The Antibacterial Effect of Cannabidiol and Hemp Seed Oil Against *Cutibacterium acnes*

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Abstract

Acne is among the top three most common skin diseases, particularly in adolescents and young adults. It is a common disorder of the pilosebaceous unit. There are four critical elements of pathogenesis, and *C. acnes* proliferation is one of the key factors involved in acne pathogenesis. *C. acnes* is an anaerobic and gram-positive microorganism that lives in the hair follicles of the skin and currently presents resistance to antibiotic-based treatments. It stimulates many inflammatory cytokines, which are the cause of acne. Cannabidiol (CBD), one of the most well-known non-psychoactive ingredients of phytocannabinoid from *Cannabis Sativa* L. It has anti-microbial, anti-inflammatory, and anti-lipogenic properties in the treatment of acne vulgaris. Therefore, this study evaluated whether concentrations of cannabidiol and hemp seed oil have the greatest antibacterial effect on *C. acnes* and studied the MIC and MBC of CBD and hemp seed oil. For the methodology, the antibacterial effect was tested by using an agar diffusion test and a broth microdilution test, in which the concentrations were evaluated: 20%,15%,10%, 5%, 1%, 0.1% CBD, and 100% hemp seed oil; Clindamycin for the positive control and Dimethyl sulfoxide for the negative control. The result showed that CBD and hemp seed oil presented antimicrobial activity against *C. acnes.* The inhibition zones in each concentration of CBD were 17.7±0.5, 17.03±0.91, 16.8±0.61, 16.7±0.17, 16.7±0.17, and 15±0.26 mm, respectively. 100% hemp seed oil measured 10.93±0.35 mm. 2 µg Clindamycin phosphate was 20.1±1.8 mm, and absolute DMSO did not form any inhibition zone on *C. acnes*. The MIC and MBC of CBD were 2 µg/mL and 4 µg/mL. In Hemp seed oil, MIC and MBC were >1000 µg/mL. In statistical analysis, there were no statistical differences among each concentration of CBD and Hemp seed oil showed a statistically significant difference compared to other groups. Therefore, our study concludes that both CBD and hemp seed oil could inhibit the growth of C. acnes, and the antibacterial effect of CBD on C. acnes has the greatest performance compared to hemp seed oil.

Keywords**:** *Cutibacterium Acnes, Cannabidiol, Hemp Seed Oil, Antibacterial Effect*

1. Introduction

Cannabis is increasingly being used to treat a variety of dermatological diseases around the world. Cannabinoids are therapeutic substances and the three main types of cannabinoids are endocannabinoids, phytocannabinoids, and synthetic cannabinoids. In Cannabis sativa, the components of cannabinoids can be divided into Δ9-tetrahydrocannabinol (Δ9-THC), Δ8-tetrahydrocannabinol (Δ8-THC), cannabigerol (CBG), cannabichromene (CBC), cannabidiol (CBD), cannabinodiol (CBND), cannabielsoin (CBE), cannabicyclol (CBL), cannabinol (CBN), and cannabitriol (CBT) (ElSohly et al., 2017). Hemp, also known as *Cannabis sativa L.*, is a crop with a variety of uses, including food, fiber, and medicine. Hemp seed is popular in the medical and cosmeceutical fields due to its high nutritional content and useful properties due to its containing many Cannabinoids (Khan, Warner, & Wang, 2014). In Jang et al., 2019), they found that CBD has the highest concentration compared to THC and CBN in hemp seeds and hempseed oil. CBD is the non-psychoactive substance in *Cannabis sativa*. Its medical properties include the decreasing pain, lowering anxiety, and treating sleep disorders (Whiting et al., 2015). Due to its potential medicinal benefits, cannabidiol

is one of the cannabinoids that is now the subject of the most investigation. The exact mechanism of action of cannabinoids against bacteria has remained undetermined, however modification of membrane permeability has been identified as one of the routes of action in cannabis. A microscopic examination of the effect of CBCA (cannabichromenic acid) on *Bacillus subtilis* reveals changes in the bacterial membrane and nucleoid, which result in cell lysis, while CBG targets gram-positive bacteria's cytoplasmic membrane (Galletta et al., 2020). CBD has been reported to cause depolarization of the cytoplasmic membrane and disruption of the membrane potential in *Staphylococcus aureus* (Wassmann, Højrup, & Klitgaard, 2020), and another function of CBD against bacteria is the modification of cell communication through inhibiting the release of membrane vesicles by bacteria (Kosgodage et al., 2019).

Acne vulgaris is one of the most common skin pathologies that causes inflammation of the sebaceous glands. One of the causes is *C. acnes*, which is an anaerobic gram-positive bacterium. It particularly populates in pilosebaceous units, due to its anaerobic growth (Lee, Byun, & Kim, 2019). In acne pathogenesis, Human keratinocytes, sebocytes, and macrophages are stimulated by *C. acnes* to produce pro-inflammatory cytokines (e.g., IL-1, IL-6, IL-8, or TNF∝) and granulocyte-macrophage colony-stimulating factor (GM-CSF) (Dessinioti, & Katsambas, 2010). On macrophages, TLR-2 activation stimulates the expression of IL-8 and IL-12 which cause hyperkeratinisation, inflammation, and oxidative stress (Dessinioti, & Katsambas, 2017).

Van Klingeren, and Ten Ham (1976), the first publication, evaluated the antibacterial activity of the purified cannabinoids of *Cannabis sativa* (Δ9-THC and CBD) for *Staphylococci* and *Streptococci*. Blaskovich et al., (2021), after some researches on Cannabinoids have been reported. More recently, in Thailand, many cosmetic and medicine industries are interested in Cannabinoids which is, especially CBD, and not much is known regarding the antimicrobial effects of cannabinoids in hemp seed extract and Isolated CBD. Therefore, it is an opportunity to study Cannabidiol and hempseed oil in antibacterial effects on C. acne by measuring substantial inhibitory effects with inhibitory zone studies and broth microdilution tests.

2. Objectives

1) To evaluate whether concentrations of cannabidiol and hemp seed oil have the greatest in antibacterial effect on *Cutibacterium acnes*

2) To study the minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of CBD and hemp seed oil on *Cutibacterium acnes*

3. Materials and Methods

3.1 Materials

Isolated CBD powder and hemp seed oil were obtained from JSP Pharmaceutical manufacturing (Thailand) Public Company Limited *Cutibacterium acnes* (DMST14916) was taken from the Department of Medical Sciences. It was grown anaerobically in Reinforced Clostridial Medium (RCM) at 37℃ for 72 hours.

3.2 Methods

3.2.1 Zone of inhibition: The bacterial isolates were grown in Reinforced Clostridial Medium. Using a sterile cotton swab, 0.1 ml of the bacterial suspension was placed onto each Reinforced Clostridial Agar plate to create the bacterial lawn. The plates were then left in contact for one minute. For disc diffusion method, each concentration of CBD (20%,15%,10%,5%,1%,0.1%) prepared in absolute DMSO, 2 µg Clindamycin phosphate as the positive control, and 20 µL absolute DMSO as the negative control were prepared to generate the paper discs. These tested compound discs (6-mm diameter) were placed on the bacterial lawn. In the case of the agar well diffusion method, agar wells of 6 mm diameter were added 100% hemp seed oil. *C. acnes* was incubated in anaerobiosis for 72 hours at 37°C, and the inhibition zone around each disc was measured in millimeters. To minimize the bias in the data, each sample assay was performed in triplicate.

3.2.2 Broth microdilution test

Minimum Inhibitory Concentration: C. acnes was cultivated in RCM under anaerobic conditions at 37 °C for 3 days, and the turbidity of the bacterial suspension was adjusted approximately to 1×10^8 colony-forming units /mL. CBD and hemp seed oil were dissolved in absolute DMSO, and the concentrations of each

compound were serially diluted twice in a two-fold series. CBD had concentrations ranging from 0.0625 µg/mL to 128 µg/mL. A positive control in this test was Clindamycin HCl, which was dissolved in sterilized distilled water. Lastly, 100 µL of bacterial culture was used per well. The plates were incubated in an anaerobic environment for 72 hours at 37°C. The lowest CBD and hemp seed oil concentrations that completely inhibited *C. acnes growth* were defined as MIC.

Minimum Bactericidal Concentration: The bacterial suspensions from the MIC were spot-plated on agar plates and incubated at 37 °C for 72 hours to determine the MBC of CBD and hemp seed oil. MBC was the lowest concentration at which no viable bacterial colonies were seen.

3.2.3 The SPSS program was used for the statistical analyses. The inhibition zone was presented as mean ± SD and analyzed by ANOVA and Multiple Comparisons by Scheffe test. A P-value <0.05 was considered statistical significance.

4. Results and Discussion

4.1 Results

CBD and hemp seed oil showed consistent antibacterial effects with a clear zone of inhibition on all the samples tested in this study. In the paper disc diffusion assay of CBD, the inhibition zone increased in a concentration-dependent manner, as shown in Figures 1 and 2. The result showed that the isolated cannabidiol and hemp seed oil presented antimicrobial activity against *C. acnes.* The inhibition zones in each concentration of CBD (20%,15%,10%,5%,1%, and 0.1%) were 17.7±0.5, 17.03±0.91, 16.8±0.61, 16.7±0.17, 16.7±0.17, and 15±0.26 mm, respectively. The largest diameter of the inhibition zone was 20% CBD (17.7 mm), and the smallest diameter was 0.1%CBD (15 mm). In the CBD group, there was no statistically significant difference in the diameter of the inhibition zone. In the case of hempseed oil, the inhibition zone was 10.93±0.35 mm, and the zone of inhibition showed a statistically significant difference compared to other groups. Clindamycin as a positive control group showed 20.1±1.8 mm against C. acnes, and DMSO as a negative control did not produce any inhibition zone on *C. acnes*, see the results in Tablel1, Figure 2 and Figure 3. CBD inhibited the growth of *C. acnes* with a MIC of 2 µg/mL, and the MBC of CBD was 4 µg/mL, whereas hemp seed oil showed more than 1000 µg/ml. For Clindamycin, the MIC and MBC were 0.25 µg/mL, see the result in Table 2. As a result, both CBD and hemp seed oil showed the inhibition zone on *C. acnes*, but CBD was larger than hemp seed oil in the zone of inhibition. The statistical analysis exhibited that each CBD concentration and hemp seed oil showed a significant difference, while each CBD concentration showed no statistically significant difference in the diameter of the inhibition zone. Thus, we concluded that CBD has the greatest antibacterial effect on *C. acne* compared to hemp seed oil. Moreover, the MIC and MBC of CBD on *C. acnes* were more effective than hemp seed oil.

Table 1 Inhibition zone of C. acnes

	Mean	**SD**	**P-value**						
	(mm)		20%CBD	15%CBD	10%CBD	5%CBD	1%CBD	0.1%CBD	100%Hemp seed oil
20%CBD	17.7	0.5	-	1.00	0.99	0.98	0.98	0.05	<0.001
15%CBD	17.03	0.91	1.00	-	1.00	1.00	1.00	0.32	<0.001
10%CBD	16.8	0.61	0.99	1.00	-	1.00	1.00	0.5	<0.001
5%CBD	16.7	0.17	0.98	1.00	1.00	-	1.00	0.59	<0.001
1%CBD	16.7	0.17	0.98	1.00	1.00	1.00	-	0.59	<0.001
0.1%CBD	15	0.26	0.05	0.32	0.5	0.59	0.59	-	<0.001
100%Hemp seed oil	10.93	0.35	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	-

Clindamycin DMSO	20.10	1.8	0.13	0.02	0.01	0.01	0.01	<0.001	<0.001

Table 2 MIC and MBC

	MIC ($\mu g/mL$)	**MBC** ($\mu g/mL$)
CBD	2	4
Hemp seed oil	>1000	>1000
Clindamycin	0.25	0.25

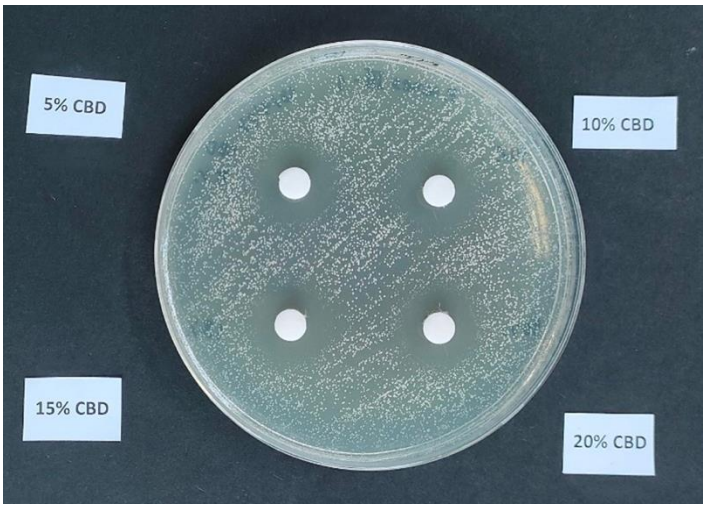


Figure1 Effect of 20%-5%CBD against the growth of *C. acnes* in the zone of inhibition

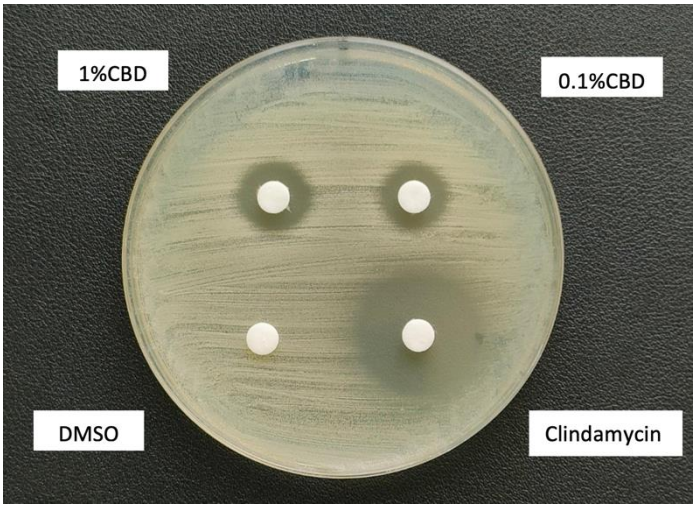
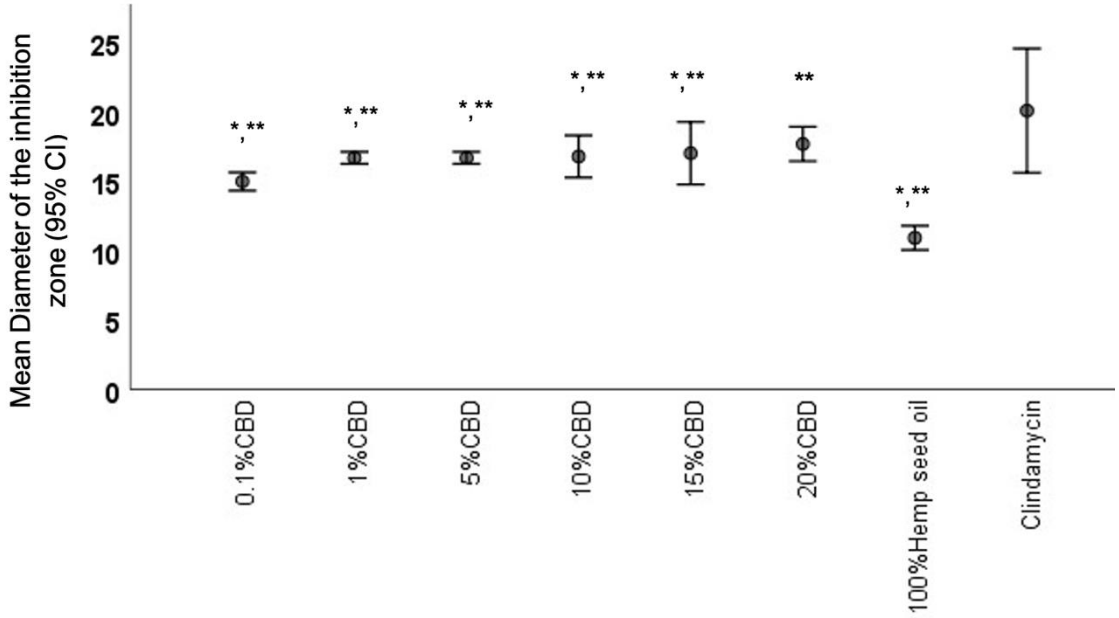


Figure 2 Effect of 1-0.1%CBD, Clindamycin (Positive control), and DMSO (Negative control) against the growth of *C. acnes* in the zone of inhibition

Figure 3 Effect of CBD and hemp seed oil against the growth of *C. acnes* in the diameter of the zone of inhibition (mm). The bars show 95%CI of Mean. (∗) p<0.05 indicates statistically significant differences compared to control



group (Clindamycin), (∗∗) the inhibition zone of each CBD concentration is a significant difference from 100% hemp seed oil

4.2 Discussion

In this study, we demonstrated the efficiency of CBD and hemp seed oil against *C. acnes*, which directly causes inflammation of keratinocytes by activating TLRs and inducing the expression of inflammatory cytokines and chemokines such as TNF-α, IL-1β, IL-6, and IL-8 (Yu et al., 2022). TLRs also trigger the activation of MAPK and NF-κB signaling pathways. Activated NF-κB induces the transcription of genes that are linked to inflammatory responses (Jin, & Lee, 2018). Cannabidiol is one of the main cannabinoids from the *Cannabis sativa* L. plant. The biological properties of CBD have been shown to reduce seizures, have anti-inflammatory properties, and be used to treat a variety of skin conditions. Furthermore, several studies have linked CBD to the treatment of acne vulgaris due to its anti-lipogenic, anti-proliferative, anti-inflammatory, and antimicrobial properties (Oláh et al., 2014). Hemp seed also contains many cannabinoids, such as CBD, that have been reported to have antibacterial effects against Gram-positive bacteria (*Staphylococcus aureus*, *Enterococcus faecalis*, and *Streptococcus pneumoniae*) (Ostapczuk et al., 2021).

The result of our study showed that CBD and hemp seed oil were able to inhibit the growth of *C. acnes*. The inhibition zone was produced by all CBD concentrations, but the maximum inhibition zone resulted in 20% CBD, which was 17.7±0.5 mm, and the minimum inhibition zone was 0.1% CBD, which showed 15±0.26 mm. Following the studies of Pila, Segarra, and Cerna (2023), they found that the concentration of cannabidiol oil at 0.8 % had an inhibition zone of 19 mm and also presented an inhibition percentage of 91.2% against *C. acnes*. The statistical analysis of our result showed that the diameter of the inhibition zone in the CBD group showed no statistically significant difference. Likewise, CBD showed a MIC of 2 µg/mL and MBC of 4 µg/mL against *C. acnes*, which is consistent with the reporting of Blaskovich et al. (2021). They found a MIC of 1-2 µg/ml against *C. acnes*. Hemp seed becomes more attractive in several fields due to its many useful properties. In our study, the antibacterial activity of 100% hemp seed oil showed 10.93±0.35 mm of the inhibition zone and more than 1,000 µg/ml of the MIC and MBC values on *C. acnes*. In a previous study, hemp seed extract showed effective growth inhibition of *C. acnes* in the MIC and MBC, which were determined in the concentration range of 256-2048 µg/mL (Sangkanu et al., 2023). Thus, our study indicated that both CBD and hemp seed oil could inhibit the growth of *C. acnes*. Based on the statistical

analysis of the inhibition zones produced by each concentration of CBD and hemp seed oil, they showed that each CBD concentration and hemp seed oil showed a statistically significant difference, while each concentration of CBD showed no statistically significant difference in the diameter of the inhibition zone. Furthermore, the MIC and MBC of CBD were more effective values against *C. acnes* than hemp seed oil. Therefore, we concluded that CBD had the greatest performance in the antibacterial effect on *C. acnes* compared to hemp seed oil in the form of the agar diffusion test and the broth microdilution test. Although CBD and hemp seed oil could inhibit *C. acnes* growth, the inhibition zones produced were smaller than Clindamycin, which were 20±1.8 mm. This is due to the antibacterial mechanism of Clindamycin, which can directly target the 50s ribosomal subunit of *C. acnes* and interfere with protein synthesis, resulting in antibacterial effect (Nguyen, & Eichenfield, 2015) that is different from CBD. The mechanisms of the antibacterial activity of CBD are depolarizing the cytoplasmic membrane and disrupting the membrane potential, which inhibit the growth of gram-positive bacteria (Wassmann et al., 2020). This study also indicated that the inhibition zone formed was completely due to CBD with no influence from the DMSO solvent because there was no formation of an inhibition zone on disc paper with DMSO. Several studies have also proven that cannabinoids play a role in antibacterial properties against both Gram- positive bacteria and Gram-negative bacteria, and some studies have shown that they improve the antimicrobial activity of conventional antibiotics against resistant microorganisms (Schofs, Sparo, & Sánchez Bruni, 2021).

5. Conclusion

Our study has identified the efficacy of cannabidiol and hemp seed oil against *Cutibacterium acnes*. The effectiveness of antimicrobial activity could be viewed as the intensity of the inhibition zone, the MIC, and the MBC values. Each CBD concentration (20%,15%,10%,5%, 1%, 0.1%) exhibited the zone of inhibition in 17.7±0.5, 17.03±0.91, 16.8±0.61, 16.7±0.17, 16.7±0.17 and 15±0.26 mm, respectively, which were not statistically significant differences and 100% hemp seed oil showed 10.93±0.35 mm. The MIC and the MBC of CBD were 2 µg/mL and 4 µg/mL and Hemp seed oil was more than 1000 µg/mL. CBD and hemp seed oil can inhibit the growth of *C. acnes*, while the performance of CBD is greater than that of hemp seed oil against C. acne. Future studies are therefore focused on the efficacy of CBD and hemp seed oil against other microorganisms in skin problems and the efficacy of CBD and hemp seed oil effect to microbial cultivation.

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