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Characterization of Main Phenolic Compounds in Eleusine indica L. (Poaceae) by HPLC-DAD and 1H NMR

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Abstract : Eleusine indica L, known as goose-grass, is considered a troublesome weed that can cause important economic losses in the agriculture worldwide. However, this grass is used as a medicinal plant in some regions of Brazil to treat influenza and pneumonia. In Africa and Asia, it is used to treat malaria and as diuretic, anti-helminthic, among other uses. Despite its therapeutic potential, little is known about the chemical composition and bioactive compounds of E. indica. Hitherto, two major flavonoids, schaftoside and vitexin, were isolated from aerial part of the species and showed inhibitory activity on lung neutrophil influxes in mice, suggesting a beneficial effect on airway inflammation. Therefore, the aim of this study was to analyze the chemical profile of aqueous extracts from aerial parts of Eleusine indica specimens using high performance liquid chromatography (HPLC-DAD) and 1H nuclear magnetic resonance spectroscopy (NMR), with emphasis on phenolic compounds. Specimens of E. indica were collected in Minas Gerais state, Brazil. Aerial parts of fresh plants were extracted by decoction (10% p/v). After spontaneous precipitation of the aqueous extract at 10-12°C for 24 hours, the supernatant obtained was frozen and lyophilized. After that, 1 g of the extract was dissolved into 25 mL of water and fractionated on a reverse phase chromatography column (RP-2), eluted with a gradient of H2O/EtOH. Five fractions were obtained. The extract and fractions had their chemical profile analyzed by using HPLC-DAD (C-18 column: 20 µL, 256 -365 nm; gradient water 0.01% phosphoric acid/ acetonitrile. The extract was also analyzed by NMR (1H, 500 MHz, D2O) in order to access its global chemical composition. HPLC-DAD analyses of crude extract allowed the identification of ten phenolic compounds. Fraction 1, eluted with 100% water, was poor in phenolic compounds and no major peak was detected. In fraction 2, eluted with 100% water, it was possible to observe one major peak at retention time (RT) of 23.75 minutes compatible with flavonoid; fraction 3, also eluted with 100% water, showed four peaks at RT= 21.47, 23.52, 24.33 and 25.84 minutes, all of them compatible with flavonoid. In fraction 4, eluted with 50%/ethanol/50% water, it was possible to observe 3 peaks compatible with flavonoids at RT=24.65, 26.81, 27.49 minutes, and one peak (28.83 min) compatible with a phenolic acid derivative. Finally, in fraction 5, eluted with 100% ethanol, no phenolic substance was detected. The UV spectra of all flavonoids detected were compatible with the flavone subclass (λ= 320-345 nm). The 1H NMR spectra of aerial parts extract showed signals in three regions: δ 0.8-3.0 ppm (aliphatic compounds), δ 3.0-5.5 ppm corresponding to carbohydrates (signals most abundant and overlapped), and δ 6.0-8.5 ppm (aromatic compounds). Signals compatible with flavonoids (rings A and B) could also be detected in the crude extract spectra. These results suggest the presence of several flavonoids in E. indica, which reinforces their therapeutic potential. The pharmacological activities of Eleusine indica extracts and fractions will be further evaluated.

Keywords : flavonoids, HPLC, NMR, phenolic compounds
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