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# Antimicrobial Effects of Cannabidiol (CBD) on Oral Pathogens: A Comparative Analysis

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**Keywords:** Cannabidiol (CBD); Oral pathogens; Antimicrobial activity; Staphylococcus aureus; Streptococcus mutans; Streptococcus pyogenes; Chlorhexidine; Disk diffusion; MIC; MBC.

## Abstract

**Background:** Cannabis has a long history of medical applications, with emerging research highlighting its antimicrobial potential. In dentistry, the search for alternative antibacterial agents is crucial due to increasing antibiotic resistance. Cannabidiol (CBD), a non-psychoactive cannabinoid, has demonstrated antimicrobial effects against various bacterial strains. This study investigates the inhibitory effect of CBD on *Staphylococcus aureus*, *Streptococcus mutans*, and *Streptococcus pyogenes* compared to chlorhexidine gluconate (CHX), a widely used antimicrobial agent in oral health.

**Methods:** CBD was prepared at concentrations of 5, 10, 15, and 20 µg/mL. The disk diffusion method was used to evaluate inhibition zones against *S. aureus AT25923, S. mutans UA159*, and *S. pyogenes* (clinical stain). Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined using the standard broth microdilution method.

**Results:** Inhibition zones at 20 µg/mL CBD were 12.60 ± 0.20 mm for *S. aureus*, 15.00 ± 0.30 mm for *S. mutans*, and 16.60 ± 0.40 mm for *S. pyogenes*, respectively. Statistical analysis revealed significant differences in inhibition zones for *S. aureus* and *S. mutans* compared to CHX ($p < 0.05$), while *S. pyogenes* at 20 µg/mL showed no significant difference from CHX ($p = 0.96$). MIC values for *S. aureus, S. mutans*, and *S. pyogenes* were 5, 2.5, and 2.5 µg/mL, respectively, while MBC was 5 µg/mL across all strains.

**Conclusion:** CBD exhibits significant antimicrobial effects against *S. aureus*, *S. mutans*, and *S. pyogenes*, with potential applications as an alternative antimicrobial agent in oral health. Further studies are required to explore its mechanism of action and possible synergy with existing antimicrobial agents.

## Introduction

Cannabis (*Cannabis sativa*), commonly known as marijuana, has been historically utilized for medicinal and industrial purposes. It has demonstrated both cognitive and physiological effects [1]. Ancient records indicate its applications in anesthesia and infection treatments, such as in India before the 10th century B.C. and in Egypt for treating eye infections in the 20th century B.C [2,3]. With its historical and modern medicinal significance, cannabis continues to be explored for therapeutic applications.

Cannabis is classified into three main species: Cannabis sativa, Cannabis indica, and Cannabis ruderalis. *C. sativa* has been

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primarily used for medicinal and recreational purposes, derived from marijuana (dried flowers and leaves), hashish (resin with high cannabinoid concentration), and hash oil (thick liquid with various terpenes and resins) [4]. The sustainability and bioactive properties of cannabis make it an attractive candidate for pharmaceutical development.

Cannabinoids, the active compounds in cannabis, originate from three primary sources: endogenous (endocannabinoids), synthetic, and phytocannabinoids (plant-derived). Among phytocannabinoids, Cannabidiol (CBD) and Delta-9-Tetrahydrocannabinol (THC) are the most studied for medicinal use. THC is the principal psychoactive component, while CBD is non-psychoactive and has been explored for its therapeutic benefits, including antimicrobial properties [5].

CBD has demonstrated anti-inflammatory, antimicrobial, antioxidant, anxiolytic, antidepressant, antipsychotic, and anticonvulsant properties. Studies have reported that CBD effectively inhibits bacterial proliferation in various clinical settings [6-8]. Given its potential as a safer alternative to conventional antimicrobials, this study aims to evaluate the inhibition zones of *Staphylococcus aureus, Streptococcus mutans, and Streptococcus pyogenes* treated with CBD preparations.

## Materials and methods

### Bacterial strains and culture conditions

*Staphylococcus aureus ATCC 25923* and *Streptococcus mutans UA159* were obtained from Chulalongkorn University.

*Streptococcus pyogenes* (clinical strain) was collected from Prof. Jintakorn Kuvatanasuchati.

Bacteria were cultured on Tryptic Soy Agar (TSA) and incubated at 37°C for 24 hours.

### Preparation of cannabidiol (CBD) solutions

CBD (1 mg/mL stock solution in methanol) was obtained from Supelco Cerilliant, Merck.

The stock solution was diluted with 0.9% NaCl to achieve final concentrations of 5, 10, 15, and 20 µg/mL.

### Antibacterial assay

### Disk diffusion method

- Bacterial suspensions were standardized to 0.5 McFarland ($10^5$ CFU/mL).
- Sterile filter paper disks were impregnated with CBD (5, 10, 15, 20 µg/mL) and placed on bacterial lawns.
- CHX (0.12%) was used as the control.
- Plates were incubated at 37°C for 24 hours, and inhibition zones were measured.

### MIC & MBC determination

- MIC was determined using broth microdilution (CLSI M07-A8 guidelines).
- MBC was identified as the lowest CBD concentration where 99.9% bacterial reduction occurred.

## Results

### Disk diffusion test

| CBD Concentration | *S. aureus* (mm) | *S. mutans* (mm) | *S. pyogenes* (mm) |
|---|---|---|---|
| 5 µg/mL | 8.6 ± 0.4 | 10.6 ± 0.5 | 9.3 ± 0.4 |
| 10 µg/mL | 10.6 ± 0.6 | 12.0 ± 0.3 | 10.6 ± 0.5 |
| 15 µg/mL | 12.0 ± 0.3 | 13.5 ± 0.4 | 14.0 ± 0.5 |
| 20 µg/mL | 12.6 ± 0.2 | 15.0 ± 0.3 | 16.6 ± 0.4 |
| CHX 0.12% | 18.6 ± 0.3 | 25.2 ± 0.5 | 17.3 ± 0.3 |

### MIC & MBC values

| Bacteria | Minimum Inhibitory Concentration (MIC) (µg/mL) | Minimum Bactericidal Concentration (MBC) (µg/mL) |
|---|---|---|
| *S. aureus* | 5 | 5 |
| *S. mutans* | 2.5 | 5 |
| *S. pyogenes* | 2.5 | 5 |

## Discussion

This study investigated the antimicrobial efficacy of Cannabidiol (CBD) against *Staphylococcus aureus, Streptococcus mutans, and Streptococcus pyogenes* using both the broth dilution method and the disk diffusion method. The findings suggest that CBD possesses significant antibacterial properties, with Minimum Inhibitory Concentration (MIC) and inhibition zone measurements varying based on bacterial species and concentration used.

### Comparison of MIC and antimicrobial studies

The MIC of CBD against *S. aureus* was determined to be 5 µg/mL, which aligns with findings by Van Klingeren & Ten Ham[9], who reported MIC values ranging between 1-5 µg/mL. Similarly, Blaskovich et al [10]. observed MIC values between 1-4 µg/mL, while Martinenghi et al [8]. reported an MIC of 1 µg/mL for *S. aureus ATCC 25923*, slightly lower than the present study. However, Abichabki et al [11]. noted higher MIC values of 64 µg/mL, which decreased to 4 µg/mL when using a different growth medium. These variations suggest that the choice of culture media significantly influences MIC outcomes.

### Disk diffusion method and inhibition zones

The inhibition zones of *S. aureus* in this study for CBD concentrations of 5, 10, 15, and 20 µg/mL were 8.6, 10.6, 12.0, and 12.6 mm, respectively. Kosgodage et al [12]. found no inhibition zone at 5 µg/mL, suggesting that CBD concentrations may need to exceed 5 µg/mL to demonstrate notable antibacterial effects. Additionally, Blaskovich et al [10]. found larger inhibition zones when testing higher CBD doses (35–100 µg/mL) against MRSA, indicating a dose-dependent antimicrobial effect.

### CBD's efficacy against *S. mutans* and *S. pyogenes*

The MIC of CBD for *S. mutans* and *S. pyogenes* was 2.5 µg/mL, consistent with findings from Barak et al. [6]. In contrast, Abichabki et al. [11] reported a higher MIC of 32 µg/mL for *S. pyogenes*, again demonstrating variations due to different culture media. Disk diffusion results revealed inhibition zones of 9.3-16.6 mm for *S. pyogenes* and 10.6-13.15 mm for *S. mutans*, reinforcing CBD's antimicrobial activity against these bacteria.

### Comparison with conventional antimicrobial agents

Although CBD exhibited significant antibacterial activity, Chlorhexidine (CHX) consistently demonstrated larger inhibition zones, indicating greater efficacy at the tested concentrations. However, CBD presents an advantage in its natural origin and lack of staining effects, a common limitation of CHX.

Implications and Future Research

The results support CBD's potential as an alternative antimicrobial agent in oral healthcare. However, future studies should explore:

- Synergistic effects between CBD and antibiotics to enhance antimicrobial efficacy.
- Long-term stability and formulation for dental applications.
- Mechanistic studies to understand how CBD interacts with bacterial cell structures.

## Conclusion

This study confirms CBD's antimicrobial efficacy against oral pathogens, particularly *S. pyogenes*. Its potential as an alternative antimicrobial agent in dental applications warrants further investigation.

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