**Research Report**

# Inhibitory effect of nano *Stolephorus insularis* and calcium hydroxide on glucosyltransferase (GTF) activity of *Lactobacillus aciophilus*

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## *ABSTRACT*

***Background:*** *Dental caries is the most common multifactorial infectious disease worldwide which referes to the process of destruction tooth hard tissue caused by bacterial by-products in the form of acids through carbohydrate fermentation. Lactobacillus acidophilus is one of the main cariogenic bacteria that causes caries by forming biofilms with the help of glucosyltransferase (GTF) enzymes. Calcium hydroxide has limited antibacterial effects. Nano brown anchovy contains fluor as the active compound which has the potential as an antibacterial alternative agent.* ***Purpose:*** *To explain the inhibition of the combination of nano Stolephorus insularis and calcium hydroxide on the activity of the glucosyltransferase enzyme of Lactobacillus acidophilus bacteria.* ***Methods:*** *This study used a combination of nano Stolephorus insularis 3.125% with calcium hydroxide as a treatment and aquades as a control. The GTF enzyme was obtained from the supernatant centrifuged by Lactobacillus acidophilus in BHIB. The activity of the GTF enzyme was considered by calculating the fructose levels using High Performance Liquid Chromatography (HPLC) in a certain formula.* ***Results:*** *Decreased levels of fructose was obtained in the treatment group. From the results of Mann- Whitney data analysis, there were significant difference in the study groups.* ***Conclusion:*** *the combination of nano Stolephorus insularis with calcium hydroxide is effective to inhibit the glucosyltransferase enzyme activity of Lactobacillus acidophilus bacteria.*

***Keywords:*** *Glucosyltransferase enzyme; Lactobacillus acidophilus; Stolephorus insularis*

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## INTRODUCTION

Dental caries is one of the most common multifactorial infectious diseases worldwide. Data Riset Kesehatan Dasar (RISKESDAS) for 2018 shows that the prevalence of the Indonesian population with active dental caries is 65.5%.[1] Dental caries is a process of destroying tooth hard tissue caused by bacterial by-products in the form of acids produced through carbohydrate fermentation.[2]

In a study of 65 samples of deep dentinal caries, the most common bacteria found was *Lactobacillus acidophilus*, this bacterium has an important role in the development and continuation of the caries process. In its pathogenesis, *Lactobacillus acidophilus* bacteria will form acids which result in demineralization of organic and inorganic materials on the teeth. In addition, these bacteria also play a role in the formation of biofilms to create an environment that can support the caries process. This biofilm is formed with the help of the enzyme glucosyltransferase (GTF) which can convert sucrose and produce glucan. Glucan is sticky and insoluble in water which can facilitate the formation of plaque and the colonization of microorganisms on the tooth surface.[3]

Calcium hydroxide ($Ca(OH)_2$) has long been used in the field of conservation dentistry and is commonly used as a root canal filling material, pulp capping material, intracanal medicament material, and pulpotomy treatment. The alkaline pH of $Ca(OH)_2$ plays a role in having antibacterial power which can influence or change the environment so that bacteria cannot develop.[4] The antibacterial activity of $Ca(OH)_2$ is due to the release of hydroxyl ions in an aqueous environment. Apart from its advantages as an antibacterial, calcium hydroxide has several disadvantages such as limited antibacterial activity and greater solubility.[5]

This research was done by combining $Ca(OH)_2$ and natural ingredients that can increase its antibacterial power without reducing the ability of calcium ions to regenerate dental hard tissue. One of them is nano *Stolephorus insularis*, The active compound in nano *Stolephorus insularis* which acts as an antimicrobial is fluoride. This is because F ions can inhibit the performance of two enzymes in bacteria, Enolase and F-ATPase enzymes. *Stolephorus* insularis has a high fluor content in the form of $CaF_2$ which is as much as 15.7-38.3 ppm.[6]

Based on research conducted by Yuanita, it was found that nano *Stolephorus insularis* has antibacterial activity


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against *Lactobacillus acidophilus* bacteria. In addition, the minimum bactericidal concentration of nano *Stolephorus insularis* against *Lacobacillus acidophilus* bacteria is 3.125%. So this study used the concentration of nano *Stolephorus insularis* of 3.125%.[6]

*Stolephorus insularis* to be studied is in nano form. Nanoparticles have components less than 100 nm in one dimension. Nanoparticles are widely used in dentistry because of their physicochemical and biological properties, including biocompatibility, size, charge, large surface area, strength, solubility, chemical and surface reactivity, color, high stability, and thermal conductivity.[7] Also nano-sized particles were used in this study because their small size makes it easier for nanoparticles to penetrate into the bacterial cell membrane thereby causing intracellular processes that cause reactions and greater antibacterial activity.[8]

This research was conducted with the aim to determine the inhibitory power of the combination of nano *Stolephorus insularis* and calcium hydroxide ($Ca(OH)_2$) in a 1 : 1 ratio on the activity of the GTF enzyme produced by *Lactobacillus acidophilus* bacteria.

## MATERIALS AND METHODS

Ethical clearance was obtained from the Health Research Ethical Clearance Commission of Universitas Airlangga Faculty of Dental Medicine (667/HRECC.FODM/IX/2022) before conducting the experiment. This study was an in vitro laboratory experimental study using the Post Test Only Control Group Design. The sample in this study was *Lactobacillus acidophilus* which was then taken by the GTF enzyme in the supernatant by centrifugation. There were 3 research groups, namely the negative control group using sterile distilled water, the positive control group using $Ca(OH)_2$ and the treatment group using a combination of nano *S. insularis* and $Ca(OH)_2$. The number of samples totaled ten in accordance with Federer's formula (1963).

Bacterial culture was taken from Lactobacillus acidophilus stock cultured in Mueller Hinton Agar (MHA) using sterile osse. then planted in a tube containing Brain Heart Infusion Broth (BHIB) and incubated for 24 hours at 37°C and the bacterial concentration adjusted to the standard 0.5 Mc Farland (1.5 x 108 CFU/ml) (6). Meanwhile, nano *Stolephorus insularis* obtained from the Surabaya Industrial Research and Consultancy Agency (BPKI) which was made into nanoparticles through a ball milling process and then diluted with distilled water up to 3.125%.[6]

In this research, 30 samples were split into three groups, they were negative control group, positive control group, and treatment group. Each group received 10 test tubes containing 0.875 ml of 0.25 M sucrose in pH 7, 0.2 M phosphate buffer, and 0.1 ml of GTF enzyme solution. In the negative control group, 0.025 ml of Aquadest was added, in the positive control group 0.025 ml of Ca(OH)2 was added, and for treatment group, 0.025 ml combination of nano *Stolephorus insularis* 3.125% + $Ca(OH)_2$ (ratio 1:1) was added. An incubation process was carried out in all control and treatment groups at 37°C for 2 hours.[9]

After incubation, enzyme activity testing was carried out at the Testing Service Unit, Faculty of Pharmacy, Airlangga University. Began by filtering the sample using 0.45 µm filter paper. Fructose levels were tested using High Performance Liquid Chromatography (HPLC), by injecting 10 µl of the treatment group solution or the control group solution, then the retention time was observed. The next step is to calculate the fructose content using the formula obtained by reading the fructose area in a fructose standard solution as follows.[9]

$$\text{Concentration}\,(\%) = \frac{\left\{\left(\frac{AC}{AS}\right) \text{x} \left(\frac{VIS}{VIC}\right)\right\}\, x\, 100\%}{\text{KS}}$$

Notes:
AC = sample area
AS = standard area
VIC = volume of sample injection
VIS = volume of standard injection
KS = standard concentration
FP = diluted factor

The study measured means and standard deviations. there were four tests performed in this study. First, The Shapiro-wilk test was performed to calculate normally distributed data. The Levene's test is performed to calculate homogeneity of the data. The next calculation uses the Kruskal Walles test to calculate the differences between groups. lastly, the Mann-Whitney test was applied to see the value of significance between groups.

## RESULTS

The results of the reading and measuring of fructose levels with HPLC are divided into 3 groups, negative control group, positive control group, and treatment group using (Figure 1.) Measurement of fructose levels in each research group was carried out through the area of fructose in chromatogram. One unit of GTF enzyme activity is defined as 1 µ mol/ml fructose of the enzyme/hour.

The normality test was carried out with the Shapiro Wilk Test. The test results in the research group had a value of $p = 0.002$ ($p < 0.05$), this indicated that the data obtained in this study were not normal. Then do the homogeneity test using Leven's Test. Based on the test results, it was found that $p=0.001$ ($p<0.05$), which indicates that the data obtained in this study were not homogeneous. From the results of these data, a non-parametric test was carried out, namely the Kruskal Wallis test. Then the test results obtained $p = 0.000$ ($P < 0.05$), these results indicate that there is a significant effect on the use of a combination of nano *Stolephorus insularis* and calcium hydroxide on the activity of the glucosyltransferase enzyme.

Then the Mann-Whitney test was carried out to see if there was a significant difference in values between the treatment groups (Table 1). From the results of the Mann-Whitney test, the data obtained between the K(-) group and the K(+) group obtained p=0.000, between the K(-) group and the P group obtained p=0.003, and between the k(+)

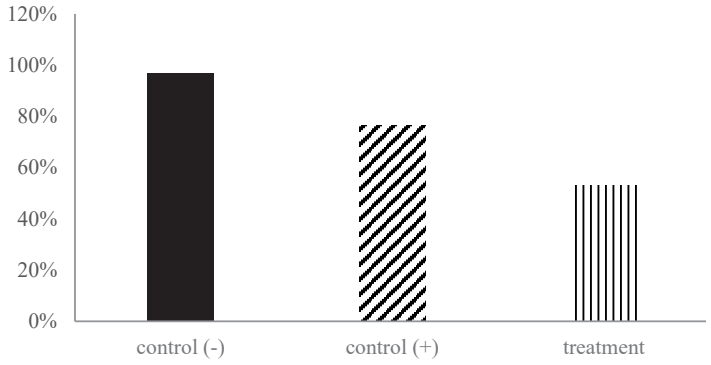


**Figure 1.** The mean and SD level of fructose in the controls and treatment groups.

**Table 1.** The results of the Mann-Whitney

| | Negative Control | Positive Control | Treatment Group |
|---|---|---|---|
| Negative control (aquades sterile) | - | p value = 0.000* | p value = 0.003* |
| Positive control ($Ca(OH)_2$) | - | - | p value = 0.000 |
| Treatment group (Combination nano *Stolephorus insularis* 3.125% + $Ca(OH)_2$ | - | - | - |

Note: significant ($p$<0.05)

group and the P obtained p=0.000. The test results showed a p value <0.05 in all treatment groups, which means that there were significant differences between treatment groups.

## DISCUSSION

The results showed that the highest average fructose level was in the negative control group (K(-)) which was 98.63% with sterile aquadest as the variable. In the positive control group (K(+)) an average decrease in fructose levels was obtained by 76.31%, in the K(+) group with the variable being $Ca(OH)_2$. Whereas in the treatment group (P) the lowest average decrease in fructose levels was 53.21%, with the variable a combination of nano *Stolephorus inslaris* and Ca(OH)2 (1:1 ratio).

The results showed that K(-) did not inhibit GTF enzyme activity, so the fructose levels produced were very high. This is because K(-) uses a variable in the form of sterile aquadest which is a neutral compound so that it does not show any inhibition on the activity of the GTF enzyme.

Whereas in K(+) there is inhibition of GTF enzyme activity, seen from the decrease in the level of fructose produced. This is because K(+) uses a variable in the form of $Ca(OH)_2$ which has antibacterial properties. $Ca(OH)_2$ has an antibacterial effect through the release of hydroxyl ions ( OH- ) in the form of high oxidant free radicals which cause extreme reactivity. Then the hydroxyl ions cause damage to the cytoplasmic membrane, protein denaturation, and damage to bacterial DNA.[10] which ultimately causes an inhibitory effect to the GTF enzymatic process.

Furthermore, in the treatment group (P) there was a large inhibition of GTF enzyme activity, which can be seen from the greatest decrease in fructose levels. This is because the treatment group uses a variable in the form of a combination of nano *Stolephorus insularis* and $Ca(OH)_2$ which provides greater antibacterial power. The $Ca(OH)_2$ material has antibacterial effect which is then combined with *Stolephorus insularis* nano which also has antibacterial properties. Nano *Stolephorus insularis* contains a calcium fluoride compound ($CaF_2$) which produces antibacterial power through the release of fluor ions which then bind to hydrogen ions to create hydrofluoric acid which is able to penetrate bacterial membranes. Hydrofluoric acid in bacteria dissociates which then causes the cytoplasmic atmosphere to become acidic and inhibits enzymatic activity in bacteria[11], so that there are greater inhibitory effect to GTF enzyme activity.

The very small nano size also makes it easier for $CaF_2$ particle material to penetrate into the bacterial cell membrane, causing intracellular processes that cause greater antibacterial reactions and activity.[8] This is mainly due to its large surface area which can present a large number of atoms on its surface, resulting in maximum contact with the environment.

In the treatment group, it can be seen that the activity of the GTF enzyme is low, this indicates that the synthesis of glucan and free fructose from the substrate sucrose is slow. In contrast, in the negative control group, the highest GTF enzyme activity could be seen which indicated that there was no inhibition of GTF enzyme activity in the use of sterile aquadest. Free fructose in this study is a product of synthesis of the GTF enzyme by *L. acidophillus* bacteria. The low concentration of fructose is assumed to be successful in inhibiting GTF enzyme activity in *L. acidophillus* bacteria.

Based on the research that has been performed, it is known that a decrease in fructose levels indicates an inhibition of the activity of the GTF enzyme in *L. acidophillus* which


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in this case is due to the combined mechanism of $CaF_2$ in nano *Stolephorus insularis* and $Ca(OH)_2$ in inhibiting the metabolism of the bacterial cell wall. Thus the combination of nano *Stolephorus insularis* and $Ca(OH)_2$ is proven to be able to inhibit the activity of the glucosyltransferase enzyme *Lactobacillus acidophilus.*

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