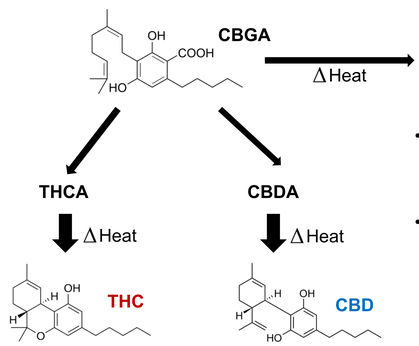
Cannabigerol (CBG): The Mother of Cannabinoids Demonstrates a Broad Spectrum of Anti-inflammatory, Antioxidant and Antimicrobial Properties Important for Skin Health

Mathias Schuetz[1], Chris Savile[1], Trevor Peters[1], Corey Webb[2], Karl Rouzard[2], José R. Fernández[2], Eduardo Pérez[2]
[1]Willow Biosciences, Suite 202, 1201 5th Street SW, Calgary, Alberta, Canada
[2]Signum Biosciences Inc., Monmouth Junction, NJ, USA

Abstract

Cannabigerol (CBG) is a minor cannabinoid present in the cannabis plant that serves as the direct precursor to cannabidiol (CBD), a building block for Tetrahydrocannabinol (THC). In most cannabis strains, CBG is present in low levels (<1% per dry weight) and is typically mixed with other cannabinoids such as THC and CBD. Therefore, purifying CBG from cannabis plant biomass is both challenging and expensive. Moreover, there has not been much dermatology focused research into CBG activity whereas the more available cannabinoid CBD has been well studied in dermatology. Utilizing our novel yeast fermentation technology platform, we can now produce cannabinoids such as CBG identical to what is produced in plants, but faster, purer, and using a more sustainable process compared to plant-based production. Thus, we sought to begin to characterize CBG's activity and safety profile for use on skin. *In vitro* results utilizing Normal Human Epidermal Keratinocytes (NHEKs) and Human Dermal Fibroblasts (HDFs) demonstrate that CBG possesses potent anti-inflammatory properties versus different environmental stressors and inhibits ultraviolet (UV) and *C. acnes*-induced pro-inflammatory cytokine release (e.g. IL-1β, IL-6). In addition to its anti-inflammatory activity, CBG also shows antioxidant properties and strong antimicrobial activity towards *C. acnes* and *S. aureus* growth, two bacteria strains shown to play a key role in the pathogenesis of acne and atopic dermatitis, respectively. Moreover, CBG also demonstrates a robust safety profile, showing no signs of skin irritation, eye irritation or phototoxicity, paving the way for its use in skin care. Additional studies including gene array analysis on a 3D human skin models provide insight into CBG and CBD function when applied to skin. Altogether, the data presented here is the first to demonstrate CBG's strong potential for use in skin care and suggest areas where it may be more effective than its better-known predecessor CBD.

Fig 1. CBG is the "mother of cannabinoids"



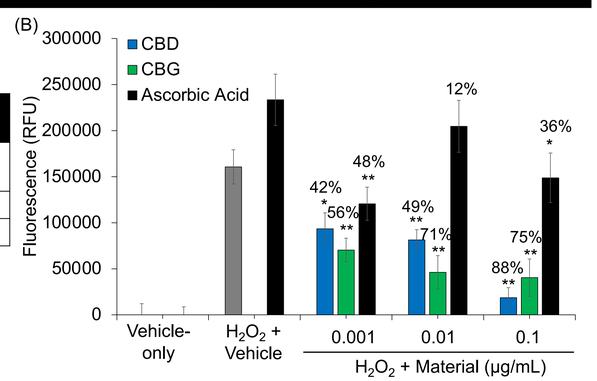
- In cannabis strains, CBG is present in low levels (<1%), therefore it is considered a minor cannabinoid.
- Willow's novel yeast fermentation technology platform produces high yields of CBG identical to plant-based sources.

Cannabigerol (CBG) has yet to match the mainstream appeal of cannabidiol (CBD), but it will soon play a significant role in everyday consumer life as it demonstrates a wide array of intriguing medicinal benefits. This bioactive ingredient activates both CB1 and CB2 cannabinoid receptors and may be the next "hero" cannabinoid to enter the consumer product space. Utilizing our novel technology platform, we can produce significant quantities of CBG with high purity (>99%).

Fig 2. CBG and CBD have antioxidant activity in skin fibroblasts outperforming Vitamin C

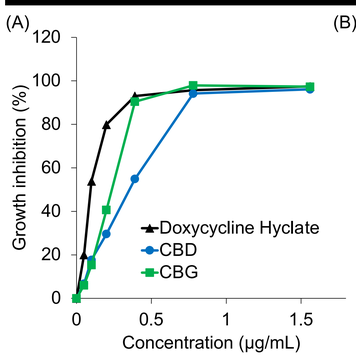
(A)

Material	IC_{50}, µg/mL
Ascorbic Acid	5
CBD	286
CBG	159



(A) Summary results table in cell-free DPPH reduction assay. IC_{50} = Test material concentration that produced 50% of DPPH radical scavenging.(B) Primary Human Dermal Fibroblasts (HDFs) were incubated with actives (≤ 0.1µg/mL) for 3 hours. Control cells received vehicle-only. Intracellular oxidative activity was determined using DCFH-DA as a marker of H_2O_2 induction (0.1mM) of oxidative stress. Data represents mean ± SE from three independent experiments. *p < 0.05; **p ≤ 0.01 compared to H_2O_2 + vehicle group. Data labels represent mean % inhibition relative to -/+ H_2O_2 control groups.

Fig 3. CBG and CBD exhibit potent antimicrobial activity vs "bad" skin bacteria

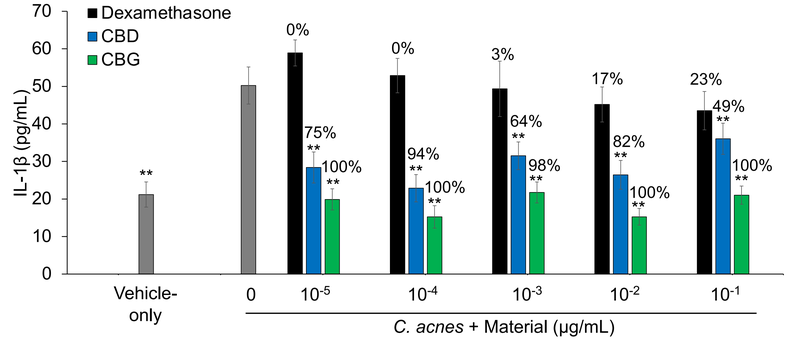


(B)

Bacteria strain	MIC, µg/mL (% w/v)		
	Doxycycline	CBD	CBG
S. aureus ATCC® 29213™	0.20	0.65	0.78
S. aureus MRSA ATCC® 33591™	20.83	1.56	1.82
S. pyogenes ATCC® 19615™	0.11	0.65	0.59
C. acnes ATCC® 6919™	0.26	0.65	0.39
C. xerosis ATCC® 373™	0.33	1.11	1.17
C. granulosum ATCC® 25564™	0.13	2.08	1.56

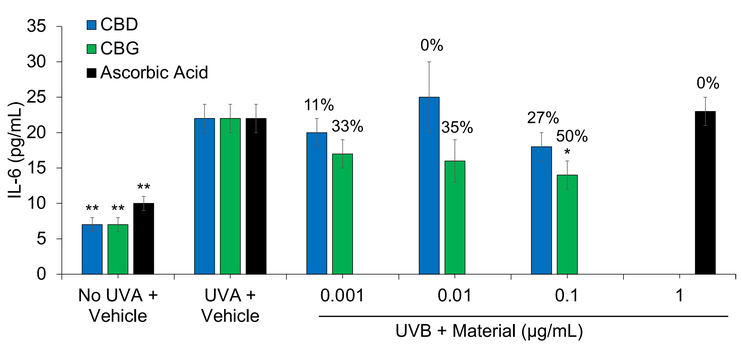
(A) Growth inhibition for *C. acnes* ATCC® 6919™ (B) Summary antimicrobial results table in skin bacteria strains. Bacteria strains were cultured as recommended by ATCC®. Test Materials were incubated at 5% in DMSO vehicle and optical density (OD 595nm) measured to determine >90% growth inhibition by broth turbidity. MIC = minimal inhibitory concentration. Data represents mean from three independent experiments.

Fig 4. CBG inhibits *C. acnes*-induced cytokine production more potently than CBD



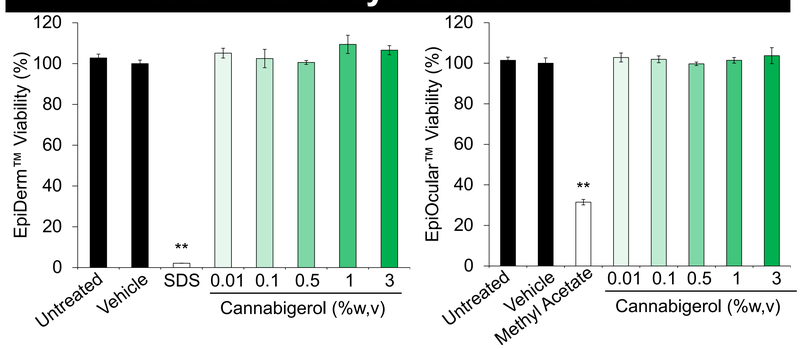
Normal Human Epidermal Keratinocytes (NHEKs) were pre-treated with CBG, CBD (<10^{-7} µg/mL) or Dexamethasone (<10µg/mL) for 1 hour, then co-treated with 10^7 CFU/mL of live *C. acnes* ATCC® 6919™ for 24 hours. Media supernatants were collected after incubation and analyzed by ELISA for Interleukin-1β (IL-1β) levels. Data represents mean ± SE from three independent experiments. *p < 0.05; **p ≤ 0.01 compared to *C. acnes* + vehicle group. Data labels represent mean % inhibition based on -/+ *C. acnes* control groups.

Fig 5. CBG inhibits UVA-induced cytokine production better than CBD



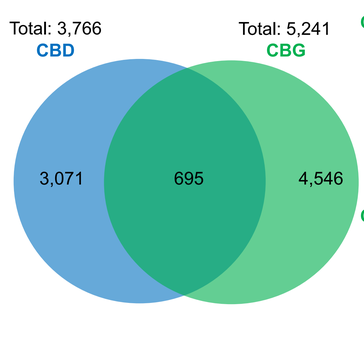
Primary Human Dermal Fibroblasts (HDFs) were cultured in the presence of CBD, CBG (0.001-0.1 µg/mL) or ascorbic acid (1 µg/mL) for 6 hours. Later, ingredients were removed, and cells were irradiated with 12.5 J/cm² UVA. Media supernatants were collected after 24 hours and analyzed by ELISA for Interleukin-6 (IL-6). Data represents mean ± SE from three independent experiments. *p < 0.05; **p ≤ 0.01 compared to UVA + vehicle group. Data labels represent mean % inhibition relative to -/+ UVA control groups.

Fig 6. CBG is safe when applied to skin and eye areas *in vitro*



CBG formulations were tested for skin and eye irritation in reconstructed human epidermis 3D models. EpiDerm™ and EpiOcular™ (MatTek®) tissues were acclimated for 1-24 hours and then treated topically with CBG formulations (≤3%) or positive controls: SDS (5%) or methyl acetate. Tissue viability levels were measured by MTT reduction assay. The levels of tissue viability after each treatment were compared to vehicle group to estimate the potential for skin or ocular irritation. **p ≤ 0.01 compared with untreated tissues.

Fig 7. CBG regulates a different set of genes than CBD in reconstituted 3D skin models



CBG promotes:
- ECM structure and Collagen biosynthesis
  - (including several different collagen genes, elastin and fibronectin)
- Cannabinoid pathway (CNCR1)
- Anti-inflammatory pathways (IL-10, PTGS2)
- Sebocyte regulation of lipid metabolism (PPARG)

CBG suppress:
- Psoriasis and androgenetic alopecia (LEP)
- Rate of ECM degradation (extracellular matrix disassembly)
- Skin pigmentation pathway
- Autophagy

Gene differential expression was quantified using a Clariom™ S assay microarray system after a 24-hour exposure in 3D skin culture model EpiDerm-FT™ (CBG & CBD were applied at 0.5%). CBG regulated 5,241 genes (2,530 up; 2,711 down) compared to the untreated group. In contrast to CBD, novel pathways modulated by CBG treatment were collagen biosynthesis (Types I, III, IV, and VI). CBG also reduced pro-inflammatory and differentiation genes, suggesting novel anti-inflammatory and epidermal keratinocyte differentiation activities.

Summary/Conclusions

- **Our novel yeast fermentation technology produces minor cannabinoids such as CBG, identical to what is produced in nature but in a more rapid, and sustainable manner with higher purity.**
- **Similar to CBD, CBG has antioxidant properties *in vitro* reducing intracellular oxidative radicals in human dermal fibroblasts and provides antimicrobial activity towards microorganisms found in skin.**
- **CBG outperforms CBD anti-inflammatory properties by reducing both *C. acnes*- and UVA-induced pro-inflammatory cytokine production in epidermal keratinocytes and dermal fibroblasts.**
- **CBG demonstrates no signs of skin or eye irritation *in vitro*, demonstrating its safe to use in skin care.**
- **Gene array analysis demonstrates that topical application of CBG on cultured reconstructed human skin modulates key signaling pathways in aging, cell signaling, and collagen production.**
- **Altogether, these results demonstrate the potential of CBG as a potent skin-care active ingredient due to its capacity to protect against environmental stressors that attack and prematurely age skin. Formulation development is underway and clinical trials will be performed in the near-term.**