

Comparative Analysis of Polyphenolic Profiles and Antioxidant and Antimicrobial Activities of Tunisian Pome Fruit Pulp and Peel Aqueous Acetone Extracts

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Pome trees, apple, pear, and quince, are classified into the subfamily Pomoideae, belonging to the Rosaceae family. Their autumnal fruits are consumed worldwide in different forms, that is, fresh or transformed into jams, jelly, juices, etc. Their well-established beneficial properties to human health were found mainly related to their phenolic content. Pulp and peel aqueous acetone extracts obtained from Tunisian fruits at commercial maturity were comparatively evaluated for their phenolic profiles and antioxidant and antimicrobial potentials. The phenolic compounds present in the extracts were identified and quantified using RP-HPLC-DAD and ESI-MS techniques. Significant differences in the chromatographic profiles among these fruits, as well as between pulp and peel extracts of each fruit, were observed. Quince, followed by 'Red Delicious', peel extracts showed the highest phenolic content (160.33 and 110.90 mg/100 g of fresh weight). The stronger inhibitory effect on DPPH radicals corresponded to those obtained from peel materials. A comparative analysis of the antimicrobial potential against a range of microorganism strains was also carried out. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus cereus* were the most sensitive to the active extracts. Among the examined phenolic extracts, 'Red Delicious' and quince peels showed the highest effects for inhibiting bacteria growth. Minimum inhibitory and bactericide concentrations ranged from 10² to 10⁴ µg of polyphenol/mL. Red skin apple and quince peels could be of great interest as important antioxidant and antimicrobial polyphenol sources.

KEYWORDS: Antimicrobial; antioxidant; ESI-MS; HPLC; pome fruit; polyphenol

INTRODUCTION

Phenolics are broadly distributed in plants and constitute their most abundant secondary metabolites (1). In general, plant materials contain complex mixtures of phenolics. These compounds are classified according to their structure as phenolic acids derivatives, flavonoids, and tannins (2). These molecules possess an aromatic ring bearing one or more hydroxyl groups (–OH). They are hydrogen-donating, antioxidants, and singlet oxygen quenchers (3). Their antioxidant potentials are superior to those of other well-known antioxidants, such as vitamin C, vitamin E, and β-carotene (4).

Polyphenols from fruits, vegetables, and beverages have received considerable interest based on positive reports of their

presumed role in the prevention of various human diseases (5–7). Apple, pear, and quince are the most of the important temperate fruits. They have been known as pome trees classified into the genus *Malus* of the Pomoideae subfamily, belonging to the Rosaceae family. Their fruits are popular because of the many ways that they can be consumed. They may be eaten off the tree or processed into sauce, slices, jams, or juices and are favored for pastries, cakes, tarts, and pies (8). In addition, apples have become the symbol of wholesomeness: "An apple a day keeps the doctor away" is a favorite aphorism. With the expanding production, the transport networks, and the requirements for high yield of commercial quality, a few cultivars have dominated all of the major growing areas. The most widely grown apple cultivars by far are the yellowish green skinned 'Golden Delicious' and the red skinned 'Red Delicious', both chance seedlings of American origin. For pear, the 'Williams' cultivar has been widely and successfully used in many countries.

Many studies have been developed in apple, pear, and quince fruits and their derivatives (5, 8–12). These works studied

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separately these fruits, essentially their phenolic constituents and the antioxidant potential of peel, pulp, and seed extracts. Given that they have been analyzed in different conditions, no reliable conclusions could be drawn about their relative potentials. In addition, little was reported about their phenolic extracts' antimicrobial activities. The work herein represents a contribution to the analysis of these pome fruits' antimicrobial activities, in correlation with their phenolic profiles and antioxidant potentials.

MATERIALS AND METHODS

Standards, Solvents, and Reagents. Phenolic compounds used as reference were purchased from Sigma-Aldrich (Milan, Italy). Standard stock and working (0.05 to 20 mg/L) solutions were prepared in methanol. HPLC-grade methanol and formic acid were obtained from Merck (Milan, Italy). The Folin–Ciocalteu reagent was from Merck (Darmstadt, Germany). Water was distilled and filtered through a Milli-Q apparatus (Millipore, Milan, Italy) before use.

Fruit Material. For each pome fruit, 10 different healthy samples homogeneously (maturity, color, size, weight) purchased from local markets were studied. They are all produced in Tunisia and included two apple cultivars, 'Golden Delicious' and 'Red Delicious'; a pear, 'Williams' cultivar; and a local quince cultivar (*Cydonia oblonga* Miller). The fruits were immediately washed and hand-peeled. Separated pulp and peel parts were cut into thin slices and frozen at $-20\text{ }^{\circ}\text{C}$ until used.

Extraction. A previously described method (13) was used. Briefly, each sample was extracted with 4 volumes (w/v) of cold acetone/water (3:1). After centrifugation at 10000g for 15 min at room temperature, the residue was re-extracted again until negative reaction with NaOH. The pooled collected supernatants were concentrated to dryness under vacuum. The obtained residue was dissolved in a final 1 volume of sterile distilled water, vortexed for 5 min, and filtered through a 0.45 μm Teflon membrane (Millipore). To prevent polyphenol oxidation, extraction was achieved rapidly and extracts were immediately used.

Colorimetric Estimation of Total Phenolic Compounds. Prior to HPLC analysis, and to estimate the phenolic amount to inject, with respect to OD values within the reference calibration curves range, total polyphenol content was evaluated spectrometrically using the Folin–Ciocalteu assay described by Singleton et al. (14) with the (+)-catechin as standard.

Reverse Phase HPLC-ESI-MS Conditions. Phenolic compounds were separated by reverse phase HPLC using a Merck-Hitachi D-7000 LaChrom equipped with a photodiode array detector (DAD) and a Shimadzu LCMS-2010 electrospray ionization mass spectrometer (ESI-MS) under conditions previously described (13). Absorbance spectra were recorded for all peaks. The injection volume for all samples was 50 μL . The identification of each phenolic compound was based on a combination of retention time and spectral matching. The quantification of individual phenolics was achieved from the areas of their peaks recorded at 280 and 350 nm, by comparison with calibration curves obtained with reference compounds solutions of phenolics. The polyphenols concentrations in the analyzed fruits are expressed in milligrams per 100 g of fresh weight (fw).

Radical Scavenging Activity. The antioxidant activity of the phenolic extracts was determined using the Trolox equivalent antioxidant capacity (TEAC), which evaluates the scavenging of the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH $^{\bullet}$) relative to Trolox, a water-soluble vitamin E analogue. An aliquot (50 μL) of sample solution was added to 2 mL of 40 μM DPPH in methanol. The mixture was shaken vigorously and left to stand for 1 h at room temperature in the dark. Controls or blanks were prepared without the sample solution. Radical scavenging activity was calculated from the difference in DPPH radical absorbance at 517 nm between a blank and a sample by the following equation: radical scavenging activity (%) = $(C - A)/C \times 100$, where A = the difference in absorbance at 517 nm between blank and sample and C = absorbance of blank. The extent of quenching of the DPPH $^{\bullet}$ radical was compared with Trolox. Results are expressed as millimolar Trolox equivalents per gram of fresh weight (TEAC).

Table 1. Main Phenolic Compounds Characterized by HPLC-DAD-MS in Apple, Pear, and Quince Pulp and Peel Aqueous Acetone Extracts

peak	t^{R} ^a HPLC-DAD [M + H] $^{+}$			identity
	(min)	λ_{max} (nm)	m/z	
1	4.3	279	273	arbutin (hydroquinone- β -D-glucoside)
2	6.8	279	291	(+)-catechin
3	7.5	325	355	neochlorogenic acid (3-O-caffeoylquinic acid)
4	8.3	279	291	(-)-catechin
5	11.6	325	355	cryptochlorogenic acid (4-O-caffeoylquinic acid)
6	12.3	326	355	chlorogenic acid (5-O-caffeoylquinic acid)
7	17.7	278	579	procyanidin B dimer
8	32.5	355	465	hyperin (quercetin-3-O-galactoside)
9	33.3	356	611	rutin (quercetin-3-O-rutinoside)
10	35.8	275	595	kaempferol-3-O-rutinoside
11	36.1	354	465	isoquercitrin (quercetin-3-O-glucoside)
12	37.3	282	437	phloridzin (phloretin 2'- β -D-glucoside)
13	38.5	278	449	kaempferol 3-O-glucoside
14	44.7	371	303	quercetin
15	48.6	311	612	quercetin glycoside acylated with p -coumaric acid
16	49.5	340	287	kaempferol

^a HPLC retention time.

Antimicrobial Analysis. The agar well (6 mm \varnothing) diffusion method (15) was used for the determination of antimicrobial activities. For each test, 100 μL of the sample was added to the well. After incubation at 37 $^{\circ}\text{C}$ (bacteria)/27 $^{\circ}\text{C}$ (*Candida albicans*) for 24 h or at 22 $^{\circ}\text{C}$ for 4–12 days (*Aspergillus niger*), the resulting inhibition zone diameters were measured. Furthermore, the minimum inhibitory and bactericide concentrations (MIC and MBC, respectively) of the phenolic extracts against the test microorganisms were determined using the broth microdilution method followed by the counting of surviving cells on PCA plates (16). All tests were performed in triplicate. A range of microorganisms were tested: *Staphylococcus aureus* (ATCC 6538 and ATCC 25923), *Staphylococcus epidermidis* (CIP 106510) and *Bacillus cereus* ATCC 11778 for Gram-positive bacteria; *Escherichia coli* (ATCC 8739 and ATCC 35218), *Pseudomonas aeruginosa* (ATCC 9027 and ATCC 27853), and a *Salmonella* sp. strain (isolated from food) for Gram-negative bacteria; the yeast *Candida albicans* (ATCC 14053); and the mold *Aspergillus niger*.

Statistical Analysis. Each experiment was repeated at least twice, and each determination was carried out in triplicate. Data presented are averages with a standard deviation of <10%. Statistical analysis (one-way ANOVA with Dunnett's post test) of data was carried out by computations using GraphPad Prism 4.03 (San Diego, CA). Differences of $P < 0.05$ were considered to be significant.

RESULTS

Identification and Quantitation of Phenolic Compounds.

The total phenolic content of all the extracts was found to be relatively high as estimated using the Folin–Ciocalteu method [1.8–4.6 mg of (+)-catechin equivalent/mL of extract]. A 10-fold dilution in water was required prior to HPLC analysis. The fractionation of the different extracts by RP-HPLC coupled to DAD followed by ESI-MS spectra analysis allowed the identification of the main phenolic compounds (Table 1) in comparison with authentic standards. Typical chromatographic separations achieved at 350 nm are shown in Figure 1. Total polyphenol contents were significantly distinct among the different fruit extracts (Figure 2). The highest (160.33 mg/100 g of fw) and the lowest contents (9.58 mg/100 g of fw) were found in the quince peel and 'Golden Delicious' pulp extracts, respectively. In addition, for each fruit, the polyphenolic content of peel extract was 1.7–4 times more important than that of the pulp extract.

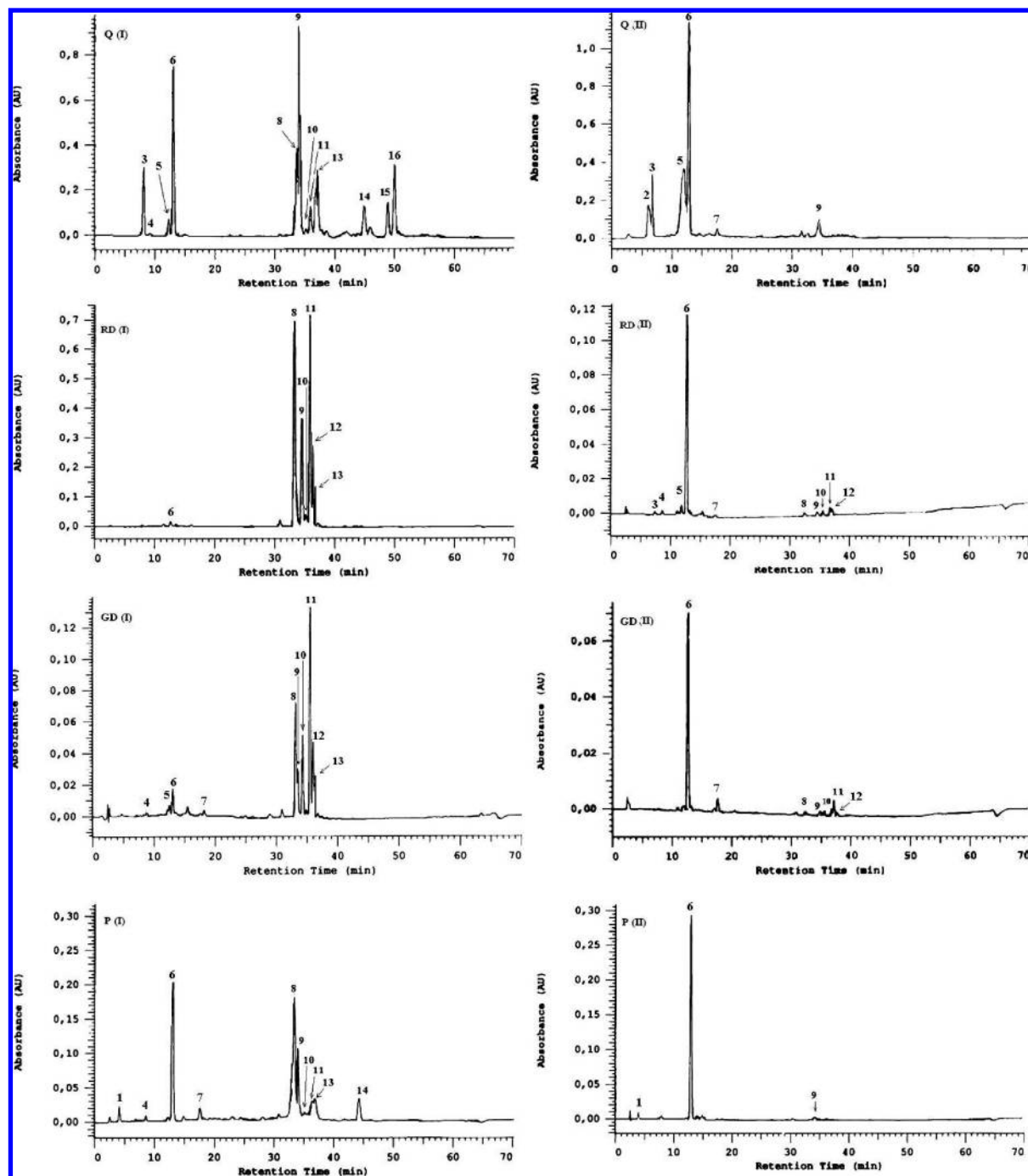


Figure 1. Representative HPLC chromatograms of apple, pear, and quince peel (I) and pulp (II) aqueous acetone extracts recorded at 350 nm: Q, quince; RD, apple 'Red Delicious'; GD, apple 'Golden Delicious'; P, pear 'Williams'. For peak identification see **Table 1**.

Antioxidant Activity. The radical scavenging activities of the reference compound Trolox and the quince, pear, and apple peel and pulp aqueous acetone extracts are presented in **Figure 3**. The lowest and greatest antioxidant effects were observed with pear pulps (4% of inhibition) and quince peels (57% of inhibition), corresponding to 0.03 and 0.44 mM Trolox equivalent, respectively. A good correlation was observed between the antioxidant potential and the total polyphenol content ($r = 0.732$), suggesting that phenolic compounds are the major contributors to these activities.

In Vitro Antimicrobial Tests. The antimicrobial properties of polyphenolic extracts have been examined, and the obtained results are summarized in **Table 2**. The agar diffusion test allowed us to select the susceptible bacteria and the active

phenolic extracts (inhibition zone $\varnothing \geq 8$ mm) for further investigation. Among the tested microorganisms, *S. aureus* and *S. epidermidis* showed susceptibility to all of the polyphenolic extracts, followed by *B. cereus* and *P. aeruginosa*, *E. coli*, and the yeast *C. albicans*. With the mold *A. niger*, no inhibition was obtained with the tested extracts. In the case of *Salmonella*, only 'Red Delicious' peel extract showed a moderate antimicrobial activity. Practically, no significant differences in susceptibility between the two species *S. aureus* and *S. epidermidis* as well as between bacterial strains within the same species were observed. The MICs and MBCs of each phenolic extract against the different bacteria were determined (**Table 2**). The values varied from 10^2 to 10^4 $\mu\text{g/mL}$. Peel extracts exhibited more antimicrobial potential than the pulp ones, reflecting their

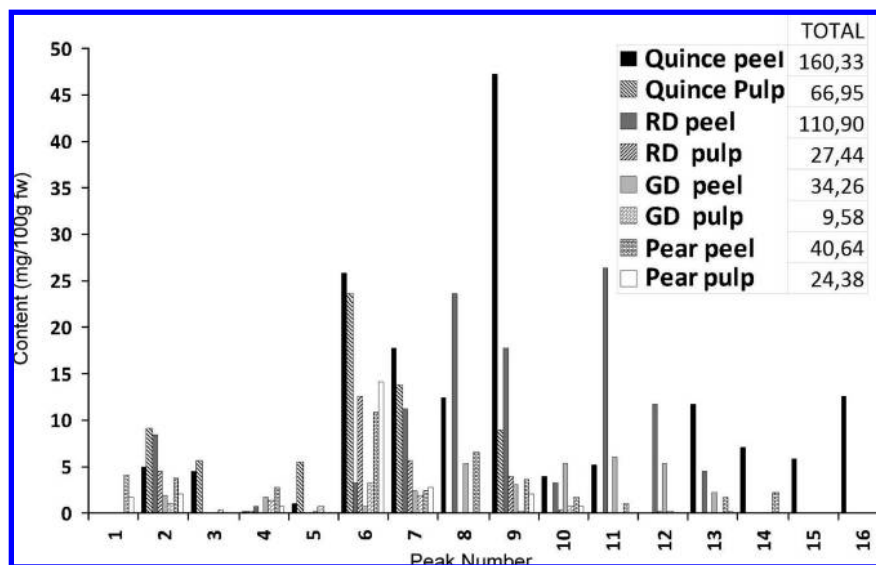


Figure 2. Phenolic content of the different aqueous acetone extracts: RD, apple 'Red Delicious'; GD, apple 'Golden Delicious'. The total phenolic contents, calculated from the sum of the determined individual compounds concentrations, are indicated. Data presented are averages with a standard deviation of <10% ($n = 3$). See **Table 1** for peak identity.

qualitative and quantitative biochemical differences. The 'Red Delicious' peel extract was the most active against all of the tested microorganisms.

DISCUSSION

To compare the physicochemical state of phenolics in the Tunisian quince, apple, and pear fruits, the polyphenols were extracted from the separated pulp and peel slices. The aqueous/acetone solvent (3:1) had been previously shown to provide a good extraction of the main polyphenols from quince peel and pulp material (13). Using the same procedure, we carried out a comparative analysis of the three fruits. The reduced number of the extraction steps, avoiding hydrolyzing reactions, should preserve the native bioavailable forms of the phenolics, which is indispensable in the evaluation of their biological significance. The analytical RP-HPLC separation of the different polyphenolic compounds in the analyzed extracts (**Figure 1**) showed typical chromatograms previously reported for these pome fruits (13, 17–20). Within each lot of samples, obtained data were homogeneous and standard deviations were found to be <10%, reflecting the uniformity of our sampling and the good repeatability and reproducibility of the method. As quince, pear, and apple belong to the same botanical family, it was not surprising to find common peaks in their respective HPLC chromatograms. Nevertheless, for each fruit, peel and pulp extracts showed different polyphenolic profiles. In this way, we were able to draw a detailed diagram containing the amount of up to 16 single compounds (**Figure 2**). The purity of the observed peaks, as determined using diode array detection, generally reached 100%. With the exception of the peaks 7 and 12, all of the compounds present in the standard mixture were identified in real samples (**Table 1**). On the basis of their UV spectra and positive ion masses, peaks 7 (m/z 579) and 12 (m/z 437) seem most likely to be a procyanidin B dimer and phloridzin, respectively, as reported in previous studies (20). Chromatograms at 280 nm were used to quantify flavanols, procyanidin B, and the phloridzin dihydrochalcone, whereas hydroxycinnamic acids and flavonols were quantified at 350 nm. Peaks 7 and 12 were estimated as (+)-catechin equivalent. The total phenolic contents, calculated from the sum of the determined individual compounds concentrations, were found to be significantly higher

in the peel than in the pulp of each fruit. This result supports conclusions of others and our previous data (13, 17, 18). When the peel extracts are analyzed, it can be seen that the highest phenolic contents were those of quince (160.33 mg/100 g of fw) and 'Red Delicious' (110.90 mg/100 g of fw), whereas the lowest contents were in the samples of 'Golden Delicious' (34.26 mg/100 g of fw). With regard to the pulp extracts, only quince showed a relatively high phenolic content (66.95 mg/100 g of fw), whereas the other fruits ranged from 10 mg/100 g of fw ('Golden Delicious') to 40 mg/100 g of fw (pear cv. 'Williams'). Furthermore, the phenolic compounds distributions among the analyzed extracts were different. Some phenolics were exclusively found in the peel extracts, namely, peaks 14–16, whereas others were present in peels at relatively higher concentrations than in pulp extracts, namely, peaks 8–13. We found that peels mainly consisted of hydroxycinnamic acids, flavonols, and flavanols, whereas the pulps consisted of only hydroxycinnamic acids, a statement in agreement with previous studies (6, 17, 21). Chlorogenic acid, especially the 5-*O*-caffeoylquinic acid (peak 6), was the main phenolic compound present in the pulps, at 35, 46, 34, and 58% (w/w) in quince, 'Red Delicious', 'Golden Delicious', and pear, respectively. Subsequently, procyanidin B (peak 7) was found at moderate proportions in all of the pulps (19–21%) except for pear, in which it was not detectable. The presence of the chlorogenic acid and procyanidin B was also noteworthy in the quince peel extracts (17 and 12%, respectively) following the main polyphenol (peak 9), rutin (31%). In the pear pulp extracts, the chlorogenic acid was also the main compound (27%) followed by hyperin (16%). With regard to the two apple cultivars, 'Red Delicious' (RD) and 'Golden Delicious' (GD), roughly the same profiles were observed with different compound concentrations. This observation is in agreement with previous reports about *Malus* species (22). The total contents of the 'Red Delicious' extracts were greater than those of the 'Golden Delicious'. In the apple pulps, chlorogenic acid was the main polyphenol (46% for RD and 34% for GD), whereas in the peels hyperin (peak 8) and isoquercitrin (peak 11) represented the highest proportions (21–24% for RD and 16–17% for GD, respectively). Arbutin (hydroquinone- β -D-glucoside) and phloridzin (phloretin 2'-glucoside), known as characteristic phenolic markers, respec-

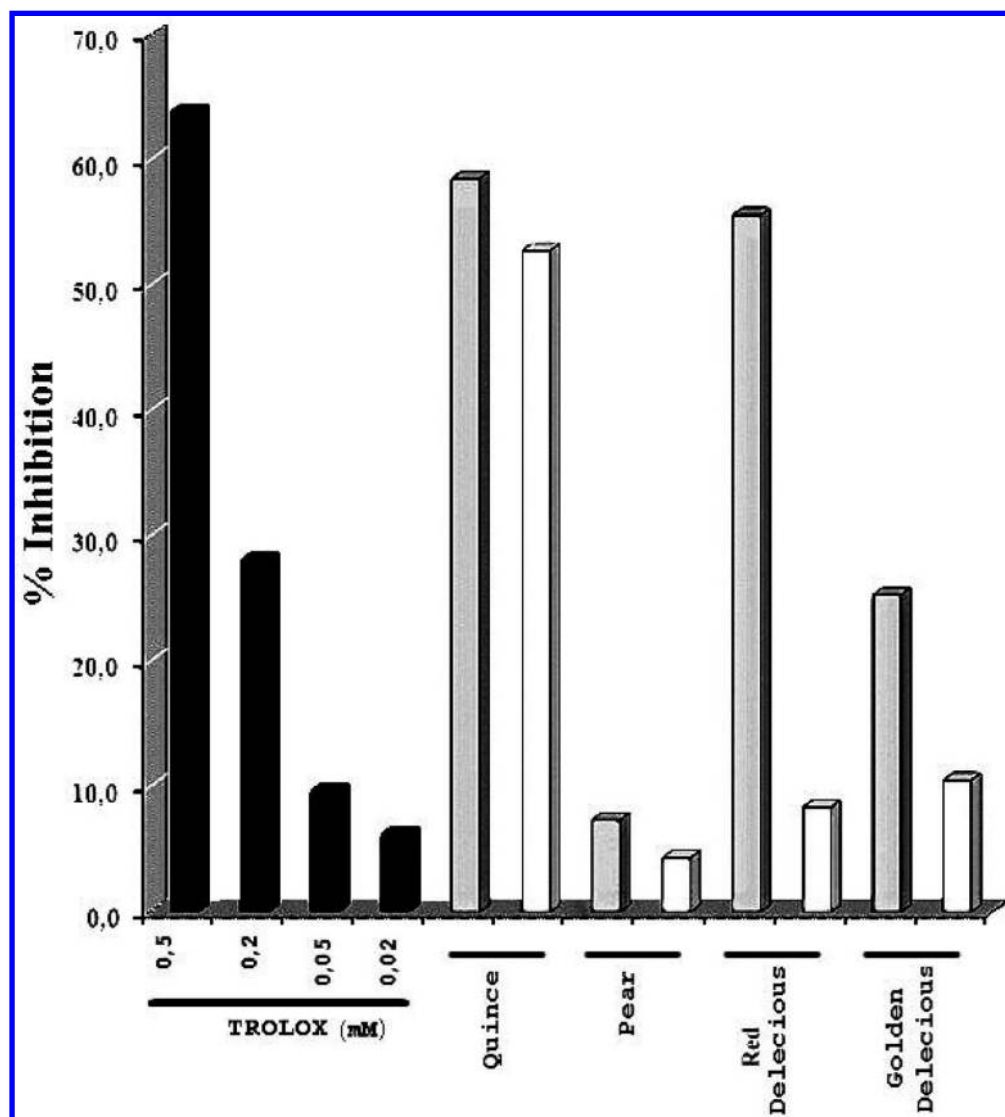


Figure 3. Comparative diagram showing average values of radical scavenging activity of Trolox (black bars), quince, pear, and apple peel (gray bars), and pulp (white bars) aqueous acetone extracts. Data presented are averages with a standard deviation of <10% ($n = 3$).

tively, for pear and apple (18), were detected in our work, reaching greatest amounts in the peels, a finding in accordance with other studies (17, 19, 21).

To assess the antioxidant potential of the prepared extracts, we used the DPPH scavenging test. As illustrated in **Figure 3**, samples varied within a wide range of values. Using the same method, Leontowicz et al. (9) determined the antioxidant activity of apple and pear fruits and found results similar to our observations. Moreover, the antioxidant activity observed in the peels, which are quantitatively and qualitatively rich in phenolic compounds, was greater than that of the corresponding pulps, confirming earlier studies (12, 13, 23). Relating the results obtained for the apple cultivars, we can conclude that the red skinned fruits are those showing the greatest antioxidant capacity. The red skinned varieties are known to contain anthocyanins, pigments having a possible influence in this activity, as suggested in the literature (11). Vrhovsek et al. (20) analyzed different apple varieties and detected anthocyanidins in 'Red Delicious' (2.52 mg/100 g of fw, essentially cyanidin-3-galactoside) but not in 'Golden Delicious'.

Furthermore, employing the well diffusion technique, we evaluated the antimicrobial activity of the phenolic extracts against a range of microorganisms that may be responsible for

foodborne diseases and/or spoilage of contaminated products. Halo zone diameter increased in the following order: pear pulp \approx pear peel < GD pulp < GD peel < RD pulp \approx quince pulp < quince peel < RD peel. Nonetheless, curiously, this activity was not found to be highly correlated to the total phenolic contents of the extracts. Alberto et al. (24) analyzed the antimicrobial effect of only apple using two cultivars, a yellowish red skinned fruit (cv. Royal Gala) and a greenish white skinned fruit (cv. Granny Smith) from Argentina, and found a direct relationship between the phenolic content and this activity. This is not contradictory to our results, because within apple peel and pulp samples we have also found a good correlation between these parameters. The fact that we did not find a good correlation between antimicrobial activity and phenolic content of all the extracts could be explained by considering qualitative more than only quantitative aspects. In addition, the inhibition of each of the pathogens tested could be the result of the synergism (or antagonism) of many components, still unknown. The broth microdilution method was also carried out to confirm and deepen the investigation. The agar diffusion test results against the sensitive bacteria were found in accord with those of the microdilution assay. The most susceptible microorganisms were *S. aureus*, *P. aeruginosa*, and *B. cereus*. The results

Table 2. Antibacterial Activity of the Apple, Quince, and Pear Pulp and Peel Aqueous Acetone Extracts

microorganism:	<i>S. aureus</i>		<i>S. epidermidis</i>	<i>B. cereus</i>	<i>P. aeruginosa</i>		<i>E. coli</i>		<i>Salmonella</i> sp.	<i>C. albicans</i>
strain/isolate:	ATCC 6538	ATCC 25923	CIP 106510	ATCC 11778	ATCC 9027	ATCC 27853	ATCC 8739	ATCC 35218	isolated from food	ATCC 14053
Diameter ^a of the Inhibition Zone										
quince peel	27.7 ± 2.4	25.5 ± 3.2	25.8 ± 1.3	25.3 ± 2.2	18.3 ± 0.9	15.5 ± 0.8	10.5 ± 1.1	09.6 ± 0.5	w	09.3 ± 0.1
quince pulp	18.5 ± 1.8	15.8 ± 1.3	16.5 ± 0.7	16.5 ± 1.5	15.2 ± 1.4	13.4 ± 0.5	08.7 ± 0.4	w	w	w
RD peel	31.2 ± 2.6	29.2 ± 2.7	28.9 ± 2.3	27.3 ± 2.1	29.3 ± 1.5	28.5 ± 2.0	10.9 ± 0.7	09.2 ± 0.3	10.4 ± 1.2	25.7 ± 2.2
RD pulp	19.8 ± 1.5	16.7 ± 1.1	17.4 ± 1.8	17.8 ± 1.3	19.5 ± 1.8	18.3 ± 1.4	w	w	w	17.3 ± 1.5
GD peel	12.8 ± 1.3	10.6 ± 1.0	11.5 ± 1.5	10.7 ± 1.2	09.1 ± 0.5	08.7 ± 0.4	09.5 ± 0.9	10.0 ± 0.5	w	10.8 ± 1.5
GD pulp	09.7 ± 0.4	08.8 ± 0.6	08.6 ± 0.5	08.8 ± 0.7	w	w	w	n	n	n
pear peel	08.5 ± 0.7	w	w	08.9 ± 0.4	w	w	n	n	n	n
pear pulp	08.3 ± 0.5	w	w	w	n	n	n	n	n	n
MIC ^b (μg/mL)										
quince peel	10 ²	10 ²	10 ²	5 × 10 ²	5 × 10 ²	5 × 10 ²	10 ³	10 ³	—	5 × 10 ³
quince pulp	10 ²	10 ²	10 ²	10 ³	10 ²	5 × 10 ²	5 × 10 ²	—	—	—
RD peel	10 ²	10 ²	5 × 10 ²	10 ²	5 × 10 ²	10 ²	10 ³	5 × 10 ³	10 ³	10 ²
RD pulp	5 × 10 ²	5 × 10 ²	10 ³	5 × 10 ²	5 × 10 ³	5 × 10 ³	—	—	—	5 × 10 ²
GD peel	5 × 10 ³	10 ⁴	5 × 10 ³	5 × 10 ³	10 ⁴	5 × 10 ³	10 ⁴	10 ⁴	10 ⁴	5 × 10 ²
GD pulp	10 ⁴	10 ⁴	10 ⁴	10 ⁴	—	—	—	—	—	—
pear peel	5 × 10 ³	—	—	5 × 10 ³	—	—	—	—	—	—
pear pulp	10 ⁴	—	—	—	—	—	—	—	—	—
MBC ^b (μg/mL)										
quince peel	10 ²	10 ²	10 ²	>	10 ²	10 ²	5 × 10 ²	5 × 10 ²	—	5 × 10 ³
quince pulp	5 × 10 ²	5 × 10 ²	5 × 10 ²	>	10 ³	10 ³	5 × 10 ³	—	—	—
RD peel	10 ²	10 ²	5 × 10 ²	>	5 × 10 ²	10 ²	10 ³	5 × 10 ³	10 ³	10 ²
RD pulp	10 ³	10 ³	10 ⁴	>	10 ⁴	10 ⁴	—	—	—	5 × 10 ³
GD peel	5 × 10 ³	10 ⁴	>	>	>	>	>	>	—	10 ³
GD pulp	>	>	>	>	—	—	—	—	—	—
pear peel	10 ⁴	—	—	>	—	—	—	—	—	—
pear pulp	>	—	—	—	—	—	—	—	—	—

^a Inhibition zone including the well diameter (mm). ^b Tested phenolic concentrations (μg of polyphenols/mL of medium culture). Values are mean ± SD of three separate experiments done in triplicate. n, no antimicrobial activity, Ø = 6 mm; w, weak antimicrobial activity, 6 mm < Ø < 8 mm; —, not determined; >, no bactericide effect until 10⁴ μg/mL; RD, 'Red Delicious'; GD, 'Golden Delicious'. No antimicrobial activities were found for the mold *A. niger* (not given in the table).

confirmed earlier findings on the susceptibility of these bacteria to plant phenolic extracts (25). *B. cereus* is a hazardous food-poisoning organism incriminated in many foodborne outbreaks. Other *Bacillus* species are being increasingly investigated, particularly the *subtilis* group, which has been associated with incidents of foodborne gastroenteritis (26). Thus, our results could be extended to these food-poisoning strains of *Bacillus* species and could lead to a promising strategy for their control. The present comparative study highlights the importance of 'Red Delicious' peel extract, which exhibits the greatest antimicrobial effect against both Gram-positive and Gram-negative bacteria. Among the studied pome fruits, 'Red Delicious' seems to attract considerable interest for sustaining human health. Because apple skin color could cover a wide range of intense red in different cultivars (8), our study might be extended to other red skinned cultivars to explore additional biological potentials. In a previous paper (13), the individual phenolic compounds were tested, and chlorogenic acid was found to be the most active substance in quince with a wide bacterial spectrum. In the case of the 'Red Delicious' peels, this compound was present in small amounts, whereas the main phenolics found were quercetin and kaempferol glycosides, suggesting their contribution to this activity. In addition, phloridzin could play a role in this effect, because it was present at noticeable proportions in 'Red Delicious' peels and was reported to be active against a variety of microorganisms (27). It is curious to observe that MBC values were found to be equal or 5–10 times more important than MICs for the examined susceptible bacteria except for *B. cereus*, for which only a bacteriostatic effect was observed even at the highest phenolic concentrations used in our experiments (10⁴ μg/mL). Bearing in mind that phenolic compounds could affect bacterial growth by adsorption to cell membranes, interaction with

enzymes, or deprivation of substrate and metal ions (28), we can assume that structural diversity of the bioavailable phenolics in the studied fruit extracts will influence their antimicrobial properties.

In conclusion, this work contributes to the knowledge of the beneficial properties of the phenolic extracts prepared from apple, pear, and quince fruits. Thus, this investigation is the first report on the comparative analysis of the antimicrobial and antioxidant properties of these pome fruits. The obtained results show that red skinned apples and, to a lesser degree, quince peels and pulps have to be used in individual consumption and for high industrial processing of dietary antioxidant formulated food products, incessantly in development nowadays.

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