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Research Report

Assessing the antibacterial efficacy of nano propolis concentrations on *Streptococcus mutans*

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ABSTRACT

Background: *Dental caries is a common disease among the Indonesian population, with a prevalence of dental caries in Indonesia reaching 88.8%. Streptococcus mutans is one of the types of bacteria found in caries lesions. Nano propolis has antibacterial properties that can help inhibit the growth of Streptococcus mutans bacteria, reducing the risk of dental caries.* ***Purpose:*** *This study aims to determine the effective concentration of nano propolis ranging from 2.5% to 0.625%.* ***Methods:*** *The research evaluates various concentrations of nano propolis, and the minimum concentration that inhibits the growth of Streptococcus mutans is observed using the colony count method.* ***Results:*** *The growth of Streptococcus mutans at a concentration of 2.5% nano propolis was 0, at 1.8% it was 6, at 1.25% it was 11.4, and at 0.625% it was 42. The positive control group containing BHIB media and Streptococcus mutans bacteria showed an average colony growth of 161.* ***Conclusion:*** *Nano propolis at a concentration of 1.25% is effective in inhibiting the growth of Streptococcus mutans.*

Keywords: *nano propolis, Streptococcus mutans, effective concentration*

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INTRODUCTION

The human oral cavity serves as a complex environment for various microorganisms, forming dynamic interactions and biofilm. Imbalances in this ecosystem, such as an overgrowth of *Streptococcus mutans*, can lead to oral diseases like dental caries. Understanding the roles of microorganisms is crucial for developing effective strategies for oral disease prevention and treatment.[1]

Streptococcus mutans, oral flora bacteria, plays a central role in dental caries due to its acid-producing ability. Efforts to prevent dental caries include reducing the quantity of *Streptococcus mutans*. Natural substances like propolis, sourced from bee hives, have been studied for their potential to inhibit bacteria, especially when used in nanoparticle form, enhancing its antibacterial effectiveness.[2]

Nanoparticles, a recent technological innovation, offer unique characteristics and significant applications in various sectors, including pharmaceuticals. Propolis, known for its low water solubility, can benefit from nano-formulation, improving its solubility and effectiveness in damaging bacterial cell walls with lower concentrations. Despite the lack of research on effective nano propolis concentrations, advancements in nano-technology warrant further investigation for its potential as an antibacterial agent against *Streptococcus mutans*.[3]

MATERIALS AND METHODS

The research design employed in this study is the post-test only control group design. The bacterial stock sample tested in this research was *Streptococcus mutans* serotype C obtained from Research Center of the Faculty of Dentistry, Universitas Airlangga, Surabaya, Indonesia. The independent variable in this study is nano propolis with concentrations of 2.5%, 1.8%, 1.25%, and 0.625%. The dependent variable is the antibacterial efficacy against *Streptococcus mutans*. The controlled variables in this study are the equipment, materials, and media, incubation temperature, incubation time, and the procedure.

The research was conducted at the Research Center, Faculty of Dental Medicine, Universitas Airlangga. The inhibitory efficacy test in this research employs a dilution method using sterile water. To assess the effectiveness of nano propolis as an antibacterial agent against *Streptococcus mutans*, statistical analysis was conducted using the Shapiro-Wilk test for normality of data, Levene's test for

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homogeneity of variance, One-Way ANOVA test, and post-hoc Games Howell test.

RESULTS

This research utilized 40 test tubes which was divided into 5 groups: 30 tubes with *Streptococcus mutans* bacteria, BHIB media, and varying concentrations of nano propolis (2.5%, 1.8%, 1.25%, 0.625%); 5 tubes as the negative control group containing BHIB media; and 5 tubes as the positive control group containing BHIB media and *Streptococcus mutans* bacteria. Colony count tests were conducted to assess the effectiveness of each nano propolis concentration against *Streptococcus mutans* bacteria. Results are shown in Table 1.

Based Table 1, it can be observed that nano propolis with a concentration of 1.25% exhibited an average bacterial growth of 11.4, considered as Minimum Inhibitory Concentration (MIC), while nano propolis with a concentration of 2.5% showed zero bacterial growth, considered as Minimum Bactericidal Concentration (MBC). This indicates that nano propolis with a concentration of 1.25% is effective in inhibiting the growth of *Streptococcus mutans* bacteria.

Table 1. *Streptococcus mutans* colony growth based on nano propolis concentration (K-: Negative control group, K+: Positive control group, P1-P4: Groups with certain concentrations of Nano propolis)

	K-	K+	P1(2.5%)	P2 (1.8%)	P3 (1.25%)	P4 (0.625%)
Mean	0	161	0	6	11.4	42

DISCUSSION

This research employed the colony count method to determine the effective concentration of nano propolis as an antibacterial agent against Streptococcus mutans. The colony count method is a technique used to quantify bacterial growth on specific media by counting the number of bacterial colonies that develop.[4,5]

Various concentrations of nano propolis ranging from 2.5% to 0.625% were used in this study. The selection of these concentrations was based on previous research indicating that conventional propolis has effective antibacterial activity against *Streptococcus mutans* at a concentration of 5%.[6,7] In this study, the concentration of nano propolis was reduced to 0.625% with the hope that nano propolis would remain effective as an antibacterial agent against *Streptococcus mutans*.

The research results showed that the lowest inhibitory concentration against *Streptococcus mutans* was 1.25%, with an average colony growth of 11.4. Conversely, the lowest concentration without *Streptococcus mutans* growth was 2.5%. In the control group containing BHIB medium and *Streptococcus mutans* bacteria, the average colony growth was 161. Based on this data, it can be concluded that nano propolis at a concentration of 1.25% has the ability to inhibit the growth of *Streptococcus mutans*.

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