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The Effectiveness of Cannabidiol as an Antibiotic against Gram-positive and Gram-negative Bacteria

Micaila Curtis

ABSTRACT

CBD is a non-psychoactive compound extracted from the stem, stalk, leaves, and flowers of the *Cannabis sativa* plant. Literature suggests a great number of medicinal benefits for CBD, including anti-inflammatory, antipsychotic for motivation disorders such depression or anxiety, and antibacterial properties. The study utilized the disk diffusion method to observe the antibacterial activity of CBD on gram-negative and gram-positive bacteria. Results suggested that CBD was not active against the strains of *S. aureus, S. epidermis,* and *E. coli* at 2.0 mg/ L concentration. Upon conclusion, an extensive literature search led to a possible explanation for the selective antibacterial properties of CBD. MDR strains of bacteria that were susceptible to CBD according to literature, all had over-expressed efflux pump genes. The bacteria in this experiment lacked the over expression of those genes. In order to understand the role of CBD, as an antibiotic, future researchers should consider the over expression of the efflux pump gene when planning their studies.

Keywords: *Cannabidiol, cannabinoids, CBD, Staphylococcus aureus, Staphylococcus epidermis, Escherichia coli, MRSA, antibiotic resistant, MDR, bacterial efflux pumps*

INTRODUCTION

Cannabidiol (CBD) is a bioactive compound extracted from the stem, stalk, leaves, and flowers of the *Cannabis sativa* plant (Kogan and Mechoulam 2007). CBD is the second most abundant cannabinoid in *C. sativa.* Compounds from *C. sativa,* are referred to as cannabinoids and have varied characteristics and effects on the human body (Appendino, et al 2008; Pisanti, et al 2017). *C. sativa* contains hundreds of compounds, some of which can cause somatic and psychic changes in humans (Adams et al 1940). The most commonly known cannabinoid is *tetra-hydrocannabinol (*THC), which is the compound responsible for the psychotropic effects of *C. sativa* (Iffland and Grotenherman 2017).

The endocannabinoid system is a network of receptors in our bodies that binds with cannabinoids and is a target of pharmacotherapy (Pacher, Batkai, and Kunos 2008; Zlebnik and Cheer 2016). THC targets CB1 receptors, which are found mainly in the central nervous system. CBD targets CB2 receptors found mainly in the immune system, gastrointestinal system, and partially in the peripheral nervous system. *C. sativa* is regularly consumed by humans for various reasons, such as medicinal or recreational purposes. Human consumption of *C. sativa* with higher THC levels and low CBD levels is correlated with depression and anxiety, as well as poor cognitive skills and memory. However, consumption of higher levels of CBD and lower THC were correlated with little to no psychosis symptoms, such as depression or anxiety, and above average recognition and memory comprehension (Atakan 2012).

CBD can be used in the form of oil, medication, supplements, inhalants, and more. Recent studies suggest CBD has an effect on neural cells and can improve the symptoms of anxiety, seizures, acne, autoimmune disorders, memory, nausea, depression, drug addiction, antibacterial properties and more (Fadda, et al 2004; Hampson, et al 1998; Iffland and Grotenherman 2017; Li, et al 2018; Mechoulam, Parker, and Galilly 2002; Zlebnik and Cheer 2016).

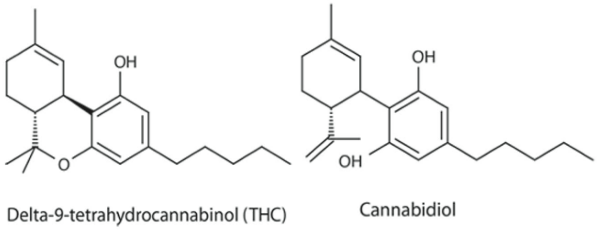

Figure 1: Molecular structures of CBD (right) and THC (left). Although they are very similar in structure and origin, their effects on the human body are extremely different (Appendino, et al 2008).

CBD has been proven to have beneficial effects on the human body (Mechoulam, Parker, and Galilly 2002). A literature study found over a thousand cited sources providing research concluding with various benefits of CBD, such as improving psychosis symptoms, lowering symptoms of autoimmune disorders, and anti-inflammatory properties aiding in pain relief (Pisanti, et al 2017). There have also been studies that found antibiotic properties of CBD (Appendino, et al 2008; Duarte 2016).

The experiment is to test various concentrations of CBD as an antibiotic against non-MDR strains of bacteria. The bacteria in this study were chosen based on their association with the healthy human microbiome and to test CBD against gram-negative

and gram-positive bacteria (Human Microbiome 2012).

Staphylococcus aureus and *Staphylococcus epidermis are* gram-positive bacteria that can be commonly found on or in healthy human bodies. *S. epidermis* comes from the same genus as *S. aureus* and has similar physiological characteristics. *S. epidermis* is most commonly found on the skin of humans, which is the largest organ of the human body (Sand, et al 2009).

Although *S. epidermis* and *S. aureus* are found in a healthy human microbiome, there are strains of these bacteria that are multiple drug resistant (MDR) and potentially dangerous if not medically treated. The methicillin-resistant strain of *S. aureus* (MRSA) is responsible for thousands of deaths per year (Chambers and DeLeo 2010, Tong, et al 2015). *S. epidermis* is sometimes referred to as an "opportunistic pathogen" due to it's presence on the human skin. It is responsible for 22% of bloodstream infections of intensive care unit patients in America each year and also for majority of the urinary tracts infections due to catheter insertion because of poor sanitization of the equipment and skin surrounding the insertion site (Otto 2009).

Escherichia coli is a gram-negative bacterium that can be found commonly in the digestive system of mammals (Winfield and Groisman 2003). Some strains of *E. coli* can be MDR and potentially harmful if left untreated. A change of diet or eating foods not cooked properly can introduce infectious strains of *E. coli* into the human body (Agus, et al 2016).

Finding new and effective antibiotics is a continuous process (Sun, Deng, and Yan 2014). Bacteria are constantly evolving to resist antibiotics (Stewart and Costerton 2001). Wounds and our natural orifices leave an open gateway for bacteria to infect our bodies and if left untreated can be life threatening (Bowler, Duerden, and Armstrong 2001; Velnar, Bailey, and Smrkolj 2009). Producing effective antibiotics against *E. coli* is a challenge because pharmacists want to kill the infectious MDR strains, but not kill the bacteria that assist in a healthy digestive system. Scientists found that when treating a specific human pathogenic strain of *E. coli* with certain antibiotics, Hemolytic Uremic Syndrome, a dangerous disease, came as a side effect (Freedman, et al 2016).

One of the most pressing issues about health is that medications are usually overprescribed and not taken correctly or for the full duration recommended by the doctor, giving bacteria the opportunity to build resistance to the antibiotic. Over periods of time where medication abuse is obvious, bacteria can become multiple drug resistant (MDR), creating an intense dynamic between pharmacists/ scientists and bacteria. Scientists race to find antibiotics that kill the bacteria, whereas the bacteria are constantly evolving in order to resist current antibiotics so they can live. The never-ending race against harmful bacteria is why finding effective, but human-friendly, antibiotics are so important.

MATERIALS AND METHODS

Microbiology Lab Materials:

Bacterial cultures were obtained from Carolina Biological Supply
E. coli (strain K12)
S. aureus (coagulase positive strain)
S. epidermis (common lab strain – no specification)
-All strains used were non-multiple drug resistant and safe to use in an undergraduate research facility

Experimental Laboratory Prep:

Before receiving the bacterial specimen and the CBD compound, proper equipment and glassware were collected and cleaned. To clean the glassware, a solution of warm water and lab detergent in a gallon bucket was used. After the volumetric flasks sat in the solution for four hours, the flasks were then removed, rinsed with distilled water and set to dry. For the glassware that still had residue after cleaning, 3M HCl was added to the container and sat for six hours before discarding the HCl and rinsing the flask again.

CBD Solutions and Controls:

CBD was purchased from Sigma-Aldrich.
The CBD arrived in a 1 mL MeOH solution. The stock solution was prepared by pouring the 1 mL CBD solution into a 100 mL volumetric flask, and then 4 mL of MeOH was added. Distilled water was used to dilute the solution up to 100mL. The stock solution contained a 5% MeOH concentration. The stock solution is referred to as A throughout the experiment.

Table 1: Concentrations of CBD for the disk diffusion method

Solution	Concentration of CBD
A (Stock)	0.1 mg / 1 L
B	0.075 mg / 1 L
C	0.050 mg / 1 L
D	0.025 mg / 1 L
E	0.010 mg / 1 L
F	0.0075 mg / 1 L
G	0.0050 mg / 1 L
H	0.0025 mg / 1 L
C1	100% H2O
C2	5% MeOH

Preparation of Nutrient Agar and Petri Plates:

100 g of nutrient agar was mixed with 2.5 L of distilled water split between two 2 L Erlenmeyer flasks. Flasks were placed on an electric plate to heat the agar solution while stirring with a magnetic stir bar. After the agar was dissolved, the flask was covered with

aluminum foil and autoclaved at 121°C for 15 minutes. 100 petri plates were then poured with the nutrient agar solution in a laminar flow hood to prevent contamination of agar with bacteria not specified in materials.

Preparation of Nutrient Broth

3.0 g of nutrient broth was mixed with 100 mL of distilled water and stirred with magnetic stir bar until the solution was clear. Broth was distributed evenly between nine test tubes: three test tubes designated to inoculate with each of the different bacterium. This was to minimize the chance of contaminating the original bacteria test tubes we received. The nine tests tubes were then set aside to be autoclaved at 121°C for 15 minutes. After they were sterilized, the tubes were set aside to adjust to 37°C before inoculating with bacteria.

Preparation of Antibacterial Paper Disks:

300 paper disks (6.35 mm in diameter) and cotton swabs for each bacteria (used to inoculate the plates) were sterilized in the autoclave at 121°C for 15 minutes.

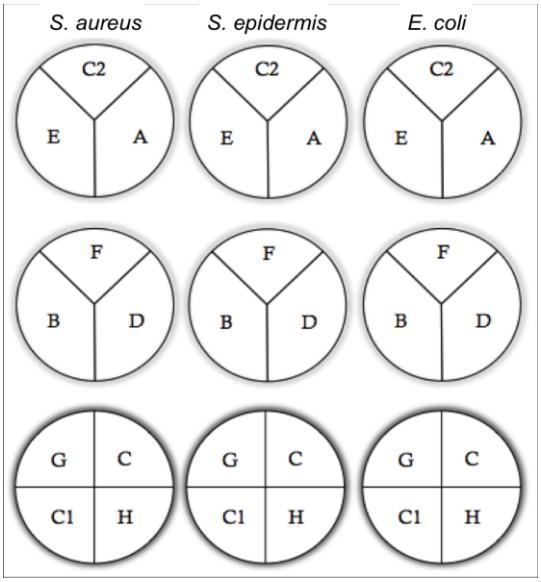

Figure 2: Labeling for Experiment 1 - Visual layout of how petri plates were labeled with the CBD concentrated paper disks and inoculated with the different bacteria.

Experiment 1:

30 plates were inoculated with *S. aureus*, 30 plates with *S. epidermis,* and 30 plates with *E. Coli* for 10 trials of each concentration with each bacterium. Paper disks were placed in correspondence with the plate labels from Figure 2. After inoculation, plates were placed in a semi-sealed bag (to minimize dehydration of the agar) and incubated at 37°C (Schmitt, Schuler-Schmid, and Schmidt-Lorenz 1990). Results were recorded at 24 and 48 hours after initial inoculation.

Experiment 2:

Stock solution, A, was used to make more concentrated CBD paper disks by pipetting 18 microliters onto one disk 5 times and to a second disk 20 times, for increased concentrations of A for solutions 1 and 2. The new control MeOH had 20 times the concentration of our 5% MeOH control. Under a sterile hood, the allotted amount of A was added to the new, more highly concentrated disks and left to dry before adding more, in hopes to reach a higher concentration. A record was kept for the time the disks had solution added to them and how much had been added so far. Three plates were inoculated with *S. aureus*, three with *S. epidermis,* and three with *E. coli.* Results were collected at 24 and 48 hours after inoculation.

RESULTS

Results were analyzed with the disk diffusion method, looking for the zone of inhibition to measure susceptibility of the bacteria to CBD as an antibiotic.

Experiment 1

Table 2: Results for experiment 1. No sign of bacterial susceptibility was shown in any of the concentrations of CBD solutions.

Solution	Concentration of CBD	Result
A	0.1 mg / 1 L	Resistant
B	0.075 mg / 1 L	Resistant
C	0.050 mg / 1 L	Resistant
D	0.025 mg / 1 L	Resistant
E	0.010 mg / 1 L	Resistant
F	0.0075 mg / 1 L	Resistant
G	0.0050 mg / 1 L	Resistant
H	0.0025 mg / 1 L	Resistant
C1 H_2O	100% H_2O	Resistant
C2 MeOH	5% MeOH	Resistant

Experiment 2

Table 3: Results for experiment 2. No sign of bacterial susceptibility was shown in any of the higher CBD concentrations.

Solution	**Concentration of CBD**	**Result**
1	0.5 mg/ 1 L	Resistant
2	2.0 mg/ 1 L	Resistant
C2 MeOH	20x MeOH 5%	Resistant

DISCUSSION

Initial reading about CBD as an antibiotic, there were not many credible sources. In designing the experiment, it was to be similar to literature studies citing CBD as an effective antibiotic (Appendino, et al 2008). However, the most significant difference in this

experiment was that the bacteria used are not drug resistant and there are three different strains used. Upon acquisition of unexpected results, a more in-depth search of literature provided a possible explanation.

Researchers are discovering more often that bacteria have multiple ways of exporting drugs from their systems. In *E. coli*, there are at least nine different known multi-drug systems, like various types of efflux pumps. These efflux pump systems are structures located in the cell membrane to identify and take in toxins from their environment and pump them back out (Paulsen 1997).

antibiotic properties of CBD could be beneficial for those who use CBD. If the hypothesis is true, it makes CBD an ideal candidate antibacterial agent to treat pathogenic MDR strains.

A possible hypothesis for future studies using CBD as an antibiotic against bacteria with the over-expression of efflux pump genes is that CBDs molecular structure is different than majority of today's antibiotics. The most obvious is the lack of nitrogen in CBD's structure. With the difference of structure, it is possible that there is still a component of CBD (i.e. the aromatic ring or alcohol groups) that could interact with the efflux pumps and potentially "clog" or further

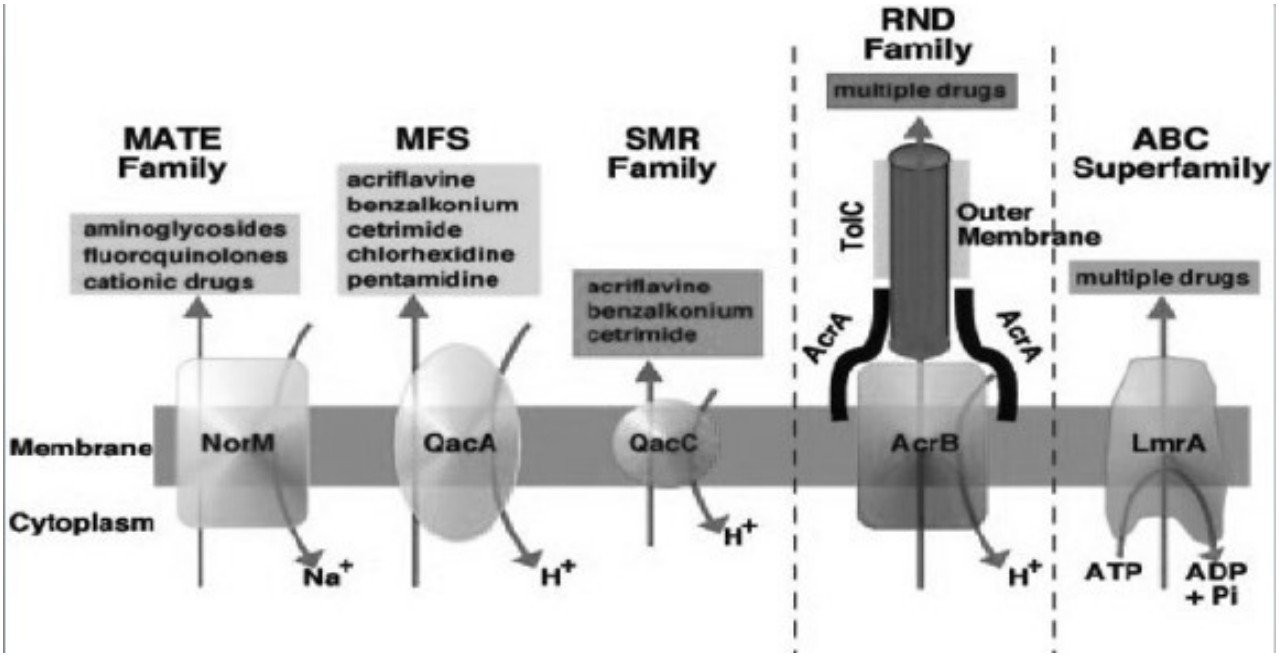

Figure 3: The five major families of bacterial efflux pumps: MFS (major facilitator superfamily), MATE (multidrug and toxic efflux, RND (resistance-nodulation-division), SMR (small multidrug resistance), and ABC (ATP binding cassette) (Piddock 2006).

The over-expression of bacterial efflux pump genes is a way that bacteria build resistance to current antibiotics. Stressors, like antibiotics, in the parent cell's environment can force the bacterial efflux pump gene to mutate in daughter cells in order to over-express (Sun, Deng, and Yan 2014; Webber and Piddock 2003). These mutations are costly and take away from the bacterium's normal metabolic functions. However, in an environment where there is a high pressure from antibiotics, it is advantageous, as the over-expression predisposes them to make more antibiotic resistant mutations to their genome, leading to MDR bacteria (Meouche and Dunlop 2018).

This over-expression can be used to understand why the bacteria in this experiment were not susceptible to CBD as an antibiotic while the bacteria in previous literature using MRSA or other multiple drug resistant bacteria were susceptible. The selective inhibit their function. With the over-expression of the efflux pump genes, this could cause a much larger area of clogged structures, being harmful or lethal to the bacterium. It can also be that any contact with a bacterial toxin from the external environment will be harmful or lethal to the bacterium's continuation of life, due to the lack of functioning efflux pumps. These are only hypotheses with no experimental data to confirm validity.

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LITERATURE CITED

Adams R., and M. Hunt, H. Clark. 1940. Structure of Cannabidiol, a Product Isolated From the Marihuana Extract of Minnesota Wild Hemp. Journal of the American Chemistry Society 62:196-200.

Agus A., Denizot J., Thevenot J., Martinez-Medina M., Massier S., Sauvanet P., Bernalier-Donadille A., Denis S., Hofman P., Bonnet R., et. al. 2016. Western diet induces a shift in microbiota composition enhancing susceptibility to Adherent-Invasive *E. coli* infection and intestinal inflammation. Scientific Reports 6:19032.

Appendino G., Gibbons S., Giana A., Pagani A., Grassi G, Stavri M., Smith E., Rahman M. 2008. Antibacterial Cannabinoids from *Cannabis sativa:* A Structure – Activity Study. Journal of Natural Products 71,8:1427-1430.

Atakan Z. 2012. Cannabis, a Complex Plant: Different Compounds and Different Effects on Individuals. Journal of Therapeutic Advances in Psychopharmacology 2(6):241-254.

Bowler P.G., Duerden B. I., Armstrong D. G. 2001. Wound Microbiology and Associated Approaches to Wound Management. Clinical Microbiology Reviews 14(2):244-269.

Chambers H., DeLeo F. 2016. Waves of Resistance: *Staphylococcus aureus* in the Antibiotic Era. Natural Revolution Microbiol Journal 7(9):629-641.

Duarte, P. 2016. Determination of the Antibiotic Properties of Cannabidiol. Journal of General Practice 4(4): 266-269.

Fadda P., Robinson L., Fratta W., et. al. 2004. Differential Effects of *THC*- or *CBD*-rich *Cannabis* Extracts on Working Memory in Rats. Neuropharmacology 47(8):1170-1179.

Freedman S., Xie J., Neufeld M., et. al. 2016. Shiga Toxin–Producing *Escherichia coli* Infection, Antibiotics, and Risk of Developing Hemolytic Uremic Syndrome: A Meta-analysis. Infectious Diseases Society of America 62(10):1251-1258.

Hampson A.J., Grimaldi M., Axelrod J., et. al. 1998. *Cannabidiol* and *(-) Delta 9 Tetrahydrocannabidiol* and Neuroprotective Antioxidants. Proceedings of the National Academy of Sciences of the United States of America 95(14):8268-8273.

Human Microbiome Project Consortium, The. 2012. Structure, Function and Diversity of the Healthy Human Microbiome. Nature International Journal of Science 486:207-214.

Iffland K., and F. Grotenherman. 2017. An Update on Safety and Side Effects of *Cannabidiol*: A Review of Clinical Data and Relevant Animal Studies. *Cannabis* and *Cannabinoid* Research 2(1):139-154.

Kogan N., and R. Mechoulam 2007. *Cannabinoids* in Health and Disease. Dialouges in Clinical Neuroscience 9(4):413-430.

Li H., Kong W., Chambers C., et. al. 2018.The Non-Psychoactive Phytocannabinoid *Cannabidiol* (CBD) Attenuates Pro-Inflammatory Mediators, T-Cell Infiltration, and Thermal Sensitivity Following Spinal Cord Injury in Mice. Cellular Immunity 329:1-9.

Mechoulam R., Parker LA., Galilly R. 2002. *Cannabidiol*: An Overview of Some Pharmacological Aspects. The Journal of Clinical Pharmacology 42(S1):11S-19S.

Meouche E., Dunlop M. 2018. Heterogeneity in Efflux Pump Expression Predisposes Antibiotic-Resistant Cells to Mutation. Science 362(6415):686-690.

Otto M. 2009. *Staphylococcus epidermis* – The "Accidental" Pathogen. Journal of National Revolution Microbiology 7(8):555-567.

Pacher P., Batkai S., Kunos G. 2008. The Endocannabidiol System as an Emerging Target of Pharmacotherapy. of Pharmacology Revolution 58(3):389-462.

Paulsen I., Brown M., Skurray R. 1997. Proton-Dependent Multidrug Efflux Systems. Microbiological Reviews 60(4):575-608.

Piddock L. 2006. Clinically Relevant Chromosomally Encoded Multidrug Resistance Efflux Pumps in Bacteria. Clinical Microbiology Reviews 19(2):382-402.

Pisanti S., Malfitano A. M., Ciaglia E., Lamberti A., Ranieri R., Cuomo G., Abate M., Faggiana G., Proto M. C., Flore D., et. al. 2017. Cannabidiol: State of the Art and New Challenges for Therapeutic Applications. Journal of Pharmacy and Therapeutics 175:133-150.

Sand M., Gambichler T., Sand D., et. Al. 2009. MicroRNAs and the Skin: Tiny Players in the Body's Largest Organ. Journal of Dermatological Science 53(3):169-175.

Schmitt M., Schuler-Schmid U., Schmidt-Lorenz W. 1990. Temperature Limits of Growth, Tnase and Enterotoxin Production of *Staphylococcus aureus* Strains Isolated From Foods. International Journal of Food Microbiology 11(1):1-19.

Stewart P., Costerton J. 2001. Antibiotic Resistance of Bacteria in Biofilms. Science Direct 358(9276):135-138.

Sun J., Deng Z., Yan A. 2014. Bacterial Multidrug Efflux Pumps: Mechanisms, Physiology and Pharmaceutical Exploitations. Biochemical and Biophysical Research Communications 453(2):254-267.

Tong S., Davis J., Eichenberger E., et. al. 2015. *Staphylococcus aureus* Infections: Epidemiology, Pathophysiology, Clinical Manifestations, and

Management. American Society for Microbiology 28(3):603-661.
University of Wisconsin. Volumetric Flasks and Pipets. Chemical Education.
Velnar T., Bailey T., Smrkolj V. 2009. The Wound Healing Process: An Overview of the Cellular and Molecular Mechanisms. Journal of International Medical Research 37(5):1528-1542.
Webber M., Piddock L. 2003. The Importance of Efflux Pumps in Bacterial Antibiotic Resistance. Journal of Antimicrobial Chemotherapy 51(1):9-11.
Winfield M., Groisman E. 2003. Role of Nonhost Environments in the Lifestyles of Salmonella and *Escherichia coli*. American Society for Microbiology 67(7):3687-3694.
Zlebnik N., Cheer J. 2016. Beyond the CB1 Recepter: Is Cannabidiol the Answer for Disorders of Motivation? Annual Review of Neuroscience 8(39)1-17.