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Antimicrobial Activity of Tunisian Quince (*Cydonia oblonga* Miller) Pulp and Peel Polyphenolic Extracts

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Quince (*Cydonia oblonga* Miller) fruit aqueous acetone extracts were evaluated. High-performance liquid chromatography−diode array detection and electrospray ionization−mass spectrometry were used for the identification and quantification of the phenolic compounds. The total phenolic content of the pulp and peel parts ranged from 37 to 47 and 105 to 157 mg/100 g of fresh weight, respectively. Chlorogenic acid (5-*O*-caffeoylquinic acid) was the most abundant phenolic compound in the pulp (37%), whereas rutin (quercetin 3-*O*-rutinoside) was the main one in the peel (36%). The radical scavenging potential of the extracts was determined and compared with that of synthetic antioxidants. The stronger properties corresponded to those obtained from peel material with a 70−80% inhibitory effect on DPPH radicals. The antimicrobial activity of the extracts against different microorganism strains was also investigated. Quince peel extract was the most active for inhibiting bacteria growth with minimum inhibitory and bactericide concentrations in the range of $10^2-5 \times 10^3$ μg polyphenol/mL. It seems that chlorogenic acid acts in synergism with other components of the extracts to exhibit their total antimicrobial activities.

KEYWORDS: Antimicrobial; antioxidant; ESI-MS; HPLC; polyphenols; quince

INTRODUCTION

The trend to view many foods not only as sustenance but also as medicine, so-called functional foods, is increasing. In addition, given that the presence of chemical residues in foods and labeling of preservatives on food packages are major concerns to consumers these days, the substitution of synthetic food additives by "naturally derived" antioxidant and/or antimicrobial agents is desirable (*1*−*3*). These components can be useful in the food industry to prevent rancidity and microbial spoilage.

Biomolecules in fruits and vegetables have attracted a great deal of attention mainly concentrated on their role in preventing diseases. Epidemiological studies have consistently shown that there is a clear significant positive association between intake of fruits and vegetables and reduced rate of heart disease mortalities, common cancers, and other degenerative diseases as well as aging (*4*−*6*). The main biomolecules, (poly)phenolic substances, are a class of higher plant secondary metabolites (*7*). Historically, these were considered as antinutrients because some "tannins" were shown to have adverse effects in human metabolism. However, recently, the recognition of the antioxidative properties of these phenolics has evoked a rethinking toward the health benefits of these compounds. These antioxidants act as reducing agents, hydrogen donators, free radicals scavengers, and singlet oxygen quenchers and, therefore, as cell saviors (*8*, *9*). In addition, the significance of these compounds for their antimicrobial activity has been established (*10*−*14*). In general, the mechanisms thought to be responsible for phenolic toxicity to microorganisms include adsorption and disruption of microbial membranes, interaction with enzymes, and substrate and metal ion deprivation (*15*).

The importance of many plants as natural cheap sources of polyphenols and as nutrition promoting human health is well-established (*4*, *9*, *16*). In the past decade, the quince fruit (*Cydonia oblonga* Miller, Rosaceae family) was found as an important source of polyphenolic antioxidants (*17*−*20*). However, there were not any data about antimicrobial activities of the quince fruit polyphenolic extracts. Therefore, the aim of the present work is to highlight the antimicrobial effect of the quince pulp and peel aqueous acetone extracts.

MATERIALS AND METHODS

Sample Preparation. Healthy quince (*C. oblonga* Miller) fruit samples were harvested at commercial maturity from the North of

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Tunisia. Twenty fruits were hand-peeled. Separated pulp and peel parts were cut into thin slices and frozen at −20 °C until used. All samples were analyzed within 6 months of collection.

Material and Solvent Standards. Phenolic compounds used as reference were purchased from Sigma-Aldrich (Milan, Italy). All standards were prepared as stock solutions in methanol. Working standards were made by diluting stock solutions in methanol to yield concentrations ranging between 0.05 and 20 mg/L. Stock/working solutions of the standards were stored in darkness at −20 °C. High-performance liquid chromatography (HPLC)-grade methanol and formic acid were obtained from Merck (Milan, Italy). Water was distilled and filtered trough a Milli-Q apparatus (Millipore, Milan, Italy) before use.

Polyphenols Extraction. Each sample (10 g) was thoroughly mixed with 40 mL of cold acetone/water (3:1 v/v). The mixture was sonicated for 20 min and centrifuged at 10000*g* for 15 min at room temperature. The residue was re-extracted with aqueous acetone until negative reaction with NaOH. The supernatants were collected, pooled, and concentrated to dryness under reduced pressure (40 °C). The residue was dissolved in a final volume of 1 mL of sterile water, vortexed for 5 min, and filtered through a 0.45 μm Teflon membrane (Millipore). To prevent oxidation of the polyphenols, extraction was achieved rapidly and extracts were immediately used.

Reverse Phase HPLC Conditions. Phenolic compounds were determined using a modification of a previously described HPLC technique (*18*). A Merck-Hitachi D-7000 LaChrom HPLC system, having a diode array detector model L-74505, an L-7200 autosampler, and an L-7100 pump (Merck), was used. Data were processed with a Merck-Hitachi D-7000 Chromatography Data Station Software. The separation was achieved on a Waters Spherisorb 5 μm ODS2 4.6 mm × 250 mm RP 18 column at ambient temperature. The mobile phase comprised (A) water−formic acid (19:1) and (B) methanol, which were previously degassed with high-purity helium. The solvent gradient started at 95% A and 5% B, reaching 75% A at 10 min, 65% A at 30 min, 55% A at 35 min, 55% A at 40 min, 50% A at 45 min, 45% A at 50 min, 30% A at 53 min, 25% A at 56 min, and 20% A at 60 min, followed by a post-time isocratic plateau for 10 min at 95% A before the next injection. The flow rate was 1 mL/min, and the injection volume was 50 μL. The monitoring wavelengths were 280 and 350 nm with a scan from 200 to 600 nm. The identification of each compound was based on a combination of retention time and spectral matching. Quantification of the identified phenolic compounds was performed by correlating the measured peak area with the calibration curves obtained with reference compounds.

LC-Electrospray Ionization (ESI)-MS Analysis. A Shimadzu LCMS-2010 System was used, equipped with an SPD-M10A vp diode array detection (DAD) detector, an SIL-10AD vp autoinjector, and an LC-10AD binary pump coupled on line with an MS-2010 mass spectrometer (Shimadzu, Milan, Italy). UV and MS data were acquired and processed using Shimadzu LCMS Solution Software. The ESI was operated in the positive mode: ESI source probe, 250 °C; CDL, 250 °C; block at 240 °C; flow gas (N_2) at 4.5 L/min; probe voltage, 4.5 kV; fragmentor voltage, 20 V; and a nominal mass range up to m/z 800.

Radical Scavenging Activity. The antioxidant activity of the polyphenolic extracts or standard pure phenolic compounds was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a free radical. The reaction for scavenging DPPH radicals was performed in polypropylene tubes at room temperature. Two milliliters of a 4×10^{-5} M methanolic solution of DPPH was added to 50 μL of the sample. The mixture was shaken vigorously and left for 60 min. The absorbance of the resulting solution was measured at 517 nm. Methanol was used as a blank solution, and DPPH solution without any sample extract served as control. The TEAC (Trolox equivalent antioxidant capacity) values were calculated from the equation determined from linear regression after plotting known solutions of Trolox with different concentrations (0.02−0.8 mM). The antiradical activity was also expressed as the inhibition percentage and was calculated using the following formula: % radical scavenging activity = (control OD − sample OD/control OD) × 100.

Antimicrobial Tests. To assess the antimicrobial activity of the extracts, the following microorganisms were used: *Staphylococcus aureus* (ATCC6538) for Gram-(+) bacteria, *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027), and a *Salmonella* sp. strain (isolated from food) for Gram-(−) bacteria, the yeast *Candida albicans* (ATCC 14053), and the mold *Aspergillus niger*. Susceptibility of the test organism to the extract was determined by employing the standard disk (*2*) or well (*21*) diffusion technique. Briefly, the bacterial suspension in potato count broth (PCB), adjusted to 0.5 McFarland turbidity and evaluated using a serial 10-fold dilution method, was spread plated on count agar medium (PCA) in order to give a population of 10^8 colony-forming units (cfu)/plate. For the disk diffusion test, sterile paper discs (6 mm Ø) were added of the test sample (20 μL) and placed onto the inoculated agar surface. After cultivation at 37 (bacteria)/ 27 °C (*C. albicans*) for 24 h or 22 °C for 4−12 days (*A. niger*), the resulting inhibition zones diameters were measured. Using the well (6 mm Ø) diffusion technique, 100 μL of the sample was tested. Inhibited microorganisms were then tested using the broth microdilution method (*22*) to determine the minimum inhibitory and bactericidal concentrations (MIC and MBC). A 100 μL amount of the diluted working extract or pure phenolic solution and 100 μL of the bacterial suspensions (5×10^5 cfu/mL) were added in the microwells. The plates were incubated aerobically at 37 °C for 24 h. Bacterial growth was revealed by the presence of turbidity and a "pellet" on the well bottom. MICs were determined as the first well in ascending order that did not produce a pellet. To confirm MIC and establish MBC, 25 μL of broth was removed from each well and inoculated on PCA plates. After overnight incubation at 37 °C, the number of surviving organisms was determined (MBC was determined when 99.9% of bacteria were dead).

Statistical Analysis. All tests and analyses were run in triplicate and averaged. Quantitative presented data are means ± standard deviations. One-way analysis of variance with Dunnett's post-test was performed using GraphPad Prism version 3.00 for Windows, GraphPad Software (San Diego, CA). Differences of $P < 0.05$ were considered significant.

RESULTS

Phenolic Profiles Analysis. The phenolic compounds of both quince pulp and peel extracts were fractionated and analyzed by reverse phase HPLC-DAD, and the corresponding chromatograms obtained at 280 and 350 nm are presented in **Figures 1** and **2**. The repeatability of the method was high, with respect to both retention times and peak areas. The chromatographic profiles at 350 and 280 nm differed mostly in the relative abundance of the various peak-forming compounds. At 280 nm, a significant rise of the baseline was observed in the chromatograms in addition to abundant various peaks (**Figure 1I** and **Figure 2I**), whereas at 350 nm reasonable chromatographic separation was achieved showing a linear baseline (**Figure 1II** and **Figure 2II**). Compounds were identified by comparison of the retention times and UV/vis spectra with the available standards corresponding peaks (**Table 1**). Seven of the 14 obtained peaks matched with the standard compounds used in this work, namely, peaks 1−7, attributed to (+)-catechin, (−)-catechin, chlorogenic acid (5-*O*-caffeoylquinic acid), rutin (quercetin 3-*O*-rutinoside), kaempherol 3-*O*-glucoside, quercetin, and kaempherol, respectively, which correspond to compounds usually found in quince fruit (*18*−*20*). The quince extracts showed a distinct total polyphenol proportions between pulp (42.17 ± 4.93 mg/100 g fw) and peel parts (131.45 ± 25.61 mg/100 g fw). Performing mass spectrometric detection in the positive ion mode resulted in mass chromatograms for protonated ions ($[M + H]^+$) at m/z ratios similar to those obtained with authentic standards. The ESI-MS technique allowed confirming and completing the identification of the phenolic compounds based on their specific and characteristic molecular ions described in the literature (**Table 1**).

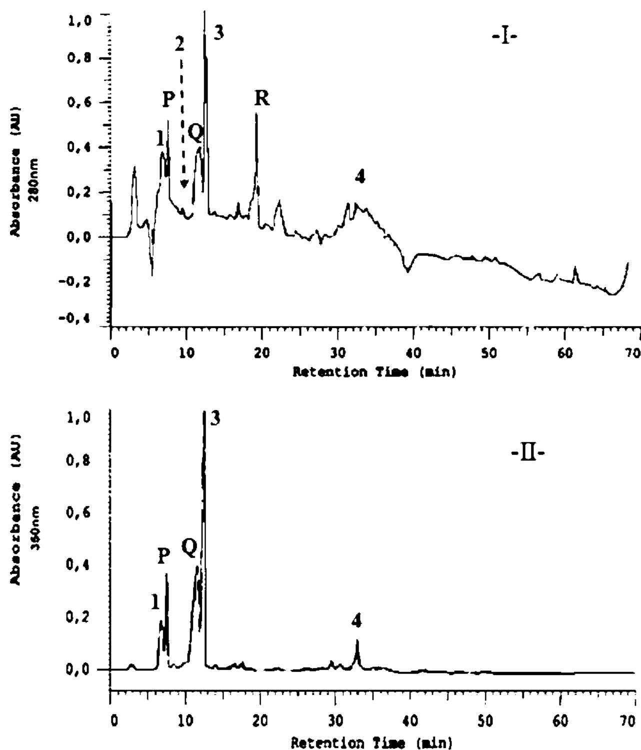

Figure 1. Typical HPLC profile of quince pulp acetone extracts with detection at 280 (**I**) and 350 nm (**II**). Identified peaks as compared to standard compounds: 1, (+)-catechin; 2, (–)-catechin; 3, chlorogenic acid; and 4, rutin. Unidentified peaks: P, Q, and R.

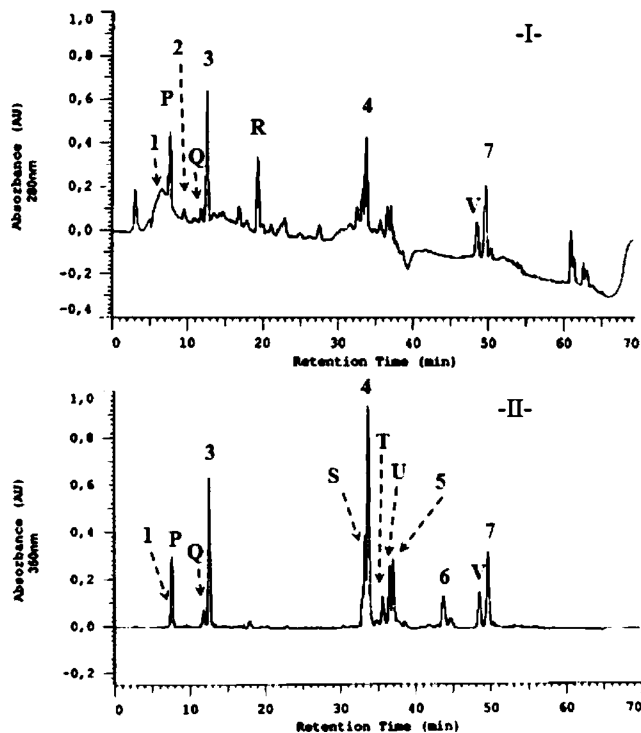

Figure 2. Typical HPLC profile of quince peel acetone extracts with detection at 280 (**I**) and 350 nm (**II**). Identified peaks as compared to standard compounds: 1, (+)-catechin; 2, (–)-catechin; 3, chlorogenic acid; 4, rutin; 5, kaempherol 3-glucoside; 6, quercetin; and 7, kaempherol. Unidentified peaks: P, Q, R, S, T, U, and V.

Antioxidant Activity. The antioxidant activity of quince pulp and peel extracts against DPPH radical was shown in **Figure 3**. Percent DPPH scavenging activities of the extracts were found to be dose-dependent. For comparative purposes, we analyzed the antioxidant activities of the standard pure phenolic compounds prepared at corresponding concentrations normally present in the fruit pulp or peel extracts in the same operating conditions. Focusing on the main phenolic constituents of each extract (**Figure 3**), we calculated the sum of the antioxidant potentials of individual standard polyphenols and retrieved about two-thirds of the total activity recorded for the corresponding whole extract.

In Vitro Antimicrobial Tests. The consistent and reproducible results obtained using the standard disk and well diffusion techniques (**Figure 4**) showed that the peel extract exhibited more antimicrobial potential than the pulp one, reflecting their qualitative and quantitative biochemical differences. The antimicrobial activity was highest against the Gram-(+) *S. aureus* and the Gram-(−) *P. aeruginosa* bacteria, somewhat weaker against the *E. coli* and the yeast *C. albicans*, while with the *Salmonella* sp. and the mold *A. niger* no inhibition was obtained (**Table 2**). *S. aureus* was the most sensitive microorganism to the extracts examined in this study. Quince pulp and peel extracts showed both bacteriostatic and batericide activities. MICs and MBCs for peel extract were found equal, whereas higher polyphenol concentrations were required to get bactericide activity for pulp extract (**Table 2**). In order to more specifically determine which components have inhibitory effects, each pure phenolic compound was examined. Chlorogenic acid was the strongest inhibitor exhibiting activity against *S. aureus*, *P. aeruginosa*, *E. coli*, and *C. albicans*. (+)-Catechins showed moderate activities and only inhibited the growth of *S. aureus* and *P. aeruginosa*. The quercetin and kaempferol antimicrobial activities were relatively lower than those of the other phenolics.

DISCUSSION

Because of the complexity of various natural mixtures of phenolic compounds, many extraction methods have been reported in the literature using different solvents and a range of selective chromatographic separation steps. In the present work, we used a simple aqueous acetone extraction method to prepare the quince fruit pulp and peel polyphenols. To find out the relationship between antioxidant potential, antimicrobial activity, and polyphenolic content, we first separated and quantified the phenolic compounds of the different extracts using RP-HPLC technique. For monitoring all classes of phenolics, no single wavelength is ideal since they display absorbance maxima at different wavelengths. In our case, 280 and 350 nm were satisfactory to detect most phenolics. The use of DAD allows not only the peak identification but also its purity determination. The purity of all identified peaks in our experiments generally reached 1.00. In addition, in the above-described conditions, the repeatability and the reproducibility of the method were good. Therefore, using the RP-HPLC-DAD and authentic standards, total polyphenols quantification was determined as the sum of individual identified compounds. According to **Table 1**, we estimated the polyphenol contents to 140 ± 16 and 438 ± 85 mg/100 g of dry quince pulp and peel, respectively. The peel extract had about three times higher amount of phenolics than that of the pulp. Comparatively, the total phenolic contents of the aqueous acetone extracts obtained in our study from both pulp and peel parts did not differ greatly from those found by

Table 1. HPLC-DAD-MS Analysis of Main Phenolics in Quince Pulp and Peel Acetonic Extracts

peak no.	R_t[a] (min)	HPLC-DAD λ_{max} (nm)	$[M + H]^+$ *m/z*	identity	concentration (mg/100 g fw)[b] pulp	peel
1	6.83	279	291	(+)-catechin	7.20 (1.26)	5.07 (2.15)
P	7.55	325	355	neochlorogenic acid (3-*O*-caffeoylquinic acid)	5.68 (0.34)	3.94 (0.17)
2	8.31	279	291	(–)-catechin	0.18 (0.03)	0.10 (0.24)
Q	11.63	324	355	cryptochlorogenic acid (4-*O*-caffeoylquinic acid)	4.49 (0.96)	0.51 (0.32)
3	12.37	326	355	chlorogenic acid (5-*O*-caffeoylquinic acid)	15.57 (0.13)	12.85 (0.16)
R	17.71	278	579	procyanidin	NQ	NQ
S	32.53	355	465	hyperin (quercetin-3-*O*-galactoside)	ND	12.40 (2.37)
4	33.31	356	611	rutin (quercetin-3-*O*-rutinoside)	9.05 (2.21)	47.21 (4.56)
T	35.84	275	595	kaempferol-3-*O*-rutinoside	ND	3.96 (1.10)
U	36.06	354	465	isoquercitrin (quercetin-3-*O*-glucoside)	ND	9.23 (2.79)
5	43.55	278	449	kaempferol 3-*O*-glucoside	ND	10.65 (3.81)
6	6.77	371	303	quercetin	ND	7.01 (2.92)
V	48.67	313	612	quercetin glycoside acylated with *p*-coumaric acid	ND	5.92 (1.15)
7	49.57	340	287	kaempferol	ND	12.60 (3.87)
total					42.17 (4.93)	131.45 (25.61)

[a] HPLC retention time. [b] On the basis of the fresh weight. The standard deviation is reported in parentheses ($n = 3$); ND, not detected; NQ, not quantified.

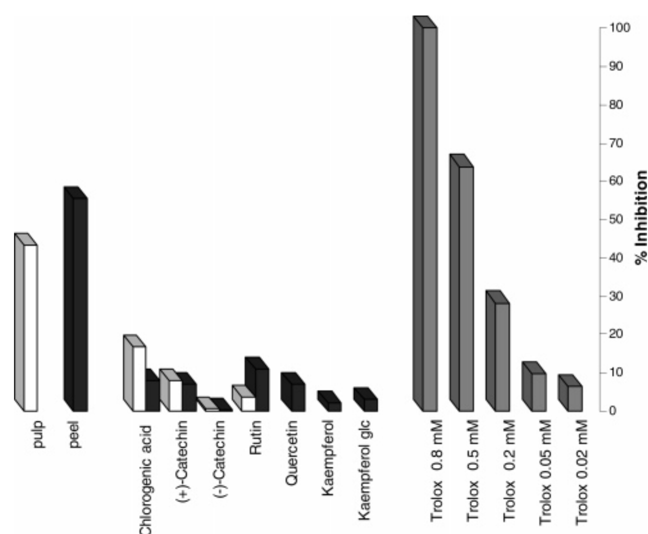

Figure 3. Radical scavenging activity (%) of Trolox (gray bar), acetone extracts, and of some standard pure phenolic compounds used at equivalent concentrations as in the quince pulp (white bar) and peel (black bar) extracts (main phenolics). Kaempferol glc, kaempferol 3-*O*-glucoside.

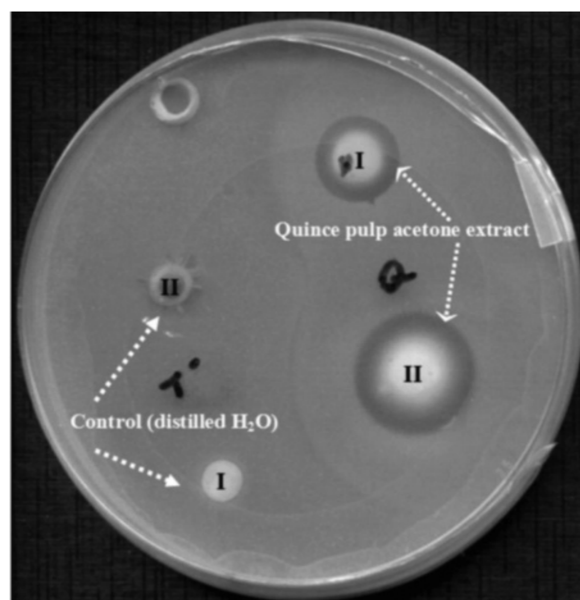

Figure 4. *S. aureus* (ATCC6538) growth inhibition: 20 and 100 μL of the acetone quince pulp extract were used in disk (I) and well (II) diffusion assays, respectively.

Silva et al. (*18*−*20*) using the extraction with aqueous methanol solvent and a solid-phase extraction purification step. In the pulp extract, chlorogenic acids represented 61% of the determined phenolics, with the 5-*O*-caffeoylquinic acid as the most abundant (37%), while in peel extract chlorogenic acids represented only 13%, with rutin as the major polyphenol (36%). Obtained results are in agreement with what was reported in literature (*18*−*20*). We assumed that major polyphenols in the Tunisian quince pulp and peel extracts are hydroxycinnamic acids, principally the chlorogenic acid. Flavonols are present as a mixture of different aglycone and glycosylated quercetin and kaempferol. Flavanols are essentially catechins and procyanidins. Flavonols and flavanols were almost restricted to peel, which is in agreement with previous studies carried out on quince fruit from Portugal (*18*−*20*). The HPLC-MS provided interesting information about the quince pulp and peel polyphenol profiles. On the basis of its UV spectra and positive ion mass at *m/z* 579, peak R seems most likely to be a procyanidin B dimer as reported for quince jam (*17*). According to previous studies (*17*−*20*), many isomers of the chlorogenic acid have been described in quince fruit. In both pulp and peel acetone extracts, we have also detected these isomers except the 3,5-dicaffeoylquinic acid. Silva et al. (*19*) analyzed quince pulp and peel from different geographical origins in Portugal and reported a dispersion in terms of caffeoylquinic acids and flavonoids composition. The authors considered that the quince phenolic profile can be influenced by various factors and the absence of some compounds can be accepted as possible. As for chlorogenic acid isomers, (+)-catechin and (−)-catechin enantiomers, which have identical *m/z* of the protonated ions, can only be distinguished by their different HPLC retention times. This shows clearly the importance of the chromatographic separation of quince peel and pulp polyphenols prior to MS detection.

The DPPH scavenging activity of the quince peel and pulp extracts was determined and found in proportion to the

Table 2. Antibacterial Activity of Quince Pulp and Peel Extracts and of Standard Pure Phenolics

microorganisms:	*S. aureus* (ATCC6538)		*P. aeruginosa* (ATCC 9027)		*E. coli* (ATCC 8739)		*C. albicans* (ATCC 14053)	
technique:	disk diffusion	well diffusion	disk diffusion	well diffusion	disk diffusion	well diffusion	disk diffusion	well diffusion
			Diameter[a] of the Inhibition Zone (mm)					
pulp extract	16.5 ± 1.3	20.2 ± 1.4	11.4 ± 0.1	16.2 ± 0.4	w	08.2 ± 0.9	n	w
peel extract	22.3 ± 2.7	27.1 ± 2.6	15.5 ± 0.8	18.3 ± 0.9	08.6 ± 1.0	10.5 ± 1.1	w	09.6 ± 0.1
(+)-catechin†	w	08.2 ± 0.9	w	08.2 ± 0.9	n	w	n	w
(−)-catechin†	n	n	n	n	n	n	n	n
chlorogenic acid†	10.3 ± 0.5	11.9 ± 0.7	10.2 ± 1.0	11.5 ± 0.6	n	w	n	
rutin‡	n	n	n	n	n	n	n	n
quercetin‡	w	10.0 ± 0.8	w	10.1 ± 0.4	w	w	n	n
kaempferol glc‡	n	n	n	n	n	n	n	n
kaempferol‡	n	w	n	w	n	n	n	n
			MIC (μg/mL)					
pulp extract	10^2		5×10^2		10^3		5×10^3	
peel extract	10^2		10^2		5×10^2		5×10^3	
(+)-catechin	5×10^3		5×10^3		–		–	
(−)-catechin	–		–		–		–	
chlorogenic acid	10^3		10^3		5×10^3		10^4	
rutin	–		–		–		–	
quercetin	10^4		10^4		10^4		–	
kaempferol glc	–		–		–		–	
kaempferol	10^4		10^4		–		–	
			MBC (μg/mL)					
pulp extract	5×10^2		10^3		5×10^3		10^4	
peel extract	10^2		10^2		5×10^2		5×10^3	
(+)-catechin	10^4		10^4		–		–	
(−)-catechin	–		–		–		–	
chlorogenic acid	5×10^3		5×10^3		10^4		–	
rutin	–		–		–		–	
quercetin	10^4		10^4		–		–	
kaempferol glc	–		–		–		–	
kaempferol	10^4		10^4		–		–	

[a] Diameter including disk or well diameter. For diffusion tests, standard compounds were used at concentrations normally present in the pulp (†) or peel (‡) extracts. Values are means ± SD of three separate experiments done in triplicate. Key: n, no antimicrobial activity, Ø = 6 mm; w, weak antimicrobial activity, 6 mm < Ø < 8 mm; –, not determined. No antimicrobial activities were found for *Salmonella* sp. and *A. niger* (not given in the table). Kaempferol glc, kaempferol 3-*O*-glucoside.

polyphenols concentrations ($r = 0.938$), suggesting that these compounds are the major contributors to the extracts antioxidant potentials. In fact, the correlation between antioxidant activity and phenolic content in quince fruit has already been reported (*18*). In the present study, the pulp (3.33 g of TEAC/100 g fw) and peel (4.27 g of TEAC/100 g fw) extracts exhibited stronger antioxidant effects as compared to the sum of those of individual pure compounds used at concentrations normally present in the whole extract (**Figure 3**). We think that interactions between different antioxidant components are likely important in terms of the overall antioxidant activity of the extracts.

Furthermore, antimicrobial activities of the quince pulp and peel extracts were evaluated. The agar diffusion assay showed that both quince pulp and peel extracts exhibited antimicrobial potentials with clear-cut inhibition zones (**Figure 4**). The diameters of these inhibition zones increased with the concentration of polyphenolic compounds indicating that these molecules were responsible of the antimicrobial effects. Our results are in line with those reported by Baydar et al. (*10*) using grape seed extracts to inhibit a bacterial collection including *E. coli*, *P. aeruginosa*, and *S. aureus*. The broth microdilution method allowed the determination of the MICs of each polyphenolic solution against the tested microorganisms as listed in **Table 2**. *S. aureus* and *P. aeruginosa* were found more susceptible to polyphenols than *E. coli* and *C. albicans*. *S. aureus* [Gram-(+)] is known for its high sensitivity to phenolic extracts (*11*). Generally, Gram-(−) are more resistant to bactericidal polyphenols than Gram-(+) bacteria. Ikigai et al. (*12*) proposed that this difference is caused by repulsion between the phenolics and the surfaces of Gram-(−) bacteria, which are coated with lipopolysaccharide. The sensitivity to the whole extracts or pure phenolic compounds varied widely among the test organisms. Rodriguez Vaquero et al. (*13*) reported that *P. aeruginosa* (ATCC 27853) and *E. coli* (ATCC 25922) were, respectively, the most sensitive and the most resistant to flavonoid compounds than the other tested bacteria. No inhibition of the mold *A. niger* growth was obtained at any concentration of the quince extracts in comparison to a control culture. This finding may be expected since microbiological analyses of quince jams, which contain mainly the same phenolics as the fruit, revealed that some samples presented a high number of yeasts and molds (*23*). Quince peel extract MBC was found equal to MIC, whereas pulp extract exhibited a bactericide effect at higher concentrations than for bacteriostatic activity. This could be explained by the differences of the qualitative composition of the two extracts. Among the standard polyphenols used at concentrations normally present in the corresponding quince extract, we found that the chlorogenic acid exhibited the greatest antibacterial activity especially against *S. aureus*. Hydroxycinnamic acids (such as chlorogenic acids) due to their propenoic side chain are much less polar than the corresponding hydroxybenzoic acids. This property might be related to the stronger inhibitory effect of hydroxycinnamic acids on Gram-(+) bacteria (*14*). Pure standard phenolics showed low antibacterial activities when used in the diffusion tests at concentrations normally present in the quince extracts. Moreover, the sum of all pure standard phenolics

activities was found relatively lower than the corresponding whole pulp or peel acetone extracts. The higher flavonols content of the peel extract could not explain its strong inhibitory effect as compared to the pulp extract. It seems that the polyphenols, especially the chlorogenic acid, act in synergism with other components of the extracts to exhibit their total antimicrobial activities. Hakkinen et al. (*24*) reported that berry extract's inhibitory effect was a synergistic effect of various phenolic compounds. Additionally, many bioactive compounds in plant extracts, alone or in combination with polyphenols, might be responsible for a total biological effect (*25*).

The present work could exalt the positive effect that the quince fruit may have on human health. Here, we demonstrate that quince peel showed a higher content of bioactive compounds than pulp. However, because of the fear of pesticides, most fruit-consuming people use peeled fruits. Thus, we must underline the importance of using whole fruit to the benefit of its total bioactive compounds. The extracts evaluated here present the advantage of being simply prepared from naturally occurring material, which could be marketed as nutraceuticals, and might be industrially exploited. Many works have focused the bioavailability and polyphenols bioefficacy in humans. The local action of polyphenols in the gastrointestinal tract may be important because the intestine is particularly exposed to oxidizing agents and may be affected by inflammation and numerous diseases such as cancer (*26*). Polyphenol concentrations in the colon can reach several hundred micromoles per liter, and together with a few carotenoids, polyphenols constitute the only dietary antioxidants present in the colon, because vitamins C and E are absorbed in the upper segments of the intestine (*27*, *28*). Dietary phenols are also known to be extensively metabolized in the body; particularly, they are mostly converted with the colonic microflora into metabolites that then reach the circulation and can produce systemic effects (*28*). Given the apparent trend toward the evolution of resistant microbial strains, our results indicate that the whole quince extracts, and not its elementary compounds, may be a useful adjuvant agent for the treatment of bacterial infections in addition to antibiotics. Understanding how these natural bioactive molecules work and inhibit the growth of microorganisms can lead to new technologies for the development of food products with particular nutritional functionalities or for food preservative purposes. Our findings bring attention to the synergistic and/or antagonistic effects of quince polyphenols that have to be considered in any application of these results.

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