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Investigation of the effect of cannabidiol, tetrahydrocannabinol, and vancomycin combination on cariogenic bacterial retention due to microleakage under prosthetic restorations

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ABSTRACT

Aims: Oral and dental infections develop due to many different bacterial activities, and with the increasing resistance mechanism against conventional antimicrobial agents, the formation of biofilms in the microleakage under prosthetic restorations, which cause dental caries, has become a major clinical challenge to overcome. This study aims to reveal the antibiofilm effect of vancomycin (VAN) combined with cannabidiol (CBD) and tetrahydrocannabinol (THC) against biofilm formation caused by *Streptococcus mutans (S. mutans)* and *Staphylococcus aureus (S. aureus)* on dental dentin under prosthetic restorations.

Methods: Freshly extracted human teeth without caries, resorption, or fracture were collected and obtained by slicing 2 mm dentin discs. A total of 64 dentin discs were inoculated with *S. mutans* and *S. aureus* and randomly allocated into control and five experimental groups consisting of CBD, THC, CBD+VAN, THC+VAN, and CBD+THC+VAN. Antibiofilm activity and combination index were analyzed by the MTT viability test and the Chou–Talalay method, respectively. Biofilm structure was assessed by scanning electron microscopy. Analysis of variance and post hoc Tukey tests were applied for comparisons.

Results: CBD and VAN showed antimicrobial effects against *S. aureus* and *S. mutans* bacteria in unary combinations, while THC was ineffective. However, no apparent synergistic interaction was observed in the binary combinations of VAN. Interestingly, a high synergistic effect (CI<1) occurred in the triple combination against both bacterial species. When this effect in the triple combination was examined, it was seen that VAN exhibited a suitable dose reduction index (DRI>1). When these synergistic and dose reduction results are evaluated together with SEM image analysis, it can be said that the triple combination of VAN+CBD+THC probably causes the most optimum antibiofilm effect on dental caries bacteria.

Conclusion: Combining VAN with CBD and THC may offer a new approach to combat microleakage-induced bacterial retention in dental dentin under prosthetic restorations.

Keywords: Cannabidiol, tetrahydrocannabinol, vancomycin, prosthetic restorations, microleakage

INTRODUCTION

Dental caries and periodontal diseases remain significant challenges in dentistry, particularly in prosthetic treatments involving crowns and bridges. While improving functionality and aesthetics, these restorations may contribute to microleakage due to improper fit or marginal discrepancies. Microleakage provides a pathway for bacterial infiltration, leading to the formation of biofilms on the dentin surface, which can ultimately result in secondary caries and restoration failure.[1,2]

Biofilms are structured microbial communities embedded in an extracellular matrix, adhering to surfaces such as dentin. Common cariogenic and pathogenic bacteria, including *Streptococcus mutans* (*S. mutans*) and *Staphylococcus aureus* (*S. aureus*), play critical roles in biofilm development and the progression of dental caries. These biofilms are particularly resistant to conventional antibacterial treatments due to their protective matrix and enhanced microbial survival mechanisms.[3-5]

Recent advancements in dental material research have focused on incorporating bioactive compounds to mitigate biofilm formation and bacterial growth. Cannabidiol (CBD) and tetrahydrocannabinol (THC), derived from cannabis plants, have demonstrated promising antibacterial and anti-inflammatory properties.[6,7] Vancomycin (VAN), a glycopeptide antibiotic, is widely used for its potent bactericidal activity against Gram-positive bacteria, including *S. mutans* and *S. aureus*.[8] In clinical practice, we chose it as an antibiotic for our study because it is a preferred agent against

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resistant strains, particularly methicillin-resistant *S. aureus*, in oral and dental infections.

In this study, *S. mutans* and *S. aureus* biofilms were cultivated on dentin surfaces to model the bacterial colonization associated with microleakage in prosthetic restorations. The biofilms were then treated with CBD, THC, and VAN to evaluate their antibacterial efficacy. Cytotoxicity assays, such as the MTT assay, and morphological analyses using scanning electron microscopy (SEM) were employed to assess the effects of these compounds on bacterial viability and biofilm integrity. This research explores the potential of these bioactive agents in reducing the bacterial populations responsible for secondary caries, thereby enhancing the longevity and success of prosthetic treatments. The findings suggest that these compounds could be incorporated into oral care products such as mouthwashes, toothpaste, or dissolvable tablets, providing an innovative approach to addressing microleakage-related challenges in prosthetic dentistry.

METHODS

Ethics

This study was approved by Yozgat Bozok University Non-interventional Clinical Researches Ethics Committee (Date: 09.04.2025, Decision No: 2025-GOKAEK-257_2025.04.09_443), and all experiments were conducted in accordance with the ethical guidelines of the Helsinki Declaration.

CBD and THC Isolation

After the hemp flowers were ground in a grinder until they became a fine powder, they were left in the oven for 1 hour at 105°C for decarboxylation. The supercritical CO_2 extraction method (230 bar, 50°C, and 50 kg/h cycle conditions) was employed for extraction following this process. The extract was then mixed with ethanol in a 1:1 (m/v) ratio to remove waxy structures and placed in a -80°C freezer for 48 hours. The sample was subsequently filtered using a vacuum funnel and filter paper to separate the extracted waxes. Finally, the molecular distillation method was utilized to separate terpenes and chlorophylls. The fractions richest in CBD and THC were obtained using the molecular distillation technique at temperatures between 180°C and 200°C.

The fractions obtained were subsequently purified with flash chromatography (BUCHI Pure C815 Flash Chromatography Systems), C18 reverse phase cartridges (40 g, C18 column), and an ethanol-water mobile phase. In this context, the 210 nm band of the CBD and THC was determined, as shown in Figure 1. Purity analysis results were carried out using HPLC (2.7 µm, 4.6x150 mm, NexLeaf CBX, PDA detector, 210 nm, mobile phase acetonitrile:water (9:1)) as illustrated in Figure 2. The yield was measured as 80% and purity as 98%.

Preparation of Dentin Discs

A total of 64 permanent molars extracted for periodontal or orthodontic reasons and with intact crown integrity were used. The teeth were mechanically cleaned with a curette to prevent any external contamination, and it was ensured that there were no organic or inorganic residues on their surfaces. The cleaned teeth were stored in a 0.01% thymol solution at room temperature to preserve their biological

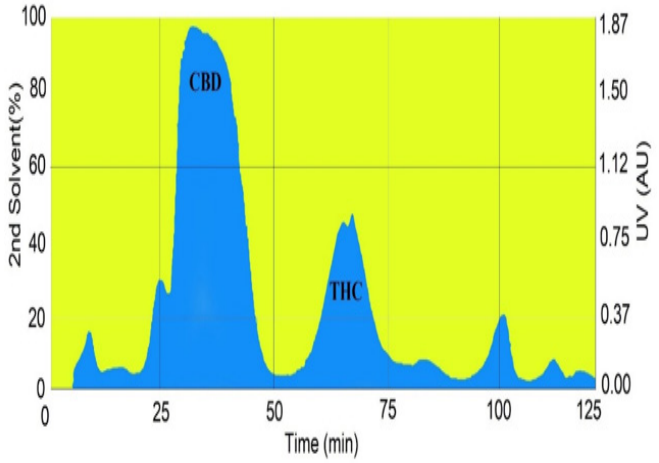

Figure 1. Flash chromatograms of CBD and THC
CBD: Cannabidiol, THC: Tetrahydrocannabinol

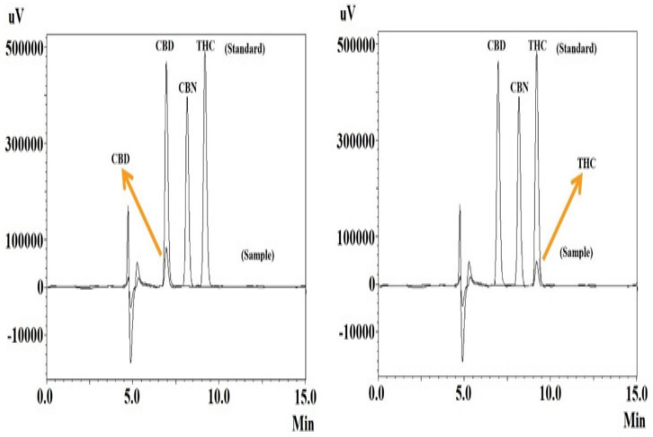

Figure 2. HPLC chromatography of CBD, THC, and standard
CBD: Cannabidiol, THC: Tetrahydrocannabinol

integrity. The teeth were embedded in polyvinyl chloride (PVC) cylinder molds of 3 cm height and 2 cm diameter using autopolymerized acrylic resin (Ortho-Jet Resin Acrylic; Lang Dental Manufacturing Co, Illinois, USA). Before completing the acrylic resin polymerization, the teeth were fixed in the molds at a height of 1 mm higher than the cementoenamel junction. During the preparation of the samples, a large-sized, water-cooled, low-speed diamond cutting saw (Metkon Microcut 201, Htp High Tech Products, Istanbul, Turkey) was used to expose the dentin layer of the teeth. Dentin discs with a thickness of 2 mm were obtained by cutting 3 mm and 5 mm below the occlusal surface. Then, the surfaces of these dentin discs were smoothed using a circular medium-grained rotary abrasive tool (Model 902; Brasseler USA). The prepared dentin discs were marked with an acetate pen using a metal mold with a 6 mm diameter circular cavity in the middle. Following the marking process, standard samples with a diameter of 6 mm and a thickness of 2 mm were obtained by an experienced dentist using a water-cooled aerator (Bien-Air Tornado; Bien-Air Dental, Bienne, Switzerland). The accuracy of the measurements was checked with a digital caliper (Mitutoyo 500-196-30; Mitutoyo Corp., Kawasaki, Japan). In the final stage, all prepared samples were cleaned with distilled water and then treated with isopropyl alcohol

for 3 minutes to remove organic and inorganic residues on the surface altogether.

Minimum Inhibitor Concentration (MIC) Determination

MIC values of the compounds against bacterial strains were determined on the basis of a micro-well dilution method. To determine the minimal inhibitory concentration (MIC) values, *S. aureus* (ATCC 700699) and *S. mutans* strains (ATCC 35668) in a 12-h Brain Heart Infusion broth culture were adjusted to 0.5 McFarland.[9-11] Each substance dissolved in dimethyl sulfoxide (DMSO) and serial twofold dilutions were made in a concentration range from 4 to 512 µg/ml in microplate wells containing nutrient broth. The growth of microorganisms was determined visually after incubation for 24 h at 35 °C. The lowest concentration at which no visible growth (turbidity) was taken as the MIC. Unary, binary, and ternary combinations of CBD, THC, and VAN were added to the wells in increasing doses and left for 24 hours of incubation. The well with no turbidity at the end of the period was selected as the MIC value.

Minimal Biofilm Inhibitory Concentration (MBIC)

Dental discs in a 24-well plate received 500 µL of a 0.5 McFarland bacterial sample and were then incubated overnight for 16 hours. At the end of the period, the dental disc samples were incubated for 72 hours for biofilm formation by changing the medium every 24 hours. After the biofilm formation, bacteria that could not adhere were gently washed with DPBS and removed from the medium. Samples were vortexed thoroughly with 500 µL of DPBS and plated in a new 24-well plate. Unary, binary, and ternary combinations of CBD, THC, and VAN were added to the wells in increasing doses and left for 24 hours of incubation. An MTT assay gives an accurate estimate of the number of viable cells. Thus, we performed an MTT assay according to AFST-EUCAST guidelines. During the experiment, one-part MTT is mixed with nine-part medium (BHI Broth for *S. mutans* and *S. aureus*) and used. First, the old medium was withdrawn. MTT solution prepared with the fresh medium was added and incubated in the dark for at least 4 hours in the incubator. Then, the MTT solution was withdrawn, and DMSO and 100 µl Sorenson's glycine buffer (glycine 0.1M, NaCl 0.1M, pH 10.5) were added to the medium and left on the mixer in the dark for 15-20 minutes. Samples were loaded onto a 96-well plate without a lid and read at 570-630 nm on a microplate reader. Unary, binary, and triple combinations were evaluated using the obtained absorbance values with the Calcusyn synergy analysis program.

Synergy Model

Synergy measurement by microplate synergy analysis was used to determine the effect of unary, binary, and triple combinations of CBD, THC, and VAN on potency compared to their activities (Table 1). The antibiofilm effects of CBD, THC, and VAN were studied for the first time on *S. aureus* and *S. mutans* dentin biofilm. The MTT cell proliferation assay for different unary, binary, and ternary drug combinations was used to evaluate the results of the *in vitro* pharmacodynamic drug interaction analysis of the selected drugs.[12] Absorbance data (CLARIOstar microplate reader) were loaded for automated calculation of the slope of the median-effect plot (m), the dose that produces 50% effect such as IC50 (Dm), and the linear correlation coefficient of the median-effect plot (r) parameters, as well as the combination index (CI) and dose reduction index (DRI) using CalcuSyn software, version 2.11, commonly used to study drug interactions described by Chou[13] and Chou and Talalay.[14]

Table 1. Concentrations of substances used (µg/ml)

THC	CBD	VAN
25	25	12.5
50	50	25
100	100	50
200	200	100
400	400	200

THC: Tetrahydrocannabinol, CBD: Cannabidiol, VAN: Vancomycin

Scanning Electron Microscopy

SEM was conducted using *S. mutans* and *S. aureus* biofilms formed on the surface of dentin discs. The samples were washed twice with DPBS and fixed in 2.5% glutaraldehyde in a phosphate buffer for 16 hours and, shortly after, refixed in 2% osmium tetroxide for two hours. Then, they were dehydrated through ethanol rinses (30, 50, 90, 95, and 100%) and mounted and sputter-coated with gold. Sample surfaces were examined using SEM (Zeiss LEO 440, Cambridge, UK).

Statistical Analysis

The statistical significance of differences was determined by the one-way analysis of variance (one-way ANOVA) followed by Tukey's test. The SPSS for Windows computer program was used for statistical analyses. The results of test values were reported as mean values ±SD of three independent assays, and differences among groups were considered to be significant at $p<0.05$.

RESULTS

Susceptibility Testing and Synergy Analysis

For the antibacterial activity studies of the test substances, the selected pathogenic Gram (+) *S. aureus* (ATCC 700699) and *S. mutans* (ATCC 35668) bacterial species were used. The plate-well technique calculated the MIC values of single molecules and combinations. Accordingly, the MIC values of the THC molecule could not be calculated since they were >512 µg/ml. CBD MIC values were measured as 8-16 µg/ml. VAN MIC value was measured as 4 µg/ml (Table 2). When we looked at the binary combinations, it was found as 4-8 µg/ml for CBD+VAN and 32 µg/ml for THC+VAN (Table 2). When we looked at the triple combinations, it was measured as 2 µg/ml (Table 2).

MTT test was performed to measure the minimal biofilm inhibitory concentration (MBIC) effects of single molecules and combinations. The ratios and effect values used for combinations are explained in Table 2, 3, 4. The activity values of the combinations were determined by the Chou-Talalay CI (mass-action law) method. After performing the MTT cell

Table 2. MIC value of unary, binary, and triple combinations of CBD, THC, and VAN

							One-way ANOVA	
MIC (µg/ml)	**THC**	**CBD**	**VAN**	**CBD+VAN**	**THC+VAN**	**CBD+THC+VAN**	**F**	**Sig.**
S.aureus	N.D.*	8[b]**	4[a]	4[a]	32[c]	2[a]	420.46	.000
S. mutans	N.D.	16[c]	4[ab]	8[b]	32[d]	2[a]	312.82	.000

MIC: Minimal inhibitory concentration, THC: Tetrahydrocannabinol, CBD: Cannabidiol, VAN: Vancomycin, *S. aureus: Staphylococcus aureus, S. mutans: Streptococcus mutans,* *N.D. Not detected
**Values followed by the same letter in the row are not significantly different

Table 3. Combination ratios used in the study

THC 2 CBD 2 VAN 1	CBD+VAN 2/1 THC+VAN 2/1	CBD+THC+VAN 2/2/1

THC: Tetrahydrocannabinol, CBD: Cannabidiol, VAN: Vancomycin

Table 4. Combination index method

Range of CI	Description	Range of CI	Description
<0.1	Very strong synergy	1.10-1.20	Mild antagonism
0.1-0.3	Strong synergy	1.20-1.45	Moderate antagonism
0.3-0.7	Synergy	1.45-3.3	Antagonism
0.7-0.85	Moderate synergy	3.3-10	Strong antagonism
0.85-0.90	Light synergy	10>	Very strong antagonism
0.90-1.10	Additiv		

CI: Combination index

proliferation test for each substance alone against bacteria, CompuSyn software was used to calculate the mass-action law parameters (Dm), (m), and (r) (Table 5, 6). Accordingly, the Dm values (IC50) of the tested substances in *S. aureus* were found to be between 205.00, 15.00, and 4.75 µg/ml for THC, CBD, and VAN, respectively. In *S. mutans*, the Dm values (IC50) of the tested substances were between 155.00, 21.00, and 3.70 µg/ml for THC, CBD, and VAN, respectively. The dose reduction index (Fa-DRI) for THC, CBD, and VAN combinations are presented in Table 7, 8, respectively. The Chou-Talalay method for drug combination is based on the median effect equation, which provides the theoretical basis for the CI, which allows the quantitative determination of drug interactions where CI <1, =1, and >1 indicate synergy, additive effect, and antagonism (Table 4). Accordingly, in *S. aureus*, the Dm values (IC50) of the tested binary and triple combinations were between 4.82-35.23 and 3.34 µg/ml, respectively. In *S. mutans*, the Dm values (IC50) of the tested binary and triple combinations were between 3.21-38.73 and 2.97 µg/ml, respectively.

This study evaluated the synergistic-antagonistic effects of THC, CBD, and VAN combinations with CI values for fa=0.5. Accordingly, when the binary and triple combinations tested in *S. aureus* were examined at fa=0.5, CBD+VAN (1.01) showed additive, CBD+THC+VAN (0.80) synergistic, and THC+VAN (4.33) antagonistic effects (Table 5). When the binary and triple combinations tested in *S. mutans* were examined at fa=0.5, CBD+VAN (1.03) showed additive, CBD+THC+VAN (0.90) synergistic, and THC+VAN (5.94) antagonistic effects (Table 6).

Table 5. Parameters were calculated from the median effect equation and median effect plot. 'm' is the slope, and m=1, >1 and <1 indicate hyperbolic, sigmoidal, and flat sigmoidal shape, respectively; 'Dm' denotes power; and 'r' is the linear correlation coefficient

S. aureus	**Combination index (CI) values at**			
	ED50	**Dm**	**m**	**r**
THC	N/A	205.00	0.63	0.92
CBD	N/A	15.00	0.90	0.92
VAN	N/A	4.75	0.72	0.93
CBD+VAN	1.01	4.82	0.87	0.94
THC+VAN	4.33	35.23	1.32	0.95
CBD+THC+VAN	0.80	3.34	0.52	0.92

S. aureus: Staphylococcus aureus, CI: Combination index THC: Tetrahydrocannabinol, CBD: Cannabidiol, VAN: Vancomycin

Table 6. Parameters were calculated from the median effect equation and median effect plot. 'm' is the slope, and m=1, >1 and <1 indicate hyperbolic, sigmoidal, and flat sigmoidal shape, respectively; 'Dm' denotes power; and 'r' is the linear correlation coefficient

S. mutans	**Combination index (CI) values at**			
	ED50	**Dm**	**m**	**r**
THC	N/A	155.00	1.34	0.95
CBD	N/A	21.00	0.99	0.97
VAN	N/A	3.70	0.77	0.94
CBD+VAN	1.03	3.21	0.74	0.94
THC+VAN	5.94	38.73	0.84	0.94
CBD+THC+VAN	0.90	2.97	0.86	0.96

S. aureus: Staphylococcus aureus, CI: Combination index, THC: Tetrahydrocannabinol, CBD: Cannabidiol, VAN: Vancomycin

Table 7. Dose reduction index (DRI), DRI=1, >1 and <1 indicate no dose reduction, appropriate dose reduction, and inappropriate dose reduction for each drug in the combination, respectively

S. aureus	**Drug alone**		**Dose reduction index (DRI)**			
Fa	**CBD**	**VAN**	**CBD**	**VAN**		
0.5	15.00	4.75	1.56	0.72		
Fa	**THC**	**VAN**	**THC**	**VAN**		
0.5	205.00	4.75	5.39	0.19		
Fa	**THC**	**CBD**	**VAN**	**THC**	**CBD**	**VAN**
0.5	205.00	15.00	4.75	49.29	3.9	1.81

S. aureus: Staphylococcus aureus, CBD: Cannabidiol, VAN: Vancomycin, THC: Tetrahydrocannabinol

This study also focused on the appropriate DRI of the dual and triple drug combination based on actual experimental data points. The Fa-DRI table shows the results (Table 7, 8). DRI, DRI=1, >1 and <1 indicate no dose reduction, appropriate

Table 8. Dose reduction index (DRI), DRI=1, >1 and <1 indicate no dose reduction, appropriate dose reduction, and inappropriate dose reduction for each drug in the combination, respectively

S. mutans	Drug alone		Dose reduction index (DRI)			
Fa	**CBD**	**VAN**	**CBD**	**VAN**		
0.5	21.00	3.70	2.19	0.70		
Fa	**THC**	**VAN**	**THC**	**VAN**		
0.5	155.00	3.70	3.68	0.17		
Fa	**THC**	**CBD**	**VAN**	**THC**	**CBD**	**VAN**
0.5	155.00	21.00	3.70	15.02	2.24	0.72

S. mutans: Streptococcus mutans, CBD: Cannabidiol, VAN: Vancomycin, THC: Tetrahydrocannabinol

dose reduction, and inappropriate dose reduction for each drug in the combination, respectively. Typically, the main aim of combination therapy is to achieve synergistic effects (CI<1) to reduce the dose of specific toxic drugs (DRI>1) and, consequently, to eliminate the possibility of drug resistance. Accordingly, when the Fa-DRI table was examined in detail at fa=0.5 (Table 7, 8), at 50% inhibition (fa=0.5) in *S. aureus*, none of the binary combinations showed an appropriate dose reduction (DRI<1). In contrast, the triple combinations THC/CBD/VAN (49.88/3.94/1.85) showed an appropriate dose reduction (DRI>1). At 50% inhibition (fa=0.5) in *S. mutans*, none of the binary combinations showed an appropriate dose reduction (DRI<1), while the triple combination THC/CBD/VAN (15.54/2.38/1.12) showed an appropriate dose reduction (DRI>1). These results show that the combined use of cannabinoids has the potential to be used in the near future to increase the effectiveness of the treatment of dental caries to a very high level.

SEM Analysis

When the SEM images in Figure 3 are evaluated with ImageJ software, it is understood that THC does not show antibiofilm properties alone. In the control dentin disc surface images for both *S. aureus* and *S. mutans*, it is seen that the area is covered with more bacteria. When the SEM images of CBD and THC are compared with CBD+VAN and THC+VAN for *S. mutans* and *S. aureus*, it is seen that the single combinations are less effective. When both bacterial biofilms are evaluated for CBD+THC+VAN, it is determined that the highest antibiofilm effect occurs. In accordance with the MIC and Synergy tests, when the SEM images are examined, the potency order of CBD+THC+VAN>CBD+VAN>THC+VAN>CBD>THC is revealed.

DISCUSSION

The facultative anaerobe *S. mutans* initiates dental caries, while other bacteria, such as S. *aureus*, contribute to periodontal disease. Previous studies have shown that *S. mutans* is one of the leading causes of dental caries and that acidic metabolites formed from sugar fermentation lead to dentin demineralization.[3,15] *S. aureus*, on the other hand, is generally associated with skin and soft tissue infections, but in recent years, it has also been detected in oral infections and has been reported to trigger periodontal diseases.[4] The pathogenicity of *S. aureus* is especially emphasized in dental root canal infections and peri-implantitis cases.[16] Managing infections caused by these bacteria requires a multifaceted approach that includes mechanical cleaning and antimicrobial agents. However, the increasing prevalence of multidrug-resistant bacteria requires innovative antimicrobial strategies. VAN, a glycopeptide antibiotic, has been used to combat Gram-positive bacterial infections, including those found in the oral cavity. Although VAN is effective against various Gram-positive bacteria, its efficacy decreases with the doses used due to evolving resistance mechanisms.

The secondary metabolites of *Cannabis sativa*, CBD, and THC have demonstrated antimicrobial properties against various bacteria, including those involved in dental infections. These compounds can disrupt bacterial cell membranes and interfere with biofilm formation, but their efficacy varies depending on the bacterial species and growth conditions. Previous studies have shown that CBD is effective against *Streptococcus* and *Staphylococcus* species.[17] Suppressing bacterial biofilm formation and inhibiting bacterial enzyme activity allow CBD to be evaluated as a potential agent in the fight against dental caries.[18] CBD effectively reduces bioactive metabolites, especially those caused by *S. mutans*.[19] Combining VAN with CBD and THC may offer a new approach to combat bacterial retention in dental dentin. The rationale for the combined use of CBD and THC with VAN is to increase antibacterial efficacy and to create the potential for synergistic effects that may reduce the risk of resistance development. Combining these drugs can exploit the antimicrobial properties of each component to disrupt bacterial cell membranes, inhibit cell wall synthesis, and interfere with biofilm formation, thereby reducing bacterial retention in dental dentin. Further research is required to elucidate the precise mechanisms of action of the combination of VAN with CBD and THC in the context of dental infections and to optimize its therapeutic potential.

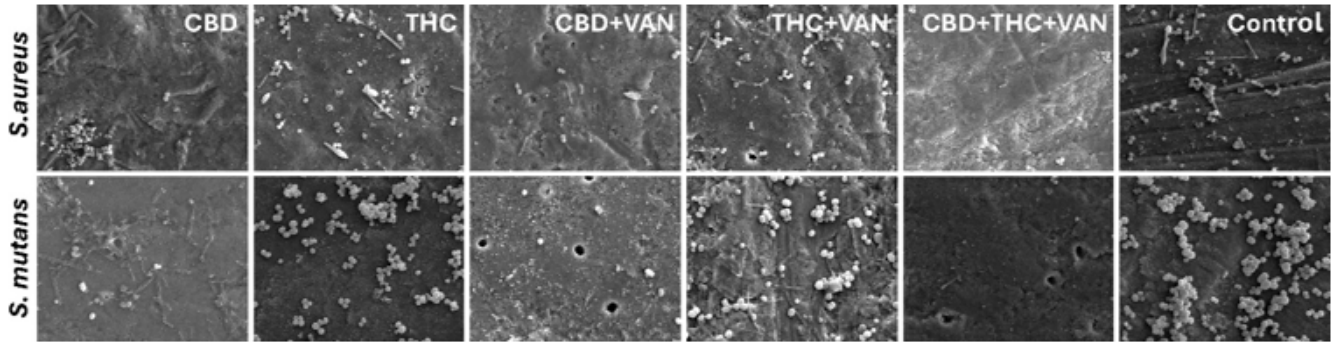

Figure 3. A scanning electron microscopic image (x25 K magnification) showing *S. mutans* and *S. aureus* biofilm formations on materials
THC: Tetrahydrocannabinol, CBD: Cannabidiol, VAN: Vancomycin, *S. aureus: Staphylococcus aureus, S. mutans: Streptococcus mutans*

Combination antibiotic therapies have various effects on bacterial survival, including additive, synergistic, and sometimes antagonistic effects, where combination therapy is less effective than single-drug therapies.[20] This is because single-drug therapies typically target a single bacterial function, whereas drug combinations are more likely to disrupt multiple bacterial processes simultaneously and produce more potent effects.[21] Combination therapies may be effective because they treat mixed infections, enhance antimicrobial activity, avoid the need for long-term antibiotic use, and prevent the emergence of multidrug-resistant bacteria.[22] One proposed theory suggests that silver nanoparticles destabilize lipopolysaccharides, increasing the permeability of the outer membrane and peptidoglycan structure, which are then recognized and captured by antibiotics, rendering resistant strains susceptible to antibiotics.[23] Here, combinations that effectively reduce the amount of VAN used prevented VAN-induced toxicity and reduced VAN resistance. This result was examined morphologically with SEM analysis, and it was shown that the findings were consistent and that the relevant combinations increased the effectiveness of VAN in the treatment of dental caries. With this study, it was added to the literature that VAN could be a safe anti-caries drug with the desired properties with an ideal combination (THC/CBD/VAN). Considering the clinical and academic importance of this study, the antibacterial activity of CBD and THC should be examined in detail, and its therapeutic potential in dentistry should be investigated. In particular, more research is needed on the pharmacokinetic profile, dose, and application methods to optimize the antibacterial activity of CBD. Future in vivo and clinical studies will more clearly demonstrate the usability of these bioactive compounds in the prevention and treatment of dental caries.

The synergistic combination of VAN, CBD, and THC identified in this study offers promising opportunities for clinical translation, particularly in developing novel antimicrobial formulations in dentistry. Given their demonstrated antibiofilm efficacy against *S. mutans* and *S. aureus*, this combination may serve as the basis for creating effective cavity disinfectants applied before prosthetic restorations, thus reducing microleakage-related bacterial retention. Furthermore, such a formulation could be adapted into antimicrobial rinses or mouthwashes aimed at preventing biofilm formation, especially in high-risk patients with extensive restorations or compromised oral hygiene. The incorporation of this combination into surface sterilization solutions or coating materials for prosthetic components may also help reduce bacterial colonization and prolong prosthesis lifespan. In endodontic or restorative procedures, this combination might function as an intracanal or intrachamber irrigant, improving disinfection while minimizing cytotoxicity by lowering the required VAN concentration.

Limitations

This study has several limitations. First, it was conducted under in vitro conditions, which do not fully mimic the complex in vivo environment of the oral cavity, including salivary enzymes, immune components, and mechanical forces. Second, the use of mono-species biofilms may not represent the polymicrobial structure of natural dental biofilms. In addition, the relatively small sample size and the limited number of tested combinations may restrict the generalizability of the results. Further in vivo and large-scale studies with more diverse combination groups are needed to confirm the clinical relevance of the findings.

CONCLUSION

Today, the powerful antimicrobial agents used in this study are essential in dentistry due to their favorable nature properties. They are of great interest in the clinic. Preservation of tooth integrity is vital for oral health. Therefore, reducing the microleakage-induced microbial load caused by pathogens such as S. mutans and S. aureus on dentin disc is essential. The results obtained in this study will help develop ideal powerful antimicrobial agents regarding oral hygiene and introduce them into the clinic.

ETHICAL DECLARATIONS

Ethics Committee Approval

This study was approved by Yozgat Bozok University Non-interventional Clinical Researches Ethics Committee (Date: 09.04.2025, Decision No: 2025-GOKAEK-257_2025.04.09_443).

Informed Consent

Since the study was designed as an experimental study, written informed consent was not required.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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