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Original Research Article

Anticariogenic Effect of Gambir (*Uncaria Gambir* [Roxb.]) Extract on Enamel Tooth Surface Exposed by *Streptococcus mutans*

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ABSTRACT

Introduction: Dental caries is the most common problem in many nations in the world, especially in Indonesia. Elimination of bacteria as one of caries causal can be done in many ways; one of them is using gambir plant extract. Gambir has chemical compounds like catechin and tannin, which have anticariogenic effect. The aim of this study was to determine anticariogenic effect of gambir extract on enamel tooth surface exposed by Streptococcus mutans.
Materials and Methods: An experimental study, in vitro, had been conducted in Integrated Laboratory Pasca Sarjana Unsri, Province's Health Laboratory of South Sumatera, dan Metalurgy's Laboratory of Engineering Faculty of University of Indonesia, and held on March to April 2015. There were 35 maxillary first premolar samples and divided into 5 groups; each groups using 40%, 50%, 60% gambir extracts, negative control or placebo, and positive control (using cefadroxil 500mg). Data were analyzed by using SPSS 22 vers.
Results: The results showed that 60% gambir extract had equal effect of reducing the decline of micropores in enamel topography to cefadroxil 500 mg, 50% gambir extract had equal effect in reducing the decline of calcium's weight to cefadroxil 500 mg and had dose-dependent effect, 40% gambir extract had equal effect in lowering colonies of Streptococcus mutans to cefadroxil 500 mg, and had dose-dependent effect.
Conclusion: It can be concluded that 60% gambir extract has equal anticariogenic effect to cefadroxil 500 mg.

Key words: dental caries, micropores, enamel topography, bacterial colonies, gambir extract.

INTRODUCTION

Dental caries is most common problem in many countries in the world. In Indonesia, dental caries and gum disease is one of major problem in health. Caries prevalence in Indonesia reached 90.06%, based on "Household Health Research", Indonesian Department of Health, 2004. Basic Research of Health (Riskesdas) in 2007 also reported that prevalence of active caries on 12 years old children reached 29, 8%, and it could increased by their following aged. [1]

Caries is a hard-tooth-surface disease; enamel, dentin, and cementum, happened as multifactorial risks, caused by bacteria activities in fermenting carbohydrate on tooth surfaces. Caries begins from the destruction of enamel minerals, called demineralization, and continues to be a cavity due to bacterial activities. [2]

The basic principle in preventing oral caries is eliminating all caries causality. One of them is bacteria. Streptococcus mutans is the most common bacteria found in caries lesion. Streptococcus mutans has ability in forming colonies quickly, fermenting carbohydrate, and lowering salivary pH. Its activities can cause demineralization and continue to be caries lesion. [3] This bacteria mostly found in patients with highly caries lesion. [4]

Eliminating these bacteria can be done in many ways, such as brushing teeth, mouth-washing with antiseptic gargle, using topical application of fluor, and utilizing plant extract, like gambir (Uncaria gambir Roxb.). In Indonesia, especially in Sumatera, gambir, used with betel leaf, is believed to "strengthen the teeth".

Gambir (Uncaria Gambir Roxb.) is an herbaceous plant with a height of 1-3 cm, straight stems, oval, serrated edge, base rounded, tapered tip, 8-13 cm long, 4-7 cm wide, and green. [5] Anticariogenic activities from this plant are due to its active compounds; catechin and tannin. Those compounds have antibacterial and antioxidant activities. [6,7] 8% catechin in gambir can inhibit the growth cycle of Streptococcus mutans, so it can prevent caries. [8] Catechin can inhibit glucosyltransferase enzyme activity and prevent extracellular glucan, which function is to attach Streptococcus mutans in tooth surface. [9] Because of the reasons above, this study aim was to determine anticariogenic effect of gambir extract on enamel surface exposed by Streptococcus mutans.

MATERIALS AND METHODS

The experiment was conducted in Integrated Laboratory of Pasca Sarjana, University of Sriwijaya, Indonesia, Province's Health Laboratory of South Sumatera, Palembang, Indonesia, and Metalurgy Laboratory in Engineer Faculty, University of Indonesia, Jakarta, Indonesia, on March to April 2015. The protocol had been approved by Health Research Review Committee, Mohammad Hoesin General Hospital and Faculty of Medicine Sriwijaya University, on March 10th, 2015. Samples were 35 maxillary premolars, already extracted, had virgin enamel, free from restoration or decay, had normal anatomic crown, the age of those teeth between 12 to 45 years. Teeth with severe attrition were excluded. The equipments used in this study were micromotor (Strong, Saeshin®, Korea), straight hand piece and contra angle (NSK, Japan), carborundum disc (Zhejiang Winking Abrasives Co., Ltd, China) diamond bur (Blu White Diamond, Kerr Co., US) plastic pots, tweezers, drop pipettes, Field Emission Scanning Electron Microscope/FESEM (FEI, Inspect^{TM}F50. Holland), Energy Dispersion Spectroscopy/EDS (EDAX, Apollo, USA), Colony counter (SC6 Plus, Stuart $^{®}$, UK). The materials used in this study were gambir extract in 40%, 50%, and 60% concentrations, sugar, artificial saliva, alcohol, Streptococcus mutans, waxes, nail polish, original product of cefadroxil 500 mg (Promed Pharma Co, Indonesia).

Preparation of teeth

Thirty five extracted maxillary first premolars were retrieved from dental practices and hospital. The teeth were sterilized with alcohol 70%, and cleaned with pumice and brushes, then dried. After that, all root surfaces of each tooth in all groups were covered with nail polish, and coated with wax to create a surface that would not be exposed to artificial saliva or experiment's solution. The 1/3 apical of roots can be cut if necessary by carborundum disc or diamond bur. The teeth were labelled with a waterproof marking pen in palatal side. Buccal surface was divided into nine columns or points, by drawing three vertical lines and three horizontal lines. These columns were used to split scanning area by SEM and to ease the calculation of porosities.

Samples were divided into five groups. Group A: samples were soaked in artificial saliva solution with 40% gambir extract (n=7); Group B: with 50% gambir

extract (n=7); Group C: with 60% gambir extract (n=7); Group D: negative control, with no gambir extract (n=7); Group E: positive control, with cefadroxil 500 mg (n=7).

Preparation of Streptococcus mutans

Bacterial strain used in this study was *S. mutans* UA159, which was grown in Blood Agar (Province's Health of Sumatera Lab, Palembang, Indonesia), had been incubated for 24 hours, 37°C. Bacterial suspension was dissolved in sodium chlorides and density was adjusted to 0.5 McFarland standards (10^8 CFU ML^{-1}).

Artificial saliva

The artificial saliva used in this study was prepared according to Macknight-Hane and Whit ford (1992) formula. The sorbitol was not used in order to reduce the viscosity happened when the sorbitol was mixed with sodium carboxymethyl cellulose (Levine, et al., 1993). The composition of artificial saliva were methyl-p-hydroxybenzoate 2 gram/ liter, sodium carboxymethyl cellulose 10 gram/liter, KCl 0.625 gram/liter, $MgCl_2.6H_2O$ 0.059 gram/liter, $CaCl_2.2H_2O$ 0.166 gram/liter, K_2HPO_4 0.804 gram/liter, KH_2PO_4 0.326 gram/liter. The pH of artificial saliva was adjusted to 6.75 with KOH. [10]

Extract gambir (Uncaria gambir [Roxb.])

1000 gr dry gambir pollen (taken from traditional market) was set in a dark with 1000 ml ethyl acetate. The mixture was swaying for 10 minutes. It was macerated for 36 hours (swayed 3 times a day) and then strained with filter paper; the waste re-macerated for three times. The filtration was evaporated by using rotary evaporator in order to get the 100% extract in dried stored.

100% gambir extract was diluted to get 40%, 60%, and 80% extract in artificial saliva, by using formula:

$$\%\ \text{gambir extract} = \frac{\text{Weifht of gambir filtrate (g)}}{\text{Artificial saliva volume (ml)}} \times 100\%$$

Pre Examination

SEM

Thirty five samples (7 per groups) were dried at room temperature and attached to a testing ring with an adhesive carbon tape (Pelco Tabs™, USA) by using FESEM (FEI, Inspect™F50 Holland) for observation the porosity. By this method, 9 points were performed per sample. The number of porosity was counted in each columns/points, and then found the average number from all columns.

EDS

Thirty five samples (7 per groups) were dried at room temperature and attached to a testing ring with an adhesive carbon tape (Pelco Tabs™, USA) by EDS (EDAX, Apollo, USA) to determine the weight percentage (wt%) of Calcium, under the following parameters: 15.0 KeV and 100 sec. live time.

All the pretest data were recorded to be analyzed after getting posttest data.

Submersion of samples

After taking pretest data, samples were soaking in the solution of artificial saliva, which consisted of sugar and Streptococcus mutans. The submersions of samples were divided based on 5 groups. Group A: 7 teeth were soaked in solution with 40% gambir extract; Group B: with 50% gambir extract; Group C: with 60% gambir extract; Group D: negative control, with no gambir extract; Group E: positive control, with cefadroxil 500 mg. All samples were soaked in artificial saliva for 60 minutes, and then the number of micropores, calcium weight (w/t %), and the number of bacteria colonies were measured. The parameters used to evaluated anticariogenic were the number of micropores, Calcium weight percentage (wt %), and Streptococcus mutans colonies.

Evaluation of the number of micropores

The evaluation data were obtained by counting the number of micropores in each columns/points and found the average. The micropores were counted by looking at the dark points of enamel surfaces characterized in SEM. Post test data were recorded.

Evaluation of calcium weight percentage (wt %)

The data were taken from EDS and noted. 9 points were executed per samples.

Counting of bacteria colonies

Petri - dish was placed on the electronic pressure pad of Colony counter (SC6 Plus, Stuart®, and UK). A transmission light array with magnifier was used to help in counting colonies. Counted CFU were marked with a felt tip pen on the plate cover to discriminate counted from uncounted colonies or to avoid double counting. Touch pressure caused a count to be registered on the digital display and an audible tone confirms each count made. The sensitivity of the electronic pressure pad is adjustable to suit the user. The examining data were recorded.

Statistical analysis

All recorded data were drawn in the form, processed, and analyzed. Before further processing, first of all, data were tested with Levene's test to know the homogeneity of samples. If $p > 0.05$, meant data were homogen. Extended Paired t-test was used to compare the changes between "before and after" experiments. Independent t-test was examined to compare the efficacy between groups in this study. One way Anova was used for significance of difference in all groups. To know the compatibility dose, Post Hoc test was used. Statistical significance was assembled as $p<0.05$. SPSS 22vs (IBM® inc.pvt ltd) and Microsoft Excel (Microsoft inc®) were used for statistical analysis.

RESULTS

Table 1: Means of micropores before and after soaking within group

Group	Mean Before	After	P
A	26.57 ± 5.85	54.00 ± 11.49	0.00
B	29.14 ± 4.14	49.71 ± 8.28	0.00
C	30.00 ± 6.93	33.43 ± 9.07	0.10
D	26.57 ± 4.72	75.43 ± 10.31	0.00
E	26.14 ± 6.12	34.29 ± 6.20	0.04

Paired t-test, p=0.05

Table 1 showed that only group C had no significant effect in forming micropores in before and after within group. This minimally forming micropore revealed that 60% gambir extract had ability in reducing the decline of micropores.

From Table 2, it could be seen that 40%, 50%, 60% gambir extract had equal effect in reducing the decline of micropores in enamel to positive control, cefadroxil 500 mg, ($p>0.05$), and had significant effect with placebo.

Table 2: The efficacy of micropores between group

Group	P
40% gambir ext - 50% gambir ext	0.44
40% gambir ext - 60% gambir ext	0.00
40% gambir ext - negative control	0.00
40% gambir ext - positive control	0.05
50% gambir ext - 60% gambir ext	0.00
50% gambir ext - negative control	0.00
50% gambir ext - positive control	0.07
60% gambir ext - negative control	0.00
60% gambir ext - positive control	0.87
Negative control - positive control	0.00

Independent t-test, p=0.05

Anova one way was done to know the significant effect of reducing the decline of micropores in 40%, 50%, 60% gambir extract, negative control, and positive control. The result was $p<0.05$, meant that there was significant effect among the groups (within and between).

Table 3: Compatibility dose in reducing the decline of microporus

Relationship between groups		P
40% gambir extract	50% gambir extract	0.42
	60% gambir extract	0.00
	Negative control	0.00
	Positive control	0.00
50% gambir extract	40% gambir extract	0.42
	60% gambir extract	0.00
	Negative control	0.00
	Positive control	0.01
60% gambir extract	40% gambir extract	0.00
	50% gambir extract	0.00
	Negative control	0.00
	Positive control	0.87
Negative control	40% gambir extract	0.00
	50% gambir extract	0.00
	60% gambir extract	0.00
	Positive control	0.00
Positive control	40% gambir extract	0.00
	50% gambir extract	0.01
	60% gambir extract	0.87
	Negative control	0.00

Post Hoc LSD, p=0.05

By using Post Hoc test, it could be described from Table 3 that only 60% gambir extract had equal effect to cefadroxil 500 mg.

Evaluation of the changes of calcium weight was done after seeing the changes of micropores in enamel premolar teeth. By

using Levene's test, we got that all data were homogen.

Table 4 showed that all data had $p<0.05$, it meant that there were significantly different within groups, before and after soaking in artificial saliva with sugar and Streptococcus mutans.

Table 4: Means of calcium weight before and after soaking within group

Group	Mean Before	After	P
A	50.27 ± 2.62	36.82 ± 3.81	0.00
B	50.45 ± 3.14	40.96 ± 5.88	0.00
C	50.27 ± 3.38	43.22 ± 4.50	0.00
D	51. 24 ± 4.29	32.51 ± 2.76	0.00
E	50.44 ± 2.42	44.59 ± 1.94	0.00

Paired t-test, p=0.05

Table 5: Effectivity test of calcium weight between groups

Group	P
40% gambir ext - 50% gambir ext	0.14
40% gambir ext - 60% gambir ext	0.01
40% gambir ext - negative control	0.03
40% gambir ext - positive control	0.00
50% gambir ext - 60% gambir ext	0.43
50% gambir ext - negative control	0.00
50% gambir ext - positive control	0.15
60% gambir ext - negative control	0.00
60% gambir ext - positive control	0.48
Negative control - positive control	0.00

Independent t-test, p=0.05

Table 6: Compatibility dose in reducing the decline of calcium weight

Relationship between groups		P
40% gambir extract	50% gambir extract	0.06
	60% gambir extract	0.01
	Negative control	0.05
	Positive control	0.00
50% gambir extract	40% gambir extract	0.06
	60% gambir extract	0.30
	Negative control	0.00
	Positive control	0.10
60% gambir extract	40% gambir extract	0.01
	50% gambir extract	0.30
	Negative control	0.00
	Positive control	0.53
Negative control	40% gambir extract	0.05
	50% gambir extract	0.00
	60% gambir extract	0.00
	Positive control	0.00
Positive control	40% gambir extract	0.00
	50% gambir extract	0.10
	60% gambir extract	0.53
	Negative control	0.00

Post Hoc LSD, p=0.05

Table 5 showed that extract gambir in all concentration had significant difference with negative control, and 50% and also 60% gambir extract had no different effect in reducing the decline of calcium weight with cefadroxil 500 mg.

One way Anova showed that there was significant effect among the groups in minimizing the decline of calcium weight.

From Table 6 showed that 50% and 60% gambir extract had compatibility dose with cefadroxil 500 mg.

The next parameter in this research was the number of bacteria colonies. Evaluation the number of bacteria colonies used posttest design only.

Table 7 showed that 40%, 50%, and 60% gambir extract were significantly effective in reducing bacteria colonies.

Table 7: The efficacy of reducing bacteria colonies between groups

Group	P
40% gambir ext - 50% gambir ext	0.57
40% gambir ext - 60% gambir ext	0.18
40% gambir ext - negative control	0.00
40% gambir ext - positive control	0.06
50% gambir ext - 60% gambir ext	0.49
50% gambir ext - negative control	0.00
50% gambir ext - positive control	0.20
60% gambir ext - negative control	0.00
60% gambir ext - positive control	0.45
Negative control - positive control	0.00

Independent t-test, p=0.05

Anova one way was done to know the significant effect in reducing bacteria colonies in 40%, 50%, 60% gambir extract, negative control, and positive control. The result was $p<0.05$, it concluded that there was significant effect among the groups.

Table 8: Compatibility dose in reducing bacteria colonies

Relationship between groups		P
40% gambir extract	50% gambir extract	0.55
	60% gambir extract	0.22
	Negative control	0.00
	Positive control	0.08
50% gambir extract	40% gambir extract	0.55
	60% gambir extract	0.51
	Negative control	0.00
	Positive control	0.24
60% gambir extract	40% gambir extract	0.22
	50% gambir extract	0.51
	Negative control	0.00
	Positive control	0.59
Negative control	40% gambir extract	0.00
	50% gambir extract	0.00
	60% gambir extract	0.00
	Positive control	0.00
Positive control	40% gambir extract	0.08
	50% gambir extract	0.24
	60% gambir extract	0.59
	Negative control	0.00

Post Hoc LSD, p=0.05

Table 8 showed that there were no different effect between 40%, 50%, 60%

gambir extract with cefadroxyl 500 mg. It concluded that 40% gambir extract was equal to cefadroxyl 500 mg. This various dose was dose dependent effect.

DISCUSSION

A micropore was formed by the result of demineralization in enamel teeth. This demineralization was formed chemically due to glycolysis process in sugar. Glycolysis is increased acid concentration. [11] This acids released hydrogen ions (H^+) and diffused to enamel, reacted with hydroxyapatite crystal, then dissolved the crystal. [12]

Extract gambir had ability in reducing the decline of micropores. The efficacy from gambir extract was just because of the antibacterial activity of catechin, so that it would decrease acidity in artificial saliva. [7] Demineralization would be decreased, micropores would be decreased too.

Ideally, the decline of calcium weight should not happen in artificial saliva or at least the decline is very minimal. It happened because of acidity formed in 3-5 minutes after artificial saliva was given with sugar and as a result of bacterial activity in fermenting sugar. [2] This activity made acidic oral environment. Bacteria fermentation produced lactic acid, that could reduce salivary pH until 5, 5, so the number of calcium weight would be declined. [13] Apatite crystal degradated its minerals soon after reducing salivary pH. One of that mineral was calcium. This condition caused demineralization and decalcification. If this process was not balanced with remineralizations, it would continue become caries. [14] The lower the decline of calcium weight, the better the extract-gambir ability in reducing it.

Gambir extract had catechin and tannin active compounds. Catechin could inhibit glucosyltransferase enzyme activity and inhibit polysaccharide extracellular glucan, so that it could prevent the attachment of Streptococcus mutans in enamel, so dental plaque would not be formed. [15,16]

Preliminary studies reported that gambir consists mostly of the flavan monomer, which contains (+)-catechin, (+) epicatechin, and alkaloids. [17,18] Characterization and quantification analysis using Fourier Transform Infrared (FTIR) spectroscopy and reverse phase-high performance liquid chromatography (RP-HPLC) had confirmed that the major chemical constituents of Uncaria gambir were mainly catechins. It was revealed that the ethyl acetate gambir extract gave the highest catechin content and antioxidant activity compared with other solvent extracts. [19]

Catechin can kill Gram positive bacteria, such as Streptococcus mutans. [20] *Streptococcus mutans* reside in mouth and, more specifically, in the multispecies biofilms on the surfaces of teeth. This bacterium can form colonies fast, and its fermentation produces lactic acid. [21,22] Streptococcus mutans is one of bacteria which always found in caries lesion, and are major cariogenic organisms. They have ability in producing large quantities of glucans as well as acid, exceeding the capacities of salivary buffering. [23] The key component of their pathogenesis is their ability to survive in acid environment by modulating pathways of sugar metabolic coupled with irreversible binding to the teeth. Its potentiality is due to the virulence of Streptococcus mutans in producing glucosyltransferase (GTFs). This enzyme synthesizes intracellular polysaccharides (IPS) and extracellular polysaccharides (EPS).The EPS, especially water-insoluble glucans, mediate the adherence of Streptococcus mutans and other oral bacterial species to tooth surfaces, contributing to the formation of dental plaque biofilms. [24] The metabolic microbial interactions taking place in the dental biofilm result in acid production and extracellular glucan formation which promote microbial attachment to teeth. [25]

In the next step, Streptococcus mutans proliferate and expand to oral mucosa with other microbial species and finally the biofilm achieves a steady state and disturbs the balance of oral ecology. This condition can cause inflammation of oral tissue, such as gingiva and periodontal tissue. [26,27] Beside that, acid production will decrease salivary pH, so that it can cause demineralization and decalcification. [28] The continuing of demineralization and decalcification, if it is not balanced with remineralization, can cause hypomineralization and micropores formed. This micropore is the early sign of caries forming. [14,29]

The mechanism of catechin in reducing Streptococcus mutans colonies are summarized into 3 steps. Firstly, catechin inhibits glycolysis process and works competitively with glucosyltransferase enzyme in saccharida reduction. So that extracellular polysaccharida glucan is not formed. [30]

Xu et al, 2011 reported that catechin inhibits the enzymatic activity of F_1F_o-ATPase and lactate dehydrogenase. It also noted that catechin is a natural anticariogenic agent that can exhibit antimicrobial activity against *Streptococcus mutans* and suppresses the specific virulence factors associated with its cariogenicity. [31]

Secondly, catechin contains polyphenol compound, that can easily bind with other organic compounds, especially proteins. It will diminish the function and the role of proteins. [16] Gradisar et al, 2007 stated that catechins inhibit bacterial DNA gyrase by binding to the ATP binding site of the gyrase B subunit. [32] Bernal, 2010. also informed that catechin binds predominantly to the cytoplasmic membrane, initially decreasing the fluidity of the bilayer, and induces changes in gene expression indicative of an attempt to preserve and repair a compromised cell wall. [33]

Thirdly, catechin contacts with peptidoglycan component in cell wall, leads bacteria membrane cell damaged. Catechin-peptide complex inhibits the ability of the bacteria to bind to host cells, restrains the ability of the bacteria to bind to each other to form biofilms, unables to secrete toxins, and damages membrane, so that can cause leakage, lysis, and died. [15,34]

Another chemical composition of gambir is tannin. Tannin disrupts cell metabolism by inducting metal ion bond and causes toxicity in Streptococcus mutans, interferences cell membrane permeability on cell wall, and protein denatured. [35,36]

Antibacterial effect from catechin reduces the number of Streptococcus mutans colonies. The more concentration of gambir extracts in artificial saliva, the higher its potential in killing bacteria.

By reducing the number of bacteria colonies, the number of sucrosa fermented is decreased, the forming of lactic acid is declined, decalcification and demineralization is minimized, so caries can be prevented.

CONCLUSION

From the results, it found that 60% gambir extract has equal effect in reducing the decline of micropores to cefadroxyl 500 mg; 50% and 60% gambir extract have equal effect in lowering the decline of calcium weight to cefadroxyl 500 mg and this dose is dose-dependent effect; and 40%, 50%, and 60% have equal effect in eliminating bacteria colonies to cefadroxyl 500 mg and this dose is dose-dependent effet.

So the conclusion is 60% gambir extract has equal anticariogenic effect to cefadroxyl 500 mg.

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Conflict of interest

There is no potential conflict of interest for any of the authors that might introduce bias or affect the judgment.

REFERENCES

1. Health Department of Republic of Indonesia. DepKes RI. Health Profile 2004. Jakarta: Departemen Kesehatan RI Pr; 2009.
2. Forssten SD, Bjorklund M, Ouwehand AC. Streptococcus mutans, caries, and stimulation models. Nutrients. 2010;2 (3):290-298.
3. Gross EL, Beall CJ, Kutsch SR, Firestone ND, Leys EJ, Griffen AL. Beyond Streptococcus mutans: Dental caries onset linked to multiple species by 16S rRNA community analysis. Plos one. 2012 Oct 16;7(10):1-11.
4. Powell LV. Caries risk assessment: Relevance to the practitioner. JADA. 1998;129(3):349-53.
5. Dhalimi A. Permasalahan gambir (Uncaria gambir L.) di Sumatera Barat dan alternatif pemecahannya. Perspektif. 2006;5(1):48-59.
6. Kozai K,Shoto N, Yamaguchi N, Nagasaka N,Pradopo S. Potential of gambir as an inhibitor of dental plaque formation. Majalah Kedokteran Gigi. 1995;23(3): 95-101.
7. Amos. Gambir as antibacterial agentin gargle formulation.Jurnal Sains dan Teknologi Indonesia. 2009;11(3):180-92.
8. Pambayun R, Gardjito M, Sudarmadji S,Kuswanto KR. Kandungan fenol dan sifat antibakteri dari berbagai jenis ekstrak produk gambir (Uncaria gambir [Roxb.]). Majalah Farmasi Indonesia.2007; 18(3):141-6.
9. Furiga A, Dols-Lafargue M, Heyraud A, Chambat G, Lonvaud-Funel A, Badet C. Effect of antiplaque compounds and mouthrinses on the activity of glucosyltransferase from Streptococcus sorbinus and insoluble glucan production. Oral Microbiol and Immun. 2008; 23(5):391-400.
10. Levine MJ. Development of artificial salivas.Critical Reviews in Oral Biol and Med. 1993; 4 (3-4):279-86.
11. Ozdamir D. Dental caries: The mostcommon disease worldwide and preventive strategies. Inter J Biol. 2013; 5(4):55-61.
12. Andhani R, Widodo, Sukmana RI, Suhartono E. Effect pH on demineralization dental erosion. Inter J Chem Eng and Appl. 2015; 6(2):138-41.
13. Matsui R, Cvitkovitch D. Acid tolerance mechanisms utilized by Streptococcus mutans. Future Microbiology.2010 Mar 5; 5(3):403-17.
14. Roberts WM, Wrights TJ. The dynamic process of demineralization and remineralization. Dimensions of Dental Hygiene. 2009 Jul;7(7):16-21.
15. Pambayun R, Gardjito M, Sudarmadji S,Rahayu K. Sensitivity of gram positive bacteria in catechins extracted from gambir (Uncaria Gambir[Roxb.]). Agritech. 2008;28(4):174-9.
16. Gerwin HM, Zuilhof H, Veldhuizen AV, van den Hauvel RHH, Hartmans S. Enhanced (+) catechin transglucosylating activity of Streptococcus mutans GS-5 glukosyl transferase-D due to fructorase removal. Applied and Environment Microbiol. 1999 Sep;65(9): 4141-7.
17. Taniguchi S, Kuroda K, Doi K,Inada K, Yoshikado N, Yoneda. Evaluation of gambir quality based on quantitative analysis of polyphenolic constituent. Yakugazu Zasshi. 2007 Aug; 127(8): 1291-300.
18. Amir M, Mujeeb M,Khan A, Ashraf K, Sharma D, Aqil M. Phytochemichal analysis and in vitro antioxidant activity of Uncaria gambir. InterJ of Green Pharm. 2012; 6(1):67-72.
19. Kassim MJ, Hussin MH, Achmad A, Dahon NH, Suan KT, Hamdan HS. Determination of total phenol, condensed tannin, and flavonoid contents and antioxidant activity of Uncaria gambir extracts. Majalah Farmasi Indonesia. 2011;22(1):50-9.
20. Kajiya K, Hojo H, Suzuki M, Nanjo F, Kniazawa S, Nakayama T. Relationship between antibacterial activity of (+)-catechn derivates and their interaction with a model membrane. JAgri and Food Chem. 2004; 52(6):1514-9.
21. Zero DTFM, Martinez-Mier EA, Ferrera-Zandona AG, Ando M, Gonzales-Cabezas C. The biology, prevention, diagnosis, all treatment of

dental caries. JAm Dent Assoc. 2009 Sep; 140:25-34.
22. Loesche WJ. Role of Streptococcus mutans in Human Dental Decay. Microbiol Rev. 1986; 50 (4): 353-80.
23. Lemos JA, Quive RG, Koo H,Abranches J. Streptococcus mutans : a new Gram positive paradigm. Microbiology 2013 Mar; 159: 436-45.
24. Griswold AR, Jameson-Lee M, Burne RA. Regulation and physiologic significance of the agmative deiminase system of Streptococcus mutans UA15. J Bacteriol. 2006 Feb;188:834-41.
25. Falsetta ML, Klein MI, Lemos JA, Silva BB, Agidi S. Novel antibiofilm chemotheraphy targets exoplysaccharide synthesis ad stress tolerance in Streptococcus mutans to modulate virulance expression in vivo. Antimicrob Agents Chemother. 2012 Dec;56:6201-11.
26. Lilijemark WF, Bloomquist C. Human oral microbial ecology and dental caries and periodontal disease. Crit Rev Oral Biol Med. 1996;7(2):180-98.
27. Isalm BKS, Khan AU. Dental caries: from infection to prevention. Med Sci Monit. 2007 Nov; 13: 196-203.
28. Sanjai K, Kumarswany J, Patil A, Papaiah L, Jayaram S, Krishnan. Evaluation and comparison of decalcification agents on the human teeth. J Oral Maxillofac Pathol. 2012 May; 16(2):222-7.
29. Lund AE. Imaging Technique promising in detecting early signs of caries. JAm Dent Assoc. 2006; 137 (5): 592-9.
30. Nakano K, Matsumura M, Kawaguchi M. Attention of glucan binding protein reduce the cariogenicity of Streptococcus mutans; analysis of strain isolated from human blood. JDent Res. 2002; 81(6):367-76.
31. Xu X, Zhou XD, Wu CD. The tea catechin, epigallocatechin gallate suppresses cariogenic virulance factor of Streptococcus mutans. Antimicrob Agents Chemother. 2011 Mar; 55 (3): 1229-36.
32. Gradisar H, Pristovsek P, Plaper A,Jerala R. Green tea catechin inhibit bacterial DNA gyrase by interaction with its ATP binding site. J Med Chem. 2007 Jan 25; 50(2):264-71.
33. Bernal P, Lemaire S, Pinho MG, Mobashery S, Hinds J, Taylor PW. Insertion of epicatechin gallate into the cytoplasmic membrane of methiacillin-resistant Staphylococcus aureus disrupts penicillin-binding-protein (PBP) 2a-mediated beta lactam resistance by delocalizing PBP2. JBiol Chem. 2010 Jul 30; 285(31):24055-65.
34. Reygaert WC. The antimicrobial possibilities of green tea. Front Microbiol. 2014 Aug 20;5:434.
35. Masduki I. Efek antibakteri ekstrak biji pinang (Areca catechu) terhadap S. aureus dan E. coli. Cermin Dunia Kedokteran.1996; 109:21-4.
36. Ho K, Tsai CC, Huang JS, Chen CP,Lin TC, Lin CC. Antimicrobial activity of tannin components from Vaccinium vitis-iduea L. J Pharm Pharmacol. 2001 Feb;53(2):187-91.

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