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ISOLATION AND STRUCTURAL ELUCIDATION OF THE FLAVONOIDS QUERCITRIN AND AFZELIN FROM THE LEAVES OF COPAIFERA OBLONGIFOLIA

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The *Copaifera* genus is a prominent source of secondary metabolites with pharmacological relevance, motivating the development of efficient phytochemical strategies for the isolation of its constituents. This study aimed to develop a comprehensive chromatographic protocol to isolate and identify the major flavonoids present in the leaves of *Copaifera oblongifolia*, a species that remains chemically underexplored. The process began with the obtention of a crude hydroalcoholic extract (64.84 g) from dried and powdered plant material (913.7 g) through five successive extractions. The extract was then subjected to liquid-liquid partitioning, yielding an enriched dichloromethane fraction (FD) of 8.9 g. This fraction was submitted to fractionation over a silica gel 60 column, using a solvent gradient of increasing polarity, which generated 110 fractions of 45 mL each. Thin-layer chromatography analysis revealed that fractions 8 to 11 showed similar chromatographic profiles and were subsequently pooled. The final purification of this group was achieved by preparative High-Performance Liquid Chromatography (HPLC) on a Gemini C-18 column, using an isocratic mobile phase of methanol and acidified water (57:43 v/v) at a flow rate of 10.0 mL/min. This procedure successfully yielded 70 mg of compound 1 and 30 mg of compound 2 in high purity. Structural elucidation, based on extensive analysis of mass spectrometry (ESI-MS) and nuclear magnetic resonance (^{1}H and ^{13}C NMR) data, and by comparison with literature values, unambiguously identified the compounds as the flavonoids quercitrin (1) and afzelin (2). The developed multi-step chromatographic methodology proved to be robust and effective for the targeted isolation of flavonoids from *C. oblongifolia*, providing pure substances in sufficient quantities for subsequent studies, such as biological activity assays.

Keywords: *Phytochemistry, Secondary Metabolites, Chromatography, Fabaceae, Spectroscopic Analysis.*