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Phenolic and other constituents of fresh water fern *Salvinia molesta*

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Abstract

Two glycosides, 6′-*O*-(3,4-dihydroxy benzoyl)-β-D-glucopyranosyl ester (**1**), and 4-*O*-β-D-glucopyranoside-3-hydroxy methyl benzoate (**2**), along with five known compounds methyl benzoate (**3**), hypogallic acid (**4**), caffeic acid (**5**), paeoniflorin (**6**) and pikuroside (**7**) were isolated for the first time from a fresh water fern *Salvinia molesta* D.S. Mitch. These compounds showed a potent antioxidant radical scavenging activity in a non-physiological assay. Their structures were determined by NMR spectroscopic and CID mass spectrometry techniques.

Keywords: *Salvinia molesta* D.S. Mitch; Salvinaceae; 6′-*O*-(3,4-dihydroxy benzoyl)-β-D-glucopyranosyl ester; 4-*O*-β-D-glucopyranoside-3-hydroxy methyl benzoate; DPPH radical scavenging assay; Collision induced dissociation (CID) mass spectrometry

1. Introduction

Ferns of the genus *Salvinia* (Salvinaceae) comprises of ten species; majority of which grow in freshwater bodies of tropical and subtropical regions, mainly in Africa and South America (Rozentsvet et al., 2005). *Salvinia molesta* is a free floating fern which is native to south eastern Brazil. In Pakistan, the fresh water lakes and mangrove areas of Sindh are adversely affected with this invasive fern, resulting into major economic losses and threat to the environment. *S. molesta* is regarded as a world's worst weed (Streever and Streever, 1999), as it doubles every 52 h. However, the ability of *Salvinia* species to bioaccumulate certain metals make it potentially useful for waste management and effluent treatment (Siriwan et al., 2006). The phytochemical investigation on the *S. natans* showed that it consists of 96% of amino compounds, such as γ-amino butyric acid, asparginine and glutamine (Lahdesmaki, 1968). The understanding of chemistry of *Salvinia* plants can help controlling their invasive growth, and promote their utilization for useful purposes.

In continuations of our studies on terrestrial and marine plants of Pakistan, we carried out a phytochemical study on *S. molesta* for the first time. This study yielded various classes of natural products, isolated from EtOAc and *n*-BuOH extracts of *S. molesta*, and explored their DPPH radical scavenging activity (Tables 2 and 3). The structures of the new glycosides **1** and **2** were deduced mainly on the basis of mass spectrometry, employing ESI-QTOF source and NMR spectroscopic techniques.

2. Results and discussion

Salvinia molesta D.S. Mitch. whole plant were collected from Haliji Lake (Sindh, Pakistan). Dried plant material was powdered and extracted with 80% aqueous methanol. The MeOH extract was partitioned into hexane, EtOAc, *n*-BuOH and water. Compounds **1**–**2** and **6**–**7** were purified from the BuOH extract by the combinations of Diaion HP-20, and Sephadex LH-20 column chromatography and preparative recycling HPLC, whereas compounds **3**–**5**

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Table 1
^{1}H and ^{13}C NMR chemical shift data for compounds **1** and **2** in CD_3OD

Assignments		Compound **1**		Compound **2**		
Aglycon		δ H (*J* in Hz)	δ C		δ H (*J* in Hz)	δ C
	1	–	122.7 / 122.6	1	–	125.7
	2	7.42 *d* (1.7)	117.5	2	7.47 *d* (2.0)	122.7
	3	–	146.1	3	–	148.7
	4	–	151.9	4	–	150.8
	5	6.79 *d* (8.7)	115.8	5	7.21 *d* (8.2)	117.1
	6	7.44 *dd* (8.3, 1.7)	123.7	6	7.45 *dd* (10.4, 2.0)	118.2
	7	–	168.3 / 168.2		–	168.7
				7-OCH_3	3.84 *br s*	52.4
Glucosyl	1′	5.17 *d* (3.5) / 4.48 *d* (8.7)	94.0 / 98.3	1′	4.89 *d* (7.8)	103.1
	2′	3.55 *dd* (7.5, 3.3) / 3.45 *dd* (7.5, 3.7)	74.8 / 73.8	2′	3.38 *t* (7.2)	74.7
	3′	3.35 *t* (7.7) / 3.3 *d* (7.9)	72.0 / 77.9	3′	3.38 *t* (7.8)	77.5
	4′	4.20 *t* (8.0) / 4.20 *t* (8.0)	76.2 / 70.9	4′	3.44 *t* (7.3)	71.3
	5′	4.47 *t* (7.3) / 3.45 *dd* (7.5, 1.9)	71.8 / 73.8	5′	3.44 *ddd* (7.3, 4.3, 2.7)	78.4
	6′	4.35 *dd* (11.9, 5.4) 4.59 *dd* (11.9, 5.4) / 4.56 *br d* (11.6) 4.49 *dd* (11.6, 5.8)	65.0 / 64.9	6′	3.72 *dd* (12.0, 5.3), 3.88 *dd* (12.1, 2.0)	62.4

^{1}H NMR at 400 MHz, ^{13}C NMR at 100 MHz.

Table 2
Antioxidant activity (DPPH radical scavenging assay) of various extracts of *S. molesta*

Sample	RSA[a](%)
Methanolic extract	86.88
Ethyl acetate extract	88.42
Butanolic extract	83.69

[a] RSA = % radical scavenging assay.

Table 3
Antioxidant activity (DPPH radical scavenging assay) of compounds **1**–**7**

Compounds	RSA[a] (%)	IC_{50} (μM) ± SEM[a]
1	79.07	350.80 ± 12.82
2	–	–
3	–	–
4	91.47921	55.35 ± 2.72
5	87.42203	302.07 ± 17.98
6	76.85654	330.80 ± 17.87
7	–	–
Propyl gallate	90.01	30.0 ± 2.7

Results are reported in ± standard error of mean of three experiments.
[a] All the samples were screened at 0.5 mM concentration.

were isolated from EtOAc extracts of *S. molesta*. Among all the isolates, compounds **1** and **2** were identified as new natural products, whereas compounds **3**–**7** were found to be already known metabolites. Significant DPPH radical scavenging activity of crude MeOH extract could be attributed to the compounds **1**, **4**, **5** and **6** (Table 2).

2.1. Characterization of the isolated compounds

Compound **1** was obtained from the BuOH fraction as a brown gum. The molecular formula of compound **1** ($C_{13}H_{17}O_9$) was deduced from ESI-QTOF-MS (+ve) (*m/z* 317.2879, calc. 317.2872). The collision induced dissociation (CID)-MS (40 eV) was found to be especially useful in compound **1** as it provided information regarding aglycon and glycon parts. Fragment ions appeared in CID-MS at *m/z* 155 (aglycon part), resulted from the loss of 162 a.m.u. from the $[M+1]^+$, which indicated that the glycon part was consist of hexose sugar. The UV spectrum of **1** showed an absorption maximum at 260 nm, characteristic of an aromatic ring with electron donating substituents (Scott, 1964). The IR spectrum showed significant absorptions at 3377, 1701, and 1600 cm^{-1}, indicating the presence of a hydroxyl group, carbonyl ester and an aromatic ring, respectively (Pavia et al., 1979). The ^{1}H NMR spectrum exhibited three aromatic protons at δ_H 7.42 (*d*, $J_{2,6}$ = 1.7 Hz), 7.44 (*dd*, $J_{6,5}$ = 8.3 Hz, $J_{6,2}$ = 1.7 Hz) and 6.79 (*d*, $J_{5,6}$ = 8.7 Hz), suggestive of a 3,4-disubstituted benzoyl moiety. Interestingly the ^{13}C NMR spectrum of **1** showed splitting of most of the carbon signals (Table 1). Similarly two sets of signals were observed for glucose protons. This indicated that compound exists as a diasteromeric mixture of α and β-D-glucopyranosides (Ding et al., 1999). The downfield value of methylene protons (2H-6′) (δ_H 4.49 and 4.56 for β and δ_H 4.35 and 4.40 for α-glucopyranoside) supported that a attachment 3-dihydroxybenzoyloxy group was attached at this position. This was also confirmed by the HMBC experiment in which the oxy-methylene protons (2H-6′) showed cross peaks with benzoyl carbon resonated at δ_C 168.2 (C-7) (see Fig. 2). The proposed structure of the **1** was supported by COSY, HMQC, and HMBC experiments, and was shown to be an isomeric mixture of disubstituted benzoyl α- and β-glycosides. Therefore, from the above evidences, the structure of **1** was deduced as the new 6′-*O*-(3,4-dihydroxy benzoyl)-β/α-D-glucopyranonsyl ester (see Fig. 1).

Compound **2** was isolated as a colorless gum and found to be a glucoside of hypogallic methyl ester. The ESI-QTOF-MS showed $[M+H]^+$ at *m/z* 331.2872, corresponding to the formula $C_{14}H_{19}O_9$ (calc., 331.2865). The

MS–MS experiment (40 eV) also showed an $[M + H]^+$ at m/z 331 and base peaks at m/z 169, due to the loss of a hexose moiety. The aromatic region in the ^1H NMR spectrum of compound **2** was similar to that of **1**, indicating a 3,4-disubstituted benzoyl group [δ_H 7.47 (*d*, $J_{2,6} = 2.0$ Hz), 7.21 (*d*, $J_{5,6} = 8.2$ Hz), and 7.45 (*dd*, $J_{5,6} = 10.4$ Hz, $J_{6,2} = 2.0$ Hz)]. The main difference being the presence of a methyl ester [δ_H 3.84 (*s*)] and the absence of signals for glucopyranoside moiety (Table 1). The ^1H NMR chemical shift (δ_H 4.89) and coupling constant ($J_{1',4} = 7.8$ Hz) of anomeric signal indicated that the compound **2** contained a β-D-glucopyranoside (Table 1). The ^{13}C NMR spectrum showed signals corresponding to a carbonyl carbon (δ_C 168.7) (See Fig. 2), three aromatic methine carbons (δ_C 122.7, 117.1, 118.2), three aromatic quaternary carbons (δ_C 125.7, 148.7, 150.8), and a methyl ester (δ_C 52.4), along with characteristic six sugar carbon signals (δ_C 62.4, 78.4, 71.3, 77.5, 74.1, 103.1) for β-D-glucopyranoside moiety (Table 1). The connectivity of sugar unit was deduced from the HMBC cross peaks between anomeric H-1′ (δ_H 4.89), and C-4 (δ_C 150.4). The glucose moiety was placed at C-4 based on the NOE between anomeric H-1′ (δ_H 4.89) and H-5 (δ_H 7.21). The signals at δ_H 7.45 (H-6) and 7.47 (H-2) showed HMBC correlations with the carbonyl methyl ester (δ_C 168.7) (see Fig. 2), suggesting that the ester group was located at C-1. These spectral data, along with the MS–MS experiments, indicated compound 2 to be 4-*O*-β-D-glucopyranoside-3-hydroxy methyl benzoate.

The known compounds, isolated from *S. molesta*, were identified as methyl benzoate (**3**) (Cook, 1989), hypogallic acid (**4**) (Scott, 1972), caffeic acid (**5**) (Kelley et al., 1976), paeoniflorin (**6**) (Lin et al., 1996), and pikuroside (**7**) (Harput et al., 2002). The presence of these diverse secondary metabolites in *S. molesta* is an interesting finding. Which has also been reported from few other species of genus *Picrorhiza* (Jia et al., 1999). The structures of **3**–**7** compounds were identified by comparing the EI MS and ^1H and ^{13}C NMR data with the literature. Isolation of paeoniflorin (**6**) and pikuroside (**7**) are of particular interest as these compounds belong to iridoid and terpenoid classes, respectively. Compound **6** is reported to have lipooxygenase inhibitory activity with an IC_{50} value of 56.9 μM (Riaz et al., 2003). On the other hand, compound **7** is reported to possess a moderate anti-inflammatory activity in mouse ear edema assay (Jia et al., 1999).

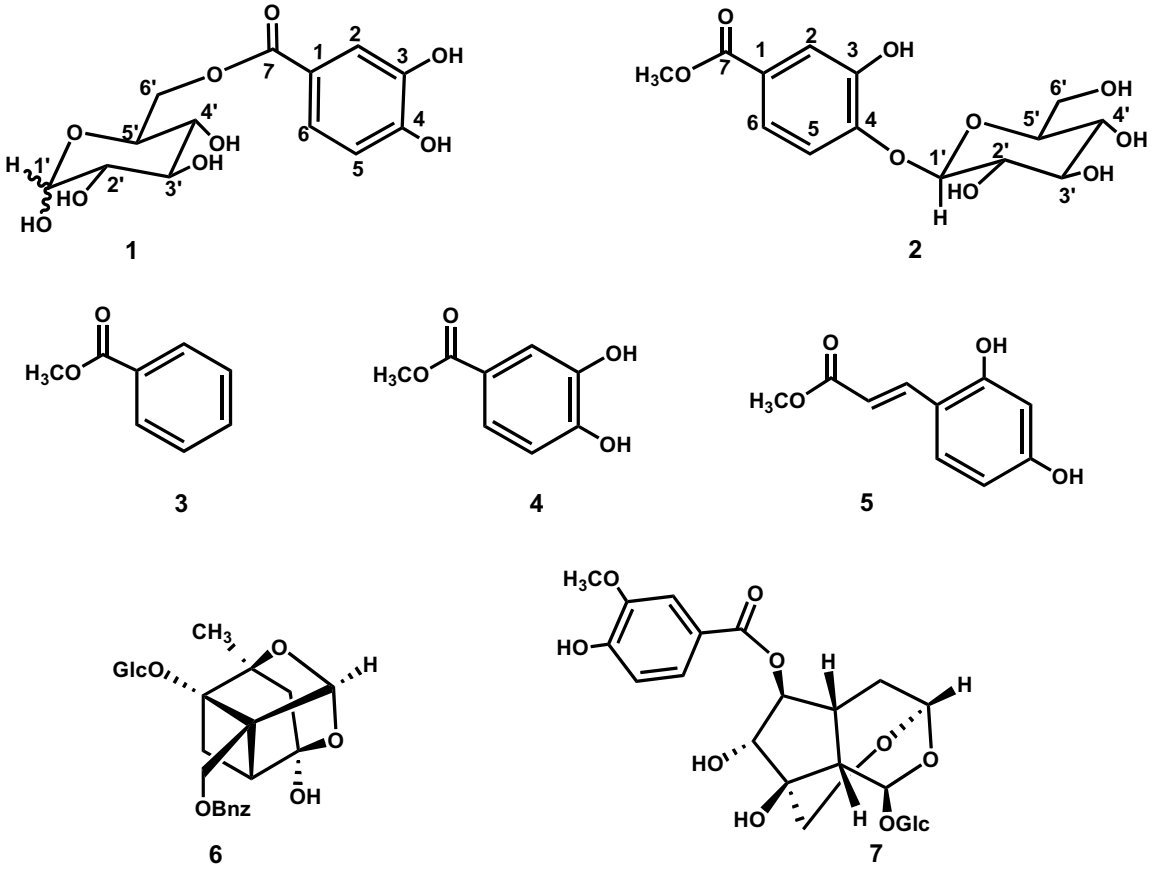

Fig. 1. Structure of compounds **1**–**7** isolated from *Salvinia molesta*.

2.2. Antioxidant activity

Free radicals play an important role in the onset of diseases. Non-physiological DPPH radical scavenging assay enables facile screening for antioxidants (Yamaguchi et al., 2006). During the current study, compounds **1**, **4**–**6** showed potent radical scavenging activity in DPPH assay (Table 3). Among these, hypogallic acid (**4**) was found to have a significant antioxidant potential with an $IC_{50} = 55.35 \pm 2.72$ μM, comparable to the standard propyl gallate ($IC_{50} = 30.0 \pm 2.7$ μM). Compound **5**, a cinnamate derivative, was also found to possess a strong antioxidant activity. The RSA value of **4** (91.4%) was in accord with those reported in the literature (98.2%) for hypogllic acid (Park et al., 2003).

3. Experimental

3.1. General experimental method

UV Spectra were measured on a Shimadzu UV240 spectrophotometer in MeOH solutions and presented as λ_{max} nm (log ε); IR spectra were recorded as KBr discs on a JASCO A-302 spectrophotometer in cm^{-1}. The melting points were recorded on a YANACO apparatus. Optical rotations were measured on a digital polarimeter JASCO DIP-360 in methanol. The ^1H and ^{13}C NMR, 1D TOCSY, HMQC, and HMBC spectra were recorded on a Bruker AV-400 spectrometer operating at 400 (^1H) and 100 (^{13}C) MHz in CD_3OD. The chemical shifts values were reported in δ (ppm), referenced with respect to the residual solvent signal of CD_3OD and coupling constants (*J*) were measured in Hz. ESI mass spectra were recorded on QSTAR XL (Applied Biosystem). For the collision induced dissociation (CID) mass spectra, the collision energy was kept at 40 eV with nitrogen as collision gas. The capillary voltage was maintained between 5 and 5.5 kV. TLC was performed on pre-coated silica gel plates (DC-Alugram 60 UV_{254} of E. Merck), and the spots were observed first under UV (254 nm) and then stained with cerium (IV) sulfate spray reagent and heated until coloration developed. Diaion HP-20 (Mitsubishi Chem. Ind., Tokyo, Japan), ODS C-18 (63–212 μm, Wako Pure Chemical Industries Ltd., Japan), polyamide-6 DF (Riedel-De Haen AG) Sephadex LH-20 (SAFC, Sweden, 25–100 μm) and silica gel (E. Merck, 230–400 μm mesh) were used. Recycling preparative HPLC (RP-HPLC) separation was performed on a JAI LC-908W (Japan Analytical Industry) with columns of YMC ODS H-80.

3.2. Plant material

The whole plant of *S. molesta* D.S. Mitch were collected from the Haliji Lake, District Thatta, Sindh Pakistan, in February 2003 and identified by Prof. Dr. Surraiya Khatoon, Taxonomist at the Department of Botany, University of Karachi.

3.3. Extraction and isolation

Dried and crushed parts (20 kg) of *S. molesta* D.S. Mitch were macerated in 80% methanol–water (150 L × 3) at room temperature. The crude methanolic extract was filtered and concentrated under reduced pressure. The concentrated extract (800 g) was dissolved in water (1 L) and successively extracted with hexane (3 L × 3), diethyl ether (3 L × 4), ethyl acetate (3 L × 3), and *n*-butanol (3 L × 4), yielding hexane (220 g), ether (180 g), ethyl acetate (25.4 g), *n*-BuOH (19.6 g) and water extracts (355 g). The butanolic extract (19.6 g) was fractionated by column chromatography on Diaion HP-20 with the mixtures of H_2O–MeOH to obtain various sub-fractions. The Sub-fraction eluted with H_2O–MeOH (1:1), was subjected to polyamide chromatography using gradients of $CHCl_3$–MeOH (100% $CHCl_3$, 10% MeOH–$CHCl_3$, 20% MeOH–$CHCl_3$, 30% MeOH–$CHCl_3$, 50% MeOH–$CHCl_3$, 100% MeOH) to afford ten fractions (P_1–P_{10}). Fractions P_5–P_9 (10 g) were combined and subjected to Sephadex LH-20 column chromatography and eluted with H_2O–MeOH in a gradient manner and afforded four sub-fractions. The final purification was carried out by reverse phase recycling HPLC, with eluting solvent H_2O–MeOH (1:1) (L-80 column). This yielded new compounds **1** (10 mg, 5×10^{-5}%), and 2 (16 mg, 8×10^{-5}%), and two unresolved fractions A and B. These fractions were reloaded on RPHPLC with modified separation conditions using H-80 column, H_2O–MeOH (1:1) which resulted in isolation of two known compounds **6** (15 mg, 7.5×10^{-5}%), and **7** (5 mg, 2.5×10^{-5}%).

The EtOAc fraction (25.4 g) was loaded on a silica gel column and eluted with EtOAc–$CHCl_3$ (10–90%) in a gradient manner. This yielded four sub-fractions A–D. Sub-fraction A was repeatedly chromatographed on silica gel by using hexane/acetone (75:25) in an isocratic manner to afford to known compounds **3** (12 mg, 6×10^{-5}%) and **4** (17 mg, 8.5×10^{-5}%). Purification of compound **5** (19 mg, 4.5×10^{-5}%) was carried on *C.C.* of silica gel column of sub-fraction C with mobile phase CH_3Cl–MeOH (90:10) in an isocratic system.

3.3.1. 6′-O-(3,4-dihydroxy benzoyl)-β-D-glucopyranosyl ester) (1)

Yellowish gum; $C_{13}H_{16}O_9$, $[\alpha]_D^{25}$: −31.4 (*c* 3.5, MeOH); UV (MeOH) λ_{max} nm (log ε): 293 (2.75), 252 (3.04), 214 (3.33), 206 (3.35); IR (KBr) ν_{max} cm^{-1}: 3381 (O–H), 1705 (C=O), 1600 (aromatic); For ^1H and ^{13}C NMR spectroscopic data, (see Table 1); ESI-QTOF (+ve) *m*/*z* (%): 317.2879 (calc. for $C_{13}H_{17}O_9$, 317.2872), ESI-QTOF MS/MS on *m*/*z* 317.28 (40 eV) *m*/*z* (%): 317 (10), 299 (61), 281 (17), 219 (12), 155 (100), 137 (10).

3.3.2. 4-O-β-D-glucopyranoside-3-hydroxy methyl benzoate (2)

Yellowish gum; $C_{14}H_{18}O_9$ $[\alpha]_D^{25}$: 1.85 (*c* 8.5, MeOH); UV (MeOH) λ_{max} nm (log ε): 261 (3.45), 221 (3.77), 203 (4.05),

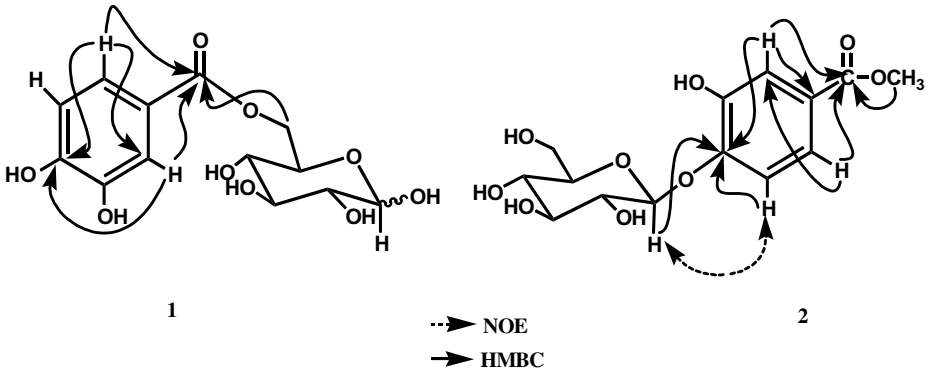

Fig. 2. Key 2D NMR correlations in compounds **1** and **2**.

189 (4.07); IR (KBr) ν_{max} cm^{-1}: 3385 (O–H), 1712 (C=O ester), and 1660 (aromatic C=C); For ^{1}H and ^{13}C NMR see Table 1; ESI-QTOF (+ve) *m/z* (%): 331.2872 (calc. for $C_{14}H_{19}O_9$, 331.2865); QTOF MS/MS on *m/z* 313.28 (40 eV) *m/z* (%): 313 (31), 261 (14), 239 (17), 169 (100), 149 (60).

3.4. Acidic hydrolysis of compounds *1* and *2*

Compounds **1** (2 mg) and **2** (3 mg) were dissolved in 5% H_2SO_4/H_2O (2 mL) and refluxed overnight at room temperature. The solution was extracted with ethyl acetate (1 mL × 3) to afford aglycons. The aqueous layer was neutralized and glucose was identified by co-TLC with authentic samples by using the standard method (Bedir and khan, 2000).

3.5. DPPH radical scavenging activity of compounds *1–7* against

Compounds **1–7** were allowed to react with stable free radical, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), for half an hour at 37 °C. The concentration of DPPH was kept as 300 μM. The test samples were dissolved in DMSO, while the DPPH solution was prepared in ethanol. After incubation, decrease in absorption was measured at 515 nm using multi-plate reader (Spectra MAX-340, Molecular Devices, CA, USA.). Percent radical scavenging activity by samples was determined in comparison with a DMSO treated control group.

% Radical scavenging activity was calculated by using the following formula:

$$\%\ \mathrm{RSA} = 100 - [(\mathrm{OD\ test\ compound}/\mathrm{OD\ control}) \times 100]$$

(Molyneux, 2004)

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References

Bedir, E., Khan, I.A., 2000. New steroidal glycosides from the fruits of *Tribulus terrestris*. J. Nat. Prod. 63, 1699–1701.

Cook, I.B., 1989. Caveat regarding the use of substituent parameters in statistical analyses of molecular properties. II Case study: carbon-13 NMR of 2-substituted pyridine and monosubstituted benzenes. Aust. J. Chem. 42, 1493–1518.

Ding, H.Y., Wu, Y.C., Lin, H.C., Chan, Y.Y., Wu, P.L., Wu, T.S., 1999. Glycosides from *Paeonia suffruticosa*. Chem. Pharm. Bull. 47, 652–655.

Harput, U.S., Saracoglu, I., Nagatsu, A., Ogihara, Y., 2002. Iridoid glucosides from *Vernica hederifolia*. Chem. Pharm. Bull. 50, 1106–1108.

Jia, Q., Hong, M-F., Minter, D., 1999. Pikuroside: a novel iridoid from *Picrorhiza kurroa*. J. Nat. Prod. 62, 901–903.

Kelley, C.J., Harruff, R.C., Carmack, M., 1976. The polyphenolic acids of *Lithospermum ruderale* II. Carbon-13 nuclear magnetic resonance of lithospermic and rosmarinic acids. J. Org. Chem. 41, 449.

Lahdesmaki, P., 1968. Free amino acids in the leaves of *Salvinia natans* and *Azolla filiculoides* grown in light and dark. Physiol. Plantarum. 21, 1097–1103.

Lin, H.C., Ding, H.Y., Wu, T.S., Wu, P.L., 1996. Monoterpene glycosides from *Paeonia suffruticosa*. Phytochemistry 41, 237–242.

Molyneux, P., 2004. The use of the stable free radical diphenylpicrylhydrazyl (DDPH) for estimating antioxidant activity. J. Sci. Technol. 26, 211–219.

Park, Y.K., Lee, H.J., Lee, S.S., Choi, D.H., 2003. Chemical components and antioxidative activity of the leaves of *Sopbora japonica*. Trend. Agric. Life Sci. 31, 43–44.

Pavia, D.L., Lampan, G.M., Kriz, G.S., 1979. Introduction to Spectroscopy. Saunders WB Co, p. 41.

Riaz, N., Anees, I., Malik, A., Ahmed, Z., Aziz-ur-Rehman, Muhammad, P., Nawaz, S.A., Choudhary, M.I., 2003. Paeonins A and B, lipoxygenase inhibiting monoterpene galactosides from *Paeonia emodi*. Chem. Pharm. Bull. 51, 252–254.

Rozentsvet, O.A., Rezanka, T., Bosenko, E.S., Uzhametskaya, E.A., Dembitskii, V.M., 2005. Fatty acids, phospholipids, and the betaine lipid DGTS from the aquatic fern *Salvinia natans*. Chem. Nat. Comp. 41, 487–490.

Scott, A.I., 1964. Interpretation of UV Spectra of Natural Products. Pergamon Press, Oxford, p. 82.

Scott, K.N., 1972. Carbon-13 nuclear magnetic resonance of biologically important aromatic acids. I. Chemical shifts of benzoic acid and derivatives. J. Am. Chem. Soc. 92, 8564–8568.

Streever, W., Streever, B., 1999. An International Perspective on Wetland Rehabitation. Kluwer Academic Publishers, The Netherlands, p. 159.

Siriwan, P., Maleeya, K., Prayad, P., Suchart, U., 2006. Toxicity and bioaccumulation of cadmium and lead in *Salvinia cucullata*. J. Environ. Biol. 27, 645–652.

Yamaguchi, L.F., Lago, J.H.G., Tanizaki, T.M., Mascio, P.D., Kato, M.J., 2006. Antioxidant activity of prenylated hydroquinone and benzoic acid derivatives from *Piper crassinervium* Kunth. Photochemistry 67, 1838–1843.