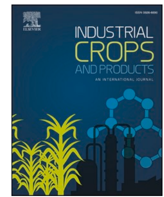
Industrial Crops & Products 219 (2024) 119007

Contents lists available at ScienceDirect

Industrial Crops & Products

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



Simple method for detecting tetrahydrocannabinolic acid and tetrahydrocannabinol in cannabis tissues using urine-based drug diagnostic device

Juyoung Kim [a], Dong-Gun Kim [a], Jin-Baek Kim [a], Jaihyuk Ryu [a], Ye-Jin Lee [a], Nam Min Woo [b], Jin Jong Sik [b], Sang Hoon Kim [a,*]

[a] Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute, 29 Geumgu-gil, Jeongeup-si, Jeollabuk-do 56212, Republic of Korea

[b] Jeonbuk National University, LED Agri-bio Technology Research Center, Specialized Campus, 79 Gobong-ro, Iksan-si, Jeollabuk-do 54596, Republic of Korea

ARTICLE INFO

Keywords:
Urine-based THC diagnostic device
Cannabis breeding
Tetrahydrocannabinolic acid (THCA)
Cannabinoids

ABSTRACT

Tetrahydrocannabinolic acid (THCA, or THC in the decarboxylated form), synthesized by cannabis plant (*Cannabis sativa* L.), is a cannabinoid that can be used as a therapeutic drug but is a dangerous psychoactive compound. Cannabis grown for industrial fiber hemp must contain little THCA to prevent misuse. Many attempts to develop new cultivars with low THCA contents have therefore been made. It is time-consuming and expensive to determine THCA in cannabis plant tissues by high-performance liquid chromatography, so we attempted to develop a simple and quick method for selected cultivars with low THCA contents. The diagnostic device, which is an immunoassay based on the principle of competitive binding of drug metabolites, was initially developed for human drug testing in urine. We aim to determine whether the diagnostic device can be applied to natural cannabinoids from cannabis plant. The devices were not suitable for aqueous extracts with methanol contents >20 %. The test (T) line on each device was less intense for leaf and flower extracts than the negative control (water). The THCA contents of water extracted leaves determined by HPLC and calculated based on the cannabidiolic acid contents were 7.5 and 15 μg mL^{-1} for SuperwomanS1 and Spectrum303 cultivars, respectively. When THCA and THC standards were loaded onto Abon devices, the T line disappeared at the THCA concentration 20 μg mL^{-1} and the THC concentration 1 μg mL^{-1}, respectively. Using extracts of 0.2 g fresh leaves, SuperwomanS1 and Spectrum303 will give faint and strong T lines, respectively, indicating that the devices could be used to test cannabis tissue THCA contents against the international regulatory limit (≤ 0.3 % w/w). The results indicated that the THCA contents of cannabis tissues at the flowering stage can readily be estimated using drug diagnostic devices, making it convenient for cannabis growers and breeders to semi-quantify THCA.

1. Introduction

Tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA) are cannabinoid components of cannabis plant (*Cannabis sativa* L.). These and other cannabinoids are terpenophenolic compounds that can be isolated from cannabis, also called marijuana (Mechoulam and Ben-Shabat, 1999). Cannabis has been used as a therapeutic drug and for fibers since before BCE 2700 because of the narcotic properties of THCA and CBDA, and the long and tough stems (Crocq, 2020). Tetrahydrocannabinol (THC), produced through decarboxylation by heat or light, is a dangerous and psychoactive compound that is strictly controlled in many countries and was included as a controlled substance under international law in 1961 as part of the United Nations Single Convention on Narcotic Drugs. Cannabinoids including THC and cannabidiol (CBD) are currently attracting attention because various therapeutic effects have been discovered by medical researchers (National Academies of Sciences, Engineering, and Medicine, 2017). Despite medicinal uses of THC, cannabis plant tissues with low THC contents are required when the plants are grown for use as industrial hemp, to prevent misuse.

THCA (or THC) contents in cannabis are determined by activity of its

Abbreviations: THCA, tetrahydrocannabinolic acid; THC, tetrahydrocannabinol; CBDA, cannabidiolic acid; CBD, cannabidiol; HPLC, high performance liquid chromatography.

* Corresponding author.
E-mail address: shkim80@kaeri.re.kr (S.H. Kim).

https://doi.org/10.1016/j.indcrop.2024.119007
Received 28 March 2024; Received in revised form 10 June 2024; Accepted 13 June 2024
Available online 26 June 2024
0926-6690/© 2024 Elsevier B.V. All rights are reserved, including those for text and data mining, AI training, and similar technologies.

biosynthetic gene, organ, and physical factors. Eliminating only THCA synthase gene activity was not sufficient to completely prevent THCA synthesis because other cannabinoid biosynthetic genes have the ability to synthesize minor amounts of THCA (Taura et al., 2007 & 2019; Zirpel et al., 2018). For instance, cannabinoid biosynthesis genes in cannabis plants have been markedly downregulated using RNA interference and agro-infiltration techniques, and Trilogene Seeds, a genetically modified hemp seed company based in Colorado, USA, produced the first hemp cultivar containing 0 % THCA, which was called Pandora (Matchett-Oates et al., 2021). However, cannabinoid biosynthesis gene expression may vary because of hormonal fluctuations and environmental changes, and the mechanisms bringing about absolute 0 % THC production have not been fully elucidated (Liu et al., 2021; Sands et al., 2023). A cannabis plant is generally classified as hemp and marijuana by its purpose and THC content. A cannabis plant is called hemp if it is intended for industrial use such as fibers and contains $\leq$ 0.3 % w/w THC (Petruzzello, 2022). Efforts to produce THC free cannabis plants for cultivation as hemp for industrial uses are continuing.

The USA and Canada require the THC contents of dry female flowers and leaves from industrial hemp to be $\leq$ 0.3 % w/w (The U.S. Department of Agriculture (USDA), 2018). It is therefore necessary to accurately determine the cannabinoid contents of cannabis plant tissues intended for various purposes, such as producing functional foods and raw materials for pharmaceuticals. After harvest, cannabis plant material needs to be stored until analyses have been performed, then material that does not meet the relevant standard, must be discarded or only sold by licensed cannabis establishments (The U.S. Department of Agriculture (USDA)). Different plant will have different THC contents because of a lack of standardized cultivars, so many analyses need to be performed. A method to allow cannabis growers to quickly assess the THC contents is required to minimize the time and cost between harvest and the material being accepted for use.

High performance liquid chromatography (HPLC) analysis is essential for developing THCA or THC-free cultivars of cannabis and detecting drugs in samples from humans (Breidi et al., 2012; Verstraete, 2004). However, it is very expensive and time-consuming to screen plant tissues from numerous cannabis cultivars for THC and THCA by HPLC. We have therefore been seeking methods for reliably confirming when the THCA contents of cannabis plant tissues are low enough to meet relevant regulations. One possible method involves diagnostic devices for marijuana's THC metabolites. Such devices were developed by two U.S companies (SYVA and Roche Diagnostics) in the 1980s (Centers for Desease Control and Prevention, 1983). The manufacturer's instructions indicate that the devices detect THC metabolites and its conjugated form both through immunological reactions. Commercially available diagnostic devices are 95 % accurate and have cut-off concentration of 50 ng mL^{-1} (or 50 ng g^{-1}) for 11-nor-Δ9-THC-COOH and 20 μg mL^{-1} for cannabinol or THCA (Yu et al., 2021). However, these diagnostic devices are only designed to test for drugs in human urine (i.e., in an aqueous solution). We tested the possibility of using these diagnostic devices to detect THC and THCA in extracts of cannabis plant tissues and then performed HPLC analyses of the THCA contents of the leaves to assess the results.

2. Materials and methods

2.1. Plant material

Spectrum303 and SuperwomanS1 cannabis (hemp) cultivars were obtained from Jeonbuk National University (Korea) and Trilogene (Boulder, CO, USA), respectively. Cannabinoids including THCA and CBDA were extracted from the leaves of four-week-old plants grown from cuttings. The 10-week-old flowers after short-day treatments were also extracted. Fresh leaves and flowers were dried at 55 °C for 24 hours. The hemp plants and three other species (sunflower, *Helianthus annuus*; kenaf, *Hibiscus cannabinus*; *Angelica gigas*) were grown in the same glass greenhouse. The growth period was from 4 April to 6 July, 2023. The light cycle and temperature followed natural conditions. For short-day conditions, a controlled shading net was applied to the greenhouse to give a 12 h light and dark cycle.

2.2. Extraction of cannabinoids for HPLC analysis and application to the THC diagnostic devices

TissueLyserII mill (Qiagen, Hilden, Germany) using stainless beads and a 2 mL tube at a frequency of 30 Hz for 2 min was used to extract approximately 100 mg of each fresh leaf sample for devices. A mortar and pestle was used to grind other leaf samples. Approximately 100 – 200 mg ground fresh leaf or flower was mixed with 1 mL of water, methanol, and water-methanol mixture for application of diagnostic devices. After 15 min, the extract was centrifuged at 3000 rpm (approximately 800 *g*) for 10 s, then 200 μL of the supernatant (no filtering) was transferred to the sample hole of each of two diagnostic devices.

For HPLC analysis using a system supplied by CTInstruments (Calgary, Canada), approximately 200 mg each replicate of a dry leaf or flower sample was mixed with 15 mL of methanol for 15 min, then 50 μL of the extract (no filtering) was mixed with 1.4 mL of pure acetonitrile and 70 μL of this was injected into the HPLC instrument. Each water extract with approximately 200 mg fresh leaf for HPLC analysis was prepared using 15 mL of water instead of methanol at above mention. Methodological comparison of extraction between diagnostic device and HPLC analysis was introduced in the S Table 4.

2.3. THC diagnostic devices

Two devices for screening human urine for drugs were used. The Abon device (Hangzhou, China) detection cut-offs were 50 ng mL^{-1} for 11-nor-Δ9-THC-COOH, 30 ng mL^{-1} for 11-nor-Δ8-THCA, 20 μg mL^{-1} for cannabinol or THCA, 15 μg mL^{-1} for Δ8-THC, and 15 μg mL^{-1} for Δ9-THC. The Asan-Pharm device (Hwaseong-si, Korea) detection cut-off for 11-nor-Δ9-THC-COOH was 50 ng mL^{-1}, and the cut-offs for other THC metabolites were not specified. The development time for each device was 5 min. Both devices were immunoassays based on competitive binding to antibodies. THCA in a leaf extract would compete with the respective THCA conjugate to bind to the relevant antibody. Positive and negative results were assessed following the instructions provided by the supplier. Each test using a diagnostic device was performed in triplicate.

2.4. HPLC running condition

The HPLC column (C18, 5 μm particle size, 150 × 4.6 mm, CTInstruments) was kept at 30 °C, the mobile phase was acetonitrile containing 20 % formate buffer (CTInstruments, Canada) and the flow rate was 1.2 mL min^{-1}. The wavelength of UV detector was 220 nm. Individual cannabinoid compounds were identified and determined by comparison with CBDA, CBD, THCA, and THC standard peaks supplied by manufacture. The HPLC software was used to determine the concentration from (in % w/w) the peak heights (Pourseyed Lazarjani et al., 2020). Each sample was analyzed three or four times. For building of calibration curves, the certified reference materials, phytocannabinoid mixture 11 (250 μg mL^{-1}, Cayman Chmical, USA), THC (100 μg mL^{-1}, Absolute Standards Inc., USA), cannabicyclol (100 μg mL^{-1}, Absolute Standards Inc., USA), THCVA-A (1 mg mL^{-1}, Cayman Chmical, USA), and benzoic acid (1 mg mL^{-1}, Absolute Standards Inc., USA) were used. Each calibration curves were performed by a 6 point and the minimum acceptable R2 was 0.9980 (provided by manufacturer). For HPLC validation, benzoic acid under the above instrument condition were used to maintain retention time and peak height at less than 5 % of total content. The low limit of detection in HPLC was 0.03 (w/w%).

2.5. Preparation of THCA and THC standards and loading onto the diagnostic devices

Standards of THCA (1 mg mL^{-1}) and THC (50 mg mL^{-1}) in acetonitrile were purchased from the Cayman Chemical Company (Ann Arbor, MI, USA). Standards were diluted by a factor of 1000 in acetonitrile. The final desired concentration was achieved by diluting a prediluted standard with water. The loading volume for a diagnostic device was 200 μL, and each standard was tested using three devices.

3. Results and discussion

Organic solvents such as methanol and its mixtures are normally used in the extraction of cannabinoids from cannabis tissues (Pourseyed Lazarjani et al., 2020; Rožanc et al., 2021). However, THC diagnostic devices are optimized for aqueous solutions. We performed tests to determine whether methanol in extracts of cannabis plant tissues with low THC contents affected the performances of the THC diagnostic devices. Cannabinoids were extracted from fresh Spectrum303 leaves using water: methanol mixtures containing between 0 % and 100 % methanol in 10 % steps. For the Abon device, the test (T) line was less intense for 100 %, 90 %, and 80 % water than for the negative control (S Fig. 1A 1–3 and Table 1). In addition, the T line was less intense for the ground leaf samples than for the negative control using the Abon device (S Fig. 1A 13). However, the T lines for 70–40 % water extracts were of similar intensities to or were more intense than the negative control T line (S Fig. 1A 4–7 and Table 1). Unfortunately, the devices did not work when the methanol concentration was >70 % (S Fig. 1A 8–11 and Table 1). SuperwomanS1 leaves were also tested to determine whether similar results to the Spectrum303 results were found. Fresh and dry SuperwomanS1 leaves gave faint T lines compared with the negative control (S Fig. 1B and Table 1). No T line was found for fresh or dry Spectrum303 flowers that had much higher THCA contents than the leaves (S Fig. 1C and Table 1). This indicated that the Abon diagnostic device for testing urine could be used to confirm that cannabis leaves had low THCA contents.

The cannabinoid extracts of the Spectrum303 samples were also tested using the Asan-Pharm device. The T line was less intense for the 100 %, 90 %, 80 %, and 40 % water extracts than the negative control (S Fig. 2 A 1–3 and 7 and Table 1). The T line was also less intense for the ground leaf samples than for negative control using the Asan-Pharm device (S Fig. 2 A 13). However, the T lines for 70–50 % water extracts were of similar intensities to or were more intense than the negative control T line (S Fig. 2 A 4–6 and Table 1). As expected, the devices did not work when the methanol concentration was >70 % (S Fig. 2 A 8–11 and Table 1). These results indicated that <20 % methanol should be used to extract cannabinoids from cannabis plant tissues for testing using both devices. Like for the Abon device, the T line intensities were similar for the Spectrum303 and SuperwomanS1 leaf extracts tested using the Asan-Pharm device (S Fig. 2B and Table1). No T line was found when Spectrum303 flower extracts were tested using the Asan-Pharm device (S Fig. 2 C and Table 1). However, the T line for the negative control was less intense for the Asan-Pharm device than the Abon device. We concluded that the Abon device performed better than the Asan-Pharm device. This conclusion was supported by the results of tests using sunflower, kenaf, and Angelica leaf extracts, for which the Abon device clearly performed the same as for the negative control (S Fig. 3). In contrast, the T lines were less intense for the sunflower, kenaf, and Angelica leaf extracts than the negative control when the Asan-Pharm device was used (S Fig. 3) even though the sunflower, kenaf, and Angelica leaves did not contain THCA. We speculated that the crude leaf extracts contained compounds that affected the Asan-Pharm device performance.

The diagnostic devices we used are suitable for testing THC metabolites in human urine. The detection limits may be different for natural THCA and THC in plant extracts. To assess this, the THCA contents of fresh and dry leaves were determined by HPLC. THCA and THC standards were also directly applied to the Abon diagnostic device. The mean THCA contents of the Spectrum303 and SuperwomanS1 leaves determined by analyzing methanol extracts by HPLC were 0.10 % w/w and 0.08 % w/w for dry leaves, respectively, and 0.12 % w/w and 0.09 % w/w for fresh leaves, respectively (Tables 2 and S1). The mean CBDA contents of the Spectrum303 and SuperwomanS1 leaves determined by analyzing methanol extracts by HPLC were 3.46 % w/w and 2.60 % w/w for dry leaves, respectively, and 2.69 % w/w and 1.73 % w/w for fresh leaves, respectively (Table 2). Only traces of THC were detected (Fig. 1). Only CBDA was detected when water extracts were analyzed by HPLC, and the contents of Spectrum303 and SuperwomanS1 leaves were found to be 0.12 % w/w and 0.05 % w/w for dry leaves, respectively, and 0.47 % w/w and 0.21 % w/w for fresh leaves, respectively (Tables 2 and S1). These results indicated that the concentrations were 7–12 times lower in the water extracts than the methanol extracts (Tables 2 and S1). Extracting dry leaves and flowers with water is not a valid method because water will inefficiently penetrate dry plant material in the restricted extraction time (Table S1).

The theoretical THCA contents of the fresh leaves can be calculated from the concentrations in the methanol extracts determined by HPLC. A 0.1 g aliquot of SuperwomanS1 fresh leaves was found to contain 90 μg of THCA and a 0.1 g aliquot of Spectrum303 fresh leaves was found to contain 120 μg of THCA. However, the actual concentrations found in the water extracts were factors of 7.26 and 12.03 lower for the for Spectrum303 and SuperwomanS1 leaves, respectively, the estimated concentrations being 7.5 and 16.7 μg mL^{-1} for SuperwomanS1 and Spectrum303, respectively (Tables 3 and S1). THCA and THC standards were tested using the diagnostic devices to test the actual performances of the devices. The T line became less intense when the 5 and 10 μg mL^{-1} THCA standards were tested and no T line appeared when the 20 μg mL^{-1} THCA standard was tested (Table S2). The T line started to become less intense when the 300 ng mL^{-1} THC standard was tested and no T line appeared when the 1 μg mL^{-1} THC standard was tested (Table S3). The Δ9-THC contents of the leaves of both cultivars were too low to be detected by HPLC (Fig. 1), but using a diagnostic device may be useful for detecting THC if cannabis leaves contain 300 ng mL^{-1} or

Table 1
Estimated tetrahydrocannabinol (THC) results for tests of cannabis plant leaf samples using the diagnostic devices. Data from S Fig. 1 and 2 are summarized. Asterisks indicate the T line intensity compared with the negative control: +, positive; -, negative; x, control C line destroyed; THCA, tetrahydrocannabinolic acid.

Detection of THCA compounds in Spectrum303 using the two devices.				
Cut-off value: 20 μg mL^{-1} for cannabinol				
No.	Percentages of mixtures		Result	
	Water	Methanol	Abon device	AsanPham device
1	100	0	+, ***	+, disappear
2	90	10	+, **	+, disappear
3	80	20	+, ***	+, ***
4	70	30	+, *	+, *
5	60	40	+, *	-
6	50	50	-	-
7	40	60	x	+,**
8	30	70	x	x
9	20	80	x	x
10	10	90	x	x
11	0	100	x	x
12	Negative control (water loading)		-	-
13	Ground with water		+, ***	+, disappear
14	Spectrum303 fresh flowers (100 % water)		+,disappear	+, ***
15	SuperwomanS1 fresh leaves (100 % water)		+, ***	+, disappear
16	SuperwomanS1 dry leaves (100 % water)		+, ***	+,**

Table 2
Cannabinoid contents of leaves and flowers and diagnostic test results. A. Spectrum303 cannabinoids. B. SuperwomanS1 cannabinoids. C. Diagnostic tests using standards and fresh tissue samples. X means the estimated tetrahydrocannabinolic acid (THCA) concentration. Each value is the mean w/w% ± standard deviation. THC, tetrahydrocannabinol; CBDA, cannabidiolic acid.

A

Tissue	Pretreatment	Average concentration determined by HPLC (w/w%), Spectrum303			
		Methanol extraction		Water extraction	
		THCA	CBDA	THCA	CBDA
Leaves	Fresh	0.12 ± 0.004	3.46 ± 0.166	trace	0.47 ± 0.012
	Dry	0.10 ± 0.000	2.69 ± 0.073	trace	0.12 ± 0.008
Flowers	Fresh	-	-	-	-
	Dry	0.21 ± 0.014	7.07 ± 0.340	trace	0.20 ± 0.036

B

Tissue	Pretreatment	Average concentration determined by HPLC (w/w%), SuperwomanS1			
		Methanol extraction		Water extraction	
		THCA	CBDA	THCA	CBDA
Leaves	Fresh	0.09 ± 0.000	2.60 ± 0.150	trace	0.21 ± 0.000
	Dry	0.08 ± 0.012	1.73 ± 0.023	trace	0.05 ± 0.004
Flowers	Fresh	-	-	-	-
	Dry	0.39 ± 0.018	12.78 ± 0.403	trace	0.59 ± 0.089

C

Detection limit in a diagnostic devices using cannabinoid standards (w/w%)				Tests in diagnostic device: Extracts (estimated Conc.)	
THC		THCA		in leaves	in flowers
1 μg mL^{-1} (0.0001)		20 μg mL^{-1} (0.002)		(0 < X < 20 μg mL^{-1})	(X > 20 μg mL^{-1})

more Δ9-THC.

The results described above indicated that the THCA and THC contents of fresh cannabis plant tissues can be estimated using THC diagnostic devices. For example, if the T line was less intense for an extract of 0.1 g of fresh leaves than the negative control, the expected THCA and THC contents X^e would be $0\ \mu g\ mL^{-1} < X^e < 20\ \mu g\ mL^{-1}$ and $0\ \mu g\ mL^{-1} < X^e < 1\ \mu g\ mL^{-1}$, respectively (Table 2C). These values were used to calculate the w/w% using an extraction factor of 10 (i.e., approximately 10 times less being extracted when testing fresh leaves using water extraction than when using methanol extraction). The THCA content would therefore be <0.02 % (200 μg mL^{-1}) and the THC content would be <0.001 % (10 μg mL^{-1}). If the fresh sample weight varies, differences between cultivars can be tested using the diagnostic devices. The expected THCA and THC contents of 0.2 g of Spectrum303 leaves (33.3 μg mL^{-1}) would not give a T line but the expected THCA and THC contents of 0.2 g of SuperwomanS1 leaves (15 μg mL^{-1}) would give a faint band (Table 3). The THCA content was approximately twice as high for Spectrum303 flowers as Spectrum303 leaves, so the expected THCA content of leaves could be doubled to estimate the THCA content of the flowers (Table 3).

4. Conclusions

The results give useful information about how THC diagnostic devices can be used to test cannabis plant tissue extracts. The results of the HPLC analyses and diagnostic device tests of standards and leaves indicated that the detection limits of the diagnostic devices for THCA and THC were 20 μg mL^{-1} (0.002 % w/w) and 1 μg mL^{-1} (0.0001 % w/w), respectively. The diagnostic devices could be used to screen cannabis cultivar tissues to determine whether the tissues have low or high THCA and THC contents. Cannabis growers could easily test the THC contents of their crops using the diagnostic devices.

Funding

This research was supported by the National Research Council of Science & Technology (NST) grant by the Korea government (MSIT) (No. CAP23051–000).

CRediT authorship contribution statement

Sang Hoon Kim: Writing – review & editing, Supervision, Conceptualization. **Dong-Gun Kim:** Writing – review & editing, Investigation,

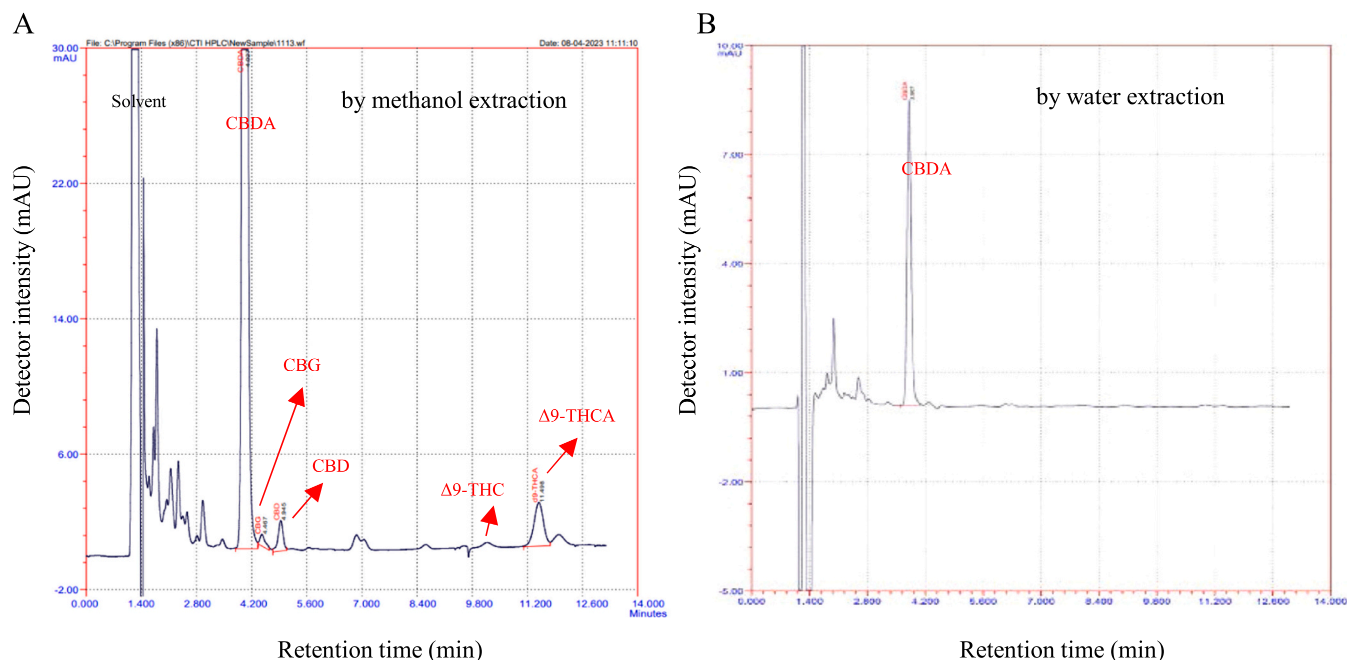


Fig. 1. Examples of high-performance liquid chromatograms of cannabinoids in leaf extracts. A. Cannabinoids in a methanol extract of fresh leaves. B. Cannabinoids in a water extract of fresh leaves. The extracts were diluted with acetonitrile and analyzed using a mobile phase of 20 % formic buffer in acetonitrile. The Y-axis is the UV photometer detector output intensity and the X-axis is the retention time (13.2 min for a complete analysis). The compounds of interest are marked in red. Only a trace of tetrahydrocannabinol (THC) was detected.

Table 3
Expected responses of the diagnostic devices. EF, extraction efficiency; THCA, tetrahydrocannabinolic acid; HPLC, high performance liquid chromatography; C line, control line; T line, test line.

Cultivar	Leaves (g)	THCA content of leaves determined by HPLC (w/w%)	THCA content of flowers determined by HPLC (w/w%)	Expected THCA content of leaves calculated from concentration in water extracts	Expected response of diagnostic device
Spectrum 303	0.1	0.12	0.21	1. 0.0012 g/g × 0.1 g = 0.00012 g 2. 120 μg / 7.2 (EF) = 16.7 μg 3. ∴ 16.7 μg / mL (at device)	C line T line
	0.2	0.12	0.21	1. 0.0012 g/g × 0.2g = 0.00024 g 2. 240 μg / 7.2 (EF) = 33.3 μg 3. ∴ 33.3 μg / mL (at device)	C line T line
Super womanS1	0.1	0.09	0.39	1. 0.0009 g/g × 0.1 g = 0.00009 g 2. 90 μg / 12 (EF) = 7.5 μg 3. ∴ 7.5 μg / mL (at device)	C line T line
	0.2	0.09	0.39	1. 0.0009 g/g × 0.2 g = 0.00018 g 2. 180 μg / 12 (EF) = 15 μg 3. ∴ 15 μg / mL (at device)	C line T line

Formal analysis. **Jin-Baek Kim:** Writing – review & editing, Conceptualization. **Juyoung Kim:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Nam Min Woo:** Resources, Methodology. **Jin Jong Sik:** Resources, Methodology. **Jaihyuk Ryu:** Writing – review & editing, Methodology. **Ye-Jin Lee:** Writing – review & editing, Resources.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

Acknowledgements

We thank Duk Man Lee and Min Soo Park for technical support cultivating the hemp plants.

Use of Generative AI

We did not use a Generative AI to prepare this work.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.indcrop.2024.119007.

References

Breidi, S.E., Barker, J., Petróczi, A., Naughton, D.P., 2012. Enzymatic digestion and selective quantification of underivatised delta-9-tetrahydrocannabinol and cocaine in human hair using gas chromatography-mass spectrometry. J. Anal. Methods Chem. https://doi.org/10.1155/2012/907893.

Centers for Desease Control and Prevention. Urine Testing for Detection of Marijuana: An Advisory. MMWR weekly. 1983. ⟨https://www.cdc.gov/mmwr/preview/mmwrhtml/00000138.htm⟩.

Crocq, M.A., 2020. History of cannabis and the endocannabinoid system. Dialog-. Clin. Neurosci. https://doi.org/10.31887/DCNS.2020.22.3/mcrocq.

Liu, Y., Zhu, P., Cai, S., Haughn, G., Page, J.E., 2021. Three novel transcription factors involved in cannabinoid biosynthesis in Cannabis sativa L. Plant Mol. Biol. https://doi.org/10.1007/s11103-021-01129-9.

Matchett-Oates, L., Spangenberg, G.C., Cogan, N.O.I., 2021. Manipulation of Cannabinoid Biosynthesis via Transient RNAi Expression. Front Plant Sci. https://doi.org/10.3389/fpls.2021.773474.

Mechoulam, R., Ben-Shabat, S., 1999. From gan-zi-gun-nu to anandamide and 2-arachidonoylglycerol: the ongoing story of cannabis. Nat. Prod. Rep. https://doi.org/10.1039/a703973e.

National Academies of Sciences, Engineering, and Medicine. The Health Effects of Cannabis and Cannabinoids: The Current State of Evidence and Recommendations for Research. Washington, DC: The National Academies Press. 2017; https://doi.org/10.17226/24625.

Petruzzello M. "What Is the Difference Between Hemp and Marijuana?". *Encyclopedia Britannica*. 2022. ⟨http://www.britannica.com/story/what-is-the-diffenence-between-hemp-and-marijuana⟩.

Pourseyed Lazarjani, M., Torres, S., Hooker, T., Fowlie, C., Young, O., Seyfoddin, A., 2020. Methods for quantification of cannabinoids: a narrative review. J. Cannabis Res. https://doi.org/10.1186/s42238-020-00040-2.

Rožanc, J., Kotnik, P., Milojević, M., Gradišnik, L., Hrnčič, M.K., Knez, Ž., Maver, U., 2021. Different *Cannabis sativa* extraction methods result in different biological activities against a colon cancer cell line and healthy colon cells. Plants. https://doi.org/10.3390/plants10030566.

Sands, L.B., Haiden, S.R., Ma, Y., et al., 2023. Hormonal control of promoter activities of Cannabis sativa prenyltransferase 1 and 4 and salicylic acid mediated regulation of cannabinoid biosynthesis. Sci. Rep. https://doi.org/10.1038/s41598-023-35303-4.

Taura, F., Sirikantaramas, S., Shoyama, Y., Yoshikai, K., Shoyama, Y., Morimoto, S., 2007. Cannabidiolic-acid synthase, the chemotype-determining enzyme in the fiber-type Cannabis sativa. FEBS Lett. https://doi.org/10.1016/j.febslet.2007.05.043.

Taura, F., Tanaya, R., Sirikantaramas, S., 2019. Recent advances in cannabinoid biochemistry and biotechnology. ScienceAsia. https://doi.org/10.2306/scienceasia1513-1874.2019.45.399.

The U.S. Department of Agriculture (USDA). Establishment of a Domestic Hemp Production Program. ⟨http://ams.usda.gov/rules-regulations/establishment-domestic-hemp-production-program⟩.

The U.S. Department of Agriculture (USDA). Section 10113 of the Agriculture Improvement Act of 2018 (2018 Farm Bill). http://congress.gov/bill/115th-congress/house-bill/5485.

Verstraete, A.G., 2004. Detection times of drugs of abuse in blood, urine, and oral fluid. Ther. Drug Monit. https://doi.org/10.1097/00007691-200404000-00020.

Yu, H., Lee, H., Cheong, J., et al., 2021. A rapid assay provides on-site quantification of tetrahydrocannabinol in oral fluid. Sci. Transl. Med. https://doi.org/10.1126/scitranslmed.abe2352.

Zirpel, B., Degenhardt, F., Zammarelli, C., et al., 2018. Optimization of Δ9-tetrahydrocannabinolic acid synthase production in Komagataella phaffii via post-translational bottleneck identification. J. Biotechnol. https://doi.org/10.1016/j.jbiotec.2018.03.008.