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Review

Ultrasound-assisted extraction of phytocannabinoids from *Cannabis sativa* L.: An update

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ABSTRACT

Introduction: Phytocannabinoids are phytochemicals that bind to cannabinoid receptors and display pharmacological properties like those of *Cannabis sativa* L. (Cannabaceae). Among well over 100 structurally related phytocannabinoids produced by *C. sativa*, cannabidiol (CBD) and Δ^9-tetrahydrocannabinol (Δ^9-THC or THC) are the two major ones. With the continuing drive for applying green, environmentally friendly, high-yielding, and simple technology for extracting phytocannabinoids, ultrasound-assisted extraction (UAE) has emerged as one of the most popular techniques.

Objectives: To provide an overview of UAE methods for phytocannabinoids extraction from *C. sativa* and its products published during 2021-25.

Material and methods: Information was obtained from Web of Science, ScienceDirect, PubMed, and Google Scholar. *Cannabis sativa*, ultrasound-assisted extraction, cannabinoid, and phytocannabinoid were used as the search terms. Only the journal articles, books, and book chapters published on the review topic during 2021-25 are included, and any doctoral theses or conference abstracts are excluded from this review.

Results: During 2021-25, there were at least 26 publications on the use of UAE for extracting phytocannabinoids from the aerial parts, flowers and inflorescences, leaves, roots, and seeds of *C. sativa* plant as well as *C. sativa*-based products. Various phytocannabinoids, e.g., CBC, CBCA, CBCV, CBD, CBDA, CBD-C4, CBDA-C4, CBDP, CBDV, CBDVA, CBG, CBGA, CBN, CBNA, THC, THCA, 11-THC-OH, 11-THC-COOH, THCV, and THCVA were successfully extracted by UAE. Ethanol and methanol were the most used solvents for UAE of phytocannabinoids. However, the use of other solvents like acetonitrile and chloroform, olive oil, and eutectic solvents was also observed.

Conclusion: UAE is an excellent green extraction technique for extracting phytocannabinoids from *C. sativa* with a high extraction yield.

Keywords: Ultrasound-assisted extraction; phytocannabinoids; *Cannabis sativa*; Cannabaceae; hemp; marijuana

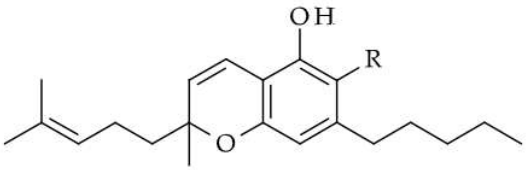
INTRODUCTION

Cannabinoids are a group of compounds that bind to cannabinoid receptors, and cannabinoids that occur in plants, e.g., *Cannabis sativa* L., are known as phytocannabinoids [1-6]. *Cannabis sativa* L., an annual herbaceous flowering plant from the family Cannabaceae, is indigenous to East Asia but cultivated widely throughout the globe [7,8]. This plant is the major source of over a hundred structurally related phytocannabinoids [1-4]. However, there are a few other plants from different genera and families, including *Acmella oleracea, Echinacea angustifolia, E. purpurea, Helichrysum umbraculigerum,* and *Radula marginata*, which also biosynthesise phytocannabinoids of different structural classes, e.g., alkamides [1-3]. Δ^9-Tetrahydrocannabinol (Δ^9-THC or THC), known to have psychoactive properties, and cannabidiol (CBD), known to counteract the psychoactive properties of THC, are (Figure 1) two main phytocannabinoids of *C. sativa* [1-5] (Figure 1). Phytocannabinoids are therapeutically [6,9,10] as well as cosmeceutically [11,13] valuable compounds. Cannabis is often clinically used for treating depression, glaucoma, nausea, neuralgia, and pain, and is used in cosmeceutical products [1, 6-13]. Cannabis-infused food products and beverages are popular in several cultures globally; for example, 'Bhang,' an Indian beverage made from *C. sativa* leaves and inflorescences, is used in religious festivals [1,14,15].

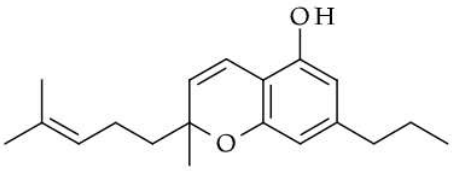
Extraction of phytocannabinoids from plant materials is one of the fundamental steps in separating and purifying individual phytocannabinoids by chromatographic methods [1-4]. For the extraction of phytocannabinoids [4], traditional extraction methods like maceration as well as modern methods, e.g., accelerated solvent extraction (ASE) [16], microwave-assisted extraction (MAE) [1,16], and ultrasound-assisted extraction (UAE) [16], are routinely used. However, the increasing pressure to use environmentally friendly laboratory practices has prompted the use of green extraction methods for phytocannabinoids, and UAE is an economically viable green extraction method for extracting phytocannabinoids from *C. sativa*. In addition to the green aspect, UAE is a rapid, usually non-thermal, and cost-effective extraction method that uses high-frequency acoustic waves to isolate valuable compounds from plant, algal, and microbial sources. Ultrasound ruptures cell walls efficiently, delivers higher yields in less time with reduced solvent use [16]. The efficiency of the UAE depends on a few variables, including ultrasound power or amplitude, frequency (usually, 20-40 kHz), extraction time, extraction solvent, extraction temperature, solid-to-liquid ratio, particle size, and duty cycle [17].

UAE depends on the principles of acoustic cavitation and involves four key steps: wave generation, bubble formation, cavitation and shear, and cell disruption [16-19]. Ultrasound waves (>20 kHz) are passed through the extracting solvent containing the extractable material (extractable). The rapid cycles of compression generate microscopic vacuum bubbles, which grow and violently collapse, resulting in intense localised shockwaves, elevated temperature, and strong shear forces. These resulting mechanical forces break rigid cell walls and membranes, affording easy penetration of any extracting solvent and effective extraction of target compounds, e.g., phytocannabinoids. UAE has distinct advantages, like quality preservation, speed of extraction, high extraction yield (high extraction efficiency), and better green technology compliance, over other extraction methods. For a UAE operation, usually an ultrasonic bath or an ultrasonic probe is used (Figure 2). Ultrasonic baths are the most used devices for UAE. Ultrasonic probes (also known as horn sonicators) (Figure 2), on the other hand, deliver direct, high-intensity ultrasound energy into the sample and are more suitable for dense materials like chitin-rich medicinal mushrooms.

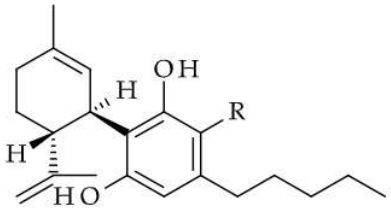
This article reviews the publications on the UAE of phytocannabinoids from *C. sativa* published during 2021-25 (Table 1) [20-45]. Since the publication of the review article on the extraction methods of phytocannabinoids in 2021 [4], which covered literature until the end of 2020, several papers have been published on the UAE of phytocannabinoids. Information was obtained from various databases, including Web of Science, ScienceDirect, PubMed, and Google Scholar. *Cannabis sativa*, ultrasound-assisted extraction, cannabinoid, and phytocannabinoid were used as the search terms. Only the journal articles, books, and book chapters published on the review topic during 2021-25 have been included, and any doctoral theses or conference abstracts have been excluded from this review. The objective of this narrative review is not to discuss the technological and mechanistic aspects of UAE in detail, but to appraise various UAE methods used for extracting phytocannabinoids from *C. sativa.*



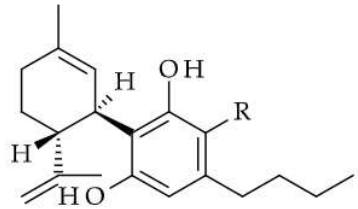
Cannabichromene (CBC), R = H
Cannabichromene acid (CBCA), R = COOH



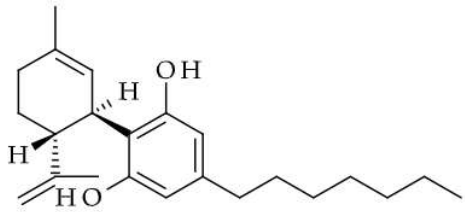
Cannabichromevarin (CBCV)



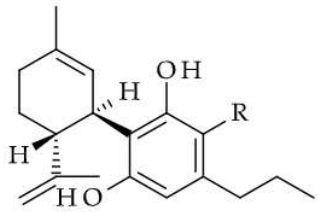
Cannabidiol (CBD), R = H
Cannabidiolic acid (CBDA), R = COOH



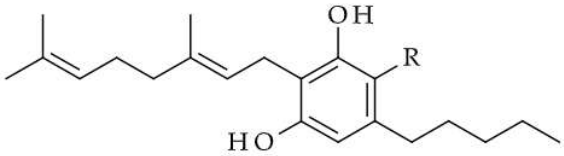
Cannabidibutol (CBD-C4), R = H
Cannabidibutolic acid (CBDA-C4), R = COOH



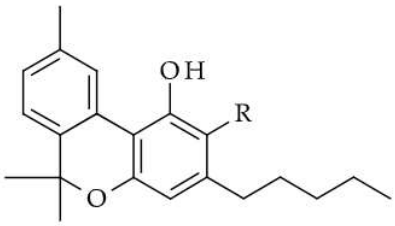
Cannabidiphorol (CBDP)



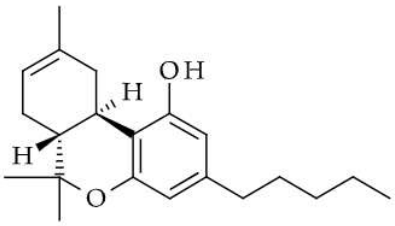
Cannabidivarin (CBDV), R = H
Cannabidivarinic acid (CBDVA), R = COOH



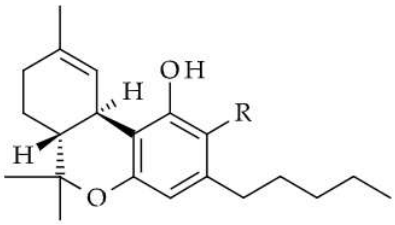
Cannabigerol (CBG), R = H
Cannabigerolic acid (CBGA), R = COOH



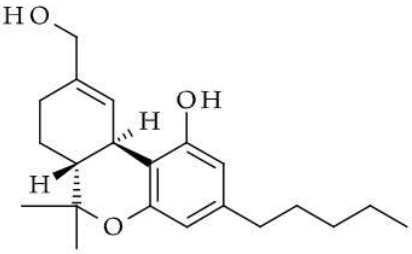
Cannabinol (CBN), R = H
Cannabinolic acid (CBNA), R = COOH

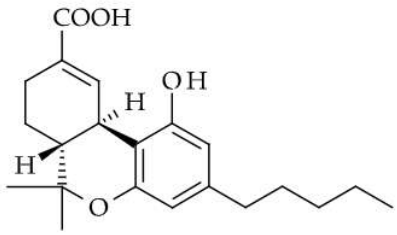


Δ^8- Tetrahydrocannabinol (Δ^8-THC)



Δ^9- Tetrahydrocannabinol

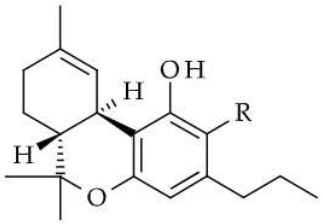




(Δ^9-THC or THC), R = H
Δ^9- Tetrahydrocannabinolic acid
(Δ^9-THCA or THCA), R = COOH

11-Hydroxy-Δ^9-tetrahydrocannabinol (11-THC-OH)

11-Nor-9-carboxy-Δ^9-tetrahydrocannabinol (11-THC-COOH)



Tetrahydrocannabivarin (THCV), R = H
Tetrahydrocannabivarin acid (THCVA), R = COOH

Figure 1. Structures of major phytocannabinoids extracted from *C. sativa* by ultrasound-assisted extraction (UAE), reported in 2021-25

ULTRASOUND-ASSISTED EXTRACTION OF PHYTOCANNABINOIDS

During 2021-25, there were at least 26 publications on the use of UAE for extracting phytocannabinoids from various parts (aerial parts, flowers and inflorescences, leaves, roots, and seeds of *C. sativa* plant as well as *C. sativa*-based products (Table 1) [20-45]. Because of low cost, operational simplicity, high extraction efficiency, and environmentally friendly nature, the UAE has become quite popular in extracting phytocannabinoids. EtOH and MeOH were the most used solvents for UAE of phytocannabinoids. However, the use of other solvents like ACN and chloroform, olive oil, and eutectic solvents was also noticed. Various phytocannabinoids, e.g., CBC, CBCA, CBCV, CBD, CBDA, CBD-C4, CBDA-C4, CBDP, CBDV, CBDVA, CBG, CBGA, CBN, CBNA, THC, THCA, 11-THC-OH, 11-THC-COOH, THCV, and THCVA (Figure 1) were successfully extracted by UAE (Table 1). Most of the UAE operations were conducted at 25-30 ^{o}C, but there were also a few instances where an elevated temperature, e.g., 60 ^{o}C was used. The extraction time was as short as 1 min, and the highest extraction time reported was 120 min for the UAE of phytocannabinoids. Ultrasonic baths used for the extraction of phytocannabinoids were mostly between 100 W and 300 W. Both bath-type and probe-type (Figure 2) were used for UAE of phytocannabinoids, albeit the bath-type was found to be more popular than the probe-type.

Aerial parts

Finely milled (a diameter of *ca.* 2.5 mm) commercially obtained hemp biomass (bulk *C. sativa* aerial parts) was extracted by probe-type UAE using EtOH as the extracting solvent to extract seven major phytocannabinoids, CBD, CBDA, CBG, CBGA, CBN, Δ^9-THC, and THCA [20] (Table 1). The UAE extraction efficiency was compared with other extraction methods, e.g., maceration, Soxhlet extraction, and supercritical CO_2 extraction. Hemp was extracted individually, as well as in combination with ginger, turmeric, and cardamom in a 1:1 ratio, using a bath-type UAE at room temperature (25 °C) for 30 min and employing EtOH as the extracting solvent [21]. This extraction successfully extracted CBC, CBD, CBDA, CBGA, CBN, Δ^9-THC, and THCA, and the extraction efficiency of the UAE method was compared with that of supercritical CO_2 extraction. An ultrasonic cleaning device was used for the extraction of CBD, CBDA, CBN, Δ^9-THC, and THCA from the aerial parts of *C. sativa* at an elevated temperature of 60°C and a prolonged extraction time of 50 min [22].

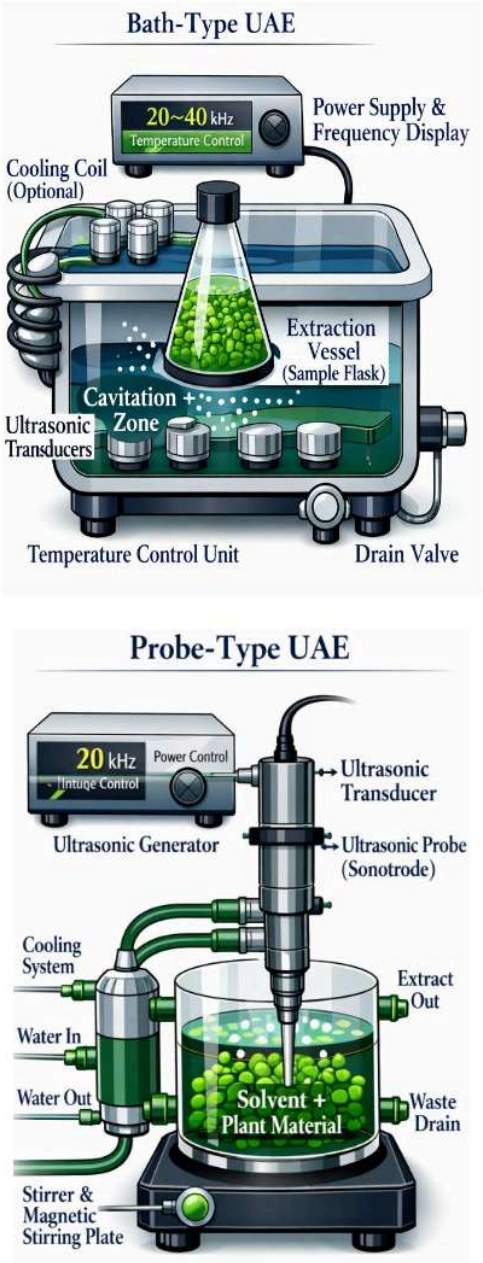


Figure 2. Ultrasound-assisted extraction devices, bath-type at the top and the probe-type at the bottom

This UAE method (0.5 g of plant material in 25 mL of EtOH) was part of a comparative study examining various methods for extracting phytocannabinoids from cannabis, including reflux and microwave-assisted extraction (MAE). None of these methods was optimised using response surface methodology (RSM) or any other mathematical or computational methods. However, Lazarevix et al. [23] used Box–Behnken experimental design and RSM [46] to optimise UAE of phytocannabinoids (CBD and Δ^9-THC) from the aerial parts of *C. sativa* using 50% aqueous EtOH and an EUP540A sonication water bath (40 kHz, 42 W). The duration of extraction was 60 min, and an elevated extraction temperature of 60 °C was used. Instead of EtOH, MeOH was used as the extraction solvent for UAE of 24 different samples of the aerial parts of *C. sativa*, obtained at different growth stages, using a simple ultrasonic bath (40 kHz), and an extraction time of 10 min at 25 °C, affording successful extraction of acidic phytocannabinoids, CBCA, CBDA, CBGA, CBNA, and THCA [24]. This study demonstrated that the

extracting capacity of MeOH in UAE was similar to that of EtOH for phytocannabinoids. The solid-to-solvent ratio for this UAE method was 100 mg in 5 mL. This study reported an interesting finding that the phytocannabinoids composition in the fibre-type *C. sativa* aerial parts was independent of the growth stage of harvesting, if they are collected before the flowering stage.

Two different solvents, 90% MeOH in chloroform, and EtOH, were used for the extraction of CBD, CBDA, CBN, Δ^8-THC, Δ^9-THC, and THCA from the aerial parts of *C. sativa* using a Soniclean 6 ultrasound bath (6-40 kHz) [25]. The solvent temperature was either 25 ^{o}C or 40 ^{o}C, and the extraction time was 15 min. Mastellone et al [26] employed an ultrasound-assisted dispersive solid–liquid microextraction using a Sonica S3 EP 2400 ultrasonic bath (40 kHz). Extraction was conducted at room temperature (25 °C) for 10 min using a eutectic solvent to extract CBD and CBDA. It can be noted that a eutectic solvent (most commonly known as a deep eutectic solvent, DES) is a mixture of two or more components that, when combined in a specific ratio, can interact through hydrogen bonding to create a liquid with a melting point significantly lower than that of any of the individual components, and is considered as an environmentally-friendly and biodegradable alternative to traditional toxic organic solvents [47]. Other advantages of eutectic solvents are their low volatility, resulting in extremely low vapour pressure, tunability (by changing the ratio of the components or the hydrogen bond donor, the viscosity, density, and acidity to fit specific applications could be altered), and solubility range (highly effective in dissolving a range of materials, including biomass, metal complexes, and enzymes). Olive oil could also be a good extraction solvent for phytocannabinoids using UAE [27]. CBD, CBDA, CBN, Δ^9-THC, and THCA were effectively extracted from the aerial parts of *C. sativa* using a UP200St ultrasonic system equipped with a sonotrode (26 kHz, 200 W); the extraction was conducted at 25-27 ^{o}C for the duration of 10-30 min. The solid-to-solvent ratio was 2 g of finely ground cannabis in 20 mL of olive oil.

Flowers and inflorescences

Flowers and inflorescences are the major parts of *C. sativa* that are used routinely for extracting phytocannabinoids [1]. During the last five years, there were at least 10 publications that described UAE methods for extracting phytocannabinoids from flowers and inflorescences, mostly from inflorescences, which are considered the primary source of phytocannabinoids [26, 28-36] (Table 1). Inflorescences, mainly from unpollinated female plants, possess dense clusters of glandular trichomes, where major phytocannabinoids, e.g., Δ^9-THC, CBD, and CBG are biosynthesized and stored [48,49]. It is worth noting that the only difference between a flower and an inflorescence is that an inflorescence is a cluster of flowers arranged on a single main stem or a complex system of branches, including the flowers themselves, their individual stalks, and the main floral axis.

CBD, CBDA, CBG, CBGA, Δ^9-THC, and THCA were successfully extracted from different varieties of fibre-type cannabis inflorescences using EtOH and an ultrasonic bath [28]. The extraction was conducted at 40 ^{o}C and for 15 min. The efficiency of the UAE method was compared with that of other techniques, including dynamic maceration and hydroalcoholic extraction (HAE). However, dynamic maceration had a better yield than UAE and HAE. The solid- to-solvent ratio in the UAE method was 0.25 g ground inflorescences in 10 mL of EtOH.

Doehlert-based optimisation [50] combined with RSM [46] was used to optimise UAE extraction (temperature and extraction time) of phytocannabinoids, e.g., CBC, CBD, CBDA, CBG, CBGA, Δ^9-THC and THCA, from inflorescences of *C. sativa* [29]. The experimental design was based on RSM, applying the two-factor Doehlert matrix. It can be noted that Doehlert-based optimisation is an efficient, space-filling experimental design, particularly used in association with RSM [50]. It allows simultaneous modelling and optimising multiple experimental variables while requiring significantly fewer experimental runs than traditional designs, e.g., central composite designs (CCD). An Arcano ultrasonic bath (200 W, 50 kHz) was used with EtOH as an extraction solvent at the optimised temperature and extraction time, respectively, 54.5 °C and 28 min 25 s. These UAE conditions allowed protection of thermolabile compounds, emphasising the suitability of UAE as a green and efficient technique for phytocannabinoids extraction [29]. A similar RSM-based optimisation but using a circumscribed CCD was applied for the UAE of two major phytocannabinoids, CBD and its acid CBDA, from *C. sativa* inflorescences using EtOH as the extraction solvent [30]. In the UAE optimisation, designed experiments were conducted under varying extraction conditions, e.g., sonication time, extraction temperature, and sample/solvent ratio, according to the selected experimental design [30]. The EU-approved varieties ‘Futura 75’, ‘Fedora 17’, and ‘Fibranova’ of *C. sativa* inflorescences were extracted.

In this study, an Elmasonic S 100H ultrasonic bath (37 kHz) was used, and the optimised extraction conditions were 40 °C and 30 min. The efficiency of the UAE method described in this study was compared with the MAE and dynamic maceration techniques, and UAE was found to be the most efficient technique among the three.

Table 1. Ultrasound-assisted extraction (UAE) of phytocannabinoids from *Cannabis sativa* L.: Progress since 2021

Plant parts	UAE details	Extraction solvent	Optimisation	Extracted phytocannabinoids	Refs
Aerial parts	A UP200Ht ultrasonic homogeniser (200 W, 26 kHz) with an S26414 sonotrode (14 mm tip diameter, 45 µm amplitude). Extraction conditions: 25 °C, ultrasound at 25 J/mL, for a final power of 2000 W.	EtOH	N/A	CBD, CBDA, CBG, CBGA, CBN, Δ^9-THC, and THCA	[20]
	Ultrasonic bath. Extraction conditions: 25 °C and 30 min.		N/A	CBC, CBD, CBDA, CBGA, CBN, Δ^9-THC, and THCA	[21]
	An ultrasonic cleaning equipment. Extraction conditions: 60 °C, 50 min.		N/A	CBD, CBDA, CBN, Δ^9-THC, and THCA	[22]
	EUP540A sonication water bath (40 kHz, 42 W). Optimised extraction conditions: 60 °C and 60 min.	50% aqueous EtOH	Box–Behnken experimental design and RSM	CBD and Δ^9-THC	[23]
	Ultrasonic bath (40 kHz). Extraction conditions: 10 min.	MeOH	N/A	CBCA, CBDA, CBGA, CBNA, and THCA	[24]
	Soniclean 6 ultrasound bath (6-40 kHz). Extraction conditions: 25 or 40 °C, 15 min.	Two different extractants: 90% MeOH in	N/A	CBD, CBDA, CBN, Δ^8-THC, Δ^9-THC, and THCA	[25]

		chloroform, and EtOH			
	Ultrasound-assisted dispersive solid–liquid microextraction using a Sonica S3 EP 2400 ultrasonic bath (40 kHz). Extraction conditions: 25 °C and 10 min	Eutectic solvent	N/A	CBD and CBDA	[26]
	A UP200St ultrasonic system equipped with a sonotrode (26 kHz, 200 W). Extraction conditions: 25-27 °C, 10-30 min, 60% ultrasonic amplitude.	Olive oil	N/A	CBD, CBDA, CBN, Δ^9-THC, and THCA	[27]
Flowers and inflorescences	An ultrasonic bath (Arcano, model PS-30A; nominal power 200 W, 50 kHz). Optimised extraction conditions: 54.5 °C and 28 min 25 s.	EtOH	Doehlert-based optimization combined with RSM	CBC, CBD, CBDA, CBG, CBGA, Δ^9-THC, and THCA	[28]
	Ultrasound bath. Extraction conditions: 40 °C and 15 min.		N/A	CBD, CBDA, CBG, CBGA, Δ^9-THC, and THCA	[29]
	An Elmasonic S 100H ultrasonic bath (37 kHz). Optimised extraction conditions: 40 °C and 30 min.		RSM and central composite design (CCD)	CBD and CBDA	[30]
	Ultrasonic bath. Optimised extraction conditions: 30 °C and 20 min.	96% EtOH	RSM	CBD, CBN, and Δ^9-THC	[31]

	An ultrasonic water bath (40 kHz). Extraction conditions: 30 min.		N/A	CBD, CBDA, Δ^9-THC, and THCA	[32]
	An ISOLAB ultrasonic bath (120 W, 40 kHz). Extraction conditions: 40 °C and 30 min	MeOH	N/A	CBC, CBCA, CBD, CBDA, CBD-C4, CBDA-C4, CBDV, CBDVA, CBG, CBGA, CBN, Δ^9-THC, and THCA	[33]
	An ultrasonic bath (500 W, 50 kHz). Extraction conditions: 40 °C and 120 min.		N/A	CBC, CBD, CBDA, CBGA, CBN, Δ^9-THC, and THCA	[34]
	A compact ultrasonic laboratory device UP 100H (30 kHz, 100 W). Optimised extraction conditions: 10-20 min, 99% ultrasonic power.	96% MeOH	RSM and central composite design (CCD)	CBD and Δ^9-THC	[35]
	TPC UC-1000 Ultrasonic Cleaner (40 kHz, 180 W). Optimised extraction conditions: 1 min, 25 °C.	Acetonitrile (ACN): EtOH = (1:1)	Design of experiment (DoE)	CBD, CBDA, CBN, Δ^8-THC, Δ^9-THC, and Δ^9-THCA	[36]
	Ultrasound-assisted dispersive solid–liquid microextraction using a Sonica S3 EP 2400 ultrasonic bath (40 kHz). Extraction conditions: 25 °C and 10 min	Eutectic solvent	N/A	CBD and CBDA	[26]
Leaves and sugar leaves	An ultrasonic bath (300 W, 20 kHz). Extraction conditions:	EtOH	N/A	(+)-Cannabidiol, CBC, CBCV, CBD, CBDA, CBDP, CBDV, CBG, CBGA, CBN, and Δ^9-THC	[37]

	30 °C and 30 min.				
	Thermo-Fisher ultrasound bath (37 kHz, 800 W). Optimised extraction conditions: 40 °C and 15 min.	MeOH	Trial and error	CBC, CBD, CBG, and Δ^9-THC	[38]
	An Erlenmeyer flask with an Elmasonic 300 H ultrasonic processor (300 W, 37 kHz). Extraction conditions: 30 °C and 25 min.	Food-grade medium-chain triglyceride oil	N/A	CBD and Δ^9-THC	[39]
Leaves and seeds blended together	Ultrasonic bath (115 W, 60 Hz). Optimised extraction conditions: room temperature and 8.7 min.	MeOH	RSM and Box-Behnken experimental design	CBC, CBD, CBG, CBN, and Δ^9-THC	[40]
	A Powersonic ultrasonic bath (132 kHz). Optimised extraction conditions: 30 °C and 20.8 min.	Ionic liquid and deep eutectic solvents	RSM and Box-Behnken experimental design	CBC, CBD, CBG, CBN, and Δ^9-THC	[41]
Roots	An Erlenmeyer flask with an Elmasonic 300 H ultrasonic processor (300 W, 37 kHz). Extraction conditions: 30 °C and 25 min	Food-grade medium-chain triglyceride oil	N/A	CBD and Δ^9-THC	[39]
Cannabis biomass	A Branson Sonifier 450 ultrasound system (20 kHz, 450 W). Optimised extraction conditions: 60 °C and 30 min.	EtOH	RSM and central composite rotatability design (CCRD)	CBCA, CBDA, CBG, CBGA, CBNA, Δ^9-THC, THCA, THCV, and THCVA	[42]

	Ultrasound-assisted extraction	ACN and 50% aqueous EtOH	N/A	CBDA and THCA	[43]
***Cannabis sativa*-based products**	An Ultrawave F0000303 ultrasonic bath (50-60 Hz, 115 W). Optimised extraction conditions: pulsed UAE with a 30 min pulsed and a mean time break of 10 min (total extraction time: 40 min)	MeOH	Trial and error	CBC, CBD, CBG, CBN, Δ^9-THC, 11-THC-OH, and 11-THC-COOH	[44]
	A 150E ultrasonic system with 4C15 probe (117 V, 150 W, 40 kHz). Extraction conditions: 1 min, 80% amplitude, room temperature.		N/A	CBD and CBG	[45]

ACN = Acetonitrile; CBC = Cannabichromene; CBCA = Cannabichromene acid; CBCV = Cannabichromevarin; CBD = Cannabidiol; CBDA = Cannabidiolic acid; CBD-C4 = Cannabidibutol; CBDA-C4 = Cannabidibutolic acid; CBDP = Cannabidiphorol; CBDV = Cannabidivarin, CBDVA = Cannabidivarinic acid; CBG = Cannabigerol; CBGA = Cannabigerolic acid; CBN = Cannabinol; CBNA = Cannabinolic acid; CCD = central composite design; CCCRD = composite rotatability design; EtOH = Ethanol; RSM = Response surface methodology; THC = Tetrahydrocannabinol; THCA = Tetrahydrocannabinolic acid; 11-THC-OH = 11-Hydroxy-Δ^9-tetrahydrocannabinol; 11-THC-COOH = 11-Nor-9-carboxy-Δ^9-tetrahydrocannabinol; THCV = Tetrahydrocannabivarin; THCVA = Tetrahydrocannabivarin acid

RSM-optimised extraction conditions (30 °C and 20 min), employing three-factor, five-level CCD, were used for the UAE of fresh *C. sativa* inflorescences using 96% aqueous EtOH to extract CBD, CBN, and Δ^9-THC [31]. A similar extraction protocol, but not optimised by RSM, was used for extracting CBD, CBDA, Δ^9-THC, and THCA from *C. sativa* female inflorescences employing an ultrasonic water bath (40 kHz) and an extraction time of 30 min [32]. Although 96% EtOH was the most efficient solvent for extracting these phytocannabinoids (2.23% CBD yield), many other solvent combinations, e.g., cold water, hot water, 20% aqueous EtOH, 40% aqueous EtOH, and 80% aqueous EtOH, were tried.

MeOH was used in the UAE of phytocannabinoids from *C. sativa* inflorescences in two published studies during 2021-25 [33,34]. An ISOLAB ultrasonic bath (120 W, 40 kHz) with extraction conditions of 40 °C and 30 min, was used to extract CBC, CBCA, CBD, CBDA, CBD-C4, CBDA-C4, CBDV, CBDVA, CBG, CBGA, CBN, Δ^9-THC and THCA from the Kompolti cultivar [33], while a standard ultrasonic bath (500 W, 50 kHz) and the extraction conditions of 40 °C and a prolonged extraction time of 120 min afforded CBC, CBD, CBDA, CBGA, CBN, Δ^9-THC and THCA, using a solid-to-solvent ratio of 15 g of ground plant material and 180 mL of MeOH [34]. Extraction efficiency of the first UAE method [33] was compared with that of supercritical CO_2 extraction. The UAE achieved greater recovery from the plant material (phytocannabinoids: 83.42 vs. 68.84 mg/g of plant material). This study reported MeOH as the most effective solvent for phytocannabinoids recovery, based on a previously published systematic evaluation of fourteen solvents employing UAE [51]. On the other hand, the second study [34] also evaluated different extraction methods, including dynamic maceration, Soxhlet, UAE, and supercritical CO_2 extraction for extracting phytocannabinoids, and UAE was established as one of the effective and environmentally friendly extraction techniques.

An RSM-optimised (using CCD) UAE method employing 96% aqueous MeOH as the extraction solvent afforded successful extraction of CBD and Δ^9-THC from the wild-grown female inflorescences of marijuana (*C. sativa*), collected from three different regions of Peru [35]. A compact ultrasonic laboratory device UP 100H (30 kHz, 100 W), and the optimised extraction conditions, 20 min and 99% ultrasonic power, were used in this study. The solid-to-solvent ratio used in this study was 5 g of plant materials in 50 mL of MeOH. RSM was used to optimise the solvent concentration, extraction time, and UAE amplitude on the yield and phytocannabinoids content.

Acetonitrile (ACN) and EtOH (1:1) were used in the UAE of dried and ground flower tops of *C. sativa* to extract CBD, CBDA, CBN, Δ^8-THC, Δ^9-THC, and Δ^9-THCA [36]. TPC UC-1000 Ultrasonic Cleaner (40 kHz, 180 W) was used in this study, and the optimised extraction conditions were 1 min and 25 ^{o}C. In this study, several extraction parameters were evaluated by different designs of experiment (DoE) and assays to facilitate sample handling and decrease the volume of solvents and time needed for phytocannabinoids extraction, and thus, to make the method more environmentally friendly [36]. A eutectic solvent, as outlined earlier in this article, was used for the extraction of two major phytocannabinoids, CBD and CBDA, from *C. sativa* inflorescences using an ultrasound-assisted dispersive solid–liquid microextraction employing a Sonica S3 EP 2400 ultrasonic bath (40 kHz) [26]. The extraction conditions were 25 °C and 10 min.

Leaves and sugar leaves

(+)-Cannabidiol, CBC, CBCV, CBD, CBDA, CBDP, CBDV, CBG, CBGA, CBN, and Δ^9-THC were successfully extracted from the dried and ground leaves of hemp (*C. sativa*) using a conventional ultrasonic bath (300 W, 20 kHz) and setting the extraction conditions to 30 °C and 30 min [37]. EtOH was used as the extraction solvent. In another study, dealing with the extraction of different samples of leaves of *C. sativa*, a Thermo-Fisher ultrasound bath (37 kHz, 800 W) was used with optimised extraction conditions of 40 ^{o}C and 15 min, resulting in the extraction of CBC, CBD, CBG, and Δ^9-THC [38]. MeOH was used as the extraction solvent, and optimisation was accomplished by trial and error. The variables such as extractants (EtOH, MeOH, and isopropanol) and their mixtures (50:50) and extraction methods (maceration and UAE) were evaluated, demonstrating considerable variations in phytocannabinoids profiles in the extracts obtained using those variables. A food-grade medium-chain triglyceride oil was used in the UAE to extract CBD and Δ^9-THC from the leaves and sugar leaves of *C. sativa* [39]. In this study, an Erlenmeyer flask with an Elmasonic 300 H ultrasonic processor (300 W, 37 kHz) using the extraction conditions 30 °C and 25 min, was employed. It can be mentioned that sugar leaves are small, frosty leaves that grow on cannabis buds; these leaves are rich in THC, making them useful for making extracts, edibles, and concentrates.

Leaves and seeds blended together

RSM and Box-Behnken experimental design [46] were used for the optimised UAE extraction of phytocannabinoids, e.g., CBC, CBD, CBG, CBN and Δ^9-THC, from samples of leaves and seeds blends of *C. sativa*; one study used MeOH as a solvent in an ultrasonic bath (115 W, 60 Hz) and optimised extraction conditions 25 ^{o}C and 8.7 min[40], while an ionic liquid and deep eutectic solvent (DES) were used in another study employing a Powersonic ultrasonic bath (132 kHz) and optimised extraction conditions 30 °C and 20.8 min [41]. The extraction efficiency of UAE was also compared with other extraction methods, such as Soxhlet extraction, MAE, and conventional-stirring extraction, and it was found that the UAE could extract Δ^9-THC, CBG, and CBC at higher amounts [40]. The study involving ionic liquid and deep eutectic solvent as the extracting solvents revealed 1-butyl-3-methyl-imidazolium chloride as a promising solvent for the recovery of phytocannabinoids using the RSM-optimised conditions, e.g., 0.071 M 1-butyl-3-methyl-imidazolium chloride, a solid-to-solvent ratio of 1 g dried hemp tea material and 24.15 mL, and an extraction time of 20.8 min [41]. In this study, four cannabis teas, sourced from two different cannabis stores located in Nicosia, Cyprus, were investigated.

Roots

There was only one report published during 2021-25 on the UAE of roots of *C. sativa* for the extraction of phytocannabinoids, CBD, and Δ^9-THC [39]. An Erlenmeyer flask with an Elmasonic 300 H ultrasonic processor (300 W, 37 kHz) with the extraction conditions 30 °C and 25 min was used in this study. A food-grade medium-chain triglyceride oil was used for this extraction.

Cannabis biomass

RSM and central composite rotatability design (CCRD) were used for the optimisation of the UAE of cannabis biomass employing a Branson Sonifier 450 ultrasound system (20 kHz, 450 W) [42]. The optimised extraction conditions were 60 ^{o}C and 30 min. The solid-to-solvent ratio was 1:15. EtOH was used as the extraction solvent to successfully extract CBCA, CBDA, CBG, CBGA, CBNA, Δ^9-THC, THCA, THCV, and THCVA. It was observed that the UAE increased phytocannabinoids concentration from 13.2 to 39.2%. It was also noted that solid-to-solvent ratios significantly influenced the secondary metabolite profiles and yields for UAE. In a similar UAE, ACN and 50% aqueous EtOH were used as the extraction solvent for extracting acidic phytocannabinoids, CBDA, and THCA from cannabis biomass [43].

Cannabis sativa-based products

As part of the evaluation of different extraction procedures for the quantification of seven phytocannabinoids, CBC, CBD, CBG, CBN, Δ^9-THC, 11-THC-OH, and 11-THC-COOH in cannabis-based edibles, UAE was used [44]. An Ultrawave F0000303 ultrasonic bath (50-60 Hz, 115 W) and optimised extraction conditions, including pulsed UAE with a 30 min pulsed and a mean time break of 10 min (total extraction time: 40 min), and MeOH as the extraction solvent were used. It was noted that the UAE could provide higher extraction efficiencies for cannabis-based edibles in solid form, and considerably high levels of phytocannabinoids were identified in cannabis tea extract prepared by the UAE procedure. The cannabis products included in this study were hemp seeds, a cannabis-infused beer, an energy drink, chocolates, a roasted coffee, and hemp tea [44]. In a similar investigation, a 150E ultrasonic system with 4C15 probe (117 V, 150 W, 40 kHz) was used with the extraction conditions of 1 min, 80% amplitude, and 25 ^{o}C to extract CBD and CBG from cannabis-based tropical products, e.g., three different commercial topicals (i.e., a water-based gel used to clear the skin, an oil-based cream used as an anti-aging treatment, and an oil-based cream used for intensive care treatment [45]. MeOH was used as the extraction solvent.

CONCLUSIONS

Well over a hundred phytocannabinoids are biosynthesized by *C. sativa*. Phytocannabinoids are concentrated the most in the inflorescences of this plant. UAE is considered a green extraction technique for phytocannabinoids from *C. sativa*, and has emerged as a safe and cost-effective method, as it reduces solvent use and energy consumption, along with various environmental hazards such as chemical waste. The use of RSM and related mathematical and computational modelling for optimisation of the UAE parameters could add further efficiency to this extraction technique. In the last five years, the use of UAE for the extraction of phytocannabinoids from *C. sativa* remained as popular as before, and most studies demonstrated higher efficiency, more cost-effectiveness, easier use, and environmental friendliness of UAE than most other available extraction techniques.

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Conflict of interest

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Supplementary materials

None

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