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# Comparative extraction of cannabinoids and terpenoids from *Cannabis Sativa L.* using three solvents

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## Abstract

Organic solvents of diethyl ether and pentane were tested against ethanol for the extraction of terpenoids and cannabinoids from hemp inflorescence. It is not well known how pretreatment of grinding and extraction conditions, that is, number of extractions, temperature, and solvent residence time affect extraction yield and product compositional profiles, so these were examined in this study. As a general trend, diethyl ether and pentane had better selectivity towards the terpenoids and cannabinoids compared to ethanol. It was found that even though grinding to reduce the particle size of the inflorescence did not dramatically increase the total extraction yield, it increased the yield of the first extraction as well as the content of terpenoids and cannabinoids in the extract. Extraction residence time trial revealed the benefit of shorter time and the different behavior of the two classes of compounds. Increasing the extraction temperature from 4 to 21°C improved the total extraction yield by all three solvents, however, no additional improvement was seen at 30°C. To achieve high concentration of the bioactive compounds in the extract, multiple extraction at 21°C with short residence time, such as 15 min, are preferred for the solvents tested, and an extract having about 6% terpenoids and 59% main cannabinoids was obtained using pentane. This systematic study provides a guide to commercial processing optimization and directions for further investigation.

**KEYWORDS**
extraction yield, solvent type, terpenoids and cannabinoids yield and composition

## INTRODUCTION

*Cannabis sativa* is a millenarian plant popularly used for its euphoric and medicinal properties. Up to date, more than 500 natural compounds have been identified in *C. sativa*, and the most interesting ones are compounds known as cannabinoids (De Backer et al., 2009). Among these, cannabidiol (CBD) is one of the main non-psychoactive but anticancer and anti-inflammatory components (Muthumalage & Rahman, 2019; Vitetta et al., 2021). It is mainly produced in the form of cannabidiolic acid (CBDA) and then transformed to CBD through decarboxylation (Russo, 2007; Takeda et al., 2012). Nonetheless, numerous international and United States regulations serve to control cannabis, and only in the recent years has the medicinal use of cannabis been again allowed (Manthey et al., 2021).

The extraction of cannabinoid compounds from cannabis has attracted the attention of many researchers (Attard et al., 2018; Casiraghi et al., 2018; Marzorati et al., 2020; Moreno et al., 2020). The most commonly reported methods of cannabis extraction are ethanol and hydrocarbon extractions, and quick wash alcohol extraction (Lewis-Bakker et al., 2019). More recently, there have been reports of small or laboratory scale extraction methods including ultrasound-assisted extraction, supercritical fluid extraction and microwave assisted extraction (Agarwal et al., 2018; Attard et al., 2018; Da Porto et al., 2014, 2015; Moreno et al., 2020; Omar et al., 2013), with supercritical $CO_2$ extraction demonstrating some selectivity towards cannabinoid compounds (Marzorati et al., 2020). In general,

[Correction added on 1 June 2022, after first online publication: The word Sativa in the article title was misspelled in the original publication and has been corrected in this version.]

  

extraction using a solvent that solubilizes and removes the compounds from the plant biomass, and at a temperature that maximizes cannabinoids recovery but minimizes the extraction of unwanted components is ideal (Lewis-Bakker et al., 2019).

Currently in CBD hemp industry, the extraction is predominately by ethanol and supercritical $CO_2$ extraction. The ethanol extraction method is relatively easy compared to the supercritical $CO_2$ method that requires more initial equipment investment and processing parameter optimization. For both aforementioned methods, the extract requires labor and energy intensive further processing and fractionation to obtain a pure CBD product. Although CBD may have been the main extraction and fractionation interest, terpenoids are also valuable compounds. They have been proven to have a synergistic action with the cannabinoids (Russo, 2011). Even though the mechanism of their therapeutic processes is not yet understood, and with their total amount recovered from *C. sativa* being about a tenth of that of cannabinoids, terpenes are believed to affect the activity of cannabinoids significantly (Namdar et al., 2018).

However, the terpenoid compounds can be molecularly altered and lost in the thermal fractionation of CBD (Leyva-Gutierrez et al., 2020). For improving the efficiency and profitability of the CBD hemp processing industry, it is desirable to maximally extract both cannabinoids and terpenoids. Very few studies are available in the literature (Namdar et al., 2018) on comparative extraction of both classes of compounds, and there is a need to study the selection of solvent for the extraction and process improvement. Therefore, this work aims to study the behavior of using solvents to extract the cannabinoids and terpenoids and product composition.

In this study, we evaluated different solvents including ethanol (as a control) and lower boiling point diethyl ether and pentane for their efficiency in extracting the terpenoids and cannabinoids. The selection of these lower boiling point solvents is to more completely remove solvent at relatively low temperature compared to ethanol to avoid the loss of the more volatile terpenoids. The effect of reducing particle size of the inflorescence by grinding was also evaluated. The impact of extraction conditions such as number of extractions, residence time of solvent in the biomass, and temperature were also investigated. To determine the best solvent and conditions, the chemical profile of the extracts obtained under various conditions were characterized.

## METHODS AND MATERIALS

The dried inflorescence of *C. sativa* cultivar Late Sue (forced air dried, 20–22°C, 60% RH) purchased from the Joe Boze Farm (Carthage, TN) was used in this study. The material was stored in dark and well-ventilated storage area below 20°C. Some samples were ground using a coffee grinder prior to the extraction and they have an average particle size of 0.45 mm. To minimize the loss of volatile compounds and facilitate grinding, the biomass samples were first dipped in liquid nitrogen for freezing treatment. Non-ground inflorescence was also used for an extraction comparison. All solvents were purchased from Fisher Scientific (Hampton, NH) or Sigma-Aldrich (Burlington, MA). Diethyl ether was ACS certified (>99% purity) and stabilized with butylated hydroxytoluene (BHT). It was distilled to remove the BHT prior to use. Pentane was ACS certified (>98% purity) and distilled before use. The ethanol was absolute grade of 200 proof and used as received.

Certified reference standards of the main classes of compounds, namely, cannabinoids and terpenoids were purchased. CBD, CBDA, $\Delta^9$-THC (tetrahydrocannabinol), THCA (tetrahydrocannabinolic acid) and CBN (cannabinol) standards were each purchased from Cerilliant (MilliporeSigma, St. Louis, MO) as 1 mg/ml solutions in either methanol or acetonitrile. Cannabis Terpene Mix 1 (lot no. AA190320009) and Cannabis Terpene Mix 2 (lot no. AA190306002) standards, representing a combined total of 42 different common *C. Sativa* terpenoids were purchased from SPEXCerti-Prep (Metuchen, NJ) as 100 μg/ml concentration for solutions in methanol, with purities in the range of 75%–100% for individual components or isomer mixtures.

### Extraction of the bioactive compounds

To investigate the effect of extraction solvent on yield, ethanol (as a control), ethyl ether, and pentane were used for the extraction. The solvent to biomass ratio was fixed at 5:1 (vol:wt, ml to g) which was the lowest level of solvent needed to submerge the sample and no stirring (only soaking) was used for all extractions. To evaluate the effect of grinding, extraction was conducted on both the ground and non-ground inflorescence. To determine the number of extractions needed for an optimal recovery, three sequential extractions were conducted for each solvent. To evaluate the effect of extraction temperature, 4, 21, and 30°C were used with a residence time of 30 min for each of the three sequential extractions. The temperature that resulted in the highest extraction yield was then selected for all of the later extractions. To investigate the effect of residence time, the soaking was done for 15, 30 and 60 min for each of the three sequential extractions at the selected extraction temperature.

The extraction was conducted using a glass syringe of 25 ml as an extraction vessel with the plunger inserted and needle attached to simulate the packed bed extraction of oilseed. This device was placed in environmental controlled incubator at the desired temperature. After the treatment time, the needle stopper

was removed and solvent was pushed out and collected. For the 2nd and 3rd sequential extraction, and same solvent to biomass ratio was used. The solvents in the extracts were evaporated at ambient temperature under a stream of nitrogen, and the extracts were weighed and stored in the freezer until analyses. The extraction yield is defined as weight ratio of the masses of the extract of a specific group of compounds to the initial biomass before extraction, expressed in percentage. For the combined yield from the three sequential extractions and the overall concentration of terpenoids or cannabinoids in the total extract, the weights were added, and concentration in the combined extract was calculated based on the mass and content of each extract. Each extraction treatment was conducted in duplicate.

## Characterization of the extracts

The composition of the extracts was characterized following a previously reported method with minor modifications (Leyva-Gutierrez et al., 2020). To detect the presence of cannabinoids in their acidic form, all the samples were silylated prior to gas chromatography (GC). The samples were also analyzed non-derivatized by dissolving in dichloromethane to prepare stock solutions and diluted with freshly distilled diethyl ether to a final concentration of 500 μg/ml. Methyl heptadecanoate was used as the internal standard for quantification, and compounds were analyzed using a Shimadzu GC-2010 with FID detector (Shimadzu, Kyoto, Japan). Separation was achieved using a nonpolar HP-5 capillary column (J&W Scientific, Folsom, CA) with a helium carrier gas flow rate of 1.5 ml/min and 1:20 split ratio. The temperature program consisted of a 1 min hold at 40°C, followed by 6°C/min ramp to 250°C and held for 10 min, with both injector and detector temperatures at 250°C, resolved the terpenoids and cannabinoids in chromatographically distinct retention windows. Terpenoids were quantitated by constructing individual six-point calibration curves (5–100 μg/ml) using non-derivatized Cannabis Terpene Mix 1 and Cannabis Terpene Mix 2 reference standards.

Cannabinoids were quantified as the trimethylsilyl (TMS) derivatives by a six-point calibration curve (5–100 μg/ml) constructed using silylated cannabinoid standards which were prepared using the same conditions as for the samples. To prepare derivatized standards, 500 μl of each cannabinoid (in either methanol or acetonitrile) were pooled into a 20 ml vial, evaporated to dryness under $N_2$, and then dissolved in 5.0 ml methanol to prepare a 100 μg/ml stock solution. Aliquots corresponding to the range of calibration concentrations were transferred to individual 1.0 ml Reacti-Vials, evaporated to dryness under $N_2$, reacted with 100 μl BSTFA (N,O-bis[trimethylsilyl]-trifluoroacetamide) with 1% TMCS (trimethylchlorosilane) at 65°C for 1 h. The reaction mixture was cooled to ambient temperature, and injected directly into GC.

Linear correlation coefficients for the standard curves ranged from 0.9897 to 0.9991 for the standards, and the results are reported as the average of two replicates. The weight percentages of terpenoids and cannabinoids were calculated as the ratios of the compounds to both the dry biomass (as extraction yield) and the solvent-free total extracts (as content or purity in the extracted product).

## Statistical analysis

Statistical analyses were performed using the JMP program (JMP Statistical Software, SAS Institute, Cary, NC). Means and standard deviations are presented in the tables. One-way analysis of variance was carried out and differences among means were compared using a Tukey test. The significance level was set at 0.05.

# RESULTS AND DISCUSSION

For accuracy of quantification, one needs to ensure that no chemical changes, such as decarboxylation, occur during sample preparation or such change is considered and accounted for. No significant decarboxylation reaction was expected during the experiment and sample handling. It was reported when plant materials were dried at 50°C, no decarboxylation conversion occurred (Knezevic et al., 2021). For cannabinoids quantification, all samples were silylated at the mild 65°C for 1 h condition prior to GC. In addition, all the internal standards, including the acidic forms of the cannabinoids were treated the same manner as for samples before GC for establishing standard curves. Therefore, any changes due to the sample derivatization was accounted for by the standards.

## Effect of grinding on extraction yield

To evaluate the effect of particle size reduction, a 30-min extraction at 21°C was conducted on both the ground and non-ground samples. Figure 1a,b show that grinding of the samples resulted in higher 1st extraction and total extraction yield, especially for diethyl ether and ethanol (about 18% total yield relative to initial biomass). More significant improvement by grinding was observed for the first extraction for all three solvents and grinding almost doubled the yield. This indicates that particle size is an important factor for extraction, particularly if only one extraction is applied. A reduction in particle size can increase the extraction yield considerably.

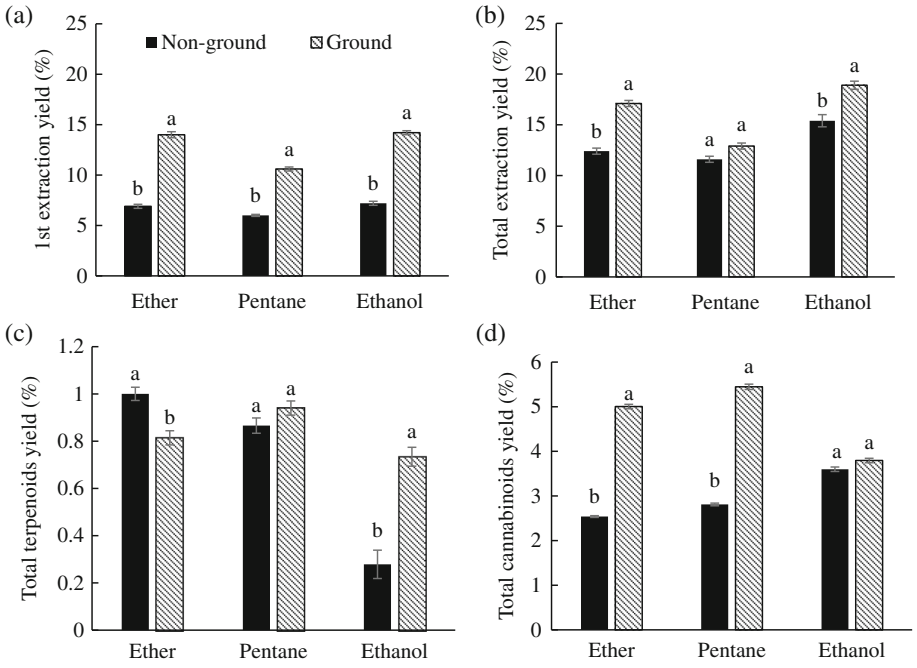


**FIGURE 1** Effect of grinding on yield of extraction (relative to initial biomass) at 21°C for 30 min; (a) 1st extraction, (b) three sequential extraction combined yield, (c) terpenoids, and (d) cannabinoids. The column legends are only shown in (a). The means of non-ground and ground samples were compared within each solvent in each chart, with the same letter indicating no statistical difference at $p = 0.05$

**TABLE 1** Content of terpenoids and cannabinoids in the combined extracts (%, relative to total extract, at 21°C for 30 min) as affected by grinding

| | Terpenoids | | Cannabinoids | |
|---|---|---|---|---|
| **Solvent** | **Non-ground** | **Ground** | **Non-ground** | **Ground** |
| Ether | $8.1 \pm 0.0^{a}$ | $4.9 \pm 0.3^{b}$ | $21.0 \pm 0.8^{b}$ | $29.5 \pm 0.3^{a}$ |
| Pentane | $7.7 \pm 0.1^{a}$ | $7.5 \pm 0.2^{a}$ | $25.1 \pm 0.6^{b}$ | $43.1 \pm 1.2^{a}$ |
| Ethanol | $1.8 \pm 0.0^{b}$ | $3.9 \pm 0.1^{a}$ | $23.0 \pm 0.5^{a}$ | $20.3 \pm 0.4^{b}$ |

*Note*: The means of non-ground and ground samples were compared within the same solvent and compound, with the same letter indicating no statistical difference at $p = 0.05$.

For oilseeds, particle size reduction disrupts the cells, leading to a much larger contact surface and easier penetration of the solvents into the seed samples (Mani et al., 2007). Similar findings were also reported by others (Shah & Roggen, 2020) on hemp extraction. More importantly, the particle size reduction treatment resulted in some improved extractions of the terpenoids and cannabinoids. Figure 1c,d show that grinding of samples resulted in higher total (combined three extracts) yield of terpenoids by ethanol compared to non-ground, and much higher yield of cannabinoids was obtained by using pentane and diethyl ether. This is likely due to the better release and solubilization of the bioactive compounds by cellular or biomass disruption by grinding treatment. Even though this result may be expected, the different behaviors of the solvents towards the two classes of compounds are very interesting to observe.

It is believed that hemp cannabinoids and terpenoids are present in glandular hairs on the inflorescences or leaf surface readily accessible by solvent. However, our experiment did show that grinding can lead to higher recovery. Marzorati et al. (2020) also used a pulverized sample for complete solvent and supercritical $CO_2$ extraction. It has been shown that spent hemp flower by supercritical $CO_2$ extraction still had 8062 ppm CBD and 1960 ppm CBDA in the biomass (Kleinhenz et al., 2020), indicating incomplete extraction. It is interesting to note that in the same study, it shows hemp leaves had as much of CBD and CBDA (3347 and 36,920 ppm) as in the un-extracted hemp flower (3509 and 32,900 ppm) (which was a different batch of material from the spent flower as mentioned above, as confirmed by an author of the paper via personal communications). Hemp leaves have become very popular ingredients for herbal teas (infusions) which had cannabinoids content of 4073 ppm CBDA and 802 ppm CBD, with the acidic form much more removed from the leaves than CBD after infusion (Knezevic et al., 2021). These reports do not show terpenoids extraction compared to that of cannabinoids.

The data of this study on effect of grinding seem to indicate that compared to terpenoids, cannabinoids are not only located in the trichomes but are also present

inside the inflorescence's intercellular tissues. The results suggest that particle size reduction will facilitate the extraction of cannabinoids by using non-polar solvent. However, if ethanol is used, cannabinoid extraction will not be affected by size reduction, but the overall yield may be lower (Figure 1d). This is a novel finding of this study. Investigations on mass distribution of these bioactive compounds in different parts of plants will be very beneficial to further conduct theory-based extraction optimization studies.

To achieve the best recovery of the targeted terpenoids and cannabinoids, it is also important to understand the selectivity of different solvents towards the bioactive compounds. Therefore, the chemical profile of the extracts obtained with the three solvents under different conditions was characterized and the content of terpenoids and cannabinoids in each extract was quantified. The content of terpenoids and cannabinoids as shown in Table 1 indicates that pentane extracted the most cannabinoids and terpenoids (43.1% and 7.5% relative to the total extract) from the ground sample compared to other extracts. Even though ethanol gave higher total extraction yield, the total terpenoids and cannabinoids yield and their contents in the extract are relatively low. This polar solvent has likely extracted other compounds from the plant biomass. Therefore, diethyl ether and pentane had better selectivity towards the terpenoids and cannabinoids compared to ethanol. The data also indicate grinding had a more significant impact on cannabinoids recovery than for terpenoids. This maybe because terpenoids are more associated with the trichomes and cannabinoids are also contained in the plant tissues. Therefore, for further investigating the effect of extraction conditions, ground samples were used in later experiments.

## Effect of residence time on extraction yield

The effect of residence time on the extraction yield from the ground biomass was determined for the three solvents at 21°C. Figure 2 shows that in general ethanol resulted in the highest total extraction yield of 19% at 30 min compared to other solvents and conditions. This is slightly lower than the 22.0% reported by others (Marzorati et al., 2020), who used higher solvent to substrate ratio (10:1), longer residence time (2 h), and constant stirring during extraction. Diethyl ether resulted in a slightly lower and pentane resulted in the lowest total extraction yield by three sequential extractions. However, the difference among the first extraction at 15 min was much smaller. Residence time of 30 min had significantly higher yield from the 1st extraction compared to 15 min for all three solvents (Figure 2a–c). However, further increasing extraction time to 60 min led to a reduction of the 1st extraction yield. The exact reason for this reduction is unknown, although it is speculated that with longer solvent and biomass contact time, the solubilization, redistribution, and absorption of the bioactive compounds by the biomass may occur. However, through the 2nd

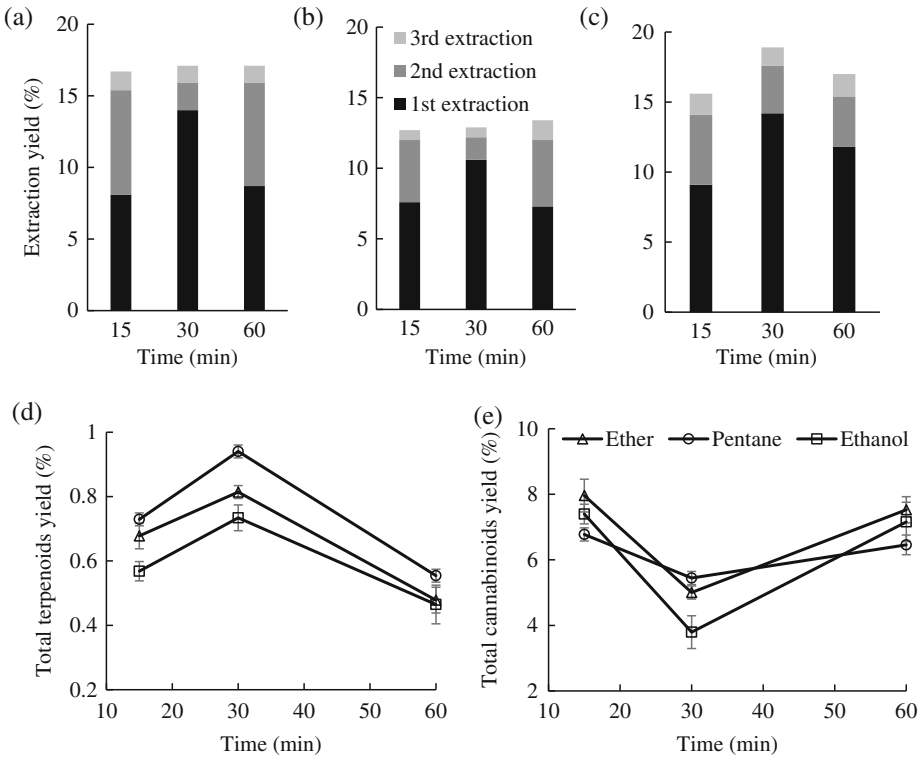


**FIGURE 2** The effect of residence time on extraction yield (relative to initial biomass) of three sequential extractions at 21°C from ground biomass, using (a) diethyl ether, (b) pentane, and (c) ethanol; (d) extraction yield of terpenoids, and (e) extraction yield of cannabinoids

and 3rd extraction with the 60-min solvent residence time, these compounds were washed out. Therefore, if only one extraction is used in practice, the identification of the best extraction time will be very important. When evaluating total yield by three sequential extractions, the effect of residence time within each solvent was much smaller. The extraction yield reaching a plateau after certain residence time was also reported by others (Gallo et al., 2020).

Figure 2d.e show the yield of terpenoids and cannabinoids relative to initial biomass by three sequential extractions (combined products) in response to extraction time. Table 2 shows their contents in the total extracts. The three solvents behaved similarly in extraction yield. The different trends of the two classes of compounds with residence time may be explained by the speed of solubilization or the different location or interactions of the terpenoids and cannabinoids with the biomass matrix. There are many unknowns in the extraction behaviors and dynamics that warrant further studies. Overall, the 15 min extraction at 21°C by pentane gave the highest cannabinoids content (52.9%, the seemingly low content was explained in later section) in the final extract as shown in Table 2.

## Effect of temperature on extraction yield

Figure 3 shows the effect of extraction temperature on recovery yield from ground biomass using 30 min solvent residence time. The temperature of 21°C resulted in higher extraction yield than that at 4°C which is due to the better solubility of the solutes in solvents at 21°C than at the low temperature. It is known that solubility increases with increased temperature (Mani et al., 2007). When the extraction was conducted at a temperature of 4°C, the three solvents resulted in almost the same extraction yield by the first extraction. While further increasing the extraction temperature to 30°C led to a reduced yield of the 1st extraction compared to that at 21°C. Higher temperature has most likely increased

**TABLE 2** Content of terpenoids and cannabinoids in the combined extracts (%, relative to total extract) as affected by solvent and residence time of three sequential extractions at 21°C from ground biomass

| | Terpenoids | | | Cannabinoids | | |
|---|---|---|---|---|---|---|
| **Solvent** | **15 min** | **30 min** | **60 min** | **15 min** | **30 min** | **60 min** |
| Ether | $4.1 \pm 0.1^{b}$ | $4.9 \pm 0.3^{a}$ | $2.8 \pm 0.1^{c}$ | $47.4 \pm 0.4^{a}$ | $29.5 \pm 0.3^{c}$ | $44.2 \pm 0.3^{b}$ |
| Pentane | $5.6 \pm 0.2^{b}$ | $7.5 \pm 0.2^{a}$ | $4.2 \pm 0.1^{c}$ | $52.9 \pm 0.7^{a}$ | $43.1 \pm 1.2^{c}$ | $48.4 \pm 0.4^{b}$ |
| Ethanol | $3.7 \pm 0.1^{a}$ | $3.9 \pm 0.1^{a}$ | $2.8 \pm 0.1^{b}$ | $47.3 \pm 0.2^{a}$ | $20.3 \pm 0.4^{c}$ | $42.4 \pm 0.4^{b}$ |

*Note*: Means were compared among the three times within the same solvent and compound, with the same letter indicating no significant difference at $p = 0.05$.

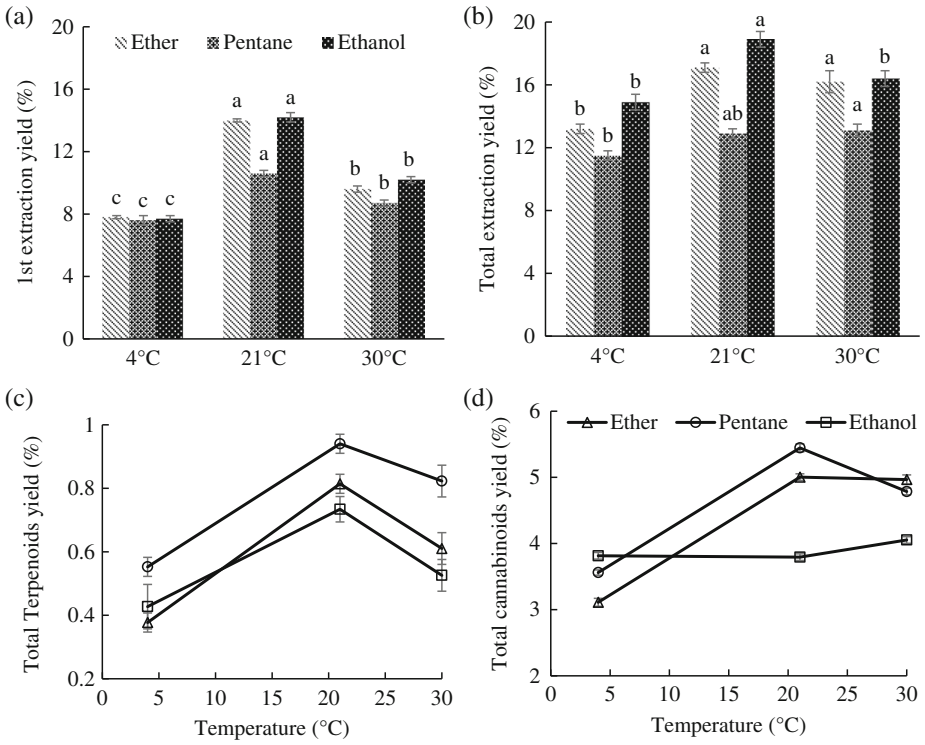


**FIGURE 3** Effect of temperature on the 30-min extraction yield (relative to initial biomass) from ground matter; (a) 1st extraction, (b) three sequential extractions combined, (c) terpenoids, and (d) cannabinoids. The means were compared among the three temperatures for each solvent in each chart, with the same letter indicating no statistical difference at $p = 0.05$

**TABLE 3** Content of terpenoids and cannabinoids in the combined extracts (%, relative to total extract) as affected by temperature on for 30-min extraction from ground biomass

| | Terpenoids | | | Cannabinoids | | |
|---|---|---|---|---|---|---|
| **Solvent** | **4°C** | **21°C** | **30°C** | **4°C** | **21°C** | **30°C** |
| Ether | $3.0 \pm 0.2^{c}$ | $4.9 \pm 0.3^{a}$ | $3.9 \pm 0.2^{b}$ | $23.9 \pm 0.5^{b}$ | $29.5 \pm 0.3^{a}$ | $30.8 \pm 0.2^{a}$ |
| Pentane | $5.0 \pm 0.2^{c}$ | $7.5 \pm 0.2^{a}$ | $6.6 \pm 0.4^{b}$ | $31.3 \pm 0.4^{c}$ | $43.1 \pm 1.2^{a}$ | $36.9 \pm 0.6^{b}$ |
| Ethanol | $2.9 \pm 0.0^{c}$ | $3.9 \pm 0.1^{a}$ | $3.3 \pm 0.1^{b}$ | $26.1 \pm 0.7^{a}$ | $20.3 \pm 0.4^{c}$ | $24.8 \pm 0.3^{b}$ |

*Note*: The means were compared among the three temperatures within the same solvent and compound, with the same letter indicating no statistical difference at $p = 0.05$.

interaction or binding of the bioactive compounds with biomass. These results are in agreement with other studies which reported lower oil extraction yield at higher extraction temperature for moringa seed kernel, celery and castor oil seeds (Akaranta & Anusiem, 1996; Mani et al., 2007; Papamichail et al., 2000).

Figure 3c,d show that low and high extraction temperatures of 4 and 30°C both negatively affected the yield of terpenoids and cannabinoids in the combined extracts, particularly at the low temperature and for terpenoids. This is likely related to the solubility of the terpenoids and cannabinoids at low temperature. The solubility of cannabinoids in pentane decreases with reduced temperature, and crystallization of cannabinoids was achieved through saturation and cooling techniques (Arora & von Salm, 2021), which is the basis for CBD isolate making in commercial practice. While at higher temperature of 30°C, the interaction of biomass with terpenoids may be stronger than for cannabinoids. In addition, more cannabinoids may be extracted from cellular structures to account for less reduction of cannabinoids extracted at 30°C relative to at 21°C, compared to the reduction of terpenoids. It is interesting to note that cannabinoids yield by ethanol extraction was less affected by temperature of extraction compared to the other two solvents. Table 3 also indicates that 21°C tends to give the highest concentrations of the two classes of compounds in the combined extracts, and overall pentane extracts have the highest concentration of terpenoids and cannabinoids.

In general, among the three solvents tested, ethanol had the lowest selectivity towards terpenoids and cannabinoids, while the non-polar solvent pentane noticeably extracted more terpenoids and cannabinoids under most of the tested extraction conditions. This is likely due to pentane's low polarity leading to a preferred extraction of the relatively non-polar cannabinoids and terpenoids without co-extracting the undesirable polar compounds. Others have also suggested that non-polar hydrocarbon solvents such as butane and propane can extract more terpenoids and result in higher purity of cannabinoids concentrate (June-Wells & Mitchell, 2018).

**TABLE 4** Identified terpenoids and cannabinoids in the extract obtained by using pentane at 21°C (three sequential extractions) at two extraction times

| | 15 min | 30 min |
|---|---|---|
| Terpenoids (%) | | |
| Alpha-humulene | 0.1 | 0.4 |
| Guaiol | 0.7 | 0.6 |
| *Trans*-caryophyllene | 0.1 | 0.3 |
| Valencene | 0.1 | 1.6 |
| *Cis*-nerolidol | — | 0.6 |
| Caryophyllene oxide | 0.9 | 0.8 |
| Cedrol | 1.2 | 1.3 |
| Alpha-bisabolol | 2.5 | 2.0 |
| Total | 5.6 | 7.5 |
| Cannabinoids (%) | | |
| CBD-diTMS | 27.3 | 21.8 |
| $\Delta^9$-THC-TMS | 1.0 | 3.5 |
| CBN-TMS | 1.3 | 0.7 |
| CBDA-TMS | 22.2 | 16.0 |
| $\Delta^9$-THCA-TMS | 1.1 | 1.1 |
| Total | 52.9 | 43.1 |
| Total quantified | 58.8 | 50.6 |

Abbreviations: CBN, cannabinol; CDB and CBDA, cannabidiol and cannabidiolic acid; THC and THCA, tetrahydrocannabinol and tetrahydrocannabinolic acid; TMS, trimethylsilyl.

## Terpenoids and cannabinoids profile of two extracts

Overall, extraction using pentane at temperature of 21°C for 15 and 30 min gave desirable yields and contents of terpenoids and cannabinoids in the extract. The compositions of these two extracts are shown in Table 4. In general, the CBD product composition is affected by plant cultivars and cultivation and post-harvest environmental factors, so this table only serves as an example of compounds quantified. There are also other minor terpenoids and cannabinoids compounds not quantified for this study. Other unidentified compounds in the extract include alkane hydrocarbon molecules as reported by Leyva-Gutierrez et al. (2020).

Even though extraction using solvent is considered as a more feasible method than supercritical $CO_2$ extraction mainly due to its initial high capital investment, the latter may be or can be modified to be more selective for the compounds of interest. Compared to extraction using methanol, supercritical $CO_2$ extraction yielded an extract with 16% CBD content and a low yield of 14%, while methanol extraction led to 22% yield but a product with much lower CBD content of 3% (Marzorati et al., 2020). These results suggest an enhanced selectivity of supercritical $CO_2$ towards CBD compared to alcoholic polar solvent. Moreno et al. (2020) also showed supercritical $CO_2$ (51%–100% yield) and liquefied propane (74%–99% yield) allow efficient and flexible extraction of cannabinoids from raw and decarboxylated plant material. Nonetheless, our extraction using solvent can lead to a product with >20% CBD and >5% total terpenoids, indicating a superior performance by solvent extraction. If the biomass is pre-decarboxylated, the CBD yield is expected to be >40% in the extract.

This is the first comparative study on how extraction yield of the terpenoids and cannabinoids is impacted by solvent type, extraction temperature and solvent residence time. This study and its observations have generated more questions than the original questions the study was designed to answer. There is a need for more theoretical or modeling studies on how these structurally diverse bioactive compounds are associated with the biomass and interacting with solvents of different polarity. Namdar et al. (2018) showed that the total amount of cannabinoids and terpenoids extracted using three different solvents (ethanol, hexane and the mixture of the two) clearly indicated preference to the mixed solution of polar and non-polar organic solvents. They suggested a need to study the selection of solvent for the extraction and process improvement. In addition to examining the type of solvent, more in-depth study under a wide range of conditions and having more data points should be conducted to fully optimize the extraction performance.

## CONCLUSION

Different solvents including ethanol and low-boiling point ethyl ether and pentane behaved differently for the extraction of terpenoids and cannabinoids from CBD hemp inflorescence. The extraction behavior of these two classes of compounds was also different, and this has not been reported. The distribution of these compounds in the inflorescence as a surface deposit or intracellular components may be different as indicated by how grinding treatment affected extraction yield. Pentane was proven to have the best selectivity towards the targeted terpenoid and cannabinoid compounds. Overall, extraction at the temperature of 21°C, and shorter residence time of 15 or 30 min for three sequential extractions were identified as the best conditions when using pentane. The extraction conditions reported in this study may serve as a benchmark comparison and a guide for manufacturers and processors to optimize their extraction systems to increase yield and purity of their crude extracts.

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## ETHICS STATEMENT

For the research presented in this study, no ethics statement is required since neither procedures nor raw materials included animals or their products.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

## AUTHOR CONTRIBUTION

Tong Wang conceived the study and in working with Tao Fei, they designed the scope of the study. Tao Fei carried out the experiment, analyzed the data and wrote the first draft. All authors contributed to and approved the final draft of the manuscript.

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