

## PAMPA Permeability, Acetylcholinesterase Inhibition and Antioxidant Activity of Pyranoisoflavones from *Polygala molluginifolia* (Polygalaceae)

Dalila Venzke,<sup>a</sup> Francieli K. Carvalho,<sup>a</sup> Ana P. Ruani,<sup>a</sup> Aldo S. Oliveira,<sup>a</sup> Inês M. C. Brighente,<sup>a</sup> Gustavo A. Micke,<sup>a</sup> Anderson Barison<sup>b</sup> and Moacir G. Pizzolatti<sup>\*,a</sup>

<sup>a</sup>Departamento de Química, Centro de Ciências Físicas e Matemáticas, Universidade Federal de Santa Catarina, Campus Universitário, Trindade, 88040-900 Florianópolis-SC, Brazil

<sup>b</sup>Departamento de Química, Centro Politécnico, Universidade Federal do Paraná, 81530-900 Curitiba-PR, Brazil

Uma investigação fitoquímica de *Polygala molluginifolia* (raiz, caule, folhas e flores) resultou no isolamento de quatro piranoisoflavonas das quais a isoflavona **1** é desconhecida na literatura. As isoflavonas **2-4**, embora sejam compostos sintéticos conhecidos são descritos neste trabalho como novos produtos naturais. Os compostos isolados foram avaliados em termos do seu efeito antioxidante e o potencial para inibir a enzima acetilcolinesterase. As isoflavonas **1** e **4** inibiram a acetilcolinesterase, exibindo valores de IC<sub>50</sub> de 68 µmol L<sup>-1</sup> e 84 µmol L<sup>-1</sup>, respectivamente. No ensaio de DPPH, os mesmos compostos e a rutina **5** apresentaram valores de EC<sub>50</sub> de 61 µmol L<sup>-1</sup>, 55 µmol L<sup>-1</sup> e 16 µmol L<sup>-1</sup>, respectivamente. Além disso, a permeabilidade das compostos **1-4** foi testada através do ensaio de permeabilidade em membrana artificial paralela (PAMPA) com as isoflavona **3** (9.25 × 10<sup>-6</sup> cm s<sup>-1</sup>) e **4** (3.48 × 10<sup>-6</sup> cm s<sup>-1</sup>) exibindo as maiores permeabilidades.

A phytochemical investigation of *Polygala molluginifolia* (root, stem, leaves and flowers) resulted in the isolation of four pyranoisoflavones, of which isoflavone **1** was previously unknown in the literature. Isoflavones **2-4**, although they are known synthetic compounds, are described in this work as new natural products. The isolated compounds were evaluated for their antioxidant effects and potential for inhibiting the acetylcholinesterase enzyme. Isoflavones **1** and **4** inhibited acetylcholinesterase, displaying IC<sub>50</sub> values of 68 µmol L<sup>-1</sup> and 84 µmol L<sup>-1</sup>, respectively. In the DPPH assay, the same compounds and rutin **5** exhibited EC<sub>50</sub> values of 61 µmol L<sup>-1</sup>, 55 µmol L<sup>-1</sup> and 16 µmol L<sup>-1</sup>, respectively. Moreover, the permeability of compounds **1-4** was evaluated using parallel artificial membrane permeability assay (PAMPA) with isoflavones **3** (9.25 × 10<sup>-6</sup> cm s<sup>-1</sup>) and **4** (3.48 × 10<sup>-6</sup> cm s<sup>-1</sup>) exhibiting the highest permeabilities.

**Keywords:** Polygalaceae, *Polygala molluginifolia*, isoflavones, *in vitro* assays

### Introduction

In the course of our research on the chemotaxonomy and biological activity of the genus *Polygala*, we investigated *P. molluginifolia* (A. St.-Hil.), a small herb found in grassland formations along the Atlantic Forest of southern Brazil.<sup>1</sup> No previous investigations have been reported regarding this species. The genus *Polygala* has been chemotaxonomically characterized by an accumulation of xanthenes,<sup>2,3</sup> coumarins,<sup>4</sup> saponins,<sup>5,6</sup> flavonoids,<sup>7,8</sup> lignans,<sup>9</sup> and more rarely, isoflavones.<sup>10</sup> In our previous studies, we detected an unusual occurrence of styrylpyrones in *P. sabulosa*.<sup>11</sup>

The *Polygala* species have significant ethnopharmacological value. For example, *P. cyparissias* is used as a local anesthetic due to the presence of methyl salicylate in its roots,<sup>12,13</sup> and *P. tenuifolia* is used in traditional Chinese medicine to improve memory and intelligence.<sup>14</sup> Biological studies have reported that *P. tenuifolia* is utilized in the treatment of Alzheimer's disease,<sup>15</sup> and also applied as an expectorant and sedative.<sup>16</sup> *P. caudate* is widely employed for treating cough and hepatitis and is used as a sedative.<sup>17</sup> Moreover, *P. radix* exhibits neuroprotective effects in Parkinson's patients,<sup>18</sup> and *P. japonica* demonstrates anti-inflammatory activity.<sup>19</sup> Our pharmacological studies indicated that *P. paniculata* possess neuroprotective effects,<sup>20</sup> whereas *P. sabulosa*

\*e-mail: moacir.pizzolatti@ufsc.br

exhibits anti-depressant, anxiolytic and anti-convulsant activity.<sup>21</sup> We now report the isolation and structural elucidation of four isoflavones from *P. molluginifolia*, their antioxidant activity, acetylcholinesterase inhibition and PAMPA (parallel artificial membrane permeability assay) permeability.

## Experimental

### General experimental procedures

Melting points were determined on a Microquímica APF-302 apparatus and are uncorrected. The infrared (IR) spectral data were acquired using Perkin-Elmer FT 16PC and Varian 3100 FT-IR instruments with KBr disks. UV spectra was measured with a Hewlett Packard 8452A instrument. The 1D and 2D nuclear magnetic resonance (NMR) data were acquired on a Varian Unity plus NMR spectrometer operating at 9.4 Tesla, observing <sup>1</sup>H and <sup>13</sup>C at 400 and 100 MHz, respectively. Thin-layer chromatography was performed on a pre-coated silica gel type-60 plate (Merck), and the spots were located by spraying with sulfuric-anisaldehyde followed by heating. For the standard chromatographic fractionation, 70-230-mesh silica gel was used, and for flash chromatographic fractionation, 230-400-mesh silica gel was employed. The electrospray ionization quadrupole time-of-flight mass spectrometry (ESI-Q-TOFMS) measurements were performed with a micrOTOF Q-II (Bruker Daltonics) mass spectrometer equipped with a KD Scientific automatic syringe pump for sample injection. The ESI-QTOF mass spectrometer was run at 4.5 kV and a desolvation temperature of 180 °C. The mass spectrometer was operated in the positive ion mode using a standard electrospray ion (ESI) source to generate the ions. The samples were injected using a constant flow rate (3 μL min<sup>-1</sup>), and the solvent was an acetonitrile/methanol mixture. The ESI-Q-TOF MS instrument was calibrated in the *m/z* 50-3000 range using an internal calibration standard (low concentration tuning mix solution) supplied by Agilent Technologies. The data were processed using Bruker data analysis software version 4.0. The acetylcholinesterase was measured using acetylthiocholine iodide (ATCI), 5,5'-dithiobis-[2-nitrobenzoic acid] (DTNB), acetylcholinesterase (AChE) type VI-S from an electric eel, [tris(hydroxymethyl)aminomethane] Tris-HCl buffer, bovine serum albumin (BSA) and magnesium chloride hexahydrate supplied by Sigma Aldrich (USA). For the 2,2-diphenyl-1-picrylhydrazyl (DPPH) analysis, DPPH (Sigma Aldrich), methanol (Vetec) and ethanol (Synth) were used. The bioassays were performed on a Perkin Elmer Lambda S spectrophotometer. High-performance

liquid chromatography (HPLC)-grade acetonitrile was purchased from J. T. Baker, and Milli-Q pure water was obtained from a Milli-Q Plus ultra-pure water purification system purchased from Millipore. The PAMPA experiments were performed using phosphatidylcholine, dimethyl sulfoxide and dodecane purchased from Sigma Aldrich and multiscreen filter plates from Millipore. The donors, acceptors and equilibrium solutions were analyzed using a Shimadzu HPLC system equipped with an LC-10AT pump and a UV detector. The analysis was performed on a Phenomenex C<sub>18</sub> column (150 × 4.6 mm; 5 μm), and elutions were obtained in isocratic mode [25% H<sub>2</sub>O (v/v) and 75% acetonitrile (v/v)] at a flow rate of 1 mL min<sup>-1</sup>, with detection at 280 nm.

### Plant material

*Polygala molluginifolia* was collected in December, 2010, near the Pitanguí River, at coordinates UTM 593095 and 7231847, in the region of Campos Gerais, Ponta Grossa, Paraná State, Brazil. The species was identified by Raquel Lüdtke (UFPel), and a sample specimen was deposited at the FLOR Herbarium (UFSC) registered under number 39551 by Rafael Trevisan.

### Extraction and isolation

The dried whole-plant material (37.0 g) was extracted (three times for seven days each) with EtOH at room temperature. The resulting extracts were combined, filtered and concentrated under reduced pressure at 50 °C to yield the crude alcoholic extract (10.45 g), which was then solid-liquid partitioned with hexane and EtOAc, and the solvents removed under reduced pressure to yield the respective extracts. The residue from the extraction was denominated water-soluble fraction. The EtOAc-soluble fraction (3.66 g) underwent chromatographic fractionation on a silica gel column and eluted with a gradient of EtOAc in hexane (1:9, 2:8, 3:7, 4:6, 5:5, 7:3), and then a gradient of EtOH in EtOAc (2:8, 4:6), to yield 41 fractions. Fraction 6 (1:9 EtOAc-hexane) underwent a successive chromatographic fractionation on a flash silica gel column and eluted with a hexane:acetone 8:2 gradient to yield isoflavones **2** (7.2 mg) and **3** (11.7 mg). Fractions 19 (3:7 EtOAc-hexane) and 31 (EtOAc) were recrystallized with MeOH to yield compounds **4** (16.4 mg) and **1** (38.7 mg).

The water-soluble fraction (3.96 g) underwent chromatographic fractionation on a silica gel column and was eluted with a scaled gradient of 7:3 hexane:EtOAc; 5:14:1 hexane:EtOAc:MeOH; 1:19:13 hexane:EtOAc:EtOH and finally MeOH to yield 30 fractions. Fractions 15 and 19

(1:19:13, hexane:EtOAc:EtOH) were combined to generate compound **5**. Fraction 28 (methanol) was recrystallized with MeOH to yield compound **6**.

3',4'-dihydroxy-6'',6''',6''',6'''-tetramethylbis(pyrano [2'',3'':5,6::2''',3''':7,8])isoflavone (**1**)

Yellow solid; mp 205–207 °C; IR (KBr)  $\nu_{\max}$  /  $\text{cm}^{-1}$  3416, 1654, 1595, 1507, 1452; UV (MeOH)  $\lambda_{\max}$  / nm ( $\epsilon$ ) 340 (2265), 302 (25604), 292 (25335), 258 (24798); for NMR data, see Table 1; HRMS (ESI+)  $m/z$  calculated for  $\text{C}_{25}\text{H}_{22}\text{O}_6$  [M+H]<sup>+</sup>: 419.1495; found, 419.1495.

3',4'-dimethoxy-5-hydroxy-6'',6''-dimethylpyrano[2'',3'':7,6] isoflavone (**2**)

Yellow solid; mp 136–138 °C; IR (KBr)  $\nu_{\max}$  /  $\text{cm}^{-1}$  3434, 1665, 1622, 1583, 1509; for NMR data, see Table 2; HRMS (ESI+)  $m/z$  calculated for  $\text{C}_{22}\text{H}_{20}\text{O}_6$  [M+H]<sup>+</sup>: 381.1338; found, 381.1328.

3'-methoxy-5,4'-dihydroxy-6'',6''-dimethylpyrano[2'',3'':7,6] isoflavone (**3**)

Yellow solid; mp 155–158 °C; IR (KBr)  $\nu_{\max}$  /  $\text{cm}^{-1}$  3434, 1662, 1624, 1581, 1516; for NMR data, see Table 2; HRMS (ESI+)  $m/z$  calculated for  $\text{C}_{21}\text{H}_{18}\text{O}_6$  [M+H]<sup>+</sup>: 367.1182; found, 367.1179.

5,3',4'-trihydroxy-6'',6''-dimethylpyrano [2'',3'':7,6]isoflavone (**4**)

Yellow solid; mp 165–167 °C; IR (KBr)  $\nu_{\max}$  /  $\text{cm}^{-1}$  3386, 1656, 1621, 1573, 1519; for NMR data, see Table 2; HRMS (ESI+)  $m/z$  calculated for  $\text{C}_{20}\text{H}_{16}\text{O}_6$  [M+H]<sup>+</sup>: 353.1025; found, 353.1022.

#### Acetylcholinesterase activity

The enzymatic activity was measured using an adaptation of the method described by Mata *et al.*<sup>22</sup> Briefly, 325  $\mu\text{L}$  of 50  $\text{mmol L}^{-1}$  Tris-HCl buffer, pH 8.0, 100  $\mu\text{L}$  of a buffer solution containing the sample at five different concentrations dissolved in EtOH and 25  $\mu\text{L}$  of an AChE solution containing 0.28 U  $\text{mL}^{-1}$  (50  $\text{mmol L}^{-1}$  Tris-HCl, pH 8.0 buffer, 0.1% BSA) were incubated for 15 min. Then, 75  $\mu\text{L}$  of an acetylthiocholine iodide solution (0.023  $\text{mg mL}^{-1}$  in water) and 475  $\mu\text{L}$  DTNB (3  $\text{mmol L}^{-1}$  in Tris-HCl, pH 8.0 buffer, 0.1  $\text{mol L}^{-1}$  NaCl, 0.02  $\text{mol L}^{-1}$   $\text{MgCl}_2$ ) were added, and the final mixture was incubated for another 30 min at room temperature. The absorbance of the mixture was measured at 405 nm. A control mixture containing ethanol instead of the sample was considered to have 100% AChE activity. The inhibition (%) was calculated as follows:  $I$  (%) =  $100 - (A_{\text{sample}}/A_{\text{control}}) \times 100$  in which  $A_{\text{sample}}$  is the

absorbance of the vegetal sample and  $A_{\text{control}}$  is the absorbance without sample. The tests were performed in triplicate, and a blank containing Tris-HCl buffer was used instead of the enzyme solution. The sample concentration with 50% inhibition ( $\text{IC}_{50}$ ) was determined by plotting the inhibition against the sample solution concentrations. Reminyl-containing galantamine was used as the positive control.

#### Antioxidant activity

The antioxidant activity was measured using the DPPH free-radical scavenging methodology described in the literature with adaptations.<sup>23</sup> A 0.004% DPPH solution in methanol was added to the test solutions in various concentrations, and the absorbances were determined using a UV-VIS (517 nm) spectrophotometer after 30 min. The absorbance at the initial time ( $A_0$ ) was obtained by measuring the absorbance of a DPPH solution. The analysis was performed in triplicate. The  $\text{EC}_{50}$  values of the samples were obtained by plotting the decreasing DPPH absorbance against the sample solution concentrations.

#### PAMPA permeability assay

PAMPA experiments for compounds **1-4** were performed according to Wohnsland and Faller with modifications using a 96-well filter plate.<sup>24</sup> The filters were coated with 10  $\mu\text{L}$  of a 1% (m/v) dodecane solution of phosphatidylcholine. The donor solutions of compounds **1-4** were prepared by diluting the DMSO stock solutions (1000 ppm) in 50% (v/v) phosphate buffered saline and stirring overnight. Then, 150  $\mu\text{L}$  of the donor solutions were added to the filter plate wells, and 300  $\mu\text{L}$  of the acceptor solutions (50% DMSO in phosphate buffer) were added to the receiver plate wells. The filter plate was coupled to the receiver plate and incubated in the dark for 5 h with agitation and then 20 min at room temperature. Next, 150  $\mu\text{L}$  of each donor solution was added to 300  $\mu\text{L}$  of the acceptor solution to yield the respective equilibrium solutions. The experiments were performed in quadruplicate. After incubation, the filter plate and the receiver plate were separated, and the donor, acceptor and equilibrium solutions were analyzed via HPLC.

The PAMPA permeability ( $P_{app}$ ) was calculated using the following equation obtained from Wohnsland and Faller:<sup>24</sup>

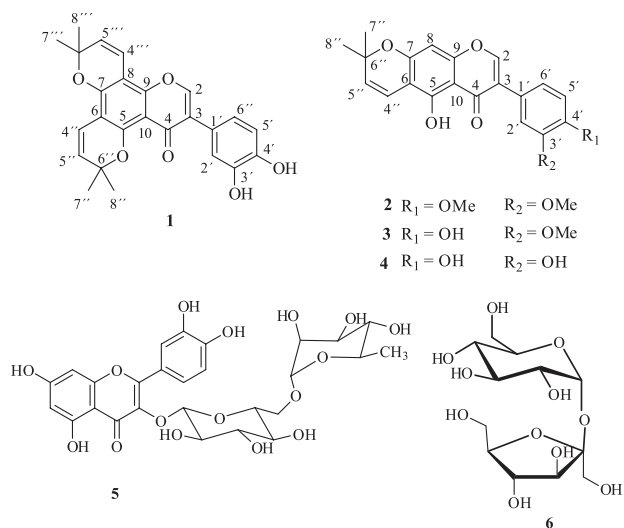
$$P_{app} = \frac{V_D V_A}{(V_D + V_A) A t} (-\ln(1 - r))$$

in which  $V_D$  is the volume in the donor well ( $\text{cm}^3$ ),  $V_A$  is the volume in the acceptor well,  $A$  is the area of the membrane

( $\text{cm}^2$ ),  $t$  is the incubation time (s) and  $r$  is the ratio between the compound concentrations in the acceptor well and equilibrium solution. The compound concentrations were obtained by integrating the HPLC peaks.

## Results and Discussion

In our investigation of the ethyl acetate fraction from *Polygala molluginifolia*, four isoflavones (**1-4**) were isolated and their structures elucidated. In addition, rutin (**5**) and sucrose (**6**) were isolated from the water-soluble fraction (Figure 1).



**Figure 1.** Structures of isolated compounds **1-6**.

The infrared spectra of compounds **1-4** demonstrated characteristic absorptions for the hydroxyl group, conjugated carbonyl, conjugated double bonds and aromatic rings. Compound **1**, a yellow solid, had the molecular formula  $\text{C}_{25}\text{H}_{22}\text{O}_6$ , as determined by HRMS (ESI+) analysis and NMR spectroscopy. The UV spectrum showed absorption maxima at 258, 292, 302 and 340 nm. The  $^1\text{H}$  NMR spectrum of compound **1** (Table 1) indicated the presence of an ortho-meta system at  $\delta_{\text{H}}$  6.79 (d, 1H,  $J$  8.0 Hz, H-5'), 6.77 (dd, 1H,  $J$  2.0, 8.0 Hz, H-6') and 6.95 (d, 1H,  $J$  2.0 Hz, H-2'), four methyl groups at  $\delta_{\text{H}}$  1.47 (s, 12H, H-7'', H-8'', H-7''', H-8'''), four *cis*-coupled olefinic hydrogens at  $\delta_{\text{H}}$  6.60 (d, 1H,  $J$  10.0 Hz, H-4''), 5.66 (d, 1H,  $J$  10.0 Hz, H-5''), 6.73 (d, 1H,  $J$  10.0 Hz, H-4''') and  $\delta_{\text{H}}$  5.69 (d, 1H,  $J$  10.0 Hz, H-5'''), and a singlet at  $\delta_{\text{H}}$  7.95 (s, 1H, H-2) corresponding to a proton attached to a  $\text{sp}^2$  carbon. The  $^{13}\text{C}$  NMR and DEPT spectroscopic data (Table 1) revealed twenty-three carbons: two quaternary  $\text{sp}^3$  carbons, two signals related to methyl groups, eight methine carbons, and eleven  $\text{sp}^2$  quaternary carbons, five of which are oxygenated and one is a carbonyl. These data, in addition to HRMS, indicated

a pentacyclic scaffold. Directly connected hydrogens and carbons were assigned via HMQC analysis. The long-range  $^1\text{H}$ - $^{13}\text{C}$  correlation map obtained from the HMBC NMR experiment (Table 1) exhibited a  $^2J_{\text{CH}}$  correlation H-2 ( $\delta_{\text{H}}$  7.95)/C-3 ( $\delta_{\text{C}}$  124.7), and  $^3J_{\text{CH}}$  correlations H-2/C-1' ( $\delta_{\text{C}}$  126.9) and H-2/C-4 ( $\delta_{\text{C}}$  177.4). The olefinic hydrogens H-4'' ( $\delta_{\text{H}}$  6.60) and H-5'' ( $\delta_{\text{H}}$  5.66) exhibited  $^3J_{\text{CH}}$  correlations with C-5 ( $\delta_{\text{C}}$  155.5) and C-6 ( $\delta_{\text{C}}$  108.5), respectively, indicating that one dimethylpyran ring was fused to the A ring at C-5 and C-6. The remaining dimethylpyran ring was fused to the A ring at carbons C-7 ( $\delta_{\text{C}}$  153.8) and C-8 ( $\delta_{\text{C}}$  103.3), as verified by the  $^3J_{\text{CH}}$  long-range correlations of these carbons with hydrogens H-4''' ( $\delta_{\text{H}}$  6.73) and H-5''' ( $\delta_{\text{H}}$  5.69), respectively. The B ring is disubstituted with two hydroxyl groups located at C-3' and C-4', as demonstrated by HMBC correlations  $^3J_{\text{CH}}$  C-3'/H-5' ( $\delta_{\text{H}}$  6.79),  $^2J_{\text{CH}}$  C-3'/H-2' ( $\delta_{\text{H}}$  6.95), and  $^3J_{\text{CH}}$  C-4'/H-2' ( $\delta_{\text{C}}$  146.5). The overall analysis of HMQC and HMBC experiments, and MS data indicated that compound **1** refers to 3',4'-dihydroxy-6'',6''',6''',6'''-tetramethylbis(pyrano[2'',3'':5,6::2''',3''':7,8]isoflavone (Figure 1), which is reported herein for the first time.

**Table 1.** NMR data (400 MHz,  $\text{CD}_3\text{OD}$ ) for compound **1**

Position	<b>1</b>		
	$\delta_{\text{H}}$ (mult., $J$ / Hz)	$\delta_{\text{C}}$ / ppm	HMBC $^2J_{\text{CH}}$ / Hz $^3J_{\text{CH}}$ / Hz
2	7.95 (s)	152.3	-    -
3	-	124.7	H-2    H-2'
4	-	177.4	-    H-2
5	-	155.5	-    H-4''
6	-	108.5	-    H-5''
7	-	153.8	-    H-4'''
8	-	103.3	-    H-5'''
9	-	154.2	-    H-2
10	-	109.9	-    H-8
1'	-	126.9	-    H-5', H-2
2'	6.95 (d, $J$ 2.0)	117.6	-    H-6'
3'	-	146.0	H-2'    H-5'
4'	-	146.5	-    H-2'
5'	6.79 (d, $J$ 8.0)	116.1	-    -
6'	6.77 (dd, $J$ 2.0, 8.0)	121.9	-    H-2'
4''	6.60 (d, $J$ 10.0)	116.4	-    -
5''	5.66 (d, $J$ 10.0)	129.8	-    H-7'', H-8''
6''	-	79.5	H-5'', H-7'', H-8''    H-4''
7'', 8''	1.47 (s)	28.3	-    -
4'''	6.73 (d, $J$ 10.0)	115.7	-    -
5'''	5.69 (d, $J$ 10.0)	128.9	-    H-7''', H-8'''
6'''	-	79.2	H-5''', H-7''', H-8'''    H-4'''
7''', 8'''	1.47 (s)	27.9	-    -





**Table 3.** Inhibitory activity of **1-4** toward acetylcholinesterase, and antioxidant effects of **1-5** in DPPH free-radical scavenging

Compounds	Acetylcholinesterase inhibition	DPPH
	IC <sub>50</sub> / (μmol L <sup>-1</sup> )	EC <sub>50</sub> / (μmol L <sup>-1</sup> )
<b>1</b>	68	61
<b>2</b>	132	838
<b>3</b>	126	208
<b>4</b>	84	55
<b>5</b>	-	16
Reminyl®	32	-
Ascorbic acid	-	52
Quercetin	-	21

rutin (**5**) and isoflavones **1** and **4**, exhibited the best results, with EC<sub>50</sub> values of 16, 61 and 55 μmol L<sup>-1</sup>, respectively, compared with ascorbic acid (52 μmol L<sup>-1</sup>) and quercetin (21 μmol L<sup>-1</sup>).

The strong antioxidant activity for rutin is in accordance with that reported in the literature,<sup>38</sup> indicating that rutin is a multifunctional agent capable of inhibiting Aβ aggregation and cytotoxicity, which can prevent mitochondrial damage, reduce free-radical and proinflammatory cytokine production and increase the levels of antioxidant enzymes.<sup>38</sup>

Given the significant acetylcholinesterase inhibition and DPPH activity of isoflavones **1-4**, the permeability of these compounds was also evaluated using the PAMPA assay. A high-throughput screening technique, PAMPA, was used to assess the passive permeability characteristics of molecules with potential pharmacological activity to predict passive transcellular molecule absorption.

In most cases, according to this assay, the hydrophobic isoflavones exhibited lower permeability than the polar isoflavones, a result consistent with that of Dreassi *et al.*<sup>39</sup> Of the compounds analyzed, isoflavone **1** exhibited the lowest permeability (1.50 × 10<sup>-6</sup> cm s<sup>-1</sup>), possibly due to the presence of two dimethylpyran rings, which increased the hydrophobicity of the molecule.

Regarding isoflavones **2** and **4**, which differ only in their B ring substitution, isoflavone **2** had the lowest permeability (2.01 × 10<sup>-6</sup> cm s<sup>-1</sup>), probably due to the presence of two methoxyl groups. Isoflavone **3** demonstrated the best permeability (9.25 × 10<sup>-6</sup> cm s<sup>-1</sup>), although it is more hydrophobic than compound **4** (3.48 × 10<sup>-6</sup> cm s<sup>-1</sup>). According to Siegel, increasing the number of hydroxyl groups can decrease the compound permeability.<sup>40</sup>

## Conclusions

This study reports the acetylcholinesterase inhibition, antioxidant activity and permeability of four isoflavones (**1-4**)

isolated and identified from *Polygala molluginifolia*. Of the compounds analyzed, isoflavones **1** and **4**, exhibited the best antioxidant activity and acetylcholinesterase inhibition, while PAMPA experiments indicated that the isoflavones **3** and **4** demonstrated the highest permeability. Results suggest that the isoflavones isolated from *P. molluginifolia* could be of interest for the treatment of neurodegenerative diseases.

## Supplementary Information

Supplementary data, including IR, MS and 1D/2D NMR, are available free of charge at <http://jbcs.sbq.org.br> as a PDF file.

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