Effect of belimbing wuluh (averrhoa bilimbi l.) extract gel exposure duration to surface roughness of enamel

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Abstract. The purpose of this study was to analyze the effect of Belimbing Wuluh Gel Extract to surface roughness of enamel. Thirty-six premolars teeth that divided into 4 groups (n = 9), were exposed to 37% phosphoric acid gel (pH = 1) for 15 seconds as a control group, and belimbing wuluh extract gel with a concentration of 80% (pH = 1.8) for 15 seconds, 20 seconds, and 25 seconds as the treatment groups. The statistical analysis of paired and unpaired T-test shows that all treatment groups experienced a significant change (p <0.05). The greatest changes in surface roughness of enamel occurred after exposed by belimbing extract gel with an exposure duration of 25 seconds, but the roughness of 37% phosphoric acid gel is still greater. There was a correlation between roughness on the surface of tooth enamel with prolonged exposure belimbing wuluh extract gel with a concentration of 80%.

1. Introduction
Indonesia has various kinds of flora, in which some have fruits with low pH. One of the famous flora among Indonesian people is Averrhoa bilimbi L. or known as “belimbing sayur” (vegetable starfruit) or “belimbing wuluh” (wuluh starfruit). This fruit is commonly used for giving sour taste into cuisines. Belimbing wuluh is rich in citric acid, oxalic acid, vitamin C, tannin, and various minerals such as sodium, potassium, calcium, phosphate, and magnesium. Belimbing wuluh is more acidic than orange [1]. Research about belimbing wuluh in dentistry had been done before, using belimbing wuluh gel extract with 70%, 80%, and 90% concentration exposed into tooth enamel for 4 hours in 14 days as home bleaching product even though the result was not effective [2]. Meanwhile with the same level of concentration and exposure duration, surface roughness test was performed with roughness level was between 0.4-1.7 µm as belimbing wuluh has pH <2 giving the demineralization effect or the dissolve of hydroxyapatite crystal in tooth enamel [3]. Therefore, high level of roughness material is not suitable for bleaching product as bleaching product only supposed to affect enamel color, not resulting in demineralisation effect; demineralisation effect and roughness increase are the purpose of etch material, concluding that belimbing wuluh extract has potential to be etch material.

Acidic etch in dentistry is used for giving high demineralisation effect in short time towards enamel or dentin surface of human tooth as the purpose is to increase enamel and dentin roughness expanding the surface to increase the adhesive bonding towards enamel by micromechanical interlocking. Acidic etch feature used is low pH to result in demineralisation. It is able to increase surface energy so adhesive can spread away and entering enamel or dentinal porous. The common etch material used is phosphoric acid with 30-50% concentration and 1-2.4 pH level, according to each product [4].
Belimbing wuluh has demineralisation effect as its acidic level is very high. In previous research, extract of belimbing wuluh using infuse technique was measured and resulted in 80.3% concentration level and pH level 2. Therefore, the aim of this research is to know the effect of 80% concentration belimbing wuluh extract towards surface roughness in human dental enamel, to know the possibility to be used as etch material in dentistry, and to know the relationship between duration of exposure and level of roughness produced by 80% gel extract of belimbing wuluh.

2. Materials and Methods
Ethical clearance was performed by Research Ethic Committee Faculty of Dentistry Universitas Indonesia, before the research began. In this research, human upper premolar teeth were used as specimens, which all had been passed the ethical clearance. All specimens had inclusion criteria such as free of caries, abrasion, erosion, and exclusion criteria of discoloration, white spot, and crack. All specimens were counted using G*Power application and earned of 36 specimens. The premolar teeth were collected and stored in saline solution.

The dental surface of each tooth was evaluated. Teeth with crack were separated and not used. The root of premolar teeth which were used as specimens was cut using rotary bur. Then the coronal part was planted into mould using 19 mm diameter and 30 mm height pipe, using decorative resin. Enamel surface was smoothed using SiC paper 2000 in grinding machine. The smoothing process was performed; the thickness of enamel disposed was not more than 100µm. Smoothed specimens then stored into plastic pot with saline solution and recorded with number. Control group in which will be exposed with 37% phosphoric acid for 15 seconds was given K initial, treatment group which will be exposed with 80% belimbing wuluh extract for 15 seconds was given A initial, treatment group in which will be exposed with 80% belimbing wuluh extract for 20 seconds was given B initial, and treatment group in which will be exposed with 80% belimbing wuluh extract for 25 seconds was given C initial. Enamel surface roughness value before exposure was measured using Surface Roughness Tester Mitutoyo SJ 301 with measurement standard JIS 2001. Measurement in each specimen was performed three times.

Belimbing wuluh which used in this research was from the same source, one tree in Cibubur area, East Jakarta. 520 gram belimbing wuluh were cleaned up then smashed using blender machine and added with 1.5 liters of aquadest. Extract was then infused in 90 °C temperature for 30 minutes. Then the extract was filtered and the waste was heated again in 90 °C for 30 minutes. Both were mixed and evaporated in 60 °C. At the end of extraction 55 grams of extract was gained, with 80.3% purity level using Grafitmetry method. Gel was made using CMC powder, by mixing 0.2 gram CMC into 10 gram belimbing wuluh extract to get belimbing wuluh extract gel with 80% concentration.

Belimbing wuluh extract gel with 80% concentration level was then checked and measured the acidity level using pH meter Thermo Orion 290A+. The result was pH level still far up from 37% phosphoric acid gel pH level, therefore decreasing process of pH level was performed. Decreasing process of pH level was done by adding HCl to make the pH level similar with 37% phosphoric acid. HCl was added gradually, 0.15cc of 0.02N HCl, 0.1cc of 0.2N HCl, and 0.2 cc of 1N HCl. pH level was then measured and gained in 1.8 level.

Nine specimens of control group were exposed with 37% phosphoric acid gel according manufacture’s instruction, for 15 seconds. The rest 27 specimens were divided into 3 groups, each 9 specimens. Each group was exposed with 80% belimbing wuluh extract for 15 seconds, 20 seconds, and 25 seconds. The enamel surface of each group was poured with gel drops and been left for 15 seconds in A group, 20 seconds in B group, and 25 seconds in C group. The surface was then cleaned from gel, sprayed with aquadest, then dried without pressure using absorbable tissue. The same procedure was performed into other specimens. Enamel surface roughness was measured for three times for each specimen to see roughness changing.

Data analysis was performed using SPSSV.20.0. Normality test was done using Saphiro-Wilk test for roughness value before and after 80% belimbing wuluh gel exposure, showed normal, however was not normal in 37% phosphoric acid gel group. Therefore, paired-T test was done to see the
increase in enamel surface roughness for each 80% belimbing wuluh extract gel group, otherwise non-parametric Mann-Whitney was done to see the increase in enamel surface roughness for 37% phosphoric acid gel group. In addition to see the differences between 37% phosphoric acid gel and each 80% belimbing wuluh extract gel groups, non-paired T test was performed. Nevertheless, to see the correlation duration exposure of 80% belimbing wuluh extract gel, Spearmen correlation test was performed.

3. Results and Discussion

3.1 Results
Analysis of belimbing wuluh content using High Performance Liquid Chromatography (HPLC) resulted in citric and oxalic acid, citric acid content in belimbing wuluh was more than 1.35%, however oxalic acid in which one of the organic acid, was not detected. Initial pH level measurement of 80% belimbing wuluh extract gel and after added using HCl solution was 2 and 1.8 respectively. Further, the pH of 37% phosphoric acid gel was 1. Surface roughness measurement in specimens before and after exposure with 37% phosphoric acid gel and 80% belimbing wuluh extract gel can be seen in Table 1.

In table 3 and figure 1 can be seen that there was increase in enamel surface roughness after exposure with 37% phosphoric acid gel and 80% belimbing wuluh extract gel. In specimens which exposed with 37% phosphoric acid gel, there were 220% increase. In specimens which exposed with belimbing wuluh extract gel for 15, 20, and 25 seconds there were increase in 20%, 50%, and 136%. By these results, there was roughness increase along with exposure duration increase. Paired T-Test statistic analysis in roughness increase for specimens which exposed by 80% belimbing wuluh extract gel stated significant difference ($p<0.05$). Result can be seen in Table 2.

Table 1. Average value of tooth enamel surface roughness before and after exposure with 37% phosphoric acid gel and 80% belimbing wuluh extract gel (µm).

|                      | 37% Phosphoric Acid Gel | 80% Belimbing Wuluh Extract Gel with HCl |
|----------------------|--------------------------|------------------------------------------|
|                      | 15 Seconds               | 15 Seconds | 20 Seconds | 25 Seconds |
| Before Exposure      | 0.224±0.065              | 0.269±0.066 | 0.253±0.058 | 0.252±0.058 |
| After Exposure       | 0.718±0.099              | 0.323±0.090 | 0.380±0.077 | 0.596±0.097 |
| Increasement Percentage | 220%                    | 20%        | 50%        | 136%       |

Table 2. Statistic result of roughness comparation before and after exposure.

| Time      | 80% Belimbing Wuluh Gel | p-value |
|-----------|-------------------------|---------|
| Start     | 15 seconds              | 0.000   |
|           | 20 seconds              | 0.000   |
|           | 25 seconds              | 0.000   |

Non-paired T-test analysis result between surface roughness increase after 37% phosphoric acid gel exposure and each 80% belimbing wuluh extract gel group showed significant differences ($p<0.05$). Result can be seen in Table 3. Spearman Correlation test to know the correlation between duration exposure and roughness level increase showed correlation as $p<0.05$ and $r = 0.848$ stated high correlation between duration exposure and roughness level.
Table 3. Statistic result of comparison between 37% phosphoric acid gel and each 80% belimbing wuluh extract gel groups.

| 37% Phosphoric Acid Gel | p-value |
|-------------------------|---------|
| BW (15 seconds)         | .000    |
| BW (20 seconds)         | .000    |
| BW (25 seconds)         | .008    |

3.2 Discussion

In this research, roughness test had been done towards enamel which exposed to 37% phosphoric acid gel and 80% belimbing wuluh extract gel with HCl addition. Enamel surface used in this research already been smoothed using Sic paper 2000 to gain smooth enamel surface to know the roughness changing after exposure with 37% phosphoric acid gel and 80% belimbing wuluh extract gel accurately.

Analysis result of belimbing wuluh extract using HPLC found 1.35% citric acid or 1.350 ppm, otherwise oxalic acid was not detected. This result was different from other research by Kenanga (2014) using the same content analysis but showed citric acid level 3.52-3.55% (3526.91- 3551.67 ppm), and oxalic acid level 0.13-0.18% (135.01-188.96 ppm) [2,3]. The technique used by Kenanga (2014) was maceration; otherwise in this research using infuse technique with heating in 90 °C for 14-20 minutes. Nevertheless, starfruit (belimbing) used in this research from Cibubur area, East Jakarta, while belimbing wuluh used by Kenanga from Aceh with pH 2.4 and from Bogor with pH 1.75; the pH of belimbing wuluh extract in this research was 2 [2,3].

Differences in belimbing wuluh content showed that even though using the same kind of belimbing wuluh, the content could be different, according to growth location, soil nutrition, and sun exposure [1,4]. Belimbing wuluh was said to be optimally grow in nutritious, moist, a little acidic, and not really wet soil.1 Weather also affect the content of belimbing wuluh. Oxalic acid level in belimbing wuluh during dry season was much lower than during rainy season [4]. This explains the undetected result of oxalic acid as the belimbing wuluh used in this research was taken during dry season.

Belimbing wuluh has various organic acid such as oxalic acid, acetate acid, malic acid, and citric acid. Citric acid is the highest amount of acid than others organic acid [1,2,3]. It is one of weak acid that has been many researched towards the effect on teeth, especially citric acid inside many kinds of drinks, like juice and fruit flavored drinks [5]. Citric acid is said to have erosive effect toward enamel, therefore influence roughness and hardness of tooth enamel [5,6]. Nevertheless, acetate acid, malic acid, and ascorbate acid may cause demineralization [6,7]. Citric acid can dissolve enamel crystal, but not as fast as phosphoric acid [8]. Phosphoric acid is a strong acid, while citric acid in belimbing wuluh is weak acid. This difference affected the attraction of atom nuclei towards hydrogen ion which bonded with phosphate in hydroxyapatite. Therefore, phosphoric acid dissolves hydroxyapatite crystal more than citric acid.

In Kenanga (2014), exposure of belimbing wuluh extract for 28 hours resulted in roughness value of 0.24 µm, therefore in this research acidity increase was done using HCl to shortened the exposure time and achieve pH level of 1.8 [3]. Dissolve of hydroxyapatite crystal is influenced by exposure duration and acid solution concentration [6,8]. This explain the result of 80% belimbing wuluh extract gel exposure result toward tooth enamel showed increase of roughness along with exposure duration increase. The biggest score of enamel roughness (0.596 µm) was after 80% belimbing wuluh extract gel exposure for 25 seconds. The added HCl into belimbing wuluh extract gel also assumed took part in demineralisation process. Even though the score of enamel roughness earned in this research still below the enamel roughness score earned by 37% phosphoric acid exposure for 15 seconds.

In other research, which correlate surface roughness with composite bond strength on tooth surface resulted in enamel surface roughness 0.352 µm gave bond strength around 40.5 MPa. Therefore, 80% belimbing wuluh extract gel has potential in demineralisation, even though clinically still not effective to be etch gel, as needed long time of exposure. Continued research is needed to know belimbing...
wuluh composition specifically and see the effect of extraction and acid etch making for much faster, safe, and effective function for tooth enamel.

4. Conclusion

This research concluded that 80% belimbing wuluh gel with HCl addition had pH 1.8 gave enamel surface roughness Ra 0.596 µm after exposure for 25 seconds. There was correlation between roughness score on tooth enamel with exposure duration of 80% belimbing wuluh gel. Researcher suggested to do further research about enamel surface morphology and microporosity depth of tooth enamel after exposure with belimbing wuluh extract. Nevertheless, further research about belimbing wuluh extract exposure effect towards smear layer dentin is needed.

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