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(54) Titre : PLANTES DE CANNABIS AYANT UN PROFIL DE CANNABINOIDES ENRICHIS POUR Δ-9-TETRAHYDROCANNABINOL ET CANNABIGEROL
 (54) Title: CANNABIS PLANTS WITH A CANNABINOID PROFILE ENRICHED FOR Δ-9-TETRAHYDROCANNABINOL AND CANNABIGEROL

(57) **Abrégé/Abstract:**

The present disclosure relates to new cannabis plants, including parts, extracts and uses thereof, comprising a cannabinoid profile enriched for total THC (i.e., Δ-9-tetrahydrocannabinol (THC) and Δ-9-tetrahydrocannabinolic acid (THCA)) and total CBG (i.e., cannabigerol (CBG) and cannabigerolic acid (CBGA)).

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CANNABIS PLANTS WITH A CANNABINOID PROFILE ENRICHED FOR Δ -9-TETRAHYDROCANNABINOL AND CANNABIGEROL

[0001] The present application claims priority from Australian Provisional Patent Applications 2018904289, 2018904291, 2018904285, and 2018904286 filed 9 November 2018 and Australian Provisional Patent Applications 2019900294, 2019900295, 2019900291 and 2019900293 filed 31 January 2019, the disclosures of which are hereby expressly incorporated herein by reference in their entirety.

FIELD

[0002] The present disclosure relates to new cannabis plants, including parts, extracts and uses thereof, comprising a cannabinoid profile enriched for total THC (*i.e.*, Δ -9-tetrahydrocannabinol (THC) and Δ -9-tetrahydrocannabinolic acid (THCA)) and total CBG (*i.e.*, cannabigerol (CBG) and cannabigerolic acid (CBGA)).

BACKGROUND

[0003] Cannabis is an herbaceous flowering plant of the *Cannabis* genus (*Rosale*), which has been used for its fibre and medicinal properties for thousands of years. The medicinal qualities of cannabis have been recognised since at least 2800 BC, with use of cannabis featuring in ancient Chinese and Indian medical texts. Although use of cannabis for medicinal purposes has been known for centuries, research into the pharmacological properties of the plant has been limited due to its illegal status in most jurisdictions.

[0004] The chemical profile of cannabis plants is varied. It is estimated that cannabis plants produce more than 400 different molecules, including phytocannabinoids, terpenes and phenolics. Cannabinoids, such as Δ -9-tetrahydrocannabinol (THC) and cannabidiol (CBD), are typically the most commonly known and researched cannabinoids. CBD and THC are naturally present in their acidic forms, Δ -9-tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA), which are alternative products of a shared precursor, cannabigerolic acid (CBGA).

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[0005] Many cannabinoids interact with the endocannabinoid system in mammals, including humans, to exert complex biological effects on the neuronal, metabolic, immune and reproductive systems. They also interact with G protein-coupled receptors (GPCRs), such as CB1 and CB2, in the human endocannabinoid system, where they are thought to play a part in the regulation of appetite, pain, mood, memory, inflammation and insulin sensitivity. Cannabinoids have also been implicated in neuronal signalling, gastrointestinal inflammation, tumourigenesis, microbial infection and diabetes.

[0006] Whilst there is an increasing body of evidence of the therapeutic potential of cannabis and cannabis-derived compounds, in particular cannabinoids, their adoption into clinical practice has been hindered, at least in part, to the fact that their mechanisms of action remain largely ill-defined, noting that different cannabinoids can exert different biological effects. The therapeutic potential of cannabis and cannabis-derived cannabinoids is further complicated by the *entourage* effect, where different cannabinoids act in combination to exert different biological effects. In view of this complexity, it is advantageous to select for new cannabis varieties that have a cannabinoid profile enriched for specific cannabinoids suitable for therapeutic use.

[0007] Previous studies of the cannabinoid content of cannabis plants have largely focused on the differentiation of cannabis varieties bred for recreational or industrial use. For example, in a study conducted by Turner *et al.* (1979, *Journal of Natural Products*, 42:319-21), leaf material from 85 cannabis varieties was screened for cannabichromene (CBC), CBD and THC in order to differentiate between recreational and industrial cannabis varieties. The recreational varieties were subjected to further cannabinoid testing to identify the correct time for sampling due to the significant variation of cannabinoid biosynthesis over the life of the plant. More recently, nuclear magnetic resonance (NMR) spectroscopy and RT-PCR analysis has been used to investigate the metabolome and cannabinoid biosynthesis in the trichomes of *Cannabis sativa* "Bebiol" (Happyana and Kayser, 2016, *Planta Medica*, 82:1217-23). The purpose of this study was to characterise the cannabinoid biosynthetic pathway over time, with the results showing that, under the conditions tested, cannabinoid biosynthesis increases in weeks five to six, but remains relatively static in the later weeks once the trichomes had matured. NMR metabolomics approaches have also been used to investigate the difference

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across 12 different cannabis varieties using leaf and flower material (Choi *et al.* 2004, *Journal of Natural Products*, 67: 953-7). Applying principal component analysis to these results, it has been shown that the major discriminator of these varieties was THCA and CBDA, however, carbohydrate and amino acid levels were also important discriminators that may be used for quality control and authentication purposes.

[0008] Despite these recent advances, there has been lack of sufficient systematic analysis for the purpose of precision breeding of cannabis plants for medicinal use. There remains, therefore, an urgent need for systematic breeding and selection of improved cannabis varieties comprising a cannabinoid profile enriched for specific cannabinoids that make them suitable for therapeutic use.

SUMMARY

[0009] In an aspect disclosed herein, there is provided a cannabis plant, or a part thereof, comprising a cannabinoid profile enriched for total THC and total CBG, wherein the cannabinoid profile comprises a level of total THC and a level of total CBG at a ratio from about 5:1 to about 50:1 (THC:CBG), wherein the total THC comprises Δ -9-tetrahydrocannabinol (THC) and Δ -9-tetrahydrocannabinolic acid (THCA), wherein the total CBG comprises cannabigerol (CBG) and cannabigerolic acid (CBGA); and wherein the level of total THC and total CBG (THC+CBG) is greater than the level of a reference cannabinoid selected from the group consisting of:

- (i) total CBD, wherein the total CBD comprises cannabidiol (CBD) and cannabidiolic acid (CBDA);
- (ii) total CBC, wherein the total CBC comprises cannabichromene (CBC) and cannabichromene acid (CBCA);
- (iii) total CBN, wherein the total CBN comprises cannabinol (CBN) and cannabinolic acid (CBNA); and
- (iv) total THCV, wherein the total THCV comprises tetrahydrocannabivarin (THCV) and tetrahydrocannabivarinic acid (THCVA).

[0010] The present disclosure also extends to seeds produced from the cannabis plant, and plants derived therefrom.

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[0011] In another aspect disclosed herein, there is provided a tissue culture of regenerable cells derived from the cannabis plant as described herein, and plants generated therefrom. In an embodiment, the plant expresses the morphological and physiological characteristics of the cannabis plant as described herein.

[0012] In another aspect disclosed herein, there is provided a method for producing an F1 hybrid cannabis plant using plant breeding techniques which employ the cannabis plant described herein, or a part thereof, as a source of plant breeding material. The present disclosure also extends to progeny plants and seeds produced from an F1 hybrid cannabis plant, as described herein.

[0013] In another aspect disclosed herein, there is provided a method for producing a cannabis plant comprising transfecting the cannabis plant described herein, or a part thereof, with a heterologous nucleic acid sequence to introduce one or more nucleic acid substitutions, deletions or additions into the genome of the cannabis plant as described above. The disclosure also extends to progeny plants and plant parts such as seeds which are produced from a transgenic cannabis plant resulting from the method disclosed herein.

[0014] In another aspect disclosed herein, there is provided a method of producing an extract comprising cannabinoids from a cannabis plant comprising harvesting plant material from the cannabis plant described herein, at least partially drying the harvested plant material, and extracting cannabinoids from the at least partially dried plant material, thereby producing an extract comprising cannabinoids.

[0015] In another aspect disclosed herein, there is provided an extract derived from the cannabis plant described herein, or part thereof, wherein the extract comprises a cannabinoid profile enriched for total THC and total CBG, wherein the cannabinoid profile comprises a level of total THC and a level of total CBG at a ratio from about 5:1 to about 50:1 (THC:CBG), and wherein the level of total THC and total CBG (THC+CBG) is greater than the level of a reference cannabinoid selected from the group consisting of: total CBD, total CBC, total CBN, and total THCV.

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[0016] In another aspect disclosed herein, there is provided a total THC- and total CBG-enriched extract derived from the cannabis plant described herein, or a part thereof, wherein the extract comprises total THC, total CBG, and one or more minor cannabinoids selected from the group consisting of: total CBD, total CBC, total CBN, total THCV, total CBL, and total Δ 8-THC, wherein the extract comprises a level of total THC and a level of total CBG at a ratio of from about 5:1 to about 50:1 (THC: CBG), and wherein the level of the one or more other cannabinoids is from about 0.01% to about 10% by weight of the total cannabinoid content of the extract.

[0017] In another aspect disclosed herein, there is provided a method for selecting a cannabis plant comprising a cannabinoid profile enriched for total THC and total CBG from a plurality of different cannabis plants, the method comprising:

- (a) harvesting plant material from a plurality of different cannabis plants;
- (b) at least partially drying the harvested plant material of step (a);
- (c) measuring in the at least partially dried plant material of step (b) a level of total THC, total CBG and one or more reference cannabinoids selected from the group consisting of THCV, CBDV, CBN, CBD, CBC, THCVA, CBDVA, CBNA, CBDA and CBCA, to generate a cannabinoid profile for each of the plurality of cannabis plants; and
- (d) on the basis of the measurements from step (c), selecting from the plurality of different cannabis plants a cannabis plant comprising a cannabinoid profile enriched for total THC and total CBG and comprising a level of total THC and a level of total CBG at a ratio from about 5:1 to about 50:1 (THC:CBG), wherein the total THC comprises THC and THCA, wherein the total CBG comprises CBG and CBGA, and wherein the level of total THC and total CBG (THC+CBG) is greater than the level of a reference cannabinoid selected from the group consisting of:
 - (i) total CBD, wherein the total CBD comprises CBD and CBDA;
 - (ii) total CBC, wherein the total CBC comprises CBC and CBCA;
 - (iii) total CBN, wherein the total CBN comprises CBN and CBNA; and
 - (iv) total THCV, wherein the total THCV comprises THCV and THCVA.

[0018] In another aspect disclosed herein, there is provided a method for selecting, from a plurality of different cannabis plants, a cannabis plant comprising a cannabinoid profile

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enriched for total THC and total CBG from a plurality of different cannabis plants, the method comprising:

- (a) harvesting plant material from a plurality of different cannabis plants;
- (b) at least partially drying the harvested plant material of step (a);
- (c) measuring in the at least partially dried plant material of step (b) a level of total THC, total CBG and one or more reference cannabinoids selected from the group consisting of THCV, CBDV, CBN, CBD, CBC, THCVA, CBDVA, CBNA, CBDA and CBCA, to generate a cannabinoid profile for each of the plurality of cannabis plants;
- (d) measuring in the at least partially dried plant material of step (b) a level of myrcene and a level of β -pinene to generate a terpene profile for each of the plurality of cannabis plants; and
- (e) on the basis of the measurements from step (c) and step (d), selecting from the plurality of different cannabis plants a cannabis plant comprising (i) a terpene profile wherein the myrcene is present at a ratio of from about 60:1 and about 1:1 to the level of β -pinene and (ii) a cannabinoid profile enriched for total THC and total CBG and comprising a level of total THC and a level of total CBG at a ratio from about 5:1 to about 50:1 (THC:CBG), wherein the total THC comprises THC and THCA, wherein the total CBG comprises CBG and CBGA, and wherein the level of total THC and total CBG (THC+CBG) is greater than the level of a reference cannabinoid selected from the group consisting of:
 - (i) total CBD, wherein the total CBD comprises CBD and CBDA;
 - (ii) total CBC, wherein the total CBC comprises CBC and CBCA;
 - (iii) total CBN, wherein the total CBN comprises CBN and CBNA; and
 - (iv) total THCV, wherein the total THCV comprises THCV and THCVA.

BRIEF DESCRIPTION OF FIGURES

[0019] **Figure 1** shows the relative intensity of CBDA and THCA in cannabis plants with a total THC- and total CBG-enriched cannabinoid profile.

[0020] **Figure 2** shows the cannabinoid content in cannabis plants with a total THC- and total CBG-enriched cannabinoid profile. (A) A graphical representation of the quantitation of

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cannabinoid content (y-axis; mg/g) against cannabinoid (x-axis), inclusive of THCA for the Cannabis-32, Cannabis-34, Cannabis-35, Cannabis-36, Cannabis-37, Cannabis-38 and Cannabis-39 strains. (B) A graphical representation of quantitation of minor cannabinoid content (y-axis; mg/g) against cannabinoid, exclusive of THCA for the Cannabis-32, Cannabis-34, Cannabis-35, Cannabis-36, Cannabis-37, Cannabis-38 and Cannabis-39 strains.

[0021] **Figure 3** shows the cannabinoid content in cannabis plants with a total THC- and total CBG-enriched cannabinoid profile. (A) A graphical representation of the quantitation of cannabinoid content (y-axis; mg/g) against cannabinoid (x-axis), inclusive of THCA for the Cannabis-40, Cannabis-41, Cannabis-42, Cannabis-43, Cannabis-44, Cannabis-45, Cannabis-46, Cannabis-47 and Cannabis-48 strains. (B) A graphical representation of to quantitation of minor cannabinoid content (y-axis; mg/g) against cannabinoid, exclusive of THCA for the Cannabis-40, Cannabis-41, Cannabis-42, Cannabis-43, Cannabis-44, Cannabis-45, Cannabis-46, Cannabis-47 and Cannabis-48 strains.

[0022] **Figure 4** shows the cannabinoid content in cannabis plants with a total THC- and total CBG-enriched cannabinoid profile. (A) A graphical representation of the quantitation of cannabinoid content (y-axis; mg/g) against cannabinoid (x-axis), inclusive of THCA for the Cannabis-49, Cannabis-50, Cannabis-51, Cannabis-52, Cannabis-53, Cannabis-54, Cannabis-55, Cannabis-56 and Cannabis-57 strains. (B) A graphical representation of to quantitation of minor cannabinoid content (y-axis; mg/g) against cannabinoid, exclusive of THCA for the Cannabis-49, Cannabis-50, Cannabis-51, Cannabis-52, Cannabis-53, Cannabis-54, Cannabis-55, Cannabis-56 and Cannabis-57 strains.

[0023] **Figure 5** shows a graphical representation of the terpene content (y-axis; counts v acquisition time (min)) against relative abundance (x-axis) in a cannabis plant. (B-G) A graphical representation of the terpene content (terpene; x-axis) against peak area (counts; y-axis) for Cannabis-31, Cannabis-32, Cannabis-34, Cannabis-35, Cannabis-36, Cannabis-37, Cannabis-38, Cannabis-39, Cannabis-40, Cannabis-41, Cannabis-42, Cannabis-43, Cannabis-44, Cannabis-45, Cannabis-46, Cannabis-47, Cannabis-48, Cannabis-49, Cannabis-50, Cannabis-51, Cannabis-52, Cannabis-53, Cannabis-54, Cannabis-55, Cannabis-56, Cannabis-57, Cannabis-58 and Cannabis-59 strains.

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[0024] **Figure 6** shows the distribution of terpene content in cannabis plants. (A) Principal component analysis (PCA) of terpene content across cannabis plants, PCA Scores on PC1 (x-axis; 69.48%) against PCA Scores on PC2 (y-axis; 16.62%). (B) High abundance terpenes across cannabis plants as determined by PCA, PCA1 (y-axis; 69.48%) against variable (x-axis).

[0025] **Figure 7** shows the relative abundance (y-axis; peak area) of (A) β -pinene and (B) myrcene in different cannabis plants.

DETAILED DESCRIPTION

[0026] Throughout this specification, unless the context requires otherwise, the word “comprise”, or variations such as “comprises” or “comprising”, will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

[0027] The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgement or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavor to which this specification relates.

[0028] Unless specifically defined otherwise, all technical and scientific terms used herein shall be taken to have the same meaning as commonly understood by one of ordinary skill in the art.

[0029] Unless otherwise indicated the molecular biology, cell culture, laboratory, plant breeding and selection techniques utilised in the present specification are standard procedures, well known to those skilled in the art. Such techniques are described and explained throughout the literature in sources such as, J. Perbal, *A Practical Guide to Molecular Cloning*, John Wiley and Sons (1984), J. Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbour Laboratory Press (1989), T.A. Brown (editor), *Essential Molecular Biology: A*

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Practical Approach, Volumes 1 and 2, IRL Press (1991), D.M. Glover and B.D. Hames (editors), *DNA Cloning: A Practical Approach*, Volumes 1-4, IRL Press (1995 and 1996), and F.M. Ausubel *et al.* (editors), *Current Protocols in Molecular Biology*, Greene Pub. Associates and Wiley-Interscience (1988, including all updates until present); Janick, J. (2001) *Plant Breeding Reviews*, John Wiley & Sons, 252 p.; Jensen, N.F. ed. (1988) *Plant Breeding Methodology*, John Wiley & Sons, 676 p., Richard, A.J. ed. (1990) *Plant Breeding Systems*, Unwin Hyman, 529 p.; Walter, F.R. ed. (1987) *Plant Breeding*, Vol. I Theory and Techniques, MacMillan Pub. Co.; Slavko, B. ed. (1990) *Principles and Methods of Plant Breeding*, Elsevier, 386 p.; and Allard, R.W. ed. (1999) *Principles of Plant Breeding*, John-Wiley & Sons, 240 p. The ICAC Recorder. Vol. XV no. 2: 3-14; all of which are incorporated by reference. The procedures described are believed to be well known in the art and are provided for the convenience of the reader. All other publications mentioned in this specification are also incorporated by reference in their entirety.

[0030] As used in the subject specification, the singular forms “a”, “an” and “the” include plural aspects unless the context clearly dictates otherwise. Thus, for example, reference to “a plant” includes a single plant, as well as two or more plants; reference to “an inflorescence” includes a single inflorescence, as well as two or more inflorescences; and so forth.

[0031] As used herein, “and/or” refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative (or).

[0032] The present disclosure is predicated, at least in part, on the inventors’ unexpected finding that a cannabis plant can be generated comprising a cannabinoid profile enriched for total THC (*i.e.*, THC and THCA) and total CBG (*i.e.*, CBG and CBGA).

[0033] Therefore, in an aspect disclosed herein there is provided a cannabis plant, or a part thereof, comprising a cannabinoid profile enriched for total THC and total CBG, wherein the cannabinoid profile comprises a level of total THC and a level of total CBG at a ratio from about 5:1 to about 50:1 (THC:CBG), wherein the total THC comprises Δ -9-tetrahydrocannabinol (THC) and Δ -9-tetrahydrocannabinolic acid (THCA), wherein the total CBG comprises cannabigerol (CBG) and cannabigerolic acid (CBGA); and

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wherein the level of total THC and total CBG (THC+CBG) is greater than the level of a reference cannabinoid selected from the group consisting of:

- (a) total CBD, wherein the total CBD comprises cannabidiol (CBD) and cannabidiolic acid (CBDA);
- (b) total CBC, wherein the total CBC comprises cannabichromene (CBC) and cannabichromene acid (CBCA);
- (c) total CBN, wherein the total CBN comprises cannabinol (CBN) and cannabinolic acid (CBNA); and
- (d) total THCV, wherein the total THCV comprises tetrahydrocannabivarin (THCV) and tetrahydrocannabivarinic acid (THCVA).

Cannabis

[0034] As used herein, the term "cannabis plant" means a plant of the genus *Cannabis*, illustrative examples of which include *Cannabis sativa*, *Cannabis indica* and *Cannabis ruderalis*. *Cannabis* is an erect annual herb with a dioecious breeding system, although monoecious plants exist. Wild and cultivated forms of *cannabis* are morphologically variable, which has resulted in difficulty defining the taxonomic organisation of the genus. In an embodiment, the cannabis plant is *C. sativa*.

[0035] The terms "plant", "cultivar", "variety", "strain" or "race" are used interchangeably herein to refer to a plant or a group of similar plants according to their structural features and performance (*i.e.*, morphological and physiological characteristics).

[0036] The reference genome for *C. sativa* is the assembled draft genome and transcriptome of "Purple Kush" or "PK" (van Bakal *et al.* 2011, *Genome Biology*, 12: R102). *C. sativa*, has a diploid genome ($2n = 20$) with a karyotype comprising nine autosomes and a pair of sex chromosomes (X and Y). Female plants are homogametic (XX) and males heterogametic (XY) with sex determination controlled by an X-to-autosome balance system. The estimated size of the haploid genome is 818 Mb for female plants and 843 Mb for male plants.

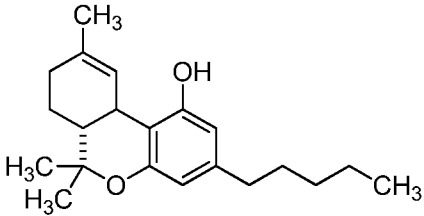
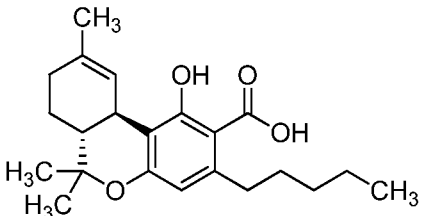
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[0037] As used herein, the term "part" refers to any part of the plant, illustrative examples of which include an embryo, a shoot, a bud, a root, a stem, a seed, a stipule, a leaf, a petal, an inflorescence, an ovule, a bract, a trichome, a branch, a petiole, an internode, bark, a pubescence, a tiller, a rhizome, a frond, a blade, pollen and stamen. The term "part" also includes any material listed in the Plant Part Code Table as approved by the Australian Therapeutic Goods Administration (TGA) Business Services (TBS). In an embodiment, the part is selected from the group consisting of an embryo, a shoot, a bud, a root, a stem, a seed, a stipule, a leaf, a petal, an inflorescence, an ovule, a bract, a trichome, a branch, a petiole, an internode, bark, a pubescence, a tiller, a rhizome, a frond, a blade, pollen and stamen. In a preferred embodiment, the part is a cannabis bud.

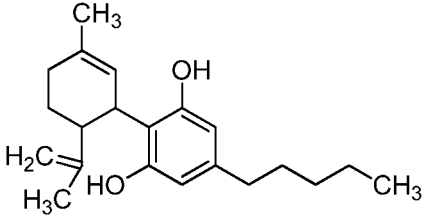
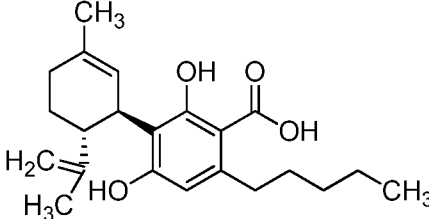
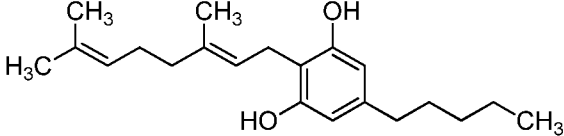
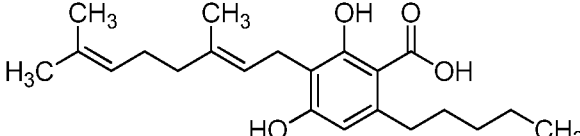
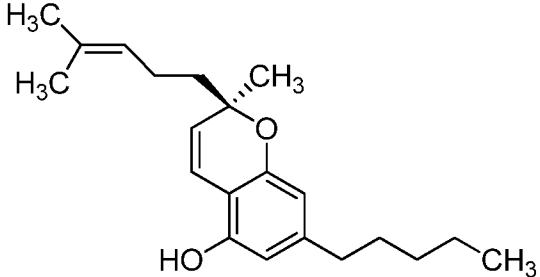
Cannabinoids

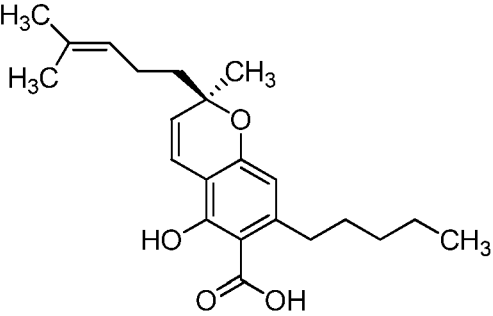
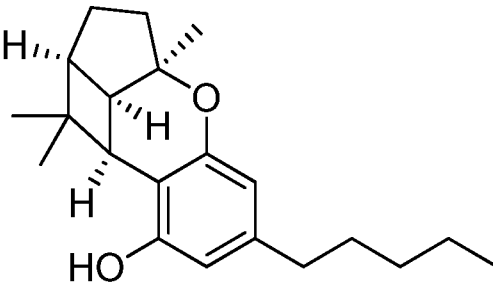
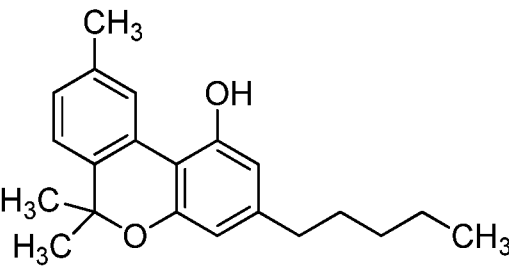
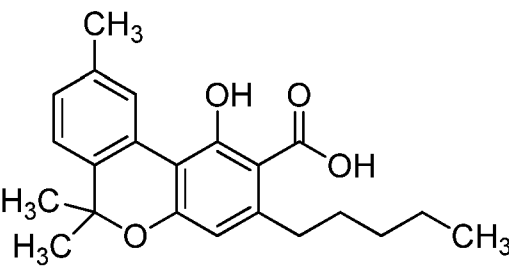
[0038] The term "cannabinoid", as used herein, refers to a family of terpeno-phenolic compounds, of which more than 100 compounds are known to exist in nature. Cannabinoids will be known to persons skilled in the art, illustrative examples of which are provided in Table 1, below, including acidic and decarboxylated forms thereof.

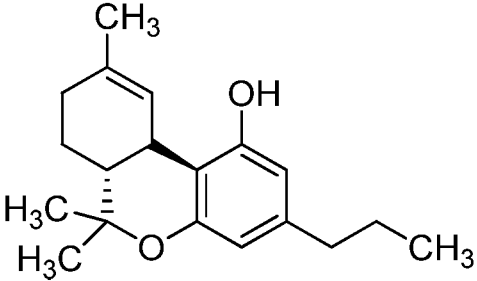
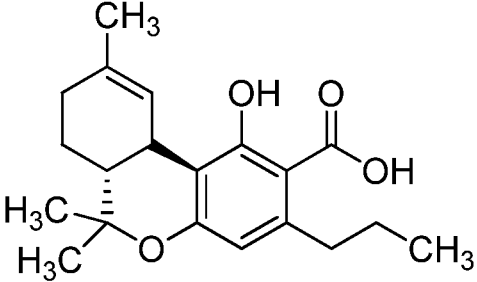
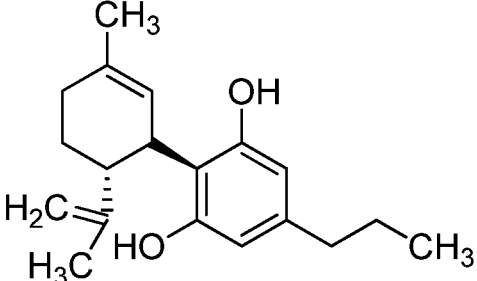
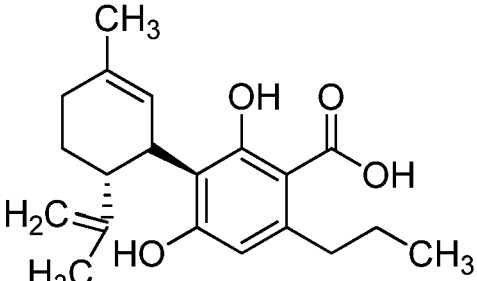
Table 1. Cannabinoids and their properties

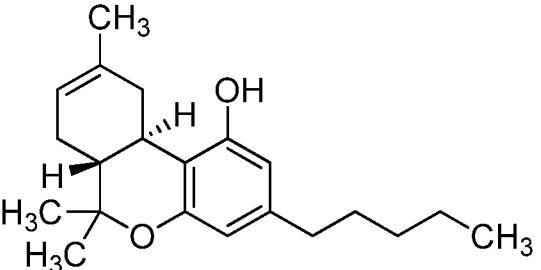
Name	Structure	Chemical properties/ [M+H] ⁺ ESI MS
<p>Δ^9-tetrahydrocannabinol (THC)</p>		<p>Psychoactive, decarboxylation product of THCA <i>m/z</i> 315.2319</p>
<p>Δ^9-tetrahydrocannabinolic acid (THCA)</p>		<p><i>m/z</i> 359.2217</p>

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Name	Structure	Chemical properties/ [M+H] ⁺ ESI MS
cannabidiol (CBD)		decarboxylation product of CBDA <i>m/z</i> 315.2319
cannabidiolic acid (CBDA)		 <i>m/z</i> 359.2217
cannabigerol (CBG)		Non-intoxicating, decarboxylation product of CBGA <i>m/z</i> 317.2475
cannabigerolic acid (CBGA)		 <i>m/z</i> 361.2373
cannabichromene (CBC)		Non- psychotropic, converts to cannabicyclol upon light exposure <i>m/z</i> 315.2319

Name	Structure	Chemical properties/ [M+H] ⁺ ESI MS
cannabichromene acid (CBCA)		<i>m/z</i> 359.2217
cannabicyclol (CBL)		Non-psychoactive, 16 isomers known. Derived from non-enzymatic conversion of CBC <i>m/z</i> 315.2319
cannabinol (CBN)		Likely degradation product of THC <i>m/z</i> 311.2006
cannabinolic acid (CBNA)		<i>m/z</i> 355.1904

Name	Structure	Chemical properties/ [M+H] ⁺ ESI MS
tetrahydrocannabivarin (THCV)		decarboxylation product of THCVA <i>m/z</i> 287.2006
tetrahydrocannabivarinic acid (THCVA)		<i>m/z</i> 331.1904
cannabidivarin (CBDV)		<i>m/z</i> 287.2006
cannabidivarinic acid (CBDVA)		<i>m/z</i> 331.1904

Name	Structure	Chemical properties/ [M+H] ⁺ ESI MS
Δ8-tetrahydrocannabinol (d8-THC)		<i>m/z</i> 315.2319

[0039] Cannabinoids are synthesised in cannabis plants as carboxylic acids. While some decarboxylation may occur in the plant, decarboxylation typically occurs post-harvest and is increased by exposing plant material to heat (Sanchez and Verpoote, 2008, *Plant Cell Physiology*, 49(12): 1767-82). Decarboxylation is usually achieved by drying and/or heating the plant material. Persons skilled in the art would be familiar with methods by which decarboxylation of cannabinoids can be promoted, illustrative examples of which include air-drying, combustion, vaporisation, curing, heating and baking.

Cannabinoid profile

[0040] The term "cannabinoid profile" refers to a representation of the type, amount, level, ratio and/or proportion of cannabinoids that are present in the cannabis plant or part thereof, as typically measure within plant material derived from the plant or part, including an extract therefrom.

[0041] The term "enriched" is used herein to refer to a selectively higher level of one or more cannabinoids in the cannabis plant or part thereof. For example, a cannabinoid profile enriched for total CBD refers to plant material in which the amount of total CBD (total CBD and total CBDA) is greater than the amount of any of the other cannabinoids that may also be present (including constitutively present) in plant material.

[0042] The cannabinoid profile in a cannabis plant will typically predominantly comprise the acidic form of the cannabinoids, but may also comprise some decarboxylated (neutral)

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forms thereof, at various concentrations or levels at any given time (*i.e.*, at propagation, growth, harvest, drying, curing, *etc.*). Thus, the term "total cannabinoid" is used herein to refer to the decarboxylated and/or acid form of said cannabinoid. For example, "total CBD" refers to CBD and CBDA, "total THC" refers to THC and THCA, "total CBC" refers to CBC and CBCA, "total CBG" refers to CBG and CBGA, "total CBN" refers to CBN and CBNA, "total THCV" refers to THCV and THCVA, "total CBDV" refers to CBDV and CBDVA, and so forth.

[0043] The terms "level", "content", "concentration" and the like, are used interchangeably herein to describe an amount of the referenced compound, and may be represented in absolute terms (*e.g.*, mg/g, mg/ml, *etc.*) or in relative terms, such as a ratio to any or all of the other compounds in the cannabis plant material or as a percentage of the amount (*e.g.*, by weight, peak area, *etc.*) of any or all of the other compounds in the cannabis plant material.

[0044] As used herein, the term "plant material" is to be understood to mean any part of the cannabis plant, including the leaves, stems, roots, and inflorescence, or parts thereof, as described elsewhere herein, as well as extracts, illustrative examples of which include kief or hash, which includes trichomes and glands. In an embodiment, the plant material is female inflorescence.

[0045] The term "inflorescence" as used herein means the complete flower head of the cannabis plant, comprising stems, stalks, bracts, flowers and trichomes (*i.e.*, glandular, sessile and stalked trichomes). In an embodiment, the plant part is female inflorescence.

[0046] " Δ -9-tetrahydrocannabinolic acid" or "THCA" is a derivative of cannabigerolic acid (CBGA), which is converted to THCA by THCA synthase. The neutral form " Δ -9-tetrahydrocannabinol" is associated with psychoactive effects of cannabis, which are primarily mediated by its activation of CB1G-protein coupled receptors, which result in a decrease in the concentration of cyclic AMP (cAMP) through the inhibition of adenylate cyclase. THC also exhibits partial agonist activity at the cannabinoid receptors CB1 and CB2. CB1 is mainly associated with the central nervous system, while CB2 is expressed predominantly in the cells of the immune system. As a result, THC is also associated with pain relief, relaxation, fatigue,

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appetite stimulation, and alteration of the visual, auditory and olfactory senses. Furthermore, more recent studies have indicated that THC mediates an anti-cholinesterase action, which may suggest its use for the treatment of Alzheimer's disease and myasthenia (Eubanks *et al.*, 2006, *Molecular Pharmaceuticals*, 3(6): 773-7).

[0047] In an embodiment, the level of total THC is at least 80%, preferably at least 81%, preferably at least 82%, preferably at least 83%, preferably at least 84%, preferably at least 85%, preferably at least 86%, preferably at least 87%, preferably at least 88%, preferably at least 89%, preferably at least 90%, preferably at least 91%, preferably at least 92%, preferably at least 93%, preferably at least 94%, preferably at least 95%, preferably at least 96%, preferably at least 97%, preferably at least 98%, or more preferably at least 99% by weight of the total cannabinoid content of the dry weight of plant material.

[0048] "Cannabigerolic acid" or "CBGA" is the common precursor of both THCA and cannabidiolic acid" or "CBDA". Its neutral form, "cannabigerol" or "CBG" has relatively weak agonistic effect on the CB1 and CB2 receptors. However, CBG acts as an AEA uptake inhibitor, α -2 adrenoceptor agonist and a moderate 5-HT1A antagonist. As a result, CBG has been suggested for use as a sedative, anti-inflammatory, anti-anxiety, anti-nausea, atypical anti-psychotic, anti-fungal and as a cancer treatment.

[0049] In an embodiment, the level of total CBG is at least 1%, preferably at least 2%, preferably at least 3%, preferably at least 4%, preferably at least 5%, preferably at least 6%, preferably at least 7%, preferably at least 9%, or more preferably at least 10% by weight of the total cannabinoid content of the dry weight of plant material.

[0050] In an embodiment, total THC and total CBG are present in a ratio of from about 5:1 to about 50:1, preferably from about 6:1 to about 50:1, preferably from about 7:1 to about 50:1, preferably from about 8:1 to about 50:1, preferably from about 9:10 to about 50:1, or more preferably from about 10:1 to about 50:1 (THC: CBG).

[0051] The cannabis plant described herein comprises plant material comprising a cannabinoid profile that is characterised by high levels of total THC, but is also relatively high levels of total CBG. Accordingly, the cannabis plant of the present disclosure may be

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variously described as “high THC/high CBG”, “high THC/CBG”, “THC/CBD-enriched” and the like. Those skilled in the art would understand this terminology to mean a cannabis plant that produced higher levels of THC and THCA and CBG and CBGA relative to the level of other cannabinoids, such as CBD and CBDA.

[0052] The reference cannabinoids disclosed herein may be alternatively described as “minor cannabinoids” or “secondary cannabinoids”.

[0053] Minor cannabinoids have been shown to exhibit unique medicinal properties. For example, CBDV has been given orphan designation by the European Medicines Agency for use in the treatment of Rett Syndrome and Fragile X Syndrome (EU/3/17/1921). THCV has also been recognised as new potential treatment against obesity-associated glucose intolerance (Wargent *et al.*, 2013, *Nutrition & Diabetes*, 3: e68). The therapeutic applications of other minor cannabinoids, such as CBC, CBG and CBN have also been reviewed by, for example, Izzo *et al.* (2009, *Trends in Pharmacological Sciences*, 30(10): 515-527) and Morabito *et al.* (2013, *Current Addiction Reports*, 3(2): 230-238).

[0054] In an embodiment, the reference cannabinoid is total CBD. In another embodiment, total THC and total CBG (THC+CBG) are present in a ratio of from about 50:1 to about 500:1 to the level of total CBD, preferably from about 50:1 to about 490:1, preferably from about 50:1 to about 480:1, preferably from about 50:1 to about 470:1, preferably from about 50:1 to about 460:1, preferably from about 50:1 to about 450:1, preferably from about 50:1 to about 440:1, preferably from about 50:1 to about 430:1, preferably from about 50:1 to about 420:1, preferably from about 50:1 to about 410:1, preferably about 50:1 to about 400:1, preferably from about 60:1 to 500:1, preferably from about 70:1 to 500:1, preferably from about 80:1 to 500:1, preferably from about 90:1 to about 500:1, or more preferably from about 100:1 to about 400:1 (THC+CBG: CBD).

[0055] In another embodiment, the level of total CBD is from about 0.01% to about 1%, preferably from about 0.02% to about 1%, preferably from about 0.03% to about 1%, preferably from about 0.04% to about 1%, preferably from about 0.05% to about 1%, preferably from about 0.06% to about 1%, preferably from about 0.07% to about 1%, preferably from about 0.08% to about 1%, preferably from about 0.09% to about 1%, or more

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preferably from about 0.1% to 1% by weight of the total cannabinoid content of the dry weight of plant material.

[0056] In an embodiment, the reference cannabinoid is total CBC. In another embodiment, THC+CBG are present in a ratio of from about 10:1 to about 120:1 to the level of total CBC, preferably from about 11:1 to about 120:1, preferably from about 12:1 to about 120:1, preferably from about 13:1 to about 120:1, preferably from about 14:1 to about 120:1, preferably from about 15:1 to about 120:1, preferably from about 10:1 to about 119:1, preferably from about 10:1 to about 118:1, preferably from about 10:1 to about 117:1, preferably from about 10:1 to about 116:1, preferably from about 10:1 to about 115:1, or more preferably from about 15:1 to about 115:1 (CBD+THC: CBC).

[0057] In another embodiment, the level of total CBC is from about 0.01% to about 10%, preferably from about 0.02% to about 10%, preferably from about 0.03% to about 10%, preferably from about 0.04% to about 10%, preferably from about 0.05% to about 10%, preferably from about 0.06% to about 10%, preferably from about 0.07% to about 10%, preferably from about 0.08% to about 10%, preferably from about 0.09% to about 10%, or more preferably from about 0.1% to about 10% by weight of the total cannabinoid content of the dry weight of plant material.

[0058] In an embodiment, the reference cannabinoid is total CBN. In another embodiment, THC+CBG are present in a ratio of from about 200:1 to about 1500:1 to the level of total CBN, preferably from about 200:1 to about 1400:1, or more preferably from about 200:1 to about 1300:1 (CBG+THC: CBN).

[0059] In another embodiment, the level of total CBN is from about 0.01% to about 1%, preferably from about 0.01% to about 0.9%, preferably from about 0.01% to about 0.8%, preferably from about 0.01% to about 0.7%, preferably from about 0.01% to about 0.6%, or more preferably from about 0.01% to about 0.5% by weight of the total cannabinoid content of the dry weight of plant material.

[0060] In an embodiment, the reference cannabinoid is total THCV. In another embodiment, THC+CBG are present in a ratio of from about 50:1 to about 700:1 to the level

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of total THC_V, preferably from about 60:1 to about 700:1, preferably from about 70:1 to about 700:1, preferably from about 80:1 to about 700:1, preferably from about 90:1 to about 700:1, preferably from about 50:1 to about 650:1, preferably from about 50:1 to about 600:1, preferably from about 50:1 to about 550:1, or more preferably from about 90:1 to about 550:1 (CBD+THC: THC_V).

[0061] In another embodiment, the level of total THC_V is from about 0.01% to about 1%, preferably from about 0.02% to about 1%, preferably from about 0.03% to about 1%, preferably from about 0.04% to about 1%, preferably from about 0.05% to about 1%, preferably from about 0.06% to about 1%, preferably from about 0.07% to about 1%, preferably from about 0.08% to about 1%, preferably from about 0.09% to about 1%, or more preferably from about 0.1% to about 1% by weight of the total cannabinoid content of the dry weight of plant material.

[0062] In an embodiment, the cannabinoid profile does not include total CBD_V, wherein total CBD_V comprises cannabidivarin (CBD_V) and cannabidivarinic acid (CBD_VA).

[0063] In an embodiment, the cannabis plant comprises:

- (i) a level of total THC of at least 80%, preferably at least 81%, preferably at least 82%, preferably at least 83%, preferably at least 84%, preferably at least 85%, preferably at least 86%, preferably at least 87%, preferably at least 88%, preferably at least 89%, preferably at least 90%, preferably at least 91%, preferably at least 92%, preferably at least 93%, preferably at least 94%, preferably at least 95%, preferably at least 96%, preferably at least 97%, preferably at least 98%, or more preferably at least 99% by weight of the total cannabinoid content of the dry weight of plant material; and
- (ii) a level of total CBG of at least 1%, preferably at least 2%, preferably at least 3%, preferably at least 4%, preferably at least 5%, preferably at least 6%, preferably at least 7%, preferably at least 9%, or more preferably at least 10% by weight of the total cannabinoid content of the dry weight of plant material;
- (iii) optionally a level of total CBD of from about 0.01% to about 1%, preferably from about 0.02% to about 1%, preferably from about 0.03% to about 1%, preferably from about 0.04% to about 1%, preferably from about 0.05% to about 1%, preferably from

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about 0.06% to about 1%, preferably from about 0.07% to about 1%, preferably from about 0.08% to about 1%, preferably from about 0.09% to about 1%, or more preferably from about 0.1% to 1% by weight of the total cannabinoid content of the dry weight of plant material;

- (iv) optionally a level of total CBC of from about 0.01% to about 10%, preferably from about 0.02% to about 10%, preferably from about 0.03% to about 10%, preferably from about 0.04% to about 10%, preferably from about 0.05% to about 10%, preferably from about 0.06% to about 10%, preferably from about 0.07% to about 10%, preferably from about 0.08% to about 10%, preferably from about 0.09% to about 10%, or more preferably from about 0.1% to about 10% by weight of the total cannabinoid content of the dry weight of plant material;
- (v) optionally a level of total CBN of from about 0.01% to about 1%, preferably from about 0.01% to about 0.9%, preferably from about 0.01% to about 0.8%, preferably from about 0.01% to about 0.7%, preferably from about 0.01% to about 0.6%, or more preferably from about 0.01% to about 0.5% by weight of the total cannabinoid content of the dry weight of plant material; and
- (vi) optionally a level of total THCV of from about 0.01% to about 1%, preferably from about 0.02% to about 1%, preferably from about 0.03% to about 1%, preferably from about 0.04% to about 1%, preferably from about 0.05% to about 1%, preferably from about 0.06% to about 1%, preferably from about 0.07% to about 1%, preferably from about 0.08% to about 1%, preferably from about 0.09% to about 1%, or more preferably from about 0.1% to about 1% by weight of the total cannabinoid content of the dry weight of plant material.

[0064] In an embodiment, the cannabis plant comprises:

- (i) a level of total THC of at least 80%, preferably at least 81%, preferably at least 82%, preferably at least 83%, preferably at least 84%, preferably at least 85%, preferably at least 86%, preferably at least 87%, preferably at least 88%, preferably at least 89%, preferably at least 90%, preferably at least 91%, preferably at least 92%, preferably at least 93%, preferably at least 94%, preferably at least 95%, preferably at least 96%, preferably at least 97%, preferably at least 98%, or more preferably at least 99% by weight of the total cannabinoid content of the dry weight of plant material; and

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- (ii) the level of total CBG of at least 1%, preferably at least 2%, preferably at least 3%, preferably at least 4%, preferably at least 5%, preferably at least 6%, preferably at least 7%, preferably at least 9%, or more preferably at least 10% by weight of the total cannabinoid content of the dry weight of plant material.

[0065] In an embodiment, the cannabis plant comprises:

- (i) a level of total THC of at least 80%, preferably at least 81%, preferably at least 82%, preferably at least 83%, preferably at least 84%, preferably at least 85%, preferably at least 86%, preferably at least 87%, preferably at least 88%, preferably at least 89%, preferably at least 90%, preferably at least 91%, preferably at least 92%, preferably at least 93%, preferably at least 94%, preferably at least 95%, preferably at least 96%, preferably at least 97%, preferably at least 98%, or more preferably at least 99% by weight of the total cannabinoid content of the dry weight of plant material;
- (ii) the level of total CBG of at least 1%, preferably at least 2%, preferably at least 3%, preferably at least 4%, preferably at least 5%, preferably at least 6%, preferably at least 7%, preferably at least 9%, or more preferably at least 10% by weight of the total cannabinoid content of the dry weight of plant material; and
- (iii) a level of total CBD of from about 0.01% to about 1%, preferably from about 0.02% to about 1%, preferably from about 0.03% to about 1%, preferably from about 0.04% to about 1%, preferably from about 0.05% to about 1%, preferably from about 0.06% to about 1%, preferably from about 0.07% to about 1%, preferably from about 0.08% to about 1%, preferably from about 0.09% to about 1%, or more preferably from about 0.1% to 1% by weight of the total cannabinoid content of the dry weight of plant material.

[0066] In an embodiment, the cannabis plant comprises:

- (i) a level of total THC of at least 80%, preferably at least 81%, preferably at least 82%, preferably at least 83%, preferably at least 84%, preferably at least 85%, preferably at least 86%, preferably at least 87%, preferably at least 88%, preferably at least 89%, preferably at least 90%, preferably at least 91%, preferably at least 92%, preferably at least 93%, preferably at least 94%, preferably at least 95%, preferably at least 96%, preferably at least 97%, preferably at least 98%, or more preferably at least 99% by

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- weight of the total cannabinoid content of the dry weight of plant material;
- (ii) the level of total CBG of at least 1%, preferably at least 2%, preferably at least 3%, preferably at least 4%, preferably at least 5%, preferably at least 6%, preferably at least 7%, preferably at least 9%, or more preferably at least 10% by weight of the total cannabinoid content of the dry weight of plant material;
 - (iii) a level of total CBD of from about 0.01% to about 1%, preferably from about 0.02% to about 1%, preferably from about 0.03% to about 1%, preferably from about 0.04% to about 1%, preferably from about 0.05% to about 1%, preferably from about 0.06% to about 1%, preferably from about 0.07% to about 1%, preferably from about 0.08% to about 1%, preferably from about 0.09% to about 1%, or more preferably from about 0.1% to 1% by weight of the total cannabinoid content of the dry weight of plant material; and
 - (iv) a level of total CBC of from about 0.01% to about 10%, preferably from about 0.02% to about 10%, preferably from about 0.03% to about 10%, preferably from about 0.04% to about 10%, preferably from about 0.05% to about 10%, preferably from about 0.06% to about 10%, preferably from about 0.07% to about 10%, preferably from about 0.08% to about 10%, preferably from about 0.09% to about 10%, or more preferably from about 0.1% to about 10% by weight of the total cannabinoid content of the dry weight of plant material.

[0067] In an embodiment, the cannabis plant comprises:

- (i) a level of total THC of at least 80%, preferably at least 81%, preferably at least 82%, preferably at least 83%, preferably at least 84%, preferably at least 85%, preferably at least 86%, preferably at least 87%, preferably at least 88%, preferably at least 89%, preferably at least 90%, preferably at least 91%, preferably at least 92%, preferably at least 93%, preferably at least 94%, preferably at least 95%, preferably at least 96%, preferably at least 97%, preferably at least 98%, or more preferably at least 99% by weight of the total cannabinoid content of the dry weight of plant material;
- (ii) the level of total CBG of at least 1%, preferably at least 2%, preferably at least 3%, preferably at least 4%, preferably at least 5%, preferably at least 6%, preferably at least 7%, preferably at least 9%, or more preferably at least 10% by weight of the total cannabinoid content of the dry weight of plant material;

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- (iii) a level of total CBD of from about 0.01% to about 1%, preferably from about 0.02% to about 1%, preferably from about 0.03% to about 1%, preferably from about 0.04% to about 1%, preferably from about 0.05% to about 1%, preferably from about 0.06% to about 1%, preferably from about 0.07% to about 1%, preferably from about 0.08% to about 1%, preferably from about 0.09% to about 1%, or more preferably from about 0.1% to 1% by weight of the total cannabinoid content of the dry weight of plant material;
- (iv) a level of total CBC of from about 0.01% to about 10%, preferably from about 0.02% to about 10%, preferably from about 0.03% to about 10%, preferably from about 0.04% to about 10%, preferably from about 0.05% to about 10%, preferably from about 0.06% to about 10%, preferably from about 0.07% to about 10%, preferably from about 0.08% to about 10%, preferably from about 0.09% to about 10%, or more preferably from about 0.1% to about 10% by weight of the total cannabinoid content of the dry weight of plant material; and
- (v) a level of total CBN of from about 0.01% to about 1%, preferably from about 0.01% to about 0.9%, preferably from about 0.01% to about 0.8%, preferably from about 0.01% to about 0.7%, preferably from about 0.01% to about 0.6%, or more preferably from about 0.01% to about 0.5% by weight of the total cannabinoid content of the dry weight of plant material.

[0068] In an embodiment, the cannabis plant comprises:

- (i) a level of total THC of at least 80%, preferably at least 81%, preferably at least 82%, preferably at least 83%, preferably at least 84%, preferably at least 85%, preferably at least 86%, preferably at least 87%, preferably at least 88%, preferably at least 89%, preferably at least 90%, preferably at least 91%, preferably at least 92%, preferably at least 93%, preferably at least 94%, preferably at least 95%, preferably at least 96%, preferably at least 97%, preferably at least 98%, or more preferably at least 99% by weight of the total cannabinoid content of the dry weight of plant material;
- (ii) the level of total CBG of at least 1%, preferably at least 2%, preferably at least 3%, preferably at least 4%, preferably at least 5%, preferably at least 6%, preferably at least 7%, preferably at least 9%, or more preferably at least 10% by weight of the total cannabinoid content of the dry weight of plant material;

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- (iii) a level of total CBD of from about 0.01% to about 1%, preferably from about 0.02% to about 1%, preferably from about 0.03% to about 1%, preferably from about 0.04% to about 1%, preferably from about 0.05% to about 1%, preferably from about 0.06% to about 1%, preferably from about 0.07% to about 1%, preferably from about 0.08% to about 1%, preferably from about 0.09% to about 1%, or more preferably from about 0.1% to 1% by weight of the total cannabinoid content of the dry weight of plant material;
- (vi) a level of total CBC of from about 0.01% to about 10%, preferably from about 0.02% to about 10%, preferably from about 0.03% to about 10%, preferably from about 0.04% to about 10%, preferably from about 0.05% to about 10%, preferably from about 0.06% to about 10%, preferably from about 0.07% to about 10%, preferably from about 0.08% to about 10%, preferably from about 0.09% to about 10%, or more preferably from about 0.1% to about 10% by weight of the total cannabinoid content of the dry weight of plant material; and
- (iv) a level of total CBN of from about 0.01% to about 1%, preferably from about 0.01% to about 0.9%, preferably from about 0.01% to about 0.8%, preferably from about 0.01% to about 0.7%, preferably from about 0.01% to about 0.6%, or more preferably from about 0.01% to about 0.5% by weight of the total cannabinoid content of the dry weight of plant material.

[0069] In an embodiment, the cannabis plant comprises:

- (i) a level of total THC of at least 80%, preferably at least 81%, preferably at least 82%, preferably at least 83%, preferably at least 84%, preferably at least 85%, preferably at least 86%, preferably at least 87%, preferably at least 88%, preferably at least 89%, preferably at least 90%, preferably at least 91%, preferably at least 92%, preferably at least 93%, preferably at least 94%, preferably at least 95%, preferably at least 96%, preferably at least 97%, preferably at least 98%, or more preferably at least 99% by weight of the total cannabinoid content of the dry weight of plant material;
- (ii) the level of total CBG of at least 1%, preferably at least 2%, preferably at least 3%, preferably at least 4%, preferably at least 5%, preferably at least 6%, preferably at least 7%, preferably at least 9%, or more preferably at least 10% by weight of the total cannabinoid content of the dry weight of plant material;

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- (iii) a level of total CBD of from about 0.01% to about 1%, preferably from about 0.02% to about 1%, preferably from about 0.03% to about 1%, preferably from about 0.04% to about 1%, preferably from about 0.05% to about 1%, preferably from about 0.06% to about 1%, preferably from about 0.07% to about 1%, preferably from about 0.08% to about 1%, preferably from about 0.09% to about 1%, or more preferably from about 0.1% to 1% by weight of the total cannabinoid content of the dry weight of plant material;
- (iv) a level of total CBC of from about 0.01% to about 10%, preferably from about 0.02% to about 10%, preferably from about 0.03% to about 10%, preferably from about 0.04% to about 10%, preferably from about 0.05% to about 10%, preferably from about 0.06% to about 10%, preferably from about 0.07% to about 10%, preferably from about 0.08% to about 10%, preferably from about 0.09% to about 10%, or more preferably from about 0.1% to about 10% by weight of the total cannabinoid content of the dry weight of plant material;
- (v) a level of total CBN of from about 0.01% to about 1%, preferably from about 0.01% to about 0.9%, preferably from about 0.01% to about 0.8%, preferably from about 0.01% to about 0.7%, preferably from about 0.01% to about 0.6%, or more preferably from about 0.01% to about 0.5% by weight of the total cannabinoid content of the dry weight of plant material; and
- (vi) a level of total THCV of from about 0.01% to about 1%, preferably from about 0.02% to about 1%, preferably from about 0.03% to about 1%, preferably from about 0.04% to about 1%, preferably from about 0.05% to about 1%, preferably from about 0.06% to about 1%, preferably from about 0.07% to about 1%, preferably from about 0.08% to about 1%, preferably from about 0.09% to about 1%, or more preferably from about 0.1% to about 1% by weight of the total cannabinoid content of the dry weight of plant material.

[0070] In an embodiment, the cannabis plant comprises:

- (i) a level of total THC of at least 80%, preferably at least 81%, preferably at least 82%, preferably at least 83%, preferably at least 84%, preferably at least 85%, preferably at least 86%, preferably at least 87%, preferably at least 88%, preferably at least 89%, preferably at least 90%, preferably at least 91%, preferably at least 92%, preferably at

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- least 93%, preferably at least 94%, preferably at least 95%, preferably at least 96%, preferably at least 97%, preferably at least 98%, or more preferably at least 99% by weight of the total cannabinoid content of the dry weight of plant material; and/or
- (ii) the level of total CBG of at least 1%, preferably at least 2%, preferably at least 3%, preferably at least 4%, preferably at least 5%, preferably at least 6%, preferably at least 7%, preferably at least 9%, or more preferably at least 10% by weight of the total cannabinoid content of the dry weight of plant material; and/or
 - (iii) a level of total CBD of from about 0.01% to about 1%, preferably from about 0.02% to about 1%, preferably from about 0.03% to about 1%, preferably from about 0.04% to about 1%, preferably from about 0.05% to about 1%, preferably from about 0.06% to about 1%, preferably from about 0.07% to about 1%, preferably from about 0.08% to about 1%, preferably from about 0.09% to about 1%, or more preferably from about 0.1% to 1% by weight of the total cannabinoid content of the dry weight of plant material; and/or
 - (iv) a level of total CBC of from about 0.01% to about 10%, preferably from about 0.02% to about 10%, preferably from about 0.03% to about 10%, preferably from about 0.04% to about 10%, preferably from about 0.05% to about 10%, preferably from about 0.06% to about 10%, preferably from about 0.07% to about 10%, preferably from about 0.08% to about 10%, preferably from about 0.09% to about 10%, or more preferably from about 0.1% to about 10% by weight of the total cannabinoid content of the dry weight of plant material; and/or
 - (v) a level of total CBN of from about 0.01% to about 1%, preferably from about 0.01% to about 0.9%, preferably from about 0.01% to about 0.8%, preferably from about 0.01% to about 0.7%, preferably from about 0.01% to about 0.6%, or more preferably from about 0.01% to about 0.5% by weight of the total cannabinoid content of the dry weight of plant material; and/or
 - (vi) a level of total THCV of from about 0.01% to about 1%, preferably from about 0.02% to about 1%, preferably from about 0.03% to about 1%, preferably from about 0.04% to about 1%, preferably from about 0.05% to about 1%, preferably from about 0.06% to about 1%, preferably from about 0.07% to about 1%, preferably from about 0.08% to about 1%, preferably from about 0.09% to about 1%, or more preferably from about

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0.1% to about 1% by weight of the total cannabinoid content of the dry weight of plant material.

[0071] As noted elsewhere herein, the inventors have surprisingly shown that the cannabis plant described herein comprises a THC- and CBG-enriched cannabinoid profile. This is unexpected because CBG is a relatively low abundance cannabinoid, particularly when compared to other major cannabinoids, such as CBD.

[0072] In another aspect disclosed herein, there is provided a seed of the cannabis plant described herein. As used herein, "seed" refers to immature seeds which are developing *in planta*. According to another aspect disclosed herein, there is provided a cannabis plant, or a part thereof, which is produced from the seed.

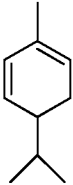
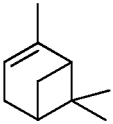
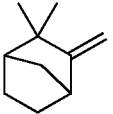
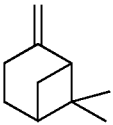
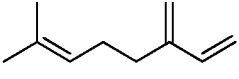
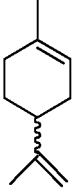
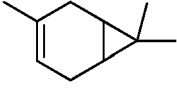
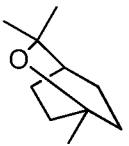
[0073] In another aspect, there is provided a seed of the cannabis plants described herein. As used herein, "seed" refers to immature seeds which are developing *in planta*. According to another aspect disclosed herein, there is provided a progeny plant, or a part thereof, which is produced from the seed.

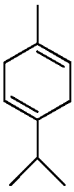
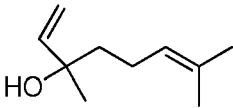
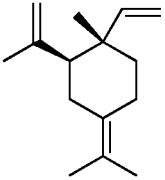
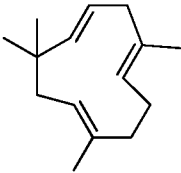
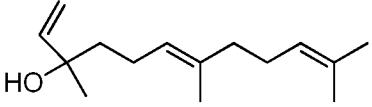
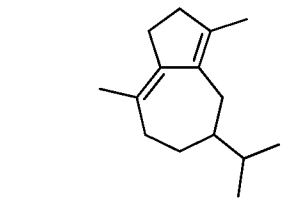
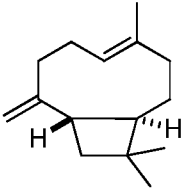
Terpenes

[0074] The term "terpene" as used herein, refers to a class of organic hydrocarbon compounds, which are produced by a variety of plants. Cannabis plants produce and accumulate different terpenes, such as monoterpenes and sesquiterpenes, in the glandular trichomes of the female inflorescence. The term "terpene" includes "terpenoids" or "isoprenoids", which are modified terpenes that contain additional functional groups.

[0075] Terpenes are responsible for much of the scent of cannabis flowers and contribute to the unique flavor qualities of cannabis products. Terpenes will be known to persons skilled in the art, illustrative examples of which are provided in Table 2.

Table 2. Terpenes and their properties

Name	Structure	Mass/Charge number (m/z)*
α -Phellandrene		m/z 93.0
α -Pinene (+/-)		m/z 93.0
Camphene		m/z 93.0
β -Pinene (+/-)		m/z 93.0
Myrcene		m/z 93.0
Limonene		m/z 68.1
3-Carene		
Eucalyptol		m/z 81.0

Name	Structure	Mass/Charge number (<i>m/z</i>)*
γ -Terpinene		<i>m/z</i> 93.1
Linalool		<i>m/z</i> 93.0
γ -Elemene		<i>m/z</i> 121.0
Humulene		<i>m/z</i> 93.0
Nerolidol		<i>m/z</i> 222.4
Guaia-3,9-diene		<i>m/z</i> 161.1
Caryophyllene		<i>m/z</i> 69.2

*The molecular ion is not necessarily seen for all compounds

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[0076] Terpene biosynthesis in plants typically involves two pathways to produce the general 5-carbon isoprenoid diphosphate precursors of all terpenes: the plastidial methylerythritol phosphate (MEP) pathway and the cytosolic mevalonate (MEV) pathway. These pathways control the different substrate pools available for terpene synthases (TPS).

[0077] Terpenes have been shown to exhibit unique medicinal properties as described, for example, by Brahmshatriya and Brahmshatriya (2013, in Ramawat and Mérillon (eds), *Natural Products*, Springer, Berlin, Heidelberg).

Terpene Profile

[0078] The term “terpene profile” as used herein refers to a representation of the type, amount, level, ratio and/or proportion of terpenes that are present in the cannabis plant or part thereof, as typically measured within plant material derived from the plant or plant part, including an extract therefrom.

[0079] The terpene profile in a cannabis plant will be determined based on genetic, environmental and developmental factors, therefore particular terpenes may be present at various amounts, levels, ratios and/or proportions at any given time (*i.e.*, at propagation, growth, harvest, drying, curing, *etc.*).

[0080] In an embodiment, the terpene profile comprises monoterpenes and sesquiterpenes.

[0081] Monoterpenes consist of two isoprene units and may be linear or contain ring structures. The primary function of monoterpenes is to protect plants from infection by fungal and bacterial pathogens and insect pests. Monoterpenes would be known to persons skilled in the art, illustrative embodiments of which include α -phellandrene, α -pinene, camphene, β -pinene, myrcene, limonene, eucalyptol, γ -terpinene and linalool.

[0082] Sesquiterpenes differ from other common terpenes as they contain one additional isoprene unit, which creates a 15 carbon structure. The primary function of sesquiterpenes is as a pheromone for the bud and flower. Sesquiterpenes would be known to persons skilled in

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the art, illustrative embodiments of which include γ -elemene, humulene, nerolidol, guaia-3,9-diene and caryophyllene.

[0083] In an embodiment, the terpene profile comprises a level of monoterpenes that correlates with the level of total THC. In a preferred embodiment, the terpene profile comprises a high level of monoterpenes that correlates to a high level of total THC.

[0084] In an embodiment, the terpene profile in the cannabis plant comprises terpenes selected from the group consisting of α -phellandrene, α -pinene, camphene, β -pinene, myrcene, limonene, eucalyptol, γ -terpinene, linalool, γ -elemene, humulene, nerolidol, guaia-3,9-diene and caryophyllene.

[0085] In a preferred embodiment, the terpene profile in the cannabis plant comprises terpenes selected from the group consisting of myrcene and β -pinene.

[0086] “Myrcene” is a monoterpinoid derivative of β -pinene. Myrcene has been associated with the therapeutic or medicinal effects of cannabis and has been suggested for use as a sedative, hypnotic, analgesic and muscle relaxant. Myrcene is also hypothesised to attenuate the activity of other cannabinoids and terpenes as part of the “entourage effect” as described in, for example, Russo, 2011, *British Journal of Pharmacology*, 163(7): 1344-1364.

[0087] “ β -pinene” is a monoterpene that is characterised by a woody-green, pine-like smell. β -pinene has been shown to act as a topical antiseptic and a bronchodilator. β -pinene is also capable of crossing the blood-brain barrier and it is hypothesised that β -pinene inhibits the influence of THC as part of the entourage effect, as described elsewhere herein.

[0088] In an embodiment, the level of myrcene is present at a ratio of from about 100:1 and about 1:1 to the level of β -pinene. The range “from about 100:1 to about 1:1” includes, for example, 100:1, 99:1, 98:1, 97:1, 96:1, 95:1, 94:1, 93:1, 92:1, 91:1, 90:1, 89:1, 88:1, 87:1, 86:1, 85:1, 84:1, 83:1, 82:1, 81:1, 80:1, 79:1, 78:1, 77:1, 76:1, 75:1, 74:1, 73:1, 72:1, 71:1, 70:1, 69:1, 68:1, 67:1, 66:1, 65:1, 64:1, 63:1, 62:1, 61:1, 60:1, 59:1, 58:1, 57:1, 56:1, 55:1, 54:1, 53:1, 52:1, 51:1, 50:1, 49:1, 48:1, 47:1, 46:1, 45:1, 44:1, 43:1, 42:1, 41:1, 40:1, 39:1, 38:1, 37:1, 36:1, 35:1, 34:1, 33:1, 32:1, 31:1, 30:1, 29:1, 28:1, 27:1, 26:1, 25:1, 24:1, 23:1,

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22:1, 21:1, 20:1, 19:1, 18:1, 17:1, 16:1, 15:1, 14:1, 13:1, 12:1, 11:1, 10:1, 9:1, 8:1, 7:1, 6:1, 5:1 and 4:1. Thus, in an embodiment, the ratio of the level of myrcene to the level of β -pinene is about preferably about 100:1, preferably about 99:1, preferably about 98:1, preferably about 97:1, preferably about 96:1, preferably about 95:1, preferably about 94:1, preferably about 93:1, preferably about 92:1, preferably about 91:1, preferably about 90:1, preferably about 89:1, preferably about 88:1, preferably about 87:1, preferably about 86:1, preferably about 85:1, preferably about 84:1, preferably about 83:1, preferably about 82:1, preferably about 81:1, preferably about 80:1, preferably about 79:1, preferably about 78:1, preferably about 77:1, preferably about 76:1, preferably about 75:1, preferably about 74:1, preferably about 73:1, preferably about 72:1, preferably about 71:1, preferably about 70:1, preferably about 69:1, preferably about 68:1, preferably about 67:1, preferably about 66:1, preferably about 65:1, preferably about 64:1, preferably about 63:1, preferably about 62:1, preferably about 61:1, preferably about 60:1, preferably about 59:1, preferably about 58:1, preferably about 57:1, preferably about 56:1, preferably about 55:1, preferably about 54:1, preferably about 53:1, preferably about 52:1, preferably about 51:1, preferably about 50:1, preferably about 49:1, preferably about 48:1, preferably about 47:1, preferably about 46:1, preferably about 45:1, preferably about 44:1, preferably about 43:1, preferably about 42:1, preferably about 41:1, preferably about 40:1, preferably about 39:1, preferably about 38:1, preferably about 37:1, preferably about 36:1, preferably about 35:1, preferably about 34:1, preferably about 33:1, preferably about 32:1, preferably about 31:1, preferably about 30:1, preferably about 29:1, preferably about 28:1, preferably about 27:1, preferably about 26:1, preferably about 25:1, preferably about 24:1, preferably about 23:1, preferably about 22:1, preferably about 21:1, preferably about 20:1, preferably about 19:1, preferably about 18:1, preferably about 17:1, preferably about 16:1, preferably about 15:1, preferably about 14:1, preferably about 13:1, preferably about 12:1, preferably about 11:1, preferably about 10:1, preferably about 9:1, preferably about 8:1, preferably about 7:1, preferably about 6:1, preferably about 5:1, preferably about 4:1, preferably about 3:1, preferably about 2:1, or more preferably about 1:1.

[0089] In an embodiment, the level of myrcene is present at a ratio of from about 60:1 and about 1:1 to the level of β -pinene.

Tissue Culture

[0090] In an aspect disclosed herein, there is provided a tissue culture of regenerable cells derived from the cannabis plant described herein, or a part thereof. In another aspect, there is provided a cannabis plant generated from the tissue culture, wherein the plant expresses the morphological and physiological characteristics of the cannabis plant described herein.

[0091] As used herein, the phrase "tissue culture" refers to a population of cells or protoplasts, including plant calli and plant tissue clumps, derived from the cannabis plant, or a part thereof described herein that are maintained *in vitro*, and from which a further cannabis plants can be generated.

[0092] Suitable techniques for establishing a tissue culture and regenerating plants therefrom will be well known to persons skilled in the art, illustrative examples of which are described in Vasil (1984), *Cell Culture and Somatic Cell Genetics of Plants*, Vol I, II, III Laboratory Procedures and Their Applications, Academic Press, New York; Green *et al.* (1987), *Plant Tissue and Cell Culture*, Academic Press, New York; Weissbach and Weissbach (1989), *Methods for Plant Molecular Biology*, Academic Press; Gelvin *et al.* (1990), *Plant Molecular Biology Manual*, Kluwer Academic Publishers; Evans *et al.* (1983) *Handbook of Plant Cell Culture*, MacMillan Publishing Company, New York.

[0093] In an embodiment, the tissue culture comprises a population of cells or protoplast of a part selected from the group consisting of seeds, leaves, stems, pollen, anthers, ovules, embryos, preferably cotyledons or hypocotyls. In a preferred embodiment, the regenerable cannabis cells are from the scutellum of immature embryos, mature embryo, callus derived from these, or meristematic tissue.

Breeding Techniques

[0094] In another aspect, there is provided a method for producing an F1 hybrid cannabis plant, the method comprising crossing the cannabis plant, as described herein, with a different cannabis plant to produce an F1 hybrid.

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[0095] By way of example, the cannabis plant described herein, is manually crossed with other cannabis plants. The resulting "Filial generation 1" or "F1" plants are self-fertilised and the resulting F2 generation plants, which will typically show large variability on account of gene segregation, are planted in a selection field. These F2 plants are observed during the growing season for phenotypic traits such as health, growth, vigour, plant type, plant structure, leaf type, flowering, maturity and inflorescent yield. F2 plants with the desirable trait(s) are selected, harvested, and the female inflorescent analysed for cannabinoid profile. The seeds of the selected F2 plants can be cleaned and stored. This procedure may be repeated, whereby the selection and testing units increase from individual plants in the F2, to multiple plants containing 'lines' (descending from one mother plant) in the F5 and the number of units decrease from approximately 500 plants in the F2 to 20 lines in the F5 by selecting about 10-20% of the units in each selection cycle. The increased size of the units, whereby more seed per unit is available, allows the selection and testing in replicated trials on more than one location with a different environment and a more extensive and accurate analysing of the cannabinoid profile. The lines or candidate varieties become genotypically more homozygous and phenotypically more homogeneous by selecting similar plant types within a line and by discarding the so-called off-types from the very variable F2 generation on to the final F7 or F8 generation. Depending on the intermediate results, the plant breeder may decide to vary the procedure such as by accelerating the process by testing a particular line earlier or retesting a line. They may also select plants for further crossing with existing parent plants or with other plants resulting from the current selection procedure.

[0096] The cannabis plant and parts thereof, as herein described, including F1 and subsequent generations derived therefrom, may be further exposed to mutagenesis and/or marker assisted selection, as is known to persons skilled in the art, to generate and/or select for new plants with desirable phenotypic, chemotypic and/or genotypic profiles. This can provide non-transgenic cannabis plants that are free of exogenous nucleic acid molecule, thereby avoiding the restrictions that otherwise apply to genetically-modified organisms (GMO), including plants, in some countries/regions. Typically, a progenitor plant cell, tissue, seed or plant is exposed to mutagenesis to produce single or multiple point mutations, such as nucleotide substitutions, deletions, additions and/or codon modification.

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[0097] Methods for performing mutagenesis on plants or plant parts will be familiar to persons skilled in the art, illustrative examples of which include chemical or radiation-induced mutagenesis, for example EMS or sodium azide treatment of seed, or gamma irradiation. Chemical mutagenesis typically favours nucleotide substitutions rather than deletions. Heavy ion beam (HIB) irradiation is known as an effective technique for mutation breeding to produce new plant cultivars. Ion beam irradiation has two physical factors, the dose (gy) and LET (linear energy transfer, keV/um) for biological effects that determine the amount of DNA damage and the size of DNA deletion, and these can be adjusted according to the desired extent of mutagenesis.

[0098] Biological agents suitable for site-directed mutagenesis include enzymes that include double stranded breaks in DNA that stimulate endogenous repair mechanisms. Illustrative examples include endonucleases, zinc finger nucleases (ZFNs), TAL effector nucleases (TALENs), transposases and site-specific recombinases. ZFNs, for example, facilitate site-specific cleavage within a genome allowing endogenous or other end-joining repair mechanisms to introduce deletions or insertions to repair the gap.

[0099] Isolation of mutants may be achieved by screening mutagenised plants or seed. For example, a mutagenised population of wheat may be screened directly for the desired genotype or indirectly by screening for a phenotype (*i.e.*, cannabinoid profile). Screening directly for the genotype preferably includes assaying for the presence of mutations which may be observed in PCR assays by the absence of markers as expected when some of the genes are deleted, or heteroduplex based assays, or by deep sequencing. Screening for the phenotype may comprise quantitative analysis of cannabinoids, as provided by the Examples. Using this methodology, large populations of mutagenised cannabis strains may be screened for a desired cannabinoid profile.

[0100] Identified mutations may then be introduced into desirable genetic backgrounds by crossing the mutant with a plant of the desired genetic background and performing a suitable number of backcrosses to cross out the originally undesired parent background.

[0101] An "induced" or "introduced" mutation is to be understood to mean an artificially induced genetic variation that may be the result of chemical or radiation treatment of a

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progenitor seed or plant. Nucleotide insertional derivatives include 5' and 3' terminal fusions as well as intra-sequence insertions of single or multiple nucleotides. Insertional nucleotide sequence variants are those in which one or more nucleotides are introduced into a site in the nucleotide sequence, either at a predetermined site as is possible with ZFNs, TALENs or homologous recombination methods, or by random insertion with suitable screening of the resulting product. Deletional variants are typically characterised by the removal of one or more nucleotides from the sequence. A mutant gene may have only a single insertion of a sequence of nucleotides relative to the wild-type gene and one or more substitution mutations. Substitutional nucleotide variants are typically those in which at least one nucleotide in the sequence has been removed and a different nucleotide inserted in its place. Preferably, the number of nucleotides affected by substitutions in a mutant gene relative to the wild-type gene is no more than 10, preferably no more than 9, preferably no more than 8, preferably no more than 7, preferably no more than 6, preferably no more than 5, preferably no more than 4, preferably no more than 3, preferably no more than 2, preferably no more than 1 nucleotide.

[0102] The term "mutation", as used herein, will typically not include a silent nucleotide substitution; that is, a mutation that does not affect the activity of the gene, and therefore includes only alterations in the gene sequence which affects the gene activity. The term "polymorphism" refers to any change in the nucleotide sequence including such silent nucleotide substitutions. Screening methods may first involve screening for polymorphisms and secondly for mutations within a group of polymorphic variants.

[0103] Marker-assisted selection is a well-recognised method of selecting for heterozygous plants required when backcrossing with a recurrent parent in a classical breeding program. The population of plants in each backcross generation will be heterozygous for the gene of interest normally present in a 1: 1 ratio in a backcross population, and the molecular marker can be used to distinguish the two alleles of the gene. By extracting DNA from, for example, young shoots and testing with a specific marker for the introgressed desirable trait, early selection of plants for further backcrossing is made whilst energy and resources are concentrated on fewer plants. To further speed up the backcrossing program, the embryo from immature seeds (25 days post anthesis) may be excised and grown up on nutrient media under sterile conditions, rather than allowing full seed maturity.

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[0104] In another aspect, there is provided a method for producing a transgenic cannabis plant, the method comprising transfecting the cannabis plant described herein, or a part thereof, with a heterologous nucleic acid sequence. In another aspect, there is provided a transgenic cannabis plant produced by the methods disclosed herein, or a seed or progeny plant derived therefrom.

[0105] In an embodiment, the transduced heterologous nucleic acid sequence introduces one or more nucleic acid substitutions, deletions, or additions into the genome of the cannabis plant.

[0106] Nucleic acid constructs useful for producing the above-mentioned transgenic plants can readily be produced using standard techniques. To ensure appropriate expression of the gene encoding an mRNA of interest, the nucleic acid construct typically comprises one or more regulatory elements such as promoters, enhancers, as well as transcription termination or polyadenylation sequences. Such elements are well known in the art. The transcriptional initiation region comprising the regulatory element(s) may provide for regulated or constitutive expression in the plant. The regulatory elements may be selected from, for example, seed-specific promoters, or promoters not specific for seed cells (such as ubiquitin promoter or CaMV35S or enhanced 35S promoters). The promoter may be modulated by factors such as temperature, light or stress. Ordinarily, the regulatory elements will be provided 5' of the genetic sequence to be expressed. The construct may also contain other elements that enhance transcription such as the nos 3' or the ocs 3' polyadenylation regions or transcription terminators.

[0107] The terms "polynucleotide", "polynucleotide sequence", "nucleotide sequence", "nucleic acid" or "nucleic acid sequence" as used interchangeably herein to designate mRNA, RNA, cRNA, cDNA or DNA. The term typically refers to polymeric form of nucleotides of at least 10 bases in length, either ribonucleotides or deoxynucleotides or a modified form or either type of nucleotide. The term includes single and double stranded forms of RNA and DNA.

[0108] As used herein, the terms "encode," "encoding" and the like refer to the capacity of a nucleic acid to provide for another nucleic acid or a polypeptide. For example, a nucleic acid

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sequence is said to "encode" a polypeptide if it can be transcribed and/or translated to produce the polypeptide or if it can be processed into a form that can be transcribed and/or translated to produce the polypeptide. Such a nucleic acid sequence may include a coding sequence or both a coding sequence and a non-coding sequence. Thus, the terms "encode," "encoding" and the like include an RNA product resulting from transcription of a DNA molecule, a protein resulting from translation of an RNA molecule, a protein resulting from transcription of a DNA molecule to form an RNA product and the subsequent translation of the RNA product, or a protein resulting from transcription of a DNA molecule to provide an RNA product, processing of the RNA product to provide a processed RNA product (*e.g.*, mRNA) and the subsequent translation of the processed RNA product.

[0109] Typically, the nucleic acid construct comprises a selectable marker. Selectable markers aid in the identification and screening of plants or cells that have been transformed with the exogenous nucleic acid molecule. The selectable marker gene may provide antibiotic or herbicide resistance to the cannabis cells, or allow the utilisation of substrates such as mannose.

[0110] Preferably, the nucleic acid construct is stably incorporated into the genome of the plant. Accordingly, the nucleic acid comprises appropriate elements which allow the molecule to be incorporated into the genome, or the construct is placed in an appropriate vector which can be incorporated into a chromosome of a plant cell.

[0111] The terms "transgenic plant" and "transgenic cannabis plant", as used herein, typically refer to a plant that contains a gene construct ("transgene") not found in a wild-type plant of the same species, variety or cultivar. That is, transgenic plants (transformed plants) contain genetic material that they did not contain prior to the transformation. A "transgene" as referred to herein has the normal meaning in the art of biotechnology and refers to a genetic sequence which has been produced or altered by recombinant DNA or RNA technology and which has been introduced into a progenitor plant cell, which cell is used to produce a new plant. The transgene may include genetic sequences obtained from or derived from a plant cell, or another plant cell, or a non-plant source, or a synthetic sequence. Typically, the transgene has been introduced into the plant by human manipulation such as, for example, by

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transformation but any method can be used as one of skill in the art recognizes. The genetic material is typically stably integrated into the genome of the plant. The introduced genetic material may comprise sequences that naturally occur in the same species but in a rearranged order or in a different arrangement of elements, for example an antisense sequence or a sequence encoding a double-stranded RNA or an artificial microRNA precursor. Plants containing such sequences are included herein in "transgenic plants". Transgenic plants as defined herein include all progeny of an initial transformed and regenerated plant (T0 plant) which has been genetically modified using recombinant techniques, where the progeny comprise the transgene. Such progeny may be obtained by self-fertilisation of the primary transgenic plant or by crossing such plants with another plant of the same species. In an embodiment, the transgenic plant comprises the introduction of one or more nucleic acid substitutions, deletions or additions into the genome of the cannabis plant of the invention. In another embodiment, the transgenic plants are homozygous for each and every gene that has been introduced (transgene) so that their progeny do not segregate for the desired phenotype. Transgenic plant parts include all parts and cells of said plants which comprise the transgene such as, for example, seeds, cultured tissues, callus and protoplasts. A "non-transgenic plant", preferably a non-transgenic cannabis plant, is one which has not been genetically modified by the introduction of genetic material by recombinant DNA techniques.

[0112] In an embodiment, the transgenic plants are produced by transfecting the cannabis plant of the invention with a heterologous nucleic acid sequence.

[0113] Transformation of a nucleic acid molecule into a cell can be accomplished by any method by which a nucleic acid molecule can be inserted into the cell. Illustrative examples of suitable transformation techniques include transfection, electroporation, microinjection, lipofection, adsorption, and protoplast fusion. A recombinant cell may remain unicellular or may grow into a tissue, organ or a multicellular organism. Transformed nucleic acid molecules of the present invention can remain extrachromosomal or can integrate into one or more sites within a chromosome of the transformed (*i.e.*, recombinant) cell in such a manner that their ability to be expressed is retained. Preferred host cells are plant cells, more preferably cells of a cannabis plant.

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[0114] Any of several methods may be employed to determine the presence of a transgene in a transformed plant. For example, polymerase chain reaction (PCR) may be used to amplify sequences that are unique to the transformed plant, with detection of the amplified products by gel electrophoresis or other methods. DNA may be extracted from the plants using conventional methods and the PCR reaction carried out using primers that will distinguish the transformed and non-transformed plants. An alternative method to confirm a positive transformant is by Southern blot hybridisation, well known in the art. Cannabis plants which are transformed may also be identified (*i.e.* distinguished from non-transformed or wild-type cannabis plants) by their phenotype, the presence of a selectable marker gene, by immunoassays that detect or quantify the expression of an enzyme encoded by the transgene, or any other phenotype conferred by the transgene.

[0115] Transgenic plants, as described herein, include plants and their progeny which have been genetically modified using recombinant techniques. This would generally be to modulate the production of at least one polypeptide defined herein in the desired plant or plant organ. Transgenic plant parts include all parts and cells of said plants such as, for example, cultured tissues, callus and protoplasts. Transformed plants contain genetic material that they did not contain prior to the transformation. The genetic material is preferably stably integrated into the genome of the plant. The introduced genetic material may comprise sequences that naturally occur in the same species but in a rearranged order or in a different arrangement of elements, for example an antisense sequence. Such plants are included herein as “transgenic plants”. A “non-transgenic plant” is one which has not been genetically modified with the introduction of genetic material by recombinant DNA techniques. In a preferred embodiment, the transgenic plants are homozygous for each and every gene that has been introduced (transgene) so that their progeny do not segregate for the desired phenotype.

Cannabis extracts

[0116] In another aspect, there is provided a method of producing an extract comprising cannabinoids from a cannabis plant, the method comprising the steps of:

- (a) harvesting plant material from the cannabis plant described herein;
- (b) at least partially drying the harvested plant material of step (a); and

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(c) extracting cannabinoids from the at least partially dried plant material of step (b), thereby producing an extract comprising cannabinoids.

[0117] In an embodiment, the extract comprises a cannabinoid profile enriched for total THC and total CBG, wherein the cannabinoid profile comprises a level of total THC and a level of total CBG at a ratio from about 5:1 to about 50:1 (THC:CBG), and wherein the level of total THC and total CBG (THC+CBG) is greater than the level of a reference cannabinoid selected from the group consisting of: total CBD, total CBC, total CBN, and total THCV.

[0118] In another embodiment, the extract comprises total THC, total CBG, and one or more minor cannabinoids selected from the group consisting of: total CBD; total CBC; total CBN; total THCV; total CBL; and total Δ 8-THC, wherein the extract comprises a level of total THC and a level of total CBG at a ratio of from about 5:1 to about 50:1 (THC:CBG), and wherein the level of the one or more other cannabinoids is from about 0.01% to about 10% by weight of the total cannabinoid content of the extract.

[0119] The term "extract", as used herein, is to be understood as including a whole cannabis extract, such as resin, hash and keif, as well as substantially purified compounds isolated from the harvested plant material, such as cannabinoids, terpenes and/or flavonoids.

[0120] As used herein, "substantially purified" refers to a compound or molecule that has been isolated from other components with which it is typically associated in its native state (*i.e.*, within plant material). Preferably, the substantially purified molecule is at least 60% free, more preferably at least 75% free, and more preferably at least 90% free from other components with which it is naturally associated. By "isolated" is meant material that is substantially or essentially free from components that normally accompany it in its native state.

[0121] Persons skilled in the art would recognise that isolated cannabinoids may exist as a number of different chemical species, illustrative examples of which include salts, solvates, prodrugs, stereoisomers or tautomers thereof.

[0122] The term "drying" as used herein refers to any method for drying the plant material. Illustrative examples include air-drying, curing, and heat drying. In an embodiment,

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the plant material is dried in a temperature, light and humidity controlled environment, such as a temperature of about 21°C and a humidity of from about 38% and 45% RH. In another embodiment, heat is applied to the plant material during the drying process to cure the dried plant material. Temperatures suitable for curing dried plant material would be known to persons skilled in the art, illustrative examples of which include a temperature from about 60°C to about 225°C, preferably from about 100°C to about 150°C, preferably from about 110°C to about 130°C, or more preferably about 120°C. In an embodiment, the dried plant material is cured by heating the dried plant material at about 120°C for 2 hours.

[0123] It is to be understood that the term “dry”, “drying” and the like is not intended to mean the absence of moisture in plant material, and therefore includes any state in which at least some moisture has been removed from the plant material. Persons skilled in the art will be familiar with the extent to which cannabis plant material can be dried to allow for extraction of the desirable compound(s), including decarboxylated cannabinoids. In an embodiment, the harvested plant material is dried under conditions and for a period of time that gives rise to a loss of at least 5%, preferably at least 10%, preferably at least 20%, preferably at least 30%, preferably at least 40%, preferably at least 50%, preferably at least 60%, preferably at least 70%, preferably at least 80%, preferably at least 90%, preferably at least 91%, preferably at least 92%, preferably at least 93%, preferably at least 94%, preferably at least 95%, preferably at least 96%, preferably at least 97%, preferably at least 98%, or more preferably at least 99% of the moisture content of the plant material at the time of harvest.

[0124] Methods of extraction would be known to persons skilled in the art, illustrative examples of which include supercritical fluid extraction (SFE). The principles of SFE relate to the disappearance of the gas-liquid boundary when the temperature of certain materials was increased by heating them in a closed glass container. This allows the material to reach its critical point, which is the temperature above which a substance or compound can co-exist in the gas, liquid and solid phases. By taking substances to their critical point and at pressure, SFE can be used as sophisticated solvents for extraction and fractionation of complex mixtures. SFE is commonly used in the processing of oil and has also been applied to the purification and separation of vegetable and fish oils. More recently SFE has been used to extract cannabinoids from plant material, for example, method for the extraction of

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pharmaceutically active cannabinoids from plant material is provided in WO/2004/016277, the contents of which is incorporated herein by reference.

[0125] In an embodiment, cannabinoids are extracted from the dried plant material by SFE.

[0126] In an embodiment, the plant material comprises female inflorescence.

[0127] In another aspect disclosed herein, there is provided an extract produced by the methods described herein.

[0128] The present disclosure provides an extract derived from the cannabis plant described herein, or a part thereof, comprising a cannabinoid profile enriched for total THC and total CBG, wherein the cannabinoid profile comprises a level of total THC and a level of total CBG at a ratio from about 5:1 to about 50:1 (THC:CBG), and wherein the level of total THC and total CBG (THC+CBG) is greater than the level of a reference cannabinoid selected from the group consisting of: total CBD, total CBC, total CBN, and total THCV.

[0129] The present disclosure also provides a total THC- and total CBG-enriched extract derived from the cannabis plant described herein, or a part thereof, wherein the extract comprises total THC, total CBG, and one or more minor cannabinoids selected from the group consisting of: total CBD, total CBC, total CBN, total THC, total CBL, and total Δ^8 -THC, wherein the extract comprises a level of total THC and a level of total CBG at a ratio of from about 5:1 to about 50:1 (THC: CBG), and wherein the level of the one or more other cannabinoids is from about 0.01% to about 10% by weight of the total cannabinoid content of the extract. In an embodiment, the extract does not comprise total CBDV.

Methods for selecting cannabis plants

[0130] The present disclosure enables the identification and selection of cannabis plants with a particular beneficial cannabinoid profile (*i.e.*, a cannabinoid profile enriched for total THC and total CBG).

[0131] In an embodiment, the selected cannabis plants, or parts thereof, can be used for medical purpose. In another embodiment, the selected cannabis plants, or parts thereof, can be

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used in the treatment, or for the amelioration of symptoms associated with, a disease. Suitable diseases will be known to persons skilled in the art, illustrative examples of which include acquired hypothyroidism, acute gastritis, agoraphobia, AIDS-related illness, alcohol abuse, alcoholism, alopecia areata, Alzheimer's Disease, amphetamine dependency, amyloidosis, amyotrophic lateral sclerosis (ALS), angina pectoris, ankylosis, anorexia, anorexia nervosa, anxiety disorders, any chronic medical symptom that limits major life activities, arteriosclerotic heart disease, arthritis, arthropathy, gout, asthma, attention deficit hyperactivity disorder (ADD/ADHD), Autism/Asperger's, autoimmune disease, back pain, back sprain, Bell's Palsy, bipolar disorder, bruxism, bulimia, cachexia, cancer, carpal tunnel syndrome, cerebral palsy, cervical disk disease, cervicobrachial syndrome, chronic fatigue syndrome, chronic pain, chronic renal failure, cocaine dependence, colitis, conjunctivitis, constipation, Crohn's Disease, cystic fibrosis, Darier's Disease, delirium tremens, dermatomyositis, diabetes, diabetic neuropathy, diabetic peripheral vascular disease, diarrhea, diverticulitis, dysthymic disorder, eczema, emphysema, endometriosis, epidermolysis bullosa, epididymitis, epilepsy, Felty's Syndrome, fibromyalgia, Friedreich's Ataxia, gastritis, genital herpes, Graves' Disease, headaches, Hemophilia A, Henoch-Schonlein Purpura, Hepatitis C, hereditary spinal ataxia, HIV/AIDS, Huntington's Disease, hypertension, hyperventilation, hypoglycemia, impotence, inflammatory autoimmune-mediated arthritis, inflammatory bowel disease (IBD), insomnia, intermittent explosive disorder (IED), Lou Gehrig's Disease, Lyme Disease, melorheostosis, Meniere's Disease, motion sickness, mucopolysaccharidosis (MPS), Multiple Sclerosis (MS), muscle spasms, muscular dystrophy, Nail-Patella Syndrome, nightmares, obesity, obsessive compulsive disorder, opiate dependence, osteoarthritis, panic disorder, Parkinson's Disease, peripheral neuropathy, pain, persistent insomnia, porphyria, Post-Polio Syndrome (PPS), Post-Traumatic Stress Disorder (PTSD), premenstrual syndrome (PMS), prostatitis, psoriasis, pulmonary fibrosis, Raynaud's Disease, Reiter's Syndrome, Restless Legs Syndrome (RLS), rosacea, schizoaffective disorder, schizophrenia, scoliosis, sedative dependence, seizures, senile dementia, severe nausea, shingles (Herpes Zoster), sinusitis, skeletal muscular spasticity, sleep apnoea, sleep disorders, spasticity, spinal stenosis, Sturge-Weber Syndrome (SWS), stuttering, Tardive Dyskinesia (TD), temporomandibular joint disorder (TMJ), tenosynovitis, thyroiditis, Tietze's Syndrome, tinnitus, tobacco

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dependence, Tourette's Syndrome, trichotillomania, viral hepatitis, wasting syndrome, Wittmaack-Ekbom's Syndrome, nausea, and vomiting.

[0132] Accordingly, in another aspect disclosed herein, there is provided a method for selecting a cannabis plant comprising a cannabinoid profile enriched for total THC and total CBG from a plurality of different cannabis plants, the method comprising:

- (a) harvesting plant material from a plurality of different cannabis plants;
- (b) at least partially drying the harvested plant material of step (a);
- (c) measuring in the at least partially dried plant material of step (b) a level of total THC, total CBG and one or more reference cannabinoids selected from the group consisting of THCV, CBDV, CBN, CBD, CBC, THCVA, CBDVA, CBNA, CBDA and CBCA, to generate a cannabinoid profile for each of the plurality of cannabis plants; and
- (d) on the basis of the measurements from step (c), selecting from the plurality of different cannabis plants a cannabis plant comprising a cannabinoid profile enriched for total THC and total CBG and comprising a level of total THC and a level of total CBG at a ratio from about 5:1 to about 50:1 (THC:CBG), wherein the total THC comprises THC and THCA, wherein the total CBG comprises CBG and CBGA, and wherein the level of total THC and total CBG (THC+CBG) is greater than the level of a reference cannabinoid selected from the group consisting of:
 - (i) total CBD, wherein the total CBD comprises CBD and CBDA;
 - (ii) total CBC, wherein the total CBC comprises CBC and CBCA;
 - (iii) total CBN, wherein the total CBN comprises CBN and CBNA; and
 - (iv) total THCV, wherein the total THCV comprises THCV and THCVA.

[0133] The terms “selecting” or “selection” as used herein means the selection of one or more cannabis plants from the plurality of different cannabis plants based on the cannabinoid profile of the individual cannabis plant. The term “plurality” is to be understood to mean more than 1 (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, *etc.*).

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[0134] In an embodiment, the method further comprises:

- (a) measuring in the at least partially dried plant material of step (b) a level of myrcene and a level of β -pinene to generate a terpene profile for each of the plurality of cannabis plants; and
- (b) on the basis of the measurements from step (e), selecting from the plurality of different cannabis plants a cannabis plant comprising a terpene profile wherein the myrcene is present in a ratio of from about 60:1 to about 1:1 to the level of β -pinene.

[0135] Thus, in an another aspect disclosed herein, there is provided a method for selecting a cannabis plant comprising a cannabinoid profile enriched for total THC and total CBG from a plurality of different cannabis plants, the method comprising:

- (a) harvesting plant material from a plurality of different cannabis plants;
- (b) at least partially drying the harvested plant material of step (a);
- (c) measuring in the at least partially dried plant material of step (b) a level of total THC, total CBG and one or more reference cannabinoids selected from the group consisting of THCV, CBDV, CBN, CBD, CBC, THCVA, CBDVA, CBNA, CBDA and CBCA, to generate a cannabinoid profile for each of the plurality of cannabis plants;
- (d) measuring in the at least partially dried plant material of step (b) a level of myrcene and a level of β -pinene to generate a terpene profile for each of the plurality of cannabis plants; and
- (e) on the basis of the measurements from step (c) and step (d), selecting from the plurality of different cannabis plants a cannabis plant comprising (i) a terpene profile wherein the myrcene is present in a ratio from about 60:1 to about 1:1 to the level of β -pinene and (ii) a cannabinoid profile enriched for total THC and total CBG and comprising a level of total THC and a level of total CBG at a ratio from about 5:1 to about 50:1 (THC:CBG), wherein the total THC comprises THC and THCA, wherein the total CBG comprises CBG and CBGA, and wherein the level of total THC and total CBG (THC+CBG) is greater than the level of a reference cannabinoid selected from the group consisting of:
 - (i) total CBD, wherein the total CBD comprises CBD and CBDA;
 - (ii) total CBC, wherein the total CBC comprises CBC and CBCA;
 - (iii) total CBN, wherein the total CBN comprises CBN and CBNA; and

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(iv) total THC_V, wherein the total THC_V comprises THC_V and THC_VA.

[0136] In an embodiment, the selected cannabis plant is crossed with a different cannabis plant to produce a F1 hybrid.

[0137] In an embodiment, regenerable cells isolated from the selected cannabis plant are transformed with a heterologous nucleic acid sequence and cultured for a time and under conditions suitable to produce a transgenic cannabis plant.

[0138] In an embodiment, regenerable cells isolated from the selected cannabis plant are transfected with a gene editing construct comprising a nucleic acid sequence encoding a DNA-recognition moiety and cultured for a time and under conditions suitable to produce a non-transgenic cannabis plant with modified gene expression.

[0139] Persons skilled in the art would understand that the DNA-recognition moiety may be DNA, RNA or a polypeptide.

[0140] Illustrative examples of suitable DNA molecules include antisense, as well as sense (*e.g.*, coding and/or regulatory) DNA molecules. Antisense DNA molecules include short oligonucleotides. Other examples of inhibitory DNA molecules include those encoding interfering RNAs, such as shRNA and siRNA. Yet another illustrative example of an inhibitor of gene expression is catalytic DNA, also referred to as DNazymes.

[0141] Illustrative examples of suitable RNA molecules include siRNA, dsRNA, stRNA, shRNA and miRNA (*e.g.*, short temporal RNAs and small modulatory RNAs), ribozymes, and guide (*i.e.*, gRNA or single-guide RNA (sgRNA)) or clustered regularly interspaced short palindromic repeats (CRISPR) RNAs used in combination with the Cas or other endonucleases (van der Oost *et al.* 2014, *Nature Reviews Microbiology*,12(7):479-92).

[0142] In an embodiment the DNA-recognition moiety is a CRISPR RNA. Suitable CRISPR RNA will be known to persons skilled in the art, illustrative examples of which include guide RNA (gRNA) and single-guide RNA (sgRNA).

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[0143] In an embodiment the DNA-recognition moiety is a polypeptide. Illustrative examples of a suitable polypeptide molecules are “Zinc finger nucleases” or “ZFN”, as described elsewhere herein.

[0144] The terms “guide RNA” or “gRNA” refer to a RNA sequence that is complementary to a target DNA and directs a CRISPR endonuclease to the target DNA. gRNA comprises crRNA and a tracrRNA. crRNA is a 17-20 nucleotide sequence that is complementary to the target DNA, while the tracrRNA provides a binding scaffold for the endonuclease. crRNA and tracrRNA exist in nature as two separate RNA molecules, which has been adapted for molecular biology techniques using, for example, 2-piece gRNAs such as CRISPR tracrRNAs (cr:tracrRNAs).

[0145] The terms “single-guide RNA” or “sgRNA” refers to a single RNA sequence that comprises the crRNA fused to the tracrRNA.

[0146] Accordingly, the skilled person would understand that the term “gRNA” describes all CRISPR guide formats, including two separate RNA molecules or a single RNA molecule. By contrast, the term “sgRNA” will be understood to refer to single RNA molecules combining the crRNA and tracrRNA elements into a single nucleotide sequence.

[0147] In a preferred embodiment, the DNA-recognition moiety is a single-guide RNA (sgRNA).

[0148] In an embodiment, the targeting gene editing construct further comprises a nucleic acid encoding an endonuclease.

[0149] Suitable endonucleases will be known to persons skilled in the art, illustrative examples of which include an RNA-guided DNA endonuclease, zinc finger nuclease (ZFN), transcription activator-like effector nuclease (TALEN), CRISPR-associated (Cas) nucleases.

[0150] In an embodiment, the nuclease is selected from the group consisting of an RNA-guided DNA endonuclease, ZFN, and a TALEN.

[0151] “Transcription activator-like effector nucleases” or “TALEN” are restriction enzymes that can be engineered to cut specific sequences of DNA. They are made by fusing a

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TAL effector DNA-binding domain to a DNA cleavage domain (a nuclease which cuts DNA strands). Transcription activator-like effectors (TALEs) can be engineered to bind practically any desired DNA sequence, so when combined with a nuclease, DNA can be cut at specific locations. The restriction enzymes can be introduced into cells, for use in gene editing or for genome editing *in situ*, a technique known as genome editing with engineered nucleases. The mechanism of TALEN-mediated cleavage of target DNA sequences would be known to persons skilled in the art and has been described, for example by Boch (2011, *Nature Biotechnology*, 29: 135-136), Juong *et al.* (2013, *Nature Reviews Molecular Cell Biology*, 14: 49-55) and Sune *et al.* (2013, *Biotechnology and Bioengineering*, 110: 1811-1821).

[0152] “Zinc finger nucleases” or “ZFN” are proteins comprising nucleic acid binding domains that are stabilised by zinc. The individual DNA binding domains are typically referred to as “fingers”, such that a ZFN has at least one finger, preferably two fingers, preferably three fingers, preferably four fingers, preferably five fingers, or more preferably six fingers. Each finger binds from two to four base pairs of a target DNA sequence, and typically comprises an about 30 amino acid zinc-chelating, DNA binding region. ZFN facilitate site-specific cleavage within a target DNA sequence, allowing endogenous or other end-joining repair mechanisms to introduce insertions or deletions to repair the gap. The mechanism of ZFN-mediated cleavage of target DNA sequences would be known to persons skilled in the art and has been described, for example, by Liu *et al.* (2010, *Biotechnology and Bioengineering*, 106: 97-105).

[0153] In an embodiment, the RNA-guided DNA endonuclease is a CRISPR-associated (Cas) endonuclease.

[0154] The CRISPR-Cas system evolved in bacteria and archaea as an adaptive immune system to defend against viral attack. Upon exposure to a virus, short segments of viral DNA are integrated in the clustered regularly interspaced short palindromic repeats (*i.e.*, CRISPR) locus. RNA is transcribed from a portion of the CRISPR locus that includes the viral sequence. That RNA, which contains sequence complementarity to the viral genome, mediates targeting of a Cas endonuclease to the sequence in the viral genome. The Cas endonuclease cleaves the viral target sequence to prevent integration or expression of the viral sequence.

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[0155] The mechanisms of CRISPR-mediated gene editing would be known to persons skilled in the art and have been described, for example, by Doudna *et al.*, (2014, *Methods in Enzymology*, 546) and Belhaj *et al.*, (2013, *Plant Methods*, 9:39) and in WO2013/188638 and WO2014/093622.

[0156] Suitable Cas endonucleases will be known to persons skilled in the art, illustrative examples of which include Cas9, Cas12a (also referred to as Cpf1), Cas12b (also referred to as C2c1), Cas13a (also referred to as C2c2), Cas13b, CasX, Cas3 and Cas10. The term “Cas endonucleases” as used herein also contemplates the use of natural and engineered Cas endonucleases, described, for example, by Wu *et al.* (2018, *Nature Chemical Biology*, 14: 642-651).

[0157] In a preferred embodiment, the Cas endonuclease is Cas9.

[0158] Those skilled in the art will appreciate that the aspects and embodiments described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that this disclosure includes all such variations and modifications which fall within the spirit and scope. The disclosure also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

[0159] Unless otherwise defined, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this disclosure belongs.

[0160] The various embodiments enabled herein are further described by the following non-limiting examples.

EXAMPLES

A. Materials

Plants

[0161] Cannabis plants were grown under an Office of Drug Control licence at the Victorian Government Medicinal Cannabis Cultivation Facility, Victoria, Australia. Indoor greenhouse growing facilities were equipped with full climate control (*i.e.*, temperature, humidity and high-intensity lighting) to ensure that crops were produced in almost identical growing conditions.

[0162] Cannabis plants were asexually propagated from cuttings taken from vegetative mother plants originating from a single seed source. Cuttings were maintained for 2 weeks at 22°C in a high humidity environment (*i.e.*, 50% relative humidity) under 18 hours day light in rooting medium to stimulate root development before being transferred to substrate medium for hydroponic growth. The plants were grown for a further 5 weeks under the same growth conditions before being transferred to a larger substrate medium to induce flowering.

[0163] Flowering conditions were identical to the rooting and growth conditions, with the exception that the daylight length was reduced to 12 hours. The plants were maintained in flowering conditions for 9 weeks to allow for flowering and maturation.

[0164] The plants were irrigated throughout their growing cycle with potable quality water and sustained release fertilizer is applied to the soil-free medium.

[0165] Upon maturation plants were harvested at the base of the plant and dried in a temperature and humidity controlled environment (*i.e.*, approximately 21°C at approximately 38-45% humidity) for between 3 to 5 weeks prior to extraction or analysis, as described below.

Reagents and standards

[0166] All HPLC grade reagents, water with 0.1% formic acid (mobile phase A), acetonitrile with 0.1% formic acid (mobile phase B) and methanol were obtained from Fisher

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Scientific (Fair Lawn, NJ). Primary standards for CBDA and THCA in acetonitrile, and CBD, CBN, CBC, THC in methanol, at 1000 µg/mL, were commercially purchased from Novachem Pty Ltd (Heidelberg West, Australia) as distributor for Cerilliant Corporation (Round Rock, Texas). A mixed stock standard at 125 µg/mL CBDA, CBN, CBC, THCA and 250 µg/mL CBD, THC in methanol was prepared with working standards at 0.05, 0.125, 0.25, 0.5, 1.25, 2.5 and 50.0 µg/mL for CBDA, CBN, CBC and THCA; and 0.1, 0.25, 0.5, 1.0, 2.5, 5.0 and 100.0 µg/mL for CBD and THC prepared from the mixed stock. Primary standards for THCV, CBDV, CBG, THCVA, CBNA, CBCA, CBGA, CBL and Δ8-THC in methanol, at 1000 µg/mL, were commercially purchased from Novachem Pty Ltd (Heidelberg West, Australia) as distributor for Cerilliant Corporation (Round Rock, Texas). These were combined to make a 100 µg/mL stock (i.e. 100µL taken and mixed from each). This mixed standard was diluted to 0.1, 0.25, 0.5, 1.0, 2.5, 5.0 and 100 µg/mL. All standards were stored at -80°C.

B. Sample Preparation

[0167] Inflorescences were separated from the plant material from 69 different female cannabis cultivars. Samples were ground to a fine powder with liquid nitrogen using a SPEX SamplePrep 2010 Geno/Grinder for 1 minute at 1500 rpm. After grinding, 10 mg of each sample was weighed into an Axygen 2.0 mL microcentrifuge tube on a Sartorius BP210D analytical balance. Each sample was extracted with 1 mL of methanol, vortexed for 30 seconds, sonicated for 5 minutes and centrifuged at 13,000 rpm for 5 minutes. The supernatant was transferred to a 2 mL amber HPLC vial and diluted 1:3 for analysis.

[0168] Where necessary, plant material was cured by heating the ground dried plant material at 120°C for 2 hours.

C. Liquid Chromatography/Mass Spectroscopy Analysis

[0169] Samples were analysed using a Thermo Scientific (Waltham, MA) Q Exactive Plus Orbitrap mass spectrometer (MS) coupled with Thermo Scientific Vanquish ultra-high performance liquid chromatography (UHPLC) system equipped with degasser, binary pump, temperature controlled autosampler and column compartment, and photodiode array detector (PDA).

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[0170] Separation was carried out using a C18 column (Phenomenex Luna Omega, 1.6 μm , 150 mm x 2.1 mm) maintained at 30°C with water and acetonitrile (both with 0.1% formic acid) as mobile phases and a flow rate of 0.3 mL/min. The separation gradient is described in Table 3.

[0171] The MS was set to acquire a full range spectrum (80 - 1,200 m/z) followed by a data independent MS2 spectrum in positive polarity with resolution set to 35,000. The capillary temperature was set to 320°C with sheath and auxiliary gas at 28 and 15 units respectively and a spray voltage of 4 kV. PDA data acquisition was set to a data collection rate of 5 Hz from 190 and 680 nm.

Table 3. Separation gradient for LCMS analysis

Time (min)	% A (Water with 0.1% FA)	% B (Acetonitrile with 0.1% FA)
0	60.0	40.0
2.0	60.0	40.0
3.0	25.0	75.0
10.0	10.0	90.0
11.0	0.0	100.0
15.0	0.0	100.0
15.1	60.0	40.0
20.0	60.0	40.0

D. Static Headspace Solid Phase Micro-Extraction. Liquid Extraction and Gas Chromatography/Mass Spectroscopy Analysis

[0172] Terpenes were extracted from 20 mg of milled, dried cannabis biomass using static headspace with direct injection, headspace solid phase micro-extraction (SPME) or liquid extraction using hexane followed by chromatographic separation on an Agilent 7000 GC-

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QQQ using a DB-5, DB-17 or VF-35 capillary GC column. The optimal column for separation of the volatiles was the DB-5 column. SPME and static headspace was effective for the analysis of extract monoterpenes, while sesquiterpenes were more effectively extracted using a hexane-based liquid extraction method. Final analytical conditions for the static headspace analysis are provided in Table 4 and final conditions for the liquid extraction are presented in Table 5.

Table 4. HSE and GC-MC parameters used for relative determination of terpenes from cannabis

GC-MS parameters for static headspace analysis		
Sample	20 mg milled, dried cannabis bud	
Incubation (pre-extraction)	5 min	
Extraction	30 min	
Desorption	2 min	
Fibre post-bake	30 min	
GC-MS Parameters		
Column	DB-5, DB-17, VF-35	
Oven	Time (min)	Temperature (°C)
	0	60
	2	60
	7	110
	12	110
	13.5	125
	18.5	125
	26	200
	28	300

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	31	300
	TOTAL RUN TIME	31 min
Carrier gas	Helium, Flow: 1.6221 mL/min at 1.5 psi	
Injector	10:1 split, 250°C, injection volume 1000 µL	
Headspace	Incubation temperature 130°C for 5 min, injection volume 1000 µL	
Scan range	29-350 <i>m/z</i>	

Table 5. Liquid extraction and GC-MS parameters used for quantitative determination of terpenes from cannabis

GC-MS parameters for liquid injection analysis		
Sample	20 mg milled, dried cannabis bud, extracted twice with 200 µL hexane. Extracts combined for analysis	
Oven	Time (min)	Temperature (°C)
	0	60
	2	60
	30	200
	31	320
	34	320
	TOTAL RUN TIME	35 min
Carrier gas	Helium, Flow: 1.6221 mL/min at 1.5 psi	
Injector	1:5 split, 250°C, 1µL	
Flow rates and Backflush settings	Main Run Column 1: flow 1.3801 mL/min; average velocity 32.251 cm/sec; Column 2: 0.50013 psi, flow 1.428 mL/min; average velocity 151.6 cm/sec Backflush Column 1: 3 min (post-run) – 1.9815 mL/min; Column 2: 3 min (post-run) – 7.0194 mL/min.	

E. Data Processing

Chemometric analysis

[0173] LCMS data was aligned, peaks picked and isotopes clustered in Genedata Refiner MS (Genedata Expressionist® 11.0.0a, Basel, Switzerland). The subsequent cluster volumes were analysed in Genedata Analyst. A total of 2,734 clusters were identified and cultivars with cannabinoid profiles enriched for total THC and total CBG were identified by comparison to 14 cannabinoids (for which standards were available), which were used as input to the clustering (Table 6).

Table 6. Cannabinoid standards used for profiling

Cannabinoid	Formula	Charge	m/z	RT
Tetrahydrocannabivarin (THCV)	C ₁₉ H ₂₆ O ₂	1	287.2004	7.90
Cannabidivarin (CBDV)	C ₁₉ H ₂₆ O ₂	1	287.2006	6.45
Cannabinol (CBN)	C ₂₁ H ₂₆ O ₂	1	311.2005	8.92
Cannabidiol (CBD)	C ₂₁ H ₃₀ O ₂	1	315.2316	7.65
(-)-Δ ⁹ -Tetrahydrocannabinol (THC)	C ₂₁ H ₃₀ O ₂	1	315.2317	9.76
Cannabichromene (CBC)	C ₂₁ H ₃₂ O ₂	1	317.2110	10.89
Cannabigerol (CBG)	C ₂₁ H ₃₂ O ₂	1	317.2473	7.52
Tetrahydrocannabivarinic acid (THCVA)	C ₂₀ H ₂₆ O ₄	1	331.1902	8.70
Cannabidivarinic acid (CBDVA)	C ₂₀ H ₂₆ O ₄	1	331.1902	6.19
Cannabinolic acid (CBNA)	C ₂₂ H ₂₆ O ₄	1	355.1901	9.73
Cannabidiolic acid (CBDA)	C ₂₂ H ₃₀ O ₄	1	359.2214	7.16

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Cannabinoid	Formula	Charge	m/z	RT
Cannabichromenic acid (CBCA)	$C_{22}H_{30}O_4$	1	359.2216	11.18
Δ^9 -Tetrahydrocannabinolic acid A (THCA)	$C_{22}H_{30}O_4$	1	359.4439	10.73
Cannabigerolic acid (CBGA)	$C_{22}H_{32}O_4$	1	361.2371	7.41

Terpene peak identification analysis

[0174] Peak identifications were assigned using MS spectral matching against reference spectra in the NIST/Wiley libraries and Kovats Indices. Confirmatory identification was done based on retention index, which was calculated for the compounds identified in each sample using an external standard analysed under the same GC conditions. The external standards (Table 7) enabled the assignment of major volatile peaks in the cannabis strains. Several peaks were not able to be identified with certainty by library matching or by comparison to the standards. These include both putative monoterpenes (M01-M13) and sesquiterpenes (S01-S08). The data was compared with the published values and peak identifications were assigned (Table 7; Figure 5).

[0175] GC-MS data was analysed by PCA using PLSToolbox (Version 8.6.1, Eigenvector Research, Inc.) running on MatLaw (Version R2018a, Mathworks).

Table 7. Terpene standards used to identify terpenes in cannabis

Peak No.	RT (min)	Name	<i>m/z</i>	Retention Index (calculated)	Status
1	8.849	α -Phellandrene	93.0	928	specID
2	9.092	α -Pinene (+/-)	93.0	937	Confirmed
3	9.590	Camphene	93.0	955	Confirmed
4	10.383	β -Pinene (+/-)	93.0	983	Confirmed
5	10.570	Myrcene	93.0	990	Confirmed
6	11.481	α -Terpinene	93.0	1021	Confirmed
7	11.848	Limonene	68.1	1033	Confirmed
8	11.930	β -Phellandrene	93.1	1036	specID
9	11.983	Eucalyptol	81.0	1038	Confirmed
10	12.264	Ocimene isomer	93.1	1047	Confirmed
11	12.700	γ -Terpinene	93.1	1061	Confirmed
12	13.088	4-Thujanol	93.1	1074	specID
13	13.531	Terpinolene	93.1	1089	Confirmed
14	13.708	Fenchone	81.1	1095	Confirmed
15	13.868	Linalool	93.0	1101	Confirmed
16	14.615	Fenchol	81.1	1126	specID
17	14.844	Trans-2-Pinanol	93.1	1133	specID
18	16.219	Borneol	95.1	1180	Confirmed
19	16.835	α -Terpineol	93.1	1200	Confirmed

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Peak No.	RT (min)	Name	<i>m/z</i>	Retention Index (calculated)	Status
20	22.511	α -Bergamotene	93.1	1406	specID
21	22.854	β -Bergamotene	119.1	1419	specID
22	23.148	trans-Caryophyllene	93.0	1431	Confirmed
23	23.280	γ -Elemene iso1	121.0	1436	specID
24	23.360	Bergamontene iso3	93.1	1439	specID
25	23.467	α -Guaiene	105.0	1443	specID
26	23.748	Farnesene	69.2	1454	Confirmed, RI
27	24.083	Humulene	93.0	1467	specID
28	24.780	(-)- α -Selinene	105.1	1494	specID
29	24.932	epi- β -selinene	93.1	1500	specID
30	25.050	sesquiT-coeluting01	93.1	1510	specID
31	25.174	δ -Guaiene	107.0	1520	specID
32	25.999	α -Bisabolene	93.1	1545	specID
33	26.099	Guaia-3,9-diene	161.1	1549	specID
34	26.210	3,7(11)-Selinadiene	161.1	1553	specID
35	26.456	β -cis-Caryophyllene	69.2	1563	specID
36	26.660	γ -Elemene iso2	121.1	1572	specID

Peak No.	RT (min)	Name	<i>m/z</i>	Retention Index (calculated)	Status
37	27.242	Caryophyllene oxide	79.0	1596	Confirmed
38	27.474	Guaiol	105.1	1606	Confirmed
39	28.187	β -Cadinene	189.1	1637	specID
40	28.922	γ -Gurjunene	59.1	1669	specID
41	29.081	sesquiterpene	107.0	1676	specID
42	29.455	α -Bisabolol	93.0	1692	Confirmed

Quantitation

[0176] Chromatograms were processed using Thermo LCQuan v.2.7 software by extracted ion using the *m/z* values specified in Table 3 with a window of 5 ppm or by PDA analysis at 280 nm. Calibration curves were developed using the serial diluted standards and the amount of each cannabinoid in the cultivars calculated.

[0177] GC-MS chromatograms were processed using Agilent MassHunter software using the retention time and *m/z* profiles of the standards specified in Table 2.

F. Supercritical Fluid Extraction (SCE) of Cannabinoids

[0178] Extract comprising cannabinoids were prepared from air dried and cured mature plant material using supercritical fluid extraction (SCE) with CO₂, as previously described in Khaw *et al.* (*Molecules*, 2017, 22:1186). Briefly, cured biomass was extracted using SFE with CO₂ using the following parameters:

- Temperature of 60°C;
- Flow rate of 150 g/min; and
- Pressure of 150 bar.

Results

Chemotyping

[0179] LCMS analysis was undertaken to identify plants with cannabinoid profiles enriched for total THC and total CBG. For untargeted analysis the intensity cut off was stringent, meaning only peaks that were relatively intense would be selected. Post peak alignment and isotope clustering a total of 2,734 isotope clusters were identified in the combined dataset. Since standards were run under the same conditions along with the plant extracts, cannabinoid profiles were generated corresponding to the known cannabinoids (Table 5). For this analysis, the plant material had not been heated so the acid forms were present at higher levels than the respective neutral species (Citti *et al.* 2018, *Phytochemical Analysis*, 29: 539-48).

[0180] Hierarchical cluster analysis of both the entire data set and the 14 cannabinoids identified 29 cannabis strains with a cannabinoid profile enriched for total CBD and total THC, where total THC and total CBG were relatively high, when compared to the other cannabinoid standards. This outcome was unexpected as CBG is generally only present in low abundance, particularly when considered relative to CBD.

Quantitative Analysis

[0181] To fully describe the cannabinoid profile enriched for total THC and total CBG, quantitative analysis was performed on the 29 cannabis strains with a cannabinoid profile enriched for total THC and CBG. The results obtained from this analysis are provided in Table 8, below.

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Table 8. Quantitative analysis of cannabinoids in THC and CBG enriched cannabis

Cannabis strain #	CBD	THC	CBG	CBC	CBN	CBDV	THCV	Total cannabinoid (mg/g)
31	0.55	80.74	4.82	2.94	0.16	0	0.27	89.48
32	0.51	110.11	6.15	3.51	0.2	0	0.24	120.72
33	0.37	66.23	2.31	3.85	0.29	0	0.13	73.18
34	0.68	84.22	2.33	3.45	0.26	0	0.16	91.1
35	0.52	76.54	2.04	4.02	0.23	0	0.19	83.54
36	0.27	66.44	3.99	3.38	0.17	0	0.3	74.55
37	0.99	119.9	5.39	4.85	0.14	0	0.66	131.93
38	0.7	134.54	6.8	3.28	0.21	0	0.75	146.28
39	0.41	134.24	6.23	2.67	0.18	0	0.93	144.66
40	0.46	119.75	8.98	2.9	0.24	0	0.91	133.24
41	0.38	99.17	4.54	1.6	0.18	0	0.49	106.36
42	0.55	93.37	4.34	0.88	0.21	0	0.62	99.97
43	0.38	129.29	8.28	4.89	0.17	0	1.41	144.42
44	0.34	105.7	3.53	1.62	0.15	0	0.78	112.12
45	0.25	71.53	2.23	1.65	0.21	0	0.29	76.16
46	0.36	81.72	1.67	1.1	0.18	0	0.38	85.41
47	0.39	124.4	3.49	2.97	0.23	0	0.71	132.19
48	0.41	115.05	4.87	2.26	0.17	0	0.69	123.45

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Cannabis strain #	CBD	THC	CBG	CBC	CBN	CBDV	THCV	Total cannabinoid (mg/g)
49	1.05	146.94	4.26	3.6	0.25	0	1.2	157.3
50	0.61	142.55	7.59	3.31	0.26	0	1.02	155.34
51	0.42	123.04	4.37	1.53	0.32	0	1.12	130.8
52	0.62	134.96	9.7	1.58	0.21	0	0.85	147.92
53	0.35	79.75	1.8	1.07	0.18	0	0.51	83.66
54	0.54	103.51	6.01	1.81	0.09	0	0.52	112.48
55	0.46	116.04	5.28	1.73	0.13	0	0.75	124.39
56	0.49	91.75	4.56	0.97	0.13	0	0.47	98.37
57	0.49	114.39	4.47	1.38	0.14	0	0.76	121.63
58	0.5	132.04	7.74	1.69	0.11	0	0.67	142.75
59	0.77	203.58	4.81	1.98	0.21	0	0.95	212.3

[0182] Using the total cannabinoid content (mg/g) for each of the analysed cannabis strains, the proportion of each cannabinoid in the total cannabinoid content of the plant material was derived to further characterise the cannabinoid profile of the cannabis strains, presented as a percentage (%) of the total cannabinoid content of the dry weight of plant material (Table 9).

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Table 9. Major and minor cannabinoid content in THC and CBG enriched cannabis

Cannabis strain #	%CBD	%THC	%CBG	%CBC	%CBN	%CBDV	%THCV
31	0.61	90.23	5.39	3.29	0.18	0.00	0.30
32	0.42	91.21	5.09	2.91	0.17	0.00	0.20
33	0.51	90.50	3.16	5.26	0.40	0.00	0.18
34	0.75	92.45	2.56	3.79	0.29	0.00	0.18
35	0.62	91.62	2.44	4.81	0.28	0.00	0.23
36	0.36	89.12	5.35	4.53	0.23	0.00	0.40
37	0.75	90.88	4.09	3.68	0.11	0.00	0.50
38	0.48	91.97	4.65	2.24	0.14	0.00	0.51
39	0.28	92.80	4.31	1.85	0.12	0.00	0.64
40	0.35	89.88	6.74	2.18	0.18	0.00	0.68
41	0.36	93.24	4.27	1.50	0.17	0.00	0.46
42	0.55	93.40	4.34	0.88	0.21	0.00	0.62
43	0.26	89.52	5.73	3.39	0.12	0.00	0.98
44	0.30	94.27	3.15	1.44	0.13	0.00	0.70
45	0.33	93.92	2.93	2.17	0.28	0.00	0.38
46	0.42	95.68	1.96	1.29	0.21	0.00	0.44
47	0.30	94.11	2.64	2.25	0.17	0.00	0.54
48	0.33	93.20	3.94	1.83	0.14	0.00	0.56

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Cannabis strain #	%CBD	%THC	%CBG	%CBC	%CBN	%CBDV	%THCV
49	0.67	93.41	2.71	2.29	0.16	0.00	0.76
50	0.39	91.77	4.89	2.13	0.17	0.00	0.66
51	0.32	94.07	3.34	1.17	0.24	0.00	0.86
52	0.42	91.24	6.56	1.07	0.14	0.00	0.57
53	0.42	95.33	2.15	1.28	0.22	0.00	0.61
54	0.48	92.03	5.34	1.61	0.08	0.00	0.46
55	0.37	93.29	4.24	1.39	0.10	0.00	0.60
56	0.50	93.27	4.64	0.99	0.13	0.00	0.48
57	0.40	94.05	3.68	1.13	0.12	0.00	0.62
58	0.35	92.50	5.42	1.18	0.08	0.00	0.47
59	0.36	95.89	2.27	0.93	0.10	0.00	0.45

[0183] Finally, as cannabis strains are often assessed and discussed in terms of their relative ratios of either major or minor cannabinoids, the THC and CBG to minor cannabinoid ratio (THC+CBG: minor cannabinoid) is described in Table 10.

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Table 10. THC+CBG: minor cannabinoid ratio for THC and CBG enriched cannabis

Cannabis strain #	Total THC+CBG (mg/g)	Ratio THC+CBG : CBD	Ratio THC+CBG : CBC	Ratio THC+CBG : CBN	Ratio THC+CBG : CBDV	Ratio THC+CBG : THCV
31	85.56	155.56	29.10	534.75	NA	316.89
32	116.26	227.96	33.12	581.30	NA	484.42
33	68.54	185.24	17.80	236.34	NA	527.23
34	86.55	127.28	25.09	332.88	NA	540.94
35	78.58	151.12	19.55	341.65	NA	413.58
36	70.43	260.85	20.84	414.29	NA	234.77
37	125.29	126.56	25.83	894.93	NA	189.83
38	141.34	201.91	43.09	673.05	NA	188.45
39	140.47	342.61	52.61	780.39	NA	151.04
40	128.73	279.85	44.39	536.38	NA	141.46
41	103.71	272.92	64.82	576.17	NA	211.65
42	97.71	177.65	111.03	465.29	NA	157.60
43	137.57	362.03	28.13	809.24	NA	97.57
44	109.23	321.26	67.43	728.20	NA	140.04
45	73.76	295.04	44.70	351.24	NA	254.34
46	83.39	231.64	75.81	463.28	NA	219.45
47	127.89	327.92	43.06	556.04	NA	180.13

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Cannabis strain #	Total THC+CBG (mg/g)	Ratio THC+CBG : CBD	Ratio THC+CBG : CBC	Ratio THC+CBG : CBN	Ratio THC+CBG : CBDV	Ratio THC+CBG : THCV
48	119.92	292.49	53.06	705.41	NA	173.80
49	151.20	144.00	42.00	604.80	NA	126.00
50	150.14	246.13	45.36	577.46	NA	147.20
51	127.41	303.36	83.27	398.16	NA	113.76
52	144.66	233.32	91.56	688.86	NA	170.19
53	81.55	233.00	76.21	453.06	NA	159.90
54	109.52	202.81	60.51	1216.89	NA	210.62
55	121.32	263.74	70.13	933.23	NA	161.76
56	96.31	196.55	99.29	740.85	NA	204.91
57	118.86	242.57	86.13	849.00	NA	156.39
58	139.78	279.56	82.71	1270.73	NA	208.63
59	208.39	270.64	105.25	992.33	NA	219.36

Terpene Profile

[0184] To further define the chemotype of the cannabis plants, terpene profiles were evaluated using GC-MS. Using Principal Component Analysis (PCA), PC1 explained 69.48% of variance, and PC2 explained 16.62% of variance in the data (total 86.1%) (Figure 6A). PC1 is characterised by plants enriched for myrcene, *i.e.*, myrcene-enriched (Figure 6B). The abundance of myrcene varied between the different cannabis strains (Figure 7B). The abundance of β -pinene was also quantified for comparative analysis (Figure 7A).

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[0185] In plants identified as comprising a cannabinoid profile enriched for total THC and total CBG, the abundance of myrcene and β -pinene was determined according to peak area (Figure 7). The relative abundance (ratio) of myrcene to β -pinene in these cannabis strains was determined to be from about 60:1 and 1:1.

Conclusion

[0186] The quantitative analysis of extracts taken from cannabis plants identified as having a THC- and CBG-enriched cannabinoid profile confirmed that these plants are characterised by high levels of THC and relatively high levels of CBG. Given that CBG has been identified as a cannabinoid with high therapeutic potential, these new cannabis varieties are likely to provide a therapeutic benefit.

Comparative Analysis

[0187] The chemotypic features of these new, THC- and CBG-enriched cannabis varieties may be used to distinguish THC- and CBG-enriched cannabis varieties from other cannabis varieties.

Cannabis plants with a cannabinoid profile enriched for total CBD and total THC

[0188] Quantitative analysis was performed on the 27 cannabis strains with a cannabinoid profile enriched for total CBD and total THC. The results obtained from this analysis are provided in Table 11, below (mg/g).

Table 11. Quantitative analysis of cannabinoids in CBD-and THC-enriched cannabis

Strain #	CBD	THC	CBG	CBC	CBN	CBDV	THCV	Total cannabinoid
2	53.33	33.96	1.21	3.12	0.1	0.23	0.24	92.19
3	91.42	57.2	2.52	5.39	0.05	0.2	0.32	157.1
6	55.49	31.36	2.38	3.08	0.09	0.3	0.35	93.05

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Strain #	CBD	THC	CBG	CBC	CBN	CBDV	THCV	Total cannabinoid
7	69.26	36.69	2.36	4.01	0.11	0.33	0.29	113.05
8	74.14	29.76	3.64	4.39	0.15	0.37	0.34	112.79
9	69.51	33.38	2.55	3.77	0.13	0.32	0.35	110.01
10	51.97	22.68	2.11	2.71	0.09	0.29	0.26	80.11
11	65.71	35.09	2.24	3.46	0.09	0.32	0.29	107.2
12	70.87	33.14	3.72	3.99	0.1	0.37	0.36	112.55
13	64.27	30.26	1.9	3.04	0.13	0.35	0.32	100.27
14	78.37	41.58	4.48	3.94	0.16	0.41	0.42	129.36
15	73.06	38.33	2.37	3.77	0.07	0.39	0.45	118.44
16	96.97	74.48	5.12	5.22	0.13	0.47	0.49	182.88
17	76.72	36.42	2.86	4.05	0.1	0.37	0.31	120.83
18	67.57	22.29	2.14	3.7	0.08	0.41	0.41	96.6
19	76.61	37.91	3.64	4.75	0.1	0.39	0.35	123.75
20	86.25	36.4	3.13	5.07	0.11	0.43	0.54	131.93
21	56.72	20.86	1.06	3.07	0.08	0.1	0.27	82.16
22	68.15	25.38	1.17	4.12	0.11	0.12	0.29	99.34
23	51.19	20.49	1.9	3.16	0.09	0.09	0.16	77.08
24	74.74	27.35	1.26	4.24	0.1	0.14	0.27	108.1

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Strain #	CBD	THC	CBG	CBC	CBN	CBDV	THCV	Total cannabinoid
25	73.92	38.55	1.95	4.27	0.12	0.13	0.25	119.19
26	82.46	43.34	1.46	6.21	0.11	0.2	0.27	134.05
27	70.43	50.77	2.77	4.04	0.08	0.41	0.33	128.83
28	65.4	33.14	0.94	3.41	0.19	0.31	0.35	103.74
29	43.1	22.39	1.17	2.56	0.11	0.2	0.21	69.74
30	42.82	28.36	1.3	2.22	0.12	0.23	0.28	75.33

[0189] In plants identified as comprising a cannabinoid profile enriched for total CBD and total THC, the abundance of myrcene and β -pinene was determined according to peak area (Figure 7). The relative abundance (ratio) of myrcene to β -pinene in these cannabis strains was determined to be from about 40:1 and about 1:1.

Cannabis plants with a cannabinoid profile enriched for total THC, total CBG and total THCV

[0190] Quantitative analysis was performed on the 12 cannabis strains with a cannabinoid profile enriched for total THC, CBG and THCV. The results obtained from this analysis are provided in Table 12, below.

Table 12. Quantitative analysis of cannabinoids in THC/CBG/THCV-enriched cannabis

Cannabis strain #	CBD	THC	CBG	CBC	CBN	CBDV	THCV	Total cannabinoid (mg/g)
60	0.73	124.09	1.83	1.77	0.21	0.02	5.47	134.12
61	0.68	96.31	2.74	2.29	0.3	0.02	4.54	106.88
62	0.46	120.87	5.3	2.13	0.24	0.02	3.26	132.28
63	0.8	104.57	8.89	1.94	0.18	0.03	6.57	122.98
64	0.42	139.17	4.47	2.32	0.2	0.02	5.63	152.23
65	0.29	85.67	1.47	1.21	0.12	0.02	2.74	91.52
66	0.66	99.73	1.37	1.32	0.12	0.02	2.79	106.01
67	0.61	86.69	1.5	0.99	0.14	0.02	3.12	93.07
68	0.38	96.7	2.08	5.74	0.25	0.02	2.68	107.85
69	0.5	81.06	3.08	1.58	0.22	0.02	2.3	88.76
70	0.27	78.86	1.5	3.23	0.3	0.02	2.23	86.41
71	0.45	83.39	2.01	1.97	0.26	0.02	2.49	90.59

[0191] In plants identified as comprising a cannabinoid profile enriched for total THC and total CBG, the abundance of myrcene and β -pinene was determined according to peak area (Figure 7). The relative abundance (ratio) of myrcene to β -pinene in these cannabis strains was determined to be from about 50:1 and 2.5:1.

Cannabis plants with a cannabinoid profile enriched for total CBD

[0192] Quantitative analysis was performed on a cannabis strain with a cannabinoid profile enriched for total CBD. The results obtained from this analysis are provided in Table 13, below.

Table 13. Quantitative analysis of cannabinoids in CBD-enriched cannabis

Cannabinoid	Concentration (mg/g)	Ratio (CBD: Cannabinoid)	% of total cannabinoid content
CBD	55.1	1	90.42
THC	1.89	29.15	3.10
CBG	0.71	77.61	1.17
CBC	2.29	24.06	3.76
CBN	0.02	2755	0.03
CBDV	0.83	66.39	1.36
THCV	0.1	551	0.16
TOTAL	60.94		

[0193] In plants identified as comprising a cannabinoid profile enriched for total CBD, the abundance of myrcene and β -pinene was determined according to peak area (Figure 7). The relative abundance (ratio) of myrcene to β -pinene in these cannabis strains was about 5:1.

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THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A cannabis plant, or a part thereof, comprising a cannabinoid profile enriched for total THC and total CBG, wherein the cannabinoid profile comprises a level of total THC and a level of total CBG at a ratio from about 5:1 to about 50:1 (THC:CBG), wherein the total THC comprises Δ -9-tetrahydrocannabinol (THC) and Δ -9-tetrahydrocannabinolic acid (THCA), wherein the total CBG comprises cannabigerol (CBG) and cannabigerolic acid (CBGA); and wherein the level of total THC and total CBG (THC+CBG) is greater than the level of a reference cannabinoid selected from the group consisting of:
 - (a) total CBD, wherein the total CBD comprises cannabidiol (CBD) and cannabidiolic acid (CBDA);
 - (b) total CBC, wherein the total CBC comprises cannabichromene (CBC) and cannabichromene acid (CBCA);
 - (c) total CBN, wherein the total CBN comprises cannabinol (CBN) and cannabinolic acid (CBNA); and
 - (d) total THCV, wherein the total THCV comprises tetrahydrocannabivarin (THCV) and tetrahydrocannabivarinic acid (THCVA).
2. The cannabis plant of claim 1, or a part thereof, wherein the part is a female inflorescence.
3. The cannabis plant of claim 1 or claim 2, or a part thereof, wherein the level of total THC is at least 80% by weight of the total cannabinoid content of the dry weight of plant material.
4. The cannabis plant of any one of claims 1 to 3, or a part thereof, wherein the level of total CBG is at least 1% by weight of the total cannabinoid content of the dry weight of plant material.
5. The cannabis plant of any one of claims 1 to 4, or a part thereof, wherein the reference cannabinoid is total CBD.
6. The cannabis plant of claim 5, or part thereof, wherein the THC+CBG and the total

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CBD are present in a ratio of from about 50:1 to about 500:1 (THC+CBG: CBD).

7. The cannabis plant of any one of claims 1 to 6, or a part thereof, wherein the level of total CBD is from about 0.1% to about 1% by weight of the total cannabinoid content of the dry weight of plant material.
8. The cannabis plant of any one of claims 1 to 7, or a part thereof, wherein the reference cannabinoid is total CBC.
9. The cannabis plant of claim 8, or part thereof, wherein the THC+CBG and the total CBC are present at a ratio of from about 10:1 to about 120:1 (THC+CBG: CBC).
10. The cannabis plant of claim 8 or claim 9, or a part thereof, wherein the level of total CBC is from about 0.01% to about 10% by weight of the total cannabinoid content of the dry weight of plant material.
11. The cannabis plant of any one of claims 1 to 10, or a part thereof, wherein the reference cannabinoid is total CBN.
12. The cannabis plant of claim 11, or a part thereof, wherein the THC+CBG and the total CBN are present at a ratio of from about 200:1 to about 1500:1 (THC+CBG: CBN).
13. The cannabis plant of claim 11 or claim 12, or a part thereof, wherein the level of total CBN is from about 0.01% to about 1% by weight of the total cannabinoid content of the dry weight of plant material.
14. The cannabis plant of any one of claims 1 to 13, or a part thereof, wherein the reference cannabinoid is total THCv.
15. The cannabis plant of claim 14, or a part thereof, wherein the THC+CBG and the total THCv are present at a ratio of from about 50:1 to about 700:1 (THC+CBG: THCv).
16. The cannabis plant of claim 14 or claim 15, or a part thereof, wherein the level of total THCv is from about 0.01% to about 1% by weight of the total cannabinoid content of the dry weight of plant material.

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17. The cannabis plant of any one of claims 1 to 16, or a part thereof, wherein the cannabinoid profile does not include total CBDV, wherein total CBDV comprises cannabidivarin (CBDV) and cannabidivarinic acid (CBDVA).
18. The cannabis plant of any one of claims 1 to 17, or a part thereof, comprising one or more terpenes selected from the group consisting of α -phellandrene, α -pinene, camphene, β -pinene, myrcene, limonene, eucalyptol, γ -terpinene, linalool, γ -elemene, humulene, nerolidol, guaia-3,9-diene and caryophyllene.
19. The cannabis plant of claim 18, or a part thereof, comprising one or more terpenes selected from the group consisting of myrcene and β -pinene.
20. The cannabis plant of claim 19, or a part thereof, wherein the level of myrcene is present at a ratio of from about 60:1 and 1:1 to the level of β -pinene.
21. A seed of the cannabis plant of any one of claims 1 to 20.
22. A cannabis plant produced from the seed of claim 21.
23. A tissue culture of regenerable cells derived from the cannabis plant, or a part thereof, of any one of claims 1 to 20.
24. A cannabis plant generated from the tissue culture of claim 23, wherein the plant expresses the morphological and physiological characteristics of the cannabis plant of any one of claims 1 to 20.
25. The tissue culture of claim 23, comprising a population of cells or protoplasts derived from the cannabis plant, wherein the population of cells or protoplasts is isolated from a part selected from the group consisting of a seed, a leaf, a stem, pollen, an anther, an ovule, an embryo, a cotyledon and a hypocotyl.
26. A method for producing an F1 hybrid cannabis plant, the method comprising crossing the cannabis plant of any one of claims 1 to 20 with a different cannabis plant to produce an F1 hybrid.

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27. A seed derived from the F1 hybrid cannabis plant produced by the method of claim 26, or from progeny plants or seeds from a subsequent generation.
28. A hybrid cannabis plant produced from the seed of claim 27.
29. A method of producing a transgenic cannabis plant, the method comprising transducing the cannabis plant of any one of claims 1 to 20, or a part thereof, with a heterologous nucleic acid sequence.
30. A method of producing a transgenic cannabis plant, the method comprising introducing one or more nucleic acid substitutions, deletions or additions into the genome of the cannabis plant of any one of claims 1 to 20.
31. The method of claim 30, wherein the heterologous nucleic acid sequence also comprises one or more regulatory sequences.
32. A transgenic cannabis plant produced by the method of any one of claims 29 to 31, or a part thereof.
33. A seed derived from the transgenic cannabis plant of claim 32.
34. A progeny plant produced from the seed of claim 33.
35. A method of producing an extract comprising cannabinoids from a cannabis plant, the method comprising the steps of:
 - (a) harvesting plant material from the cannabis plant of any one of claims 1 to 20;
 - (b) at least partially drying the harvested plant material of step (a); and
 - (c) extracting cannabinoids from the at least partially dried plant material of step (b), thereby producing an extract comprising cannabinoids.
36. The method of claim 35, wherein the extract comprises a level of total THC and a level of total CBG at a ratio from about 5:1 to about 50:1 (THC:CBG), and wherein the level of total THC and total CBG (THC+CBG) is greater than the level of a reference cannabinoid selected from the group consisting of: total CBD, total CBC, total CBN, and total THCV.

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37. The method of claim 35, wherein the extract comprises total THC, total CBG, and one or more minor cannabinoids selected from the group consisting of: total CBD, total CBC, total CBN, total THCV, total CBL, and total Δ 8-THC, wherein the extract comprises a level of total THC and a level of total CBG at a ratio of from about 5:1 to about 50:1 (THC: CBG), and wherein the level of the one or more other cannabinoids is from about 0.01% to about 10% by weight of the total cannabinoid content of the extract.
38. The method of any one of claims 35 to 37, wherein the plant material comprises female inflorescence.
39. The method of any one of claims 35 to 38, further comprising curing the dried plant material of step (b) before step (c).
40. The method of any one of claims 35 to 39, wherein cannabinoids are extracted from the dried plant material in step (c) by supercritical fluid extraction.
41. An extract produced by the method of any one of claims 35 to 40.
42. An extract derived from the cannabis plant of any one of claims 1 to 20, or a part thereof, wherein the extract comprises a cannabinoid profile enriched for total THC and total CBG, wherein the cannabinoid profile comprises a level of total THC and a level of total CBG at a ratio from about 5:1 to about 50:1 (THC:CBG), and wherein the level of total THC and total CBG (THC+CBG) is greater than the level of a reference cannabinoid selected from the group consisting of: total CBD, total CBC, total CBN, and total THCV.
43. A total THC- and total CBG-enriched extract derived from the cannabis plant of any one of claims 1 to 20, or a part thereof, wherein the extract comprises total THC, total CBG, and one or more minor cannabinoids selected from the group consisting of: total CBD, total CBC, total CBN, total THCV, total CBL, and total Δ 8-THC, wherein the extract comprises a level of total THC and a level of total CBG at a ratio of from about 5:1 to about 50:1 (THC: CBG), and wherein the level of the one or more other

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cannabinoids is from about 0.01% to about 10% by weight of the total cannabinoid content of the extract.

44. A method for selecting a cannabis plant comprising a cannabinoid profile enriched for total THC and total CBG from a plurality of different cannabis plants, the method comprising:
- (a) harvesting plant material from a plurality of different cannabis plants;
 - (b) at least partially drying the harvested plant material of step (a);
 - (c) measuring in the at least partially dried plant material of step (b) a level of total THC, total CBG and one or more reference cannabinoids selected from the group consisting of THCV, CBDV, CBN, CBD, CBC, THCVA, CBDVA, CBNA, CBDA and CBCA, to generate a cannabinoid profile for each of the plurality of cannabis plants; and
 - (d) on the basis of the measurements from step (c), selecting from the plurality of different cannabis plants a cannabis plant comprising a cannabinoid profile enriched for total THC and total CBG and comprising a level of total THC and a level of total CBG at a ratio from about 5:1 to about 50:1 (THC:CBG), wherein the total THC comprises THC and THCA, wherein the total CBG comprises CBG and CBGA, and wherein the level of total THC and total CBG (THC+CBG) is greater than the level of a reference cannabinoid selected from the group consisting of:
 - (i) total CBD, wherein the total CBD comprises CBD and CBDA;
 - (ii) total CBC, wherein the total CBC comprises CBC and CBCA;
 - (iii) total CBN, wherein the total CBN comprises CBN and CBNA; and
 - (iv) total THCV, wherein the total THCV comprises THCV and THCVA.
45. A method for selecting a cannabis plant comprising a cannabinoid profile enriched for total THC and total CBG from a plurality of different cannabis plants, the method comprising:
- (a) harvesting plant material from a plurality of different cannabis plants;
 - (b) at least partially drying the harvested plant material of step (a);

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- (c) measuring in the at least partially dried plant material of step (b) a level of total THC, total CBG and one or more reference cannabinoids selected from the group consisting of THCV, CBDV, CBN, CBD, CBC, THCVA, CBDVA, CBNA, CBDA and CBCA, to generate a cannabinoid profile for each of the plurality of cannabis plants;
 - (d) measuring in the at least partially dried plant material of step (b) a level of myrcene and a level of β -pinene to generate a terpene profile for each of the plurality of cannabis plants; and
 - (e) on the basis of the measurements from step (c), selecting from the plurality of different cannabis plants a cannabis plant comprising (i) a terpene profile wherein the myrcene is present at a ratio of between about 60:1 and about 1:1 to the level of β -pinene and (ii) a cannabinoid profile enriched for total THC and total CBG and comprising a level of total THC and a level of total CBG at a ratio from about 5:1 to about 50:1 (THC:CBG), wherein the total THC comprises THC and THCA, wherein the total CBG comprises CBG and CBGA, and wherein the level of total THC and total CBG (THC+CBG) is greater than the level of a reference cannabinoid selected from the group consisting of:
 - (i) total CBD, wherein the total CBD comprises CBD and CBDA;
 - (ii) total CBC, wherein the total CBC comprises CBC and CBCA;
 - (iii) total CBN, wherein the total CBN comprises CBN and CBNA; andtotal THCV, wherein the total THCV comprises THCV and THCVA.
46. The method of claim 44 or claim 45, wherein the plant material comprises female inflorescence.
47. The method of any one of claims 44 to 46, wherein the level of total THC is at least 80% by weight of the total cannabinoid content of the at least partially dried weight of the plant material.
48. The method of any one of claims 44 to 47, wherein the level of total CBG is at least 1% by weight of the total cannabinoid content of the at least partially dried weight of the plant material.

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49. The method of any one of claims 44 to 48, wherein the reference cannabinoid is total CBD.
50. The method of claim 49, wherein the THC+CBG and the total CBD are present in a ratio of from about 50:1 to about 500:1 (THC+CBG: CBD) in the plant material
51. The method of claim 49 or claim 50, wherein the level of total CBD is from about 0.1% to about 1% by weight of the total cannabinoid content of the at least partially dried weight of the plant material.
52. The method of any one of claims 44 to 51, wherein the reference cannabinoid is total CBC.
53. The method of claim 52, wherein the THC+CBG and the total CBC are present at a ratio of from about 10:1 to about 120:1 (THC+CBG: CBC) in the plant material.
54. The method of claim 52 or claim 53, wherein the level of total CBC is from about 0.01% to about 10% by weight of the total cannabinoid content of the at least partially dried weight of the plant material.
55. The method of any one of claims 44 to 54, wherein the reference cannabinoid is total CBN.
56. The method of claim 55, wherein the THC+CBG and the total CBN are present at a ratio of from about 200:1 to about 1500:1 (THC+CBG: CBN) in the plant material.
57. The method of claim 55 or claim 56, wherein the level of total CBN is from about 0.01% to about 1% by weight of the total cannabinoid content of the at least partially dried weight of the plant material.
58. The method of any one of claims 44 to 57, wherein the reference cannabinoid is total THCV.
59. The method of claim 58, wherein the THC+CBG and the total THCV are present at a ratio of from about 50:1 to about 700:1 (THC+CBG: THCV) in the plant material.

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60. The method of claim 58 or claim 59, wherein the level of total THCV is from about 0.01% to about 1% by weight of the total cannabinoid content of the at least partially dried weight of the plant material.
61. The method of any one of claims 44 to 60, wherein the cannabinoid profile does not include total CBDV, wherein total CBDV comprises cannabidivarin (CBDV) and cannabidivarinic acid (CBDVA).
62. The method of claim 44, further comprising measuring in the at least partially dried plant material of step (b) one or more terpenes selected from the group consisting of α -phellandrene, α -pinene, camphene, β -pinene, myrcene, limonene, eucalyptol, γ -terpinene, linalool, γ -elemene, humulene, nerolidol, guaia-3,9-diene and caryophyllene.
63. The method of claim 62, wherein the one or more terpenes are selected from the group consisting of myrcene and β -pinene.
64. The method of claim 45 or claim 63, wherein the level of myrcene is present at a ratio of from about 60:1 and 1:1 to the level of β -pinene.
65. The method of any one of claims 44 to 64, wherein the selected cannabis plant is crossed with a different cannabis plant to produce an F1 hybrid.
66. The method of any one of claims 44 to 64, wherein regenerable cells isolated from the selected cannabis plant are transformed with a heterologous nucleic acid sequence and cultured for a time and under conditions suitable to produce a transgenic cannabis plant.
67. The method of any one of claims 44 to 64, wherein regenerable cells isolated from the selected cannabis plant are transfected with a gene editing construct comprising a nucleic acid sequence encoding a DNA-recognition moiety and cultured for a time and under conditions suitable to produce a non-transgenic cannabis plant with modified gene expression.

FIGURE 1

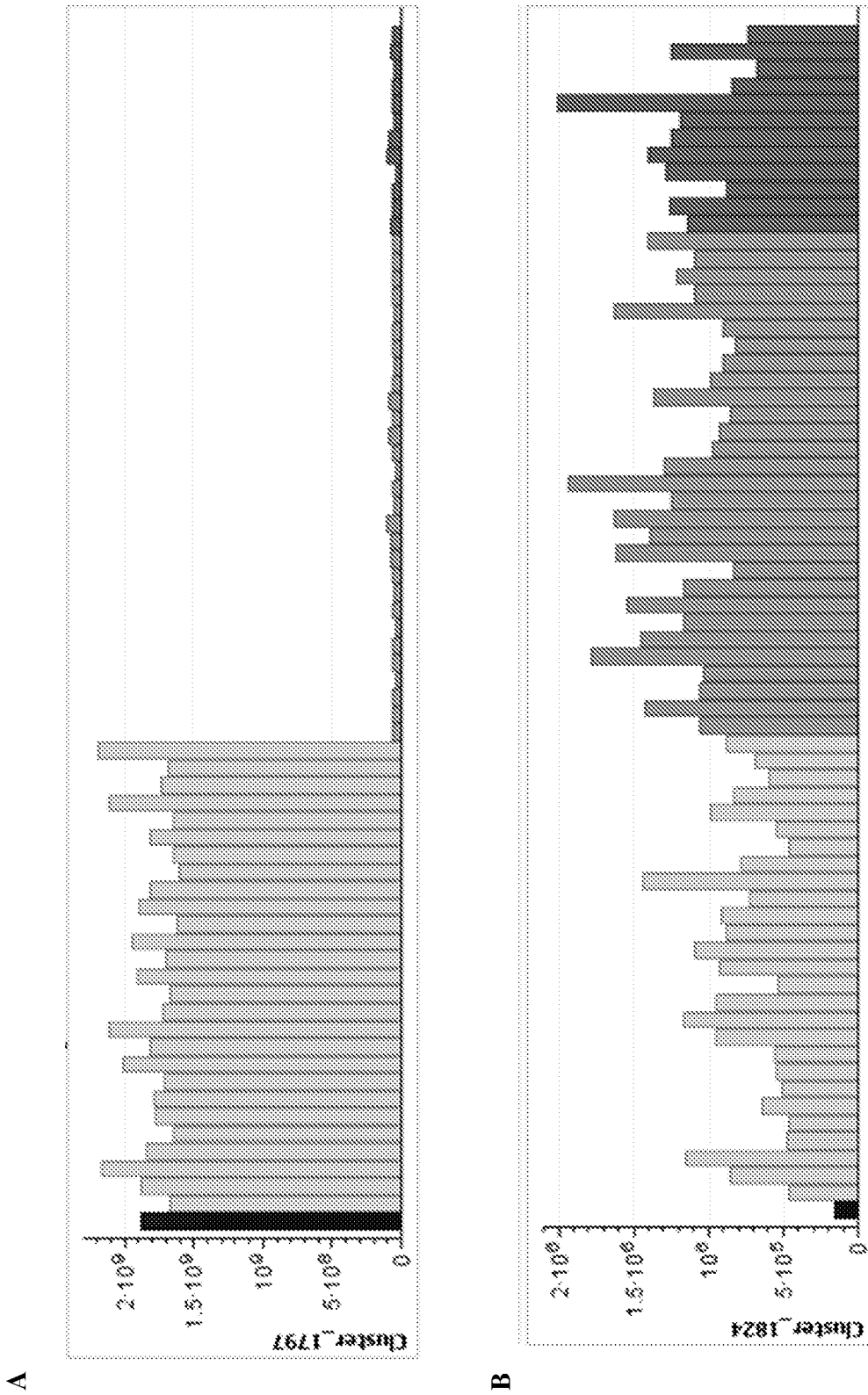
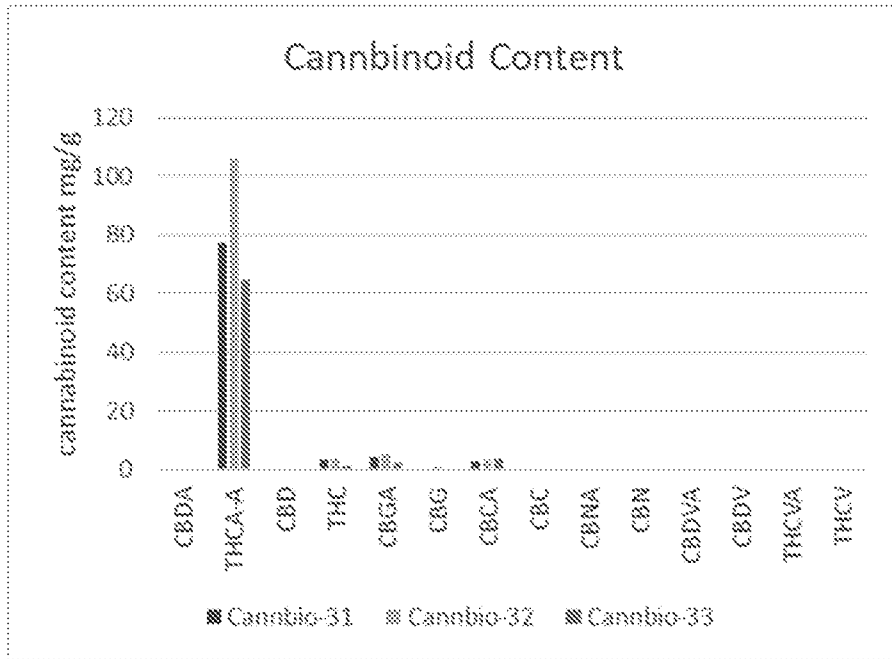


FIGURE 2

A



B

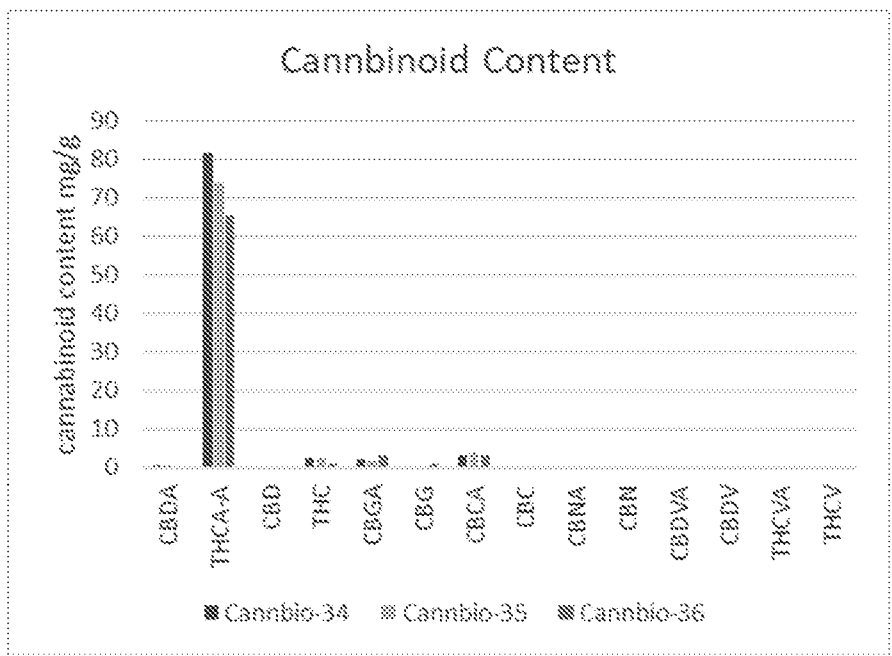
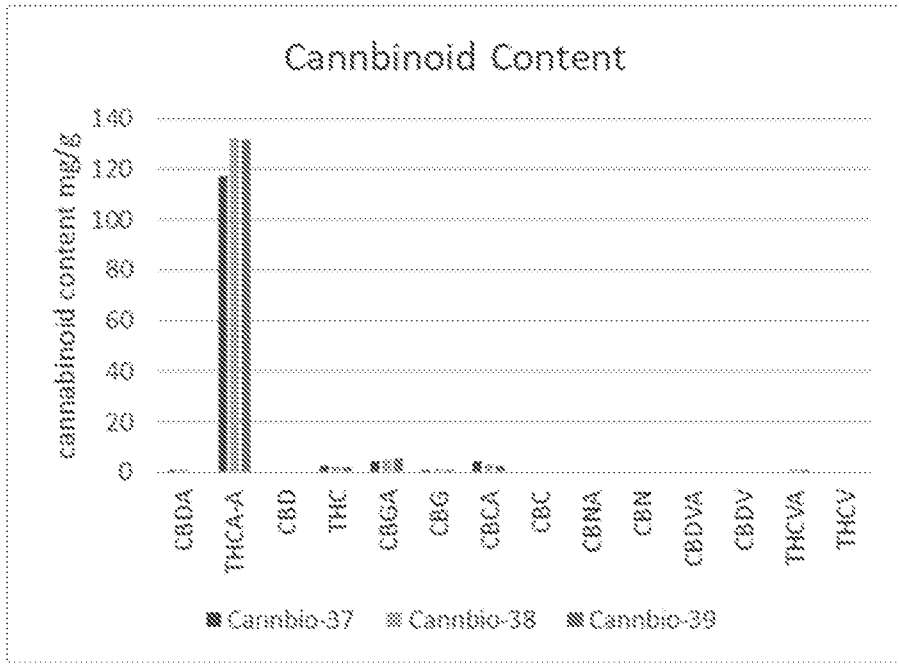


FIGURE 2 (CONTINUED)

C



D

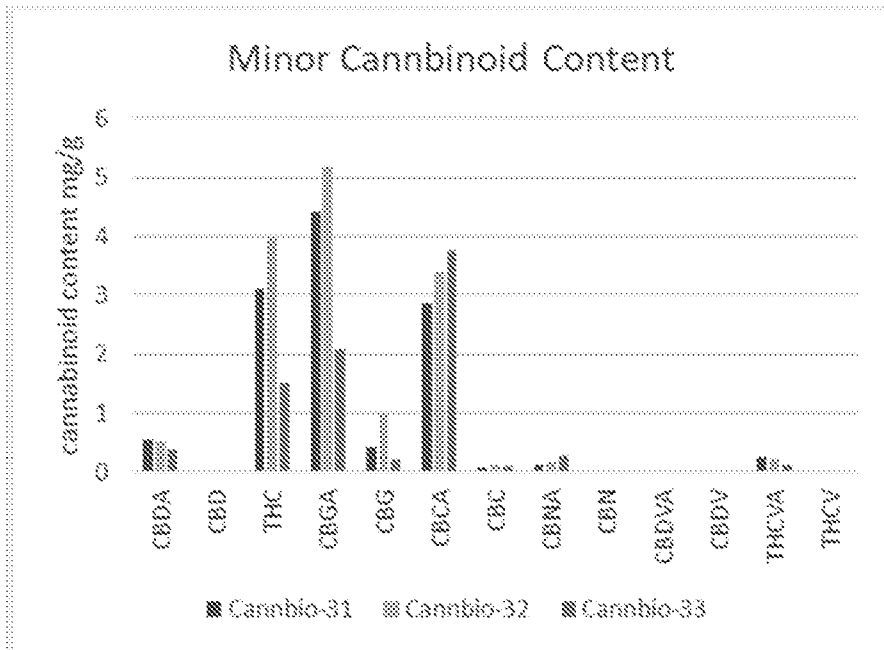
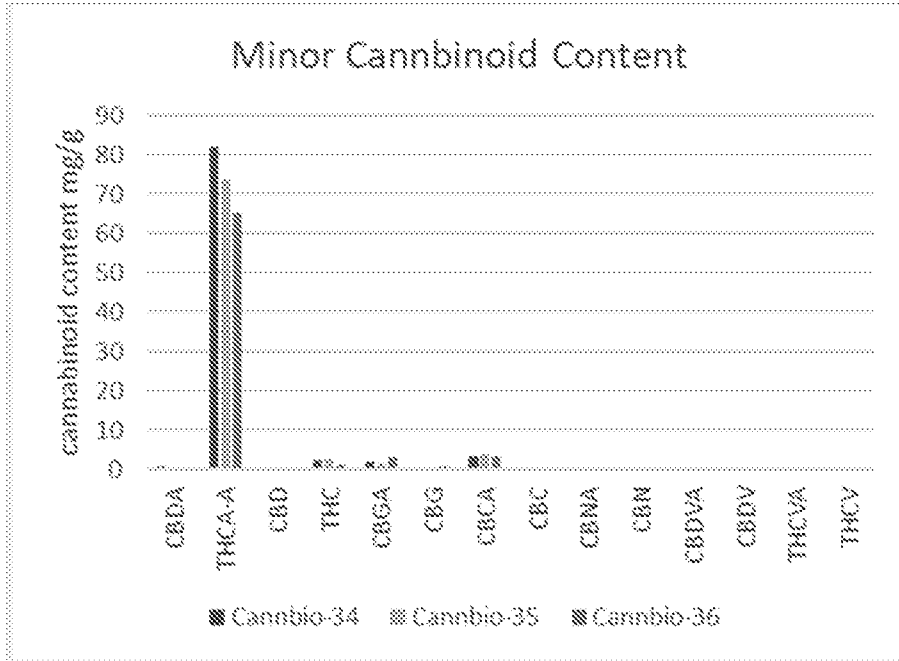
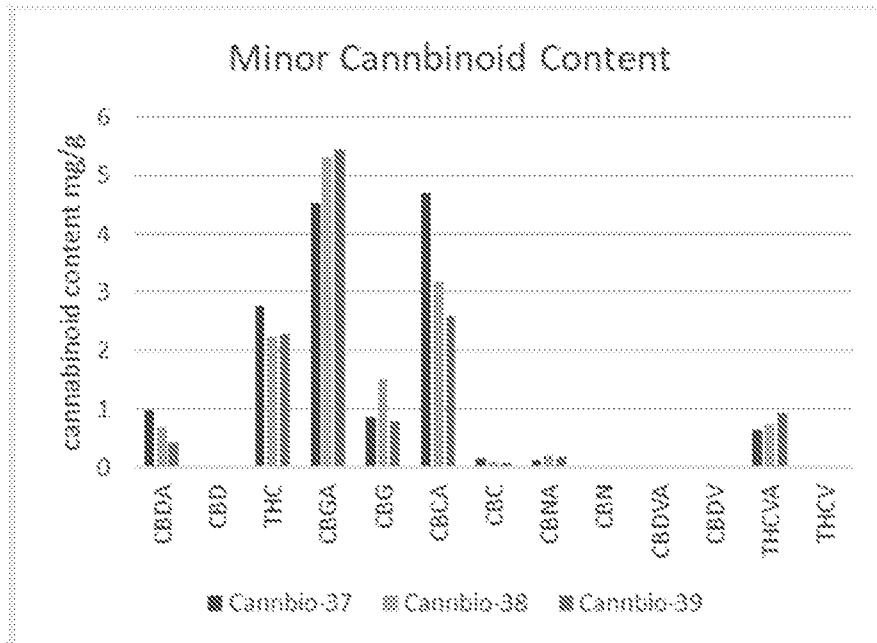


FIGURE 2 (CONTINUED)

E



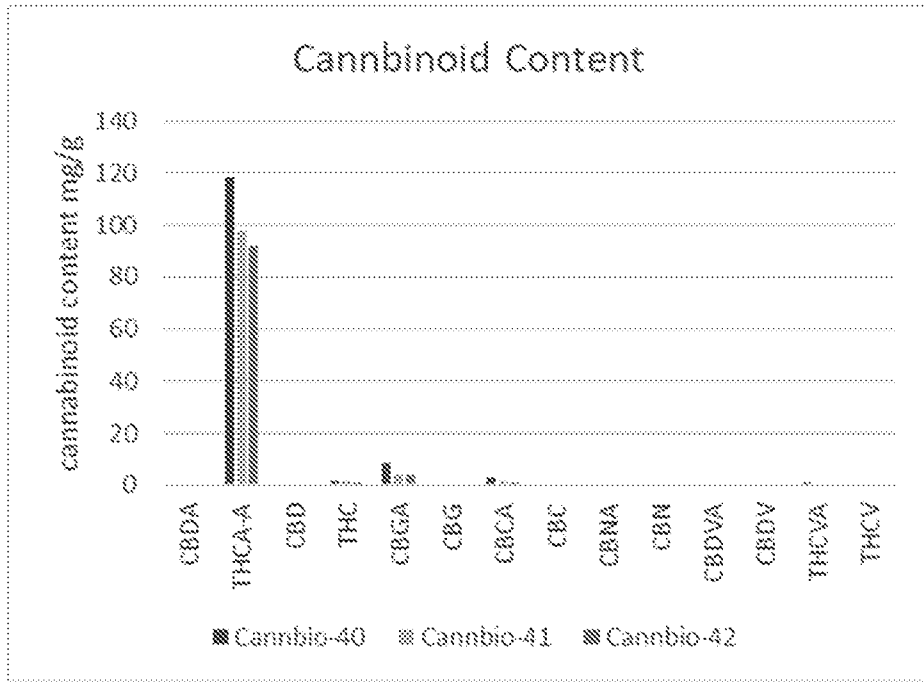
F



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FIGURE 3

A



B

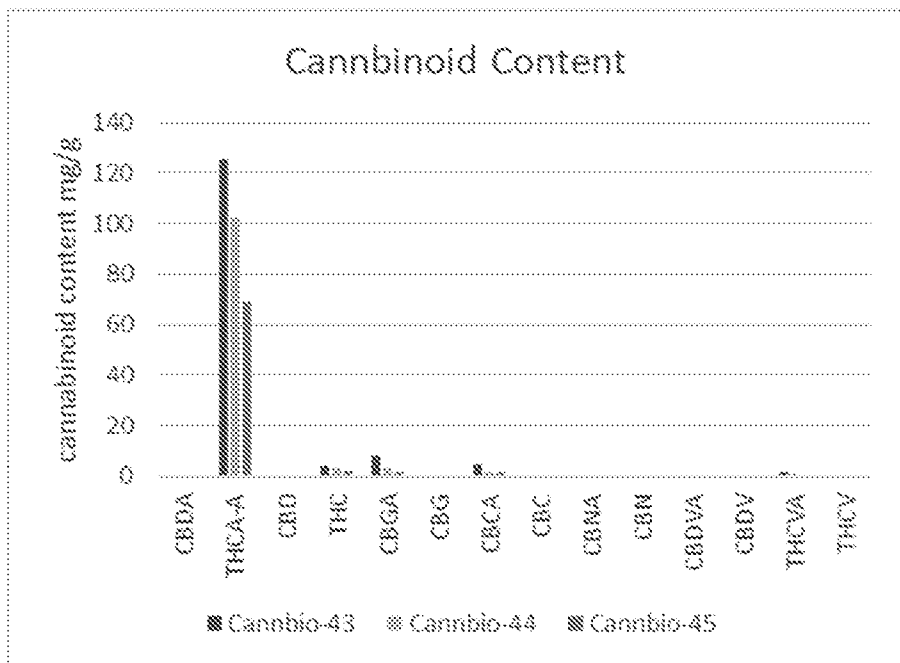
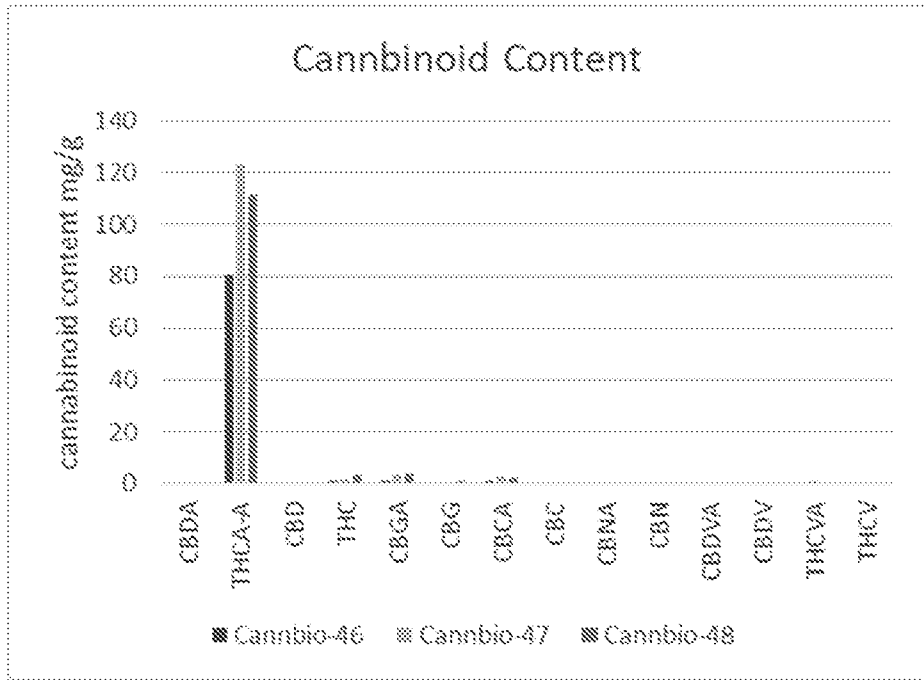
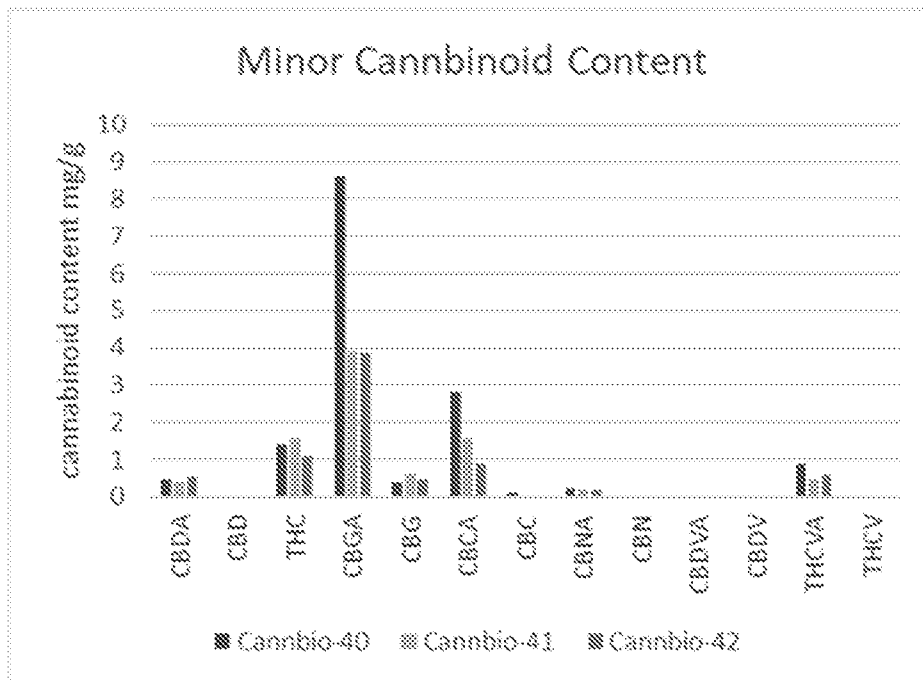


FIGURE 3 (CONTINUED)

C



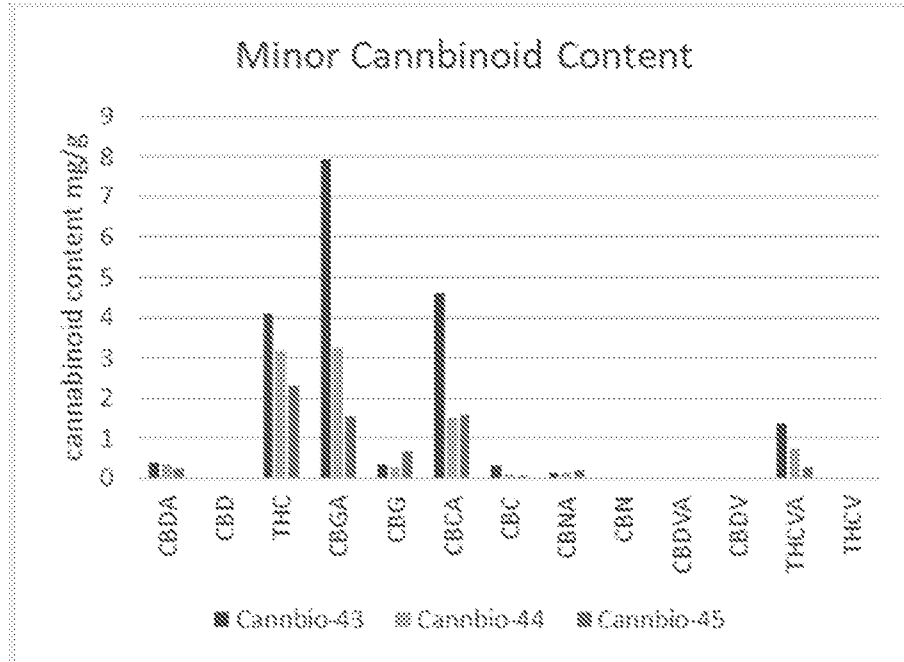
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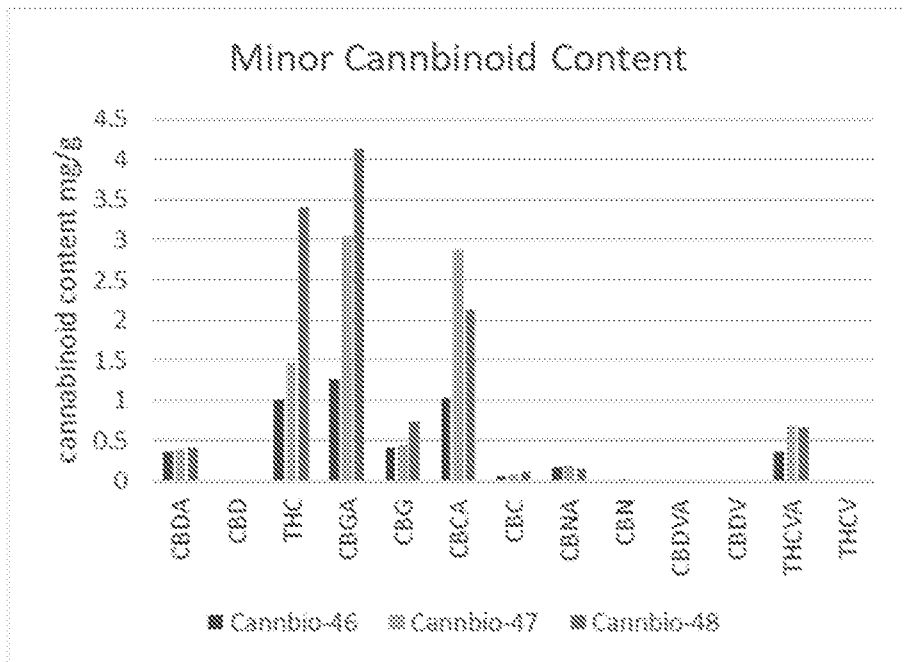
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FIGURE 3 (CONTINUED)

E



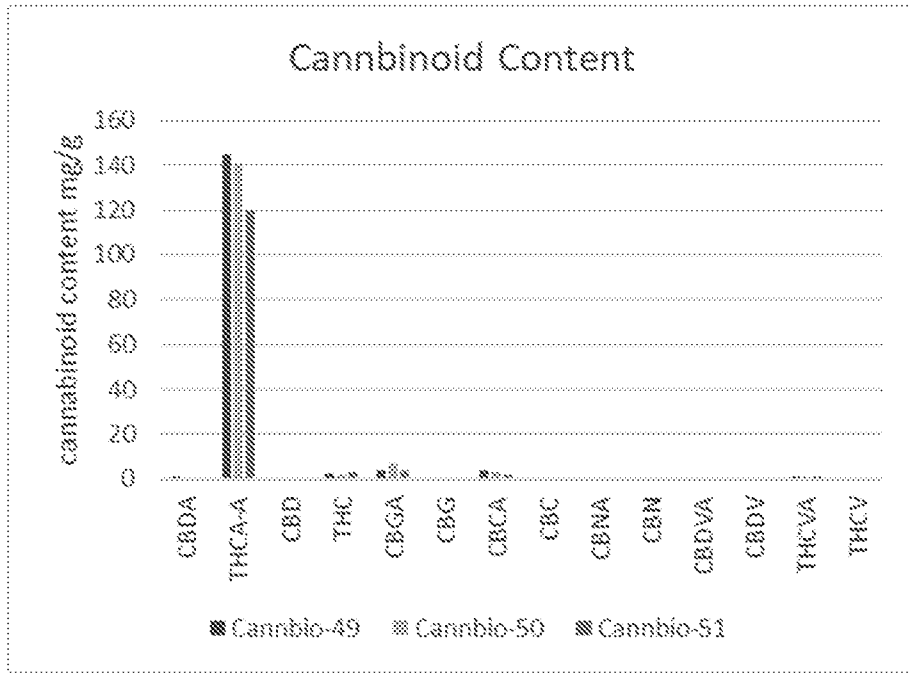
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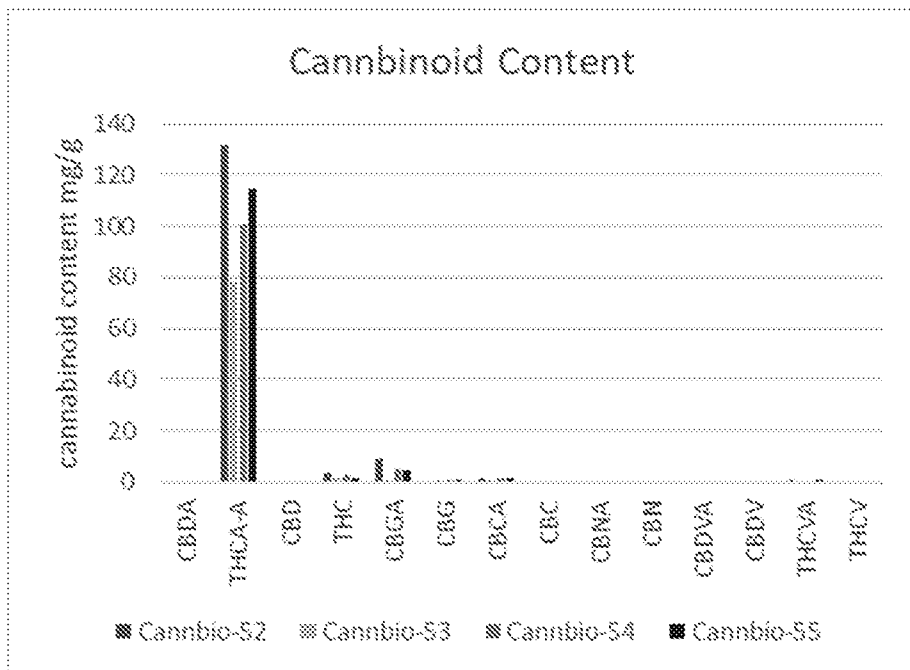
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FIGURE 4

A



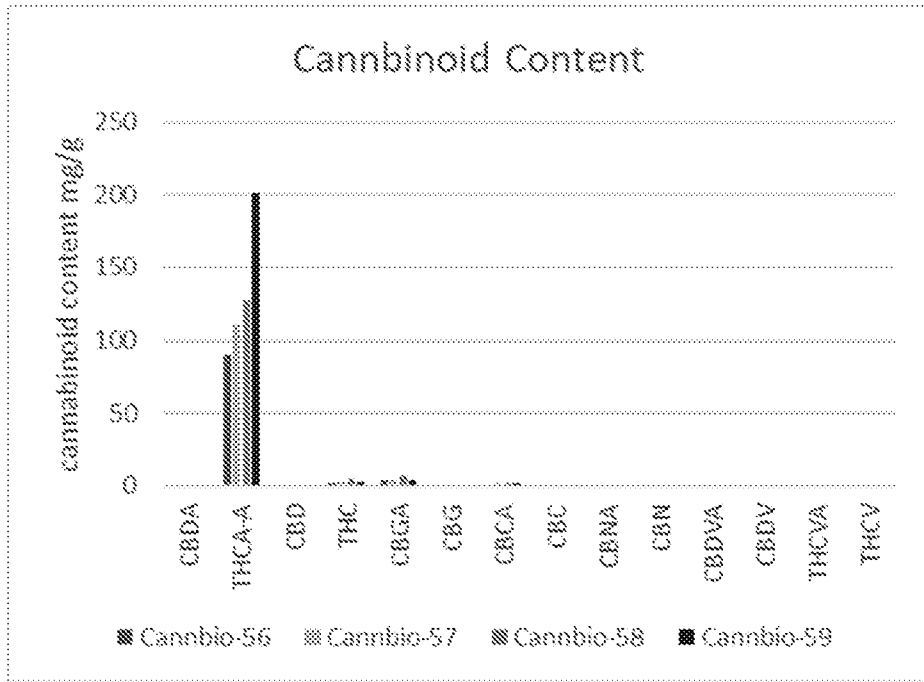
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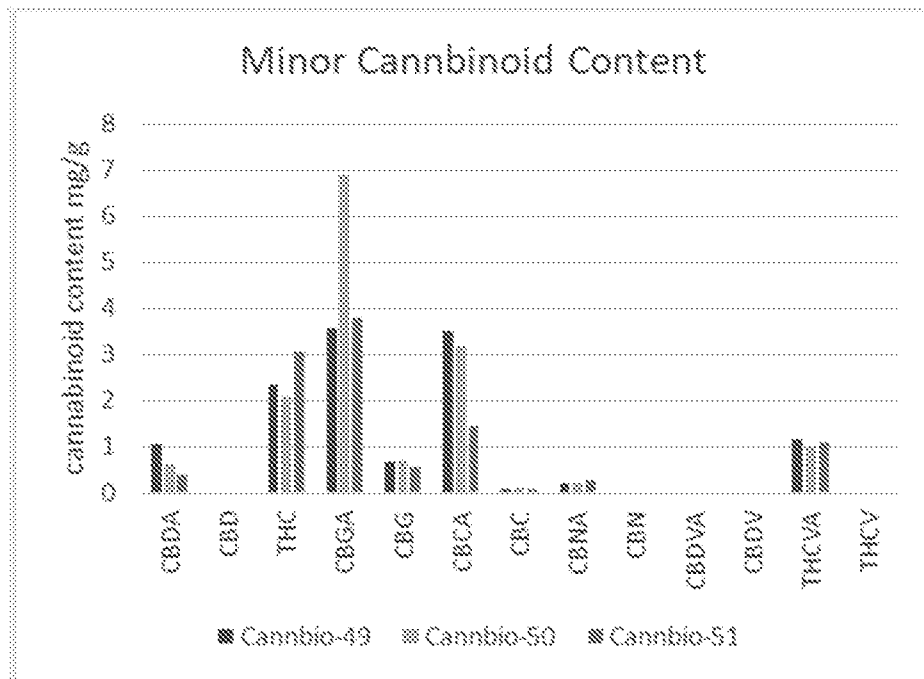
9/20

FIGURE 4 (CONTINUED)

C



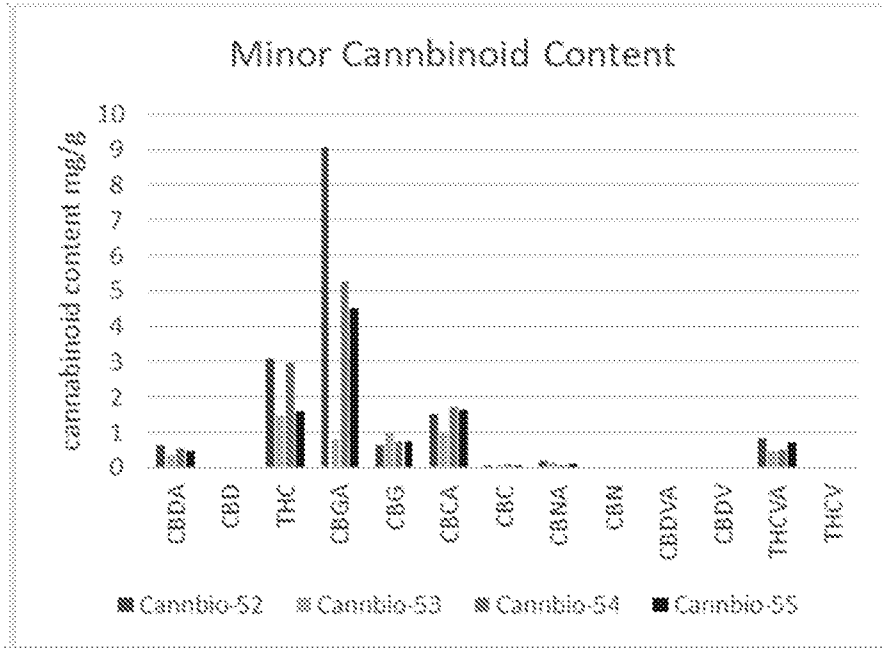
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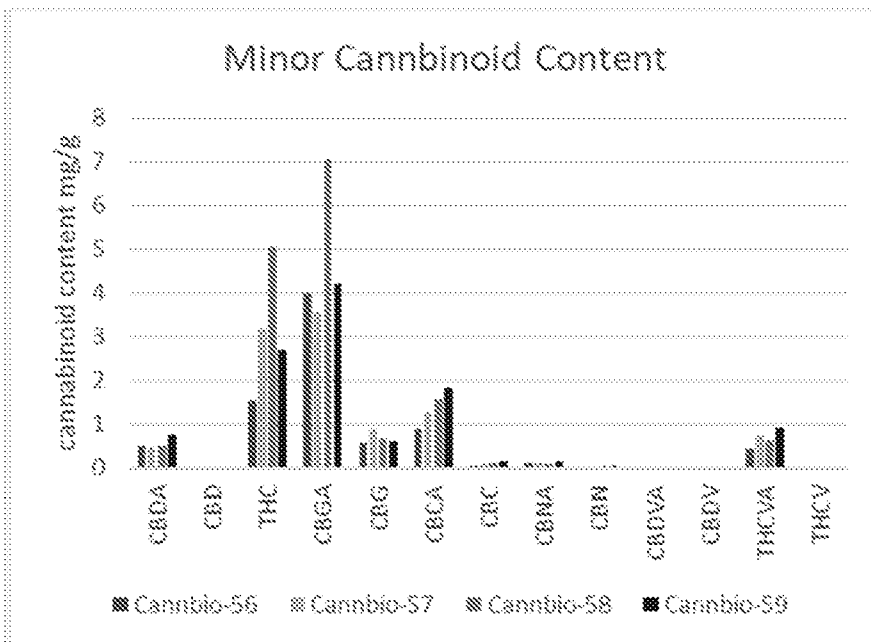
10/20

FIGURE 4 (CONTINUED)

E



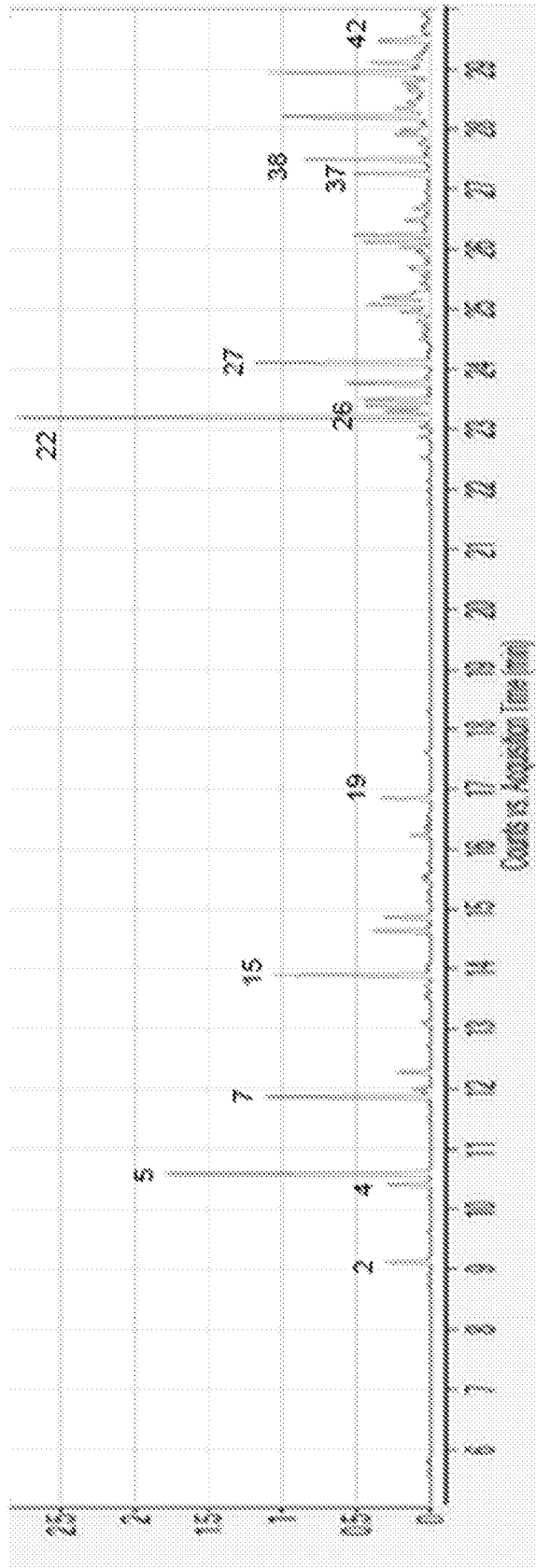
F



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FIGURE 5

A



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FIGURE 5 (Continued)

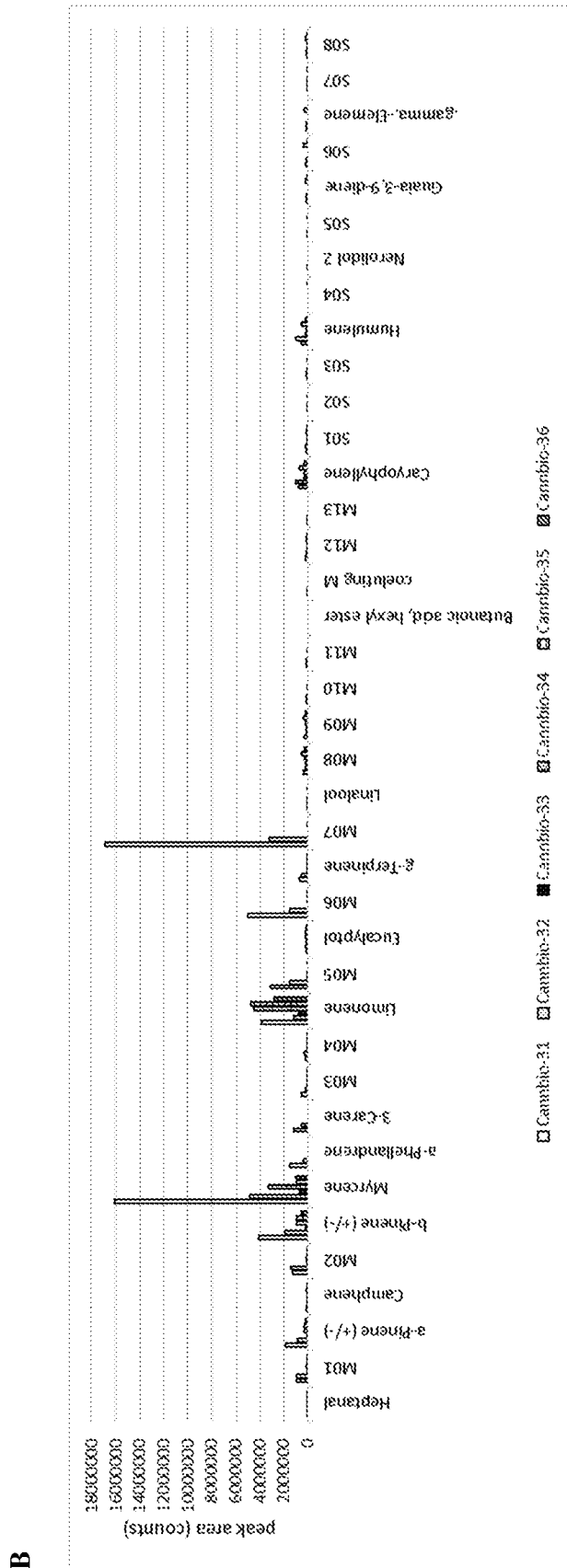


FIGURE 5 (Continued)

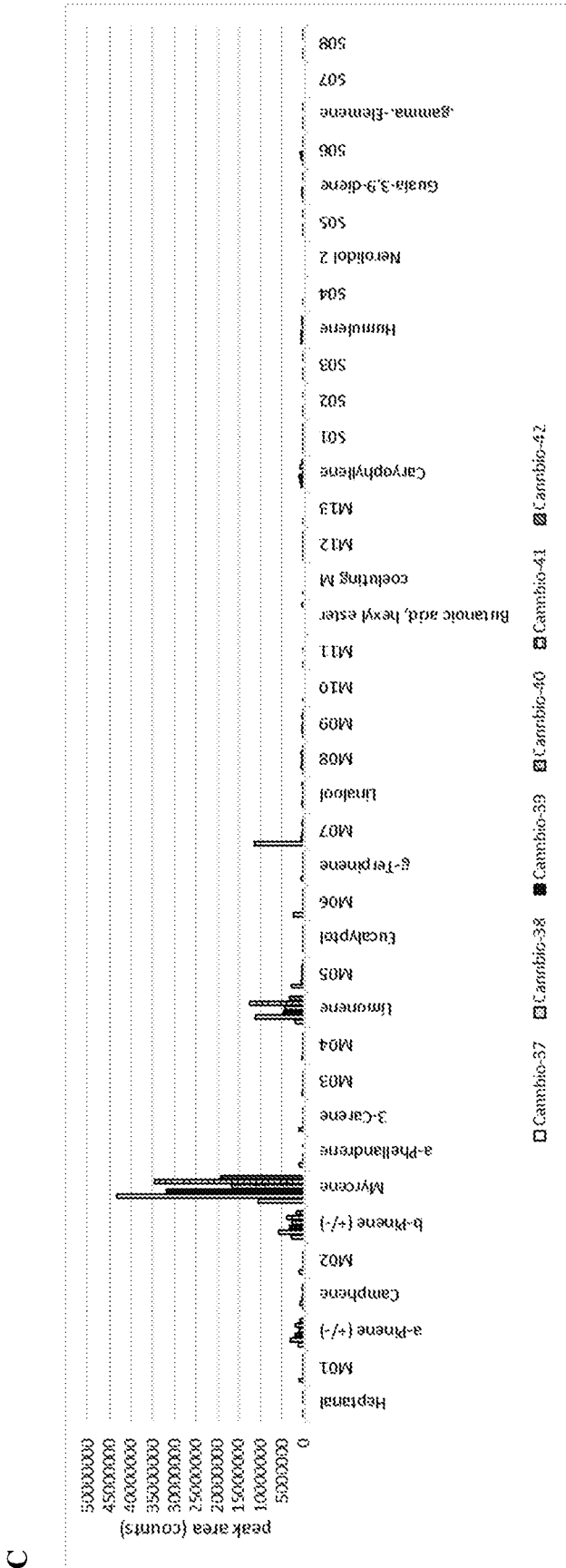
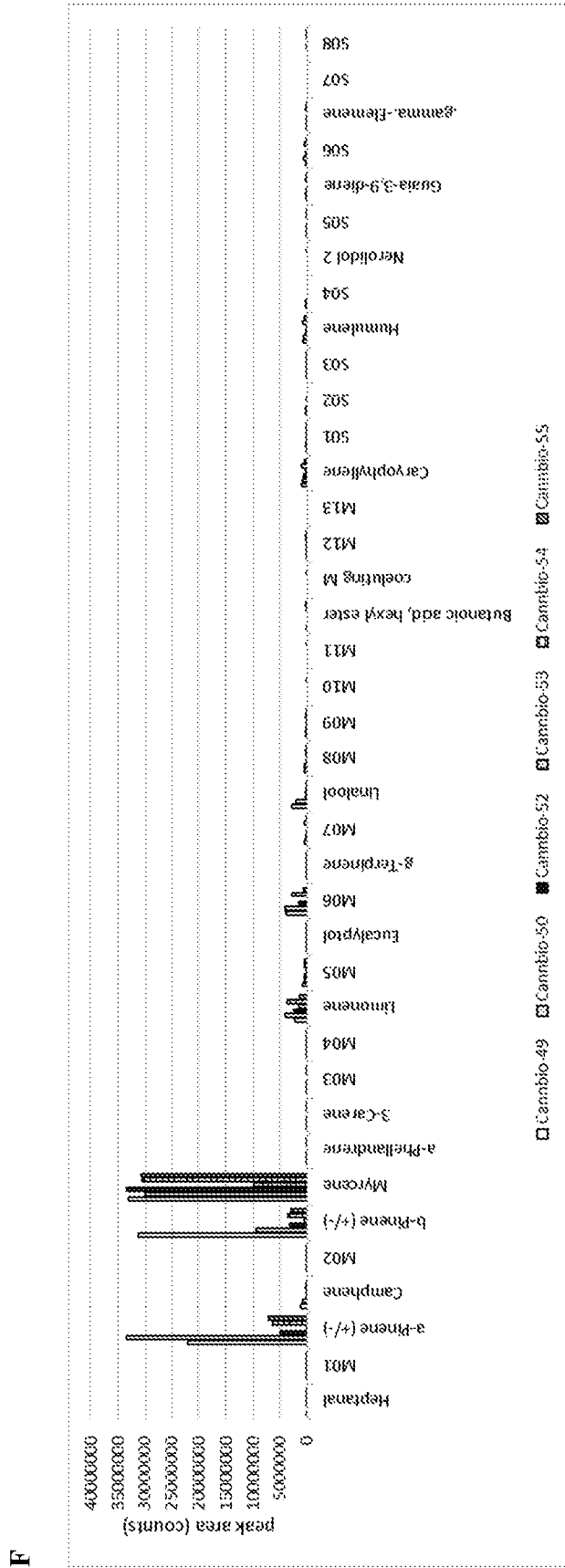
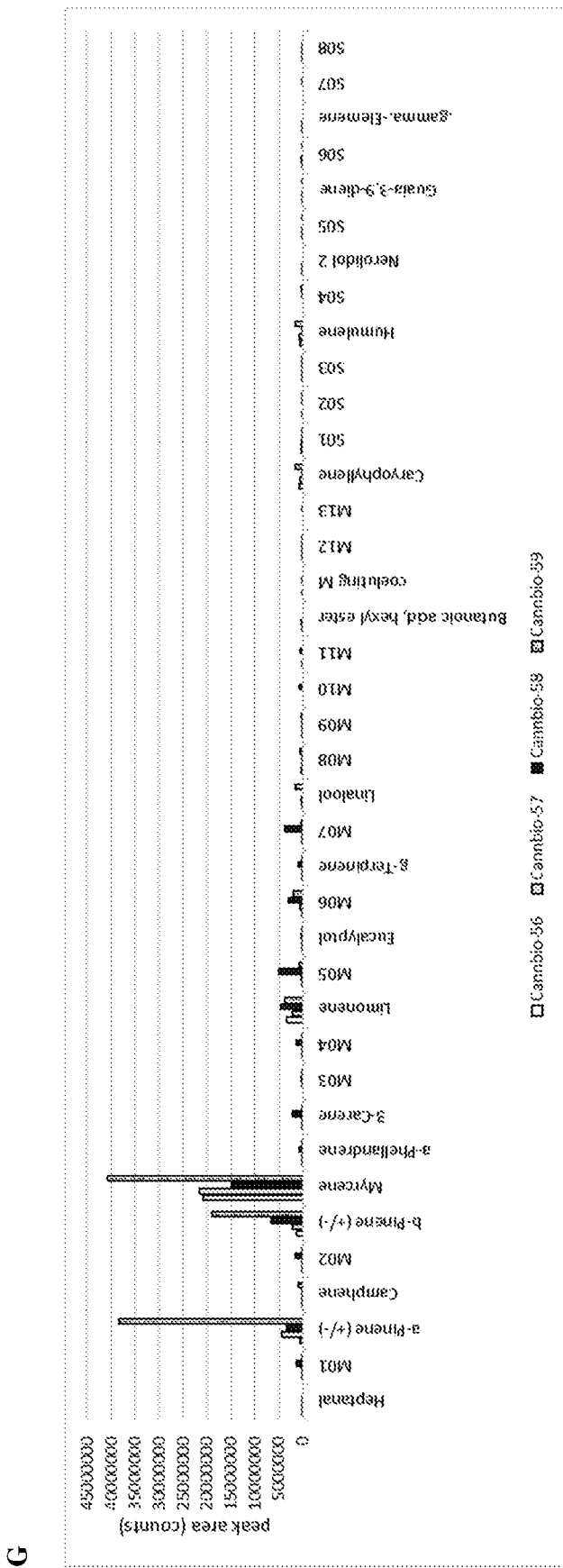


FIGURE 5 (Continued)



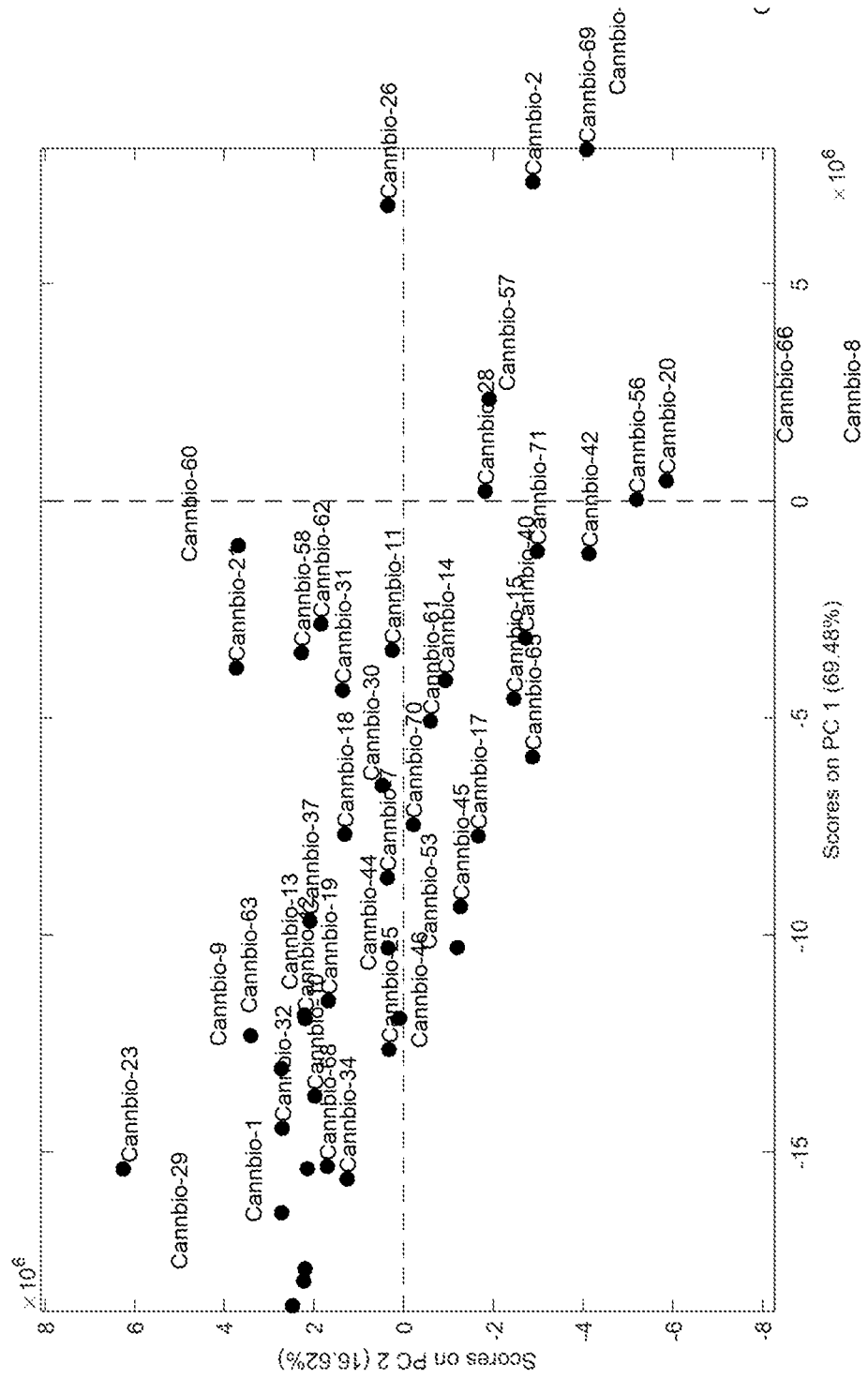
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FIGURE 5 (Continued)



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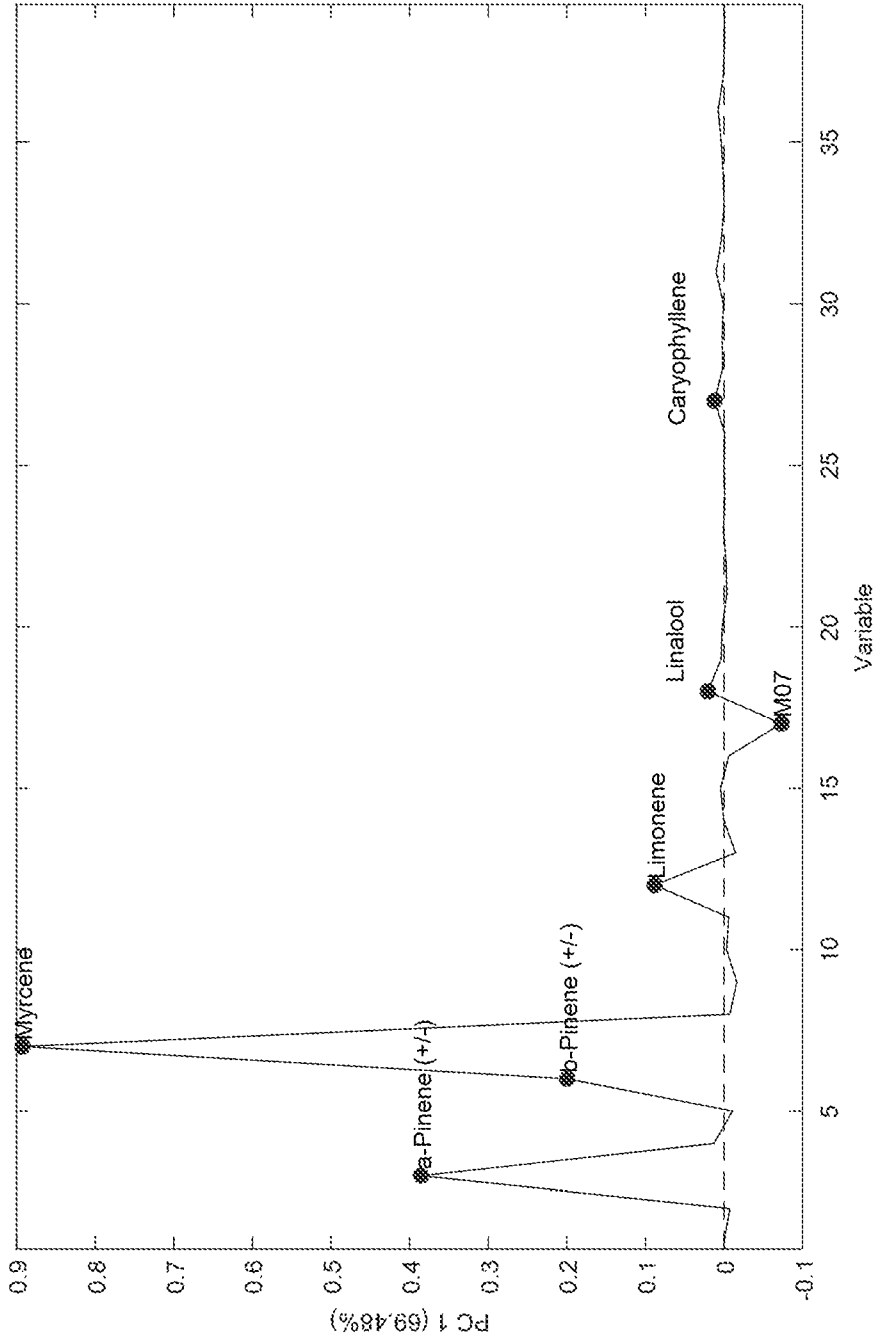
FIGURE 6



A

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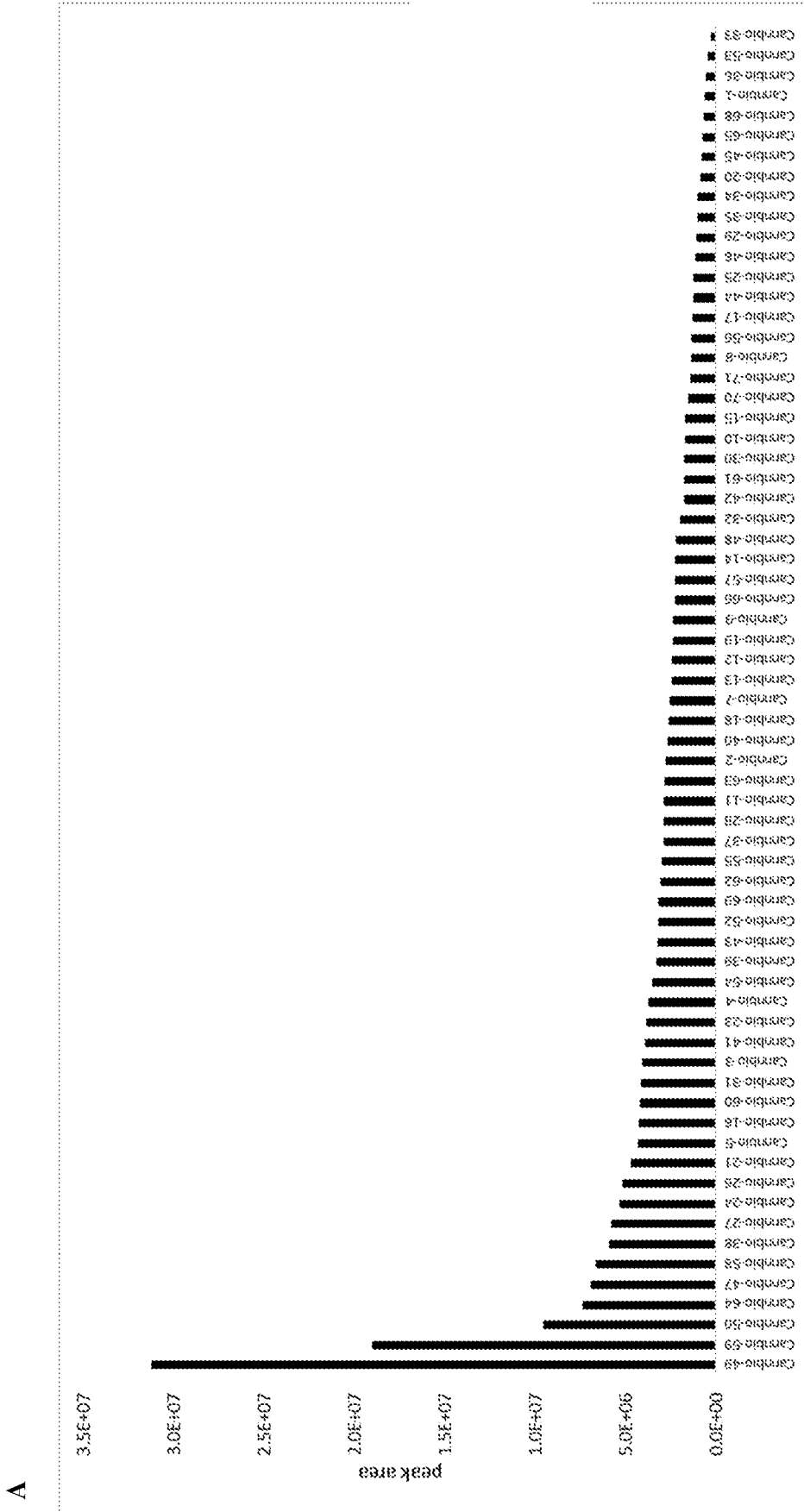
FIGURE 6 (Continued)



B

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FIGURE 7



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FIGURE 7 (Continued)

