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Isolation and Antioxidative Activities of Caffeoylquinic Acid Derivatives and Flavonoid Glycosides from Leaves of Sweet Potato (*Ipomoea batatas* L.)

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Abstract – Bioassay-directed chromatographic fractionation of an ethyl acetate extract from leaves of sweet potato (*Ipomoea batatas* L.) afforded six quinic acid derivatives: 3,5-*epi*-dicaffeoylquinic acid (**1**), 3,5-dicaffeoylquinic acid (**2**), methyl 3,5-*O*-dicaffeoylquinate (**3**), methyl 3,4-dicaffeoylquinate (**4**), methyl 4,5-dicaffeoylquinic acid (**5**), 4,5-dicaffeoylquinate (**6**), and two phenolic compounds: caffeic acid (**7**) and caffeic acid methyl ester (**8**) together with three flavonoids: quercetin 3-*O*-β-D-glucopyranoside (**9**), quercetin 3-*O*-β-D-glucopyranoside, isoquercitrin (**10**) and kaempferol 3-*O*-β-D-glucopyranoside (**11**). The structures of these compounds were elucidated by the aid of spectroscopic methods. These compounds were assessed for antioxidant activities using three different cell-free bioassay systems. All isolates except 11 showed potent DPPH and superoxide anion radicals scavenging, and lipid peroxidation inhibitory activities. 3,5-*epi*-DCQA (**1**) and methyl quinates (**3-5**) along with flavonoide 9 were isolated for the first time from this plant.

Key words □ *Ipomoea batatas*, Convolvulaceae, sweet potato, dicaffeoylquinic acid derivatives, flavonoid glycosides, Antioxidants

INTRODUCTION

Most free radical reactions involve the reduction of molecular oxygen leading to the formation of reactive oxygen species (ROS) including superoxide anion and hydroxyl radicals. ROS can cause oxidative damage to cell components and may, therefore, play an important role in various pathological conditions. They attack biological molecules such as lipids, proteins, enzymes, DNA, and RNA leading to cell or tissue injury associated with degenerative diseases (Jung *et al.*, 1999). Excessive free radical production and lipid peroxidation are also known to be involved in several pathological conditions including atherosclerosis, aging, nephritis, diabetes mellitus, rheumatic disease, cardiac and cerebral ischemia, cancer, and adult respiratory distress syndrome (Miyake *et al.*, 2000). Antioxidants have been used as food additives to avoid degradation. Currently, there is considerable interest in new natural antioxidants to replace synthetic ones such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), because of their possible activity as promoters of carcinogenesis (Barlow, 1990). Consequently, in recent years, there has been much interest in the antioxidant activity of naturally occurring substances (Kähkönen *et al.*, 1999; Robards 2003). Therefore, antioxidants could be useful as prophylactic and therapeutic agents for the treatment of diseases in which oxidants or free radicals are implicated (Tseng *et al.*, 1997).

In our continued search for biologically active compounds from traditional medicine, we have found that the ethyl acetate fraction from leaves of sweet potato (*Ipomoea batatas*) showed potent antioxidant activities in the DPPH and superoxide anion radicals scavenging assay systems. The *Ipomoea batatas* (Convolvulaceae) has been reported to possess antiobesity, radical-scavenging capacity, anti-cardiovascular diseases, anti-mutation, antiaging, and anti-tumor effects as well as other potential health benefits (Islam, 2006). *I. batatas* is known to contain flavonoids, caffeoylquinic acid derivatives, anthocyanins as well as polyphenolics (Yoshimoto *et al.*, 2002; Luo *et al.*, 2005).

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In this paper, we describe the non-nutritional components and physiological functions of sweet potato leaves when used as a vegetable, and as a resource for products with these functions.

MATERIALS AND METHODS

Reagents

Xanthine, nitroblue tetrazolium (NBT), xanthine oxidase, 1,1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, resveratrol, vitamin E and other chemical reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Plant material

Sweet potato (*Ipomoea batatas* L.) leaves were collected at Yeongju (Gyeongbuk, Korea) in August 2001 and identified by an Emeritus Prof. Chang Soo Yook at Kyung Hee University in Korea. Voucher specimens (924-29A) have been deposited in our laboratory at Korea Institute of Science and Technology (KIST).

Extraction and isolation

Fresh leaves of *I. batatas* (2.1 kg) were extracted three times with methanol (10.5 l) to give a methanol-soluble extract. The dried extract residue (180.5 g) was suspended in water (0.7 l) and then partitioned in turn with dichloromethane (0.5 l×3), ethyl acetate (0.5 l×3) and *n*-butanol (0.5 l×3). The ethyl acetate fraction was evaporated under reduced pressure to yield 3.8 g of a residue. This residue was separated into 8 fractions (EA-EH) by column chromatography over silica gel using CH_2Cl_2/MeOH/H_2O (30:10:1) as eluent. Fraction EF (1.3 g) was purified by column chromatography over Sephadex LH-20 using EtOH as eluent to give eleven sub-fractions (EFA-EFK). Sub-fraction EFF (222.1 mg) was further purified by column chromatography over Toyopearl HW-40 (MeOH) and preparative TLC (40% MeOH) on RP-18 to give compounds **1** (40.5 mg), **2** (18.1 mg) and **5** (18.0 mg). The fraction ED (455.1 mg) was subjected to column chromatography on silica gel eluting with CH_2Cl_2/MeOH/H_2O (40:10:1→30: 15: 1) to afford eight fractions (EDA-EDH). Sub-fraction EDB (62.1 mg) was further purified by preparative TLC (60% MeOH) on RP-18 to give compounds **3** (27.7 mg) and **6** (13.7 mg). Sub-fraction FDD (110.5 mg) was further purified by column chromatography over Toyopearl HW-40 (MeOH) and preparative TLC (48% MeOH) on RP-18 to give compounds **9** (2.5 mg) and **10** (32.8 mg). Fraction EF (291.8 mg) was subjected to column chromatography over silica gel (CH_2Cl_2/MeOH/H_2O, 70:10:1) and LiChroprep® RP-18 (60% MeOH) to give compound **8** (56.6 mg). Fraction EE3-6 (139.3 mg) was subjected to column chromatography over Toyopearl HW-40 (MeOH) and preparative TLC (60% MeOH) on RP-18 to give compounds **4** (6.1 mg) 7 (70.8 mg) and **11** (21.8 mg).

3,5-Dicaffeoyl-*epi*-quinic acid (**1**): m.p. (uncorrected) 184-187°C (dec); UV (MeOH): λ_{max} (log ε) = 218 (3.81), 243 (2.91), 297sh (3.53), 328 (4.59); IR (KBr): ν_{max} = 3455, 3200, 1715, 1686, 1621, 1594, 1526, 1374, 1286, 1184, 1118, 978 cm^{-1}; $[\alpha]^{21}_D$ –166.0° (*c* 0.27, MeOH); HR-FAB-MS (negative-ion mode): *m/z* = 512.1202 (calcd. for $C_{25}H_{23}O_{12}$: 515.1190); CD nm (MeOH) 338 (Δε, -41.0), 289 (26.7); ^{1}H NMR (300 MHz, CD_3OD): δ 2.04 (m, 1H, H-6), 2.11 (m, 2H, H-2), 2.28 (dd, 1H, *J* = 15.2, 3.4 Hz, H-6), 3.91 (dd, 1H, *J* = 9.9, 3.4 Hz, H-4), 5.39 (m, 1H, H-5), 5.55 (dt, 1H, *J* = 5.8, 10.0 Hz, H-3), 6.31, 6.43 (d, each 1H, *J* = 15.8 Hz, H-8'), 6.78 (d, 2H, *J* = 8.2 Hz, H-5'), 6.96, 6.97 (dd, each 1H, *J* = 8.2, 2.0 Hz, H-6'), 7.06, 7.08 (d, each 1H, *J* = 2.0 Hz, H-2'), 7.59, 7.62 (d, each 1H, *J* = 15.8 Hz, H-7'); ^{13}C NMR (75 MHz, CD_3OD): δ 37.5 (C-6), 40.6 (C-2), 72.4 (C-3), 73.0 (C-4), 74.4 (C-5), 76.3 (C-1), 115.2 (C-2'), 115.4, 115.9 (C-8'), 116.4 (C-5'), 122.9 (C-6'), 127.8, 128.0 (C-1'), 146.6, 146.6 (C-7'), 146.8, 146.9 (C-3'), 149.2, 149.4 (C-4'), 169.0, 169.4 (C-9'), 181.3 (COOH).

3,5-Dicaffeoylquinic acid (**2**): a pale yellow powder; $[\alpha]^{21}_D$ -154.4° (*c* 0.71, MeOH); CD nm (MeOH): 340 (Δε, -39.6), 289 (23.1); ^{1}H NMR (300 MHz, CD_3OD): δ 2.15 (dd, 1H, *J* = 14.2, 6.40 Hz, H-2), 2.21 (m, 2H, H-6), 2.31 (dd, 1H, *J* = 14.1, 3.4 Hz, H-2), 3.96 (dd, 1H, *J* = 7.8, 3.2 Hz, H-4), 5.42 (m, 2H, H-3, 5), 6.27, 6.35 (d, each 1H, *J* = 15.9 Hz, H-8'), 6.77 (d, 2H, *J* = 8.2 Hz, H-5'), 6.96, 6.97 (dd, each 1H, *J* = 8.2, 1.9 Hz, H-6'), 7.06, 7.07 (d, each 1H, *J* = 1.8 Hz, H-2'), 7.58, 7.62 (d, each 1H, *J* = 15.9 Hz, H-7'); ^{13}C NMR (75 MHz, CD_3OD): δ 35.1 (C-6), 36.9 (C-2), 69.9 (C-4), 71.1 (C-5), 71.7 (C-3), 73.9 (C-1), 114.1, 114.2 (C-2'), 114.3, 114.6 (C-5'), 115.5, 115.5 (C-8'), 122.1, 122.1 (C-6'), 126.8, 127.0 (C-1'), 145.7, 145.7 (C-7'), 146.1, 146.3 (C-3'), 148.4, 148.4 (C-4'), 167.5, 168.0 (C-9'), 176.6 (COOH).

Methyl 3,5-dicaffeoylquinate (**3**): ^{1}H NMR (300 MHz, CD_3OD): δ 2.17 (m, 1H, H-2), 2.22 (m, 1H, H-6), 2.34 (m, 1H, H-6), 2.38 (m, 1H, H-2), 3.71 (s, 3H, H-OMe), 4.00 (dd, 1H, *J* = 6.52, 3.16 Hz, H-4), 5.41 (m, 1H, H-3), 5.35 (m, 1H, H-5), 6.25, 6.37 (d, each 1H, *J* = 15.9 Hz, H-8'), 6.80, 6.81 (d, each

1H, J = 8.2 Hz, H-5'), 6.97, 7.00 (dd, each 1H, J = 8.2, 1.7 Hz, H-6'), 7.08, 7.09 (d, each 1H, J = 1.7 Hz, H-2'), 7.57, 7.61 (d, each 1H, J = 15.9 Hz, H-7'); ^{13}C NMR (75 MHz, CD_3OD): δ 34.7 (C-2), 35.9 (C-6), 52.1 (OMe), 68.9 (C-4), 71.1 (C-5), 71.2 (C-3), 73.7 (C-1), 113.9, 114.5 (C-8'), 114.2 (C-2'), 115.5, 115.6 (C-5'), 122.1, 122.2 (C-6'), 126.7, 126.9 (C-1'), 145.7, 145.8 (C-3'), 146.2, 146.5 (C-7'), 148.5, 148.7 (C-4'), 167.1, 167.8 (C-9'), 174.7 (COOH).

Methyl 3,4-dicaffeoylquinate (4): ^{1}H NMR (300 MHz, CD_3OD): δ 2.11 (m, 1H, H-2), 2.16 (m, 2H, H-6), 2.37 (dd, 1H, J = 14.5, 3.9 Hz, H-2), 3.77 (s, 3H, OMe), 4.33 (m, 1H, H-5), 5.04 (dd, 1H, J = 8.2, 3.3 Hz, H-4), 5.63 (m, 1H, H-3), 6.27, 6.29 (d, each 1H, J = 15.9 Hz, H-8'), 6.75, 6.77 (d, each 1H, J = 8.2 Hz, H-5'), 6.89, 6.92 (dd, each 1H, J = 8.5, 1.9 Hz, H-6'), 7.03, 7.04 (d, each 1H, J = 2.1 Hz, H-2'), 7.55, 7.58 (d, each 1H, J = 15.9 Hz, H-7'); ^{13}C NMR (75 MHz, CD_3OD): δ 36.8 (C-2), 41.3 (C-6), 52.9 (OMe), 66.1 (C-5), 69.9 (C-3), 75.2 (C-4), 75.6 (C-1), 114.9, 115.0 (C-8'), 115.1, 115.2 (C-2'), 116.5 (C-5'), 123.1, 123.2 (C-6'), 127.7, 127.8 (C-1'), 146.8 (C-3'), 147.4 (C-7'), 149.6, 149.7 (C-4'), 168.4, 168.5 (C-9'), 176.1 (COOMe).

Methyl 4,5-dicaffeoylquinate (5): ^{1}H NMR (300 MHz, CD_3OD): δ 2.09 (dd, 1H, J = 13.9, 5.9 Hz, H-2), 2.25 (m, 2H, H-6), 2.31 (dd, 1H, J = 13.9, 3.1 Hz, H-2), 3.72 (s, 3H, OMe), 4.34 (m, 1H, H-3), 5.11 (dd, 1H, J = 8.1, 2.7 Hz, H-4), 5.55 (m, 1H, H-5), 6.16, 6.29 (d, each 1H, J = 15.8 Hz, H-8'), 6.75 (d, 2H, J = 8.1 Hz, H-5'), 6.90 (m, 2H, overlapped with each H-6'), 7.00, 7.02 (br s, each 1H, H-2'), 7.55, 7.59 (d, each 1H, J = 15.8 Hz, H-7'); ^{13}C NMR (75 MHz, CD_3OD): δ 37.4 (C-2), 39.0 (C-6), 52.1 (OMe), 67.7 (C-3), 68.1 (C-5), 74.0 (C-4), 74.8 (C-1), 113.6, 113.8 (C-8'), 114.2 (C-2'), 115.5 (C-5'), 122.2 (C-6'), 126.6, 126.7 (C-1'), 145.8 (C-7'), 146.7, 146.7 (C-3'), 148.7, 148.7 (C-4'), 167.0, 167.5 (C-9'), 174.2 (COOMe).

4,5-Dicaffeoylquinic acid (6): ^{1}H NMR (300 MHz, CD_3OD): δ 2.06 - 2.30 (m, 4H, H-2, 6), 4.36 (m, 1H, H-3), 5.11 (dd, 1H, J = 9.5, 2.6 Hz, H-4), 5.67 (m, 1H, H-5), 6.16, 6.27 (d, each 1H, J = 15.9 Hz, H-8'), 6.72, 6.73 (d, each 1H, J = 8.2 Hz, H-5'), 6.87, 6.90 (dd, each 1H, J = 8.3, 1.9 Hz H-6'), 6.99, 7.01 (d, each 1H, J = 1.8 Hz, H-2'), 7.49, 7.58 (d, each 1H, J = 15.9 Hz, H-7'); ^{13}C NMR (75 MHz, CD_3OD): δ 37.5 (C-2), 38.6 (C-6), 68.1 (C-3), 68.6 (C-5), 75.0 (C-4), 75.5 (C-1), 113.7 (C-8'), 114.2 (C-2'), 115.5 (C-5'), 122.1 (C-6'), 126.6 (C-1'), 145.7 (C-7'), 146.6, 146.7 (C-3'), 148.6 (C-4'), 167.3, 167.6 (C-9'), 176.5 (COOH).

Caffeic acid (7): ^{1}H NMR (300 MHz, CD_3OD): δ 6.14 (d, 1H, J = 15.9 Hz, H-8), 6.69 (d, 1H, J = 8.2 Hz, H-5), 6.86 (dd, 1H, J = 8.2, 1.9 Hz, H-6), 6.96 (d, 1H, J = 1.9 Hz, H-2), 7.45 (d, 1H, J = 15.9 Hz, H-7).

Caffeic acid methyl ester (8): ^{1}H NMR (300 MHz, CD_3OD): δ 3.64 (s, 3H, H-OMe), 6.14 (d, 1H, J = 15.9 Hz, H-8), 6.67 (d, 1H, J = 8.2 Hz, H-5), 6.82 (dd, 1H, J = 8.2, 1.4 Hz, H-6), 6.93 (d, 1H, J = 1.7 Hz, H-2), 7.43 (d, 1H, J = 15.9 Hz, H-7); ^{13}C NMR (75 MHz, CD_3OD): δ 52.0 (C-OMe), 114.8 (C-8), 115.1 (C-2), 116.4 (C-5), 122.9 (C-6), 127.6 (C-1), 146.7 (C-3), 146.9 (C-7), 149.5 (C-4), 169.7 (C-9).

Quercetin 3'-*O*-β-D-glucopyranoside (9): yellowish amorphous powder; IR ν_{max} (KBr): 3424, 2917, 1656, 1608, 1510, 1362, 1312, 1202, 1086 cm^{-1}; UV (MeOH): λ_{max} (log ε) = 362 (4.21), 258 (4.27); ^{1}H NMR (300 MHz, CD_3OD): δ 3.21-3.75 (m, 6H, H-2", 3", 4", 5", 6"), 5.28 (d, 1H, J = 7.4 Hz, H-1"), 6.22 (d, 1H, J = 2.1 Hz, H-6), 6.41 (d, 1H, J = 2.1 Hz, H-8), 6.87 (d, 1H, J = 8.5 Hz, H-5'), 7.60 (dd, 1H, J = 8.5, 2.1 Hz, H-6'), 7.72 (d, 1H, J = 2.1 Hz, H-2'); ^{13}C NMR (75 MHz, CD_3OD): δ 179.5 (C-4), 166.1 (C-7), 163.0 (C-5), 158.8 (C-9), 158.4 (C-2), 149.9 (C-4'), 145.8 (C-3'), 135.8 (C-3), 122.9 (C-6'), 122.8 (C-1'), 117.8 (C-2'), 116.1 (C-5'), 105.6 (C-10), 105.4 (Glc-1), 99.9 (C-6), 94.7 (C-8), 77.2 (Glc-5), 75.1 (Glc-3), 73.2 (Glc-2), 70.0 (Glc-4), 61.9 (Glc-6).

Quercetin 3-*O*-β-D-glucopyranoside (10): yellowish amorphous powder; IR ν_{max} (KBr): 3422, 2370, 1654, 1604, 1560, 1356, 1302, 1200, 1066 cm^{-1}; UV (MeOH): λ_{max} (log ε) = 358 (4.16), 257 (4.68); ^{1}H NMR (300 MHz, CD_3OD): δ 3.50-3.63 (m, 4H, H-2", 3", 4", 5"), 3.69 (dd, 1H, J = 11.1, 6.2 Hz, H-6"), 3.88 (dd, 1H, J = 11.1, 2.3 Hz, H-6"), 5.23 (d, 1H, J = 7.8 Hz, H-1"), 6.26 (br s, 1H, H-6), 6.45 (br s, 1H, H-8), 6.91(d, 1H, J = 8.5 Hz, H-5'), 7.62 (dd, 1H, J = 8.4, 1.7 Hz, H-6'), 7.89 (d, 1H, J = 1.8 Hz, H-2'); ^{13}C NMR (75 MHz, CD_3OD): δ 62.5 (C-6"), 71.1 (C-4"), 75.6 (C-2"), 78.0 (C-3"), 78.2 (C-5"), 94.7 (C-8), 99.9 (C-6), 104.5 (C-1"), 105.5 (C-10), 115.9 (C-5'), 117.6 (C-2'), 122.9 (C-1'), 123.2 (C-6'), 135.6 (C-3), 145.7 (C-3'), 149.7 (C-4'), 158.2 (C-2), 159.0 (C-5), 162.8 (C-9), 165.8 (C-7), 179.3 (C-4).

Kaempferol 3-*O*-β-D-glucopyranoside (11): yellowish amorphous powder; ^{1}H NMR (300 MHz, CD_3OD): δ 3.26-3.76

(m, 6H, H-2", 3", 4", 5", 6"), 5.30 (d, 1H, J = 7.3 Hz, H-1"), 6.23 (d, 1H, J = 1.4 Hz, H-6), 6.43 (br s, 1H, H-8), 6.92 (d, 2H, J = 8.8 Hz, H-3', 5'), 8.09 (d, 2H, J = 8.9 Hz, H-2', 6'); ^{13}C NMR (75 MHz, CD_3OD): δ 62.7 (C-6"), 71.5 (C-4"), 75.9 (C-2"), 78.2 (C-3"), 78.6 (C-5"), 94.8 (C-8), 100.0 (C-6), 104.1 (C-1"), 105.7 (C-10), 116.2 (C-3', 5'), 122.7 (C-1'), 132.3 (C-2', 6'), 135.4 (C-3), 158.3 (C-2), 158.6 (C-9), 161.7 (C-4'), 163.0 (C-5), 166.1 (C-7), 179.3 (C-4).

DPPH radical scavenging assay

The potential antioxidant activity of pure compounds was assessed on the basis of scavenging activity of the DPPH free radicals. Reaction mixtures containing test samples (dissolved in EtOH) and 100 μM DPPH ethanolic solution in 96-well microtiter plates were incubated at 37°C for 30 min. Absorbances were then measured at 515 nm using VERSAmax, microplate reader (Molecular Devices, USA). Percent inhibition was determined by comparison with an ethanol-treated control. IC_{50} values denote the concentration of samples required to scavenge 50% DPPH free radicals (Lee *et al.*, 2002).

Assay for scavenging activity of superoxide anion radicals produced by xanthine/xanthine oxidase

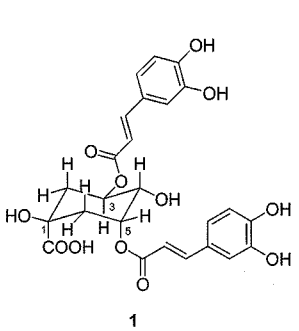
The reaction mixture consisted of 40 mM sodium carbonate buffer (pH 10.2) containing 0.1 mM xanthine, 0.1 mM EDTA, 50 μg protein/ml bovine serum albumin, 25 mM NBT and 1.4 $\times 10^{-8}$ units xanthine oxidase (EC 1.2.3.2) in final volume of 200 μl. After incubation at 25°C for 20 min, the reaction was terminated by addition of 6.6 μl of 6 mM $CuCl_2$. The absorbance of formazan was determined at 560 nm using VERSAmax, microplate reader (Molecular Devices, USA). IC_{50} values denote the concentration of samples required to scavenge 50% superoxide anion radicals (Toda *et al.*, 1991).

Inhibition of lipid peroxidation by ferric thiocyanate

Inhibitory activity for lipid peroxidation was evaluated using 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH)-induced lipid peroxidation of a tween-emulsified linoleic acid system and measured by the ferric thiocyanate assay (Son *et al.*, 2002). Briefly, 1.0 ml of 0.2 M phosphate buffer, pH 7.0, and 15 mg/ml tween-20 (in buffer solution) were mixed with 3 mg/ml linoleic acid in ethanol. The peroxidation was initiated by the addition of AAPH (17 mM) solution. The serially diluted ethanolic solution of inhibitors was then added, and the reaction was carried out at 37°C for 150 min in the dark. The degree of inhibition of oxidation was measured by ferric thiocyanate. To 0.1 ml of peroxidation reation mixture, 0.1 ml of 30% ammonium thiocyanate and 0.1 ml of 2×10^{-2} M freshly prepared $FeCl_2$ (in 3.5% aqueous HCl) were added. Precisely, 3 min after addition, the absorbance of the red complex $[Fe(SCN)]^{+2}$ (Ramette, 1963) was measured at 500 nm. The control for the assay was prepared in the same manner by mixing all of the chemicals and reagents except the test compound. Ascorbic acid, vitamin E and resveratrol served as positive controls.

RESULTS AND DISCUSSION

Compound **1** was obtained as a white amorphous powder with a negative optical rotation ($[\alpha]_D^{21}$: -166.0°). The molecular formula $C_{25}H_{24}O_{12}$ for **1** was established by high resolution FAB-MS. The ^{1}H-NMR spectrum of **1** exhibited signals for two caffeic acids and a quinic acid moiety. Four doublets with coupling constants of 15.8 Hz appeared for the *trans* olefinic protons H-7' and H-8' of **1**. The signals at δ = 7.08 and 7.06 (both d, J = 2.0 Hz, H-2'), 6.78 (d, J = 8.2 Hz, H-5') and 6.97 and 6.96 (dd, 2H, J = 2.0 and 8.2 Hz, H-6') can be assigned to two groups of three aromatic protons in characteristic ABX spin system. The signals of H-5 (m) at δ = 5.39 and H-3 (dt, J = 10.0, 5.8 Hz) at δ = 5.55 and the doublet of doublet signal of H-



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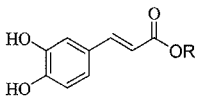
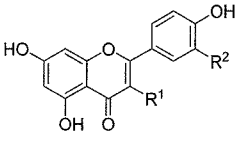
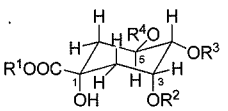


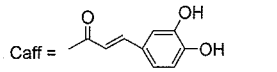
7 R = H
8 R = Me



	R^1	R^2
9	OH	*O*-β-D-glc.
10	*O*-β-D-glc.	OH
11	*O*-β-D-glc.	H



	R^1	R^2	R^3	R^4
2	H	Caff	H	Caff
3	Me	Caff	H	Caff
4	Me	Caff	Caff	H
5	Me	H	Caff	Caff
6	H	H	Caff	Caff



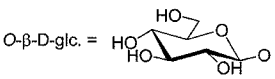


Fig. 1. Chemical structures of compounds isolated from leaves of *Ipomoea batatas* L.

4 (dd, J = 9.9, 3.4 Hz) at δ 3.91 of quinic acid moiety were assigned according to their multiplicity and coupling pattern. From these observations, the structure of **1** was expected to be a quinic acid derivative having two caffeoyl groups substituted at C-3 and C-5 position. However, the ^{1}H-NMR spectrum of **1** showed slight different peak patterns of H-2' and 5' of caffeoyl group and at H-3 and H-5 positions when compared to those of 3,5-dicaffeoylquinic acid (**2**), which was also isolated in this investigation. Furthermore, physical properties (solubility, optical rotation, apparent color, *etc.*) of compounds **1** were different from those of **2**. The solubility of compound **1** was very low in methanol while compound **2** was freely soluble in the same solvent. The structure was also identified by comparing their physical and spectral data with the literature values (Kim *et al.*, 2005).

Based on these findings and considerations, the structure of **1** was established as 3,5-dicaffeoyl-*epi*-quinic acid (3,5-*epi*-DCQA). The absolute configuration of **1** was further determined by examining CD spectrum. Since the CD spectrum of **1** showed negative first and positive second Cotton effects, two caffeoyl residues should constitute a left-handed screw (Harada *et al.*, 1983). Hence, it was confirmed that the parent quinic acid moiety of **1** is (-)-quinic acid.

By means of bioassay-guided fractionation of MeOH extract from leaves of *I. batatas*, we found that the EtOAc fraction showed potent antioxidant activity. Six quinic acid derivatives: 3,5-*epi*-dicaffeoylquinic acid (**1**), 3,5-dicaffeoylquinic acid (**2**) (Pauli *et al.*, 1998), methyl 3,5-*O*-dicaffeoylquinate (**3**), methyl 3,4-dicaffeoylquinate (**4**), methyl 4,5-dicaffeoylquinate (**5**), 4,5-dicaffeoylquinic acid (**6**) (Basnet *et al.*, 1996), and two phenolic compounds: caffeic acid (**7**) and caffeic acid methyl ester (**8**) were isolated from *I. batatas* together with three flavonoids: quercetin 3'-*O*-β-D-glucopyranoside (**9**) (Zhong *et al.*, 1997), quercetin 3-*O*-β-D-glucopyranoside, isoquercitrin (**10**) and kaempferol 3-*O*-β-D-glucopyranoside (**11**) (Popoff *et al.*, 1977). 3,5-*epi*-DCQA (**1**) and methyl quinates (**3-5**) along with flavonoide **9** were isolated for the first time from this plant.

All the isolates were assessed for antioxidant activities in the DPPH and superoxide anion radicals scavenging and lipid peroxidation inhibition assay systems (Table I). For comparisons, the antioxidant activity data of ascorbic acid, vitamin E, and resveratrol were included as positive controls. Most of the isolated compounds showed strong inhibitory activities in these assay systems. It was reported that the antioxidant activity is mainly due to the catechol moiety in the structure (Parejo *et al.*, 2004). Two phenolic compounds (**7** and **8**) strongly scavenged superoxide anion radicals produced in the xanthine/xanthine oxidase system (IC_{50} = 0.54 ± 0.03 and 0.58 ± 0.02 μg/ml, respectively) and showed potent DPPH radical scavenging activities (IC_{50} = 3.22 ± 0.08 and 4.56 ± 0.17 μg/ml, respectively) when compared to the positive controls. Moreover, these compounds also showed potent lipid peroxidation inhibition with % inhibition values of 54.1 and 54.7 at 3.12 μg/ml concentration, respectively, comparable to positive controls. Six quinic acid derivatives (**1** - **6**) also exhibited potent inhibitory activities in our assay systems indicating that location of caffeoyl moiety in the quinic acid and methyl group were not important for antioxidant activities. Two quercetin glucopyra-

Table I. Antioxidant activities of compounds (**1** - **11**) isolated from leaves of *I. batatas*.

Compound	IC_{50} (μg/ml) on DPPH[a]	IC_{50} (μg/ml) on superoxide anion[a]	Inhibitory effect on linoleic acid oxidation (%) at 3.12 μg/ml
1	5.70 ± 0.06	3.82 ± 0.36	51.19
2	5.64 ± 0.11	2.90 ± 0.14	51.52
3	5.53 ± 0.11	2.53 ± 0.44	53.78
4	6.07± 0.15	1.07 ± 0.13	54.16
5	5.36 ± 0.13	3.04 ± 0.20	53.72
6	5.89 ± 0.21	3.29 ± 0.42	51.69
7	3.22 ± 0.08	0.54 ± 0.03	54.09
8	4.56 ± 0.17	0.58 ± 0.02	54.73
9	11.60 ± 0.83	2.81 ± 0.39	49.88
10	16.56 ± 1.04	3.41 ± 0.27	49.96
11	>50	>50	10.92
Ascorbic acid	5.53 ± 0.13	>50	44.11
Vitamin E	9.42 ± 0.26	>50	61.16
Resveratrol	17.05 ± 1.08	>50	39.05

[a]All values are averages of at least three runs.

nosides (**9** - **10**) showed moderate DPPH radical scavenging activities (IC_{50} = 11.60 ± 0.38 and 16.56 ± 1.04 μg/ml, respectively) and effectively scavenged superoxide anion radicals with IC_{50} values of 2.81 ± 0.39 and 3.41 ± 0.27 μg/ml, respectively, due to the catechol moiety in the B-ring of quercetin. But, kaempferol 3-*O*-*β*-D-glucopyranoside (**11**) was inactive in the three different assay system.

In conclusion, the present study demonstrated that the ploy phenolics such as dicaffeoylquinic acid derivatives and flavonoids contribute on the antioxidative activities of the leaves of *I. batatas*. Therefore, the leaves of *I. batatas* may be helpful for maintaining and promoting human health as antioxidative functional food.

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