Variability in sourcing and potency of Cannabidiol (CBD) and Hemp-based extracts impact gene expression changes in-vitro, highlighting the need for reliable analytical methods and standards

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Introduction of research

Skincare products claiming benefits from CBD (cannabidiol) and other hemp-based extracts remain a rapidly growing trend in the US and other global markets. However, research in this area is only emerging and scientific validation for the safety and benefits of topical application is not well established. Benefit claims seem to be based on anecdotal testimony, animal model studies, or clinical trials without defined quality standards or analytical characterization of test articles. To properly investigate the potential for skin benefits from the topical application of cannabinoids we understood it is equally important to verify the composition of the test articles being screened. Prior to the Agriculture Improvement Act of 2018, any cannabis material containing Tetrahydrocannabidiol (THC) was considered a schedule-1 drug, so standards and methods were mainly used to detect the presence of certain cannabinoids not for accurate and reliable measures of concentrations. Now the law requires accurate concentration measurements and according to the National Institute of Standards (NIST), determining accurate analytical numbers at levels as low as 0.3% can be a particular challenge for laboratories (1). This challenge may explain and possibly contribute to the infamous findings of the 2017 JAMA study which analyzed 84 products from 31 categories and found that 70 percent of them had labeled the incorrect percentage of CBD (2). So, our research outlined below is composed of two parts; 1. An independent analytical evaluation of several hemp-based cannabinoid extracts, and 2. An investigation of gene expression modulation from these extracts in a human skin cell model. Our hypothesis was that differences in composition and concentration of hemp-based cannabinoid extracts would affect gene expression modulation.

Methodology

In this work, we analyzed six hemp-based extracts including CBD isolates from three separate sources, one broad-spectrum distillate, and one full-spectrum distillate. Our results were then compared to the values declared on the supplier's COA. The instrument used is a Bruker Q-ToF (Quadrupole-Time-of-Flight) mass spectrometer. Data was acquired with LC/MS, where the LC was done with a reverse-phase column using a methanol gradient, in which the mobile phase contained 5 mM ammonium formate and 0.01% formic acid. Cannabinoids found in samples were compared to authentic standards. Three separate Certified Reference Materials (CRMs) of CBD and THC were sourced from Cerilliant/Sigma, Chromadex and Restek.

The potential for skin benefits was investigated in-vitro using a qPCR based model with human keratinocyte and fibroblasts (Genemarkers). Test articles were screened for their influence on 5 endogenous control genes and 163 gene targets known for their important roles in skin biology. We tested CBD isolates from three separate sources (A, B and C) at 0.1% and source (A) at 0.5%. We also tested one broad-spectrum distillate and one full-spectrum distillate, both at 0.1%.

Results

The results from our analysis of the three sources of CBD Isolate are shown in (Fig. 1) with the supplier's COA values to the side for comparison. Measured CBD values are significantly lower than the supplier declared values and samples contain significant diversity in secondary cannabinoid content.

CBD Isolate (A)
Lot: xxxxxx
Sample appearance: White powder

Cannabinoid	Cannabinoid Sample Purity (%)	COA Claim %
CBD	55.7	102.16
CBDV	0.10	0.00
CBN	1.11	0.00
CBG	ND	
CBC	ND	
delta-9 THC	ND	
delta-8 THC	ND	
11-OH-THC	ND	
THCV	ND	
CBDVA	ND	
CBD/THC isomers and other cannabinoids	<<1	
pesticides/toxic contaminants	ND	

CBD Isolate (B)
Lot: xxxxxx
Sample appearance: White powder

Cannabinoid	Cannabinoid Sample Purity (%)	COA Claim %
CBD	56.0	99.8
CBDV	0.23	0.28
CBN	ND	
CBG	ND	
CBC	ND	
delta-9 THC	ND	
delta-8 THC	ND	
11-OH-THC	ND	
THCV	ND	
CBDVA	ND	
CBD/THC isomers and other cannabinoids	<<1	
pesticides/toxic contaminants	ND	

CBD Isolate (C)
Lot: xxxxx
Sample appearance: White powder

Cannabinoid	Cannabinoid Sample Purity (%)	COA Claim %
CBD	53.5	99.6
CBDV	0.19	0.39
CBN	ND	
CBG	ND	
CBC	ND	
delta-9 THC	ND	
delta-8 THC	ND	
11-OH-THC	ND	
THCV	ND	
CBDVA	ND	
CBD/THC isomers and other cannabinoids	<<1	
pesticides/toxic contaminants	ND	

Figure 1. Analytical results for CBD Isolates A, B and C versus Suppliers COA Claims

Results of the characterizations of both the broad-spectrum and full-spectrum distillates are shown in (Fig. 2). Again, the values for CBD are significantly lower than the COA's and additional cannabinoids are present at significant levels. Of note, the full-spectrum distillate in an undiluted state exceeds the legal limit of 0.3% for THC.

Broad-Spectrum Distillate
Lot: xxxxxxx
Sample appearance: Yellow-brown paste

Cannabinoid	Cannabinoid Sample Purity (%)	COA Claim %
CBD	44.3	65
CBDV	0.09	Not Tested
CBN	0.01	Not Tested
CBG	ND	
CBC	ND	
delta-9 THC	0.01	0.1
delta-8 THC	ND	
11-OH-THC	ND	
THCV	ND	
CBDVA	ND	
CBD/THC isomers and other cannabinoids	0.5	
pesticides/toxic contaminants	ND	

Full-Spectrum Distillate
Lot:xxxxxx
Sample appearance: dark-brown paste

Cannabinoid	Cannabinoid Sample Purity (%)	COA Claim %
CBD	47.2	75.5
CBDV	0.23	0.18
CBN	0.31	0.37
CBG	ND	1.09
CBC	ND	2.61
delta-9 THC	1.17	3.46
delta-8 THC	ND	0.26
11-OH-THC	ND	
THC-V	ND	0.00
CBDVA	ND	
CBD/THC isomers and other cannabinoids	6.4	
pesticides/toxic contaminants	ND	

Figure 2. Analytical results and COA values for Broad-Spectrum and Full-Spectrum distillates

An example of several genes differentially affected by varying concentrations, sources and types of extracts is shown in (Fig. 3).

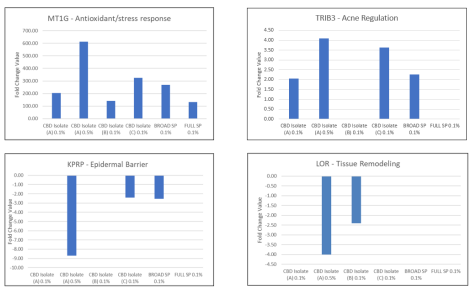

Figure 3. Examples of variability in gene modulation (MT1G, TRIB3, KPRP, LOR) affected by concentration, source and type of extract.

The potential for biphasic responses (3) as a result of differing concentrations is shown in (Fig. 4).

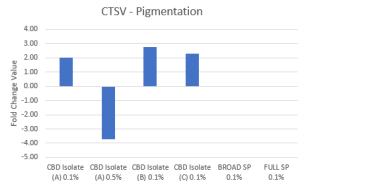

Figure 4. An example of the biphasic potential of gene modulation (CTSV) from different extract concentrations.

And finally, (Fig. 5) demonstrates two examples of genes with modulation suggesting the potential for an 'entourage effect' where biological contributions may be derived from the addition of terpenes (4).

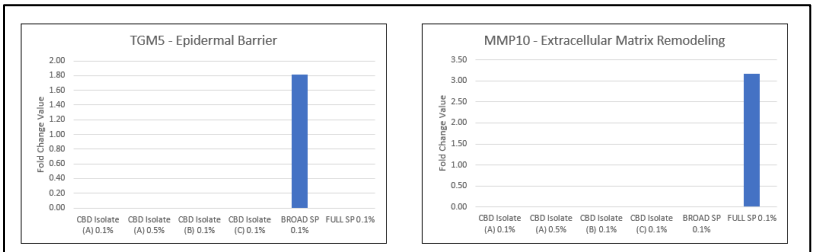

Figure 5. Examples of potential 'Entourage effect' on TGM5 and MMP10.

Conclusion

From the outset, we discovered that the standards and methods for reliable analytical evaluations of CBD and other cannabinoids presented a challenge. Our analytical results showed significant discrepancies between the vendor supplied COA's and our own measurements (Fig. 1-2). In fact, we even found up to 20% variation between the three CRM's sourced as analytical standards (data not shown). Again, this is a well-known challenge and one actively being addressed by the NIST.

The significance of these challenges in analytical accuracy and reproducibility are demonstrated further in out in-vitro gene modulation studies. Dramatic difference in the responses from genes exposed to CBD and other hemp-based extracts arise dependent upon concentration, source and type of extract (Fig. 3-5). The significance of additional known and unknown cannabinoids is a potential cause as well as the biphasic nature of their effects in biological systems. In some cases, these additional cannabinoids are not even tested as a part of the COA. It is an obstacle and a fact that over 100 different cannabinoids have been discovered (5), but most have not been isolated or closely studied to date. It is quite clear, the industry must overcome this challenge of reliable methods and standards for the proper investigation not only of potential benefits, but also risks for humans in the topical and oral use of these cannabinoids.

References

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About the speaker

Brian Cook received his Bachelor of Science degree in Biology and Master of Science in Biochemistry and Molecular Biology from the University of Georgia. His 20+ years of experience in product development and research has covered polymer science, personal care formulation, and skin & hair research innovations. Brian joined Nu Skin in 2013 first as a senior formulation chemist and currently is a senior associate scientist.

Since joining Nu Skin, Brian has contributed to countless brands, including Epoch, Nutriol, Clear Action, and AP-24. Brian currently serves as the development scientist over ageLOC Galvanic Spa & Body Spa, ageLOC LumiSpa, ageLOC Me, ageLOC Transformations, ageLOC Nutriol, and also supports Nu Skin science through new ingredient research.