



# Cannabinoids as Antimicrobial Agents

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The United States Food and Drug Administration currently prohibits the addition of CBD to food and dietary supplement product



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## Executive Summary

Antibiotic resistance has emerged as a critical issue among the global healthcare community. In a call to action, the World Health Organization (WHO) released a list of twelve pathogens that have developed antibiotic resistance and thus present a serious threat to public health. Eight of these pathogens are classified as gram-negative and are notoriously difficult to treat due in part to their characteristic protective polysaccharide shell that surrounds their outer lipid membrane. Of the remaining four pathogens, which are classified as gram-positive and lack a protective outer membrane, methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most prevalent infectious pathogens, with US infections topping 300,000 every year since 2005, resulting in more than 10,000 deaths and \$1.7B in healthcare costs in 2017. Similarly, the Canadian Antimicrobial Resistance Surveillance System reported that the rate of MRSA infections within sentinel hospitals increased since 2015 from 2.8 to 3.17 cases per 10,000 patient-days in 2017. During the same time period, community-associated MRSA infections increased by over 60%, while the rate of community-associated MRSA bloodstream infections (BSI) doubled. These infectious threats extend beyond developing nations where infectious disease remains a leading cause of death, as these resistant pathogens are also the major cause of infections and mortality within developed healthcare settings.

Current in-vitro scientific evidence suggests that major cannabinoids THC, CBD, CBG, CBN, and CBC possess potent activity against gram-positive bacteria, as well as activity against gram-negative bacteria when supplemented with outer membrane penetrating agents. In particular, CBG presents superior activity in the real-world applications of biofilm inhibition, biofilm eradication, and antibacterial activity at concentrations well below toxic levels that are achievable with topical formulations with utility in OTC and acute care clinical settings. These cannabinoids, along with the barely explored minor cannabinoids, present an opportunity to develop a novel and highly effective topical and potentially systemic antibiotic pipeline pending further in-vivo investigations. Further in-vivo work is required to validate the systemic antibacterial efficacy of cannabinoids when administered orally, intravenously, or through other routes for systemic treatment.

At the forefront of cannabinoid cellular agriculture, LAVVAN utilizes yeast fermentation technology to produce high-quality, reliably sourced, natural cannabinoid ingredients. LAVVAN will provide cannabinoids with unparalleled purity, consistency, potency and sustainability at a scale capable of serving a range of industries including health, beauty, food and beverage, and pharmaceuticals. LAVVAN's cannabinoids are identical to those found in nature, and produced in a cGMP facility in accordance with the most stringent standards, including being devoid of pesticides, mold, bacteria, and other contaminants often found in traditional cannabis agriculture. In addition to providing high purity cannabinoid ingredients, LAVVAN will leverage its cannabinoid formulations expertise to support its industry partners with integrating cannabinoids into formulations for various end products that require specific utility.

## Summary of Scientific Evidence

### Gram-Positive Antibacterial Activity

- Non-psychotropic cannabinoids CBD, CBG, CBN and psychotropic cannabinoid THC demonstrated potent antibacterial activity at non-cytotoxic concentrations of 2 µg/mL against various strains of gram-positive methicillin-resistant *Staphylococcus aureus* (MRSA) during in-vitro susceptibility tests

### Biofilm and Persister Cell Effects

- CBG demonstrated superior antimicrobial potency as a biofilm inhibitor at 4 µg/mL and persister cell eradicator at 5 µg/mL

### Gram-Negative Antibacterial Activity

- 1 µg/mL THC, CBD, CBG, CBN, and CBC displayed potent antibacterial activity against *E. coli* when co-administered during in-vitro susceptibility tests with sub-lethal doses of Polymyxin B, a gram-negative specific antibiotic known to disrupt the bacterial outer membrane
- Co-administration of Polymyxin B also enabled CBG's antibacterial activity against additional gram-negative pathogens *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*
- 1µM and 5 µm of CBD inhibited the release of membrane vesicles from *E. coli* and altered their metabolic processes, cellular respiration and antibiotic functions protein profile
- 100 mg/kg CBG demonstrated in-vivo efficacy when administered intraperitoneally in a mouse systemic infection model against MRSA

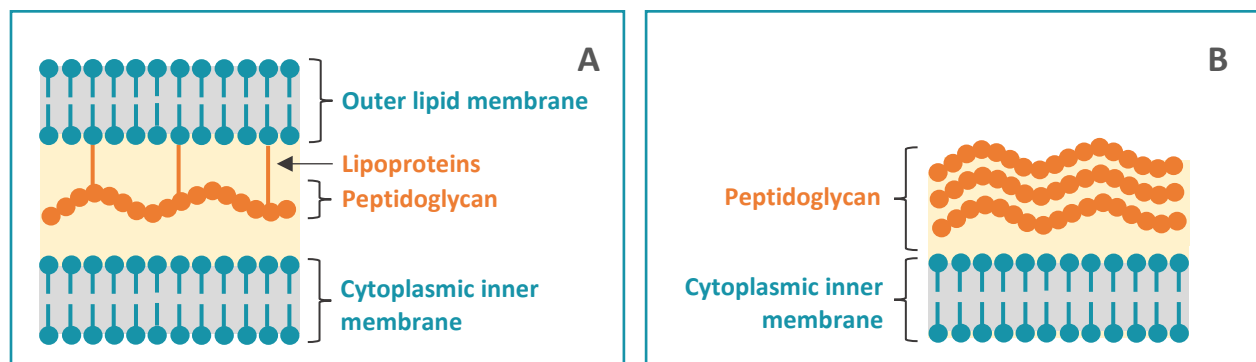
### Mechanism of Antibacterial Action

- The suggested mechanism of these cannabinoids' antibacterial action is weakening of the cytoplasmic membranes
- In the case of gram-negative pathogens, co-administration of an antibiotic that disrupts the outer membrane is required to enable this antibacterial action
- CBD disrupts gram-negative survival mechanisms by inhibiting the release of membrane vesicles

## Introduction to Bacteria and Other Microbes

A microbe is any single-cell organism, which includes bacteria, archaea, fungi, and single-celled eukaryotes related to both plants and animals. Bacteria, the most numerous and diverse microbes, represent roughly 15% of the Earth's biomass and 50% of the cells on and in a human body, located mostly in the gut and on the skin – an ecosystem known as the 'Microbiome'<sup>1</sup>. Bacteria are characterized as either gram-negative or gram-positive based on the structure of their bacterial cell wall (Figure 1). Gram-positive bacteria have a cytoplasmic membrane surrounded by a thick peptidoglycan layer, while the more numerous and varied gram-negative bacteria have three layers, including a thin peptidoglycan layer surrounded by an elaborate outer membrane studded with complex sugars.

Bacteria can exist in different states. While free-growing bacterial populations reproduce very quickly, a fraction of the population may consist of 'persister cells' that reproduce extremely slowly or not at all. Some bacterial species and sub-species (also known as 'strains') are capable of attaching to natural or artificial surfaces and aggregating into biofilms, which are contiguous hydrogel phases that are highly protective and difficult to perturb. Certain gram-positive bacteria can enter a dormant state called an 'endospore', rendering them inert to ultraviolet radiation, desiccation, high temperature, extreme freezing and chemical disinfectants.



**Figure 1:** Bacterial cell wall structure of gram-negative (A) and gram-positive (B) bacteria.

## Antibiotic Resistance: A Global Healthcare Emergency

Bacteria were the harbingers of molecular biology, with the late 19th century German dye industry birthing antibiotics by happenstance and the germ theory of disease birthing modern medicine. Antibiotics made previously lethal injuries banal and became the first cancer chemotherapies. It is understood today that of the bacteria humans interact with, only some are pathogenic, more are beneficial, and most are neutral as far as humans are concerned. Unfortunately, a century spent casting these organisms as villains has contributed to an unsustainable infrastructure that provides potent antibiotics in great excess, prophylactically, over the counter, and in animal diets.

Derived from molecules found in nature, standard antibiotics are small molecules that interfere with a single protein essential for the bacteria's survival, with the protein's identity differing between antibiotic classes. As predicted by scientists and doctors soon after the release of Penicillin, antibiotics impose an acute selective pressure that promotes the emergence of genetic mutations in the bacteria under attack. While most bacteria are successfully killed, a single lucky organism may evolve to develop antibiotic resistance and rapidly expand in the human host, killing the patient and spilling into the healthcare

system. By infecting other patients and forming biofilms on hospital supplies and infrastructure, these resistant bacteria take root and become a source of Hospital or Healthcare Acquired Infections (HAIs or HCAs). Simultaneously, patients returning home may bring resistant bacteria with them, leading to Community-Acquired Infections (CAIs).

Antimicrobial resistance has emerged as a critical issue among the global healthcare community, with bacterial strains demonstrating resistance to a range of first-line antibiotic treatments including fluoroquinolones, macrolides, tetracyclines, beta-lactams, vancomycin, linezolid and daptomycin. In a call to action, the World Health Organization (WHO) released a list of 12 pathogens that have developed antibiotic resistance and thus present a serious threat to public health<sup>2</sup>. This threat extends beyond developing nations where infectious disease remains a leading cause of death, as these resistant pathogens are also the major cause of infections and mortality within developed healthcare settings. The odds of acquiring an infection once admitted to a hospital range from 4-10% in the developed world and 10-20% in the developing world<sup>3</sup>. In the United States, nearly 6% of HAIs are fatal and the Centers for Disease Control and Prevention (CDC) estimates that more than 35,000 Americans die each year from antibiotic-resistant infections<sup>4,5</sup>. This risk is of particular concern for immunocompromised patients due to their increased susceptibility to infection. The prospect of losing lives to avoidable infections is real, particularly during spikes in healthcare use which may overburden healthcare systems, compromise sanitation practices, and further exacerbate the emergence of antimicrobial resistance.

Gram-negative bacteria are frequently to blame for infections and are often difficult to treat in part due to the protective polysaccharide shell that surrounds their outer lipid membrane. Indeed, 8 of the 12 WHO Priority Pathogens are gram-negative. Within the gram-positive pathogens, *Staphylococcus aureus* is of particular concern, having developed high rates of resistance to the beta-lactam Methicillin throughout the world, giving rise to the moniker MRSA (Methicillin-Resistant *S. aureus*). MRSA's ability to survive on people and surfaces has allowed it to thrive and travel between hospital, community, and animal agriculture settings. Today, MRSA is one of the most prevalent infectious pathogens, with US infections topping

300,000 every year since 2005, leading to more than 10,000 deaths and \$1.7B in healthcare costs in 2017<sup>5</sup>. The Canadian Antimicrobial Resistance Surveillance System reported that the rate of MRSA infections within sentinel hospitals increased since 2015 from 2.8 to 3.17 cases per 10,000 patient-days in 2017<sup>6</sup>. During the same time period, community-associated MRSA infections increased by over 60%, while the rate of community-associated MRSA bloodstream infections (BSI) doubled<sup>6</sup>.

There is an urgent need for new antibacterial treatment options, and it is essential these new classes of antibiotics be developed and deployed with resistance in mind. This can be done by employing "broad spectrum" antibiotics that affect molecular targets found throughout the bacterial kingdom, by seeking multi-faceted molecular mechanisms that simultaneously perturb multiple targets, by deploying cocktails of antibiotics, each of which delivers a different effect at the same time, and by ensuring that drugs are effective against all bacterial states, including persisters and biofilms.

## Cannabinoids as Antibacterial Agents

In response to this aforementioned healthcare challenges, researchers have focused their efforts on evaluating cannabinoids as potential antibacterial agents against gram-negative and gram-positive bacteria. One of the first studies in this domain evaluated the antibacterial properties of delta-9-THC and

CBD against 7 different pathogens that included both gram-negative and gram-positive bacteria in nutrient broth agar and horse blood agar<sup>7</sup> (Table 1). The minimum inhibitory concentration (MIC) of THC and CBD required to prevent bacterial growth was reported as 1-5 µg/mL against gram-positive staphylococci and streptococci pathogens in broth. No effect against gram-negative pathogens was observed, suggesting that cannabinoids are unable to cross the bacterial outer membrane unassisted. Interestingly, the MIC was 10-fold higher in horse blood agar, possibly due to sequestration by plasma proteins. At 2–10 µg/mL, both cannabinoids were also found to be bactericidal against *S. aureus* when administered in saline solutions containing 4% horse serum.

Pathogen	Nutrient Broth Agar		Horse Blood Agar	
	THC	CBD	THC	CBD
<i>S. aureus</i>	2 – 5 µg/mL	1 – 5 µg/mL	20 – 50 µg/mL	20 – 50 µg/mL
<i>Streptococcus pyogenes</i>	5 µg/mL	2 µg/mL	50 µg/mL	50 µg/mL
<i>Streptococcus milleri</i>	2 µg/mL	1 µg/mL	50 µg/mL	50 µg/mL
<i>Streptococcus faecalis</i>	5 µg/mL	5 µg/mL	Not tested	
<i>E. coli</i>	> 100 µg/mL	> 100 µg/mL		
<i>Salmonella typhi</i>	> 100 µg/mL	> 100 µg/mL		
<i>Proteus vulgaris</i>	> 100 µg/mL	> 100 µg/mL		

**Table 1:** The MIC (ug/mL) of THC and CBD against various gram-positive (grey shading) and gram-negative (gold shading) pathogens in nutrient broth and horse blood agar<sup>7</sup>.

This early work suggested that THC and CBD possess both bacteriostatic (prevent further growth) and bactericidal (kill existing cells) properties against gram-positive bacteria but have no effect on gram-negative bacteria. These findings were in agreement with more recent research, which utilized in-vitro susceptibility models to assess the antibacterial activity of five major cannabinoids against gram-positive methicillin-resistant *S. aureus* (MRSA)<sup>8</sup>. In this work, the MIC of THC, CBD, CBG, CBC and CBN was reported as < 2 µg/mL (Table 2). This activity was found to be comparable to several commonly used antibiotics, suggesting that cannabinoids are potent antibacterial compounds when applied directly to the pathogen site, as is the case with topical administration. A separate study reported similar MIC values for the same cannabinoids against a different strain of MRSA (USA300), with the exception of CBC which had a higher MIC<sup>9</sup> (Table 2). The agreement between these two studies serves as promising evidence of the potential that cannabinoids offer as topical antibacterial agents.

Lastly, 10µM CBD was found to upregulate LL-37 cathelicidin, an antimicrobial peptide, in an immortalized human SZ95 sebocyte cell model<sup>10</sup>. This finding suggests that CBD, and potentially other cannabinoids, may possess antibacterial activity against virulent strains of *Propionibacterium acne*, which grow optimally when nestled in hair shafts next to sebaceous (sweat) glands and are responsible for a plurality of acne outbreaks.

Cannabinoid	ATCC259238	SA-1199B <sup>8</sup>	RN-4220 <sup>8</sup>	XU212 <sup>8</sup>	EMRSA-15 <sup>8</sup>	EMRSA-16 <sup>8</sup>	MRSA USA300 <sup>9</sup>
<b>CBD</b>	0.5 µg/mL	1 µg/mL	1 µg/mL	1 µg/mL	1 µg/mL	1 µg/mL	2 µg/mL
<b>CBG</b>	1 µg/mL	1 µg/mL	1 µg/mL	1 µg/mL	2 µg/mL	1 µg/mL	2 µg/mL
<b>CBC</b>	2 µg/mL	2 µg/mL	2 µg/mL	1 µg/mL	2 µg/mL	2 µg/mL	8 µg/mL
<b>THC</b>	1 µg/mL	2 µg/mL	1 µg/mL	1 µg/mL	2 µg/mL	0.5 µg/mL	2 µg/mL
<b>CBN</b>	1 µg/mL	1 µg/mL	1 µg/mL	1 µg/mL	1 µg/mL	Not tested	2 µg/mL
<b>Norfloxacin</b> (Fluoroquin.)	1 µg/mL	32 µg/mL	1 µg/mL	4 µg/mL	0.5 µg/mL	128 µg/mL	Not tested
<b>Erythromycin</b> (Macrolide)	0.25 µg/mL	0.25 µg/mL	64 µg/mL	>128 µg/mL	>128 µg/mL	>128 µg/mL	Not tested
<b>Tetracycline</b>	0.25 µg/mL	0.25 µg/mL	0.25 µg/mL	128 µg/mL	0.125 µg/mL	0.125 µg/mL	Not tested
<b>Oxacillin</b> (β-lactam)	0.125 µg/mL	0.25 µg/mL	0.25 µg/mL	128 µg/mL	32 µg/mL	>128 µg/mL	Not tested

**Table 2:** The MIC (µg/mL) of the five major cannabinoids against various drug-resistant strains of gram-positive *S. aureus*<sup>8,9</sup>. Strains include SA-1199B (multi-drug resistance, particularly for fluoroquinolones), RN-4220 (macrolide resistant), XU2-2 (tetracycline resistant), EMRSA-15 and EMRSA-16 (methicillin resistant) and ATCC25923 (standard lab strain). 4 antibiotics from the fluoroquinolones, macrolide, tetracycline, and penicillin families were included as benchmarks. The antibiotic that each strain is resistant to is highlighted in gold.

## Cannabinoids as Inhibitors and Eradicators of Bacterial Biofilms

While bacterial growth is a concern for bacterial spread, biofilm formation is regarded as a critical factor contributing to infection duration because of its heartiness in the face of traditional hygiene and subsequent accumulation on surfaces and in reservoirs, particularly in hospitals and other healthcare settings. In this context, one study explored the ability of THC, CBC, CBG, CBN, and CBC to inhibit MRSA biofilm formation, and to eradicate preformed biofilms of MRSA<sup>9</sup>. The results indicated that these cannabinoids were able to inhibit biofilm formation, and that this inhibitory activity was correlated with their antibacterial activity as measured by MIC.

In particular, CBG was identified as the most effective cannabinoid capable of inhibiting 50% of biofilm formation at 0.5 µg/mL. CBG was also able to eradicate pre-formed biofilms (minimum biofilm eradication concentration of 4 µg/mL) and kill MRSA persister cells (MIC of 5 µg/mL). Furthermore, MRSA did not develop resistance to CBG, even when treated with lethal concentrations of CBG ranging from 2 to 16X MIC and cultured for 15 days, an important finding in the context of antibacterial resistance. Collectively, these findings suggest that the antibacterial activity of CBG may extend beyond topical antibiotic applications and be suited for antibacterial coating applications for medical equipment, such as stents.

## Cannabinoids Against Gram-Negative Pathogens

While previous findings suggested that the antibacterial activity of cannabinoids was limited to gram-positive pathogens, a more recent study aimed to evaluate the effect of co-administration of a commonly used gram-negative antibiotic on cannabinoid anti-bacterial activity against gram-negative pathogens<sup>9</sup>.



This study determined the MIC of CBG, CBN, CBD, CBC, and THC against a laboratory strain of gram-negative *E. coli* following co-administration of sub-lethal concentrations of Polymyxin B, a topical antibiotic that disturbs the outer membrane characteristic to gram-negative bacteria and used in the commercially available topical ointment Polysporin®. In all cases, co-administration of Polymyxin B enabled the cannabinoids to act as effective anti-bacterial agents against the lab strain at MIC values of 1 µg/mL (Table 3). However, the dose of Polymyxin B required to enable this activity varied with each cannabinoid. In particular, CBC and THC required the lowest amount of Polymyxin B (0.032 µg/mL, or 13% the lethal dose) to prevent bacterial growth, while all other cannabinoids required twice the amount of Polymyxin B to achieve the same synergistic effect.

CBG was demonstrated as the most effective cannabinoid against biofilms and persister cells, and therefore was selected for further testing against other gram-negative pathogens (Table 3). Co-administration of sub-lethal concentrations of Polymyxin B proved to enable CBG's antibacterial activity against a clinical strain of *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii* at varying concentrations not exceeding 1 µg/mL.

Treatment	Lab Strain	Clinical Isolates			
	<i>E. coli</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>	<i>A. baumannii</i>
<b>Polymyxin B</b> (lethal dose)	0.25 µg/mL	0.3 µg/mL	1 µg/mL	0.3 µg/mL	0.5 µg/mL
<b>CBG</b> (Polymyxin B)	1 µg/mL (0.061 µg/mL)	1 µg/mL (0.061 µg/mL)	0.5 µg/mL (0.32 µg/mL)	1 µg/mL (0.32 µg/mL)	0.5 µg/mL (0.32 µg/mL)
<b>CBN</b> (Polymyxin B)	1 µg/mL (0.061 µg/mL)	Not tested			
<b>CBD</b> (Polymyxin B)	0.5 – 1 µg/mL (0.061 µg/mL)				
<b>CBC</b> (Polymyxin B)	1 µg/mL (0.032 µg/mL)				
<b>THC</b> (Polymyxin B)	1 µg/mL (0.032 µg/mL)				

**Table 3:** The MIC of CBG, CBN, CBD, CBC, and THC against various gram-negative pathogens when co-administered with sublethal-doses of Polymyxin B<sup>9</sup>. Lethal doses of Polymyxin B against each pathogen are listed in row 1.

### CBG as an In-Vivo Antibacterial Agent Against MRSA

When exposed to mammalian cells, CBG was hemolytic at 32 µg/mL (16x the bacterial MIC) as evidenced by the destruction of red blood cells, demonstrating a selectivity for bacterial cells at therapeutically relevant doses<sup>9</sup>. In a validation study, in-vivo results obtained from a mouse infection model demonstrated that a single dose of CBG at a concentration of 100 mg per kg body weight prevented MRSA infection, resulting in 1000-fold lower bacterial spleen levels, comparable to those observed following treatment with the positive control antibiotic vancomycin<sup>9</sup>. A more conservative dose of 25mg/kg CBG drove a 100-fold reduction in MRSA spleen levels. At all doses tested, no adverse effects or lethality was observed in the mouse population. This finding is the first to validate the in-vitro

results suggesting that the systemic antibacterial potency of CBG against MRSA cells is comparable to antibiotic treatments. However, further research is required to fully elucidate the mechanism of action and clinical utility of CBG and other cannabinoids as oral antibiotics with systemic efficacy.

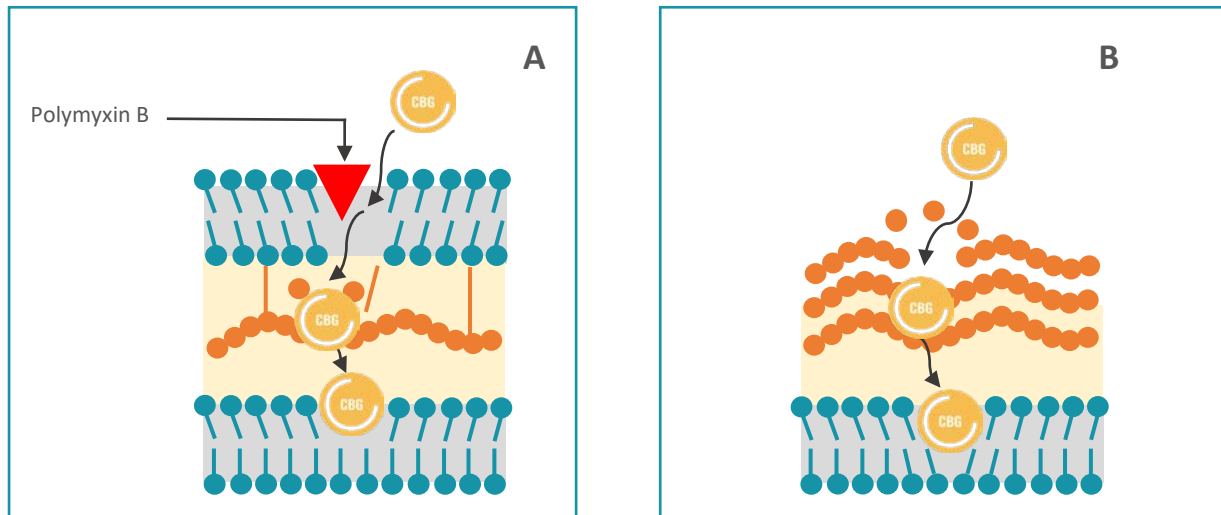
A review of CBG’s interactions with key cell surface receptors may offer further insights into the clinical utility of this cannabinoid. In particular, CBG is regarded as a fair TRPM8 antagonist (EC<sub>50</sub> 160 nM), a potent  $\alpha$ 2-adrenoceptor agonist (EC<sub>50</sub> = 0.2 nM), and a strong binder of the serotonin receptor 5-HT1A (KB = 51.9 nM)<sup>11,12</sup> (Table 4). The TRPM8 ion channel, also known as the menthol receptor, senses cold stimuli and mediates subsequent thermoregulation,  $\alpha$ 2-adrenoceptor agonists are employed as analgesics, sedatives, and anxiolytics, and 5-HT1A is key to inhibitory neurotransmission<sup>13,14</sup>. These interactions are being actively researched and may represent synergistic targets in certain applications as well as relevant biomarkers when developing products.

Target	Target Biology	Effect	Potency	CBG Purity	Test System
$\alpha$ 2-adrenoceptor <sup>12</sup>	Vascular modulation via (nor)epinephrine	Potent Agonist	EC <sub>50</sub> = 0.2 nM	Unknown	Ex Vivo Mouse Tissue
5-HT1A receptor <sup>12</sup>	Neuromodulation via serotonin	Antagonist	KB = 51.9 nM	Unknown	Ex Vivo Mouse Tissue
TRPM8 channel <sup>11</sup>	Thermoregulation – cold stimuli	Antagonist	EC <sub>50</sub> = 160 nM	≥95%	In Vitro Cell Line
TRPA1, TRPV1, TRPV2 channels <sup>11</sup>	Somatosensory – pain, itch, heat, cold	Weak Agonist	EC <sub>50</sub> = 700 nM, 1.3 $\mu$ M, 1.72 $\mu$ M	≥95%	In Vitro Cell Line
COX 1/2 enzymes <sup>15</sup>	Generation of inflammatory prostanoids	Negligible	300 $\mu$ M / 25 $\mu$ M	≥92% (botanical extract)	In Vitro Enzyme Assay

**Table 4:** CBG receptor pharmacology. EC<sub>50</sub> is defined as the concentration required to induce a 50% maximal response. KB is the apparent dissociation constant, the concentration of ligand at which 50% of receptors are bound.

## Mechanism of Antibacterial Action

It has been proposed that cannabinoids’ mechanism of action against gram-positive bacteria is disruption of the cytoplasmic membranes (Figure 2). Correspondingly, the MIC of CBG, THC, CBD, CBC and CBN against gram-negative E. coli when co-administered with sub-lethal concentrations of Polymyxin B, a topical antibiotic that disturbs the outer membrane of gram-negative bacteria, was 1  $\mu$ g/mL, comparable to their activity against gram-positive bacteria<sup>9</sup>.



**Figure 2:** Schematic of cannabinoid mechanism of antibacterial action against gram-negative (A) and gram-positive (B) bacteria, with CBG as an example. CBG weakens the cytoplasmic membrane of gram-positive bacteria. For gram-negative bacteria, a chemical perturber is required to disrupt the protective outer lipid membrane and enable CBG to reach the cytoplasmic inner membrane<sup>9</sup>.

It is possible that different cannabinoids employ unique mechanisms of action against gram-negative pathogens. For instance, one study reported that 1  $\mu\text{m}$  and 5  $\mu\text{m}$  of CBD inhibited the release of membrane vesicles from gram-negative *E. coli*, with a stronger inhibitory effect observed at the lower dose<sup>16</sup>. Furthermore, CBD altered the protein profile of released membrane vesicles, with marked changes among proteins associated with metabolic processes, cellular respiration and antibiotic functions.

Membrane vesicles are produced and secreted predominately by gram-negative bacteria as part of their stress response and play a crucial role in bacterial survival through mechanisms such as inter-bacterial communication, toxin release, and biofilm formation. They are also known to promote antibiotic resistance through shielding of bacterial biofilms. As thus, in the context of antibacterial treatments, inhibition of membrane vesicle release presents a viable target for reducing bacterial biofilm formation, pathogen-host interactions, and antibiotic resistance.

Further work is required to confirm if these mechanisms of action are common to other cannabinoids beyond CBG and CBD, or if other antibacterial modalities exist. This knowledge will enable the development of novel cannabinoid cocktails that, when combined with antibiotics, may render highly effective antibiotic treatments against gram-negative pathogens when applied as topical formulations.

The cannabinoids described thus far represent a small subset of naturally occurring cannabinoids. Small structural differences can lead to large changes in cannabinoid functionality. In the context of antibiotic research, few studies have explored these variants. To this end, two separate studies corroborated a structure-function relationship between cannabinoid structure and antimicrobial potency. The first study

tested several CBD variants and found that they were less potent than CBD against MRSA<sup>8</sup>. A second study further evaluated the antibacterial and antifungal activity of CBC, as well as 2 of its synthesized homologs and isomers against a range of bacteria and fungi<sup>17</sup>. In general, CBC outperformed its variants and the positive antibiotic control, with the exception of one variant which was more effective against *B. Subtilis*. All cannabinoids displayed mild antifungal activity that was inferior compared to Amphotericin B as a control. This limited data highlights the need for further exploration of numerous cannabinoids that can translate into diversified and innovative product pipelines of both topical and oral antibiotics.

## LAVVAN's Cannabinoid Solutions

At the forefront of cannabinoid cellular agriculture, LAVVAN utilizes yeast fermentation technology to produce high-quality, reliably sourced, natural cannabinoid ingredients. LAVVAN will provide cannabinoids with unparalleled purity, consistency, potency and sustainability at a scale capable of serving a range of industries including health, beauty, food and beverage, and pharmaceuticals. LAVVAN's cannabinoids are identical to those found in nature, and produced in a cGMP facility in accordance with the most stringent standards, including being devoid of pesticides, mold, bacteria, and other contaminants often found in traditional cannabis agriculture. In addition to providing high purity cannabinoid ingredients, LAVVAN will leverage its cannabinoid formulations expertise to support its industry partners with integrating cannabinoids into formulations for various end products that require specific utility.

## Conclusions

Current scientific evidence suggests that major cannabinoids THC, CBD, CBG, CBN, and CBC possess potent activity against gram-positive bacteria, as well as gram-negative bacteria when supplemented with outer membrane penetrating agents. CBG presents superior activity in the real-world applications of biofilm inhibition, biofilm eradication, and treatment of infection at concentrations well below toxic levels that are easily achieved in topical formulations with utility in OTC and acute care clinical settings. While these results are promising in the context of topical antibiotic applications, further in-vivo work is required to determine the systemic antibiotic efficacy of cannabinoids when administered orally.

Cannabinoids are uniquely positioned to circumvent antibiotic resistance for three key reasons. First, unlike the majority of antibiotics in development, which target a specific molecule with high affinity and impose a selective pressure on that molecule to mutate, cannabinoids appear to disrupt the bacterial inner membrane in a distributed fashion that involves multiple interactions, such that a single mutation is unlikely to confer resistance, while the many mutations required for resistance would be highly unlikely to occur<sup>18</sup>. Secondly, given the efficacy of multiple cannabinoids, cocktails of cannabinoids may be optimized for particular applications to further reduce the likelihood of resistance. Lastly, as evidenced with the outer membrane disruptor Polymyxin B, cannabinoids can be synergistically combined with low levels of existing antibiotics, thereby reducing their use and flattening the curve of resistance against these front-line drugs.

Crucially, the existing pool of antibiotic cannabinoids – together with the barely explored minor cannabinoids – form an extensive and coherent pipeline that awaits development. The current research suggests that cannabinoids are well-suited antibiotic and sanitizing agents when applied topically,

particularly against gram-positive pathogens, while action against gram-negative bacteria is enabled by co-administration of a secondary antibiotic. Ongoing in-vitro and in-vivo work will undoubtedly further our understanding of the topical and systemic antibiotic properties of cannabinoids and cement their utility in the global fight against antibiotic resistance.

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