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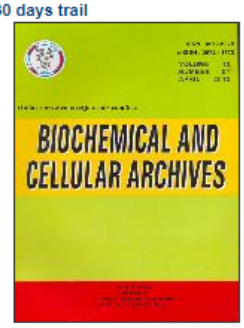

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ANTIBACTERIAL ACTIVITY OF CLOVE BUD ESSENTIAL OIL (*SYZYGIUM AROMATICUM*) AS COOLANT AGENT IN DENTAL ULTRASONIC SCALER AGAINST *ACTINOBACILLUS ACTINOMYCETEMCOMITANS*

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ABSTRACT : Dental clinicians prefer the use of ultrasonic scalers over manual scalers for dental treatment. The aim of this study is to examine the concentration of clove bud essential oil (CBEO) as an ultrasonic scaler coolant that could inhibit the growth of *Actinobacillus actinomycetemcomitans,* a key periodontal pathogen. Three replicates of 5 mL of 0.04%, 0.08%, 0.16%, 0.32%, and 0.64% CBEO, 5% PEG and 0.0005% chlorhexidine gluconate mixed with an ultrasonic scaler were sprayed into A. *actinomycetemcomitans* cultures (1.5×10^4 colony forming units/mL in 5 mL of 0.9%NaCl). A dilution of the bacterial–antibacterial mixture (200 µL) was inoculated on blood agar plates and then incubated for 3 × 24 h. Inhibitory effects were examined by the colony count method. The Kruskal–Wallis test showed significant differences in the antibacterial activity of different CBEO concentrations ($p < 0.05$). Multiple comparisons showed significant differences (1) between the negative control and 0.04% and 0.08% CBEO and (2) between 0.64% CBEO and the positive control. No significant difference was observed between 0.16%, 0.32% and 0.64% CBEO and the positive control. In conclusion, CBEO has potential application as an antibacterial ultrasonic scaler coolant. Further research on the optimum concentration and toxicity of CBEO is necessary.

Key words : *Actinobacillus actinomycetemcomitans*, antibacterial, bacterial inhibition, clove bud essential oil, dental scaling, ultrasonic scaler coolant.

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INTRODUCTION

The prevalence of periodontal disease in the global population is approximately 20%–50% (Nazir, 2017; Aprilianti *et al*, 2020). The disease begins with bacterial colonization in the plaque and calculus covering tooth surfaces (Preshaw, 2018; Nugraha *et al*, 2020). One of the causative pathogens of periodontal disease is *Actinobacillus actinomycetemcomitans* (Johansson and Kalfas, 2012; Ramadhani *et al*, 2020a). *A. actinomycetemcomitans* involves in the disease progression of periodontitis by inducing the inflammation process, increasing tissue disruption and producing leukotoxins that destroy immune cells, leading to therapy failure (Preshaw, 2018; Johansson and Kalfas, 2012; Ridwan *et al*, 2018). The initial therapy of periodontal disease begins with scaling to remove plaque and calculus, control the infection, improve gingival tissue healing, and reform periodontal tissues (Preshaw, 2018; Ramadhani *et al*, 2020b). Dental clinicians often prefer the use of ultrasonic scalers rather than hand instruments for treatment because the former offers less time and energy consumption (Krishna and De Stefano, 2016). Ultrasonic scalers have a cooling system that typically consists of distilled water flowing along the device to reduce the heat generated by ultrasonic vibration; this water is called coolant water (Ibiyemi *et al*, 2012).

As scaling treatment is performed, aerosols could be produced from the combination of water spray, compressed air and organic particles, such as tissue, blood, and saliva (Singh *et al*, 2016). Therefore, bacteria from the oral cavity and ultrasonic scaler waterline can generate airborne contaminants that may be harmful to the dentist and patient (Acharya *et al*, 2010; Molinari, 2012; Singh *et al*, 2016). Clove bud essential oil (CBEO) contains

antibacterial substances, such as eugenol, eugenyl acetate, and caryophyllene, which have been proven to be effective against Gram-positive and Gram-negative bacteria (Moon *et al*, 2011; Pandey and Singh, 2011). Therefore, addition of CBEO as an ultrasonic scaler coolant may be expected to decrease the number of bacteria in the dental unit waterline and aerosols and potentially improve the initial treatment of patients with periodontal disease. The aim of the current study is to investigate the concentration of CBEO as an ultrasonic scaler coolant that could inhibit the growth of *A. actinomycetemcomitans*.

MATERIALS AND METHODS

Clove bud essential oil distillation

Clove bud was obtained from Hargotirto Village, Kokap District, Kulon Progo Regency, D.I. Yogyakarta Province and identified by Laboratorium Sistematik Tumbuhan, Faculty of Biology, Universitas Gadjah Mada as *Syzygium aromaticum* (L.) Merr. & Perry. Steam distillation of CBEO was conducted at Laboratorium Penelitian dan Pengujian Terpadu (LPPT) Unit III Universitas Gadjah Mada (Damayanti and Setyawan, 2012). Briefly, ground clove buds were placed in a distillation pot with 7 L of water and then heated to 100°C. The vaporized essential oil was condensed into liquid form and placed in a sterile container.

In vitro antibacterial activity assay

CBEO was diluted with distilled water to concentrations of 0.04%, 0.08%, 0.16%, 0.32% and 0.64% with 5% PEG as the solvent. The CBEO samples, 5% PEG as the negative control and 0.0005% chlorhexidine gluconate as the positive control were filtered through 0.2 µm sterile syringe filters prior to streaming in an ultrasonic scaler (UDS-G Woodpecker) as antibacterial solutions. *In vitro* antibacterial examination was conducted in Balai Laboratorium Kesehatan Yogyakarta. Five-milliliter suspensions of 1.5 × 10^4 colony forming units (CFUs)/mL of *A. actinomycetemcomitans* in 0.9% NaCl were placed in a reaction tube (Ridwan *et al*, 2021). Approximately 5 mL of the antibacterial solutions was sprayed into the bacterial suspensions (bacterial:antibacterial = 1:1) at a pressure of 0.1–0.5 bar and vibration frequency of 30 ± 3 kHz. The tubes were vortexed for 10 s and allowed to stand for ±30 min to allow complete contact between the essential oil and bacteria. Each treatment group was replicated thrice. Aliquots of 0.2 mL were obtained from the bacterial–antibacterial mixtures, inoculated on blood agar plates via the spread plate method (Sanders, 2012) and then incubated at 37°C for 3 × 24 h in a CO_2 incubator.

Data collection and statistical analysis

Inhibition of *A. actinomycetemcomitans* growth was examined by direct bacterial colony counting of the blood agar plates (Fig. 1) (Sutton, 2011). The significance of the antibacterial effect of different concentrations of CBEO as an ultrasonic scaler coolant on *A. actinomycetemcomitans* was analyzed by the Kruskal–Wallis test followed by multiple comparisons ($\alpha = 0.05$). Statistical analyses were performed using IBM SPSS Statistics version 25 for Windows.

Fig. 1 : Visible growth of *A. actinomycetemcomitans* colonies on blood agar plates after 3 × 24 h of incubation.

RESULTS

The numbers of CFUs in treatments of different concentrations of CBEO, the negative control, and the positive control are presented in Table 1. The number of *A. actinomycetemcomitans* colonies decreased as the CBEO concentration increased (Fig. 2). The negative control (5% PEG) and 0.64% CBEO respectively showed the highest and lowest numbers of *A. actinomycetemcomitans* colonies. Kruskal–Wallis analysis confirmed the significant antibacterial effect of different concentrations of CBEO as an ultrasonic scaler coolant against *A. actinomycetemcomitans* ($p < 0.05$). The results of multiple comparisons between different treatment groups are presented in Table 2. Although, a general decline in *A. actinomycetemcomitans* CFU number was observed, differences among the CBEO concentrations of 0.04%, 0.08%, 0.16% and 0.32% were not significant ($p > 0.05$). No significant difference was found among the CBEO concentrations of 0.16%, 0.32%, and 0.64% and the positive control ($p > 0.05$). Significant differences in colony number were observed between

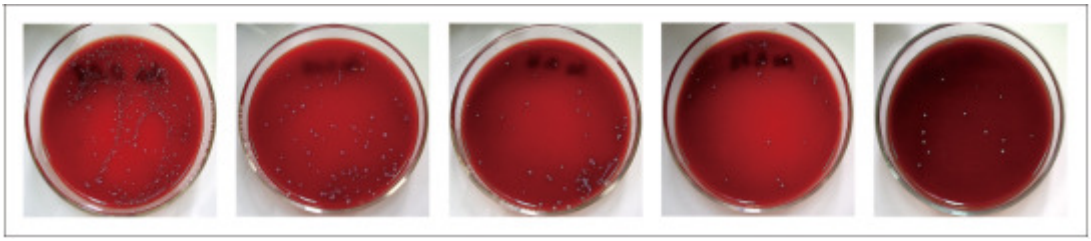

Fig. 2 : From left to right: Colony growth of *A. actinomycetemcomitans* in clove bud essential oil at concentrations of 0.04%, 0.08%, 0.16%, 0.32% and 0.64% in ultrasonic scaler coolant water.

Table 1 : Numbers of colony forming units of *A. actinomycetemcomitans* in each treatment.

Treatment	Mean of colony forming unit
Control (–)	55.87 ± 31.44
CBEO 0.04%	42.66 ± 13.38
CBEO 0.08%	36.22 ± 2.44
CBEO 0.16%	24.67 ± 2.12
CBEO 0.32%	15.11 ± 8.03
CBEO 0.64%	4.67 ± 4.34
Control (+)	10.11 ± 3.09

CBEO: Clove bud essential oil; Control (–): 5% PEG; Control (+): 0.0005% Chlorhexidine gluconate; S.E.M: Standard error of the mean.

for antiplaque agents and has an antibacterial mechanism similar to that of CBEO (Balagopal and Arjunkumar, 2013). The concentration of chlorhexidine in this work was based on its MIC against *Haemophilus influenza*, which belongs to the same family (*Pasteurellaceae*) as *A. actinomycetemcomitans* (Denton, 2001).

The main antibacterial compound in CBEO is eugenol (Moon *et al*, 2011). Eugenol is a phenol derivative that inhibits bacterial activity by partitioning bacterial cell membrane lipids and rendering them permeable. Antibacterial compounds can then penetrate the cell and

Table 2 : Cross-tabulation of multiple comparisons of the number of CFUs of *A. actinomycetemcomitans* in different treatments.

Treatment	Mean rank difference					
	CBEO 0.04%	CBEO 0.08%	CBEO 0.16%	CBEO 0.32%	CBEO 0.64%	Control (+)
Control (–)	**1.00**	**1.67**	**3.67**	**3.33**	**11.67***	**9.67**
CBEO 0.04%	-	0.67	4.67	4.33	12.67*	10.67*
CBEO 0.08%	-	-	5.33	5.00	13.33*	11.33*
CBEO 0.16%	-	-	-	0.33	8.00	6.00
CBEO 0.32%	-	-	-	-	8.33	6.33
CBEO 0.64%	-	-	-	-	-	2.00
Control (+)	-	-	-	-	-	-

*significant difference ($p < 0.05$)CBEO: Clove bud essential oil; Control (–): 5% PEG; Control (+): 0.0005% Chlorhexidine gluconate.

(1) the negative control and 0.64% CBEO, (2) CBEO at concentrations of 0.04% and 0.64% and the positive control, and (3) CBEO at concentrations of 0.08% and 0.64% and the positive control ($p < 0.05$).

DISCUSSION

Antibacterial agents are often added to ultrasonic scaler coolant water to reduce the presence of bacteria in the waterline and improve treatment effects (Jawade *et al*, 2016; Yilmaz and Bayindir, 2012). Therefore, the present study evaluated the potential use of different concentrations of CBEO as an antibacterial agent for ultrasonic scaler coolants. The range of CBEO concentrations used in this work was based on the study of Moon *et al* (2011), which indicated that the minimum inhibitory concentration (MIC) of CBEO against *A. actinomycetemcomitans* is 0.08%. Chlorhexidine was used as a positive control because it is the gold standard

cause precipitation, protein denaturation, cytoplasmic coagulation, and tissue disruption leading to lysis and cell death (Hyldgaard *et al*, 2012; Ridwan *et al*, 2020). This mechanism shows effective results against periodontopathogens (Moon *et al*, 2011; Pramod *et al*, 2016; Zhang *et al*, 2017). In present study, the number of *A. actinomycetemcomitans* colonies decreased as the CBEO concentration in the ultrasonic scaler coolant increased. Therefore, future studies using only eugenol as an antibacterial agent should be considered.

Ultrasonic scalers produce cavitation, which refers to the streaming motion of microscopic gas bubbles; this motion causes the bubbles to grow larger and eventually explode, resulting in hydrodynamic tension and stress (Vyas *et al*, 2017; Walmsley *et al*, 2013). High pressure and shear stress could cause cell damage and disruption (Capocelli *et al*, 2014). Miller *et al* (1985) demonstrated

that the microstreaming effect of stable cavitation at ultrasound frequencies could modify the membrane structure of cells and induce irreversible cell damage because of exposure to critical shear stress. In the present research, ultrasonic cavitation appeared to provide additional antibacterial effects against *A. actinomycetemcomitans.* Unfortunately, the antibacterial effect of ultrasonic cavitation alone could not be examined in the present study because a control experiment without the use of the ultrasonic scaler was not conducted. Future research could compare antibacterial activities with and without the application of an ultrasonic scaler.

Multiple comparisons indicated that the antibacterial effect of 0.08% CBEO was not significantly different compared with that of 0.0005% chlorhexidine gluconate. This result proves that CBEO at concentrations as low as 0.08% may be used an alternative antibacterial ultrasonic scaler coolant. The present study did not examine the MIC or minimum bactericidal concentration (MBC) of CBEO in ultrasonic scaler coolant water against *A. actinomycetemcomitans*. However, the decrease in *A. actinomycetemcomitans* growth observed at the maximum CBEO concentration of 0.64% indicates that further research involving the MBC of CBEO as an ultrasonic scaler coolant is likely greater than 0.64%. Interactions between the chemical components of CBEO and the ultrasonic scaler waterline should also be investigated to prevent tract defects due to continuous usage.

CONCLUSION

In conclusion, CBEO has potential to be applied as antibacterial coolant for ultrasonic scalers because it shows antibacterial activity. Further research to determine the optimal concentration of CBEO against *A. actinomycetemcomitans* and assess its toxicity is necessary to promote the use of the essential oil as an antibacterial agent in periodontal therapy.

Conflict of interest

The authors declare no conflict of interest related to the data contained in the manuscript.

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