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Cannabis - from cultivar to chemovar

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The medicinal use of Cannabis is increasing as countries worldwide are setting up official programs to provide patients with access to safe sources of medicinal-grade Cannabis. An important question that remains to be answered is which of the many varieties of Cannabis should be made available for medicinal use. Drug varieties of Cannabis are commonly distinguished through the use of popular names, with a major distinction being made between Indica and Sativa types. Although more than 700 different cultivars have already been described, it is unclear whether such classification reflects any relevant differences in chemical composition. Some attempts have been made to classify Cannabis varieties based on chemical composition, but they have mainly been useful for forensic applications, distinguishing drug varieties, with high THC content, from the non-drug hemp varieties. The biologically active terpenoids have not been included in these approaches. For a clearer understanding of the medicinal properties of the Cannabis plant, a better classification system, based on a range of potentially active constituents, is needed. The cannabinoids and terpenoids, present in high concentrations in Cannabis flowers, are the main candidates. In this study, we compared cultivars obtained from multiple sources. Based on the analysis of 28 major compounds present in these samples, followed by principal component analysis (PCA) of the quantitative data, we were able to identify the Cannabis constituents that defined the samples into distinct chemovar groups. The study indicates the usefulness of a PCA approach for chemotaxonomic classification of Cannabis varieties.

Keywords: cannabis; cannabinoids; terpenoids; chemical profiling; cultivar; principle component analysis

Introduction

Cannabis as a medicine

Although there are an estimated 165 million frequent users of Cannabis worldwide,[1] it is presently unclear how many of these are medicinal users. Nevertheless, through persistent lobbying by patients as well as through mounting scientific evidence, the medicinal use of Cannabis is slowly gaining acceptance from authorities. Over the last decade, both the Netherlands [2] and Canada [3] have implemented state-run medicinal Cannabis programmes, and other countries are considering a similar move (Israel, Brazil) or are importing herbal material from the Dutch programme (Italy, Finland, Germany; pers. comm. Office of Medicinal Cannabis, the Netherlands).[4] In the USA, a growing number of states have adopted medical marijuana laws to provide safer access of Cannabis for medicinal use to patients, despite the fact that this is vehemently opposed by the Federal government.[5]

Besides obvious legal implications, Cannabis as an herbal medicine poses serious challenges to modern medicine, which operates according to the 'single compound, single target' paradigm of pharmacology. An obvious question therefore is how the chemical constituents found in Cannabis reflect different medicinal properties, and what types of Cannabis should consequently be made available to patients. In fact, the Canadian programme is currently under review, after increasing complaints from patients that the single variety of Cannabis that is currently available is not effective for a large proportion of patients.[6]

It is now widely accepted that Cannabis is monotypic and consists only of a single species *Cannabis sativa*, as described by Leonard Fuchs in the sixteenth century.[7,8] But as a result of centuries of breeding and selection, a large variation of cultivated varieties (cultivars) have been developed. These are commonly distinguished, by plant breeders, recreational users, and medical Cannabis patients alike, through the use of popular names such as White Widow, Northern Lights, Amnesia, or Haze. Already, over 700 different varieties have been described [9] and many more are thought to exist. However, it is unclear whether or not these names reflect any relevant differences in chemical composition.

Classification systems

Some attempts have been made to classify Cannabis varieties based on chemical composition. A first study was done by Grlic,[10] who recognized different ripening stages. Later, Fettermann [11] described different phenotypes based on quantitative differences in the content of main cannabinoids and he was the first to distinguish the drug- and fibre-type. Further extension of this approach was done by Small and Beckstead,[12] Turner,[13] and Brenneisen.[14] However, it was found that a single plant could be classified into different phenotypes, according to plant age. More recently, a classification system was developed by de Meijer,[15] who recognized five different Cannabis types based on the (relative) content of three major cannabinoids.

For forensic and legislative purposes, the most important classification of Cannabis types is that into the drug-type and the fibre-type (hemp). The main difference between these two is found in the content of the psychotropic component *delta*-9-tetrahydrocannabinol (THC): a high content of THC classifies as drug-type Cannabis, while a low content is found in fibre-type Cannabis (max. 0.2–0.3% THC on basis of dry matter in the upper reproductive part of the plants), which

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may also be cultivated for its seeds for human or animal consumption. The content of the closely related but psychotropically inactive cannabidiol (CBD) is not regulated by law, and its levels tend to be higher in Cannabis cultivated for seed or fibre. [16]

With almost no exception, Cannabis varieties presently used for medicinal purposes in official programs belong to the drug-type, because of their high content of the biologically active THC. But although fibre-type Cannabis is not commonly used for either medicinal or recreational purposes, it does contain components that were found to be biologically active such as CBD,[17] indicating that the distinction between the two types has limited relevance for medicinal research into Cannabis. Moreover, it is becoming increasingly clear that components in Cannabis beyond THC and CBD, such as other minor plant-cannabinoids and volatile secondary metabolites called terpenoids are involved in the drug's overall effect. [18]

All major terpenoids present in Cannabis (including e.g. myrcene, *alpha*-pinene, *beta*-caryophyllene) can be found ubiquitously in nature. [19] For this reason these components did not receive much scientific attention, until it was suggested that the terpenoid profile of Cannabis products may help in determining the origin of Cannabis in custom seizures.[20] However, no further reports on this approach were subsequently made in the scientific literature, most likely because of limited accuracy for forensic use.

The most common way currently used to classify Cannabis cultivars is through plant morphology (phenotype) with two types typically recognized: Sativa and Indica. Cannabis cultivars of the Indica type are smaller in height with broader leaves, while Sativa types are taller with long, thin-fingered leaves. [21,22] Indica plants typically mature faster than Sativa types under similar conditions, and the types tend to have a different smell, perhaps reflecting a different profile of terpenoids. [23,24]

Bridging the gap between culture and science

As a result of limited understanding and support from the medical community, medicinal users of Cannabis generally adopt the terminology derived from recreational users to describe the therapeutic effects they experience. Although it is hard to study the popular Cannabis literature and come to a single clear conclusion, the following general picture emerges about the difference between typical Sativa and Indica effects upon smoking.

The Sativa high is often characterized as uplifting and energetic. The effects are mostly cerebral (head-high), also described as spacey or hallucinogenic. This type gives a feeling of optimism and well-being, as well as providing a good measure of pain relief for certain symptoms. Although Indicas are generally said to contain more THC, a few pure Sativa types are also very high in THC content. Sativas are considered a good choice for daytime smoking. In contrast, the Indica high is most often described as a pleasant body buzz (body-high). Indicas are primarily enjoyed for relaxation, stress relief, and for an overall sense of calm and serenity. Indicas are supposedly effective for overall body pain relief, and often used in the treatment of insomnia; they are the late-evening choice of many smokers as an aid for uninterrupted sleep. A few pure Indica strains are very potent in THC, and may cause the 'couchlock' effect, enabling the smoker to simply sit still and enjoy the experience of the Cannabis. [22]

It has not been properly confirmed whether subjective descriptions such as these are correlated in any way to the morphological distinctions between Indica and Sativa cultivars, or to any other chemical classification described above. It is obvious that a better understanding of chemical differences between Cannabis cultivars could help to bridge the gap between the vast knowledge on Cannabis that exists within the community of recreational users, and the information needed by medicinal users and health professionals. However, the high number of (potential) active components present in Cannabis significantly complicates a conventional reductionist approach using analytical chemistry, animal studies, and clinical trials, where an active ingredient needs to be identified before further study is possible.

From cultivar to chemovar

An alternative approach to this multiple component problem may be to simultaneously identify and quantify all major components present in various Cannabis types, and use a multi-variant data analysis tool such as principal component analysis (PCA) to classify cultivars in a small number of chemically distinct groups. With well-designed animal studies and/or clinical trials, and using a range of distinct chemical varieties, correlations may then be observed between specific chemical characteristics, and potentially beneficial biological effects. Of course, such an approach fits exactly within the paradigm of Systems Biology, which is recognized as a way to better understand the complex interactions that can be involved in the effects of medicinal plants with multiple active ingredients.[25] With this approach it may be possible to move away from Cannabis *cultivars*, with often vague and unsubstantiated characteristics, towards a new classification using *chemovars* with a complex, but nevertheless well-defined chemical composition (also known as a chemical 'fingerprint').

In this study we attempt for the first time to directly compare cultivars obtained from official as well as illicit sources through a comprehensive chemical profile, including cannabinoids such as THC and CBD, but also minor cannabinoids and a range of mono- and sesqui-terpenoids. Two popular and widely available Cannabis cultivars were obtained from a number of Dutch coffee shops (highly regulated outlets of small amounts of Cannabis for recreational use, tolerated under Dutch law).[2] All samples were chemically profiled using gas chromatography with flame ionization detection (GC/FID). Because major components are more likely to be involved in medicinal effects, only compounds present above a 0.5 mg/gram threshold level were selected for further analysis, in order to determine the chemical variation present in both varieties. This approach resulted in the quantification of 28 different sample components. The results were then compared to those obtained by analyzing three medicinal-grade Cannabis varieties currently available from Dutch pharmacies. [26] The study indicated the usefulness of PCA analysis for chemotaxonomic classification of Cannabis varieties, and may assist in a better identification of Cannabis cultivars with a potential for medicinal use.

Materials and methods

Sample collection

In order to evaluate the chemical variation found in some major Cannabis cultivars, ten coffee shops in four major, geographically dispersed, cities in the Netherlands (Amsterdam, Utrecht, Groningen, and Maastricht) were selected for sample collection. All locations were visited a second time about two months later to obtain a larger variation (different batches) of Cannabis samples. Two popular strains were selected for analysis, because they were generally available at all locations throughout the year: Amnesia (a Sativa-dominant type Cannabis) and White Widow (an Indica-dominant type). [27] One gram of each sample was purchased. All samples were delivered in small zip-lock bags, as typically provided by Dutch coffee shops.

Three varieties of pharmaceutical-grade Cannabis (official product name: Bedrocan®, Bedrobinol®, and Bedica®) were obtained directly from the official Dutch cultivator, Bedrocan BV (Veendam, the Netherlands). Plants were grown from clones under standardized indoor conditions, and quality-controlled for chemical composition, water content, and the absence of adulterants. [26] Flowertops were harvested and air-dried for one week at controlled temperature and humidity. Samples were delivered in airtight, triple-laminate bags. All samples were kept in a freezer until analyzed.

Solvents and chemicals

Authentic standards for *alpha*-bisabolol, myrcene, *alpha*-pinene, *beta*-pinene, *gamma*-terpineol and *beta*-caryophyllene were purchased from Sigma-Aldrich (Steinheim, Germany). *alpha*-Humulene was from Fluka (Steinheim, Germany). Terpinolene, *gamma*-cadinene, *cis*-ocimene and *beta*-phellandrene were from a chemical database of the authors.

Calibrated standards for THC, CBD, cannabigerol (CBG), cannabichromene (CBC), *trans*-(−)-*delta*-9-tetrahydrocannabivarin (THCV), and cannabinol (CBN) were purified and quantified as previously described. [28,29] All cannabinoid references were >98% pure in ethanol. For the structures of the cannabinoids (Figure 1). A standard for cannabigerol-monomethylether (CBGM) was not available.

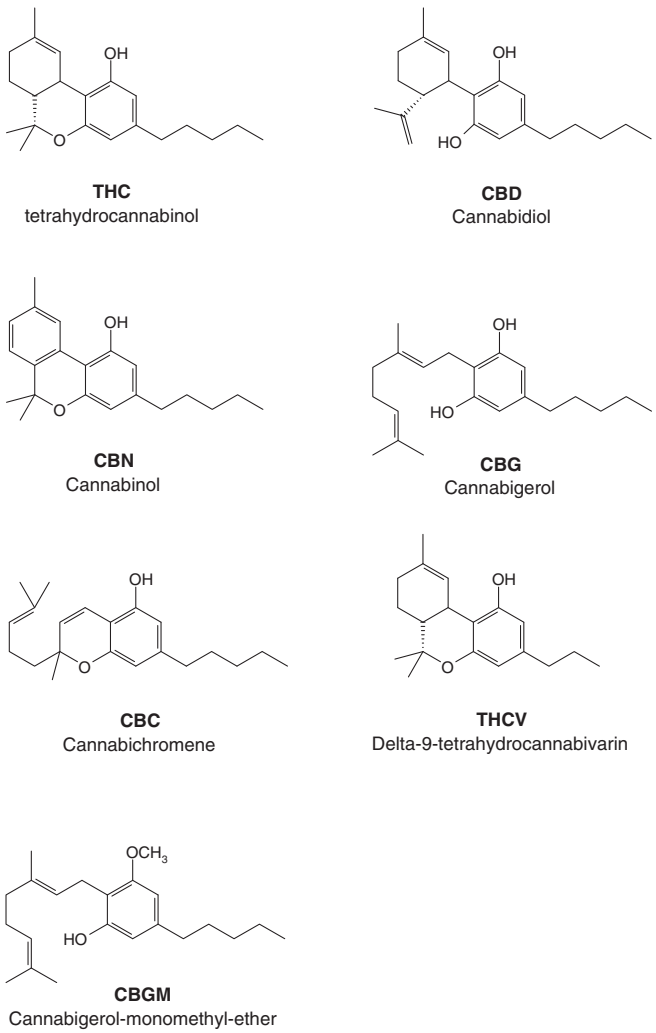

Figure 1. Structures of main cannabinoids.

All organic solvents used were of analytical reagent (AR) grade (Biosolve BV, Valkenswaard, the Netherlands).

Extraction

Extracts were prepared as described recently by Fischedick *et al.* [30] In short, 500 mg (+/− 2 mg) of each sample was extracted with 40 ml of absolute ethanol in plastic serum tubes (total volume 50 ml) while mechanically shaking for 10 min. Tubes were centrifuged and clear supernatant was transferred to a 100-ml volumetric flask. For exhaustive extraction, the procedure was repeated twice more with 25 ml of ethanol, and supernatants were combined. Volumes were adjusted to 100 ml and mixed well. Finally, the solution was filtered through a 0.45 μm PTFE syringe filter. Filtrates were stored at −20 °C until analysis.

GC-analysis

An Agilent GC 6890 series (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with a 7683 autosampler and a flame ionization detector (FID) was used for the simultaneous analysis of monoterpenoids, sesquiterpenoids, and cannabinoids, as previously described by Fischedick. [30] The instrument was equipped with a DB5 capillary column (30 m length, 0.25 mm internal diameter, film thickness 0.25 μm; J&W Scientific Inc., Folsom, CA, USA). The injector temperature was 230 °C, with an injection volume of 4 μl, a split ratio of 1:20 and a carrier gas (N_2) flow rate of 1.2 ml/min. The temperature gradient started at 60 °C and increased at a rate of 3 °C/min until 240 °C which was held for 5 min making a total run time of 65 min/sample. The FID detector temperature was set to 250 °C. The GC-FID was controlled by Agilent GC Chemstation software version B.04.01.

All terpenoids were quantified from a 4-point standard curve of *gamma*-terpinene while all cannabinoids were quantified from a 4-point standard curve of THC, which was previously validated to give adequate quantitative results. [30] The final result, expressing the concentration of each component in mg/gram of plant material, was used for PCA data analysis. Values were not corrected for water content of the samples.

In order to confirm peak identification, samples and available standards were further analyzed by gas chromatography–mass spectrometry (GC-MS) as recently described [30] using a single quadrupole MS in Total Ion Count (TIC) mode. Compounds were identified by comparing their mass spectra and retention times with authentic references as well as literature reports. [23,31–34] The NIST library was also used to assist in compound identification (version 2.0f; Standard Reference Data Program of the National Institute of Standards and Technology, as distributed by Agilent Technologies).

PCA data analysis

All compounds present at a mean concentration of ≥ 0.5 mg/gr in at least one of the Cannabis varieties were identified and quantified as described above. Resulting data was subjected to principal component analysis (PCA) with the aid of SIMCAP + version 12.0 software (Umetrics, Umeå, Sweden). Unit variance scaling was used. The first two principal components (PC1 and PC2) were visualized in a scatter-plot, in combination with its accompanying loading-plot. For more information on interpreting PCA data, you are referred to the tutorials section of the Umetrics website. [35]

Results and discussion

Sampling and analysis

In total, we intended to obtain 40 Cannabis samples for our study (10 coffee shops x 2 visits x 2 varieties). However, in five cases the desired product was not available from the coffee shop visited (2x Amnesia, 3x White Widow), which resulted in a total of 18 Amnesia samples and 17 White Widow samples available for analysis. In total, 28 different components were found to be present in one or more Cannabis samples at a mean level of ≥ 0.5 mg/gr. These are indicated in Table 1 (coffee shop samples) and Table 2 (pharmacy samples). Values were not corrected for water content of the samples as this may require more sample (cost) and it may induce the loss of volatile constituents. All components could be positively identified with the help of retention time, mass-data and authentic standards, except one single compound (*m/z*: 356 [M+], 313, 297 [base], 243, 231). Because we expected this component to be a cannabinoid, it was used in the final data analysis (compound 24 in Tables 1 and 2).

The use of only one terpenoid (*gamma*-terpinene) and one cannabinoid (THC) for quantification of all sample components may demand some further explanation. In a previously published report, we showed that the differences in FID-detector response between different types of cannabinoids, or between a range of mono- and sesqui-terpenoids, were relatively small. [30] Obviously, quantifying all 28 sample components with their own standard curves would be preferred, but this is a tremendous and costly task. Our current approach may give small deviations from actual concentrations, but the method is easy to perform and it does not invalidate our findings. In our case, the method is used for selecting the main sample components (above ca. 0.5 mg/gr) and to subsequently compare the relative differences between samples. Because our intention is to keep extending our database of cultivars on a regular basis, and therefore would need a rapid and simple methodology, we believe the limitations of our method are acceptable.

The data presented in Table 1 prompt some intriguing questions about the popular name used for these cultivars. For example, Amnesia has a mean titer of *alpha*-pinene that is less than half that of White Widow. If Amnesia actually has more amnesic effects, this distinction is noteworthy, since that component is an anticholinesterase inhibitor, and may reduce associated short-term memory deficits engendered by THC. [18] Similarly, the myrcene titre of White Widow is more than four times that in Amnesia. If White Widow, being of the Indica chemotype, is indeed noted for its sedating properties (see Introduction), this could be correlated, since myrcene has been associated with 'couch-lock'. [18]

Table 1. GC quantitative data of coffee shop samples.

ID#	Compound	RRT	white widow			amnesia		
			MEAN	STDEV	RSD%	MEAN	STDEV	RSD %
1	alpha-pinene	*0.257*	1.1	0.24	21.1	0.5	0.04	6.9
2	beta-pinene	*0.306*	0.5	0.08	16.3	0.7	0.16	22.7
3	myrcene	*0.319*	2.9	1.16	39.5	0.7	0.16	23.4
4	beta-phellandrene	*0.371*	0.7	0.13	17.9	0.7	0.13	19.9
5	cis-ocimene	*0.397*	—	—	—	0.7	0.14	19.3
6	terpinolene	*0.458*	—	—	—	1.9	0.77	39.6
7	terpineol	*0.622*	0.6	*	0.0	—	—	—
8	beta-caryophyllene	*1.000*	1.2	0.39	31.1	2.1	0.59	27.6
9	alpha-guaiene	*1.030*	—	—	—	0.5	*	0.0
10	humulene	*1.054*	0.6	0.11	19.2	0.8	0.21	25.2
11	beta-farnesene	*1.057*	0.5	0.06	11.2	0.5	0.00	0.7
12	gamma-selinene	*1.136*	—	0.7	0.25	—	34.6	—
13	delta-guaiene	*1.136*	—	—	—	—	—	—
14	gamma-cadinene	*1.180*	0.6	0.22	34.3	0.6	0.08	14.2
15	eudesma-3,7(11)-diene	*1.190*	0.8	0.27	34.9	0.6	0.09	15.2
16	elemene	*1.214*	0.7	0.18	27.2	0.5	0.07	13.8
17	guaiol	*1.274*	0.6	0.12	20.0	—	—	—
18	gamma-eudesmol	*1.307*	0.6	0.21	35.7	—	—	—
19	beta-eudesmol	*1.355*	0.5	0.06	11.2	—	—	—
20	alpha-bisabolol	*1.398*	0.5	*	0.0	—	—	—
21	THCV	*2.179*	1.0	0.22	23.1	1.2	0.21	17.2
22	CBD	*2.292*	0.7	0.10	14.3	0.6	0.05	9.1
23	CBC	*2.303*	1.8	0.33	18.4	2.8	0.73	26.3
24	Unknown compound	*2.348*	0.7	0.26	36.2	0.7	0.12	17.4
25	CBGM	*2.358*	1.9	*	0.0	—	—	—
26	THC	*2.399*	159.5	26.76	16.8	167.5	21.88	13.1
27	CBG	*2.456*	5.4	2.34	43.2	12.5	2.95	23.6
28	CBN	*2.461*	0.9	0.19	21.0	1.4	0.41	29.6

All levels are expressed in mg of analyte per gram of cannabis sample.
* Analyte detected in only one sample, so standard deviation could not be calculated. RRT: relative retention time compared to beta-caryophyllene.

Table 2. GC quantitative data of pharmacy samples.

ID#	Compound	BEDROCAN			BEDROBINOL			BEDICA		
		MEAN	STDEV	RSD %	MEAN	STDEV	RSD %	MEAN	STDEV	RSD %
1	alpha-pinene	0.5	0.03	6.3	1.9	0.03	1.8	1.1	0.02	2.0
2	beta-pinene	0.8	0.03	3.8	0.5	0.02	3.3	0.3	0.28	86.7
3	myrcene	2.9	0.11	3.6	5.9	0.24	4.1	3.7	0.18	4.8
4	beta-phellandrene	1.2	0.05	4.0	—	—	—	—	—	—
5	cis-ocimene	2.0	0.07	3.8	—	—	—	—	—	—
6	terpinolene	5.8	0.27	4.6	—	—	—	—	—	—
7	terpineol	—	—	—	—	—	—	—	—	—
8	beta-caryophyllene	1.5	0.05	3.3	0.9	0.04	4.2	1.3	0.01	0.6
9	alpha-guaiene	—	—	—	—	—	—	—	—	—
10	humulene	0.5	0.03	5.8	—	—	—	—	—	—
11	beta-farnesene	—	—	—	—	—	—	—	—	—
12	gamma-selinene	—	—	—	—	—	—	—	—	—
13	delta-guaiene	0.7	0.02	3.5	—	—	—	—	—	—
14	gamma-cadinene	0.6	0.03	5.2	—	—	—	—	—	—
15	eudesma-3,7(11)-diene	0.8	0.00	0.6	—	—	—	0.6	0.03	5.5
16	elemene	0.9	0.03	3.0	—	—	—	—	—	—
17	guaiol	—	—	—	—	—	—	0.6	0.03	5.3
18	gamma-eudesmol	—	—	—	—	—	—	0.6	0.04	6.8
19	beta-eudesmol	—	—	—	—	—	—	—	—	—
20	alpha-bisabolol	—	—	—	—	—	—	0.7	0.03	3.6
21	THCV	1.3	0.04	3.2	0.9	0.08	8.4	1.0	0.09	8.5
22	CBD	0.8	0.03	4.3	—	0.6	0.01	—	—	1.7
23	CBC	2.8	0.02	0.8	2.5	0.02	0.6	2.6	0.11	4.2
24	Unknown compound	—	—	—	—	—	—	—	—	—
25	CBGM	—	—	—	—	—	—	—	—	—
26	THC	211.9	6.48	3.1	135.0	5.02	3.7	174.5	6.14	3.5
27	CBG	12.0	0.64	5.3	2.4	0.11	4.4	12.4	0.47	3.8
28	CBN	0.8	0.16	19.0	—	—	—	0.7	0.08	11.1

All levels are expressed in mg of analyte per gram of cannabis sample.

PCA and quantitative analysis of coffee shop samples

PCA is a mathematical way of identifying patterns in data, and expressing the data in such a way as to highlight their similarities and differences. Since patterns can be hard to find in data of higher dimension, where the luxury of graphical representation (i.e. a 2- or 3-dimensional diagram) is not available, PCA is a powerful tool for analyzing data. [36] In this study, PCA was used to analyze our data set, which consisted of 44 samples with up to 28 compounds each, equaling 1232 data points. Each compound represented a variable while its quantity represented the observation. Figure 2 shows an evaluation of the first two principal components of all samples in the form of a scatter plot (Figure 2a) and its related loading plot (Figure 2b).

The data as represented in the PCA scatter plot clearly shows that Cannabis cultivars Amnesia and White Widow cluster in two chemically distinct groups. Principal component 1 (PC1; 27.2%) and PC2 (17.0%) together explained 44.2% of the total variance found in the sample set, indicating a relatively high degree of confidence. Analysis of PC3 added only another 11.4%. Interestingly, the mean THC content found in Amnesia (167.5 mg/gr, or 16.75 %) and White Widow (159.5 mg/gr, or 15.95 %) samples showed no significant difference, providing no basis for distinguishing both popular varieties. However, the loading plot (Figure 2b) helps us understand how exactly the two cultivars differ from one another. Based on our PCA analysis, it becomes clear that monoterpenoids, sesquiterpenoids, and minor cannabinoids are important in distinguishing the varieties.

Amnesia samples were characterized mainly by the presence of the terpenoids terpinolene, *alpha*-guaiene and *gamma*-selinene, which were not detected (i.e. below threshold levels) in White Widow. Levels of *beta*-caryophyllene, as well as cannabinoids THCV, CBC and, particularly, CBG were higher in Amnesia than in White Widow. In contrast, White Widow samples showed significantly higher content of myrcene and *alpha*-pinene. Samples of this variety were further characterized by the presence of guaiol, *beta*-eudesmol, *gamma*-eudesmol, and *alpha*-bisabolol, which were not detected in Amnesia samples. Such sesquiterpene alcohols have previously been reported to be important for the chemotaxonomic discrimination of Indica Cannabis varieties originating from Afghanistan. [24]

In three cases, chemical analysis showed that the obtained sample was likely not of the desired cultivar type. More specifically, two samples that were purchased as White Widow were found to resemble the typical chemical fingerprint of Amnesia, while for one Amnesia sample the opposite was true. Although these samples are shown in Figure 2, we assumed that a mix-up had occurred at the coffee shop, and these outliers were not included in our final evaluation of chemical diversity. However, it is also possible that these samples represented actual specimens of the desired cultivars, which would even further increase the chemical diversity (i.e. STDEV) found in both cultivar groups (Table 1). Naturally, a mix-up of samples

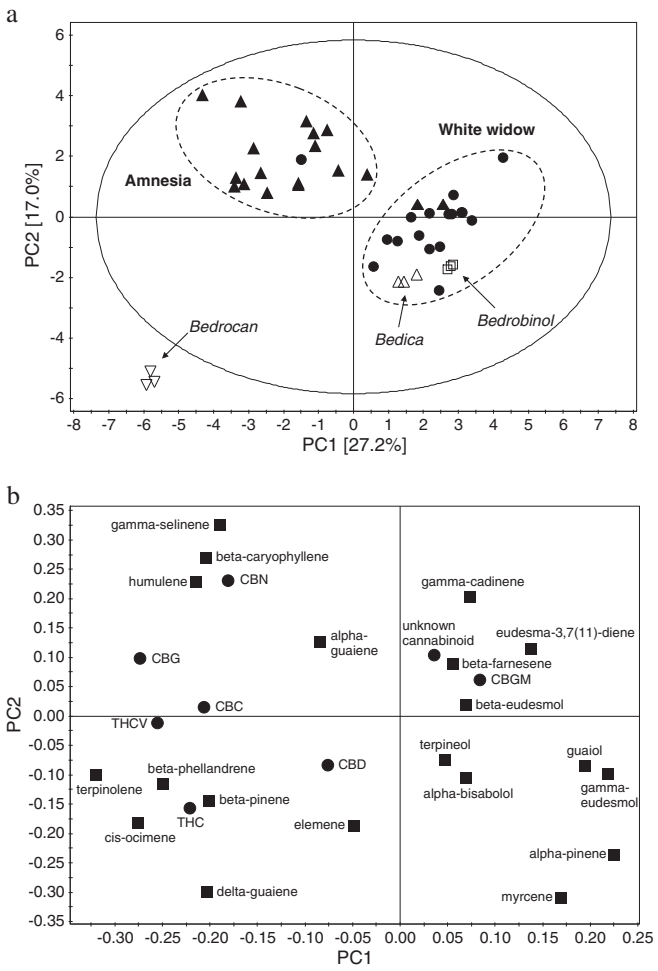

Figure 2. PCA scatter plot. Solid ellipse indicates 95% confidence interval. Dotted lines were drawn by the authors to facilitate visual interpretation of this figure only; it does not signify significance or confidence intervals. Note that 2 samples purchased as Amnesia (closed triangles) were chemically similar to White Widow (closed circles), while for one sample the opposite was observed. It was assumed these samples were mixed up by the coffee shop, and they were not used for final statistical evaluations. Amnesia and White Widow data all represents individually purchased samples from different batches. Data for Bedrocan, Bedrobinol and Bedica represents triplicate analyses of the same batch.

during extraction or processing may also be considered as a possible reason for the observed effect. However, this risk was minimized by clearly marking all samples directly after purchase, while keeping them in their original packaging. Samples were then extracted and processed in groups of eight sequential samples by two people checking each other.

It may be concluded that, based on the overall chemical profile consisting of 28 major components, it is indeed possible to distinguish between two cultivars that are popularly recognized by recreational Cannabis users: Amnesia and White Widow. However, an important issue when using Cannabis as a medicine is the reproducibility of the chemical composition of the product that the patient is trying to obtain. For both Cannabis varieties analyzed in this study, chemical variability was considerable, as shown in Table 1 and visualized in the PCA score plot. Deviations (relative standard deviation) of more than ± 25% were found for 14 analyzed compounds. To put this variability in perspective, it makes sense to compare these results to the chemical variation commonly observed in Cannabis obtained from standardized sources (see next section).

Comparing coffee shop to pharmacy samples

Three different types of medicinal-grade Cannabis, currently available on prescription in the Netherlands, were analyzed according to the procedures mentioned. In order to obtain better statistical results, these analyses were done on triplicate samples for each variety (compared to obtaining samples in 20-fold for both coffee shop varieties). Chemical profiles were then compared to determine similarities and differences between coffee shop and pharmacy samples.

Although varieties Bedrobinol and Bedica have quite different genetic backgrounds, PCA analysis indicated that both closely resemble the typical fingerprint of the White Widow variety. Indeed, variety Bedica is genetically derived from an Indica type, so this would explain their similarity in chemical profile, such as the presence of sesquiterpene alcohols. The fact that variety Bedrobinol resembles White Widow is more surprising, as it was genetically derived from a Haze variety, which has a large proportion of Sativa genes in its background. [27] A potential explanation is the fact that variety Bedrobinol has very low content of terpenes in general, with only a few components detectable above our 0.5 mg/gram threshold level. As a result, there may not have been enough distinguishing features to clearly show differences with the other analyzed samples. Finally, the difference between Bedrobinol and Bedica is mainly found in the presence of sesquiterpenes such as guaiol, *gamma*-eudesmol, and *alpha*-bisabolol in Bedica, while Bedrobinol was largely devoid of these compounds.

Bedrocan is currently the most popular medicinal Cannabis variety in the Netherlands. It is interesting to conclude that it does not resemble either coffee shop type, or the two other pharmacy varieties. In Figure 2, the data for Bedrocan (left-lower corner) even falls outside the 95% confidence interval region, underscoring the fact that its composition is significantly different from any of the other four varieties analyzed. Bedrocan was originally developed from a Jack Herer genetic background, which supposedly is a slightly Sativa dominant hybrid. [27] Indeed, Bedrocan is closer to Amnesia, which is also considered Sativa dominant, than to White Widow. The main distinguishing feature of Bedrocan compared to Amnesia is the markedly higher content of myrcene, *cis*-ocimene and terpinolene by at least a factor of 2. In contrast, a few components found in Amnesia were not detected (below threshold levels) in Bedrocan (i.e. *beta*-farnesene, *gamma*-selinene, *gamma*-cadinene and eudesma-3,7(11)-diene).

It is clear that plant-based products can never be perfectly standardized for content of active components, as they are dependent on too many environmental factors to completely predict the chemical composition of the final product. For that reason, a variability of up to ± 15% is allowed for the THC and CBD levels in the Dutch medicinal Cannabis. Although currently no regulations exist for the levels of minor cannabinoids or terpenoids, any producer of a medicinally used product should strive for a minimum of variation in chemical composition. In our previous study on medicinal-grade Cannabis [30] using the same analytical methodology, an average variation (RSD) of about ± 7.6% was found for cannabinoids, and ± 11.0% for terpenoids in a standard batch of variety Bedrocan; for variety Bedica these numbers were ± 4.3% (terpenoids) and ± 5.5% (cannabinoids). In contrast to the coffee shop samples discussed above, none of the components analyzed in the pharmacy varieties showed a variability of over 25%. Obviously, this result may be expected based on the fact that the Cannabis samples of pharmaceutical grade were cultivated under

strictly controlled indoor conditions, and each batch was analyzed for chemical content by validated laboratory methods.

Conclusion

The psychotropic compound THC is often considered to be the main component responsible for (medicinal) effects of Cannabis products, through activation of specific cannabinoid-binding receptors. [37] But increasingly, other, non-psychotropic cannabinoids are showing their medicinal potential, [38,39] sometimes even through mechanisms that do not involve binding to the known cannabinoid receptors. [17] With a growing number of medicinal Cannabis users worldwide, it therefore may be time to define the 'potency' of Cannabis products in a more comprehensive manner than by THC content alone. With modern analytical techniques, a full- range analysis of cannabinoids and terpenoids has become possible.

In previous studies, our group showed that a range of Cannabis cultivars grown by clonal propagation under controlled environmental conditions could be accurately distinguished based on their cannabinoid and terpenoid content. We also observed that the chemical profile of cannabinoids and terpenoids in different batches of the same cultivar (grown sequentially over the period of several months) was highly reproducible. [30] These data suggest it is possible to fully standardize the chemical content of cannabinoids as well as terpenoids in Cannabis, when cultivated in a controlled setting.

In this study we applied the same analytical methodology to evaluate the chemical variation of two popular Cannabis cultivars obtained in Dutch coffee shops. With this study we intended to (1) determine whether there is a chemical basis to consider the obtained varieties as truly different products, and (2) evaluate the chemical variability that exists within each cultivar type. In order to bridge the gap between recreational and medicinal use of Cannabis, we then compared the overall chemical composition of the obtained coffee shop samples with three types of medicinal Cannabis from a government-licensed facility in the Netherlands. The fourth variety of Cannabis currently available on prescription in the Netherlands, called Bediol ®, was not included in this study because its high content of CBD (7.5%) completely changes its pharmacological spectrum, compared to the varieties analyzed in this study (CBD $< 0.1\%$).

In a total of 40 visits to the coffee shop, the desired product was not available on five occasions, while in three cases a product was obtained that significantly differed from the average composition of the requested variety. As we assumed these three samples had been mixed up in the coffee shop (either intentionally or accidentally), we chose not to include them in our final analysis. If our experiences can be considered as representative, it means that in 20% of cases (8/40) a consumer is not able to receive a reliable service when visiting an average coffee shop. The observed variability in chemical composition may also be a cause of concern for medicinal users. As it is often claimed that the exact combination of active constituents (synergy) gives each Cannabis variety its unique (medicinal) properties, medicinal Cannabis users may inadvertently purchase a product that has unexpected effects on their health and/or psyche. It should be noted that changes in chemical composition may not only be derived from genetic differences between batches of the same Cannabis variety, but could also be caused by differences between coffee shops (or their suppliers) in, for example, cultivation conditions, drying, processing, and storage. However, no matter what the cause, Cannabis products from a standardized and quality-controlled source may be the safer choice for medicinal users.

An important reason for patients to keep purchasing their materials from illicit markets is the fact that, by trial and error, they have found a strain that works optimally for treatment of their specific symptoms. With the limited choice of Cannabis varieties currently available from official sources, it is hard to deny the value of such choice. By making use of the comprehensive chemical fingerprint of herbal Cannabis, our results may help medicinal users of Cannabis and their doctors to switch from a beneficial variety obtained through the street market, to a strain that is available through official state-run programmes. It may also help these official programmes to narrow down the search for future Cannabis varieties to be standardized and introduced as an official medicine.

As this study intended to compare chemical data about Cannabis samples with knowledge available from Cannabis popular culture, we decided to use information from the Sensi Seed Bank (the Netherlands) website as a reference for the Sativa or Indica background of cultivars. Sensi Seed Bank is perhaps the largest supplier of Cannabis seeds in the world, and an authoritative voice among producers and users of recreational Cannabis. In a next phase of our ongoing studies, we would like to obtain typical 'pure' Sativa and Indica strains to determine the two extreme ends of the chemical diversity scale. Although this was our intention for the present study, we were not able to identify such strains that were available from all ten coffee shops we visited for sample collection. According to Sensi Seed Bank, a typical Sativa type Cannabis would be *Durban* (90% Sativa), while some well-known examples of Indica types would be *Hindu kush* (100% Indica), *Afghani #1* (95%), or *Northern lights* (90%). As an example of pure Sativa we could also select a non-drug variety (i.e. hemp) available in the Netherlands, and grow it under standardized conditions.

We consider this study a work in progress, and wish to add a larger number of popular strains from the recreational scene, as well as Cannabis varieties from official programmes to our database. Over time, we hope this will lead to a better understanding of the overlap between medicinal use of Cannabis, and the street culture around the same plant. It is our personal belief that, with such an approach, the endless number of popular cultivars could be reduced to perhaps two dozen chemically distinct chemical varieties, or chemovars. Further study into the chemical similarities between popular coffee shop (or other illicit sources) Cannabis types, and available medicinal-grade strains may help medical users to accurately and efficiently find a more reliable source of medicine within the various national medicinal Cannabis programmes. Exchange of cultivars and information between the various national programmes, in countries including the Netherlands, Canada, and the USA, may greatly facilitate such a transition.

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Disclaimer

AH has been employed by Bedrocan BV as the head of Research and Development since February 2011.

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