

PHYTOCHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES OF TWO CANNABIS SATIVA L. BIOTYPES EXTRACTS

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Introduction: Non-drug *Cannabis sativa* L. varieties, known to contain less than 0.2% of Δ^9 -tetrahydrocannabinol (Δ^9 -THC) in the whole plant, exhibit high contents of non-psychoactive phytocannabinoids that could be promising for its possible use in therapy, free of the well-known side psychoactive effects of Δ^9 -THC. The aim of this study was to examine and compare the phytochemical composition of four different hexane extracts obtained from two *C. sativa* L. biotypes, a new Chinese accession (G-309) and a fibrate variety, with Δ^9 -THC content <0.2%, evaluating also their antioxidant and antimicrobial properties.

Materials and methods: Cannabis flowering tops dried as such and after hydrodistillation of the essential oil were extracted with acidic hexane producing the cannabis hexane extracts 1 and 2 both from *C. sativa* Chinese accession and *C. sativa* var. fibrate named CChHE1 and CChHE2, CFHE1 and CFHE2, respectively. The extracts were characterized by HPLC-DAD and GC-MS analyses and their antioxidant properties investigated by *in vitro* assays. In addition, the bacteriostatic and bactericidal activity was tested against standard and clinical isolates of *Staphylococcus aureus*. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of CChHE1 and CChHE2, CFHE1 and CFHE2 were determined using a broth microdilution method according to Clinical and Laboratory Standards Institute.

Results: A preliminary phytochemical screening showed high total phenols content in all extracts (10820 \pm 316 mg GAE/100 g FW and 11930 \pm 912 mg GAE/100 g FW for CChHE1 and CChHE2, respectively vs 19108 \pm 523 mg GAE/100 g FW and 8587 \pm 132 mg GAE/100 g FW for CFHE1 and CFHE2, respectively). HPLC-DAD analysis highlighted a different phytochemical profile of the extracts under examination with a high titer of both acid and neutral phytocannabinoids, whose presence remains however conspicuous as neutral derivatives in the plant samples after hydrodistillation. An *in depth* phytochemical profile elucidation by GC-MS analysis led to identification of 73 and 43 compounds into CChHE1 and CChHE2, and 88 and 56 compounds into CFHE1 and CFHE2, respectively. Cannabinoids are the most abundant compounds in all extracts investigated (54.23% and 70.81% into CChHE1 and CChHE2, respectively vs 80.83% and 82.92% into CFHE1 and CFHE2, respectively).

C. sativa biotypes extracts showed remarkable antioxidant activity with the following order of potency: TEAC > β -carotene bleaching > ORAC > FRAP > DPPH. However, the Cannabis var. fibrate showed the best antioxidant activity probably due to the highest content of cannabinoids and in particular of cannabidiol (275.694 \pm 8.56 mg/100 g FW into CFHE1 vs 11.197 \pm 0.44 mg/100 g FW into CChHE1). The antimicrobial activity results showed a bactericidal effect of all tested cannabis extracts, the fibrate variety being more active (MIC range between 2.44 and 9.77 μ g/ml) than Chinese accession (MIC range between 9.77 and 39.06 μ g/ml).

Discussion and conclusion: The differences found in the relative abundance of phytocannabinoids between the hexane extracts 1 and 2 respectively, are mainly attributable to the loss of sesquiterpenes, sesquiterpene oxides and alkanes during the distillation process. However, the results suggest that most likely the remarkable antioxidant and antimicrobial properties highlighted by cannabis extracts are mainly due to the synergy of phytocannabinoids and terpenoids.