

The Effect of *Uncaria gambir* Extract in Inhibiting *Streptococcus Mutans* Glucosyltransferase Enzyme

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Abstract

Gambir (*Uncaria gambir*) is a type of Indonesian plant used by many people as traditional medicine. It contains catechin and tannin that have antimicrobial properties.

One of the main bacteria in the oral cavity that plays a significant role developing dental caries is *Streptococcus mutans* (*S. mutans*). In the process of tooth decay, these bacteria produce glucosyltransferase enzyme (gtf) that has the ability to catalyze glucan synthesis.

The aim of this study was to know the effect of gambir in inhibiting glucosyltransferase enzyme. Samples were divided into 3 groups, Group A was a sample with 5 % gambir extract, Group B was a sample with 5% xylitol as positive control, and Group C was distilled water as negative control. High-Performance Liquid Chromatography (HPLC) was used to analyze and assess the fructose region to determine the gtf enzyme. The extensive fructose area was conducted based on each group's retention duration.

The results showed that *Uncaria gambir* demonstrated a higher ability to reduce the activity of the gtf enzyme than the negative control group (distilled water) ($p < 0.05$). Still, it had a similar ability compared to positive control ($p > 0.05$). It can be concluded that *Uncaria gambir* extract has an effect in inhibiting *Streptococcus mutans* glucosyltransferase enzyme.

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Introduction

Uncaria gambir is typically a kind of plant that is mostly found in Asia. People usually use this plant as a mixture of chewing betel which is useful for strengthening teeth so that the teeth do not easily decay.¹ Previous study reported that this plant has medical benefits with limited side effects because it contains catechin (59-90%) and tannin.² Catechin in *Uncaria gambir* is effective mostly in reducing Gram-positive bacteria, followed by Gram-negative bacteria.³ Harnis *et al* revealed that mouthwash with extract Gambir can inhibit the growth of *Staphylococcus aureus*.⁴ Dewi *et al* also reported that *Uncaria gambir* extract had an anti-cariogenic effect because this extract had the ability to reduce *S.*

mutans colonies, the decline of calcium weight, and micropores in enamel.⁵

Streptococcus mutans is an acid-producing bacteria that plays an important role in dental caries.⁶ *S. mutans* colonizes in dental surface and together with fermentable carbohydrates leads to demineralizing the hard tooth structure.⁷ This type of bacteria is commonly found to be the dominant microbes in the oral cavity.⁶ Other factors that make this bacteria have a central role in the etiology of dental caries are its ability to adhere to the hard tooth surface, capacity to build up glycogen reserves, strongly produce acidic metabolites, and synthesize extra-cellular polysaccharides.⁸ Previous studies reported that the appearance of *S. mutans* in the cavity of the tooth was followed by dental caries after 6 to 24 months. Since *S. mutans* adheres to the hydroxyapatite matrix of the tooth surface, the pH of the tooth surface is easily reduced to below the critical pH of hydroxyapatite demineralization.⁷

One of the virulence factors of dental caries of *S. mutans* is the glucosyltransferase (gtf) enzyme. This enzyme catalyzes the

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arrangement of soluble and insoluble glucan inferred from fructose and sucrose, while moreover playing a critical part in the polysaccharides contexture of the dental plaque.⁹ Glucan helps the attachment of oral bacteria and forms the accumulation of plaque on the tooth surface.¹⁰ The inhibiting of this enzyme can prevent tooth decay.¹¹ According to Dewi *et al* (2023), chewing candy containing catechin from *Uncaria gambir* extract could inhibit the synthesis of insoluble glucan produced by the glucosyltransferase enzyme.¹² The purpose of this study was to know the effect of *Uncaria gambir* in inhibiting *S. mutans*' glucosyltransferase enzyme.

Materials and methods

This research used a randomized post-test-only control group design of experimental investigation. The samples were *Streptococcus mutans* taken from the Microbiology Laboratory of the Faculty of Medicine, Universitas Sriwijaya, Indonesia. The samples were divided into three groups, Group A was samples with 5% gambir extract, Group B was samples with 5% xylitol, and Group C was samples with distilled water. The total samples were 30 samples. Gtf enzyme was derived from *S. mutans* and tested using HPLC on Polytechnic of Negeri Sriwijaya.

Uncaria gambir was obtained from gambir field in Babatoman Village, Musi Banyuasin district, South Sumatera Province, Indonesia. This material was identified and authenticated by Faculty of Agriculture, Universitas Sriwijaya, Indonesia.

Gambir leaves were dried in the sun for 48 hours without direct sunlight. The drying process was followed by an oven (CutLab Vacuum, USA) for 90 minutes.

The dried leaves were crushed and blended until smooth to become simplicia. A total of 60 grams of simplicia was wrapped in filter paper and cotton as a base and then inserted into a soxhlet tube (Biobase Biolin, China). The soxhlet tube was filled with 700 mL of 96% ethanol solvent and heated at 78.3°C (at the boiling point of the solvent) for 10 hours. The filtration was evaporated using a rotary evaporator at a temperature of 50°C to obtain 100% extract.¹³ The extraction results were placed in a glass container and aired until a dry extract was obtained. The gambir extract was

stored in the refrigerator and then ground using a mortar and pestle. The extract was weighed with an analytical balance and diluted to get a concentration of 5%.

Streptococcus mutans taken from Laboratory were cultured into a test tube containing 10 mL of liquid Brain Heart Infusion Broth (BHIB) media. After that *S. mutans* were incubated for 24 hours at 37°C. The culture medium was vibrated at a speed of 150 rpm with Thermoshaker (AMTAST, Indonesia), and centrifuged for 30 minutes at 1500 rpm by using Beckman Coulter Centrifuge, USA at 4°C. A Supernatant containing the glucosyltransferase enzyme was obtained.

Thirty test tubes were provided in this study, ten of which were used for each group, namely the treatment group (5% gambir extract), positive control (xylitol), and negative control (distill water). All groups were given a mixture of 0.875 ml of 0.25 M sucrose in 0.2 M phosphate buffer pH 7, added 0.1 ml of gtf enzyme solution. Group A was added with 5% concentration of gambir extract, while group B was added with xylitol. All samples were incubated in a water bath at 37°C for 120 minutes. Following incubation and filtration with 0.45 µm filter paper, 5 µl of the treatment solution or control solution was injected, and the retention time was then monitored to determine the fructose concentration using High-Performance Liquid Chromatography (HPLC) (Buchi, Sweden). To calculate the fructose content, the formula used is obtained by reading the area of fructose in the standard fructose solution as follows:¹⁴

Explanation:

SPA = the area of samples

STA = the standardized area

VIP = the volume of sample injection (5mL)

VIS = the volume of standard injection (5mL)

SC = the standard concentration (1%)

DF = dilution factor

One unit of gtf enzyme activity was defined as 1 µmol/mL fructose from the enzyme/hour.

The data was obtained based on the amount of fructose calculation using the SPSS program. Normality test to determine whether the data is normally distributed or not using the Shapiro-Wilk test. Homogeneity of variance test to determine the homogeneity of data variation in each group using Levene's test. The Kruskal-

Wallis test determined the differences between groups with a significance value of $p < 0.05$. After that, a Mann-Whitney test was continued to perform the differences between groups.

Results

The results showed that the normality test was $p > 0.05$, it meant that the data were normally distributed, while Levene's test result revealed that $p = 0.00$, indicating that the data were not homogeneous. The Kruskal-Wallis test indicated $p = 0.00$ ($p < 0.05$), which meant that there were significant differences between groups. Table 1 showed that negative control had a high means of fructose, followed by *Uncaria gambir* extract and positive control.

Groups	Means $\pm$ SD	P value
5% <i>Uncaria gambir</i> extract	5.77 $\pm$ 0.60	0.00*
Positive Control	5.02 $\pm$ 0.91	
Negative Control	23.67 $\pm$ 1.79	

Table 1. The Means of the area of fructose.

* Significant level, Kruskal Wallis-test, $p < 0.05$.

The analyzing data were continued with Mann-Whitney test to perform the differences between groups (Table 2).

From Table 2, it could be seen that *Uncaria gambir* extract had a similar effect with xylitol as a positive control group in inhibiting glucosyltransferase enzyme derived from *S. mutans*. However, those groups had significant differences compared to the negative control. Both groups had higher inhibition ability than negative control.

Groups/p value	<i>Uncaria gambir</i> extract	Positive control	Negative control
<i>Uncaria gambir</i> extract		P=0.054	P=0.00
Positive control			P=0.00
Negative control			

Table 2. Result of Mann-Withney test.

* Significant level, Mann Whitney-test, $p < 0.05$.

Discussion

This study showed that the concentration area of fructose in the positive control and treatment group had higher inhibition than the negative control group. The lower concentration was due to their ability to inhibit *S. mutans* enzyme. Distilled water was neutral, so *S.*

mutans was capable to colonizing and producing the gtf enzyme. While in *Uncaria gambir* extract group and xylitol group (positive control group), the enzyme was inhibited so the sucrose was not broken down into fructose.

Uncaria gambir is one of the plants that grows well in Southeast Asia. People in this country have consumed and also used this plant as traditional medicine.¹ *Uncaria gambir* extract had the same ability to reduce the gtf enzyme with xylitol as a positive control group. Liyana *et al* (2023) stated that xylitol was effective in inhibiting the biofilm formation of *Streptococcus mutans*. *S. mutans* cannot ferment xylitol, so it will change the mass of *S. mutans* biofilm.¹⁵ This bacteria can transport xylitol in an energy-consuming cycle.^{15,16} The phosphoenolpyruvate system converts xylitol to xylitol-5-phosphate. The dephosphorylation process of xylitol-5-phosphate needs a lot of energy so that *S. mutans* will starve and die.¹⁶ Loimaranta *et al* (2020) investigated that xylitol modified the expression levels of glucosyltransferase B, C, and D genes that were important in polysaccharide-mediated adherence of *S. mutans*.¹⁷

Uncaria gambir has a high concentration of catechin, which possesses antibacterial properties. Dewi *et al* mentioned that catechin in *Uncaria gambir* had three mechanisms as an antibacterial agent. Gambir inhibited nucleic acid synthesis and energy metabolism, damaged the cytoplasmic membrane, and changed the bacterial cell wall. This condition also caused the inhibition of the gtf enzyme.¹⁸ Aragao *et al* (2022) reported that catechin interfered with quorum sensing (QS), a regulator of gene expression to acclimate the environmental disadvantage.¹⁹ Kriswandini *et al* (2020) concluded that derivatives of catechin blocked the activity of gtf, which serves to assist biofilm formation. This inhibition may lead to a decrease in glucan synthesis so the colonization process was stopped.²⁰

The catechin and tannin contents of gambir extract inhibited the formation of insoluble glucan from sucrose by glucosyltransferase enzyme derived from *Streptococcus mutans*.⁸ This inhibition prevented the formation of extracellular polysaccharides. It hindered *S. mutans* from adhering to the tooth surface of hard tooth surface and prevented plaque production.⁷ Wu *et al* (2022) reported that

catechin had an inhibitory effect against biofilm formed from *Streptococcus mutans*.²¹ Another study, Schneider-Rayman *et al* (2021) explained that catechin derivatives reduced the dental biofilm of *S. mutans* because this active compound had down-regulation of genes responsible for extra-polysaccharides production.²²

Catechin disrupts the formation of biofilm through some different mechanisms. Catechin inhibits bacterial metabolism, promotes bacterial aggregation, and reduces gtf enzyme activity.³ Previous studies affirmed that catechin derivatives reduced the expression of three genes that code for glucosyltransferase enzyme.²³

Another active compound found in *Uncaria gambir* was tannin. Tannins are oligomers and polymers that have the ability to bind with proteins and polysaccharides in bacterial cell membranes.²⁴ This complexional binding inhibits extracellular enzyme of bacteria and oxidative phosphorylation. So that the bacterial metabolism will lose substrate availability and cause cell death.²⁵ Dharsono *et al* (2023) investigated the potency of tannin derived from *Uncaria gambir* against the bacterial enzyme.²⁶ The investigation of docking studies showed that tannin had high affinity and conformity to bacterial enzymes due to its biological activity of tannin, in which the hydrogen bonds to amino acids in bacterial enzymes.²⁷ Pribadi *et al* (2017) reported that tannin from *Mangosteen pericarp* extract inhibited the *S. mutans* gtf enzyme activity effectively.¹⁴

Conclusions

The conclusion of this study was *Uncaria gambir* extract inhibited *S. mutans* glucosyltransferase enzyme.

Declaration of Interest

The authors report no conflict of interest.

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