JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

ANTILISTERIAL ACTIVITY OF CBD FOR THE PREVENTION OF *LISTERIA MONOCYTOGENES* IN DAIRY PRODUCTS

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https://doi.org/10.55251/jmbfs.10659

ARTICLE INFO

Received 14. 10. 2023
Revised 11. 10. 2024
Accepted 16. 10. 2024
Published xx.xx.201x

Regular article

OPEN ACCESS

ABSTRACT

Research background. Herbal antimicrobials exist in plants and their derived compounds. One such compound is cannabidiol (CBD) extracted from the plant, *Cannabis Sativa*, also referred to as hemp or marijuana, has drawn interest for its alleged antibacterial and antioxidant abilities. With the increasing problem of antibiotic resistance and the desire to reduce antibiotics in the food industry, researchers are exploring the potential of cannabinoids as an alternative antimicrobial agent. The use of cannabidiol (CBD) in food and beverage products is a growing trend, it is also important for manufacturers to approach this new area with more studies. Milk is a key component in the production of most dairy produce. The products made from milk usually undergo various processes such as pasteurization, fermentation, curdling, and aging to create the final product. This study aims to examine the potential of CBD isolate as a antimicrobial agent which can be used in dairy products to reduce microbial growth and extend their shelf life.
Experimental approach. This paper investigated the antilmicrobial properties of CBD against *L. monocytogenes* in milk as the key solvent to perform studies. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of CBD in TSB was carried out at 37 °C in 24 h. CBD's antilisterial activity was found for whole milk and skim milk at 4°C for 3 days by analyzing their respective growth and kill curves.
Results and conclusions Both the MIC and MBC for *L. monocytogenes* was found to be 2 µg/mL measured by dilution series and plating, respectively. CBD significantly slowed the growth of listeria populations in milk but its effectiveness of CBD was dependant on the fat content of milk. In this paper, we have discussed the potential for using and improving CBD as a preservative to combat *L. monocytogenes* in dairy products that use milk as a primary ingredient.

Keywords: CBD, *Listeria Monocytogenes*, antimicrobial studies, dairy produce

INTRODUCTION

The Food and Drug Administration (FDA) has currently published a listeria infection epidemic that has been linked to ice cream produced in 11 states **(Center for Food Safety and Applied Nutrition. U.S. Food and Drug Administration, 2019).** Unlike other bacteria, *L. Monocytogenes* can survive freezing temperatures and other preservation techniques. People who consume food contaminated with L. monocytogenes pose the risk of developing the condition known as listeriosis**(Center for Food Safety and Applied Nutrition. U.S. Food and Drug Administration, 2022).** Listeriosis are rare but can be fatal to pregnant women, infants, and adults with compromised immune systems**(Center for Food Safety and Applied Nutrition. U.S. Food and Drug Administration, 2022).** High-risk foods involve ready-to-eat seafood, pre-prepared or pre-packaged fruit and vegetable salads, deli meats, unpasteurized milk or dairy produce from contaminated milk, ice-creams, cheeses – such as brie, camembert, ricotta and feta, dips and salad dressings **(Center for Food Safety and Applied Nutrition. U.S. Food and Drug Administration, 2022)**. With the increasing problem of microbial antibiotic resistance and the desire to reduce antibiotic use in the food industry, alternative antimicrobial methods have become a focus of research and development. The majority of ongoing research is to ensure food security without sacrificing the flavour and nutrition of foods **(Gandhi *et el.*, 2007)**. Herbal antimicrobials exist in plants and their derived compounds. One such plant is the cannabis sativa plant, also referred to as hemp or marijuana, has drawn interest for its alleged antibacterial and antioxidant abilities. The *derivatives consists of C. Sativa* majorly of the intoxicating compound tetrahydrocannabinol (THC), then non-psychotropic cannabidiol (CBD) and low amounts of cannabinol (CBN) **(ElSohly *et el.*,2005)**. CBD has garnered significant attention due to its diverse range of therapeutic and pharmacological properties **(Fernández-Ruiz *et el.*,2013)**. CBD is a non-psychotropic compound, has a safe profile in terms of cell viability, and it does not appear to cause significant damage to healthy cells even at therapeutic doses **(Pagano *et el.*,2020)**. The availability of diverse flavours and the legalisation of cannabis for medical or recreational purposes are major drivers of the cannabis food and beverage market's expansion in North America and Europe. Many countries including Canada, allow manufacturers to produce and sell food / drinks that are infused with cannabinoid extracts such as baked goods, candies, chocolates, lozenges and beverages so long as they have a licence from Health Canada **(Province of Manitoba – Agriculture, n.d)**. While hemp-derived cannabinoid extracts are well known for their therapeutic and psychological effects, many still fail to realize that hemp extracts may have nutritional benefits and anti-microbial capacities. Research on the antimicrobial properties of cannabidiol (CBD) has shown promising results, including its ability to inhibit the growth of various Gram-positive bacteria, including drug-resistant strains **(Blaskovich *et al.*,2021)** making it an excellent candidate for inhibiting bacterial growth in food and drinks.

Several dairy food chains, including the renowned ice cream brand Ben & Jerry's, have declared their new innovation of integrating cannabidiol (CBD) into their ice cream **(Helmore, 2019)**. MariMed, a cannabis company, partnering with Emack & Bolio's will create a lineup of cannabis edibles, which includes various products infused with cannabis, such as chocolates, gummies, beverages, and now, ice cream **(Canning, 2021)**. These companies along with many others intend to make CBD-infused dairy produce. Many countries have been using CBD infused in dairy for centuries, e.g. CBD infused dairy in India called bhang. It is a blend of the marijuana plant's buds, leaves, and flowers, added to food and beverages at religious ceremonies for centuries (**H, R.B., 2023**). Female cannabis leaves, buds, and flowers are ground into a paste and is then mixed with milk (lassi), ice cream (kulfi) and dairy weets (laddoos). Bhang has been taken in India for generations. That being said, cannabis has already developed a large market potential for milk products and more research needs to carried out in order to speed up legalizing processes. In this paper, we have discussed the potential for using and improving CBD as a preservative to combat *L. monocytogenes* in the dairy environment. Since CBD flavored milk and its products has an increase in demand and companies are awaiting licensing approvals this novel study will bring light to dairy food preservation using CBD. The capacity of CBD to inhibit *L. monocytogenes* growth and induce bacterial cell death has not been previously investigated in scientific studies up our knowledge. There are no past papers on cannabinoids as antilisterial agents and studies on *L. Monocytogenes* need to be performed. *L. monocytogenes*

is bacterium that cultivate in dairy products even under refrigerated temperatures. Milk is the primary ingredient for several dairy products like icecream, yougurt, cheese and many more which is why for simplicity we used milk as the primary medium for our studies. So the objective of this paper was to study the antilisterial activity of CBD in Tryptic Soy Broth (TSB), whole and skim milk at over a range of temperatures.

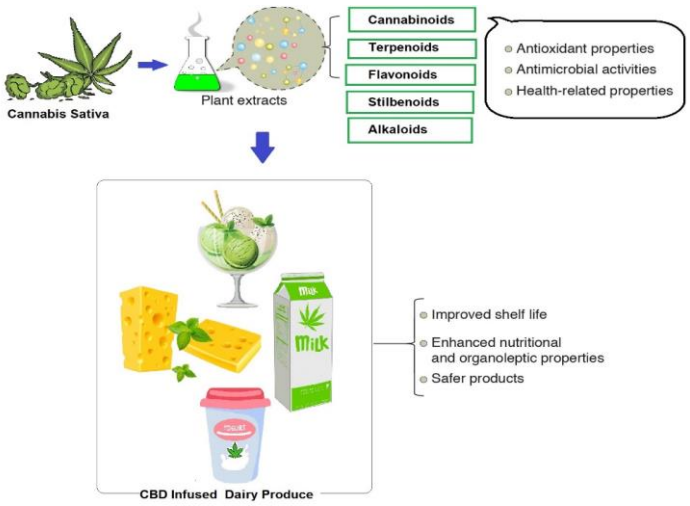

Figure 1 Application of CBD in dairy produce

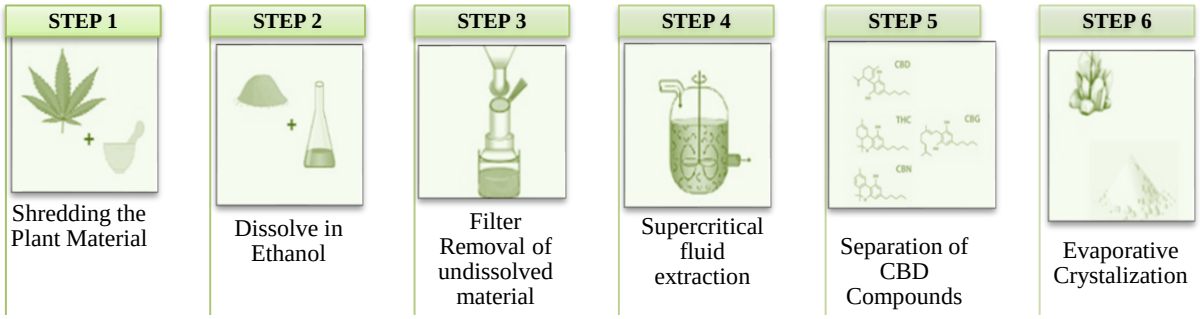

Figure 2 Industrial Extraction of CBD

MATERIAL AND METHODS

CBD Extraction

Using a similar extraction method as in ***(*Aari *et el., 2017)***, industrial samples of CBD isolate and oils were kindly provided by Mera Cannabis Corporation, St. Thomas, Canada. Their industrial extraction process as shown in Fig 2, included shredding the plant material, soaking it in ethanol to create a mixture of polar solvent and cannabinoids, further filtering the mixture to remove any remaining solid plant material, and then subjecting the mixture to a supercritical fluid to segmentate and purify into individual compounds of cannabinoids. The extracts produced a 95% CBD orange-colored oil. Highly purified CBD powder was further refined by evaporative crystallization of CBD.

Characterization of CBD Extracts

Purity analysis of the CBD crystal samples was measured by ^{1}H NMR. The determination of cannabinoids of interest was achieved by peak evaluation of our sample CBD sample to literature **(Barthlott *et el.*,2021)** as measured using ^{1}H and ^{13}C NMR spectroscopy, Bruker Neo 600.

Antimicrobial Activity of CBD in TSB

Bacterial Strains and Culture

The *L. monocytogenes* EGD strain is from the Trudeau Institute (NCTC7973) supplied from the Heinrichs lab stock. The media used were broth (TSB) and nutrient agar (NA) medium, which were purchased from Sigma Aldrich Canada. The lab stock bacterial culture was grown in TSB for 24 hours at 37°C. The stock culture was streaked onto agar plates to isolate individual colonies. One colony was transferred to TSB (Lysogeny Broth) and incubated for 28 hours at 37°C. Fresh cultures for the experiments were prepared using a small amount of the pure culture that had been incubated into standardized Optical Density, OD_{600} 1 sample and diluted in the 3 mediums : whole milk, skim milk and TSB respectively to obtain a bacterial concentration of between 2 and 5×10^{5} CFU/mL.

Antilisterial Treatment

Antilisterial compositions were as follows: Isolate Low Dose (0.2 mg/mL CBD); Isolate High Dose (1 mg/mL CBD) and Oil Based (20 µL CBD oil, 1 mL canola oil). The crystalized cannabis powder extracts of respective doses were dissolved in 1mL solvent Dimethyl Sulfoxide (DMSO) and sonicated. The orange oil extracts obtained before crystallization were of lesser purity (95%), 20 µL, were dissolved in pure canola oil (Saporito foods). The difference in antimicrobial effect of isolated CBD of higher purity and oil based CBD of lower purity will be observed.

Bacteriostatic Activity

MIC was measured as described in the literature **(Elshikh *et al.*, 2016)** with a small modification. The test strain were removed from the freezer at −80 °C and followed the procedure given in the previous section for the inoculum to be OD_{600} 1. The MIC of CBD isolate was found through the dissolution of the particles in DMSO to create varying concentrations. To begin with, a solution of different solutions were made by the CBD/DMSO solution to 2 mL of TSB. This prepared sample was then diluted to make concentrations of 2, 1, 0.5, 0.25, 0.1, 0.06 µg/mL CBD and a no CBD control, using the two-fold series of. 10µL of L. monocytogenes EGD at an OD_{600} 1 was added to each concentration of CBD sample, making a starting sample of OD_{600} 0.01. After incubation and shaking for 24 hours, the OD of each sample was measured using a 96 well plate reader from Biotek instruments (USA), and the MIC was determined as the lowest concentration with no discernible growth. Column 11 contained DMSO as control and column 12 contained only TSB. A concentration–effect of the preservatives was observed and the statistical significance between the groups of different concentrations were recorded. The experiment was conducted in triplets, with the mean result being used and plotted using GraphPad Prism software (California, USA).

Bactericidal Activity

The Minimum Bactericidal Concentration for CBD isolate was also determined by preparing a solution in DMSO. Prepared CBD samples of concentrations ranged from 2-0.06 µg/mL was tested using two-fold dilution series in TSB. L. monocytogenes was added to each sample at an initial OD_{600} 0.01. The samples underwent incubation at 37 °C for 24 hours. After incubation the samples were platted, and their respective colonies were counted and recorded. The MBC was defined as the least concentration of CBD which cause 99.9% of the original inoculum perished and no colony was observed when 10µL of the TSB sample was plated and incubated. The experiment was replicated in three distinct trials, and the outcomes were graphed using GraphPad Prism (California, USA).

Zone of Inhibition Studies

Zone of Inhibition (ZOI) studies were carried out using the oil-based CBD extracts and isolated CBD to compare their antimicrobial potency. Dissolving CBD oil-based extracts in canola oil posed challenges when attempting to dissolve them into the broth solution for dilution and conduct MIC studies. The solubility challenge was addressed by employing the disk diffusion method to assess CBD oil. Agar plates treated with a standardized OD_{600} 1 inoculum of L. monocytogenes EGD. Next, filter paper discs with a diameter of 6 mm, each loaded with 10 µl of a CBD oil/canola mixture at a concentration of 20 mg/mL, were placed into agar surface. Subsequently, the petri dishes were subjected to overnight incubation at a temperature of 37 ºC. The antimicrobial properties of CBD diffused into the agar media and hindered the growth of the microorganism, which was quantified by measuring the diameter of the inhibition growth zone. The measured inhibition zones were recorded in mm on the agar plates. For control, the solvents, DMSO and canola oil, were used. For statistical analysis of ZOI experiments, Microsoft Office-Excel (2013) was used in the calculation of averages and standard deviation.

Anti Microbial Activity of CBD in Milk

The antimicrobial activity of CBD was compared in skim (0.3% fat) and whole milk (3.25 % butter fat) following the same procedure as given in Section 2.3.3 and 2.3.4 for TSB. Powdered milk was prepared by adding sterilized water to whole milk powder and skim milk powder bought from Medallion whole milk powder - dried milk from Canada (free of antibiotics). Utilizing sterile milk samples, different CBD concentrations were formulated for assessment and inoculation purposes. In accordance with the MIC determined in TSB beforehand, the subsequent CBD concentrations were examined in milk: 0.2 mg/mL and 1 mg/mL. Control test tubes containing 1 mL of DMSO and others with neither DMSO nor CBD were included.
The solutions were created by injecting 200 µl of the prepared CBD/DMSO mixture into the test tubes containing sterile milk. These tubes were then vigorously vortexed to ensure complete dissolution. After 10µL of Listeria at an OD_{600} 1 was added to final milk solutions of 0.2 mg/mL and 1 mg/mL , the tubes were incubated at 4 °C for 3 days and another group was incubated at 37 °C for 24 h; the experiments were carried out in triplicate. Negative control samples with no CBD were always used as reference. The count of the remaining population was determined by placing 10 µl of the sample on TSA plates at intervals of 0, 1, 2, and 3 days for the tubes incubated at 4 °C, and after a 24-hour period for the tubes incubated at 37 °C. The growth curves were established by graphically representing the logarithm of CFU/mL against the time using GraphPad Prism software (California, USA) .

Statistical Analysis

All experiments were replicated three times, and the mean of the results were presented along with the standard deviation (SD). Statistical analysis of the data was conducted using Dunnett's Multiple Comparison test within an Analysis Of Variance (ANOVA) framework in GraphPad PRISM Software (Origin 9.5.1 Version). Statistical significance was considered when $p < 0.05$. The significant differences between the treated groups with CBD and non CBD (indicated as $P < 0.05$) were graphically represented. CBD and non CBD groups are expected to be statistically significantly different from each other, and CBD concentrations should have an effect on the log CFU of the cells in both TSB and milk for it to be considered effective as a preservative. For the results of ZOI experiments, we used statistical software Microsoft Office-Excel (2013) in the calculation of averages, error, and standard deviation

RESULTS

Quality and purity of CBD Extracts

As the leaf extracts gave an orange-colored oil of 95% CBD, which was further purified by evaporative crystallization to produce a CBD purity of >99%. To verify the high quality of the industrial CBD crystal samples, the CBD fractions were tested through Shimadzu HPLC (Kyoto, Japan) in Charpentier's laboratory. The quantification of the desired cannabinoids was accomplished by comparing the peaks and integration values of the sample CBD to those documented in the literature **(Barthlott *et al.*,2021)**. NMR spectrum in Fig. 3 showed peaks corresponding to [M]+ and [M-C6H11]+ at m/z values, which are compatible with the known cannabinoid structure. In addition, the ^{1}H-Nuclear Mangentic Resonace Spectroscopy (NMR) and ^{13}C NMR spectrum of CBD in deuterochloroform shown in Table 1 assigned the peaks that corresponded to its structure in Fig 3. It is also noted that no other peaks were observed that did not correspond to the CBD compound. This suggests that the sample of CBD used was 99.9 % pure and did not contain significant impurities or contaminants that might have contributed to the observed NMR signals. This suggested that the NMR spectra of CBD are consistent with its known chemical structure and properties.

Table 1 ^{1}H and ^{13}C-NMR peak assignments for cannabidiol in $CDCl_3$

Position	^{1}H NMR	^{13}C NMR
1	3.87 (1H, br. d, 9.1 Hz)	37.1
2	5.58(1H, br. s)	124.1
3		140.0
4	2.12 (1H, m), 2.24 (1H, m)	30.4
5	1.83 (2H, m)	28.4
6	2.41 (1H, m)	46.1
7	1.80 (3H, s)	23.6
8		149.2
9	4.57(1H, s, cis), 4.67(1H, s, trans)	110.8
10	1.67 (3H, s)	20.4
1’		113.7
2’		153.8
3’	6.30 (1H, br. s)	109.7
4’		143.0
5’	6.17 (1H, br. s)	107.9
6’		156.0
1”	2.45 (2H, m)	35.5
2”	1.57 (2H, quin, 7.48 Hz)	30.6
3”	1.30 (2H, m)	31.5
4”	1.32 (2H, m)	22.5
5”	0.89 (3H, t, 7.04 Hz)	14.0
2’-OH	6.01 (1H, br. s)	
6’-OH	4.82 (1H, br. s)	

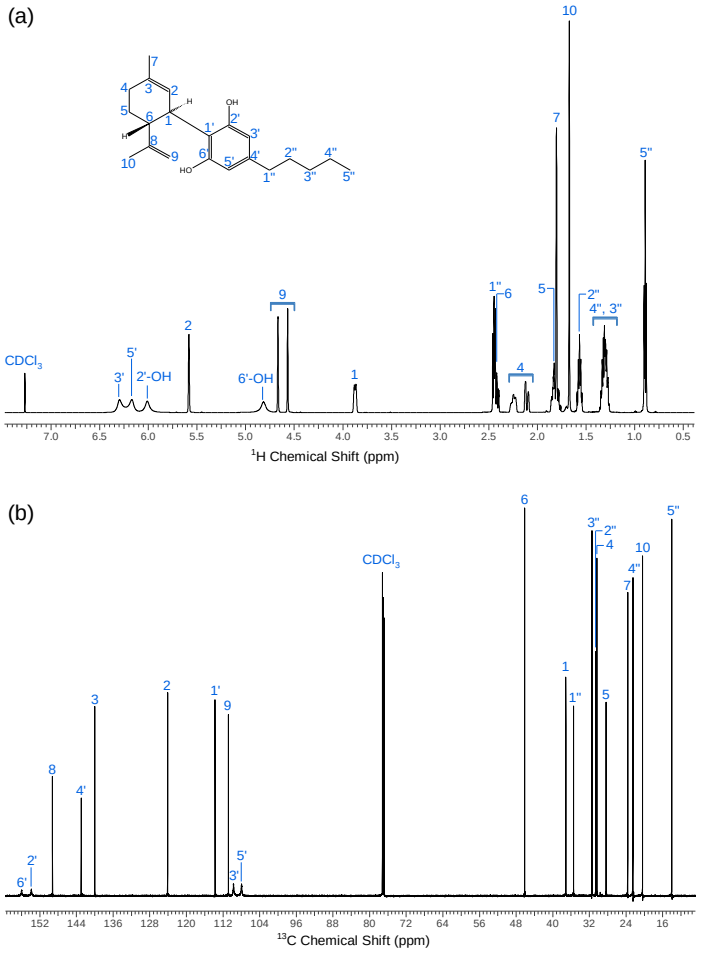

Figure 3 (a) ^{1}H and **(b)** ^{13}C NMR spectra of cannabidiol in $CDCl_3$.

Minimum Inhibitory Concentration in TSB

Table 2 MIC & MBC of *L.Monocytogenes* in TSB of CBD (n = 4) on TSB/TSA after 24 hrs at 37 ºC

MIC	MBC
2 µg/mL	2 µg/mL

The minimum inhibitory concentration (MIC) of CBD against *L. Monocytogenes* (EGD) was determined in TSB. We used a one-way ANOVA test running Dunnet's multiple comparisons by PRSM, to find the significant difference of the control column (DMSO only) and the different concentrations of CBD columns in TSB. We saw changes in OD value accounting for growth in all concentrations treated

with CBD except for 2 µg/mL. After comparing the mean of OD readings of each column with control column, the P values are shown in Fig. 4. The highest p – value ($P < 0.0001$) was found when comparing 2 µg/mL of CBD group with the DMSO reading. On the other hand, there is no statistical significance found when when comparing 0. 25 µg/mL of CBD group with the DMSO group. Overall, CBD acted as a good growth inhibitor as the minimum inhibitory concentration(MIC) was found to be 2 µg/mL as it corresponds to the group with the highest significance difference when compared with the control as show in Fig. 4.The presence of CBD appears to have a notable effect on the growth and that it was concluded that the CBD concentration has an inverse relationship with the growth of Listeria populations.

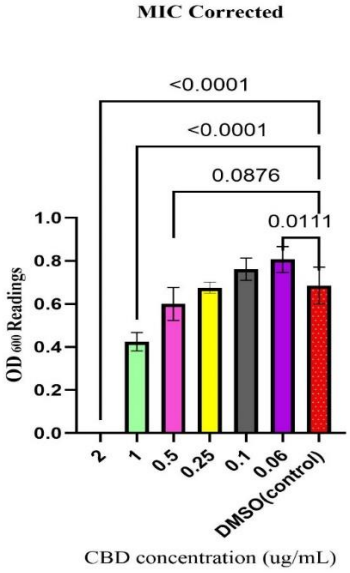

Figure 4 Corrected O.D readings of groups of different concentrations of CBD and their corresponding significant difference (P values) with the Control.

Zone Of Inhibition based on CBD Quality

To evaluate the changes in the growth for CBD of different purities, their inhibition zone were measured with calibration to 0.1 mm. Due to the low solubility of the oil based CBD (95 % purity) in TSB, it was difficult to carry out other antimicrobial tests apart from disk diffusion studies. The antimicrobial effectiveness of CBD and its respective solvents was assessed, and the outcomes were documented in Table 3. The findings indicate that the solvents, namely DMSO and canola oil, did not significantly impact the antimicrobial efficacy of CBD extracts. Among the tested substances, CBD oil exhibited the most potent inhibitory activity, with an average inhibition zone diameter [DIZ] of 8.6 ± 0.41 mm, in contrast to CBD isolate, which had a [DIZ] of 6.5 ± 0.4 mm. The DIZ investigations revealed that CBD oil displayed a greater potency in inhibiting Gram-positive Listeria compared to CBD isolate. CBD oil has small amounts, 5 %, composed of terpenes, flavonoids, and different cannabinoids like cannabigerol (CBG) and cannabinol (CBN). Hemp essential oils or terpenes such α-Pinene α-Terpinolene, β-Caryophyllene, β-Pinene, β-Myrcene have showed antibacterial activity against Listeria monocytogenes. This shows that terpenes have the ability to limit or prevent bacterial multiplication, so lowering the chance of microbiological contamination, particularly in the food industry **(Iseppi *et al.*, 2019).**
Thus, CBD with low purity benefits the synergistic effect, which occurs when different cannabis chemicals are combined, enhances the antimicrobial effects of the plant. CBD isolates may be useful for people who are very sensitive to other compounds in the plant but will not benefit from the synergistic effect.

Table 3 ZOI of CBD (n=3)

Isolated Compound	Zone of Inhibition of CBD against Listeria-EGD, (Average ± SD)
CBD (powder)	6.5 ± 0.4 mm
CBD (oil)	8.6 ± 0.41 mm
DMSO (control)	None
Canola (control)	None

Legend: CBD – cannaboidal, DMSO – Dimethyl sulfoxide, n – number of replications, SD – standard deviation

Minimum Bactericidal Concentration

The MBC, which was recorded as 2 µg/mL, reflecting the lowest value at which no bacterial colonies could be seen. While not completely eradicated in the case of the 1 µg/mL sample, the quantity of colony-forming units (CFUs) was noticeably decreased, as seen in Fig. 5. In this case, the MBC of CBD was found to be 2 µg/mL, which means that this concentration was effective in killing all the bacteria in the sample.
We used a one-way ANOVA test running Dunnet's multiple comparisons by PRSM, to find the significant difference of the control column (DMSO only) and the different concentrations of CBD columns in TSB. We recorded the changes in counts of the colonies accounting for cell death respective to the concentrations of CBD. There was no colony observed at 2 µg/mL. After comparing the mean of the colony counts/ mL of each column with the control column, the P values are shown in Fig. 5. The highest p – value ($P < 0.0001$) was found when comparing 2 µg/mL of CBD group with the DMSO reading. On the other hand, there is no statistical significance found when when comparing 0. 06 µg/mL of CBD group with the DMSO group. The presence of CBD appears to have a notable effect on the death of populations and that it was concluded with a CBD concentration of 2 µg/mL it was possible to completely eradicate Listeria populations.
The exact mechanism by which CBD exerts its antimicrobial effects is not yet fully understood. One potential mechanism is CBD's interaction with the bacterial cell membrane. CBD has been shown to disrupt the integrity of bacterial membranes, which can lead to leakage of cellular contents and ultimately bacterial death (Blaskovich et al.,2021). Concentrations near the MIC levels hindered multiple cellular processes in bacteria, such as the synthesis of proteins, DNA, RNA, and peptidoglycan. This aligns with a fast-acting bactericidal effect that effectively disrupts all synthesis pathways (Blaskovich et al.,2021) .

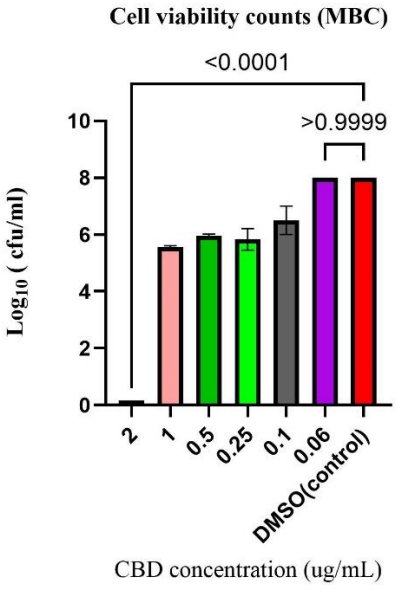

Figure 5 Kill curve of *L. monocytogenes* treated with isolated CBD. The logarithm of colony counts per ml of groups of different concentrations of CBD and their corresponding significant difference (P values) with the Control.

Anti-Microbial Activity of CBD in Milk

In order to evaluate the effect of CBD on Listeria populations in skim milk and whole milk, we needed to count the log colony forming units per mL on each day, which are indicators of food preservation. To test this, CBD extract was added to skim milk and whole milk and stored at 4°C for 3 days as shown in Fig 6(a) and 6(b). Our findings revealed that the growth rate of Listeria in both treated milk samples was slower than in the control samples for each day. As expected, the concentration of 1 mg/mL showed a slower growth curve than 0.2 mg/ml in both skim and whole milk. We can conclude that higher concentrations of CBD could show a greater effect as a preservative for longer storage intervals. Fig 6(b) suggests that while CBD may have had some initial antimicrobial effect in whole milk, it did not result in a significant reduction in Listeria populations over the course of the 3-day storage period. On the other hand, effect of CBD in skim milk showed a much significant slower growth rate in Listeria populations over the course of the 3-day storage period. In skim milk, the growth rate reduction after 24 hrs treated with CBD of concentration 0.2 mg/mL was 90 % less whereas for 1 mg/mL it was 99 % reduced compared to the DMSO control. After 48 hours, the growth rate reached the same value for 0.2 mg/mL in skim milk but for 1 mg/mL it was still 90 % slower. To compare the growth curve of control column (DMSO only) and the growth in skim milk and whole milk for different days, we conducted a one-way ANOVA test with the PRISM tool. Based on the results in Table 4, we found a p-value of 0.0018 for skim milk, denoted by **, with CBD compared to control (DMSO only). A p-value of 0.0494 for whole milk with CBD compared to control (DMSO only) denoted by *.

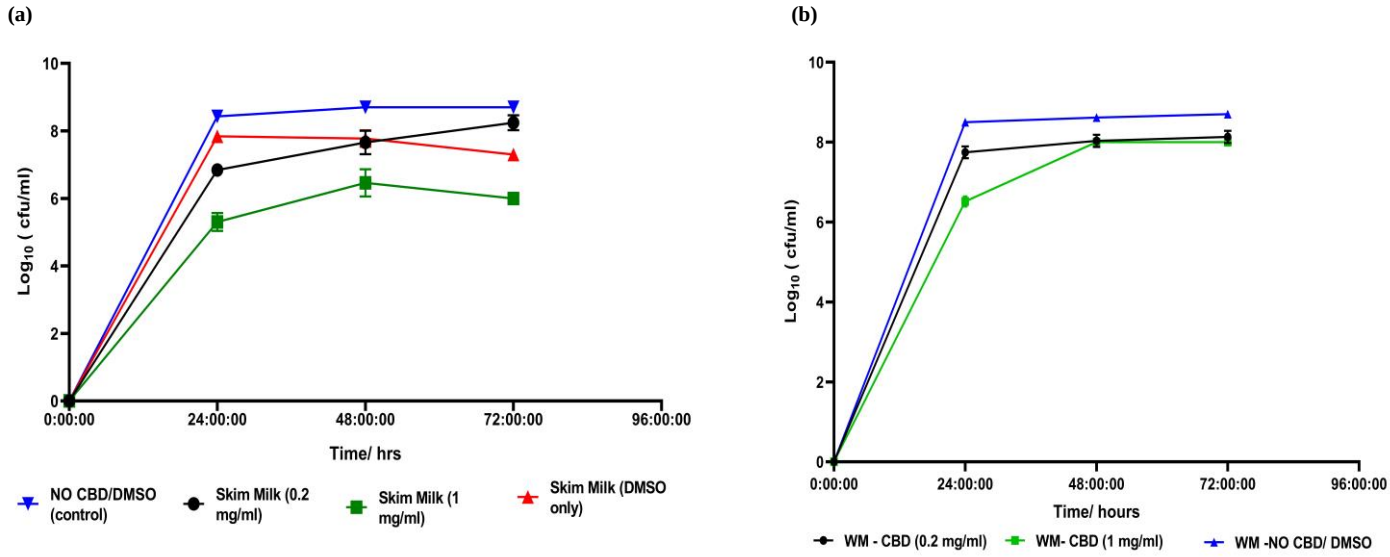

Figure 6 Growth curves of L. monocytogenes at 4 °C in (a) semi-skim and (b) whole milk in 3 days.

A higher p-value was generally considered statistically significant, which means that the probability of obtaining the observed difference by chance alone is less than 5%. Therefore, the result for skim milk with CBD is more statistically significant than the result for whole milk with CBD. Based on the ANOVA test results, we can conclude that CBD had a more significant effect on cell growth in skim milk than in whole milk.

Table 4 ANOVA Analysis of Different Milks treated with CBD compared with Control (n = 3)

	Whole Milk ANOVA Test	Skim Milk ANOVA Test
F	4.280	13.91
P value	0.0494	0.0018
P value summary	*	**
Significant diff. among means (P < 0.05)	Yes	Yes

ANOVA test of each type of milk was compared with Control Column only

The reduced antibacterial potency of CBD in whole milk can be explained by its interaction with the fat molecules present. CBD is considered a lipophilic molecule, indicating its natural affinity for fats and oils. Due to this lipophilicity and CBD's inherent instability, when exposed to high-fat content milk, the compound tends to partition into the lipid-rich fat phase. In whole milk, the higher fat content may have bound more of the CBD, reducing its availability to interact with the bacteria. This can significantly reduce the resultant dose of the antibiotic at the site of infection.

CONCLUSION

CBD contains a number of properties that make it an excellent antibacterial agent. Nonetheless, the usage of CBD shows intriguing potential, and given the development in antibiotic resistance, it is critical to investigate its use as a viable alternative to existing antimicrobials. Plants seldom develop resistance to germs and antimicrobial drugs (Apendino et al., 2008; Lopez-Romero et al., 2015). As a result, CBD might be a potential alternative for treating antibiotic-resistant microorganisms. However, no products containing CBD are currently being utilised to increase the shelf life of food goods, despite rising customer desire for ecologically safe and natural products. Our study showed CBD could inhibit the growth of Listeria populations in skim milk, but it showed a low effectiveness in whole milk. The MIC and MBC for *L. monocytogenes* was found to be 2 µg/mL measured in TSB. Even though the presence of fat in whole milk may interfere with the solubility and absorption of CBD, leading to a decrease in its antimicrobial activity it can still be useful for milk with low fat. According to this study, cannabis may be used as a preserving agent for dairy produce with low fat content, such as fat-free dairy (skim), low-fat (1%) milk, reduced fat (2%) milk, fat-free yogurt, processed cheeses, low fat ice creams, puddings, etc, in the future, it will be utilised for more than merely flavouring. This helps to reduce product waste and improves product quality and safety for a longer duration.

Future studies can be done to assess the quality while using CBD in milk products. Tests can be done to analyze color, flavor, and aroma of CBD infused milk. If the addition of CBD effects the microbial characteristics of milk, it could also have an effect on it‘s sensory, chemical and physical properties as well. Although **Jeremy *et el.*, 2023**, published the toxic effects of oral CBD on human reproductive organs, more studies are needed to be done on its’ side effects on the human body. Before CBD cab be be used as food additives or as an antimicrobial agent in milk, they must meet safety requirements. Additives must be anticipated to be safe for all customers throughout their lives. As a result, assessing safety necessitates taking into account long-term usage as well as use among diverse population segments, including sensitive subpopulations such as pregnant women, the conceptus, the foetus, young children, and the elderly. Foods intended to deliver pharmacologically active chemicals are susceptible to inadvertent intake by both adults and children. To avoid this, in many countries, particularly in Canada, cannabis products are not permitted for sale and have strict controls to reduce the appeal of such products to youth; risk of accidental consumption by vulnerable age groups. For marketing commercial products made from Cannabis, products containing CBD should only be sold to adults, through legally authorized sources, such as Authorized Cannabis Stores. These authorized stores also serve as a highlighting the side effects of such products to consumers who are in high risk of medical issues. Other countries can also implement such regulation in the possession, production, distribution and sale of CBD dairy products, until research confirms that CBD infused products outweigh the advantages over their demerits for the safety of children and it’s vulnerable population. CBD infused products could be also kept under observation like the CBD infused drug Epidiolex and can be limited to foods demanding use of large amounts of preservatives. There is a growing consumer demand for natural, plant-based and clean-label preservatives. Using CBD extracted from hemp plants aligns with this trend, as they are perceived as more natural and less processed compared to synthetic preservatives. CBD could be used effectively to prolong the shelf life of dairy products by inhibiting the main spoilage microorganism, *L. monocytogenes.*

Acknowledgement: This work was supported by Canadian Natural Science and Engineering Research Council (NSERC) for funding the research. The first author was funded by Ontario Graduate Scholarship (OGS) Scheme. We would like to thank Mera Cannabis Corp. for kindly being our industrial partner.

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