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A single-step isolation of glaucolide B from *Lepidaploa chamissonis* by centrifugal partition chromatography

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Sesquiterpene lactones (SL), commonly found in Asteraceae, have many biological applications.[1] *Lepidaploa chamissonis* (Less) H. Rob., known in Brazil as "cambarazinho",[2] have not been previously chemical and biologically studied. Considering the cytotoxicity and antiparasitic SL effects, this work aimed to develop a simple method for the SL glaucolide B purification from *L. chamissonis* leaves extract by centrifugal partition chromatography (CPC). Glaucolide B was formerly isolated from *Lepidaploa* species[3] and was moderately leishmanicidal.[4] Entire and dried leaves of *L. chamissonis* (278.38 g) were submitted to a washing extractive process with acetone (1:50), during 10 min. The obtained extract (4.11 g) was defatted by a liquid-liquid partition, yielding *n*-hexane (0.98 g), ethyl acetate (EtOAc, 2.15 g) and aqueous (1.08 g) fractions. SL enriched EtOAc fraction (2 g) was purified by CPC on an Armen Instrument SCPC-250-L coupled to a Spot Prep II system (equipped with a quaternary pump, PDA detector and an automatic collector). Data was processed with *Armen Glider CPC v.5.0* software. The CPC separation was developed in descending mode using the biphasic solvent system Arizona M (hexane/EtOAc/methanol/water, 5/6/5/6, v/v/v/v). The lipophilic upper phase was loaded into to the CPC 250 ml-column at flow rate of 30 $ml.min^{-1}$, at 500 rpm, during 10 min. The hydrophilic mobile lower phase was pumped through the stationary phase for 10 min, at flow rate of 8 $ml.min^{-1}$, at 1600 rpm (analytical conditions), until equilibrium (83% of stationary phase retention). The sample was dissolved in 10 ml of upper/lower phase mixture (1/1, v/v) and injected into the system. The mobile phase was pumped at the analytical conditions during 70 min, with a collection of 7 ml/fraction. Subsequently, the upper phase was pumped at flow rate of 30 $ml.min^{-1}$, at 1600 rpm, during 15 min, for the extrusion step, with a collection of 10 ml/fraction. CPC eluted fractions were monitored at 287 and 330 nm and pooled according to its TLC profile, yielding nine fractions (A-I). Fraction G afforded 1.04 g of glaucolide B corresponding to 25% yield of the extract. Its structure was characterized by NMR spectroscopy, HR-ESIQToF-MS and in comparison with reported data. The leaf washing turned to be a selective method for the extraction of SL whereas CPC technique proved to be a simple and efficient tool for the isolation of glaucolide B within few hours. Glaucolide B has been isolated for the first time from this species.

Keywords: *Lepidaploa chamissonis*; glaucolide B; centrifugal partition chromatography
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