Effects of Indian *Sandalwood* Essential Oil (*Santalum album Linn*) in Inhibiting the Growth of Biofilm *Streptococcus Mutans ATCC 25175*

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Abstract. *Streptococcus mutans* is a major cause of dental caries. The gold standard for antimicrobial mouthwash is *chlorhexidine gluconate* 0.2%. If used long term, *chlorhexidine gluconate* might induce an allergic response as well as tooth discoloration. The essential oil of Indian *Sandalwood* is a natural product with antimicrobial qualities and little adverse effects. The objective of this research was to see how Indian *Sandalwood* essential oil (*Santalum album Linn*) extract affected the biofilm formation of *Streptococcus mutans ATCC 25175*. *Streptococcus mutans ATCC 25175* was cultivated in nutrient broth for 24 hours at 37 °C. Inoculating the culture into a 96-well plate resulted in the formation of biofilm. After that, the well plate was incubated for 24 hours. It was prepared essential oils of Indian *Sandalwood* with concentrations of 12.5%, 6.25%, 3.125%, and 1.56%. The biofilm assay technique was used to evaluate the biofilm for 15 minutes. Positive controls used 0.2% *chlorhexidine gluconate*. The optical density values at 595 nm wavelength were observed using a microplate reader to achieve the result. The optimal concentration of Indian *Sandalwood* essential oil is 1.56%, which can limit the formation of *Streptococcus mutans ATCC 25175* biofilm. The *ANOVA* test reveals significant changes in each concentration group when compared to 0.2% *chlorhexidine gluconate*. The essential oil of Indian *Sandalwood* (*Santalum album Linn*) can suppress the biofilm formation of *Streptococcus mutans ATCC 25175*.

Keywords: Dental Caries, Indian *Sandalwood* Essential Oil (*Santalum album Linn*), *Streptococcus mutans*, Biofilm.

1 Introduction

The most common disease sequence in Indonesia is oral health problems. Dental caries is one of the most frequent preventable diseases and the leading cause of oral pain and tooth loss. According to the results of the National Health Survey (SKRT), 46% of the Indonesian group aged 10 years and older had gum disease and 71.2% had dental caries, while 76.2% of those aged 12 years had dental caries [1]. Dental caries is an infectious disease that begins with demineralization of the tooth hard tissues surface induced by organic acids produced from sugary diets [2]. *Streptococcus sobrinus*, *Streptococcus mutans*, and *Lactobacillus acidophilus* are some of the cariogenic bacteria [3].

M. Setiyo et al. (eds.), *Proceedings of the 4th Borobudur International Symposium on Science and Technology 2022 (BIS-STE 2022)*, Advances in Engineering Research 225,
https://doi.org/10.2991/978-94-6463-284-2_91

The study's findings that the major bacteria of dental caries is *Streptococcus mutans* in tooth plaque [4]. Dental caries is caused by the combination of four factors: bacteria, host, diet, and time [5]. Caries is characterized by pain, discomfort biting, eating, smiling, and expressing due to lost, discolored, or broken teeth. Caries' microbial community is varied, including many facultative anaerobes and obligately anaerobic bacteria [2].

Dental caries is the localized deterioration of susceptible dental hard tissues caused by acidic products of microbial fermentation of carbohydrates [2]. Bacteria appear in two types: planktonic and biofilm. The oral microflora on the tooth surface forms polymicrobial colonies known as dental biofilm [6]. The biofilm extracellular polymer (EPS) matrix creates a pathogenic environment for cariogenic bacteria. Dental caries is typically a biofilm-induced diseases rather than an infectious disease, with the disease process starting in the biofilm that covers the tooth's surface. The biofilm's structure gives it particularly resistant to antimicrobial agents such as antibiotics, disinfectants, and germicides [7].

Santalum album Linn (*Santalaceae*), often called as Indian *Sandalwood*, is a natural substance with a fragrant aroma that is effective as a medication [8]. Indian *Sandalwood* oil is used in herbal medicine as an antibacterial, antipyretic, and to treat bronchitis and urinary tract infections [9].

Sandalwood has sedative, astringent, diuretic, expectorant and stimulant effects. It is useful to treat irritation of the stomach, jaundice, dysentery, tension, anxiety and is also used as a remedy for the heart, digestion tract, liver, anti-toxic, shivers and stage blood. vessel8

2 Methods

2.1 *Santalum album linn* oil preparation

Essential oil is extracted from Indian *Sandalwood* powder. Extraction using microwave air-hydro distillation method. Indian *Sandalwood* powder is dissolved with deionized water for 2 hours in a microwave oven. The essential oil is then evaporated to eliminate any remaining water. Dilution of oil with a tween 20 solution produced concentrations of 12.5%, 6.25%, 3.125%, and 1.56%.

2.2 *Streptococcus mutans* culture

Streptococcus mutans bacterium samples were collected from the Faculty of Dentistry's Microbiology Core Laboratory (MiCORE). A test tube is filled with a suspension of McFarland standard bacteria equivalent to 1.5 x 108 CFU/mL in 0.65 gram nutritional broth and 50 mL of *aquades*. It was then cultured for 24 hours at 37°C.

2.3 Biofilm assay

Streptococcus mutans biofilm was extracted by using up to 20 L of bacterial solution (1.5 × 108 CFU/mL) and artificial saliva. The culture is then homogenized using a vortexer before being placed in a 96-well plate and cultured for 24 hours at 37 °C.

Following incubation, the bacterial medium is removed with a micropipette. Each sample is loaded onto a micro well-plate with 12.5%, 6.25%, 3.125%, and 1.56% concentrations of Indian *Sandalwood* essential oil. After incubating for 15 minutes at 37°C, it is rinsed twice with *aquades*. Furthermore, fixation sample by passing it through the fire and stained with violet crystal stain (0.5% w/v), which was then dispersed into a well-plate and cultured for 15 minutes. It is then removed and washed with *aquades*. The microplate is then filled with 96% ethanol and left to incubate for 15 minutes. The results of biofilm calculations were measured using a microplate reader with a wavelength of 595 nm. The positive control used was 0.2% *chlorhexidine*.

2.4 Data analysis

The *anova* one-way statistical test was used to examine the distributed data and the Tukey-HSD test was used to see significance of the differences data. The significance level was set at $p<0.05$.

3 Results

The findings of the experiments suggest that the concentration of Indian *Sandalwood* essential oil is the most effective in suppressing the biofilm *Streptococcus mutans ATCC 25175*, specifically during the incubation period of 15 minutes concentration of 1.56% with OD of 0.1704 0.0072. Then following concentrations of 3.13%, 6.25%, and 12.50%. The OD of the positive control is 0.2901 0.0318. The OD of the negative control is 0.354 0.021. The experiment lasted 15 minutes. (Table 1) Based on the findings of descriptive statistical analysis on OD data with a 15-minute incubation time, the optimal concentration is at a concentration of 1.56% with OD of 0.1704 0.0072. (See Fig. 1).

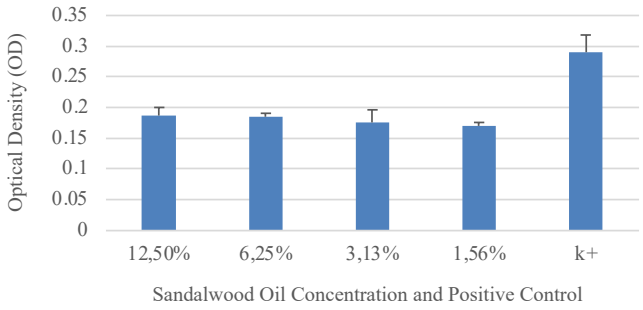

Fig. 1. *Streptococcus mutans ATCC 25175* optical density biofilm after a 15-minute incubation period with Indian *Sandalwood* essential oil

Table 1. Average *Streptococcus mutans ATCC 25175* optical density biofilm after a 15-minute incubation period with Indian *Sandalwood* essential oil

Sandalwood concentration	Optical Density 15-minute 595 nm
12.50%	0.1868 ± 0.0159
6.25%	0.1858 ± 0.0059
3.13%	0.1767 ± 0.0217
1.56%	0.1704 ± 0.0072
K+	0.2901 ± 0.0318

The normality test findings on OD data with a 15-minute incubation period show that all measurement data in each concentration group are normally distributed since the whole data group produces a p>0.05 value. So that the parametric statistical approach, especially the One Way *Anova* test, may be used for the comparison test. The One Way *Anova* statistical test findings revealed that there was a significant difference in the data investigated, with Sig. =.000 (p0.05).

The data continued with the Post Hoc LSD test, there was a significant difference in the 15-minute incubation period in the negative control with the average OD of the positive control. The mean OD on positive controls differed significantly from the average OD at concentrations of 12.5%, 6.25%, 3.125%, and 1.56%.

4 Discussion

The most frequent bacterial infections in the oral cavity are dental caries and periodontal disease. Dental caries and periodontal disease are the most common microbial infections.10 *Streptococcus mutans* bacteria are very significant numbers of dental plaque bacteria that may create extracellular polysaccharides and colonize the tooth surface at acidic pH levels, considerably influencing caries development [11].

Positive control used in this study was 0.2% *chlorhexidine gluconate*, *chlorhexidine* is the most effective for antiplaque. *Chlorhexidine* as a mouthwash can help prevent tooth plaque and gingivitis [12]. The use of *chlorhexidine gluconate* 0.2% mouthwash has side effects if used for a long time, namely such as tooth discolouration and loss of taste sensitivity [13].

This study used Indian *Sandalwood* plant (*Santalum album Linn*.) which has several active compounds *α-santalol*, *β-santalol*, and *α-bergamotol* [14]. In previous studies, it was known that The essential oil of Indian *Sandalwood* has antibacterial properties against gram-positive bacteria such as, *S. epidermidis* and *S. pyogenesada* in previous studies, it was known that The essential oil of Indian *Sandalwood* has antimicrobial properties against gram-positive bacteria. as, *S. aureus*, *S. epidermidis*, and *S. pyogenes* [15].

Indian *Sandalwood* essential oil is obtained using the microwave water-hydrodistillatio method, according to previous research [16]. Tween 20 serves as an emulsifier in oils, which can improve solubility in essential oils [17]. Tween 20 has no antibacterial [18].

There are several methods for testing the production of biofilms, including method of the congo red agar, dilution method, microtiter plate assay, SEM, and Transmission Electron Microscopy. Based on prior research, this study used the microtiter plate assay method as a biofilm production test since it is quick, simple, and sensitive [19].

The active compounds in Indian *Sandalwood* essential oil are *α -santalol*, *β -santalol*, and *α -bergamotol*. The essential oil of Indian *Sandalwood* contains 50-70% santalol [16]. The main component of *Sandalwood* plant oil is *alpha-santalol*, which has the ability to reduce incidences of *papillomas* (skin tumors) and has a sedative effect because it can affect the central nervous system [20]. *Alpha-santalol* is also known to have anticancer properties [21]. *Beta-santalol* has antihyperlipidemic properties [22].

5 Conclusion

According to the results, the Indian *Sandalwood* essential oil (*Santalum album Linn*.) inhibited the formation of the biofilm *Streptococcus mutans ATCC 25175*.

Acknowledgments. This research was performed in the Microbiology Center of Research and Education (MiCORE) laboratory of the Faculty of Dentistry at Universitas Trisakti.

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