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METABOLOMIC ANALYSIS AND ANTIMICROBIAL ACTIVITY OF FRACTIONS FROM THE ETHYL ACETATE PHASE OF CASEARIA SYLVESTRIS

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Phytochemical studies on *Casearia sylvestris* Sw. (Salicaceae), popularly known as "guaçatonga", have revealed a predominance of clerodane diterpenes. These metabolites are taxonomic markers for the genus *Casearia* and are associated with diverse biological activities, including anti-inflammatory, antitumor, and antiulcerogenic effects[1]. The chemical profile of CSA fractions, obtained from chromatographic separation of the ethyl acetate phase, was characterized by UHPLC-HR-ESI-MS/MS. Fragmentation data were processed using the *Global Natural Products Social Molecular Networking* (GNPS2) platform, enabling molecular networking and putative metabolite annotation. The analysis revealed clusters consistent with clerodane diterpene spectra, showing characteristic neutral losses of butanoic acid (88 Da) and acetaldehyde (44 Da). The antimicrobial activity of CSA fractions was assessed by the agar diffusion method against clinically relevant strains: *Acinetobacter baumannii* ATCC 19606, *Escherichia coli* ATCC 25922, *Enterobacter cloacae* clinical isolate 79101, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, methicillin-resistant *Staphylococcus aureus* (MRSA) 29213, and *S. aureus* ATCC 43300. Notably, zones of inhibition were observed for *P. aeruginosa*, a pathogen classified by the World Health Organization (WHO) as a critical-priority target for new antimicrobial development due to high resistance rates and limited therapeutic options[2]. Samples CSA-16B-1, CSA-16B-2, and CSA-21-5, derived from chromatographic fractionation of the ethyl acetate fraction, exhibited inhibition zones of 12.9 mm, 16.1 mm, and 8.0 mm, respectively, indicating a potential inhibitory effect against this difficult-to-treat microorganism. According to the WHO, multidrug-resistant *P. aeruginosa* is a major cause of severe hospital-acquired infections, associated with high morbidity and mortality, particularly in immunocompromised patients. The detection of inhibitory activity against this species in the ethyl acetate fractions of *C. sylvestris* highlights the relevance of further studies aimed at isolating and characterizing active compounds for the development of novel antimicrobial agents. The authors acknowledge the support of the participating institutions and the financial assistance of CEPID-ARIES, FAPESP, CAPES, CNPq, and CEMUD-UNIFESP.

Keywords: *Casearia sylvestris, clerodane diterpene, GNPS2, antimicrobial activity*

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