ORIGINAL ARTICLE

# Antimicrobial potency of toothpaste containing gambir (*Uncaria gambir)* extract

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## ABSTRACT

**Introduction**: The biggest problem in oral health is dental caries and periodontal disease. The way to prevent dental and mouth problems is to keep oral hygiene. One of them is adequate plaque control. Plaque control can be done by brushing teeth and using toothpaste. Currently, many herbal ingredients have been used as antibacterials such as gambir (*Uncaria gambir*). Gambir mainly contains catechins and tannins, which have the ability to inhibit the growth of bacteria. The purpose of this study was to determine the antimicrobial potential of toothpaste containing gambir (*Uncaria gambir*). **Methods**: The total sample was 30, and it divided into three groups. Samples were toothpaste containing ten percent of *Uncaria gambir* extract, fluoride, placebo toothpaste, *Streptococcus mutans* (Gram-positive), *Phorphyromonas gingivalis* (Gram-negative), and Candida albicans. Antimicrobial activity of toothpaste with gambir (*Uncaria gambir*) extract against microorganisms was observed with the formation of inhibitory zone diameter in agar. Data were analyzed by using SPSS with one-way ANOVA and Tukey test. **Results**: The results showed that toothpaste containing gambir extract could inhibit the *Streptococcus mutans* ($p<0.05$), *Phorphyromonas gingivalis* ($p<0.05$), *Candida albicans* ($p<0.05$) compared significantly with placebo ($p<0.05$). Antimicrobial toothpaste containing gambir extract had no different effect from toothpaste containing fluoride. However, toothpaste containing gambir extract had significantly different effect than toothpaste containing fluoride. **Conclusion**: It can be concluded that toothpaste containing gambir *(Uncaria gambir*) extract has antimicrobial potential against *Streptococcus mutans, Phorphyromonas gingivalis,* and *Candida albicans.* These findings showed that the extract of *Uncaria gambir* possesses potent antimicrobial activity.

**KEYWORDS**
antimicrobial, *candida albicans, phorphyromonas gingivalis, streptococcus mutans, uncaria gambir*

## INTRODUCTION

Dental caries, Periodontal disease, and oral mucosal lesions are the main problems worlwide.[1] Those diseases are caused by microbes that live in the oral cavity. Dental caries is a chronic disease caused by complex interactions among acid-producing bacteria, tooth surfaces, fermentable carbohydrates, and time.[2] *Streptococcus mutans* is a bacterium that is mostly found in the dental cavity.[3] *Streptococcus mutans* is a Gram-positive, cocci-shaped, and facultative anaerobic bacterium. This type of bacteria can survive in an acidic environment, produce glycosyltransferases (GTFs), and form glucan. Glucan plays a role in the attachment and increases bacteria colonies, then causes dental plaque.[4]

The most common bacteria involved in the pathogenesis of periodontal disease is *Phorphyromonas gingivalis*.[5,6] *Phorphyromonas gingivalis* is an anaerobic Gram-negative bacterium that plays an important role in the third phase of plaque formation of secondary colonization and microbial maturation. These bacteria produce virulence factors such as proteases that can destroy immunoglobulins, complement factors, heme-sequestering, hemolysin, and collagenase. Other virulence factors are fimbriae (FimA), gingipain, and lipopolysaccharide (LPS).[7]

Gingipain and lipopolysaccharide (LPS) are virulence factors of *Porphyromonas gingivalis* used to invade the periodontium tissue. Fimbriae A (FimA) helps *Porphyromonas gingivalis* adhere to subgingival bacteria such as *Streptococcus* and *Actinomyces* species.[6,7] Various surface components of *Porphyromonas gingivalis* enable it to interact with host cells, such as neutrophils, epithelial cells, fibroblasts, etc., thus making it easier for bacteria to grow, acquire nutrients, colonize, and form plaque biofilms.[7] Their productions influence the migration of PMN leukocytes, weaken the immune system, and lead to the damage of periodontal tissue.[6,7]

The prevalence of oral mucosal lesions in the global population is approximately 64.7%.[8] Lesions of the oral mucosa are characterized by the alteration of color, surface, shape, mucosal integrity, and swelling. Microbial infections, such as candida, mostly contribute to these lesions.[8] *Candida albicans* is an opportunistic microbe that is found in oral candidiasis. This lesion can be found in every oral mucosa, but the most common locations are buccal, buccal folds, oropharynx, and tongue.[9] The pathogenicity of this fungus as a human pathogen is determined through several mechanisms, such as biofilm formation, mediating adhesion and invasion of human cells, secreting the hydrolyses, yeast-to-hypha transition, contact sensing, thigmotropism, and phenotypic switching.[10]

To prevent oral and dental problems, people should improve oral hygiene by reducing the accumulation of plaque, called plaque control.[2] The aim of plaque control is to eliminate bacteria in dental plaque.[2,3] Plaque control is classified into mechanical plaque control and chemical plaque control. Mechanical plaque control can be done by brushing teeth, using dental floss, and interdental brush, while chemical plaque control can be achieved by using toothpaste and mouthwash.[11] Optimal plaque control can be achieved by the combination of mechanical and chemical plaque control. Brushing teeth with toothpaste is the first step to controlling oral hygiene.[2,11]

The functions of toothpaste are to eliminate microbes that live in the oral cavity, control the plaque, polish the tooth surface, strengthen the teeth, prevent bad breathing, and maintain the hardness of soft tissue in the oral cavity.[12] Toothpaste is a thick-mass mixture of powder and liquid. Toothpaste contains active and inactive ingredients.[13] The active ingredients of toothpaste provide a therapeutic effect, and the inactive ingredients thicken toothpaste formulations, bind to toothpaste components, and have a certain color or taste. Among the inactive ingredients include abrasive agents, humectants, binding agents, surfactants, preservatives, sweeteners, fragrances, and solvents.[14] The active ingredients used are antibacterial agents, such as fluoride or herbs.[15] Several studies have proven that herbal ingredients have benefits for reducing the number of bacteria and fungi that cause oral disease.[16] Nuraskin et al. showed that herbal toothpaste was more effective compared to conventional toothpaste.[17] The American Dental Association (ADA) and the Indonesian National Standard (SNI) recommend the use of herbal ingredients because herbal ingredients are relatively safe, cause no side effects, and are cheap and easy to find.[18] One of herbal ingredients that can be used to improve the function of toothpaste and have high biocompatibility is Gambir (*Uncaria gambir*).[19]

Gambir (*Uncaria gambir*) is a tropical plant that is mostly found in Indonesia. The biochemical compounds in Gambir extract are catechins (7-33%), catechin tannic acid (20-55%), pyrocatechol (20-30%), fluorescent Gambir (1-3%), red catechu (3-5%), quercetin (2-4%), certain oils (1-2%), wax (1-2%), and small concentrations of alkaloids.[20] Catechins, the main contents of Gambir, can damage the cell membranes or cell walls by interfering with the cell permeability of bacteria.[21] Melia et al. (2015) reported that *Uncaria gambir* extract has antimicrobial activity against some Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative (*E. coli* and *Salmonella* sp).[22] Catechins are antioxidants that have a mechanism of action in the form of decreasing the mechanism of ergosterol synthesis in *Candida albicans* cell walls which can further suppress the growth of *Candida albicans*.[23] Ifora[24] stated that ethanol extract from gambir leaves (*Uncaria gambir* Roxb) could inhibit the growth of *Candida albicans.* Another study (2016) also reported that *Uncaria gambir* extract was capable of reducing the decline of micropores on the enamel surface, minimizing the decline of calcium weight, and reducing bacteria colonies.[25] It was also stated that best conditions of gambir extraction process was by using a solvent ethyl acetate[20] , the addition of gambir extract to toothpaste can be used in preventing dental caries. Besides that, the addition of gambir toothpaste can also be used as an alternative to toothpaste for users who are sensitive to fluoride. The purpose of this study was to know the antimicrobial potential of toothpaste containing gambir extract (*Uncaria gambir*).

## METHODS

The study was *in vitro* experimental laboratory with a post-test-only control group design. It was conducted at Balai Besar Laboratorium Kesehatan (BBLK) of South Sumatra Province. *Streptococcus mutans* ATCC 25175, *Porphyromonas gingivalis* ATCC 33277, and *Candida albicans* ATCC 10231 obtained from BBLK Palembang. The object of this study was toothpaste containing gambir 10%. Isromarina et al stated that ethyl acetate *Uncaria gambir* extracts at 10% concentration could inhibit the growth of *Vibrio cholera.*[26] Dewi et al.[27] Reported that 10% *Uncaria gambir* extract has an antiseptic effect on gingival wounds in rats. Gambir leaves were collected from Babat Toman, Sekayu District of South Sumatera, and had been identified and authenticated by Faculty of Agriculture, Universitas Sriwijaya, Indonesia.

The samples size was 90, it was divided into 3 groups. Each group consisted of 3 subgroups. The sample size of the subgroup was 10. Group I was Gram-positive bacteria (*Streptococcus mutans*), Group II was Gram-negative bacteria (*Porphyromonas gingivalis*), and Group III was *Candida albicans.* Those 3 groups were divided into 3 subgroups; subgroup 1 was toothpaste without active ingredients, or a placebo as negative control; subgroup 2 was toothpaste containing fluoride as positive control; and subgroup III was toothpaste containing Gambir extract (*Uncaria gambir).*

The preparation of Gambir extract started when Gambir leaves were dried in the sun for 48 hours without direct exposure to sunlight. 60 grams of dried Gambir was mashed with a size of 40-60 mesh. The mashed Gambir was then wrapped in filter paper and put into the soxhlet tube. 98% of ethyl acetate, 300 ml, was put into the soxhlet tube, and heated at 77 C for 7 hours. The solution

obtained was evaporated using a rotary evaporator for 2 days. The process of evaporation produces 100% of Gambir extract. The extract was diluted with distilled water to produce 10% of the extract.[20]

The preparation of toothpaste was accomplished by heating a mortar with hot water, and drying it. Sodium Carboxymethylcellulose (CMC) was dissolved in hot water (p to u20 times the weight of sodium CMC) and left in the mortar for 15 minutes while stirring until homogeneous (mass A). Calcium carbonate and sodium benzoate were crushed, and glycerin was added, and then mixture was stirred until homogeneous (mass B). Mass B was added to the mortar containing mass A, and they were thoroughly combined. In this part, natrium fluoride was added for Group II and Gambir extract for Group III, and they were stirred until homogeneous. Saccharin was added to the pasta mass and stirred until homogeneous. Sodium lauryl sulfate was added to the paste mass. The toothpaste mixture was blended and then distributed evenly to be homogenous. The toothpaste homogeneity test was carried out by smearing the toothpaste on a glass object before putting it in the ointment pot. [28]

To prepare *Streptococcus mutans* in agar media, 40 grams of blood agar base were dissolved in 1000 ml of distilled water and then heated and stirred until homogeneous. The agar media was then sterilized using an autoclave at 121°C for 15 minutes. 5% of the total base medium (blood agar base) of Sheep blood was added to the agar medium at 45-60ºC and then shaken until homogeneous. 10-15 ml of blood medium was poured into a petri dish and left to became solid.[3]

Isolates of *Streptococcus mutans* were taken as 1-2 oese and suspended in a 0.9% sodium chloride (NaCl) solution in a sterile test tube to meet Mc Farland standard of 0.5 (10-8 CFU/ml). To avoid excess liquid, a sterile cotton swab was dipped into the suspension and then squeezed into a test tube. In a petri dish, the sterile cotton swab was smeared evenly on the blood agar medium.[3]

The preparation of Phorphyromonas media began by placing the brucella media to dissolve and homogenize. The media was sterilized at $121^0$℃ with a pressure of 2 atm for 15 minutes using an autoclave. The sterile media was cooled at a temperature of $40^0$-$45^0$℃, then mixed with 5% sheep blood and homogenized. The media was transferred to a petri dish.[29]

Isolates of *Porphyromonas gingivalis* were taken and put in a test tube. 2 ml of 0.45% NaCl was added to the test tube. Next, the test tube was put in an anaerobic jar and then incubated for 48 hours at $37^0$℃ using an incubator. After that, the dilution was carried out by adding sterile distilled water and homogenizing it using a vortex. Bacterial turbidity was equivalent to 0.5 McFarland or 1.5 X 108 CFU/ml.[30]65 gr of the medium was suspended in a liter of distilled water to prepare the *Candida albicans* medium. The medium was heated with frequent agitation and boiled for one minute to completely dissolve it, then put into an autoclave at 121° C for 15 minutes. It was cooled to 45 to 50°C and poured into Petri dishes or tubes for slanting.[31]

The culture of *Candida albicans* on Sabouraud Dextrose Agar medium was incubated at room temperature for 2 x 24 hours (or two 24-hour cycles). The cultures were then extracted with an ose needle and suspended in a test tube filled with 0.9% NaCl. The fungal suspension in the test tube was homogenized, and the turbidity was adjusted using a densitometer with a MacFarland standard of 0.5 (1-5 x 106 CFU/ml).[31]

1.5 gr of toothpaste was diluted with 5 ml of distilled water using a sterile spoon. The toothpaste solution was put in a Petri dish. The paper disc was taken using tweezers and dipped in a Petri dish filled with toothpaste solution for 30 minutes. The disc paper is placed in a petri dish containing each medium and microbe according to the treatment group. The Petri dish that had been treated with disc paper was incubated for 24 hours at 37°C for bacteria and 48 hours at 37°C for candida. The Petri dish was removed from the incubator, then the inhibition zone was measured using a calliper.[32]

Data analysis was performed with the SPSS program. The obtained data were analyzed by normality and homogeneity tests using the Shapiro-Wilk test and Levene's test. The parametric test was carried out using the One-Way ANOVA statistical test to determine whether there was an effect of Gambir toothpaste (*Uncaria gambir* Roxb.) on the Gram-positive, Gram-negative, and Candida bacteria. The analysis was continued with the post-hoc Tukey test to determine the significance. The results of the study were stated to be statistically significant if the p-value <0.05.

## RESULTS

The inhibitory zones of each sample were evaluated. All the groups and subgroups showed inhibitory zones, except placebo toothpaste.

**Table 1.** The average diameter of zone inhibition against microbes

| Groups | Subgroups | n | Zone of inhibition (mm) |
|---|---|---|---|
| *Streptococcus mutans* | Placebo toothpaste | 10 | 0.001 ± 001 |
| | Toothpaste containing fluoride | 10 | 9.58 ± 0.52 |
| | Toothpaste containing Gambir | 10 | 9.13 ± 0.62 |
| *Porphyromonas gingivalis* | Placebo toothpaste | 10 | 0.001 ± 0.01 |
| | Toothpaste containing fluoride | 10 | 14.22 ± 0.42 |
| | Toothpaste containing Gambir | 10 | 7.65 ± 0.12 |
| *Candida albicans* | Placebo toothpaste | 10 | 0.001 ± 001 |
| | Toothpaste containing fluoride | 10 | 1.37 ± 0.66 |
| | Toothpaste containing Gambir | 10 | 1.82 ± 0.48 |

Table 1 described the average diameter of inhibitory zone of toothpaste against Gram-positive (*Streptococcus mutans*), Gram-negative (*Porphyromonas gingivalis*), and *Candida albicans.* According to the results, significant antimicrobial activities of toothpaste with fluoride and toothpaste with Gambir extract were shown against these bacteria and *Candida*. were shown on toothpaste containing fluoride-containing toothpaste showed the highest zones of inhibition of *Streptococcus mutans* and *Porphyromonas gingivalis*, while toothpaste with Gambir extract came in second. While the highest zone of inhibition of *Candida albicans* was given by toothpaste containing Gambir, followed by toothpaste containing fluoride. Placebo did not show any inhibitory zones in all subgroups.

The normality test using Shapiro Wilk and the homogeneity test using Levene's test showed p-values >0.05. It meant that all data were normally distributed and homogenous (identical). Data analysis was continued using the One-way ANOVA test. According to Table 2, the toothpaste treatment in all groups obtained p<0.05, which meant that there was a significant difference in the inhibitory zone among the treatment subgroups. The analysis was continued with the post-hoc Tukey test to find out which of the four treatment groups had statistically significant differences (Tables 3,4, and 5).

**Table 2.** One-way ANOVA test results

| Groups | p |
|---|---|
| *Streptococcus mutans* | 0.001* |
| *Porphyromonas gingivalis* | 0.001* |
| *Candida albicans* | 0.001* |

*significant (p<0.05)

Table 3 showed that there was a significant difference between placebo and toothpaste containing fluoride; and placebo with toothpaste containing Gambir. However, there was no significant difference between toothpaste containing fluoride and toothpaste containing Gambir. It can be concluded that the potency of the antibacterial agent of toothpaste with Gambir extract had the same ability as one with fluoride against Gram-positive bacteria (*Streptococcus mutans)*.

**Table 3.** Post-Hoc Tukey test from the toothpaste against *Streptococcus mutans*

| Sub groups | Placebo | Toothpaste containing fluoride | Toothpaste containing gambir |
|---|---|---|---|
| Placebo | | 0.001* | 0.001* |
| Toothpaste with fluoride | 0.001* | | 0.68 |
| Toothpaste with Gambir | 0.001* | 0.68 | |

*significant (p<0.05)

Table 4 showed that the comparison of all subgroups had significant differences (p<0.05). It described that toothpaste containing gambir had a potential effect as an antibacterial agent against Gram-negative (*Porphyromonas gingivalis)* but not as strong as toothpaste containing fluoride.

**Table 4.** Post-Hoc Tukey test from the toothpaste against *Porphyromonas gingivalis*

| Sub groups | Placebo | Toothpaste containing fluoride | Toothpaste containing gambir |
|---|---|---|---|
| Placebo | | 0.001* | 0.001* |
| Toothpaste containing fluoride | 0.001* | | 0.001* |
| Toothpaste containing Gambir | 0.001* | 0.001* | |

*significant (p<0.05)

Table 5 showed that there was significant difference among all subgroups. In contrast, there was no significant difference between toothpaste containing fluoride and toothpaste containing Gambir. Therefore, the antibacterial effect of Gambir-containing toothpaste had the same ability as fluoride-containing toothpaste against *Candida albicans.*

**Table 5**. Post-Hoc Tukey test from the toothpaste against *Candida albicans*

| Sub groups | Placebo | Toothpaste containing fluoride | Toothpaste containing gambir |
|---|---|---|---|
| Placebo | | 0.001* | 0.001* |
| Toothpaste containing fluoride | 0.001* | | 1.00 |
| Toothpaste containing gambir | 0.001* | 1.00 | |

*significant (p<0.05)

## DISCUSSION

According to Davis and Stout (1971), the diameter of the inhibition zone is determined by the antimicrobial activity of active substances. Substances with an inhibition zone diameter of less than 5 mm were categorized as weak antimicrobials. Substances with inhibition zone diameters of 5-10 mm were categorized as having moderate antimicrobial properties. And substances with an inhibition zone diameter of 11-20 mm were categorized as strong antimicrobial agents.

The study indicated that toothpaste containing Gambir was categorized as having moderate antibacterial properties against *Streptococcus mutans* and *Porphyromonas gingivalis,* and was categorized as having weak antimicrobial properties against *Candida albicans.* Toothpaste containing fluoride was categorized as having moderate antibacterial properties against *Streptococcus mutans,* strong antibacterial agents against *Porphyromonas gingivalis,* and weak antimicrobial properties against *Candida albicans.*

The results from Table 1 showed that Gambir-containing toothpaste increased the antimicrobial ability against *Streptococcus mutans, Porphyromonas gingivalis*, and *Candida albicans.* The main components of Gambir extracts were catechins and tannins.[33] Inmawaty et al. stated that the extract of Gambir containing catechins had an inhibition zone against *Streptococcus mutans*.[34] Catechins are phenolic group compounds that play important role in denaturing and precipitating bacterial cell proteins, and inactivating *Streptococci* enzymes.[35] Catechins interact with peptidoglycan and change the structure of the bacterial cell wall.[36] This process increases the permeability of the bacterial cell membrane, which is followed by cell membrane damage, and then the bacteria will lyse and die.[35]The different results of antimicrobial activities among different microbes (Table 3, 4 and 5) were due to the distinction of cell wall and its composition. There was a difference between Gram-positive and Gram-negative bacterial cell walls. The composition of the peptidoglycan layer of Gram-positive bacteria has a thicker peptidoglycan layer than that of Gram-negative[37] Therefore, the toothpaste containing Gambir extract is more effective against *Streptococcus mutans* than against *Porphyromonas gingivalis,* as described in this study. Taylor stated that catechins tend to have less activity against Gram-negative than they are against Gram-positive bacteria.[38]

Catechins are able to work competitively with glucosyltransferase (GTF) enzymes, preventing the bacteria from synthesizing extracellular polysaccharides (glucans) necessary for *Streptococcus mutans* attachment and colonization.[39] Catechins also play a role in inhibiting the metabolism of *Streptococcus mutans* at the glycolysis stage.[35] Catechins bind to the catalyst to inhibit the activity of the enzyme lactate dehydrogenase.[36] This activity makes it difficult for bacteria convert pyruvate to lactic acid.[35,36] Xu et al. reported that catechins inhibited not only lactate dehydrogenase activity but also F1Fo-ATPase.[40] F1Fo-ATPase activity plays an important role in the virulence factor of *Streptococcus mutans*.[37] This role is carried out by pumping protons from the cytoplasm to the bacterial cell membrane to maintain pH homeostasis and preserve the bacteria's enzymatic function..[37] Catechins in gambier extract inhibit the activity of the F1Fo-ATPase enzyme, resulting in an increase in cytoplasmic acidity, which in turn interferes with bacteria's cell membrane.[39]

Catechins inhibit the expression of some genes involved in host colonization, such as fimbriae A (*fimA*), tissue destruction, and heme acquisition *(hem*).[6] Reducing the expression of *fimA* may also help reduce inflammation because this virulence factor has the ability to induce host cells to produce cytokines.[7] Catechins also increased the expression of the stress protein *htrA* gene. This gene is known to be responsible for resisting oxidative stress in *Porphyromonas gingivalis*, helping them survive under stressful conditions and causing stress on the bacteria.[40]

Catechins penetrate deeper into the lipid bilayer interface by absorption. It suggests that catechins regulate membrane partitioning by hydrogen bonding between the catechins and membrane lipids, specifically between the hydroxyl groups of the catechin and the oxygen atom of the lipids.[36] Catechins not only interfere with the cell membrane and cell wall of *Porphyromonas gingivalis*, but also affects the growth and adhesion of *Porphyromonas gingivalis*, interrupt the biofilm formation, weaken its invasiveness to host cells and tissues, and reduce the production of volatile sulfur compounds and causing oral halitosis by inhibition of relevant virulence factors such as fimbriae, collagenase, gingival protease, toxic end metabolites.[36,41] Lestari reported that Gambir catechins affected the amount of *Actinobacillus actinomycetemcomitans* (AA) in periodontitis mice.[42] Additionally, Sari and Deynalisa also reviewed that gargling with 1 gram gambir-boiling water was effective in treating gingivitis.[43]

Other active substances found in Gambir extract (*Uncaria gambir Roxb.)* are tannins.[26] Tannins have an antibacterial action due to their ability to deactivate bacterial adhesins, inhibit both the action of enzymes and protein transport in the cell envelope.[44] The mechanism of action of tannins as an antibacterial agent, among others, is through the destruction of bacterial cell membranes due to the toxicity of tannins and the formation of metal ion complex bonds from tannins, which play a role in tannin toxicity.[44] Tannins also have the ability to neutralize toxins in bacteria.[45]

Dewi et al, Reported that tannins play a role in inhibiting bacterial cell metabolism by inducing ionic bonds and causing toxicity, thereby disrupting cell membrane permeability and denaturing proteins.[25,27] Eolia reported that tannins isolated from *Ficus carica Linn*. had the ability to destroy *Porphyromonas gingivalis*.[45] Ho et al, Revealed that tannin compounds were proven to be able to inhibit the growth of bacteria that cause periodontitis, one of which is *Porphyromonas gingivalis.*[46] Choi et al. stated that tannins could reduce the cytotoxicity of bacterial toxins so that bacterial pathogenesis decreased.[47]

This study also presented that toothpaste containing gambir had the ability to inhibit *Candida albicans* and statistically had no different significant effect on toothpaste containing fluoride. The antimycotic activity of gambir is carried out through several underlying mechanisms, including plasma membrane disruption, the induction of mitochondrial dysfunction, inhibiting cell wall formation and division, interfering with RNA and protein synthesis, and affecting the efflux-mediated pumping system.[48] Catechins, an active metabolite from gambir, are known as antioxidants, which are capable of inhibiting the growth of *Candida albicans* by reducing the mechanism of ergosterol synthesis.[24] The mechanism of action of phenol is based on denaturation and precipitating microbial cell proteins, so the antifungal effect of gambir is effective against *Candida albicans*.[25] Furthermore, excess production of reactive oxygen species (ROS) induces severe oxidative stress on the cell, which increases the

permeability of membrane cells, injures nucleic acids, and leads to the oxidation of fatty acids and amino acids.[48] ROS produces lipid peroxidation, resulting in disruption of lipid bilayer, altering membrane potentials, progressing membrane permeability, and interfering with phospholipids.[49] Catechins and tannins found in gambir can change cell wall formation by inhibiting the synthesis of b-glucans and chitin.[48]

Catechins inhibit *Candida albicans* nucleic acid synthesis. Study analysis by flow cytometry showed that catechins exhibited the inhibition of FCS-induced hyphal formation.[48] Catechins enter the cell through active transport. After they reach the nucleus, they disrupt DNA, RNA, and protein synthesis.[49] Tannins have the ability to inhibit the mitochondrial electron transport chain and diminish the potential of cell membranes. Tannins obstruct the proton pumps and reduce ATP synthesis. This condition causes cell death.[50] Rahayuningsih et al, stated that gambir was effective as antifungal agents.[51] Another study also reported that polyphenolic compounds found in *Uncaria gambir* had a potential effect as antibacterial and antifungal agents.[24]

Toothpaste containing fluoride as positive control had been proven as an antimicrobial agent to maintain oral hygiene. Fluoride inhibits glycolytic enzyme enolase from acidic bacteria and heme-based peroxidase enzymes by binding fluoride to heme.[52] Flouride involves the formation of metal-fluoride complexes, such as AlF4-.[53] These complexes are responsible for fluoride inhibition of proton-translocating F-ATPases and are thought to act by mimicking phosphate to form complexes with ADP at the reaction centers of the enzymes.[53] Another mode of action is to enhance membrane permeabilities to protons and disrupts the functioning of F-ATPases in exporting protons. Fluoride reduces the acid tolerance of oral bacteria, especially *Streptococcus mutans*.[52] In Gram-negative, fluoride inhibits heme catalase, penetrates cells, and causes cell death.[53] A good penetration of bacteria cell walls will ease the active compounds to kill the cell. Fluorides are compounds that combine with some positively charged counterparts.[54] The anion porphyrins of Gram-negative bacteria will bind with the positive charged compounds, and disorganize the outer membrane structure, so the fluor ion killing effect can take place.[53,54]

Toothpaste containing fluoride has long been known as an antifungal agent. Fluoride blocks the functions of key metabolic enzymes and leads to the growth of candida. The mode of action is by interfering with cell membrane integrity, causing leakage of cytoplasmic constituents so that cell growth will be interrupted.[55] Mozayeni et al. Reported that chlorhexidine had significantly higher anticandidal activity against *Candida albicans* compared to fluoride.[56] Adwan et al. stated that parodontax (toothpaste containing herbal ingredients) showed a significant difference ($P< 0.001$) against *Candida albicans* isolates compared to toothpaste containing sodium fluoride.[57]

Resistance of *Candida albicans* is due to its ability to increase expression or decrease susceptibility of the target, spontaneous mutations, and escape host immune defenses.[58] Zaidi et al, indicated that the evolutionary changes in the total protein profile enhanced the resistance of *Candida albicans.*[59]

This study showed that toothpaste containing gambir had antibacterial and antifungal activities against *Streptococcus mutans, Porphyromonas gingivalis*, and *Candida albicans.* So that it also showed that *Uncaria gambir* plants, which are widely distributed in South Sumatera and are traditionally used to treat a variety of diseases, were effective in reducing bacteria and candida in the oral cavity.

## CONCLUSION

It concluded that toothpaste containing gambir (*Uncaria gambir*) has antimicrobial potency.

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