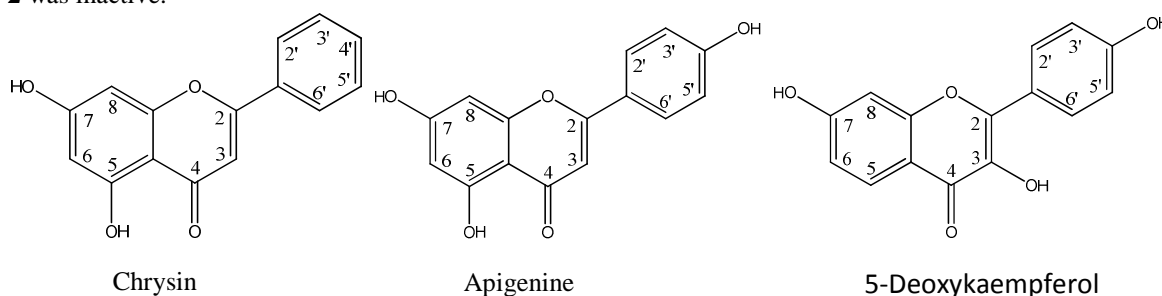


Anti-Leishmanial Effects of Flavonoids from *Crotolaria spectabilis* Roth

Pedro H. Arato Ferreira¹, Karina F. Peres¹, Luiz Felipe D. Passero², João Henrique G. Lago¹, Thiago A. M. Veiga¹

¹Instituto de Ciências Ambientais, Químicas e Farmacêuticas - Universidade Federal de São Paulo, Diadema, Brasil; ²Departamento de Patologia - Faculdade de Medicina da Universidade de São Paulo
 E-mail: pedro_henrique228@hotmail.com

Crotolaria genus is a member of the *Fabaceae* family, and comprises about 550 species; originally from China and Africa, but currently dispersed throughout the world, majority well adapted to tropical climate. This genus is widely used in nitrogen fixation, protection against soil erosion and control of nematodes [1]. In species of *Crotolaria* are commonly found specialized metabolites belonging to the class of flavonoids, saponins, tannins and steroids [2]. Previous reports showed the anti-leishmanial potential of several flavonoids and derivatives: sakuranetin [3], 5,6,7-trihydroxy-4'-methoxyflavanone [4], orientin [5] and kaempferol 3-*O*- α -L-arabinopyranosyl (1 \rightarrow 2) α -L-rhamnopyranoside [6]. Based on this context, in our work the roots, aerial parts and fruits of *Crotolaria spectabilis* were collected at the “Mina do Sossego-Canaã dos Carajás – PA”. The dried and ground roots material (1.0 Kg) has been extracted with ethanol. The extract was submitted to a liquid-liquid extraction with hexane, dichloromethane and ethyl acetate, leading to respective fractions. The dichloromethane fraction was chromatographed under SPE-C18 (solid phase extraction), resulting in three sub-fractions (MeOH, AcOEt and CH₂Cl₂). Methanolic sub-fraction was purified by preparative HPLC (phenyl-hexyl column, 150 x 5.00 mm, 5 μ ; mobile phase milli-Q water/MeOH, 4:6). This procedure allowed us to isolate three known flavonoids (**1** – **3**), that were analyzed by spectrometric techniques (¹H NMR and MS). ¹H NMR spectra (300 MHz, acetone-d₆) of compounds **1** and **2** showed similarities. The region between δ 6.44 and 8.00 presented signals for aromatic hydrogens. Data also presented two doublets with J values characteristics for *meta* substitution on their rings A. Flavonoid **3** showed a different pattern of substitution on its A-ring: two doublets (*d*) at δ 7.92 (*J*= 8.7 Hz) and 6.82 (*J*= 1.6 Hz), and one double doublet (*dd*) at δ 6.91 (*J*= 1.6 and 8.7 Hz), that were assigned to **H-5**, **H-8** and **H-6**, respectively. The pattern of substitution of ring B for the flavonoids presented a little difference. Compounds **2** and **3** showed a hydroxyl group at **C-4'**, while compound **1** is non-substituted at this position. The structural differences between the flavonoids were confirmed by LC-MS analysis. Comparison of our spectrometric data with those reported in the literature [7, 8] allowed the identification of the compounds: 5,7-dihydroxyflavone or chrysin (**1**), 4',5,7-trihydroxyflavone or apigenine (**2**) and 5-deoxykaempferol (**3**). The compounds were tested against promastigote forms of *Leishmania (L.) braziliensis* and *Leishmania (L.) amazonensis*. At 1,0 μ g/mL, flavonoids **1** and **3** caused the death of 60% of *L. braziliensis*; while compound **2** was inactive.



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