New diterpenoid glucoside and flavonoids from *Plectranthus scutellarioides* (L.) R. Br.

R. Kubínová [a], M. Gazdová [a,*], Z. Hanáková [a], S. Jurkaninová [a], S. Dall'Acqua [b], J. Cvačka [c], O. Humpa [d]

[a] Department of Natural Drugs, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences Brno, Palackého 1/3, 612 42 Brno, Czech Republic
[b] Department of Pharmaceutical Sciences, University of Padua, Via F. Marzolo 5, 351 31 Padua, Italy
[c] Mass Spectrometry Group, Institute of Organic Chemistry and Biochemistry, v.v.i, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, 166 10 Prague, Czech Republic
[d] Central European Institute of Technology, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic

ARTICLE INFO

ABSTRACT

Three new compounds, diterpenoid glucoside (13S,15S)-6β,7α,12α-trihydroxy-13β,16-cyclo-8-abietene-11,14-dione 7-O-β-D-glucoside **1**, flavonoids apigenin 7-O-(3''-O-acetyl)-β-D-glucuronide **2** and apigenin 5-O-(3''-O-acetyl)-β-D-glucuronide **3**, together with known compounds caffeic acid **4**, luteolin 5-O-β-D-glucoside **5** and rosmarinic acid **6** were isolated from the aerial parts of *Plectranthus scutellarioides* (L.) R. Br. The structures of the new compounds were elucidated by spectroscopic and mass-spectrometric analyses, including 1D- and 2D-NMR. Compound **1** inhibited hyaluronidase by 25% at the concentration of 200 μM, compounds **2** and **3** showed inhibitory activity on butyrylcholinesterase better than standard galanthamine at the concentration of 100 μM, and compound **6** is a potent antioxidant with an ORAC value of 2.15 ± 0.12.

© 2018 SAAB. Published by Elsevier B.V. All rights reserved.

1. Introduction

Plectranthus scutellarioides (L.) R. Br. (syn. *Coleus blumei* Benth., *Solenostemon scutellarioides* (L.) Codd) is a member of the Lamiaceae family and is highly prized for its vibrant and colorful foliage. It is a perennial native to Java, but now spread over many parts of Asia, America and Europe and widely cultivated in Southern Africa as an ornamental plant (Codd 1975).

People living in the Tlanchinol region of Mexico use *P. scutellarioides* infusion to treat gastrointestinal illnesses (Andrade-Cetto 2009). Hot water extract of *P.scutellarioides* mixed with citrus fruit juice and applied on skin after scorpion bite has reportedly been used in the Lohit community of Arunachal Pradesh in India (Namsa etal. 2009).

Previous studies on *P.scutellarioides* have reported the isolation of the terpenoid coleon O and a mixture of diastereomers of a new abietane type diterpene with antibacterial activity (Ragasa etal. 2001). Phytochemical analysis of the plant has been carried out and the secondary metabolites rosmarinic acid, caffeic acid, sterols and flavonoids with antioxidant activity, especially luteolin and its glycosides (Li 2006), have been detected.

Oxidative and inflammatory processes are among the pathological features associated with the central nervous system in Alzheimer's Disease (AD) (Fawole etal. 2010). Apart from the fact that compounds with antioxidant and anti-inflammatory activity could be used in the treatment of AD, increasing the levels of acetylcholine through the inhibition of cholinesterases is also necessary. Acetycholinesterase (AChE) predominates in the healthy brain and along with butyrylcholinesterase (BuChE) plays a crucial role in the regulation of brain acetylcholine levels. However, BuChE activity progressively increases in patients with AD. It may be disadvantageous for a cholinesterase inhibitor to be selective for AChE as is demonstrated by the administration of the mixed AChE/BChE inhibitor rivastigmine in AD patients (Greig etal. 2002).

Preventive and symptomatic treatment of AD needs a multitarget drug strategy. Therefore, after isolation of the compounds from *P. scutellarioides*, we investigated their activity against AChE and BuChE. The antioxidant potential of the compounds was expressed as the ORAC value (Trolox equivalents) in the oxygen radical absorbance capacity assay. Inhibition of hyaluronidase that is associated with inflammatory processes was also tested.

Table1
^{1}H NMR (at 500 MHz in CDCl$_3$, δ in ppm, J in Hz) and ^{13}C NMR data (at 500 MHz in CDCl$_3$, δ in ppm) for compound **1**.

Position	^{1}H NMR	^{13}C NMR
1	1.29 (ov)	36.8, CH$_2$
	2.06, br dt (12.5)	
2	1.56, m	18.4, CH$_2$
	1.88, m	
3	1.33, m (ov)	42.0, CH$_2$
	1.41, br dt (12.9)	
4		33.3, C
5	1.68, s	47.6, CH
6	4.52, br s	65.3, CH
7	4.71, d (2.0)	72.8, CH
8		138.5, C
9		156.9, C
10		38.2, C
11		199.0, C
12	3.76, s	76.8, CH
13		35.6, C
14		197.1, C
15	2.14, dt (6.6, 9.0)	20.4, CH
16	0.90, dd (3.7, 6.6)	26.3, CH$_2$
	1.14, dd (3.7, 9.0)	
17	1.28, d (6.6)	12.1, CH$_3$
18	1.26, s	22.9, CH$_3$
19	1.08, s	32.6, CH$_3$
20	1.71, s	21.1, CH$_3$
1´	4.53, d (6.7)	101.3, CH
2´	3.15, dd (9.0, 6.7)	73.2, CH
3´	3.36, dd (9.0, 9.8)	76.4, CH
4´	3.26, t (9.8)	70.4, CH
5´	3.24, m	76.3, CH
6´	3.66, dd (5.6, 12.0)	61.4, CH$_2$
	3.88, dd (2.0, 12.0)	

ov = signals overlapped.

2. Experimental

2.1. General experimental procedures

The methanol extract and ethyl acetate fraction were analyzed by HPLC-DAD-MS (HP 1100, Agilent Technologies, Czech Republic, column ABZ + Plus 150 × 4.6 mm, 3 μm particle size, flow rate 1 mL/min, temp.40 °C; LC-MSD Trap VL combined with electrospray ionization, negative mode, nebulizer pressure 50 psi, dry gas 10 L/min, dry temp.350 °C). The components were isolated by semi-preparativeHPLC (HP 1100, column ABZ + Plus, 250 × 10 mm, 5 μm particle size, flow rate 5 mL/min, temp.40 °C, with the same gradient elution). Compounds **1**, **2**, and **3** were characterized by 1D- and 2D-NMR (Bruker Avance 400 Ultrashield and Bruker Avance 500 Ultrashield; TMS as internal standard, COSY, NOESY) and HRMS analysis (Orbitrap ESIHRMS, Thermo Scientific for **1**; TOFESIHRMS, Applied Biosystems, for **2** and **3**). Compounds **4**, **5**, and **6** were identified by co-HPLC/DAD with standards, HPLC-MS and by comparison with data published previously (Pereira etal. 2012; Kubínová etal. 2013). The bioassay measurements were performed using a microplate-reader (Bio-Tek, Germany).

2.2. Chemicals and reagents

AChE from electric eel, BuChE from equine serum, hyaluronidase from bovine testes, dithio-bis-(2-nitrobenzoic acid) (DTNB), acetyl/butyryl thiocholine iodide (ATCI/BTCI), phosphate buffer, potassium hyaluronate, _p_-dimethylaminobenzaldehyde(_p_-DMAB), CaCl$_2$, NaOH, potassium tetraborate, acetate buffer, fluorescein, 2,2′-azo-bis(2-amidinopropane) (AAPH), galanthamine, qurcetin, and Trolox Cwere purchased from Sigma–Aldrich (Prague, Czech Republic). Organic solvents (of analytical grade) were purchased from Merck (Czech Republic).

2.3. Plant material

The plant _P.scutellarioides_ was grown in a greenhouse at the Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences Brno. A dried voucher specimen has been deposited in the herbarium of this faculty (CB-BD/2013).

2.4. Extraction and isolation of compounds

The aerial parts of the plant were collected (420 g), frozen at −60 °C, ground, and extracted with methanol (2 L) for 24 h at room temperature. After evaporation of the solvent, the residue (2.7 g) was suspended in H$_2$O and extracted three times with ethyl acetate using a separatory funnel to give ethyl acetate fraction (1 g).

The ethyl acetate fraction was separated by semi-preparativeHPLC, with gradient elution. The mobile phase consisted of ACN and 0.2% aqueous HCOOH, with the ACN component increasing from 10 to 100% over 36 min, and a flow rate of 5 mL/min. The new compounds **1**(8 mg), **2** (8 mg), and **3** (5 mg) were eluted with retention times 12.2, 13.3 and 14.6 min, compounds **4** (4 mg), **5** (3 mg), and **6** (10mg) with retention times 7.0, 8.3 and 11.1 min, respectively. After evaporation of the solvent, samples were lyophilized to give solid compounds.

2.5. Inhibition of AChE and BuChE

The inhibitory activities of AChE and BuChE were measured by the Ellman method with a slight modification (Kubínová etal. 2016). The reaction mixture contained: 120 μL of 0.1 M phosphate buffer (pH7.0), 20 μL of 2.3 U/mL AChE/BChE solution in buffer, 20 μL of the test sample, and 20 μL of 10 mM DTNB. After incubation at 37 °C (15min), 20 μL of 7.5 mM ATCI/BTCI was added to initiate the reaction. The hydrolysis was monitored at a wavelength of 405 nm by the formation of the yellow 5-thio-2-nitrobenzoate anion resulting from the enzyme-catalyzed reaction of DTNB with thiocholines. A set of mixtures prepared with an equivalent volume of methanol, replacing the test sample was used as a control ($A_{control}$). Another set of mixtures, prepared with an equivalent volume of phosphate buffer instead of the enzyme, was used as a blank (A_{blank}). The inhibitory rates (%) were calculated according to the formula: $(1 - (A_{sample} - A_{blank}) / A_{control}) \times 100$.

2.6. Inhibition of hyaluronidase

The assay of hyaluronidase inhibition was performed according to the Morgan-Elson method with a slight modification (Rahman etal. 2001). The reaction mixture contained: 40 μL of hyaluronidase enzyme (400 U/mL) in 0.1 M acetate buffer (pH 3.5) and 10 μL of 12.5 mM CaCl$_2$ as hyaluronidase activator. After incubation (20 min, 37 °C), 20 μL of the test sample was added to the activated enzyme. This mixture was incubated again for 20 min. Then the substrate, 50 μL of potassium hyaluronate (1.2 mg/mL), was added and the mixture incubated for 20 min. The enzyme reaction was stopped by adding 10 μL of 0.4 M NaOH and 10 μL of 0.2 M potassium tetraborate. The mixture was heated in a boiling water bath for exactly 3 min. After it had cooled completely, 200 μL of _p_-DMAB reagent was added, and the solution was mixed and incubated at 37 °C for 20 min to allow the color to develop. The absorbance of the mixture was measured at 585 nm. One set of mixtures prepared with an equivalent volume of methanol instead of a test sample was used as a control ($A_{control}$). Another set of mixtures prepared with an equivalent volume of acetate buffer instead of the enzyme was used as a blank (A_{blank}). The inhibitory rates (%) were calculated according to the formula: $(1 - (A_{sample} - A_{blank}) / A_{control}) \times 100$.
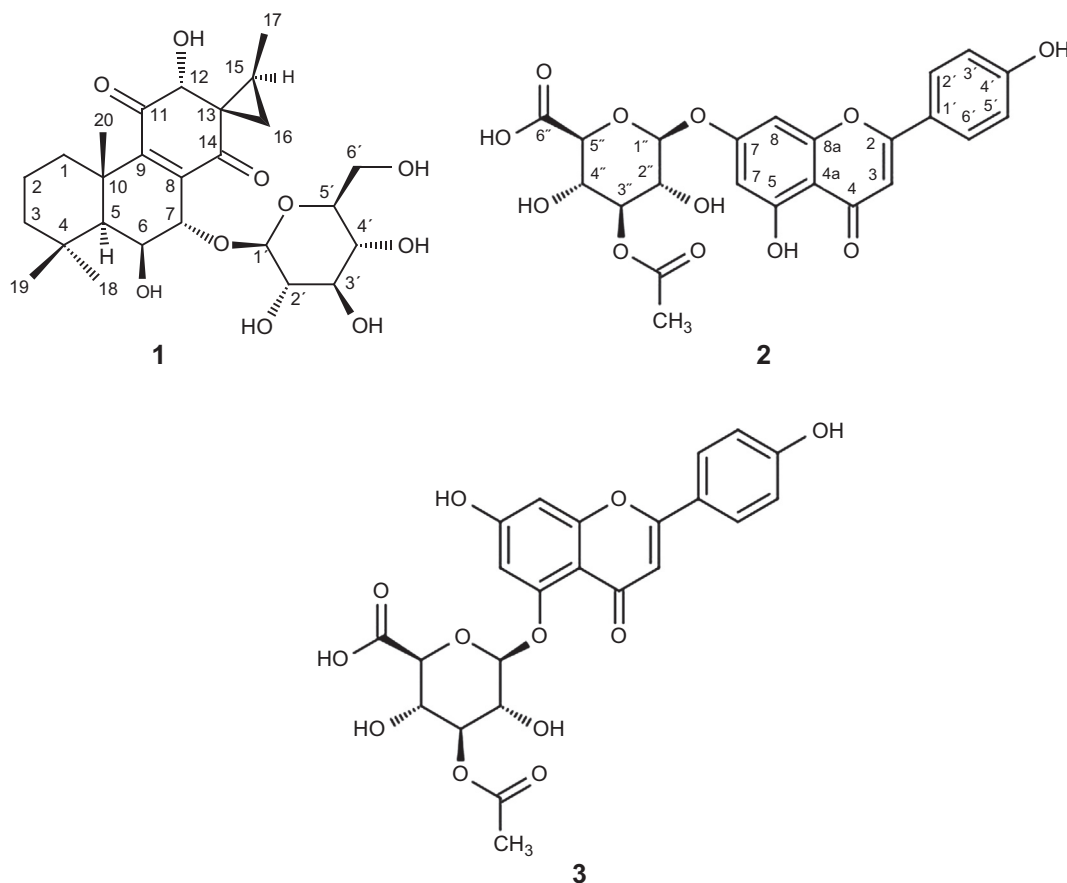
Fig.1. Chemical structures of the new compounds **1**, **2**, and **3** obtained from *P.scutellarioides* (L.) R. Br.

2.7. ORAC assay

The ORAC assay was conducted according to the literature with a slight modification (Matsutomo etal. 2013). The reaction mixture contained: 150 μL of fluorescein sodium salt dissolved in 75 mM phosphate buffer (pH 7.0) and 25 μL of the test sample. After incubation at 37 °C (3 min), 25 μL of 0.1 M AAPH was added. The fluorescence at the excitation wavelength of 485 nm and the emission at 528 nm were monitored every minute for 180 min. A standard curve of Trolox C was plotted (1–50 μM). The antioxidant capacity was calculated based on the area under the fluorescence curve. The effect was expressed as a Trolox equivalent (μM Trolox/μM compound).

3. Results and discussion

3.1. Elucidation of structure

Investigation of the aerial parts of *P.scutellarioides* (L.) R. Br. led to the isolation of three new compounds from a methanolic extract. Compound **1** was the diterpenoid (13S,15S)-6β,7α,12α-trihydroxy-13β,16-cyclo-8-abietene-11,14-dione 7-O-β-D-glucoside, compound **2**was the flavonoid apigenin 7-O-(3''-O-acetyl)-β-D-glucuronide, and compound **3** was the flavonoid apigenin 5-O-(3''-O-acetyl)-β-D-glucuronide. Caffeic acid **4**, luteolin 5-O-β-D-glucoside **5**, and rosmarinic acid **6** had been previously reported in *P.scutellarioides*(Li 2006). Compounds **4**, **5**, and **6** were identified by co-HPLC/DAD with standards, HPLC-MS, and by comparison of their spectroscopic data with those in the literature.

Compound **1** was isolated as an amorphous yellowish powder. The molecular formula was determined to be $C_{26}H_{38}O_{10}$ by Orbitrap HR-MS analysis (m/z 509.2386 $[M–H]^-$, calcd. 509.2387). The IR
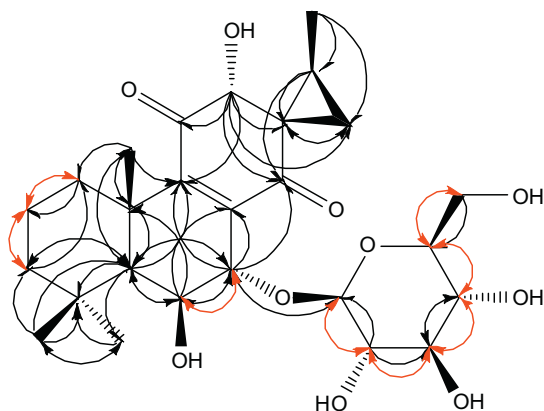

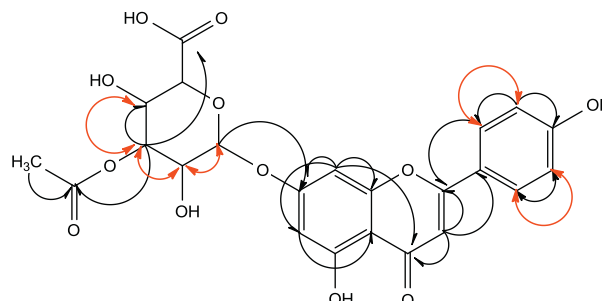**Fig.2.** Key COSY and HMBC correlation of **1**.

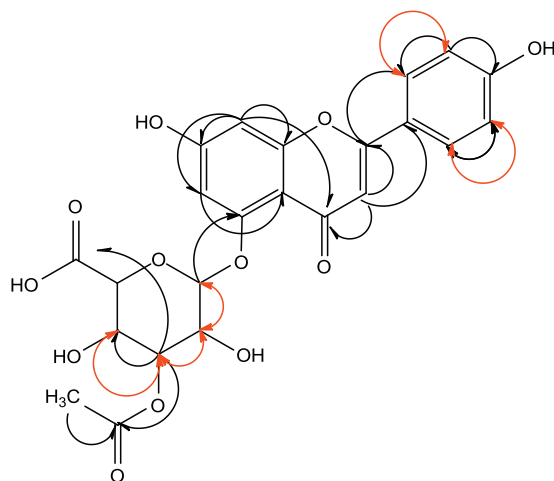**Fig.3.** Key COSY and HMBC correlation of **2**.

Fig.4. Key COSY and HMBC correlation of **3**.

spectra showed absorptions for OH groups (3387 cm^{-1}), quinone (1663cm^{-1}), and the glycosidic moiety (1038 cm^{-1}). The ^{1}H and ^{13}C NMR spectra of **1**(Table1) were very similar to those of spirocoleon 5 and spirocoleon 2 obtained from *P.saccatus* and *P.porcatus*(Simões etal. 2010). One hydroxy-substitutedCH group was missing and one more CH$_3$ group was found. These differences were assigned to substitutions at C-4 of ring A of cycloabietane, resulting in a basic dimethyl substitution (C-18 and C-19). Futhermore, the ^{1}H NMR spectrum and the MS/MS fragmentation pattern clearly showed the presence of a glucosidic moiety. HMBC correlations helped to assign the attachment of glucose through the oxygen at C-7. In addition to the proton and carbon chemical shifts, the coupling constants of the methylcyclopropane moiety and the abietane skeletone were also in accordance with the spirocoleons reported previously (Simões etal. 2010). This fact, together with observations in the NOESY spectrum, convinced us to assign the stereochemistry of **1** as identical to that of spirocoleon 2 (Simões etal. 2010). Thus, this new diterpenoid glycoside was elucidated as (13S,15S)-6β,7α,12α-trihydroxy-13β,16-cyclo-8-abietene-

11,14-dione 7-O-β-D-glucoside and was named as spirocoleon 7-O-β-D-glucoside (Fig.1).

Compounds **2** and **3** were both isolated as amorphous yellowish powders. The IR absorption of **2** indicated the presence of OH groups (3159 cm^{-1}), an aromatic ring (1603 cm^{-1}), a carbonyl group (1750cm^{-1}), and a glycosidic moiety (1036 cm^{-1}). The molecular formula of compound **2** was determined to be C$_{23}$H$_{20}$O$_{12}$ by HR-MS analysis (m/z 487.0997 [M- H]$^-$, calcd. 487.0955). The ^{1}H and ^{13}C-NMR signals of **2**(Table2) suggested the occurrence of a flavone skeleton substituted with a glycosidic moiety. In the sp2 region, the four proton signals of the B-ring appeared as two doublets at δ 7.95 and 6.94 with $J = 8.5$ Hz. Because of *ortho*-coupling, those signals were assigned to H-2′,6′ and H-3′,5′ and the two protons at δ 6.87 and 6.48 with $J = 2$ Hz (suggesting *meta*-coupling) were assigned to H-8 and H-6, respectively. The characteristic olefinic signal at δ 6.85 was ascribed to the C-3 proton on the ring C of the flavone, as was confirmed by the HMBC correlations from δ 6.85 to C-2, C-4, and C-1′. These findings suggested that **2** contains a 5,7,4′-trihydroxyflavone unit and were in agreement with data previously published for apigenin (Ersözetal. 2002). In addition, the glycosidic C-1″ signal was evident [δ_H 5.30 (d, H1″), δ_C 99.8 (C1″)] in the ^{1}H- and ^{13}C-NMR spectra. ^{1}H and 2D-NMR observations indicated the presence of an acetate group [δ_H 2.06 (s), δ_C 21.5, 170.1] and its linkage through the oxygen at position 3″ of the sugar was deduced. Further HMBC correlations of the acetate showed the presence of a carboxy group at position 5″. Together with the above corroboration, the structure of the glycosidic moiety was characterized as 3″-O-acetyl-β-D-glucuronide. The attachment of this moiety was deduced to be at the either 5-O or 7-O position of apigenin, according to the glycosylation rule. The HMBC correlations further confirmed that the 3″-O-acetyl-β-D-glucuronyl group is connected by an acetal linkage *via* the 7-O position of apigenin, as the anomeric proton at δ 5.30 (H-1″) correlated with the carbon signal at δ 163.5 (C-7). Thus, compound **2** was identified as the apigenin 7-O-(3″-O-acetyl)-β-D-glucuronide (Fig.1).

The IR,HR-MS, ^{1}H- and ^{13}C-NMR data for **3** were similar to those for **2**, with the HMBC correlations differing from the anomeric proton at δ_H 5.26 (H-1″) to δ_C 162.4 (C-5), which indicated that the 3″-O-acetyl-β-D-glucuronyl was attached at C-5(Table2). HR-MS analysis of compound **3** revealed a molecular formula C$_{23}$H$_{20}$O$_{12}$ based on the presence of a pseudomolecular ion [M–H]$^-$ at m/z 487.1017, calcd. 487.0955. Thus, compound **3** was identified as the apigenin 5-O-(3″-O-acetyl)-β-D-glucuronide (Fig.1). (See Figs.2–4.)

Diterpenoids and flavonoids appear to have a wide distribution in the genus *Plectranthus*, but **1**, **2**, and **3** have not been reported from other sources.

3.2. Inhibition of hyaluronidase, cholinesterases and antioxidant activity of compounds

Compound **1** showed a good inhibition effect on hyaluronidase with 24.5% at the concentration of 200 μM (Table3) and was also a better hyaluronidase inhibitor than flavonoids **2**, **3**, and **5**. Some flavonoid glycosides have been reported as active substances, mainly 7-O-

Table2
^{1}H NMR (at 400 MHz in DMSO, δ in ppm, J in Hz) and ^{13}C NMR data (at 400 MHz in DMSO, δ in ppm) for compounds **2** and **3**.

	2		3	
Position	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR
2		164.9, C		162.3, C
3	6.85, s	103.5, CH	6.86, s	103.3, CH
4		182.0, C		182.4, C
5		162.8, C		162.4, C
6	6.48, d (2.0)	99.8, CH	6.47, d (2.2)	99.9, CH
7		163.5, C		163.9, C
8	6.87, d (2.0)	95.1, CH	6.87, d (2.2)	95.1, CH
4a		105.7, C		105.4, C
8a		157.6, C		157.0, C
1′		121.3, C		121.0, C
2′	7.95, d (8.5)	128.8, CH	7.96, d (8.6)	128.9, CH
3′	6.94, d (8.5)	116.4, CH	6.94, d (8.6)	116.5, CH
4′		161.9, C		161.9, C
5′	6.94, d (8.5)	116.4, CH	6.94, d (8.6)	116.5, CH
6′	7.95, d (8.5)	128.8, CH	7.96, d (8.6)	128.9, CH
1″	5.30, d (7.7)	99.8, CH	5.26, d (7.7)	99.5, CH
2″	3.49 (ov)	70.5, CH	3.50 (ov)	70.2, CH
3″	4.92, t (9.4)	77.3, CH	4.93, t (9.4)	77.4, CH
4″	3.42 (ov)	70.7, CH	3.39 (ov)	70.6, CH
5″	3.96, d (9.4)	74.9, CH	3.95, d (9.4)	74.5, CH
6″		170.5, C		170.7, C
AcO-3″		170.1, C		170.2, C
AcO-3″	2.06, s	21.5, CH$_3$	2.05, s	21.6, CH$_3$

ov = overlapped with H$_2$O signal.

Table3
Inhibition of hyaluronidase at the concentration of 200 μM and the ORAC values.

Compounds	Inhibition of hyaluronidase (%)	ORAC value
Spirocoleon 7-O-β-D-glucoside (**1**)	24.5 ± 2.1	0.27 ± 0.03
Apigenin 7-O-(3″-O-acetyl)-glucuronide (**2**)	10.0 ± 4.3	1.57 ± 0.02
Apigenin 5-O-(3″-O-acetyl)-glucuronide (**3**)	13.9 ± 4.4	1.30 ± 0.03
Caffeic acid (**4**)	11.7 ± 0.9	1.95 ± 0.04
Luteolin 5-O-glucoside (**5**)	10.6 ± 2.0	1.47 ± 0.01
Rosmarinic acid (**6**)	29.5 ± 0.7	2.15 ± 0.12
Quercetin	89.5 ± 0.7	2.50 ± 0.14

Values mean ± SD, $n = 4$.

Table 4
Acetyl and butyrylcholinesterase inhibitory activities of isolated compounds.

Compounds	AChE (%)		BuChE (%)	
	100 μM	50 μM	100 μM	50 μM
Spirocoleon 7-O-β-D-glucoside (**1**)	36.4 ± 8.7	21.6 ± 2.0	40.6 ± 5.8	19.2 ± 3.4
Apigenin 7-O-(3″-O-acetyl)-glucuronide (**2**)	41.8 ± 0.8	25.4 ± 1.8	59.7 ± 4.1	26.7 ± 7.1
Apigenin 5-O-(3″-O-acetyl)-glucuronide (**3**)	40.5 ± 2.9	22.5 ± 3.1	63.1 ± 4.2	22.8 ± 5.6
Caffeic acid (**4**)	16.3 ± 1.7	7.8 ± 0.4	12.4 ± 3.6	5.2 ± 4.0
Luteolin 5-O-glucoside (**5**)	36.5 ± 5.9	26.1 ± 0.6	23.3 ± 1.3	8.8 ± 1.4
Rosmarinic acid (**6**)	21.6 ± 5.1	13.9 ± 0.1	13.5 ± 5.9	5.3 ± 2.7
Galanthamine	95.7 ± 3.2	92.3 ± 2.5	57.9 ± 4.8	55.3 ± 5.5

Values mean ± SD, $n = 4$.

glucuronides and the aglycones quercetin and kaempferol (Li et al. 1997; Murata et al. 2010). The activity of compound **1** is comparable to that of rosmarinic acid (**6**). Rosmarinic acid was determined as hyaluronidase inhibitor with IC_{50} 309 μM (Murata et al. 2010). In our study, quercetin was used as a control. Rosmarinic acid (**6**), which showed the highest ORAC value among the compounds isolated, can represent an effective anti-inflammatory drug. In addition, the anti-inflammatory effect of **6** was also determined in vivo (Boonyarikpunchai et al. 2014) and together with compound **1** contributed to the pharmacological action of the aqueous extract of *P. scutellarioides* used in traditional medicine to treat inflammation-related diseases (Namsa et al. 2009).

Among the classes of flavonoids, the best inhibition potential against AChE was reported for quercetin. Galangin and apigenin were the most effective against BuChE (Katalinić et al. 2010). The other findings showed that glycosides exerted a moderate inhibition of cholinesterases (Orhan et al., 2007) or were even inactive at the test concentration (Khan et al. 2009). Despite the fact that a previous study had established that glycosides are compounds with lower inhibitory activity than free aglycones, glucuronides of apigenin (**2** and **3**) still had good anticholinesterase effects (Table 4). It is well known that this good balance may result in a higher efficacy (Khan et al. 2009).

Flavonoids from species that are highly efficient in inhibiting cholinesterases have also been demonstrated to exert neuroprotection, expressed as significant antioxidant activity (Şenol et al. 2010). In our study, the isolated compounds **2** and **3** were good antioxidants (with ORAC values of 1.57 and 1.30, respectively), but they were weaker than rosmarinic acid or the standard quercetin (Table 3).

4. Conclusion

This study was focused on widely cultivated *P. scutellarioides*. Our results may suggest that this ethno-medicinal anti-inflammatory plant is a good source of phenolic compounds with the antioxidant property and hyaluronidase inhibition activity. Therefore, it should be reasonable to conclude that anti-inflammatory potential of drug might be related to the presence especially of these phytocompounds. Isolated flavonoid glycosides showed a good balance between the inhibiton of AChE and BuChE. *P. scutellarioides* is also a source abietane diterpenoids and further studies need to aim at isolation of these bioactive compounds.

Acknowledgments

References

Andrade-Cetto, A., 2009. Ethnobotanical study of the medicinal plants from Tlanchinol, Hidalgo, México. Journal of Ethnopharmacology 122, 163–171.

Boonyarikpunchai, W., Sukrong, S., Towiwat, P., 2014. Antinociceptive and anti-inflammatory effects of rosmarinic acid isolated from *Thunbergia laurifolia* Lindl. Pharmacology Biochemistry and Behavior 124, 67–73.

Codd, L.E., 1975. *Plectranthus* (Labiatae) and allied genera in Southern Africa. Bothalia 11, 371–442.

Ersöz, T., Harput, S., Saracoğlu, I., Çalı, İ., 2002. Phenolic compounds from *Scutellaria pontica*. Turkish Journal of Chemistry 26, 581–588.

Fawole, O.A., Amoo, S.O., Ndhlala, A.R., Light, M.E., Finnie, J.F., Van Staden, J., 2010. Anti-inflammatory, anticholinesterase, antioxidant and phytochemical properties of medicinal plants used for pain-related ailments in South Africa. Journal of Ethnopharmacology 127, 235–241.

Greig, N.H., Lahiri, D.K., Sambamurti, K., 2002. Butyrylcholinesterase: an important new target in Alzheimer's Disease therapy. International Psychogeriatrics 14, 77–91.

Katalinić, M., Rusak, G., Domaćinović Barović, J., Sinko, G., Jelić, D., Antolović, R., Kovarik, Z., 2010. Structural aspects of flavonoids as inhibitors of human butyrylcholinesterase. European Journal of Medicinal Chemistry 45, 186–192.

Khan, M.T.H., Orhan, İ., Şenol, F.S., Kartal, M., Şener, B., Dvorská, M., Šmejkal, K., Šlapetová, T., 2009. Cholinesterase inhibitory activities of some flavonoid derivatives and chosen xanthone and their molecular docking studies. Chemico-Biological Interactions 181, 383–389.

Kubínová, R., Švajdlenka, E., Schneiderová, K., Hanáková, Z., Dall'Acqua, S., Farsa, O., 2013. Polyphenols and diterpenoids from *Plectranthus forsteri* ʹMarginatus. Biochemical Systematics and Ecology 49, 39–42.

Kubínová, R., Švajdlenka, E., Jankovská, D., 2016. Anticholinesterase, antioxidant activity and phytochemical investigation into aqueous extracts from five species of *Agrimonia* genus. Natural Product Research 30, 1174–1177.

Li, T.S., 2006. Taiwanese Native Medicinal Plants: Phytopharmacology and Therapeutic Values. Taylor and Francis Group, New York, p. 27.

Li, M.W., Yudin, A.I., Vandevoort, C.A., Sabeur, K., Primakoff, P., Overstreet, J.W., 1997. Inhibition of monkey sperm hyaluronidase activity and heterologous cumulus penetration by flavonoids. Biology of Reproduction 56, 1383–1389.

Matsutomo, T., Stark, T.D., Hofmann, T., 2013. In vitro activity-guided identification of antioxidants in aged garlic extract. Journal of Agricultural and Food Chemistry 61, 3059–3067.

Murata, T., Miyase, T., Yoshizaki, F., 2010. Cyclic spermidine alkaloids and flavone glycosides from *Meehania fargesii*. Chemical and Pharmaceutical Bulletin 58, 696–702.

Namsa, N.D., Tag, H., Mandal, M., Kalita, P., Das, A.K., 2009. An ethnobotanical study of traditional anti-inflammatory plants used by the Lohit community of Arunachal Pradesh, India. Journal of Ethnopharmacology 125, 234–245.

Orhan, İ., Kartal, M., Tosun, F., Sener, B., 2007. Screening of various phenolic acids and flavonoid derivatives for their anticholinesterase potential. Zeitschrift für Naturforschung C 62, 829–832.

Pereira, O.R., Silva, A.M.S., Domingues, M.R.M., Cardoso, S.M., 2012. Identification of phenolic constituents of *Cytisus multiflorus*. Food Chemistry 131, 652–659.

Ragasa, Y., Templora, V.F., Rideout, J.A., 2001. Diastereomeric Diterpenes from *Coleus blumei*. Chemical & Pharmaceutical Bulletin 49, 927–929.

Rahman, A.U., Choudhary, M.I., Thomsen, W.J., 2001. Bioassay Techniques for Drug Development. Harwood Academic Publishers, Singapore, p. 139.

Şenol, F.S., Orhan, İ., Yilmaz, G., Çiçek, M., Şener, B., 2010. Acetylcholinesterase, butyrylcholinesterase, and tyrosinase inhibition studies and antioxidant activities of 33 *Scutellaria* L. taxa from Turkey. Food and Chemical Toxicology 48, 781–788.

Simões, M.F., Rijo, P., Duarte, A., Barbosa, D., Matias, D., Delgado, J., Cirilo, N., Rodríguez, B., 2010. Two new diterpenoids from *Plectranthus* species. Phytochemistry Letters 3, 221–225.