Veterinary Medicine: Research and Reports

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ORIGINAL RESEARCH

Cannabinoid, Terpene, and Heavy Metal Analysis of 29 Over-the-Counter Commercial Veterinary Hemp Supplements

This article was published in the following Dove Press journal:
Veterinary Medicine: Research and Reports

Joseph J Wakshlag[1]
Stephen Cital[2]
Scott J Eaton[3]
Reece Prussin[2]
Christopher Hudalla[3]

[1]Department of Clinical Sciences, Cornell University College of Veterinary Medicine, Ithaca, NY 14853, USA; [2]ElleVet Sciences, Product Development and Scientific Communications, Portland, ME, USA; [3]ProVerde Laboratories, Milford, MA 01757, USA

Purpose: The use of veterinary low tetrahydrocannabinol (THC) *Cannabis sativa* (ie, hemp) products has increased in popularity for a variety of pet ailments. Low-THC *Cannabis sativa* is federally legal for sale and distribution in the USA, and the rise in internet commerce has provided access to interested consumers, with minimal quality control.

Materials and Methods: We performed an internet word search of "hemp extract and dog" or "CBD product and dog" and analyzed 29 products that were using low-THC *Cannabis sativa* extracts in their production of supplements. All products were tested for major cannabinoids including cannabidiol (CBD), Δ9-tetrahydrocannabinol (THC), cannabigerol (CBG), and other minor cannabinoids, as well as their carboxylic acid derivatives (CBDA, THCA, CBGA) using an ISO/IEC 17025 certified laboratory. Products were also tested for major terpenes and heavy metals to understand constituents in the hemp plants being extracted and distributed.

Results: All products were below the federal limit of 0.3% THC with variable amounts of CBD (0–88 mg/mL or g). Only two products did not supply a CBD or total cannabinoid concentration on their packaging or website, while 22/29 could supply a certificate of analysis (COA) from a third-party laboratory. Ten of the 27 products were within 10% of the total cannabinoid concentrations of their label claim with a median concentration of 93% of claims (0–154%). Heavy metal contamination was found in 4/29 products, with lead being the most prevalent contaminant (3/29).

Conclusion: The products analyzed had highly variable concentrations of CBD or total cannabinoids with only 18 of 29 being appropriately labeled according to current FDA non-medication, non-dietary supplement or non-food guidelines. Owners and veterinarians wanting to utilize CBD-rich *Cannabis sativa* products should be aware of low-concentration products and should obtain a COA enabling them to fully discuss the implications of use and calculated dosing before administering to pets.

Keywords: cannabinoid, hemp, supplement, cannabidiol, pet, terpene, oral

Introduction

The recent federal legalization and deregulation of low-THC *Cannabis sativa*, otherwise known as hemp, as a commercial crop in the USA has created a new supplement market for humans and pets alike that is largely unregulated.[1] The de-scheduling of low-THC *Cannabis sativa* derived extracts forced any oversight of products containing hemp derived CBD, and other cannabinoids, to the Food and Drug Administration (FDA).[2] The lack of clear FDA regulations and inconsistent state regulations being

Correspondence: Joseph J Wakshlag
Cornell University College of Veterinary Medicine, Veterinary Medical Center C2-009, 930 Campus Road, Ithaca, NY 14853, USA
Email Dr.joesh@gmail.com

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DovePress
http://doi.org/10.2147/VMRR.S248712

implemented leaves many practitioners contemplating the legality of low-THC *Cannabis sativa* distribution in each state, even though federally legal. Some associations and organizations refer to the Dietary, Supplement, Health and Education Act (DSHEA) of 1994 for guidelines regarding marketing and labeling of *Cannabis sativa*-derived CBD products, when in fact, the US Congress clarified the intent of DSHEA as not relevant to animals.[3] Instead, this lack of oversight responsibility has left a legal gray zone where animal supplements are not illegal, but are self-regulated with enforcement discretion maintained by the FDA. The FDA currently only oversees three defined categories when it comes to animal products, being medicines, medical devices and food.

Currently, at the time of writing, compliant labeling and marketing of low-THC CBD products must not state or imply the prevention, mitigation or curing of disease. This mandate mirrors all other human or animal supplements and nutraceuticals on the market today. Until the FDA resolves the issue regarding guidelines of "hemp" CBD products many manufacturers will likely continue illegal and dishonest marketing and labeling, possibly weighing the earning potential against the unlikely event of FDA enforcement in a saturated market.

The use of CBD-rich extracts in pets is commonplace, as identified by Kogan et al in a range of survey work, leaving veterinarians in a tenuous place as health professionals due to the paucity of clinical or safety studies. Client survey work suggests that cannabidiol (CBD) rich *Cannabis* extracts are currently being used for disorders including anxiety, cancer and cancer chemotherapy side effects, inflammatory bowel disease, osteoarthritis and seizures.[4,5] The primary cannabinoid of interest is CBD due to the tremendous amount of preclinical and human clinical research suggesting it may have utility in a range of inflammatory and neurologic disease processes.[6–10] Other cannabinoids can also be found in many of these preparations including Δ9-tetracannabinol (THC), Δ8-tetrahydrocannabinol (Δ8-THC), cannabichromene (CBC), cannabinol (CBN), cannabigerol (CBG), cannabidivarin (CBDV), exo-THC, tetrahydrocannabivarin (THCV) and their derived acids such as cannabidiolic acid (CBDA), cannabigerolic acid (CBGA), and tetrahydrocannabinolic acid (THCA); as well as terpenes. Terpenes are a class of mono and dicyclical volatile compounds that lead to the aroma of the extract and may also have modest medicinal properties, but are typically found at lower concentrations than cannabinoids (less than 1% dry weight of plant material).[11]

Unfortunately, due to a lack of regulation, the quality control is suspect in all human and animal supplements, *Cannabis* and non-*Cannabis* supplements alike. Two recent publications examining selected cannabinoid concentrations in human over-the-counter products showed a tremendous disparity between labeling claims and analysis of the products, with over 40% having less than the labeled amount and over 40% having more than the labeled amount.[12,13] The THC concentrations in a Canadian study were less than 0.01% for all products showing compliance with the Canadian standard for Cannabis CBD products.[12] In a study examining 14 European products, all with THC concentrations being below the 0.2% allowable limit, CBD concentrations varied from either total cannabinoids or specific CBD concentrations as labeled.[14]

This disparity in products can be from a range of issues including batch to batch variation, intentional improper labeling, degradation over time, poor extraction techniques and lack of certification of the laboratories being used to measure cannabinoids. That said, in the veterinary literature there has been some initial pilot pharmacokinetic, safety and pilot clinical trials that provide some insights for veterinarians regarding dosing regimens.[15–19]

The objectives of this study were to provide information regarding the important plant constituents including cannabinoids, terpenes and heavy metal contamination (lead, arsenic, mercury and cadmium) in commercial products obtained through internet commerce using liquid chromatography with diode array detection and mass spectroscopy (LC-DAD/MS), headspace gas chromatography (HS-GC-FID) and inductive coupled plasma mass spectroscopy (ICP-MS), respectively. The cannabinoids analyzed included CBD, THC, Δ8-THC, CBG, CBN, CBC, THCV, CBDV and derived acids CBDA, THCA, and CBGA. Major terpenes in the analysis included β-myrcene, linalool, limonene, β-caryophyllene, pinenes and other lesser terpenes. A second objective was to examine labels to determine if manufacturers complied with current FDA supplement guidelines, relative consistency between analyzed concentrations versus labeled CBD or total cannabinoids, and the manufacturer's ability to produce a certificate of analysis regarding cannabinoid analysis for the lot purchased.

Materials and Methods

Product Selection and Preparation

Pet-specific products were obtained from an internet search which included the Google search engine input of

"hemp extract and dog" or "CBD product and dog". The first 30 products out of 65 products specifically for dog use were identified and often came in multiple forms (dry capsule, oil tincture, soft chew or powder). Products were not purchased if the advertisements were from hemp seed rather than whole plant extract. If multiple forms were identified then an oil product was chosen for analysis. If an oil was not available then a powdered capsule form was chosen, and if a chew was the only form available then it was chosen for analysis. All products were paid for in US dollars and the retail price was recorded minus the shipping and handling costs.

After purchase all companies were contacted to provide acertificate of analysis (COA) related to the product purchased based on lot number. If an original third-party COA was not provided then marketing material or label concentrations were used to assess against laboratory analysis by a certified 3rd party laboratory. After mixing well,three separate 2 mL or 2 g aliquots were prepared for analysis within a month of receipt, were within the labeled expiratory date, and were then sent to an ISO/IEC 17025 Certified laboratory (ProVerde, Milford, MA, USA) for analysis of products for common cannabinoids including CBD, CBDA, CBDV, THC, THCA, Δ8-THC, exo-THC, THCV, CBC, CBG, CBGA, and CBN. Products were also tested for a range of common terpenes found in cannabis including camphene, β-linene, 3-careen, α-terpenine, α-pinene, ocimene, limonene, P-cymene, eucalyptol, γ-terpenine, terpinolene, linalool, β-myrcene, β-caryophyllene, humulene, caryophyllene oxide and α-bisabolol. Lastly, one aliquot from each product was tested for four major heavy metals found in cannabis including lead, mercury, cadmium and arsenic.

Cannabinoid Analysis

All samples submitted for testing were visually and microscopically inspected prior to analysis for foreign material with no remarkable findings. Samples were homogenized in their entirety. Solid samples were mechanically reduced to a free-flowing powder, while oil samples were vortexed for 1 minute prior to subsampling. Aliquots for testing were made either at 20, 100 or 1000 mg to achieve a lower detection limit of 0.0025 wt % for chewables and 0.01 wt% for orals. Cannabinoids were extracted into either isopropanol, acetonitrile or a 60/40 (vol/vol) mixture of acetonitrile and water, filtered to 0.2 µm and diluted in a 60/40 (vol/vol) mixture of acetonitrile and water prior to quantitation.

Chromatography was achieved using a Waters ACQUITY H-Class ultra-performance liquid chromatography (UPLC) with diode array detector (DAD) and quadrupole mass spectrometer. The system was calibrated for 12 cannabinoids including seven major cannabinoids (CBGA, CBG, CBDA, CBD, THCA, THC, and CBN) and five minor cannabinoids (exo-THC, Δ8-THC, CBC, THCV and CBDV) using 5-point linear regression over the range of 0.0005–0.05 mg/mL with a minimum coefficient of determination of 0.999 using 1/X weighting. Quantitation utilized the 225 nm extracted absorbance from the 3-D DAD spectra (190–500 nm) with confirmed peaks compared to reference library UV spectra as well as mass fragmentation patterns from 200–400 m/z for identification.

Terpene Analysis

All terpene samples followed the homogenization and subsampling procedures described in the cannabinoid analysis section. Aliquots of nominally 20 mg, irrespective of matrix type, were placed in 20 mL borosilicate headspace vials. Samples were analyzed for terpene profiles using headspace gas chromatography with flame ionization detection (HS-GC-FID). An Agilent 7694 headspace autosampler was used for sample injection and utilizing nitrogen vial pressurization and carrier gas. A heated transfer line carried analytes to a split injection port of a Shimadzu GC-2014 gas chromatograph. Split ratio was maintained at a constant 10:1 ratio under column velocity control with overall nitrogen flow rate of approximately 80 mL/min. The instrument was calibrated to analyze 16 terpene compounds using a 6-point linear calibration over the range of 0.625– 37.5 µg (31–1875 ppm) with a minimum coefficient of determination of 0.98.

Heavy Metal Analysis

Heavy metals were determined utilizing an Agilent 7800 inductively coupled plasma mass spectrometer (ICP-MS). Samples were homogenized with approximately 100 mg of sample and aliquoted into 25 mL MARSXpress microwave digestion tubes. After addition of 2 mL of a 9:1 concentrated mixture nitric and hydrochloric acid in water, the samples were digested with microwave assist (CEM, Mars6) at 210°C for 20 minutes and allowed to cool prior to centrifuging and filtering. The resulting digest was diluted to a final volume of 20 mL with 0.5% hydrochloric acid in water prior to analysis. The analyzer was calibrated using a 6-point linear calibration from 0 ppb to 5.00 ppb using 71 element standard mix. Continuing calibration verifications were performed every 5–10 samples.

Results

Of the original 30 products identified as hemp extract containing products that were purchased, all but one used hemp extract in the formulation of the product. One product was labeled as a "hemp chew", however the ingredients contained no hemp-derived cannabinoid; only hemp seed oil. This product was excluded from the analysis since it is well known that hemp seed contains nominal cannabinoids and terpenes.[20] Of the 29 products that were analyzed two were powders and one was a soft chew format; the remaining 26 were oil tinctures.

Labeling and COA

All of the boxes and labels were examined according to FDA compliant supplement guidelines, to determine any reference to cannabidiol or CBD concentration in the product and claims of mitigating a specific ailment. Eleven of the 29 products had reference to CBD as a constituent of their product, while the remaining products referred to their products concentrations as "total cannabinioids" or "total hemp extract" (Table 1). A COA was available from 22 of the 29 manufacturers; however, three of the COAs were likely from the raw materials rather than the actual final oil tincture (represented by asterisk products in Table 1); therefore, those were considered to be a flawed COA taking the number of actual product COAs to 19 of the 29 manufacturers. When examining the analysis of total cannabinioids of the product (which were predominantly CBD), the total cannabinoid concentration calculation was divided by the cost of the bottle (minus shipping), revealing a mean cost per mg of cannabinoid at $0.19/mg (median $0.14/mg – range $0.05-$0.58/mg); this calculation did not account for the two products that had no cannabinoid present on analysis (Table 1).

Cannabinoid analysis compared to the COA provided by the manufacturer was calculated as a mean percent and median of the COA (mean 75%, median 90%, range 0–129%), showing that COA concentrations were often lower than the actual certified laboratory analysis. These calculations were performed without the three companies with inappropriate COAs being factored into this calculation (19/29 products). Cannabinoid analysis compared to the label claim appeared to be more accurate (26/29 with label claims; mean 93%; median 99% – range 0–154%). Three of the manufacturer's provided COAs that were done at the laboratory chosen for our comprehensive analysis and our cannabinoid results were identical (bolded results on Table 1 – column labeled company COA provided).

Cannabinoid Concentrations

The cannabinoids that were identified in products based on mean abundance (and presence in products) in descending order were CBD (27/29 products), CBDA (12/29 products), CBC (19/29 products), CBG (18/29 products), Δ9-THC (19/29 products), THCA 2/29 (2/29 products), CBDV (17/29 products), and CBN (8/29 products). There was no detection of Δ8-THC, exo-THC or CBGA in any of the products tested. As the major cannabinoids marketed in low-THC *Cannabis sativa*, CBD (and CBDA) concentrations are shown in Figure 1 revealing a large degree of variability in CBD and CBDA concentrations. Table 2 shows the entire cannabinoid profiles and reveals that all products are below the USDA limit of 0.3% dry weight (combined Δ9-THC and THCA).

Terpene Concentrations

Terpene analysis revealed their concentration to be far lower than cannabinoids; the values are reported as mg/L or mg/kg. Although all of the analyzed terpenes could be found in some of the products at over 1 mg/kg, the only terpenes included in the table were those that could be found at over 100 mg/L or mg/kg in a product, representing the major terpenes found in hemp products (Table 3). Terpene abundance (and presence in products) based on mean concentrations in descending order were β-caryophyllene (29/29 products), humulene (28/29 products), β-myrcene (25/29 products), limonene (25/29 products), linalool (27/29 products), α-pinene (22/27 products), β-pinene (26/29 products), eucalyptol (19/29 products) and 3-carene (18/29).

Heavy Metal Concentrations

Of the 29 products analyzed four were positive for heavy metals at above the lower limit of detection (products #1, 2, 23 and 24). Product one contained 2104 µg/kg of arsenic, 209 µg/kg of cadmium and 157 µg/kg of lead. Only arsenic was considered above the limit for oral consumption based on the laboratory analysis guidelines and this product was a hemp powder supplement. Product 2 was an oil that contained 2296 µg/mL of lead which would not pass for oral consumption according to laboratory analysis guidelines and current USP guidelines. Products 23 and 24 were both oils that contained 262 µg/mL arsenic and 8 µg/mL of lead, respectively; which would both be considered safe for oral consumption. No other products

Table 1 Low-THC *Cannabis sativa* Products Selected Including Label Claims Regarding CBD, 0.3% THC Limits, COA Provided by Company, Laboratory Reported Total Cannabinoid Value, % of Label Claim, Percent of Company COA Compared to Analysis and Pricing per mg of Cannabinoid Based on Analysis

Product Form (Assigned #)	CBD on Label	Company-Provided COA Reported Total Cannabinoids (mg/mL or mg/g)	< 0.3% THC	Company-Reported COA Cannabinoid Conc. (mg/mL or mg/g)	Lab-Reported Total Cannabinoid Conc. (mg/mL or mg/g)	Label Claim Cannabinoid (mg/mL or mg/g)	% of Label Claim Total Cannabin.	% of Company COA	Price per mg Cannabin.
Powder (1)	No	No	Yes	NA	1	NA	NA	NA	$ 0.09
Oil (2)	Yes	Yes	Yes	114	61	50	122	53	$ 0.06
Oil (3)	Yes	Yes	Yes	33	30	***33***	91	90	$ 0.14
Capsule (4)	No	Yes	Yes	68	88	NA	NA	129	$ 0.37
Oil (5)	No	Yes	Yes	9	9	8	103	95	$ 0.12
Oil (6)	Yes	Yes	Yes	61	60	***48***	124	98	$ 0.13
Oil (7)	No	Yes	Yes	18	23	NA	NA	126	$ 0.12
Oil (8)	No	Yes	Yes	893[a]	13	15	87	NA	$ 0.34
Oil (9)	No	Yes	Yes	242[a]	8	8	99	NA	$ 0.21
Oil (10)	No	No	Yes	NA	0	33	0	NA	NA
Oil (11)	No	Yes	Yes	**18**	18	17	110	102	$ 0.10
Oil (12)	No	Yes	Yes	10	10	10	103	103	$ 0.15
Oil (13)	No	No	Yes	NA	3	3	88	NA	$ 0.39
Oil (14)	No	Yes	Yes	7	6	4	154	92	$ 0.18
Oil (15)	Yes	Yes	Yes	24	15	15	100	63	$ 0.19
Oil (16)	No	Yes	Yes	21	20	17	117	95	$ 0.11
Oil (17)	No	Yes	Yes	25	19	20	96	77	$ 0.16
Oil (18)	No	Yes	Yes	**66**	66	50	132	100	$ 0.06
Oil (19)	Yes	Yes	Yes	12	11	***9***	118	90	$ 0.13
Oil (20)	No	Yes	Yes	18	16	17	98	91	$ 0.08
Oil (21)	Yes	No	Yes	NA	19	17	111	NA	$ 0.12
Oil (22)	Yes	Yes	Yes	10	7	10	74	74	$ 0.40
Chew (23)	Yes	Yes	Yes	1000[a]	4	***5***	80	NA	$ 0.17
Oil (24)	No	No	Yes	NA	25	20	123	NA	$ 0.05
Oil (25)	Yes	Yes	Yes	**5**	5	117	4	97	$ 0.58
Oil (26)	Yes	Yes	Yes	18	13	17	77	71	$ 0.22
Oil (27)	No	No	Yes	NA	0	117	0	NA	NA
Oil (28)	Yes	Yes	Yes	5	4	***4***	92	76	$ 0.35
Oil (29)	No	No	Yes	NA	9	10	93	NA	$ 0.12
			Mean (mg)	29	20	26	92	91	$ 0.19
			St.Dev (mg)	29	22	30	38	19	$ 0.13
			Median (mg)	18	13	17	99	92	$ 0.14
			Range (mg)	0–114	0–88	0–117	0–154	0–129	$0.05–0.58

Notes: [a]COA results that were based on dry materials used in preparation of final product; these products were not used in final calculations of percentage of company COA. Company reported COA bolded numbers represent COA analysis from the same company we utilized in this study (#11,18,25). Label claim % numbers bolded and italicized were products that only contained a CBD label claim as the primary cannabinoid and no total cannabinoid claims for comparative purposes. NA is non-applicable calculation due to lack of cannabinoid, lack of proper COA or lack of finite calculation related to a numerator or denominator being equal to zero.

contained lead, mercury, arsenic, and cadmium at above the lower limit of detection for these heavy metals.

Discussion

This pet product examination is the first of its kind to utilize a certified laboratory in the analysis of cannabinoids, terpenes and heavy metals in commercially available low-THC *Cannabis sativa* pet supplements. Prior work for human products has examined a smaller profile of cannabinoids in over-the-counter hemp products, showing numerous discrepancies with what was tested versus labeling concentrations.[12–14]

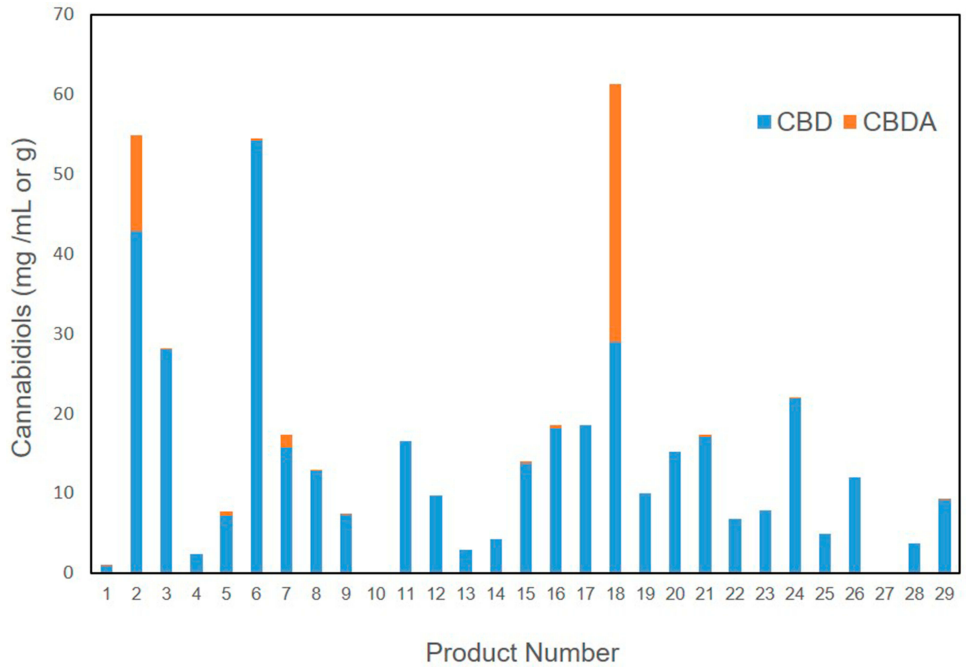

Figure 1 Concentrations of cannabidiols (CBD and CBDA) bar graph representation of 29 hemp derived pet marketed supplements.

Our analysis showed that all products complied, containing less than 0.3% THC as either THC or THCA (the precursor acid to THC). These THC results were encouraging regarding relative safety from THC intoxication to pets using these products. Issues related to USDA regulation suggest that all products should be processed from certified "hemp farms", yet this is difficult to determine as most companies could not supply us with paperwork related to the low-THC *Cannabis sativa* sourcing. Just over 75% of companies could supply us with a COA based on lot number of the product that was purchased and three of those COAs were from the base low-THC *Cannabis sativa* utilized to prepare the product and not the final product itself. Obtaining a COA is an important part of understanding the necessary dosing of the product if using cannabinoids for wellness issues. It is also critical that veterinary staff become accustomed with interpreting COAs for pet owners. The presence of a heavy metals, residual solvents, or other contaminants does not automatically disqualify a product from being safe to use, but the product should at minimum comply with USP standards for orally consumed products. The fiscal nature of product selection shows that price paid per mg of cannabinoid can be a 10-fold difference depending on product choice. Interestingly, two of the 29 products had absolutely no cannabinoids detectable in the product showing the lack of uniformity and fraud being perpetuated by some manufacturers.

Beyond the CBD and THC values represented it is important to note that due to the extensive nature of our testing we can confirm that there were no other forms of THC represented in any of the products (Δ8-THC or exo-THC) that can still impart psychotropic properties.[21] Other cannabinoids of interest that may have implications in neurology and inflammation are CBC, CBG and CBN which could not be found in over 50% of products. In products that did contain some of these cannabinoids the concentrations were less than 1 mg/mL or gram on average with concentrations at high as 1.6 mg/mL in an occasional product. Further examination of the other THC and CBD forms including THCA and CBDV show that there is less than 1 mg/mL or gram on average and that these are not major cannabinoids found in products. More interestingly, CBDA was a major cannabinoid identified in two products. This is likely due to the use of lower temperatures during extraction and processing, which is not commonly found, as higher heat processing is typical, and will result in the decarboxylation conversions of the native CBDA to the neutral CBD.[20,22] It is likely that this is intentional as CBDA has been associated with an anti-inflammatory effect and is thought to be involved in improved bioavailability of cannabinoids.[23–25] The remainder of the products and the two higher CBDA products also contained CBD as the primary cannabinoids with a range of 0–54 mg/mL or gram and a median of 10 mg/mL or gram. In fact, there are few products that have over 20 mg/mL or gram

Table 2 Cannabinoids Represented as mg/mL or mg/g of Product. Mean and Standard Deviations as Well as Medians and Ranges Across Products Represented. Total Cannabinoids Detected for Each Product in Right Hand Column

Product #	Δ9-THC	THCA	CBD	CBDV	CBDA	CBG	CBC	CBN	Total
1	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.9
2	2.0	0.0	42.8	0.2	12.1	1.6	1.9	0.1	60.7
3	0.1	0.0	28.0	0.6	0.0	0.1	0.6	0.0	29.5
4	0.1	0.0	2.4	0.0	0.0	0.0	0.2	0.0	2.7
5	0.3	0.0	7.1	0.2	0.5	0.1	0.3	0.0	8.5
6	1.4	0.0	54.2	0.9	0.3	0.9	1.9	0.2	59.5
7	1.4	0.9	15.7	0.5	1.6	1.2	1.2	0.1	22.6
8	0.0	0.0	12.9	0.2	0.1	0.0	0.0	0.0	13.1
9	0.2	0.0	7.3	0.1	0.1	0.3	0.3	0.0	8.3
10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
11	0.7	0.0	16.6	0.2	0.0	0.2	0.6	0.0	18.3
12	0.4	0.0	9.8	0.0	0.0	0.1	0.0	0.0	10.3
13	0.0	0.0	2.9	0.0	0.0	0.0	0.0	0.0	2.9
14	0.0	0.0	4.3	0.2	0.0	0.4	0.8	0.8	6.4
15	0.6	0.0	13.8	0.1	0.2	0.1	0.7	0.1	15.4
16	0.6	0.0	18.1	0.1	0.4	0.1	0.6	0.0	19.9
17	0.0	0.0	18.5	0.3	0.0	0.3	0.0	0.1	19.2
18	1.3	0.9	29.0	0.1	32.2	0.4	1.4	0.1	65.3
19	0.3	0.0	10.0	0.0	0.0	0.1	0.5	0.0	10.8
20	0.4	0.0	15.2	0.1	0.0	0.3	0.3	0.0	16.4
21	0.7	0.0	17.1	0.0	0.2	0.0	0.5	0.0	18.6
22	0.2	0.0	6.8	0.0	0.0	0.1	0.3	0.0	7.4
23	0.0	0.0	7.9	0.0	0.0	0.0	0.0	0.0	7.9
24	0.9	0.0	21.8	0.1	0.3	0.6	0.9	0.0	24.6
25	0.0	0.0	4.9	0.0	0.0	0.0	0.0	0.0	4.9
26	0.3	0.0	12.0	0.1	0.0	0.2	0.2	0.1	12.8
27	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
28	0.0	0.0	3.7	0.0	0.0	0.0	0.1	0.0	3.8
29	0.0	0.0	9.2	0.0	0.1	0.0	0.0	0.0	9.3
Mean (mg)	0.4	0.1	13.5	0.1	1.7	0.3	0.5	0.1	16.6
St.Dev. (mg)	0.5	0.2	12.4	0.2	6.3	0.4	0.5	0.2	17.4
Median (mg)	0.2	0.0	10.0	0.1	0.0	0.1	0.3	0.0	10.8
Range (mg)	0–2	0–0.9	0.0–54.2	0–0.9	0–32.2	0–1.6	0–1.9	0–0.8	0.0–65.3

(6/29) or 50 mg/mL (3/29) or gram of CBD or total cannabinoids. The implications of low concentration products is important since dosing appears to be in the 1–3 mg/kg range based on current knowledge,[16–18] which would suggest that minimally 1 mL or gram of product would be needed per 10 kg body weight using products that are 20 mg/mL, while the products that are 50 mg/mL would only require 0.3 mL per 10 kg body weight. These concepts regarding dosing are necessary for veterinary health professionals to consider when discussing the logistics of safety, efficacy, value and dosing surrounding product selection.

The fact that only 10 of the 29 products were within 90–110% of the label claims regarding cannabinoid concentrations provides little comfort to the consumer that products are labeled appropriately. Nine of the products were over 110% of the label claim on analysis and 10 were under 90% of the label claim, with two of these containing no cannabinoids. This is not atypical of what has been found in human hemp products, where less than 50% were found to be within 90–110% of label claims.[13] Reasons for low-THC *Cannabis sativa* discrepancies involve many factors such as poor formulation, degradation of cannabinoids and bioconversion over time, or inappropriate laboratory analysis. In addition, we did not test multiple batches of product, which may be another reason for drift in cannabinoid concentrations within certain products, bringing to light the need for further examination of such issues in the industry.

Table 3 Terpenes Identified as Having Over 100 mg/mL or mg/kg in Any Product. Mean and Standard Deviation and Median and Ranges as Well as Total Terpene Concentrations per Product

Product #	β-Myrcene	Linalool	β-Caryophyllene	Humulene	β-Pinene	Eucalyptol	3-Carene	β-Pinene	Limonene	Tot. Terp.
1	0	10	24	4	2	0	2	0	3	44
2	6	13	127	47	28	13	1	2	4	242
3	390	485	105	14	15	4	35	2	104	1153
4	1	10	110	46	1	1	3	0	0	173
5	28	6	17	8	13	4	0	5	13	96
6	14	28	165	68	4	4	2	4	7	296
7	400	360	229	83	200	25	316	365	925	2903
8	9	5	16	2	12	0	0	4	4	54
9	18	0	6	5	3	0	0	6	7	45
10	23	1	4	4	9	0	4	5	10	60
11	57	20	284	78	4	23	6	6	22	500
12	18	1	2	1	4	1	0	6	10	41
13	21	1	5	2	3	0	0	7	8	48
14	4	3	1	1	0	0	0	1	4	15
15	3	4	45	16	2	2	1	2	2	76
16	5	6	206	71	0	19	0	1	5	314
17	723	322	268	18	21	82	37	358	741	2570
18	1254	90	154	39	188	36	3	414	154	2331
19	781	5	154	230	281	184	117	2975	2062	6790
20	38	34	68	19	43	111	37	40	365	756
21	8	5	70	19	26	3	1	5	20	156
22	11	46	63	0	21	4	1	14	44	203
23	0	4	8	3	2	0	3	0	1	22
24	64	16	126	38	1	6	2	0	19	272
25	0	3	3854	560	3	0	2	0	0	4422
26	1	1	182	35	1	2	0	1	2	225
27	2	0	2	2	1	0	0	6	1	15
28	0	222	1	1	0	0	0	6	0	229
29	2	1	6	1	5	0	0	11	0	25
Mean (mg)	134	59	217	49	31	18	20	146	156	830
St.Dev. (mg)	56	23	131	20	13	8	11	103	79	291
Median (mg)	11	6	68	16	4	2	1	5	7	203
Range (mg)	0–1254	0–485	0–3854	0–560	0–281	0–184	0–316	0–2975	0–2062	15–6790

Currently, there are no established federal standards for low-THC *Cannabis sativa* testing. However, the ISO/IEC 17025 standard for analytical testing laboratories has become a requirement for labs providing testing services in multiple states, and has become a foundational component of the recent interim USDA Hemp Farming and Testing guidelines.[26,27] Laboratories accredited to this standard are routinely assessed by accrediting bodies to ensure that laboratories adhere to good laboratory practices, utilizing validated methods on calibrated instruments. Analyst and technician training must be well documented to support the individual activities performed by laboratory personnel. Laboratories are also required to demonstrate their competency by participation in proficiency testing programs, in which they are challenged with blind samples to maintain accreditation. Results from this testing are collected with statistical comparison to other laboratories and/or to the established values for the testing performed. Practitioners and consumers should rely on product COAs provided only by an accredited laboratory, to ensure that the data provided is reliable.

A further novelty of our investigation is not only the range of cannabinoids tested, but also the extensive terpene analysis of products. Of the over 20 terpenes assessed there were only 9 that had over 100 mg/kg of one or more of the terpenes in any one product (0.01%) reported in Table 3. The primary terpenes of interest were β-caryophyllene, β-myrcene, pinenes, humulene, linalool and limonene; and depending on the product, the profiles can be dramatically different. Terpenes are the major volatile products of hemp that provide a distinct odor. Interestingly, due to similar backbone precursor molecules

total terpenes can often, but do not always, follow cannabinoid concentrations. The enzymatic machinery of the plant cultivars do dictate terpene formation to a large degree (Table 3). Our data suggest that in some cases there were abnormally high total terpene concentrations in some products which are likely due to manufacturers "spiking" products with terpenes to either provide some natural medicinal properties that have been attributed to terpenes,[28,29] or to enhance the aroma, thereby misleading the consumer into thinking that the product was highly enriched with cannabinoids, despite phytocannabinoids having no scent or flavor.

Terpenes may have some therapeutic advantages since it has been observed that whole plant extracts can be superior to single molecule constituents, which is known as the "entourage effect".[30–33] In general, the milligram quantity of total terpenes across products ranges from 0.015–6.7 mg/mL or gram with only 6 products with over 1 mg/mL or gram of total terpenes. Further examination for any single terpene at concentrations higher than 1 mg/mL leaves only three products (#18, 19, 25) that have a single terpene at that level. Overall, total terpenes for most products would be similar in concentrations to some of the minor cannabinoids observed, making it difficult to elucidate exactly whether alternate cannabinoids and/or terpenes are exhibiting some of the synergy observed as the "entourage effect" discussed in the literature.

As with any plant material, accumulation of minerals from soil is part of the nutritional benefits of plant consumption; however, chronic consumption of any plant material with accumulation of heavy metals is an important health consideration. Most concerning is that hemp as a crop has been utilized in polluted areas to help with bioremediation of soils due to its ability to grow in heavily contaminated soils.[34–36] Crop growth in variable geographic regions leads to variable mineral and heavy metal accumulation. Lead and arsenic accumulation appear to be most relevant as potential contaminants leading to health concerns. Our analysis of four common heavy metals did show contamination in 4 of 29 products (12%). For two of these products, the recorded levels of arsenic and lead exceed the regulatory limits established by the Massachusetts Department of Public Health for cannabis products.[27] In one product with excessive arsenic accumulation, the product was a dried hemp powder (#1). The product with excessive lead above the limit for oral consumption was an oil product (#2). We cannot comment on whether this lead contamination was from the hemp, the carrier oil used as a diluent/solvent or the processing equipment or materials used for extraction. Regardless, it becomes critical for pet owners to have products tested, or to insist on COA results for heavy metals before supplementation.

Our study did not examine other possible sources of contamination in pet hemp consumables including solvents used in the extraction process, pesticides used on crops, mycotoxins that can accumulate in dried crops, and microbiological contaminants. For each of these issues there have been reports of contaminants in hemp production and extraction, which is why consumers must be aware and solicit this information from manufacturers.[36] The scope of this assessment was to study the constituents that might accumulate in the plant tissues themselves, not focusing on crop management or extraction related contamination, yet research assessing products for these contamination issues is sorely needed.

In summary, until further guidelines can be defined by the FDA, state specific laws, Federal Trade Commission and the USDA, there is a need for intervention by veterinarians and technicians into this ever-expanding world of low-THC *Cannabis sativa* supplements. Practitioners need to become versed in product selection and utilization, and should be asking questions of manufacturers regarding CBD and THC concentrations in the products minimally; with further inquiries into potential contaminants including heavy metals, solvents, pesticides, microbials and mycotoxins. The range and variability of products in the veterinary market is alarming and veterinary professionals should only consider manufacturers providing product safety data in the form of a COA, pharmacokinetic, and clinical application data when clients solicit information regarding product selection.

Acknowledgments

The authors would like to thank Ms. Amanda Howland for her help with the logistics involved in obtaining the products and results from ProVerde Labs.

Author contributions

All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

Joseph J Wakshlag is a paid consultant of ElleVet Sciences and reports grants, personal fees from Ellevet Sciences,

outside the submitted work. Stephen Cital and Reece Prussin are employed full-time at ElleVet Sciences, which does research, manufactures and sells products containing cannabinoids. The authors report no other conflicts of interest in this work.

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