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Comparison of the Efficiency of Deep Eutectic and Organic Solvents in the Extraction of Phytochemicals from *Cannabis sativa* L.

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Abstract: Industrial hemp (*Cannabis sativa* L.) is an attractive candidate for sustainable pest management due to its abundance of bioactive compounds with potential pesticidal properties. Solvent choice has a significant impact on the extraction efficiency of bioactive compounds. Deep Eutectic Solvents (DESs) are gaining popularity in extraction because they are safe and environmentally friendly, making them viable alternatives to organic solvents (OSs). This research first compared the extraction efficiency of OSs in the extraction of phytochemicals from the infloresences of two hemp varieties, Citrus and Cherry Dwarf. Inflorescences were extracted using three OSs, ethanol, ethyl acetate, and hexane. The highest level of cannabidiol (CBD; 0.69%) was extracted from Cherry Dwarf using ethanol, while the level of delta-9 tetrahydrocannabinol THC (0.19%) was essentially the same in both. Therefore, Cherry Dwarf was selected to compare the extraction efficiency of DESs with OSs. The DESs were choline chloride/ethylene glycol, citric acid/ethylene glycol, menthol/lauric acid, choline chloride/urea, and choline chloride/glycerol. In the targeted analysis, choline chloride/ethylene glycol extracted the highest amount of CBD (0.87%) followed by choline chloride/urea (0.78%). As some DESs outperformed ethanol, the popular solvent for extracting cannabinoids, DESs are viable candidates for replacement of organic solvents.

Keywords: phytochemical; deep eutectic solvent; terpenes; cannabinoids; organic solvents; extraction; industrial hemp; *Cannabis sativa* L.

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1. Introduction

Industrial hemp (*Cannabis sativa* L.) is found in the Cannabaceae family, which contains only one genus (Cannabis) and one highly variable species, *C. sativa* [1]. The genus is one of the oldest crops grown for food, fiber, and medicinal purposes. *Cannabis sativa* L. plants have been used around the world since antiquity due to their multifunctional properties, including their use for oil and protein, food, and feed production; fiber, paper, textiles, and resins [1–3]. The genus contains more than 500 chemical constituents, 125 of which are classified as cannabinoids [1]. Cannabinoids, a class of terpenophenolic compounds, are the most psychoactive, accumulating primarily in the trichome cavity of female flowers along with non-cannabinoids that include phenols, flavonoids, and alkaloids [1,4]. Delta 9-tetrahydrocannabinol (THC), which is naturally present in the form of an acid (delta 9-tetrahydrocannabinolic acid, THCA), is the psychoactive cannabinoid component of cannabis with the highest concentration. To form the pharmacologically active THC, the acid must be decarboxylated with time or heat [4]. Cannabidiol (CBD), another cannabinoid of interest, is the most promising compound from a pharmaceutical perspective as it exhibits antioxidant, anti-inflammatory, antibacterial, antiproliferative, and

neuroprotective properties. *C. sativa* contains four major cannabinoids in addition to delta 9-THC and CBD: tetrahydrocannabivarin (THCV), cannabinol (CBN), cannabigerol (CBG), and cannabichromene (CBC), which exhibit remarkable antibacterial, anti-inflammatory, and antiproliferation properties [1].

Different extraction methods and solvents have been used to recover hemp phytochemicals and are grouped as conventional and modern techniques; some use organic solvents and some are solventless. Conventional techniques include liquid–liquid, Soxhlet, and reflux extraction, as well as maceration followed by extraction [5]. These techniques employ organic solvents, the selection of which depends on the polarity of the target compounds. These traditional methods are effective, but time consuming and require a large quantity of organic solvents that are toxic, flammable, and unfriendly to the environment [5].

Modern techniques include microwave-assisted extraction and ultrasound-assisted extraction [5]. Ultrasound-assisted extraction uses ultrasonic waves, which are mechanical vibrations that pass through the extraction medium. Waves induce acoustic cavitation by generating cycles of expansion and compression, causing the formation of expanding and collapsing bubbles [5]. These effects destroy the cell walls, releasing the contents of the cell. This technique is mostly performed in an ultrasonic bath containing organic solvents or their aqueous mixture. Microwave-assisted extraction, pressurized liquid extraction, extrusion, and rapid solid–liquid dynamic extraction are other modern green extraction techniques. Microwaves are electromagnetic radiations that can interact with polar molecules and penetrate plant biomass. The water in the biomass absorbs microwave energy and rapidly heats the cells, causing their disruption and the release of the desired substances [4–7].

Supercritical fluid extraction, an environmentally friendly technique that uses supercritical CO_2, has many benefits, including chemical stability, low toxicity, inflammability, and affordability. Two other benefits are that supercritical carbon dioxide has a low critical temperature (31 °C) and pressure (73.8 bar) for safe extraction of thermolabile components, which makes it an effective solvent for extracting volatile compounds such as terpenes from plant sources [4,8] and its simple separation from the extract.

Ionic liquids have been used with and without supercritical CO_2 to extract cannabinoids. The synergistic combination of ILs and supercritical CO_2 enables a reduced use of resources and improves the extraction of the main cannabinoids [9]. ILs are preferred mainly for their ability to stabilize major cannabinoids such as CBD and to accelerate the decarboxylation of CBDA [10]. Ionic liquids are made up of cations and anions that can be selected for hydrophilic and hydrophobic properties that result in dissolution and extraction at various temperatures and time [10]. Apart from cannabinoids, ILs are also used to extract other phytochemicals such as phenolic compounds, alkaloids, glycosides, flavonoids, and essential oils [11].

Hydrodistillation and steam distillation are other methods commonly used to extract terpenes, including essential oils from plant sources [12]. These techniques reduce the boiling point of molecules by using water vapor pressure. The water vapor permeates the biomass and dissolves the volatile substances. Condensing the solvent and solutes causes their separation, with the essential oil usually occupying the upper phase of the liquid. In steam distillation, the plant material is brought directly into contact with steam, whereas in hydrodistillation, the plant material is soaked in water and brought to a boil prior to exposure to steam [7,12]. Water is a precious resource and heating for extended periods consumes a lot of energy, so the environmental impact is reduced when these methods incorporate microwave heating [13].

Despite modern extraction techniques, organic solvents (e.g., chloroform, ethyl acetate, ethanol, and methanol) are frequently used to extract bioactive components from plant materials. Because organic solvents are expensive, flammable, toxic, and not environmentally friendly, their excessive use not only harms the natural environment but also poses a health risk to humans [14].

Therefore, it is essential to explore alternative methods for extracting phytochemicals. DESs have emerged as a new class of environmentally friendly solvents to replace organic solvents [15]. As extraction solvents, DESs have the advantage of higher efficiency, shorter extraction time, lower cost, lack of toxicity, biodegradability, and improved product purity, making them suitable for different applications [16]. DESs combine a halide salt or another hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD) (e.g., urea, carboxylic acid, sugar, amide, or another Lewis acid) at a specified molar ratio. The components are mixed and heated at a specified temperature with magnetic stirring until a transparent liquid is produced [16,17].

Choline chloride (ChCl) is the most widely used HBA combined with an HBD, the most popular of which are urea, ethylene glycol, and glycerol; however, other alcohols, amino acids, carboxylic acids, and sugars have also been widely used [16–18]. These solvents have a well-defined composition, and they exhibit a unique, minimum melting point in the solid/liquid phase diagram, which is significantly lower than the melting points of the individual components, highlighting noncovalent molecular affinities. In most cases, DESs can be used as a liquid at room temperature [17]. DESs are used to extract many secondary metabolites of plant materials, including phenolics, flavonoids, isoflavonoids, terpenoids, alkaloids, anthocyanins, anthraquinones, and polysaccharides [17].

Although DESs have been reported to be environmentally friendly and have been used to extract various phytochemicals, little is known about the efficacy of DESs in the extraction of cannabinoids, THC, and terpenes from industrial hemp. Consequently, this study aims to compare the efficiency of different DESs and organic solvents in extracting phytochemicals from industrial hemp.

2. Materials and Methods

2.1. Plant Materials, Reagents, and Instruments

Industrial hemp inflorescences were harvested from the George Washington Carver Agricultural Experiment Station (GWCAES) at Tuskegee University in 2022, air dried, ground using a heavy-duty laboratory grinder, and stored in amber containers. Choline chloride (AR, 98%), ethylene glycol (AR, 99.5%), glycerin (AR, 99%), citric acid (AR, >99.5), lauric acid (AR, 99%), menthol (AR, 99.9%) ethanol, ethyl acetate, and hexane (Fischer Scientific, Roswell, GA, USA), and reference standards (Sigma Aldrich, Burlington, MA, USA) were used as received. The instruments used include a pH meter, viscosity measuring cup, shaker bath, centrifuge, magnetic stirring hot plate, GC-MS, and LC-HRMS.

2.2. DES Preparation and Properties Determination

Based on the literature references, choline chloride, citric acid, and menthol were selected as HBAs and ethylene glycol, glycerol, urea, and lauric acid were selected as HBDs. The molar ratios of the five different DESs are given in Table 1. The HBA and HBD reagents were mixed in an appropriate molar ratio and heated on the magnetic stirring hot plate at 80 °C until a clear and homogeneous liquid was formed. No water was added to any of these DESs, and all of the DESs were used the same day to prevent significant water absorption.

Table 1. Properties of DESs, M (menthol), LA (lauric acid), CA (citric Aacid), EG (ethylene glycol, CCL (choline chloride), U (urea), and G (glycerol).

HBA	HBD	Molar Ratio	Color	pH	Density (g/mL)	Viscosity (cP)	Polarity
M	LA	2:1	Transparent colorless	5.61	0.85	10.24	Nonpolar
CA	EG	1:4	Transparent colorless	1.23	1.22	102.39	Polar
CCL	EG	1:2	Yellowish	5.05	1.07	20.09	Medium polarity
CCL	U	1:2	Transparent colorless	8.95	1.13	250.07	Medium polarity
CCL	G	1:2	Yellowish	5.03	1.11	196.45	Medium Polarity

The pH of each DES was measured at 25 °C; viscosity was measured using a viscosity determination cup according to the procedure in the literature. Density was calculated by using the mass and volume of the solvent, and the polarity was determined qualitatively using information from the literature. Each DES property was measured in triplicate.

2.3. Extraction Using DES

An accurate weight sample (1 g) of industrial hemp powder was added to 20 mL of DES in a 50 mL centrifuge tube. The extraction was carried out with the help of a shaker bath set at 37 °C, 75 rpm for 24 h. The extract was centrifuged at 4000× *g* for ten minutes and the supernatant was collected [16]. The collected supernatant was stored at room temperature until further analysis. The extraction was carried out in triplicate to validate the results. The extracts were visually compared based on color to determine the qualitative efficiency of extracting colored compounds from industrial hemp.

2.4. Extraction Using Organic Solvents

Organic solvents used were ethanol, ethyl acetate, and hexane; the choice was based on the variation in polarity. The extraction procedure followed the method previously used with DESs detailed above with little modification. The extract was filtered using Whatman No.1 filter paper followed by a syringe filter of 0.22 µm. The extraction was carried out in triplicate.

2.5. Characterization of Extract Using LC/MS

Analysis was performed on a Vanquish UHPLC system (Thermo Fisher, Waltham, MA, USA) coupled with a quadrupole orbitrap mass spectrometer (Orbitrap Exploris 120, Thermo) with electrospray ionization (H-ESI) in positive mode using Xcalibur software (V4.4.16.14). The samples were diluted to 0.1% (*vol*/*vol*) in 50% methanol 50% water containing 500 ng/mL of CBD-D3 and (−)-Δ9-THC-D3, each. Injection of 10 µL of the sample was made into a C18 column (ACQUITY UPLC® BEH C18, 1.7 µm, 2.1 × 50 mm, Waters) maintained at 40 °C with a 400 µL/min flow rate of the mobile phase solution A (20 mM ammonium carbonate pH 3.2) and the mobile phase B (100% acetonitrile) beginning at 40% B, held for 1 min, increased to 85% B at 12 min, increased to 95% B, and held for 2 min, followed by reequilibration to 40% B for 3 min for a total analysis time of 17 min. Samples were chilled to 10 °C while the column was heated to 25 °C. The MS scan range was 100–1000 m/z with resolution of 120,000, standard AGC target, 70% RF lens, maximum injection time of 100 ms, with EASY-IC run start on. The spray voltage was 3500 V in positive mode and 2500 V in negative mode, the ion transfer tube temperature was 320 °C and the vaporizer temperature was 290 °C, the sheath and aux gases were 30 and 7, respectively. There was a targeted fragmentation analysis of several compounds, including CBDVA, CBDV, CBDA, CBGA, CBG, CBD, THCV, CBN, THCA, and internal standards. The isolation window was 1.3, collision energy was normalized to 30%, and the orbitrap resolution was 15,000 with other parameters set to auto. Analysis of variance (ANOVA), principal component analysis (PCA), and partial least squares discriminant analysis (PLS-DA) were used to compare the metabolic differences among different solvents using MetaboAnalyst 5.0

2.6. Characterization of Extracts Using GC/MS

Benzophenone (0.5 mg/mL) was added to the samples at 1% (*vol*/*vol*) and a terpene standard (Restek catalog No. 34095) was used for quantification. Terpenes were analyzed by gas chromatography-mass spectrometry on an Agilent 6890N GC and 5975 MS with a Restek Rxi-5Sil MS column with Integra-guard (15 m × 0.25 mm ID × 0.25 µm df). The injection port and MS transfer line temperature was set at 310 °C and the flow was constant at 1.0 mL/min. 0.2 µL of the sample was injected in splitless mode. The temperature of the GC oven was programmed as follows: initial temperature of 40 °C held for 3 min, ramp to 180 °C at 10 °C/min, ramp to 320 °C at 50 °C/min, with a hold of 5 min, thus requiring

a total run time of 24.8 min. The EI was set to 70 eV and the mass range was from 35 to 350 m/z. The MS source was set to 230 and the quad was set to 150. Raw data were processed and analyzed using MS-DIAL. Statistical analysis was performed with GraphPad Prism 10.1 and MetaboAnalyst 5.0.

3. Results

3.1. Properties of DESs

Each DES was prepared as a homogeneous liquid and remained liquid at room temperature (Figure 1). The properties of the DESs varied widely (Table 1), as density, acidity, alkalinity, viscosity, and polarity depended on the composition of each. The ethylene glycol/citric acid DES had the highest acidity, while the menthol/lauric acid DES had the lowest viscosity and density. The choline chloride/urea DES possesses the highest pH and viscosity. Evaluating these properties will facilitate the efficient extraction of target compounds from natural materials.

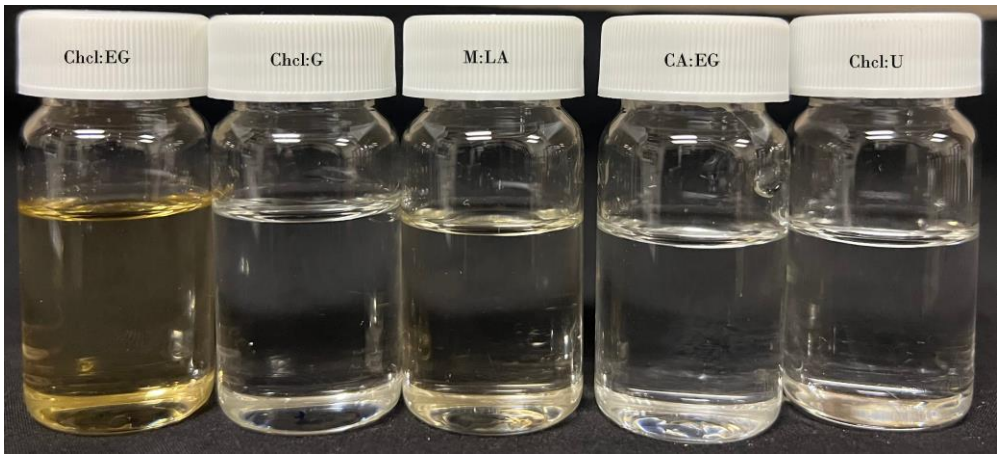


Figure 1. DESs, which were used in the extraction. ChCl:EG (Choline Chloride:Ethylene Glycol), ChCl:G (Choline Chloride:Glycerol), M:LA (Menthol:Lauric Acid), CA:EG (Citric acid:Ethylene Glycol) ChCl:EG (Choline Chloride:Urea).

3.2. Comparison of Extract Color

The color of the choline chloride/ethylene glycol, choline chloride/glycol, citric acid/ethyl glycol, and choline chloride/urea extracts were similar. In contrast, menthol/lauric acid produced a greenish color distinct from that of the other extracts Figure 2a. With organic solvents, the color of the extracts was similar (green), except for the hexane extract, which does not extract chlorophyll Figure 2b. These color differences demonstrate the turnability of DESs to selectively extract classes of compounds.

3.3. Targeted Analysis of Industrial Hemp Extracts

3.3.1. Analysis of Cannabinoids

Two varieties of industrial hemp, Cherry Dwarf (CD) and Citrus (CT), were analyzed for tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabinol (CBN) and targeted using their corresponding reference standards. The two varieties were extracted using ethanol, hexane, ethyl acetate, and the combination of three solvents in a ratio of 1:1:1. Overall, there was a significant difference between CD and CT in all compounds ($p = 0.004$) and there was a significant difference between solvents ($p < 0.0001$). The highest percentage of CBD was detected in Cherry Dwarf (0.69%) extracted with ethanol, followed by Citrus (0.61%) and Cherry Dwarf extracted by ethyl acetate (0.59%). The lowest percentage of CBD was obtained from Citrus samples extracted by hexane (0.04%), while it was 0.3 in Cherry Dwarf (Figure 3a). All solvents extracted a similar percentage of CBN (0.08%) from Cherry Dwarf, while Citrus ethanol extracted a similar amount (0.08%). The combination and ethyl acetate extracted very low amounts of 0.01% and 0.03%, respectively, while

hexane did not extract any detectable amount (Figure 3c). The highest percentage of THC was extracted with ethanol and was similar for both varieties (0.19%), followed by Cherry Dwarf extracted with ethyl acetate (0.18%). For both varieties, the remaining solvents extracted very low percentages of THC and the least was hexane in Citrus (Figure 3b). Based on the results, Cherry Dwarf was shown to contain the highest percentage of CBD, CBN, and THC compared to Citrus.

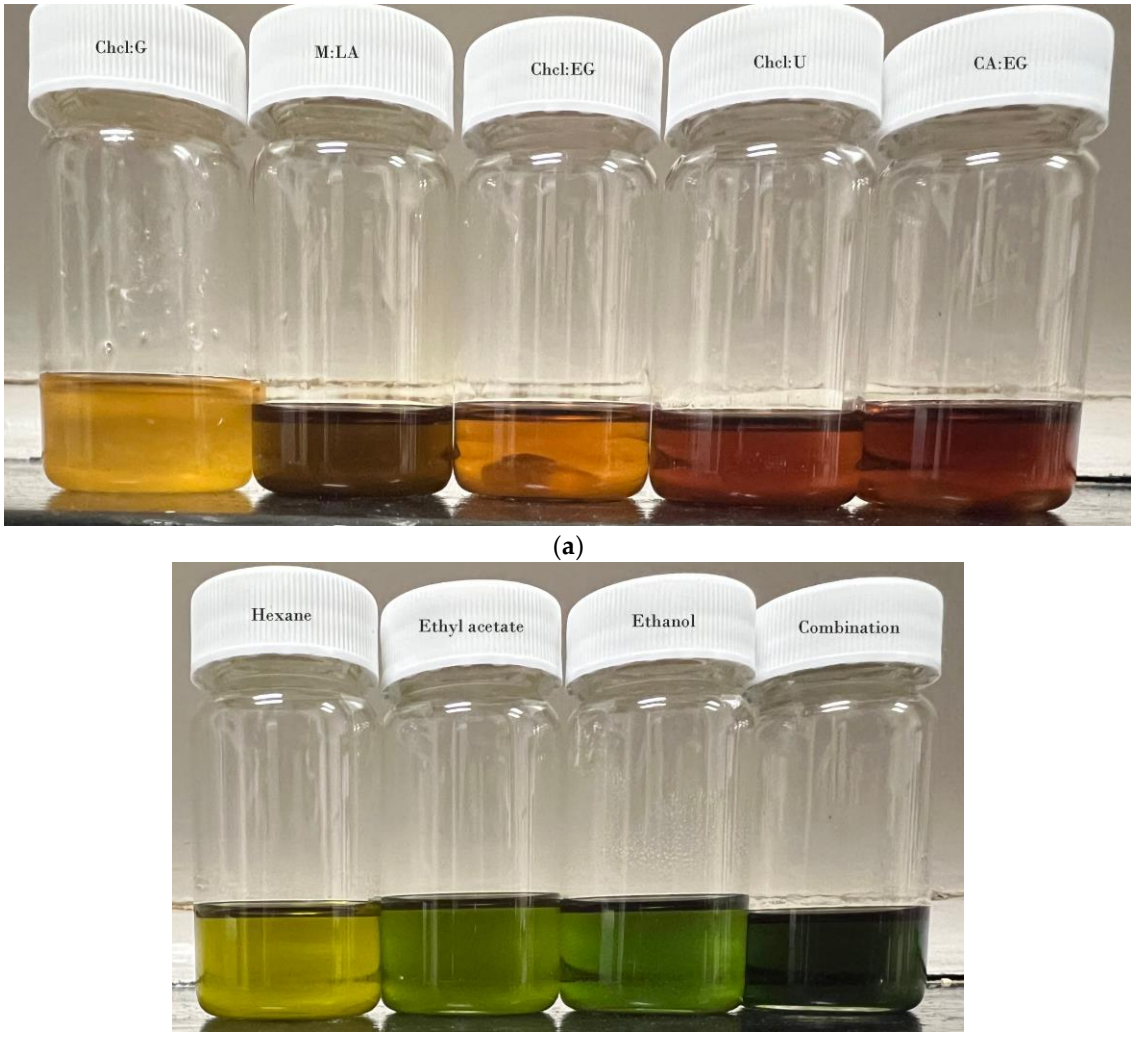


Figure 2. Industrial hemp extracts showing the variation in color depending on the solvent used. (**a**) DES extracts: ChCl:G (Choline Chloride:Glycerol), M:LA (Menthol:Lauric Acid), ChCl:EG (Choline Chloride:Ethylene Glycol), ChCl:U (Choline Chloride:Urea) CA:EG (Citric Acid:Ethylene Glycol); (**b**) organic solvent extracts: H-Hexane, EA-Ethyl Acetate, E-Ethanol, and M-Methanol.

Cherry Dwarf had the highest percentage of extracted cannabinoids and therefore was selected for comparing the extraction efficiency of Oss and DESs. The Oss used were ethanol, ethyl acetate, hexane, and combination, while the DESs used were menthol/lauric acid, citric acid/ethylene glycol, choline chloride/ethylene glycol, choline chloride/urea, and choline chloride/glycerol. There was a significant interaction between compounds and solvents ($p < 0.0001$). DESs showed a high ability to interact with CBD, resulting in two DESs (choline chloride/ethylene glycol and choline chloride/urea) outperforming ethanol, the OS usually used to extract CBD. Choline chloride/ethylene glycol extracted the highest percentage of all solvents (0.87%), followed by choline chloride/urea (0.78%), citric acid/ethylene glycol (0.48%), choline chloride/glycerol (0.39%), and menthol/lauric

acid (0.04%), followed by ethanol extracted (0.69%), ethyl acetate (0.59%), hexane (0.32%), and a combination of the three solvents (0.29%) (Figure 4).

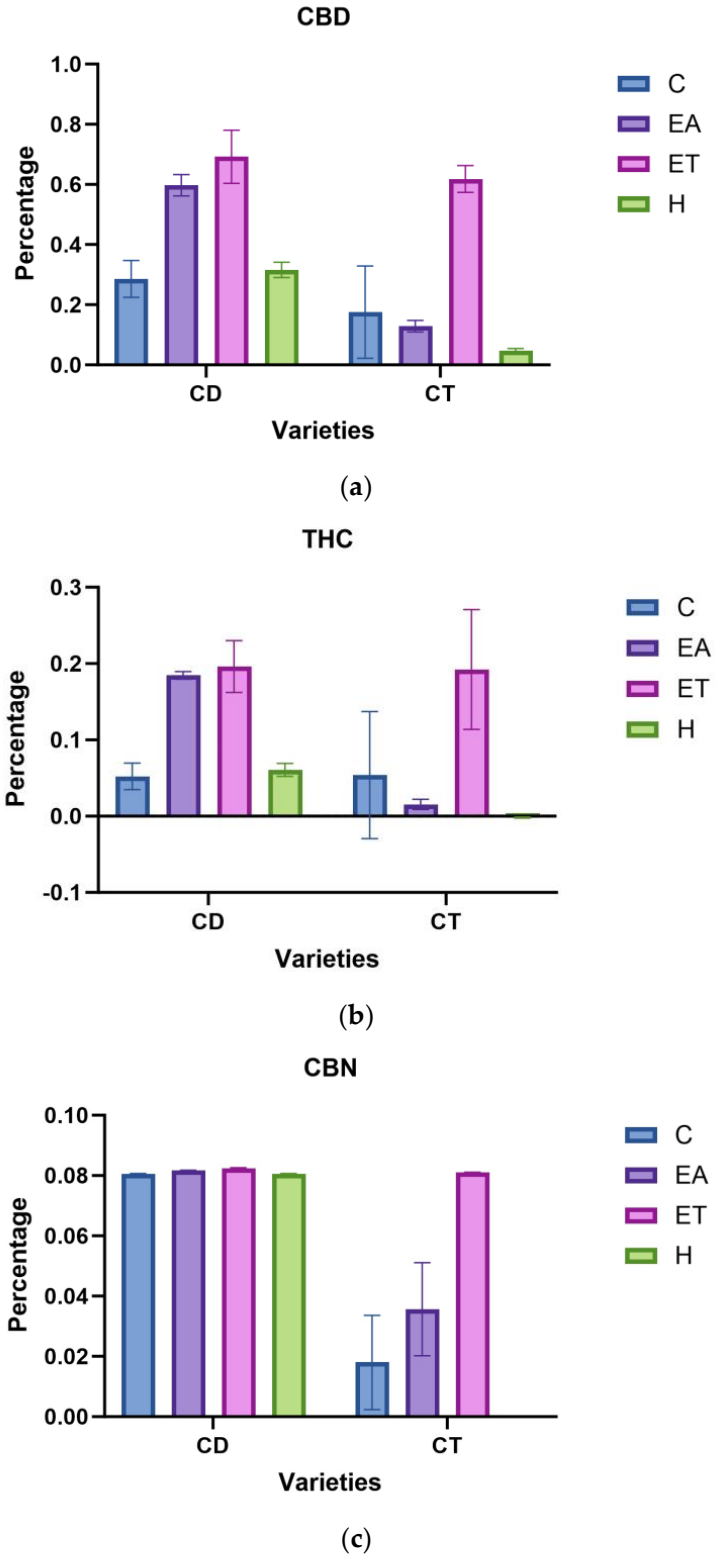


Figure 3. Comparison of the percentage concentration of (**a**) CBD, (**b**)THC, and (**c**) CBN cannabinoids in the Cherry Dwarf (CD) and Citrus (CT) industrial hemp varieties extracted using organic solvents H-Hexane, ET--Ethanol, EA-Ethyl Acetate, and C-Combination of three solvents at 1:1:1 using targeted analysis.

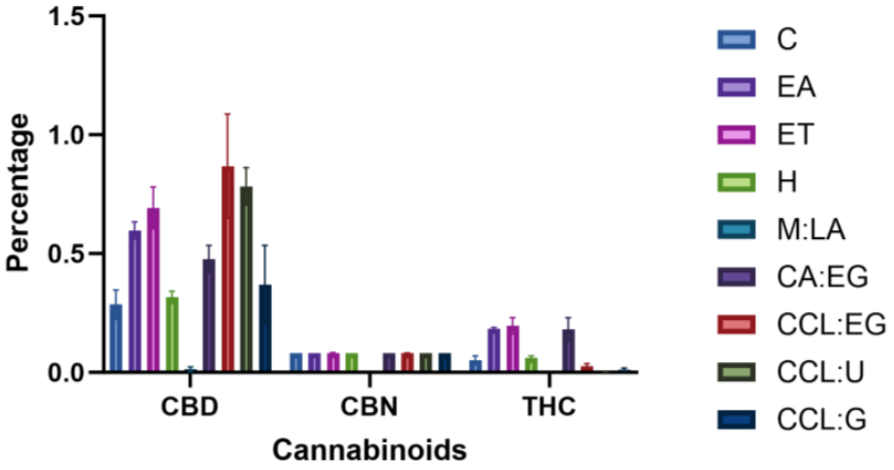


Figure 4. Percentage concentration of CBD, CBN, and THC extracted by organic solvents (H-Hexane, ET-Ethanol, EA-Ethyl Acetate, and C-Combination of H, ET, and EA at 1:1:1) and DESs (Menthol/Lauric Acid, Citric Acid/Ethylene Glycol, Choline Chloride/Ethylene Glycol, Choline Chloride/Urea and Choline Chloride/Glycerol).

3.3.2. Analysis of Terpenes

A refence standard with 19 terpenes was used in the targeted analysis to determine the concentration of these terpenes in Cherry Dwarf and Citrus industrial hemp varieties. The targeted 19 terpenes in the extracts of both OSs and DESs were using gas chromatography mass spectrophotometry. Terpenes were detected in samples extracted using OSs; however, DESs produced large broad peaks in the gas chromatography; therefore, they were excluded from the determination process. After the initial analysis of the GC/MS data, two terpenes were excluded from further statistical analysis as they could not be detected in any of the varieties. Eight terpenes that had significant concentrations in both varieties were selected for further comparison between varieties and between solvents. There was a significant interaction between terpenes and solvents ($p < 0.001$) in Citrus, while in Cherry Dwarf the terpenes differed significantly at ($p < 0.001$), while the solvents differed significantly ($p = 0.02$). In general, there were no significant differences between varieties and solvents for all terpenes except for beta caryophyllene in Citrus ($p = 0.03$) with the concentration of 0.074 mg/mL. Although no statistically significant difference was detected between the solvents, Figure 5 shows that hexane extracted the highest amount of terpenes.

3.4. Untargeted Analysis of Hemp Extracts

3.4.1. Univariate Statistical Analysis of Cannabinoids

Untargeted analysis of cannabinoids was performed using LC-MS, resulting in 90 compounds. Screening based on the 90% best match of the MS/MS fragmentation data compared to mzCloud resulted in nine compounds that were used for further comparison based on peak area. These included cannabichromene, cannabichromevarin, cannabicitran, cannabidiol, cannabidiolic acid, cannabidivarin, cannabinodiol, cannabinol, and THC. There was a significant interaction between varieties and solvents in all cannabinoids except cannabichromevarin and cannabinodiol, which were not significant. Cherry Dwarf showed the highest peak areas in all compounds except cannabichromevarin, which was similar in Citrus and Cherry Dwarf; however, Citrus has the highest peak area of cannabinodiol (Figure 6). These findings are consistent with those obtained from the targeted analysis, where Cherry Dwarf has the highest concentration of all the selected compounds. Therefore, Cherry Dwarf was used to compare OSs and DESs on untargeted multivariate analysis.

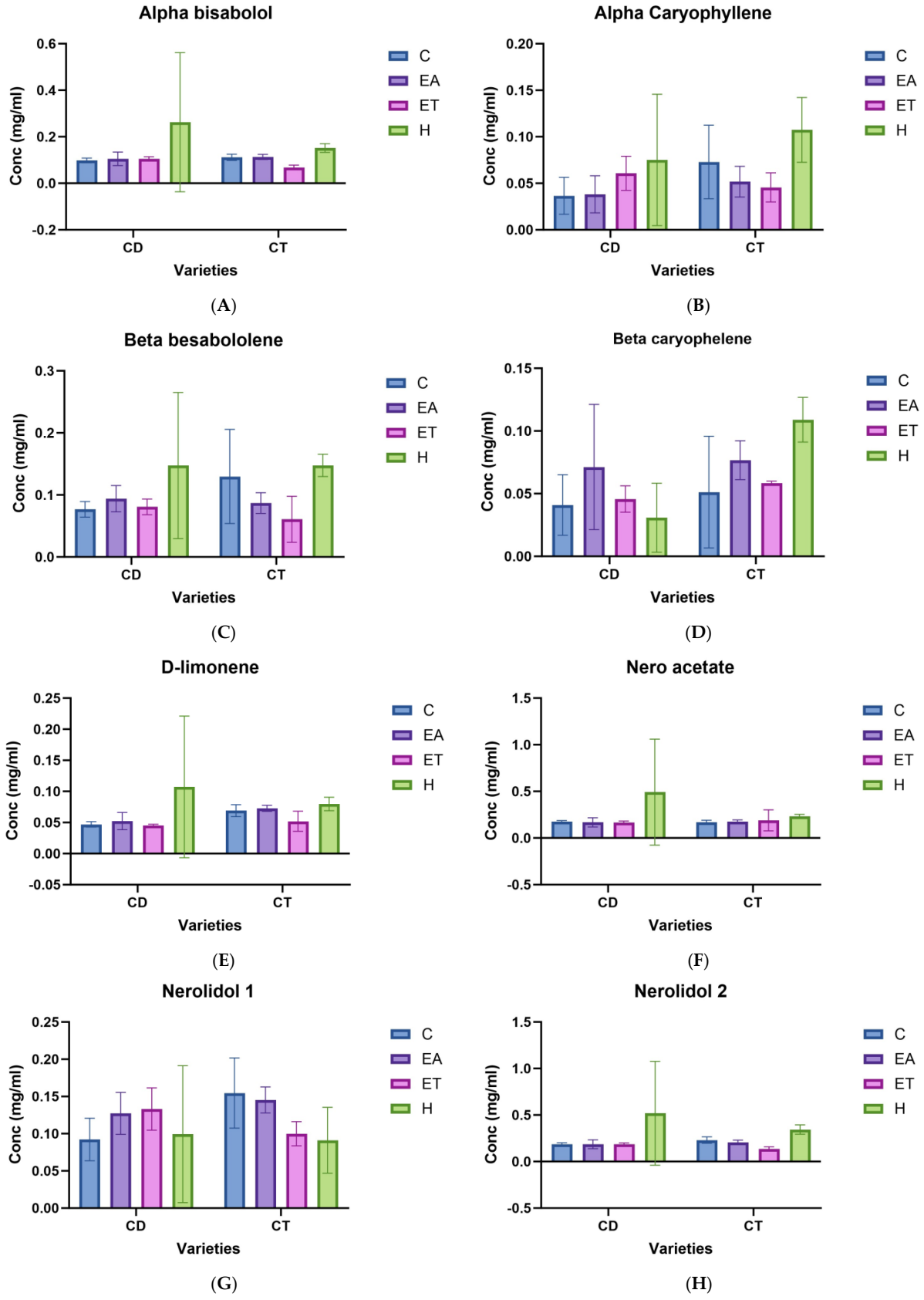


Figure 5. Comparison of the terpene concentrations (**A**) Alpha bisabolol, (**B**) Alpha caryophyllene, (**C**) Beta bisabololene, (**D**) Beta caryophyllene, (**E**) D-limonene, (**F**) Nero acetate, (**G**) Nerolidol 1, and (**H**) Nerolidol 2 extracted using OSs hexane, ethanol, ethyl acetate, and combination of the three solvents at 1:1:1 extracted from Cherry Dwarf and Citrus industrial hemp varieties.

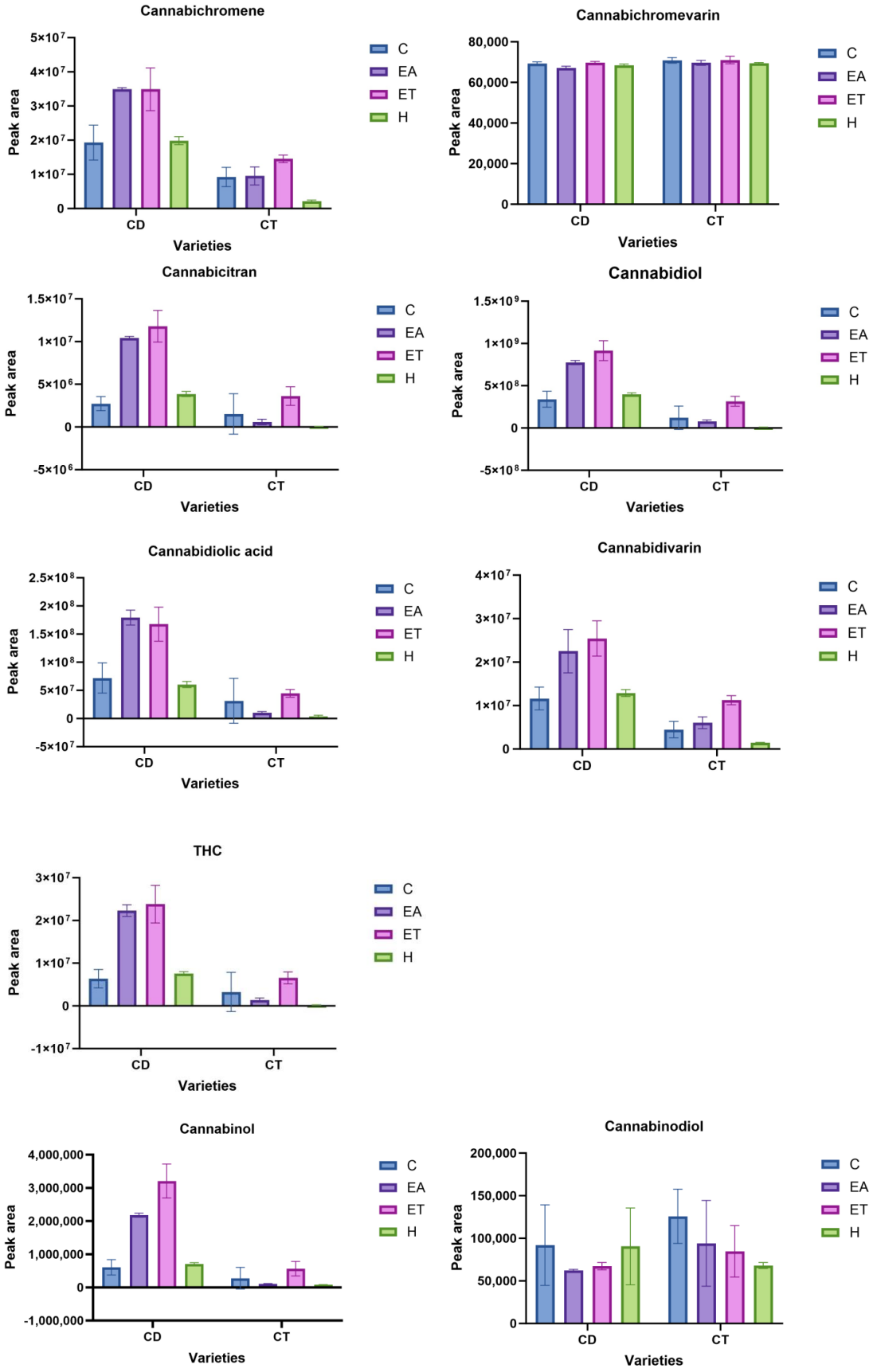
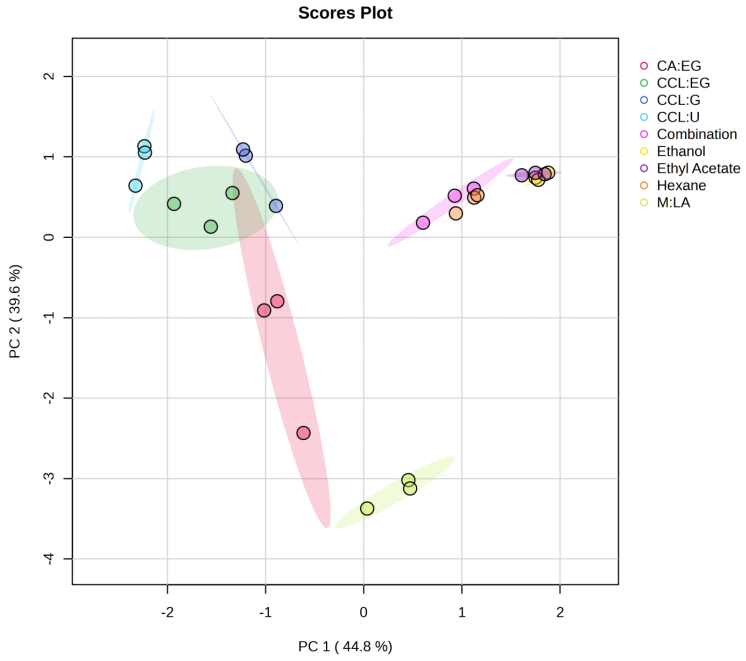


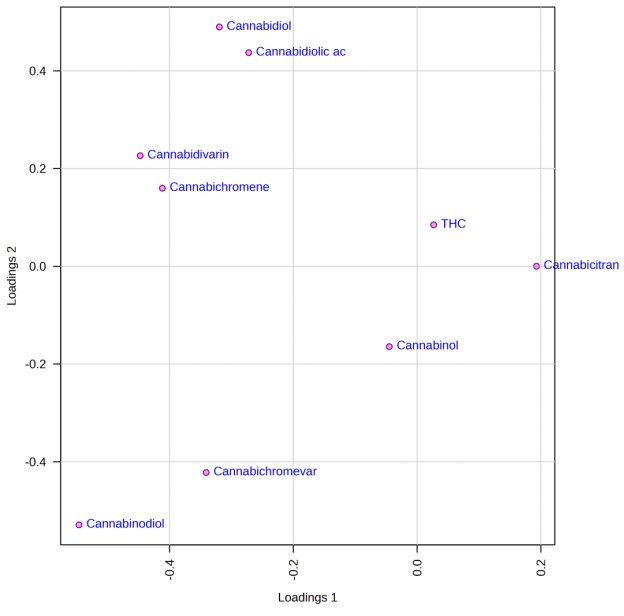
Figure 6. Comparison of the peak areas of cannabinoids from the nontargeted analysis. The Cherry Dwarf and Citrus industrial hemp varieties were extracted using OSs hexane, ethanol, ethyl acetate, and combination of the three solvents at 1:1:1.

3.4.2. Multivariate Statistical Analysis of Cannabinoids

PCA was carried out to better visualize, within the nine extraction solvents, the behaviors of the extracted compounds. The first PCA (Figure 7a–c) was performed to identify the correlation groups for solvents based on LC/MS results. The plots had 44.8% data variability on the first principal component (PC 1) and 39.6% on the second one (PC 2). The variance was caused mainly by (menthol/lauric acid), which contained a very low amount of all extracted compounds, and to some extent by citric acid/ethylene glycol DES which has the highest cannabinodiol in PC 1. The variation in the second principal component (PC 2) was mainly caused by ethanol and ethyl acetate which have the highest cannabicitran peak areas and very small peak areas from hexane and the combination of solvents. These findings are further illustrated by the heat map (Figure 7d) which shows the peak areas of each extracted compound in each solvent used for extraction.



(**a**)



(**b**)

Figure 7. *Cont.*

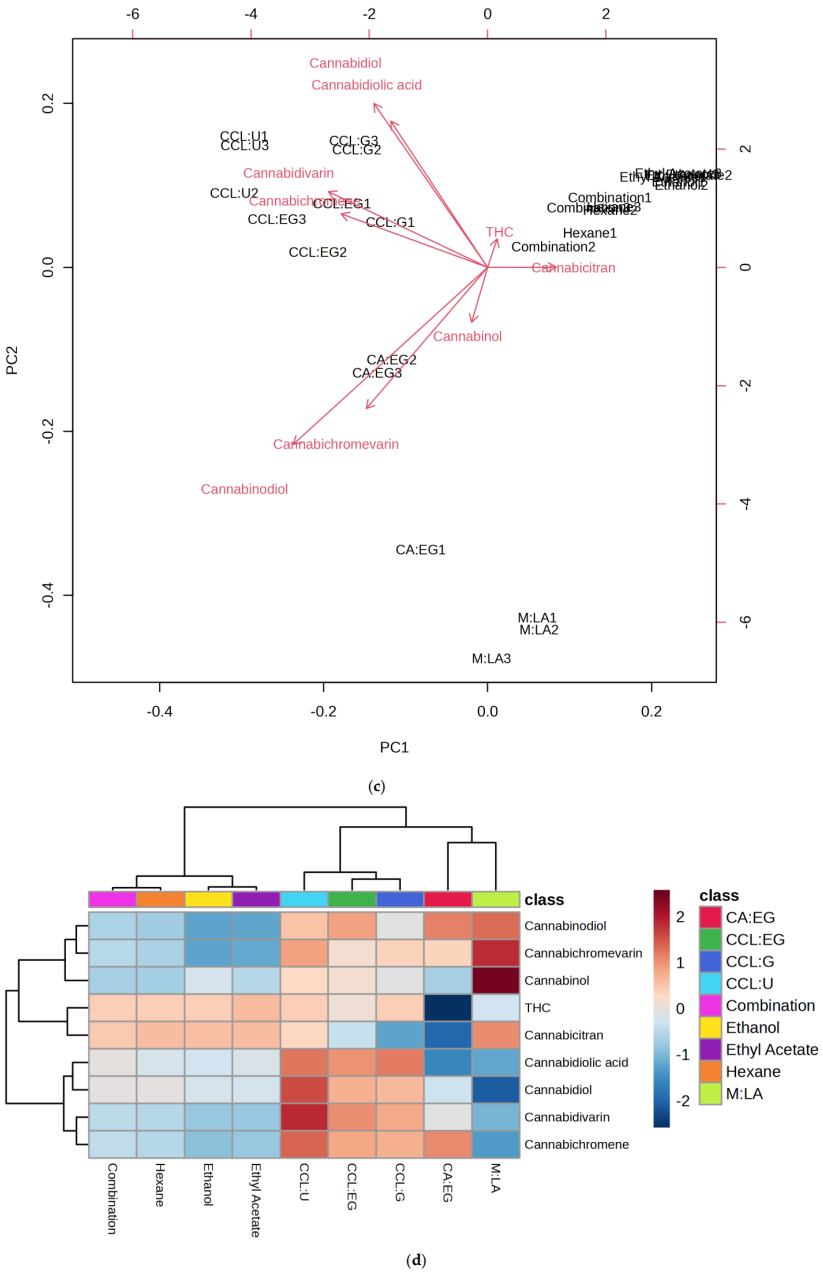


Figure 7. (**a**) PCA score plot of solvents used in extraction and (**b**) loadings of compounds extracted, (**c**) biplot showing distribution of compounds and solvents, and (**d**) heat map showing variability of OSs and DESs in extracting cannabinoids using untargeted analysis.

4. Discussion

The physical properties of DESs (e.g., viscosity, polarity, and pH) may influence the extraction efficiency and, consequently, the type and amount of phytochemical compound obtained. In this study, no water was added to the DESs, so their properties were determined in their pure form. Menthol/lauric acid DES was the least viscous (10.24 cP) of the DESs in this study and was slightly acidic (5.61). Similar properties were obtained by [18] who also identified menthol/lauric acid as a hydrophobic DES [18]. The pure menthol/lauric acid DES was transparent, while the extract color was greenish, indicating a green compound or mixture was extracted. Despite menthol/lauric acid DES being less viscous, the extraction efficiency of cannabinoids (CBD, THC, and CBN) was very low. Menthol/lauric acid was unable to extract THC or CBN and could only extract 0.04% of CBD. In contrast, Tiago et al. found that menthol/lauric acid extracted a very high

amount of CBD+CBDA from leaves, flowers, and seeds of the Futura 75 hemp variety [19]. However, this discrepancy has several possible explanations as Tiago et al. only used HPLC analysis with external standards, while this study used both targeted and untargeted LC/MS analysis with internal standards; in addition, Tiago et al. did not differentiate between CBDA and CBD. This study does match some findings of Tiago et al. as their reported properties of the menthol/lauric acid DES including results density, molar ratio, and viscosity are similar to those reported in Table 1. Also, Tiago et al. reported menthol/lauric acid extracted the most chlorophyll of all DESs used, similar to what can be observed in Figure 2a.

Comparison of ethanol, ethyl acetate, hexane, and their combination revealed that ethanol extracted the highest amount of CBD in both Cherry Dwarf (0.69%) and Citrus (0.61%). The concentration of CBD, CBN, and THC was variety dependent. These findings match those of Wongwailikhit et al. [20] who revealed that ethanol outperformed isopropanol in extracting CBD and THC. Furthermore, the findings correspond to De Vita et al. [21], who found that CBD and THC concentrations increased with increasing ethanol concentration. Therefore, this finding is consistent with other reports [21,22] that ethanol is preferred for extraction of CBD over nonpolar organic solvents.

A further comparison of OSs with DESs was performed on the extraction of CBD, CBN, and THC from Cherry Dwarf. The OSs were ethanol, ethyl acetate, hexane, and their combination, while the DESs used were menthol/lauric acid, citric acid/ethylene glycol, choline chloride/ethylene glycol, choline chloride/urea, and choline chloride/glycerol. The results revealed that DESs based on choline chloride (choline chloride/ethylene glycol and choline chloride/urea) extracted a higher amount of CBD, 0.87% and 0.78%, respectively, than ethanol (0.69%), the organic solvent most used in hemp extraction. These findings are consistent with those of Liu et al. [23] in which DESs with choline chloride/urea extracted the highest amount of flavonoids from lotus leaves. However, these findings are contrary to those of Tiago et al., in which they observed a large amount of CBD extracted using menthol/lauric acid [19]. The DES made from menthol/lauric acid in this study did not extract any CBN or THC and extracted a very small amount of CBD. One reason for the difference could be the extraction method (ultrasonic bath, temperature at 60 °C) employed by Tiago et al., which possibly improved the extraction efficiency [19], while in our study, maceration/shaker water bath at 37 °C was used. This contradiction was addressed earlier in more detail.

Eight terpenes were found in high concentrations in both varieties and identified as alpha bisabolol, alpha caryophyllene, beta bisabolene, beta caryophyllene, d-limonene, nerolidol 1, nerolidol 2, and nero acetate. These results are similar to the terpenes identified in a study by Namdar et al. of the hemp line CS12 that also reported that hexane extracted more beta caryophyllene and that ethanol extracted more beta bisabolene and nerolidol [24]. Benelli et al. reported 49 terpenes from fresh inflorescences of Felina 32 using stem distillation and these include all the terpenes found in our study [25]. However, it can not be concluded that Cherry Dwarf and Citrus hemp varieties contain fewer terpenes than Felina 32 or CS12, as the differences in terpene compositions can result from differences in extraction and analysis methods.

Other solvents have been used to extract phytochemicals and showed better extraction efficiency than organic solvents. For example, ionic liquids (ILs) have been used in several studies. Sillero et al. extracted flavonoids using ILs and the results revealed that 779 mgCE/g was extracted which was 23 times higher than the water extracts [26]. In addition, Liu et al. extracted five tashnones using ILs and found that the extraction was enhanced by 35% compared to methanol extraction [27]. These findings justify the search for other extraction solvents that could be commercially exploited. However, ILs have problems with biodegradability as many have chains that are biodegradable but also a core structure that is poorly biodegradable. During biodegradation, this can result in intermediates that are more toxic than the original molecule [28]. Also, ILs are highly stable and bio-accumulative, often resulting in serious threats to the environment and human

health [29]. Although a search is ongoing for safer ILs, DESs are currently among the best alternative extraction solvents.

5. Conclusions

This study aimed to evaluate the extraction efficiency of DESs and OSs on cannabinoids and terpenes from industrial hemp inflorescence. Among the OSs, ethanol extracted the highest amount of CBD and THC with more extracted from the Cherry Dwarf variety than the Citrus variety. There was no significant difference between the CBN extracted by all OSs in both varieties. The comparison of DESs and OSs on the extraction of cannabinoids indicated that choline chloride/ethylene glycol extracted the highest amount of CBD followed by choline chloride/urea, and these DESs outperformed ethanol, a popular solvent for the extraction of cannabinoids. There was no significant difference between DESs and OSs when extracting CBN. Citric acid/ethylene glycol DES extracted a comparable amount of THC as ethanol and ethyl acetate. Eight terpenes were identified in the two hemp varieties, these terpenes were also identified in other varieties in various studies. DESs are known to be safe, cheap, and environmentally friendly compared to OSs, and the findings suggest that DESs can be used to extract cannabinoids replacing Oss. However, there is a need for further research on the effective and efficient means of separating DESs from the extract to obtain pure crude extracts. In addition, analysis of terpenes extracted with DESs needs to be optimized and validated. Since identified cannabinoids and terpenes are known to be antimicrobial, antifungal, and with some pesticide properties, the findings will be the basis for continued research on the use of hemp as a biopesticide.

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