

HYBRID PERCOLATION-ULTRASOUND TECHNOLOGY TO IMPROVE EXTRACTION OF BIOACTIVE SUBSTANCES FROM HEMP BUDS FOR AGRICULTURAL APPLICATIONS

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Abstract

The increasing interest in hemp (Cannabis sativa L.) as a source of high-value bioactive compounds has driven the development of efficient and environmentally responsible extraction technologies. Hemp buds contain substantial levels of polyphenols with antioxidant, anti-inflammatory, and antimicrobial properties, making them valuable for functional food, nutraceutical, and cosmetic applications. Conventional extraction methods often require long processing times, high solvent volumes, and provide limited recovery of thermolabile compounds. This study evaluates a hybrid extraction technique that combines percolation with ultrasound-assisted extraction to improve the yield and functionality of polyphenolic fractions from hemp buds. Key parameters - solvent type, percolation rate, ultrasound intensity, and extraction duration - were systematically varied and optimized using response surface methodology. Extraction performance was assessed through total polyphenol content, total flavonoid content, and antioxidant activity, with chromatographic analyses used to quantify major phenolic constituents. The hybrid system outperformed individual techniques, achieving higher polyphenol yields with shorter extraction times and reduced solvent consumption. The resulting extracts showed strong antioxidant capacity associated with their enriched chemical profiles.

Key words: ultrasound-assisted extraction, bioactive substances, polyphenols, percolation, flavonoids.

INTRODUCTION

Hemp buds and inflorescences represent the anatomical structures of the plant with the highest concentrations of bioactive secondary metabolites, including terpenes (α -pinene, myrcene, limonene, β -caryophyllene), polyphenolic compounds, and phytocannabinoids such as cannabidiol (CBD) and cannabidiolic acid (CBDA). These compounds are predominantly localized within glandular trichomes, conferring a broad and complex biological activity profile (Ascrizzi et al., 2024; Yusoff et al., 2021). The extracts obtained from the inflorescences have been shown to be biostimulants, stimulating the germination and development of plants,

increasing biomass, chlorophyll and carotenoid content, and improving the antioxidant activity of the treated crops, which suggests an improved photosynthetic efficiency and stress response (Varga et al., 2025). At the same time, hydrophilic extracts from buds can manifest significant inhibitory effects on fungi due to their high content of polyphenols and antioxidants (Kursa et al., 2024). The essential oils extracted from the inflorescences showed insecticidal and repellent effects, causing high mortality in the larvae of some insect pests and over 80% effectiveness against the *Tetranychus urticae* mite, due to the high concentrations of volatile terpenes (Rossi et al., 2020; Prvulović et al., 2023). At higher concentrations, inflorescence extracts may exhibit allelopathic

activity, suppressing the germination of certain plant species. This suggests their potential applicability in the development of bioherbicide formulations and their use in sustainable soil management practices (Kojić et al., 2025).

Given this diversity of bioactive compounds and their application potential, the process of obtaining extracts becomes an essential element for the optimal use of plant resources. However, traditional extraction methods have several disadvantages: they often use toxic solvents in large quantities, are time-consuming and consume a lot of energy (Cao et al., 2025). In contrast, modern hybrid technologies, such as ultrasonically assisted percolation extraction, offer a much more efficient solution for obtaining these valuable substances. Ultrasound facilitates the rapid release of compounds from plant cell structures, reduces extraction time and increases yield, while requiring lower energy consumption (Nenciu et al., 2023; Tăbărașu et al., 2025).

In the study (Kobus et al., 2022), the extraction of bioactive compounds from hemp leaves and buds was performed by ultrasonically assisted extraction, using an aqueous solution of 60% ethanol as a solvent. The extraction process was carried out at a frequency of 20 kHz and at a controlled temperature of 30°C. The efficiency of the extraction was evaluated by determining the total flavonoid content, the values obtained varying between 3.02 and 9.35 mg QE/g plant material. In another study (Tiago et al., 2022), the extraction of total phenols from hemp was achieved by both ultrasound-assisted extraction and conventional Soxhlet extraction. Ultrasonic extraction was performed at 60°C for 90 minutes and a solid-liquid ratio of 1:10, resulting in a high polyphenol yield of approximately 7.76 mg GAE/g plant material. In contrast, the Soxhlet ethanol extraction, carried out for 90 minutes, generated a significantly lower content of phenolic compounds, of about 0.26 mg GAE/g plant material.

In the study (Lazarević et al., 2025), the extraction of total phenols from the flower buds was performed by conventional extraction and ultrasonically assisted extraction. Conventional extraction was performed at room temperature for 24 hours using a solid-liquid ratio of 1:10. The results showed that 50% ethanol led to the highest concentrations of phenolic compounds,

with the total phenol content varying, depending on the stage of harvest, between approximately 0.4996 and 1.7045 mg GAE/mL extract. For ultrasonically assisted extraction, a 40 kHz ultrasonic bath was used, maintaining the same solid-liquid ratio (1:10) and choosing 50% ethanol as solvent. The varied process parameters were temperature (40-80°C), extraction time (20-60 min) and ultrasonic power density (24-60 W/L). Under these conditions, the total phenol content of the extracts ranged from 1.0552 to 1.7795 mg GAE/mL.

In the study (Chemat et al., 2025), the extraction of phenolic compounds from *Cannabis sativa* L. seeds was achieved by subcritical extraction with microwave-assisted water. The extraction process was carried out at essential parameters such as temperature (100-180°C), extraction time (5-60 min) and solid-liquid ratio (1:5-1:30 g/v). The maximum reported value was approximately 6.57 g GAE/100 g extract.

The synergistic integration of cannabinoids and polyphenols into crop protection strategies represents a novel approach to sustainable agriculture, transforming the secondary metabolite spectrum of the *Cannabis sativa* L. plant into a multipurpose biochemical shield. While cannabinoids exert direct insecticidal and repellent pressure on pests by disrupting neurophysiological processes, the polyphenolic fraction adds an essential layer of protection through its robust antioxidant and antimicrobial properties (Benelli et al., 2018). These phenolic compounds, including flavonoids and phenolic acids, act as inhibitors of insect digestive enzymes and strengthen the structural barriers of treated plants, while simultaneously providing protection against oxidative stress induced by environmental factors (Bass et al., 2014). Unlike synthetic pesticides, this hybrid phytochemical complex exhibits superior biodegradability and minimal toxicity to non-target organisms, facilitating the ecological management of agroecosystems (Lupi et al., 2013). Utilizing these bioactive fractions not only minimizes dependence on conventional chemical inputs but also valorizes industrial biomass in the spirit of a circular economy, transforming extraction by-products into renewable solutions for restoring soil health and optimizing horticultural productivity (Benelli et al., 2017).

From the analyzed studies, it appears that conventional extraction methods are associated with longer process times and lower yields of phenolic compounds caused by the increased temperatures affecting certain classes of thermosensitive polyphenols (Tăbărașu et al., 2024; Oprescu et al., 2023).

The use of hemp extracts is a promising solution for organic farming, due to the high content of bioactive compounds with antifungal and insecticidal effect. These properties allow crops to be protected from pathogens and pests, reducing the need for the use of synthetic chemical pesticides (Ona et al., 2022; Selçuk et al., 2022). In the study (Kursa et al., 2024), hemp extracts, rich in polyphenols, flavonoids and cannabinoids, demonstrated pronounced antifungal activity against phytopathogenic fungi such as *Botrytis cinerea*, *Alternaria alternata* and *Colletotrichum coccodes*, supporting their potential as natural alternatives to conventional chemical fungicides.

The aim of the present research was to assess the potential of a hybrid ultrasound-percolation technology for extracting polyphenols from hemp buds, for an improved recovery of polyphenolic compounds, total cannabinoids, nitrogen, flavonoids, and minerals. The integration of acoustic cavitation with continuous solvent displacement provides a superior extraction kinetic, significantly enhancing process efficiency while maintaining a low-cost, scalable operational profile.

MATERIALS AND METHODS

The hybrid extraction equipment consists of a Timatic DUO percolator (Tecnolab SRL, Spello, Italy) (Figure 1) and a UP400St ultrasonic generator (Hielscher Ultrasonics GmbH, Teltow, Germany). The percolator includes an extraction chamber, two hydropneumatic cylinders, a pump, solenoid valves, an expansion vessel, a pneumatic distributor, a compressor, a control panel, a pressure sensor and a tank for collecting the extract. For the realization of the hybrid extraction, the extraction chamber of the percolator was technically adapted by integrating the ultrasonic probe of the ultrasonic generator UP400St (Figure 2).



Figure 1. Timatic DUO percolator: 1 - extraction chamber; 2 - control panel; 3 - extract collection vessel; 4 - expansion vessel; 5 – compressor

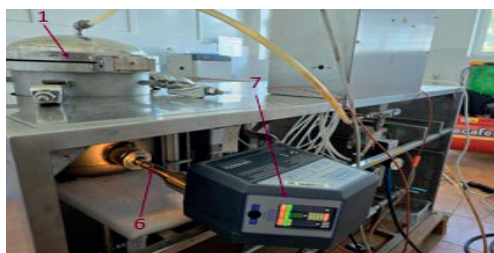


Figure 2. UP400St Ultrasonic Generator: 1 - chamber extractive; 6 - sonotrode; 7 - panel ultrasonic generator control

The hybrid extraction process begins by supplying the system with compressed air generated by the compressor, distributed through the pneumatic distributor to the solenoid valves and hydropneumatic cylinders. The solvent is pumped out of the expansion vessel with the help of the pump and introduced into the extraction chamber, where the plant material is located. Here the pressure extraction process takes place, monitored by the pressure sensor, and assisted by ultrasound generated by the ultrasonic device. After the extraction is completed, the extract is discharged through the automatically controlled valves and collected into the extract tank. The total polyphenols were extracted in a water/methanol mixture (1:1), followed by centrifugation and analysis by the Folin-Ciocalteu method. A permeable bag was used to immobilize the plants in the extraction chamber (Figure 3).

The hybrid ultrasound-assisted percolation (UAP) extraction process optimizes the recovery of bioactive compounds by integrating acoustic cavitation forces into a continuous solvent flow.

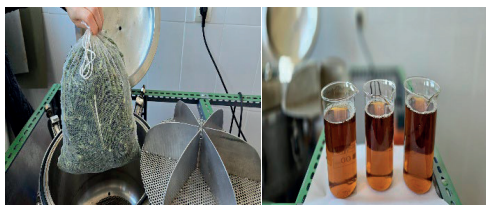


Figure 3. Biomass sample introduction into the extraction chamber and the obtained extracts

Simultaneously, percolation (pressure increase to 7 bar, maintaining it for 5 minutes, followed by a rapid decreasing in pressure to 0.15 bar and maintaining it for 2 minutes) maintains a constant concentration gradient, transporting the extracted metabolites out of the plant matrix and preventing solvent saturation. This synergy drastically reduces processing time and solvent consumption while preserving the thermal integrity of sensitive molecules through controlled power output.

To evaluate the biochemical profile, extracts were obtained from the buds and inflorescences of the Marideea hemp variety, a monoecious industrial cultivar developed at SCDA Secuieni, utilizing an optimized ultrasound-assisted percolation framework. This variety constitutes an ideal feedstock for industrial-scale extraction, providing chemical uniformity and high biomass availability, while being characterized by its adaptability to Eastern European pedoclimatic conditions and a THC profile strictly compliant with current European regulations. Its significant concentration of secondary metabolites, particularly polyphenols and cannabinoids within the floral by-products, makes it an ideal candidate for intensification through hybrid ultrasound-percolation modalities.

The total phenolic content was quantified using the Folin-Ciocalteu spectrophotometric assay and expressed as Gallic Acid Equivalents (GAE). Antioxidant capacity was evaluated through the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay, measuring the percentage of radical inhibition. For the nutritional profile, total nitrogen was determined via the Kjeldahl method, involving sulfuric acid digestion followed by distillation and titration. Total cannabinoids (CBD and CBDA), were analyzed using High-Performance Liquid Chromatography (HPLC-UV) to ensure the separation of neutral and

acidic forms without thermal degradation. vitamin C levels were assessed through redox titration, while the structural and lipid components, specifically crude fiber and crude fat, were quantified using enzymatic-gravimetric digestion and Soxhlet extraction with non-polar solvents, respectively. Soluble solids were monitored using refractometry and expressed in degrees Brix.

RESULTS AND DISCUSSIONS

By utilizing ultrasound-assisted percolation, the research evaluates the technical capacity to mobilize bioactive compounds into a highly bioavailable liquid format. The objective of this study was not the analysis of the hemp specie (which would have required a multi-year analysis), but prioritized the efficiency of the extraction method considering the plant genetic limitations.

Table 1. Evaluation of bioactive compounds from Marideea (Secuieni) cultivar biomass and ultrasound-assisted percolates extraction method

Compound Group	Values in Plant	Ultrasound-Percolation Extraction	Role as Fertilizer / Biostimulator
Total Cannabinoids (CBD/CBDA)	0.73%	0.61%	Antifungal effect and pest repellent.
Total Polyphenols	34.71 mg GAE/g	29 mg/g	UV protection and resistance to pathogens.
Organic Nitrogen (N)	3.68%	3.15%	Primary source for protein synthesis in new crops.
Amino Acids (glutamic, aspartic)	155 mg/g	105 mg/g	Natural chelation of micronutrients and cold resistance.
Phenolic Acids & Flavonoids	34.71 mg GAE/g	31.8 mg/g	Strong antioxidant; protects plants from thermal stress.
Chelated Minerals (K, Mg, Ca)	K: 1.8%	K: 1.1%	Essential for photosynthesis and cell wall rigidity.
	Mg: 0.4%	Mg: 0.25%	
	Ca: 1.2%	Ca: 0.9%	
Phytohormones (Cytokinins, Auxins)	9 µg/g	6 µg/g	Stimulates cell division and root development.
Vitamin C	65 mg/100 g	52 mg/100g	Enzyme cofactor; helps detoxify plants under stress.
Sugars (Brix)	14.06 °Bx	12.9 °Bx	Energy source for soil microorganisms.

The integration of ultrasonic cavitation effectively bypasses the plant's significant fiber

matrix to release a high-performance concentration of bioactive compounds. The high recovery rate ensures that the resulting liquid serves as a potent nitrogen source for protein synthesis, while the vitamin C acts as a critical enzyme cofactor to help plants detoxify under environmental stress. Furthermore, the extraction process successfully captures amino acids like glutamic and aspartic acid at concentrations which are essential for the natural chelation of micronutrients and increasing a crop's resistance to cold temperatures. Beyond basic nutrition, the Marideea variety extract stands out due to its massive antioxidant capacity, evidenced by the 34.71 mg GAE/g of total polyphenols measured in the sample. The ultrasound-assisted percolation efficiently transfers these into the extract at levels between 31.0 and 33.0 mg/g, providing a "biological shield" that offers UV protection and increased resistance against pathogenic agents. This is complemented by the

CONCLUSIONS

Experimental data showed that the hybrid ultrasound-assisted percolation (UAP) technology is suitable for the extraction of target bioactive substances from the Marideea hemp variety. Analytical assessments of the resulting extracts indicate a superior recovery rate for both cannabinoids and polyphenols, surpassing the yields typically achieved through conventional maceration or simple percolation. This increased efficiency is attributed to the mechanical effects of acoustic cavitation, which ensures the total disruption of glandular trichomes and parenchymal tissues, thereby maximizing the mass transfer of secondary metabolites into the solvent. Furthermore, the continuous flow nature of the system maintained a high kinetic gradient, preventing the degradative thermal accumulation often associated with prolonged sonication. The stability of the phytochemical profile confirms that the UAP parameters were optimally calibrated to balance extraction power with molecular integrity. Given that monoecious hemp varieties exhibit lower cannabinoid concentrations compared to high-resin dioecious cultivars, the present research focused primarily on optimizing extraction

0.61% total cannabinoids successfully extracted from the initial 0.73% found in the plant, which contributes an antifungal effect and acts as a natural repellent for various pests.

The mineral profile is equally impressive, with the process yielding 1.6% Potassium, 0.35% Magnesium, and 0.9% Calcium in chelated forms that are vital for maintaining photosynthesis and structural cell wall rigidity in treated plants. When these minerals are combined with the extracted phytohormones (up to 12 µg/g) and a sugar content of 13.5 °Bx, the resulting biostimulant not only triggers root development and cell division but also provides an immediate energy source for beneficial soil microorganisms. Ultimately, the high fiber content of 25.91% and ash content of 8.30% in the base plant material are effectively bypassed by the ultrasonic method to create a concentrated, bioactive fertilizer that far exceeds the performance of standard organic inputs.

efficiency rather than on the inherent genetic limitations of the plant material.

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