Bioorganic & Medicinal Chemistry Letters 21 (2011) 633–637

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Antioxidant activity of a new *C*-glycosylflavone from the leaves of *Ficus microcarpa*

Phan Van Kiem [b], Nguyen Xuan Cuong [b], Nguyen Xuan Nhiem [a,b], Vu Kim Thu [b], Ninh Khac Ban [b], Chau Van Minh [b], Bui Huu Tai [a,b], Truong Nam Hai [c], Sang Hyun Lee [d], Hae Dong Jang [d], Young Ho Kim [a,*]

[a] College of Pharmacy, Chungnam National University, Daejeon 305-764, Republic of Korea
[b] Institute of Marine Biochemistry, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Caugiay, Hanoi, Vietnam
[c] Institute of Biotechnology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Caugiay, Hanoi, Vietnam
[d] Department of Food and Nutrition, Hannam University, Daejeon 305-811, Republic of Korea

ARTICLE INFO

Article history:
Received 26 September 2010
Revised 6 December 2010
Accepted 7 December 2010
Available online 10 December 2010

Keywords:
Ficus microcarpa
Moraceae
Ficuflavoside
Ficumegasoside
Antioxidant
Oxygen radical absorbance capacity

ABSTRACT

By bioactive-guided fractionation of methanol extract of the *Ficus microcarpa* leaves, one new *C*-glucosylflavone, ficuflavoside (**1**), one new megastigmane glycoside, ficumegasoside (**8**), and twelve known compounds including flavonoids (**2–6**), phenylpropanoids (**7**), megastigmanes (**9–11**) and sterol derivatives (**12–14**) were isolated. Their chemical structures were elucidated by mass, 1D, and 2D NMR spectroscopies. The antioxidant activities of these compounds were measured using the oxygen radical absorbance capacity methods. Compounds **1–6** exhibited potent antioxidant activities of 6.6–9.5 μM Trolox equivalents at the concentration of 2.0 μM. The results indicated **2**, **3**, and **5** having meaningful reducing capacity of copper (I) ions concentration of 6.1–8.4 μM.

Natural antioxidants function as free radical scavengers, reducing agents, chelators of pro-oxidant metals, or as quenchers of singlet oxygen. Natural antioxidants are also present in many health-promoting supplements.[1] Consumption of foods rich in natural antioxidants has been reported as being protective against certain types of cancer[2] and coronary disease,[3] and may also reduce the risk of cardiovascular and cerebrovascular events. These actions of antioxidants have been attributed to their ability to scavenge free radicals, thereby reducing oxidative damage of cellular biomolecules such as lipids, proteins, and nucleic acids.[4] Oxygen radical absorbance capacity (ORAC) is a method of measuring antioxidant capacities in biological samples *in vitro*. It has been recently accepted as a standard method to analyze the antioxidant potential of active substances of plants.[5,6] The ORAC assay involves the completion of free radicals activities as a mean of quantization, and combines both the extent of inhibition and the length of inhibition time of free radical action by antioxidants into a single quantity. The ORAC assay provides important information regarding the antioxidant capacity of various biological samples from pure compounds such as phenolic acids and flavonoids to complex matrices such as tea, fruits, vegetables, and animal tissues.[5]

Ficus (Moraceae) constitutes one of the largest species of flowering plants with about 800 genera of deciduous trees, hemiepiphytes shrubs and climbers occurring in tropical and subtropical regions of both hemispheres. The genus is remarkable for the large variation in the habits of its species.[7] *Ficus microcarpa* L.f. (Moraceae) is widely distributed as an ornamental plant and is one of the most common street trees in warm climates.[8] The dried leaves, aerial roots, and barks from *F. microcarpa* have been used as folk herbs for perspiration, alleviating fever, and relieving pain in Vietnam.[8] Previous phytochemical and biological investigations of *F. microcarpa* have yielded a number of triterpenoids,[9–11] flavonoids,[12] monoterpenoids, and other class compounds.[13,14] In our screening project for antioxidant agents from natural sources, we found *F. microcarpa* to possess antioxidant effects. As part of our continuing research to evaluate the biological activities of this plant, here we report the isolation, structural elucidation, and evaluation of the antioxidant activity of one new flavonoid, one new megastigmane glycoside and twelve known compounds from the methanol extract of the *F. microcarpa* leaves.

Dried leaves of *F. microcarpa*[15] were extracted with methanol and then fractionated with chloroform, ethyl acetate and water. From these extracts and by using combined chromatographic separations, two new and twelve known compounds were isolated.[16] Their structures were elucidated using physicochemical and spectroscopic methods.

* Corresponding author. Tel.: +82 42 8215933; fax: +82 42 8236566.
E-mail address: yhk@cnu.ac.kr (Y.H. Kim).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.12.025

Compound **1** was obtained as a yellow amorphous powder. Its basic ion peak at *m/z* 565.1 $[M+H]^+$ was observed in the positive-ion electrospray ionization mass spectrometry (ESI-MS), and HR-ESI-MS analysis revealed the molecular formula to be $C_{26}H_{28}O_{14}$, with a cluster ion peak at *m/z* 565.1568 $[M+H]^+$ (calcd for $C_{26}H_{29}O_{14}$: 565.1557). The ^{1}H NMR spectrum of **1** (in methanol-d_4) showed the following signals: four aromatic protons were at δ_H 6.84 (2H, d, J = 8.4 Hz) and 7.88 (2H, d, J = 8.4 Hz), assigning to the AA′BB′ coupling system in B ring, one singlet proton of A ring was at δ_H 6.16 (1H, s), and two anomeric protons were at δ_H 4.87 (1H, d, J = 9.0 Hz) and 5.03 (1H, d, J = 1.2 Hz), suggesting the appearance of two sugar units. The ^{13}C NMR and DEPT spectra revealed 26 carbon signals, of which, 15 signals were assigned to a flavone aglycone and 11 signals were assigned to two monosaccharide moieties. The heteronuclear multiple bond correlations (HMBC) cross peaks from H-3 (δ_H 6.49) to C-1′ (δ_C 123.7), C-2 (δ_C 166.7), C-4 (δ_C 183.1), and C-10 (δ_C 105.7); from H-6 (δ_H 6.16) to C-5 (δ_C 162.7), C-7 (δ_C 164.1), C-8 (δ_C 105.4), and C-10 (δ_C 105.7) confirmed that the two singlet protons were at C-3 and C-6, respectively. Acid hydrolysis of **1** revealed D-apiose (identified as trimethylsilyl (TMS) derivatives by a gas chromatography method).[17] Furthermore, the ^{13}C NMR spectral data of **1** showed the presence of one β-D-apiofuranose and one *C*-β-D-glucopyranose moieties.[18] The HMBC between the glc H-1″ (δ_H 4.87) and C-7 (δ_C 164.1), C-8 (δ_C 105.4), C-9 (δ_C 158.5) of the aglycone, between api H-1‴ (δ_H 5.03) and glc C-2″ (δ_C 77.4), between glc H-2″ (δ_H 4.13) and api C-1‴ (δ_C 111.2) were observed (see Fig. 2). These observations indicated the sequence of sugar linkages of **1** and sugar moiety located at C-8 of the aglycone, which was also supported by the good agreement of ^{13}C NMR chemical shifts for the sugar moieties with those of abrusin 2″-*O*-apioside.[19] Consequently, the structure of **1** was determined to be 8-*C*-(2″-*O*-β-D-apiofuranosyl)-β-D-glucopyranosyl apigenin, a new compound named ficuflavoside.

Compound **8** was obtained as a white amorphous powder and its molecular formula was determined to be $C_{24}H_{38}O_{11}$ by ESI-MS at *m/z* 525 $[M+Na]^+$ (positive) and HR-ESI-MS at *m/z* 525.2317 $[M+Na]^+$ (calcd for $C_{24}H_{38}O_{11}Na$ 525.2312). The ^{1}H NMR spectrum of **8** (methanol-d_4) showed signals for three tertiary methyl groups at δ 0.98, 1.00, and 1.91 (each 3H, s), one secondary methyl group at δ 1.26 (3H, d, J = 6.6 Hz), and two anomeric protons at δ_H 4.32 (1H, d, J = 7.8 Hz) and 4.94 (1H, d, J = 1.2 Hz) suggesting the presence of two sugar units, as listed in Table 1. The ^{13}C NMR and DEPT spectra revealed 24 carbon signals, 13 of which were assigned to a megastigmane aglycone moiety and the remaining 11 assigned to the two monosaccharide moieties. The aglycone of **8** was concluded to be 9-hydroxy-4,7-megastigmadien-3-one.[20] The HMBC cross peaks from H-4 (δ_H 5.86) to C-3 (δ_C 202.1), C-5 (δ_C 166.0), C-6 (δ_C 56.6), and C-13 (δ_C 23.8); from H-13 (δ_H 1.91) to C-4 (δ_C 126.1), C-5 (δ_C 166.0), and C-6 (δ_C 56.6) confirmed that a double bond was at C-4/C-5, ketone and tertiary methyl groups were at C-3 and C-5, respectively. On the other hand, the chemical shift of C-1 (δ_C 37.1), C-5 (δ_C 166.0), C-6 (δ_C 56.6), and C-7 (δ_C 129.1) in the aglycone confirmed the stereochemistry at C-6 to be *R*-configuration by comparing with the corresponding data of the (6*S*,7*E*,9*S*)-9-hydroxy-4,7-megastigmadien-3-one 9-*O*-β-D-glucopyranoside[21] [δ_C values for C-1 (37.5), C-5 (165.9), C-6 (57.3), and C-7 (131.8)] and salvionoside A[22] ([δ_C values for C-1

Table 1
The ^{1}H and ^{13}C NMR data of compounds **1** and **8** in CD_3OD

Position	1		Position	8	
	δ_C[a]	δ_H[b] mult. (J in Hz)		δ_C[a]	δ_H[b] mult. (J in Hz)
Aglycone			Aglycone		
2	166.7	—	1	37.1	—
3	103.5	6.49 (s)	2	48.2	2.02 (d, 16.8)
					2.42 (d, 16.8)
4	183.1	—	3	202.1	—
5	162.7	—	4	126.1	5.86 (s)
6	99.3	6.16 (s)	5	166.0	—
7	164.1	—	6	56.6	3.80(d, 9.0)
8	105.4	—	7	129.1	5.63 (dd, 9.0, 15.0)
9	158.5	—	8	138.0	5.73 (dd, 6.0, 15.0)
10	105.7	—	9	77.4	3.34 (m)
1′	123.7	—	10	21.1	1.26 (d, 6.6)
2′	130.1	7.88 (d, 8.4)	11	27.6	0.98 (s)
3′	117.0	6.84 (d, 8.4)	12	28.0	1.00 (s)
4′	162.6	—	13	23.8	1.91 (s)
5′	117.0	6.84 (d, 8.4)			
6′	130.1	7.88 (d, 8.4)			
8-*C*-Glc			9-*O*-Glc		
1″	73.8	4.87 (d, 9.0)	1′	102.5	4.32 (d, 7.8)
2″	77.4	4.13 (t, 9.0)	2′	75.1	3.15 (t, 7.8)
3″	80.9	3.53 (t, 7.5)	3′	77.9	3.32[c]
4″	72.2	3.53 (t, 7.5)	4′	71.8	3.25 (t, 9.0)
5″	82.9	3.33 (m)	5′	76.7	3.36 (m)
6″	63.0	3.70 (d, 11.4)	6′	67.9	3.56 (dd, 6.0, 10.8)
		3.90 (dd, 2.0, 11.4)			3.95[c]
Api (1→2)Glc			AAra (1→6)Glc		
1‴	111.2	5.03 (d, 1.2)	1″	109.9	4.94 (d, 1.2)
2‴	77.8	3.67 (d, 1.2)	2″	83.2	3.96[c]
3‴	80.6	—	3″	78.9	3.78 (m)
4‴	74.7	3.04 (d, 9.6)	4″	85.9	3.93 (m)
		2.45 (d, 9.6)			
5‴	65.8	3.29 (d, 11.4)	5″	63.1	3.62 (dd, 5.4, 10.8)
		3.18 (d, 11.4)			3.72 (dd, 3.6, 10.8)

[a] 150 MHz.
[b] 600 MHz.
[c] Overlapped signal. Assignments were done by HMQC, HMBC, and ^{1}H–^{1}H COSY experiments, Glc: D-glucopyranosyl, Api: D-apiofuranosyl, Ara: L-arabinofuranosyl.

(37.1), C-5 (165.8), C-6 (56.7), and C-7 (128.7)]. Moreover, the configuration at C-9 was identified to be *R*-configuration by comparing chemical shifts at C-8 (δ_C 138.0), C-9 (δ_C 77.4), and C-10 (δ_C 21.1) of the aglycone with the corresponding data of the salvionoside A[22] [δ_C values for C-8 (138.1), C-9 (δ_C 76.5), and C-10 (δ_C 20.9)] and salvionoside B[22] [δ_C values for C-8 (136.8), C-9 (δ_C 74.8), and C-10 (δ_C 22.2)]. The ^{13}C NMR spectral data of **8** showed the presence of one α-L-arabinofuranose and one β-D-glucopyranose moieties.[18] Furthermore, acid hydrolysis of **8** revealed L-arabinose and D-glucose as sugar components (identified as TMS derivatives by a gas chromatography method).[17] The HMBC between the glc H-1′ (δ_H 4.32) and C-9 of the aglycone (δ_C 77.4), between ara H-1″ (δ_H 4.94) and glc C-6′ (δ_C 67.9), between glc H-6′ (δ_H 3.56 and 3.95) and ara C-1″ (δ_C 109.9) were observed (see Fig. 2). These observations indicated the sequence of sugar linkages of **8** and sugar moiety located at C-9 of the aglycone, which was also supported by the good agreement of ^{13}C NMR chemical shifts for the sugar moieties with those of megastigmane glycosides from *Erythroxylum cuneatum*.[18] Consequently, the structure of **8** was determined to be (6*R*,7*E*,9*R*)-9-hydroxy-4,7-megastigmadien-3-one 9-*O*-[α-L-arabinofuranosyl-(1→6)-β-D-glucopyranoside], a new compound named ficumegasoside.

The other compounds were characterized as (+)-catechin (**2**),[23] (−)-epicatechin (**3**),[23] isovitexin (**4**),[24] luteolin 6-*C*-β-D-glucopyranoside (**5**),[25] isosaponarin (**6**),[26] syringin (**7**),[27] (3*S*,5*R*,6*R*,7*E*,9*S*)-megastigman-7-ene-3,5,6,9-tetraol (**9**),[28] bridelionoside B (**10**),[29] dihydroalangionoside A (**11**),[30] β-sitosterol (**12**),[31] daucosterol (**13**),[31] and β-sitosterol 3-*O*-(6′-octadecanoyl) β-D-glucopyranoside (**14**)[32] (see Fig. 1) on the basis of spectral data and chemical evidence, which were in good agreement with those reported in the literature. Of these, dihydroalangionoside A (**11**) was isolated from nature for the first time. Compounds **4**–**7**, **9**, **10**, and **14** were isolated from this genus for the first time.

The antioxidant capacities of methanol extract and other fractions (chloroform, ethyl acetate and water fractions) from the leaves of *F. microcarpa* were tested by ORAC assay (see Fig. 3).[33] Trolox was used as a control standard and prepared fresh daily. The results showed that methanol extract showed significant peroxyl radical-scavenging activity with 2.6 μM Trolox equivalent (TE)

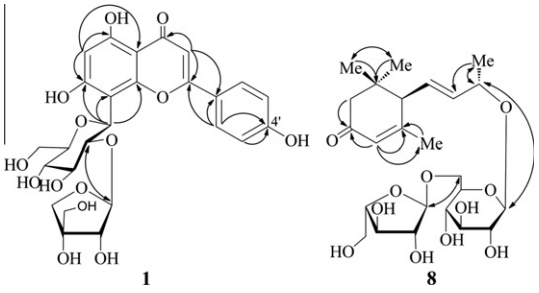

Figure 2. Selected HMBC spectrum of compounds **1** and **8**.

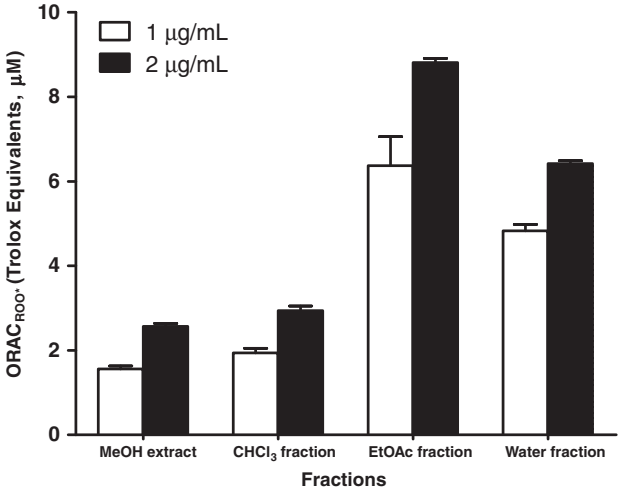

Figure 3. Peroxyl radical-scavenging activity (Trolox equivalent, μM) of extracts. The ORAC value is calculated by dividing the area under the sample curve by the area under the Trolox curve, with both areas being corrected by subtracting the area under the blank curve. One ORAC unit is assigned as the net area of protection provided by Trolox at a final concentration of 1 μM. The area under the curve of the sample is compared to the area under the curve for Trolox, and the antioxidant value is expressed in micromoles of Trolox equivalent per liter. The results represent the mean ± S.D. of values obtained from three measurements.

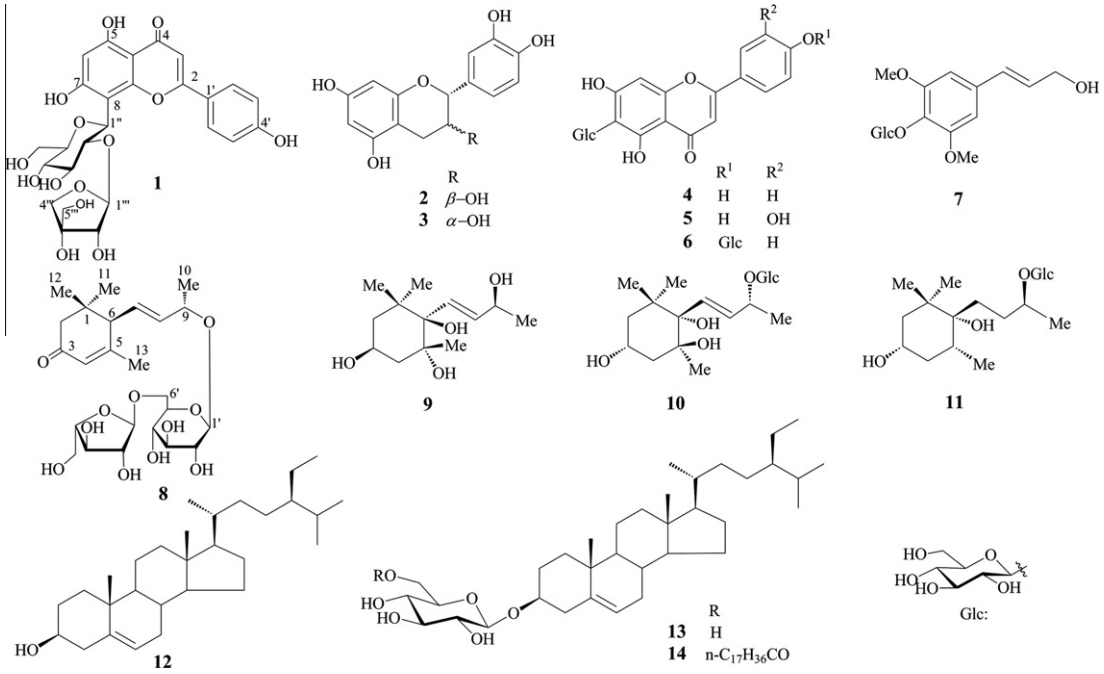

Figure 1. Structures of isolated compounds (**1**–**14**) from the leaves of *F. microcarpa*.

at the concentration of 2.0 μg/mL. The ethyl acetate fraction exhibited potent antioxidant activity followed by water fraction and chloroform fraction with values of 8.8, 6.4, and 3.0 μM TE at the concentration of 2.0 μg/mL, respectively. Subsequently, all isolates from *F. microcarpa* were measured by ORAC assay at the concentration of 1.0 and 2.0 μM (see Fig. 4). With regard to peroxyl radical-scavenging activity, six flavonoids (**1**–**6**) exhibited potent antioxidant activity compared with that of positive control, Trolox. Among them, isosaponarin (**6**) was reported antioxidant activity for the first time, isovitexin (**4**) was evaluated antioxidant activity using ORAC method for the first time. However, catechin (**2**), epicatechin (**3**), and luteolin 6-*C*-β-D-glucopyranoside (**5**) was reported antioxidant activity using ORAC method.[34,35] Another compounds as phenylpropanoid (**7**), megastigmanes (**8**–**11**), and sterol derivatives (**12**–**14**) showed weak or no activity. Our results suggest that hydroxyl groups on aromatic rings may contribute to peroxyl radical-scavenging activity by donating hydrogen atoms to peroxyl radicals. We next tested reducing capacity of all compounds by measuring the concentration of Cu (I) ions reduced from Cu (II) ions.[36] The results indicated **2**, **3**, and **5** having meaningful reducing capacity of copper (I) ions of 6.1–8.4 μM, at the concentration of 2 μM (see Fig. 5). From the results of antioxidant activities of all compounds, we may conclude compounds **2**, **3**, and **5**

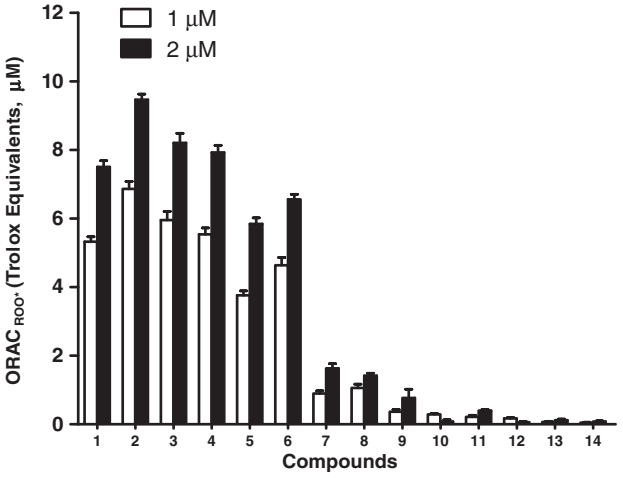

Figure 4. Peroxyl radical-scavenging activity (Trolox equivalent, μM) of compounds (**1**–**14**).

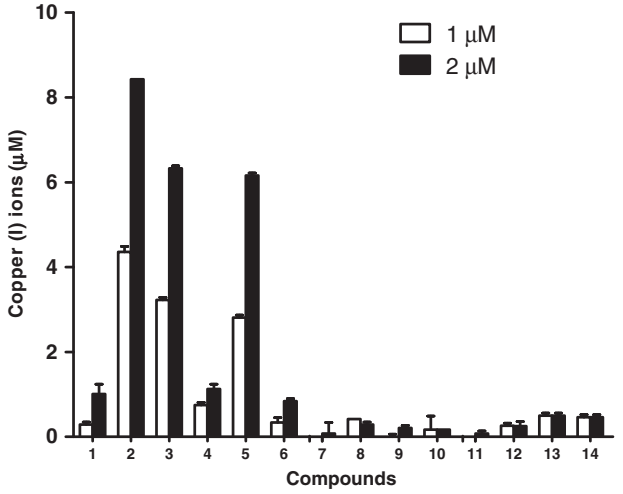

Figure 5. Reducing capacity of compounds **1**–**14**. The results represent the mean ± S.D. of values obtained from three measurements.

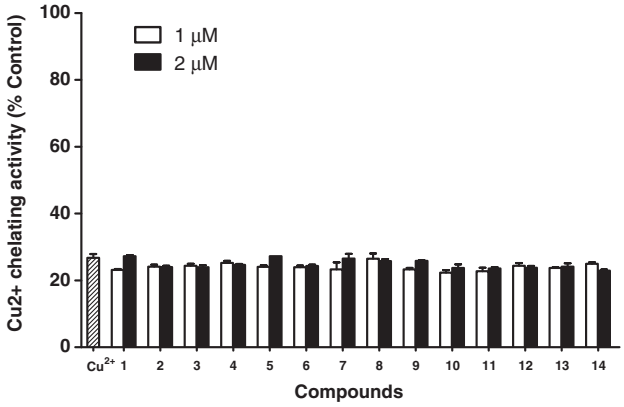

Figure 6. Metal chelating activity of compounds **1**–**14**. The results represent the mean ± S.D. of values obtained from three measurements.

having two hydroxyl groups at C-3 and C-4 in B ring appear as a strong antioxidant to donate their hydrogens or electrons to peroxyl radicals and Cu (II) ions, respectively. Although compounds **1**, **4**, and **6** showed potent peroxyl radical-scavenging activity, they appear to have low reducing capacity compared to compounds **2**, **3**, and **5**. This result may conclude that the hydrogen donating activity of compounds **1**, **4**, and **6** is strong, but their single electron transferring activity is weak due to one hydroxyl group in B ring resulting in unfavorable electron transfer to Cu (II) ion. Moreover, all compounds showed very weak metal chelating activity.[37] This suggested antioxidant activity of these compounds is not due to their metal chelating activities with transition metal ions (see Fig. 6).

Acknowledgments

This work was financially supported by Vietnam National Foundation for Science & Technology Development (Project No: 104.01.31.09) and Priority Research Center Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0093815), Republic of Korea. The authors are grateful to Institute of Chemistry, VAST and KBSI for recording the NMR and mass spectra.

References and notes

1. Lee, J. C.; Kim, H. R.; Kim, J.; Jang, Y. S. *J. Agric. Food Chem.* **2002**, *50*, 6490.
2. Lee, H. P.; Gourley, L.; Duffy, S. W.; Esteve, J.; Lee, J.; Day, N. E. *Lancet* **1991**, *337*, 1197.
3. Hertog, M. G.; Feskens, E. J.; Hollman, P. C.; Katan, M. B.; Kromhout, D. *Lancet* **1993**, *342*, 1007.
4. Aruoma, O. I. *J. Am. Oil Chem. Soc.* **1998**, *75*, 199.
5. Cao, G.; Sofic, E.; Prior, R. L. *Free Radical Biol. Med.* **1997**, *22*, 749.
6. Kurihara, H.; Fukami, H.; Asami, S.; Toyoda, Y.; Nakai, M.; Shibata, H.; Yao, X. S. *Biol. Pharm. Bull.* **2004**, *27*, 1093.
7. Herre, E. A.; Jander, K. C.; Machado, C. A. *Ann. Rev. Ecol. Evol. Syst.* **2008**, *39*, 439.
8. Bich, D.H.; Chung, D.Q.; Chuong, B.X.; Dong, N.T.; Dam, D.T.; Hien, P.V.; Lo, V.N.; Mai, P.D.; Man, P.K.; Nhu, D.T.; Tap, N.; Toan, T. Hanoi Science and Technology Publishing House: Hanoi, 2004; Vol. 1, p 875.
9. Kuo, Y. H.; Chiang, Y. M. *Chem. Pharm. Bull.* **1999**, *47*, 498.
10. Chiang, Y. M.; Kuo, Y. H. *J. Nat. Prod.* **2000**, *63*, 898.
11. Chiang, Y.-M.; Chang, J. Y.; Kuo, C. C.; Chang, C. Y.; Kuo, Y. H. *Phytochemistry* **2005**, *66*, 495.
12. Li, Y. C.; Kuo, Y. H. *J. Nat. Prod.* **1997**, *60*, 292.
13. Li, Y. C.; Kuo, Y. H. *Phytochemistry* **1998**, *49*, 2417.
14. Li, Y. C.; Kuo, Y. H. *Chem. Pharm. Bull.* **2000**, *48*, 1862.
15. The leaves of *F. microcarpa* were collected in Hanoi, Vietnam in November, 2009, and identified by Dr. Ninh Khac Ban (Institute of Ecology and Biological Resources, VAST, Vietnam). A voucher specimen (FM1109) was deposited at the Herbarium of Institute of Marine Biochemistry, VAST, Vietnam.
16. The dried leaves of *F. microcarpa* (3.0 kg) were extracted with MeOH three times under reflux for 15 h to yield 280 g of a dark solid extract, which was then suspended in water and successively partitioned with chloroform ($CHCl_3$) and ethyl acetate (EtOAc) to obtain $CHCl_3$ (F1, 68.0 g), EtOAc (F2, 90.5 g), and

water (F3, 127.5 g) extracts after removing solvent *in vacuo*. The F1 extract was chromatographed on a silica gel column and eluted with *n*-hexane–acetone gradient (100:1–1:1, v/v) to obtain fractions F1A–F1D. The F1B fraction was chromatographed on a silica gel column eluting with $CHCl_3$–MeOH (30:1, v/v) to yield **12** (10.0 mg) and **14** (8.0 mg). The F1C fraction was further chromatographed on a silica gel column eluting with CH_2Cl_2–MeOH (15:1, v/v) to give fractions F1C1–F1C3. The F1C1 fraction was chromatographed on an YMC RP-18 column eluting with acetone–water (10:1, v/v) to yield **13** (5.0 mg). The F1C3 fraction was chromatographed on a silica gel column eluting with MeOH–water (5:1, v/v) to yield **9** (7.1 mg). The F2 extract was chromatographed on a silica gel column and eluted with $CHCl_3$–MeOH gradient (50:1→1:1, v/v) to obtain fractions F2A–F2D. The F2B fraction was chromatographed on an YMC RP-18 column eluting with MeOH–H_2O (10:1, v/v) to yield **2** (6.3 mg) and **3** (12.0 mg). The F2C fraction was chromatographed on a silica gel column eluting with $CHCl_3$–MeOH (7:1, v/v) to give fractions F2C1–F2C3. The F2C1 traction was chromatographed on an YMC RP-18 column eluting with MeOH–water (5:1, v/v) to yield **4** (5.0 mg) and **5** (11.5 mg). The F2D fraction was chromatographed on a silica gel column eluting with CH_2Cl_2–MeOH–H_2O (6:1:0.07, v/v/v) to yield **7** (6.0 mg). The water soluble fraction F3 was chromatographed on a Diaion HP-20P column (Mitsubishi Chem. Ind. Co., Tokyo, Japan) eluting with water containing increasing concentrations of MeOH (0%, 25%, 50%, 75%, and 100% MeOH) to give four fractions, F3A–F3D. The F3B fraction was chromatographed on a silica gel column eluting with $CHCl_3$–MeOH–H_2O (5:1:0.1, v/v/v) to give fractions, F3B1–F3B3. The F3B2 fraction was chromatographed on a silica gel column eluting with CH_2Cl_2–MeOH–H_2O (5:1:0.1, v/v/v) to yield the new compound **8** (4.5 mg). The F3B3 fraction was chromatographed on a silica gel column eluting with CH_2Cl_2–acetone–H_2O (1:2.5:0.1, v/v/v) to yield the new compound **1** (4.0 mg). The F3D fraction was chromatographed on a silica gel column using CH_2Cl_2–MeOH–H_2O (6:1:0.05, v/v/v) to give fractions F3D1–F3D4. The F3D2 fraction was further separated on an YMC RP-18 column eluting with acetone–MeOH–H_2O (1:1:2, v/v/v) to yield **10** (5.0 mg) and **11** (8.0 mg). The F3D4 fraction was separated on an YMC RP-18 column eluting with MeOH–H_2O (1:2, v/v) to yield **6** (5.5 mg).

17. Each compound (**1** and **8**, 2.0 mg) was dissolved in 1.0 N HCl (dioxane–H_2O, 1:1, v/v, 1.0 mL) and then heated to 80 °C in a water bath for 3 h. The acidic solution was neutralized with silver carbonate and the solvent thoroughly driven out under N_2 gas overnight. After extraction with $CHCl_3$, the aqueous layer was concentrated to dryness using N_2 gas. The residue was dissolved in 0.1 mL of dry pyridine, and then L-cysteine methyl ester hydrochloride in pyridine (0.06 M, 0.1 mL) was added to the solution. The reaction mixture was heated at 60 °C for 2 h, and 0.1 mL of trimethylsilylimidazole solution was added, followed by heating at 60 °C for 1.5 h. The dried product was partitioned with *n*-hexane and H_2O (0.1 mL, each), and the organic layer was analyzed by GC: Column: column of SPB-1 (0.25 × 30 m); detector FID, column temp 210 °C, injector temp 270 °C, detector temp 300 °C, carrier gas He (2.0 mL/min). The retention times of persilylated glucose, apiose, and arabinose were founded to be 14.11, 6.70, and 9.16 min, respectively, when compared with the standard solutions prepared by the same reaction from the standard monosaccharides. The retention times of persilylated D-glucose, L-glucose, D-apiose, L-apiose, D-arabinose, and L-arabinose were 14.11, 14.26, 6.70, 6.95, 4.72, and 9.16 min, respectively.
18. Kanchanapoom, T.; Sirikatitham, A.; Otsuka, H.; Ruchirawat, S. *J. Asian Nat. Prod. Res.* **2006**, *8*, 747.
19. Markham, K. R.; Wallace, J. W.; Babu, Y. N.; Murty, V. K.; Rao, M. G. *Phytochemistry* **1989**, *28*, 299.
20. Cutillo, F.; Dellagreca, M.; Previtera, L.; Zarrelli, A. *Nat. Prod. Res.* **2005**, *19*, 99.
21. Lee, E. H.; Kim, H. J.; Song, Y. S.; Jin, C.; Lee, K. T.; Cho, J.; Lee, Y. S. *Arch. Pharm. Res.* **2003**, *26*, 1018.
22. Takeda, Y.; Zhang, H.; Matsumoto, T.; Otsuka, H.; Oosio, Y.; Honda, G.; Tabata, M.; Fujita, T.; Sun, H.; Sezik, E.; Yesilada, E. *Phytochemistry* **1997**, *44*, 117.
23. Cai, Y.; Evans, F. J.; Roberts, M. F.; Phillipson, J. D.; Zenk, M. H.; Gleba, Y. Y. *Phytochemistry* **1991**, *30*, 2033.
24. Lin, C. N.; Kuo, S. H.; Chung, M. I. *J. Nat. Prod.* **1997**, *60*, 851.
25. Rayyan, S.; Fossen, T.; Nateland, H. S.; Andersen, Ø. M. *Phytochem. Anal.* **2005**, *16*, 334.
26. Hosoya, T.; Yun, Y. S.; Kunugi, A. *Tetrahedron* **2005**, *61*, 7037.
27. Kiem, P. V.; Minh, C. V.; Dat, N. T.; Cai, X. F.; Lee, J. J.; Kim, Y. H. *Arch. Pharm. Res.* **2003**, *26*, 1014.
28. Takeda, Y.; Okada, Y.; Masuda, T.; Hirata, E.; Shinzato, T.; Takushi, A.; Yu, Q.; Otsuka, H. *Chem. Pharm. Bull.* **2000**, *48*, 752.
29. Sueyoshi, E.; Liu, H.; Matsunami, K.; Otsuka, H.; Shinzato, T.; Aramoto, M.; Takeda, Y. *Phytochemistry* **2006**, *67*, 2483.
30. Otsuka, H.; Kamada, K.; Ogimi, C.; Hirata, E.; Takushi, A.; Takeda, Y. *Phytochemistry* **1994**, *35*, 1331.
31. Chang, I. M.; Yun, H. S.; Yamasaki, K. *Korean J. Pharmacogn.* **1981**, *12*, 12.
32. Jung, K.; Chin, Y. W.; Kim, Y. C.; Kim, J. *Arch. Pharm. Res.* **2005**, *28*, 1381.
33. The ORAC assay was carried out on a Tecan GENios multi-functional plate reader with fluorescent filters using excitation and emission wavelengths of 485 and 535 nm, respectively. In the final assay mixture, 40 nM fluorescein was used as a target of free radical attack with 20 mM 2,2-azobis dihydrochloride (AAPH) as a peroxyl radical generator in a peroxyl radical-scavenging capacity ($ORAC_{ROO.}$) assay. Trolox (1 mM) was used as a control standard and prepared fresh daily. The analyzer was programmed to record the fluorescence of fluorescein every 2 min after addition of AAPH or H_2O_2–$CuSO_4$. All fluorescence measurements were expressed relative to the initial reading. Final results were calculated based on the difference in the area under the fluorescence decay curve between the blank and each sample. $ORAC_{ROO.}$ was expressed as micromoles of Trolox equivalents (TE). One ORAC unit is equivalent to the net protection area provided by 1 mM of Trolox.
34. Wolfe, K. L.; Liu, R. H. *J. Agric. Food Chem.* **2008**, *56*, 8404.
35. Hoyweghen, L. V.; Karalic, I.; Calenbergh, S. V.; Deforce, D.; Heyerick, A. *J. Nat. Prod.* **2010**, *73*, 1573.
36. The 40 μL of different concentrations of the compounds in distilled water were mixed with 160 μL of the mixture containing 0.5 mM $CuCl_2$ and 0.75 mM neocuproine in 10 mM phosphate buffer, pH 7.4. The absorbance was measured with a micro-plate reader at 454 nm for 1 h. Increased absorbance of the reaction mixture indicates increased reducing capacity.
37. One hundreds micro-liters of different concentration of the compounds were mixed with 100 μL of 0.1 μM $CuSO_4$. After one hundreds micro-liters of mixture solution was added to 100 μL of 0.1 μM calcein, the fluorescence of mixture solution was measured using a Tecan GENios multi-functional plate reader with fluorescent filters (excitation wavelength: 485 nm and emission filter: 535 nm) and compared to the fluorescence intensity of control which contained only calcein.