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Chemical Constituents of the *Ficus elastica* Leaves and Their Antioxidant Activities

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Ficus elastica Roxb. Ex Hornem also known as rubber tree, is a tree species belonging to the Moraceae family. This plant grows profusely without any agronomic management and survives well under extreme environmental conditions such as high temperature and limited water supply. *F. elastica* possesses antimicrobial activity and the leaves extract is used for the treatment of skin infections and skin allergies, as well as a diuretic agent. In addition, several chemical constituents from *F. elastica* have been investigated.[1] Previously, we reported the antioxidant activities of compounds from the leaves of *F. microcarpa* and *F. callosa*.[2,3] In our screening project for antioxidant activities from *Ficus* species, the leaves of *F. elastica* was found to exhibit significant antioxidant activities. As part of our continuing research into the biological activities of *F. elastica* leaves, we report herein the isolation, structural elucidation, and evaluation of antioxidant activities of extracts, as well as two new compounds, ficuselastic acid (**2**) and (1'*S*,6'*R*)-8-*O*-β-D-glucopyranosyl abscisate sodium (**7**), along with twelve known compounds from the leaves of *F. elastica*.

Compound **2** was obtained as a white amorphous powder and its molecular formula was determined to be $C_{14}H_{16}O_6$ by ESI-MS at *m/z* 279 $[M–H]^-$ (negative) and HR-ESI-MS at *m/z* 279.0858 (Calcd $C_{14}H_{15}O_6$ for 279.0874). The ^{1}H-NMR spectrum of **2** showed signals for a secondary methyl at δ_H 1.23 (d, 7.0), an acetyl methyl at δ_H 2.04 (s), an oxygenated proton at δ_H 5.19 (m), and an olefinic proton at δ_H 6.19 as listed in Table 1. The ^{13}C-NMR and DEPT spectra of **2** revealed seven non-protonated carbons, consistent with two carbonyls (δ_C 172.7 and 174.9) and five olefinics (δ_C 105.2, 122.3, 139.1, 161.9, and 164.9), together with seven protonated carbons (see Table 1). The ^{1}H- and ^{13}C-NMR spectra of **2** were similar to those of feroxidin (6*S*,8*S*-5,6,7,8-tetrahydro-8-methyl-1,3,6-naphthalenetriol) (**1**)[4] with the addition of carboxylic and acetyl groups at C-4 and C-6, respectively. Alternatively, the low field chemical shift of proton H-6 (δ_H 5.19) suggested that the acetate group was at C-6 and further confirmed by HMBC correlations between H-6 (δ_H 5.19), methyl (δ_H 2.04) and carbonyl (δ_C 172.7). The

Table 1. ^{1}H and ^{13}C NMR data for compound **2**

Pos.	δ_C[a,b]	δ_H[a,c] (mult., *J* in Hz)	HMBC (H→C)
1	161.9	-	
2	101.7	6.19 (s)	1, 3, 4, 9
3	164.9	-	
4	105.2	-	
4a	174.9	-	
5	36.4	2.81 (dd, 10.0; 18.0, *ax*) 3.59 (dd, 4.8, 18.0, *eq*)	4, 6, 7, 9, 10
6	69.6	5.19 (m, *ax*)	C=O (Ac)
7	35.7	1.74 (dt, 5.8, 12.0, *ax*) 1.90 (brd, 12.0, *eq*)	5, 6, 8, 8a, 9
8	29.0	3.25 (ddq, 2.0, 5.8, 7.0, *eq*)	1, 6, 7, 8a, 9, 10
8a	21.4	1.23 (d, 7.0, *ax*)	7, 8, 9
9	122.3	-	
10	139.1	-	
6-*O*-Ac			
$\underline{CO}CH_3$	172.7	-	
$CO\underline{CH_3}$	21.3	2.04 (s)	

[a]Measured in CD_3OD. [b]100 MHz. [c]400 MHz. Assignments were done by HMQC, HMBC, and ROESY experiments

HMBC cross peaks from proton H-8 (δ_H 3.25) to C-1 (δ_C 161.9), C-6 (δ_C 69.6), C-7 (δ_C 35.7), C-8a (δ_C 21.4), C-9 (δ_C 122.3), and C-10 (δ_C 139.1); and from methyl protons H-8a (δ_H 1.23) to C-7 (δ_C 35.7), C-8 (δ_C 29.0), and C-9 (δ_C 122.3) confirmed that the methyl group was at C-8. The HMBC correlations between H-2 (δ_H 6.19) and C-1 (δ_C 161.9), C-3 (δ_C 164.9), C-4 (δ_C 105.2), and C-9 (δ_C 122.3) were also observed, while the chemical shift of remaining carboxylic carbon (δ_C 174.9) suggested the carboxylic group was at C-4. Based on the above evidence, the planar structure of **2** was elucidated (see Figure 1). The proton-proton coupling constants $J_{5a–6}$ = 10.0 Hz and $J_{5e–6}$ = 4.8 Hz confirmed *axial* position of proton H-6 as well as *equatorial* position of the acetate group at C-6. In addition, ROESY correlations between protons of H_e-5 (δ_H 3.59), H_a-6 (δ_H 5.19), and H_e-7 (δ_H 1.90); between protons of H_a-5 (δ_H 2.81), H_a-7 (δ_H 1.74),

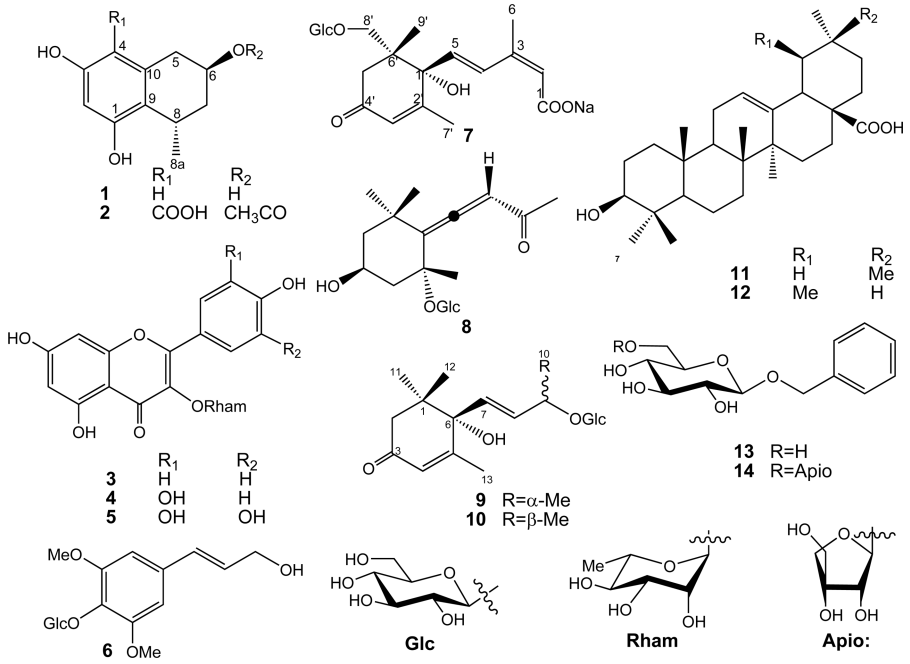

Figure 1. Structures of isolated compounds (**1**-**14**) from the leaves of *F. elastica.*

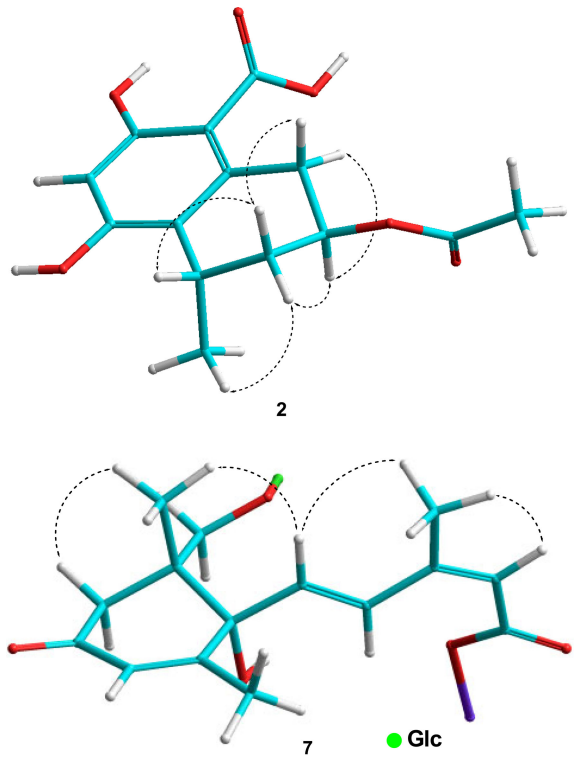

Figure 2. The strong ROESY correlations of compounds **2** and **7**.

and H_e-8 (δ_H 3.25) were observed (see Figure 2), suggesting the *equatorial* and *axial* orientations of 6-acetate groups and 8-methyl, respectively. This represented the configurations in **2** to be (6*S*,8*S*) or (6*R*,8*R*). On the other hand, the negative optical rotation of **2** ($[\alpha]_D^{25}$ = −15.0) indicated the both configurations at C-6 and C-8 to be *S* by comparing optical rotations of (6*S*,8*S*) 5,6,7,8-tetrahydro-1,3-dimethoxy-8-methyl-6-naphthalenol ($[\alpha]_D^{25}$ = −11.5)[4] and (6*R*,8*R*) 5,6,7,8-tetrahydro-1,3-dimethoxy-8-methyl-6-naphthalenol ($[\alpha]_D^{25}$ = +10.0).[5] This was further supported by a positive peak at 285 nm in CD spectrum of **2**, similarly with those of feroxidin (**1**), (a positive peak at 279 nm).[4] Thus, the structure of **2** was determined to be (6*S*,8*S*) 5,6,7,8-tetrahydro-1,3,6-trihydroxy-6-acetyl-8-methyl-naphthalene-4-carboxylic acid, a new compound named ficuselastic acid.

Compound **7** was obtained as a white amorphous powder and its molecular formula was determined to be $C_{21}H_{29}O_{10}Na$ by ESI-MS at *m/z* 465 $[M + H]^+$ and 487 $[M + Na]^+$ (positive) and HR-ESI-MS at *m/z* 487.1571 (Calcd $C_{21}H_{29}O_{10}Na_2$ for 487.1556). The ^{1}H-NMR spectrum of **7** showed signals for three tertiary methyl at δ_H 1.09, 1.93, and 1.95, two singlet olefinic protons at δ_H 5.87 and 5.94, two *trans* olefinic protons at δ_H 5.96 and 7.58 (each doublet and 16.0 Hz), and an anomeric proton at δ_H 4.17 as listed in Table 2. The ^{13}C-NMR and DEPT spectra of **7** revealed 21 carbon signals, 15 of which were assigned to the abscisic acid aglycone and the remaining 6 carbons were assigned to the glucose moiety. The ^{1}H and ^{13}C-NMR data of **7** were similarly to those of (1'*S*,6'*R*)-abscisic acid 8-*O*-β-D-glucopyranoside[6] except for the difference of sodium carboxylate group at C-2 of the aglycone moiety. The HMBC cross peaks from H-5 (δ_H 5.96) to C-3 (δ_C 140.2), C-1' (δ_C 80.0), C-2' (δ_C 170.0), and C-6' (δ 46.7); from H-6 (δ_H 1.93) to C-2 (δ_C 128.7), C-3 (δ_C 140.2), and C-4 (δ_C 131.0); and from H-2 (δ_H 5.87) to C-1 (δ_C 175.4), C-3 (δ_C 140.2), and C-6 (δ_C 20.5) confirmed that two double bonds were at C-2/C-3 and C-4/C-5 (see Figure 2). Furthermore, the proton-proton coupling constant, $J_{5\text{-}6}$ = 16.0 Hz confirmed the *E*-configuration at C-4/C-5. Alternatively, the chemical shift of C-6 (δ_C 20.5) confirmed *Z*-geometric isomerism for the double bond at C-2/C-3 by comparison with that of *sec*-hydroxyaeginetic acid[7] (δ_C for C-6: 14.3) and abscisic acid 8-*O*-β-D-glucopyranoside[6] (δ_C

Table 2. ^{1}H and ^{13}C NMR data for compound **7**

Pos.	δ_C[a,b]	δ_H[a,c] (mult., *J* in Hz)	HMBC (H→C)
Aglycone			
1	175.4	-	
2	128.7	5.87 (s)	1, 3, 4, 6
3	140.2	-	
4	131.0	7.58 (d, 16.0)	2, 3, 5, 6, 1'
5	132.8	5.96 (d, 16.0)	3, 1', 2', 6'
6	20.5	1.93 (d, 1.0)	2, 3, 4
1'	80.0	-	
2'	170.0	-	
3'	127.9	5.94 (s)	7', 2', 1', 5'
4'	201.1	-	
5'	45.4	2.72 (d, 17.0) 2.42 (d, 17.0)	1', 3', 4', 6', 8', 9'
6'	46.7	-	
7'	19.7	1.95 (d, 1.0)	1', 2', 3'
8'	74.6	3.97 (d, 10.0) 3.63 (d, 10.0)	1', 5', 6', 9'
9'	20.3	1.09 (s)	1', 5', 6', 8'
8'-*O*-Glc			
1"	104.7	4.17 (d, 7.5)	8'
2"	75.2	3.17 (dd, 7.5, 9.0)	
3"	78.0	3.37*	1", 5"
4"	71.6	3.30 (t, 8.5)	
5"	77.9	3.25 (m)	
6"	62.5	3.86 (dd, 2.3, 12.0) 3.68 (dd, 5.5, 12.0)	4", 5"

[a]Measured in CD_3OD. [b]125 MHz. [c]500 MHz. Assignments were done by HMQC and HMBC, and ROESY experiments

for C-6: 19.7). The HMBC cross peaks from H-3' (δ_H 5.94) to C-1' (δ_C 80.0), C-2' (δ_C 170.0), C-4' (δ_C 201.1), and C-7' (δ_C 19.7); and from H-7' (δ_H 1.95) to C-1' (δ_C 80.0), C-2' (δ_C 170.0), and C-3' (δ_C 127.9) confirmed that the double bond was at C-2'/C-3'; while ketone and tertiary methyl groups were at C-4' and C-2', respectively. The ROESY correlation between H-5 (δ_H 5.96) and H-9' (δ_H 1.09) confirmed that the side-chain and methyl group at C-1' and C-6' were same side. In addition, the CD spectrum of 7 showed one negative peak at λ 231 nm and one positive peak at λ 271, similarly to those of abscisic acid 8-*O*-β-D-glucopyranoside,[6] suggested that the configurations at C-1' and C-6' were *S* and *R*, respectively. Acid hydrolysis of **7** revealed D-glucose (identified as a TMS derivative by a gas chromatography method). Furthermore, the HMBC between the glc H-1" (δ_H 4.17) and C-8' of the aglycone (δ_C 74.6), and between H-8' (δ_H 3.63 and 3.97) and C-1" (δ_C 104.7) suggested that the glucopyranosyl moiety was at C-8'. Consequently, the structure of **7** was determined to be (1'*S*,6'*R*)-8-*O*-β-D-glucopyranosyl abscisate sodium.

The remaining compounds were identified as feroxidin (**1**),[8] quercitrin (**3**)[9] kaempferin (**4**),[10] myricitrin (**5**),[9] syringin (**6**),[11] citroside B (**8**),[12] corchoionoside C (**9**),[13] (6*S*,9*R*)-roseoside (**10**),[13] oleanolic acid (**11**),[14] ursolic acid (**12**),[14] benzyl *O*-β-D-glucopyranoside (**13**),[15] icariside F_2 (**14**),[16] (see Figure 1). Their structures were established on the basis of spectral and chemical evidence, which were in agreement with those reported in literature. All these compounds were isolated from *F. elastica* for the first time.

The antioxidant capacities of methanol extract and other fractions (*n*-hexane, ethyl acetate and water fractions) from the leaves of *F. elastica* were tested by the oxygen radical absorbance capacity (ORAC) assay (see Figure 3). Trolox was used as a control and prepared fresh daily. The methanol extract displayed significant peroxyl radical-scavenging activity with 4.2 μM Trolox equivalent (TE) at a concentration of 5.0 μg/mL. The water fraction exhibited potent antioxidant activity followed by ethyl acetate fraction and *n*-hexane fraction with values of 23.4, 17.9, and 5.8 μM TE at the concentration of 5.0 μg/mL, respectively (see Figure 3). Bioassay-guided fractionation led us to choose water and ethyl acetate fractions for further isolation of bioactive compounds. Subsequently, all isolates from *F. elastica* were measured by the ORAC assay at concentrations of 1.0 and 5.0 μM (see

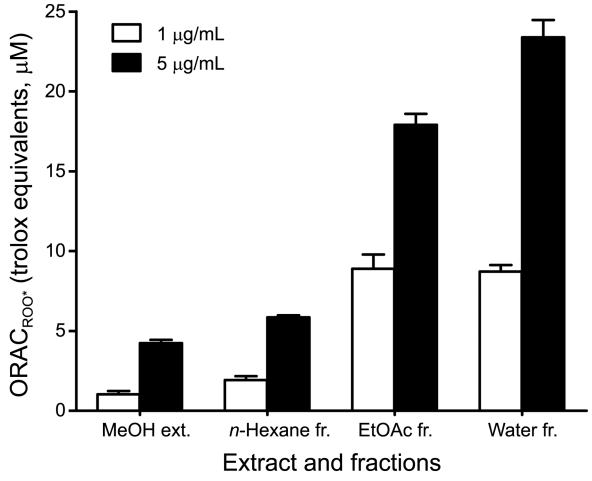

Figure 3. Peroxyl radical-scavenging activity (Trolox equivalent, μM) of methanol extract (MeOH ext.) as well as *n*-hexane (*n*-Hexane fr.), ethyl acetate (EtOAc fr.), and water (Water fr.) fractions from the leaves of *F. elastica*.

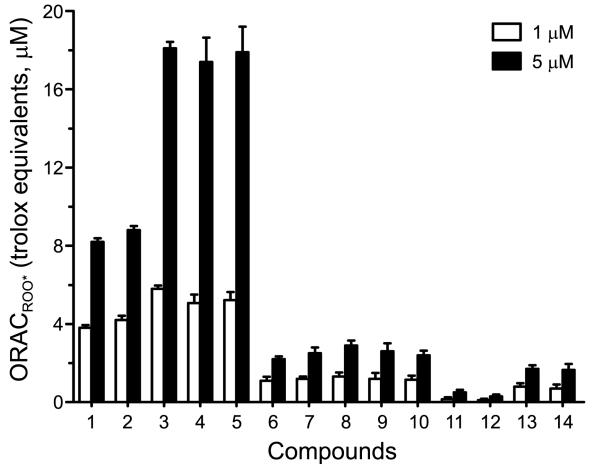

Figure 4. Peroxyl radical-scavenging activity (Trolox equivalent, μM) of compounds (**1**-**14**).

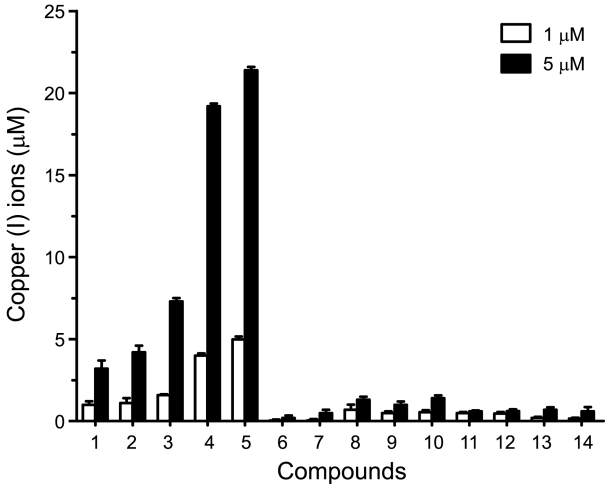

Figure 5. Reducing capacity of compounds **1**-**14**. The results represent the mean ± S.D. of values obtained from three measurements.

Figure 4). With regard to peroxyl radical-scavenging activity, three flavonoids **3**-**5** exhibited potent antioxidant activity compared with that of the positive control, Trolox. Compounds **1** and **2** showed significant antioxidant activities with values of 8.2 and 8.8 μM TE at a concentration of 5.0 μM. We next tested the reducing capacity of all compounds by measuring the concentration of Cu (I) ions reduced from Cu (II) ions. Compounds **4** and **5** exhibited meaningful reducing capacity of Cu (I) ions with values of 19.2 and 21.4 μM TE at a concentration of 5.0 μM (see Figure 5). Moreover, all compounds revealed very weak metal chelating. These data suggest that the antioxidant activities of each compound are not due to metal chelating activity with transition metal ions (see Supporting information). Instead, the antioxidant activity from the *F. elastica* leaves determined by the ORAC and reducing capacity assays may be attributed to the water and ethyl acetate fractions in which compounds **3**-**5** potently donate a hydrogen atom and a single electron is contained.

Experimental

Plant Material. The leaves of *F. elastica* were collected in Caugiay, Hanoi, Vietnam in March, 2011, and identified by Dr. Ninh Khac Ban, Institute of Marine Biochemistry, VAST, Vietnam. A voucher specimen (FE0311) was deposited at the Herbarium of Institute of Marine Biochemistry.

Ficuselastic Acid (2): A white amorphous powder, $[\alpha]_D^{25}$: -15^0 (c = 0.1, MeOH), negative ESI-MS: *m/z* 279 $[M-H]^-$, $C_{14}H_{16}O_6$, HR-ESI-MS found *m/z* 279.0858 $[M-H]^-$ (Calcd $C_{14}H_{15}O_6$ for 279.0869), CD (c = 1.5 × 10^{-5}, MeOH), [θ] –43485 (214), +3372 (243), –11576 (263), +34628 (285), ^{1}H- and ^{13}C-NMR: see Table 1.

(1'*S*,6'*R*)-8-*O*-β-D-Glucopyranosyl Abscisate Sodium (7): A white amorphous powder, $[\alpha]_D^{25}$: $+60^0$ (c = 0.1, MeOH), positive ESI-MS: *m/z* 465 $[M+H]^+$, 487 $[M+Na]^+$, $C_{21}H_{29}O_{10}Na$, HR-ESI-MS found *m/z* 487.1571 $[M+Na]^+$ (Calcd $C_{21}H_{29}O_{10}Na_2$ for 487.1556), CD (c = 1.5 × 10^{-5}, MeOH), [θ] –43681 (231), +54730 (271), ^{1}H- and ^{13}C-NMR: see Table 2.

Supporting Information. General procedures, extraction, isolation, hydrolysis procedure, antioxidant assays, and NMR spectra of **2** and **7** are available as Supporting Information.

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References

1. Ogunwande, I. A.; Flamini, G.; Adefuye, A. E.; Lawal, N. O.; Moradeyo, S.; Avoseh, N. O. *S. Afr. J. Bot.* **2011**, *77*, 645.
2. Kiem, P. V.; Cuong, N. X.; Nhiem, N. X.; Thu, V. K.; Ban, N. K.; Minh, C. V.; Tai, B. H.; Hai, T. N.; Lee, S. H.; Jang, H. D.; Kim, Y. H. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 633.
3. Kiem, P. V.; Cuong, N. X.; Nhiem, N. X.; Hang, D. T. T.; Nam, N. H.; Ban, N. K.; Minh, C. V.; Bing, Z.; Jang, H. D.; Kim, Y. H. *Nat. Prod. Commun.* **2011**, *6*, 159.
4. Speranza, G.; Manitto, P.; Pezzuto, D.; Monti, D. *Chirality* **1991**, *3*, 263.
5. Aina, G.; Nasini, G.; Vajna de Pava, O. *J. Mol. Catal. B- Enzym.* **2001**, *11*, 367.
6. Ramos, M. D. R.; Gerold, J.; Socorro, V.; Dellamary, F. L.; Reiner, W.; Peter, W. *Phytochemistry* **2004**, *65*, 955.
7. Sasaki, H.; Morota, T.; Nishimura, H.; Ogino, T.; Katsuhara, T.; Sugama, K.; Chin, M.; Mitsuhashi, H. *Phytochemistry* **1991**, *30*, 1997.
8. Speranza, G.; Manitto, P.; Monti, D.; Lianza, F. *Tetrahedron Lett.* **1990**, *31*, 3077.
9. Zhong, X.-N.; Otsuka, H.; Ide, T.; Hirata, E.; Takushi, A.; Takeda, Y. *Phytochemistry* **1997**, *46*, 943.
10. Mourad, K. *Phytochemistry* **1990**, *29*, 2295.
11. Kiem, P. V.; Minh, C. V.; Dat, N. T.; Cai, X. F.; Lee, J. J.; Kim, Y. H. *Arch. Pharm. Res.* **2003**, *26*, 1014.
12. Zhang, Z.; Zhang, W.; Ji, Y.-P.; Zhao, Y.; Wang, C.-G.; Hu, J.-F. *Phytochemistry* **2010**, *71*, 693.
13. Yoshikawa, M.; Shimada, H.; Saka, M.; Yoshizumi, S.; Yamahara, *J. Chem. Pharm. Bull.* **1997**, *45*, 464.
14. Seebacher, W.; Simic, N.; Weis, R.; Saf, R.; Kunert, O. *Magn. Reson. Chem.* **2003**, *41*, 636.
15. Ky, P. T.; Anh, P. T.; Kiem, P. V.; Minh, C. V.; Nhiem, N. X.; Tai, B. H.; Ngan, N. T. T.; Quang, T. H.; Thao, N. P.; Kim, Y. H. *Bull. Korean Chem. Soc.* **2012**, *43*, 3763.
16. Miyase, T.; Ueno, A.; Takizawa, N.; Kobayashi, H.; Oguchi, H. *Chem. Pharm. Bull.* **1988**, *36*, 2475.