



The Effectiveness of Propolis Antibacterial Against *Staphylococcus aureus* as an Alternative to Root Canal Irrigation

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Abstract

Introduction: Irritation by bacteria can cause infection of the dental pulp, including *Staphylococcus aureus* which is the most resistant facultative microorganism that can cause root canal treatment failure. Root canal treatment is necessary to eliminate infection and protect the decontaminated tooth. Eliminating microorganisms from infected root canals is a complex task that requires various instrumentation techniques such as root canal irrigation, and the selection of irrigation materials that have antibacterial criteria, one of which is propolis. The antimicrobial properties of propolis are related to the presence of flavonoids. The antimicrobial activity of propolis is very effective against gram-positive and gram-negative bacteria. **Purpose:** To explain the inhibition of propolis extract as an alternative to irrigation solution in habituating the growth of *Staphylococcus aureus* bacteria in root canal treatment. **Methods:** This research is true experimental research conducted using the disc method in a laboratory. The samples tested were 24 samples in the form of *Staphylococcus aureus* bacteria in Tryptic Soy Agar (TSA) media. Variations of treatment concentration were propolis extract 2.5%, 5.25%, NaOCl 5.25% (positive control) and sterile distilled water (negative control). **Results:** The average inhibition of *Staphylococcus aureus* bacteria in propolis *Trigona sp.* 5.25% was 8.6 ± 0.5 mm. In positive control NaOCl 5.25% was 9.5 ± 0.9 mm. Hypothesis testing has a value of $p=0,000$ ($p<0,05$). **Conclusion:** There is an inhibitory activity against *Staphylococcus aureus* bacteria at a concentration of 5.25% propolis extract.

Keywords: Propolis; root canal treatment; root canal irrigant; *Staphylococcus aureus*

Introduction

Dental pulp is a vital/complicated tissue that is protected by the dentin of the tooth, as the pulp tissue is damaged or dead. Irritation by bacteria can also affect infection of the dental pulp, which includes bacteria in caries that gradually reach the pulp which can lead to the infection of the pulp.^{1,2} According to Yamin et al. (2014) bacteria identified from necrotic root canals are: *Acinobacter calcoaceticus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Actinomyces spp* and *Streptococcus spp.*²

Staphylococcus aureus is a Gram-positive bacterium which is the main pathogen for humans that can cause various clinical infections.^{3,4} *Staphylococcus aureus* is also a facultative microorganism that is considered as the most resistant species in the oral cavity and a possible



cause of root canal treatment failure.^{5,6} Root canal treatment is required to eliminate infection and protect the decontaminated tooth further.⁷

Eliminating microorganisms from infected root canals is a complex task that requires various instrumentation techniques such as root canal irrigation.^{8,9,10} Good root canal irrigation using the appropriate irrigating agent is able to clean the smear layer with minimal toxicity.¹¹ NaOCl is the gold standard for root canal irrigation, but NaOCl has disadvantages such as unpleasant taste and corrosiveness. Therefore, a more practical and affordable alternative material has emerged from herbal ingredients, namely propolis^{12,13,14}. Propolis has antimicrobial, antioxidant, anti-inflammatory and antiproliferative properties. The antimicrobial characteristics of propolis are associated with the presence of flavonoids. The present study was undertaken to assess the potential of propolis extract against microorganisms present in root canals.^{15,16}

Methods

This study is an experimental laboratory research conducted in a laboratory setting. The research design employed is the post test-only design. The research sample used was *Staphylococcus aureus*. The variables tested included propolis extract at concentrations of 2.5% and 5.25%, NaOCl at 5.25%, and sterile distilled water. Raw propolis, obtained from *Trigona sp.* bees in Cibubur, was extracted using the maceration method.

The sample size was calculated using the Federer formula, resulting in 6 samples per group and a total of 24 samples. The inhibition zone was measured using the paper disc diffusion method. Data were analyzed using the SPSS software.

Normality of the data was assessed using the Shapiro-Wilk test to evaluate the differences in the inhibition zones for the different concentrations of propolis extract, NaOCl, and sterile distilled water. If the data were not normally distributed ($p < 0.05$), the Kruskal-Wallis test was used for analysis. Post hoc analysis was conducted using the Mann-Whitney test for pairwise comparisons.



Results

The inhibitory power of propolis extract at concentrations of 2.5% and 5.25%, NaOCl at 5.25%, and sterile distilled water against *Staphylococcus aureus* was measured using the paper disc diffusion method, as presented in Table 1. The inhibition of bacteria was indicated by the absence of *Staphylococcus aureus* growth around the paper disc, forming an inhibition zone or a clear zone. The size of the inhibition zone was measured using a digital calliper in millimetres.

Table 1. Descriptive statistics

Groups	Mean	Median	Standard Deviation
Extract propolis 2.5%	6	6	0.0
Extract propolis 5.2%	8.6	8.3	0.5
K+ (NaOCl 5.25%)	9.5	9.5	0.9
K- sterile Aquades	6	6	0

Table 1 showed the initial analysis involved descriptive statistical analysis to determine the characteristics of the data from the research results.

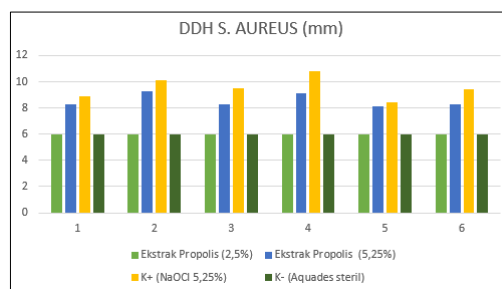


Figure 1. The DDH test results

Based on the data from the DDH test results in Fig.1, it can be seen that the highest DDH was found in the K+ group with an average of 9.5 mm, a median of 9.5 mm and a standard deviation of 0.9 mm, while based on propolis extract treatment, the highest DDH was obtained



in 5.2% propolis extract treatment with an average of 8.6 mm, a median of 8.3 mm and a standard deviation of 0.5 mm.

Based on Table 2, the normality test for the growth inhibition data of *Staphylococcus aureus* using the Shapiro-Wilk test showed a statistic of 0.049 with $p < 0.05$, indicating that this data group was not normally distributed. For the 5.25% NaOCl group, the test yielded a statistic of 0.967 with $p > 0.05$, indicating that this data group was normally distributed.

The normality test for the inhibition of *Staphylococcus aureus* after administering 2.5% propolis extract and sterile distilled water could not be calculated due to a lack of variation (constant data). Therefore, these data groups are considered not normally distributed.

Table 2. Normality Test

Groups	P-Value
Propolis Extract (2.5%)	-
Propolis Extract (5.25%)	0.049
K + (NaOCl 5.25%)	0.967
K-(sterile distilled water)	-

Based on the test results listed in the data in Table 2, it can be seen that $p\ value < 0.05$, then H_0 is rejected, indicating that there is a significant difference in the inhibition of growth of the *Staphylococcus aureus* after being given 5.25% propolis extract and 5.25% NaOCl.

Discussion

The results showed that propolis extract at a concentration of 5.25% and NaOCl at 5.25% both had inhibitory zones around the paper disc, indicating inhibition against the growth of *Staphylococcus aureus*. Mann-Whitney test results showed that the 5.25% propolis extract had inhibitory power, but it was not significantly different from the inhibitory power of 5.25% sodium hypochlorite (NaOCl) on the growth of *Staphylococcus aureus*. This inhibition is due to the presence of flavonoid compounds in the propolis extract, which have antibacterial properties that can inhibit bacterial growth. Flavonoids, identified as polyphenolic compounds,



can exert antibacterial activities through various mechanisms of action, including suppression of nucleic acid synthesis, cytoplasmic membrane function, and energy metabolism.^{14,15}

According to David and Stout (1971), antibacterial activity can be categorized based on the inhibition zone formed around the paper disc. The assessment criteria are as follows: an inhibition zone diameter of 5 mm or less is categorized as weak, 5-10 mm as moderate, 10-20 mm as strong, and 20 mm or more as very strong. Referring to these criteria, propolis extract at a concentration of 5.25% exhibited moderate antibacterial activity, while sodium hypochlorite (NaOCl) at 5.25% exhibited strong antibacterial activity.¹⁷

This study is in line with research conducted by Lestari et al., which stated that propolis had antimicrobial activity on *S. aureus*, as well as research conducted by Mentari et al., which found that the average antibacterial inhibition zone of propolis against *Staphylococcus aureus* was greater than that of honey.

Conclusions

The results of this study stated that there was an inhibition of propolis extract concentration of 5.25% and NaOCl 5.25% against the growth of *Staphylococcus aureus*. This research needs to be studied further because phytochemical tests were not carried out to determine more clearly about the components of bioactive compounds contained in propolis extract.

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