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ORIGINAL

Quantification of Cannabinoids in Cultivars of *Cannabis sp*. by Gas Chromatography–Mass Spectrometry

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Abstract

In the chemical characterization of medically valued *Cannabis*, the present work has used a gas chromatography (GC) method coupled with mass spectrometry (MS) for identification and quantification of cannabinoids. We have modified a GC–MS method for chemical analysis of cannabinoids extracted from dried flowers of different *Cannabis* varieties. The method allows for quantification of major cannabinoids, i.e. cannabidiol (CBD), cannabichromene (CBC), tetrahydrocannabinol (THC), cannabigerol (CBG), and cannabinol (CBN) simultaneously. We found that the *Cannabis* cultivar called *Cannabis* 5-CW had the highest amount of CBD. We used a modified GC–MS method for chemical profiling of cannabinoids extracted from a variety of *Cannabis* samples, especially the high CBD, CBC, THC, CBG and CBN-producing ones. The method was successfully applied for identification and quantification of cannabinoids in a short time with better separation resolution among the reported methods.

Keywords *Cannabis* · Chemical analysis · Cannabinoids · Medicinal plants · CBD · THC

Introduction

Cannabis has been used for medical and recreational purposes for thousands of years. Medical *Cannabis* is legally available for patients in a number of countries [27]. The science of *Cannabis* is rapidly developing and recent evidence supports its therapeutic applications [5]. A number of studies described the biological potential of *Cannabis* for the treatment of pain, glaucoma, nausea, asthma, depression, insomnia and neuralgia [30], multiple sclerosis [33], together with inflammatory diseases [11, 16], epilepsy [12], and movement disorders [36]. *Cannabis* is a chemically rich plant of unparalleled versatility exhibiting a unique variety of natural compounds [40]. It contains over 500 chemical entities of which more than 100 are cannabinoid compounds [14]. Cannabinoids are a group of compounds bearing a C21 terpenophenolic skeleton, generate the medically important chemicals of the *Cannabis* plant. These compounds have been previously analyzed by LC-UV [1, 3], LC–MS [6, 41], GC-FID [18, 23, 35], GC–MS [2, 4, 8, 10, 15, 21, 34] and SFC-MS [40].

This work presents a GC–MS method which was modified from the method by Cardenia et al. [10] because this 2018 method was quick in identification of cannabinoids over the other methods. The modified method, which provides a fast detection of cannabinoids from a variety of samples, allows us to verify the presence of a high-CBD producing *Cannabis* sample.

Materials and Methods

Standards and Reagents

Cannabinoid standards such as cannabidiol (CBD), cannabichromene (CBC), tetrahydrocannabinol (THC), cannabigerol (CBG), cannabinol (CBN), cannabicyclol (CBL) in 1 mg/mL, as well as CBD-d3 and THC-d3 in 100 microgram/mL, were purchased from Cerilliant Corporation (Round Rock, TX) as drug enforcement agency-exempt solutions, in methanol (MeOH). The chemical structures of the cannabinoids are shown in Fig. 1. All other chemicals and solvents used were of analytical grade.

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Gas Chromatography (GC–MS) Analysis

The cannabinoids were identified by GC–MS. The GC (Agilent 6890 series) was equipped with a HP-5MS column (30 m × 0.25 mm, 0.25 µm film thickness); hydrogen was used as the carrier gas (constant flow of 1.6 mL/min). The oven temperature was programmed from 180 °C (for 0.50 s) to 250 °C at 5 °C/min, and then to 325 °C (at 30 °C/min); final temperature was maintained for 2 min. One µL of sample was injected using autosampler and the injector port temperature was set to 280 °C. The MS (model 5973 N) used electron impact ionization and transmission quadrupole mass spectrometer. For quantification, MS data were obtained using the selected ion monitoring (SIM) method based on fragment ions (in *m/z*) for CBL (231), CBD (246), CBC (231), THC (193), CBG (193), and CBN (295).

Cannabis Plant Extraction

Dried flowers of different *Cannabis* varieties were obtained from Medcan Biotechnologies Inc. (Vancouver, BC, Canada). Flowers were grounded using the mortar and pestle, and samples of 200 mg were accurately weighed. For extraction, the samples were suspended in 2 mL methanol, followed by vortexing, sonication for 10–20 min and centrifugation at 4,000 rpm for 5 min. The supernatants were transferred to a 10-mL glass vial. The extraction procedure was repeated two more times and the respective supernatants were combined. Thereafter, supernatants were filtered by passing through a 0.22-µm sterile syringe filter. Extracts were dried under a gentle stream of nitrogen gas. Dried extracts (with weights) obtained were *C. sativa* (92.2 mg), *C. indica* (35.1 mg), Cannalope Kush (72.5 mg), *Cannabis* 5-CW (56.3 mg), Rock Star (74.2 mg) and Super Silver (66.8 mg). Dried extracts were reconstituted with 200 µL MeOH for GC–MS analysis.

Standard Solutions of Cannabinoids

Stock solutions of individual standards and internal standards were prepared separately at concentration of 100 µg/mL in methanol. A standard mixture of the cannabinoid standards and internal standard (100 µg/mL) was also prepared.

Method Validation

GC–MS method was validated for specificity (selectivity), precision (repeatability and intermediate precision), limit of detection (LOD), limit of quantification (LOQ) and recovery. Specificity (selectivity) was determined by injecting a solvent blank to confirm that there were no false signal peaks at the targeted retention times. Statistical analysis was applied to the linear regression lines to determine the standard deviation (SD) and slope (S). From these values, LOD and LOQ were calculated using the equations (LOD = 3 × SD/S; LOQ = 10 × SD/S). Recoveries were determined by the method described previously [17].

Spiking of *Cannabis* Extracts with Cannabinoid Standards

Cannabinoids were quantified using internal standard and standard addition methods. For standard addition, extracts of *C. indica*, *C. sativa*, Cannalope Kush, *Cannabis* 5-CW, Rock Star and Super Silver (14 mg/mL) were spiked with cannabinoid standards i.e. CBC, CBG, and CBN (50 µg/mL and 100 µg/mL), and CBD-d3 (50 µg/mL). For THC quantification, *C. indica*, *C. sativa*, Cannalope Kush, *Cannabis* 5-CW, Rock Star, Super Silver extracts (14 mg/mL) were spiked with THC-d3 (50 µg/mL). For CBD quantification, *C. indica*, *C. sativa*, Cannalope Kush, Rock Star, Super Silver extracts (14 mg/mL) and *Cannabis* 5-CW extract (0.2815 mg/mL) were spiked with CBD-d3 (50 µg/mL). CBL was below

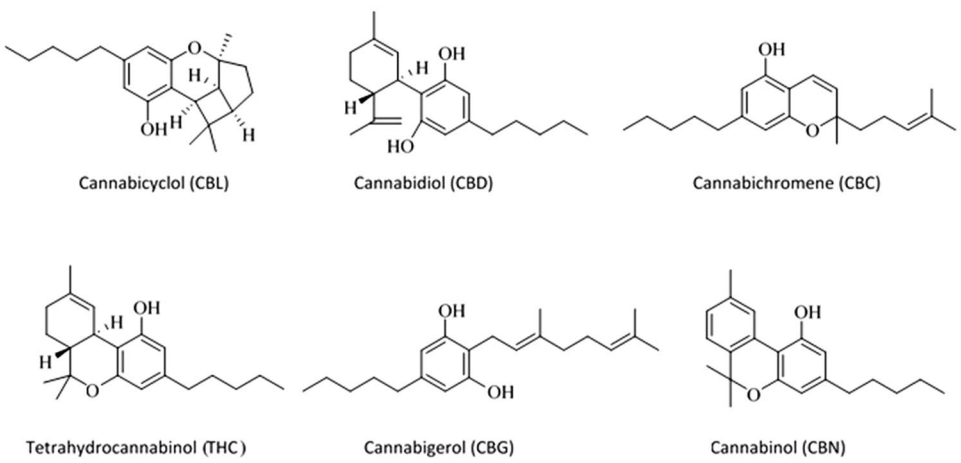

Fig. 1 Chemical structures of compounds in *Cannabis*

the detection level and so it was not quantified. MS data were obtained using selected ion monitoring (SIM). The determined amounts of cannabinoids were expressed as a percentage of the dry weights of *Cannabis* samples.

Results and Discussion

The goal of the present work was to separate, identify, and quantify various cannabinoids from different *Cannabis* varieties using a GC–MS method. Cannabinoids were identified by comparing their mass spectra with an online compound database and the retention times with those of the corresponding standard compounds. Our method provided faster separations of CBD, THC, CBG, and CBC (within 13 min) than the methods reported by Richins et al. [34] (24 min), Leghissa et al. (18 min) [26], Mariotti et al. (20.5 min) [29], Cadola et al. [8] (19 min), and and Hillig and Mahlberg [21] (26.5 min). Moreover, CBD and CBC were baseline-separated (resolution of 1.5), which was better than in methods previously published [21], and [24, 29] (resolution less than 1), and the resolution for CBG–CBN peaks was (~ 1.3).

Identification of Cannabinoids by GC–MS

Gas chromatography-mass spectrometry (GC–MS) is a useful approach for identification and quantification of cannabinoids. For identification, individual cannabinoids were run on GC–MS, and mass spectra were obtained and compared with compound database search data, and published literature (Figs. 2, 3 and 4). The identified compounds were CBL, CBD, CBC, THC, CBG, and CBN (Figs. 2, 3). Mass spectra data showed the base and parent ion peaks for cannabinoids, CBL, CBD, CBC (*m/z* 231, 314), THC (*m/z* 299, 314), CBG (*m/z* 193, 316), and CBN (*m/z* 295, 310) (Fig. 3a–f). Typical chromatograms of deuterated CBD (m/z 231, 234) and THC (m/z 193, 196) showing the natural abundance of ions are represented in (Fig. 5). These target cannabinoids were analyzed from six samples: *C. sativa*, *C. indica*, Cannalope Kush, *Cannabis* 5-CW, Super Silver and Rock Star. CBD, CBC, THC, CBG, and CBN were identified from tested *Cannabis* samples. Target compounds were identified in the elution order of (i) CBD ($C_{21}H_{30}O_{2;}$ molecular mass 314.469 g/mol), (ii) CBC ($C_{21}H_{30}O_{2;}$ molecular mass 314.469 g/mol), (iii) THC ($C_{21}H_{30}O_{2;}$ molecular mass 314.469 g/mol), (iv) CBG ($C_{21}H_{32}O_{2;}$ molecular mass 316.485 g/mol), and (v) CBN ($C_{21}H_{26}O_{2;}$ molecular mass 310.4319 g/mol). CBD is abundant and the major compound of *Cannabis* 5-CW; whereas THC is the major compound in the rest of the samples. Five different cannabinoids (CBD, CBC, THC, CBG, and CBN) were analyzed simultaneously in our method, but other studies have focused on different cannabinoids using GC–MS and GC-FID techniques. Literature revealed that researchers published their work on cannabinoids and terpenes but excluded one or more cannabinoids that were analyzed in the current method. For instance, the recent work by Pellati et al. [31] analyzed cannabinoids and

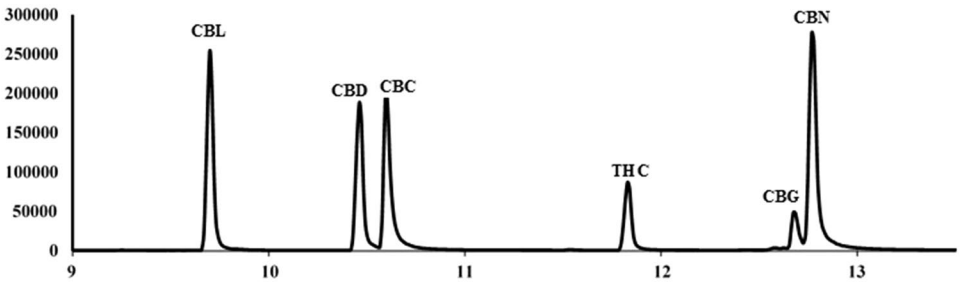

Fig. 2 GC–MS chromatogram of cannabinoid standards. SIM data were obtained for CBD (231), CBC (231), THC (193), CBG (193), and CBN (295). The *x*-axis represents time in minutes, and *y*-axis represents ion abundance

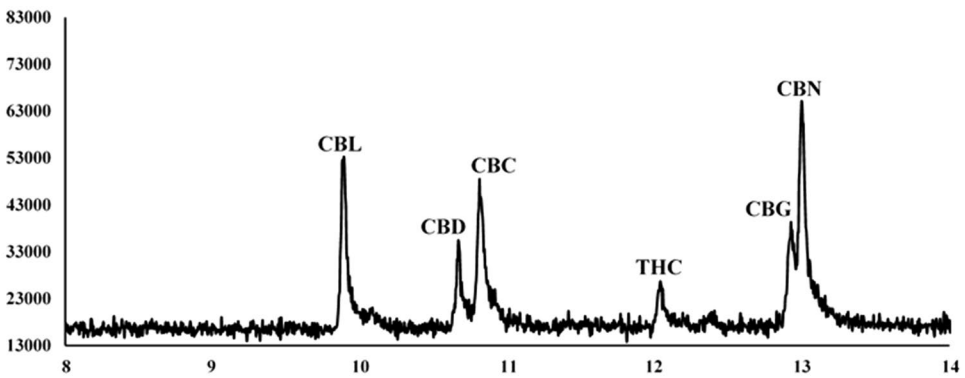

Fig. 3 *Cannabis* 5-CW extract spiked with standard compounds (CBL, CBC, CBG, CBN). CBD and THC were not spiked. The *x*-axis represents time in minutes, and *y*-axis represents ion abundance

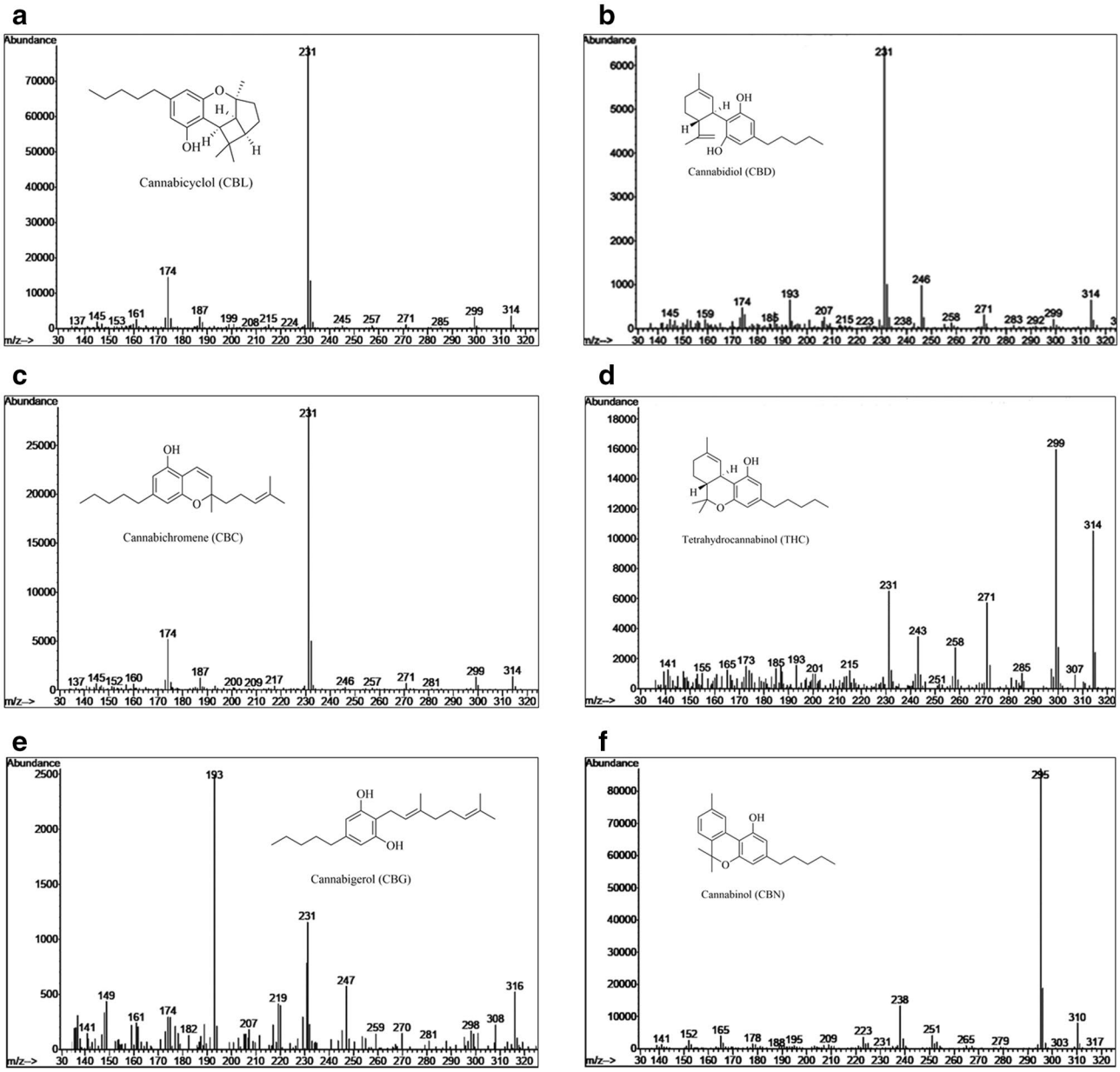

Fig. 4 **a** Mass spectra of cannabicyclol (CBL). **b** Mass spectra of cannabidiol (CBD). **c** Mass spectra of cannabichromene (CBC). **d** Mass spectra of Tetrahydrocannabinol (THC). **e** Mass spectra of cannabigerol (CBG). **f** Mass spectra of cannabinol (CBN)

terpenes but they excluded THC, CBC and CBN. Similarly, some previous reports [7, 39], and [37, 38] only analyzed CBD, THC, CBN, but not CBC and CBG.

Method Validation of Cannabinoids

The linear ranges of all cannabinoids were determined over the concentrations of 0.0125 to 0.1 mg/ml. Linearity was sufficient for all cannabinoids, with regression coefficients (> 0.98). Results of GC method validation were determined for intraday precision the percent relative standard deviation (RSD) of cannabinoids (1.49–8.35%). Intermediate precisions of cannabinoids were determined from intraday and interday analyses. All compounds had RSD ranges of 1.32–5.14%. These low RSD values indicate that the method is reproducible for the analysis of *Cannabis* extracted cannabinoids. In terms of detection and quantitation limits, LOD ranged between 0.006 and 0.008 mg/ml, while the LOQ varied between 0.018 and 0.026 mg/ml.

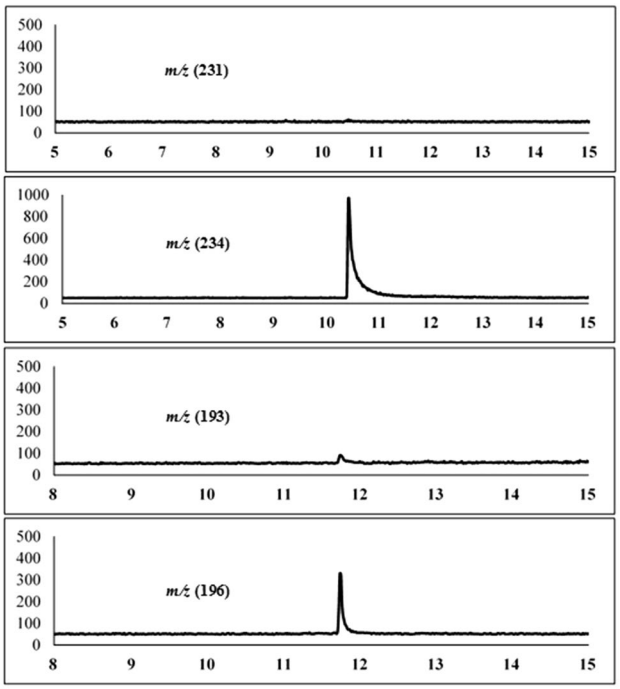

Fig. 5 GC–MS chromatograms of isotope standards, CBD-d3 at *m/z* 231 and 234, THC-d3 at *m/z* 193 and 196. The *x*-axis represents time in minutes, and y-axis represents ion abundance

Recovery

The recoveries of cannabinoids were determined to range between 80 and 100%. All the recoveries were good and consistent with the literature values [20, 25, 26]. Moreover, Calzolari et al. [9] explained that methanol is an excellent solvent for extraction of cannabinoids and flavonoids. The *Cannabis* monograph of the American Herbal Pharmacopoeia also indicated this solvent for preparation of standard solutions of cannabinoids [28].

Quantification of Cannabinoids from Cannabis Samples

The method described herein was successfully applied for quantification of cannabinoids from different *Cannabis* samples. Phytocannabinoids were quantified by standard addition and internal standard methods. THC and CBD were quantified using isotope standards: THC-d3 and CBD-d3, respectively. On the other hand, CBC, CBG and CBN were quantified by standard addition method. CBL was below the detection limit in all samples, and so their amounts should be less than 0.02% dry weight. Table 1 showed the quantitation data of cannabinoids extracted from different *Cannabis* samples. As listed in Table 1, *Cannabis* 5-CW showed the highest CBD content (4.6%) and *C. indica* the lowest (0.077%). The THC was highest in *C. sativa* (7.96%) and lowest in *C. indica* (2.81%). The CBC levels were the highest in *Cannabis* 5-CW (4.93%) and the lowest in *C. indica* (0.34%). Interestingly, CBG was recorded the highest level for Cannalope Kush (7.58%) and the lowest for *Cannabis* 5-CW (0.55%). The highest amount of CBN was seen in the case of Rock Star (2.39%) and the lowest level was found in *C. indica* (0.33%). It becomes evident from Table 1 that Cannalope Kush, Super Silver are rich in CBG and Rock Star is rich in CBN, and so these three samples should be further tested for their pharmacological effects. Though other samples have high contents of either CBD or THC, none of those samples showed high concentrations of CBG and CBN.

In the literature, different *Cannabis* cultivars showed cannabinoid contents in the ranges of CBD (9.84–0.01%), THC (21.53–0.26%), CBC (0.62–0.03%), CBG (2.08–0.05%), and CBN (7.25–0.18%) [34, 40]. Chemical composition of *Cannabis* varieties depends upon several factors such as genetic variation, soil, climate, maturity of plants at harvest and storage conditions. Seasonal variations also affect the levels of CBN and THC in Indiana varieties of *Cannabis* [32]. Moreover, plant age, time of collection and geographic location are among the factors affecting chemical composition of *Cannabis* [22].

Conclusion

Herein we reported a modified GC–MS method for chemical profiling a variety of *Cannabis* samples, resulting in quantitation of various cannabinoids. Among the tested samples, CBD and THC were predominant constituents. *Cannabis*

Table 1 Cannabinoids contents (% dry weight of flowers) determined in *Cannabis* samples by GC–MS

Samples	GC–MS estimated cannabinoids (Mean ± SD)					
	% CBD	% THC	% CBC	% CBG	% CBN	% CBL
Cannabis sativa	0.254 ± 0.006	7.96 ± 0.43	2.13 ± 0.21	1.67 ± 0.38	2.02 ± 1.01	< 0.02
Cannabis indica	0.077 ± 0.004	2.81 ± 0.08	0.34 ± 0.02	0.73 ± 0.20	0.33 ± 0.11	< 0.02
Cannalope Kush	0.25 ± 0.02	6.40 ± 0.07	1.98 ± 0.37	7.58 ± 2.27	0.98 ± 0.19	< 0.02
Cannabis 5-CW	4.6 ± 0.2	3.82 ± 0.16	4.93 ± 1.57	0.55 ± 0.18	0.51 ± 0.23	< 0.02
Rock Star	0.142 ± 0.005	6.49 ± 0.10	0.83 ± 0.04	1.09 ± 0.10	2.39 ± 0.71	< 0.02
Super Silver	0.13 ± 0.01	5.68 ± 0.34	1.16 ± 0.29	3.76 ± 1.17	1.13 ± 0.08	< 0.02

5-CW exhibits the highest CBD level and therefore could be a promising cultivar from the medical perspective. On the other hand, *C. sativa* showed the high THC levels as compared to other *Cannabis* samples. In addition, Cannalope Kush, Super Silver are rich in CBG, and *C. sativa* and Rock Star contain high amounts of CBN. Results from this study help to understand the complex nature of *Cannabis* species and its chemotypes.

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Author contributions AQA performed the experimental work. AQA, PCL and DN analyzed the data. The manuscript was written through the contributions of all authors. All authors have given approval to the final version of the manuscript.

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Availability of Data and Material Not applicable.

Code Availability Not applicable.

Declarations

Conflict of interest DN is president of Medleaf Biotechnologies and other authors declare that they have no competing interests. The paper is the subject of a PCT application: CA2021050503.

Ethical Approval and Consent to Participate Not applicable.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

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