

# The effectiveness of betel leaf (*piper betle* Linn) extract gel and cocoa bean (*theobroma cacao* L) extract gel application against the hardness of enamel surface *in vitro*.



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## Abstract

**Objective:** Nowadays several ways have been evolved to increase the hardness of the enamel surface as an effort to prevent caries. One of the alternatives that can be used is application of gel with herbal basic material.

**Material and Methods:** Use of herbal basic material is preferred by people because the side effects are relatively small compared to synthetic drugs. Piper betle and cocoa beans are medicinal plants that is often used by people to inhibit caries. This is because piper betle and cocoa beans contain hardness that may influence the enamel surface. Maxillary first premolar teeth, which has been extracted and does not have caries, were divided into 3 treatment groups: piper betle extract gel, cocoa bean extract gel and distilled water as a negative control.

Each treatment group consisted of 8 samples. Samples before and after the application in each treatment group were measured using Universal Hardness Tester.

**Results:** Data were collected and analyzed using ANNOVA Repeated test. Showed there were significant differences ( $p < 0.05$ ) of enamel surface hardness before and after the application of cocoa bean extract gel. There were no significant differences ( $p > 0.05$ ) of enamel surface hardness before and after the application of piper betle extract gel and distilled water.

**Conclusion:** That cocoa bean extract gel is more effective to increase the hardness of email surface.

**Keywords:** Cocoa bean, Hardness of email surface, Piper betle linn

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## Introduction

Teeth are a chewing tool in the digestive system of the human body. Dental disease that is often suffered by almost all of Indonesian people is caries. Basic Health Research or Riset Kesehatan Dasar 2007 showed that the prevalence of active caries in Indonesia is 46,5%.<sup>1-4</sup>

Caries is an inflectional disease caused by email and dentin demineralization which is closely linked with the consumption of foods that are cariogenic. There are several ways to prevent caries, started from brushing teeth regularly, mouthwash and fluoride application to the use of toothpaste made from herbs, such as betel leaf.<sup>1,5,6</sup>

Betel leaf contains many organic compounds which are essential oil, alkaloid, flavonoid, tannin and polyphenol. Polyphenols in betel leaf act as an antiseptic agent and can inhibit biological activity of *S. mutans*, bacteria that causes tooth caries.<sup>7</sup> The increase in the hardness of email surface after the application of betel leaf extract gel. This is due to the binding reaction between calcium hydroxyapatite and tannin in betel leaf.<sup>5</sup>

Theobromine is an alcaloid compound from metilxantina group contained in cocoa bean or

chocolate (*teobroma cacao*). The last few years, theobromine has been researched for its use in the dental health field, as it has anti-cariogenic effect which is higher than fluoride in reducing email solubility after phosphoric acid exposure. The effect of distilled water mixed with theobromine powder were effective to increase the hardness of email surface.<sup>8,9</sup>

Email as the outermost and hardest layer in the teeth consists mostly of hydroxyapatite crystals. This crystal is permeable to ions and molecules in direct contact to the surface. When email come into contact with acid, the calcium ion will dissolve and cause a decrease in the hardness of dental surface so that this become early caries.<sup>10-12</sup>

Hardness is defined as object's endurance against penetration. Email hardness is one of the email physical characteristic which is affected by the number of inorganic materials such as calcium. The dilution of calcium and hydroxyapatite crystals can decrease email hardness making it susceptible to caries. The hardness of email surface is one of the important factors in the physical characteristic of teeth.<sup>12,13</sup>

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Several studies have shown that betel leaf and cocoa bean have an influence on the increase in the hardness of email surface. But there has been no research that suggests comparison on the effectiveness of betel leaf extract and cocoa bean extract in the form of gel on the hardness of email surface.

Based on the description above, the authors wanted to do a research about the effectiveness of using betel leaf (*piper betle linn*) extract gel and cocoa bean (*teobroma cacao L*) extract gel against the hardness of enamel surface *in vitro*.

## Material and Methods

Study type which was applied was experimental laboratories. The research was conducted at Conservation Laboratory in Faculty of Dentistry Hasanuddin University, Biology Laboratory UNM and Mechanic Laboratory Politeknik Negeri Ujung Pandang from September to October 2016. Studied samples were maxillary first premolar teeth. Criteria of the samples was maxillary first premolar teeth which was extracted with orthodontic reason, rooted in two and the apex tip has closed perfectly.

Tools and materials used in this study: Handpiece high speed (Pana-Air® Japan), carborundum disk drill (Edenta®, Swiss), individual plastic containers, tweezer, blender, funnel and filter paper, evaporation machine (Hanshin®, Japan), Vickers Hardness Tester, analytical balance, mortar and pestle, disposable 1 cc, glass tools, betel leaf extract gel 15%, distilled water (Aqua®, Indonesia), cocoa bean extract gel 15% and gel making materials (carbomer 2%, TEA 2%, calcium carbonate 20%, sodium lauryl sulfate 1%, AgNO<sub>3</sub> solution 70%, sodium saccharine 0.1%, menthol 0.4%, sodium benzoate 0.1%, distilled water).

### Sample preparation

The entire sample of 24 teeth soaked in saline. Teeth were then separated from root and crown at 2 mm below the CEJ using carborundum disc. Teeth were cleaned by using a brush and pumis, then dried with an air blow. Each sample was divided into three treatment groups and each was given a serial number. Group 1: betel leaf extract gel, Group 2: cocoa beans extract gel, Group 3: distilled water. After that the orthoplast beams was made with 1 cm high then the samples were planted on orthoplast mold with labial surface of teeth facing upward.

### Preparation of betel leaf extract and cocoa bean extract

Betel leaf that has been cleaned then weighed 100 grams each for the manufacture of dry powder. It dried for 24 hours then weighed to determine the weight of powdered betel leaf that has been dried.

Dried leaf then blended for 3 minutes and placed into erlenmeyer flask before added ethanol 70%. Filtration was performed using fine filter paper. The solvent evaporation was conducted by using evaporation machine for 45 minutes. Betel leaf crude extract was then stored in glass bottle.

Cocoa bean was dried with aerated for approximately 24 hours. Cocoa bean then coarsely ground and aerated again until dried for approximately 48 hours and then pulverized to a powder in a blender. Powdered cocoa beans were weighed using an analytical balance as much as 200 grams. Cocoa powder then soaked with 80% ethanol of 300 ml for 24 hours and then macerated with constant stirring and then filtered using a filter paper. The filtrate then concentrated by rotary evaporation machine for 2 hours to separate the solvent from cocoa beans extract, and then the filtrate was transferred to a water bath for 4×24 hours to evaporate residual ethanol in the extract to obtain crude and viscous extract. After that the extract was diluted by series dilution using sterile aqua bidestilata solvent to obtain a concentration of 15%.

### Preparations of betel leaf extract gel and cocoa bean extract gel

The preparation of this teeth gel cleansing was done by adding carbomer 940 and hot water into a mortar and then stirred it. Added TEA, homogeneously stirred until the gel swelled, kept it for 24 hours (M-1). After that, put in AgNO<sub>3</sub> solution little by little while stirred. Added calcium carbonate and stirred. Then added menthol which has been diluted in ethanol into the mortar contained gel base and homogenized it. After that, put in sodium benzoate, sodium lauryl sulfate and sodium saccharine that have been diluted before by using hot water then homogenized it (M-2). Combined M-1 and M-2, homogeneously stirred and added betel leaf extract and cocoa bean with concentration of 15%, homogenized it.

### Hardness test of email surface.

Hardness test was conducted on every sample by using Vickers Hardness Tester and then recorded. First measurement was conducted without treatment on 24 samples (pre-test). Score obtained an initial hardness score before being treated. The next hardness test performed as follows: sample placed on universal hardness tester table, then performed indentation on the enamel surface using steel balls corresponding emphasis brinell method. First, performed the installation of the penetrator, which is a steel ball with a diameter of 2.5 mm and a ring nut on the tool. Then, handwheel contained in the tool was rotated slowly until the labial surfaces of

the teeth in contact with penetrator. The measurement results can be viewed on the monitor screen after 10 seconds.

Having obtained the initial hardness score, the next stage was testing stage of the sample in the test material. Each sample of 8 samples in group 1 was polished with betel leaf extract gel until the entire labial surface was covered and leaved it for 5 minutes. After 15 minutes the sample was measured twice to get the average value of hardness of each sample. Samples then irrigated by using saline to clean the gel has been applied before. Then applied the polishing betel leaf extract gel and leaved it for 15 minutes then gave the same treatment with intervention time for 35 minutes. Group II with cocoa beans extract gel was given the same treatment in group I. Group III soaked in a container containing distilled water until the entire surface of the tooth labial submerged and left it for 5 minutes, 15 minutes and 35 minutes.

## Results

Table 1 showed the means of enamel hardness surface used betel leaf extract gel. Table above can be seen that enamel hardness increased in interval time 5 minutes. While enamel hardness decreased in interval time 15 minutes and 35 minutes. Enamel hardness surface in pre is 117.713, in post 1 is 120.673, in post 2 is 108.625 and in post 3 is 101.438. Based on result of repeated ANNOVA test, obtained  $p$ -value = 0.185 ( $p < 0.05$  significant).

It means that betel leaf extract gel has no significant effect of enamel hardness surface.

Table 2 showed the means of enamel hardness surface used cocoa seed extract gel. The table above shows that enamel hardness surface increased significantly in time interval pre through post 1, nevertheless it decreased in time interval on post 2 and post 3. Enamel hardness surface in pre time is 116.025, in post 1 is 132.800, in post 2 is 122.600 and post 3 is 117.063. Based on the result of repeated ANNOVA test, obtained  $p$ -value = 0.031 ( $p < 0.05$ ; significant). It means that cocoa seed extract gel has significant effect in the enamel hardness surface.

Table 3 shows the means of enamel hardness surface uses aquades. It can be seen that enamel hardness decreased on time interval pre, post 1 and post 2 and increased in post 3, however no surpass level of before experiment hardness. Enamel hardness surface in pre time is 148.288, post 1 is 137.550, post 2 is 135.575 and post 3 is 140.925. Based on the result of repeated ANNOVA test, obtained  $p$ -value = 0.321 ( $p < 0.05$ ; significant). It means that aquades has no significant effect to enamel hardness surface.

## Discussion

Enamel is a tissue which undergo high mineralization and susceptible acid. Enamel contains, based on its weight, 95-98% anorganic, 1-2% organic and 4% water. High mineral provides hardness and solidity, yet brittleness. Mineral on human tooth, which provides it, is calcium hydroxyapatite

**Table 1** The differences from enamel hardness between time intervention and betel leaf extract gel

Material	Pre (0 minute)	Post 1 (5 minute)	Post 2 (15 minute)	Post 3 (35 minute)	P-value
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	
Betel leaf extract gel	117.713 $\pm$ 28.4744	120.637 $\pm$ 23.6197	108.625 $\pm$ 19.6479	101.438 $\pm$ 26.7899	0.185*

\*Repeated ANNOVA Test:  $p > 0.05$  : not significant

**Table 2** The differences from enamel hardness surface between time intervention and cocoa seed extract gel

Material	Pre (0 minute)	Post 1 (5 minute)	Post 2 (15 minute)	Post 3 (35 minute)	P-value
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	
cocoa seed extract gel	116.025 $\pm$ 17.9194	132.800 $\pm$ 28.4177	122.600 $\pm$ 28.1209	117.063 $\pm$ 30.6776	0.031*

\*Repeated ANNOVA Test:  $p < 0.05$  : Significant

**Table 3** The differences of enamel hardness surface between time intervention and aquades

Material	Pre (0 minute)	Post 1 (5 minute)	Post 2 (15 minute)	Post 3 (35 minute)	P-value
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	
Aquades	148.288 $\pm$ 14.8307	137.550 $\pm$ 8.7158	135.575 $\pm$ 14.1243	140.925 $\pm$ 17.1674	0.321*

\*Repeated ANNOVA Test:  $p > 0.05$  : not significant

(Ca<sub>10</sub>(PO<sub>4</sub>)<sub>6</sub>(OH)<sub>2</sub>). Enamel can solve when contacts with acid, which produce half or all mineral of enamel dissolve and decrease enamel hardness.

Electing time for gel application in 5, 15 and 35 minutes for 5 minutes are time estimation used for applies gel per day. So that the total time estimation needed for applying gel in 3 days are 15 minutes while apply gel for 7 days need 35 minutes.

This study is using Universal Hardness Tester tools for hardness test by performing indentation on enamel surface using steel ball pressure according to brinell method. Vickers Hardness Number (VHN) determined by standard table referention which is defined by convert result value of brinell method analysis.

Table 1 showed no significant effect of increased enamel hardness surface after application of betel leaf extract gel. Decrease of enamel hardness surface related to 0.1-1.3% tannin acid on betel leaf extract.<sup>17</sup> According to a study by Hagerman<sup>18</sup> tannin acid can easily bonded with protein that produce protein complex. There are some proteins, called amelogenin, on enamel mineralization. Protein establish approximately 58% organic on enamel. It is indicate that tanin and protein bond on enamel and affect enamel hardness surface. There is chemical bonded between tanin acid and protein on enamel when measuring enamel hardness surface. The bond covered enamel surface and caused enamel hardness decrease.

Decrease of enamel hardness surface consort that betel leaf extract contains tanin acid, lead to increase of demineralization on enamel surface. Solubility of enamel surface, caused by acid is reaction between hydrogen ion and anorganic material of enamel forms. The increase of phosphat ion and H<sup>+</sup> ion caused pH of hydroxiapatite crystal decrease up to 4.5 which indicates porosity form on enamel. The decrease of enamel hardness surface is affected tanin acid and gel bond as well. If tanin acid is blended with gelatin, it would be a sedimentation. This sedimentation covers enamel surface and causes enamel hardness surface decrease. Application of betel leaf extract gel approximately 5 minutes, leads to increase of enamel hardness surface. It is caused by reaction between tanin acid and calcium hydroxiapatite. Bond between tanin acid and calcium hydroxiapatite form new layer leads to enamel hardness surface increase, yet the increase is not significant.<sup>5</sup>

Table 2 showed the increase of enamel hardness surface significantly after used cocoa extract gel. This ensued because the contents of teobromin. Theobromin is a chemical substances from alcaloid. This study used silver nitrate solvent (AgNO<sub>3</sub>). Theobromin and silver nitrate can establish sediment in alkaline environment by releasing H ion.

This sedimentation established new layer on enamel surface. Although teobromin is a weak acid, pH 8.6 and 9.9 pKa, when it bonds with silver nitrate, they will release H ion which causes remineralization increase thus presence calcium hydroxiapatite and leads to the increase of enamel hardness surface.<sup>19</sup>

Enamel hardness surface decreased in minutes 15th and 35th of application, which was caused by unfermented cocoa. It consort with study by Widayat<sup>20</sup> reported that unfermented cocoa seed contains 9.1-9.6% sugar. The more the cocoa fermented, the more the sugar decreased. It was occurred because sugar level in cocoa seed, used by khamir, establish alcohol, that caused sugar level high and split alcohol became lactac acid and acetic acid.<sup>20</sup> That alcohol drinks is able to decrease enamel hardness surface because it contains acid, lactac acid and acetic acid are chelating agents, which bond calcium into saliva and decrease remineralization on enamel surface.<sup>11</sup>

Table 3 showed no significant increase differences on enamel hardness surface sample after soaked into aquades, although it increased on 35th minutes soaked. Aquades has ability to dissolves enamel calcium and decrease enamel solubility based on applying time. It is based on pH alkaline of aquades.

## Conclusion

Application of 15% cocoa seed extract gel effective to increase enamel hardness surface compared to 15% betel leaf extract gel and aquades. The more 15% betel leaf extract gel and 15% cocoa seed extract gel apply, the more enamel hardness surface decrease. The more it soaked into aquades, the more enamel hardness surface increase.

## Conflict of Interest

The authors report no conflict of interest.

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