This paper is submitted to

Conference
The 1st International Electronic Conference on Plant Science
Session
Phytochemistry and Bioremediation

Submission details

Article ID
sciforum-038927
Status
abstract accepted

Title
Optimization of non-psychotropic *Cannabis sativa* L. extraction and evaluation of anti-inflammatory activity on microglial cells

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Abstract

Cannabis sativa L. (Cannabaceae) is an annual flowering plant well known worldwide since ancient times. After the discovery and elucidation of cannabinoids and of the endocannabinoid system, *C. sativa* has attracted the interest of the scientific community more and more in the last fifteen years, as new evidences of its potential role in medicine has arisen; nevertheless, the presence of the psychoactive compound Δ^9-tetrahydrocannabinol (THC) still represents an important issue for clinical uses. For this reason, many authors have taken into account the biological effects of the non-psychotropic constituents of *C. sativa*, in particular cannabidiol (CBD). Some clinical trials and animal models have shown that CBD could be considered in the treatment of central and peripheral inflammation, gastrointestinal upset, epilepsy and neurodegenerative diseases.

THC containing *C. sativa* is approved in many countries in the world for medical purposes and some herbal preparations are enlisted in monographs of official Pharmacopoeias. On the contrary, non-psychotropic *C. sativa* has still a confuse regulatory status, very variable between different countries and herbal preparations are not specified.

With the aim of defining the best way to extract CBD and other secondary metabolites from non-psychotropic *C. sativa*, starting from the female inflorescences of *C. sativa* L. var. *carmagnola*, we tested different extraction methods by using ethanol (30-60-96% V/V) and olive oil as solvents, by varying the extraction time and the heat-decarboxylation of the herbal material. We applied two methods obtained from the Italian Pharmacopoeia FUI XII (maceration and percolation) and an automatic extraction method by means of Naviglio Estrattore®.

Each extract was compared for the content in cannabinoids by means of TLC/HPTLC and HPCL-DAD, total phenolic compounds and total flavonoids by means of colorimetric methods and volatile constituents using a GC-MS analysis.

Each extract was evaluated for its antiradical capacity, assessed through the 2,2-diphenyl-1-picrylhydrazyl (DPPH) test.

The extract with the best phytochemical profile and antiradical capacity was then compared to CBD for its *in vitro* anti-inflammatory activity by dosing the production of TNF-α in LPS-stimulated microglial cells (BV2), through non-competitive sandwich ELISA.

The 96% V/V ethanolic extract (drug:extract ratio 1:10) obtained by maceration of the decarboxylated herbal material gave the best yield of active compounds: cannabidiol 466 mg/l, total flavonoids 90 mg/l (of which vitexin 12 mg/l), sesquiterpenes (being beta-caryophyllene the most representative) and very few amount of monoterpenes (<0.1 mg/l).

The better phytochemical profile correlated well with the antiradical capacity, with the IC_{50} of the ethanolic extract being 18.7 mg/ml.

Differently from cannabidiol alone (1 μg/ml), the selected extract, used at the same concentration, was able to significantly reduce by 28.9 % the LPS-induced production of TNF-α in BV2 microglial cells.

Keywords
Cannabis sativa; cannabidiol; extraction method; non-psychotropic cannabis; terpenes