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Industrial hemp as a promising source of anti-Inflammatory, anti-proliferative and antimycotic agents: results from in silico, in vitro and ex vivo studies

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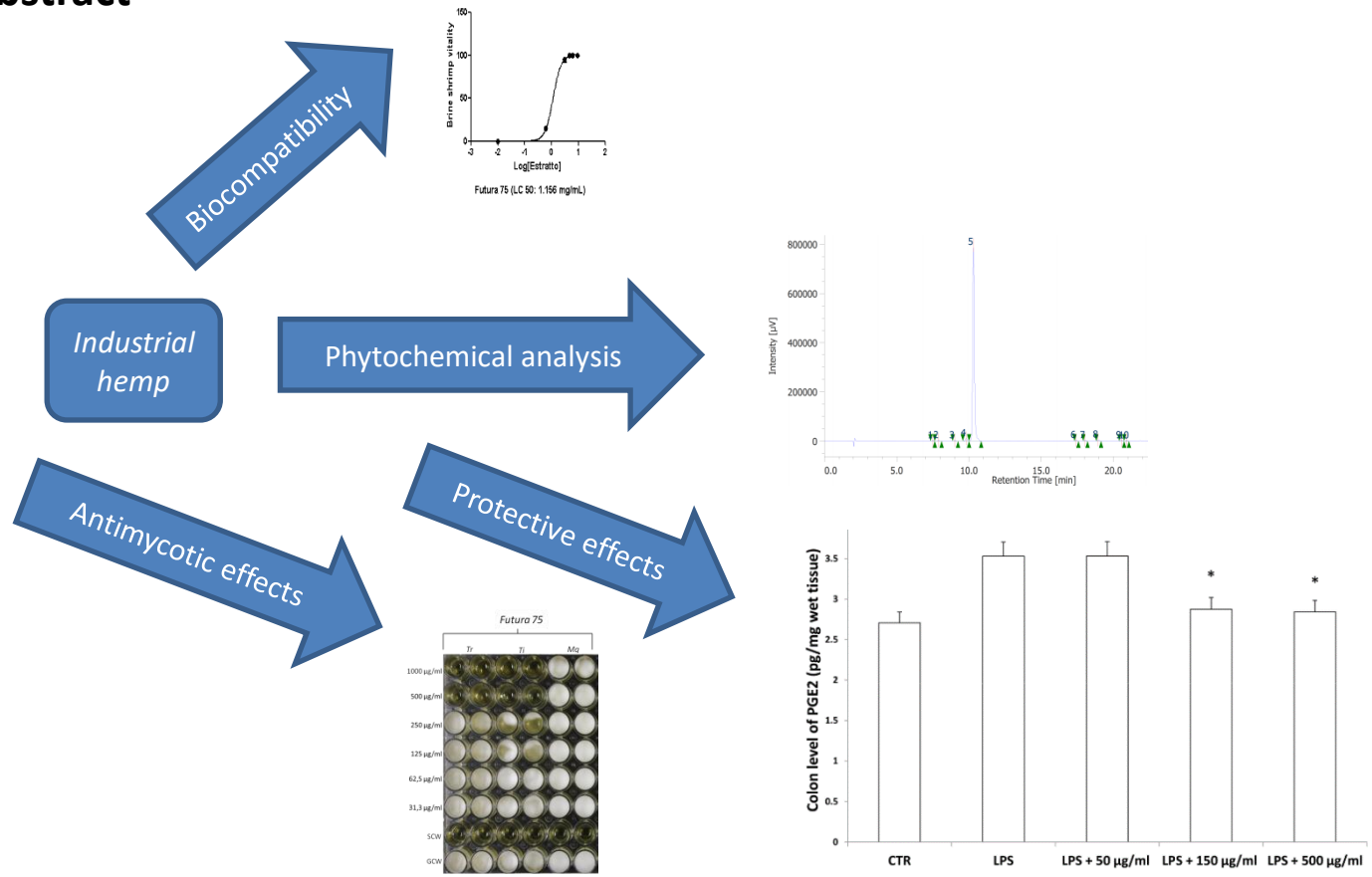
Graphical Abstract



Abstract:
Industrial hemp is cultivated as a source of fibers and nutrients. Multiple studies demonstrated antimicrobial, anti-proliferative, phytotoxic and insecticide effects of the essential oil. On the other side, only a few studies explored the potential pharmacological application of polar extracts. In the present study, we investigated the water extract from inflorescences of industrial hemp. The water extract was assayed for antiradical and anti-tyrosinase effects. We explored the extract effects on LPS-induced production of colon serotonin, dopamine, kynurenine pathways and (PG)E2. Anti-proliferative effects were also evaluated against human colon cancer HCT116 cell line. Antimycotic effects were investigated against *Trichophyton rubrum*, *Trichophyton interdigitale*, *Microsporum gypseum*. Finally, *in silico* studies were conducted in order to predict the putative targets. Hemp water extract was able to blunt LPS-induced reduction of serotonin and increase of dopamine and kynurenine turnover. Additionally, the reduction of PGE2 levels was observed as well. The extract inhibited the HCT116 cell viability, the growth of *T. rubrum* and *T. interdigitale* and the activity of tyrosinase, whereas docking studies highlighted the inhibitions of cyclooxygenase-1, carbonic anhydrase IX, and lanosterol 14-α-demethylase. The present findings suggest female inflorescences from industrial hemp as high quality by-products, thus representing promising sources of nutraceuticals and cosmeceuticals .

Keywords: Hemp; anti-inflammatory; anti-mycotic; anti-proliferative.

Introduction

Industrial hemp (*Cannabis sativa*) has been long cultivated throughout history as a valuable source of fibers and nutrients. Specifically, the fibers, isolated from the stalk, are used for manufacturing ropes, paper, clothing and construction materials (thermal and acoustic insulation), whereas hemp seeds demonstrated a high nutritional value, due to their richness in minerals, vitamins (i.e. A, C and E complexes), carbohydrates, proteins and lipids, these last consisting of linoleic (ω-6) and α-linolenic acid (ω-3) in the ideal ratio 3:1. Additionally, industrial hemp varieties are also bred to produce tetrahydrocannabinol (THC) in traces (<0.3%), and only certified varieties, with THC content < 0.2%, are admitted to cultivation, according to National and International Regulations (EU Regulation N°. 1124/2008-12 November 2008; Italian Regulation n°172/2017). Although the hemp chain production was principally focused on fiber production and seed-deriving foods, in the last years there has been a renewed interest in the study and valorization of hemp-deriving extracts, that are sources of terpenes, terpenophenolics, amino acids, fatty acids, sugars, hydrocarbons, flavonoids, with promising health-promoting and pharmacological effects. In this regard, multiple studies focused on the antimicrobial, anti-proliferative, phytotoxic and insecticide effects of the essential oil from hemp female inflorescences, sold dried for technical use and usually considered as a waste material of industrial hemp chain production. On the other side, only a few studies explored the potential pharmacological application of polar extracts from inflorescences. In our previous study, we showed antioxidant/anti-inflammatory and antimycotic properties of the certified hemp variety "Futura 75", whose essential oil from inflorescences was also reported as an antibacterial and anti-proliferative agent. In the present study, we aimed to further characterize the water extract from the inflorescences of this hemp variety, through a qualitative phytochemical analysis and a pharmacological investigation aimed to evaluate protective effects in a toxicological experimental paradigm constituted by isolated rat colon and liver exposed to lipopolysaccharide (LPS). In this regard, we explored the effects of water hemp extract on serotonin, dopamine and kynurenine pathways, in rat colon, whereas prostaglandin (PG)E_2 levels were measured in both colon and liver. Anti-proliferative effects were also assayed against the human colon cancer HCT116 cell line. Taking into consideration the inhibitory effects induced by this extract on both *Candida albicans* and *C. tropicalis* [10], we also explored the antimycotic effects of Futura 75 water extract on multiple dermatophytes species, namely *Trichophyton rubrum, Trichophyton interdigital, Microsporum gypseum.* In parallel, we assayed the enzyme inhibition effect of the extract against tyrosinase, whose increased activity is related to skin hyperpigmentation, following mycotic infectious diseases, as well. Finally, *in silico* studies, including bioinformatics, network pharmacology and docking approaches were conducted in order to predict the putative targets underlying the observed pharmacological and microbiological effects.

Results and discussion

The extract displayed a content of rutin (>9 µg/mg extract), that was considered putatively responsible, at least in part, of the protective effects exerted in the colon. Chlorogenic acid, gallic acid and carvacrol were also measured in the extract. The phenolic profile is also consistent with the scavenger, reducing, chelating and anti-tyrosinase properties, thus suggesting intrinsic antioxidant and enzyme inhibition effects that could account for multiple pharmacological applications.

Table 1. Total phenolic, flavonoid content, antioxidant parameters and tyrosinase inhibitory effect

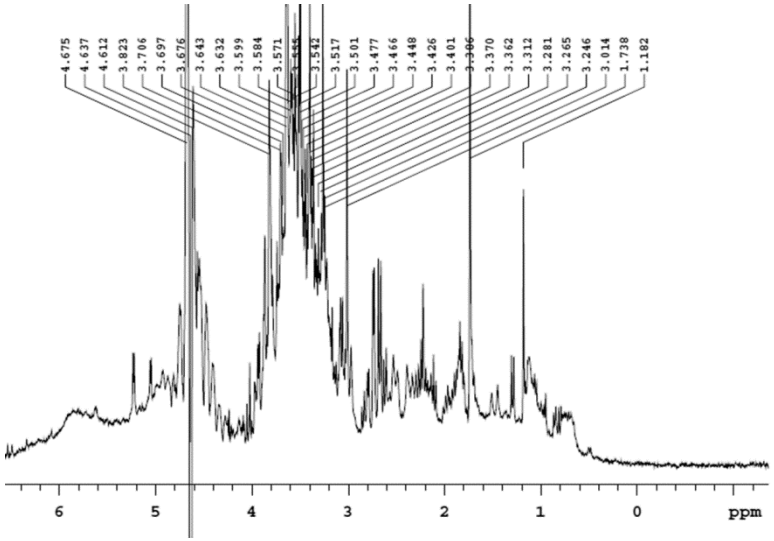
Parameters	Results
Total phenolic content (mg GAE/g)	21.16±0.10
Total flavonoid content (mg RE/g)	7.05±0.63
DPPH (mg TE/g)	14.87±0.59
ABTS (mg TE/g)	39.00±0.43
CUPRAC (mg TE/g)	47.53±0.18
FRAP (mg TE/g)	27.53±0.27
Tyrosinase (mg KAE/g)	18.67±0.28

GAE: Gallic acid equivalents; RE: Rutin equivalents; TE: Trolox equivalents. KAE: Kojic acid equivalents. Values are reported as mean ± SD. of three parallel experiments.

	Futura 75
Gallic acid	0.37 ± 0.03
Catechin	
Chlorogenic acid	0.30 ± 0.02
p-OH-Benzoic Acid	0.39 ± 0.04
Vanillic acid	0.03 ± 0.01
Epicatechin	0.94 ± 0.07
P-Coumaric acid	
Rutin	9.4 ± 0.70
2,3-diMeOH Benzoic acid	
Benzoic acid	0.30 ± 0.02
Naringenin	0.02 ± 0.01
Carvacrol	0.65 ± 0.06

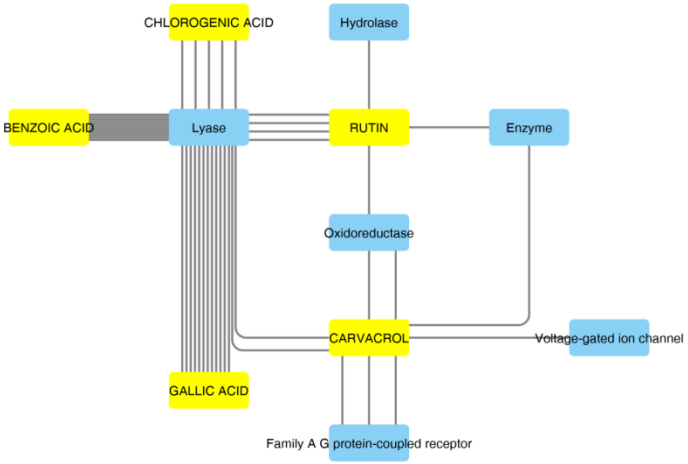
Results and discussion

The extract was also analyzed through ^{1}H-NMR. The suppression of water signal was directly applied, in order to explore the qualitative composition of the water extract itself. The chemical shift values, in the range 3-4 ppm, indicate that sugar fraction represents most of the extract phytocomplex,



Results and discussion

According to the aforementioned fingerprint analysis, a network pharmacology profile was built, plotting the selected compounds towards the putative targets yielded by SwissTagetPrediction software. The results of network pharmacology approach indicate the putative interaction of most selected phenolic compounds towards multiple carbonic anhydrase (CA) isoforms, and this was consistent with our recent findings. Intriguingly, the sole carvacrol showed potential capability to interact with cyclooxygenase-1 (COX-1).



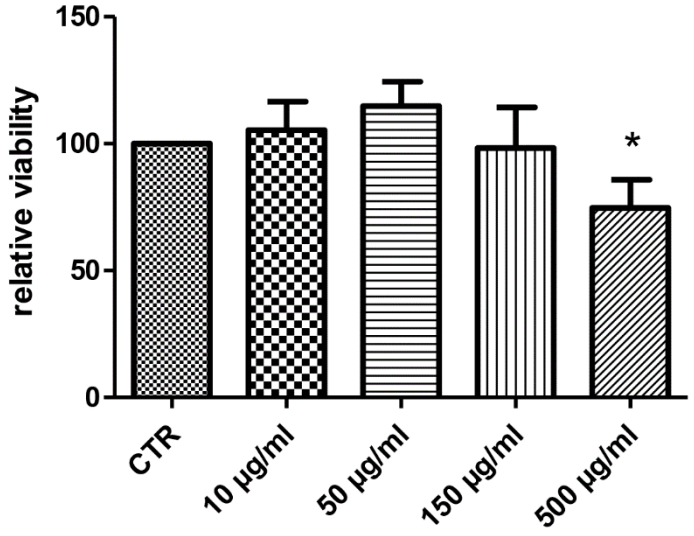
Results and discussion

Considering the up-regulated levels of both CA IX and COX-1 in colon cancer, thus suggesting the inhibition of these enzymes as a promising pharmacological tool to counteract colon cancer, we further investigated the putative interactions between gallic acid, chlorogenic acid and carvacrol towards these targets, through a docking approach. Chlorogenic acid has shown higher binding energy against carbonic anhydrase IX enzyme in comparison with gallic acid.

Targets	ΔG (K_i)	Key residues	no. of HB
Carbonic Anhydrase IX			
Chlorogenic Acid	-7.30 (4.5 μM)	His64 (HB), Asn62 (HB), Thr199 (HB), His119 (HB), Glu106 (HB), Pro201 (HB), Trp5 (HB), Leu198, Thr200	**8**
Gallic Acid	-4.97 (228.8 μM)	Thr199 (HB), His119 (HB), Thr200 (HB), Gln92 (HB), Leu198	**4**
Cyclooxygenase-1			
Carvacrol	-6.03 (38.2 μM)	Met522 (HB), Trp387, Tyr385, Phe381, Leu384, Ile523, Val349, Leu352, Gly526	**1**

Results and discussion

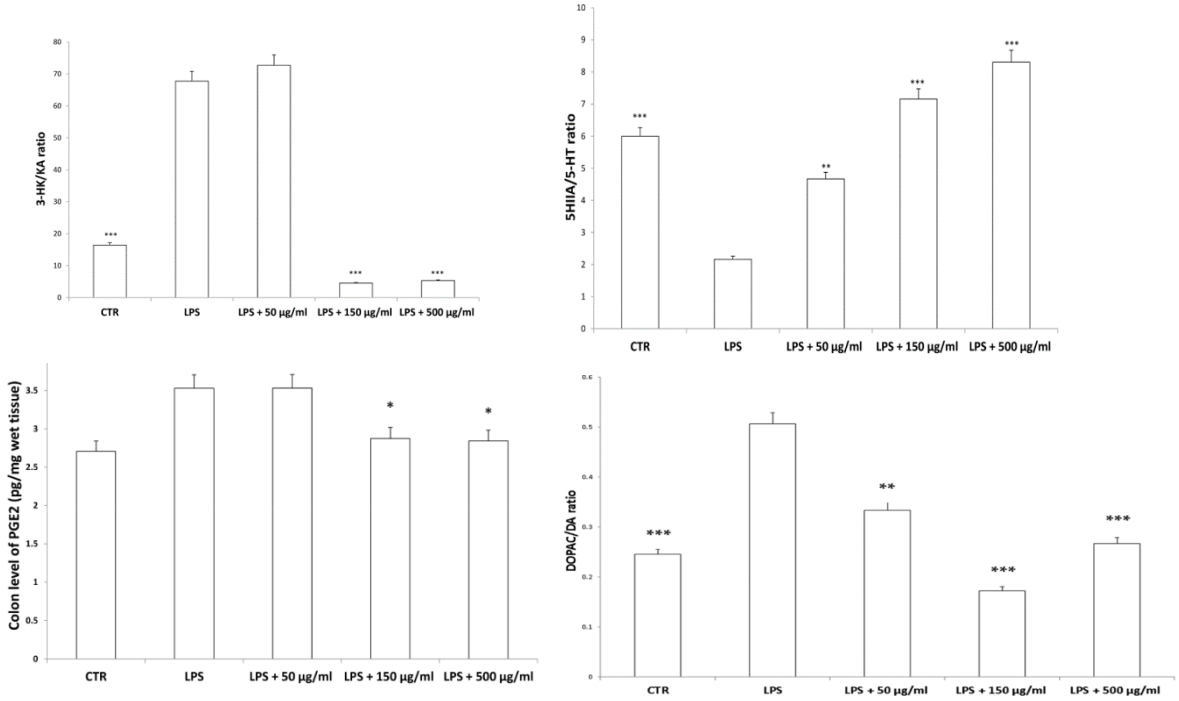
Considering the docking results on CA IX and COX-1 enzymes, the water extract was also investigated for evaluating anti-proliferative effects towards the human colon cancer HCT116 cell that displayed a significant reduction of viability at the upper tested concentration (500 µg/mL), thus suggesting mild anti-proliferative effects.

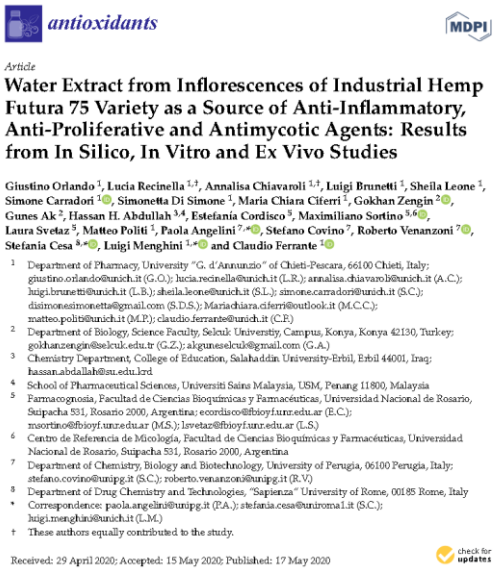


Results and discussion

The anti-inflammatory and antioxidant effects induced by hemp water extract were also investigated in isolated colon stimulated with LPS, and anti-oxidant effects were determined as 3-HK/KA and 5HIIA/5-HT ratio, respectively.

In the present study, we investigated for the first time the influence of hemp extracts on the two major tryptophan degradative pathways, namely kynurenine and and serotonin. In this context we hypothesize that hemp water extract is able to counteract the LPS-induced shift of tryptophan metabolism towards the production of pro-inflammatory metabolites.





antioxidants

MDPI

Article

Water Extract from Inflorescences of Industrial Hemp Futura 75 Variety as a Source of Anti-Inflammatory, Anti-Proliferative and Antimycotic Agents: Results from In Silico, In Vitro and Ex Vivo Studies

Giustino Orlando [1], Lucia Recinella [1,†], Annalisa Chiavaroli [1,†], Luigi Brunetti [1], Sheila Leone [1], Simone Carradori [1], Simonetta Di Simone [1], Maria Chiara Ciferri [1], Gokhan Zengin [2], Gunes Ak [2], Hassan H. Abdullah [3,4], Estefanía Cordisco [5], Maximiliano Sortino [5,6], Laura Svetaz [5], Matteo Politi [1], Paola Angelini [7,*], Stefano Covino [7], Roberto Venanzoni [7], Stefania Cesa [8,*], Luigi Menghini [1,*] and Claudio Ferrante [1]

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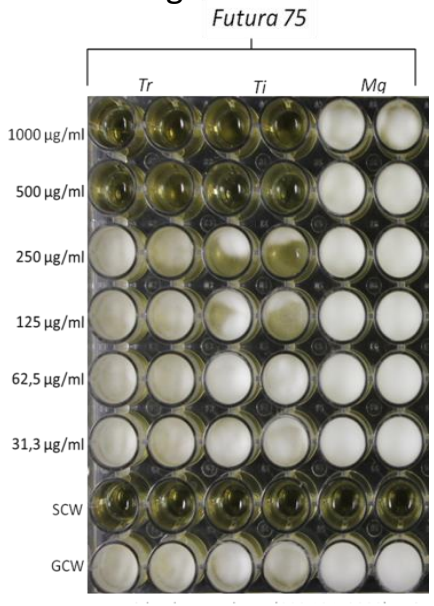
Results and discussion

In the present study we also evaluated the effects of the extract (500-1000 µg/mL) on different fungi strains, namely *T. rubrum*, *T. interdigitale* and *M. gypseum*, and involved in multiple skin alterations, including infectious granuloma and hyperpigmentation. No activity was observed against *M. gypseum.* These results could be added to the aforementioned inhibitory effect of the extract against tyrosinase, whose inhibition represents a cornerstone in the management of skin hyperpigmentation. The anti-mycotic effects could be also related, albeit partially, to the good submicromolar affinity of rutin towards lanosterol 14$^{\alpha}$-demethylase, that plays a key role in fungal metabolism.



Targets	ΔG (K_i)	Key residues	no. of HB
Lanosterol 14-α-demethylase			
Rutin	-8.74 (390.0 nM)	Gly303 (HB), Tyr132 (HB), Phe463 (HB), His468 (HB), Arg381 (HB), Tyr118 (HB), Ser378 (HB), Leu376, Pro375.	**10**

Conclusions

Concluding, the present findings highlight the potential of water extracts from industrial hemp inflorescences as sources of natural compounds that, besides the intrinsic antiradical activity, possess discrete mechanisms related to anti-inflammatory, anti-proliferative and antimycotic effects. In this context, and also in view of a more sustainable circular economy, it is desirable an improvement of industrial hemp chain production, taking into consideration the female inflorescences as high quality by-products with putative nutraceutical and cosmeceutical applications.

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