

Research

The Antimicrobial Activity of Plant-Derived Cannabinoid Suspensions from *Cannabis sativa* L. Against the Mixed Oral Microflora of Humans and Canine

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Abstract:

Cannabis sativa L. is a dioecious annual plant that is widely cultivated for its industrial, nutritional and medicinal uses. Historically used as a fibre plant and for therapeutic purposes, its modern importance lies in the production of biologically active compounds, particularly cannabinoids. Cannabis-derived compounds, particularly cannabidiol (CBD), have shown antimicrobial activity against both Gram-positive and Gram-negative bacteria. Preliminary studies suggest that cannabinoids may be more effective in reducing dental plaque than conventional oral care products. This study aims to evaluate the *in vitro* inhibitory effects of "pure" plant CBD and a full-spectrum cannabis isolate on polymicrobial cultures derived from the oral surfaces of healthy humans and canine to investigate their potential to prevent periodontal disease in both human and veterinary medicine. The cannabis isolate showed a slightly stronger activity, which is probably due to the synergistic effect of several cannabinoids, terpenes and other bioactive compounds. Factors such as potentially better solubility, the presence of minor cannabinoids (e. g. cannabigerol, CBG) and the membrane disruption caused by terpenes could contribute to this stronger effect. These results suggest that full-spectrum cannabis extracts may offer greater potential for oral antimicrobial applications than "pure" CBD alone.

Keywords: Plant, *Cannabis sativa* L., Cannabinoids, Human medicine, Veterinary medicine, Mixed oral microflora, Antimicrobial activity

1. Introduction

1.1. *Cannabis sativa* L.

Cannabis (*Cannabis sativa* L.) is a dioecious, annual flowering plant belonging to the family Cannabaceae, widely distributed due to its adaptability to environmental conditions. Historically valued for its industrial and medicinal properties, cannabis has been cultivated by humans since antiquity, serving as a source of fiber, oil, and therapeutic agents. In recent decades, scientific interest in this plant has significantly intensified due to its wide spectrum of biologically active compounds and therapeutic potential (Pečan et al., 2021).

The pharmacological properties of cannabis are primarily attributed to a unique group of secondary metabolites known as cannabinoids, of which over 140 have been identified to date (Figure 1). These compounds are synthesized and stored in trichomes, glandular structures especially abundant on the female inflorescences of the plant. Cannabinoids interact with the endocannabinoid system in the human body, modulating various physiological processes (Pečan et al., 2023; Appendino et al., 2008). Among them, Δ^9 -tetrahydrocannabinol (THC) and cannabidiol (CBD) are the most extensively studied. THC is known for its psychoactive effects and therapeutic use in conditions such as chronic pain and nausea, acting as a partial agonist at CB1 and CB2 receptors (Pečan et al., 2021). In contrast, CBD exhibits no psychoactivity but offers anti-inflammatory, analgesic, antineoplastic, and neuroprotective effects, partly through modulation of oxidative stress and autophagic pathways (Pečan et al., 2023).

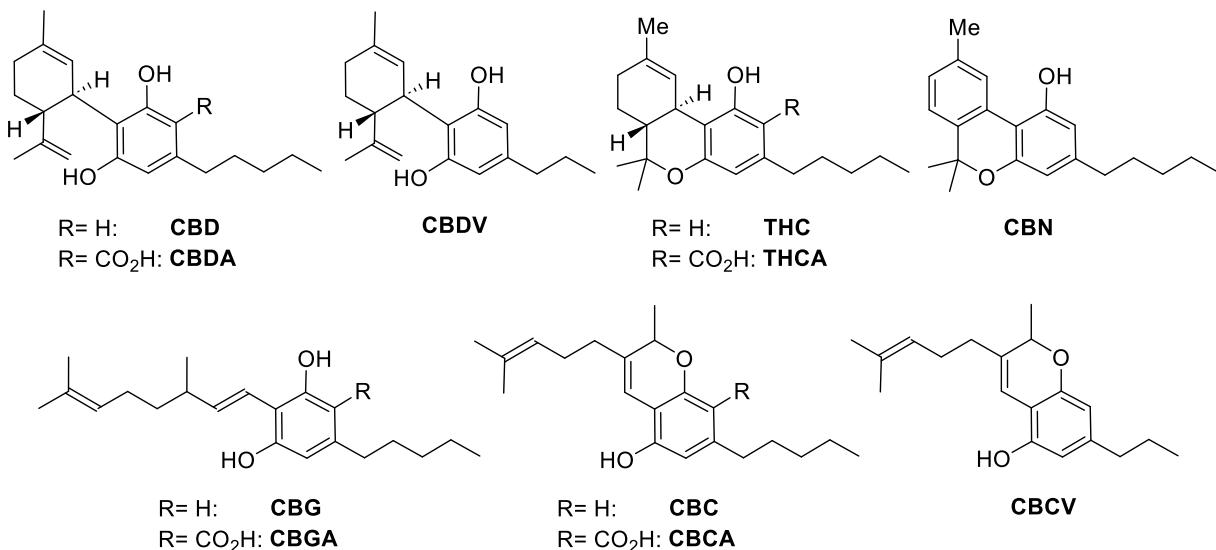


Figure 1. Structural formulas of some terpenes that may occur in cannabis isolate.

Beyond these two key molecules, cannabis also contains terpenes, flavonoids, and other phytochemicals with synergistic or individual bioactivity. These compounds contribute to the plant's potential in treating a range of conditions including antibiotic-resistant infections, dermatological disorders, and chronic inflammation. As a result, cannabis is being increasingly recognized not only in human medicine but also in veterinary science, particularly for dermatological applications. However, its therapeutic use remains subject to complex legal regulations that vary widely across jurisdictions (Mišič Jančar et al., 2024).

1.2. *Oral microbiome, cannabinoids and antimicrobial activity*

Oral microbiome is a complex microbial community composed by a variety of bacteria, archaea, viruses and fungi (Rajasekaran et al., 2024). Human and canine oral microbiome differs significantly, with 16.4% of taxa shared (Dewhirst et al., 2012). In healthy dogs, oral microbiota is composed by hundreds of bacterial species. *Porphyromonas* and *Corynebacterium* are the most prevalent genera (Šakarnytė et al., 2023). *Porphyromonas* species are increased in dogs suffering from periodontal diseases along with

Bacteroides and *Fusobacterium* (Santibáñez et al., 2021). Similar results were found in humans affected with periodontal disease, where *Fusobacterium*, *Porphyromonas*, *Leptotrichia* and *Prevotella* among others, were the most abundant genera (López-Martínez et al., 2020).

Inflammatory periodontal disease (gingivitis and periodontitis) is the most common disease involving the oral cavity of human adults and companion animals, and it is frequently induced by bacterial plaque and its derivative byproducts (Bellows et al., 2019; Martínez-García et al., 2021). As in humans, tooth brushing is the “gold standard” procedure to maintain a good oral health in companion animals. However, due to the difficulty to be performed and the low owner compliance, alternative chemical anti-plaque retardants are needed (Gawor et al., 2025).

Plants are a wide source of natural bioactive compounds with antimicrobial activity (Chassagne et al., 2021). The resin obtained from the inflorescence of the plant *Cannabis sativa* L. contains more than 500 molecules (ElSohly et al., 2017). Cannabinoids and terpenes are the most abundant chemical groups in cannabis extracts, and both have proven to exert antimicrobial activity against pathogenic bacteria (Schofs et al., 2021). In particular, cannabidiol, a 314 Da phytocannabinoid, is the most studied cannabinoid in view of its safety profile and wide range of pharmacological effects (Izzo et al., 2009). CBD has shown an antibacterial activity against Gram-positive and Gram-negative bacteria (Blaskovich et al., 2021). Additionally, cannabinoids were found to be more effective than conventional commercial oral care products in reducing the bacterial burden of dental plaques from human sources *in vitro* (Stahl & Vasudevan, 2020). Hence, cannabis extracts and purified cannabinoids could be useful to reduce bacterial plaque formation and therefore to prevent periodontal disease in human and veterinary medicine.

The main goal of the present work was to preliminary assess the *in vitro* inhibitory effect of pure CBD and a complete cannabis isolate on a polymicrobial culture obtained from dental surfaces of a healthy human and dog, respectively.

2. Materials and Methods

2.1. Experimental suspensions of plant material

Two polymicrobial culture samples, collected from the dental surfaces of a healthy human and dog were studied. The CBD sample was a white powder with 99.3% purity. In contrast, the full-spectrum isolate—free of psychoactive components such as THC—was partially crystalline and amber-colored. Both samples were donated by Herbana, Ltd.

Fourier transform infrared (FTIR) spectroscopy is used to determine the molecular structure and composition of organic compounds by analyzing their absorption of infrared light. It helps to identify functional groups such as hydroxyl (−OH), cyano (−CN), carbonyl (−C=O) and amino (−NH₂) based on their characteristic absorption bands (Larkin, 2011). This technique can be used to confirm the identity of compounds, detect impurities, analyze the composition of polymers, etc. In our case, it was used to confirm the identity of compounds. In this study FTIR spectroscopy (Bruker, Tensor II) was used to determine the cannabinoid content in the full spectrum isolate sample (Figure 2).

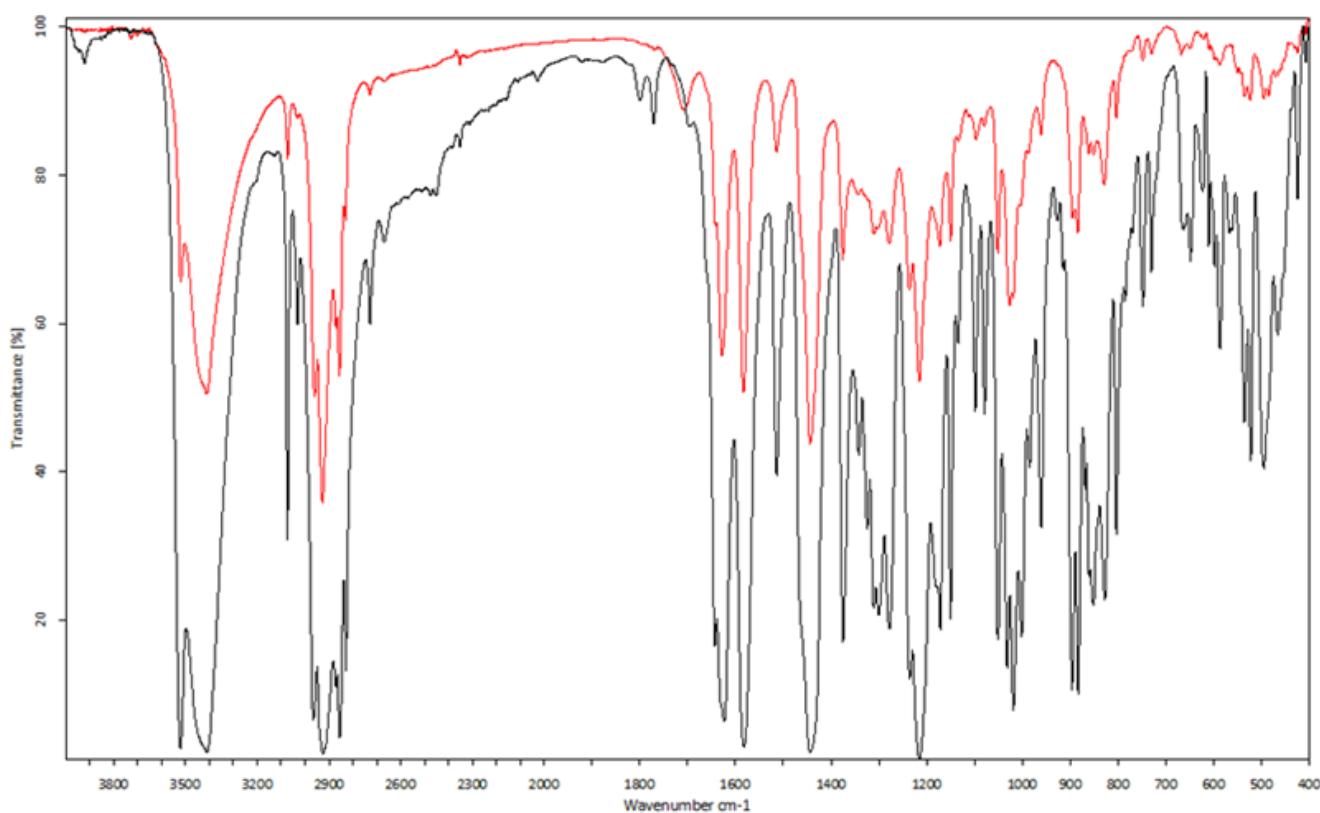


Figure 2. FTIR spectrum of the chemically "pure" CBD compound (black) and of isolate of cannabinoids (red).

FTIR spectra of a chemically pure cannabidiol (CBD) sample (black) and a full-spectrum cannabinoid isolate lacking psychoactive components such as THC (red) are presented in **Figure 2**. The spectral profiles of both samples exhibit strong similarities, particularly in key absorption regions corresponding to characteristic functional groups. A broad absorption band around 3400 cm^{-1} in both spectra indicates the presence of hydroxyl ($-\text{OH}$) groups, consistent with the phenolic structure of CBD and related cannabinoids. The C–H stretching vibrations observed between 2950 and 2850 cm^{-1} suggest aliphatic hydrocarbon chains, which are typical features of cannabinoids. Furthermore, the presence of peaks near 1600 – 1500 cm^{-1} is attributed to C=C stretching in aromatic rings, a core component of the cannabinoid structure. The fingerprint region (1500 – 500 cm^{-1}), which contains complex vibrational patterns unique to molecular structure, shows highly overlapping signals between the two samples. This spectral similarity implies that the full-spectrum isolate contains CBD and likely other non-psychoactive cannabinoids with comparable structural features. The lack of distinct additional peaks suggests the absence of unrelated impurities, supporting the hypothesis that the isolate is composed primarily of structurally similar phytocannabinoids. Overall, the FTIR data confirm the chemical relatedness of the two samples and support the use of the full-spectrum isolate in comparative bioactivity studies.

3.2. Antibacterial activity

The antimicrobial activity of the plant-derived cannabinoid samples was evaluated using the agar diffusion method. Suspensions of the active compounds were prepared in a 0.1% aqueous solution of the co-solvent dimethyl sulfoxide (DMSO) and applied onto a solid growth medium inoculated with the model microbial culture.

The antimicrobial activity of the cannabinoid samples on the oral microflora of human and canine was therefore tested on nutrient agar (solid medium) in Petri dishes. 22.5 g Plate Count Agar (PCA; Vigitone, Millipore) was suspended in 1 L of deionized water. The contents were heated with intensive stirring until a homogeneous suspension was formed, then sterilized at $121\text{ }^{\circ}\text{C}$ for 30 minutes and poured onto Petri dishes in an aseptic environment.

Oral swabs were collected from the tooth surfaces of a healthy 34-year-old male (co-author of this study) and a healthy 1-year-old Weimaraner dog. The collected biocultures were transferred onto agar plates using sterile cotton swabs moistened with physiological saline solution.

Then, under an aseptic atmosphere, round filter discs (*diameter* = 0.5 mm) made of quantitative filter paper (MN 640W, Macherey-Nagel, Germany) were placed on each culture medium. A 10 µL of the respective samples were applied directly onto the surface of each disc as follows:

- different concentrations (5, 50 and 100 µg/mL) of suspensions of (i) cannabinoid CBD and (ii) full spectrum isolate prepared in 0.1 % aqueous DMSO solution;
- negative control: (i) saline solution (0.9% NaCl, B. Braun, Germany), (ii) deionised water, (iii) 0.1% aqueous DMSO solution (Merck, Germany); and
- positive control: 10% aqueous hydrogen peroxide solution (H₂O₂) (Merck, Germany), which has a proven antimicrobial effect.

The culture samples prepared as described were incubated in a Heratherm IGS60 GP incubator at 37 °C and evaluated after 24 and 48 hours. Antimicrobial activity was assessed by measuring the diameter of the inhibition zones, defined as the clear areas surrounding the filter discs where microbial growth was absent. Each concentration of the cannabinoid samples was tested in triplicate against the model oral microflora to ensure reproducibility and statistical reliability of the results.

3. Results

In our study, the DMSO was used as a co-solvent at a final concentration of 0.1%, a level widely recognized in the literature as having negligible effects on biological systems. Higher concentrations have been shown to adversely affect cell viability and metabolic activity (Tunçer et al., 2018). Our results showed normal microbial growth in the negative control in the absence of active compounds (**Table 1**). This proves that that DMSO at this concentration did not interfere with microbial viability.

As expected, the microorganisms on the oral swab outgrew the diffusion discs with three different negative controls (saline, deionised water, 0.1% DMSO solution) after 24 hours of observation, as these samples provide the microorganisms with an environment in which they can grow and multiply undisturbed (**Table 1**). The highest zone of inhibition of microbial growth on diffusion discs was achieved with a positive control (10% H₂O₂). Interestingly, 10% H₂O₂ inhibits the growth of microorganisms in dogs better than in humans (inhibition zone after 24 hours 8.5 cm *vs.* 3.6 cm).

Table 1. The results of the inhibition zones for individual samples, taking into account the measurement error.

No.	Sample	Inhibition zone diameter [cm]			
		Human		Canine	
		24 h	48 h	24 h	48 h
1	10% H ₂ O ₂	3.6 ± 0.1	3.0 ± 0.1	8.5 ± 0.0	8.5 ± 0.0
2	5 µg/mL CBD	0.6 ± 0.1	0.4 ± 0.1	0.6 ± 0.1	0.5 ± 0.1
3	50 µg/mL CBD	0.8 ± 0.2	0.8 ± 0.1	0.7 ± 0.1	0.6 ± 0.0
4	100 µg/mL CBD	0.8 ± 0.1	0.7 ± 0.1	1.5 ± 0.1	1.0 ± 0.2
5	5 µg/mL isolate	0.7 ± 0.1	0.6 ± 0.1	0.9 ± 0.2	0
6	50 µg/mL isolate	0.8 ± 0.1	0.6 ± 0.1	0.9 ± 0.1	0
7	100 µg/mL isolate	1.0 ± 0.1	0.8 ± 0.1	1.5 ± 0.1	0.8 ± 0.1
8	Saline	0	0	0	0
9	Deionized water	0	0	0	0
10	0.1% DMSO	0	0	0	0

In both samples, the chemically "pure" CBD and the isolate, the inhibition zone of microbial growth were larger at higher concentrations. Both samples showed the greatest effect on the model microorganisms at the highest concentration tested (100 µg/mL) and the least at the lowest concentration (5 µg/mL).

The CBD concentration of 5 µg/mL had a moderate effect on human microorganisms after 24 hours (inhibition zone 0.6 cm), and after 48 hours the microorganisms grew slightly beyond the inhibition zone (0.4 cm). A similar effect was also observed on the growth of canine microorganisms after 24 hours (inhibition zone 0.6 cm) and after 48 hours (inhibition zone 0.5 cm).

Compared to pure CBD, the isolate with a concentration of 5 µg/mL had a slightly stronger effect on the growth of microorganisms in both humans (inhibition zone after 24 hours 0.7 cm, after 48 hours 0.6 cm) and canine (inhibition zone after 24 hours 0.9 cm), but in canine the effect was no longer present after 48 hours (inhibition zone 0 cm); the microorganisms had outgrown the inhibition zone.

At a CBD concentration of 50 µg/mL, the effect was better than at a concentration of 5 µg/mL and was the same after 24 hours as after 48 hours; in humans, the inhibition zone at both time points – 24 and 48 hours – was 0.8 cm and in canine 0.7 cm after 24 hours and 0.6 cm after 48 hours.

Taking into account the measurement error, the isolate with a concentration of 50 µg/mL had a very similar effect on human microorganisms as CBD (inhibition zone 0.8 cm), but after 48 hours the microorganisms had grown slightly more in humans (inhibition zone 0.6 cm) and beyond the inhibition zone in canine.

A CBD concentration of 100 µg/mL had the same effect on human microorganisms after 24 hours as a concentration of 50 µg/mL (inhibition zone 0.8 cm), but after 48 hours the microorganisms grew slightly beyond the inhibition zone (0.7 cm). In canine, the activity after 24 hours was twice as high as at a concentration of 50 µg/mL (inhibition zone 1.5 cm compared to 0.7 cm), but after 48 hours the difference decreased (1.0 cm compared to 0.6 cm).

The isolate with a concentration of 100 µg/mL was slightly more active in humans after 24 hours (inhibition zone 1.0 cm); after 48 hours, the zone of inhibition decreased by about 20% (inhibition zone 0.8 cm). In canine, the isolate was as active as CBD (inhibition zone 1.5 cm), and after 48 hours the inhibition zone was reduced by almost half (to 0.8 cm).

From these results we can conclude that the cannabis isolate is slightly more antimicrobially active against microorganisms from the oral cavity of humans and canine than pure CBD.

4. Discussion and Conclusion

The results of this study indicate that the full-spectrum cannabis isolate demonstrated slightly greater antimicrobial activity against oral microflora of both human and canine origin compared to pure cannabidiol (CBD). This enhanced effect is likely attributable to the synergistic interaction between cannabinoids, terpenes, and other bioactive constituents present in the isolate, supporting the hypothesis that complex phytochemical mixtures may exert broader or more potent antimicrobial effects than isolated compounds. These findings suggest that full-spectrum hemp extracts could offer increased utility in oral antimicrobial applications relative to purified CBD alone. Future studies should investigate the antimicrobial efficacy of concentrations exceeding 100 µg/mL to determine dose-dependent effects. Additionally, expanding the sample size to include oral swabs from a larger and more diverse cohort of individuals would be essential to validate and generalize these preliminary findings.

There are several reasons why hemp isolate, which contains more than just pure CBD, may be more effective in fighting bacteria. Cannabis extracts contain a mixture of cannabinoids such as CBD, CBG and CBC, along with terpenes and other natural plant compounds (Farha et al., 2020). These components can work together to enhance the antibacterial effect, a phenomenon known as a synergistic effect. Some terpenes, such as pinene, limonene and caryophyllene, have natural antibacterial properties and can interact with cannabinoids to enhance their overall effect. However, the presence of these terpenes in an isolate depends on how it is processed, as some volatile compounds may be lost during isolation (Sionov

et al., 2022; Palmieri et al., 2021; Khan et al., 2014). In addition, certain minor cannabinoids such as cannabigerol (CBG) have shown a strong antibacterial effect, even against antibiotic-resistant bacteria such as MRSA, which is not the case with "pure" CBD (Farha et al., 2020). Some studies also suggest that full-spectrum cannabis extracts are more effective at breaking down bacterial cell walls (membranes) or disrupting quorum sensing; the way bacteria communicate – making them less resistant and enhancing the antibacterial effect (Sionov et al., 2022; Coelho et al., 2025; Aqawi et al., 2020). Since microorganisms play a central role in oral health and disease in both humans and animals, finding ways to control harmful bacteria while supporting beneficial ones is important for improving dental health in all species.

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Conflicts of Interest: The authors declare no conflict of interest.

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