10th Brazilian Conference on Natural Products
XXXVI RESEM
4-7 November 2025, Belo Horizonte, MG, Brazil
Section: 02

ISOLATION OF AMYRINIC TRITERPERNES FROM PROTIUM HEPTAPHYLLUM OLEORESIN

Stefany Marques da Silva (IC)[1*], Michelle Frazão Muzitano (PQ)[1], Danilo Ribeiro de Oliveira (PQ)[2], Shaft Côrrea PINTO (PQ)[1].

*stefolegario@gmail.com

1-Laboratório de Produtos Bioativos, UFRJ-Macaé, Av. Aloísio da Silva Gomes, 50, Macaé, RJ, 27930-560, Brazil. 2-Laboratório de Bioprospecção e Etnofarmacologia Aplicada, UFRJ-Fundão, Av. Carlos Chagas Filho, 373, Rio de Janeiro, RJ, 21941-170, Brazil.

The oleoresin of *Protium* has been traditionally employed in the treatment of several patologies and has attracted pharmacological and cosmetic interest (Albino et al., 2021). In this context, the present study aims to fractionate this oleoresin in order to isolate and identify amyrinic triterpenes, providing material for quality control and future biological assay. For this purpose, the oleoresin (401.6 mg) was fractionated by HSCCC under the following conditions: biphasic solvent system Hex:AcN (1:1, v/v), mobile phase = lower phase, column volume (Vc) = 113 mL, flow rate 2.0 mL/min, rotation = 850 rpm (stopped at KD = 2, VR = 207.36 ~ tube 52), Sf = 84%, 4.0 mL/tube, extrusion with the stationary phase (SP). Fractions were screened by TLC (silica gel F254, $CHCl_3$ 100% v/v, vanillin–sulfuric acid), compared with α-amyrin standard. Fractions containing amyrins were analyzed by HPLC-DAD: isocratic elution (95% AcN, 5% H_2O + 0.05% TFA); λ = 202 nm; flow = 1.0 mL/min; column: Luna C18 (250mm×4.6mm×5µm); T=40°C. Isolation of amyrins was performed by semipreparative HPLC-DAD under the same conditions as for the qualitative analysis, except for the use of a Luna C18 column (250mm×10mm×5µm) and a flow rate of 3.0 mL/min. Fractions 67–77 showed indications of amyrin presence when compared to the commercial standard and were analyzed individually by HPLC-DAD, and those with similar patterns were combined in order to increase the mass available. So, fractions were grouped into three sets: 68–69, 70–72, and 73–77. Comparing the chromatographic profile of fraction 70–72 with the results of Haralampidis et al. (2001), the presence of γ-amyrin, α-amyrin, and β-amyrin was suggested. Continuing with the separation process by HPLC-DAD, fraction 70–72 resulted four isolated substancies, which will be subjected to NMR. The remaining combined fractions will also undergo semipreparative separation. In conclusion, the biphasic solvent system employed for resin partitioning led to the obtainment of an amyrin mixture, as suggested by TLC and HPLC-DAD results. Finally, semipreparative HPLC-DAD enabled the isolation of four peaks, which will be characterized by NMR techniques, including ^{1}H, ^{13}C, HSQC, HMBC, COSY, and NOESY, and compared with existing literature data.

Keywords: *Protium, oleoresin, isolation, amyrin, chromatography.*