THE EFFECT OF *Averrhoa blimbi* L (BELIMBING WULUH) GEL EXTRACT ON INCREASING DEGREE OF TEETH BRIGHTNESS (*IN VIVO*)

(PENGARUH GEL EKSTRAK *Averrhoa blimbi* L (BELIMBING WULUH) TERHADAP PENINGKATAN DERAJAT KECERAHAN GIGI (IN VIVO))

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ABSTRACT

Discoloration (extrinsic and intrinsic) reduce the beauty of someone's appearance and confidence. Discoloration affected the increased aesthetic treatment needs in dentistry. Discoloration can be treated by teeth whitening procedures. Teeth whitening can be done with dental bleaching from the application of chemicals on the surface of the teeth. However, dental bleaching has side effects such as reduce the amount of calcium, phosphate and fluoride in tooth enamel, reduce enamel hardness, enamel surface roughness, and dental hypersensitivity. Belimbing wuluh is one of the herbal ingredients to minimise the risk; it contains oxalic acid, a natural peroxide compound. This study purposed to determine the effect of belimbing wuluh gel extract in increasing the degree of teeth brightness colour by in vivo in rabbits. This study used an experimental analytic method with 27 rabbits' incisors, divided into three groups: 50% belimbing wuluh gel, 40% oxalic acid gel, and negative control group. Rabbits' incisors were previously coated in a transparent varnish on the specified area. Teeth bleaching is done for 4 hours in 14 days. Colour measurements used camera and ring-light, which MATLAB will convert. The data were analysed statistically with One way ANOVA followed by a post-hoc NSK test (p <0.05). The result showed that
belimbing wuluh gel extract caused an increase in the teeth colour's brightness (p = 0.0030). So, it can be concluded that belimbing wuluh gel extract affects increasing the degree of teeth brightness.

**Keywords**: belimbing wuluh, dental bleaching

**ABSTRAK**

Perubahan warna (ekstrinsik dan intrinsik) mengurangi keindahan penampilan dan kepercayaan diri seseorang. Diskolorasi mempengaruhi peningkatan kebutuhan perawatan estetik dalam kedokteran gigi. Perubahan warna dapat diatasi dengan prosedur pemutihan gigi. Pemutihan gigi dapat dilakukan dengan pemutihan gigi dari aplikasi bahan kimia pada permukaan gigi. Namun, pemutihan gigi memiliki efek samping seperti mengurangi jumlah kalsium, fosfat dan fluorida dalam email gigi, mengurangi kekerasan email, kekasaran permukaan email, dan hipersensitivitas gigi. Belimbing wuluh adalah salah satu bahan herbal untuk meminimalkan risiko; mengandung asam oksalat, senyawaperoksida alami. Penelitian ini bertujuan untuk mengetahui pengaruh ekstrak gel belimbing wuluh dalam meningkatkan derajat kecerahan warna gigi secara in vivo pada kelinci. Penelitian ini menggunakan metode eksperimental analitik dengan gigi seri 27 kelinci yang dibagi menjadi tiga kelompok yaitu gel belimbing wuluh 50%, gel asam oksalat 40%, dan kelompok kontrol negatif. Gigi seri kelinci sebelumnya dilapisi pernis transparan pada area yang ditentukan. Pemutihan gigi dilakukan selama 4 jam dalam 14 hari. Pengukuran warna menggunakan kamera dan ring-light, yang akan dikonversi oleh MATLAB. Data dianalisis secara statistik dengan One way ANOVA dilanjutkan dengan uji NSK post-hoc (p <0,05). Hasil penelitian menunjukkan bahwa ekstrak gel belimbing wuluh menyebabkan peningkatan kecerahan warna gigi (p = 0,0030). Sehingga dapat disimpulkan bahwa ekstrak gel belimbing wuluh berpengaruh terhadap peningkatan derajat kecerahan gigi.

**Kata Kunci**: belimbing wuluh; pemutihan gigi

**INTRODUCTION**

Having an attractive appearance is an important thing in a person's life because it can increase self-confidence to trigger people to improve their aesthetics in dentistry. Since the end of the 19th century, teeth whitening treatments have been
recognised by the public. Dental bleaching is the process of applying chemicals to the surface of the teeth so that they can brighten the colour. Dental bleaching performed by dentists in their practice is called in-office bleaching. There is a tooth whitening technique that patients can do at home called home bleaching.\textsuperscript{1-4}

Chemicals that are often used in tooth whitening treatments include carbamide peroxide with varying concentrations in dosage forms in the form of gels or pastes. The teeth whitening mechanism (carbamide peroxide) occurs because an oxidation reaction converts peroxide into dihydroxyl and oxygenase. These substances are free radicals that will bind to substances that cause tooth staining. Apart from whitening teeth, these ingredients also have disadvantages, so they must be done properly and carefully. The use of teeth whitening agents with high concentrations to increase efficiency is believed to impact the state of the tooth structure. Several studies have stated that tooth hypersensitivity and irritation to soft tissues are the most frequent tooth whitening treatments using these ingredients.\textsuperscript{5,6}

Several research attempts were made to use other natural ingredients to replace carbamide peroxide as a dental bleaching agent, including those containing oxalic acid levels such as belimbing wuluh. They contain carboxylic compounds and peroxide compounds that are thought to whiten teeth that experience discoloration. Fauziah et al. (2012) conducted a study that proved an increase in teeth brightness after applying belimbing wuluh compared to 10% carbamide peroxide in vitro on human premolar teeth and measured qualitatively with the VITA Classical Shade Guide. After being applied, belimbing wuluh is for 2 hours in 14 days. Based on research by Medina M. et al. In 2014, it has been reported that belimbing wuluh extract gel has an effect on tooth discoloration based on the concentration level applied to the surface of cattle teeth in vitro. The study applied Aceh and Bogor belimbing wuluh extract gels with various concentrations of 70%, 80%, 90% for 4 hours for 14 days. The higher the concentration, it can cause discoloration. The discoloration of the enamel surface was due to the presence of tannin in the gel (the specimens, which were originally greyish translucent colour, turned brownish yellow after application of the belimbing wuluh extract gel). Because of the tannin content in the gel, tannin is a chromogenic agent; namely, the substrate produces colour products that can cause dark discoloration of the teeth. Until now, there has been no research on the effect of tooth discoloration using belimbing wuluh extract gel in vivo, namely on rabbit teeth using a 50% concentration of belimbing wuluh. Gel preparations are made because the thickening element affects the effectiveness of the peroxide compound so that it can cause good adhesion. Rosy Husaini, in 2019, conducted a study that proved that there was an increase in the brightness of the teeth after applying belimbing wuluh in vitro with qualitative measurements using the VITA Classical Shade Guide and quantitatively using Dino-lite, which was converted to MATLAB. The result
obtained 50% belimbing wuluh extract gel affected the degree of brightness of the stained teeth.⁷-⁹

Therefore, researchers are interested in knowing the effect of 50% belimbing wuluh extract gel in increasing the degree of brightness of the teeth in vivo in rabbits because the size of the rabbit's teeth has a size that can be seen the difference is also the easy extraction method.

METHOD
This research is an analytical laboratory experimental research focusing on 50% concentration of belimbing wuluh extract gel to increase the degree of brightness of teeth in vivo. The object of this research was the rabbit's upper right incisor. The study was conducted on rabbit teeth, and the experiment was carried out by making moulded spoons for rabbit teeth made of shellac to protect and minimise the entry of saliva. Then apply transparent varnish to the designated area, which is the left half of the size of the rabbit's upper right incisor, as a measurement before exposure to gel because the varnish has good protection. After that, the upper right incisor of the rabbits was applied with a 50% concentration of belimbing wuluh extract gel for 4 hours within 14 days. It was measured using the VITA Classical Shade Guide and quantitatively using a camera assisted by a ring light converted into MATLAB to determine the effect of gel exposure. The results of the pre-exposure data taken in the transparent varnish area and after being given exposure to the gel were statistically analysed by the Chi-Square test followed by the ANOVA test and ending with the Post-hoc SNK test.¹⁰⁻¹⁵

RESULT
This study used 25 samples divided randomly into three groups: nine samples of 50% belimbing wuluh gel, 7 samples of 40% oxalic acid gel, and nine negative control samples. The measurement on the rabbit teeth divided into two parts, for the left of the measurement and the right after. Measurement of color brightness is measured qualitatively and quantitatively. Qualitatively, measurements were made by assessing the degree of brightness of the teeth based on the VITA Classical Shade guide with the results shown in Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Before</th>
<th>After</th>
<th>Rate of Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>A₁</td>
<td>A₁</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>C₁</td>
<td>C₁</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>C₂</td>
<td>C₂</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>C₁</td>
<td>C₁</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>D₁</td>
<td>D₁</td>
<td>6</td>
</tr>
<tr>
<td>50% Belimbing Wuluh Gel</td>
<td>C₁</td>
<td>C₁</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>C₂</td>
<td>C₂</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>B₁</td>
<td>B₁</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>B₂</td>
<td>B₂</td>
<td>2</td>
</tr>
<tr>
<td>40% Oxalic Acid Gel</td>
<td>G₁</td>
<td>&gt;B₁</td>
<td>&gt;5</td>
</tr>
<tr>
<td></td>
<td>C₁</td>
<td>&gt;B₁</td>
<td>&gt;5</td>
</tr>
<tr>
<td></td>
<td>D₁</td>
<td>&gt;B₁</td>
<td>&gt;7</td>
</tr>
<tr>
<td></td>
<td>B₁</td>
<td>&gt;B₁</td>
<td>&gt;2</td>
</tr>
</tbody>
</table>

Table 1. Qualitative measurement results
The results and measurements contained in Table 1 were compared after exposure, then the level of change is seen. In the group exposed to the belimbing wuluh extract gel, the rate of change ranged from 1-2 (VITA Classical Shade Guide).

Quantitative research data showed a homogeneous and varied color change in the specimens applied with 50% belimbing wuluh extract gel and a non-homogeneous color change in the 40% oxalic acid gel application group. The varied color changes that occurred in the specimens applied with 50% belimbing wuluh extract gel were thought to be related to the thickness of the enamel layer and the age of the rabbits.

Table 2 shows the mean increase in the degree of brightness of the teeth affected by the type of gel exposed. The group exposed to 40% oxalic acid gel had the highest growth in tooth color brightness, namely 4.61 and the group with low values were negative control with a value of 0.00. In Figure 2, it is clear that the increase in the degree of color brightness of each treatment group is influenced by the type of gel exposed.

Colour change data was tested for normality using the Chi-square test; the normality test results listed in the attachment show that the data is normally distributed, followed by the One-way ANOVA parameter test, then the SNK post-hoc test is carried out to determine which variables have significant differences. Table 3 shows the statistical analysis data analysis results using the ANOVA test for exposure of 50% belimbing wuluh gel and 40% oxalic acid gel. Based on the tests carried out, the ANOVA test results obtained with a value of p = 0.0030 or p <0.05, it can be concluded that there is a significant difference in the value of the degree of brightness of the tooth color after treatment. Next is to see which groups differ, using the SNK post-hoc test. The post-hoc test is a further differential test conducted to determine which group has the most significant increase in the degree of brightness of the teeth. The results of the post-hoc test are presented in Table 4.

**Table 1. The mean value of changes in the degree of brightness of the teeth**

<table>
<thead>
<tr>
<th>Specimen Group</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>50% Belimbing Wuluh Gel</td>
<td>9</td>
<td>3.16</td>
<td>3.19</td>
</tr>
<tr>
<td>40% Oxalic Acid Gel</td>
<td>7</td>
<td>4.61</td>
<td>2.89</td>
</tr>
<tr>
<td>Negative Control</td>
<td>9</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>
Based on Table 4 above, it can be seen that there are differences in specimens exposed to 50% belimbing wuluh gel and negative control (sig. <0.05).

**Table 4.** The difference in the degree of brightness of the teeth

<table>
<thead>
<tr>
<th>Group (I)</th>
<th>Group (J)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Belimbing</td>
<td>Oxalic Acid</td>
<td>0.3652*</td>
</tr>
<tr>
<td></td>
<td>Negative Control</td>
<td>0.0089*</td>
</tr>
<tr>
<td>Oxalic Acid</td>
<td>Belimbing</td>
<td>0.3652*</td>
</tr>
<tr>
<td></td>
<td>Negative Control</td>
<td>0.0003*</td>
</tr>
<tr>
<td>Negative Control</td>
<td>Belimbing</td>
<td>0.0089*</td>
</tr>
<tr>
<td></td>
<td>Oxalic Acid</td>
<td>0.0003*</td>
</tr>
</tbody>
</table>

Note: Post-hoc SNK test * p <0.05 = Significant

**DISCUSSION**

The group exposed to the oxalic acid gel could not be assessed after exposure because the appearance that appeared on the teeth was opaque white. At the same time, the brightest colour of the VITA Classical Shade Guide was B1 and did not match the opaque colour that appeared on the labial surface where the oxalic acid gel was applied—grouped these teeth. The negative control group did not show any changes because they were not given treatment. From the results of these qualitative measurements, it can be concluded that 50% belimbing wuluh extract gel can increase the degree of tooth color brightness in vivo.¹⁶

The teeth used in this study came from New Zealand rabbits, so that there were different variations in enamel thickness. The thicker the tooth enamel, the smaller the ability of the 50% belimbing wuluh extract gel to carry out the whitening reaction; this is because belimbing wuluh has a large molecular size so that it is unable to penetrate the enamel and dentin matrix. The process occurs due to differences in the thickness of the enamel. Figure 1 shows the clinical picture of each treatment sample of 50% belimbing wuluh extract gel and 40% oxalic acid gel. Sample number 1 shows a homogeneous color change, while in sample number 2, there is a color change that is not homogeneous. Quantitatively, the teeth were measured by taking pictures using a camera assisted by a ring light then reading the images analysed using MATLAB. The value of the degree of color brightness in each sample was obtained. The test results of 25 rabbit teeth samples after exposure to each gel for 14 days, because there was a drop out in 2 rabbits applied by 40% oxalic acid gel because they died due to illness. Meanwhile, if the rabbit is sick, it will be difficult to recover and die. Then based on the data obtained, the change in the value of the degree of brightness of the tooth color after treatment. The data obtained based on samples of rabbit teeth were divided into three groups, namely groups of exposure to 50% belimbing wuluh gel, 40% oxalic acid gel and negative control; the average value of the degree of brightness of the teeth in Table 2 was obtained.¹⁷-²¹

There was no statistically significant difference in samples exposed to 50% wuluh belimbing wuluh gel and 40% oxalic acid gel because it was a positive control. There was also a difference in specimens
exposed to 40% oxalic acid gel with negative control because the p-value = 0.0003. Based on the table above, it can be concluded that the belimbing wuluh extract gel affects increasing the degree of brightness of the teeth in an in vivo study of rabbit teeth. Research conducted on the teeth of New Zealand rabbits in vivo, but when viewed on the thickness of the enamel on the incisors, the enamel thickness varied.\textsuperscript{22,23}

Big changes (the brightness, degrees of red-green, and degrees of yellow and blue) influence color changes after application. Ascorbic acid also affects the pH measurement results showing that 50% belimbing wuluh extract gel has a pH> 2 and <3. The low pH value of belimbing wuluh extract the gel and below the critical pH of tooth enamel (<5.5) causes the possibility of increasing enamel surface roughness and dissolving calcium in the enamel after gel application conditions, the acid ion will bind to the phosphate group in the mineral. Hydroxyapatite, so that there will be an imbalance and there will be demineralisation.\textsuperscript{24-26}

Demineralisation appeared to be greater in the group of applied 40% oxalic acid gel specimens, marked by a color change that was not homogeneous, such as the appearance of white spots on the enamel surface. The process of dissolving hydroxyapatite causes lesions in the form of white spots or white spots to occur due to demineralisation, which is not balanced with the remineralisation process. This clinical white appearance is an optical phenomenon caused by the loss of mineral content on the surface and subsurface of the enamel.\textsuperscript{1,7}

CONCLUSION
Based on the results of data processing and discussion, the conclusion obtained from this study is that 50% belimbing wuluh extract gel affects in increasing the degree of brightness of the teeth in an in vivo study, namely the teeth of New Zealand rabbits.

CONFLICT OF INTEREST
The authors reported no potential conflict of interest.

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