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RESEARCH ARTICLE

The Effect of Adding Propolis to Formula Milk on The *Streptococcus Sobrinus* Inhibition Zone (In Vitro)

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ABSTRACT

Background: *Caries is one of the dental and oral health problems that are often suffered by children. One of the main causes is the habit of children consuming formula milk as it contains sucrose. Streptococcus sobrinus is an oral pathogenic bacteria that has a role in the initial process of dental caries. One of the natural ingredients that function as anti-caries is propolis.* ***Objective:*** *Therefore, this study aims to determine the effect of adding propolis to formula milk on the inhibition zone of Streptococcus sobrinus bacteria.* ***Methods:*** *This type of research is a Laboratory Experiment with a Post-Test control Group Design. It consists of 4 groups, namely the formula milk group without propolis as a negative control, the formula milk treatment group with propolis concentrations of 5%, 10%, and 20% carried out with 7 replications of each group. The sample is a pure isolate of Streptococcus sobrinus bacteria.* ***Results:*** *The results showed the average inhibition zone was the formula milk group without propolis was 0.00±0.00 mm, the formula milk group with propolis concentration of 5% was 12.87±1.18 mm, the formula milk group with propolis concentration of 10% was 16.65±1.51 mm, and the formula milk group with propolis concentration of 20% was 24.87±0.39 mm.* ***Conclusion:*** *It means the addition of propolis to formula milk has been proven to be effective in inhibiting the growth of Streptococcus sobrinus bacteria.*

Keywords: *Formula milk, Inhibition zone, Propolis, Streptococcus sobrinus.*

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INTRODUCTION

Caries are one of the most common oral health problems suffered by various social and ethnic groups.[1] The prevalence of pediatric dental caries in Indonesia is still quite high, especially at the age of 3-4 years as much as 81.5% with an average def-t index of 6.2 and age 5-9 years occurs as much as 92.6% with a def-t index of 8.1.[2] The main microorganism that causes dental caries is mutans streptococci (MS), which are Streptococcus mutans and Streptococcus sobrinus. Previous studies have shown that the presence of these two cariogenic microorganisms in dental plaque is closely related to the beginning of the process of dental caries formation.[3] Based on several epidemiological studies and in vitro studies, Streptococcus sobrinus can be more cariogenic than Streptococcus mutans.[4]

Milk formula is an industrially produced milk that is used to fulfill the nutritional needs of children. fulfilled. Formula milk has a fairly high nutritional value because most of the essential nutrients are present in formula milk.[5] Parents' habits in serving formula milk that are not correct can contribute to ccurrence of dental caries in children.[6] The media for giving formula milk using a pacifier bottle until the child falls asleep for a long time can cause the surface of the primary teeth to be exposed to glucose-rich milk so that carbohydrate fermentation occurs and causes demineralization of tooth enamel.[7]

The use of natural materials in dentistry can support dental care programs, especially for the prevention and treatment of caries. One of the natural ingredients that functions as an antibacterial is propolis.[8] There are differences in the composition of substances contained in propolis depending on where the resin comes from, the climate, the type of bee and the time of collection. The content contained in propolis is resin and bioactive ingredients including flavonoids, tannins, artepicillins, saponins, phenols and apigenin. The content contained in propolis functions as antibacterial, anti-inflammatory, antiviral, antioxidant, antifungal, and immunomodulator.[9]

Based on the results of previous research on the effect of propolis extract and propolis candy solution on the growth of Streptococcus sobrinus, it was found that propolis with a concentration of 5%, 10% and 20% could inhibit the growth of Streptococcus sobrinus bacteria.[10] Effect of probiotic milk combined with propolis has a significant effect on the growth of Bifidobacterium infantis.[11] There has been no research that combines formula milk with the addition of propolis and its relationship to Streptococcus sobrinus. Therefore, the purpose of this study was to determine the effect of propolis addition to formula milk in various concentrations of 5%, 10%, and 20% on the growth inhibition zone of Streptococcus sobrinus bacteria.

MATERIALS AND METHODS

This type of research is *Laboratories experimental* with *Post Test Only Control Group Design.* Seven repetitions were carried out to determine the effect of adding propolis with the Brintish Propolis brand to formula milk with the Nestle Lactogrow 3 brand on the *inhibition* zone of *Streptococcus sobrinus* bacteria *(In vitro).*

Mixing of propolis milk formula was carried out by making propolis concentrations of 5%, 10%, and 20% propolis. Then dissolve 40 grams of formula powder into 400 ml of sterile distilled water heated to a temperature of 73 ° C until boiling. Then cool to room temperature ± 25°C. From each propolis concentration, namely 5%, 10%, and 20% concentrations. Take 2 ml of propolis solution to be added to 100 ml formula milk. Then put it in different plastic bottles.

Analysis of Results

The data obtained were then analyzed using SPSS to determine the clear zone formed on Petri dishes. The normality of the data was evaluated using the *Shapiro Wilk* test and the

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homogeneity of the data was evaluated using the *Levene Statistic test.* If the data obtained is normally distributed and homogeneous, the *One-Way ANOVA* parametric test is carried out with the *Post Hoc* test. If the test results obtained are not normal and not homogeneous, the *Kruskal Wallis* non-parametric test is continued with the *Mann Whitney test*.

RESULT

The smallest inhibition zone average in the formula milk group without propolis is 0.00 ± 0.00 and the largest inhibition zone average in the formula milk + propolis 20% group is 24.87 ± 0.39. Based on the Shapiro-Wilk test, the data of the formula milk group without propolis cannot be processed because it has a value of 0. The value in the formula milk group without propolis does not meet the assumption of normality so that the data is not normally distributed. While the 5% propolis + formula milk group, 10% propolis + formula milk group, and 20% propolis + formula milk group have a p>0.05 value. So that the data is normally distributed. So it can be concluded that the results of the normality test show that the data are not all normally distributed. The formula milk group without propolis cannot be processed in the homogeneity test because it has a value of 0. In the 5% propolis + formula milk group, 10% propolis + formula milk group, and 20% propolis + formula milk group, the Sig. value is 0.001 so it is concluded that the variance of the data of the 5% propolis + formula milk group, 10% propolis + formula milk group, and 20% propolis + formula milk group is not homogeneous. Thus, the requirements for parametric tests using *One-Way ANOVA are* not met because the data are not normal and not homogeneous, as an alternative, the *Kruskal Wallis* non-parametric test is used to determine the significant effect of adding propolis to formula milk on the *inhibition* zone of *Streptococcus sobrinus* bacteria *(In vitro)*.

Table 1. *Kruskal Wallis Statistical Result*

Value result sign	Standard sign	Description
0.000	< 0.05	There is a difference

The results of the *Kruskal Wallis* statistical test showed a *p* value of 0.000 which is <0.05, which means that there is a significant difference in influence between the formula milk group without propolis, formula milk + 5% propolis group, formula milk + 10% propolis group, and formula milk + 20% propolis group on the inhibition zone of *Streptococcus sobrinus* bacteria *(In vitro)*. Data were tested with the *Mann Whitney* test to determine which groups were different.

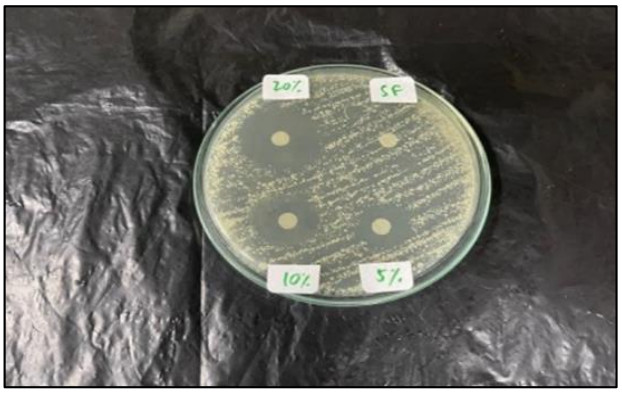

Figure 1. Diameter of inhibition zone formed from the addition of propolis to infant formula

Table 2. *Mann Whitney Test Result*

Group	Total N	P-value
Milk formula without propolis Milk formula + 5% propolis	14	0.001
Milk formula without propolis Formula milk + 10% propolis	14	0.001
Milk formula without propolis Formula milk + 20% propolis	14	0.001
Milk formula + 5% propolis Formula milk + 10% propolis	14	0.002
Milk formula + 5% propolis Formula milk + 20% propolis	14	0.002
Formula milk + 10% propolis Formula milk + 20% propolis	14	0.002

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Mann Whitney test results obtained *p* value <0.05 which shows the comparison of the average diameter of the inhibition zone between each group shows a significant difference (significant).

DISCUSSION

The clear area formed on the Petri dish indicates the diameter of the inhibition zone. The higher the concentration of propolis in formula milk, the greater the content of active substances in it so that the clear area formed is greater, which indicates that the inhibition zone formed is also greater. This is evidenced by the increase in the average inhibition zone in *Streptococcus sobrinus* bacteria, namely (12.87, 16.65, and 24.87). The addition of propolis with a concentration of 20% in formula milk has the largest inhibition zone and in formula milk without propolis there is no inhibition zone formed as shown in Figure 1.

The results of this study indicate that the effect of adding propolis to formula milk has an inhibitory effect on *Streptococcus sobrinus* bacteria. The decrease in the growth of *Steptococcous sobrinus bacteria is* due to propolis containing flavonoid chemical compounds. Brazilian propolis shows a high antibacterial effect on the growth of *Steptococcous sobrinus bacteria*. The result of the interaction of DNA and flavonoids results in damage to the permeability of microsomes, bacterial cell walls and lysosomes.[12]

Flavonoids function by inhibiting cell membrane function, inhibiting nucleic acid synthesis, and bacterial energy metabolism.[13] Inhibition of bacterial cell membrane function results in the formation of complex compounds from flavonoids that will damage the cell membrane of *Streptococcus sobrinus bacteria* and intracellular compounds of *Streptococcus* bacteria.

When inhibiting nucleic acid synthesis, rings A and B of the compounds in this flavonoid play a role in the interclassification process or hydrogen bonding process, namely the accumulation of nucleobases, resulting in inhibition of the formation of DNA and RNA.[15] Propolis is effective in inhibiting the growth of *Streptococcus sobrinus* during cell division. During cell division, the thin cell surface layer allows flavonoids to easily penetrate the cell wall and damage the cell contents of *Streptococcus sobrinus* bacteria.[10]

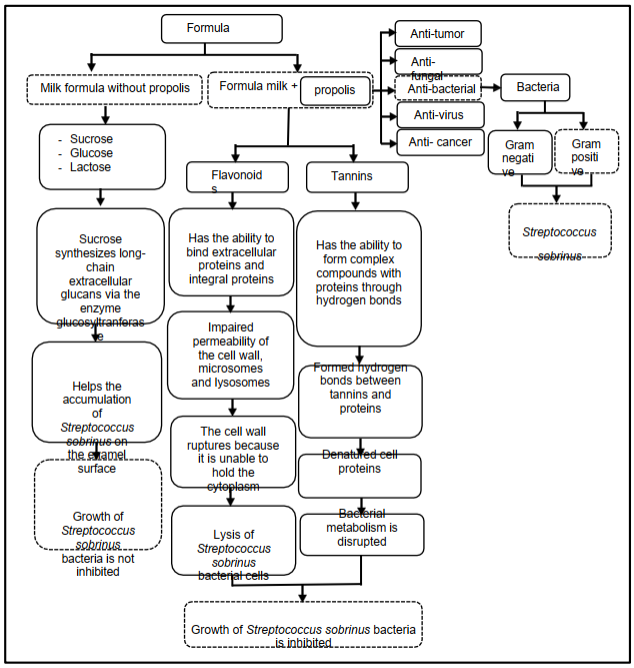

Figure 2. The role of propolis in inhibiting the growth of Streptococcus sobrinus

Propolis besides containing flavonoids also contains phenols that inhibit the growth of *Streptococcus sobrinus* bacteria.[14] Phenolic acid inhibits the growth of *Streptococcus sobrinus* bacteria by reducing the surface tension of *Streptococcus* bacteria cells. *sobrinus. The* OH group on phenol is toxic to the original nature of bacterial cells and can damage cells, penetrate the cell wall of *Streptococcus sobrinus bacteria* and denature enzyme proteins in the cytoplasm by forming hydrogen bonds at the active site of the enzyme.[16] This phenol compound can bind to *Streptococcus sobrinus* bacterial proteins. *This* weak bond between protein-phenol is formed in low concentrations that allow phenol penetration into cells which

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change protein properties and inhibit bacterial growth. Whereas in high concentrations phenol can cause protein coagulation and the lysis of *Streptococcus sobrinus* cell membranes. The active substances contained in propolis depend on the compound species and the collection site. Propolis extracts with higher levels of active substances can inhibit bacterial growth more effectively.[10]

Propolis also contains antibacterial compounds in the form of tannin. Tannin is one of the polyphenol class antibacterial compounds, soluble in water and organic solvents found in plants that can form protein complexes.[8] the way tannin works in inhibiting the growth of *Streptococcus sobrinus* bacteria is by lysing the bacteria. This is because tannin targets the wall polypeptides in the bacterial cell wall resulting in less the formation of bacterial cell walls so that bacterial cells will die.[14]

CONCLUSIONS

Formula milk with the addition of propolis at a concentration of 20% has the largest inhibition zone against the growth of *Streptococcus sobrinus* bacteria. Therefore it can be used as an alternative to reduce the risk of dental caries in children and formula milk without the addition of propolis does not have an inhibition zone on *Streptococcus sobrinus.*

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