EFFICACY IN REDUCING BACTERIAL CONTENT IN ORAL CAVITY BY CANNABINOIDS IN ORAL CARE PRODUCTS-A COMPARATIVE STUDY

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ABSTRACT
Aim: Purpose of our research was to assess the efficacy of cannabinoids in oral care products in reducing bacterial content inside the mouth.
Methodology: Dental plaques of 30 healthy adults were collected using paro-toothpick sticks and spread on three Petri dishes (A, B and C) containing cannabinoids containing toothpaste, oral B and Colgate respectively. The Petri dishes were sealed and incubated at 37°C for twenty-four hours, followed by counting the amount of colonies using colony counter.
Results: By evaluating the colony count of the dental bacteria isolated from six groups, it absolutely was found that cannabinoids were simpler in reducing the bacterial colony count in dental plaques as compared to the well-established synthetic oral care products like Oral B and Colgate.
**Conclusion:** Cannabinoids have the potential to be used as an efficient antibacterial agent against dental plaque-associated bacteria. Moreover, it provides a safer alternative for synthetic antibiotics to reduce the event of drug resistance. 

**Keywords:** Cannabinoids, bacterial plaque, Antibacterial, Oral Care Products, Personalized attention.

**INTRODUCTION**

Cannabis, also referred to as marijuana, is one in every of the foremost commonly used substances for medical and recreational purposes globally. Cannabis has been provided to patients for various sorts of pain. However, over-dose use of cannabis has adverse effects on health including, inducing tachycardia, impairment of immune function, interference with cognitive function, and increasing the chance of developing cancer.\(^1\) Cannabinoids are phytochemicals / secondary metabolites naturally produced by cannabis plant (Cannabis sativa L.) which include some psychoactive compounds like Δ9-tetrahydrocannabinol (Δ9-THC) and various non-psychoactive compounds like cannabichromene (CBC), cannabidiol (CBD), cannabinergic (CBG) and cannabinol (CBN).\(^2,3\) Cannabinoids are reported to possess antibacterial activity against several gram-positive further as gram-negative bacterial species.\(^4,7\) An interesting detailed molecular study reported that synthetic cannabinoid interferes in AI-2 quorum sensing signal cascade in Vibrio harveyi.\(^8\) The periodontal pathogenic bacteria also are reported to possess AI-2 quorum sensing system to speak and to control various function including biofilm formation, stress response and virulence factor expression.\(^9,10\) The combinatorial ability of cannabinoids as antimicrobial agent along with ability to interfere in AI-2 quorum sensing signal cascade makes cannabinoids an ideal candidate to use in aid. Cannabinoids are gaining enormous research interest within the recent late to its various beneficial properties within the field of pharmaceutical and cosmetic industry. additionally, the antibacterial properties of cannabis essential oils and cannabinoids are being reported \(^5,11\) including antibacterial activity against methicillin-resistant Staphylococcus aureus (MRSA).\(^12\) an artificial cannabinoid HU-210 has been demonstrated to own inhibitory effect on quorum sensing (QS) and QS-dependent virulence properties in Vibrio harveyi.\(^8\) Additionally, a recent study reported that CBD strongly inhibit the membrane vesicle formation in gram-negative bacteria and enhance the efficiency of bactericidal activity of antibiotics on both gram-positive and gram-negative bacteria.\(^7\) These properties make cannabinoids as potential candidates for various applications including but not limited to inhibit bacterial plaque bacteria. Wasim et al. reportedly tested ethanol and petroleum extracts of cannabis leaves for effects against different microorganisms. The results showed that the extracts obtained from cannabis leaves, have strong inhibitory effects on both gram-positive bacteria (Bacillus subtilis, Bacillus pumilus, S. aureus, and Micrococcus flavus) and gram-negative bacteria (Proteus vulgaris and Bordetella bronchiseptica).\(^13\) Therefore, to check the efficacy of well-established commercial oral care products and cannabinoids in reducing the bacterial content of the plaque, this study was undertaken. Reducing the bacterial content could significantly decrease and stop gum diseases that became an enormous global burden as a result of their direct relation with systemic diseases.
AIM OF THE STUDY
Purpose of our research was to assess the efficacy of cannabinoids in oral care products like mouthwashes, dentifrices etc. in reducing bacterial content inside the mouth.

METHODOLOGY
A randomised control trial was conducted on 30 medically fit patients in the age group of 18-45 years; selected for the current study who reported to our institution. The study protocol and also the purpose were explained to the participants, and consent from each participant was obtained before the beginning of the study. Their gingival condition was also categorized and noted with scores given as –
GI 1- Mild Gingivitis
G2- Moderate Gingivitis
G3- Severe gingivitis
The participants were divided into three groups who had to use oral care products for continuous 3 months –
A – Patients utilizing cannabino1 containing toothpaste
B- Patients using Oral B toothpaste
C- Patients using Colgate toothpaste
After regular usage of those oral care products for 3 months, plaque samples were collected. Before plaque sampling, saliva on the tooth surface was removed by water spray, and therefore the sampling target was dried with cotton. Plaque samples were collected from interdental spaces employing a paro-toothpick stick consisting of red velvet on the active part that might easily pick up the bacterial plaque. The collected plaque samples were directly spread on three Petri dishes (marked as A, B, and C) consisting of lysogeny broth agar and pre-treated with test components. The plaque sample was spread/streaked over the identical area of the agar plate pre-treated. The Petri dishes were sealed with paraffin film and incubated at 37°C for twenty-four hours. After 24 hours, colony counting was performed in automated colony counter. The colony count values from 10 individuals of every of the three groups were respectively combined to get a cumulative value for every group against each product tested.

RESULTS
We evaluated the colony count of plaque samples of 10 candidates from each of the three research groups on exposure to cannabinoids or toothpastes. The colony count in cannabinoid treatments were all significantly not up to that recorded in any of the toothpaste tested. Among the cannabinoids tested, CBN and CBC were effective as compared to oral health care. It was noted that maximum bacterial colonies were noted in the age group of 41-45 years of age and lowest in case of 18-30 years of age. (Table 1) We pooled the colony count data of all 10 candidates from each research group respectively to study the outcome. Of course, the bacterial colony count was much higher in Colgate, Oral B treatments, whereas significantly less colony count was observed altogether cannabinoid treatments. However, the efficiency of cannabinoids varied from individual to individual because the level of gingivitis varied all told the study subjects. More number of colonies were evident in case of plaque samples of severe gingivitis cases. (Table 2) Noteworthy was cannabinoids were very
effective in controlling the bacterial population as compared to Oral B as well as Colgate and the result was statistically significant. (Table 3)

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Mean Colony count (Mean ±SD)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Cannabinoid toothpaste</td>
</tr>
<tr>
<td>18-30 years</td>
<td>2±0.39</td>
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<tr>
<td>30 -40 years</td>
<td>5±0.47</td>
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<td>41-45 years</td>
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<table>
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<th>Gingival status</th>
<th>Mean Colony count (Mean ±SD)</th>
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<td></td>
<td>Cannabinoid toothpaste</td>
</tr>
<tr>
<td>GP1</td>
<td>1±0.44</td>
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<td>GP2</td>
<td>6±1.34</td>
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<td>GP3</td>
<td>11±1.87</td>
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<table>
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<th>Cannabinoid toothpaste</th>
<th>Oral B</th>
<th>Colgate</th>
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<tr>
<td>t test</td>
<td>p value</td>
<td>t test</td>
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<tr>
<td>0.853</td>
<td>0.0103</td>
<td>0.671</td>
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DISCUSSION
Oral biofilm may be a complex structure formed by sequential accumulation of over 600 species of bacteria. The plaque includes, supragingival plaque and subgingival plaque. The supragingival plaque contains mainly of aerobic bacteria, in contrast to subgingival plaque which contains mostly of anaerobic bacteria. The buildup of supragingival plaque eventually results in the establishment of subgingival plaque. It's been suggested that supragingival plaque act because the reservoir of periodontal pathogens which potentially spread to and infect the subgingival sites. Regular self-oral hygiene practices can effectively help to get rid of supragingival accumulation thereby suppresses periodontopathogens in subgingival plaque. Post brushing rinsing/mouthwash helps to cut back plaque and gingivitis. Most of the popular mouthwash products contain fluoride, CPC, alcohol or extreme pH. Cannabinoids (CBD / CBG) infused mouthwashes along with other natural key ingredients shows promising bactericidal activity in vitro against total-culturable aerobic bacterial content in bacterial plaque, expeditiously adore or better than that of the gold standard (0.2% chlorhexidine). CannIBite mouthwash products with cannabinoids infusion offer a safer and effective alternative with none fluorides or alcohol. The endocannabinoid (EC), anandamide (AEA), and arachidonoyl serine (AraS) exert antibacterial properties against MRSA strains. Moreover, they need the potential to switch the
bacterial membrane and forestall biofilm formation.\textsuperscript{19} C. sativa extracts exert antimicrobial activity on gram-positive bacteria, such as B. subtilis, B. pumilus, S. aureus, M. flavus; gram negative bacteria like P. vulgaris, B. bronchiseptica, Pectobacterium carotovorum, and Pseudomonas savastanoi, additionally as certain fungi like Aspergillus niger and Monilia albicans.\textsuperscript{4,20} Feldman et al. demonstrated that the tested compounds (AEA in particular) could impair the pathogenicity of MRSA by inhibiting their ability to create biofilm, reducing the metabolic activity of mature biofilm, and modifying the bacterial cell surface characteristics without killing the bacteria. They concluded that ECs and EC-like compounds may function a natural line of defense against MRSA or other antibiotic-resistant bacteria. Such cannabinoids, thanks to their anti-biofilm action, might be a promising alternative to antibiotic therapeutics against biofilm-associated MRSA infections.\textsuperscript{19} In the present study, we compared the efficacy of oral care products and cannabinoids in reducing the bacterial content of dental plaques. In our study, cannabinoids were found to be more practical in reducing the colony count of the bacterial strains as compared to the well-established synthetic oral care products like Oral B or Colgate. The selection of appropriate oral health care products could play a critical role in improving oral health and in preventing dental diseases. However, the foremost common problem faced by people is that the difficulty in selecting the proper oral care product. As shown within the present study, even the foremost commonly used commercial toothpastes lack the efficacy to completely reduce bacterial count from the mouth. The bacterial composition of oral biofilm varies from person to person. As shown within the present study, even the efficiency of cannabinoids may vary from individual to individual thanks to the character of individual oral biofilm. Hence, a personalised approach would be appropriate to spot the simplest formulation of oral care that matches into the necessity and nature of biofilm of a personal. Moreover, a scheduled repetitioan of oral care hygiene procedures may be a must to get the required results because it takes a lifetime of care to realize a healthy mouth.

**CONCLUSION**

Although popular oral hygiene products are somewhat effective in maintaining the oral health of the typical population, our study found that cannabinoids are substantially effective in reducing the colony count of the bacterial strains of the plaque as compared to the well-established synthetic oral care products like Oral B and Colgate. Additionally, our results suggest that the efficiency of cannabinoids could vary from individual to individual plausibly because of the microbial diversity of oral biofilms.

**REFERENCES**

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