ISOLATION OF CANNABINOIDS BY PREPARATIVE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY: TETRAHYDROCANNABINOL AND CANNABIDIOLIC ACID

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Cannabis sativa L. (Cannabaceae) is a plant of growing pharmacological interest due to its wide range of bioactive compounds, with emphasis on its phytocannabinoids.[1] This study aimed to isolate cannabinoids from resin samples extracted from *C. sativa* inflorescences using preparative high-performance liquid chromatography (preparative HPLC), followed by structural identification. 2,4-dihydroxy-3-[(*1R,6R*)-3-methyl-6-prop-1-en-2-ylcyclohex-2-en-1-yl]-6-pentylbenzoic acid (cannabidiolic acid or CBDA) was isolated from the non-decarboxylated resin of the Feno Swiss variety, while (−)-*trans*-Δ^9-tetrahydrocannabinol (tetrahydrocannabinol or THC) was obtained from the decarboxylated resin of the Northern Lights variety. Initial analyses were performed separately by analytical HPLC for each resin. To improve separation efficiency, fractionation with Sephadex LH-20 using methanol as eluent was carried out individually. The resulting eluates were pooled into three fractions based on chemical similarity, assessed by thin-layer chromatography (ethyl acetate:hexane 1:1). These fractions were further analyzed by analytical HPLC, and fraction 2, the most abundant in both cases, was selected for preparative HPLC using a 71-minute method. The samples were prepared by dissolving in 10 mL of acetonitrile and 5 mL of water, both containing 0.1% formic acid, with acetonitrile as solvent B and water as solvent A. A small sample of the collected peaks were reanalyzed by analytical HPLC, and those with sufficient purity and content were dried and dissolved in deuterated chloroform for ¹H and ¹³C NMR analysis. Identification, based on comparison with spectral data reported by Brenneisen *et al.* (2004)[2], confirmed the successful isolation of CBDA and THC from their respective resin samples. This work contributes to the development of strategies for cannabinoid isolation, especially when commercial standards are not available. The authors thank their institution for support and CAPES for financial assistance.

Keywords: *Cannabis sativa, cannabidiolic acid, tetrahydrocannabidiol, preparative HPLC, ¹H NMR, ¹³C NMR.*

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