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QUANTIFICATION OF METHOXYFLAVONES IN THE AQUEOUS EXTRACT OF *Ageratum conyzoides* (ASTERACEAE)

Larissa Gabriela Faqueti[1], Vincent Brieudes[2], Maria Halabalaki[2], Alexios Leandros Skaltsounis[2], Maique Weber Biavatti[1].

[1]Universidade Federal de Santa Catarina, Florianópolis, Brazil; [2]National and Kapodistrian University of Athens, Athens, Brazil; larissafaqueti@gmail.com

Abstract: *Ageratum conyzoides* L., known as "mentrasto", is used in Brazilian folk medicine as analgesic, anti-inflammatory and antirheumatic [1]. The aqueous extract of the leaves of this plant in a clinical trial in patients with arthrosis revealed substantial reduction of chronic pain and improvement of articular mobility [2]. Among the compounds isolated in this specie are the methoxyflavones, a very rare class of flavonoids [3]. The present work has the objective of develop and validate an analytical method for quantification of the methoxyflavones in the aqueous extract of the leaves of this specie by Ultra Pressure Liquid Chromatography (UPLC). The leaves were collected in Florianópolis, Brazil and the aqueous extract was prepared by infusion of the fresh leaves with boiling purified water for 15 minutes. The qualitative analyses were performed with mass spectrometer LQT-Orbitrap Discovery XL coupled to Accela system and the quantitative analyses performed on an ACQUITY UPLC system equipped with photo diode array detector (PDA), both using an Acquity UPLC BEH C18 column (150 mm x 2.1 mm, 1.7 µm) at 25ºC. The mobile phase consisted of a gradient system combining 0.1% aqueous formic acid and acetonitrile for 11 minutes, at a flow rate constant of 300 µL/min. The UV detector was set at a monitoring wavelength 330 nm. The method was validated following the linearity, detection (DL) and quantification (QL) limits, specificity, precision (repeatability and intermediate precision) and accuracy parameters according to ICH (2005) [4]. Qualitative analyses revealed the presence of eleven methoxyflavones, 5,6,7,3',4',5'-hexametoxyflavone (**1**); nobiletin (**2**); 5'-methoxynobiletin (**3**) and eupalestin (**4**) were quantified. The results of method validation showed correlation coefficients higher than 0,999 for the four standards tested indicating a good linearity**,** with DL between 0,004-0,0075 µg/g and QL between 0,015-0,02 µg/g. The method also is accurate (recovery of 100-101%) and precise (reproducibility RSD of 10-14%; intermediate precision RSD of 9-14%). The quantitative analyses of the *A. conyzoides* aqueous extract showed a content of 2,84 ± 0,5 mg/g, 1,50 ± 0,1 mg/g, 6,17 ± 0,5 mg/g and 1,24 ± 0,2 mg/g, respectively to **1**, **2**, **3** and **4**. Therefore, a simple and rapid UPLC-PDA method to quantify the methoxyflavones in *A. conyzoides* aqueous extract was developed and validated, which can be applied at the quality control of this specie preparation.

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