5th BCNP Brazilian Conference on Natural Products
XXXI RESEM
Atibaia – SP – Brazil
ISBN: 978-85-66836-10-3
Oct. 26–29th 2015

Structure-activity relationship of anti-allergic activity of flavonoids isolated from *Bidens sulphurea* and the assessment of the intestinal absorption of avicularin

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Abstract:

The aim of this study was to determine the anti-allergic activity of extract, fractions and isolated flavonoids of *Bidens sulphurea*, establishing their structure-activity relationship, as well as to assess the intestinal absorption rate of active flavonoid avicularin.

The aerial parts of *B. sulphurea* were dried and submitted to ethanol extraction (BsfcEt). Then, it was partitioned with hexane, dichloromethane and methanol (BsfcEt/Hx, BsfcEt/DCM and BsfcEt/Me fractions). Extract and fractions were submitted to anti-allergic activity assay using the β-hexosaminidase quantification method [1]. BsfcEt/DCM fraction was submitted to dereplication with LC-DAD-MS/MS. The major compounds were isolated by classic column chromatography and semipreparative HPLC-DAD, then the structures were confirmed by NMR and HRMS [2]. Thirteen flavonoids were isolated and also evaluated the anti-allergic activity. The four major compounds were flavonoids with similar polarity, and avicularin, the major constituent from BsfcEt/DCM, was chosen to perform an intestinal perfusion assay in rats in order to evaluate its intestinal absorption by the rat gut *in situ* technique [3] in the doses of 1mg, 5mg/kg and 1mg/kg with the efflux inhibitor verapamil 0.05 mM [4]. The study was approved by the Animal Use Ethics Committee. The samples were assayed by a validated UPLC-DAD-MS/MS method.

The inhibitory effect (IC_{50}) of extract and fractions on the release of β-hexosaminidase were 9.2±1.2, stimulates, 5.8±1.1 and 9.5±1.1mg/mL for BsfcEt, BsfcEt/Hx, BsfcEt/DCM, BsfcEt/Me, respectively. The substances identified as the major compounds of BsfcEt/DCM were: 3-O-β-D-galactopyranosylquercetin, 3-O-β-D-glicopyranosylquercetin, 3-O-β-L-arabinofuranosylquercetin and 3-O-α-L-ramnopyranosylquercetin, and their results are shown in figure 1, along with the other flavonoids and their chemical structures. The intestinal absorption rate for avicularin 1mg, 5mg/kg and 1mg/kg with verapamil were 30.56 ± 10.5; 39.49±7.7 and 87.43 ± 9.4, respectively.

The anti-allergic activity assay revealed that BsfcEt/DCM was the most active fraction and its major compounds presented a lower inhibition isolated, what suggests a synergism of these constituents. Also analyzing the fourteen isolated compounds we can establish a structure-activity relationship between them that explains the variations in their activity. A quantitative structure-activity relationship model was estimated using 13 molecular descriptors and showed a multivariated linear structure-activity relationship what supports the variance of the experimental activity of the flavonoids.

The absorption assay with avicularin showed that the increase of dose didn´t affect the rate of absorption, however with the presence of verapamil the absorption was higher, indicating that avicularin is a P-gp substrate. That data becomes significant once avicularin is a flavonoid of dietary intake.

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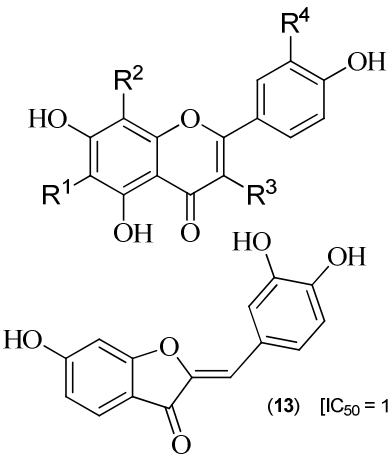

(**1**) R^1 = *C*-β-glicopyranosyl, R^2 = H, R^3 = H, R^4 = H [IC_{50} = 11.2 ± 2.5 μM]
(**2**) R^1 = H, R^2 = H, R^3 = *O*-β-galactopyranosyl, R^4 = OH [IC_{50} = 9.4 ± 1.4 μM]
(**3**) R^1 = H, R^2 = H, R^3 = *O*-β-glicopyranosyl, R^4 = OH [IC_{50} = 13.0 ± 1.2 μM]
(**4**) R^1 = H, R^2 = H, R^3 = *O*-β-xylopyranosyl, R^4 = OH [IC_{50} = 17.6 ± 1.3 μM]
(**5**) R^1 = H, R^2 = H, R^3 = *O*-α-arabinofuranosyl, R^4 = OH [IC_{50} = 21.3 ± 1.2 μM]
(**6**) R^1 = H, R^2 = H, R^3 = *O*-α-rhamnopyranosyl, R^4 = OH [IC_{50} = 55.4 ± 1.4 μM]
(**7**) R^1 = H, R^2 = H, R^3 = *O*-α-arabinofuranosyl, R^4 = H [IC_{50} = 29.2 ± 2.3 μM]
(**8**) R^1 = H, R^2 = H, R^3 = H, R^4 = OH [IC_{50} = 23.3 ± 1.5 μM]
(**9**) R^1 = H, R^2 = H, R^3 = OH, R^4 = OH [IC_{50} = 5.1 ± 1.3 μM]
(**10**) R^1 = H, R^2 = *C*-β-glicopyranosyl, R^3 = H, R^4 = H [IC_{50} = 28.6 ± 2.0 μM]
(**11**) R^1 = *C*-β-glicopyranosyl, R^2 = H, R^3 = H, R^4 = OH [IC_{50} = 38.4 ± 1.2 μM]
(**12**) R^1 = H, R^2 = H, R^3 = *O*-β-glicopyranosyl-coumaroyl, R^4 = OH [IC_{50} = 42.6 ± 1.4 μM]

(**13**) [IC_{50} = 10.5 ± 1.0 μM]

Figure 1. Chemical structures of the isolated flavonoids and their inhibitory effect on the release of the β-hexominidase of RBL-2H3 mast cells lineage [IC_{50}].

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