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Research Article

The effect of *Averrhoa bilimbi* L. gel as a natural teeth whitening ingredient on the hardness of tooth email: study experimental

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ABSTRACT

Introduction: *Averrhoa bilimbi* L, known for its oxalic acid and peroxide content, may serve as a natural alternative to synthetic whitening agents. Synthetic teeth-whitening agents, such as carbamide peroxide, are effective but often cause adverse effects like enamel demineralization and sensitivity. This study aimed to evaluate the effect of 50% *Averrhoa bilimbi* L gel on enamel hardness compared to a synthetic whitening agent and a negative control. **Methods:** This experimental laboratory study utilized 36 teeth from 18 New Zealand rabbits (*Oryctolagus cuniculus*). *Averrhoa bilimbi* L extract was obtained via maceration with 96% ethanol and formulated into a gel. Eighteen New Zealand rabbits (36 teeth) were divided into three groups: negative control, 10 % carbamide peroxide, and 50% *Averrhoa bilimbi* gel. Each group underwent a 4-hour daily treatment for 14 days, following which enamel hardness was evaluated using the Vickers hardness test. Data were analyzed using ANOVA ($p < 0.05$). **Results:** There were no significant differences in enamel hardness among the three groups ($p = 0.424$), indicating that *Averrhoa bilimbi* L gel had comparable effects on enamel hardness to those of carbamide peroxide and the negative control. **Conclusions:** 50% *Averrhoa bilimbi* L gel is a safe natural alternative for teeth whitening, as it does not significantly affect enamel hardness. Further research is recommended to explore its effects on soft tissues and overall dental safety.

KEY WORDS: *Averrhoa bilimbi* L, hardness, whitening

Pengaruh gel *Averrhoa bilimbi* L. sebagai bahan pemutih gigi alami terhadap kekerasan email gigi: studi eksperimental

ABSTRAK

Pendahuluan: *Averrhoa bilimbi* L, yang dikenal karena kandungan asam oksalat dan peroksidanya, dapat menjadi alternatif alami pengganti bahan pemutih gigi sintetis. Bahan pemutih gigi sintetis, seperti karbamid peroksida, efektif tetapi sering menimbulkan efek samping seperti demineralisasi email gigi dan sensitivitas. Penelitian ini bertujuan untuk mengevaluasi efek gel *Averrhoa bilimbi* L 50% terhadap kekerasan email gigi dan membandingkannya dengan bahan pemutih sintetis serta kontrol negatif. **Metode:** Studi laboratorium eksperimental ini menggunakan 36 gigi dari 18 kelinci New Zealand (*Oryctolagus cuniculus*), ekstrak *Averrhoa bilimbi* L diperoleh melalui maserasi dengan etanol 96% dan diformulasikan menjadi gel. Delapan belas kelinci New Zealand (36 gigi) dibagi menjadi tiga kelompok: kontrol negatif, karbamid peroksida (10%), dan gel *Averrhoa bilimbi* 50%. Setiap kelompok menjalani perlakuan harian selama 4 jam selama 14 hari, setelah itu kekerasan email dievaluasi menggunakan uji kekerasan Vickers. Data dianalisis menggunakan ANOVA ($p < 0,05$). **Hasil:** tidak ada perbedaan signifikan dalam kekerasan enamel gigi di antara ketiga kelompok ($p = 0,4240$), yang menunjukkan bahwa gel *Averrhoa bilimbi* memiliki efek yang sebanding dengan karbamid peroksida dan kontrol negatif. **Simpulan:** Gel *Averrhoa bilimbi* dengan konsentrasi 50% merupakan alternatif alami yang aman untuk pemutih gigi, karena tidak memengaruhi kekerasan enamel secara signifikan. Penelitian lebih lanjut disarankan untuk mengeksplorasi efeknya pada jaringan lunak dan keseluruhan keamanan gigi.

KATA KUNCI: *averrhoa bilimbi*, kekerasan, pemutih

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INTRODUCTION

Teeth are the most important part of a person's facial aesthetics, especially when smiling to increase self-confidence. The patient desires whiter teeth, because it can increase the patient's confidence. Teeth whitening is one of the treatments needed for aesthetic care.¹ One of the problems affecting aesthetic function is discoloration which can have a psychological impact on the affected individuals.² For patients with tooth discoloration, especially in the anterior teeth, it can significantly impact their appearance and cause concern.³ Discoloration is one of the consequences of aesthetic problems and will motivate patients to plan dental treatment.⁴ Discoloration can be divided into two types, namely extrinsic and intrinsic.⁵ Treatments for patients with tooth discoloration include making the front side of the tooth (veneering), making crowns and teeth whitening or bleaching.⁶ In this modern era, teeth whitening is very important for society. One of the treatments that has become the most popular choice for people to perform aesthetic treatments is teeth whitening or bleaching.⁷ Teeth whitening is a treatment that removes stains using chemical oxidizing agents, which can be applied either at home (home bleaching) or in a dental clinic (in-office bleaching).^{8,9}

Several studies have also proven natural teeth whitening ingredients that can be used as an alternative to bleaching such as apples.³ Apart from apples and strawberries, *Averrhoa bilimbi* L. can serve as an alternative for dental bleaching due to its content of carboxylic compounds, including oxalic acid and peroxide compounds, which help whiten discolored teeth, making it a potential natural dental bleaching agent.¹⁰ *Averrhoa bilimbi* L. has many ingredients that can be used for health herbal medicines such as coughs, rheumatism, diabetes, aphthous ulcers, bleeding gums, cavities, and improve digestive function.¹¹ Previous research stated that there was a change in tooth color after 50 gram of *Averrhoa bilimbi* L extract and 10% carbamide peroxide were applied.¹⁰ However, its high acidity level (pH 4.7) can cause the pH of the oral cavity to become critical.¹² If our oral cavity is becomes acidic and has a pH below 5.5 it will result in the dissolution of enamel minerals or what can be called demineralization and if it occurs continuously it will cause damage to tooth enamel tissue.¹³

Averrhoa bilimbi L gel at a 50% concentration has been shown to increase the brightness of tooth color in rabbit teeth. However, no studies have yet examined its effect on enamel hardness following the application of *Averrhoa bilimbi* L gel.¹⁴ *Averrhoa bilimbi* L has a pH of 4.7 but contains high levels of calcium and phosphate, which are essential for repairing hydroxyapatite and promoting tooth remineralization.¹⁵

These minerals make *Averrhoa bilimbi* L a potential natural dental whitening agent. However, its acidity can lower the oral cavity's pH which can lead to demineralization of teeth. So, the authors are interested in researching *Averrhoa bilimbi* L. gel as a tooth whitening agent using rabbit teeth as research samples. Rabbit teeth were selected due to their suitable size that the difference is visible and the extraction method is easy. The novelty of this research lies in the use of *Averrhoa bilimbi* L. gel as a natural teeth-whitening agent and its effect on enamel hardness. This study aimed to analyze the effect of *Averrhoa bilimbi* gel as a natural teeth-whitening agent on enamel hardness compared to 10% carbamide peroxide and a negative control.

METHODS

The design of this study was an analytical in vivo laboratory experimental study with the focus to determine the effect of *Averrhoa bilimbi* L extract as an alternative material for teeth whitening on the hardness of tooth enamel in vivo in rabbit teeth. The object of this research is using rabbits (*Oryctolagus cuniculus*) obtained from Biofarma for animal development. The inclusion criteria for New Zealand rabbits in this study required healthy rabbits with no pre-existing oral or systemic health conditions, intact and structurally normal teeth free from cracks or caries, and an age and weight range (2–3 kg) ensuring physiological uniformity. Rabbits had to be tolerant of handling and able to undergo daily gel application for 14 days. Exclusion criteria disqualified rabbits with oral diseases (e.g., enamel erosion or periodontal issues), systemic conditions, recent dental treatments, behavioral issues such as excessive stress or aggression.

This research utilized *Averrhoa bilimbi* L processed at a temperature below 100°C to preserve its oxalic acid content, as higher temperatures may degrade this compound. The extract was then concentrated by evaporating the water content until it thickened. After concentration, the extract was formulated into a gel, using Hydroxypropyl Methylcellulose (HPMC) as the gelling agent. HPMC was used at an 80% concentration to provide the necessary viscosity and stability. After that, a personal impression tray/custom tray was made from Shellac to accommodate 50% *Averrhoa bilimbi* L gel in contact with the

labial part of the maxillary incisor. First, a maxillary impression of the rabbit's teeth was taken using alginate, then a plaster/gypsum working model was prepared and shaped, red wax/base plate was placed on the labial surface of the maxillary incisor to maintain a 4 mm thick space for 50% *Averrhoa bilimbi* L gel, after that it was molded. back to using alginate and make a plaster model again.¹⁶

A layer of 50% *Averrhoa bilimbi* L gel was placed on the labial surface of the maxillary incisors, then the personal impression spoon was filled with 50% *Averrhoa bilimbi* L gel covering the labial surface of the teeth thoroughly. The application of *Averrhoa bilimbi* L gel lasted for 4 hours daily and was repeated for 14 days. Before changing the gel, the teeth were brushed using fluoride-free toothpaste for 3 minutes and rinsed using distilled water and repeated for 14 days. After the above procedure the rabbit's front teeth will be extracted then sterilized and stored in deionized water.¹⁶ Rabbits are exterminated using a scalpel and blade. The incisors were moved using an excavator and then removed using modified pliers in the form of a hemostat. After the tooth socket was extracted, it was emphasized using a tampon for 1 minute until the bleeding stopped.^{17,18} The enamel hardness was measured using the Micro Vickers Hardness Tester on a diamond pyramid with a dihedral angle of 136 degrees compressed with an "F" force on the surface of the material.¹⁹ (Figure 1).



Figure 1. Micro Vickers Hardness Tester

The results of the diagonal length measurement are then taken on average and entered into the formula. The statistical test used was one-way ANOVA, which was used to test the average comparison between several data groups to test the effect of exposure to *Averrhoa bilimbi* L extract gel, 10% carbamide peroxide exposure group and control group on tooth enamel hardness.

After confirming the distribution of normal data and homogeneity of variance, the data were analyzed using a parametric test, specifically ANOVA. If the p-value is less than 0.05, it indicates that there is a significant difference between at least two of the groups. The analysis will be conducted using the Student-Newmann-Keuls (SNK) test). Data analysis was performed using the independent t-test to test for significance between groups. The results of the normality and homogeneity tests indicated that the data were normal and homogeneous, so the analysis continued with an ANOVA test..

RESULTS

The results of the study provide a comparison of enamel hardness among the three treatment groups: 50% *Averrhoa bilimbi* L. gel, 10% carbamide peroxide, and the negative control. The data, as presented in Table 1, illustrate the effects of each treatment on tooth enamel hardness.

Table 1. Description of email hardness in A *Averrhoa bilimbi* L gel, carbamide peroxide, and negative control

Treatment	n	Std. Def	Average
<i>Averrhoa bilimbi</i> L	9	9.85	56.3
Carbamide Peroxide	9	9.24	50.4
Negative Control	9	10.05	52.1

The treatment with 50% *Averrhoa bilimbi* L. gel on a sample of 9 rabbit teeth resulted in an average hardness of 56.3 VHN. In comparison, the carbamide peroxide treatment on 9 rabbit teeth produced an average hardness of 50.4 VHN, while the negative control group, also with 9 rabbit teeth, showed an average hardness of 52.1 VHN. The results of the normality and homogeneity tests indicated that the

data were normal and homogeneous, so the analysis continued with an ANOVA test. The results of the normality and homogeneity tests indicated that the data were normal and homogeneous, so the analysis continued with an ANOVA test.

To assess changes in enamel hardness following the application of 50% *Averrhoa bilimbi* L. gel, a one-way ANOVA test was conducted to compare the average values across the treatment groups: 50% *Averrhoa bilimbi* L. gel, 10% carbamide peroxide, and the negative control. The results of the one-way ANOVA test are presented in Table 2.

Table 2. The effect of *Averrhoa bilimbi* L gel on enamel hardness compared to 10% carbamide peroxide and a negative control

	N	Square mean	F	p-value
Treatment	9	84.053	0.89	0.4240
Total	26			

One-way ANOVA test, *p < 0.05 significant

From Table 2, the p-value of 0.4240 (> 0.05) indicates that the test is statistically non-significant. This demonstrates that all three treatments had a similar effect on enamel hardness, as reflected in their average values.

DISCUSSION

The findings of this study highlight the potential differences in the impact of bleaching agents on enamel hardness, particularly between natural and synthetic formulations. Vasconcelos *et al.*,²⁰ reported that the hardness of teeth that had been bleached by Carbamide peroxide showed results that were not much different in the conditions before and after bleaching. In the research of Carvalho *et al.*,²¹ it was found that bleaching materials, one of which is carbamide peroxide, can reduce enamel hardness, with the lowest percentage causing a decrease observed at a concentration of 20%. This indicated that higher concentrations, such as 20%, may result in substantial demineralization. This is attributed to the disruption of the enamel's organic and inorganic matrix, causing the release of carbon dioxide and water, which weakens the hydroxyapatite structure.

These results align with the current study, which showed that even at a lower concentration (10%), carbamide peroxide caused greater enamel demineralization compared to *Averrhoa bilimbi* L gel. The importance of this comparison lies in the growing demand for safer alternatives to synthetic whitening agents.²¹ The dental matrix is organic and inorganic until only carbon dioxide and water remain. While enamel remineralization is expected to occur through the action of saliva during bleaching, several *in situ* studies have reported a decrease in the hardness of bleached enamel. Reduced tooth hardness can be associated with loss of mineral content due to demineralization. Therefore, microhardness tests are often applied to evaluate the side effects of bleaching agents on enamel.²⁰

According to Insan,⁷ *Averrhoa bilimbi* L. is widely used as a natural source of sour flavor due to its rich acid content, including oxalic acid. The acidic properties of *Averrhoa bilimbi* L. have the potential to influence enamel integrity, as higher concentrations of the extract can significantly lower the pH of formulations, increasing the risk of demineralization or hydroxyapatite breakdown in tooth enamel. This is consistent with the chemical behavior of acids, which can dissolve calcium and phosphate ions from enamel, weakening its structure.

However, Rahaju¹⁰ also emphasized that the effects of *Averrhoa bilimbi* L. on enamel depend on its concentration. While high concentrations may cause enamel damage, low to moderate concentrations, such as the 50% concentration used in this study, appear to strike a balance that minimizes these adverse effects. This study further supports this claim, as the results showed no significant difference in enamel hardness between the 50% *Averrhoa bilimbi* L. gel, the 10% carbamide peroxide, and the negative control groups.

Additionally, the findings suggest that the natural components in *Averrhoa bilimbi* L., including antioxidants and other bioactive compounds, may contribute to its protective effects. These compounds could potentially neutralize free radicals and mitigate oxidative stress, which are known to play a role in enamel degradation. This could explain why, despite its acidic nature, the 50% *Averrhoa bilimbi* L. gel did not cause notable enamel demineralization.

Moreover, the results highlight an important distinction between natural and synthetic whitening agents. Carbamide peroxide, a commonly used whitening agent, can lead to enamel surface alterations with prolonged or excessive use due to its oxidative properties. In contrast, the 50% *Averrhoa bilimbi* L. gel provided comparable whitening effects without significantly affecting enamel hardness, suggesting that it may be a gentler alternative for long-term use.

Furthermore, saliva plays a critical role in mitigating enamel hardness reduction, as emphasized by Farooq et al.,¹⁰ saliva contains key minerals such as calcium, phosphate, and fluoride, which not only buffer acidic conditions but also promote the natural remineralization of enamel. In this study, the statistically non-significant differences between treatments may indicate that saliva effectively neutralized the potential enamel damage caused by both carbamide peroxide and *Averrhoa bilimbi* L gel. This underscores the importance of natural oral defenses in maintaining enamel integrity during whitening treatments. The study used *Averrhoa bilimbi* L gel with a concentration of 50%, carbamide peroxide 10% and the negative control showed that the results were statistically non-significant which means that there was no significant difference between the three treatments.

The findings also have significant implications for clinical practice. *Averrhoa bilimbi* L gel presents a natural and safer alternative to carbamide peroxide, especially for individuals seeking whitening treatments without compromising enamel hardness. Its high mineral content and remineralizing properties make it a promising option for integrating into dental care routines. However, while *Averrhoa bilimbi* L gel showed reduced enamel damage, optimizing its pH and concentration could further enhance its safety and effectiveness. Future formulations might explore combining *Averrhoa bilimbi* L extract with buffering agents or additional remineralizing components to improve its clinical application.

Carbamide peroxide causes greater enamel demineralization compared to the use of *Averrhoa bilimbi* L gel. This study proves that *Averrhoa bilimbi* L gel with a concentration of 50% is safer to use when compared to carbamide peroxide and does not cause major changes in the hardness of rabbit teeth. Research by Triputra Abidin et al.,¹⁵ indicates that although *Averrhoa bilimbi* L has a pH of 4.7, it has a high calcium and phosphate content which is important for repairing hydroxyapatite and can stimulate remineralization of teeth.

Despite its slightly acidic nature, *Averrhoa bilimbi* L gel proved to be less detrimental to enamel hardness. This can be attributed to its high calcium and phosphate content, as noted by Abidin et al.,¹⁵ which may offset the potential demineralization associated with its acidity. These minerals are essential for repairing hydroxyapatite and stimulating enamel remineralization. Therefore, the dual mechanism of action—gentle demineralization counterbalanced by remineralization—positions *Averrhoa bilimbi* L gel as a safer and effective alternative.

This proves that *Averrhoa bilimbi* L does not cause tooth decay but instead has offers beneficial effects in low concentrations. Overall, this study highlights the need for further research into natural teeth-whitening agents that prioritize both efficacy and safety. Long-term studies and clinical trials are essential to validate the use of *Averrhoa bilimbi* L gel in humans and to explore its potential synergistic effects when combined with other remineralizing agents.

By combining the dual benefits of whitening and enamel protection, natural alternatives like *Averrhoa bilimbi* L gel can revolutionize teeth-whitening treatments, offering a more sustainable and less invasive solution. The results of this study, which compared 50% *Averrhoa bilimbi* L gel, 10% carbamide peroxide, and the negative control, showed statistically non-significant differences, indicating no substantial enamel damage between the three treatments. This finding holds clinical significance as it suggests that *Averrhoa bilimbi* L gel may serve as a natural and safer alternative to carbamide peroxide for individuals seeking whitening treatments without compromising enamel hardness. Furthermore, the high mineral content and remineralizing properties of *Averrhoa bilimbi* L gel make it a promising option for integration into dental care routines.

CONCLUSION

50% *Averrhoa bilimbi* L. gel does not significantly demineralize tooth enamel hardness when compared to 10% carbamide peroxide and the negative control in this eksperimental laboratory study. This indicates that *Averrhoa bilimbi* L. gel at this concentration is a potentially safer alternative to synthetic whitening agents, as it does not cause notable enamel demineralization. The findings highlight the potential of *Averrhoa bilimbi* L. gel as a natural, gentler teeth-whitening option. Further studies are recommended to investigate its long-term safety, efficacy, and synergistic potential with other natural ingredients to enhance whitening performance and dental health benefits. The findings of this study imply that 50% *Averrhoa bilimbi* L. gel is a safer, natural alternative to synthetic teeth-whitening agents like carbamide peroxide, as it does not significantly demineralize enamel. This suggests that *Averrhoa bilimbi* L. gel could be a viable option for individuals seeking a gentler, more natural approach to teeth whitening.

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