



ISOLATION OF CASCARA SAGRADA (*Rhamnus purshiana*) CONSTITUENTS AND CYTOTOXICITY EVALUATION

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Abstract: The *Rhamnus purshiana*, known as cascara sagrada, is a medicinal plant widely used to constipation treatment. Despite the extensive use of anthranoids laxatives to treat chronic constipation, this active principles lead to melanosis of patient's rectum [1]. Therefore, the aim of this study is isolate the cascarosides stereoisomers and evaluate their toxicity against cancer cell lines. The powder of cascara bark was extracted by percolation process. The crude extract (CASCrExt) obtained was dried and submitted to a partition with hexane, ethyl acetate and methanol/water (1:1 – CASMeWFas). After lyophilized, 1.5137 g of CASMeWFas was chromatographed in classical column using silica gel (35-75 Mesh) and ethyl acetate: methanol as mobile phase. The fractions were analyzed in LC-MS (ESI-qTOF) using a C₁₈ column and flow of 1 mL.min⁻¹. Water and methanol were used in all chromatographic analysis as mobile phase, both with 0.1% of TFA (Analytical method: 20% (B) – 0 min, 20% (B) – 20 min, 30% (B) – 26 min, 30% (B) – 50 min, 50% (B) – 66 min, 100% (B) – 86 min). The fraction CSCFr124-145 (672.7 mg) was fractionated in HPLC-semiprep using a C₁₈ column and flow of 9 mL.min⁻¹ (Method: 20% (B) – 0 min, 20% (B) – 39 min, 30% (B) - 49 min, 30% (B) – 55 min, 50% (B) – 90 min, 100% (B) – 91 min). Cascarosides A, B, C and D were isolated, identified by MS and NMR (¹H, ¹³C, Dept, NOESY) and submitted to cytotoxicity evaluation using HeLa, T98-G, K562 and HL-60 cell lines at concentrations of 1, 10, 50, 100, 500 and 1000 µg.ml⁻¹/ µm.ml⁻¹. The developed method was efficient to isolate cascara constituents and the yield was 2.1%, 2.1%, 0.9% and 1.4%, for cascarosides A, B, C and D, respectively. The IC₅₀ for CASCrExt was 124-129 µg.ml⁻¹ against HeLa and T98-G cell lines, while the cascarosides were not toxic (over 1600 µm.ml⁻¹). CASCrExt exhibit an IC₅₀ of 7.8 and 2.9 µg.ml⁻¹ for K562 and HL-60 cells, respectively; while the cascarosides were less toxic (for K562 cells were 461 to 754 µm.ml⁻¹ and for HL-60 cells were 20.63, 103.9, 103.1 and 743.7 µm.ml⁻¹ for cascarosides B, A, D and C, respectively). No toxicity difference was founded between the cascarosides isomers, except for HL-60 cells. Hence, cascarosides were less cytotoxic compared to CASCrExt, indicating the cytotoxic compounds may not be them or they are acting together with other toxic small molecules [2-3] to promote cytotoxicity of herbal medicines and in cancer cells.

References

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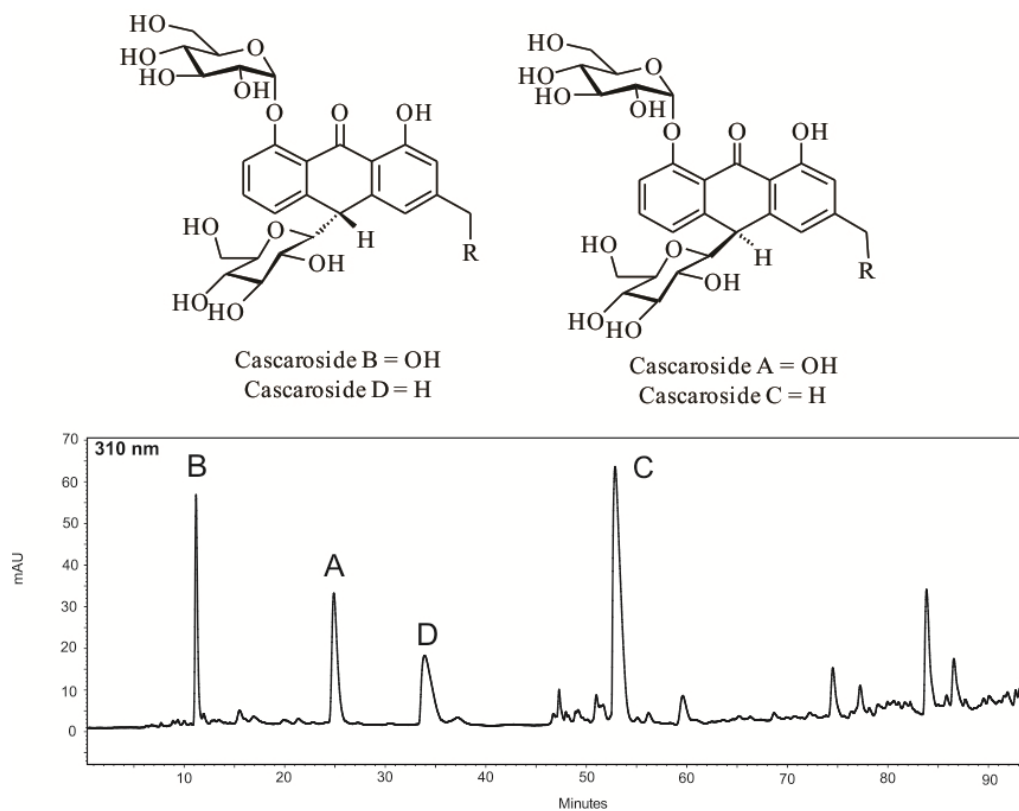


Fig 1 Chromatogram of CASMeWFs submitted to HPLC-semiprep and the cascariosides isolated.