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Enrichment fraction of polymethoxyflavones from *Ageratum conyzoides* by centrifugal partition and size-exclusion chromatographies purification steps

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Ageratum conyzoides L., Asteraceae, is a plant widely used in folk medicine for its anti-inflammatory and analgesic properties.[1] Regarding its chemical composition, polymethoxyflavones are abundant and also have demonstrated significant anti-inflammatory activity.[2,3] This study aim to optimize the extraction and to develop a standard purification method to obtain an enriched fraction of polymethoxyflavones from *A. conyzoides* (EFP). In order to obtain a selective method for the extraction of polymethoxyflavones, aliquots of the dried aerial parts (10 g) were extracted using two extraction techniques (Soxhlet extractor and static maceration) with four different solvents (hexane, dichloromethane, ethyl acetate and methanol, 200 ml). Polymethoxyflavones content was established by UPLC-ESI-QToF-MS quantitative method in positive mode. A calibration curve was built with 5'-methoxynobiletin using the extracted ion (*m/z* 433.1499) chromatograms. One-way ANOVA followed by Games-Howell test was used to evaluate the quantification results. Thus, the aerial parts (1.8 kg) were extracted using this optimized selective extraction methodology, with *n*-hexane at 80 ºC in a Soxhlet extractor during 3 h, yielding 33.5 g. The hexane extract (1 g) was subjected to purification using a centrifugal partition chromatography (CPC) instrument Armen SCPC-250 coupled to an Armen Spot Prep II. CPC separation was performed in ascending mode using a biphasic solvent system consisting in hexane-ethyl acetate-ethanol-water (1:1:1:1 v/v/v/v). The stationary phase (lower phase) was pumped into the CPC rotor at a flow rate of 30 ml/min and 500 RPM for 10 min. The mobile phase (upper phase) was conditioned through the rotor at a flow rate of 8 ml/min, at 1800 rpm for 10 min and then the sample was injected into the system. The elution of the fractions was carried out at the analytical conditions for 75 min followed by an extrusion phase lasting 12 min, yielding seven fractions (I-VII). For the final purification step, fractions IV-VII containing polymethoxyflavones of interest were pooled (135.4 mg) based on their TLC profile and separated through a Sephadex LH-20 column with dichloromethane and methanol (7:3, v/v). The resulting EFP corresponds to 7.4% of the hexane extract. The present study allowed a rapid (two steps) purification method for polymethoxyflavones of *A. conyzoides*.

Keywords: *Ageratum conyzoides*, polymethoxyflavones, centrifugal partition chromatography
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Referências

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