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Antiproliferative and antioxidant effect of polar hemp (*Cannabis sativa L*) extracts

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The health benefits and nutritional values of hemp products are attributed to its unique fatty acid composition and bioactive minor components, including polyphenols. Polyphenolic compounds from a large variety of plant foods, spices and beverages have been shown to inhibit or attenuate the initiation, progression and spread of cancers both in cells *in vitro* and in animals *in vivo*. In the present investigation, we aimed at extracting and characterizing the phenolic compounds from seed, flour and oil obtained from a single hemp cultivar, Fedora, which is a monoecious EU registered hemp genotype. Furthermore, we assessed their potential effects on modulating cell death and cell growth in Caco-2 cell line, deriving from a human adenocarcinoma and largely used as a model resembling the intestinal epithelium. Phenolics from seeds, flour and hemp oil were extracted with MeOH / H_2O (80/20, v/v). The total polyphenol content determined by the Folin-Ciocalteu method ranged from 767, 744 and 21 mg GAE (gallic acid equivalents)/ 100 g for seeds, flour and oil, respectively. However, the HPLC-based determination yielded higher values for the total polyphenol content, being 1540, 1078 and 23.5 mg CAE (caffeic acid equivalents) / 100 g, respectively. The antioxidant activity determined with the DPPH method exhibited intense profiles of radical scavenging properties, accounting for 51.5, 46.8 and 8.2% for seeds, flour and oil respectively. The HPLC-DAD and LC-MS profiles of the extracts from flour and seeds were, as expected, strictly similar whereas they significantly differed from the oil counterpart. The most abundant components of seeds and flour extracts were lignanamides, namely N-trans-caffeoyltyramine and cannabisin B along with structurally correlated compounds (e.g. N-trans-feruloyl and coumaroyl tyramine), as confirmed by high-resolution MS/MS analysis. In contrast, the oil extracts were particularly complex and their characterization is still in progress. The effect on cell proliferation was measured by the CyQuant viability assay. Among the three extracts tested, only hemp oil was able to reduce significantly cell proliferation in Caco-2 cells of about 40% after 48 h of treatment at a concentration of 70-100 mg/ml (w/v). The same preparation doubled caspase-3 activity, a biochemical marker of apoptotic cell death and reduced ROS (reactive oxygen species) intracellular levels of about 30%, while hemp seed and flour preparations showed a pro-oxidant behavior. Considering that the latter extracts possessed higher phenolic content and antioxidant capacity, our data suggest that the biological effects of hemp oil do not exclusively depend upon its antioxidant activity and total polyphenolic content. Probably, an important role is played by the specific composition and relative concentrations of single components.

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