Development of the Industrial Chain for the Production of Cannabigerol (CBG): from Plant to Pure Compound

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Phytoplant Research S.L. is a privately-owned Spanish company founded in 2008 and active in the field of phytotherapy research.

The company specializes in developing the industrial chain of medicinal plants, from selection and breeding to registration (for example, at Community Plant Variety Office (CPVO)), cultivation of registered varieties and obtaining derived products.

Phytoplant Research S.L. focuses on research and development products that contain plant material and extracts, as well as essential and seed oils. These products represent significant economic potential for the pharmaceutical, nutraceutical and dermocosmetic industries.

The company also wants to ensure the industrial supply of phyto-pharmaceutical quality raw materials through the cultivation of medicinal plants and extraction of plant material, with the objective of isolating, purifying and manufacturing naturally active ingredients (psychotropic and non psychotropic), semi-synthetic, biotransformed, pharmaceutically acceptable salts and derivatives.

Phytoplant Research S.L. is committed to the quality, safety and innovation of the full range of its products and services:
SPECIALIZATION AREAS

- Selection and cultivation of medicinal plants
- Extraction of plant material
- Following best industry standards and certifications
- Consulting and technical advice on crop industrialization
Métodos de extracción y purificación

- Proceso de producción – extracto / cannabinoides purificados
Vivacell, is the owner of the monoecious hemp variety registered at the CPVO (file nº 16305) identified with the denomination CARMA (chemotype CBG 3% (max) - THC<0,2%) and the rights of explotation have been ceded to Phytoplant.

**INTRODUCTION**

- Joint Venture with Vivacell Biotechnology España since 2012:
The strategy of Vivacell is to improve the biological activity of the phytocannabinoids and ensure the intellectual property of the NCE.

INTRODUCTION

**Cannabis sativa**
CARMA (CPVO 16305)

**PHYTOPLANT RESEARCH S.L.**

**EXTRACTS**

**CDE-001**
ATOPIC DERMATITIS
SKIN INFECTIONS

**HERBAL DRUG**

**VCE-003**
HUNTINGTON
PARKINSON
LATERAL AMIOTROPHIC SCLEROSIS

**VCE-004**
SCLEROSIS MULTIPLE
SCLERODERMIA

**NEW CHEMICAL ENTITIES (NCE)**

**CBG**

**CBD**

**PHYTOCANNABINOIDs**
AS A CHEMICAL PRECURSORS
INTRODUCTION

- Mechanism of action and effects of CBG

**CHRONIC and INFLAMMATORY PAIN**

**CANCER**

**METABOLIC DISORDERS**

**ANTI INFLAMMATORY**

**ANTIBIOTIC**

**BONE STIMULANT**

**ANTIDEPRESANT**

**METABOLIC DISORDERS HABIT CESSATION**

**(Cascio et al., 2009) (De Petrocelis et al., 2010) (Granja et al., 2012)**

- TRPA1, TRPM8, TRPV1, TRPV2
- α-2-adrenergic
- CB1
- CB2
- PPAR-γ
- 5-HT-1β
INTRODUCTION

- **Biosynthesis of cannabinoids in *Cannabis sativa* L.**

(Flores Sanchez & Verporte, 2009)
INTRODUCTION

Genetic theory of cannabinoid biosynthesis:

Codominant Alleles encoding for THCAS ($B_T$) or CBDAS ($B_D$)
Receive Allele that encodes for a non functional CBDAS ($B_0$) that leads to CBG accumulation

(de Meijer, et al. 2003)
Heterogeneity in the Cannabinoid synthases encoded in the Locus B:

INTRODUCTION

Mutation that produces a non functional THCA synthase

Mutations that produces a non functional CBDA synthase

Onofri et al. (2015)
Phytoplant CBG varieties with protection at CVPO

Denomination: CARMA
CPVO file N: 2003/0046
CPVO grant N: 16305
Sex expression: mainly monoecious, but presence of dioecious male and female plants
Propagation: sexual (by seeds)
Chemotype: CBG (most of the plants)
CBG: 1,5 – 3,0%
Yield: 2 - 5 tons/Ha (flowers and leaves)
Cultivation in open field

Denomination: CARMA C80
Sex expression: dioecious female plant
Propagation: asexual (by cuttings)
Chemotype: CBG
CBG: 1,5 – 3,0%
Yield: 250 - 300 gr/planta (flowers and leaves)
Cultivation in high tunnel

Denomination: CARMA C54
Sex expression: dioecious female plant
Propagation: asexual (by cuttings)
Chemotype: CBG
CBG: 1,5 – 3,0%
Yield: 225 - 275 gr/planta (flowers and leaves)
Cultivation in high tunnel
**Denomination:** AIDA  
CPVO file N: 2016/0167  
Chemotype: CBG  
CBG: 3,5 - 8,5%  
Yield: **500 - 700 gr/plant**  
(flowers and leaves)  
Cultivation in high tunnel

**Denomination:** JUANI  
CPVO file N: 2016/0117  
Chemotype: CBG+CBGV  
CBG: 2,0 - 4,5%, CBGV: 0,3-0,6%  
Yield: **700 - 900 gr/plant**  
(flowers and leaves)  
Cultivation in high tunnel

**Denomination:** OCTAVIA  
CPVO file N: 2017/0148  
Chemotype: CBG  
CBG: 3,5 - 7,0%  
Yield: **700 - 900 gr/plant**  
(flowers and leaves)  
Cultivation in high tunnel

**BREEDING OF CANNABIS VARIETIES OF CHEMOTIPE IV (CBG)**

- Phytoplant CBG varieties with provisional protection at CVPO
**BREEDING OF CANNABIS VARIETIES OF CHEMOTYPE IV (CBG)**

- **Phytoplant CBG varieties – other secondary metabolites**

**Denomination: AIDA**  
*Major terpenoids: Myrcene dominant*  
*Secondary terpenoids: Guaiol : Phytol- 1 > β Caryophyllene : α Pinene- 1*  
*Cannflavin A: 470.3 µg/g*  
*Cannflavin B: 292.6 µg/g*  
*Canniprene: 5.5 µg/g*

**Denomination: JUANI**  
*Major terpenoids: Phytol dominant*  
*Secondary terpenoids: Myrcene > β Caryophyllene : guaiol- 1 > α Pinene : Ocimene- 1*  
*Cannflavin A: 316.3 µg/g*  
*Cannflavin B: 99.8 µg/g*  
*Canniprene: 11.2 µg/g*

**Denomination: OCTAVIA**  
*Major terpenoids: Phytol dominant*  
*Secondary terpenoids: Myrcene > β Caryophyllene : Ocimene- 1*  
*Cannflavin A: 106.0 µg/g*  
*Cannflavin B: 55.0 µg/g*  
*Canniprene: 81.3 µg/g*
Different Agro-technics studied as a function of variety and growing conditions

CARMA is a sexually propagated (by seeds) monoecious variety

- Open Field (1 cycle/year)
- High Tunnel (1 cycle/year)
- High technology greenhouse (4-5 cycles/year)
- Indoor CEA (4-5 cycles/year)
Different Agro-technics studied as a function of variety and growing conditions

- **Open Field**
  - (1 cycle/year)

- **High technology greenhouse**
  - (4-5 cycles/year)

- **High Tunnel**
  - (1 cycle/year)

- **Indoor CEA**
  - (4-5 cycles/year)

*C54, C80, AIDA, JUANI and OCTAVIA are asexually propagated (by cuttings) dioecious varieties.*
Yields of CARMA cultivated in open field from seeds

Figure 1. Yield values in Ermes (A) and Carma (B). Bars with different letters significantly differ (p < 0.05) by Tukey’s test. IR1, irrigated at 100% of crop evapotranspiration (ETc); IR2, irrigated at 80% of ETc; PD, plant density; FW, fresh weight; DW, dry weight.

Garcia-Tejero et al. (2014)
CULTIVATION

- Cultivation of experimental varieties in High tunnel. Comparison of yields in cultivation on pots or in the soil directly.
EXTRACTION & PURIFICATION OF CBG

Typical extraction/purification protocol of cannabinoids from Cannabis sativa L.

Step 1: Decarboxylation of PM (120 °C for 1 hour).

Step 2: Extraction with supercritical fluid or solvent from PM.

Step 3: "Winterization" (Dissolve in EtOH and cooling to -20 °C) of the extract.

Step 4: Chromatography of the "winterized" extract to obtain the purified extract.

Step 5: Dissolve the purified extract fractions in a first solvent (polar or non-polar), filter off any insoluble material, and remove the solvent from the filtrate.

Step 6: Dissolve the filtrate in a second (non-polar or polar) solvent, filter off any insoluble material and remove the solvent from the filtrate to obtain a substantially pure cannabinoid.

Step 7: Optional treatment with activated charcoal or Florisil.

Step 8: Optional flash chromatography or recrystallization.

Step 9: Optional Chemical Derivation and Crystallization.


(Galal, et al. 2009)
**Basic methods of extraction of cannabinoids from Cannabis sativa L. plant:**

- Extraction by infussion in water and milk or with solubilizers as cyclodextrins or lecitine.

- Extraction by direct maceration in food oil.

**Extraction with liquid organic solvents:**
- Extraction by percolation or maceration with organic solvents.
- Hot extraction with organic solvent, Soxhlet type.
- Auxiliar energy assisted extraction (Ultrasounds or Microwaves).

**Extraction with pressurized gas:**
- Extraction with CO2 in any state, liquid, subcritic or supercritical state.
- Extraction with butane or propane.
- Extraction with refrigerant gas.
Solvent and extraction technique comparative in the yield in % of extraction of cannabinoids from *Cannabis sativa* L.
PURIFICATION OF CBG

Basic methods of purification of cannabinoids from *Cannabis sativa* L. plant:

- Purification by molecular distillation.
- Purification by derivates formation and recrystallization.

Purification by solid-liquid chromatography methods:
- Purification by column chromatography (Silica, C18 or other solid phases) by gravity or Flash.
- Purification by high pressure liquid chromatography (HPLC) in column of Silica, C18 or other solid phases.
- Purification by supercritical chromatography (SFC) using CO$_2$ as a solvent.

Purification by liquid-liquid chromatography methods:
- Purification by centrifugal partition chromatography (CPC).
- Purification by counter current chromatography (CCC).

Purification by recrystallization.
PURIFICATION OF CBG

- Purification by liquid-liquid chromatographic methods (CPC & CCC):

- Pump: Isocratic or gradient up to 250 bars, 10 to 500 ml/min
- Injection valve: 1 to 100ml, Option: Autosampler
- Detector: UV-Visible, ELSD, MS
- Fraction collector

Separatory funnel

\[ K_D = \frac{[A]_{\text{upper}}}{[A]_{\text{lower}}} \]

- \( K_D > 1 \)
- \( K_D = 1 \)
- \( K_D < 1 \)
Purification of CBG

- Purification by liquid-liquid chromatographic methods (CPC & CCC):
Comparation of cannabinoid purity of isolated cannabinoids purified by liquid-liquid chromatographic methods (CPC Vs CCC):

<table>
<thead>
<tr>
<th>Isolated cannabinoid</th>
<th>Isolated in this study (mg)</th>
<th>Relative yield a)</th>
<th>purity GC b)</th>
</tr>
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<tbody>
<tr>
<td>Δ⁹-THC</td>
<td>90,0</td>
<td>0.83</td>
<td>93.1%</td>
</tr>
<tr>
<td>THCA</td>
<td>1590</td>
<td>8.34</td>
<td>94.0%</td>
</tr>
<tr>
<td>CBD</td>
<td>232</td>
<td>0.46</td>
<td>92.7%</td>
</tr>
<tr>
<td>CBDA</td>
<td>326</td>
<td>0.65</td>
<td>90.2%</td>
</tr>
<tr>
<td>CBG</td>
<td>40,3</td>
<td>0.54</td>
<td>92.2%</td>
</tr>
<tr>
<td>CBGA</td>
<td>37,9</td>
<td>0.46</td>
<td>92.9%</td>
</tr>
<tr>
<td>CBN</td>
<td>99,4</td>
<td>1.38</td>
<td>95.0%</td>
</tr>
</tbody>
</table>

(Hazekamp et al., 2004)

<table>
<thead>
<tr>
<th>Compuesto</th>
<th>Concentración (% peso seco ± SD)</th>
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<tbody>
<tr>
<td>CBD</td>
<td>98.76 ± 0.45</td>
</tr>
<tr>
<td>CBDV</td>
<td>100.00 ± 206</td>
</tr>
<tr>
<td>CBDA</td>
<td>98.24 ± 2.81</td>
</tr>
<tr>
<td>THCA</td>
<td>98.14 ± 0.12</td>
</tr>
<tr>
<td>THC</td>
<td>95.04 ± 0.18</td>
</tr>
<tr>
<td>CBGA</td>
<td>95.88 ± 0.28</td>
</tr>
<tr>
<td>CBG</td>
<td>99.06 ± 0.08</td>
</tr>
</tbody>
</table>

(Internal data of Phytoplant Research SL)
Purification patent “Methods of Purifying Cannabinoids, Compositions and Kits Thereof”
US 20160214920 A1

- Easy to Scale Up method
- Non expensive equipment
- Quick and efficient method
- Environmentally friendly

<table>
<thead>
<tr>
<th>APPLICATION NUMBER</th>
<th>FILING or 371(c) DATE</th>
<th>GRP ART UNIT</th>
<th>FIL FEE REC'D</th>
<th>ATTY.DOCKET.NO</th>
<th>TOT CLAIMS</th>
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<td>15/004,848</td>
<td>01/22/2016</td>
<td>1629</td>
<td>730</td>
<td>31PPP1.0001US</td>
<td>20</td>
<td>2</td>
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</tbody>
</table>
Step 1: Incubating the PM with non-polar solvent

Step 2: Reducing the volume of the 1st solvent mixture to about 50%

Step 3: Incubating the reduced 1st solvent mixture (-70-40°C) to crystalize the cannabinoids

Step 4: Dissolving the crystalized cannabinoids with non-polar solvent (being the 2nd solvent mixture)

Step 5: Incubating the 2nd solvent mixture (-70-40) to crystalize the cannabinoids. Getting a purification of cannabinoids > 95%.
Sampling

Identification

Chemical determinations
  - Foreign matter
  - Water quantification
  - Total ash quantification
  - Acid insoluble ash quantification
  - Determination of the purity of the APIs (cannabinoids)
  - Quantification of mycotoxins (aflatoxins and ochratoxins)
  - Determination of heavy metals
  - Determination of residual solvents
  - Determination of pesticides

Microbiological determination
# TESTING AND QUALITY CONTROL

## Determination of the purity of the APIs (cannabinoids)

<table>
<thead>
<tr>
<th>Name</th>
<th>Component</th>
<th>Purity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBG &gt; 98 %</td>
<td>Cannabigerol (CBG)</td>
<td>99.67 ± 0.46</td>
</tr>
</tbody>
</table>

Column: InfinityLab Poroshell, Ec-C18, 2.7μm size particule, 150 x 2.1mm
Movil phase: Water and Acetonitrile with formiate ammonium
Det.: DAD, 210nm
Inj.: 3 μL
Oven: 30 ºC
Date: 25/08/2016
Thank you for your attention