Food Bioscience 62 (2024) 105344

Contents lists available at ScienceDirect

Food Bioscience

journal homepage: www.elsevier.com/locate/fbio

Phytochemical profiling and biological evaluation of the residues from industrial hemp (*Cannabis sativa* L.) inflorescences trimming: Focus on water extract

Simonetta Cristina Di Simone [a,1], Maria Loreta Libero [a,1], Riccardo Pulcini [b], Nilofar Nilofar [a], Annalisa Chiavaroli [a], Fatma Tunali [a], Paola Angelini [c], Giancarlo Angeles Flores [a,c], Roberto Venanzoni [c], Gaia Cusumano [c], Gokhan Zengin [d], Luigi Brunetti [a], Lucia Recinella [a], Sheila Leone [a], Giustino Orlando [a], Luigi Menghini [a], Claudio Ferrante [a,*], Alessandra Acquaviva [a]

[a] *Botanic Garden "Giardino dei Semplici", Department of Pharmacy, "G. d'Annunzio" University, 66100, Chieti, Italy*
[b] *Center of Advanced Studies and Technology, Department of Innovative Technology in Medicine and Dentistry, "G. d'Annunzio" University, 66100, Chieti, Italy*
[c] *Department of Chemistry, Biology and Biotechnology, University of Perugia, 06122, Perugia, Italy*
[d] *Physiology and Biochemistry Research Laboratory, Department of Biology, Science Faculty, Selcuk University, 42130, Konya, Turkey*

ARTICLE INFO

Keywords:
Industrial hemp
Water extract
Terpenophenols
Phenolic compounds
Anti-inflammatory effects
Antimicrobial effects

ABSTRACT

The study focused on investigating the phytochemical composition and bio-pharmacological properties of a water extract obtained from industrial hemp by-products, after trimming female inflorescences of *Cannabis sativa* L., cultivar Kompolti. The extract was found to be rich in phenolic compounds, particularly gallic acid, catechin, coumaric acid, ferulic acid, and benzoic acid. It also contained significant amounts of cannabidiolic acid and cannabigerolic acid as the main terpenophenols. To assess the biological potential of the extract, various eco-toxicological assays were conducted. The extract did not show significant phytotoxic effects on seedling germination in the allelopathic assay. In the brine shrimp (*Artemia salina*) lethality test, the LC_{50} value was 1.726 mg/mL, and in the *Daphnia magna* test, the extract showed no cardiotoxicity effects at the same concentration; thus, confirming its biocompatibility with these eukaryotic organisms. Additionally, the extract did not induce cytotoxicity in the murine C2C12 cell line till to the concentration of 1000 μg/mL. In isolated mouse prostate specimens, the extract (10–1000 μg/mL) also prevented LPS-induced gene expression of pro-inflammatory cytokines, namely tumor necrosis factor (TNF)α, interleukin (IL)-6, and IL-1β. Furthermore, the extract exhibited inhibitory effects on the growth of pathogenic bacterial, fungal, and dermatophyte species commonly associated with prostatitis. These results suggest that the residual powder from female inflorescence trimming can be considered an innovative plant material from the industrial hemp supply chain that deserves to be valorised in terms of recycling and upcycling; thus, reducing the environmental impact of the by-products and also opening the way for innovative health-promoting agents.

1. Introduction

Cannabis sativa L. is an annual plant that belongs to the Cannabaceae family and is native to Central Asia, particularly India and China (Hourfane et al., 2023). It has been cultivated since ancient times and has been used as a source of fibers, food, oil, and medicine (Bonini et al.,

* Corresponding author.
E-mail addresses: simonetta.disimone@unich.it (S.C. Di Simone), maria.libero@unich.it (M.L. Libero), riccardo.pulcini@unich.it (R. Pulcini), annalisa.chiavaroli@unich.it (A. Chiavaroli), fatma.tunali@unich.it (F. Tunali), paola.angelini@unipg.it (P. Angelini), giancarlo.angelesflores@unich.it (G.A. Flores), roberto.venanzoni@unipg.it (R. Venanzoni), gaia.cusumano@dottorandi.unipg.it (G. Cusumano), gokhanzengin@selcuk.edu.tr (G. Zengin), luigi.brunetti@unich.it (L. Brunetti), lucia.recinella@unich.it (L. Recinella), sheila.leone@unich.it (S. Leone), giustino.orlando@unich.it (G. Orlando), luigi.menghini@unich.it (L. Menghini), claudio.ferrante@unich.it (C. Ferrante), alessandra.acquaviva@unich.it (A. Acquaviva).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.fbio.2024.105344
Received 13 September 2024; Received in revised form 22 October 2024; Accepted 23 October 2024
Available online 28 October 2024

2018). Recently, the female inflorescences of *C. sativa* have been recognized as sources of bioactive extracts and biomolecules, including terpenes, terpenophenolics, and phenolic compounds, which have potential health-promoting applications (Ferrante et al., 2019). These metabolites are synthesized and accumulated in the trichomes, which are clusters of pistils surrounded by bracts (Andre et al., 2016). The capitate-stalked trichomes consist of two parts: the gland (head) and the stem. The head contains disk cells which are presumed to be the site of cannabinoid production, while the stem is not yet functionally characterized (Happyana et al., 2013).

The color transition of trichome heads is often used as an indicator of the maturation stage of the plant (Sutton et al., 2023). The clear to milky to brown color change is used to approximate the stage of maturity, with milky representing the optimal state and brown indicating over-maturation (Sirikantaramas et al., 2005). The glandular trichomes achieve maturation over a 7–8 week period during *Cannabis* flower development and presumably contain the highest content of specialized metabolites (Sutton et al., 2023). Depending on their color, hemp glandular trichomes show different secretory phases: the mature secreting gland appears translucent, while aging glands are yellow and senescing brown (Mahlberg & Kim, 2004).

In *Cannabis*, over 20 flavonoids have been found, which are mainly categorized into two classes: flavones and flavonols (Pollastro et al., 2018). Additionally, three flavanones named cannaflavin A, B, and C have also been isolated (Flores-Sanchez & Verpoorte, 2008; Radwan et al., 2008). Flavonoids have been acknowledged for their ability to promote health in both human and animal nutrition (Ferguson, 2001). This is due to their various biomedical and pharmacological properties, which include the activation or inhibition of specific enzymes such as lipoxygenase and cyclooxygenase (Schewe et al., 2002). They are also known to aid in the detoxification of carcinogens and in the prevention of chemotherapy (Birt et al., 2001). Our recently published paper highlights that polar extracts from hemp pollen are rich in phenolic compounds, including hydroxytyrosol, coumaric acid, and hesperetin. These extracts have been proven effective in inhibiting different bacterial and fungal strains (Acquaviva et al., 2022). The water extract obtained from female inflorescences of the variety *Futura 75* was able to protect human keratinocytes and fibroblasts from cytotoxicity and apoptosis induced by oxidative stress (Orlando et al., 2020), that could be related to its phytochemical composition, characterized by the prominent presence of cannabidiol (CBD) and its acid form, cannabidiolic acid (CBDA). Moreover, in isolated rat skin, the extract showed anti-inflammatory and antioxidant effects, by reducing hydrogen peroxide-induced l-dopa turnover, prostaglandin-E2 production and the ratio of kynurenine/tryptophan; thus, corroborating the efficacy of hemp water extract from *Futura 75* as a skin protective agent (di Giacomo et al., 2021). The polar extract from inflorescences of the cultivar *Strawberry* displayed antimicrobial and antimycotic effects. The phenolics detected in the extracts, including benzoic acid, have contributed to these effects, although partially. Additionally, CBDA and CBD, the main identified terpenophenolics, may have also mediated the antioxidants and antimicrobial effects of the extracts (Serventi et al., 2023); thus, supporting the growing interest in polar extracts from industrial hemp as innovative products with health-promoting applications. They could also be advantageous for optimizing processes and reducing waste in the supply chain. Water and hydroalcoholic solutions have been found to be effective and safe, as extraction solvents, in combining pharmacological benefits. Investigating polar extracts of plant materials that are conventionally considered as secondary products could open up new potential for improving the whole botanical process (di Giacomo et al., 2021). Overall, these findings suggest that the inflorescences of *C. sativa* could be a valuable source of plant material for the development of bioactive extracts with antioxidant and antimicrobial effects, also in a more sustainable perspective, as the inflorescences are still considered as waste material in the botanical supply chain of industrial hemp (Bertoli et al., 2010).

In this regard, we explored the phytochemical composition and biological properties of a water extract obtained from industrial hemp female inflorescences of the cultivar Kompolti. The innovation in this study is based on using waste powder obtained after trimming female inflorescences. Trimming is a crucial process in the production of hemp inflorescences, which consists of removing excess leaves from the flowers to improve the appearance, quality and preservation of the final product. The effects are related to the improvement of aesthetics and quality. The highest density of trichomes is on the flowers, while the leaves, although showing trichomes on the surface, represent a by-product that could be further studied for unravelling innovative applications.

There are currently no studies in the literature that thoroughly examine the phytochemical and bio-pharmacological aspects of the water extract from this powder by-product. Previous studies have mainly focused on the pharmacological properties of industrial hemp trichomes-deriving cannabinoids (Tanney et al., 2021), essential oils after hydrodistillation (Mazzara et al., 2022), and inflorescences- and leaves-deriving organic and hydroalcoholic extracts (Donati et al., 2024).

In particular, in this multidirectional study, we explored the phenolic and terpenophenolic composition of the extract. Subsequently, the extract was subjected to a preliminary ecotoxicological test, as the allelopathy assay, to determine the non-phytotoxic concentration range in the seedling germination from seeds of commercial plants, including *Cichorium* and *Dichondra* genera. Furthermore, to better define the limits of biocompatibility in eukaryotic organisms, the extract was tested on *Daphnia magna* and *Artemia salina* crustacean species, and in an *in vitro* model constituted by the murine C2C12 cell line. Additionally, anti-inflammatory effects were studied in isolated prostate specimens exposed to *Escherichia coli* lipopolysaccharide (LPS) and antimicrobial effects were assayed against bacterial, *Candida* and dermatophytes strains also involved in prostatitis and in lower urinary tract symptoms (LUTS) (Ferrante et al., 2020).

2. Materials and methods

2.1. Plant material

The plant material consists of a residual powder obtained from trimmed female inflorescences of *Cannabis sativa* L., cultivar Kompolti. Plants were cultivated in the Abruzzo Region (Italy) avoiding chemical additives. At the end of the full blooming state, female inflorescences were manually harvested from plants and dried in a ventilated oven at 25 °C until reached the constant weight. Trimming is a crucial process in the production of hemp inflorescences, which consists of removing excess leaves from the flowers to improve the appearance, quality and preservation of the final product. The effects are related to the improvement of aesthetics and quality. The highest concentration of trichomes is on the flowers, while the external part of leaves.

Subsequently, inflorescences were chopped and plant residuals, including bracts and trichomes (Fig. S1: supplementary materials), were separated mechanically from the dried inflorescences through a vibration sieve under 200 μm. The obtained powder was stored in airtight plastic bags, in a dark and dry place at room temperature (22–24 °C), before performing phytochemical and biological assays.

Samples were kindly supplied by Veridia Italia S.R.L. from Pescara - Italy, during the cultivation season in 2022. The production of inflorescences is dedicated to further extraction processes, whilst the powder represents a byproduct. All the production is certified for THC that is <0.3% w/w, according to the European Regulation EC no. 1124/2008–12 November 2008.

2.2. Extract preparation

The powder (1 g) was weighted using a Precisa XT220A balance

(Micro Precision Calibration Inc., Grass valley, CA, USA) in 50 mL Falcon tubes and then immediately homogenized together with 25 mL of extraction solvent. Ultrasound-assisted extraction (UAE) of the homogenate was carried out at 80 °C for 20 min, maximum power. Distilled water was used as extraction solvent to mimic potential homemade use in decoctions or infusions. After centrifuge, samples were filtered with PTFE 0.45 μm before performing analyses.

2.3. Phytochemical analyses

The extract was subjected to a reversed-phase HPLC-UV-MS analysis, in gradient elution mode, to quantitatively determine the phenolic and terpenophenolic composition. The HPLC apparatus consisted of a two PU-2080 PLUS chromatographic pump, a DG-2080-54-line degasser, a mix-2080-32 mixer, UV, and mass spectrometer (MS) detector (expression compact mass spectrometer, CMS, Advion, Ithaca, NY 14850, USA), an AS-2057 PLUS autosampler and a CO-2060 PLUS column thermostat (all from Jasco, Tokyo, Japan). ChromNAV2 Chromatography software was used for integration. Terpenophenolic compound standards were purchased from Focus Analytics Srl (Arcore (MB), Italy). For terpenophenolic determination, the separation was conducted within 30 min, starting from the following conditions: 0.007% formic acid, 7% water, 93% acetonitrile. The details about gradient are listed in Table S1 in supplementary materials. The separation was performed on an Infinity lab Poroshell 120-EC reverse phase column (C18, 150 mm × 4.6 mm i.d., 2.7 μm; Agilent Santa Clara, CA, USA). Column temperature was set at 25 °C. Quantification was done through 7-point calibration curves, with linearity coefficients (R2) > 0.999, in the concentration range 2–160 μg/mL. Detection was performed at 230 nm, via UV detector. Before injecting in the HPLC apparatus, hemp extract was centrifuged at 1500 g for 15 min, and supernatant diluted at 10 mg/mL with acetonitrile.

The separation to determine the polyphenolic composition was conducted within 60 min of the chromatographic run, starting from the following separation conditions: 97% water with 0.1 % formic acid, and 3% methanol with 0.1 % formic acid. The details about gradient are listed in Table S2 in supplementary materials. The separation was performed on an Infinity lab Poroshell 120-SB reverse phase column (C18, 150 × 4.6 mm i.d., 2.7 μm; Agilent, Santa Clara, CA, USA). Column temperature was set at 30 °C. Quantitative determination of phenolic compounds was performed via UV detector at 254 nm. The injection volume was 5 μL. Quantification was done through 7-point calibration curves, with linearity coefficients (R2) > 0.999, in the concentration range 2–140 μg/mL. The final concentration of the extract was 40 mg/mL before injecting in HPLC system. The extract was also qualitatively analyzed using an expression compact mass spectrometer (Advion, Ithaca, NY, USA) in negative and positive ion mode (m/z scan mode: 100–1200). MS signal identification was realized through comparison with standard solution and MS spectra present in the MassBankEurope database. The details of the MS analysis are reported in supplementary materials (Table S3).

2.4. Determination of total bioactive components and antioxidant activity

Total phenolic and flavonoid contents were determined according to Folin-Ciocalteu assay, and the results were expressed as gallic acid (mg GAE/g dry extract) and rutin (mg RE/g dry extract) equivalents. The antioxidant activity of the hemp extract was determined by neutralization methods like DPPH and ABTS assay. The comprehensive procedures are reported in the literature (Öztürk et al., 2011).

2.5. Ecotoxicological investigation

The possible phytotoxicity of the extract was evaluated through the allelopathy assay. For the test, commercial seeds of three different varieties were used: *Dicondra repens* (DR), *Cichorium intybus* (CI), and *Cichorium endivia* (CE).

Toxicity limits of the extract were evaluated on eukaryotic organisms, namely *Artemia salina* and *Daphnia magna*, to establish the biocompatibility thresholds of the extract (Chiavaroli et al., 2024). The procedures are fully reported in supplementary materials.

2.6. In vitro study

The effect of the water extract on cell viability was investigated through MTT test on murine C2C12 cells. Cells were cultured in DMEM supplemented with 10% (v/v) heat-inactivated fetal bovine serum, and 1.2% (v/v) penicillin G/streptomycin, maintained in a humidified incubator with 5% CO_2 at 37 °C. Then, cells were incubated with the extract in the range concentration of 5–1000 μg/mL for 24 h. Cells were added with 10 μL of MTT solution at the concentration of 5 mg/mL and incubated for 3 h. The formazan product was solubilized with DMSO, and the absorbance of the solutions was measured (Ferrante et al., 2019).

2.7. Ex vivo study

In agreement with the recognized principles of "replacement, refinement and reduction in animals in research", prostate specimens were obtained as residual material from vehicle-treated mice randomized in our previous experiments, approved by the local ethical committee ('G. d'Annunzio' University, Chieti, Italy) and Italian Health Ministry (Project no. 885/2018-PR).

The prostate specimens were maintained in a humidified incubator at 37 °C and challenged with scalar concentrations of the extract (10–1000 μg/mL). Samples were then subjected to gene expression analyses of IL-6, IL-1β, and TNF-α, as previously reported (Ferrante et al., 2020).

2.8. Antimicrobial activity

The extract was tested for *in vitro* antifungal and antimicrobial activity against different species: *Candida albicans* (YEPGA 6183), *C. tropicalis* (YEPGA 6184), *C. albicans* (YEPGA 6379), *C. parapsilopsis* (YEPGA 6551), *Arthroderma curreyi* (CCF 5207), *A. gypseum* (CCF 6261), *A. insingulare* (CCF 5417), *A. quadrifidum* (CCF 5792), *Trichophyton mentagrophytes* (CCF 4823), *T. mentagrophytes* (CCF 5930), *T. rubrum* (CCF 4933), *T. tonsurans* (CCF 4834), *Escherichia coli* (ATCC 10536), *E. coli* (PeruMycA 2), *E. coli* (PeruMycA 3), *Pseudomonas aeruginosa* (ATCC 15442), *Salmonella typhi* (PeruMyc 7), *Bacillus subtilis* (PeruMyc 6), and *Staphylococcus aureus* (ATCC 6538). Ciprofloxacin, fluconazole, and griseofulvin were used as reference drugs (Serventi et al., 2023).

2.9. Statistical analysis

Statistical analysis was performed by GraphPad Prism™ (Version 5.01) software (GraphPad Software, Inc., San Diego, CA, USA). The statistical significance ($P < 0.05$) was evaluated through analysis of variance (ANOVA) followed by Newman–Keuls comparison multiple test.

3. Results and discussion

3.1. Phytochemical composition

The water extract obtained from the residual powder following female inflorescences trimming was analyzed for phytochemical composition using HPLC coupled to UV detector. The results of the chromatographic analysis revealed the presence of cannabidiolic acid (CBDA, peak #1) and cannabigerolic acid (CBGA, peak #2) in larger quantities in comparison with other terpenophenols identified, such as cannabidiol (CBD, peak #3) and tetrahydrocannabinolic acid (THCA, peak #4), which were only present in traces (Fig. 1). Quantitative

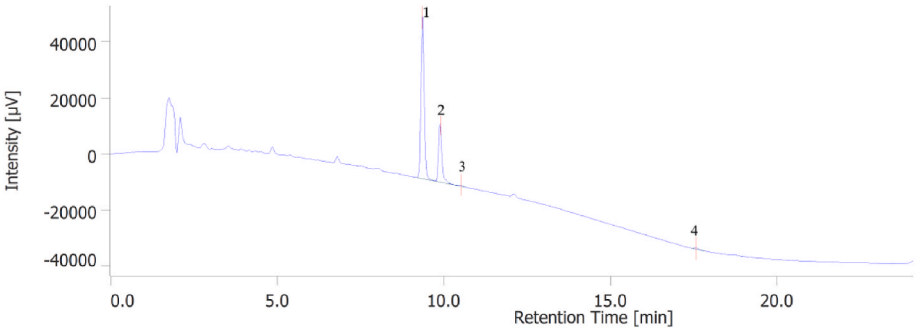

Fig. 1. Chromatogram of terpenophenolic compounds of water extract from residual female inflorescences trimming. CBDA (peak #1) and CBGA (peak #2) were the prominent, compared with the other identified terpenophenolics, CBD (peak #3) and THCA (peak #4). The separation of the terpenophenols was conducted in gradient elution mode on an Infinity lab Poroshell 120-EC reverse phase column (C18, 150 mm × 4.6 mm i.d., 2.7 µm; Agilent Santa Clara, CA, USA). Details about chromatographic conditions are reported in supplementary materials (Tables S1–S5).

determination is reported in Table S4 of supplementary materials. Previous studies have shown the dominant presence of CBDA, CBGA (Palmieri et al., 2023), and CBD (Orlando et al., 2021) in several extracts of the *Kompolti* variety, while the water extracts of *Futura 75* and *Strawberry* were characterized by a high content of CBDA and CBD (di Giacomo et al., 2021; Serventi et al., 2023). These findings align with the results obtained from the terpenophenolic analysis of the water extract in this study.

CBGA has minimal binding affinity for CB1 and CB2 receptors (Navarro et al., 2018). High levels of CBGA in *Cannabis* have shown cytotoxic activity against colon cancer cells, indicating potential benefits in treating certain types of cancer (Hazekamp & erkelens, 2014). CBDA has a weak binding affinity for cannabinoid CB1 and CB2 receptors (Mechoulam & Gaoni, 1965; Navarro et al., 2018; Zagzoog et al., 2020). Reported assays have been conducted with CHO cells *in vitro*, which have indicated that CBDA, CBGA, and CBG activate PPARα and PPARγ. These two receptors are critical for regulating energy homeostasis and metabolism (D'Aniello et al., 2019). Administration of CBDA in a rodent model with carrageenan-induced hind paw inflammation produces dose-dependent anti-hyperalgesic and anti-inflammatory effects (Rock et al., 2018).

The Folin-Ciocalteu colorimetric assays permitted to quantify total phenolic and flavonoid compounds, whose content in the extract can be related, albeit partially, with antiradical effects, as indicated by ABTS and DPPH assays (Table 1).

Phenolic and flavonoid compounds were also measured through a targeted chromatographic analysis that permitted to identify and quantify, through comparison with pure standards, 34 phenolic compounds. Gallic acid (peak #1), catechin (peak #4), p-coumaric acid (peak #15), t-ferulic acid (peak #1 16), and benzoic acid (peak #17) were the most abundant phenols (Fig. 2). Details about quantitative analysis are fully reported in supplementary materials (Table S5). *Cannabis* phenolics have been reported to exert anti-inflammatory, anticancer, and neuro-protective effects (Andre et al., 2010). In many cancers, phenolic acids such as gallic and ferulic acids can inhibit key enzyme actions, prevent angiogenesis, and activate caspase-mediated apoptosis, resulting in reduced tumor growth and progression (Carocho & Ferreira, 2013). Also, flavonoids and phenolic acids have been found to inhibit the inflammation process (Dos Santos et al., 2006; Sur et al., 2008; Tipoe et al., 2007). Interestingly, in the present study the level of cannaflavin A, a characteristic *C. sativa* flavonoid, has been evaluated in the extract (peak # 34), although the concentration of the phytochemical (about 2% compared with the prominent phenolic compound, namely gallic acid) pointed to a minor role of cannaflavin A in influencing the extract biological effects, as detailed below.

Table 1
Total phenolic content (TPC) and total flavonoid content (TFC) of the industrial hemp water extract from residual material after trimming the female inflorescences (WE). Values are reported as mean ± S.D. of three parallel measurements. Intrinsic antioxidant properties of the tested extract evaluated with ABTS and DPPH assays. GAE: Gallic acid equivalents; RE: Rutin equivalents. TE: Trolox equivalents; dm: dry material.

Treatment	TPC (GAE) (mg/g_{dm})	TFC (RE) (mg/g_{dm})	ABTS (TE) (mg/g_{dm})	DPPH (TE) (mg/g_{dm})
Water Extract	7.29 ± 0.15	2.37 ± 0.12	8.61 ± 0.31	3.51 ± 0.01

3.2. Biocompatibility limits

In order to explore the potential health-promoting effects of the extract, an eco-toxicological study was formerly approached to define the limits of biocompatibility in eukaryotic organisms using different plant and animal alternative toxicological models. Specifically, an allelopathy assay was conducted to predict the influence of the extract on seedling germination of different herbaceous and commercial plants, namely *C. inthybus, C. endivia,* and *D. repens*. It is well-known that specialized metabolites working as allelochemicals, among which phenolic compounds, are released by plants in the surrounding environment to compete with other species (Khamare et al., 2022). In this regard, phytotoxic effects were considered in terms of seedling germination delay of the tested species measured as root and hypocotyl elongation during the 96 h seed exposure to the extract, *in vitro*. In the concentration range 0.039–10.0 mg/mL, the extract did not alter significantly ($P > 0.05$) the seedling germination compared with the untreated control group (Fig. 3); thus, indicating the absence of any phytotoxic effect and confirming biocompatibility against eukaryotic organisms that was further validated with independent toxicity assays, namely *A. salina* lethality and *D. magna* toxicity assays.

Artemia salina, also known as brine shrimp, is a zooplanktonic crustacean ubiquitous in saline aquatic environments ranging from lakes to oceans and it is extensively utilized as a model system for the evaluation of acute toxicological responses. In particular, the brine shrimp lethality assay is widely used in preliminary screenings for bioactive compounds due to its simplicity, rapidity, reliability, and cost-efficiency (Meyer et al., 1982). This assay also demonstrates a high correlation of results with cytotoxic activity in higher organisms, in particular with the toxicity data of rodents and humans and shows a good correlation with cytotoxicity tests; thus, making these measurements suitable as preliminary results (da Silveira Carvalho et al., 2017; Živković et al., 2016). *Artemia* species have been used in testing acute toxicity of toxic materials, such as heavy metals and pesticides (Ates et al., 2013), nanoparticles, bioactive molecules, plant extracts, and metal complexes (Zhu et al., 2018). In this context, the present study was conducted to determine the toxicity limits of the extract that were evaluated in terms of LC_{50} (lethality concentration) value, in the same concentration range

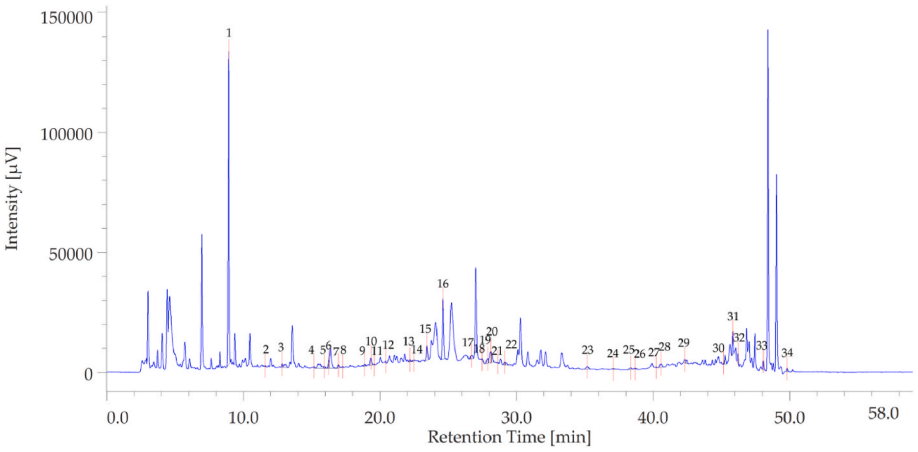

Fig. 2. Chromatogram of phenolic analysis of water extract from industrial hemp residuals. The prominent detected phenolic compounds are gallic acid (peak #1), catechin (peak #4), p-coumaric acid (peak #15), t-ferulic acid (peak #1 16), and benzoic acid (peak #17). The separation of the phenolic compounds was conducted in gradient elution mode on an Infinity lab Poroshell 120-SB reverse phase column (C18, 150 × 4.6 mm i.d., 2.7 µm; Agilent, Santa Clara, CA, USA). Details about chromatographic conditions are reported in supplementary materials (Tables S1–S5).

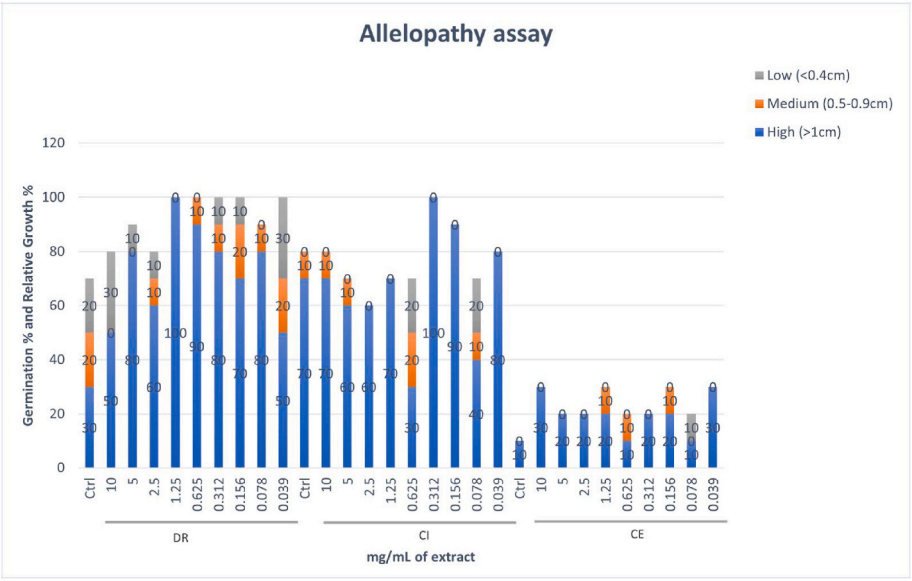

Fig. 3. Null effects of *C. sativa* water extract (0.039–10 mg/mL) on the seedling germination of *C. inthybus* (CI), *C. endivia* (CE), and *D. repens* (DR) from seeds exposed to the extracts for 96 h. The extract is considered phytotoxic only in case of seedling germination reduction >30%.

selected for the allelopathy assay (0.039–10 mg/mL). The determination of the toxicity level was based on LC_{50} values, using the standard toxicity indices established by Meyer and Clarkson. This classification helps in assessing the potential toxicity of various substances, including plant extracts, chemicals, and pharmaceuticals. According to Meyer's classification, extracts are considered toxic if the LC_{50} is less than 1000 µg/mL, and non-toxic if the LC_{50} is greater than 1000 µg/mL (Meyer et al., 1982).

Clarkson's classification categorizes substances as non-toxic for LC_{50} values above 1000 µg/mL, low toxic for LC_{50} values between 500 and 1000 µg/mL, medium toxic for LC_{50} values between 100 and 500 µg/mL, and highly toxic for LC_{50} values between 0 and 100 µg/mL (Clarkson et al., 2004). Therefore, considering the LC_{50} value of 1.726 mg/mL (Table 2; Fig. 4) the water extract from trimming-deriving material can be considered biocompatible and non-toxic in the selected eco-toxicological model. The same LC_{50} value was indicative for the set-up of the *D. magna* cardiotoxicity assay. *Daphnia magna*, a widely used model in biology, is known for its sensitive behavioural and physiological reactions that serve as biomarkers for environmental and chemical impacts, including herbal extracts and pharmaceuticals

Table 2
Brine shrimp lethality assay results in terms of LC_{50} value and toxicity levels according to Meyer's and Clarckson's classifications. Tested sample: water extract from *C. sativa* female inflorescences residuals after trimming, in a concentration range between 0.039 and 10 mg/mL.

	Concentration range [mg/mL]	LC_{50} (mg/mL)	95% confidence interval	R^2	Toxicity class	
					Meyer's classification	Clarkson's classification
Water extract	[0.039–10]	1.726	1.318–2.260	0.97	non-toxic	non-toxic

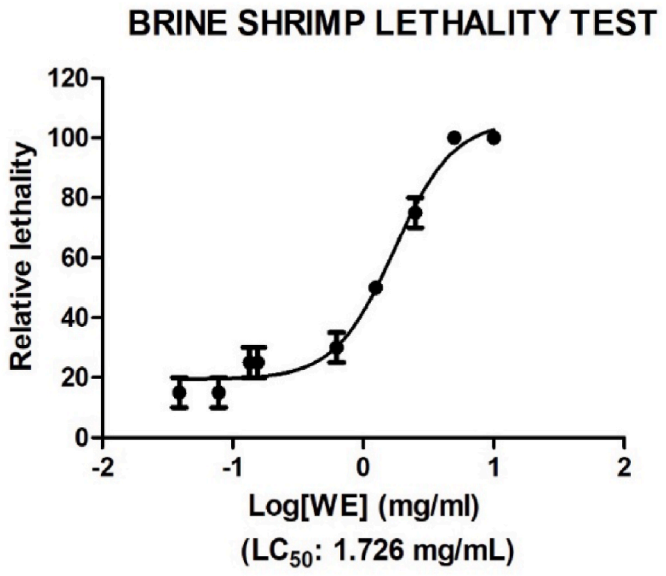

Fig. 4. Dose-response curve related to the effect induced by industrial hemp water extract (0.039–10 mg/mL) on the viability of *Artemia salina (*Brine shrimp lethality assay). LC_{50}: 1.726 mg/mL.

(Tkaczyk et al., 2021). At the concentration of 1.726 mg/mL the extract did not alter significantly the hearth rate beat, either in basal conditions or in ethanol-induced hearth rate beat reduction (Fig. 5); thus, ruling out any cardiotoxic effect. The biocompatibility limits of the extract were also investigated in mouse C2C12 cells. A concentration limit at least ten-fold lower (1000 μg/mL) compared with *A. salina* assay was selected in this *in vitro* model. Specifically, in C2C12 cells the extract was tested in the concentration range 10–1000 μg/mL evaluating its impact on cell viability. As shown by MTT assay (Fig. 6), the extract did not influence cell viability; thus, further corroborating the extract biocompatibility.

3.3. Anti-inflammatory and antimicrobial activity

After defining the limits of biocompatibility, the extract (10–1000 μg/mL) was tested on isolated mouse prostate specimens exposed to *E. coli* LPS, an *ex vivo* experimental paradigm mimicking the burden of

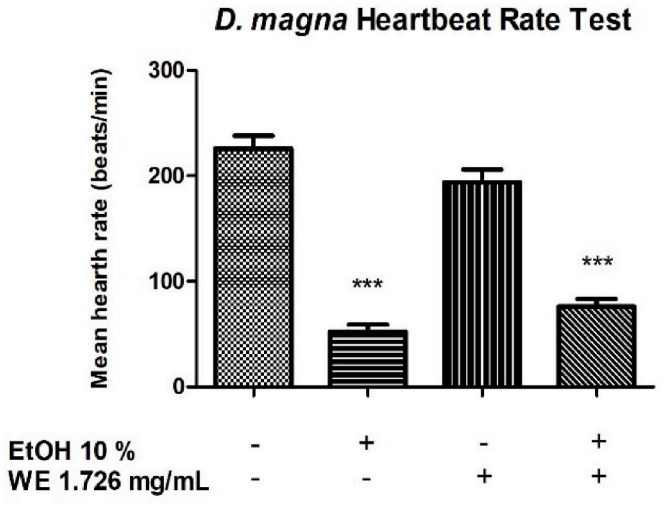

Fig. 5. Null effect induced by industrial hemp water extract (1.726 mg/mL) on *Dapnia magna* hearth rate beat in both basal and ethanol-induced cardiotoxic conditions. ANOVA, $P < 0.0001$; ***$P < 0.001$ vs. untreated control group (−/−).

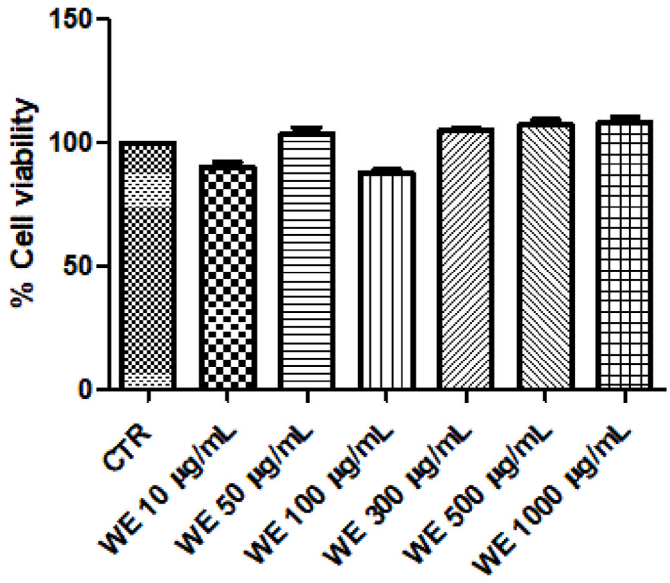

Fig. 6. Null effect induced by industrial hemp water extract (WE) 10–1000 μg/mL on murine C2C12 cell line viability.

inflammation occurring in bacterial prostatitis (Chiavaroli et al., 2022; Ferrante et al., 2020). In the present study, the extract was effective in preventing the LPS-induced gene expression of different cytokines, namely TNFα, IL-6, and IL-1β (Fig. 7) that are known to be involved in prostatitis (Jang & Schaeffer, 2003). It has been reported that phenolic compounds (Chiavaroli et al., 2022; Ferrante et al., 2020; Locatelli et al., 2018) and cannabinoids (O'Reilly et al., 2023; Piao et al., 2024) exert a protective role in the prostate, with both anti-inflammatory effects and reduction of prostate cancer cell viability *in vitro*. The present extract conserved the anti-inflammatory effects typical of both phenolics and cannabinoids. But, considering the high degree of biocompatibility observed in eco-toxicological models, a cytotoxic effect against prostate cancer cells is questionable. This hypothesis is also supported by our previous observation of null effect on human prostate cancer PC3 cells viability induced by polar extracts from industrial hemp pollen (Acquaviva et al., 2022). Nevertheless, considering the involvement of pro-inflammatory cytokines in the so-called inflammatory to cancer transition (Chen et al., 2019), the present results pointed to the potential of the extract to contrast not only the clinical symptoms occurring in bacterial prostatitis but also to prevent the prostate carcinogenesis process. This protective role is also consistent with the results of the antimicrobial activity shown by the extract (Tables 3–5), that demonstrated bacteriostatic and mycostatic effects at MIC values, expressed as μg/mL, that were in the range of biocompatibility. The extract was indeed effective against bacterial (*E. coli, P. aeruginosa, B. subtilis, S. typhi,* and *S. aureus*) and *Candida* (*C. albicans. C. parapsilosis* and *C. tropicalis*) species playing a key role in prostatitis (Delcaru et al., 2017; Mishra et al., 2016; Odabasi & Mert, 2020). Furthermore, a significant inhibitory effect was observed against different dermatophytes species (*Trichophyton rubrum*, T. *tonsurans, T. mentagrophytes*, *A. quadrifidum*, *A. gypseum*, *A. currey*, and *A. insingulare*), as well. This is of particular relevance considering the higher incidence of dermatophytes infections in patients suffering from urinary tract diseases (Irimie et al., 2014). Also in this case, the pattern of antimicrobial effects can be related, albeit in part, to the content of both cannabinoids and phenolic compounds (Bottari et al., 2017; de Camargo et al., 2017; Schofs et al., 2021).

4. Conclusions

The residual plant material derived from the trimming process of *Cannabis* inflorescences is typically regarded as waste in the industrial

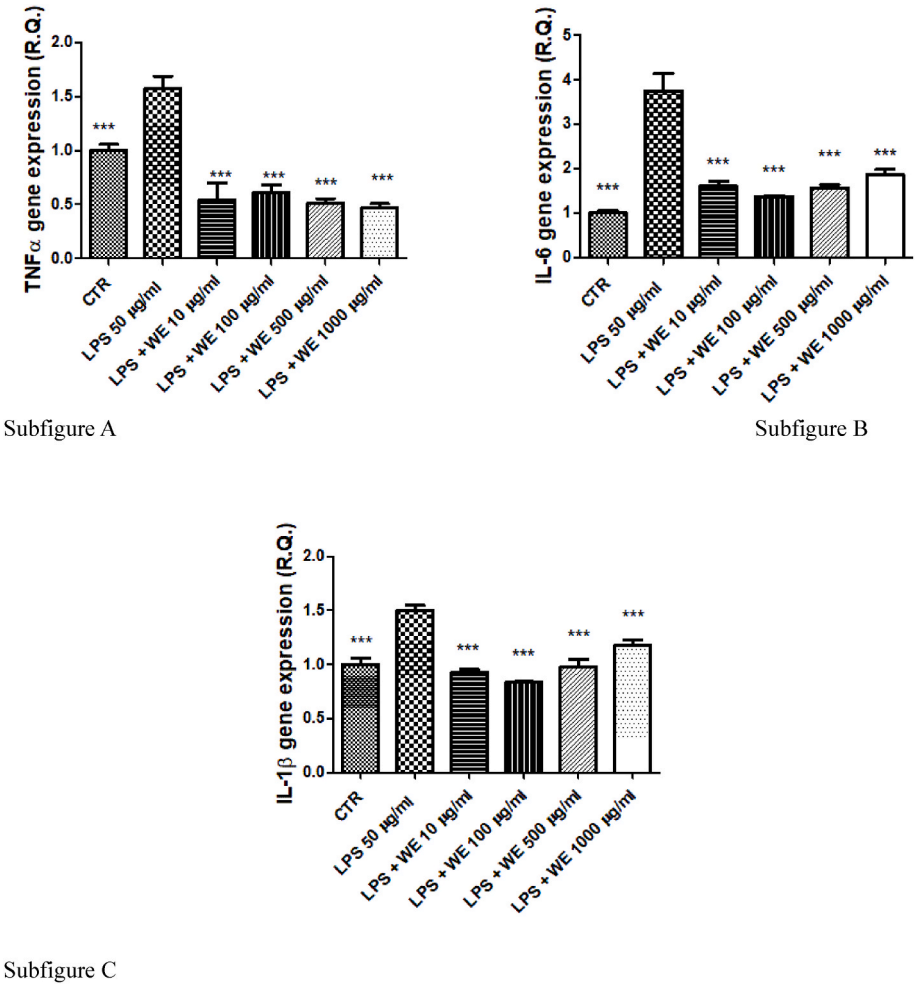

Fig. 7. Inhibitory effects induced by industrial hemp water extract (WE) 10–1000 μg/mL on *E. coli* LPS (50 μg/mL)-induced gene expression of TNFα (Subfigure A), IL-6 (Subfigure B), and IL-1β (Subfigure C), in isolated mouse prostate specimens. ANOVA, $P < 0.0001$; ***$P < 0.001$ Vs. respective CTR (Control) group.

Table 3
Minimal inhibitory concentrations (MICs) of hemp extract against bacterial strains. MIC (μg/mL). The MIC values are reported as geometric means of three independent replicates (n = 3). The MIC range concentrations are reported within brackets.

Bacteria	*Escherichia* coli (ATCC 10536)	*Escherichia* coli (PeruMycA 2)	*Escherichia* coli (PeruMycA 3)	*Bacillus* cereus (PeruMycA 4)	*Pseudomonas* aeruginosa (ATCC 15442)	*Bacillus* subtilis (PeruMycA 6)	*Salmonella* typhi (PeruMycA 7)	*Staphylococcus* aureus (ATCC 6538)
Hemp extract	>200	>200	>200	>200	>200	>200	>200	>200
Ciprofloxacin	31.49 (25–50)	9.92 (6.25–12.5)	79.37 (50–100)	125.99 (100–200)	125.99 (100–200)	125.99 (100–200)	9.37 (50–100)	>200

Table 4
Minimal inhibitory concentrations (MIC, μg/mL) of industrial hemp water extract against yeast strains. The MIC values are reported as geometric means of three independent replicates (n = 3). The MIC range concentrations are reported within brackets.

Yeast strains	*Candida* tropicalis (YEPGA 6184)	*Candida* albicans (YEPGA 6379)	*Candida* parapsilosis (YEPGA 6551)	*Candida* albicans (YEPGA 6183)
Hemp extract	125.99 (100–200)	>200	>200	>200
Fluconazole	2	1	4	2

hemp supply chain. However, in the interest of promoting sustainability within impactful chains, such as industrial hemp, there is potential to repurpose this by-product as an additional ingredient for the formulation of health-promoting agents. Indeed, the water extract of this powder, which has been shown to contain trichome glands as a putative source of its content in specialized metabolites, namely cannabinoids and phenolic compounds, demonstrated anti-inflammatory and antimicrobial properties; thus, deserving to be considered as an innovative secondary raw material that can be recycled and upcycled. Furthermore, employing an environmentally friendly extraction solvent, such as water, on one side aligns with the objectives outlined in the UN 2030 agenda for modern sustainable development, on the other hand, is consistent with a traditional phytotherapeutic use of medicinal plants whose preparation very often involves the extraction with simple homemade methods like infusion and decoction. In conclusion, the polar extract obtained from the residual powder of the inflorescence trimming

Table 5
Minimal inhibitory concentrations (MICs) of hemp extract against dermatophyte isolates. MIC (μg/ml). The MIC values are reported as geometric means of three independent replicates (n = 3). The MIC range concentrations are reported within brackets.

Dermatophytes	*Trichophyton* mentagrophytes (CCF 4823)	*Trichophyton* tonsurans (CCF 4834)	*Trichophyton* rubrum (CCF 4933)	*Trichophyton* mentagrophytes (CCF 5930)	*Arthroderma* quadrifidum (CCF 5792)	*Arthroderma* gypseum (CCF 6261)	*Arthroderma* curreyi (CCF 5207)	*Arthroderma* insingulare (CCF 5417)
Hemp extract	62.99 (50–100)	158.74 (100–200)	158.74 (100–200)	125.99 (100–200)	158.74 (100–200)	>200	>200	>200
Griseofulvin	2.52 (2–4)	0.198 (0.125–0.25)	1.26 (1–2)	3.174 (2–4)	>8	1.587 (1–2)	>8	>8

offers a new and promising product that could have a positive impact on the industrial hemp supply chain, particularly due to its efficacy as anti-inflammatory and antimicrobial and the high degree of biocompatibility, as shown by the ecotoxicological study. Furthermore, the collection of this plant material, through the reduction of the waste material produced with the trimming process, can be regarded as a further smart strategy to optimize the efficiency of the whole supply chain.

CRediT authorship contribution statement

Simonetta Cristina Di Simone: Writing – original draft, Investigation, Data curation. **Maria Loreta Libero:** Investigation, Formal analysis, Data curation. **Riccardo Pulcini:** Data curation, Conceptualization. **Nilofar Nilofar:** Investigation. **Annalisa Chiavaroli:** Investigation. **Fatma Tunali:** Investigation. **Paola Angelini:** Investigation. **Giancarlo Angeles Flores:** Investigation. **Roberto Venanzoni:** Visualization, Supervision. **Gaia Cusumano:** Investigation. **Gokhan Zengin:** Writing – review & editing, Software, Formal analysis, Data curation. **Luigi Brunetti:** Visualization, Supervision. **Lucia Recinella:** Investigation. **Sheila Leone:** Investigation. **Giustino Orlando:** Resources, Project administration, Methodology, Funding acquisition. **Luigi Menghini:** Resources, Project administration, Methodology, Formal analysis, Data curation, Conceptualization. **Claudio Ferrante:** Writing – review & editing, Writing – original draft, Software, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Alessandra Acquaviva:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization.

Funding

The study was conducted within the joint project (2020–2023) between Department of Pharmacy of "G. d'Annunzio" University and Veridia S.r.l. aimed to the valorization of industrial hemp cultivation in the Middle Italy, Abruzzo Region. Project coordinators are Giustino Orlando, Luigi Menghini, and Claudio Ferrante. In this context, the Veridia S.r.l. also funded the industrial PhD project of Dr. Alessandra Acquaviva entitled: "*Cannabis and circular economy: enhancing an agricultural supply chain with a high social impact, for the development of agro-industry through botanical innovations and related applications in the nutraceutical, pharmacological, cosmetic/cosmeceutical and health sectors in general*". Academic tutors are Giustino Orlando and Claudio Ferrante.

The study was also supported by the Cristalfarma S.r.l. that funded the PhD project of Dr. Simonetta Cristina Di Simone entitled "From the Botanical Garden "Giardino dei Semplici" to modern rational phytotherapy: Enhancement of biodiversity and study of plant extracts in complex mixtures for innovative phytotherapeutic applications". Academic tutors are Luigi Menghini and Claudio Ferrante.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The present study is part of the third mission activities of the Botanic Garden "Giardino dei Semplici".

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2024.105344.

Data availability

Data will be made available on request.

References

Acquaviva, A., Cristina Di Simone, S., Canini, A., Braglia, R., Di Marco, G., Campana, C., Angelini, P., Angeles Flores, G., Venanzoni, R., Loreta Libero, M., Tirillini, B., Zengin, G., Chiavaroli, A., Recinella, L., Leone, S., Nilofar, Brunetti, L., Orlando, G., Menghini, L., & Ferrante, C. (2022). Phytochemical and biological investigations on the pollen from industrial hemp male inflorescences. *Food Research International, 161*, Article 111883. https://doi.org/10.1016/j.foodres.2022.111883

Andre, C. M., Hausman, J.-F., & Guerriero, G. (2016). *Cannabis sativa*: The plant of the thousand and one molecules. *Frontiers in Plant Science, 7*, 19. https://doi.org/10.3389/fpls.2016.00019

Andre, C. M., Larondelle, Y., & Evers, D. (2010). Dietary antioxidants and oxidative stress from a human and plant perspective: A review. *Current Nutrition & Food Science, 6*(1), 2–12. https://doi.org/10.2174/157340110790909563

Ates, M., Daniels, J., Arslan, Z., Farah, I. O., & Rivera, H. F. (2013). Comparative evaluation of impact of Zn and ZnO nanoparticles on brine shrimp (*Artemia salina*) larvae: Effects of particle size and solubility on toxicity. *Environmental Science. Processes & Impacts, 15*(1), 225–233. https://doi.org/10.1039/c2em30540b

Bertoli, A., Tozzi, S., Pistelli, L., & Angelini, L. G. (2010). Fibre hemp inflorescences: From crop-residues to essential oil production. *Industrial Crops and Products, 32*(3), 329–337. https://doi.org/10.1016/j.indcrop.2010.05.012

Birt, D. F., Hendrich, S., & Wang, W. (2001). Dietary agents in cancer prevention: Flavonoids and isoflavonoids. *Pharmacology & Therapeutics, 90*(2–3), 157–177. https://doi.org/10.1016/s0163-7258(01)00137-1

Bonini, S. A., Premoli, M., Tambaro, S., Kumar, A., Maccarinelli, G., Memo, M., & Mastinu, A. (2018). *Cannabis sativa*: A comprehensive ethnopharmacological review of a medicinal plant with a long history. *Journal of Ethnopharmacology, 227*, 300–315. https://doi.org/10.1016/j.jep.2018.09.004

Bottari, N. B., Lopes, L. Q. S., Pizzuti, K., Filippi Dos Santos Alves, C., Correa, M. S., Bolzan, L. P., Zago, A., de Almeida Vaucher, R., Boligon, A. A., Giongo, J. L., Baldissera, M. D., & Santos, R. C. V. (2017). Antimicrobial activity and phytochemical characterization of *Carya illinoensis*. *Microbial Pathogenesis, 104*, 190–195. https://doi.org/10.1016/j.micpath.2017.01.037

Carocho, M., & Ferreira, I. C. F. R. (2013). The role of phenolic compounds in the fight against cancer—a review. *Anti-Cancer Agents in Medicinal Chemistry, 13*(8), 1236–1258.

Chen, X., Xu, C., Hong, S., Xia, X., Cao, Y., McDermott, J., Mu, Y., & Han, J.-D. J. (2019). Immune cell types and secreted factors contributing to inflammation-to-cancer transition and immune therapy response. *Cell Reports, 26*(7), 1965–1977.e4. https://doi.org/10.1016/j.celrep.2019.01.080

Chiavaroli, A., Di Simone, S. C., Acquaviva, A., Libero, M. L., Campana, C., Recinella, L., Leone, S., Brunetti, L., Orlando, G., Nilofar, Vitale, I., Cesa, S., Zengin, G., Menghini, L., & Ferrante, C. (2022). Protective effects of PollenAid Plus soft gel capsules' hydroalcoholic extract in isolated prostates and ovaries exposed to lipopolysaccharide. *Molecules, 27*(19). https://doi.org/10.3390/molecules27196279

Chiavaroli, A., Masciulli, F., Ingallina, C., Mannina, L., Loreta Libero, M., Di Simone, S. C., Acquaviva, A., Nilofar, Recinella, L., Leone, S., Brunetti, L., Carradori, S., Cantò, L., Orlando, G., Zengin, G., Ibrahim Uba, A., Cakilcioğlu, U., Mukemre, M., Elkiran, O., … Ferrante, C. (2024). Comprehensive metabolite and biological profile of "Sulmona Red Garlic" ecotype's aerial bulbils. *Food Research International, 175*, Article 113654. https://doi.org/10.1016/j.foodres.2023.113654

Clarkson, C., Maharaj, V. J., Crouch, N. R., Grace, O. M., Pillay, P., Matsabisa, M. G., Bhagwandin, N., Smith, P. J., & Folb, P. I. (2004). In vitro antiplasmodial activity of

medicinal plants native to or naturalised in South Africa. *Journal of Ethnopharmacology, 92*(2–3), 177–191. https://doi.org/10.1016/j.jep.2004.02.011
da Silveira Carvalho, J. M., de Morais Batista, A. H., Nogueira, N. A. P., Holanda, A. K. M., de Sousa, J. R., Zampieri, D., Bezerra, M. J. B., Stefânio Barreto, F., de Moraes, M. O., Batista, A. A., Gondim, A. C. S., Paulo, T.de F., de França Lopes, L. G., & Sousa, E. H. S. (2017). A biphosphinic ruthenium complex with potent anti-bacterial and anti-cancer activity. *New Journal of Chemistry, 41*(21), 13085–13095. https://doi.org/10.1039/C7NJ02943H
D'Aniello, E., Fellous, T., Iannotti, F. A., Gentile, A., Allarà, M., Balestrieri, F., Gray, R., Amodeo, P., Vitale, R. M., & Di Marzo, V. (2019). Identification and characterization of phytocannabinoids as novel dual PPARα/γ agonists by a computational and in vitro experimental approach. *Biochimica et Biophysica Acta. General Subjects, 1863*(3), 586–597. https://doi.org/10.1016/j.bbagen.2019.01.002
de Camargo, A. C., Regitano-d'Arce, M. A. B., Rasera, G. B., Canniatti-Brazaca, S. G., do Prado-Silva, L., Alvarenga, V. O., Sant'Ana, A. S., & Shahidi, F. (2017). Phenolic acids and flavonoids of peanut by-products: Antioxidant capacity and antimicrobial effects. *Food Chemistry, 237*, 538–544. https://doi.org/10.1016/j.foodchem.2017.05.046
Delcaru, C., Podgoreanu, P., Alexandru, I., Popescu, N., Marutescu, L., Bleotu, C., Mogosanu, G. D., Chifiriuc, M. C., Gluck, M., & Lazar, V. (2017). Antibiotic resistance and virulence phenotypes of recent bacterial strains isolated from urinary tract infections in elderly patients with prostatic disease. *Pathogens (Basel, Switzerland), 6*(2), E22. https://doi.org/10.3390/pathogens6020022
di Giacomo, V., Recinella, L., Chiavaroli, A., Orlando, G., Cataldi, A., Rapino, M., Di Valerio, V., Politi, M., Antolini, M. D., Acquaviva, A., Ak, G., & Ferrante, C. (2021). Metabolomic profile and antioxidant/anti-inflammatory effects of industrial hemp water extract in fibroblasts, keratinocytes and isolated mouse skin specimens. *Antioxidants, 10*(1), 1–21. https://doi.org/10.3390/antiox10010044
Donati, L., Casagrande Pierantoni, D., Conti, A., Calzoni, E., Corte, L., Santi, C., Rosati, O., Cardinali, G., & Emiliani, C. (2024). Water extracts from industrial hemp waste inhibit the adhesion and development of *Candida* biofilm and showed antioxidant activity on HT-29 colon cancer cells. *International Journal of Molecular Sciences, 25*(7). https://doi.org/10.3390/ijms25073979
dos Santos, M. D., Almeida, M. C., Lopes, N. P., & de Souza, G. E. P. (2006). Evaluation of the anti-inflammatory, analgesic and antipyretic activities of the natural polyphenol chlorogenic acid. *Biological and Pharmaceutical Bulletin, 29*(11), 2236–2240. https://doi.org/10.1248/bpb.29.2236
Ferguson, L. R. (2001). Role of plant polyphenols in genomic stability. *Mutation Research, 475*(1–2), 89–111. https://doi.org/10.1016/s0027-5107(01)00073-2
Ferrante, C., Chiavaroli, A., Angelini, P., Venanzoni, R., Flores, G. A., Brunetti, L., Petrucci, M., Politi, M., Menghini, L., Leone, S., Bacchin, F., & Orlando, G. (2020). Phenolic content and antimicrobial and anti-inflammatory effects of *Solidago virgaaurea, Phyllanthus niruri, Epilobium angustifolium, Peumus boldus*, and *Ononis spinosa* extracts. *Antibiotics, 9*(11), 1–21. https://doi.org/10.3390/antibiotics9110783
Ferrante, C., Recinella, L., Ronci, M., Menghini, L., Brunetti, L., Chiavaroli, A., Leone, S., Di Iorio, L., Carradori, S., Tirillini, B., Angelini, P., Covino, S., Venanzoni, R., & Orlando, G. (2019). Multiple pharmacognostic characterization on hemp commercial cultivars: Focus on inflorescence water extract activity. *Food and Chemical Toxicology, 125*. https://doi.org/10.1016/j.fct.2019.01.035
Flores-Sanchez, I. J., & Verpoorte, R. (2008). Secondary metabolism in cannabis. *Phytochemistry Reviews, 7*(3), 615–639. https://doi.org/10.1007/s11101-008-9094-4
Happyana, N., Agnolet, S., Muntendam, R., Van Dam, A., Schneider, B., & Kayser, O. (2013). Analysis of cannabinoids in laser-microdissected trichomes of medicinal *Cannabis sativa* using LCMS and cryogenic NMR. *Phytochemistry, 87*, 51–59. https://doi.org/10.1016/j.phytochem.2012.11.001
Hazekamp, A., & erkelens, jaap (2014). Exploring the sativa indica dilemma. *Cannabinoids, 9*, 9–15.
Hourfane, S., Mechqoq, H., Bekkali, A. Y., Rocha, J. M., & El Aouad, N. (2023). A comprehensive review on *Cannabis sativa* ethnobotany, phytochemistry, molecular docking and biological activities. *Plants (Basel, Switzerland), 12*(6). https://doi.org/10.3390/plants12061245
Irimie, M., Tataru, A., Oanta, A., & Moga, M. (2014). In vitro susceptibility of dermatophytes isolated from patients with end- stage renal disease: A case-control study. *Mycoses, 57*(3), 129–134. https://doi.org/10.1111/myc.12114
Jang, T. L., & Schaeffer, A. J. (2003). The role of cytokines in prostatitis. *World Journal of Urology, 21*(2), 95–99. https://doi.org/10.1007/s00345-003-0335-2
Khamare, Y., Chen, J., & Marble, S. C. (2022). Allelopathy and its application as a weed management tool: A review. *Frontiers in Plant Science, 13*, Article 1034649. https://doi.org/10.3389/fpls.2022.1034649
Locatelli, M., Macchione, N., Ferrante, C., Chiavaroli, A., Recinella, L., Carradori, S., Zengin, G., Cesa, S., Leporini, L., Leone, S., Brunetti, L., Menghini, L., & Orlando, G. (2018). Graminex pollen: Phenolic pattern, colorimetric analysis and protective effects in immortalized prostate cells (PC3) and rat prostate challenged with LPS. *Molecules, 23*(5). https://doi.org/10.3390/molecules23051145
Mahlberg, P. G., & Kim, E. S. (2004). Accumulation of cannabinoids in glandular trichomes of cannabis (Cannabaceae). *Journal of Industrial Hemp, 9*(1), 15–36. https://doi.org/10.1300/J237v09n01_04
Mazzara, E., Torresi, J., Fico, G., Papini, A., Kulbaka, N., Dall'Acqua, S., Sut, S., Garzoli, S., Mustafa, A. M., Cappellacci, L., Fiorini, D., Maggi, F., Giuliani, C., & Petrelli, R. (2022). A comprehensive phytochemical analysis of terpenes, polyphenols and cannabinoids, and micromorphological characterization of 9 commercial varieties of *Cannabis sativa* L. *Plants (Basel, Switzerland), 11*(7). https://doi.org/10.3390/plants11070891
Mechoulam, R., & Gaoni, Y. (1965). Hashish. IV. The isolation and structure of cannabinolic cannabidiolic and cannabigerolic acids. *Tetrahedron, 21*(5), 1223–1229. https://doi.org/10.1016/0040-4020(65)80064-3
Meyer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., Nichols, D. E., & McLaughlin, J. L. (1982). Brine shrimp: A convenient general bioassay for active plant constituents. *Planta Medica, 45*(5), 31–34. https://doi.org/10.1055/s-2007-971236
Mishra, P. P., Prakash, V., Singh, K., Mog, H., & Agarwal, S. (2016). Bacteriological profile of ısolates from urine samples in patients of benign prostatic hyperplasia and or prostatitis showing lower urinary tract symptoms. *Journal of Clinical and Diagnostic Research: Journal of Clinical and Diagnostic Research, 10*(10), DC16–DC18. https://doi.org/10.7860/JCDR/2016/21973.8734
Navarro, G., Varani, K., Reyes-Resina, I., Sánchez de Medina, V., Rivas-Santisteban, R., Sánchez-Carnerero Callado, C., Vincenzi, F., Casano, S., Ferreiro-Vera, C., Canela, E. I., Borea, P. A., Nadal, X., & Franco, R. (2018). Cannabigerol action at cannabinoid CB(1) and CB(2) receptors and at CB(1)-CB(2) heteroreceptor complexes. *Frontiers in Pharmacology, 9*, 632. https://doi.org/10.3389/fphar.2018.00632
Odabasi, Z., & Mert, A. (2020). Candida urinary tract infections in adults. *World Journal of Urology, 38*(11), 2699–2707. https://doi.org/10.1007/s00345-019-02991-5
O'Reilly, E., Khalifa, K., Cosgrave, J., Azam, H., Prencipe, M., Simpson, J. C., Gallagher, W. M., & Perry, A. S. (2023). Cannabidiol ınhibits the proliferation and ınvasiveness of prostate cancer cells. *Journal of Natural Products, 86*(9), 2151–2161. https://doi.org/10.1021/acs.jnatprod.3c00363
Orlando, G., Adorisio, S., Delfino, D., Chiavaroli, A., Brunetti, L., Recinella, L., Leone, S., D'antonio, M., Zengin, G., Acquaviva, A., Menghini, L., & Ferrante, C. (2021). Comparative investigation of composition, antifungal, and anti-inflammatory effects of the essential oil from three industrial hemp varieties from Italian cultivation. *Antibiotics, 10*(3). https://doi.org/10.3390/antibiotics10030334
Orlando, G., Recinella, L., Chiavaroli, A., Brunetti, L., Leone, S., Carradori, S., Simone, S. D., Ciferri, M. C., Zengin, G., Ak, G., Menghini, L., & Ferrante, C. (2020). 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. *Antioxidants, 9*(5). https://doi.org/10.3390/antiox9050437
Öztürk, M., Duru, M. E., Kivrak, S., Mercan-Doğan, N., Türkoglu, A., & Özler, M. A. (2011). In vitro antioxidant, anticholinesterase and antimicrobial activity studies on three *agaricus* species with fatty acid compositions and iron contents: A comparative study on the three most edible mushrooms. *Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association, 49*(6), 1353–1360. https://doi.org/10.1016/j.fct.2011.03.019
Palmieri, S., Fanti, F., Oliva, E., Viteritti, E., Sergi, M., Pepe, A., & Compagnone, D. (2023). Chemical characterization and evaluation of antioxidant activity from different cultivars of *Cannabis sativa* L. of Abruzzo's region. *Natural Product Research, 37*(15), 2591–2595. https://doi.org/10.1080/14786419.2022.2051704
Piao, J. J., Kim, S., Shin, D., Lee, H. J., Jeon, K.-H., Tian, W. J., Hur, K. J., Kang, J. S., Park, H.-J., Cha, J. Y., Song, A., Park, S.-H., Rajasekaran, M., Bae, W. J., Yoon, S. K., & Kim, S. W. (2024). Cannabidiol Alleviates Chronic prostatitis and chronic pelvic pain syndrome via CB2 receptor activation and TRPV1 desensitization. *The World Journal of Men's Health*. https://doi.org/10.5534/wjmh.230352
Pollastro, F., Minassi, A., & Fresu, L. G. (2018). *Cannabis* phenolics and their bioactivities. *Current Medicinal Chemistry, 25*(10), 1160–1185. https://doi.org/10.2174/0929867324666170810164636
Radwan, M. M., Elsohly, M. A., Slade, D., Ahmed, S. A., Wilson, L., El-Alfy, A. T., Khan, I. A., & Ross, S. A. (2008). Non-cannabinoid constituents from a high potency *Cannabis sativa* variety. *Phytochemistry, 69*(14), 2627–2633. https://doi.org/10.1016/j.phytochem.2008.07.010
Rock, E. M., Limebeer, C. L., & Parker, L. A. (2018). Effect of cannabidiolic acid and Δ(9)-tetrahydrocannabinol on carrageenan-induced hyperalgesia and edema in a rodent model of inflammatory pain. *Psychopharmacology, 235*(11), 3259–3271. https://doi.org/10.1007/s00213-018-5034-1
Schewe, T., Kühn, H., & Sies, H. (2002). Flavonoids of cocoa inhibit recombinant human 5-lipoxygenase. *The Journal of Nutrition, 132*(7), 1825–1829. https://doi.org/10.1093/jn/132.7.1825
Schofs, L., Sparo, M. D., & Sánchez Bruni, S. F. (2021). The antimicrobial effect behind *Cannabis sativa*. *Pharmacology Research & Perspectives, 9*(2), Article e00761. https://doi.org/10.1002/prp2.761
Serventi, L., Flores, G. A., Cusumano, G., Barbaro, D., Tirillini, B., Venanzoni, R., Angelini, P., Acquaviva, A., Di Simone, S. C., Orlando, G., Zengin, G., Menghini, L., & Ferrante, C. (2023). Comparative ınvestigation of antimicrobial and antioxidant effects of the extracts from the ınflorescences and leaves of the *Cannabis sativa* L. cv. strawberry. *Antioxidants, 12*(2), Article Scopus. https://doi.org/10.3390/antiox12020219
Sirikantaramas, S., Taura, F., Tanaka, Y., Ishikawa, Y., Morimoto, S., & Shoyama, Y. (2005). Tetrahydrocannabinolic acid synthase, the enzyme controlling marijuana psychoactivity, is secreted into the storage cavity of the glandular trichomes. *Plant and Cell Physiology, 46*(9), 1578–1582. https://doi.org/10.1093/pcp/pci166
Sur, R., Nigam, A., Grote, D., Liebel, F., & Southall, M. D. (2008). Avenanthramides, polyphenols from oats, exhibit anti-inflammatory and anti-itch activity. *Archives of Dermatological Research, 300*(10), 569–574. https://doi.org/10.1007/s00403-008-0858-x
Sutton, D. B., Punja, Z. K., & Hamarneh, G. (2023). Characterization of trichome phenotypes to assess maturation and flower development in *Cannabis sativa* L. (cannabis) by automatic trichome gland analysis. *Smart Agricultural Technology, 3*, Article 100111. https://doi.org/10.1016/j.atech.2022.100111
Tanney, C. A. S., Backer, R., Geitmann, A., & Smith, D. L. (2021). Cannabis glandular trichomes: A cellular metabolite factory. *Frontiers in Plant Science, 12*, Article 721986. https://doi.org/10.3389/fpls.2021.721986

Tipoe, G. L., Leung, T.-M., Hung, M.-W., & Fung, M.-L. (2007). Green tea polyphenols as an anti-oxidant and anti-inflammatory agent for cardiovascular protection. *Cardiovascular & Hematological Disorders Drug Targets, 7*(2), 135–144. https://doi.org/10.2174/187152907780830905

Tkaczyk, A., Bownik, A., Dudka, J., Kowal, K., & Ślaska, B. (2021). *Daphnia magna* model in the toxicity assessment of pharmaceuticals: A review. *The Science of the Total Environment, 763*, Article 143038. https://doi.org/10.1016/j.scitotenv.2020.143038

Zagzoog, A., Mohamed, K. A., Kim, H. J. J., Kim, E. D., Frank, C. S., Black, T., Jadhav, P. D., Holbrook, L. A., & Laprairie, R. B. (2020). In vitro and in vivo pharmacological activity of minor cannabinoids isolated from *Cannabis sativa*. *Scientific Reports, 10*(1), Article 20405. https://doi.org/10.1038/s41598-020-77175-y

Zhu, B., Zhu, S., Li, J., Hui, X., & Wang, G.-X. (2018). The developmental toxicity, bioaccumulation and distribution of oxidized single walled carbon nanotubes in *Artemia salina*. *Toxicology Research, 7*(5), 897–906. https://doi.org/10.1039/c8tx00084k

Živković, M. B., Matić, I. Z., Rodić, M. V., Novaković, I. T., Sladić, D. M., & Krstić, N. M. (2016). Synthesis, characterization and in vitro cytotoxic activities of new steroidal thiosemicarbazones and thiadiazolines. *RSC Advances, 6*(41), 34312–34333. https://doi.org/10.1039/C6RA01516F