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Measuring the bioactivity of phytocannabinoid cannabidiol from cannabis sources, and a novel non-cannabis source.

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Abstract:

Phytocannabinoid Cannabidiol (CBD) has been shown to elicit a great many immunological benefits. It acts on the endocannabinoid system, namely through interactions with cannabinoid receptor 2 (CB2). CBD-CB2 affinity, which we refer to as bioactivity, is rarely tested for clinical samples. We believe that uncontrolled variation in bioactivity levels have been silently confounding many CBD experiments. In our four-part study, we validate an efficient bioactivity test that can enable greater scientific control over CBD studies. We use it to compare the bioactivity of CBD obtained from different plant organs, and we also studied whether processing methods play a role in determining bioactivity. We also examine the bioactivity and processing factors of a novel non-cannabis plant capable of producing CBD in commercial quantities, named *Humulus Kriya* (*H. Kriya*, U.S. Patent No. 15/932,529, 2018). We also test the bioactivity of some CBD isolates/extracts currently sold in the market, and compare them with a CBD product called ImmunAG, which was extracted from the inflorescence of *H. Kriya*. We find that the CBD from the inflorescence of the plant produces the highest bioactivity, followed by the apical buds/leaves, the petioles, and finally the stalk. We find that *H. Kriya* has a bioactivity profile similar to *Cannabis Sativa*. We find that the bioactivity levels among cannabis-based commercial CBD products are quite low, and variable. We find significantly higher bioactivity levels in ImmunAG.

In recent years, there has been a great surge in scientific research involving the Phytocannabinoid Cannabidiol (CBD) (Burstein, 2015; Zuardi, 2008). CBD appears to have an acceptable, if not favorable safety profile (Iffland, & Grotenhermen, 2017; Devinsky et al., 2016) and has been shown to be anxiolytic, antidepressant, antipsychotic, anticonvulsant, anti-nausea, antioxidant, anti-inflammatory, anti-arthritic, and anti-neoplastic (Ligresti, De Petrocellis, & Di Marzo, 2016). It has shown to be protective in animal models of epilepsy, anxiety, psychosis, and basal ganglia diseases (Ligresti, De Petrocellis, & Di Marzo, 2016). Anti-cancer effects have also been shown (Pisanti et al., 2017).

Of the receptors upon which CBD acts, cannabinoid receptor 2 (CB_2) has the most ubiquitous, well-studied presence in the immune system (Malfitano, Basu, Maresz, Bifulco, & Dittel, 2014). CB_2 presents in NK cells, B cells, monocytes, CD4+ cells, CD8+ cells, T cells, and neutrophils (Malfitano, Basu, Maresz, Bifulco, & Dittel, 2014; Tanasescu, Gran, & Constantinescu, 2013; Pacher & Mechoulam, 2011), and it appears to be the key mediator for cannabinoid regulation of inflammation and other immune functions (Ashton & Glass, 2007; Xiong et al., 2012; Lunn et al., 2006; McKallip et al., 2002; McKallip, Lombard, Martin, & Nagarkatti, 2002).

We refer to the affinity for CBD to interact with CB_2 as its bioactivity[1,2]. Historically, CBD bioactivity tests have relied on costly and short-lived biological tools (e.g., transfected CHO membranes). Experiments in which the bioactivity of a CBD sample was tested, and subsequently used in clinical trials, have been prohibitively expensive and time consuming to carry out. We believe that uncontrolled variation in bioactivity levels have been silently confounding many CBD experiments.

The factors underlying variability in CBD bioactivity have never been publicly identified. Possible candidates include organ source within the plant, and the extraction/processing methodology. These factors vary wildly among suppliers. Many legalities surrounding *Cannabis Sativa*, the plant from which CBD has traditionally been extracted, reinforce idiomatic peculiarities. In the United States, despite a federal ban on all cannabis-based CBD extractions (Mead, 2017), some state governments have unique laws sanctioning the supply of cannabis-based CBD products (Cambron, Guttmannova, & Fleming, 2017). State-funded research utilizes cannabis plants with different cannabinoid profiles than what is grown and used in the legal market (Vergara et al., 2017). Research that is funded by, independently run, private organizations, in the commercial sector often utilize their own CBD isolates/extracts (e.g., French et al., 2017). Taken together, laws, regulations, and practices reinforce the problem of variability in CBD bioactivity.

In the present article, we validate a more efficient bioactivity test that can enable greater scientific control over CBD studies. We explore two factors responsible for cannabis-based CBD bioactivity: the plant organ source from which the CBD is extracted, and the extraction method. We also examine the bioactivity, per organ, and extraction method of a novel, proprietary, non-cannabis plant capable of producing CBD in commercial quantities, named *Humulus Kriya* (*H. Kriya*, U.S. Patent No. 15/932,529, 2018). We then test the bioactivity of some CBD isolates/extracts currently sold in the market, and compare them with CBD extracted from *H. Kriya* with knowingly controlled bioactivity factors.

Experiment 1: A novel, valid, scalable CBD bioactivity test

Highly pure, naturally occurring CBD molecules were extracted from the *Avidekel* plant, obtained in 2014 from Tikun Olam, Israel, via sonic fractionation and ultra centrifugal separation.
[3h]-CP55940 displacement assays were performed for this reference sample using membrane fractions of CHO cells expressing recombinant human CB_2. Additionally, binding of an MCA (described in U.S. Patent No. 62,599,501, 2017) was tested for this

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[1] Bioactivity refers to the intensity of the biological response that results when a ligand makes contact with its intended target. Generally, a more bioactive ligand causes a more pronounced effect. Bioactivity should not be confused with bioavailability, which is the rate of diffusion of a substance through membranes to reach the intended target in the body.

[2] CBD displays potent antagonism of CB2 receptor agonists (Pertwee, 2008; Thomas et al., 2007) and has also been shown to function as an inverse agonist at CB_2 (Pertwee, 2008; Pertwee et al., 2010). CBD acts as an antagonist preventing [35S]GTPγS binding and Rho activation (Ryberg et al., 2007; Whyte et al., 2009; Ford et al., 2010), modulating Ca2+ mobilization (Lauckner et al., 2008) and β-arrestin recruitment (Yin et al., 2009). CB2 inverse agonism can block migration of immune cells and decrease inflammation (Lunn et al., 2006). CBD potently inhibits migration of macrophages, microglial cells and neutrophils (Walter et al., 2003; Sacerdote et al., 2005). CBD-induced block of chemotaxis of macrophages can be prevented by SR144528, a CB2 selective antagonist (Sacerdote et al., 2005). CBD potently inhibits forskolin-stimulated cyclic AMP production by human CB2 receptor-expressing CHO cells (Gauson, Stevenson, Thomas, Baillie, Ross, & Pertwee, 2007).

reference sample. The resulting displacement and binding values were used as a reference standard against which 26 CBD samples (acquired from Natural Hemp Solutions, Atlanta Georgia) were compared.

We looked for a correlation between the CHO CB_2 binding, and the MCA binding across 26 samples. If the MCA binding correlated to the CHO-CB_2, it could be used instead for quicker, more efficient testing.

Results:

Pearson correlation analysis was performed using R on the CB_2 and CAC binding values of the 26 CBD samples. The binding affinities to both the recombinant human CB_2 and the highest-affinity MCA are listed in Table 1 as a proportion relative to the binding affinity shown by a highly pure CBD molecule. Using the Pearson correlation analysis, we found that these were highly correlated (Pearson coefficient = .97). Thus, we can predict the bioactivity of CBD using the MCA with high accuracy.

Discussion:

We have validated a successful and scalable bioactivity test for CBD. Bioactivity values are expressed as a proportion between 0 and 1 as compared to the CHO-CB_2 binding of the purest CBD molecule we could isolate. The lower the number, the lower the bioactivity. If a CBD molecule has a bioactivity below 0.5, one could expect to observe CBD-CB2 binding at half the strength of a molecule with a bioactivity of 1. If a molecule had an observed bioactivity of 0.2, one could expect the binding affinity to be at 1/5 the strength of a molecule with a bioactivity of 1. This test will illuminate the distribution of bioactive molecules throughout various parts of the plant.

Experiment 2: Cannabis CBD bioactivity by plant organ

It is well known, among growers, that the yield of CBD is variable across different organs in the cannabis plant, with the inflorescence producing the

Sample	MCA Affinity	CB2 Affinity
1	0.78	0.81
2	0.34	0.42
3	0.25	0.29
4	0.32	0.3
5	0.34	0.36
6	0.31	0.33
7	0.24	0.25
8	0.29	0.34
9	0.32	0.3
10	0.29	0.31
11	0.25	0.25
12	0.31	0.32
13	0.35	0.33
14	0.31	0.31
15	0.25	0.26
16	0.3	0.31
17	0.27	0.24
18	0.25	0.24
19	0.32	0.29
20	0.21	0.24
21	0.26	0.24
22	0.44	0.41
23	0.33	0.41
24	0.81	0.8
25	0.3	0.22
26	0.19	0.22
27	0.78	0.81
28	0.34	0.42

Table 1: Binding affinities for the MCA versus the CB2 complex in 26 CBD-producing plant samples. They were highly correlated ($r = .97$).

highest output[3]. (We have replicated this in a to-be-published study.) However, the bioactivity of CBD extracted from different organs has never been studied before.

Using the bioactivity test, validated in experiment 1, we examined 4 regions from 48 different cultivars of cannabis obtained from the USA, India, China, and the Czech Republic. Inflorescence, petioles, apical buds/leaves, and stalks were tested separately. A combination of sonic fractionation and ultra centrifugal separation was used on the inflorescence to obtain purified samples. We also used cold solvent extraction to obtain CBD from the inflorescence, petioles, apical buds/leaves, and stalks.

Results:

A 1x5 ANOVA and appropriate post-hoc comparisons were conducted on bioactivity with plant organ as the only factor. Bioactivity means and standard errors were plotted.

Levene's test indicated heteroscedastic variances between the organs $F(4,235) = 6.05$, $p < .001$. As such, we conducted a robust ANOVA as described by Wilcox (2012). It found a significant difference between the bioactivities of centrifuge-extracted inflorescence CBD ($M = .96$, $SD = .02$, solvent-extracted CBD from the inflorescence ($M = .86$, $SD = .04$), solvent-extracted CBD from the petioles ($M = .54$, $SD = .03$), solvent-extracted CBD from the apical buds/leaves ($M = .4$, $SD = .04$), and solvent-extracted CBD from the stalks ($M = .19$, $SD = .02$), $F(4, 70.38) = 9885.21$, $p < .001$. (20% trimmed means are presented above.)

Robust post-hoc comparisons (Mair & Wilcox, 2016), revealed significant differences between each of the CBD source categories. (See table 2 for the psihat values of each comparison, and their associated

	Inflorescence	Petioles	Apical Buds/Leaves	Stalks
Inflorescence Centrifuge	-.205 [-.222 to -.187]	-.769 [-.781 to -.756]	-.669 [-.685 to -.654]	-.344 [-.360 to -.329]
Inflorescence		-.563 [-.583 to -.545]	-.464 [-.486 to -.443]	-.140 [-.161 to -.119]
Petioles			.099 [.082 to .117]	.424 [.407 to .441]
Apical buds/Leaves				.325 [.305 to .344]

Table 2. Psihat and corresponding confidence interval values (in brackets) for robust one-way ANOVA post-hoc comparisons of bioactivity. Psihat values for each post-hoc comparions were obtained using 20% trimmed means. Corresponding 95% confidence interval values are presented in brackets. All associated p-values were $< .001$.

[3] The tip of secreting hairs located mainly on female-plant contain resin glands that have a considerable amount of cannabinoids. These glands are fewer in number in the leaves (Zuardi, 2008).

confidence intervals.) In summary, the highest bioactivity CBD was found in the pods of each plant, with decreasing bioactivity in the petioles, apical buds/leaves, and stalks respectively (See figure 1). (Individual bioactivity scores obtained per plant are provided in Appendix 1.)

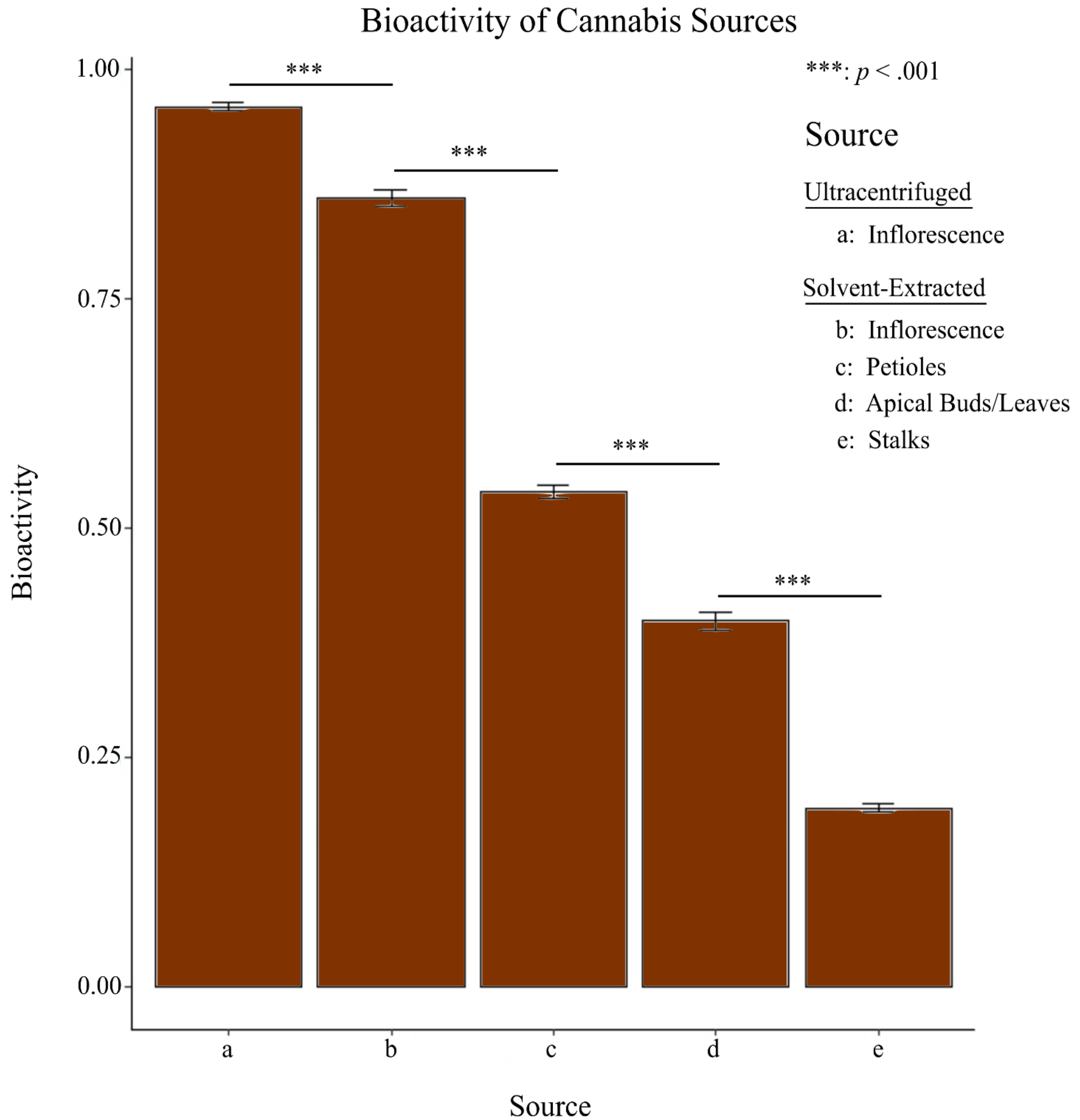

Figure 1: A highly canonical pattern emerged with inflorescence producing the highest bioactivity. CBD source a was obtained through ultra centrifugal separation. CBD sources b, c, d, and e were obtained through cold-solvent ethanol extraction. Standard errors shown above are from untrimmed means to show the most statistically conservative estimates.

Discussion

Our results showed a difference between extraction methodologies. The combination of sonic fractionation and ultra centrifugal separation produced CBD with the highest bioactivity. Sonic fractionation and ultra centrifugal extraction are labor- and equipment-intensive laboratory procedures, not fit for large-scale manufacture. By contrast, ethanol solvent extraction causes a small amount of degradation in bioactivity, but is scalable and relatively inexpensive to carry out. Ergo, it is a far more common procedure for commercial CBD production[4].

Among plant organs subjected to ethanol extraction, our results indicate a canonical pattern of CBD bioactivity. The inflorescence produced the highest bioactivity CBD molecules, with levels five times higher than CBD extracted from the stalk. Inflorescence should be used exclusively for the production of high bioactivity CBD. If commercial CBD suppliers have mixed in biomass from the stem and bark of the plant before extraction, it has lily led to low bioactivity in their products.

Experiment 3: Testing the bioactivity of a novel, non-cannabis, plant source of CBD

Using a plant from the Humulus family that produces CBD, a new plant was developed called *Humulus Kriya*. It does not produce THC, is from a family of plants considered GRAS (FDA Title 21, Volume 3, Sec 182.2- CAS 8060-28-4) and has been certified by FSSAI (Food Safety and Standards Authority of India) as a "Food Ingredient". It should not fall under the Scheduled List classification. We tested the bioactivity profile of the various parts of *H. Kriya* using the same methods as in experiment 2.

Results:

Our samples were made of Six *H. Kriya* plants, provided by ImmunAG, LLP, India, and thirty one samples of ImmunAG oil extract.

We used Welch's t to compare CBD bioactivity of *H. Kriya* from all five groups to the cannabis samples (Individual bioactivity scores obtained per plant are provided in Appendix 2.) The centrifuged pod CBD from *H. Kriya* (M = .95, SD = .01) showed no difference in bioactivity compared to cannabis samples, $t(8.6594) = 1.74$, $p = .12$. The solvent extracted pod CBD (M = .86, SD = .06) showed no difference, $t(5.3803) = 0.007$, $p = .99$. The solvent-extracted petiole CBD (M = .54, SD = .01) showed no difference, $t(14.123) = 0.373$, $p = .715$. The solvent-extracted leaf CBD (M = .41, SD = .04) showed no difference, $t(6.164) = -1.0212$, $p = .346$. The solvent-extracted stem CBD (M = .20, SD = .01) showed no difference, $t(7.322) = -1.143$, $p = .289$. It appears as though *H. Kriya* has an identical CBD bioactivity profile to the cannabis strains we tested. Comparisons are shown in figure 3.

Discussion:

We found identical CBD bioactivity between *H. Kriya* and Cannabis for CBD extracted from various parts of the plant. *H. Kriya* appears to be a viable cannabis alternative for CBD research. CBD from *H. Kriya* has no risk of THC contamination. It has been certified as a food ingredient by the Food Safety and Standards Authority of India.

Experiment 4: Examining the Bioactivity of commercially available CBD products

The bioactivity of commercial CBD samples has never been examined. We are publishing results of commercial, cannabis-based, products analyzed over the past 2 years. These samples were sent to us directly by vendors (Natural Hemp Solutions, Centuria Foods, BSPG, Isodiol, Hammer Enterprises, etc.) or sent to us by 3rd parties. We have deliberately not published the bioactivity results for individual vendors and have anonymously presented the bioactivity results for all of the vendors together.

There are many cannabimimetic molecules other than CBD. The two announced sources of CBD

[4] It is worth noting that many other extraction processes exist (e.g., CO_2 and freon extraction). Inflorescence should produce the most bioactive CBD regardless of extraction methodology but further research needs to be done to confirm this.

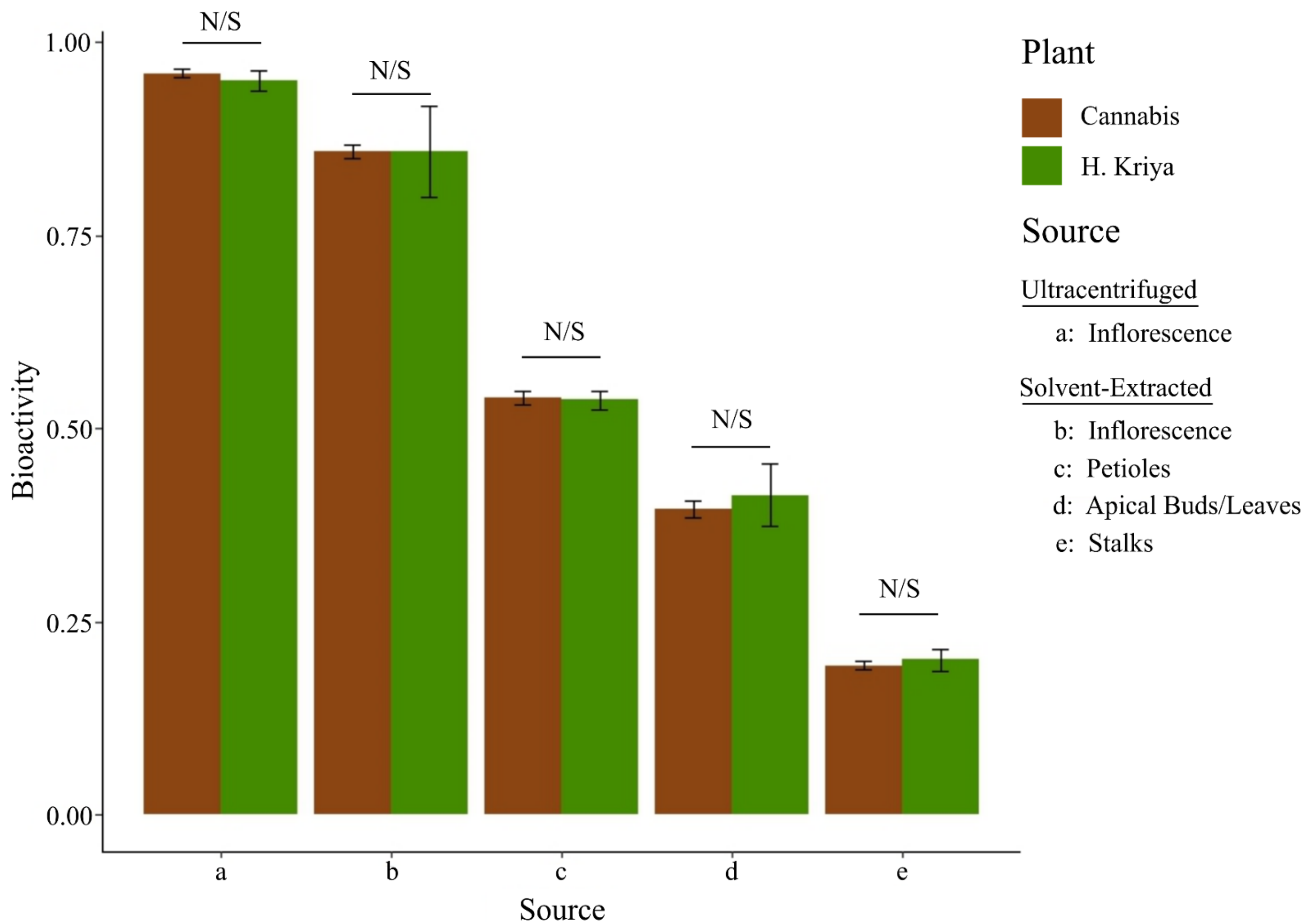

Figure 2: No significant differences were found between *H. Kriya* and cannabis for any of the organs.

from non-hemp/cannabis sources are yeast and humulus. We attempted for a while but could not get samples of CBD extracted from yeast. We tested the bioactivity of CBD extracted from *H. Kriya* (ImmunAG), and compared it to commercial cannabis products.

Results:

The minimum bioactivity in commercial samples was 0.11 and the maximum was 0.41. The minimum bioactivity in ImmunAG was 0.72, and the maximum was 0.98. Bioactivity scores for both classes of product are shown in figure 3.

When comparing the CBD bioactivity in ImmunAG (M = .88, SD = .06) to products on the market (M = .23, SD = .07), Welch's t found a significant difference in bioactivity, $t(41.288) = 53.41$, $p < .001$.

Discussion:

Commercial CBD bioactivity were low, having values consistent with the lower bioactive organs— stalks, stems, barks and leaves. It is possible that suppliers have been using biomass rich in stalk, stem and leaves to comply with regulations and increase mass. The caution is that low bioactive CBD

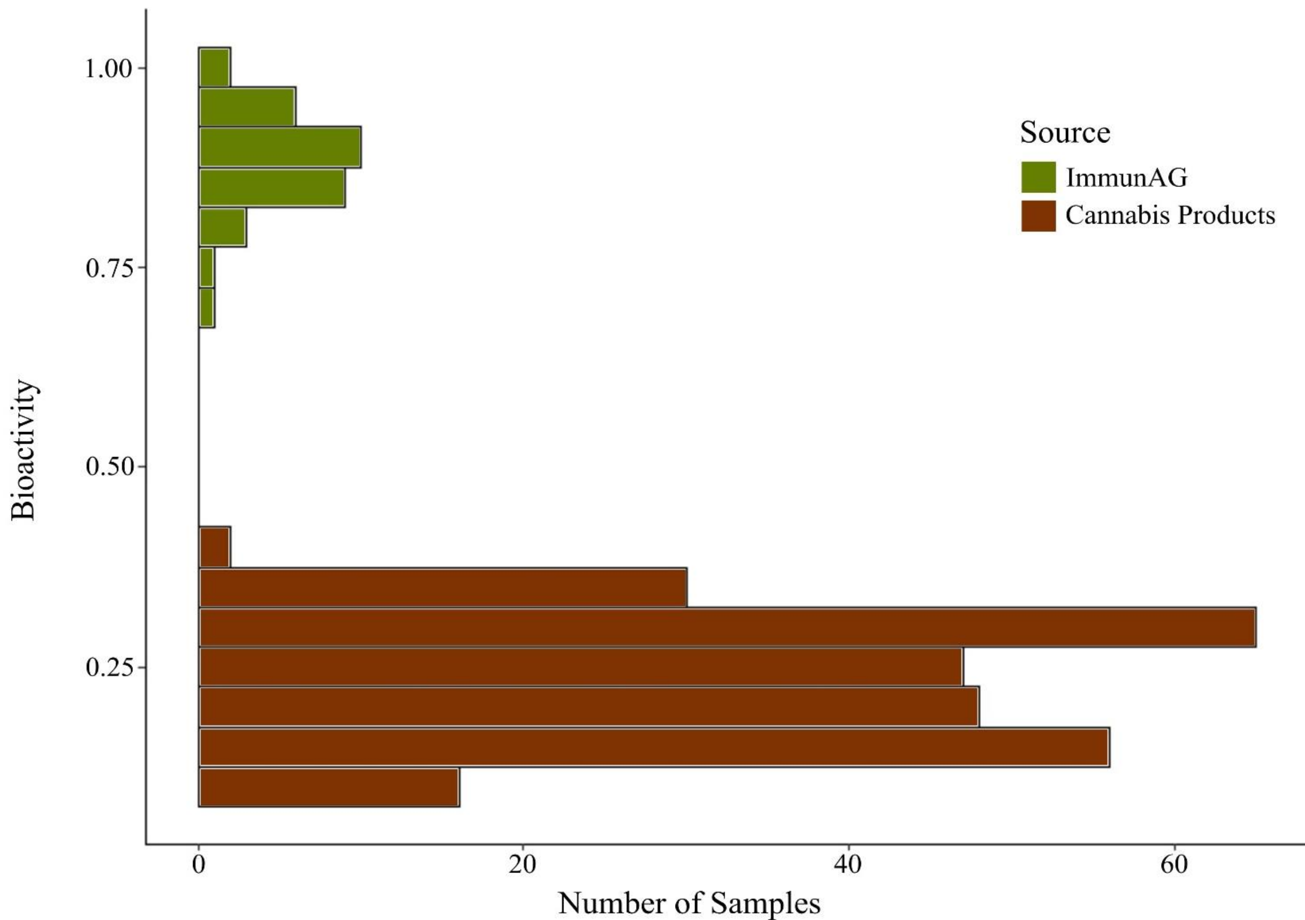

Figure 3: *H. Kriya*-based ImmunAG shows higher bioactivity across all samples than cannabis-based commercial products.

may not produce desirably intense immunologic cell signals. Commercial CBD bioactivity were also quite variable, with a minimum of 0.11 and a maximum of 0.41. The highest commercial sample had almost four times the potency of the lowest sample. Left unchecked, low bioactivity CBD are likely to confound medical use or research and produce spurious results.

ImmunAG samples ranged from 0.72 to 0.98, with the lowest ImmunAG bioactivity higher than the highest commercial cannabis-based CBD bioactivity. This is not surprising because ImmunAG is only made from the inflorescence of *H. Kriya*. An audit revealed that carefully regulated processing conditions also enabled ImmunAG to maintain significantly high bioactivity. The effects of processing conditions on CBD bioactivity will be published in a subsequent paper.

Conclusions:

We found that use of mono clonal antibody testing of CBD bioactivity was viable. We found that CBD extracted from different plant organs had different bioactivity, with inflorescence having the highest bioactivity, and stalks/stems having the lowest. We evaluated a non cannabis CBD-producing plant, *H. Kriya*, that has a bioactivity profile similar to

cannabis. We found that hemp/cannabis based CBD products sold commercially have low bioactivity. We found that commercial CBD products made from it *H. Kriya* had the highest bioactivity.

CBD-CB2 interactions are responsible for a wide range of immunologic effects. The samples we studied had widely varying levels of bioactivity. We believe it is likely that bioactivity levels have been silently confounding historical research results. Scientific studies utilizing CBD for medical research should strive to use products with the highest bioactivity levels.

Method:

CHO cells and membrane preparation

These were stably transfected with cDNA encoding human CB2 receptors. The CB_2-transfected cells used in binding assays with [$_3$H]-CP55940, [$_3$H]-WIN55212-2 or [$_{35}$S]-GTPγS (Bmax=72.5 pmol mg−1 protein). The clones used in the assays were the same as those used in the sPAP reporter assay described by Green *et al.* (1998). Cells were maintained at 37°C and 5% CO2 in DMEM (f-12 HAM) with 2 mm Glutamine, Geneticin (600 µg ml−1) and Hygromycin (300 µg ml−1). Because receptor over expression may lead to the activation of effector mechanisms to which receptors in natural membranes are not normally coupled (see Kenakin, 1995), the assays were performed with cells expressing fewer CB_2 receptors than the cells used in the binding assays.

CHO cells were suspended in 50 mm Tris buffer (pH 7.4) and 0.32 m sucrose and homogenized with an Ultra-Turrex homogenizer. The homogenate was diluted with 50 mm Tris buffer (pH 7.4) and centrifuged at 50,000×*g* for 1 h to isolate the membranes.

CHO-CB_2 binding

A filtration procedure was used to measure [$_3$H]-CP55940 and [$_3$H]-WIN55212-2 binding. This is a modification of the method described by Compton *et al*. (1993). Binding assays were performed with [$_3$H]-CP55940 or [$_3$H]-WIN55212-2, 1 mm MgCl2, 1 mm EDTA, 2 mg ml−1 bovine serum albumin (BSA) and 50 mm Tris buffer, total assay volume 500 µl. Binding was initiated by the addition of cell membranes (20–30 µg protein). Assays were carried out at 30°C for 90 min before termination by addition of ice-cold wash buffer (50 mm Tris buffer, 1 mg ml−1 BSA) and vacuum filtration using a 12-well sampling manifold (Brandel Cell Harvester) and Whatman GF/B glass-fibre filters that had been soaked in wash buffer at 4°C for 24 h. Each reaction tube was washed three times with a 4 ml aliquot of buffer. The filters were oven-dried for 60 min and then placed in 5 ml of scintillation fluid (Ultima Gold XR, Packard). Radioactivity was quantified by liquid scintillation spectrometry. Specific binding was defined as the difference between the binding that occurred in the presence and absence of 1 µm reference cannabidiol. Protein assays were performed using a Bio-Rad Dc kit. Unlabeled and radio labelled cannabidiol were each added in a volume of 50 µl following dilution in assay buffer (50 mm Tris buffer containing 10 mg ml−1 BSA). The concentration of [$_3$H]-CP55940 or [$_3$H]-WIN55212-2 used in displacement assays was 0.5 nm. The concentrations of cannabidiol that produced a 50% displacement of radio ligand from specific binding sites (IC50 values) were calculated using GraphPad Prism (GraphPad Software, San Diego, U.S.A.). Competitive binding curves were fitted with minimum values for displacement of radio ligand from specific binding sites constrained to zero. Dissociation constant (*K*i) values were calculated using the equation of Cheng & Prusoff (1973) and dissociation constant values of [$_3$H]-CP55940 and [$_3$H]-WIN55212-2 shown in the footnote to Table 1.

Generation and binding of the anti-CBD antibody

Reference CBD was extracted from the inflorescence of the *Avidekel* plant. 5% reference CBD was dissolved in caproic acid ($C_5H_{11}COOH$). 0.2 ml of this solution was injected with a 27-28 mm gage needle into the lateral tail vein of BALB/Lac mice. The injections were repeated every other day for 14 days for a total of 7 injections. Each injection was followed by an *in vivo* electroporation of 80 pulses of 100 microseconds at 0.3 Hz with an electrical field magnitude of 2500 V/cm. Following cannabidiol immunization, mouse splenocytes were extracted and isolated. They were fused with myeloma cells by

Labelled cannabinoid	Unlabelled cannabinoid	CB2 K_i (nM)
[^{3}H]-CP55940	CP55940	1.8±0.2
	L759633	6.4±2.2
	L759656	11.8±2.5
	AM630	31.2±12.4
	SR144528	5.6±1.1
[^{3}H]-WIN55212-2	AM630	37.5±15.4
	SR144528	4.1±1.3

Table 3: K_i values were calculated by the Cheng & Prusoff equation (n = 3 or 4) using K_D values of 0.8 nm for [^{3}H]-CP55940 in membranes of CB2 cells and a Kd value of 2.1 nm for [^{3}H]-WIN55212-2 in membranes of CB2 cells (Ross & Pertwee, unpublished).

dielectrophoresis using a BTX ECM 2001 Electrofusion Generator, manufactured by BTX Harvard Apparatus, Holliston, MA, USA. The fused cells were incubated in a hypoxanthine-aminopterin-thymidine medium (with respective concentrations 0.1 mM, 0.4 μM, and 0.016 mM) for between ten and fourteen days, resulting in the survival of only the B cell-myeloma hybrids. Following limiting dilution to one cell per plate, ELISA was used to select hybridomas that produced antibodies with higher binding to our pure CBD molecule. The antibody was linked to a Cytochrome P450 enzyme. We used Pentalenolactone as our Cytochrome P450 substrate. The hybridoma producing the antibody with the highest binding affinity, as measured by a molar weight increase in the Cannabidiol Antibody Complex (CAC), was cloned using supplemental media cultures containing interleukin-6. These procedures are additionally described in patent X.

Cloned hybridomas grew in culture medium RPMI-1640 with antibiotics and fetal bovine serum. A/G purification was used to extract monoclonal antibodies from hybridomas. The culture supernatant contained 46 micrograms/milliliter to 72 micrograms/milliliter of Cannabidiol monoclonal antibody (MCA). This antibody was maintained at -20ºC or lower until used. Fluorescence labelled ELISA was used to measure binding for each sample. The molar weight of the CAC was divided by the molar weight of the gold standard reference CAC to derive binding affinity values.

Ultracentrifugal CBD extraction:

Plant tissue (from the inflorescence) was ultrasonically fractioned. The pulp and plasma were separated by centrifugation. The plasma fraction was further fractionated and studied by analytical ultra centrifuge to obtain the sedimentation coefficient of CBD. Isopycnic density gradient preparative ultracentrifugation (up to 130,000 RPM), using sodium bromide and cesium chloride, was then done to collect the purified CBD samples. This is not a commercially viable process but it provided enough mg of CBD to conduct the bioactivity test.

Solvent CBD extraction:

For the solvent procedure, we extracted dried plant material at around 20ºC with ethanol, followed by methylene chloride, and separated uncarboxylated cannabinoids from carboxylated cannabinoids.

CBD isolation and analysis:

Each fraction was identified by using the following methods: Silica gel eluting with $CHCl_3$; silica gel eluting with C_6H_6-MeOH-AcOH (88%: I 0%: 2%) (as in Mechoulam, Ben-Zvi, Yagnitinsky, & Shani, 1969); Korte and Sieper's (1964) published method; Cannabinoid reference standards.

Following CBD isolation and identification, Fast Blue B Salt colors were used for qualitative analysis. The cannabinoids were then analyzed after trimethyl-sililation, by GLC using OV225 (50' SCOT column) or OV17 (2% on Chromosorb W, 5' column). Acid cannabinoids were estimated after decarboxylation by heating in pyridine.

Fluorescence labelled ELISA was used to measure the bioactivity of sample

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Appendix 1: 48 cultivars of cannabis, and their associated bioactivity levels by plant organ.

Cultivar	Ultracentrifuged Inflorescence	Inflorescence	Petiole	Apical Bud/Leaf	Stalk
Uniko B	0.956	0.902	0.517	0.36	0.169
Kompolti	0.98	0.881	0.538	0.383	0.192
Fedora 17	0.976	0.865	0.52	0.43	0.191
Fedora 17	0.919	0.892	0.509	0.468	0.194
Fedora 17	0.95	0.907	0.569	0.409	0.201
Ferimon 12	0.92	0.866	0.517	0.392	0.19
Santhica 27	0.985	0.85	0.557	0.453	0.162
Epsilon 68	0.991	0.859	0.575	0.37	0.171
Futura 75	0.958	0.823	0.557	0.41	0.189
Futura 75	0.963	0.85	0.53	0.443	0.176
Felina 32	0.974	0.88	0.57	0.378	0.189
Felina 34	0.946	0.817	0.588	0.392	0.183
Juso 14	0.956	0.832	0.505	0.428	0.199
Bialobrzeskie	0.973	0.836	0.561	0.391	0.216
Beniko	0.984	0.844	0.516	0.434	0.175
Chamaeleon	0.969	0.85	0.525	0.384	0.17
Chamaeleon	0.972	0.907	0.513	0.397	0.226
Carmagnola	0.961	0.894	0.533	0.283	0.224
Carmagnola	0.973	0.862	0.565	0.431	0.21
Carmagnola selezionata	0.932	0.877	0.561	0.387	0.185
Tiborszallasi	0.94	0.882	0.531	0.359	0.192
Fibranova	0.974	0.893	0.51	0.408	0.23
Delta-llosa	0.949	0.852	0.54	0.397	0.228
Delta-405	0.982	0.858	0.561	0.378	0.179
Novgorod-Seversky, cv	0.947	0.888	0.569	0.393	0.204
Bernburgskaya Odnodomnaya, bm	0.97	0.805	0.552	0.385	0.193
Szegedi 9	0.936	0.88	0.521	0.373	0.166

Fibrimulta 151	0.971	0.876	0.531	0.358	0.189
Glukhovskaya 10 Zheltostebel'naya	0.989	0.807	0.554	0.378	0.192
Krasnodarsky 10 FB	0.965	0.876	0.576	0.43	0.191
Alpine Rocket	0.951	0.841	0.62	0.427	0.198
Alpine Rocket	0.947	0.79	0.517	0.436	0.206
Hindu Kush	0.935	0.887	0.529	0.425	0.183
Northern Light	0.993	0.871	0.546	0.36	0.221
Snow White	0.931	0.817	0.506	0.349	0.159
Top 44	0.973	0.839	0.515	0.38	0.189
Top 44	0.934	0.861	0.553	0.325	0.188
F1 Fraise	0.966	0.863	0.514	0.431	0.197
B52	0.943	0.914	0.543	0.429	0.226
Peace Maker	0.946	0.848	0.534	0.346	0.197
Big Bud	0.951	0.9	0.536	0.379	0.2
Big Skunk	0.967	0.867	0.509	0.369	0.184
F Fraise	0.931	0.875	0.517	0.405	0.213
Hawaii Maui Waui	0.985	0.84	0.485	0.382	0.194
Haze	0.993	0.884	0.596	0.457	0.158
Swaziland	0.981	0.834	0.579	0.397	0.192
Mexican Sativa	0.963	0.825	0.528	0.451	0.207
Ruderalis Indica	0.942	0.789	0.499	0.398	0.186

Appendix 2: 6 samples of ImmunAG, and their associated bioactivity levels by plant organ.

Cultivar	Ultracentrifuged Inflorescence	Inflorescence	Petiole	Apical Bud/Leaf	Stalk
H. Kriya #3	0.964	0.829	0.536	0.465	0.212
H. Kriya #5	0.947	0.798	0.532	0.414	0.195
H. Kriya #6	0.956	0.883	0.549	0.399	0.215
H. Kriya #11	0.961	0.96	0.551	0.445	0.188
H. Kriya #14	0.941	0.835	0.519	0.402	0.21
H. Kriya #17	0.932	0.851	0.536	0.355	0.182

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