ORIGINAL ARTICLE

A C G publications

*Rec. Nat. Prod.*7:4 (2013) 325-331

records of natural products

A New Bezoxepine Derivative from *Senecio desfontainei*

Syed Hamid Hussain[1,*], Abdul Latif [2,†], Mohammad Arfan[3], Mumtaz Ali[2], Thomos J Simpson[4], Russell J Cox[4], Farzana Shaheen[5] and Ghias Uddin[3]

[1] *Department of Chemistry, Bacha Khan University, Charsadda, Pakistan*
[2] *Department of Chemistry, University of Malakand, Chakdara Dir (L), Pakistan*
[3] *Institute of Chemical Sciences, University of Peshawar, Peshawar, Pakistan* [4] *School of Chemistry, University of Bristol, Bristol, United Kingdom*
[5] *International Center for Chemical and Biological Sciences, University of Karachi, Pakistan*

(Received April 27, 2013; Revised July 9, 2013; Accepted July 24, 2013)

Abstract: Phytochemical investigation of the aerial parts of *Senecio desfontainei* afforded one new benzoxepine derivative, senecioside (**1**) along with eleven known compounds (**2-12**). Their structures were assigned from ^{1}H and ^{13}C NMR spectra, DEPT and 2D COSY, NOE, HSQC and HMBC NMR experiments. All these compounds were tested for antioxidant activity in the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. Among them, compounds **2**, **3**, **4**, **6** and **9** showed significant antioxidant activity against DPPH radicals.

Keywords: *Senecio desfontainei*; Compositae; Senecioside; Antioxidant activity

1. Introduction

The genus *Senecio* comprises of nearly 3000 species, of which about 37 occur in Pakistan. The importance of genus *Senecio* is based on its botanical, pharmacological and toxicological properties [1,2]. The genus *Senecio* is widely known for its pyrrolizidine alkaloids and some of these reveal a potentially helpful level and spectrum of antitumor activity [3]. Many species of this genus are widely used in Chinese traditional medicine and as folk remedy for inflammatory and infectious diseases [4]. They are also used as blood regulator, for relieving stomach pain, in curing of ulcer, to counteract mountain sickness and are also used as emenagogue, digestive and cough suppressant [5]. In this paper we report the isolation and complete ^{1}H and ^{13}C NMR assignment of the new senecioside (**1**) together with the known compounds ethyl 3,5-di-*O*-caffeoylquinic acid (**2**), ethyl 3,4-di-*O*-caffeoylquinic acid (**3**), quercetin-3-*O*-rutinoside (**4**), isorhamentin 3-*O*-*β*-D-rutinoside (**5**), 3*β*,23-dihydroxy-lup-20(29)-en-28-oic acid-3*β*-caffeate (**6**), 5,7,4'-trihydroxy-3,8-dimethoxyflavone (**7**), 3,5-di-*O*-caffeoylquinic acid (**8**), 4,5-di-*O*-caffeoylquinic acid (**9**), 2-(1,4-dihydroxy cyclohexanyl)-acetic acid (**10**), *p*-hydroxyphenylacetic acid (**11**), *β*-sitosterol-3-*O*-*β*-D-glucopyranoside (**12**) from *S. desfontainei* collected form Kaghan, Khyber Pakhtunkhwa, Pakistan.

2. Materials and Methods

2.1. General

Optical rotations were measured using an ADP 220 polarimeter at 589 nm. IR spectra were recorded using a Perkin-Elmer FTIR instrument for compounds mounted directly on the diamond cell. NMR spectra were recorded on Varian VNMRS-400 and Varian VNMRS-500 spectrometers with

* Corresponding authors: E-mail: hussainjan2@yahoo.com;
† Corresponding authors: E-mail: dralatif@uom.edu.pk

TMS as internal standard and CD_3OD as solvent. HRESIMS were carried out on an APEX IV Fourier-transform Ion Cyclotron resonance instrument (Bruker Daltonics, Coventry, UK) using an Apollo ESI source. The nebulizer and drying gas was nitrogen at 180C. Samples were infused from solution (50% MeOH/DCM) by syringe pump at 100 microlitres per hour. Accurate masses to within 5ppm were obtained using a broad range external calibration using a mixture of PEG polymer grades. Reverse phase HPLC was conducted using a Waters mass-directed autopurification system comprising of a Waters 2767 autosampler, Waters 2545 pump system, a Phenomenex LUNA column (5µ, C_{18}, 100 Å, 10 x 250 mm) equipped with a Phenomenex Security Guard precolumn (Luna C_5 300 Å) eluting at 4 mL/min. Solvent A, HPLC grade H_2O + 0.05% formic acid; Solvent B, HPLC grade CH_3CN + 0.045% formic acid or HPLC grade CH_3OH + 0.045% formic acid. The post-column flow was split (100:1) and the minority flow was made up with MeOH + 0.045% formic acid to 1 mL/min for simultaneous analysis by diode array (Waters 2998), evaporative light scattering (Waters 2424) and ESI mass spectrometry in positive and negative modes (Waters Quatro Micro). Silica gel (220-440 mesh) was used for column chromatography and Merck keiselgel 60 F254 pre-coated silica gel glass plates were used for analytical and preparative TLC. TLCs were visualized in UV light (λ = 254 nm, 366 nm) or by heating after spraying with 15% aqueous sulfurikc acid saturated with ceric sulfate.

2.2. Plant Material

The plant (*S. desfontainei*) was collected from Kaghan, Khyber Pakhtunkhwa, Pakistan, in August 2008, which was identified and authenticated by Prof. Dr. Habib Ahmad, Department of Botany, Hazara University, Pakistan. A voucher specimen (No. 123) has been deposited in the herbarium of the Department of Botany, Hazara University, Mansehra, Pakistan.

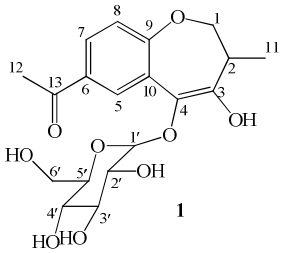
2.3. Extraction and Isolation

The air dried aerial parts of *S. desfontainei* (12 kg) were extracted at room temperature with methanol (MeOH) (4 days x 3). The combined methanolic extract was evaporated under vacuum yield 300 g of residue, which was then partitioned with organic solvent (hexane, DCM, EtOAc and MeOH) by addition of H_2O to obtained *n*-hexane (70 g), dichloromethane (100 g), ethyl acetate (90 g), and aqueous methanolic (40 g) fractions.

The EtOAc fraction (90 g) was chromatographed on silica gel (70-230 mesh, 1500 g, 9 x 120 cm) column, eluting with hexane, ethyl acetate and methanol, in order to increase the polarity of solvent to methanol 100%. As a result twenty major semi pure fractions (SD/E-1A to SD/E-20A) were obtained. These fractions were analyzed by TLC and reunited in 17 new fractions. Fraction SD/E-16A (10 g) was chromatographed on silica gel (70-230 mesh, 300 g, 6 x 100 cm) column, eluting successively with a gradient of *n*-hexane, ethyl acetate and methanol, increasing the polarity to methanol 100%. Seventeen sub-fractions (SD/E-16A-1B to SD/E-16A-17B) were collected. These fractions were analyzed by TLC and reunited in 14 new fractions. SD/E-16A-16B (2 g) was subjected to HPLC mass-directed preparative purification using H_2O- CH_3CN as a mobile phase. 200 μL was injected during successive rounds of a 20 minute program (0 min, 25% B; 0.01 min, 25% B; 15 min, 35% B; 16 min, 95% B; 18.50 min, 95% B; 19 min, 25% B; 20 min, 25% B). Evaporation of the collected fractions under a stream of dry nitrogen yielded 7.8 mg of **1**, 25.5 mg of **4** and 87.5 mg of **5**. Fraction SD/E-12A (5 g) was chromatographed on silica gel (220-440 mesh, 200 g, 4 x 90 cm) column, eluting successively with a gradient of DCM-MeOH (100:00, 98:02, 96:04, 94:06, 90:10, 85:15 & 80:20). As a result, eleven sub-fractions SD/E-12A-1B to SD/E-12A-11B) were collected based on TLC profile. SD/E-12A-8B (825 mg) was subjected to HPLC mass-directed preparative purification using deionized H_2O and MeOH as a mobile phase. 200 μL was injected during successive rounds of a 20 minute program (0 min, 40% B; 13.00 min, 95% B; 17 min, 95% B; 19.50 min, 40% B; 20 min, 40% B). Evaporation of the collected fractions under high vacuum yielded 12 mg of **2** and 10 mg of **3**. SD/E-12A-5B (617 mg) yielded a crystalline compound containing minor impurities. The impurities were washed off with dichloromethane. Transparent needles of the compound **10** (212 mg) were obtained by re-crystallization using a mixture of EtOAc:MeOH (85:15). SD/E-18A (1 g) was subjected to HPLC mass-directed preparative purification using H_2O- MeOH as a mobile phase. 200

μL was injected during successive rounds of a 20 minute program (0 min, 40% B; 13.00 min, 95% B; 17 min, 95% B; 19.50 min, 40% B; 20 min, 40% B), yielded 15.3 mg of **6**. Fraction SD/E-14A (11 g) was chromatographed on silica gel (70-230 mesh, 300 g, 6 x 100 cm) column, eluting successively with a gradient of *n*-hexane, ethyl acetate and methanol, increasing the polarity to methanol 100%. Twenty five sub-fractions SD/D-14A-1B to SD/D-14A-25B were collected based on TLC profile. SD/E-14A-17B (51 mg) was subjected to HPLC mass-directed preparative purification using H_2O-MeOH as a mobile phase. 200 μL (0 min, 25% B; 13.00 min, 95% B; 15 min, 95% B; 17.00 min, 25% B; 20 min, 25% B), furnished 23.7 mg of **7**. SD/E-14A-16B (315 mg) was also subjected to HPLC (injected volume 200 μL; H_2O-MeOH mobile phase; 0 min, 25% B; 13.00 min, 95% B; 15 min, 95% B; 17.00 min, 25% B; 20 min, 25% B), to afford compounds **8** (12 mg) and **9** (15 mg)**.** Fraction SD/E-7A (2 g) was chromatographed on silica gel (70-230 mesh, 300 g, 4 x 90 cm) column, eluting successively with a gradient of *n*-hexane, ethyl acetate and methanol, increasing the polarity to methanol 100%. Twenty sub-fractions (SD/E-7A-1B to SD/E-7A-20B) were collected, which were analyzed by TLC and reunited in 11 major fractions. SD/E-7A-10B (55 mg) was subjected to HPLC (injected volume 200 μL; H_2O-MeOH mobile phase; 0.00 min, 20% B; 0.01 min, 20% B; 6.00 min, 30% B; 13.50 min, 60% B; 14.50 min, 95% B; 18.50 min, 95% B; 19.00 min, 20% B; 20.00 min, 20% B), to obtained **11** (16.6 mg).

The DCM fraction (100 g) was chromatographed on silica gel (70-230 mesh, 1500 g, 9 x 120 cm) column, eluting with *n*-hexane, dichloromethane, ethyl acetate and methanol, increasing the polarity to methanol 100%. Thirteen sub-fractions SD/D-1A to SD/D-13A were collected based on TLC profile. Fraction SD/D-9A (*n*-hexane:EtOAc (2:8)-EtOAc:MeOH (0.3:9.7)) was precipitated which was purified by washing with acetone and methanol, yielded **12** (4 mg).



1

Figure 1. Structure of compound **1**

*2.4. Senecioside (**1**)*:

Brownish yellow solid (7.8 mg); $[\alpha]_D^{20}$ -12.9 (c = 0.15, MeOH); UV λ_{max} (MeOH) = 240 nm; IR(neat) ν_{max} 3336, 1714, 1661 cm^{-1}; ^1H NMR and ^{13}C NMR spectroscopic data, see Table-1; HRESIMS *m/z* 419.1306 $[M + Na]^+$ (calcd for $C_{19}H_{24}O_9Na$, 419.1313).

2.5. Free radical scavenging activity

Assays were performed in flat bottom polystyrene 96-well micro-titer plates. The DPPH radical-scavenging effects were measured using a modified previously established methodology [6,7]. To 100 μL of each sample at different concentrations in MeOH were added 150 μL of DPPH (0.25 mM) in MeOH. The resultant mixture was briefly shaken and maintained at room temperature in the dark for 30 min. The absorbance was measured at 517 nm by using a Spectra Max 190 96-well plate reader and data was processed on SOFTmax pro 4.0 software.

Calculations: The percentage of scavenging of DPPH radicals from a sample at a given concentration can be calculated from the following equation.

$$\% \text{ scavenging} = 100 - \frac{(A_{sample} - A_{sample\ black}) x100}{A_{control} - A_{blank}}$$

3. Results and Discussion

3.1. Structure elucidation

Senecioside (**1**) was obtained as a brownish yellow solid. The IR spectrum showed absorption bands of hydroxyl group (3329 cm^{-1}), double bonds (1661 cm^{-1}) and carbonyl group (1714 cm^{-1}). The molecular formula was deduced as $C_{19}H_{24}O_9$ (eight degrees of unsaturation) from its positive-mode HRESIMS (*m/z* 419.1306, calcd. for $C_{19}H_{24}O_9Na$ $[M + Na]^+$ 419.1313). The ^{1}H-NMR spectrum (Table-1) of **1** exhibited the presence of a trisubstituted coupled aromatic ring (δ_H 7.46, 7.92 and 8.46), a methyl group as a doublet (δ_H 1.32) and acetyl protons as a singlet (δ_H 2.68). The existence of glucose moiety in the molecule was confirmed from the signal of anomeric proton doublet (δ_H 4.75, J = 7.6 Hz) in the ^{1}H-NMR spectrum and six carbon signals of the pyranose ring (δ_C 106.4, 75.1, 77.9, 71.2, 78.0 and 62.4) in the ^{13}C-NMR spectrum (Table-1). The deshielded resonances in the ^{13}C-NMR spectrum [δ_C 152.1 (C-3), 138.2 (C-4) and 200.4 (C-13)] were ascribed to two olefinic carbons and a ketone, respectively. The positions of the methyl, acetyl and glucose moieties were fixed to C-2, C-6 and C-4, respectively, on the basis of HMBC NMR correlations (Figure-2). The β-configuration of the glucose moiety was based on coupling constant of 7.6 Hz for the anomeric proton (H-1′).

Comparison of the NMR spectroscopic data (Table-1) with those of the reported compound [8] indicated that **1** was a benzoxepine glucoside. The only difference between **1** and the known compound [8] was presence of the double bond between C-3 and C-4 in the oxepine ring of **1**. The position of the double bond was decided on the basis of HMBC correlations (Figure-2) of H-11 to C-3 and H-5 to C-4. Thus, the structure of **1** was characterized as a new 1-(4-hydroxy-3-methyl-3,4,5-trihydroxy-6-hydroxymethyl-tetrahydro-2*H*-pyran-2-yl)oxy)-2,3-dihydrobenzo[b]oxepin-7-yl)ethanone (senecioside).

The known compounds (**2-12)** were readily identified from their spectral data and by comparison with reported literature, as ethyl 3,5-di-*O*-caffeoylquinic acid (**2**), ethyl 3,4-di-*O*-caffeoylquinic acid (**3**) [9], quercetin-3-*O*-rutinoside (**4**) [10], isorhamentin 3-*O*-β-D-rutinoside (**5**) [11], 3β,23-dihydroxy-lup-20(29)-en-28-oic acid-3β-caffeate (**6**) [12], 5,7,4′-trihydroxy-3,8-dimethoxyflavone (**7**) [13], 3,5-di-*O*-caffeoylquinic acid (**8**) [14], 4,5-di-*O*-caffeoylquinic acid (**9**) [15], 2-(1,4-dihydroxy cyclohexanyl)-acetic acid (**10**) [16], *p*-hydroxyphenylacetic acid (**11**) [17], β-sitosterol-3-*O*-β-D-glucopyranoside (**12**) [18].

3.2. Antioxidant activity

The isolated compounds (**1-11**) were tested for their anti-oxidative properties in the DPPH radical scavenging assay. As a result (Table 2), compounds **2**, **3**, **4**, **6** and **9** have significant radical-scavenging effect comparable to that of ascorbic acid, that is, almost complete scavenging at a concentration of 250-500 μM (93 – 96% scavenging for compounds **2**, **3**, **4**, **6** and **9** as compared to 98.2% scavenging for ascorbic acid) and > 46% scavenging was still observed at 5 μM while compounds **1**, **5**, **7**, **8**, **10** and **11** showed activities less than ascorbic acid at higher concentration but still comparable to that of ascorbic acid at lower concentrations (5-30 μM). Compound **12** was not tested in the current study due to insufficient quantity.

Table 1. ^{13}C and ^{1}H-NMR spectroscopic data for Senecioside (**1**) in CD_3OD, 125 MHz for ^{13}C and 500 MHz for ^{1}H.

C/H	δ_C ppm	DEPT	δ_H ppm (*J*, Hz)
1	65.9	CH_2	3.75 m 3.82 m
2	35.1	CH	3.53 m
3	152.1	C	-
4	138.2	C	-
5	122.2	CH	8.46 dd (1.9/0.6)
6	133.5	C	-
7	125.4	CH	7.92 dd (8.7/1.9)
8	112.3	CH	7.46 dd (8.7/0.6)
9	156.3	C	-
10	125.5	C	-
11	15.2	CH_3	1.32 d (7.0)
12	26.9	CH_3	2.68 s
13	200.4	C	-
1′	106.4	CH	4.75 d (7.6)
2′	75.1	CH	3.51 m
3′	77.9	CH	3.45 m
4′	71.2	CH	3.46 m
5′	78.0	CH	3.30 m
6′	62.4	CH_2	3.77 m 3.89 m

Table 2. Antioxidant activity of compounds **1-11** showing % scavenging at different concentrations and 50% inhibitory concentrations (IC_{50}) in the DPPH radical scavenging assay[a].

Compound	**% Scavenging[b]**							
	5[c]	**15**	**30**	**60**	**125**	**250**	**500**	**IC_{50}**
1	49.2 ± 1.6	51.6 ± 3.1	52.6 ± 2.1	53.5 ± 2.1	57.3 ± 2.1	61.3 ± 2.9	71.4 ± 1.5	13.8
2	52.9 ± 2.8	57.5 ± 3.0	66.2 ± 4.1	77.7 ± 2.1	95.5 ± 1.0	95.5 ± 2.1	96.1 ± 0.7	N.C [f]
3	51.2 ± 2.7	57.0 ± 3.5	64.3 ± 2.0	78.4 ± 2.1	93.9 ± 1.8	94.1 ± 1.8	95.3 ± 1.0	N.C
4	48.2 ± 1.7	51.3 ± 1.7	57.3 ± 2.1	66.4 ± 2.9	83.5 ± 2.2	93.2 ± 2.7	95.3 ± 1.1	8.3
5	46.4 ± 2.9	46.4 ± 2.1	48.2 ± 1.7	49.2 ± 0.4	51.3 ± 2.7	59.4 ± 3.1	77.5 ± 2.1	75.5
6	46.5 ± 0.8	51.5 ± 2.0	54.6 ± 3.1	62.3 ± 1.9	75.4 ± 1.1	96.1 ± 0.7	96.4 ± 1.6	7.7
7	46.3 ± 1.6	46.4 ± 1.2	47.4 ± 2.8	49.9 ± 1.7	47.8 ± 3.0	52.0 ± 1.9	56.6 ± 2.1	166.5
8	46.1 ± 2.4	46.6 ± 0.9	46.6 ± 2.1	48.3 ± 1.7	52.3 ± 3.9	54.2 ± 3.1	61.6 ± 2.9	114.6
9	50.3 ± 2.7	54.3 ± 3.2	65.3 ± 2.2	78.6 ± 3.0	96.4 ± 1.2	96.6 ± 0.9	96.6 ± 0.8	N.C
10	43.6 ± 1.7	43.4 ± 1.8	43.7 ± 1.2	44.4 ± 2.6	44.5 ± 2.1	44.8 ± 3.1	45.4 ± 1.3	N.C
11	46.1 ± 2.8	46.3 ± 1.9	47.3 ± 3.1	47.8 ± 1.2	48.4 ± 1.4	49.1 ± 2.8	49.4 ± 1.9	N.C
Asorbic acid[d]	43.0 ± 0.8	45.7 ± 1.1	46.7 ± 2.4	57.5 ± 0.9	73.6 ± 2.2	98.1 ± 0.5	98.2 ± 0.7	33.8 ± 0.9

[a] Values are mean ± SD (n = 3).
[b] Scavenging % = 100-(A sample[e] x 100/A control[e]).
[c] Concentrations in μmol/L.
[d] Ascorbic acid used as positive control.
[e] Absorbance of sample and control measured at 517 nm.
[f] N.C = Not calculated

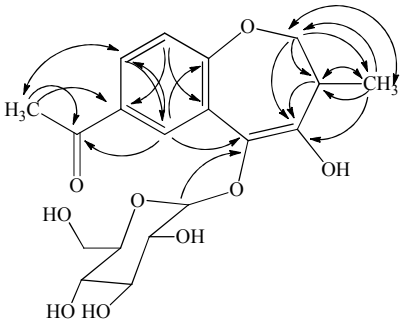


Figure 2. Selected HMBC and NOESY correlations of senecioside (**1**)

Acknowledgments

The authors acknowledge the financial support provided by the Higher Education Commission of Pakistan. We also acknowledge the School of Chemistry, University of Bristol, UK, for providing bench space in their labs and access to spectroscopic instruments.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

References

[1] D. N. Shargh, S. Raftari and F. Deyhimi (2008). Analysis of the essential oil of *Senecio vernalis* Waldst. & Kit. from Iran, *Flavour Fragr. J.* **23,** 357-359.

[2] R. R. Stewart (1972). *Flora of West Pakistan*, ed: E. Nasir, S. I. Ali, Karachi, Fakhri Printing Press. p. 781.

[3] G. R. Pettit, J. J. Einck, P. Brown, T. B. Harvey, R. H. Ode and C. P. Pase (1980). Antineoplastic Agents, *J. Nat. Prod*. **43,** 609-616.

[4] J. Shi, L. Yang, C. H. Wang, G. Chou and Z. Wang (2007). A new lactone from *Senecio scandens*, *Biochem, Syst. Ecol.* **35,** 901-904.

[5] W. Toma, J. R. Trigo, A. C. B. Paula and A. R. S. M. Brito (2004). Preventive activity of pyrrolizidine alkaloids from *Senecio brasiliensis* (Asteraceae) on gastric and duodenal induced ulcer on mice and rats, *J. Ethnopharmacol*. **95,** 345-351.

[6] M. S. Blois (1958). Antioxidant determinations by the use of a stable free radical, *Nature*. **26,** 1199-1200.

[7] A. Yildirim, A. Mavi and A. A. Kara (2003). Antioxidant and antimicrobial activities of *Polygonum cognatumi* Meissn extracts, *J. Sci. Food Agr*. **83,** 64-69.

[8] K. Nishikawa, T. Munechika, M. Ibaraki, T. Fujioka, H. Fujii, K. Mihashi, K. Shimomura and K. Ishimaru (2000). New benzoxepin glucoside from transformed root cultures of *Helianthus annuus* L, *Nat. Med*. **54,** 93-96.

[9] Y. Wang, M. Hamburger, J. Gueho and K. Hostettmann (1992). Cyclohexanecarboxylic-acid derivatives from *Psiadia trinervia*, *Helv. Chim. Acta*. **75,** 269-275.

[10] K. N. Vinod, Puttaswamy, K. N. N. Gowda and R. Sudhakar (2011). Isolation of colour components from flowers of *Tabebuia argentea*: kinetic and adsorption studies on silk yarn, *Color. Technol*. **127,** 205-209.

[11] H. Liu, Y. Mou, J. Zhao, J. Wang, L. Zhou, M. Wang, D. Wang, J. Han, Z. Yu and F. Yang (2010). Flavonoids from *Halostachys caspica* and their antimicrobial and antioxidant activities, *Molecules*. **15,** 7933-7945.

[12] H. Pan, L.N. Lundgren and R. Andersson (1994). Triterpene caffeates from bark of *Betula pubescens*, *Phytochemistry*. **37,** 795-799.

[13] E. Wollenweber, V. H. Dietz, G. Schilling, J. F. Bonvin and D. M. Smith (1985). Flavonoids from chemotypes of the goldback fern, *Pityrogramma triangularis*, *Phytochemistry*. **24,** 965-971.

[14] C. Wu, F. Chen, X. Wang, Y. Wu, M. Dong, G. He, R. D. Galyean, L. He and G. Huang (2007). Identification of antioxidant phenolic compounds in feverfew (*Tanacetum parthenium*) by HPLC-ESI-MS/MS and NMR, *Phytochem. Analysis*. **18,** 401-410.

[15] X. F. Zhu, H. X. Zhang and R. Lo (2009). Three di-*O*-caffeoylquinic acid derivatives from the heads of *Cynara scolymus* L. *Nat. Prod. Res.* **23,** 527-532.

[16] G. G. Zhang, S. J. Song, J. Ren and S. X. Xu (2002). A new compound from *Forsythia suspense* (Thunb.) Vahl with antiviral effect on RSV, *J. Herb. Pharmacother*. **2,** 35-40.

[17] G. B. S. Reddy and N. Dhananjaya (2000). Chemical investigation of *Mycale mytilorum* and a study on a toxicity and antidiabetic activity of 5-octadecylpyrrole-2-carboxaldehyde, *Bioorg. Med. Chem.* **8,** 27-36.

[18] S. Faizi, M. Ali, R. Saleem, Irfanullah and S. Bibi (2001). Complete ^{1}H and ^{13}C NMR assignments of stigma-5-en-3-*O*-*β*-glucoside and its acetyl derivative, *Magn. Reson. Chem.* **39,** 399-405.

A C G
publications

© 2013 Reproduction is free for scientific studies