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ARTICLE

Identification and Assessment of Antioxidant Capacity of Phytochemicals from Kiwi Fruits

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The kiwi fruit is the edible berry of a cultivar group of the woody vine of several *Actinidia* species. The most common commercially available, green-fleshed kiwi fruit is the cultivar 'Hayward', which belongs to the *Actinidia deliciosa* species. An antioxidative screening of kiwi fruit components (peel and pulp) crude extracts was carried out using specific assay media characterized for the presence of highly reactive species such as 2,2′-diphenyl-1-picrylhydrazyl radical (DPPH$^{\bullet}$), H_2O_2, and $O_2^{\bullet-}$. The Mo(VI) reducing power of the samples was also determined. The phenol and flavonoid contents were quantified. Phytochemical analysis of kiwi peel crude extracts led to the isolation of vitamin E, 2,8-dimethyl-2-(4,8,12-trimethyltridec-11-enyl)chroman-6-ol, as well as α- and δ-tocopherol, 7 sterols, the triterpene ursolic acid, chlorogenic acid, and 11 flavonoids. Chemical fractionation of pulp crude extracts led to the isolation of two caffeic acid glucosyl derivatives and two coumarin glucosydes, besides the three vitamin E, β-sitosterol, stigmasterol, and its Δ^7 isomer, campesterol, chlorogenic acid, and some flavone and flavanol molecules. All of the compounds were tested for their radical scavenging and antioxidant capabilities by measuring their capacity to scavenge DPPH and anion superoxide radical and to reduce a Mo(VI) salt.

KEYWORDS: *Actinidia deliciosa* 'Hayward'; kiwi fruit; phytochemicals; antioxidants; radical scavenging capacity

INTRODUCTION

Epidemiological research, analyzing the relationship between diet and health state, suggests that the regular intake of fruits and vegetables is associated with a lower incidence of cancer, diabetes, and cardiovascular and neurodegenerative pathologies (*1*). In fact, vegetables, besides providing essential nutrients, are a precious source of bioactive compounds, called phytochemicals, that are effective safeguards for human health. The protective effects on health of these substances seem to be strongly related to their structure. In fact, nowadays great interest is turned to phenol food constituents and their capabilities to prevent and/or limit stress oxidative damages (*2*, *3*) due to radical species. These oxidants are normal cell metabolites, but their production can be increased under several pathologic conditions (such as the inflammation process) or after exposure to different agents (such as UV light, X-rays, carcinogens, antineoplastic drugs, pollutants) (*4*). The overproduction of these highly reactive chemicals triggers off-chain reactions that result in an irreversible chemical structure change of the main biomolecules. The beneficial effects of phenols are due to their massive ability to scavenge free radicals. The natural antioxidant vitamins, as tocols and ascorbic acid, have significant health-promoting properties; tocols, for example, act as neuroprotective and anticancer molecules and inhibitors of cholesterol synthesis (*5*). The serum cholesterol level decrease is aided by phytosterols, plant secondary metabolites with a structure closely similar to that of cholesterol (*6*).

Recently, fruits of eight *Actinidia* genotypes were evaluated for antioxidant capability by several assays and tested for their polyphenol composition and vitamin C contents (*7*). Although kiwi fruit has generally high antioxidant capacity, the antioxidant capacity of *Actinidia* is strongly influenced by species and cultivar. *Actinidia deliciosa* 'Hayward' is the most popular commercial kiwi fruit variety; it is a heavy cropper with supreme flavor.

In the assessment of antioxidant properties of Mediterranean fruits, we reported the chemical study and biological activity of several phytochemicals isolated from fruit cultivated in Italy (*8*−*14*). In this context we carried out the chemical investigation of *A. deliciosa* 'Hayward' fruits, and in this study the phytochemical composition is evaluated as is the antioxidant value.

MATERIALS AND METHODS

General Experimental Procedures. NMR spectra were recorded on a Varian 300 spectrometer Fourier transform NMR in $CDCl_3$ or CD_3OD at 25 °C. UV spectra were performed on a UV-1700 Shimadzu spectrophotometer. Optical rotations were measured on a Perkin-Elmer 141 (Perkin-Elmer Co., Norwalk, CT) in MeOH solution. The preparative HPLC apparatus consisted of Knauer Smartline 31/40 module equipped with Knauer Smartline 1000 pump, a UV Knauer Smartline 2500 detector, an RI Knauer Smartline 2300 detector, and PC

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CromGate software. Analytical HPLC was performed using Luna SiO_2 (5 μm, 250 × 4.6 mm i.d., Phenomenex, Torrance, CA), Luna RP-8 (5 μm, 250 × 4.6 mm i.d., Phenomenex), Polar-RP-80A (4 μm, 250 × 4.6 mm i.d., Phenomenex,), and Maxsil 10 silica (10 μm, 250 × 10.0 mm i.d., Phenomenex).

Analytical TLC was performed on Merck Kieselgel 60 F_{254} or RP-8 F_{254} plates with 0.2 mm layer thickness. Spots were visualized by UV light or by spraying with H_2SO_4/AcOH/H_2O (1:20:4). The plates were then heated for 5 min at 110 °C. Preparative TLC was performed on Merck Kieselgel 60 F_{254} plates, with 0.5 or 1.0 mm film thickness. Column chromatography (CC) was performed on Merck Kieselgel 60 (70−240 mesh) or NH_2 silica (LiChroprep NH_2, 40−63 μm, Merck, Darmstadt, Germany).

GC-MS quali-quantitative analysis of *A. deliciosa* 'Hayward' metabolites was carried out using an HP 6890 GC instrument (Agilent Technologies, Little Falls, DE) equipped with a 5975B VL MSD detector. The GC was equipped with a fused silica capillary column (Zebron ZB5MS, 30 m × 0.25 mm i.d., film thickness = 0.25 μm, Phenomenex), with He as carrier gas (flow = 1.2 mL/min). The column head pressure was set at 10.27 psi. Temperature conditions were as follows: injector port at 250 °C; initial oven temperature, 60 °C for 1 min, then increased at 20 °C/min linearly to 180 °C, then increased at 2.5 °C/min linearly to 275 °C, and held for 23 min. Sample solutions were injected using the splitless mode. Full-scan mass spectra were collected between 15 and 600 amu at 2 scan/s. The MS was operated in the electron impact (EI) ionization mode with electron energy of 70 eV. The ion source and quadrupole temperatures were maintained at 230 and 150 °C, respectively.

The compounds' concentration was determined by proper calibration curves of the three pure standards previously set. The concentrations were calculated as milligrams per 100 g of fresh weight using the average peak area compared between standard and sample after triplicate injections.

Fruit Sample Collection. *A. deliciosa* 'Hayward' fruits were collected in Rotondi, near Caserta (Italy), in October 2007, when the fruit had just been harvested. Unripe fruits were stored in a climatic cell at 4 °C and 98% humidity.

Extraction and Isolation. The kiwi fruits were sliced into their components. The peels (4.2 kg) were dried in a ventilated thermostat at 45 °C overnight. The dried material (1.1 kg) was infused first in hexane for 5 days, then in ethyl ether (Et_2O) for 5 days, and finally in EtOH for 5 days in a refrigerated chamber at 4 °C, in darkness. After removal of the solvents, we obtained crude hexane (8.1 g), Et_2O (7.2 g), and EtOH (250.1 g) extracts, which were stored at −80 °C until their purification.

Fresh pulps of kiwi fruits (12.8 kg) were infused first in EtOH for 5 days, then in Et_2O for 5 days, and finally in hexane for 5 days in a refrigerated chamber at 4 °C in darkness. After removal of the solvents, we obtained three crude extracts: EtOH (291.4 g), Et_2O (12.0 g), and hexane (15.1 g).

Peel Organic Extract Fractionation. The peel hexane extract was chromatographed on silica gel eluting with petroleum ether (PE) and Me_2CO solutions (**Figure 1**). The fraction eluted with PE/Me_2CO (9:1) was rechromatographed by CC on NH_2 silica gel, eluting with PE/EtOAc solutions to give seven fractions A−G. Fraction A, eluted with PE/EtOAc (24:1), was constituted by pure β-sitosterol (**4**, 16.3 mg). Fraction B, eluted with PE/EtOAc (16:1), contained α-tocopherol (**2**, 45.0 mg). Fraction C, eluted with PE/EtOAc (47:3), furnished a fraction that was purified by RP-8 HPLC eluting with MeOH/MeCN/H_2O (15:4:1) to give pure compounds **1** (22.0 mg) and **3** (30.0 mg). Fraction D, eluted with PE/EtOAc (23:2), was definitively purified by RP-8 HPLC [MeOH/MeCN/H_2O (15:4:1)] to obtain pure metabolites **5** (13.0 mg), **6** (8.1 mg), **7** (7.0 mg), and **8** (10.0 mg). Fraction E, eluted with $CHCl_3$/EtOAc (4:1), was composed of compound **9** (5.0 mg), whereas fraction F contained pure metabolite **10** (8.2 mg). Fraction G, eluted with $CHCl_3$/EtOAc (1:1), gave a fraction that when purified by SiO_2 HPLC [MeCOEt/hexane (2:23)] furnished pure ursolic acid **11** (29.6 mg).

The peel ethanol extract was dissolved in water and shaken with EtOAc in a separator funnel. The obtained aqueous fraction was chromatographed on Amberlite XAD-4 and XAD-7 eluting first with H_2O and then with MeOH. The methanol organic fraction was chromatographed on Sephadex LH-20 eluting with H_2O/MeOH solutions to obtain two fractions H and I. Fraction H, eluted with MeOH/H_2O (1:1), gave two fractions: the first was purified by preparative TLC eluting with $CHCl_3$/MeOH (4:1) to give gallocatechin **27** (1.1 mg) and catechin **26** (20.3 mg); the second was chromatographed by RP-8 HPLC [MeOH/MeCN/H_2O (1:4:15)] to give epicatechin **25** (6.5 mg), quercetin 3-*O*-β-D-glucopyranoside **22** (2.0 mg), rutin **24** (18.0 mg), and chlorogenic acid **14** (1.0 mg). Fraction I, eluted with MeOH/H_2O (4:1), was purified by preparative TLC eluting with $CHCl_3$/MeOH (4:1) to give quercetin 3-*O*-α-L-rhamnopyranoside **21** (59.0 mg) and kaempferol 3-*O*-α-L-rhamnopyranoside **20** (67.0 mg).

As the preliminary metabolic fingerprinting of the Et_2O extract and the organic (EtOAc) fraction of the EtOH extract revealed a similar composition for both fractions, they were joined together and chromatographed on SiO_2 with $CHCl_3$/MeOH solutions as eluent to obtain two fractions J and K.

Fraction J, eluted with $CHCl_3$/MeOH (19:1), was chromatographed by CC on Sephadex LH-20 eluting with hexane/$CHCl_3$/MeOH (1:1:1) to give pure tricin **19** (2.0 mg) and quercetin **18** (34.5 mg). Fraction K, eluted with $CHCl_3$/MeOH (4:1), was chromatographed by FCC on SiO_2 under N_2 pressure, eluting with the lower organic phase of a $CHCl_3$/MeOH/H_2O (13:7:3) biphasic solution. One of the obtained fractions chromatographed by CC on RP-8 silica gel, eluting with MeOH/MeCN/H_2O (1:2:7), furnished two fractions: the first, purified by TLC (0.5 mm) eluting with EtOAc/EtOH/H_2O (24:4:1), supplied pure naringenin **17** (36.0 mg); the second, purified by RP-8 HPLC eluting with MeCN/H_2O (1:4), gave pure metabolite **23** (61.1 mg).

Pulp Organic Extract Fractionation. The pulp hexane extract was chromatographed by SiO_2 FCC eluting with the PE/Me_2CO solutions, to furnish two fractions A′ and B′ (**Figure 2**). The first one was purified, by CC, on NH_2 silica gel eluting with PE/EtOAc solutions, to furnish pure **2** (30.7 mg), and a mixture that was chromatographed by RP-8 HPLC [MeOH/MeCN/H_2O (15:4:1)] to give pure metabolites **1** (3.6) and **3** (23.5 mg). Fraction B′ was chromatographed by SiO_2 HPLC [MeCOEt/hexane (2:23)] to obtain compounds **4** (21.8 mg), **5** (6.8 mg), **6** (7.2 mg), and **7** (8.0 mg).

The pulp ethanol extract was suspended in water and shaken with EtOAc to obtain organic (12.1 g) and aqueous (181.0 g) fractions. The aqueous fraction was chromatographed on Amberlite XAD-4 eluting with H_2O. The eluate was directly passed on Amberlite XAD-7. After both columns had been washed with 4 volumes of water, organic compounds were eluted with MeOH. The MeOH fraction obtained from Amberlite XAD-7 was chromatographed on Sephadex LH-20 eluting with MeOH/H_2O solutions to obtain four fractions C′−F′. Fraction C′, eluted with H_2O, was chromatographed on a SiO_2 column by flash chromatography. One of the obtained fractions, eluted with the organic phase of $CHCl_3$/MeOH/H_2O (13:7:5) biphasic solution, was purified by TLC, eluting with the same solution, to give pure compound **16** (9.0 mg). Fraction D′, eluted with MeOH/H_2O (1:4), was chromatographed by TLC eluting with the lower phase of $CHCl_3$/MeOH/H_2O (13:7:2) biphasic solution, to obtain compounds **15** (6.7 mg), **12** (44.7 mg), and **13** (35.6 mg). Fraction E′, eluted with MeOH/H_2O (1:1), was purified by RP-8 HPLC eluting with MeOH/MeCN/H_2O (1:4:15) to give pure compounds catechin **26** (16.0 mg), epicatechin **25** (25.4 mg), quercetin 3-*O*-β-D-glucopyranoside **22** (9.2 mg), and chlorogenic acid **14** (11.5 mg). Fraction F′, eluted with pure MeOH, gave three fractions: the first contained pure gallocatechin **27** (23.1 mg); the second, chromatographed by RP-8 HPLC eluting with MeOH/MeCN/H_2O (1:1:8), gave pure kaempferol 3-*O*-β-D-rutinoside **23** (10.0 mg) and rutin **24** (23.1 mg).

The EtOAc organic fraction (7.4 g) of the EtOH extract was joined together with the Et_2O crude extract and chromatographed on SiO_2 eluting with $CHCl_3$/Me_2CO solutions to obtain two further fractions G′ and H′. Fraction G′, eluted with $CHCl_3$/Me_2CO (1:1), was chromatographed by CC on Sephadex LH-20 eluting with hexane/$CHCl_3$/MeOH (1:1:1) to give pure tricin **19** (1.0 mg) and quercetin **18** (44.6 mg). Fraction H′, eluted with Me_2CO, was purified on an RP-8 column with MeOH/MeCN/H_2O (4:1:5) to give pure metabolites **20** (72.1 mg) and **21** (33.2 mg).

Extraction Procedure for GC-MS Analyses. Three aliquots of about 100 mg of lyophilized peels and pulps of kiwi fruits were extracted by ultrasound-assisted extraction for 1 h with 1.0 mL of hexane/EtOAc

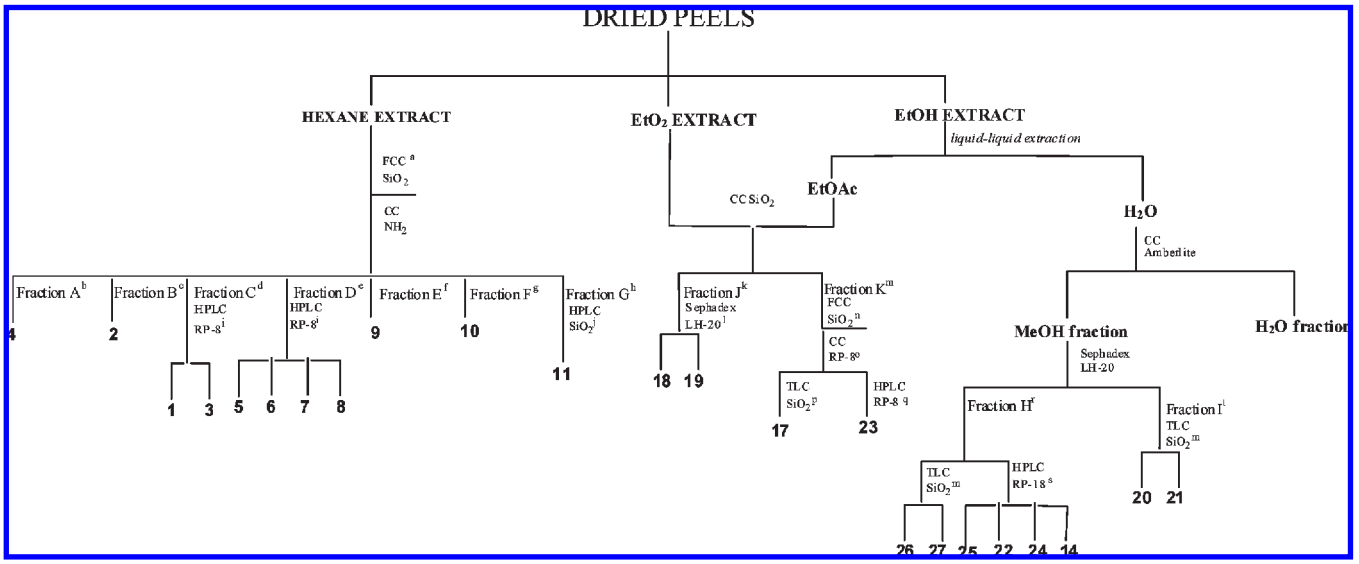

Figure 1. *A. deliciosa* 'Hayward' dried peel extraction and fractionation. [a] Petroleum ether/Me_2CO (9:1); [b] petroleum ether/EtOAc (24:1); [c] petroleum ether/EtOAc (16:1); [d] petroleum ether/EtOAc (47:3); [e] petroleum ether/EtOAc (23:2); [f] $CHCl_3$/EtOAc (4:1); [g] $CHCl_3$/EtOAc (7:3); [h] $CHCl_3$/EtOAc (1:1); [i] MeOH/MeCN/H_2O (15:4:1); [j] hexane/MeCOEt (23:2); [k] $CHCl_3$/MeOH (19:1); [l] hexane/$CHCl_3$/MeOH (1:1:1); [m] $CHCl_3$/MeOH (4:1); [n] lower phase of $CHCl_3$/MeOH/H_2O (13:7:3); [o] MeOH/MeCN/H_2O (1:2:7); [p] EtOAc/EtOH/H_2O (24:4:1); [q] H_2O/MeCN (4:1); [r] MeOH/H_2O (1:1); [s] MeOH/MeCN/H_2O (1:4:15); [t] MeOH/H_2O (4:1).

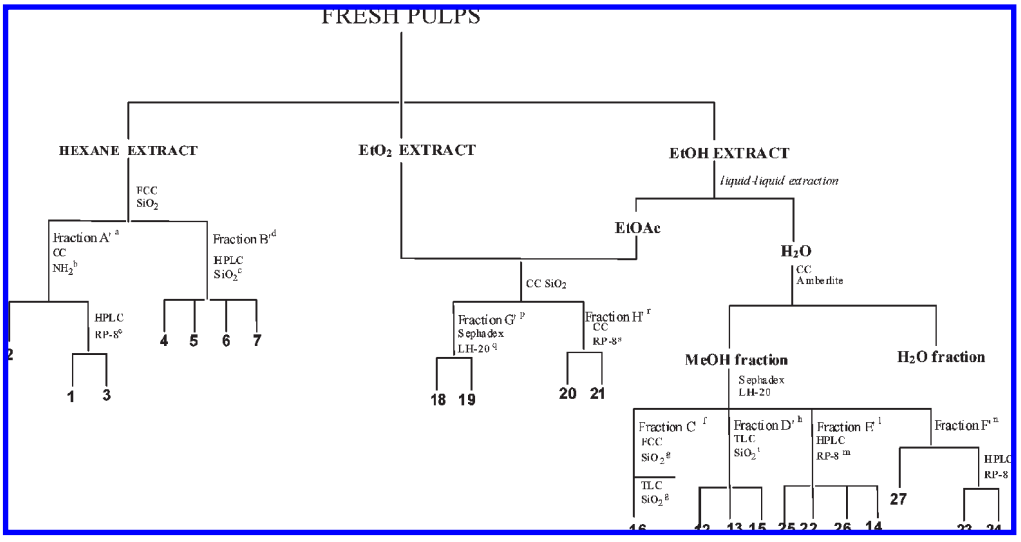

Figure 2. *A. deliciosa* 'Hayward' fresh pulp extraction and fractionation. [a] Petroleum ether/Me_2CO (8:1); [b] petroleum ether/EtOAc (15:1); [c] MeOH/MeCN/H_2O (15:4:1); [d] petroleum ether/Me_2CO (7:2); [e] hexane/MeCOEt (23:2); [f] H_2O; [g] lower phase of $CHCl_3$/MeOH/H_2O (13:7:5); [h] MeOH/H_2O (1:4); [i] lower phase of $CHCl_3$/MeOH/H_2O (13:7:2); [l] MeOH/H_2O (1:1); [m] MeOH/MeCN/H_2O (1:4:15); [n] MeOH; [o] MeOH/MeCH/H_2O (1:2:7); [p] $CHCl_3$/Me_2CO (1:1); [q] hexane/$CHCl_3$/MeOH (1:1:1); [r] Me_2CO; [s] MeOH/MeCN/H_2O (4:1:5).

(17:3) solution containing 50 μg/mL of butylated hydroxytoluene (BHT) as antioxidant. After removal of the solvent, the residue was dissolved in heptane (500 μL) and analyzed by GC-MS. Each analysis was performed in triplicate. The retention times (**Figure 3**) of the compounds were as follows: 46.36 min (**2**), 41.64 (**3**), 42.69 (**1**), 51.50 (**4**), 49.43 (**5**), 53.66 (**7**), and 48.59 (**6**).

Extraction Procedure for Phenol, Flavonoid Contents, and Extract Antioxidant Activities. The *A. deliciosa* 'Hayward' pulps and peels were frozen in liquid nitrogen, powdered in a mortar, and lyophilized. The freeze-dried products were extracted within a Soxhlet apparatus for 4 h with hexane. The solutions were dried using a rotary evaporator system to obtain crude peel and pulp extracts. After the removal of solvent, the residues were re-extracted for 4 h first with Et_2O and then with EtOH.

Total Phenol Determination. The amount of total phenols in *A. deliciosa* 'Hayward' crude extracts was determined according to the Folin−Ciocalteu procedure reported by Kähkönen et al. (*15*). Each crude extract (100 μL, 10 mg/mL) was introduced into test tubes: 0.5 mL of Folin−Ciocalteu reagent (Fluka Chemie, Buchs, Switzerland) and 4 mL of Na_2CO_3 (7.5%, w/v) were added. The contents of the tubes were mixed and allowed to stand for 3 h. Then, each aliquot (750 μL) was poured into 750 μL of deionized water. Absorption at 765 nm was measured. The total phenols of the samples are expressed as milligrams of gallic acid equivalents (GAE) per 100 g of fresh material and reported as phenol milligrams per gram of fresh product.

Total Flavonoid Determination. The flavonoid content of methanolic extracts was measured using a colorimetric assay developed by Zhishen et al. (*16*). A known volume (0.5 mL) of the extract or standard solution of catechin was added to a 10 mL volumetric flask. Distilled water was added to make a volume of 5 mL. At zero time, 0.3 mL of $NaNO_2$ (5%, w/v) was added to the flask. After 5 min, 0.6 mL of $AlCl_3$ (10%, w/v) was added, and after 6 min, 2 mL of NaOH (1 M) was added to the mixture followed by the addition of 2.1 mL of distilled water. Absorbance was read at 510 nm against the blank (water), and flavonoid content was expressed as milligrams of catechin equivalents (CATE) per 100 g of fresh material.

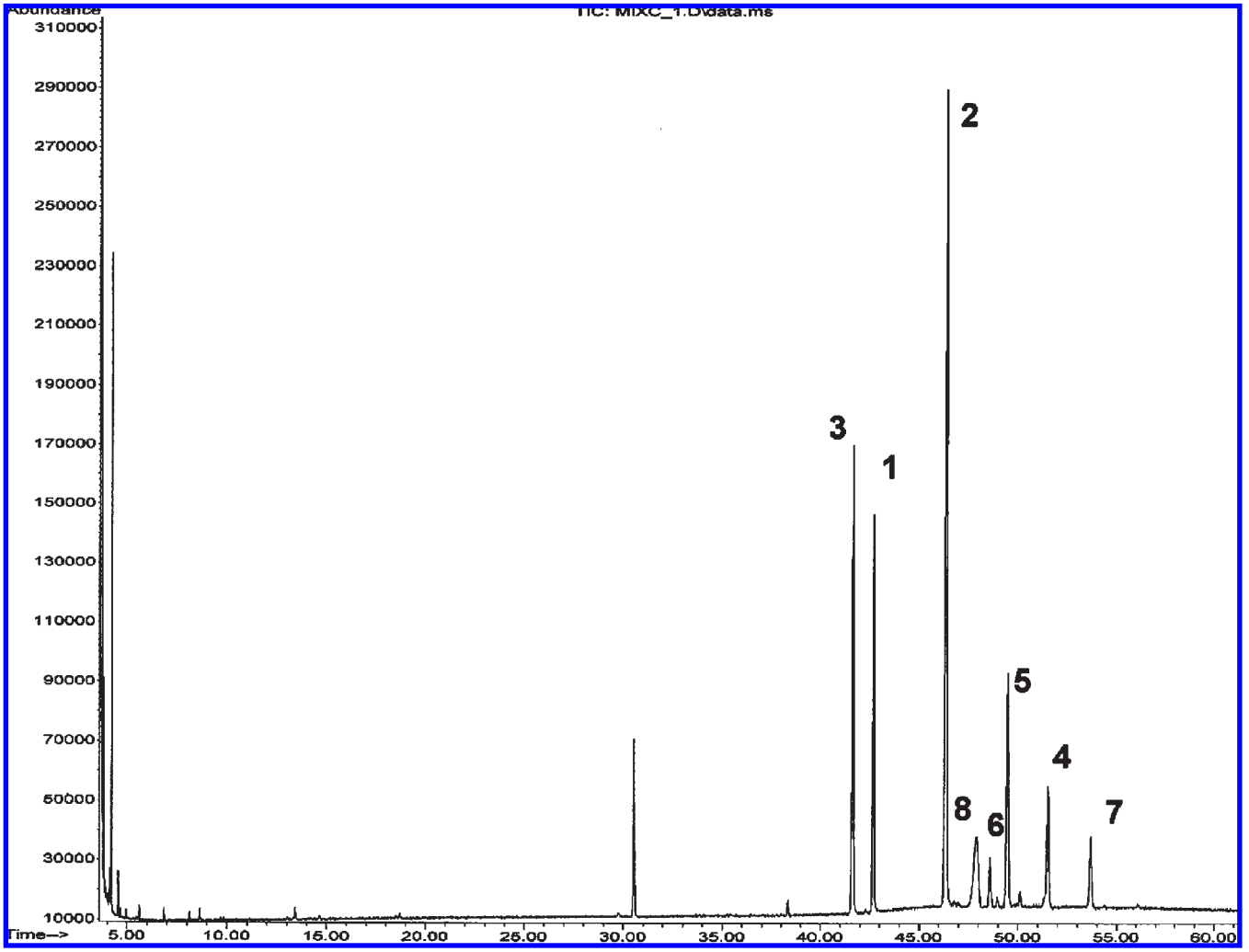

Figure 3. Elution profile on GC of tocopherols and steroids from kiwi fruit extracts.

DPPH Radical Scavenging Capacity. The DPPH radical scavenging capacity of *A. deliciosa* 'Hayward' extracts was measured according to the method of Brand-Williams et al. (*17*). Aliquots of each extract (100, 200, 300, 500, and 800 μg) or pure isolated metabolites (100 μL, 0.4 mM) were dissolved in 1.0 mL of MeOH. An aliquot (100 μL) was combined with 1.40 mL of 9.4×10^{-5} M methanolic DPPH$^{\bullet}$ solution (Fluka, Buchs, Switzerland) at room temperature. The absorbance at 517 nm was measured at 30 min versus a blank (100 μL of MeOH in 1.40 mL of DPPH$^{\bullet}$ solution). The analyses were carried out in triplicate. The results are expressed in terms of the percentage reduction of the initial DPPH radical adsorption by the test samples. The DPPH radical scavenging activity of the extracts has been compared with those exercised by ascorbic acid and α-tocopherol used as standards. The IC_{50} value, which is defined as the amount of antioxidant necessary to decrease the initial DPPH radical concentration by 50%, was also determined from the plotted graph of scavenging capacity versus each extract dose. The DPPH radical scavenging activity of the investigated metabolites has been compared with that exercised by ascorbic acid.

H_2O_2 Scavenging Capacity. The H_2O_2 scavenging capacity evaluation was carried out as reported from Sroka and Cisowski (*18*), with some modifications. Aliquots of each extract (100, 200, 300, 500, and 800 μg) were dissolved in 1 mL of H_2O/DMSO (19:1, v/v). One hundred microliters of sample was added to100 μL of H_2O_2 (0.002%) and 400 μL of NaCl (0.1 M) solution. After incubation at 37 °C for 30 min, to each sample was added 700 μL of Red Phenol−HRP (Red Phenol, 1.5 mg/mL; horseradish peroxidase, 1.0 mg/mL) in phosphate buffer (0.2 M, pH 7.8). After incubation at room temperature for 15 min, the samples were transferred in a polycarbonate cuvette, and NaOH (1 M, 50 μL) was added. The absorbance at 560 nm was measured versus blank.

Table 1. Total Phenol Content (TPC) and Total Flavonoid Content (TFC) of Kiwi Fruit Crude Extracts

	TPC[a]		TFC[b]	
	pulp	peel	pulp	peel
hexane	1.22 ± 0.17	0.44 ± 0.06		
Et_2O	52.8 ± 2.46	29.5 ± 2.02	38.7 ± 1.75	20.2 ± 1.37
EtOH	269.5 ± 2.07	267.3 ± 1.18	131.7 ± 3.42	108.9 ± 2.27

[a] Values are reported as GAE mg/100 g of fw ± SD. [b] Values are reported as CATE mg/100 g of fw ± SD.

The analyses were carried out in triplicate. The scavenging capacity was expressed as reduction percentage of Red Phenol absorbance sample induced and compared with those exercised by ascorbic acid and α-tocopherol used as standards. IC_{50} values were also determined.

Superoxide Radical Scavenging Capacity. Superoxide radical scavenging capacity assay was based on the capacity of crude extracts to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) (Fluka, Buchs, Switzerland) in the riboflavin−light−NBT system (*19*). Aliquots of each extract (100, 200, 300, 500, and 800 μg) or sample solutions (100 μL) containing reducing metabolites (0.4 mM) were dissolved in 1 mL of H_2O/dimethyl sulfoxide (19:1, v/v). Each 3 mL of reaction mixture contained sodium phosphate buffer (50 mM, pH 7.8), methionine (13 mM, Fluka), riboflavin (2 μM, Riedel-de Haën, Seelze, Germany), EDTA (100 μM, Carlo Erba Reagents, Rodano, Milano, Italy), NBT 75 μM, and extract solution (100 μL). The increase in absorbance at 560 nm was monitored. The analyses were carried out in triplicate. The scavenging capacity was expressed as

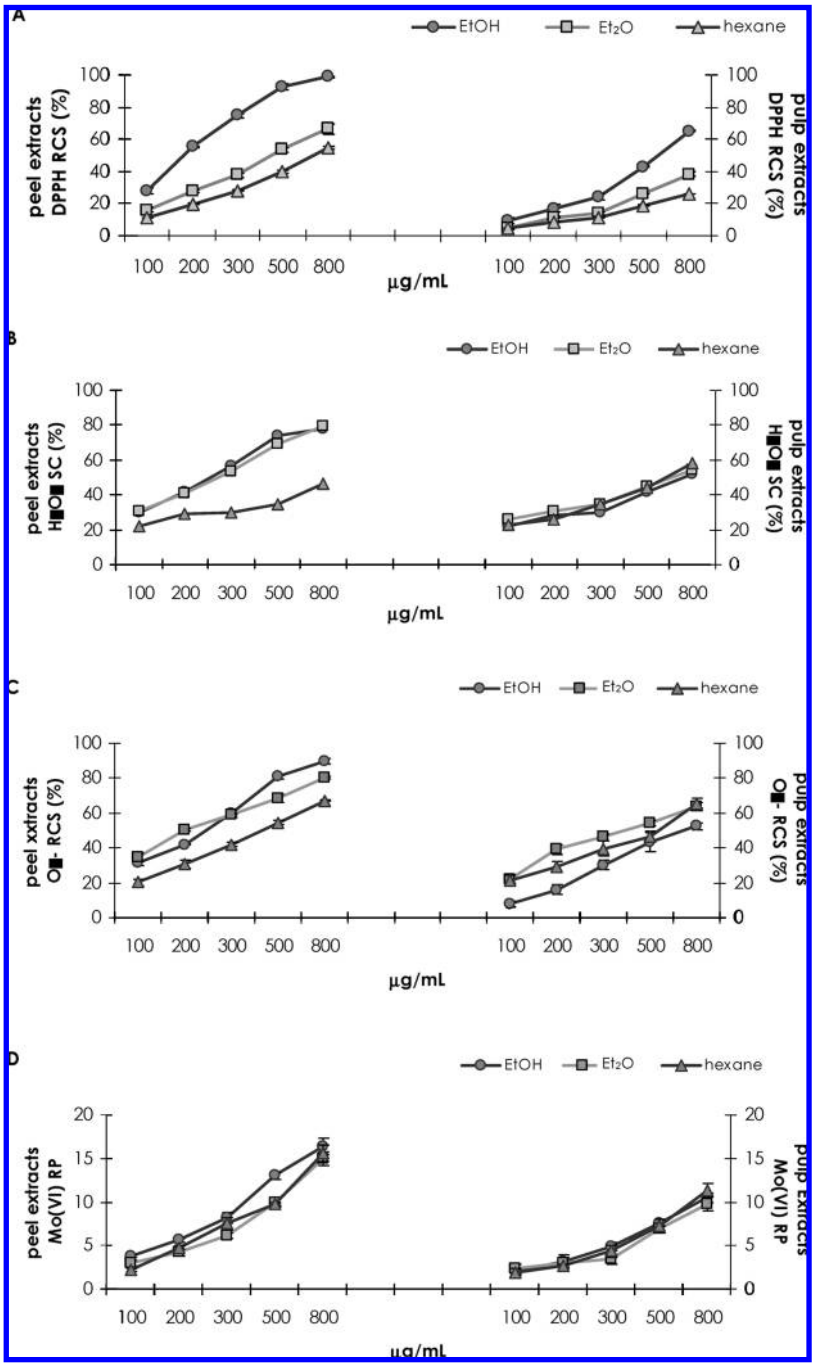

Figure 4. Antioxidant properties of *A. deliciosa* 'Hayward' organic extraction: (**A**) DPPH radical scavenging capacity (RSC); (**B**) H_2O_2 scavenging capacity (SC); (**C**) $O_2^{\bullet-}$ RSC; (**D**) Mo(VI) reducing power (RP). In **A**−**C** values are reported as percentage versus blank ± SD. In **D** values are reported as microgram caffeic acid equivalents ± SD.

reduction percentage of NBT absorbance sample induced. The registered scavenging capacity of the extracts was compared with those exercised by ascorbic acid and α-tocopherol used as standards. The extract's IC_{50} value was also determined. The $O_2^{\bullet-}$ scavenging activity of the investigated metabolites has been compared with that exercised by ascorbic acid.

Evaluation of Antioxidant Capacity. The quantitative determination of antioxidant capacity was carried out according to the method of Prieto et al. (*20*). Sample extract (100 μL, 100, 200, 300, 500, and 800 μg in 1 mL of H_2O/DMSO, 19:1 v/v) or sample solutions (100 μL) containing reducing metabolites (0.4 mM) were combined in an Eppendorf tube with 2.4 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). Tubes were capped and incubated in a thermal block at 95 °C for 90 min. After the samples had cooled at room temperature, the absorbance was measured at 650 nm versus a blank. The absorption value increase blank related is antioxidant capacity estimate. The analyses were carried out in triplicate. The antioxidant capacity was expressed as caffeic acid equivalent (CAE). Extracts registered activities that were compared to those exercised by α-tocopherol and ascorbic acid used as standards in the assay. The antioxidant activity of the investigated metabolites has been compared with that exercised by ascorbic acid.

RESULTS AND DISCUSSION

Many papers indicate kiwi fruits as one of the healthiest food products due to their high content of antioxidant compounds such as ascorbic acid and polyphenols (*7*, *21*). Continuing the investigation of health-protecting factors from fruits produced in

the Mediterranean region (*8*−*14*), we undertook the phytochemical investigation of this species.

In a preliminary approach, crude organic extracts underwent a chemical screening evaluating their phenol and flavonoid contents as well as their antioxidant capacity by using different activity tests. The analysis confirmed the massive presence of phenolic substances of the most polar type in both ethanolic extracts (**Table 1**). The phenol content, expressed as gallic acid equivalents (GAE mg/100 g of fw), was 269.5 ± 2.07 for EtOH pulp extract and 267.3 ± 1.18 for EtOH peel extract.

The determination of the flavonoid content of Et_2O and EtOH extracts, expressed as catechin equivalents (CATE mg/100 g of fw), established the peculiar EtOH pulp extract flavonoid abundance (**Table 1**).

Determination of the fruit antioxidant properties (**Figure 4**) was carried out by measuring its reducing power against a molybdenum salt through the formation of a phosphomolybdenum complex and its ability to scavenge the DPPH radical, the anion superoxide radical, and the pro-oxidant hydrogen peroxide. The detected activities were compared to those exercised by the natural standards ascorbic acid and α-tocopherol. The reducing Mo(VI) efficacy is expressed as caffeic acid microgram equivalents (CAE); the scavenging capacities are reported as percentage reduction of radical ($DPPH^{\bullet}$, $O_2^{\bullet-}$) and no radical (H_2O_2) species in the assay media. The relative IC_{50} values are also reported (**Table 2**).

The results of the DPPH radical, the anion superoxide radical, and the pro-oxidant hydrogen peroxide scavenging capacity assays showed that all of the extracts scavenged massively the radical target species. The peel hexane extract is responsible for a strong antioxidant ability; its calculated IC_{50} values relative to the chemical species DPPH and H_2O_2 are 114.4 and 106.7 μg/mL. Peel Et_2O extract has equally marked antioxidant properties: it determined a strong reduction (80.0%) at the highest tested dose of Red Phenol and nitroblue tetrazolium salt, used as probes in H_2O_2 and $O_2^{\bullet-}$ scavenging capacity tests, respectively. The extracts promoted a striking Mo(VI) reducing power; the antioxidant efficacy of the three extracts 800 μg dose was comparable to that exerted by ascorbic acid 15 μg average dose. Kiwi fruit pulp extracts carried on weak reducing and scavenging capabilities. Spectrophotometric evaluation of antioxidant capacity through the formation of a phosphomolybdenum complex evidenced that the tested samples at 800 μg dose were able to reduce Mo(VI) to Mo(V) comparably to ascorbic acid 10 μg average dose. All of the extracts brought about dose−response scavenging abilities and defined at the highest share a strong percentage reduction. In fact, the hexane, diethyl ether, and ethanolic extracts reduced $O_2^{\bullet-}$ by 52.5, 63.7, and 65.6%, respectively, and scavenged 52.2, 54.6, and 58.3% of the pro-oxidant H_2O_2.

Kiwi fruit crude extracts were separated by chromatographic techniques into their constituents. The 27 pure compounds were characterized by spectroscopic analysis using, in particular, 1D and 2D NMR experiments. Structures of phytochemicals isolated from *A. deliciosa* 'Hayward' extracts are reported in **Figures 5** and **6**. Compound **1** was identified as δ-tocomonoenol, a new vitamin E, whereas compounds **2** and **3** were identified as α-tocopherol and δ-tocopherol, respectively (*22*). The sterols **4**−**10** were characterized as stigmastane sterols. Compound **4** was β-sitosterol, whereas compounds **5**−**10** were identified as stigmasterol, campesterol, stigmast-7-en-3β-ol (*23*), ergosterol (*24*), its peroxide derivative (*25*), and 5,7,14,22-ergostatetraen-3β-ol (*26*), respectively.

Compound **11** was identified as ursolic acid. Compounds **12** and **13** were identified as caffeic acid glucosyl derivatives and

Table 2. IC_{50} Values of *A. deliciosa* 'Hayward' Extracts

		IC_{50} (μg/mL)		
		$DPPH^{\bullet}$	H_2O_2	$O_2^{\bullet-}$
α-tocopherol		6.69		17.6
ascorbic acid		4.58	2.48	5.0
peel	hexane	753.7	>1000	406.4
	Et_2O	444.7	246.6	202.4
	EtOH	171.3	234.1	209.9
pulp	hexane	>1000	630.5	475.0
	Et_2O	>1000	701.6	385.0
	EtOH	596.7	856.0	726.3

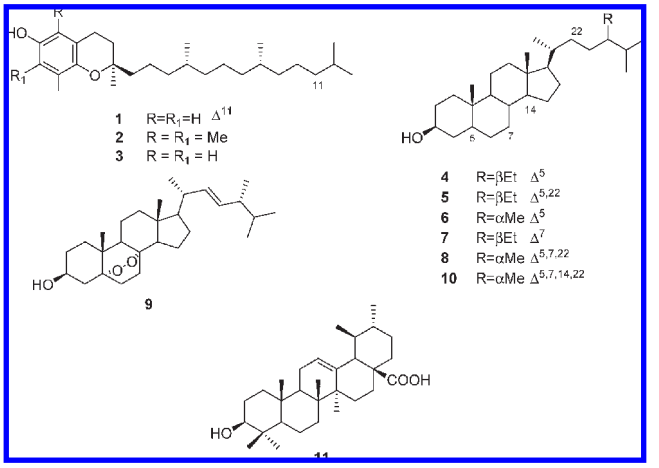

Figure 5. Chemical structures of compounds **1**−**11** from *A. deliciosa* 'Hayward'.

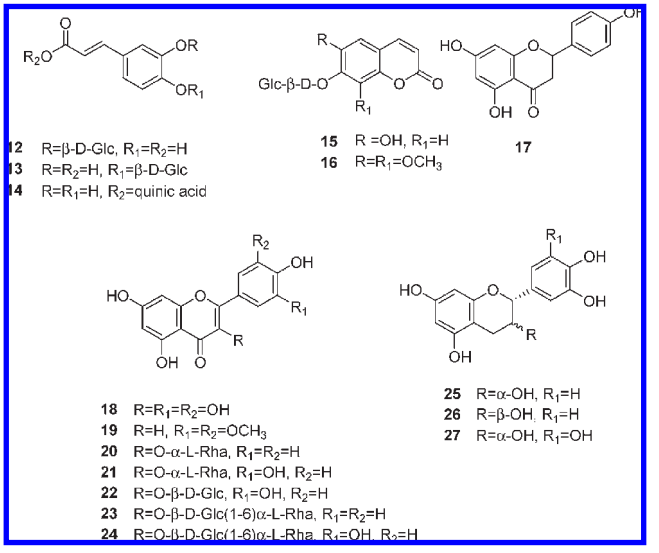

Figure 6. Chemical structures of compounds **12**−**27** from *A. deliciosa* 'Hayward'.

characterized as caffeic 3-*O*-β-D-glucopyranoside acid (*27*) and caffeic 4-*O*-β-D-glucopyranoside acid (*28*), respectively. Compound **14** was identified as chlorogenic acid.

Polyphenols **15** and **16** were characterized as coumarin derivatives. In particular, compound **15** was identified as 6-hydroxy-7-(β-D-glucopyranosyloxy)coumarin (*29*) and metabolite **16** as 6,8-dimethoxy-7-(β-D-glucopyranosyloxy)coumarin (*30*). Metabolites **17**−**27** were characterized as flavonoid molecules. Compound **17**, present only in peel matrix, was naringenin (*31*), and

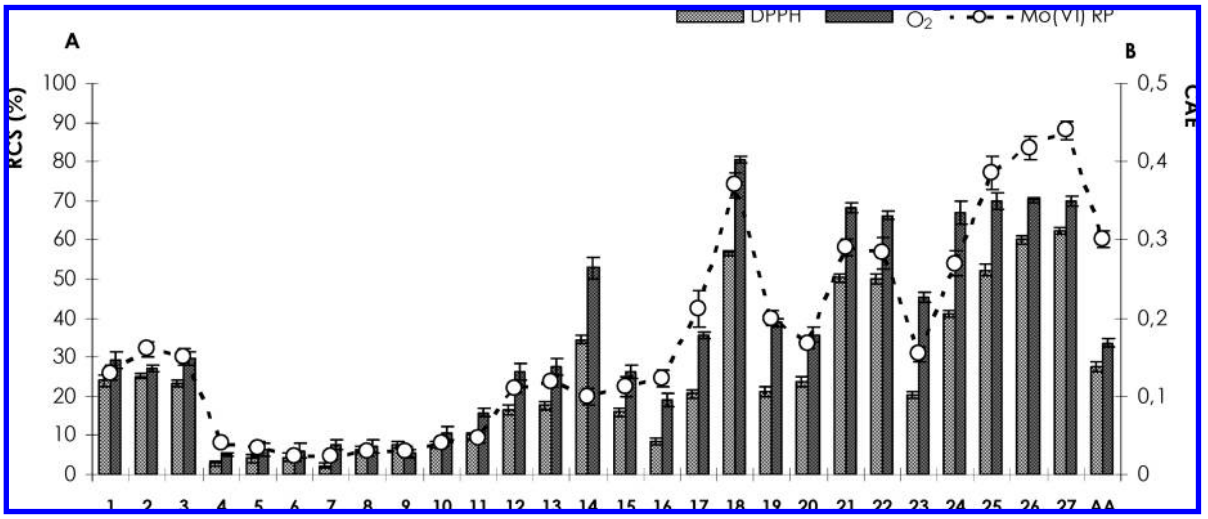

Figure 7. (**A**) Radical scavengig capacity (RCS) of metabolites **1**−**27** from *A. deliciosa* 'Hayward' on DPPH radical and $O_2^{\bullet-}$ radical. Values are reported as percentage versus a blank ± SD. (**B**) Mo(VI) reducing power (RP). Values are reported as micrograms of caffeic acid equivalents (CAE) ± SD.

compounds **18** and **19** were quercetin and tricin, respectively. Compounds **20** and **21** were identified as 3-*O*-α-L-rhamopyranosyl derivatives of flavones kaempferol and quercetin, respectively. Compound **22** was quercetin-3-*O*-*β*-D-glucopyranoside, whereas compounds **23** and **24** were kaempferol-3-*O*-*β*-rutinoside and rutin. Compounds **25** and **26** were identified as epicatechin and catechin, respectively. Compound **27** was gallocatechin.

To investigate the presence of compounds **1**−**7** in the kiwi fruits, a GC-MS analysis was performed. To this aim, aliquots of lyophilized pulps and peels were extracted in hexane/EtOAc (17:3) solution, in the presence of BHT. The extracts were dried and dissolved in heptane and analyzed by GC-MS. All of the analyses were carried out in triplicate. The identification of the analytes was obtained by comparison of the retention times with those of pure standards and by the fragmentation patterns in the EIMS spectra. The results (**Table 3**) are expressed in milligrams per 100 g of fresh fruit matrix. *δ*-Tocopherol and *δ*-tocomonoenol are more abundant in peel than in pulp, whereas α-tocopherol is at similar levels in both fruit components. The presence of phytosterols is registered as being more massive in pulp than in peel.

All of the metabolites isolated from *A. deliciosa* 'Hayward' fruits were tested for their antioxidant activity by measuring their ability to scavenge free radicals, DPPH• and $O_2^{\bullet-}$, and by evaluating their capacity to induce the formation of a phosphomolybdenum complex. The antioxidant capacity of compounds was compared with that shown by the known natural antioxidant, ascorbic acid (AA), treated in the same way. The results are reported in **Figure 7**. The strongest antioxidant activity was observed.

It is currently accepted that the consumption of kiwi fruits has a preventive effect against certain cancers and vascular diseases. The total antioxidant efficacy of commonly consumed fruits has been rated in the order strawberry > orange > plum > kiwi > grapefruit > apple > pear (*32*). In particular, *A. deliciosa* is a significant source of the antioxidants ascorbic acid and α-tocopherol (*33*). One large peeled kiwi fruit contains 84.4 mg (141% of the USDA Recommended Daily Value) of ascorbic acid and 1.3% (4% RDV) of α-tocopherol.

The observed high antioxidant and radical scavenging capabilities allow us to define kiwi fruits precious cocktail of protective phytochemicals. It is notable that bone health can be improved and maintained by involvement in proper physical activity and eating a nutritious well balanced diet. Among foods utilized for their beneficial properties, kiwi fruits have been one of the most recommended. Our research demonstrates the high antioxidant and radical scavenging capabilities of kiwi fruits, revealing a unique and precious cocktail of protective phytochemicals. The massive presence of vitamin E and flavonoids ensures the antioxidant protective effect in both lipophilic and hydrophilic conditions. Moreover, the presence of phytosterols and ursolic acid could contribute to reduce the hemeatic cholesterol level and to inhibit carcinogenesis processes.

Table 3. Amount of Compounds **1**−**7** Found in Pulp and Peel of Kiwi Fruits[a]

	peel	pulp
δ-tocomonoenol (**1**)	1.45 ± 0.08	0.85 ± 0.02
α-tocopherol (**2**)	1.05 ± 0.06	1.02 ± 0.03
δ-tocopherol (**3**)	2.49 ± 0.12	0.64 ± 0.01
β-sitosterol (**4**)	4.84 ± 0.20	9.28 ± 0.45
stigmasterol (**5**)	3.35 ± 0.11	2.40 ± 0.11
campesterol (**6**)	1.75 ± 0.04	2.77 ± 0.10
stigmast-7-en-3*β*-ol (**7**)	1.81 ± 0.07	5.32 ± 0.29

[a] Values are reported as mg/100 g of fw.

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