Chemical constituents and allelopathic activity of *Machaerium eriocarpum* Benth.

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Abstract

The analysis by HPLC-PDA of the hydroalcoholic extract of the leaves of *M. eriocarpum* together with the injection of the fractions containing the already identified metabolites allowed the detection of at least 5 flavonoids, of which two are derived from apigenin and three from luteolin. After isolating larger amounts of isovitexin (I), assays were performed to evaluate the allelopathic activity together with the crude extract.

The results show that the initial inhibition indexes were very similar to those observed in the treatments with F17 (Fraction enriched in isovitexin) and F18 (isovitexin), mainly in the concentrations of 500 and 1000 $mg.L^{-1}$. The index of the number of lateral roots, an increase of the inhibitory effect

Experimental

Chemicals

HPLC-grade chloroform and methanol were purchase from J.T. Baker (Baker Mallinckrodt, USA). HPLC-grade water (18 mV) was prepared using a Milli-Q purification system (Millipore Corp., USA).

Mature and undamaged leaves of *Machaerium eriocarpum* were collected at Campo Florido Farm, Porto Murtinho City, Mato Grosso do Sul, Brazil, in December 2011 by T.E. Lima, A.L.B. Sartori, E.S.S. Lima and F.J. Kochanovski. A voucher specimen of *M. eriocarpum* (CGMS 411323) was deposited at the Herbarium CGMS of Universidade Federal do Mato Grosso do Sul *campus* Campo Grande. The leaves were air-dried and powdered in a mill. The plant material was extracted with ethanol (EtOH) 70% and the extract was protected from light and percolate in 20 drops/minute, resulting in a hydroalcoholic extract. The solvent was evaporated in vacuum to give a dark residue (11.2 % of yield).

Extraction and Isolation

The extract (2.2 g) was dissolved in 15 mL of methanol and was fractionated by gel permeation column chromatography. The column was packed with Sephadex (LH-20, 57 cm x 3,0 cm i.d.) and soaked with methanol. The column was eluted with the same solvent yielded 57 fractions (10 mL each one). The compound (**1**) (10 mg) was obtained from the subfraction 3 (110 mg) through the column chromatography (silica gel) using as eluent the mixture of $CHCl_3$/methanol/ H_2O 80:18:02 v/v. Subfraction 2 was fractionated by column chromatography using as eluent the mixture of $CHCl_3$/methanol/H_2O 43:37:20 (organic phase, v/v) furnishing a yellow precipitated (18 mg, compounds (**2**-**3**). The isolation of the compound (**4**) (5 mg) was obtained by the fractionation of the subfraction 1 using a silica gel column chromatography and as mobile phase the mixture of $CHCl_3$/methanol (90:10, v/v).

Biological assays

The hydroethanolic extract, at doses of 100, 500 and 1000 mg/l, chromatographic fractions at a dose of 1 gl^{-1}, and the isolated flavonoid and fraction enriched with a flavonoid at concentrations at 10^{-7} M, were tested for their effects on the

germination and the radical elongation of *Cucumis sativus* L. (cucumber) and *Sorghum bicolor* (L.) Moench (sorghum). During the test seeds were utilized surface-sterilized in 2% sodium hypochlorite solution for 2 min and then washed using distilled water and sown in Petri dishes (Ø=90 mm), containing two layers of Whatman filter paper, impregnated with 10 ml of distilled water (control) or 10 ml of tested solution. The germination for cucumber and sorghum seeds was performed at 25 ± 1°C with natural photoperiod in growth chamber. Seed germination process was observed when the protrusion of the radical became evident directly in Petri dishes, each 24 h and the final percentage of germination, root growth and number of lateral roots were analyzed in seedlings after 120 h (De Feo et al. 2003; Franco et al. 2015; Ahmed 2017). Each determination was repeated three times, using Petri dishes containing 10 seeds each.

Statistical analysis

The obtained data were submitted to the mean variance analysis and polynomial regression.

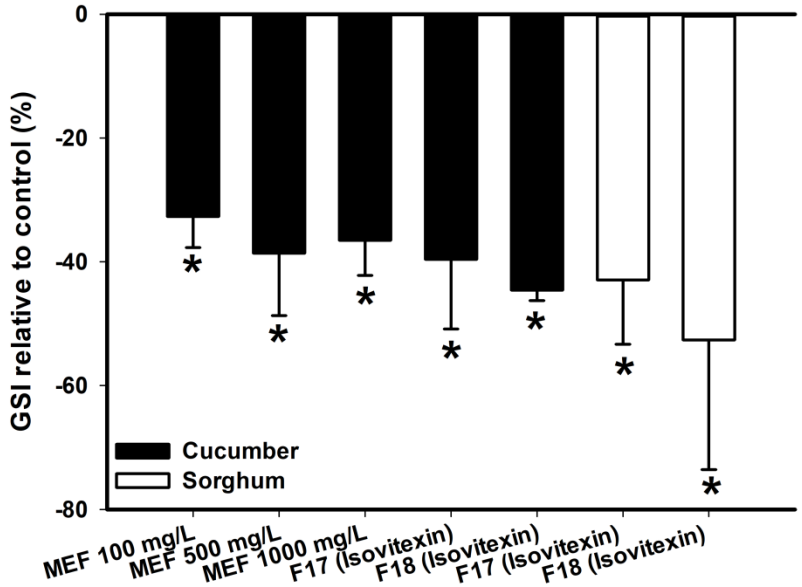

Figure S1: Germination speed index (GSI) of cucumber (black columns) and sorghum seeds (white columns) under effect of leaf extracts of *Machaerium eriocarpum* (MEF) and isovitexin enriched fraction (F17) and flavonoid isovitexin (F18). * Significant difference in relation to control. Significance level $p < 0.01$ by analysis of variance followed by Holm-Sidak test.

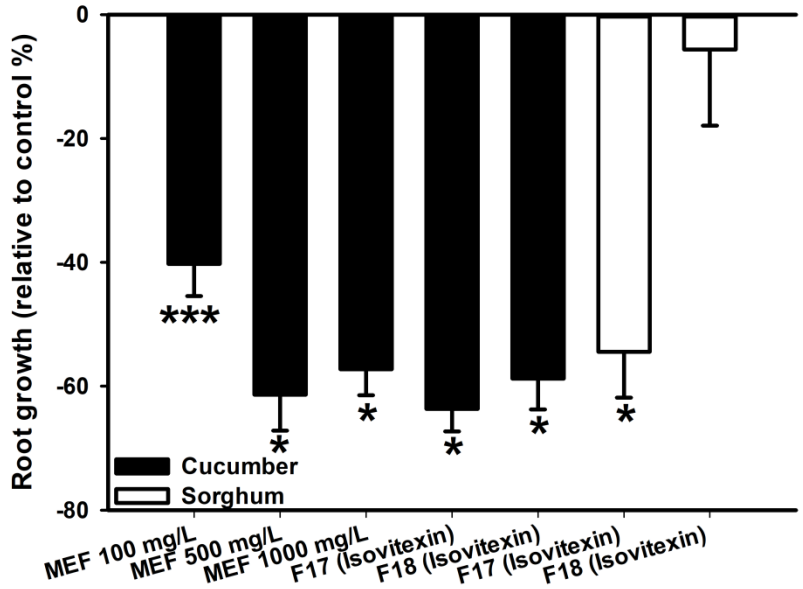

Figure S2: Growth of cucumber (black columns) and sorghum roots (white columns) under effect of *Machaerium eriocarpum* leaf extracts and flavonoid isovitexin. * Significant difference in relation to control. *** Significant difference with respect to isovitexin enriched fraction (F17) and isovitexin isolated (F18). Significance level $p < 0.01$ by analysis of variance followed by Holm-Sidak test.