

Characterization of novel compounds from black beans (*Phaseolus vulgaris L.*): selective supercritical extraction

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Background: phytochemicals extracted from black beans (*Phaseolus vulgaris L.*), have been studied especially for their antioxidant, anti-inflammatory, hypocholesterolemic, and inhibitory effects in the absorption of lipids. Saponins, anthocyanins, and flavonoids are among the compounds identified with these bioactivities. Nonetheless, it is known the presence of unidentified compounds in the black beans that may have a synergistic effect with the well-known phytonutrients. **Objective:** this research aimed at the characterization of novel compounds extracted from a germinated black bean mixture of hulls and sprouts with the implementation of supercritical fluid extraction with CO₂ (SFE). Furthermore, the obtained profile was evaluated towards the potential effect in lowering cholesterol absorption. **Materials and Methods:** samples containing a mixture of hulls, sprouts, and cotyledons in a proportion of 64:16:20, respectively, were used. Selective extraction was performed using 20 g of material in a Thar SFE 1 L equipment team with CO₂ at 99.9% purity. A response surface methodology with three levels of pressure (100- 400 bar), temperature (40- 70 °C) and a co-solvent flow rate (aqueous ethanol, 70%; 5- 15 %) was evaluated. The CO₂ flow rate was fixed at 20 g/min, for 50 min. Conventional methanolic and hexane extractions (1:10 w/v) were used to compare the phytochemical profile. Characterization and quantification of novel compounds were performed using a GC-MS and an HPLC-DAD-ELSD. The hypocholesterolemic effect of the novel compound profiles was evaluated using the inhibition of *in vitro* micellar solubility of cholesterol. **Results:** selective extraction at 250 bar, 55 °C and 10% co-solvent, exhibited a similar profile compared to the conventional extraction with hexane. The pressure, temperature and percentage of co-solvent significantly affected the global yield and phytochemical profile. The treatment at 100 bar, 40 °C and 10% co-solvent, selectively extracted non-polar compounds with different profile compared to the conventional extraction with hexane; whereas 100 bar, 70 °C and 10% co-solvent, also extracted triterpenes compounds. The novel compounds profile significantly affected the inhibition of cholesterol micellization of the extracts. **Conclusions:** the SFE allowed the extraction of novel non-polar compounds and different phytochemical profile of black beans (*Phaseolus vulgaris L.*) hulls and sprouts than the conventional extraction with hexane. The selective SFE of novel non-polar compounds with bioactivity can be used as health-promoting for medical applications, i.e., in the metabolic syndrome ailments; or cosmetic applications without further purification steps.

Keywords: non-polar compounds; *Phaseolus vulgaris L.*; selective extraction; supercritical fluids; GC-MS; HPLC-DAD-ELSD.