

STUDYING THE CHEMOPREVENTIVE PROPERTIES OF INFLORESCENCES FROM INDUSTRIAL HEMP VARIETY FELINA 32 IN RELATION TO THE PHENOLIC AND TERPENOID COMPOSITION

Antonella Di Sotto¹, Caterina Frascchetti², Marcello Locatelli³, Giada Freddoni¹, Antonello Filippi², Simone Carradori³, Cinzia Ingallina², Luisa Mannina², Gabriela Mazzanti¹, Silvia Di Giacomo¹

¹Department of Physiology and Pharmacology "V. Erspamer", Sapienza University, Rome - Italy, ²Department of Chemistry and Technology of Drugs, Sapienza University, Rome - Italy, ³Department of Pharmacy, University "G. D'Annunzio" of Chieti-Pescara, Chieti - Italy

Introduction: Industrial hemp (*Cannabis sativa* L.) is a rich source of materials to be exploited in different fields, including textile, nutraceutical and pharmaceutical industries. Inflorescences are a hemp by-product, with potential interest as a source of bioactive compounds, including nonpsychoactive terpenoids, sesquiterpenes and phenolics. In line with this evidence and within a Lazio Regional project for valorizing local hemp cultivations, the inflorescences of *C. sativa* var. Felina 32, harvested in June and September, were chemically characterized and their possible chemopreventive properties were evaluated.

Material and methods: Different organic and hydroalcoholic Bligh-Dyer extracts were prepared. Phenolic and terpenoid composition was characterized by an integrated spectrophotometric and chromatographic (i.e. HPLC and GC-MS) approach. Preliminarily, the radical scavenging power and the antimutagenic activity towards the oxidative DNA-damage induced by tert-butyl hydroperoxide (t-BOOH) were evaluated. Furthermore, the cytotoxicity of the extracts towards a panel of different human cancer cell lines, including MDA-MB-468 (triple negative breast cancer), H358 (non-small cell lung cancer) and Caco2 (epithelial colorectal adenocarcinoma) was assessed and compared to that of nonpsychoactive terpenoids (i.e. cannabidiol and cannabichromene) and caryophyllene sesquiterpenes (i.e. β -caryophyllene, β -caryophyllene oxide and α -humulene). Specific cannabinoid and adrenergic inhibitors were also included in order to support preliminary mechanistic hypotheses. At last, the ability of the extracts to inhibit glucose-6-phosphate dehydrogenase (G6PD), known to control intracellular redox potential and to sustain cell proliferation and metastasization of cancer cells, was assessed.

Results: Phytochemical analysis highlighted the presence of cannabidiol, β -caryophyllene, α -humulene, caryophyllene oxide as major terpenoids, while catechin, rutin and carvacrol were the major phenolics. The organic samples achieved the higher amount of total polyphenols and terpenoids. Cannabidiol and total polyphenols increased from June to September, while sesquiterpenes and flavonoids slightly decreased. All the extracts produced radical scavenging effects and inhibited the tBOOH-induced mutagenicity, with a strong antimutagenicity of organic extracts. Conversely, hydroalcoholic extracts were the most potent radical scavenging samples. Despite a null cytotoxicity of hydroalcoholic extracts, the organic samples were also able to inhibit cancer cell proliferation, with higher potency towards MDA-MB-468 cells. Analogously, both nonpsychoactive terpenoids and caryophyllene sesquiterpenes exerted cytotoxic effects, being cannabidiol and β -caryophyllene the most potent compounds in each group. The pre-treatment with AM281 and AM630, inhibitors of CB1 and CB2 receptors respectively, did not affect the cytotoxicity of the extracts. Conversely, AM630 slightly inhibited the antiproliferative activity of β -caryophyllene. Surprisingly, the cytotoxicity of hemp extracts, cannabidiol and β -caryophyllene was significantly inhibited by the β 2-adrenergic antagonist ICI 118,551.

Discussion and conclusions: A broad spectrum of chemopreventive properties for the organic extracts of Felina 32 hemp inflorescences, were found. According to the literature, carvacrol, cannabidiol and caryophyllene sesquiterpenes could contribute to the antimutagenic and antiproliferative effects. The last ones appear slightly mediated by CB1/CB2 receptors, whilst a greater contribution of β 2-adrenoceptors could be hypothesized. In conclusion, present results highlight the possible interest for Felina 32 hemp inflorescences as a source of bioactive molecules and stimulate further investigations about the possible synergistic contribution of nonpsychoactive terpenoids and sesquiterpenes to the chemopreventive properties of this hemp by-product.