Detection of Diterpenes in the Oleoresin of *Eperua oleifera* Ducke (Fabaceae) and Isolation of a Naturally Occurring Methyl Ester of a Diterpenic Acid

Rayssa Ribeiro[1*], **Anna Cristina da Costa e Souza**[1], Henrique Marcelo G. Pereira[2], Monica Costa Padilha[2], Valdir Florencio da Veiga Junior [1]

annasouz@gmail.com

1-Military Institute of Engineering, Rio de Janeiro, Praça General Tibúrcio, 80, Praia Vermelha, Urca, 22290-270, Brazil, 2-Federal University of Rio de Janeiro, Chemistry Institute, Brazilian Doping Control Laboratory (LBCD/IQ – UFRJ), Rio de Janeiro, RJ 21941-909, Brazil;

Oleoresins are plant exudates composed solely of terpenes, typically consisting of volatile monoterpenes and sesquiterpenes that solubilize heavier diterpenes and triterpenes. Studies on *Eperua* species, such as *E. purpurea* and *E. leucantha*, revealed compositions mainly of diterpenic acids and minor alcohols, particularly labdane-type diterpenes (copalic, cativic, and eperuic acids). Other plant parts of *Eperua* species have been reported to contain phenolic acids, flavonoids, sesquiterpenes, triterpenes, and abundant diterpenes. The oleoresin of *Eperua oleifera* Ducke, commonly known as "copaíba-jacaré," is traditionally used in folk medicine for wound healing and as an antifungal and antibacterial agent, similar to species of the genus *Copaifera* (Fabaceae). Nevertheless, despite its pharmacological potential, few studies have investigated *E. oleifera* to confirm its bioactivity in relation to its chemical composition. In this context, the present study examined the oleoresin of *E. oleifera* collected in the Brazilian Amazon region. After derivatization, the sample was analyzed by GC-MS to identify its chemical constituents and characterize the volatile fraction, with the data compared against the literature, mass spectral libraries, and previously isolated standards. Furthermore, neutral and acidic fractions were separated using open-column chromatography and ion-exchange chromatography (KOH-impregnated silica as the stationary phase). This approach resulted in the identification of twelve diterpenes, including nine carboxylic acids and three alcohols, with hardwickiic and copalic acids as the primary components, which NMR fully characterized. Ion-exchange chromatography proved to be more selective, enabling the direct isolation—without subsequent steps—of a methyl ester identified by HRMS and NMR as the methyl ester of hardwickiic acid. This represents the first report of such a compound in oleoresins. We conclude that GC-MS analysis enabled the identification of diterpenes based on known fragmentation patterns. Furthermore, ion-exchange chromatography combined with infusion experiments allowed the isolation and detection of a naturally occurring methyl ester that, to our knowledge, had not been previously reported in oleoresins. This novel finding highlights the potential for discovering additional esters in oleoresins through infusion-based analyses.

Keywords: *Eperua oleifera Ducke, GC-MS, diterpene acids, HRMS, Hardwickiic acid methyl ester*