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ERIODICTYOL: ISOLATION, QUANTIFICATION FROM THE LEAVES OF *Vernonanthura tweedieana* AND ANTIPROTOZOAL EVALUATION

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Abstract: *Vernonanthura tweedieana* (Baker) H. Rob. is known in Brazil as "assapeixe" and its leaves used popularly for the treatment of respiratory diseases [1]. The aim of this work was develop and validate an UPLC-PDA method for quantification of eriodictyol in ethanol extract of *V. tweedieana* leaves and evaluate the antiprotozoal effect of eriodictyol *in vitro*. Fresh leaves (2.6 kg) were macerated with ethanol 75% at room temperature, for 56 days. The resulting crude extract (172.0 g) was partitioned, yielding *n*-hexane (17.9 g), dichloromethane (3.6 g), ethyl acetate (EtOAc, 10.4 g) and the aqueous residual (104.3 g) fractions. The EtOAc fraction was subjected to successive silica gel chromatographic procedures allowing the isolation of eriodictyol (104.7 mg), whose elucidation was established by ^{1}H and 2D NMR spectroscopy and in comparison with published data. The quantitative analyses of eriodictyol were performed in an *Acquity UPLC* system equipped with photo diode array (PDA) detector, using an *Acquity UPLC BEH* C_{18} (2.1 x 50 mm i.d., 1.7 µm) column at 40 ºC. The mobile phase consisted of a 7 min gradient system combining 0.1% aqueous formic acid and acetonitrile with a flow rate of 0.4 ml.min^{-1}. The chromatograms were recorded at wavelength of 287 nm. The UPLC-PDA method was validated in terms of specificity, linearity, precision (repeatability and intermediate precision), detection (LOD) and quantification (LOQ) limits, accuracy and robustness (variables evaluated were flow, column temperature and modifier content) parameters [2]. Eriodictyol was tested against *Leishmania amazonensis* and *Trypanosoma cruzi* amastigotes expressing β-galactosidase, according method described by Lima et al. [3], and the results were expressed in percentage of protozoal growth inhibition. The correlation coefficient (0.9999) revealed a great linearity between 1.5-30.0 µg/g, with LOD and LOQ of 0.02344 and 0.06214 µg/g, respectively. The results showed a good precision (RSD=2% for repeatability; RSD=8% for intermediate precision) and accuracy (average recovery from 98.6% to 99.7%, RSD<3%). The robustness revealed that all variables were critical for the method and should be carefully controlled. The quantitative analysis of *V. tweedieana* extract showed an eriodictyol content of 41.40 ± 0.13 mg/g (RDS=0.31%). Eriodictyol presented a weak trypanocidal activity (22.65% of growth inhibition) and demonstrated no leishmanicidal activity. A simple and fast UPLC-PDA method for quantification of eriodictyol in ethanol extract from *V. tweedieana* leaves was developed and validated, which can be used to the quality control of *V. tweedieana* preparations.

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