbH, Bad Grund, Germany), at a pressure of 0.01 mbar (temperature of condenser −85°C) for 24 h. The lyophilized fruits, leaves were grounded to a fine powder with a knife mill GM (Retsch GmbH, Haan, Germany). The seeds were removed and dried in a convection dryer for 24 hours at 60°C, after seeds were crushed and divided into five

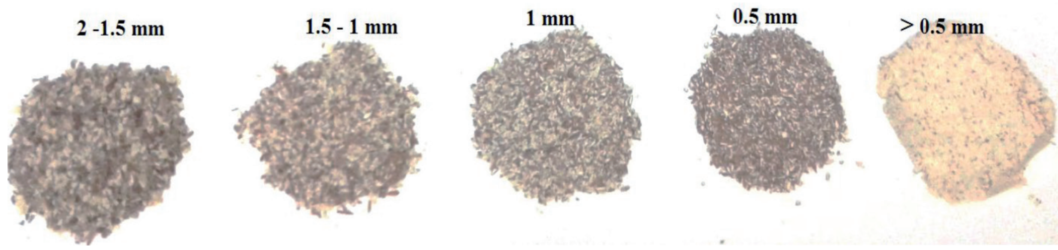

Figure 2.
Different fractions of grounded Japanese quince seeds.

fractions according to seeds particle size: 2–1.5 mm, 1.5 mm–1 mm, 1 mm, 0.5 mm, > 0.5 mm (**Figure 2**).

2.3 Phenolic compounds extraction optimization

First, the influence of different solvents on the extraction efficiency was initially determined, 0.5 g of the seeds or leaves powder with 10 mL solvent in different concentrations (ratio 1:20, w/v) were mixed and left at room temperature 22°C in the dark for 24 h. After extraction, the samples were centrifuged and filtered through a Whatman filter paper. For phenolic compounds extraction efficiency analysis, three solvents (ethanol, methanol, and acetone) and three concentrations (100%, 70%, and 50%) were chosen. After selecting the most efficient solvent system, ultrasound extraction (UE) of phenolic compounds was carried out with Sonorex Digital 10 P ultrasonic bath (Bandelin Electronic GmbH & Co. KG, Berlin, Germany). Response surface methodology (RSM) was used to examine the influence of UE processing variables on phenols extraction, and three parameters were selected for optimization—temperature, extraction time, and ultrasonic power regarding methodology described by Urbanavičiūte et al. [10].

2.4 Determination of dry matter (DM), total soluble solids (TSS), firmness, sugar, and fiber content

Dry matter content was determined after forced air convention drying at 105°C to a constant weight. The total soluble solids were determined using a digital refractometer (ATAGO PR-32, Atago Co., Ltd., Tokyo, Japan). Fruit firmness was determined by the texture analyzer TA.XTPlus (Stable Micro Systems, UK) using the P/2 probe. Total sugar content was determined using the Bertrand method. Fiber content was determined using.

Fiber analyzer Ankom 2000 (Ankom Technologies, Madison NY, USA) and expressed as a percentage.

2.5 Determination of biochemical composition, antioxidant and antimicrobial activities

Spectrophotometric measurements were performed using a Genesys-10 UV/Vis spectrophotometer (Thermo Spectronic, Rochester, NY, USA). The total amount of phenols was assessed using Folin–Ciocalteu method according [41] at 765 nm wavelength, and was expressed in mg 100 g^{-1}, the equivalent of gallic acid. The antiradical activity was determined using two methods—the DPPH (515 nm) method described by Brand-Williams et al. [42], and ABTS (734 nm) assay was applied according to the methodology described by Re et al. [43]. Antiradical activities were expressed in μmol of Trolox equivalents in g^{-1} dry extracts. Total proanthocyanidins (640 nm) were determined using the technique described by Heil et al. [44]. According to the methodology described by Liaudanskas et al. [45], the high-performance liquid chromatography (HPLC) method was used for the determination of phenolic compounds. The antimicrobial activity against three Gram-positive and three Gram-negative bacteria was evaluated by the agar well diffusion method described by Urbanavičiūte et al. [10].

2.6 Statistical analysis

Data collected are expressed as mean ± standard deviation. The results of three replicates were presented and univariate analysis of variance (ANOVA) was applied. Tukey's HSD (honest significant difference test) was used for multiple mean comparisons. Statistical significant differences were considered at $p < 0,05$. The statistical analysis was performed using Statistica 10 software (StatSoft, Inc., Tulsa, OK, USA).

3. Results and discussion

3.1 Japanese quince fruit characteristics and biochemical composition

The cultivars Darius, Rondo, and Rasa are characterized by the absence of needles, their leaves are resistant to blemishes, the whole plant is resistant to frost and the fruit to rot. The fruits finish ripening evenly in early September. Rondo is characterized by large shrubs with orange flowers. Shrubs of Darius are small, with several main branches, blooming in orange-dense inflorescences. Shrubs of the Rasa are of medium size, the flowers are pink and not dense. The characteristics of the fruit listed in **Table 1**, may help for better adoption of quince processing techniques, and for farmers to select cultivars with desired properties.

Significant differences were found between the three cultivars in fruit size and their number of seeds. Rasa had the largest fruit and highest number of ripening seeds, almost twice as much as others (**Table 1**). The skin and flesh firmness were not significantly different between cultivars, but the fruits of the Rondo were slightly softer. Moreover, Rasa had the lowest amount of sugars and sucrose content.

Our previous studies have shown that the biochemical composition significantly varied between genotypes. Rasa had the highest amount of rutin and lowest vitamin C, while Rondo and Darius had higher catechin and chlorogenic acid, respectively [10]. Genotypic differences in the biochemical composition of JQ juices were also reported [46].

Moreover, it was found that different solvents and their ratio to water had a significant impact on phenols extraction efficiency using the simple maceration method from freeze-dried JQ fruit powder [10]. Besides, using ultrasound power decreased extraction time while increasing phenol yield from JQ fruit by 14.5% [10]. Five phenols (−)-epicatechin, (+)-catechin, chlorogenic acid, rutin, and isoquercitrin were identified in all the three cultivars Rasa, Darius, and Rondo, using the HPLC method [10]. The predominant phenols in all cultivars were flavan-3-ols (catechin and epicatechin), which account for around 94% of the total amount [10]. It was also reported that all cultivars have accumulated high levels of proanthocyanidins [10]. Similar results were demonstrated where 11 phenols were determined with a distribution of procyanidins (57.8%), (−)-epicatechin (33%), and chlorogenic acid (4.4%) [47]. In addition, 24 phenols were identified in the study of five *Chaenomeles* species including

Characteristic	*C. japonica*		
	Darius	**Rondo**	**Rasa**
Average yield, kg/shrub	9 ± 0.6[a]	10 ± 0.5[a]	8 ± 0.8[a]
Fruit weight, g	46.2 ± 8.3[a]	75.7 ± 13.1[b]	97.7 ± 21.2[b]
Diameter of fruit, mm	44.4 ± 2.1[a]	53.0 ± 2.7[b]	58.7 ± 3.4[b]
Seeds weight, g	2.5 ± 0.8[a]	3.4 ± 0.9[a]	7.8 ± 1.9[b]
Number of seeds	59.2 ± 19.9[a]	62.0 ± 15.6[a]	127.2 ± 36.3[b]
Flesh thickness, mm	11.6 ± 1.3[a]	13.5 ± 0.6[a]	12.0 ± 1.9[a]
Diameter of the core, mm	22.6 ± 1.7[a]	24.8 ± 2.9[a]	39.6 ± 5.0[b]
Dry Matter of fruit, %	9.2 ± 0.1[a]	9.6 ± 02[a]	9.2 ± 0.1[a]
Dry Matter of leaves, %	46.1 ± 0.1[a]	43.1 ± 0.1[a]	43.7 ± 0.1[a]
Dry Matter of seeds, %	60.2 ± 0.1[a]	53.8 ± 0.1[b]	54.1 ± 0.3[b]
Skin firmness, N cm^{-2}	329.3 ± 31.1[a]	315.5 ± 14.3[a]	324.3 ± 38.6[a]
Flesh firmness, N cm^{-2}	180.6 ± 13.3[a]	150.8 ± 12.2[a]	171.2 ± 20.1[a]
Total sugar content, %	3.45 ± 0.02[a]	4.0 ± 0.34[a]	2.69 ± 0.22[b]
Sucrose, %	1.08 ± 0.07[a]	1.05 ± 0.04[a]	0.77 ± 0.03[b]
Fiber content, %	19.3 ± 2.3[a]	17.9 ± 3.1[a]	21.1 ± 2.1[a]
Dry soluble solids, %	9.9 ± 0.2[a]	9.4 ± 0.3[a]	8.1 ± 0.1[b]

Table 1.
The characteristics of Chaenomeles japonica cultivars. Different letters (a, b, and c) in the same row indicate significant differences between samples ($p < 0.05$).

JQ, and they reported variations in their antioxidant activity and the quantity of compounds, such as chlorogenic acid, catechin, epicatechin, procyanidins B1 and B2 [2].

Moreover, phenols' quantity and their biological activity expand the uses of JQ fruit as a promising substitute for chemical preservatives in the food and cosmetic industry due to demonstrated antibacterial activity [48]. The cultivars Rasa, Darius, and Rondo showed antimicrobial activity against three Gram-positive and three Gram-negative bacteria, in a concentration-dependent manner [10]. However, they have not shown antifungal activity against *Candida albicans* yeast [10].

Nevertheless, it was mentioned that phenol-rich extracts obtained from JQ fruit could replace aggressive synthetic drugs with side effects through demonstrated anticancer activity [13, 14, 49, 50]. Also, JQ fruit extracts can be used as an antioxidant drug for the prevention of diseases caused by inflammation or oxidative stress [47, 51]. Moreover, JQ phenols-rich extracts as modulators of carbohydrates metabolism showed a promising hypoglycemic effect and decreased intracellular ROS accumulation [52]. Despite all these health benefits and pharmaceutical properties of JQ fruits, till now it is mostly used for the food industry. Moreover, exploiting significant unavoidable amounts of waste left after industrial processing can offer potential economic and ecological benefits.

3.2 Japanese quince by-products biochemical composition and possible utilizations

Due to the firmness, sourness, and astringency of JQ fruit, they are not suitable for fresh consumption, mostly used for syrup and candied production. The JQ

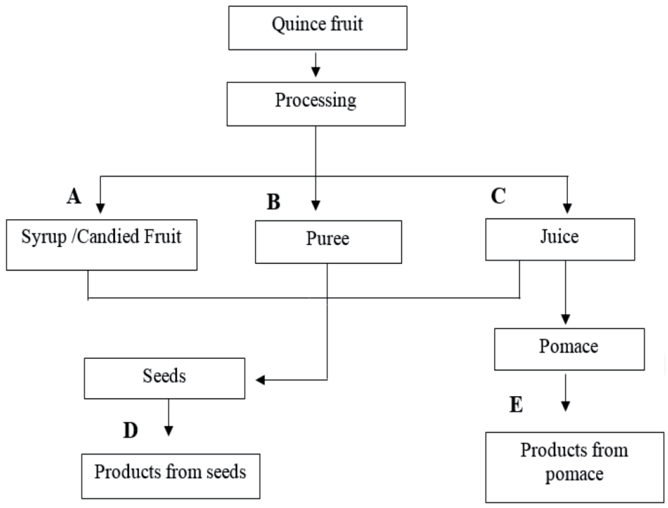

Figure 3.
General scheme of quince processing.

by-products after processing were received from Puree/juice manufacturer, collected after three different processing methods—syrup and candied fruits, puree, and juice (**Figure 3**). The seeds separation and preparation for analysis were performed following methods described by Urbanavičiūtė et al. [29]. The amount of by-products after JQ fruits processing depended on the manufacturing technology, ranged from 20–40%, and consisted mostly of pomace and seeds [29].

Most of the by-products remain after juicing (**Figure 2C**), range 40–60% of fresh fruit weight [7, 29]. The pomace left after juicing showed a significant amount of phenols, even 13 times more than juice (**Table 2**). Juicing is a rapid process that releases only a fraction of the biologically active compounds from the fruit, so most of them remain in the pomace. The JQ pomace also showed strong antioxidant activity and high amounts of proanthocyanidins.

Numerous studies have reported an important role of phenols in inhibiting the growth of microorganisms [53–55]. Our results showed that JQ pomace extracts had antimicrobial activity against three Gram-positive and three Gram-negative bacteria in a concentration-dependent manner, where the strongest inhibition effect was obtained using 5% concentration pomace extracts (**Figure 4**).

The greatest inhibitory effect of pomace extracts was found on the gram-positive *Enterococcus faecalis* (ATCC 29212) strain. In general, this strain was most sensitive for treatment with JQ pomace extracts, using all concentrations (**Figure 4**). Moreover, dried pomace is a promising source of pectin and an excellent raw material for fiber-rich products [33, 34].

Parameters	Juice	Pomace
Total Phenols, mg 100 g^{-1}	488 ± 11^{b}	6645.6 ± 211^{a}
RSA (DPPH), µmol TE 100 g^{-1}	14.7 ± 1.1^{b}	152.2 ± 13^{a}
RSA (ABTS), µmol TE 100 g^{-1}	69.7 ± 2.1^{b}	938.2 ± 33^{a}
Content of proanthocyanidins, mg 100 g^{-1}	218.5 ± 7.1^{b}	1368.7 ± 55.4^{a}

Table 2.
The total phenols, proanthocyanidins, and RSA - Radical scavenging activity DPPH, and ABTS of JQ juice and pomace. Different letters (a, b, and c) in the same row indicate significant differences between samples ($p < 0.05$).

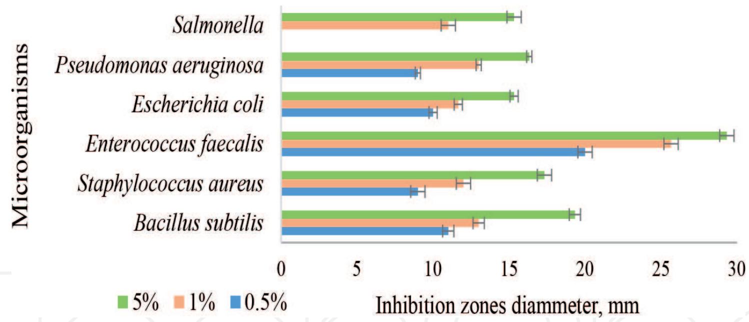

Figure 4.
Antibacterial activity of Chaenomeles japonica *pomace extract.*

In the food industry, JQ seeds are mostly discarded as waste, while new utilization can reduce losses for producers, especially when the seeds accounted for more than 30% of the total waste [29]. Moreover, it was reported that seeds from different species of the *Chaenomeles* genus including JQ are potential phenols sources, especially proanthocyanidins, triterpenes, essential amino acids, K, and microelements such as Fe, Cu, Zn, and Mn [56].

Before determining the content and diversity of phenolic compounds in Rasa, Darius, and Rondo seeds, the impact of different extraction parameters on their efficiency was performed. To determine the most efficient solvent system for phenols extraction, three solvents in different concentrations were used. After maceration with methanol, the highest content of phenolic compounds was extracted with a solvent concentration of 70% (**Figure 5**).

Pure ethanol has been shown to be the wrong choice for extracting phenols from quince seeds, and no significant differences between fractions were found (**Figure 6**). Extraction efficiency with ethanol was the highest when the ratio of water/alcohol was 1:1 (**Figure 6**).

The extraction of crushed JQ seeds with pure acetone also was not appropriate, while 70% concentration showed the highest amount of extracted phenols, five and six times more than using ethanol and methanol, respectively (**Figure 7**).

Our results showed that solvent type and its concentration had significant effects on phenol extraction efficiency from JQ seeds. Moreover, our results agreed with previously reported studies that dual solvent systems are more efficient for phenols extraction than with pure solvents [37, 57, 58]. Besides, phenols dissolved differently

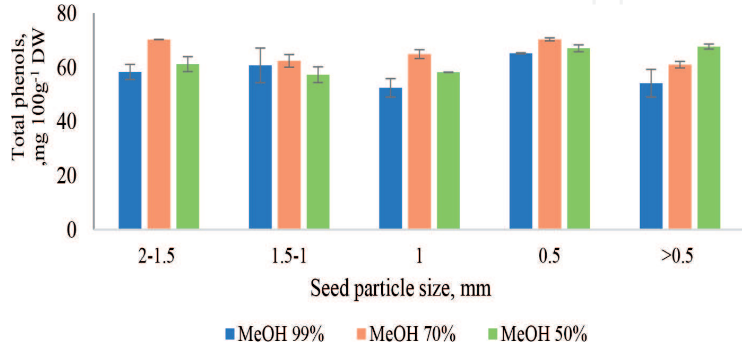

Figure 5.
Impact of methanol concentration and seeds particle size on the total content of total phenols compounds in quince seeds.

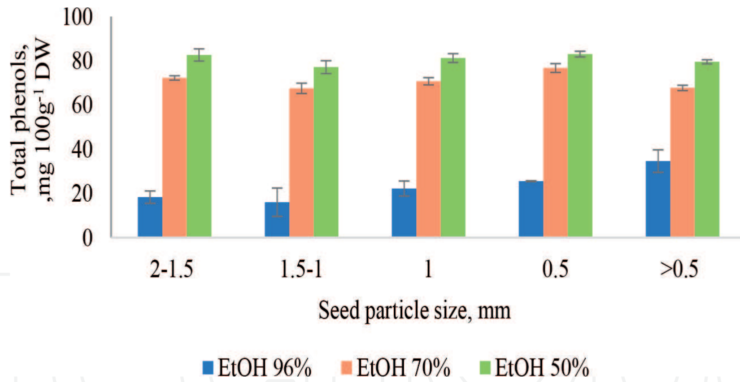

Figure 6.
Impact of ethanol concentration and seeds particle size on the total content of phenolic compounds in quince seeds.

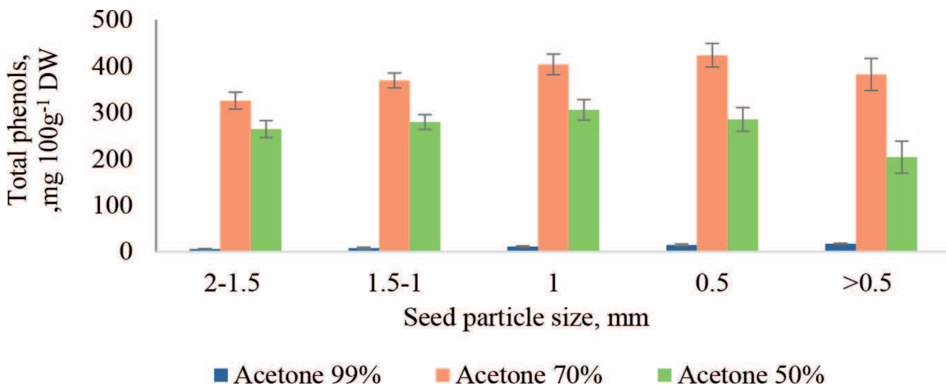

Figure 7.
Impact of acetone concentration and seeds particle size on the total content of phenolic compounds in quince seeds.

regarding the solvent system, for example, it was reported that methanol is the best for extraction of catechin, epicatechin, and epigallocatechin, then 70% acetone provides the highest content of proanthocyanidins and total phenolic compounds, while the highest content of gallic acid was extracted with 75% ethanol [59].

The most efficient solvent system (70% acetone) was used for ultrasound extraction of phenolic compounds with an ultrasonic bath. Response surface methodology (RSM) was used to examine the influence of ultrasound processing variables on phenols extraction, and three parameters were selected for optimization—temperature (15–50°C), extraction time (15 min–60 min), and ultrasonic power (48 W–480 W) following the methodology described by Urbanavičiūte et al. [10].

Using RSM, the highest amount of phenols was obtained when the samples were extracted for 60 min, at 50°C with 480 W ultrasonic power (**Figure 8**).

The temperature had the highest impact on phenols extraction, the efficiency increased around 80%, and time was reduced to 23 hours in comparison with simple maceration. Such a strong effect may have been due to the ultrasound power ability to destroy complexes of mucus with phenols, which makes their extraction more difficult from seeds [60]. Using the previously determined optimal parameters for phenol extraction, qualitative and quantitative analysis for Rasa, Rondo, and Darius seeds extracts were performed. The highest total phenolic compounds and antioxidant activity in seed extracts were found in Rondo and the lowest in Darius (**Table 3**).

Twelve phenolic compounds in Rasa, Rondo, and Darius seeds extracts using high-performance liquid chromatography (HPLC) were identified (**Table 4**).

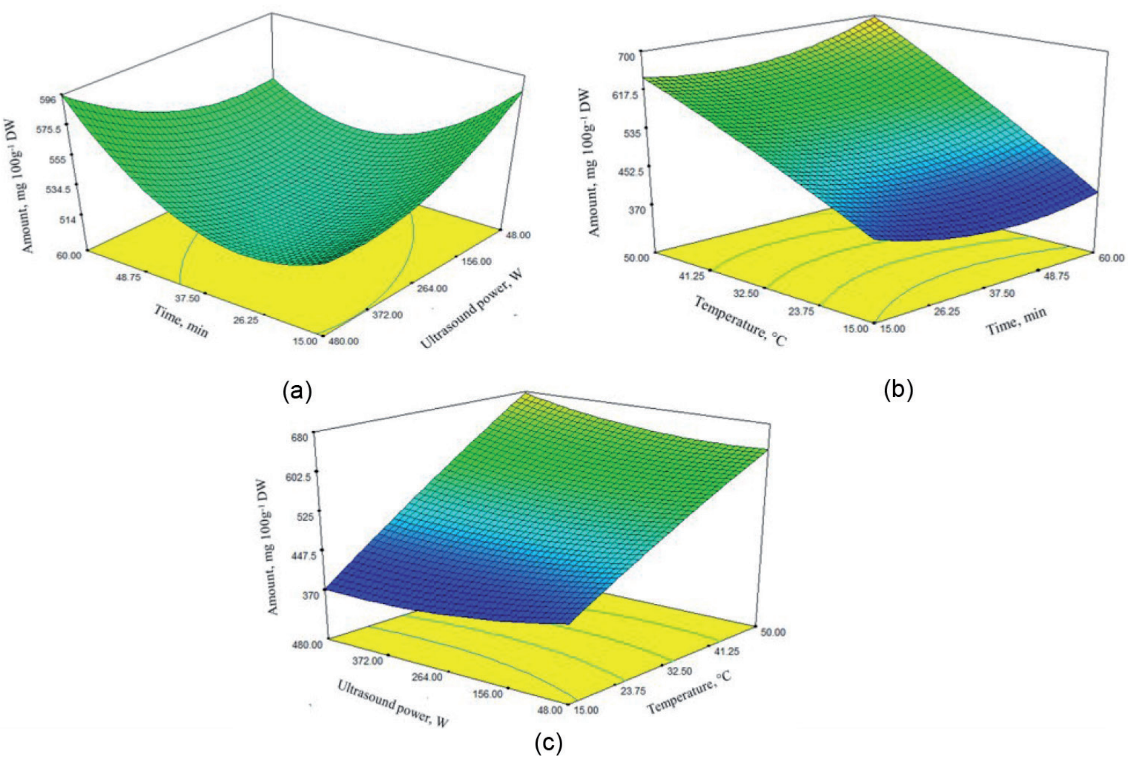

Figure 8.
The influence of (a) – Time and ultrasound power, (b) – Temperature and time, (c) – Ultrasound power and temperature on extraction efficiency of phenols from JQ seeds.

Cultivars	Total phenols mg/100 g	RSA (DPPH) µmol TE g^{-1}	RSA (ABTS) µmol TE g^{-1}
Rasa	427.6 ± 13.2^{b}	20.4 ± 1.4^{b}	41 ± 2.1^{b}
Rondo	499.6 ± 14.5^{a}	26.4 ± 1.2^{a}	50.9 ± 2.4^{a}
Darius	283.5 ± 9.4^{c}	14.7 ± 0.8^{c}	27.7 ± 1.1^{c}

Table 3.
The antioxidant activity, and total phenols of JQ seeds cultivars cultivated in Lithuania. Different letters (a, b, and c) in the same column indicate significant differences between samples ($p < 0.05$).

The predominant compounds were procyanidin B2, procyanidin C1, and chlorogenic acid, their amounts varied between genotypes. Rasa and Darius had a higher amount of chlorogenic acid than Rondo, while Rasa with Rondo had a higher amount of procyanidins than Darius (**Table 4**).

Phenols quantitative analysis using HPLC was applied for seeds left after different industrial processing. Seeds, which were left after puree production had significantly the highest amount of phenols (**Table 5**). From 12 detected phenols, procyanidin B2 and C1, and (−)-epicatechin were predominant. Epicatechin levels could be explained as a consequence of the production method, as JQ fruit (reach in epicatechin) was treated without removing the seeds.

3.3 Japanese quince leaves biochemical composition and possible utilization

To optimize the extraction of phenolic compounds from JQ leaves, three solvents and five concentrations were selected. The lowest concentration of phenolic compounds in quince leaves was determined by the maceration with pure solvents (**Figure 9**). The

Compound, μg g^{-1} DW	***Chaenomeles japonica***		
	Darius	**Rondo**	**Rasa**
Rutin	6.0 ± 0.3[a]	6.7 ± 0.4[a]	6.5 ± 0.3[a]
(+)-Catechin	4.2 ± 0.2[b]	4.2 ± 0.1[b]	7.5 ± 0.5[a]
Chlorogenic acid	20.7 ± 1.5[b]	17.2 ± 1.3[a]	24.2 ± 2.3[b]
Caffeic acid	9.7 ± 0.7[a]	9.4 ± 0.5[a]	9.3 ± 0.6[a]
Syringic acid	3.9 ± 0.2[a]	3.9 ± 0.2[a]	4.3 ± 0.5[a]
Hyperoside	5.3 ± 0.5[a]	5.1 ± 0.4[a]	ND
Procyanidin B2	22.5 ± 1.5[b]	41.5 ± 3.5[a]	45.0 ± 1.4[a]
Procyanidin C1	29.1 ± 2.1[b]	41.0 ± 3.2[a]	37.4 ± 1.8[c]
(−)-Epicatechin	14.1 ± 0.7[b]	19.8 ± 0.5[a]	16.7 ± 1.3[c]
Isoquercitrin	10.4 ± 1.5[a]	9.7 ± 1.2[a]	9.7 ± 1.5[a]
Quercitrin	4.4 ± 0.2[a]	4.6 ± 0.2[a]	4.6 ± 0.1[a]
p-Coumaric acid	6.4 ± 0.5[a]	5.8 ± 0.6[a]	6.8 ± 0.7[a]
Total	136.8 ± 9.7[b]	169.1 ± 4.5[a]	172.1 ± 3.7[a]

Table 4.
The quantitative composition (HPLC) of phenolic compound in JQ seeds. Different letters (a, b, and c) in the same row indicate significant differences between samples (p < 0.05).

Compound, μg g^{-1} DW	**Seeds left after different JQ processing**		
	After juicing	**After puree**	**After syrup**
Rutin	5.6 ± 0.3[a]	8.4 ± 0.4[a]	6.6 ± 0.3[a]
(+)-Catechin	13.9 ± 0.2[a]	4.2 ± 0.1[a]	7.5 ± 0.5[b]
Chlorogenic acid	20.7 ± 1.5[a]	25.0 ± 1.3[b]	8.8 ± 2.3[b]
Caffeic acid	9.1 ± 0.7[a]	10.3 ± 0.5[a]	9.6 ± 0.6[a]
Syringic acid	5.7 ± 0.2[a]	8.2 ± 0.2[b]	4.3 ± 0.5[a]
Hyperoside	5.0 ± 0.5[a]	8.2 ± 0.4[b]	ND
Procyanidin B2	24.6 ± 1.5[a]	790.9 ± 43.5[b]	62.0 ± 1.4[c]
Procyanidin C1	27.6 ± 2.1[a]	317.6 ± 23.2[b]	65.8 ± 4.8[c]
(−)-Epicatechin	14.4 ± 0.7[a]	709.8 ± 36.5[b]	43.3 ± 2.3[c]
Isoquercitrin	9.7 ± 1.5[a]	14.3 ± 1.2[b]	10.0 ± 1.5[a]
Quercitrin	4.6 ± 0.2[a]	7.6 ± 0.2[b]	4.1 ± 0.1[a]
p-Coumaric acid	8.0 ± 0.5[a]	21.9 ± 0.6[a]	6.8 ± 0.7[a]
Total	146.2 ± 9.7[a]	2041.4 ± 84.5[b]	250.7 ± 13.7[c]

Table 5.
Impact of JQ processing on the quantitative composition of phenols in seeds. Different letters (a, b, and c) in the same row indicate statistically significant differences between the individual compounds (p < 0.05).

proportion of water in solvents had no significant effect on the extraction efficiency. Significant higher content of phenolic compounds was obtained by maceration of quince leaves with 50% acetone (**Figure 9**).

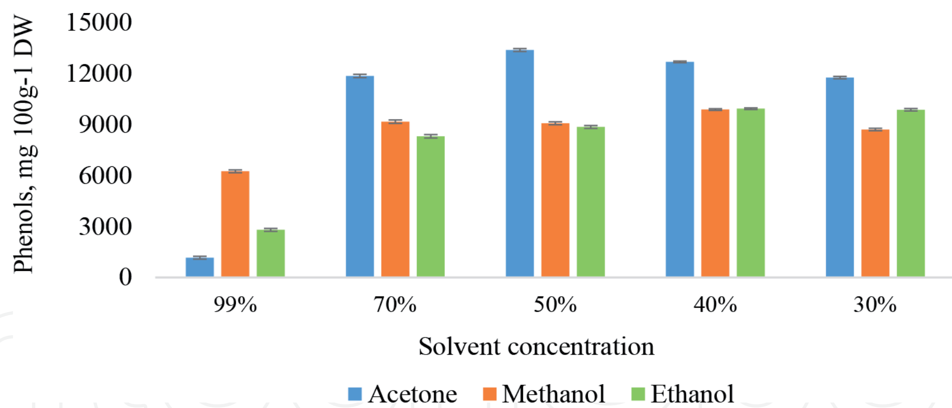

Figure 9.
Impact of solvents and their concentration on the total content of phenolic compounds in quince leaf.

The following ranges of independent variables were chosen for the extraction of phenolic compounds from quince leaves in the ultrasonic bath—ultrasonic power 48 W–480 W, temperature 30–60°, and time 20–60 minutes (**Figure 10**).

Based on the developed model, the obtained results showed that the effect of all the three variables was not significant and had no effect on the extraction efficiency, except that the process was shortened by 23 hours compared to simple maceration. The highest extraction yield of phenolic compounds was obtained when the samples were extracted with 480 W ultrasonic power for 60 min, at 60°C temperature (**Figure 10**).

Our previous studies revealed that leaves of Rasa, Darius, and Rondo cultivated in both Latvia and Lithuania were rich in biologically active compounds [11, 35]. The highest amount of phenols were found in Darius and the lowest in Rondo leaves. The identified compounds belong to three main groups—hydroxycinnamic acid derivatives, flavonols, and flavan-3-ols. The chlorogenic acid was the most common

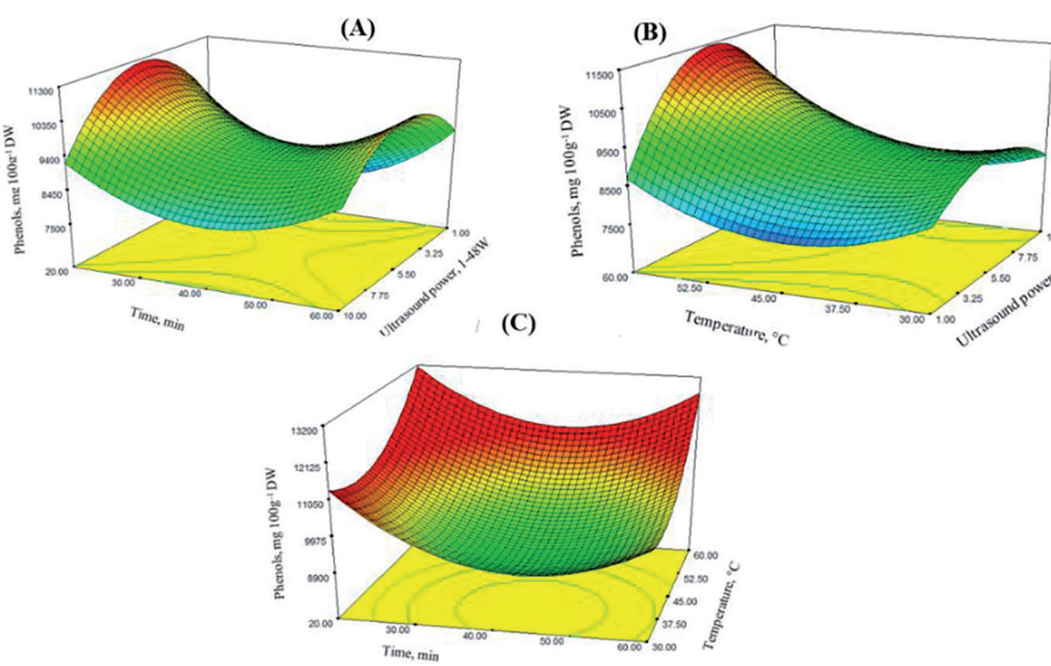

Figure 10.
The influence of (A) – time and ultrasound power, (B) – temperature and ultrasound power, (C) – time and temperature on extraction efficiency of phenols from JQ leaves.

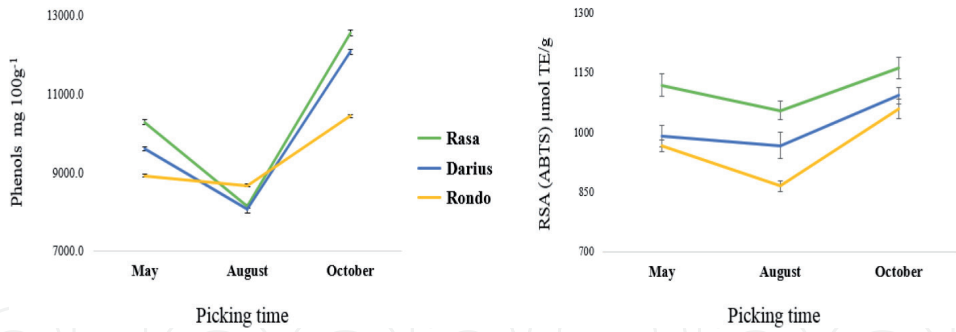

Figure 11.
Effect of cultivar and climate conditions on the accumulation of phenolic compounds and radical scavenging activity in quince leaves.

in leaves of all three cultivars, which accounts for about 80% of the total phenolic compound [11, 35].

The environmental conditions in different seasons also significantly influenced the total amount of phenolic compounds and antiradical activity in leaves. They accumulate higher levels of phenolic compounds in spring and autumn in adverse weather conditions such as frosts and temperature fluctuations, and less levels in summer under more favorable conditions (**Figure 11**). To summarize, the development of new needleless cultivars Rasa, Darius, and Rondo has facilitated collecting promising and long-undervalued raw materials, such as *Chaenomeles japonica* leaves. Moreover, the maximum phenols content and the best time to collect was at a very convenient time after harvesting fruits without affecting the quality or yield parameters.

Due to the significant amount of phenols in JQ leaves, the antimicrobial activity against three Gram-positive and three Gram-negative bacteria, and one yeast strain, in Rasa, Darius, and Rondo was identified. JQ leaves have shown antibacterial activity against microorganisms, the strongest inhibition effect was obtained for the *Enterococcus faecalis* (ATCC 29212) strain using all cultivars extracts (**Table 6**). Extracts of Rasa leaves had a stronger inhibitory effect on *Bacillus subtilis* and *Pseudomonas aeruginosa*, while Rondo inhibited *Staphylococcus aureus* stronger. However, extracts have not shown antifungal activity against *Candida albicans* (ATCC 10231) yeast strain.

A previous study has been reported that *C. japonica* leaves extracts with similar predominant phenolic compounds had antibacterial activity against the same four strains, however, antifungal activity against yeast strain *C. albicans* (ATCC 10231) was demonstrated [48].

Although no significant differences were detected between cultivars in fiber content, leaves had a higher amount than their fruits (**Figure 12**).

3.4 Japanese quince applications and health benefits

Recent research on *Chaenomeles japonica* and cultivars Rasa, Darius, and Rondo have extended their range of applications, which are summarized in **Table 7**. Fruits of these cultivars are promising for pharmaceutical industries due to their biochemical composition, anticancer, antibacterial, anti-inflammation properties, and hypoglycaemic effect.

JQ fruit extracts rich in proanthocyanidins showed proapoptotic activity in Caco-2 cells [13]. Their extracts rich in flavanols had an antiproliferative effect against various cancer cells, decreased their invasiveness by regulating several genes involved in apoptosis, angiogenesis, and metastasis [14, 49]. Their extracts also demonstrated

	Microorganism	Extract Conc., %	*C. japonica*		
			Rasa	Darius	Rondo
			Inhibition zone size, mm		
Gram-positive	*Bacillus subtilis* (ATCC 6633)	0.5	10.0 ± 0.0[a]	11.3 ± 0.5[a]	11.0 ± 0.0[a]
		1	15.0 ± 0.0[a]	15.3 ± 0.5[a]	12.0 ± 0.0[b]
		5	18.3 ± 0.5[a]	17.0 ± 0.0[a]	14.7 ± 0.5[b]
	Enterococcus faecalis (ATCC 29212)	0.5	17.0 ± 0.0[b]	17.3 ± 0.5[b]	20.0 ± 0.0[a]
		1	20.0 ± 0.0[b]	20.3 ± 0.5[b]	23.0 ± 0.0[a]
		5	25.3 ± 0.5[a]	24.0 ± 0.0[a]	25.7 ± 0.5[a]
	Staphylococcus aureus (ATCC 25923)	0.5	9.0 ± 0.0[a]	9.3 ± 0.5[a]	0.0
		1	14.0 ± 0.0[a]	10.3 ± 0.5[b]	11.0 ± 0.0[b]
		5	16.3 ± 0.5[b]	16.0 ± 0.0[b]	17.7 ± 0.5[a]
Gram-negative	*Escherichia coli* (25922 ATCC)	0.5	9.0 ± 0.0[a]	9.3 ± 0.5[a]	10.0 ± 0.0[a]
		1	10.0 ± 0.0	10.3 ± 0.5	11.0 ± 0.0
		5	15.3 ± 0.5	15.0 ± 0.0	15.6 ± 0.5
	Pseudomonas aeruginosa (27853 ATCC)	0.5	0.0	0.0	9.0 ± 0.0[a]
		1	11.7 ± 0.8[a]	10.3 ± 0.5[a]	11.0 ± 0.0[a]
		5	16.3 ± 0.5[a]	15.0 ± 0.0[a]	12.7 ± 0.5[b]
	Salmonella Typhimurium (ATCC 14028)	0.5	9.0 ± 0.0[a]	0.0	0.0
		1	10.0 ± 0.0[a]	9.3 ± 0.5[a]	9.0 ± 0.0[a]
		5	14.3 ± 0.5[a]	15.0 ± 0.0[a]	12.7 ± 0.5[b]
	Candida albicans (ATCC 10231)	0.5	0	0	0
		1	0	0	0
		5	0	0	0

Table 6.
Antibacterial activity of Chaenomeles japonica cultivars leaves extracts. Different letters (a, b, and c) in the same row indicate statistically significant differences between the individual compounds ($p < 0.05$).

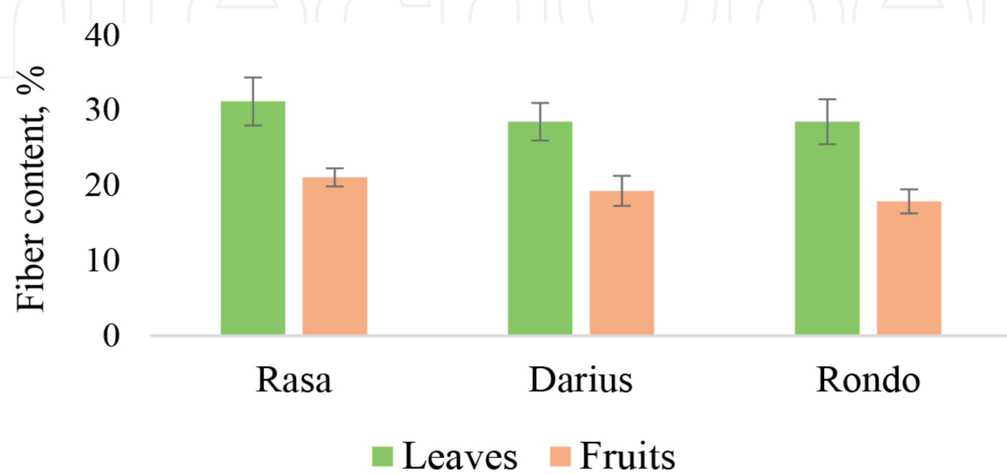

Figure 12.
The total fiber content in Japanese quince leaves and fruit.

Source	Bio compounds	Possible utilization	References
Fruits	Phenols, triterpenes	Functional food, Cosmetic	[2, 10, 26]
		Pharmaceutical (Anti-inflammation)	[47, 51]
		Pharmaceutical (Anticancer)	[14, 49, 50]
		Pharmaceutical (Antibacterial)	[10, 48]
		Pharmaceutical (hypoglycaemic)	[52]
	vitamin C	Functional food, cosmetic	[3, 7, 9]
	Fibers	Functional food	[8, 23]
By-Products			
Pomace	Phenols, fibers	Functional food, Cosmetic	(**Table 2**), [33]
		Pharmaceutical (Antibacterial)	(**Figure 4**), [48]
Seeds	Phenols	Functional food, Cosmetic	(**Tables 3, 4, 5**), [56]
	Tocopherols, carotenoids, squalene, phytosterols	Cosmetic	[29, 31, 61]
	Mucilage	Food safety	[62, 63]
		Pharmaceutical (wound healing)	[64–67]
		Cosmetic (emulsion stabilization)	[68]
Leaves	Phenols, triterpenes	Functional food, Cosmetic	[11, 35]
		Pharmaceutical (Anti-inflammation)	[69]
		Pharmaceutical (Anticancer)	[11, 70]
		Pharmaceutical (Antibacterial)	(**Table 6**)
	Fibers	Functional food	(**Figure 12**)

Table 7.
Promising utilisation of quince Chaenomeles japonica plant.

an inhibitory effect on the MMP-2 and MMP-9 enzymes activity and can be used in cancer chemoprevention [50]. Besides, they showed the ability to protect biological membrane lipids from oxidation, and usage of those extracts as an antioxidant drug can be a prevention for diseases caused by inflammation or oxidative stress [47, 51]. Moreover, the human cells of hepatoma HepG2 pretreated with JQ phenols-rich extracts as modulators of carbohydrates metabolism showed a promising hypoglycemic effect and decreased intracellular ROS accumulation [52].

Due to the high content of bio-compounds with positive effects on human health, fruits, leaves, and by-products of cultivars Rasa, Darius, and Rondo are excellent raw materials for functional food, which allows increasing antioxidants in final products (**Table 7**). Moreover, extracts from fruit and their pomace, leaves, seeds, could be used as an antibacterial agent and a substitute for chemical preservatives in both the food and cosmetic industry. The importance of antioxidants for skincare is as essential as their internal consumption, so all JQ organs are a great source for cosmetic products as plant-based raw material.

Several studies showed that seeds of JQ are suitable for obtaining the oil rich in α-tocopherol, carotenoids, squalene, phytosterols, and phenols [29, 31, 61]. It was reported that seeds remaining after industrial manufacturing are still suitable for oil recovery. The yield and biochemical composition of oils depended on both the processing of seed pretreatment and the oil extraction method [29]. The biochemical composition varied between cultivars, especially the profile of fatty acids and accumulation of bioactive compounds, while oil yield was affected by the extraction technique [71]. The lowest oil yield was obtained using the cold-press method from Rondo seeds, and the largest from Rasa using ultrasonic extraction [71]. Despite the fact that JQ seed oils contain strong antioxidants it is not recommendable for the food industry regarding a very high omega-6/omega-3 ratio [29, 61, 72]. However, JQ seed oil is well suited for skincare products regarding the high content of linoleic acid and perfect fitness of linoleic acid/oleic acid ratio [73]. In addition, studies have shown that quince seeds are suitable for other products, such as mucilage preparation, which have various biological activities and possible applications [32, 64]. Quince seed mucilage has been used successfully for wounds and toxin-damaged skin treatment [64–67]. Quince seed's ability to form edible films together with oregano or thyme essential oil can be used to ensure food safety, and extend the shelf life of food products [62, 63]. Ethanol extract from quince seeds protected skin from allergen-induced Th2-type inflammation and reduced the effects of atopic dermatitis [74]. In addition, quince seed can be used for skincare products as an excellent emulsifier and stabilizer [68].

The same as fruits and seeds of these cultivars, leaves are promising raw material due to the high amount of chlorogenic acid that has shown many biological properties, including antibacterial, antioxidant, anticarcinogenic activities, hypoglycaemic and hypolipidemic effects [75–77]. The compounds with strong biological activity such as triterpenes have been detected in these three cultivar leaves as well [35]. Among the four identified triterpenes, ursolic and oleanolic acid were predominant, whose functions for human health have been investigated including cardioprotective [78], strong immunomodulatory [79], suppress tumorigenesis [80], valuable antimetastatic agent [81], and prevention or alleviation of glycation-associated renal diseases [82]. Finally, other *C. japonica* species leaves extracts, and extracts from Rasa, Darius, and Rondo demonstrated anticancer and anti-inflammatory activities [11, 69, 70].

4. Conclusion

This work about *C. japonica* and new cultivars Rasa, Darius, and Rondo will expand the knowledge about their new possible application in the food, cosmetic and pharmaceutical industries. As well as the utilization of additional materials, such as waste (pomace and seeds) and leaves, could provide economic and ecological advantages.

Conflict of interest

The authors declare no conflict of interest.

Author details

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