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High Cannabigerol Hemp Extract Moderates Colitis and Modulates the Microbiome in an Inflammatory Bowel Disease Model[S]

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ABSTRACT

Cannabis sativa L. has a long history of medicinal use, particularly for gastrointestinal diseases. Patients with inflammatory bowel disease (IBD) report using cannabis to manage their symptoms, despite little data to support the use of cannabis or cannabis products to treat the disease. In this study, we use the well-described dextran sodium sulfate (DSS) model of colitis in mice to assess the impact of commercially available, noneuphorigenic, high cannabigerol (CBG) hemp extract (20 mg/mL cannabigerol, 20.7 mg/mL cannabidiol, 1 mg/mL cannabichromene) on IBD activity and the colonic microbiome. Mice were given 2% DSS in drinking water for 5 days, followed by 2 days of regular drinking water. Over the 7 days, mice were dosed daily with either high CBG hemp extract or matched vehicle control. Daily treatment with high CBG hemp extract dramatically reduces the severity of disease at the histological and organismal levels as measured by decreased disease activity index, increased colon length, and decreases in percent colon tissue damage. 16S rRNA gene sequencing of the fecal microbiota reveals high CBG hemp extract treatment results in alterations in the microbiota that may be beneficial for colitis. Finally, using metabolomic analysis of fecal pellets, we find that mice treated with high CBG hemp extract have a normalization of several metabolic pathways, including those involved in inflammation. Taken together, these data suggest that high CBG hemp extracts may offer a novel treatment option for patients.

SIGNIFICANCE STATEMENT

Using the dextran sodium sulfate model of colitis, the authors show that treatment with high cannabigerol hemp extract reduces the severity of symptoms associated with colitis. Additionally, they show that treatment modulates both the fecal microbiota and metabolome with potential functional significance.

Introduction

Inflammatory bowel disease (IBD) is a group of chronic inflammatory diseases characterized by abdominal pain, diarrhea, and weight loss. The etiology of IBD is complex, involving both environmental and genetic components; however, it is believed that IBD results from a combination of dysregulated immune responses against the microbiota of a genetically susceptible individual (Tavakoli et al., 2021). The two major forms of IBD include ulcerative colitis (UC), which can affect any portion of the colon and Crohn's disease that can affect the entire alimentary canal. Current therapies to treat UC (steroids, immunosuppressants, and 5-aminosalicylic acid) have limited effectiveness and frequently have undesirable

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ABBREVIATIONS: ASVs, amplicon sequence variants; CBC, cannabichromene; CBD, cannabidiol; CBG, cannabigerol; DAI, disease activity index; DSS, dextran sodium sulfate; FDR, false discovery rate; IAA, indole 3-acetic acid; IBD, inflammatory bowel disease; LC-MS, liquid chromatography–mass spectrometry;; OD, optical density; THC, tetrahydrocannabinol; UC, ulcerative colitis.

side-effects (Calvino-Suarez et al., 2022; Sleiman et al., 2022).

Recent surveys of patients with IBD suggest that between 10%–12% use cannabis or cannabis products to treat the symptoms of their disease (Bogale et al., 2021). Additionally, there is growing interest among patients with IBD in the potential of cannabis to treat their disease. Although some evidence exists to support the hypothesis that cannabinoids can be an effective treatment of UC, these studies have been largely limited to tetrahydrocannabinol (THC) and cannabidiol (CBD)—both in patients and animal models. The cannabinoid receptors (CB1 and CB2), are upregulated in tissues from UC patients and higher levels of endocannabinoids have also been reported (Di Sabatino et al., 2011). Studies in animals have generally reported an improvement in colitis symptoms in animals treated with cannabinoids (Izzo et al., 2001; Jamontt et al., 2010; Bento et al., 2011; Borrelli et al., 2013; Pagano et al., 2016). However, in human studies, the data remain inconclusive (Naftali et al., 2013, 2017; Storr et al., 2014; Irving et al., 2018). This discrepancy is likely due to multiple reasons including small individual study sample sizes and the use of variable plant material. Moreover, the psychoactive activities of THC make it difficult to have a valid placebo control. Still, most patients report that use of cannabis reduced the pain associated with IBD. Importantly, changes in inflammatory markers and overall disease pathology have generally not been assessed. Alternatively, studies have found marijuana (THC-dominant cannabis) use improved symptoms but increased the risk for future surgery in Crohn's disease patients and an increase in emergency department visits (Storr et al., 2014; Glickman et al., 2023). Overall, these studies suggest that marijuana may improve at least some symptoms and the quality of life for patients but may not be effective for the treatment of the underlying disease in patients.

Cannabis produces over 100 cannabinoids. Specifically, the plant produces a precursor cannabinoid, cannabigerol (CBG), from which the other cannabinoids are produced (Gülck and Møller, 2020; Nachnani et al., 2021). Typically, CBG is found in very low amounts in both hemp and marijuana, due to the rapid conversion of cannabigerolic acid into the other three major cannabinoids. However, through selective breeding and due to increased interest from the public, several new cultivars of hemp with high levels of CBG have been produced; however, the biological activity of extracts from these cultivars remains to be determined. Here, we assessed the therapeutic potential of a commercially available, full spectrum hemp extract high in CBG in the well-characterized dextran sodium sulfate (DSS) model of colitis in mice. We find that daily administration of high CBG extract dramatically reduces the development of colitis following DSS treatment. Therapeutic effect is coupled with concomitant reshaping of the microbiota and metabolome within the colon of DSS-treated mice compared with vehicle-treated animals.

Materials and Methods

Materials. All solvents and chemicals used, unless otherwise noted, were of reagent or spectroscopic grade, as required, and obtained from VWR (Radnor, PA) or Sigma Aldrich (St. Louis, MO).

Animals and Induction of Colitis. For this study, 6- to 8-week-old, male C57BL/6 J (Jackson Laboratories, Bar Harbor, ME) mice (n = 31) were treated with 2% DSS (ThermoFisher, Waltham, MA) in their drinking water for 5 days to induce colitis as previously described (Konsavage et al., 2012, 2013; Raup-Konsavage et al., 2016). Following the 5-day treatment, animals were returned to normal drinking water for 2 days. A group of animals (n = 10) were maintained on normal drinking water during the study to serve as controls. Animals were individually housed in a barrier facility and had ad libitum access to food and water. The Pennsylvania State University College of Medicine Institutional Animal Care & Use Committee approved the animal protocols used in this study.

TABLE 1
Cannabinoid content of high CBG hemp extract

Cannabinoid	Concentration (mg/mL)
CBC	1.74
CBD	36.56
CBG	35.63
Total cannabinoid content	73.74

Administration of Hemp Extract. High CBG hemp extract (Extract Laboratories, Lafayette, CO) was administered at a daily dose of 20 mg/kg CBG, 20.7 mg/kg CBD, and 1 mg/kg cannabichromene (CBC) via intraperitoneal injection (n = 16). The full cannabinoid and terpene composition of the oil is provided in Tables 1 and 2. Fractionated coconut oil (Pursonic, New York, NY) was used for the vehicle-treated animals (n = 15). All animals were assessed for colitis damage and a subset (as indicated in the results) was used for microbiome and metabolome analysis. Oils were diluted in a vehicle of DMSO:Tween: Saline (1:1:18) as previously reported (Sepulveda et al., 2022a,b). Control animals also received daily injections of vehicle (n = 5) or high CBG hemp extract (n = 5).

Disease Activity Index (DAI) Scoring. DAI scoring following DSS-treatment was performed as previously described (Konsavage et al., 2012; Raup-Konsavage et al., 2016). Animals were scored daily based on percent weight loss, stool consistency, and blood in stool/rectum, with a score of 0–4 for each category. Weight loss was scored as 0 if no weight loss occurred, 1 for 1%–5% loss, 2 for 5%–10% loss, 3 for 10%–20% loss, and 4 for weight loss >20% of initial weight. Stool was scored as 0 for normal, 2 for soft/loose, and 4 for diarrhea, while bleeding was scored as 0 for absent, 2 for Hemoccult-positive, and 4 for visible/gross blood in the stool/hindquarters. The three scores are added together for a DAI score, and data are presented as average daily DAI scores. DAI scores are presented as mean ± S.D. and analyzed by 2-way ANOVA and Šídák's multiple comparison test.

Sample Collection and Histology. Fresh stool samples were collected prior to the start of DSS treatment, at the end of the DSS treatment (day 5), and at the time of tissue collection (day 7). Stool was frozen at –80°C until analysis. Animals were killed via isoflurane overdose followed by cervical dislocation. Colon tissue was collected and fixed in 4% paraformaldehyde overnight and then transferred to 70% ethanol. H&E staining, paraffin embedding, and slide preparation were all performed by the Comparative Medicine Histology Core at Penn State College of Medicine (Konsavage et al., 2012, 2013;

TABLE 2
Terpene content of high CBG hemp extract

Terpene	Concentration (mg/mL)
β-Caryophyllene	0.091
α-Humulene	0.023
(–) α-Bisabolol	0.040
β-Myrcene	0.001
R (+) limonene	0.003
3-Carene	0.003
Endo-fenchyl alcohol	0.003
α-Terpineol	0.001
Guaiol	0.040
Total terpene content	0.205

Raup-Konsavage et al., 2016). Damage to the colonic epithelium was measured using ImageJ software and the total length of damaged tissue was divided by 2× the total colon length for that animal to generate a percent damaged. Colon lengths were compared using a two-way Student's *t* test and colonic damage comparisons were made using 2-way ANOVA and Šídák's multiple comparison test.

Microbiota Sequencing. A previous published protocol described at github.com/bisanzlab/AmpliconSeq was used. Briefly, frozen stool samples were extracted using a ZymoBIOMICs 96 MagBead DNA Kit with 5 minutes of lysing with a FastPrep 96 (MPBio, Santa Anna, CA). Primary polymerase chain reaction was performed using 515F and 806R primers with partial sequencing extensions. Primary polymerase chain reaction was performed using KAPA HiFi hot start polymerase with 25 cycles. A 10× dilution series of each sample was performed with amplification monitored in real time (BioRad CFX384 Opus). One dilution was selected per sample in late exponential phase amplification selected for indexing. Indexing was performed using dual 12-bp indexes derived from the Earth Microbiome Project error-correcting Golay indexes using the same polymerase. Amplicons were quantified using Picogreen (Life Technologies, Carlsbad, CA) and pooled at equimolar concentrations before a final size selection and clean up (Ampure XP). Most liquid handling steps were performed using an OT-2 pipetting robot (Opentrons). The final library was quantified by quantitative polymerase chain reaction and loaded on a MiSeq (v3 600 cycle reagent kit) run at 270 × 12 × 12 × 270, indicating paired 270 nt reads with dual 12 nt indexing reads.

Data were processed using QIIME2 v 2023.5 following the script available in the previously linked GitHub repository (Bolyen et al., 2019). Primers were trimmed allowing for an error rate of 15% and adapter-less reads were discarded. Denoising, overlapping, merging of paired reads, and chimera read removal was performed using q2-DADA2 (Callahan et al., 2016). Resulting amplicon sequence variants (ASVs) went through in silico size selection (250–255 nt). Taxonomic assignment of ASVs was performed through DADA2 using SILVA (nr99 v138.1) (Quast et al., 2013). This method uses a naive Bayesian classifier to assign kingdom, phylum, class, order, family, and genus level taxonomic assignments. Species level assignments are based on exact matches of ASV sequences to the 16S rRNA sequences of reference strains. The mean number of denoised processed reads per sample was 36,189 ± 15,125.17 (mean ± S.D.). Data were subsequently imported into R v4.3.2 using qiime2R (v0.99.6). Samples were rarified at an even depth without replacement based on the sample with the lowest read count using qiime2R::subsample_table prior to calculating α diversity calculations. Samples were normalized by center log ratio transformation before calculating Euclidean distances for β diversity calculations, diversity metrics and PERMANOVAs (Fernandes et al., 2013, 2014; https://cran.r-project.org/web/packages/vegan/index.html). Data were visualized using ggplot2 and relevant extensions (Wickham, 2016). Where applicable, statistical analysis was performed using either Welch's *t* test, Mann-Whitney *U* test, or a linear mixed effects model (lmerTest v3.1-3, with model: Abundance ~ CBG_Treatment * Time_Days + (1|MouseID)) (Kuznetsova et al., 2017). The interaction term of CBG_Treatment and Time_Days was taken to represent the effect of treatment over time accounting for differences in baseline composition. Differential abundance of microbes was carried out using genus-summarized abundances (qiime2R::summarize_taxa) followed by centered log2-ratio transformation. Models that reported singular fit warnings were excluded from downstream analysis/reporting. Multiple testing correction was carried out using Benjamini Hochberg's false discovery rate with a significance threshold of a false discovery rate (FDR) <0.1 unless otherwise noted.

Bacterial Culture Screening. Strains derived from the Broad Institute-OpenBiome Microbiome Library were cultured in brain heart infusion + 0.05 w/v% cysteine, *5* μg/mL hemin, *1* μg/mL vitamin K3 (BHI CHV) before being inoculated 0.5 v/v% into fresh media containing either 0.75% DMSO vehicle or a range of CBD/CBG (Cayman Chemical, Ann Arbor, MI) in equimolar concentrations (32 μg/mL for the collection screening) (Poyet et al., 2019). All growth assays were performed in an anaerobic chamber (Coy Anaerobic Systems, 5% H_2, 20% CO_2, 75% N_2) with growth at 37°C. Kinetic growth data were collected using a Multiskan spectrophotometer (Thermo Fisher) at 600 nm and reads every 15 minutes. Bacterial culture screening assay was performed in two 384-well plates in parallel with 80 μL of media and sealed with Breathe-Easy sealing membranes and the lid was taped (Diversified Biotech). The 384-well plates contained 24 total sterile control wells, 12 DMSO control, and 12 CBD/CBG wells. Microbroth dilution assays were carried out in 96-well plates with 200 μL of media and were sealed with Breathe-Easy sealing membranes and the lid was taped (Diversified Biotech). The 384-well plates contained 12 total sterile control wells, three wells of each CBD/CBG concentration used. Disk diffusion assays were performed by diluting bacterial cultures to an optical density (OD) of 0.1 and spreading on BHI CHV plates using a sterile cotton swab. Disks (6 mm; Cytiva) were placed before being loaded with 60 μg equimolar CBD/CBG mixture, 15 μg erythromycin (control), or DMSO (vehicle), which were subsequently incubated for 48 hours under anaerobic conditions.

Bacterial Culture Growth Kinetics Analysis. Growth kinetics analysis was performed using R (v4.3.2). Strains with inconsistent growth (growth in fewer than three vehicle control wells) were excluded from analysis. Carrying capacity was calculated by determining the highest median OD_{600} within 1-hour intervals. Time to mid log and maximal growth rate were determined by fitting growth data to a logistic equation using growthcurveR with background correction from the blank well OD_{600} at each time point (0.3.1) (Sprouffske and Wagner, 2016). Fold changes were calculated and mapped to a phylogenetic tree using ggtree (3.18) (Yu et al., 2018). Phylogenetic tree was constructed using PhyloPhlAn (used amino acid sequences, low diversity, and accurate assignments) (3.0), and the RAxML best scoring tree used (Asnicar et al., 2020). Taxonomic assignments generated via Genome Taxonomy Database Toolkit using default settings (Release 214.1) (Chaumeil et al., 2019).

Metabolomics Analysis. Stool samples were charged with ice-cold 80% aqueous methanol (with 0.1% formic acid) at a ratio of 10 μL/mg. Samples were sonicated at 60 minutes at 10°C, then centrifuged at 12,000 × g for 10 minutes at 4°C. Supernatants were removed and extraction was repeated a second time. Supernatants from the two rounds of extraction were combined and evaporated to dryness in a Speedvac. Extracts were reconstituted at 1 mg/mL in 50% aqueous methanol with 1 μM chlorpropamide (Santa Cruz Biotechnology, Dallas, TX) added as an internal standard.

Untargeted metabolomic analyses were performed on a Vanquish Duo UHPLC system connected to a Thermo Orbitrap Exploris 120 Mass Spectrometer (Thermo Fisher Scientific, Waltham, MA). A Waters Acquity UPLC BEH C18 (1.7 μm, 2.1 × 150 mm) column was used with a flow rate of 0.1 mL/min at 55°C. Solvent A was liquid chromatography–mass spectrometry (LC-MS) grade H_2O with 0.1% formic acid (v/v); solvent B was LC-MS grade acetonitrile with 0.1% formic acid (v/v). The mobile phase gradient of solvent B was: 2% B for 2 minutes, increase to 15% B in 1 minute, increase to 50% B in 3 minutes, increase to 98% B in 1.5 minutes, hold for 4 minutes, return to original conditions in 0.1 minute, and hold for 3.4 minutes. All samples were injected at 5 μL.

The mass spectrometer was operated with an electrospray ionization source with a positive ion spray voltage of 3500 V and negative ion spray voltage of 2700 V, sheath gas, and auxiliary gas flow of 25 and 5 units, respectively. The ion transfer temperature of 275°C, and vaporizer temperature of 75°C. MS1 data were acquired with an Orbitrap resolution of 120,000, scan range of 100–1000 Da, and radio frequence (RF) lens of 50% in the centroid mode. MS^2 data were collected in a data-dependent acquisition mode with an intensity threshold of 2.5e4 and 30-second dynamic exclusion.

The LC-MS/MS data were analyzed and processed using MZmine 3.9 software (Schmid et al., 2023). Peaks were detected with a noise level of 3E4 counts. The ADAP algorithm was used to build chromatograms with the following parameters: minimum scan size = 5, group intensity threshold = 9E4, minimum highest intensity = 1.5E5, and scan-to-scan accuracy = 0.005 Da. Chromatograms were smoothed

via 5-point Savitzky-Golay method and resolved using a local minimum feature resolver with a chromatographic threshold of 90%, minimum feature height of 5E4, minimum ratio of peak top/edge of 1.80, peak duration range of 0.00–0.75 minutes, and minimum number of scans of 4. Isotopes were filtered before integrating all features with the join aligner algorithm with the following parameters: m/z tolerance = 0.005 Da, weight for m/z = 3, retention time tolerance = 0.15 minutes, weight for retention time = 1. Features not present at an intensity higher than 5-fold the average intensity for the given feature in the 75% of the blank samples were removed.

Spectral data were annotated via MS/MS comparisons using the Global Natural Products Social Molecular Networking platform, with a precursor mass window of 0.1 Da, fragment ion mass tolerance of 0.2 Da, and minimum cosine score of 0.6 with minimum matched fragments 4. Identified metabolites were cross-referenced against the human metabolome database (https://hmdb.ca/) for relevant identifiers, and then analyzed with the Pathway Analysis and Statistical Analysis modules in MetaboAnalyst 6.0 (https://www.metaboanalyst.ca/home.xhtml) (Pang et al., 2021; Shah et al., 2023). Principal component analysis, metabolite comparisons, and significance were calculated in R 4.3.2 using the *prcomp* and *car* packages.

Results

High CBG Hemp Oil Reduces Colitis Severity and Colonic Damage. The DSS-model of acute colitis was used to assess the impact of high CBG hemp extract on disease in mice. Animals were treated with 2% DSS for 5 days followed by 2 days of normal drinking water and received a daily intraperitoneal injection of hemp extract or vehicle (coconut oil) at 20 mg/kg CBG (Fig. 1A). CBG treatment had no impact on noncolitic animals (Fig. 1A). However, a marked improvement in DAI was observed for animals receiving CBG hemp extract compared with vehicle-treated animals (Fig. 1B). Additionally, it is well documented that DSS-induced colitis results in a shortening of the colon and this shortening was prevented in animals that received hemp extract (Fig. 1, C and D).

Damage to the colonic epithelium was assessed by examining the entire H&E stained colonic section for each animal. No significant difference in damage was noted between treatment groups at day 5 (end of DSS treatment), and damage on this day was absent or minimal, as has been previously reported for DSS-treatment (Fig. 2, A and B) (Konsavage et al., 2012, 2013; Raup-Konsavage et al., 2016). Significant differences were noted at day 7 (2 days post DSS treatment), when damage typically peaks in this model (Fig. 2, A and B). Importantly, CBG hemp oil appears to reduce the colonic damage in this model of colitis.

High CBG Hemp Oil Modulates Microbiota Composition through Clade-Specific Antimicrobial Activity. To assess the gut microbiota composition changes during high CBG hemp oil treatment, and to gain insights on how DSS treatment may alter these changes, the fecal microbiota of a subset of animals were analyzed based on controlling for animal batch effects (n = 26 mice). The high CBG hemp extract-treated mice had a significantly lower microbial diversity (number of observed ASVs) compared with vehicle-treated mice on day 7 (Fig. 3A) with significantly different trajectories in their microbial compositions during the course of treatment (Fig. 3, B and C). Comparison of Aitchison distances suggests that CBG-treated mice have a greater change in microbial composition after CBG treatment (Fig. 3C; Supplemental Fig. 1). Contrasting high CBG hemp oil-treated and untreated microbiotas, there was an enrichment of *Rombustia* spp. with a concomitant decline in *Oscillospiraceae* spp. and Bacilli class RF39.

In mice that were treated with DSS, administration of high-CBG hemp oil or vehicle control resulted in distinct microbiota composition on day 7 compared with day 0. Although the number of observed ASVs was not significantly different between hemp oil and vehicle control-treated mice, the compositions did change significantly after 7 days of high CBG hemp oil or vehicle treatment (Fig. 3, E–G). A greater change in Aitchison distance was observed in CBG-treated mice than in the

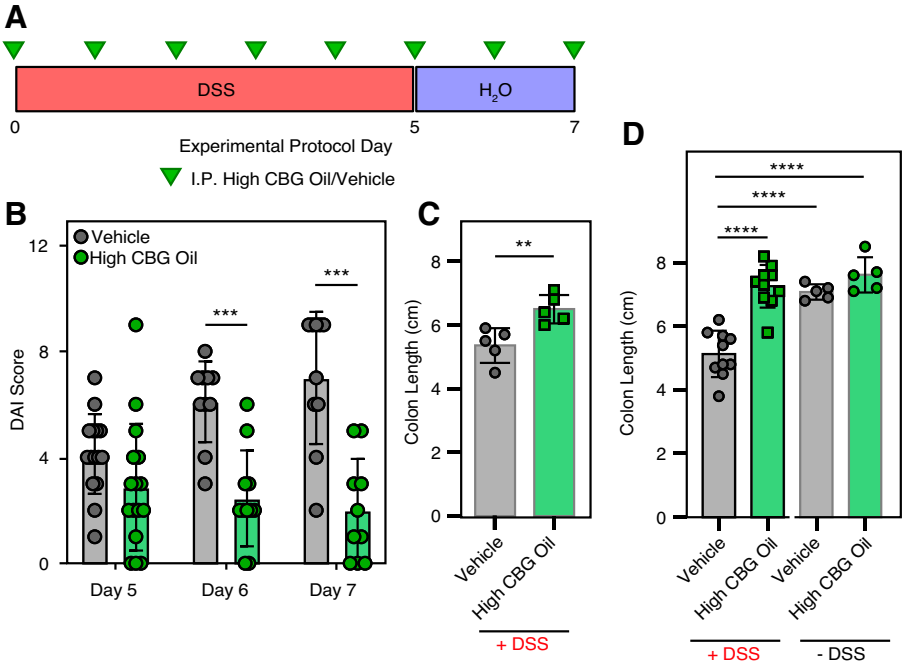

Fig. 1. High CBG hemp extract reduces severity of colitis. (A) Schematic showing the DSS-induced colitis protocol. Animals received a daily dose of high CBG hemp extract (CBD/CBG oil) starting on day 0, as indicated by the green arrows. (B) Composite DAI scores on days 5, 6, and 7 of the DSS colitis protocol. (C) Colon length from animals receiving either vehicle or high CBG hemp extract at the end of DSS-treatment (day 5). (D) As in (C) except day 7 of protocol and includes tissue collected from noncolitic (water-treated) mice at this time point. Data are presented as mean ± S.D. with individual mice shown (N = 16 mice colitic CBG-treated, N = 15 mice colitic-treated, N = 5 noncolitic CBG-treated, N = 5 noncolitic-treated), $^{**}P < 0.01$, $^{***}P < 0.005$, and $^{****}P < 0.001$. Student's t test was used for (C), a 2-way ANOVA with Šídák's multiple comparison test was used for (B), and Tukey's multiple comparison test was used for (D) to assess differences.

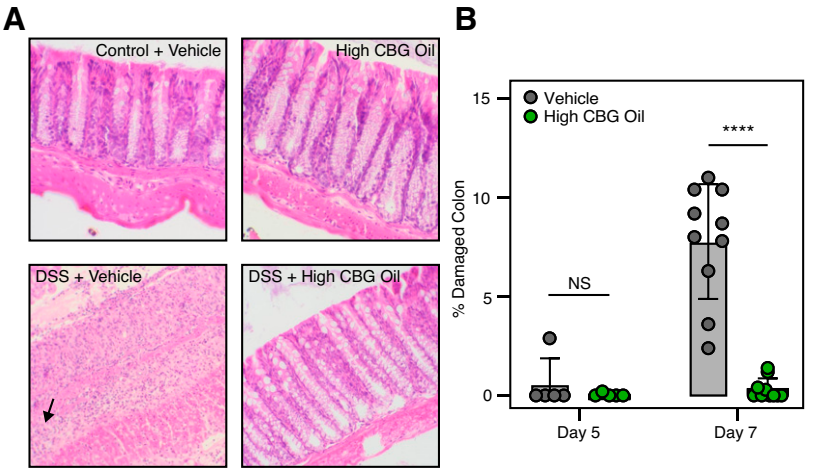

Fig. 2. High CBG hemp extract reduces colonic damage. (A) Representative images are shown for tissue collected on day 7 of the DSS protocol for both vehicle and high CBG hemp extract-treated animals, tissue taken from control (water-treated) animals is also shown. Normal crypt architecture of the colon is seen in control animals regardless of treatment (vehicle or CBG hemp extract) and is also observed in DSS-treated animals that received CBG hemp extract. This architecture is lost in DSS-treated animals that received vehicle treatment. Additionally, in the vehicle-treated animals, there is a marked increase in the space between the two muscle layers, due to immune cell infiltration (arrow). (B) Quantitation of colon damage for vehicle and high CBG hemp extract-treated mice. Data are presented as mean ± S.D., $N = 10$ mice/group, $^{****}P < 0.001$. NS represents not significant based on 2-way ANOVA with Šídák's multiple comparison test.

vehicle-treated colitic mice (Fig. 3G). Five bacterial genera displayed differential shifts in abundance contrasting CBG hemp oil-treated versus vehicle animals (Fig. 3H; Supplemental Fig. 2). CBG treatment enriched *Aldercreutzia*spp. and Anaerovoracaceae Family XIII AD3011 group compared with controls while depleting the Lachnospiraceae FCS020 group, Clostridia vadin BB60 group, preventing an enrichment of unidentified Clostridia class members (Supplemental Fig. 2).

Although mice are a valuable model system, the taxonomic composition varies significantly from those found in humans.

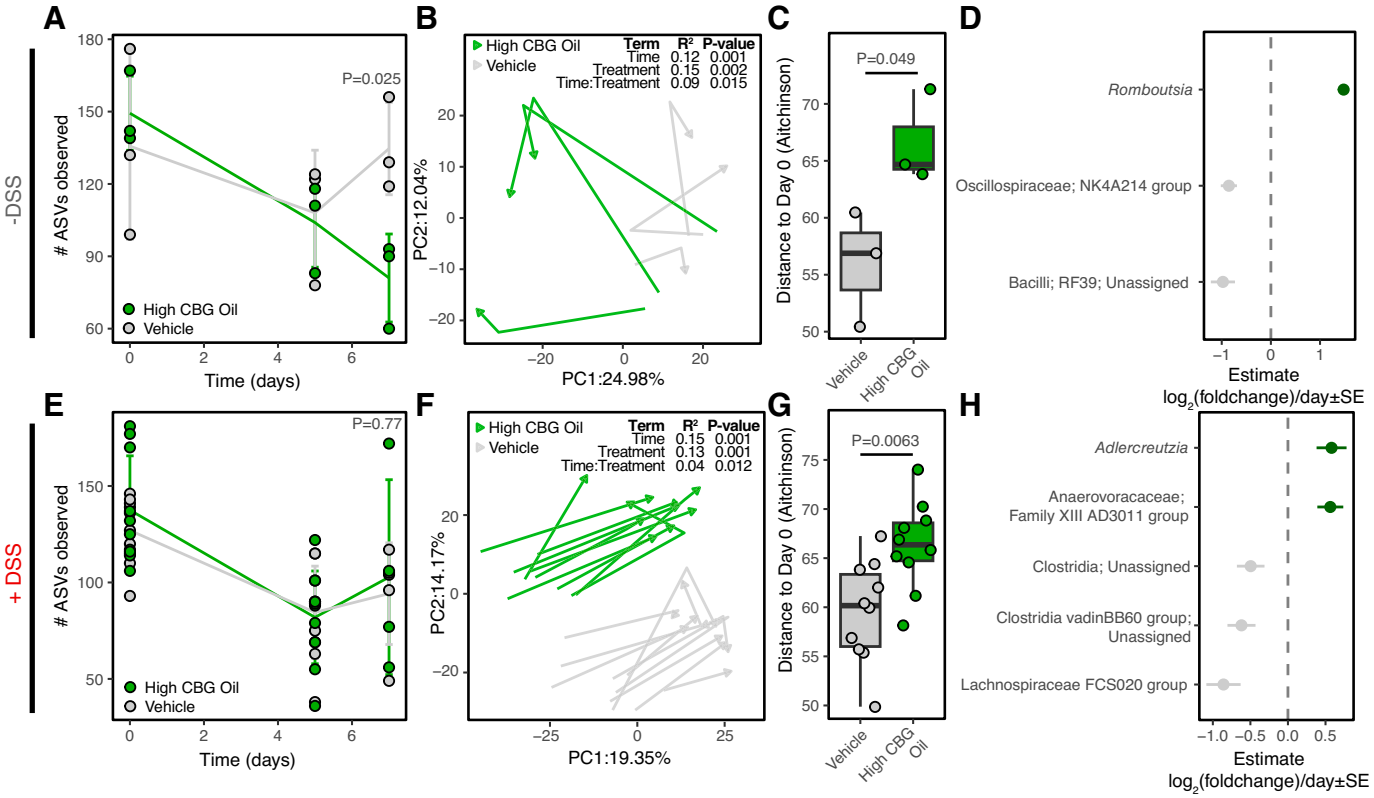

Fig. 3. High CBG hemp extract modifies the structure of the gut microbiota in both DSS-untreated (A–D) and DSS-treated mice (E–H). (A) Seven-days exposure to high CBG hemp extract results in a significant decrease in microbial α diversity measured by ASV richness compared with vehicle control in DSS-untreated mice ($N = 3$ mice/group, Welch's t test). (B) Community composition is reproducibly altered by high CBG hemp extract treatment in DSS-untreated mice ($N = 3$ mice/group, PERMANOVA). (C) Five days of high CBG hemp extract treatment results in communities that are more dissimilar to their starting state than vehicle controls in DSS36-untreated ($N = 3$ mice/group, Welch's t test). (D) Exploratory analysis of in DSS-untreated mice reveals modulation of major bacterial genera ($N = 3$ mice/group, interaction of time and treatment, linear mixed effects model FDR <0.1). (E) Seven days exposure to high CBG hemp extract does not significantly alter microbial α diversity measured by ASV richness compared with vehicle control in DSS-treated mice ($N = 10$ mice/group, Welch's t test). (F) Community composition is reproducibly altered by high CBG hemp extract treatment in DSS-treated mice ($N = 10$ mice/group, PERMANOVA). (G) Five days of high CBG hemp extract treatment results in communities that are more dissimilar to their starting state than vehicle controls in DSS-treated mice ($N = 10$, Welch's t test). (H) Longitudinal analysis in DSS-treated mice reveals modulation of multiple keystone genera within the gut microbiota ($N = 10$ mice/group, interaction of treatment and time, linear mixed effects model, FDR <0.1).

To understand the spectrum of interactions including potential antibacterial activity of cannabinoids on human-associated microbes, we leveraged a high-throughput screening assay to test 38 representative gut microbes with an equimolar mixture of CBD and CBG mimicking the composition of high CBG extract. The members of this collection were selected for testing because they have a diverse phylogenetic background and represent the major phyla and genera within the human gut microbiota. Inhibitory effects, indicated by changes in carrying capacity, growth rate, and time to midexponential growth, show that closely related taxa have similar responses to cannabinoid treatment (Fig. 4A). Most Bacteroidota (Bacteroidetes) and Actinomycetota (Actinobacteria) were inhibited by CBD/CBG treatment, whereas Pseudomondota (Proteobacteria) were not. Differential susceptibility within the subphyla of the former Firmicutes (now Bacillota) demonstrated clade-specific effects with the Bacilloat_A and Bacillota_C largely resistant. Taken together, these data suggest high-level conserved responses to growth inhibition by cannabinoids in gut microbes. These results matched our observations of mouse microbiotas where members of the Clostridia (Bacillota) were depleted during high CBG oil treatment (Supplemental Figs. 2 and 3). We further selected three representative strains, *Bacteroides eggerthii*, *Bifidobacterium longum*, and *Escherichia coli*. Monoculture growth experiments show dose-dependent growth inhibition for *B. eggerthii*and *B. longum*, but not *E. coli* (Fig. 4B). Disk diffusion assays showed an increased zone of inhibition for *B. eggerthii*and *B. longum* compared with *E. coli* (Fig. 4, C and D) further illustrating differential susceptibility. Taken together, these data demonstrate that cannabinoid

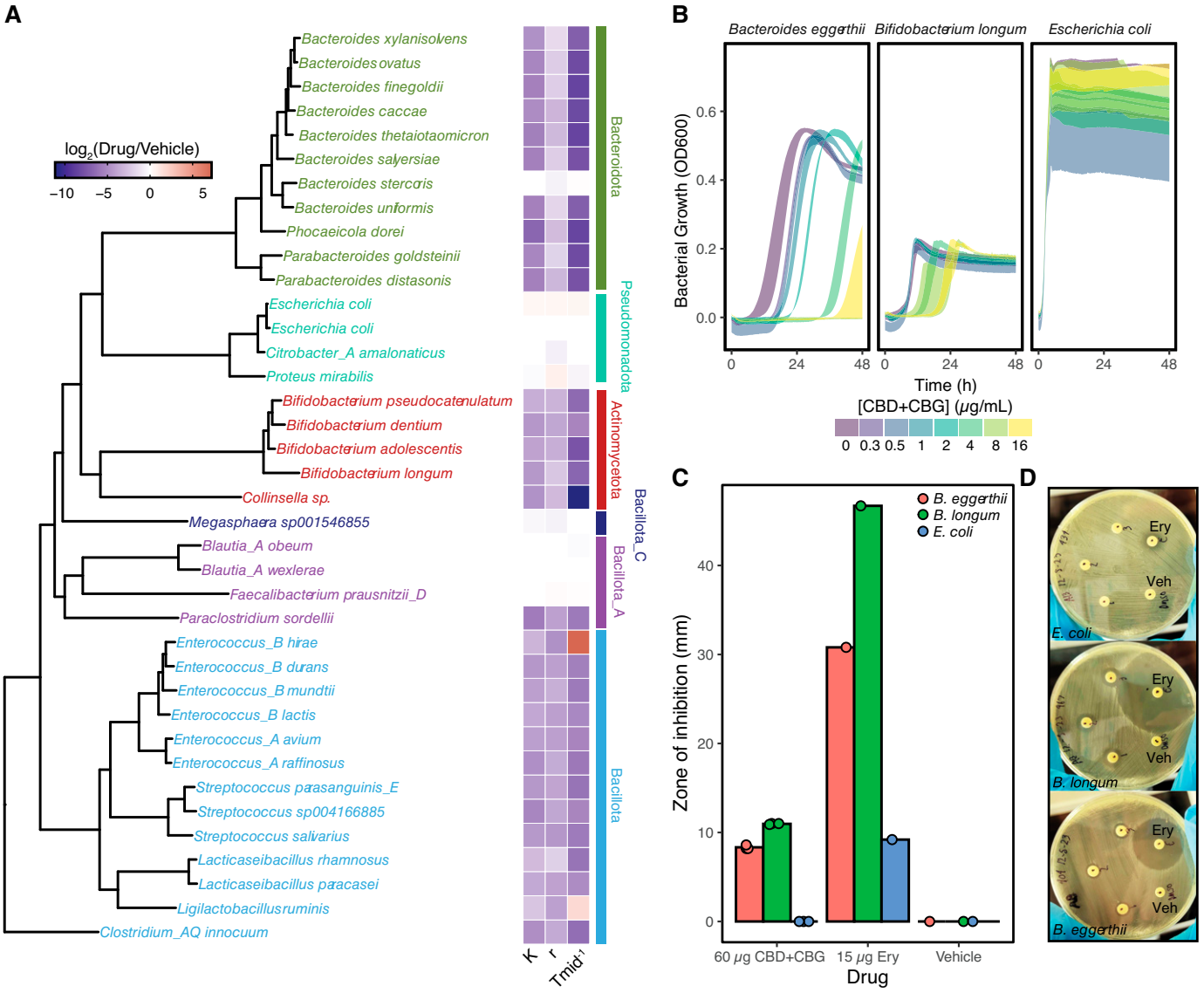

Fig. 4. CBD/CBG mixtures exhibit mild clade-specific antimicrobial activities. (A) Bacterial growth kinetics were determined in 32 μg/mL CBD + CBG and compared with vehicle demonstrating broad spectrum antimicrobial activity without significant impacts on the Pseudomonadota (Proteobacteria) and Bacillota A (Firmicutes) phyla. Strains are displayed on a whole-genome phylogenetic tree. K = carrying capacity, r = growth rate, and Tmid = time to mid exponential growth. $N = 4$ replicate wells per strain per condition. (B) Broth microdilution assays of representative strains reveal dose-dependent responses in growth inhibition consistent with antimicrobial activity for *Bacteroides* and *Bifidobacterium*, but not *E. coli*. $N = 3$ replicate wells per strain per drug concentration with ribbon width representing mean ± S.E. (C) Disk diffusion assays confirm clade-variable antimicrobial activity with erythromycin included as a positive control with known poor efficacy against *E. coli* ($N = 3$ disks per strain). (D) Representative images of disk diffusion plates.

treatment elicits relatively broad range antimicrobial activities against common gut microbes that may underlie modulation of colitis activity.

High CBG Hemp Oil Impacts Metabolic Pathways Associated with Colitis. Shifts in the metabolome of DSS-treated mice were observed via untargeted LC-MS analysis of stool samples. No significant difference between vehicle and CBG-treated fecal metabolomes was observed pretreatment at day 0. However, significant differences were observed at day 7, with high CBG extract and vehicle control groups displaying statistically significant clusters (FDR corrected $P < 0.05$). Notably, the day 7 CBG group was positioned closer to the day 0 group, suggesting a reduction in the impact of DSS-treatment on the metabolome (Fig. 5).

Annotation of the MS/MS fragmentation data yielded 652 putative identified compounds that were input as the independent variables in a sparse partial least squares-discriminant analysis supervised model. Among the metabolites detected in the stool samples, those that significantly contributed to clustering between the vehicle and high CBG hemp extract groups were identified according to the magnitude of their variable importance in projection >1.5, $P < 0.05$. Comparing the high CBG hemp extract-treated-day 7 DSS mice versus the vehicle control, 270 variables were significant in differentiating the two groups. To better understand the physiological role of these metabolites, they were cross-referenced against metabolic pathways for significance.

Metabolic pathway analysis was performed to identify significant metabolic pathways that were modulated by supplementation with high CBG hemp extract in DSS-treated mice. The annotated metabolites were assigned to 43 different pathways according to the Kyoto Encyclopedia of Genes and Genomes database. Key pathways were identified based on their pathway impact calculations and −log(p value) (Fig. 6; Supplemental Table 1). Treatment with DSS induced changes in multiple metabolic pathways from day 0 to day 7 compared with the control group (Fig. 6A), including tryptophan metabolism, steroid hormone biosynthesis, long-chain fatty acid metabolism, and glycine/serine/threonine metabolism. Supplementation with high-CBG hemp in DSS-treated mice yielded a significant overlap in affected pathways (Fig. 6B) as compared against the vehicle-supplemented mice; pathways affected included histidine, tryptophan, alanine/aspartate/glutamate, steroid, and long-chain fatty acid metabolism. Principal metabolites from these pathways were also included in the variable importance in projection list and highlight the effect of CBG treatment on the mice (Fig. 6C). For asparagine (amino acid metabolism pathway), histidine (histidine metabolism), indole-3-acetic acid (tryptophan metabolism), and linoleic acid (LCFA metabolism), DSS treatment resulted in a significant reduction in concentration by day 7 ($P < 0.05$); however, supplementation by high-CBG hemp mitigated the decrease in all four of these metabolites, suggesting an ameliorating effect against the progression of IBD.

Discussion

Here, we show that high CBG hemp extracts reduce the severity of colitis in the DSS-induced model of colitis in terms of DAI scores, colon length, and colonic damage. A previous report observed that pure CBG reduced symptoms of colitis in the DNBS model of colitis (Borrelli et al., 2013). Our findings not only confirm this finding using a different murine model, but also provide more translatable results using a commercially available, over-the-counter, high CBG hemp extract. Both CBD and CBC, also present in the extract used, have also been reported to reduce inflammation in the colon and colitis symptoms in murine models (Izzo et al., 2012; Pagano et al., 2016; Bacalia et al., 2022; Sun et al., 2024). It should be noted that despite these studies in mice, studies in human patients with IBD using cannabis fail to observe significant impacts on clinical symptoms despite reports of improved quality of life (Naftali et al., 2013, 2017; Storr et al., 2014; Irving et al., 2018). Importantly, all of the human studies focused on cannabis products with high levels of THC, as opposed to non-euphorigenic cannabinoids. The three cannabinoids present in the high CBG hemp extract in this study, CBG, CBD, and CBC, all have reported anti-inflammatory, antinociceptive, and antibacterial properties that could be beneficial in combatting IBD (Eisohly et al., 1982; Appendino et al., 2008; Romano et al., 2013; Ward et al., 2014; Sepulveda et al., 2022a; Hong et al., 2023; Nachnani et al., 2023; Raup-Konsavage et al., 2024).

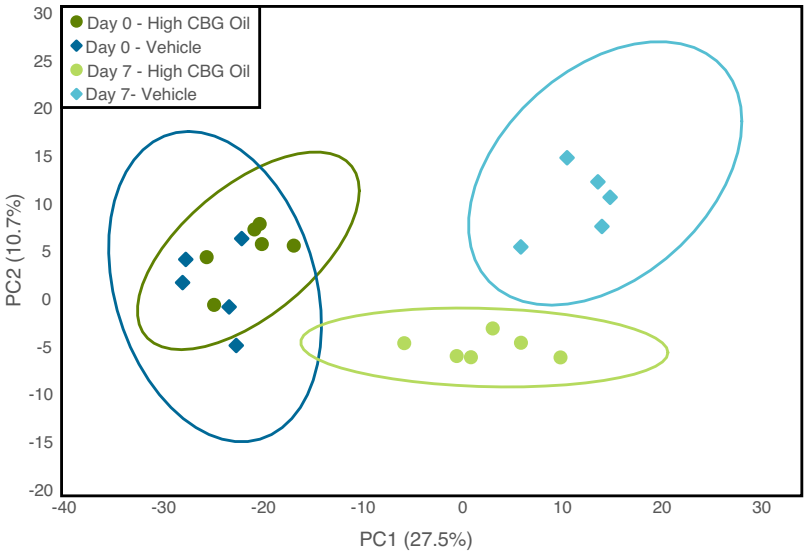

Fig. 5. High CBG hemp extract impacts metabolite pools following DSS-induced colitis. Principal component analysis scores plot of DSS-stimulated mice at day 0 and day 7, treated either with high CBG hemp extract (circle markers) or vehicle (diamonds). Ellipses represent Hotelling's 95% confidence ellipses (N = 5–6 mice/group, Hotelling's T^2 test, FDR corrected $P < 0.05$). Intervention groups are indistinguishable at baseline; however, following DSS-induced colitis the vehicle and treatment groups results in two significantly different compositions.

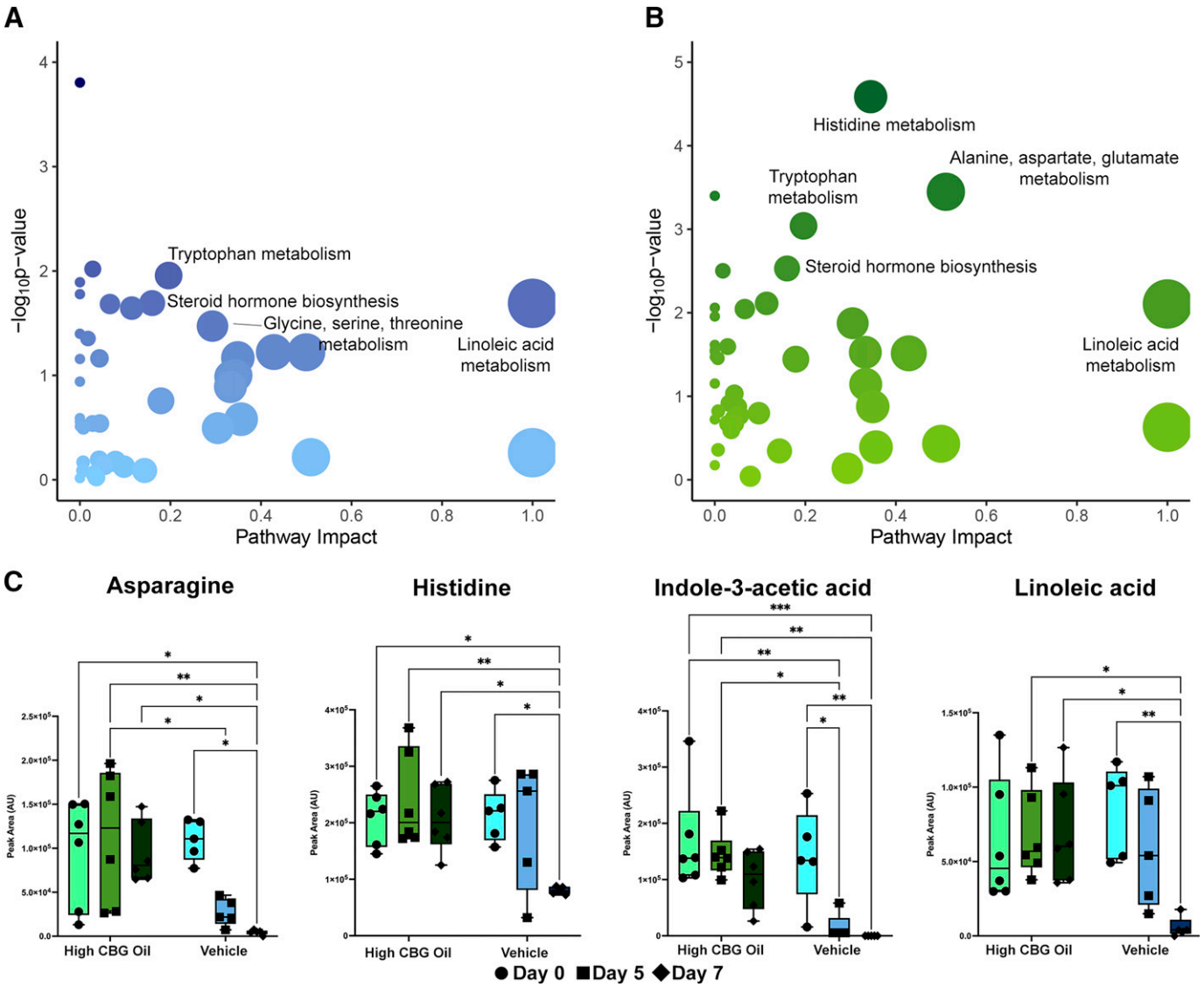

Fig. 6. High CBG hemp extract reduces metabolomic pathway changes associated with DSS-induced colitis. Pathway analysis, combining pathway enrichment and pathway topology analysis, of annotated metabolites in DSS-treated mice contrasting day 7 and day 0 for (A) vehicle and (B) high CBG hemp extract-treated. The x-axis marks the pathway impact, and the y-axis represents pathway enrichment. Each node marks a specific pathway, with larger sizes and darker colors representing higher impact values and enrichment, respectively. (C) Boxplot of representative metabolites from differentially expressed pathways at days 0 (●), 5 (■), and 7 (◆) with treatment by vehicle (blue) or high-CBG hemp oil (green). N = 5–6 mice/group, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$ FDR corrected Mann-Whitney test.

Although patients with IBD continue to report the use of high THC cannabis for self-medication and management of their disease, the data presented here suggest that noneuphorigenic cannabis extracts may offer a better treatment option, especially in light of the lack of an effect of high-THC products in clinical trials.

The hallmark of IBD is chronic unresolved inflammation that results from genetic susceptibility and environmental triggers including the gut microbiota (Adolph et al., 2022). Many of the genetic polymorphisms associated with IBD susceptibility are in loci related to inflammatory responses to microbiota, particularly those of the innate response. No single microbial trigger of IBD has been found; however, associations with altered microbiota composition in humans have been reported including increases in Enterobacteriaceae like *E. coli* (Caruso et al., 2020). Whether these alterations are a cause or an effect of the chronic inflammation remains unclear; however, they may further potentiate disease activity. This provides an opportunity for microbiota-targeted interventions for treatment of IBD. Among the most extreme of these interventions is fecal microbiota transplant, which has shown some efficacy in human studies (Imdad et al., 2023); however, more targeted and safe interventions are needed. In this work, we characterized the microbiota-modulating activity of high CBG hemp extracts in both animals and in vitro. Although the translational relevance of the microbes modulated in the mouse microbiota is limited due to the limited taxonomic similarities between human and mice microbes, direct demonstration of clade-specific antimicrobial activity against human isolates in vitro suggests these hemp extracts may be valuable tools for microbiota modulation.Previous research has explored the antibacterial effects of CBD in the context of human pathogens rather than commensals (Appendino et al., 2008; Farha et al., 2020; Blaskovich et al., 2021). Potent

antibacterial activity was noted against Gram-positive organisms, but Gram-negative bacteria had more varied responses. Some bacterial species were susceptible to CBD treatment, but others required administration of cell membrane permeabilizing agents to be effective (Farha et al., 2020). These results corroborate previous results that show CBD having activity against *Clostridium* species (Blaskovich et al., 2021). Future studies will leverage humanized gnotobiotic mice to examine modulation of human microbiotas in a surrogate mammalian host. Furthermore, they will establish causality of CBG extract treatment's effects on the microbiota in modulating disease activity through experiments involving fecal transplant of treated animals to treatment-naive animals and provide a model system for mechanistic study.

Metabolomic analyses of DSS-treated mice suggested differences in metabolic profiles of mice treated with high CBG hemp extract or vehicle control. DSS treatment impacted multiple biochemical pathways, including histidine, linoleic acid, tryptophan, and amino acid metabolism. These pathways were shown to be depressed in metabolomics analysis in patients with ulcerative colitis, as well as a study using urine metabolomics from irritable bowel syndrome patients (Diab et al., 2019; Yu et al., 2019).

Representative metabolites from these pathways decreased significantly over the time course in the colitis mice, but that decrease was attenuated in the high CBG hemp extract-treated mice. Asparagine was significantly depleted in colitis mice; previously this has been shown to improve intestinal integrity during inflammatory conditions by modulating TLR4 expression (Chen et al., 2016). Indole 3-acetic acid (IAA), a microbial tryptophan metabolite, was also depleted in vehicle control-treated mice. IAA has been shown to alter inflammatory response in hepatocytes by its interactions with the aryl hydrocarbon receptor (Krishnan et al., 2018). This suggests that increased IAA levels in high CBG hemp extract-treated mice may function to decrease the inflammatory tone and suppress inappropriate responses. Linoleic acid was also significantly lowered in the DSS-treated mice. A product of lipid metabolism, linoleic acid has been suggested as a key biomarker in colitis patients and as an indicator of progression to colorectal cancer (Tang et al., 2020). Previous work on non-hemp phytochemical supplementation demonstrated an impact on lipid metabolism and linoleic acid (Zhang et al., 2024). The conditionally essential amino acid histidine has been shown to alleviate inflammation when supplemented to colitis mice (Andou et al., 2009; Liu et al., 2017). Decreased levels of histidine indicated potentially higher inflammation and oxidative processes, and high-CBG supplementation restored the levels of histidine. This was shown in a previous study treating colitis mice with the alkaloid berberine (from *Hydrastis canadensis*) (Liao et al., 2019). Taken together, this suggests that supplementation with high-CBG hemp mitigates the effects of DSS treatment on essential biochemical pathways in the colon and could result in a decrease in the disease severity and downstream impacts on the physiology of the organism.

However, mice were given access to food ad libitum, but the consumption of food by the mice was not assessed. Future mouse experiments using metabolic cages and monitoring blood glucose levels would provide a powerful insight into how food consumption contributes to the observed metabolic differences. There is not a single model system for studying IBD that fully recapitulates the disease in humans, although the DSS-model is one of the most widely used, it has limitations. Animals used in the experimental design are typically between 4–10 weeks old with 6–8 weeks being the most common (Wirtz and Neurath, 2007; Konsavage et al., 2012, 2013; Low et al., 2013; Raup-Konsavage et al., 2016; Baydi et al., 2021). As such, these animals are approximately in early adulthood that corresponds to average age of diagnosis of IBD; however, IBD elicits a multimodal distribution with a second maxima of diagnosis later in life (Dutta and Sengupta, 2016; Ministro et al., 2021). However, changing microbiota composition with age may further complicate the interpretation of this model to later onset IBD (Ghosh et al., 2022). Future experiments could explore how age contributes to the effects of high CBG hemp extract has on DSS treatment. Additionally, male animals tend to be used more frequently because females are less susceptible to this model that may limit the translational potential of this model (Chassaing et al., 2014; Eichele and Kharbanda, 2017; Baydi et al., 2021).

In conclusion, these data demonstrate that high CBG hemp oil treatment decreases colitis in DSS-treated mice, altering the microbial communities and metabolic pools. These observations create both translational and mechanistic opportunities to further understand the interplay between natural products, microbes, and the host. Future studies will be required to understand the directionality of effects in this model system and the utility of high CBG hemp extract treatment in patients with IBD; however, its over-the-counter availability addresses urgent unmet needs for patients who suffer with the associated abdominal pain and quality of life issues associated with IBD.

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Data Availability

Histological images will be available at ScholarSphere. Sequencing data were deposited to the NCBI Sequence Read Archive under PRJNA1111247. Raw LC-MS spectral data were deposited in the MASSive database (ID: MSV000094050; https://doi.org/10.25345/C5XW4869Q).

Authorship Contributions

Participated in research design: Bisanz, Kellogg, Raup-Konsavage.

Conducted experiments: Anderson, Sepulveda, Nachnani, Cortez-Resendiz, Beckett, Bisanz, Kellogg, Raup-Konsavage.

Performed data analysis: Anderson, Beckett, Bisanz, Kellogg, Raup-Konsavage.

Wrote or contributed to the writing of the manuscript: Anderson, Coates, Beckett, Bisanz, Kellogg, Raup-Konsavage.

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