

Effects Of Occlusal Splint Cleanser Containing Clove Bud Essential Oil

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ABSTRACT

Background/Objectives: The purpose of this study was to prepare an occlusal splint cleanser containing natural extract clove bud essential oil (CBEO) to evaluate its antimicrobial effect and to analyze the changes in the properties of the resin.

Methods/Statistical analysis: A cleanser containing 5, 10, and 15 vol% CBEO was prepared, and antimicrobial evaluation was performed using the disc diffusion method and microbial viability rate evaluation. In addition, after storing the resin specimens in the experimental and control groups for 24 h, the change in the physical properties of the resin was confirmed through the change in surface hardness (Hv) and color difference (ΔE^*).

Findings: In the disc diffusion method and microbial viability rate evaluation, antimicrobial effects of cloves on both strains of *S. mutans* and *C. albicans* showed superior as the concentration of cloves increased ($p < 0.05$). In addition, there was no significant difference from the control distilled water in the evaluation of the change in physical properties; therefore, there was no change in the surface hardness and color difference of the resin ($p > 0.05$).

Improvements/Applications: The occlusal splint cleanser containing CBEO shows an excellent antimicrobial effect without any physical change to the resin; therefore, it can be used as basic data for the application of occlusal splint cleansers containing natural ingredients such as CBEO.

Keywords: Anti-microbial effect, Occlusal splint cleanser, Clove, Essential oil

1. INTRODUCTION

Dental caries and periodontal disease are two major oral diseases that severely affect oral health [1]. The main causative bacterium of dental caries is *S. mutans*, which is known to cause dental caries by producing lactic acid during the decomposition of sucrose or glucose contained in ingested food as a

facultative anaerobic bacterium [2]. *C. albicans* is a fungus that occurs as an opportunistic infection in patients with weakened immunity or serious systemic diseases, stimulates the oral mucosa, and reproduces on oral devices or denture bases, causing harm to the oral mucosa [3].

Various studies have reported that the number of bacteria increases after the installation of oral prostheses and intraoral devices [4–6]. In patients with removable devices, it is difficult to expect a self-purification effect due to plaque remaining between the teeth and the device and changes in saliva secretion and flow in the oral cavity [7]. One of these removable devices, the occlusal splint, is worn in the oral cavity for a certain period to express its function. At this time, the anaerobic condition of the teeth surrounded by the device is strengthened and the microorganisms residing therein can be freed from the natural cleaning power of saliva [8]. According to a study by Byun and Seo [8], the number of anaerobic bacteria and *S. mutans* in the oral cavity changed when wearing an occlusal splint.

To suppress the growth of microorganisms in the oral device and prevent oral diseases, cleaning and disinfection of the oral device is essential, and both mechanical and chemical cleaning methods are available. However, the mechanical method changes the surface of the device, and the synthetic materials used in the chemical method are harmful to the eyes and skin. Its disadvantages are highlighted by causing a corrosive effect on the metal and discoloration of the resin [9]. In addition, antibiotics have been mainly used to inhibit various pathogens; however, since long-term use can create resistant bacteria, the use of natural extracts has recently been recommended to complement the shortcomings of these chemical agents and to derive excellent antibacterial activity [10].

The cloves used in this study are buds of the clove family and are evergreen trees [11]. Eugenol is a natural substance extracted from cloves in the form of essential oil and is beneficially used in the food and medical fields, including the dental field, as a spice, toothache analgesic, and toothpaste fragrance component [12]. There are also studies on the antiviral [13] and anti-stress [14] effects of clove, and many previous studies have investigated the antibacterial activity of clove against oral bacteria [15–17].

The cleaning method recommended by most dentists for patients with occlusal splints is to clean only with a toothbrush using dishwashing detergent or water without toothpaste and use a commercially available denture cleaner to clean. However, denture cleaners have the disadvantage of changing the surface or not tasting and smelling good [18]. More importantly, various previous studies [19–20] have shown the study results of the lack of antibacterial effects of denture cleansers. In addition, denture cleansers mainly focus on fungi that inhabit dentures rather than *S. mutans*, and studies on cleansing agents that can simultaneously control the growth and proliferation of *S. mutans* and *C. albicans* are limited. In addition, research on microorganisms present in occlusal splints or proposals for cleaning management methods are also insufficient. Therefore, this study was conducted to evaluate the antimicrobial effect of occlusal splint cleaning by using a self-developed cleaner containing clove bud essential oil (CBEO), a natural extract with reported antimicrobial effect, and evaluate the change in the physical properties of the resin.

2. MATERIALS AND METHODS

2.1. MATERIALS

2.1.1. Essential oil solubilization

CBEO (Indonesia) was solubilized and used to confirm the antimicrobial effect and physical properties of the resin used to manufacture occlusal splints. The concentrations were 5, 10, and 15 vol. %. Moreover, Tween 20 (Tween 20, Samchun, Seoul, Korea) and Tween 80 (Tween 80, Samchun, Seoul, Korea) were added as emulsifiers. The concentrations of CBEO were 50, 100, and 150 µl/ml (v/v), based on 100 µl/ml (v/v), which showed excellent results in the evaluation of antibacterial properties and efficacy and safety according to pH, heat, and UV rays in the study by Choi et al. [21]. As a negative control, distilled water was used for both strains, and as a positive control, 0.12 % Chlorhexidine (CHX, Hexamedine, Bukwang Pharmacy, Seoul, Korea) was used for *S. mutans* and 0.6 % Na OCl (Sense Clenser, NaOCl 6 %, Sunjin Bio, Seoul, Korea) was used for *C. albicans* by diluting 6 % Na OCl in distilled water.

2.1.2. Microbial viability rate evaluation

The strains used were obtained from lyophilized strains (*S. mutans* KCCM 40105 and *C. albicans* KCCM 11282) from the Korea Microorganism Conservation Center (KCCM). *S. mutans* culture medium was brain heart infusion (BHI, BD Difco, Franklin Lakes, NJ, USA) broth and agar medium. *C. albicans* culture medium was yeast malt (YM; MB Cell, Seoul, Korea) broth and agar medium was used.

2.1.3. Physical property evaluation

The specimens used in this study were 3D printed to produce an occlusal splint. The test material was “Ortho Rigid” of Next Dent and the 3D printer was a DLP 3D printer (DLP 3D printers, ZENITH D, Dentis, Daegu, Korea). An ultrasonic cleaner (Ultrasonic cleaner, SAE HAN, Korea) was used for cleaning after printing, and a Veltz 3D curing machine (Veltz 3D Curing Machine, Veltz3D, Incheon, Korea) was used for curing. The specimens were prepared with a diameter of 12 mm and a height of 2 mm.

2.2. METHODS

2.2.1. Antimicrobial evaluation

1) Disc diffusion method

The research method of Choi et al. [21] was modified and applied in the experiment. After diluting the activated strains in liquid medium to a concentration of 1×10^6 colony forming units (CFU)/ml, 100 µl of the bacteria was dispersed on an agar plate with a triangular rod. Then, 20 µl of CBEO by concentration and control groups were dispersed on a sterilized 6 mm paper disc and then absorbed and dried at approximately 28 °C room temperature for 10 min. Afterward, the diameter of the clear zone (mm) around the disc was measured. The experiment was repeated four times, with 10 plates per experiment.

2) Microbial viability rate evaluation

The research method of Choi and Kang [18] was modified and applied in the experiment. After making the activated strains at a concentration of 1×10^6 CFU/ml, 100 µl each of bacteria and CBEO were injected into 96 wells to adjust the total volume to 200 µl and stored in a 37 °C incubator. After 24 h, absorbance was measured at 550 nm using a microplate spectrophotometer (Bio Tek EPOCH, Bio tek, Winooski, VT, USA). The microbial viability rate (%) in the experimental group was

determined when the negative control group was expressed based on 100 % viability. The experiment was repeated five times.

2.2.2. Physical property evaluation

Ten specimens for each experimental group were placed in 24 wells, and 1 ml of each of the prepared CBEO cleansers and control group for each concentration, and stored by precipitating them in a 37 °C constant temperature water bath for 24 h. The surface hardness (Hv) was measured using a hardness tester (microhardness tester, Dmh-2, Matsuzawa Seiki, Tokyo, Japan), and a spectrophotometer (CM-3500d, Minolta, Kyoto, Japan) was used to measure the color difference.

For the hardness load value, referring to the study of Lee et al. [22], a 50 g load was applied on the surface of the specimen for 5 s with a diamond pyramid indenter with a facing angle of 136 ° with the same load and indentation time. For the color tone measurement, referring to the study by Choi et al. [23], L*, a*, and b* values were obtained, and then the ΔE^* value, which is a color difference value, was calculated. A negative control group, in which the specimen was stored in distilled water, was set as a reference value, and the change was measured by comparing the reference value with the result value of the experimental group. In both experiments, the results were rounded off from the third decimal place and expressed as the second decimal place.

2.2.3. Statistical analysis

The experimental results were analyzed using IBM SPSS Statistics ver. 21.0 (IBM Co., Armonk, NY, USA) and statistical analysis was performed with one-way analysis of variance (ANOVA) for group-specific significance test, and the significance between groups was analyzed with Tukey's post-hoc test. All analyses were performed at a 95 % confidence level.

3. RESULTS AND DISCUSSION

For microorganisms to survive in the oral cavity, they must be attached to the hard or soft tissues of the oral cavity [24]. Various researchers [6, 25-26] have studied the use various kinds of chemical or antibacterial agents to inhibit and interfere with the adhesion of these microorganisms or to remove microorganisms, such as mouthwashes and denture cleansers. Most mouthwashes and denture cleansers are manufactured using chemically synthesized ingredients. However, it has been reported that these ingredients change the surface of the resin, and when they come into contact with the tissues in the oral cavity, they can cause a toxic action, which affects the physical strength, color tone, and surface of the resin [20,27]. Accordingly, research has recently been carried out using various natural extracts [28-29].

However, research on the occlusal splint cleaning method has been insufficient so far, and although a detailed study on this is necessary, a denture cleanser is recommended as a cleaning method of the occlusal splint, and no other appropriate cleaning method has been proposed. However, these denture cleansers focus on fungi, which are microorganisms mainly present in dentures, rather than dental caries-causing bacteria. It can be said that a cleanser capable of simultaneously inhibiting fungi and caries-causing bacteria in the occlusal splint is needed. This study aimed to investigate the effect of applying CBEO as a cleaning agent for the occlusal splint by referring to the results of previous studies that confirmed the antimicrobial effect of CBEO among various natural extracts.

3.1. Antimicrobial evaluation

3.1.1. Disc diffusion method

As a result of the disc diffusion method analysis for antimicrobial evaluation, antimicrobial effects of cloves on both strains of *S. mutans* and *C. albicans* showed weaker than the positive control group and higher antimicrobial effect than the negative control group in both strains. The higher the concentration in the experimental group, the higher the antimicrobial effect. All results were significant ($p < 0.05$, Table 1, Figures 1–2). The study by Choi et al. [21] also showed results similar to those of this study, but at a concentration of 10 %, it showed a larger inhibitory ring than the one obtained by this study, showing a higher antimicrobial effect. In addition, Cho et al. [30] found that even at a concentration of 0.2 %, an antibacterial effect was exhibited, and an excellent antimicrobial effect was exhibited even at a lower concentration than that used in this study.

Table 1. Results of the disc diffusion method (Mean±standard deviation)

	Zone of Inhibition (mm) M±SD				
	Negative control *	5 %*	10 %*	15 %*	Positive control *
<i>S. mutans</i>	0.00 mm ^a	7.81±1.39 mm ^b	10.93±2.24 mm ^c	12.85±3.15 mm ^d	17.32±2.52 mm ^e
<i>C. albicans</i>	0.00 mm ^a	9.64±1.54 mm ^b	13.58±2.78 mm ^c	20.40±3.77 mm ^d	24.73±3.14 mm ^e

p value by one-way ANOVA and Tukey’s test
Different alphabetic letters indicate statistically significant differences
* $p < 0.05$

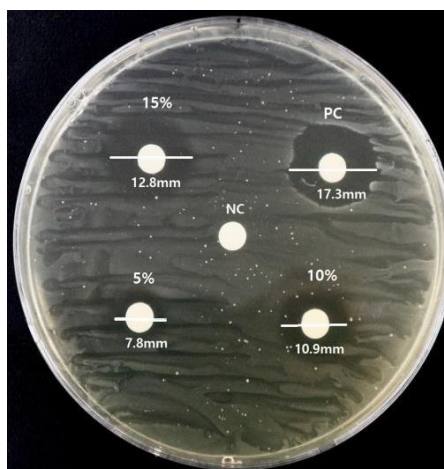


Figure 1. *S. mutans*

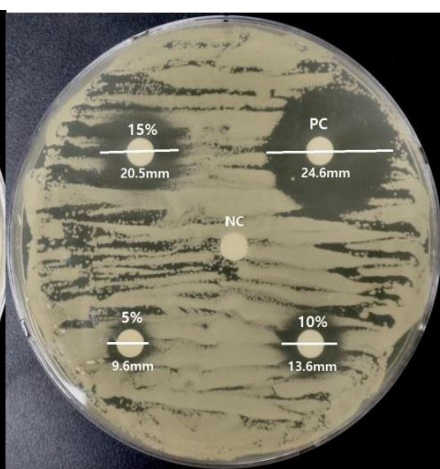


Figure 2. *C. albicans*

3.1.2. Microbial viability rate evaluation

The microbial viability rate revealed that both strains were affected significantly by the antimicrobial effect of CBEO ($p < 0.05$, Figure 3). In the case of *S. mutans*, the viability rate was approximately 50 % at a concentration of 10 %, and in the case of *C. albicans*, the survival rate was 45 % at a concentration of 5 %. According to Cui et al. [31], CBEO disrupts the cell membrane of bacteria and affects the synthesis of monocytogenes nucleic acids and the expression of related genes, which in turn interferes with the synthesis of specific proteins and enzymes. CBEO has been reported to exert antibacterial properties by reducing protein expression in cells. Therefore, it is considered that the antimicrobial effect was mediated by the above mechanism.

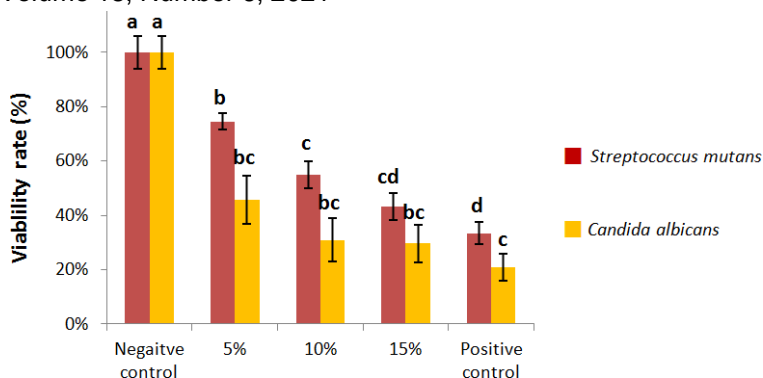


Figure 3. Viability rate of strains.

Different alphabetic letters indicate statistically significant differences.

3.2. Physical property evaluation

3.2.1. Hardness evaluation

The surface hardness of the experimental group was higher than that of the negative and positive control groups, but the difference was not statistically significant ($p > 0.05$, Table 2). In the study by Kim et al. [32], when Polident and Cleadent were used, the surface hardness decreased in the heat-curable resin compared to the control. In addition, in the study by Choi et al. [33], the surface hardness was measured after immersing the resin acrylic tooth in hexamedin, and the acrylic resin tooth showed a significantly high hardness decrease. However, there was no statistically significant difference between the cleanser containing CBE0 and the negative control group used in this study; therefore, it is considered that it does not affect the surface hardness of the resin.

Table 2. Vickers microhardness analysis results (Mean±standard deviation)

Group	Hardness (Hv)
Negative control (distilled water)	21.12±2.34
5 % Clove bud essential oil	22.57±3.50
10 % Clove bud essential oil	22.81±2.84
15 % Clove bud essential oil	23.00±2.80
Positive control - 0.12 % Chlorhexidine	20.69±2.36
Positive control - 0.6 % Na OCl	18.27±2.36

3.2.2. Color changes

The color stability analysis of the resin revealed that the ΔE^* value of the surface after storage for 24 h in the 5, 10, and 15 % experimental groups was not significantly different from that of the negative control group ($p > 0.05$, Table 3). Ruyter et al. [34] stated that it is possible to observe color changes visually when the change in the ΔE^* value becomes 1 or more, and it is acceptable when it is less than 3.3 in the dental area. Moreover, Johnston and Kao [35] reported that when ΔE^* was less than 1, the color change was mild, and when ΔE^* was between 1 and 2, it was clinically acceptable. In a study by Yang et al. [27], when a hypochlorite-based denture cleanser was applied to denture base resin, the resin specimens showed a color change that could be observed with the naked eye, and

alkaline peroxide-based Polident also showed a moderate color change in the resin specimens. The results of this study showed color changes of 1 or more and less than 2 in all experimental groups, indicating color changes in L^* , a^* , b^* , and ΔE^* values. However, based on the criteria of Ruyter et al., and Johnston and Kao, it can be said that although it is possible to observe the change visually, it is clinically acceptable.

Table 3. Color changes (ΔE^*) (Mean \pm standard deviation)

Group	ΔE^*
Negative control (distilled water)	0.00
5 % Clove bud essential oil	1.05 \pm 0.60
10 % Clove bud essential oil	1.47 \pm 0.78
15 % Clove bud essential oil	1.21 \pm 0.72
Positive control - 0.12 % Chlorhexidine	1.09 \pm 0.80
Positive control - 0.6 % Na OCl	1.17 \pm 0.48

The results of this study showed that an occlusal splint cleanser containing CBEO exhibited antimicrobial effects against *S. mutans* and *C. albicans* without any physical changes in the resin. In the future, it will be necessary to further study the extent to which the cleaning agent stimulates the oral mucosa for its use as an occlusal splint cleanser and to evaluate its efficacy. In addition, follow-up studies, such as efficacy over time, should be performed by further subdividing the concentrations of the components.

4. CONCLUSION

This study evaluated the antimicrobial effect on *S. mutans* and *C. albicans* by preparing an occlusal splint cleanser containing 5, 10, and 15 vol% CBEO. In addition, the physical properties of the resin were analyzed by examining the changes in the surface hardness and color of the resin.

1. The antimicrobial effect of CBEO against *S. mutans* and *C. albicans* was confirmed using the disc diffusion method and microbial viability rate evaluation, with a statistically significant difference of ($p < 0.05$).
2. As a result of confirming the change in the physical properties of the resin, there was no statistically significant difference between the surface hardness and the amount of color change ΔE^* compared to the negative control group ($p > 0.05$).

Therefore, the occlusal splint cleanser containing CBEO shows excellent antimicrobial effect without any physical change to the resin; therefore, it is believed that it can be used as a basic data for the development of a cleanser containing natural ingredients, such as CBEO.

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