



PURIFICATION AND ISOLATION OF FLAVONOIDS FROM LEAVES OF *Ziziphus joazeiro* BY pH-ZONE-REFINING HIGH-SPEED-COUNTER-CURRENT-CHROMATOGRAPHY AND SEMIPREPARATIVE HPLC

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Abstract: *Ziziphus joazeiro* Martius (Rhamnaceae), popularly known as “joazeiro” and “juá” is a plant from the Caatinga biome, whose leaves and inner bark are traditionally used as toothpaste, hair washer and for stomachache relief [1]. The phytochemical studies mainly focus in the isolation and structural elucidation of triterpenoids and triterpene saponins from the inner bark [2,3]. However, the studies regarding the secondary metabolites of leaves are scarce. In this context, the aim of this work was the isolation of flavonoids from the leaves of *Ziziphus joazeiro* by means of pH-zone-refining high-speed-counter-current-chromatography (HSCCC) followed by semipreparative HPLC. The leaves of *Ziziphus joazeiro* were collected in Rio Grande do Norte, Brazil, air-dried, powdered and extracted by maceration in ethanol-water (70:30, v/v). The extract was filtered, concentrated under reduced pressure and partitioned with petroleum ether, chloroform, ethyl acetate (EtOAc) and n-butanol. The EtOAc (1.5 g) fraction was selected for further analysis because of its rich content in flavonoids, being submitted to a pH-zone refining HSCCC separation on a PC Inc[®] HSCCC equipment, coupled with a 110 mL multilayer coiled column. The conditions were a two-phase solvent system in reverse mode composed of ethyl acetate: n-butanol: water (1:0,4:1, v/v/v), with a two-step pH gradient (pH 8 to pH 10), under 600 rpm and flow of 1.2 mL/min. The process afforded 19 fractions, and fraction 13, which contained the major flavonoids, was selected for further purification in a RP-18 column, followed by a two-step semipreparative HPLC separation in a Hypersil GOLD Cyano column (250x10 mm, 5 µm). The separation afforded 5 compounds. Compound 1, 3,3 mg, purity = 99,96 %, λ_{max} UV = 257, 354 nm; Compound 2: 1.5 mg, purity = 99,99 %, λ_{max} UV = 266, 345; Compound 3: 1.4 mg, purity = 100 %, λ_{max} UV = 255, 354. Compound 4: 4,7 mg purity = 99,98 %, λ_{max} UV = 255, 353. Compound 5: 0,9 mg, purity = 99,93 %, λ_{max} UV = 257, 348. According to previous results, compound 1 may be rutin and the extract may contain quercetin and kaempferol derivatives. In conclusion, the pH-zone-refining HSCCC showed to be a useful technique for preparative separation of flavonoids from *Ziziphus joazeiro* leaves, affording purified fractions which, with a few more steps, yielded isolated compounds. The leaves of this specie have a complex flavonoid profile, but the techniques used in the study may allow the isolation and identification of new compounds.

References:

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