Flavonoid Glycosides from *Chromolaena odorata* Leaves and Their *in Vitro* Cytotoxic Activity

Tran Manh Hung,*[,a,b] To Dao Cuong,[a,c] Nguyen Hai Dang,[c] Shu Zhu,[b] Pham Quoc Long,[c] Katsuko Komatsu,[b] and Byung Sun Min[a]

[a] *College of Pharmacy, Catholic University of Daegu; Gyeongsan 712–702, Korea:* [b] *Division of Pharmacognosy, Department of Medicinal Resources, Institute of Natural Medicine, University of Toyama; 2630 Sugitani, Toyama 930–0194, Japan: and* [c] *Institute of Natural Product Chemistry, Vietnamese Academic of Science and Technology; 18 Hoang Quoc Viet, Hanoi, Vietnam.* Received September 6, 2010; accepted October 8, 2010

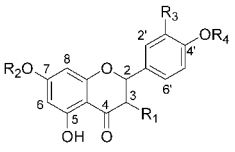
Two new flavonoid glycosides, (1, 2), and eleven known compounds, (3—13), were isolated from from a 70% EtOH extract of the leaves of *Chromolaena odorata* (Asteraceae). Their structures were elucidated by 1D and 2D NMR spectroscopic interpretation as well as by chemical studies. The newly isolated compounds were tested *in vitro* for their cytotoxic activities against the LLC and HL-60 cancer cell lines. Compound 1 showed cytotoxicity against LLC and HL-60 cancer cell lines with IC_{50} values of 28.2 and 11.6 μM, respectively. Compound 2 exhibited significant cytotoxic activity in the inhibition of HL-60 cancer cell lines with IC_{50} value of 10.8 μM.

Key words *Chromolaena odorata*; Asteraceae; flavonoid glycoside; cytotoxic

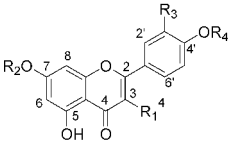
Chromolaena odorata (L.) King et Robinson (*Eupatorium odoratum* L., family Asteraceae) is a diffuse, scrambling shrub that is mainly a weed of plantation crops and pastures of southern Asia and western Africa. Traditionally, fresh leaves or a decoction of *C. odorata* have been used throughout Vietnam for many years as well as in other tropical countries for the treatment of inflammation, leech bite, soft tissue wounds, burn wounds, skin infection and dento-alveolitis.[1)] A number of studies demonstrated that the extract of the leaves of *C. odorata* inhibited the growth of bacteria,[2—5)] enhanced hemostasis and blood coagulation,[2,6)] inhibited the contraction of collagen,[7)] enhanced fibroblasts and endothelial cell proliferation.[8)] The antifungal,[9)] and antiinflammation[10)] of the plant extract were also investigated. Previous phytochemical investigations of this plant described the presence of flavonoids,[11—15)] phenolics,[16)] alkaloids,[17)] terpenoids,[18)] and essential oil.[19,20)] Recently, chromomoric acids were isolated and showed significant effect on proliferator-activated receptor (PPAR)-γ activation.[21)] However, no study has been specifically investigated the ability of extract or isolated compounds from this plant on cytotoxic activity. In our ongoing research on the chemistry and biological activity of the traditional herbs, we found that the 70% EtOH extract of the leaves of *C. odorata* showed significant cytotocxicity against cancer cell lines. A phytochemical study led to the isolation of two new flavonoid glycosides (**1**, **2**) and the eleven known compounds (**3**—**13**). This article reports the isolation and structural determination of isolated components, as well as an evaluation of their cytotoxic activity against LLC and HL-60 cancer cell lines.

Repeated column chromatography of the ethyl acetate soluble fraction of the 70% EtOH extract of *C. odorata* leaves resulted in the purification of thirteen compounds (**1**—**13**). The known compounds were identified as aromadendrin 4′-methyl ether (**3**), eriodictyol 7,4′-dimethyl ether (**4**), naringenin 4′-methyl ether (**5**), isosakuranetin (**6**), quercetin 7,4′-dimethyl ether (**7**), kaempferide (**8**), acacetin (**9**), rhamnazin (**10**), quercetin 3-*O*-rutinoside (**11**), kaempferol 3-*O*-rutinoside (**12**), and kaempferol 3-*O*-glucopside (**13**) (Fig. 1) on the basis of spectroscopic analysis, chemical evidence and comparison of spectral data with the literature data.[12—16,18,21)]

Compound **1** was obtained as a yellowish powder. The molecular composition of **1** was determined as $C_{28}H_{33}O_{14}$ by the positive high resolution (HR)-FAB-MS (*m/z* 539.5185 $[M+H]^+$, Calcd for 539.5191 $[M+H]^+$). The IR absorptions at 3320 and 1660 cm^{-1} showed the presence of hydroxy and carbonyl groups, respectively. The UV spectrum displayed two maximum bands at 262 and 381 nm, characteristic of a flavone.[12,13)] The ^{13}C and distortionless enhancement by polarization transfer (DEPT) NMR spectra revealed 28 signals, of which 12 were assigned to the sugar moieties and 16 to a flavone moiety. The ^1H-NMR spectrum of **1** indicated the presence of a methoxy group at δ 3.68 (3H, s), and an aromatic proton at δ 6.95 (1H, s, H-3). The proton signals at δ 6.47 (1H, d, J=2.4 Hz) and 6.89 (1H, d, J=2.4 Hz) were assigned to H-6 and H-8 in A ring, respectively, and a pair of proton signals with coupling constants as 8.0 and 1.8 Hz at δ



	R_1	R_2	R_3	R_4
2	H	CH_3	H	Glc(2-1)Glc
3	OH	H	H	CH_3
4	OH	CH_3	OH	CH_3
5	H	H	H	CH_3



	R_1	R_2	R_3	R_4
1	H	CH_3	H	Glc(2-1)Rha
6	H	CH_3	H	CH_3
7	OH	OH	H	CH_3
8	H	H	H	CH_3
9	OH	CH_3	OCH_3	H
10	OH	CH_3	OCH_3	CH_3
11	Glc(6-1)Rha	H	OH	H
12	Glc(6-1)Rha	H	H	H
13	Glc	H	H	H

Fig. 1. Chemical Structures of Isolated Compounds **1**—**13**

* To whom correspondence should be addressed. e-mail: tmhung1810@yahoo.com

8.11 (H-2′, 6′) and 7.35 (H-3′, 5′) indicated the presence of 4-substituted phenyl in B ring of flavone.[12,15,18] On the basis of the ^{1}H- and ^{13}C-NMR data, the aglycon of **1** was identified as genkwanin.[22,23] Additionally, signals for two anomeric protons at δ 5.43 (1H, d, J=7.5 Hz), and 5.15 (1H, d, J=3.5 Hz) were observed in the ^{1}H-NMR spectrum, suggesting the presence of two sugar units. In comparison with the corresponding signals in a model compound reported in the literature,[23] glycosidations at C-4′ was indicated by the significant downfield shift observed for this carbon signals in **1**. Acid hydrolysis of **1** yielded D-glucose and L-rhamnose (1 : 1), which were confirmed by GC analysis. The chemical shifts, the signal multiplicities, the absolute values of the coupling constants, and their magnitude in the ^{1}H-NMR spectrum, as well as the ^{13}C-NMR data, indicated that the glucose unit has a β-configuration, and rhamnose unit has α-configuration. The linkage sites and sequences of the two saccharides and the aglycon were deduced from a heteronuclear multiple bond connectivity (HMBC) experiment as observed correlations between H-1″ of Glc and C-4′ of the aglycon, and between H-1‴ of the Rha and C-2″ of Glc (Fig. 2). Thus, the structure of **1** was elucidated as genkwanin 4′-*O*-[α-L-rhamnopyranosyl(1→2)-β-D-glucopyranoside].

Compound **2** was isolated as white powder. Its molecular formula was established as $C_{28}H_{35}O_{15}$ from the HR-FAB-MS quasimolecular ion at *m/z* 611.5674 (Calcd for 611.5679 $[M+H]^+$). Compound **2** displayed aromatic signals downfield at δ 7.65 (2H, d, J=8.0 Hz, H-2′, 6′), 7.24 (2H, d, J=8.0 Hz, H-3′, 5′), δ 128.7 (C-2′, 6′), 118.5 (C-3′, 5′), and 162.4 (C-4′) in the ^{1}H- and ^{13}C-NMR spectra, suggesting the hydroxygenated position to be at C-4′. The ^{1}H-NMR spectrum of **2** indicated the presence of a methoxy group at δ 3.68 (3H, s), two proton signals at δ 6.47 (1H, d, J=2.4 Hz) and 6.89 (1H, d, J=2.4 Hz) were observed and assigned for H-6 and H-8 in A ring, respectively. The remarkable differences could be observed between the ^{1}H- and ^{13}C-NMR spectra of compound **2** and those of **1**. The three one-proton coupled double doublets at δ 5.47 (1H, dd, J=2.8, 11.8 Hz, H-2), δ 3.21 (1H, dd, J=11.8, 16.0 Hz, H-3ax), and δ 2.85 (1H, dd, J=2.4, 16.0 Hz, H-3eq) suggested that ring C was saturated.[12,24] This splitting pattern was due to the coupling between H-2 axial proton and the H-3 geminal protons.[24] The absolute configuration at the C-2 stereocenter was established to be *S* on the basic of high-amplitude negative Cotton effect in the 270—300 nm region and the weak positive Cotton effect in the 325—350 nm region,[24,25] which indicated stereospecificity was achieved in the process of C-ring cyclization of sakuranetin.[24,25] Additionally, the ^{1}H- and ^{13}C-NMR spectra of **2** showed characteristic signals of two sugar moiety in the region ranging from δ 3.80 to 4.92 and from δ 60.5 to 105.4, respectively. These sugars were assigned to be glucoses on the basis of NMR data and the *Rf* value compared with authentic glucose after acid hydrolysis. The absolute configuration was determined to be D-glucose by gas chromatography. The signals for two anomeric protons at δ 4.85 (1H, d, J=7.6 Hz, H-1″), and 4.72 (1H, d, J=7.0 Hz, H-1‴) indicated that these glucoses were linked *via* β-linkage. The locations of glucoses were confirmed by HMBC correlations between H-1″ and C-4′, and between H-1‴ and C-2″ (Fig. 2). On the basis of these data, the structure of **2** was assigned as sakuranetin 4′-*O*-[β-D-glucopyranosyl(1→2)-β-D-glucopyranoside].

The new metabolites, **1** and **2**, were evaluated *in vitro* for their cytotoxic activity against cancer cell lines LLC and HL-60 with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay method.[26] Compound **1** showed cytotoxicity against LLC and HL-60 cancer cell lines with IC_{50} values of 28.2 and 11.6 μM, respectively. Compound **2** exhibited weak cytotoxic activity against LLC (IC_{50} value >50 μM), however, it showed significant cytotoxic activity in the inhibition of HL-60 cancer cell lines with IC_{50} value of 10.8 μM.

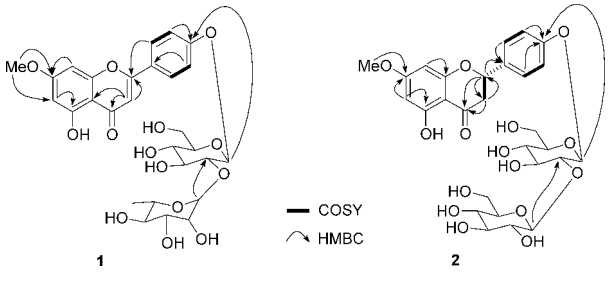


Fig. 2. Key COSY and HMBC Correlations of Compounds **1** and **2**

Experimental

General Procedures Optical rotations were determined on a JASCO P-1020 polarimeter using a 100 mm glass microcell. IR spectra (KBr) were recorded on a Bruker Equinox 55 Fourier transform (FT)-IR spectrometer. NMR spectra were obtained on a Varian Inova 400 MHz spectrometer with TMS as the internal standard. FAB-MS and HR-FAB-MS data were obtained on a Micromass QTOF2 (Micromass, Wythenshawe, U.K.) mass spectrometer. For column chromatography, silica gel (63—200 μm particle size), RP-18 (75 μm particle size, Merck, Germany), and YMC gel (ODS-A, S-150 μm, YMC Co., Ltd., Japan) were used. TLC was carried out with Merck silica gel 60 F254 and RP-18 F254 plates. HPLC was carried out using a Gilson system with a UV detector and an Optima Pak C_{18} column (10×250 mm, 10 μm particle size, RS Tech, Korea). GC system: GC-14BPF column, 5% OV-225/AW-DMCS-Chromosorb W (80—100 mesh), 3 mm i.d.×2.5 m; column temperature, 210 °C; injection temperature, 250 °C; carrier gas, N_2 at a flow rate of 25 ml/min; detector, FID (Shimadzu, Japan). Solvents were purchased from Samchun Chemicals Co., Korea, RPMI 1640, fetal bovine serum (FBS), phosphate buffered saline (PBS) buffer, penicillin–streptomycin, and 10% trypsin-ethylenediaminetetraacetic acid (EDTA) were purchased from Gibco (Carlsbad, CA, U.S.A.). MTT reagent and dimethyl sulfoxide (DMSO) were obtained from Sigma Aldrich.

Plant Material The leaves of *Chromolaena odorata* were collected from Hoa Binh province, Vietnam, in July 2009 and identified by Dr. Tran Cong Luan, Ginseng and Plant Materials Center, Ho Chi Minh City, Vietnam. A voucher specimen (TMH-0012) was deposited in the herbarium of the center.

Extraction and Isolation The dried leaves (1.0 kg) of *C. odorata* were extracted with 2 l of 70% ethanol, three times. The 70% EtOH extract was combined and concentrated to yield a residue that was suspended in water and then successively partitioned with $CHCl_3$, EtOAc, and *n*-BuOH to afford $CHCl_3$-, EtOAc-, and *n*-BuOH-soluble fractions. The EtOAc-soluble fraction (28.7 g) was separated by silica gel (63—200 μm particle size) column chromatography using a gradient of $CHCl_3$–MeOH–H_2O (from 60 : 1 : 0.1 to 5 : 1 : 0.1) to yield eleven sub-fractions (E.1—E.11) according to their TLC profiles. Sub-fraction E.4 was chromatographed over a YMC column (150 μm particle size) using a gradient of MeOH–H_2O (from 5 : 1 to 10 : 1), to yield six further sub-fractions E.4.1—E.4.6. The sub fraction E.4.2 was further fractionated and purified using repeated column chromatography employing Sephadex LH-20 (MeOH–$CHCl_3$, 60 : 40) to yield compounds **3** (14.4 mg) and **4** (87 mg). The sub fraction E.4.3 was fractionated and purified using repeated silica gel column chromatography with $CHCl_3$–MeOH gradient (from 92 : 8 to 85 : 15) to give compounds **6** (8 mg), **8** (14.8 mg) and **9** (118 mg). The E.6 sub fraction was purified by semi-preparative HPLC [RS Tech Optima Pak C_{18} column (10×250 mm, 10 μm particle size); mobile phase MeOH–H_2O (63 : 37) with 0.1% formic acid; flow rate 2 ml/min;

UV detection at 205 nm], and resulted in the isolation of compounds **5** (6.8 mg), and **7** (4.1 mg). Sub fraction E.7 was purified by column chromatography employing MCI gel CHP20P (MeOH–H_2O gradient, 5 : 5→5 : 1) and silica gel ($CHCl_3$–MeOH gradient, from 9 : 1 to 3 : 1) to give compounds **2** (10.2 mg) and **10** (20.7 mg). The E8 sub fraction was also purified by semi-preparative HPLC using the above conditions resulting in the isolation of compounds **1** (9.1 mg), **12** (8.8 mg) and **13** (6.2 mg). Repeating the reversed phase column chromatography (YMC) eluted with MeOH–H_2O (4 : 1) for sun fraction E.9 yielded compound **11** (19.8 mg).

Genkwanin 4′-*O*-[α-L-Rhamnopyranosyl(1→2)-β-D-glucopyranoside] (1) Yellowish powder, mp 216—218 °C; $[\alpha]_D^{26}$ −16.7 (c=0.2, MeOH); UV (MeOH): 270, 325 nm; IR (KBr) ν_{max}: 3512, 3490, 2960, 2881, 1750, 1148 cm^{-1}; FAB-MS $[M+H]^+$ 593.5 m/z; HR-FAB-MS $[M+H]^+$ 593.5185 m/z ($C_{28}H_{33}O_{14}$ Calcd 593.5191). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 6.95 (s, H-3), 6.47 (1H, d, J=2.4 Hz, H-6), 6.89 (1H, d, J=2.4 Hz, H-8), 8.11 (2H, dd, J=1.8, 8.0 Hz, H-2′, 6′), 7.35 (2H, dd, J=1.8, 8.0 Hz, H-3′, 5′), 3.68 (3H, s, 7-MeO); Glc: 5.43 (1H, d, J=7.5 Hz, H-1″), 3.68—4.55 (m, H-2″, 3″, 4″, 5″), 3.48 (1H, m, H-6″a), 3.71 (1H, dd, J=5.4, 11.2 Hz, H-6″b), Rha: 5.15 (1H, d, J=3.5 Hz, H-1‴), 4.20—4.85 (m, H-2‴, 3‴, 4‴, 5‴), 1.35 (3H, d, J=5.7 Hz, H-6‴); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 163.7 (C-2), 105.4 (C-3), 184.5 (C-4), 161.1 (C-5), 101.3 (C-6), 163.3 (C-7), 96.8 (C-8), 156.5 (C-9), 105.7 (C-10), 124.7 (C-1′), 129.4 (C-2′, C-6′), 116.3 (C-3′, 5′), 162.2 (C-4′), 55.4 (7-MeO); Glc: 100.2 (C-1″), 75.4 (C-2″), 78.6 (C-3″), 71.2 (C-4″), 75.8 (C-5″), 61.2 (C-6″); Rha: 100.6 (C-1‴), 70.8 (C-2‴), 71.3 (C-3‴), 72.7 (C-4‴), 67.4 (C-5‴), 17.6 (C-6‴).

Sakuranetin 4′-*O*-[β-D-Glucopyranosyl(1→2)-β-D-glucopyranoside] (2) White powder, mp 197—199 °C; $[\alpha]_D^{26}$ −114.4 (c=0.2, MeOH); UV: 230, 287 nm; IR (KBr) ν_{max}: 3412, 2908, 1626, 1584, 1557, 1286 cm^{-1}; FAB-MS $[M+H]^+$ 611.5 m/z; HR-FAB-MS $[M+H]^+$ 611.5674 m/z ($C_{28}H_{35}O_{15}$ Calcd 611.5679); ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 5.47 (1H, dd, J=2.8, 11.8 Hz, H-2), 3.21 (1H, dd, J=11.8, 11.6 Hz, H-3ax), 2.85 (1H, dd, J=2.4, 16.0 Hz, H-3eq), 6.40 (1H, d, J=2.0 Hz, H-6), 6.62 (1H, d, J=2.0 Hz, H-8), 7.65 (2H, d, J=8.0 Hz, H-2′, 6′), 7.24 (2H, d, J=8.0 Hz, H-3′, 5′), Glc-1: 4.85 (1H, d, J=7.6 Hz, H-1″), 3.75—4.80 (m, H-2″, 3″, 4″, 5″), 4.40 (1H, m, H-6″a), 4.60 (1H, br d, J=11.5 Hz, H-6″b), Glc-2: 4.72 (1H, d, J=7.0 Hz, H-1‴), 3.80—4.52 (m, H-2‴, 3‴, 4‴, 5‴), 4.38 (1H, m, H-6‴a), 4.57 (1H, br d, J=11.0 Hz, H-6‴b); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 78.1 (C-2), 46.8 (C-3), 192.4 (C-4), 161.6 (C-6), 99.3 (C-6), 164.5 (C-7), 96.7 (C-8), 158.3 (C-9), 108.8 (C-10), 129.5 (C-1′), 128.7 (C-2′, 6′), 118.5 (C-3′, 5′), 162.4 (C-4′), 56.0 (7-MeO); Glc-1: 100.5 (C-1″), 70.4 (C-2″), 77.3 (C-3″), 69.6 (C-4″), 75.7 (C-5″), 60.5 (C-6″); Glc-2: 105.4 (C-1‴), 73.5 (C-2‴), 78.2 (C-3‴), 70.5 (C-4‴), 76.3 (C-5‴), 61.2 (C-6‴).

Determination of Sugar Components The monosaccharide subunits of **1** and **2** were obtained by acid hydrolysis. Compounds **1** and **2** (4 mg each) in 10% HCl–dioxane (1 : 1, 1 ml) were each heated at 80 °C for 4 h in a water bath. The reaction mixtures were neutralized with Ag_2CO_3, filtered, and then extracted with $CHCl_3$ (1 ml×3). After concentration, each H_2O layer (monosaccharide portion) was examined by TLC with $CHCl_3$–MeOH–H_2O (55 : 45 : 10) and compared with authentic samples. Each sugar residue was then dissolved in 2 ml of H_2O, 15 mg of $NaBH_4$ was added, and the mixture left to stand for 2 h at ambient temperature. Several drops of 25% HOAc were added until the pH value was 4—5. After co-distillation with CH_3OH to remove the extra boracic acid and water, the resulting products were put into a vacuum-desiccator overnight and then heated at 110 °C for 15 min to further remove the water. Next, 3 ml of acetic anhydride were added and the solution was kept at 100 °C for 1 h. Then the solution was cooled and co-distillated with toluene several times. The acetate derivatives were dissolved in $CHCl_3$ and washed with distilled water and then anhydrous sodium sulfate, filtered, and concentrated to 0.1 ml. The acetate derivatives were subjected to GC analysis to identify the sugars.[27] Column temperature 210 °C; injection temperature 250 °C; carrier gas N_2 at a flow rate of 25 ml/min; D-glucose, L-rhamnose, 17.40, and 6.18 min, respectively.

Cytotoxic Activity The cancer cell lines (LLC, and HL-60) were maintained in RPMI that included L-glutamine with 10% FBS and 2% penicillin–streptomycin. Cells were cultured at 37 °C in a 5% CO_2 incubator. Cytotoxic activity was measured using a modified MTT assay.[26] Viable cells were seeded in the growth medium (100 μl) into 96 well microtiter plates (1×10^4 cells per well) and incubated at 37 °C in a 5% CO_2 incubator. The test sample was dissolved in DMSO and adjusted to final sample concentrations ranging from 5.0 to 150 μM by diluting with the growth medium. Each sample was prepared in triplicate. The final DMSO concentration was adjusted to <0.1%. After standing for 24 h, 10 μl of the test sample was added to each well. The same volume of DMSO was added to the control wells. Removing medium after 48 h of the test sample treatment, MTT 10 μl was also added to the each well (final concentration, 5 mg/ml). After 4 h incubation, the plates were removed, and the resulting formazan crystals were dissolved in DMSO (150 μl). The OD was measured at 570 nm. Adriamycin (Sigma, purity >98%) was used as positive control. The IC_{50} value was defined as the concentration of sample that reduced absorbance by 50% relative to the vehicle-treated control.

Acknowledgment This work was supported by a grant on Studies on the identification of the biological active components from Vietnamese traditional medicines from Cuchoa Pharmaceutical Co., Ltd. (Vietnam).

References

1) Thang P. T., See P., Lee S. T., Chang S. Y., *Burns*, **27**, 319—327 (2001).
2) Akah P. A., *Int. J. Crude Drug Res.*, **28**, 233—235 (1990).
3) Bamba D., Bessiere J. M., Marion C., Pelissier Y., Fourate I., *Planta Med.*, **59**, 184—185 (1993).
4) Irobi O. N., *J. Ethnopharmacol.*, **37**, 81—83 (1992).
5) Caceres A., *J. Ethnopharmacol.*, **48**, 85—88 (1995).
6) Triaratana T., Suwannuraks R., Naengchomnong W., *J. Med. Assoc. Thailand*, **74**, 283—286 (1991).
7) Phan T. T., Hughes M. A., Cherry G. W., *J. Alt. Comp. Med.*, **2**, 335—343 (1996).
8) Phan T. T., Hughes M. A., Cherry G. W., *Plast. Recontr. Surg.*, **101**, 756—765 (1998).
9) Ngono N. A., Ebelle Etame R., Ndifor F., Biyiti L., Amvam Z. P. H., Bouchet P., *Chemotherapy*, **52**, 103—106 (2006).
10) Owoyele V. B., Adediji J. O., Soladoye A. O., *Inflammopharmacology*, **13**, 479—484 (2005).
11) Wollenweber E., Dörr M., Muniappan R., *Biochem. Syst. Ecol.*, **23**, 873—874 (1995).
12) Barua R. N., Sharma R. P., Thyagarajan G., Hertz W., *Phytochemistry*, **17**, 1807—1808 (1978).
13) Suksamrarn A., Chotipong A., Suavansri T., Boongird S., Timsuksai P., Vimuttipong S., Chuaynugul A., *Arch. Pharm. Res.*, **27**, 507—511 (2004).
14) Pisutthanan N., Liawruangrath B., Liawruangrath S., Bremner J. B., *Nat. Prod. Res.*, **20**, 1192—1198 (2006).
15) Ling S. K., Pisar M. M., Man S., *Biol. Pharm. Bull.*, **30**, 1150—1152 (2007).
16) Srinivasa R. K., Chaudhury P. K., Pradhan A., *Food Chem. Toxicol.*, **48**, 729—732 (2010).
17) Biller A., Boppre M., Witte L., Hartmann T., *Phytochemistry*, **35**, 615—619 (1994).
18) Sunil K., Durga T., Bhar S., Talapatra B., *Phytochemistry*, **13**, 284—285 (1974).
19) Baruah R. N., Leclercq P. A., *Planta Med.*, **59**, 283 (1993).
20) Pisutthanan N., Liawruangrath B., Liawruangrath S., Baramee A., Apisariyakul A., Korth J., Bremner J. B., *Nat. Prod. Res.*, **20**, 636—640 (2006).
21) Dat N. T., Lee K., Hong Y. S., Kim Y. H., Minh C. V., Lee J. J., *Planta Med.*, **75**, 803—780 (2009).
22) Greenaway W., English S., May J., Whatley F. R., *Phytochemistry*, **30**, 3005—3308 (1991).
23) Tan G. T., Pezzuoto J. M., Kinghorn A. D., Hughes S. H., *J. Nat. Prod.*, **54**, 143—154 (1991).
24) Hurabielle M., Eberle J., Paris M., *Planta Med.*, **46**, 124—125 (1982).
25) Valant-Vetschera K. M., Bhutia T. D., Wollenweber E., *Phytochemistry*, **71**, 937—947 (2010).
26) Thao N. T., Hung T. M., Lee M. K., Kim J. C., Min B. S., Bae K., *Chem. Pharm. Bull.*, **58**, 121—124 (2010).
27) Hung T. M., Thu C. V., Cuong T. D., Hung N. P., Kwack S. J., Huh J. I., Min B. S., Choi J. S., Lee H. K., Bae K., *J. Nat. Prod.*, **73**, 192—196 (2010).