HEMP BYPRODUCTS

Optimizing Supercritical Extraction and Unveiling Antimicrobial Potential

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RESEARCH CONTEXT

The background:

In the last few years, because of the diffusion of non-psychotropic varieties, hemp market has been skyrocketing, with an increase in hemp biomass and consequent byproducts [1].

The project:

"NOrCa - Not Ordinary Cannabis"
PRIN 2022 PNRR
Exploring the chemical space around hemp (*Cannabis sativa* L.) waste and by-products from a circular economy perspective

It aims at the valorisation of *C. sativa* waste and by-products, in view of a green chemistry and environmental compatibility.

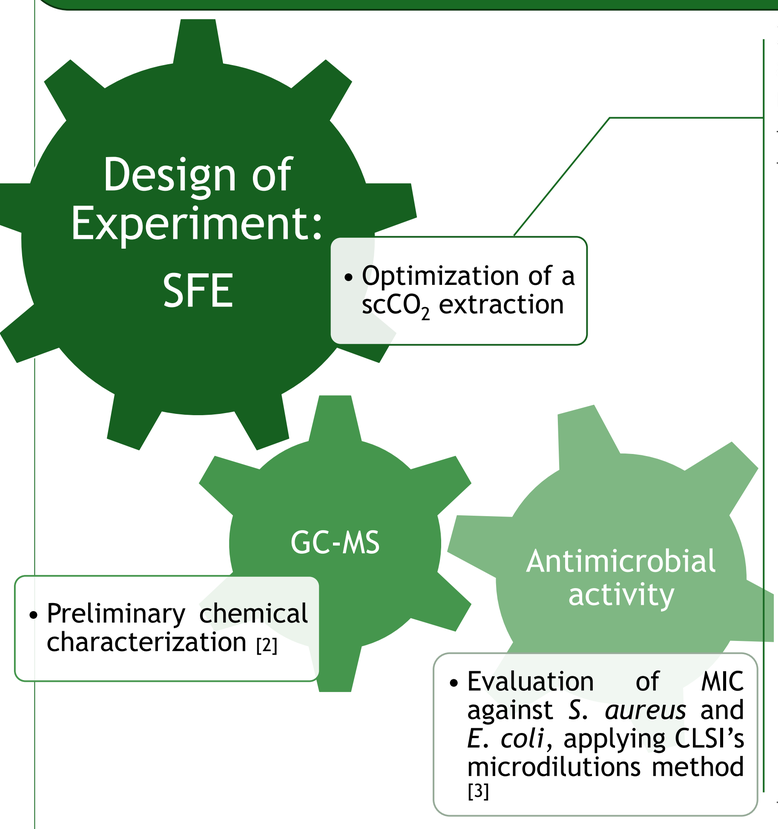
The protagonist:

Aerial parts of non-psychotropic hemp, a byproduct of industrial seed cleaning, were provided by Whole Lotta Hemp Srl (Parma).

Current aims:

- Optimisation a sustainable extractive method;
- Chemical characterisation of extracts' bioactive components;
- Bioactivity evaluation for potential health, nutraceutical or agronomic applications.

METHODOLOGY

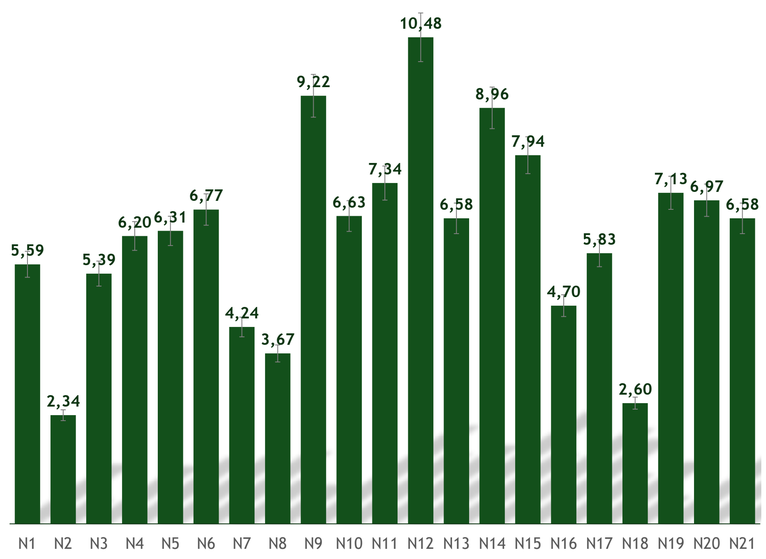


21 experiments performed in the DoE, at different conditions of temperature, time, flow, pressure and EtOH %:

No.	°C	hs	L/min	% EtOH	bar
1	40	1	2	0	350
2	70	1	2	0	150
3	40	2	2	0	150
4	70	2	2	0	350
5	40	1	4	0	150
6	70	1	4	0	350
7	40	2	4	0	350
8	70	2	4	0	150
9	40	1	2	5	150
10	70	1	2	5	350
11	40	2	2	5	350
12	70	2	2	5	150
13	40	1	4	5	350
14	70	1	4	5	150
15	40	2	4	5	150
16	70	2	4	5	350
17	40	1	2	0	350
18	70	1	2	0	150
19	55	1.5	3	2.5	250
20	55	1.5	3	2.5	250
21	55	1.5	3	2.5	250

RESULTS

Extractive yield (%)

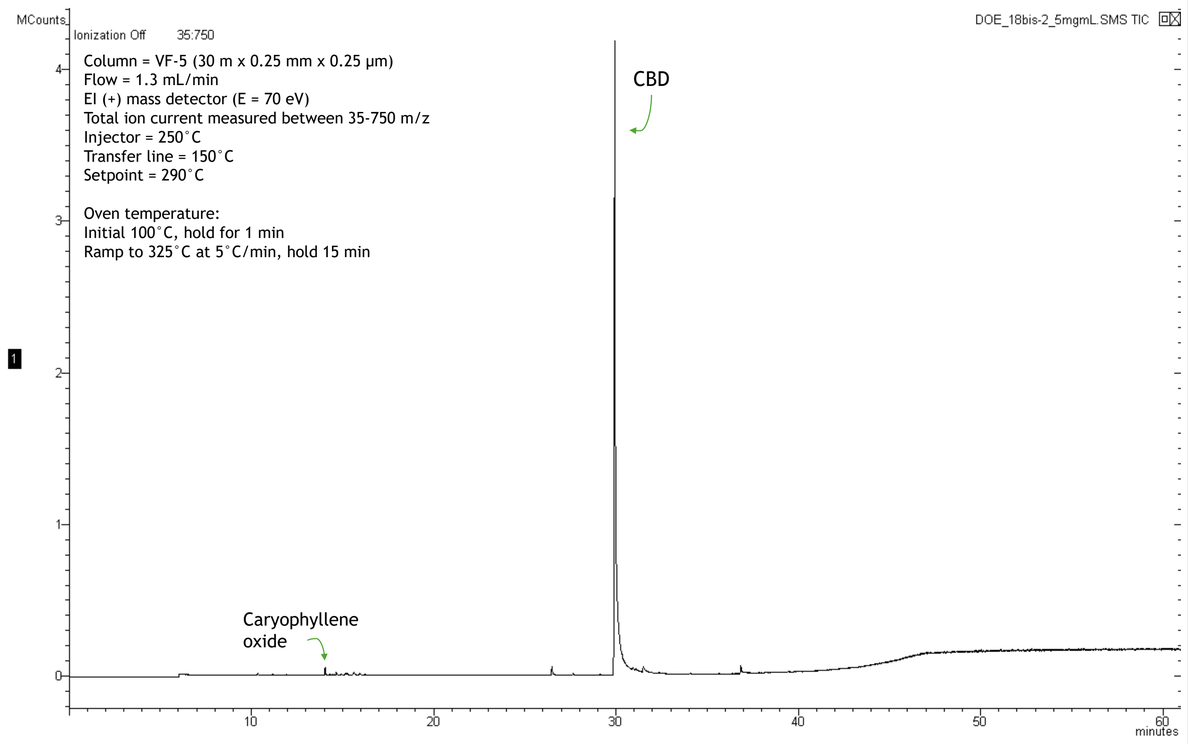


Highest yield was obtained in exp. n.12 (10.48%), followed by n.9 and 14. These have in common a 5% of EtOH added as a co-solvent and a pressure of 150 bar.

Lowest yield was obtained in exp n.2 and its replicate, n.18, followed by n.8, featuring no EtOH added, 70°C and 150 bar.

The only variable with statistical relevance in increasing the yield is the presence of EtOH as a co-solvent, while high flow and pressure seem to be predictive of a lower yield. Other variables don't represent a significant predictive method.

GC-MS



There is no significant difference between GC-MS spectra of all experiments.

Compound	Area
CBD	88.02-95.76 %
β-caryophyllene	0.01-1.34 %
caryophyllene oxyde	0.28-2.44 %

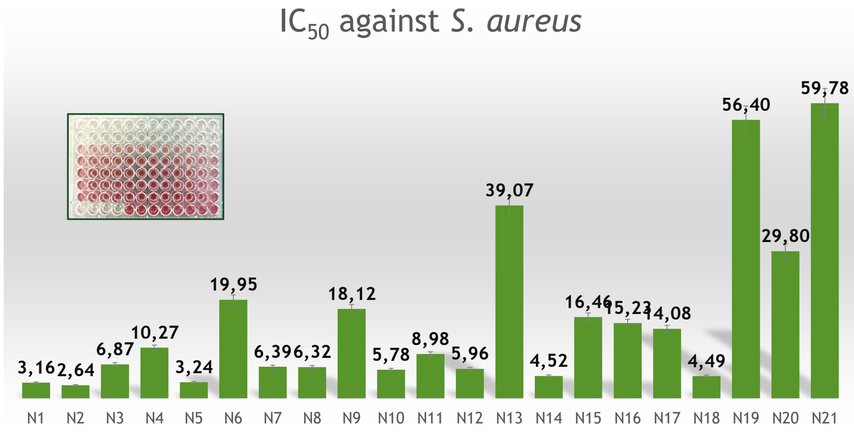
Spectra all share a strong cannabinoid prevalence (mainly CBD) and a characteristic presence of sesquiterpenes (mostly caryophyllene oxide and other caryophyllene derivates).

Antibacterial activity

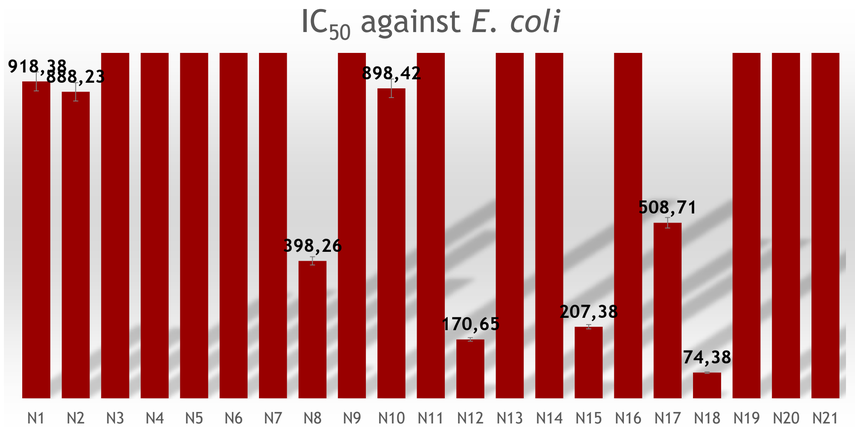
While none of the extracts showed a Minimum Inhibitory Concentration against *Escherichia coli*, with IC_{50} values rarely below the highest concentration evaluated of 1 mg/mL, much more interesting results were obtained against *Staphylococcus aureus*: all extracts demonstrated a MIC below 100 µg/mL. Extract n.2 achieved a MIC as low as 6.25 µg/mL (IC_{50} 2.64 µg/mL), better than Chloramphenicol used as a positive control during the analysis.

Extracts obtained without ethanol addition seem more active against *S. aureus*, but a proper statistical correlation between antimicrobial activity and extractive conditions is still under evaluation.

IC_{50} against *S. aureus*



IC_{50} against *E. coli*



CONCLUSIONS AND FUTURE PERSPECTIVES

Ethanol addition as a co-solvent during SFE seems to improve extractive yield, while reducing antimicrobial activity against *S. aureus*. Other than this, a proper statistical correlation between extracts characteristics (in terms of both composition and bioactivity) and extractive conditions is still unclear and under evaluation.

It's interesting noting that all extracts, obtained from industrial aerial part byproducts, showed a good activity against the Gram + bacteria, comparable to data reported in literature regarding raw hemp inflorescence [4], while the similarity wasn't maintained against the Gram – bacteria.

S. aureus and *E. coli* represent a preliminary analysis. Assays will continue against other human pathogens, both Gram + and Gram – bacteria and dermatophytes, before moving on to phytopathogen bacteria and fungi. At the same time, the current aim is to optimise a chromatographic separation of DoE extracts, to identify and purify the main components responsible for their biological activity.

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