

Why you and your family should use Divine Noni?

Reason 2

Noni has very good antioxidant activity, which helps to improve human health by inhibiting oxidative stress and cellular damage of vital organs like lungs

Scientific Reference :

Chemical Constituents of the Fruits of *Morinda citrifolia* (Noni) and Their Antioxidant Activity

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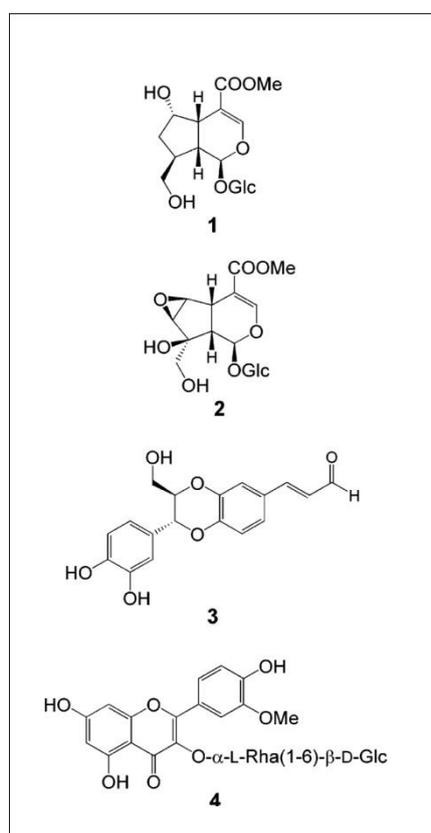
Purification of a n-BuOH-soluble partition of the MeOH extract of *Morinda citrifolia* (Noni) fruits led to the isolation of two new iridoid glucosides, 6R-hydroxyadoxoside (1) and 6 $\hat{\alpha}$,7 $\hat{\alpha}$ -epoxy-8-epi-splendoside (2), as well as 17 known compounds, americanin A (3), narcissoside (4), asperuloside, asperulosidic acid, borreriagenin, citrifolinin B epimer a, citrifolinin B epimer b, cytidine, deacetylasperuloside, dehydromethoxygaertneroside, epi-dihydrocornin, D-glucose, D-mannitol, methyl R-D-fructofuranoside, methyl $\hat{\alpha}$ -D-fructofuranoside, nicotifloroside, and $\hat{\alpha}$ -sitosterol 3-O- $\hat{\alpha}$ -D-glucopyranoside. The structures of the new compounds were determined by spectroscopic data interpretation. Compound 4, borreriagenin, cytidine, deacetylasperuloside, dehydromethoxygaertneroside, epi-dihydrocornin, methyl R-D-fructofuranoside, and methyl $\hat{\alpha}$ -D-fructofuranoside were isolated for the first time from *M. citrifolia*. The antioxidant activity was evaluated for all isolates in terms of both DPPH and ONOO- bioassays. The neolignan, americanin A (3), was found to be a potent antioxidant in these assays.

Morinda citrifolia L. (Rubiaceae), commonly called Noni or Indian mulberry, is a small evergreen tree or shrub of Polynesian origin.¹ *M. citrifolia* bears a lumpy, green to yellowish-white fruit, normally 5 to 10 cm in length, with a surface covered in polygonal-shaped sections.^{1,2} *M. citrifolia* has a long history of use as a medicinal plant in parts of Southeast Asia, Polynesia, and Australia and is

considered to be the second most important medicinal plant in the Hawaiian Islands.^{1,3} The leaves, roots, bark, and fruits have all been used medicinally to treat a wide range of ailments. These include, but are not limited to, diabetes, diarrhea, hypertension, malaria, pain, and topical infections.^{1,4} The fruits are also eaten as a food, but primarily only in times of famine.⁴ Today, the use of *M. citrifolia* in the United States is becoming more widespread and Noni products are commercially available in health food stores, chain grocery stores specializing in natural foods, and on the Internet. Both the leaves and the fruits are being sold in tablet, tea, and juice form, although the fruit as a juice is the predominant formulation sold. This growth in popularity in the United States may in part be attributed to claims of Noni being a “cure-all” or aid in relieving symptoms for a host of chronic conditions such as arthritis, cancer, diabetes, and hypertension.¹ A number of in vitro biological activities have been reported, such as angiogenesis inhibition,⁵ antioxidant,⁶ cyclooxygenases-1 and -2 inhibition,^{7,8} and tyrosine kinase inhibition.⁹ Most of these studies have involved crude extracts or fractions of *M. citrifolia*, and the compound(s) responsible for the biological activities have not been determined. In addition, an in vivo study using the ethanol-insoluble precipitate of *M. citrifolia* fruits, given intraperitoneally to mice with Lewis lung carcinoma implanted cells, demonstrated a significant life-prolonging effect that was increased when given in conjunction with several chemotherapeutic agents.¹⁰ Currently, a freeze-dried Noni fruit extract is in phase I clinical trials at the Cancer Research Center of Hawaii in cancer patients for which there is no other standard treatment available. The aim of this trial is to determine if Noni extract can be useful to cancer patients for antitumor and/or symptom control.¹¹ To date, the major chemical constituents of this plant have been found to be anthraquinones,¹² flavonol glycosides,¹³ iridoid glycosides,^{13,14} lipid glycosides,¹⁵ and triterpenoids.¹⁴ In the present study, the n-BuOH-soluble partition part of the MeOH extract of *M. citrifolia* fruits was found to have moderate antioxidant activity in a free radical (DPPH) scavenging bioassay. This partition was purified by repeated chromatography, which led to the isolation of two new iridoid glucosides, 6R-hydroxyadoxoside (1) and 6 \hat{a} ,7 \hat{a} -epoxy-8-epi-splendoside (2), and 17 known compounds. All isolates obtained in this study were evaluated for their antioxidant activity, and the neolignan, americanin A (3), demonstrated significant antioxidant activity in two antioxidant bioassays.

Compound 1, [R]23D -50.7° (c 0.28, MeOH), was obtained as a colorless gum by repeated chromatography and finally purified by reversed-phased HPLC. A molecular formula of C₁₇H₂₆O₁₁ was established for 1 on the basis of the observed sodiated molecular ion peak at m/z 429.1378 [M + Na]⁺ in its

HRESIMS (calcd for C₁₇H₂₆O₁₁Na, 429.1373). The IR absorption bands at 3355 and 1692 cm⁻¹, respectively, indicated the presence of hydroxyl groups and a carbonyl group in the molecule. The characteristic ¹H NMR (in CD₃OD) signals at δ_H 7.46 (1H, d, J) 1.2 Hz, H-3), 5.15 (1H, d, J) 6.0 Hz, H-1), and 4.65 (1H, d, J) 7.9 Hz, Glc-1) and ¹³C NMR (in CD₃OD) signals at δ_C 169.5 (C, C-11), 153.1 (CH, C-3), 113.0 (C, C-4), 100.6 (CH, Glc-1), and 99.1 (CH, C-1) suggested that compound 1 is an iridoid glucoside.^{16,17} The observed HMBC correlation from the anomeric proton (Glc-1) of glucose to the acetal carbon at δ_C 99.1 (C-1) indicated that the location of the glucose unit is at C-1 in the molecule of 1.



The presence of a methyl ester group at C-4 was readily assigned on the basis of the chemical shifts of H-3, C-3, C-4, and C-11, as well as the observed correlations from both H-3 (δ_H 7.46) and the methoxy (δ_H 3.69) signals to the carbonyl carbon (δ_C 169.5) in the HMBC spectrum of 1. Generally, the five- and six-membered rings of iridoids are cis-fused,¹⁸ although a few trans-fused iridoids also have been reported.^{16,19} The chemical shifts of C-1 are about 103 ppm and 96-100 ppm for trans- and cis-fused iridoids, respectively.¹⁶ Therefore, the rings are cis-fused in the molecule of 1, since the C-1 signal was detected at 99.1 ppm in the ¹³C NMR spectrum of this isolate. Further analysis of the observed 2D NMR (¹H-¹H COSY, HMQC, and HMBC) correlations indicated the gross structure of compound 1 to be 6-hydroxyadoxoside.^{16,17} A literature survey revealed that 6â-

hydroxyadoxoside has been previously isolated from *Bessaya plantaginea*, and a doublet of doublets with the coupling constants of 3.7 and 7.8 Hz was reported for H-6 of this compound.¹⁷ However, in the ¹H NMR spectrum of 1, the resonance signal of H-6 was displayed as a broad singlet at δ H 4.29. This suggested that compound 1 is 6*R*-hydroxyadoxoside, which was confirmed by a ROESY NMR correlation from H-6 to H-9.

Compound 2 was isolated as a colorless gum, $[\alpha]_D^{23} -108.4^\circ$ (c 0.17, MeOH). A molecular formula of C₁₇H₂₄O₁₂, indicating six degrees of unsaturation, was assigned to 2 on the basis of its HRFABMS (found m/z 443.1147, calcd for C₁₇H₂₄O₁₂Na, m/z 443.1166). Seventeen carbon signals, including one methoxy group, two methylenes, 11 methines, and three quaternary carbons, were evident from the ¹³C NMR and DEPT data of 2. Similar to 1, the resonance signals of two doubly oxygenated methines were displayed at δ C 99.9 (Glc-1) and 93.8 (C-1)

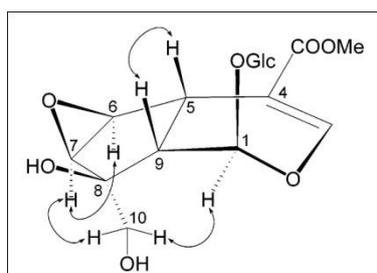


Figure 1. Selected NOESY correlations for 6 α ,7 α -epoxy-8-*epi*-splendoside (2).

in the ¹³C NMR and DEPT spectra of 2. This, in combination with the observed ¹H NMR signals at δ H 5.76 (1H, s, H-1) and 4.56 (1H, d, J = 8.0 Hz, Glc-1), suggested that compound 2 is also an iridoid glucoside.¹⁶⁻¹⁸ In the same manner as for 1, the locations of the glucose unit and the methyl ester group in 2 were also determined at C-1 and C-4 on the basis of the observed HMBC correlations from the anomeric proton (Glc-1) of glucose to the acetal carbon at δ C 93.8 (C 1) and from both H-3 (δ H 7.48) and the methoxy (δ H 3.73) signals to the carbonyl carbon (δ C 168.5), respectively. The presence of an epoxy group between C-6 and C-7 and the hydroxyl groups at C-8 and C-10 was assigned by further analysis of the chemical shifts of the remaining protons and carbons, the observed 2D NMR correlations (1H-1H COSY, HMQC, and HMBC), and the determined unsaturation value of 2. In the NOESY NMR spectrum (Figure 1) of 2, correlations from H-5 to H-9 and from H-6 to H-7 were evident, but there was no correlation from H-5 to H-6 or from H-9 to H-7, indicating the α -, *R*-, *R*-, and α -orientations of H-5, H-6, H-7, and H-9, respectively. Another key correlation

observed in the NOESY spectrum (Figure 1) of 2 was observed between H-1 (δ H 5.76, 1H, s) and H-10a (δ H 3.68, 1H, d, J) 11.7 Hz), which was suggestive of an R-orientation of the hydroxymethylene group at C-8. This was consistent with absence of the NOESY correlation from H-9 to both H-10a and H-10b. Accordingly, the structure of this new iridoid glucoside was determined as 6 \hat{a} ,7 \hat{a} epoxy-8-epi-splendoside (2).

In addition to compounds 1 and 2, 17 known compounds, americanin A (3),^{20,21} narcissoside (4),²² asperuloside,²³ asperulosidic acid,¹⁵ borreriagenin,²⁴ citrifolinin B epimer a,¹³ citrifolinin B epimer b,¹³ cytidine,²⁵ deacetylasperuloside,²³ dehydromethoxygaertneroside,²⁶ epi-dihydrocornin,²⁷ D-glucose,²⁸ D-mannitol,²⁹ methyl R-D-fructofuranoside,³⁰ methyl \hat{a} -D-fructofuranoside,³⁰ nicotifloroside,³¹ and \hat{a} -sitosterol 3-O- \hat{a} -D-glucopyranoside, were also isolated in the present study. The structures of these known compounds were identified by comparing their physical and spectroscopic data ([R]_D, ¹H NMR, ¹³C NMR, DEPT, 2D NMR, and MS) with those of published values or by comparing with an authentic sample (\hat{a} -sitosterol 3-O- \hat{a} -D-glucopyranoside) directly. Americanin A (3) was initially isolated from *Phytolacca americana* in 1978,²⁰ and its structure was revised in 1986.²¹ The structures of americanin A (3) and its regioisomer, isoamericannin A, were confirmed by total synthesis.³² The ¹H and ¹³C NMR data of these two neolignans are very similar.³² Compound 3 obtained in the present study was assigned as americanin A on the basis of the observed key correlation from H-7 to C-4' in the HMBC spectrum acquired on a 600 MHz NMR spectrometer using a cryoprobe (Supporting Information). Among these isolates, compound 4, borreriagenin, cytidine, deacetylasperuloside, dehydromethoxygaertneroside, epi-dihydrocornin, methyl R-D-fructofuranoside, and methyl \hat{a} -D-fructofuranoside were isolated from *M. citrifolia* for the first time. One of the major compounds obtained in the present study is an iridoid glucoside, asperuloside (~0.08% w/w). The presence of this compound in the n-BuOH-soluble extract was confirmed by LC-MS analysis (Supporting Information).

The antioxidant ability of all isolates obtained in this study to scavenge DPPH, authentic ONOO-, and 3-morpholinopyridone (SIN-1)-derived ONOO- was evaluated. As summarized in Table 1, the neolignan, americanin

Table 1. Antioxidant Activity of Compounds Isolated from *M. citrifolia* Fruits

compound	DPPH	peroxynitrite	
		authentic ONOO-	SIN-1-derived

	(IC50 μ M)	(IC50 μ M)	ONOO- (IC50 μ M)
3	16.9	3.3	25.8
4	NDb	3.8	9.6
gallic acidc	2.7		
penicillaminec		3.3	4.4

a All compounds obtained in this study were evaluated in both the DPPH and peroxy nitrite free-radical scavenging assays. Except for compounds 3 and 4, all other isolates were indicated to be inactive (IC50 values over 30 μ M). b Not determined, since compound 4 was not active in the DPPH assay. c Positive controls used.

A (3), was found to be a potent antioxidant in these bioassays. The flavonol glycoside, narcissoside (4), exhibited evident scavenging activity against both authentic ONOO⁻ and SIN-1-derived ONOO⁻. However, another structurally similar flavonol glycoside, nicotifloroside (unsubstituted instead of having a methoxy group at C-3' as in 4), was found to be inactive. The isolation and characterization of 3 as an antioxidant constituent of *M. citrifolia* fruits thus provides a basis for the previous literature reports of the antioxidant effects of extracts of this species.^{6,33} Several lignans and neolignans including compound 3 were reported from *M. citrifolia* fruits very recently.³⁴ The copper-induced low-density lipoprotein oxidation inhibition activities of these isolates were determined.³⁴

Experimental Section

General Experimental Procedures. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were measured on a PerkinElmer 241 automatic polarimeter. The UV spectra were obtained with a Beckman DU-7 spectrometer, and the IR spectra run on an ATI Mattson Genesis Series FT-IR spectrophotometer. NMR spectroscopic data were recorded at room temperature on a Bruker Advance DPX-300, 360, 400, or DRX500 MHz spectrometer with tetramethylsilane (TMS) as internal standard. The HMBC spectrum of americanin A (3) was acquired on a Bruker Advance DRX-600 NMR spectrometer with a TXI cryoprobe. Standard pulse sequences were employed for the measurement of 2D NMR spectra (1H-1H COSY, HMQC, HMBC, and NOESY). FABMS was obtained on a VG 7070E-HF sector-field

mass spectrometer, while CIMS, EIMS, and ESIMS were performed on a Finnigan/MAT 90/95 sector-field mass spectrometer. A YMC-pack ODC-AQ column (5 μm , 25 \times 2 cm i.d., YMC Co., Wilmington, NC) was used for semipreparative HPLC, along with two Waters 515 HPLC pumps and a Waters 2487 dual λ absorbance detector (Waters, Milford, MA). Column chromatography was carried out with silica gel G (Merck, 70-230 or 230-400 mesh). Analytical thin-layer chromatography (TLC) was performed on 250 μm thickness Merck Si gel 60 F254 aluminum plates. Plant Material. The freeze-dried fruit powder of *M. citrifolia* (lot number 229) used in this study was obtained from Nature's Sunshine Products, Inc. A representative sample (# N0001) was deposited in the Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University. LC-MS traces of the n-BuOH-soluble extract of *M. citrifolia* fruits are included in the Supporting Information. Extraction and Isolation. The freeze-dried fruit powder (1 kg) was extracted by maceration with MeOH three times (3 \times 4.5 L) at room temperature, for 3 days each. After filtration and evaporation of the solvent under reduced pressure, the combined crude methanolic extract was suspended in H₂O (800 mL), then partitioned in turn with petroleum ether (3 \times 1000 mL), CHCl₃ (4 \times 1000 mL), and n-BuOH (4 \times 500 mL), to afford dried petroleum ether- (102 g), CHCl₃- (12.5 g), n-BuOH (41.2 g), and H₂O-soluble (~55 g) extracts. The n-BuOH-soluble partition part of the MeOH extract was found to be active (56.2% at 200 $\mu\text{g}/\text{mL}$) in a DPPH free-radical scavenging bioassay. Therefore, the n-BuOH-soluble extract was subjected to chromatography over a silica gel column (9 \times 45 cm), eluted with CHCl₃-MeOH (15:1 to 1:1, then pure MeOH), to give seven fractions (F01-F07). Compound 3 (6.5 mg; 0.00065% w/w) was obtained as a white amorphous powder from a CHCl₃-MeOH mixture (ca. 10:1) of fraction F01, eluted with CHCl₃-MeOH (15:1). Fraction F04 (9.17 g), eluted with CHCl₃-MeOH (8:1), was chromatographed over a silica gel column (5 \times 45 cm), eluted with EtOAc-MeOH (10:1 to 2:1), and five combined subfractions were obtained (F0401-F0405). F0401 was further purified over a Sephadex LH-20 column (2.8 \times 45 cm), using pure MeOH for elution, and afforded borrieriagenin (200 mg; 0.02% w/w) and cytidine (58 mg; 0.0058% w/w), and a mixture. This mixture was purified by semipreparative HPLC, by eluting with MeOH-H₂O (40:60; 8 mL/min), to afford pure asperuloside (t_R) 6.5 min, 64 mg; 0.0064% w/w) and dehydromethoxygaertneroside (t_R) 28.5 min, 1.6 mg; 0.00016% w/w). Asperuloside (750 mg; 0.075% w/w) was obtained as a white amorphous powder from a solution (EtOAc-MeOH, ca. 8:1) of F0402 as a major component. Fraction F05 (4.0 g), eluted with CHCl₃-MeOH (6:1), was chromatographed over a silica gel column (5 \times 40 cm), eluted with EtOAc-MeOH (10:1 to 2:1), to give pure

asperulosidic acid (400 mg; total yield 0.0435% w/w; an additional 35 mg was isolated from F0602) and four subfractions (F0501- F0504). F0502 was further fractionated over a Sephadex LH20 column (2.8 × 45 cm), eluted using pure MeOH, and gave three fractions (F050201-F050203). F050201 was finally purified by preparative TLC (500 μM; 20 × 20 cm), with CHCl₃-MeOH (4:1) as developing solvent, to give epi-dihydrocornin (R_f) 0.54, 4.5 mg; 0.00045% w/w) and a mixture of citrifolinin B epimer a and citrifolinin B epimer b (R_f) 0.50, 6.0 mg; 0.0006% w/w) in a ratio of 1:1 (integration of ¹H NMR signals). F0503 was purified by HPLC using MeOH-H₂O (40: 60; 8 mL/min) as solvent, to afford two flavonol glycosides, nicotifloroside (t_R) 35.0 min, 16 mg; 0.0016% w/w) and compound 4 (t_R) 39 min, 11 mg; 0.0011% w/w). β -Sitosterol 3-O- β -D-glucopyranoside (58 mg; 0.0058% w/w) was obtained as an amorphous solid from a CHCl₃-MeOH (~6:1) solution of F0504. Fraction F06, eluted with CHCl₃-MeOH (4:1), was separated over a Sephadex LH-20 column (2.8 × 45 cm), eluted with MeOH, and afforded three subfractions (F0601-F0603). F0602 was then purified by semipreparative HPLC using MeOH-H₂O (30:70; 7 mL/min) as eluent, to afford compound 1 (t_R) 16.7 min, 7.5 mg; 0.00075% w/w) and asperulosidic acid (t_R) 22.0 min, 35 mg; total yield 0.0435% w/w; an additional 400 mg was isolated from F05). F0603 was chromatographed over a silica gel column (5.0 × 40 cm) using CHCl₃-MeOH:H₂O (from 8:1:0.05 to 2:1:0.1) as solvent system, to give methyl R-D-fructofuranoside (35 mg; 0.0035% w/w) and methyl β -D-fructofuranoside (105 mg; 0.011% w/w), five subfractions (F060301-F060305), and the mixture of R- and β -D-glucopyranoses (250 mg; 0.025% w/w) in a ratio of 10:3 (based on the integrations of ¹H NMR spectra in DMSO-d₆), in order of polarity. F060302 was finally purified by semipreparative HPLC using MeOH-H₂O (30:70; 6 mL/min) as solvent, to give deacetylasperuloside (t_R) 19.5 min, 13 mg; 0.0013% w/w) and compound 2 (t_R) 21.5 min, 1.8 mg; 0.00018% w/w). D-Mannitol (180 mg; 0.018% w/w) was obtained as colorless needles from a CHCl₃-MeOH (ca. 2:1) solution of fraction F07. 6r-Hydroxyadoxoside (1): colorless gum; [R]_D²³ -50.7° (c 0.28, MeOH); UV (MeOH) λ_{max} (log) 231 (3.55) nm; IR (dried film) ν_{max} 3355, 1692, 1635, 1294, 1077, 807 cm⁻¹; ¹H NMR (360 MHz, in CD₃OD, TMS) δ 7.46 (1H, d, J) 1.2 Hz, H-3), 5.15 (1H, d, J) 6.0 Hz, H-1), 4.65 (1H, d, J) 7.9 Hz, Glc-1), 4.29 (1H, brs, H-6), 3.87 (1H, dd, J) 12.2, 1.5 Hz, Glc-6a), 3.79 (2H, brd, J) 5.4 Hz, H2-10), 3.69 (3H, s, -COOMe), 3.66 (1H, dd, J) 12.2, 5.6 Hz, Glc-6b), 3.35 (1H, m, Glc-5), 3.27- 3.31 (Glc-3 and Glc-4, overlapped with solvent signal), 3.19 (1H, dd, J) 9.0, 7.9 Hz, Glc-2), 3.13 (1H, m, H-8), 2.26 (1H, m, H-7a), 2.04-2.09 (2H, m, H-5, and H-9), 1.54 (1H, m, H-7b); ¹³C NMR (90 MHz, in CD₃OD, TMS) δ 169.5 (C, C-11), 153.1 (CH, C-3), 113.0

(C, C-4), 100.6 (CH, Glc-1), 99.1 (CH, C-1), 78.3 (CH, Glc-3), 78.0 (CH, Glc-5), 74.7 (CH, Glc-2), 73.2 (CH, C-6), 71.5 (CH, Glc-4), 62.7 (CH₂, Glc-6), 62.3 (CH₂, C-10), 51.7 (CH₃, -COOMe), 49.9 (CH, C-9), 43.1 (CH₂, C-7), 42.4 (CH, C-8), 35.5 (CH, C-5); LRESIMS *m/z* 835 (100) [2M + Na]⁺, 429 (55) [M + Na]⁺, 360 (15), 339 (13); HRESIMS *m/z* 429.1378 [M + Na]⁺ (calcd for C₁₇H₂₆O₁₁Na, 429.1373). 6,7-Epoxy-8-epi-splendoside (2): colorless gum; [R]_D -108.4° (c 0.17, MeOH); UV (MeOH) λ_{max} (log ε) 232 (4.00), 280 (3.05) nm; IR (dried film) ν_{max} 3397, 1700, 1639, 1440, 1293, 1178 cm⁻¹; ¹H NMR (400 MHz, in CD₃OD, TMS) δ 7.48 (1H, d, J = 1.5 Hz, H-3), 5.76 (1H, s, H-1), 4.56 (1H, d, J = 8.0 Hz, Glc-1), 3.87 (1H, dd, J = 11.9, 1.8 Hz, Glc-6a), 3.80 (1H, d, J = 2.5 Hz, H-6), 3.73 (3H, s, -COOMe), 3.68 (1H, d, J = 11.7 Hz, H-10a), 3.65 (1H, dd, J = 11.9, 5.8 Hz, Glc-6b), 3.50 (1H, d, J = 2.6 Hz, H-7), 3.47 (1H, d, J = 11.7 Hz, H-10b), 3.23- 3.33 (H-5, Glc-3, Glc-4, and Glc-5, overlapped with solvent signal), 3.13 (1H, dd, J = 8.9, 8.1 Hz, Glc-2), 2.32 (1H, d, J = 8.8 Hz, H-9); ¹³C NMR (100 MHz, in CD₃OD, TMS) δ 168.5 (C, C-11), 154.2 (CH, C-3), 107.7 (C, C-4), 99.9 (CH, Glc-1), 93.8 (CH, C-1), 80.7 (C, C-8), 78.4 (CH, Glc-3), 78.0 (CH, Glc-5), 74.6 (CH, Glc-2), 71.6 (CH, Glc-4), 65.2 (CH₂, C-10), 62.8 (CH₂, Glc-6), 60.8 (CH, C-7), 57.9 (CH, C-6), 51.9 (CH₃, -COOMe), 46.4 (CH, C-9), 33.3 (CH, C-5); LRCIMS *m/z* 438 (80) [M + NH₄]⁺, 258 (55), 241 (15), 198 (35), 180 (40), 75 (100); HRFABMS *m/z* 443.1147 [M + Na]⁺ (calcd for C₁₇H₂₄O₁₂Na, 443.1166).

Measurement of DPPH Free-Radical Scavenging Activity. This assay used the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) to determine the potential scavenging activity of extracts and pure compounds, as previously described.³⁵ Briefly, extracts and pure compounds, in DMSO, were plated in triplicate to give a final concentration of 200 μg/mL and incubated in 200 μM DPPH in EtOH at 37 °C for 30 min in the dark. DMSO was used as the negative control and gallic acid as the positive control. Absorbance was measured at 515 nm, and the percent scavenging activity was determined by comparison with the DMSO negative control. Active compounds were tested in triplicate in the same manner at final concentrations of 2.5, 5, 10, and 20 μg/mL. The IC₅₀ values were obtained through extrapolation from linear regression analysis.³⁵

Measurement of ONOO⁻ Scavenging Activity. ONOO⁻ used in this study was purchased from Cayman Chemical Co. or derived by 3-morpholinopyrrolidone (SIN-1). ONOO⁻ scavenging ability was measured by monitoring the oxidation of DHR 123 using the modified method of Kooy et al.³⁶ Briefly, a stock solution of DHR 123 (5 mM) purged with nitrogen was prepared in advance and stored at -20 °C. A working solution of DHR 123 (5 μM) diluted from the stock solution was placed on ice in the dark immediately prior to each experiment. The rhodamine buffer

(sodium phosphate dibasic, 50 mM; sodium phosphate monobasic, 50 mM; sodium chloride, 90 mM; potassium chloride, 5 mM) including diethylenetriaminepentaacetic acid (DTPA, 5 mM) was purged with nitrogen and placed on ice before use. The ONOO--scavenging ability was determined at room temperature by a microplate fluorescence spectrophotometer FL500 (Bio-Tek Instruments) with excitation and emission wavelengths of 485 and 530 nm, respectively. The background and final fluorescent intensities were measured 5 min after treatment with or without native ONOO- (10 μ M) in 0.3 N sodium hydroxide or SIN-1 (10 μ M). Oxidation of DHR 123 by decomposition of SIN-1 gradually increased, whereas native ONOO- rapidly oxidized DHR 123 with its final fluorescent intensity being stable over time on the inhibition of DHR 123 oxidation by ONOO-. Penicillamine was used as a positive control.

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