

Chemical Study and Cytotoxic Activity of Extracts from *Erythroxylum* Genus against HeLa Cells

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Abstract : Recognized as a global biodiversity hotspot, the Cerrado (Brazil) presents an extreme abundance of endemic species and it is considered to be one of the biologically richest tropical savanna regions in the world. *Erythroxylum* genus is found in Cerrado and chemically is characterized by the presence of tropane alkaloids, among them cocaine, a natural alkaloid produced by *Erythroxylum coca* Lam., which was used as a local anesthetic in small surgeries. However, cocaine gained notoriety due to its psychoactive activity in the Central Nervous System (CNS), becoming one of the major problems of public health today. Some species of *Erythroxylum* are referred to in the literature as having pharmacological potential, which provide alkaloids, terpenoids, and flavonoids. *E. vacciniifolium* Mart., commonly known as 'catuaba', is used as a central nervous system stimulant and has aphrodisiac properties and *E. pelleterianum* A. St.-Hil. in the treatment of stomach pains. Already *E. myrsinites* Mart. and *E. suberosum* A. St.-Hil. are used in the tannery industry. Species of *Erythroxylum* are also used in folk medicine for various diseases, against diabetes, antiviral, fungicidal, cytotoxicity, among others. The Cerrado is recognized as the richer savannah in the world in biodiversity but little explored from the chemical view. In our on-going study of the chemistry of *Erythroxylum* genus, we have investigated four specimens collected in central Cerrado of Brazil: *E. campestre* (EC), *E. deciduum* (ED), *E. suberosum* (ES) and *E. tortuosum* (ET). The cytotoxic activity of extracts was evaluated using HeLa cells, in vitro assays. The chemical investigation was performed preparing the extracts using n-hexane (H), dichloromethane (D), ethyl acetate (E) and methanol (M). The cells were treated with increasing concentrations of extracts (50, 75 and 100 µg/mL) diluted in DMSO (1%) and DMEM (0.5% FBS and 1% P/S). The IC₅₀ values were determined measured spectrophotometrically at 570 nm, after incubation of HeLa cell line for 48 hours using the MTT (SIGMA M5655), and calculated by nonlinear regression analysis using GraphPad Prism software. All the assays were done in triplicate and repeated at least two times. The cytotoxic assays showed some promising results with IC₅₀ values less than 100 µg/mL (ETD = 38.5 µg/mL; ETM = 92.3 µg/mL; ESM = 67.8 µg/mL; ECD = 24.0 µg/mL; ECM = 32.9; EDA = 44.2 µg/mL). The chemical profile study of ethyl acetate (E) and methanolic (M) extracts of *E. tortuosum* leaves was performed by LC-MS, and the structures of the compounds were determined by analysis of ¹H, HSQC and HMBC spectra, and confirmed by comparison with the literature data. The investigation led to six substances: α-amyrin, β-amyrin, campesterol, stigmastan-3,5-diene, β-sitosterol and 7,4'-di-O-methylquercetin-3-O-β-rutinoside, with flavonoid the major compound of extracts. By alkaline extraction of the methanolic extract, it was possible to identify three alkaloids: tropacocaine, cocaine and 6-methoxy-8-methyl-8-azabicyclo[3.2.1]octan-3-ol. The results obtained are important for the chemical knowledge of the Cerrado biodiversity and brought a contribution to the chemistry of *Erythroxylum* genus.

Keywords : cytotoxicity, *Erythroxylum*, chemical profile, secondary metabolites

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