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New Polyphenol Derivative in *Ipomoea batatas* Tubers and Its Antioxidant Activity

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Four different polyphenolic compounds were isolated by chromatographic methods from methanolic and hydromethanolic extracts of *Ipomoea batatas* tuber flour. On the basis of UV, mass, and NMR analysis procedures, the structure of the isolated compounds were determined as 4,5-di-*O*-caffeoyldaucic acid (**1**), 4-*O*-caffeoylquinic acid (**2**), 3,5-di-*O*-caffeoylquinic acid (**3**), and 1,3-di-*O*-caffeoylquinic acid (**4**). To the best of our knowledge, this is the first report of isolation and characterization of compound **1**. Then, we evaluated the antioxidant activity of daucic acid derivative by using DPPH and FRAP methods together with authentic antioxidant standards, L-ascorbic acid, tert-butyl-4-hydroxy toluene (BHT), and gallic acid. The activity of compound **1** in both methods was higher than that of all standards used at the same molar concentration.

KEYWORDS: Camote; sweet potato; *Ipomoea batatas*; caffeoyl; convolvulaceae; NMR; antioxidant

INTRODUCTION

Ipomoea batatas Lam, known as sweet potato or camote, belongs to the Convolvulaceae family, but despite its name, it is not related to the potato (Solanaceae family). It is used for human consumption, as livestock feed, and in industrial processes to make alcohol and starch and products such as noodles, candy, desserts, and flour. The camote tubers are rich in carotenoids, vitamin B, vitamin E, iron, calcium, zinc, fiber, and protein and contain virtually no fat and are low in sodium (*1*). Camote is one of the few vegetables that can be grown easily during the monsoon seasons of the tropics, are usually the only greens available in some countries after a flood or a typhoon, and are tolerant to diseases, pests, and high moisture. Furthermore, these tubers have the advantage of being harvested several times in a year (*2*−*5*). The aim of this work was the analytical studies of phenols to provide information to establish nutritional values of the sweet potato tuber, because in some developing countries the camote is being incorporated into the diet of children to solve the serious problem of infant malnutrition. In particular, it is now being used in Africa to combat a widespread vitamin A deficiency that results in blindness and even death for 250 000−500 000 African children a year. Sweet potato is also a candidate root crop for production in the Advanced Life Support System (ALSS) program of the National Aeronautic and Space Administration (NASA) (*6*). We have been interested in the plant phenols because they are an important group of natural antioxidants and because they contribute to the organoleptic and nutritional qualities of fruit and vegetables. Polyphenol compounds have attracted special attention because they may protect the human body from oxidative stress, which can contribute to many diseases including cancer, cardiovascular diseases, and aging (*7*−*16*). Caffeoyl derivatives have received special attention because of their ability as anti-HIV and antiviral agents (*17*) and to increase hepatic glucose utilization (*18*−*21*). Therefore, we have evaluated the phenolic quality and the antioxidant property of the unknown compound in *Ipomoea batatas*. Research on antioxidants has increased considerably during the past 10 years (*22*−*26*). Actually, there is no simple universal method by which antioxidant capacity can be measured accurately and quantitatively, because multiple reaction characteristics and mechanisms are usually involved. In the literature, several in-vitro analytical methods are reported to characterize the antioxidant properties of bioactive compounds in plant foods. The mechanism of antioxidant action in vitro may involve direct inhibition of the generation of reactive oxygen species or the scavenging of free radicals. The antioxidative activity of daucic acid derivative was evaluated by using DPPH and FRAP methods together with authentic antioxidant standards, L-ascorbic acid, BHT, and gallic acid.

MATERIALS AND METHODS

Plant Material. The plant material was collected in Peru in 2005 and was identified by Dr. Santago Antunez De Mayolo, Casella Postale 18-1125, Lima 18, Peru, and reference specimens were deposited at the Dipartimento di Chimica delle Sostanze Naturali, Napoli (Italy).

General Experimental Procedures. Spectroscopic Apparatus and Methods. FABMS spectra, recorded in a glycerol matrix, were measured on a Prospec Fisons mass spectrometer (Danvers, NJ). ESIMS experiments were performed on an Applied Biosystem API 2000 triple-quadrupole mass spectrometer (Warrington, Cheshire, United Kingdom). The spectra were recorded by infusion into the ESI source using MeOH as the solvent. NMR spectra were determined at 25 °C on a Varian

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Unity Inova 500 NMR spectrometer (Palo Alto, CA) and were processed using the Varian VNMR software package; chemical shifts were referenced to the residual solvent signal (CH_3OD: δ_H 3.34, δ_C 49.0). Homonuclear ^1H connectivities were determined by COSY experiments. The reverse-detected, gradient-enhanced single-quantum heteronuclear correlation (HSQC) spectra were optimized for an average $^1J_{CH}$ of 140 Hz. The gradient-enhanced multiple-bond heteronuclear correlation (HMBC) experiments were optimized for a $^3J_{CH}$ of 8 Hz. Optical rotations were determined on a Jasco P-100 polarimeter (Tokyo, Japan) equipped with a sodium lamp (589 nm) and a 10-mm microcell.

Chromatographic Apparatus and Methods. HPLC analysis in linear gradient mode was performed on a Hewlett-Packard HP 1100 series apparatus with a UV-photodiode detector (λ 200 nm) (Waldbronn, Germany), equipped with a 300 × 7.8 mm i.d. μ-Bondapack C_{18} column (Waters, Milford, MA). DCCC (droplet counter current chromatography) separation was performed on a Buchi apparatus (Flawil, Switzerland) equipped with 300 tubes (2.0 mL, 2.2 mm i.d.). TLC was performed on Si-gel plates in *n*-BuOH/HOAc/H_2O (60:15:25).

Determination of Ferric Reducing/Antioxidant Power (FRAP method). The total antioxidant potential of the new compound was determined using the ferric reducing ability of plasma (FRAP) assay of Benzie and Strain (*27*). A solution of 10 mM TPTZ (2,4,6-tris-2,4,6-tripyridyl-2-triazine) and 12 mM ferric chloride was diluted in 300 mM sodium acetate buffer (pH 3.6) at a ratio of 1:1:10. Aliquots (20 μL) of the new compound and the antioxidant standard solution (both 1 mM) were added to 3 mL of the FRAP solution, and the absorbance at 593 nm was determined at 37 °C for 110 min.

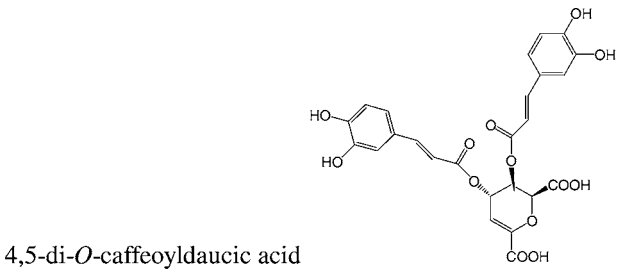
Determination of Free-Radical-Scavenging Ability (DPPH method). The ability of a new compound and new standards to scavenge the DPPH (1,1-diphenyl-2-picrilhydrazyl) radical was measured using the method of Brand-Williams (*28*). Aliquots (20 μL−1 mM) were added to 3 mL of DPPH (6×10^{-2} mM), and the absorbance was determined at 515 nm for 110 min.

Statistics. Triplicate analyses for each measurement were conducted for each sample. Differences between the means were evaluated with ANOVA, using the Graf Pad Instat 3 (Microsoft Software) statistics program. The significance of the model was evaluated by ANOVA. The significance of the regression coefficients was evaluated by Student's *t* test. The significance level was fixed at 0.05 for all the statistical analysis.

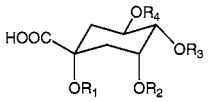
Extraction and Isolation. The whole flour from the tubers without skin (1 kg) was extracted with MeOH, MeOH/H_2O (90:10), and MeOH/H_2O (80:20). The last extract was partitioned between *n*-BuOH and H_2O. The butanol extract (6.0 g) was further separated by DCCC using *n*-BuOH/Me_2CO/H_2O (60:12:28) as stationary phase and *n*-BuOH/Me_2-CO/H_2O (14:12:74) as descending phase. DCCC fractions 45−245 (9.0 mL each) containing the crude phenolic mixture were chromatographed by reversed-phase HPLC using a linear gradient of H_2O/MeOH from 100% H_2O at 0 min to 100% MeOH at 20 min, at a flow rate of 2.5 mL/min to yield four pure compounds: **1** (23.5 mg, R_f 0.51), **2** (2.3 mg, R_f 0.66), **3** (5 mg, R_f 0.80), and **4** (4.9 mg, R_f 0.79). Their R_f values were determined by using silica gel thin layer chromatography with *n*-BuOH/HOAc/H_2O (60:15:25) as the mobile phase.

Compound **1**. 4,5-di-*O*-caffeoyldaucic acid (**Figure 1**). Amorphous powder. Melting point 210−212. $[\alpha]^{25}_D$ + 35.0° (c = 0.003 in MeOH); HRFABMS negative ion, found *m*/*z* 527.08254 [M − H]$^-$; calculated for $C_{25}H_{20}NO_{13}$ *m*/*z* 528.09039. ESI-MS (negative ion): *m*/*z* 527 [M − H]$^-$; ESI-MS/MS fragments: *m*/*z* 179, *m*/*z* 186, *m*/*z* 202, *m*/*z* 186, *m*/*z* 365. UV λ max (MeOH) nm (log ϵ): 227 (3.02), 251 (2.93), 278 (2.79), 300 (3.03), 352 (2.88) (**Figure 2**).

Compound **2.** 4-*O*-caffeoyl quinic acid (**Figure 1**). White powder. ESI-MS (negative ion): *m*/*z* 353 [M − H]$^-$, ESI-MS/MS fragments: *m*/*z* 191, *m*/*z* 179, *m*/*z* 135. UV λ max (MeOH) nm (log ϵ): 225 (2.73), 247 (2.65), 280 (2.57), 328 (2.79). ^1H NMR (500 MHz, CD_3OD): (**2**) δ 7.04 (1H, d, J = 2.0 Hz, H-2 caffeoyl), δ 6.77 (1H, d, J = 8.0 Hz, H-5 caffeoyl), δ 6.94 (1H, dd, J = 2.0 and 8.0 Hz, H-6 caffeoyl), δ 7.57 (1H, d, J = 16.3 Hz, H-7 caffeoyl), δ 6.29 (1H, d, J = 16.3 Hz, H-8 caffeoyl), δ 1.98-δ 2.13 (2H, m, J = 5.0 Hz, H-2 quinic), δ 5.36 (1H, m, J = 5.0 Hz, H-3 quinic), δ 3.55 (1H, dd, J = 3.0, 9.0 Hz, H-4 quinic), δ 4.11 (1H, dd, J = 3.0, 9.0, H-5 quinic), δ 2.13−2.28 (2H, m, H-6 quinic); ^{13}C NMR (500 MHz, CD_3OD): (**2**) δ 127.9 (C-1 caffeoyl), δ 115.6 (C-2 caffeoyl), δ 146.8 (C-3 caffeoyl), δ 149.5 (C-4 caffeoyl), δ 116.5 (C-5 caffeoyl), δ 122.9 (C-6 caffeoyl), δ 146.8 (C-7 caffeoyl), δ 115.1 (C-8 caffeoyl), δ 169.1 (C-9 caffeoyl), δ 76.6 (C-1 quinic), δ 39.0 (C-2 quinic), δ 69.8 (C-3 quinic), δ 79.3 (C-4 quinic), δ 65.5 (C-5 quinic), δ 42.5 (C-6 quinic), δ 176.9 (C-7 quinic) (*29*).



4,5-di-*O*-caffeoyldaucic acid



4-*O*-caffeoyl quinic acid, **2**: R_1 =R_2 = R_4= H; R_3= caffeoyl
3,5-di-*O*-caffeoylquinic acid, **3**: R_1 =R_3 = H; R_2 =R_4= caffeoyl
1,3-di-*O*-caffeoylquinic acid, **4**: R_3 =R_4 = H; R_1= R_2 caffeoyl

Figure 1. Compounds **1**–**4** isolated from *Ipomoea batatas* tubers.

Compound **3.** 3,5-di-*O*-caffeoylquinic acid (**Figure 1**). Yellow powder. ESI-MS (negative ion): *m*/*z* 515 [M − H]$^-$, ESI-MS/MS fragments: *m*/*z* 191, *m*/*z* 179, *m*/*z* 135, *m*/*z* 353. UV λ max (MeOH) nm (log ϵ): 225 (3.08), 247 (3.02), 280 (2.98), 343 (3.03). ^1H NMR (500 MHz, CD_3OD): (**3**) δ 7.06 (1H, d, J = 2 Hz, H-2 caffeoyl), δ 6.78 (1H, d, J = 8.0 Hz, H-5 caffeoyl), δ 6.96 (1H, dd, J = 2.0 and 8.0 Hz, H-6 caffeoyl), δ 7.57 (1H, d, J = 16.3 Hz, H-7 caffeoyl), δ 6.27 (1H, d, J = 16.3 Hz, H-8 caffeoyl), δ 7.02 (1H, d, J = 2 Hz, H-2′ caffeoyl) δ 6.78 (1H, d, J = 8.2 Hz, H-5′ caffeoyl), δ 6.96 (1H, dd, J = 2.0 and 8.2 Hz, H-6′ caffeoyl), δ 7.45 (1H, d, J = 16.3 Hz, H-7′ caffeoyl), δ 6.18 (1H, d, J = 16.3 Hz, H-8′ caffeoyl), δ 2.13−δ 2.34 (2H, m, J = 5.0 Hz, H-2 quinic), δ 5.43 (1H, m, J = 5.0 Hz, H-3 quinic), δ 3.97 (1H, dd, J = 3.4, 7.6 Hz, H-4 quinic), δ 5.39 (1H, ddd, J = 5.9, 7.6, 10.8, H-5 quinic), δ 2.13−2.34 (2H, m, H-6 quinic); ^{13}C NMR (500 MHz, CD_3OD): (**3**) δ 127.9 (C-1 caffeoyl), δ 115.7 (C-2 caffeoyl), δ 146.8 (C-3 caffeoyl), δ 147.8 (C-4 caffeoyl), δ 116.3 (C-5 caffeoyl), δ 123.0 (C-6 caffeoyl), δ 147.0 (C-7 caffeoyl), δ 115.1 (C-8 caffeoyl), δ 168.1 (C-9 caffeoyl), 127.8 (C-1′ caffeoyl), δ 115.7 (C-2′ caffeoyl), δ 146.9 (C-3′ caffeoyl), δ 147.7 (C-4′ caffeoyl), δ 116.2 (C-5′ caffeoyl), δ 122.9 (C-6′ caffeoyl), δ 146.9 (C-7′ caffeoyl), δ 115.0 (C-8′ caffeoyl), δ 168.0 (C-9′ caffeoyl), δ 73.0 (C-1 quinic), δ 35.7 (C-2 quinic), δ 72.1 (C-3 quinic), δ 71.5 (C-4 quinic), δ 73.0 (C-5 quinic), δ 37.0 (C-6 quinic), δ 175.8 (C-7 quinic) (*30*).

Compound **4.** 1,3-di-*O*-caffeoylquinic acid (**Figure 1**). Yellow powder. ESI-MS (negative ion): *m*/*z* 515 [M − H]$^-$, ESI-MS/MS fragments: *m*/*z* 191, *m*/*z* 179, *m*/*z* 135, *m*/*z* 353. UV λ max (MeOH) nm (log ϵ): 225 (2.95), 247 (2.81), 280 (2.75), 343 (2.76). ^1H NMR (500 MHz, CD_3OD): (**4**) δ 6.81 (1H, d, J = 2.0 Hz, H-2 caffeoyl), δ 6.50 (1H, d, J = 8.2 Hz, H-5 caffeoyl), δ 6.58 (1H, dd, J = 2.0 and 8.2 Hz, H-6 caffeoyl), δ 7.46 (1H, d, J = 16.3 Hz, H-7 caffeoyl), δ 6.18 (1H, d, J = 16.3 Hz, H-8 caffeoyl), δ 6.92 (1H, d, J = 2 Hz, H-2′ caffeoyl) δ 6.63 (1H, d, J = 8.2 Hz, H-5′ caffeoyl), δ 6.74 (1H, dd, J = 2.0 and 8.2 Hz, H-6′ caffeoyl), δ 7.48 (1H, d, J = 16.3 Hz, H-7′ caffeoyl), δ 6.18 (1H, d, J = 16.3 Hz, H-8′ caffeoyl), δ 1.83- δ 2.87 (2H, m, J = 5 Hz, H-2 quinic), δ 5.36 (1H, m, J = 5.0 Hz, H-3 quinic), δ 3.61 (1H, dd, J = 3.6, 9.6 Hz, H-4 quinic), δ 4.22 (1H, ddd, J = 4.4, 9.6, 11.2, H-5 quinic), δ 1.83−2.87 (2H, m, H-6 quinic); ^{13}C NMR (500 MHz, CD_3OD): (**4**) δ 127.5 (C-1 caffeoyl), δ 115.4 (C-2 caffeoyl), δ 146.5 (C-3 caffeoyl), δ 149.3 (C-4 caffeoyl), δ 116.1 (C-5 caffeoyl), δ 122.0 (C-6 caffeoyl), δ 147.2 (C-7 caffeoyl), δ 115.1 (C-8 caffeoyl), δ 167.8 (C-9 caffeoyl), 127.4 (C-1′ caffeoyl), δ 115.5 (C-2′ caffeoyl), δ 146.7 (C-3′ caffeoyl), δ 149.7 (C-4′ caffeoyl), δ 116.6 (C-5′ caffeoyl), δ 123.0 (C-6′ caffeoyl), δ 147.8 (C-7′ caffeoyl), δ 115.0 (C-8′ caffeoyl), δ 168.9 (C-9′ caffeoyl), δ 79.3 (C-1 quinic), δ 33.1 (C-2 quinic), δ

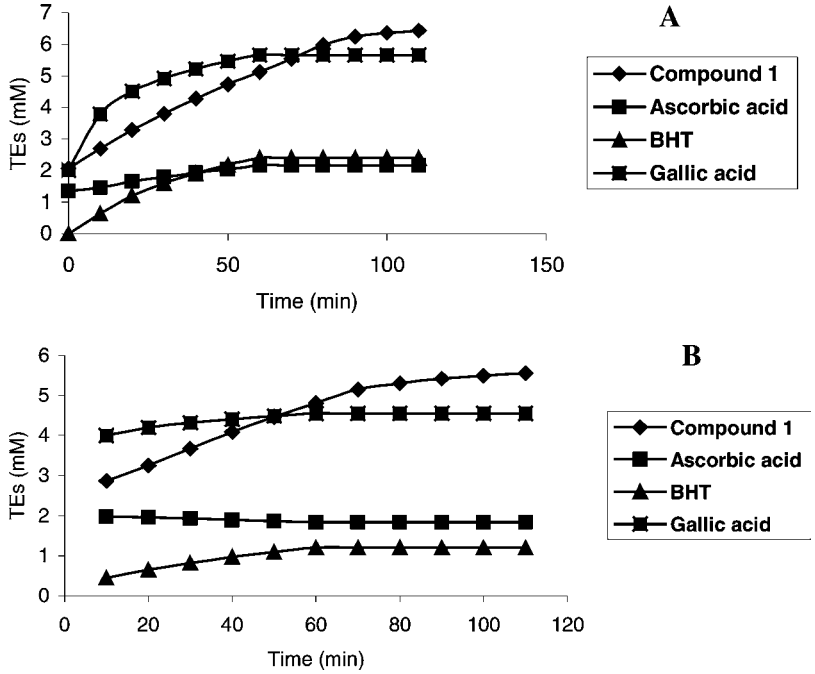


Figure 2. Antioxidant capacity of new compound (**1**) vs three antioxidant standards measured by using A, the FRAP method, and B, the DPPH method.

73.2 (C-3 quinic), δ 75.7 (C-4 quinic), δ 67.9 (C-5 quinic), δ 41.3 (C-6 quinic), δ 175.0 (C-7 quinic) (*30*).

RESULTS AND DISCUSSION

Identification of 4,5-di-*O*-caffeoyldaucic Acid (1). The flour from camote tubers was exhaustively extracted with MeOH, MeOH:H_2O/90:10, MeOH:H_2O/80:20, and the latter extract was partitioned beetwen BuOH and H_2O and was then purified by sequential chromatographic techniques, affording the caffeoyl derivatives. The negative ESI-MS spectrum of compound **1** showed a pseudomolecular ion peak at *m*/*z* 527 $[M - H]^-$. High-resolution measurements on the pseudomolecular ion peak indicated the molecular formula $C_{25}H_{20}NO_{13}$.

The ^{1}H NMR spectrum of **1** (CD_3OD, (**Table 1**) exhibited four doublets with coupling constants of 16.6 Hz characteristic for trans olefinic protons (δ 7.44, δ 6.12, δ7.48, δ 6.27), and the coupling pattern of two 1,3,4-trisubstituted benzenes 6.73 (1H, dd, J = 2.0; 8.3 Hz), 6.65 (1H, d, J = 8.3 Hz), 6.96 (1H, d, J = 2.0 Hz) and 6.88 (1H, dd, J = 2.0; 8.3 Hz), 6.74 (1H, d, J = 8.3 Hz), 7.01 (1H, d, J = 2.0 Hz) indicated the presence of two (*E*)-caffeic acid moieties, whose presence was confirmed in ^{13}C spectrum and was also supported by characteristic UV absorptions at 330 and 352 nm and ESI-MS-MS peaks at *m*/*z* 179. Moreover, the spectrum showed the presence of another olefinic proton at δ 5.71 (1H, dd, J = 1.5, 2.6) with an upfield shift with respect to those previously described and three protons α to oxygen: 6.04 (1H, ddd, J = 1.5, 2.6, 4.3), 5.93 (1H, ddd, J = 1.5, 1.7, 4.3), 4.66 (1H, dd, J = 1.5, 1.7), which along with the values of coupling constants $J_{3,4}$ (2.6 Hz), $J_{4,5}$ (4.3 Hz), $J_{5,6}$ (1.7 Hz), and $J_{3,5}$ (1.5 Hz) and ^{13}C data (**Table 1**) were comparable with those reported for a daucic acid derivative (*31*). All the proton resonances of each compound were unambiguously associated with the relevant carbon atoms by using the HSQC spectrum. The proton sequence within each spin system was elucidated by the series of cross-peaks of the COSY spectrum, while data arising from the HMBC experiment were used to interconnect the partial structures. HMBC cross-peaks were detected between the daucic acid proton H-4 (δ 6.04) and caffeic acid carboxyl group C-9″ (δ 168.3) and between the daucic acid proton H-5 (δ 5.93) and the caffeic acid carboxyl group C-9′ (δ 168.0), indicating the substitution positions on the daucic acid moiety. From these data, the structure of **1** was established as 4,5-di-*O*-caffeoyldaucic acid (**Figure 1**).

Table 1. NMR Data of 4,5-di-*O*-caffeoyldaucic Acid[a]

position	^{1}H	^{13}C
2		150.7
3	5.71 dd (1.5, 2.6 Hz)	103.9
4	6.04 ddd (1.5, 2.6, 4.3 Hz)	66.0
5	5.93 ddd (1.5, 1.7, 4.3 Hz)	68.0
6	4.66 dd (1.5, 1.7 Hz)	78.1
$COOH_{in\ 2}$		173.2
$COOH_{in\ 6}$		168.8
1′		127.6
2′	6.73 dd (8.3; 2.0 Hz)	123.4
3′	6.65 d (8.3 Hz)	116.5
4′		146.8
5′		149.8
6′	6.96 d (2.0 Hz)	114.8
7′	7.44 d (16.6 Hz)	147.3
8′	6.12 d (16.6 Hz)	114.7
9′		168.0
1″		127.8
2″	6.88 dd (8.3; 2.0 Hz)	123.1
3″	6.74 d (8.3 Hz)	116.3
4″		146.6
5″		149.6
6″	7.01 d (2.0 Hz)	115.0
7″	7.48 d (16.6 Hz)	147.5
8″	6.27 d (16.6 Hz)	115.0
9″		168.3

[a] Spectra were measured in CD_3OD.

Determination of Antioxidant Capacity of 4,5-di-*O*-caffeoyldaucic Acid (1). In general, caffeic acid derivatives are well-known to have potent antioxidant properties because the catechol structure donates the phenolic hydrogens or electrons to acceptors such as reactive oxygen species or lipid peroxyl radicals (*32*−*33*). The antioxidant capacity of all the known compounds from *Ipomoea batatas* tubers has been reported (*34*). Hence, we evaluated the antioxidative activity of daucic acid derivative by using DPPH and FRAP methods together with authentic antioxidant standards, L-ascorbic acid, BHT, and gallic

Table 2. Near Equilibrium Steady-State Antioxidant Capacity[a]

	assay method	
	FRAP	DPPH
4,5-di-*O*-caffeoyldaucic acid	6.44 ± 0.4	5.56 ± 0.2
ascorbic acid	2.16 ± 0.3	1.84 ± 0.1
tert-butyl-4-hydroxy toluene (BHT)	2.40 ± 0.3	1.2 ± 0.0
gallic acid	5.67 ± 0.5	4.55 ± 0.3

[a] Expressed as TEs (mM) of new compound and three antioxidant standards measured by FRAP and DPPH assays after a 110-min reaction time.

acid. We have used the FRAP assay because it is the only one that directly estimates the capacity of antioxidants or reductants in a sample and is based on the ability of the analyte to reduce the Fe^{3+}/Fe^{2+} couple (*35*). In this case, it was possible to use this test because the new compound does not show functional groups whose reduction potentials is below that of the Fe^{3+}/Fe^{2+} half reaction. The antioxidant capacity was evaluated from 0 to 110 min every 10 min, and the results confirmed those obtained by the DPPH test, which determines radical-scavenging activities of compounds by measuring the inactivation potential of radicals in an aqueus media. It is simple and rapid and needs only a UV/vis spectrophotometer to be performed, but DPPH generally has a relatively small linear reaction range of only 2−3-fold, and small molecules that have better access to the radical site have higher apparent antioxidant activity with this test. DPPH also is decolorized by reducing agents as well as hydrogen transfer, which also contributes to inaccurate interpretations of antioxidant capacity (*36*−*37*). The activity of compound **1** in both methods was higher than that of all standards used at the same molar concentration, and the steady state was reached after 110 min. The results are shown in **Table 2** and in **Figure 2**

In conclusion, we have isolated and characterized four caffeoyl derivatives in *Ipomoea batatas* tubers of which the 4,5-di-*O*-caffeoyldaucic acid was isolated for the first time and 1,3-di-*O*-caffeoylquinic acid was not previously described in *I. batatas* as in the Convolvulaceae family. Furthermore, their finding is important from a nutritional point of view, because they may have beneficial health effects because of their high antioxidant activity.

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