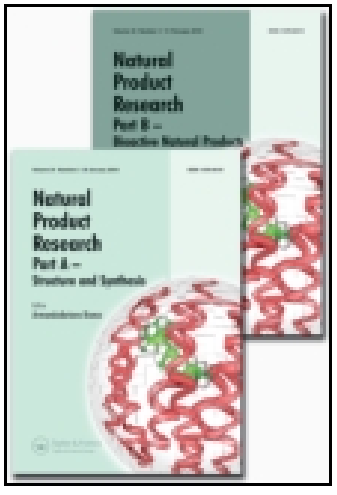

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Flavonoids from *Lupinus texensis* and their free radical scavenging activity

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Seventeen flavonoids including one new compound were isolated from Texas bluebonnet (*Lupinus texensis*), the state flower of Texas. Their structures were determined by extensive nuclear magnetic resonance and high-resolution electrospray ionization mass spectrometry analyses. High-performance liquid chromatography analytic method for simultaneous determination of the 17 compounds was established and validated. Eleven isolated flavonoids were first evaluated for their free radical scavenging activity using α,α-diphenyl-β-picrylhydrazyl scavenging assay and they showed activity with EC_{50} 48.6–172.5 $\mu g\,mL^{-1}$.

Keywords: *Lupinus texensis*; Fabaceae; flavonoids; HPLC analysis; free radical scavenging activity

1. Introduction

The genus *Lupinus* L. (Fabaceae) has over 400 species worldwide. It is well known that alkaloids (Reinhard, Rupp, Sager, Streule, & Zollern, 2006), flavonoids (García-López, Kachlicki, Zamora-Natera, Ruiz-Moreno, & Stobiecki, 2006; Tahara, Ingham, Nakahara, Mizutani, & Harboren, 1984) and triterpenoid saponins (Woldemichael, Montenegro, & Timmermann, 2003; Woldemichael & Wink, 2002) are the main secondary metabolites of this genus. To date, more than 150 alkaloids of the quinolizidine, piperidine and indole groups have been found in lupins (Reinhard et al., 2006). Lupin alkaloids such as lupanine, sparteine, multiflorine and N-methylcytisine have been reported to possess hypoglycemic activity (Kubo et al., 2000; Mohamed, Kamel, El-Moghazy, & Murakoshi, 1993), while the triterpenoid saponins from lupins have antifungal activity against *Candida albicans* (Woldemichael & Wink, 2002) and hepatoprotective property (Kinjo, Udayama, Okawa, & Nohara, 1999).

Lupins have long been used as a source of protein and oil in the Andean region of South America (Woldemichael et al., 2003). Because lupins and soya have comparable nutritive characteristics, they are possible substitutes for genetically

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modified soya in human foodstuffs (Reinhard et al., 2006; van Barneveld, 1999). Four lupine species are of agronomic interest: *Lupinus albus* (white lupin), *Lupinus angustifolius* (blue lupin), *Lupinus luteus* (yellow lupin) and *Lupinus mutabilis* (Andean lupin) (Reinhard et al., 2006).

Lupinus texensis Hook., known as Texas bluebonnet, Texas lupine, buffalo clover, wolf flower and (in Mexican) el conejo, is the state flower of Texas. It is an annual plant endemic to Texas. This species has larger more sharply pointed leaves and more numerous flower heads than similar lupins and is often planted by the Texas Department of Transportation and garden clubs. To the best of our knowledge, there has been no report on the chemical constituents, nor a recorded traditional use or agronomic interest of this plant. Our interest in the discovery of novel bioactive natural products (Zhang & Li, 2007; Zhang, Wang, Yuan, & Li, 2008) from native plants in Texas prompted us to investigate the chemical constituents of *L. texensis*. This study described the isolation and structure determination of 17 flavonoids including one new compound from *L. texensis* and reported the free radical scavenging activities of 11 flavonoids for the first time. In addition, a validated HPLC method for simultaneous analysis of the 17 compounds was also presented.

2. Results and discussion

Repeated column chromatography following by HPLC purification of an ethanol extract of the whole plant of *L. texensis* yielded one new flavonoid (**2**), together with 16 known ones (**1**, **3–17**) (Figure 1). On the basis of the nuclear magnetic resonance (NMR) analysis and the comparison of the reference data, the known compounds were identified as genistein 7,4′-di-*O*-*β*-D-glucopyranoside (**1**) (Watanabe, Kinjo, & Nohara, 1993), pratensein 7-*O*-*β*-D-glucopyranoside (**3**) (Anhut et al., 1984), orientin (**4**) (Burns, Ellis, & March, 2007), vitexin (**5**) (Burns et al., 2007), 2′-hydroxygenistein (**6**) (Tahara et al., 1984), diosmetin 7-*O*-*β*-D-glucopyranoside (**7**) (Wang, Yang, & Guo, 2008), genistein (**8**) (Tahara et al., 1984), acacetin (**9**) (Rahman & Moon, 2007), diosmetin (**10**) (Domínguez, Marín, Esquivel, & Céspedes, 2007), crotarin (**11**) (Chaturvedi, Pant, Garg, & Bhakuni, 1987), 3-[2,3-dihydro-4-hydroxy-2-(1-hydroxy-1-methylethyl)-7-benzofuranyl]-5,7-dihydroxy-4H-1-benzopyran-4-one (**12**) (Tahara, Nakahara, Mizutani, & Ingham, 1985), erythrinin C (**13**) (Deshpande, A. Pendse, & R. Pendse, 1977), sophoraisoflavone A (**14**) (Yoshiaki, Ichiro, Mami, Tsuyoshi, & Manki, 1988), erythrinin B (**15**) (Deshpande et al., 1977), licoisoflavone A (**16**) (Kinoshita, Saitoh, & Shibata, 1978) and lupinisoflavone A (**17**) (Tahara et al., 1984).

Compound **2**, a yellow powder, had a molecular formula of $C_{22}H_{22}O_{11}$ as deduced from its HRESIMS at m/z 463.1229 $[M+H]^+$ and NMR data. The ^{1}H-NMR of **2** showed one methoxy signal at δ 3.89 (3H, s, H-4′) and six aromatic proton signals, of which one appeared as a singlet at δ 8.09 and was assigned to H-2, two were doublets at δ 6.53 and 6.73 ($J = 2.1$ Hz, each) and attributed to H-6 and H-8, and three at δ 6.40 (d, $J = 2.3$ Hz, H-3′), 6.37 (1H, dd, $J = 8.3/2.3$ Hz, H-5′), and 7.05 (d, $J = 8.3$ Hz, H-6′) corresponded to an 1′,2′,4′-oxygenation-aromatic ring. These data indicated that the aglycone of **2** was 5,7,2′-trihydroxy-4′-methoxyisoflavone (dehydroferreirin) (Aparecida dos Santos, Geraldo de Carvalho, & Braz-Filho,

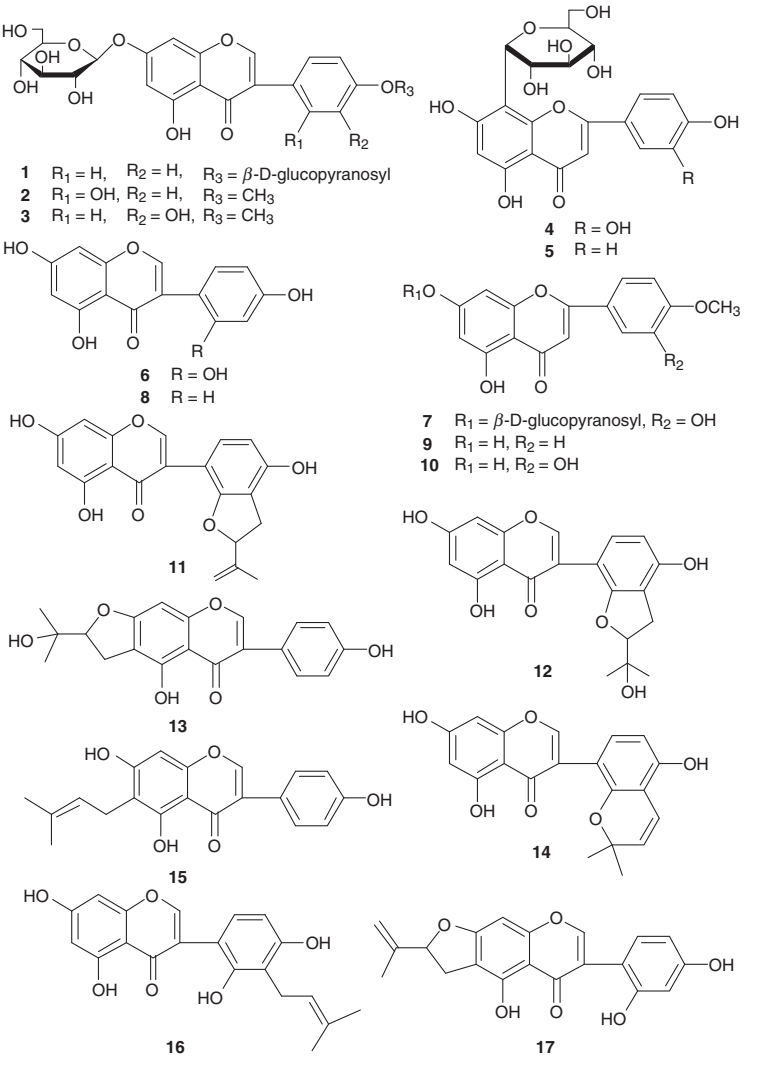

Figure 1. Chemical structures of flavonoids **1–17**.

1995). The ^{13}C-NMR spectra of **2** exhibited 22 carbon signals, 16 of these signals were identical to those reported for dehydroferreirin (Aparecida dos Santos et al., 1995). The remaining six carbon signals were indicative of the presence of one hexose. Acidic hydrolysis of **2** produced dehydroferreirin identified by NMR spectroscopic analysis with the reference data (Aparecida dos Santos et al., 1995), and D-glucose confirmed by co-HPLC analysis with authentic sugar and by measurement of optical activity after separation by HPLC. The monosaccharide was assigned to be in the pyranose form from analysis of its ^{13}C-NMR data. The β-anomeric configuration of glucose was defined from its $^{3}J_{H1,H2}$ (7.3 Hz) and $^{1}J_{CH}$ (161 Hz). A key HMBC correlation between δ 5.06 (glc-H-1) and δ 164.1 (C-7) established that the glucose was attached to the assigned position. The full assignments of the protons and carbons were achieved by a combination of ^{1}H, ^{13}C, HMQC and HMBC

experiments. The structure of **2**, a new isoflavone glycoside, was determined as 5,7,2′-trihydroxy-4′-methoxyisoflavone 7-*O*-*β*-D-glucopyranoside (dehydroferreirin 7-*O*-*β*-D-glucopyranoside).

The chemical profiles of *L. texensis* were characterised by defining and verifying the chemical ingredients and determining the concentration of 17 individual flavonoids using HPLC method. The simultaneous separation of these compounds was achieved on a reverse-phase HP Hypersil C_{18} column (250 × 4.6 mm, 5 μm) with grade mobile system consisting of methanol and 0.5% HOAc in water in 90 min. Fourteen compounds isolated and identified from the whole plant of Texas bluebonnet were used as the marker compounds. The content of compounds **9**, **13** and **15** were calculated from the linear regression equation of compounds **10**, **12** and **16**, respectively. Table 1 presented the concentration of individual and total flavonoid compounds. The results showed that Texas bluebonnet contained a total of over 7.79% flavonoids with vitexin (**5**) as the major one (3.70%), following by genistein (**8**, 0.84%).

This HPLC method was validated for linearity and reproducibility. The linearity of the standard curves was studied for compounds **1**–**8**, **10**–**12**, **14**, **16**, **17**. The calibration curve of each compound was investigated between the peak area (y) and the quantity of each reference compound (x, μg). The linearity is expressed in terms of the correlation coefficient (r). The correlation coefficient was found to be better than 0.999 for most of the reference compounds in the range of 25–300 $\mu g\,mL^{-1}$. The reproducibility of the method was assessed by within and between run validations. RSD% and RE% were found to be $\leq$2.05% and 1.93%, respectively.

Accelerated solvent extraction (ASE) was used for the preparation of sample solution for HPLC analysis. The recovery of the 14 reference compounds extracted using ASE with 95% ethanol as solvent was investigated. The results showed that the recovery for each analysed compound was over 98.1%, indicating that using ASE with 95% ethanol as extract solvent is a validated method to prepare the sample solution for HPLC analysis.

All tested flavonoids (**1**–**3**, **6**, **11**–**17**) have free radical scavenging activities with EC_{50} 48.6–172.5 $\mu g\,mL^{-1}$ (Table 1). To the best of our knowledge, the free radical scavenging activities of these flavonoids were reported for the first time.

Table 1. Contents of flavonoids and their free radical scavenging activities (mean ± SD, $n = 3$).

Compound	Content (%)	EC_{50} ($\mu g\,mL^{-1}$)	Compound	Content (%)	EC_{50} ($\mu g\,mL^{-1}$)
1	0.427 ± 0.005	172.5 ± 2.3	10	0.290 ± 0.008	–
2	0.320 ± 0.022	93.1 ± 1.9	11	0.113 ± 0.005	71.8 ± 1.8
3	0.203 ± 0.009	74.2 ± 1.2	12	0.053 ± 0.005	82.8 ± 2.3
4	0.670 ± 0.022	–	13[a]	0.163 ± 0.005	98.1 ± 1.9
5	3.707 ± 0.009	–	14	0.053 ± 0.005	78.5 ± 1.6
6	0.207 ± 0.017	66.9 ± 1.8	15[a]	0.083 ± 0.005	73.8 ± 1.7
7	0.163 ± 0.012	–	16	0.143 ± 0.005	56.3 ± 1.5
8	0.843 ± 0.009	–	17	0.123 ± 0.005	48.6 ± 2.0
9[a]	0.227 ± 0.005	–	Total	7.790 ± 0.043	

Notes: [a]The content of compounds **9**, **13** and **15** was calculated from the linear regression equation of compounds **10**, **12** and **16**, respectively.

3. Experimental

3.1. *General experimental procedures*

NMR experiments were performed on a Bruker 600 MHz NMR instrument. NMR data were reported as δ (ppm) values and referenced to the solvent used. HRESIMS were acquired on an electrospray instrument (MDS Sciex Pulsar Qstar). The UV spectra were recorded in MeOH with a μQuant spectrophotometer (Bio-Tek Instruments, Inc.). Optical rotation values were measured on a Jasco P-1010 polarimeter. Octadecyl-functionalised silica gel (ODS, Aldrich) and silica gel (60–200 mesh, 60 Å, Aldrich) were used for low-pressure chromatography. HPLC analysis was performed on an Agilent 1100 HPLC system with an Agilent 1100 diode array detector or an Agilent 1100 refractive index detector using a Hypersil ODS column (column A, 250 × 4.6 mm, 5 μm, Supelco) or a SupelcoGel CA column for sugar (column B, 300 × 7.8 mm, Supelco). Preparative HPLC was performed with an Acuflow Series III pump connected with an Acutect 500 UV/VIS detector using an Econosil ODS column (250 × 22 mm, 10 μm, Alltech; Detection: 256 nm; flow rate: 3.5 mL min^{-1}). ASE (Dionex model ASE 200) was used to prepare the sample solution for HPLC analysis.

3.2. *Plant material*

The whole plants of *L. texensis* were collected in Nacogdoches, Texas, USA in April 2007 and were identified by Greg Grant. A voucher specimen (TX-NAC-SFATC-20070402-#0002-LT) and a sample of the experimental plant material (NCPC-LT20070402) were deposited at the National Center for Pharmaceutical Crops at Stephen F. Austin State University, USA.

3.3. *Extraction and isolation*

The air-dried whole plant materials (500 g) were ground to a coarse powder and percolated with 95% ethanol thrice (each 4000 mL). The combined percolated solution was concentrated under vacuum to give an ethanol extract (40.5 g). This extract was suspended in MeOH–H_2O (1 : 1, v/v), and then partitioned successively with hexanes, chloroform and *n*-butanol to yield hexane (5.0 g), chloroform (10.1 g), *n*-butanol (15.1 g) and aqueous parts (10.1 g). The *n*-butanol part was applied to a column (60 × 5 cm) of ODS (300 g) eluting with water (1000 mL) and then 30% methanol (2000 mL). The fractions 8–13 (each 100 mL) collected from the 30% methanol eluate were combined and then dried to afford fraction A (1.6 g). Part of fraction A (300 mg) was separated by preparative HPLC using methanol/0.5% HOAc in water (35:65) as flow phase to yield compounds **1** (18.9 mg, t_R 30 min), **2** (15.8 mg, t_R 43 min), **3** (9.6 mg, t_R 48 min), **4** (22.6 mg, t_R 56 min) and **5** (123.8 mg, t_R 70 min). The chloroform part (5.0 g) was chromatographed on a column (60 × 5 cm) of silica gel (500 g) eluting with a mixture of chloroform and methanol (19 : 1, 3000 mL) to give fractions B_1 (200 mg, 300–350 mL), B_2 (100 mg, 450–550 mL), B_3 (60 mg, 600–750 mL), B_4 (600 mg, 800–900 mL), B_5 (126 mg, 1150–1200 mL), B_6 (208 mg, 1450–1500 mL), B_7 (300 mg, 1600–1700 mL) and B_8 (150 mg, 1900–2000 mL). Compounds **8** (13.6 mg), **6** (12.2 mg), **17** (8.6 mg) and **7** (9.5 mg) were crystallised from fractions B_1, B_2, B_7 and B_8, respectively. Fraction B_3 was purified by preparative

HPLC (MeOH/0.5% HOAc in water, 70:30) to yield **9** (6.1 mg, t_R 36.0 min). Compounds **10** (13.5 mg, t_R 49.7 min), **11** (7.9 mg, t_R 58.6 min) and **12** (7.3 mg, t_R 66.6 min) were separated by preparative HPLC (MeOH/0.5% HOAc in water, 65:35) from fraction B_4. Similarly, fraction B_5 was separated by preparative HPLC (MeOH/0.5% HOAc in water, 70 : 30) to give **13** (11.7 mg, t_R 53.6 min) and **14** (7.8 mg, t_R 61.7 min); and fraction B_6 was separated by preparative HPLC (MeOH/0.5% HOAc in water, 70:30) to give **15** (8.3 mg, t_R 63.2 min) and **16** (9.1 mg, t_R 68.5 min). All isolated compounds have purity of over 95% determined by HPLC analysis.

3.3.1. *5,7,2′-Trihydroxy-4′-methoxyisoflavone 7-O-β-D-glucopyranoside (**2**)*

Yellow powder; $[\alpha]_D^{25} + 39.6^\circ$ (*c* 0.1, MeOH); ^{1}H-NMR (DMSO-d_6, 600 MHz): δ 8.09 (1H, s, H-2), 7.05 (1H, d, $J = 8.3$ Hz, H-6′), 6.73 (1H, d, $J = 2.1$ Hz, H-8), 6.53 (1H, d, $J = 2.1$ Hz, H-6), 6.40 (1H, d, $J = 2.3$ Hz, H-3′), 6.37 (1H, dd, $J = 8.3/2.3$ Hz, H-5′), 5.06 (1H, d, $J = 7.3$ Hz, glc-H-1), 3.91 (1H, dd, $J = 12.2/2.0$ Hz, glc-H-6a), 3.89 (3H, s, OCH_3-4′), 3.70 (1H, dd, $J = 12.2/5.6$ Hz, glc-H-6b), 3.53 (1H, m, glc-H-5), 3.50 (1H, m, glc-H-3), 3.47 (1H, m, glc-H-2) and 3.40 (1H, m, glc-H-4)); ^{13}C-NMR (DMSO-d_6, 150 MHz): δ 182.2 (C, C-4), 164.1 (C, C-7), 163.3 (C, C-5), 159.7 (C, C-2′), 158.9 (C, C-9), 156.8 (C, C-4′), 156.5 (CH, C-2), 132.6 (CH, C-6′), 122.1 (C, C-3), 109.8 (C, C-1′), 107.4 (CH, C-5′), 106.8 (C, C-10), 103.1 (CH, C-3′), 100.3 (CH, $^1J_{CH} = 161$ Hz, glc-C-1), 100.1 (CH, C-6), 94.7 (CH, C-8), 78.0 (CH, glc-C-5), 77.6 (CH, glc-C-3), 74.9 (CH, glc-C-2), 70.6 (CH, glc-C-4), 62.1 (CH_2, glc-C-6), 56.0 (CH_3, OCH_3-4′); HRESIMS: *m/z* 463.1229 $[M + H]^+$ {Calcd for $[M(C_{22}H_{22}O_{11}) + H]$, 463.1240}.

3.4. *Acidic hydrolysis of compound 2*

Compound **2** (7.0 mg) was refluxed with 2 mL of 1 M HCl (dioxane–H_2O, 1 : 1) at 80°C for 2 h. Following dioxane removal, the solution was extracted with EtOAc (5 mL × 3). The extraction was washed with water and then concentrated. The residue was purified by preparative HPLC using methanol/0.5% HOAc in water (55 : 45) to give 5,7,2′-trihydroxy-4′-methoxyisoflavone (dehydroferreirin, 3.6 mg). The monosaccharide portion was neutralised by passing through an ion-exchange resin (Amberlite MB-3) column, and then concentrated for analysis. The monosaccharide of **2** was determined to be glucose (t_R 11.6 min) based on co-HPLC analysis with reference sugar (Column B; Detector: refractive index; Mobile phase: deionised water; Flow rate: 0.5 mL min^{-1}; Temperature: 78°C). The monosaccharide portion of **2** was then separated by HPLC using the above HPLC conditions and the optical activity of the monosaccharide (D-glucose, +46.6°, c. 0.18, H_2O) was measured.

3.5. *HPLC analysis*

HPLC analysis was performed on an Agilent 1100 HPLC system with an Agilent 1100 diode array detector using a Hypersil ODS column (250 × 4.6 mm, 5 μm, Supelco). The water with 0.05% acetic acid was employed as mobile phase A, and methanol as mobile phase B. The gradient procedure was 0–25 min with 10–30% B,

25–39 min with 30–40% B, 39–40 min with 40% B, 40–60 min with 40–65% B, 60–80 min with 65–90% B, 80–85 min with 90% B, 85–86 min with 90–10% B and 86–90 min with 10% B. The flow rate was 1.0 mL min^{-1}. The column temperature was set at 36°C, and detection was carried out at 256 nm. Data collection and integration were performed using HP ChemStation software.

3.5.1. *Preparation of sample solution for HPLC analysis*

ASE (Dionex model ASE 200) was applied to prepare the sample solution for HPLC analysis; 95% ethanol was used as the extract solvent. To prepare a single extraction cartridge, about 20 g of 30–40-mesh sand SX0075-3 (EM Sciences, Gibbstown, NJ) was mixed with 2.0 g of plant materials. Extractions were conducted at temperature 85°C with a pressure of 800 psi and 120 s nitrogen purges for a total of 30 min with three 10 min cycles. The solution of each extraction was transferred into a 50 mL volumetric flask and diluted with DMSO to make a final solution of 40 mg mL^{-1}. The solution of each sample was filtered through a 0.2 μm nylon cartridge before HPLC analysis. Extraction for each sample was performed in triplicate.

3.5.2. *Reference compounds*

A total of 14 flavonoids (**1**–**8**, **10**–**12**, **14**, **16** and **17**; purity: 95–98%) isolated and purified from *L. texensis* were used as reference compounds. Each reference compound (3.0 mg) was accurately weighed into a 1.0 mL volumetric flask and dissolved in methanol to make a stock solution (3.0 mg mL^{-1}) of the 14 components. The final solution for HPLC analysis was prepared from the stock solution.

3.5.3. *Linear regression equation and calibration curve*

The linear regression equation and the calibration curve were investigated between peak area (y) and the quantity (x, μg) of each component. Seven injections were performed to obtain the absorption plots ranging from 25–300 μg mL^{-1} in seven increments.

3.5.4. *Reproducibility of the HPLC method*

The precision and accuracy of the HPLC method were assessed by within and between run validations. The variation was evaluated by three injections of a mixture of 14 reference compounds each day on three consecutive days after the preparation of reference compound solution. By substituting the peak area into the linear regression equation from the same run, the measured concentrations were obtained. The coefficient of variance RSD% was calculated by comparing the measured concentrations. The relative error RE% was obtained by comparing calculated and theoretical concentrations.

3.5.5. *Recovery of the 14 reference compounds*

A certain amount of each reference compound was added into the plant material (2.0 g) of *L. texensis*, and the mixtures were extracted by ASE using 95% ethanol as solvent as described previously. The extract solution was transferred into a 50 mL volumetric flask, diluted with DMSO to make a final solution of 40 mg mL^{-1} and

then filtered through a 0.2 μm nylon cartridge prior to HPLC analysis. For comparison, a blank sample without spiking with marker compounds was prepared and similarly analysed.

3.6. *Determination of the free-radical-scavenging activity*

The α,α-diphenyl-β-picrylhydrazyl (DPPH) scavenging assay was carried out according to the procedure described previously (Tadić et al., 2008). Briefly, different concentrations of the samples (100 μL) were mixed with 900 μL of 0.04 mg mL^{-1} methanolic solution of DPPH. The mixture was kept at room temperature for 20 min and then the UV absorbance at 517 nm was measured. The inhibition percentage was calculated using the following equation: $I = [(A_c - A_s)/A_c] \times 100$, where I was the inhibition percentage, A_c the absorbance of the negative control (containing 100 μL of methanol instead of the tested samples) and A_s the absorbance of the samples. The inhibition percentage was plotted against the concentration of the samples, and EC_{50} values (mean ± SD) were determined by linear regression analysis of three determinations.

Acknowledgements

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References

Anhut, S., Zinsmeister, H.D., Mues, R., Barz, W., Mackenbrock, K., Köster, J., & Markham, K.R. (1984). The first identification of isoflavones from a bryophyte. *Phytochemistry, 23*, 1073–1075.

Aparecida dos Santos, S., Geraldo de Carvalho, M., & Braz-Filho, R. (1995). Unambiguous ^{1}H- and ^{13}C-NMR assignments of isoflavones from *Virola caducifolia*. *Journal of the Brazilian Chemical Society, 6*, 349–352.

Burns, D.C., Ellis, D.A., & March, R.E. (2007). A predictive tool for assessing ^{13}C-NMR chemical shifts. *Magnetic Resonance in Chemistry, 45*, 835–845.

Chaturvedi, R., Pant, N., Garg, H.S., & Bhakuni, D.S. (1987). Isoflavanoids of *Crotalaria madurensis*. *Journal of Natural Products, 50*, 266–269.

Deshpande, V.H., Pendse, A.D., & Pendse, R. (1977). Erythrinins A, B and C, three new isoflavones from the bark of *Erythrina variegata*. *Indian Journal of Chemistry B, 15*, 205–207.

Domínguez, M., Marín, J.C., Esquivel, B., & Céspedes, C.L. (2007). Pensteminoside, an unusual catalpol-type iridoid from *Penstemon gentianoides* HBK (Plantaginaceae). *Phytochemistry, 68*, 1762–1766.

García-López, P.M., Kachlicki, P., Zamora-Natera, F., Ruiz-Moreno, J., & Stobiecki, M. (2006). Profiling isoflavone conjugates in different organs of *Lupinus exaltatus* Zucc. *Phytochemical Analysis, 17*, 184–191.

Kinjo, J., Udayama, M., Okawa, M., & Nohara, T. (1999). Study of structure – hepatoprotective relationships of oleanene-type triterpenoidal glucuronides obtained from several fabaceous plants on rat primary hepatocyte cultures. *Biological and Pharmaceutical Bulletin, 22*, 203–206.

Kinoshita, T., Saitoh, T., & Shibata, S. (1978). Chemical studies on the oriental plant drugs. XLIII. A new isoflavone from licorice root. *Chemical and Pharmaceutical Bulletin, 26*, 141–143.

Kubo, H., Kobayashi, J., Higashiyama, K., Kamei, J., Fuji, Y., & Ohmiya, S. (2000). The hypoglycemic effect of (7R,9aS)-7-phenyloctahydroquinolizin-2-one in mice. *Biological and Pharmaceutical Bulletin, 23*, 1114–1117.

Mohamed, M.H., Kamel, M.S., El-Moghazy, S.A., & Murakoshi, I. (1993). The hypoglycemic effect of some lupin alkaloids. *Bulletin of Faculty of Pharmacy (Cairo University), 31*, 107–111.

Rahman, A.A., & Moon, S.S. (2007). Antimicrobial phenolic derivatives from *Dendranthema zawadskii var. latilobum* Kitamura (Asteraceae). *Archives of Pharmacal Research, 30*, 1374–1379.

Reinhard, H., Rupp, H., Sager, F., Streule, M., & Zollern, O. (2006). Quinolizidine alkaloids and phomopsins in lupin seeds and lupin containing food. *Journal of Chromatography A, 1112*, 353–360.

Tadić, V.M., Dobric, S., Markovic, G.M., Dordevic, S.M., Arsic, I.A., Menkovic, N.R., & Stevic, T. (2008). Anti-inflammatory, gastroprotective, free-radical-scavenging, and antimicrobial activities of hawthorn berries ethanol extract. *Journal of Agricultural and Food Chemistry, 56*, 7700–7709.

Tahara, S., Ingham, J.L., Nakahara, S., Mizutani, J., & Harboren, J.B. (1984). Fungitoxic dihydrofuranoisoflavones and related compounds in white lupin, *Lupinus albus*. *Phytochemistry, 23*, 1889–1890.

Tahara, S., Nakahara, S., Mizutani, J., & Ingham, J.L. (1985). Fungal metabolism of the prenylated isoflavone licoisoflavone A. *Agricultural and Biological Chemistry, 49*, 2605–2612.

van Barneveld, R.J. (1999). Understanding the nutritional chemistry of lupin (*Lupinus* spp.) seed to improve livestock production efficiency. *Nutrition Research Reviews, 2*, 203–230.

Wang, Y.J., Yang, X.W., & Guo, Q.S. (2008). Studies on chemical constituents in Huangjuhua (flowers of *Chrysanthemum morifolium*). *Zhongguo Zhongyao Zazhi, 33*, 526–530.

Watanabe, K., Kinjo, J., & Nohara, T. (1993). Leguminous plants. XXXIX. Three new isoflavonoid glycosides from *Lupinus luteus* and *L. polyphyllus* × *arboreus*. *Chemical & Pharmaceutical Bulletin, 41*, 394–396.

Woldemichael, G.M., Montenegro, G., & Timmermann, B.N. (2003). Triterpenoidal lupin saponins from the Chilean legume *Lupinus oreophilus* Phil. *Phytochemistry, 63*, 853–857.

Woldemichael, G.M., & Wink, M. (2002). Triterpene glycosides of *Lupinus angustifolius*. *Phytochemistry, 60*, 323–327.

Yoshiaki, S., Ichiro, Y., Mami, N., Tsuyoshi, T., & Manki, K. (1988). Studies on the constituents of *Sophora* species. XXII. Constituents of the root of *Sophora moorcroftiana* Benth. ex Baker. (1). *Chemical & Pharmaceutical Bulletin, 36*, 2220–2225.

Zhang, Z.Z., & Li, S.Y. (2007). Cytotoxic triterpenoid saponins from the fruits of *Aesculus pavia* L. *Phytochemistry, 68*, 2075–2086.

Zhang, Z.Z., Wang, P., Yuan, W., & Li, S.Y. (2008). Steroids, alkaloids, and coumarins from *Gelsemium sempervirens*. *Planta Medica, 74*, 1818–1822.