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# The Application of Apple (*Malus sylvestris*) Extract-Based Gel: Effects on Teeth Colour Change and Hardness

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**ABSTRACT**

Manalagi apple extract, which contains malic acid, has the potential to be used as an alternative teeth whitening. Therefore, this study aimed to investigate the effect of Manalagi Apple (*Malus sylvestris*) extract-based gel on teeth colour change and hardness. It included an experimental laboratory with a pre-test, post-test, and control group design. The samples consisted of thirty anterior extracted teeth which were divided into five groups, including negative control, 10% carbamide peroxide, 25%, 50%, and 75% extract. Teeth were discoloured for seven days using a coffee solution, and gel was applied for 8 hours a day for 14 days. Teeth colour change was assessed using colourimeter device, while hardness was measured using a micro Vicker hardness tester. The data analysis was carried out using the Kruskal-Wallis test followed by Mann Whitney test, and the t-dependent test. The result showed that there was a significant change in teeth colour after treatment with 75% apple extract gel compared to the negative control and carbamide peroxide 10%. The extracted gel significantly increased teeth hardness value. These results showed that apple extract-based gel improves teeth colour without negatively affecting the hardness, suggesting that apple extract-based gel can potentially be used as a dental whitening agent.

**Keywords:** Discolouration, Teeth Colour Change, Tooth Hardness, Apple Extract Gel

**ABSTRAK**

Ekstrak apel manalagi mengandung asam malat. Dapat digunakan sebagai alternatif bahan pemutih gigi. Tujuan penelitian ini adalah untuk mengetahui pengaruh pengolesan gel ekstrak apel manalagi (*Malus sylvestris*) terhadap perubahan warna dan kekerasan gigi. Penelitian ini termasuk eksperimental laboratoris dengan *pre-test post-testcontrol group design*. Sebanyak 30 gigi anterior yang telah dicabut dibagi ke dalam 5 kelompok (kontrol negatif, karbamid peroksida 10%, perlakuan ekstrak 25 %, 50% dan 75%) digunakan sebagai sampel penelitian ini. Gigi dilakukan diskolorasi selama 7 hari menggunakan larutan kopi. Pengolesan gel dilakukan selama 8 jam perhari selama 14 hari. Perubahan warna gigi diuji menggunakan alat kolorimeter, sedangkan kekerasan gigi diukur dengan alat *micro vicker hardness tester*. Analisis data pada penelitian ini menggunakan uji *Kruskal Wallis* dilanjutkan dengan uji *Mann Whitney* dan uji *t-dependen*. Hasil penelitian menunjukkan bahwa terdapat perubahan warna gigi yang signifikan setelah pengolesan gel ekstrak apel manalagi 75% dibandingkan dengan kontrol negatif dan karbamid peroksida 10%. Selain itu, terdapat peningkatan nilai kekerasan gigi yang signifikan setelah aplikasi gel ekstrak apel. Berdasarkan hasil tersebut dapat disimpulkan bahwa gel ekstrak apel dapat meningkatkan perubahan warna gigi tanpa memberikan efek buruk terhadap kekerasan gigi, sehingga bahan ini berpotensi sebagai alternatif agen pemutih gigi.

**Kata kunci** Diskolorasi, Perubahan Warna Gigi, Kekerasan Gigi, Gel Ekstrak Apel

## 1. Introduction

Aesthetics have recently become a significant concern for many people, particularly regarding the appearance of their teeth. This concern can have a psychological impact, leading to a lack of self-confidence and adversely affecting quality of life.[1,2] Teeth discolouration is an abnormal condition where colour of teeth darkens. Two factors contribute to teeth discolouration, namely the extrinsic factors caused by habits, intake of food and beverages such as tea, coffee, and chocolate, and long-term use of mouthwash; while the intrinsic factors including the use of drugs, dental trauma, non-vital teeth, and dental caries.[3] Teeth discolouration can be addressed through dental bleaching treatments. However, the goal of teeth whitening is to restore the natural colour through a chemical process [3,4]. Currently, there are two primary methods for bleaching, including in-office and home bleaching.[4–6] The in-office bleaching technique performed by professionals in the clinic enables a rapid lightening of teeth color. In contrast, home bleaching is carried out by the patient at home under the observation of a dentist.[4,7]

The use of dental bleaching agents such as carbamide peroxide has some side effects on the soft tissues in the oral cavity, as well as change in teeth structure. Common symptoms include gingival irritation, hypersensitivity reactions, changes in teeth surface roughness, and loss of teeth minerals.[8–10] Carbamide peroxide-containing acidic substances induce teeth demineralization, leading to pores formation on the enamel surface.[11] This eventually allows the bleaching materials to enter the pulp chamberthrough the open dentinal tubules. This process triggers fluid movement in the tubules, which indirectly stimulates pulp nerve endings and transmits sensations of pain or aching to the brain.[11,12] In addition, these chemicals alter the surface of teeth enamel by changing its texture, causing mineral loss, and reducing hardness.[13] Changes in teeth structure can increase enamel surface roughness, facilitating the adhesion of *Streptococcus mutans*, which is the main microorganism causing teeth caries.[14]

Various natural ingredients have been extensively studied for their effects on change teeth colour.[15–17] Apple fruit is an organic material that has the potential to be utilized as teeth-whitening agent, characterized by a relatively elevated concentration of malic acid.[18] Organic acids, including malic acid, used as active substances have an essential role in altering teeth colour and possess the capability to effectively eliminate dental stains.[19] However, the influence of apple extract gel on change of teeth colour and enamel hardness remains undetermined. Therefore, this study aimed to investigate the effect of apple extract gel on colour changes and teeth hardness.

## 2. Materials and Methods

### *2.1 Preparation of Apple Extract Gel*

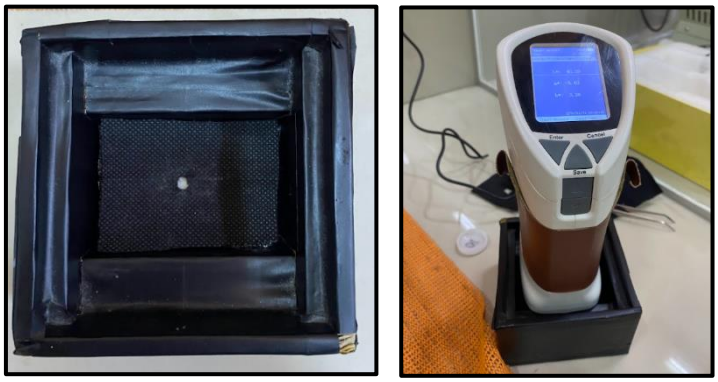
Fresh apple fruits were obtained from Batu, East Java, Indonesia, and apple extract was prepared using a maceration method. A total of 400 grams of Manalagi Apples (*Malus sylvestris*) were washed, chopped and then smoothed. Four litres of ethanol were added and mixed with apple fruits for the first 6 hours. The solution was filtered using cotton and filter paper, then evaporated using a rotavapor at a temperature of 40°C until the crude thick extract was obtained. The crude extract was then mixed with carbomer to make gel based on apple extract and prepared into various concentrations of 75%, 50%, and 25%. The pH of the extracted gel was adjusted to 6.5 by adding triethanolamine.[20]

### 2.2 *Application of Extract Gel*

In this study, all samples selected were the extracted anterior teeth collected from patients at several hospitals and dental clinics in Medan, North Sumatera. The inclusion criteria for teeth were normal morphology and extracted less than 3 months. Teeth with restoration, caries, fracture, non-vital, and any abnormal condition were excluded. Before gel application, teeth underwent a discolouration process. All samples were soaked in a coffee solution at room temperature for 7 days and the liquid was changed daily. Apple extract-based gel with concentrations of 75%, 50% and 25% was then applied on teeth surface. Gel was applied over teeth surface for 8 hours for 14 days. Samples in negative control were treated with gel base without extract, while carbamide peroxide 10% (Opalescence) treated teeth were used as a positive control. After 8 hours of application, gel was washedoff with running water and the sample was stored in artificial saliva at 37°C in the incubator.[21]

*2.3 Measurement of tooth colour change*

Teeth colour was measured before and after apple extract-based gel were applied using colourimeter (AMTAST AMT501, China). Colourimeter was positioned vertically on the surface of the sample. The instrument was held in the opposite direction, 90 degrees from the sample's middle surface, and the button was pressed until a sound came out, showing the measurement was complete (Figure 1).



a b

Figure 1. Teeth were placed on the base of colourimeter device (a). Then the instrument was positioned perpendicular to teeth surface (b).

The evaluation results were showed on the screen in L*a*b* format. The L value shows the brightness of colour in the range of 0 (black) to ± 100 (white). The notation value "a" was a value showing the chromatic colour of the mixture red-green, with colour range red "+a" from 0 to (+100) and the green colour range green "-a" between 0 and (-80). The notable value "b" was the value showing a mixture of blue-yellow colour with colour range of yellow "+b" from zero to (+70) and a blue colour range "-b" of 0 to (-80). The total measurement of the resulting colour change was carried out using the following formula:

$$\Delta E^{*}\text{ab} = \sqrt{(\Delta L^{*})^2 + (\Delta a^{*})^2 + (\Delta b^{*})^2}$$

Description:
ΔL* (L* sample - L* standard) = brightness change
Δa* (a* sample - a* standard) = red-green change
Δb* (b* sample - b* standard) = yellow-blue change
ΔE* = total colour change

*2.4 Tooth hardness assessment*

Before performing the test, teeth were planted in a 1.5cm x 1cm PVC using an inlay wax. The strength of teeth enamel was measured before and after application of apple extract gel using a Micro Vickers Hardness Tester, and the measurement was performed at three points. The result of the diagonal length and weight generated on the device will be automatically showed with the Vickers Hardness Number unit.

*2.5 Ethics*

This study was approved by the Health Research Ethics Committee of Universitas Sumatera Utara with No. 320/KEPK/USU/2023.

*2.6 Statistics*

Data were represented as mean ± SD and analysed using SPSS software. The Kruskal-Wallis test followed by the Mann-Whitney was used to evaluate the difference of teeth colour change among various groups. The dependent t-test was selected to determine the difference in teeth surface hardness before and after gel treatment. A p-value < 0.05 showed a significance of the result.

## 3. Results

According to Figure 2, the study showed that all treated groups exhibited a significant improvement in teeth colour change compared to base gel (negative control). Subsequently, application of 75% apple extract-based gel had the highest value of colour change (7.83 ± 0.53) while gel base had the lowest value of teeth colour alteration (1.68 ± 0.35). Colour change in apple extract of 75% treated teeth was significantly higher than 10% carbamide peroxide, while the 25% extract group showed a significant reduction compared to 10%

carbamide peroxide. Teeth colour change from apple extract of 50% was not significant to 10% carbamide peroxide (positive control), showing the lowest concentration of apple extract that showed a similar effect to positive control.

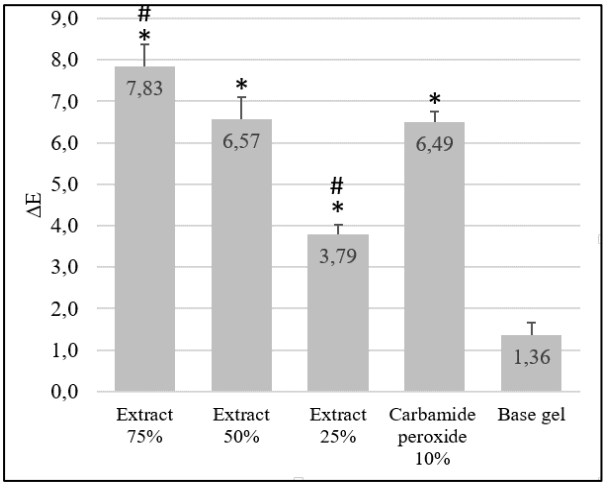



Figure 2. The difference in teeth colour alteration is shown in the graphic above. The improvement of colour change in extract of 75% treated teeth is significantly higher than 10% carbamide peroxide, while extract of 50% showed a significant increase compared to negative control. Thirty extracted anterior teeth used (*) showed a significant difference compared to base gel. (#) denotes the significance of 10% carbamide peroxide.

The results showed that a 75% apple extract-based gel led to a significant increase in average teeth hardness. As shown in Table 1, all treatment groups experienced an increase in tooth surface hardness after application of gel. Subsequently, application of gel base exhibited enhanced teeth hardness. The dependent t-test showed significant differences between the various gel tested. Specifically, the 75% apple extract gel showed a p-value of 0.017 ($p < 0.05$), while the 50% apple extract gel had a p-value of 0.073 ($p > 0.05$), and the 25% apple extract-based gel showed a p-value of 0.150 ($p > 0.05$). Comparatively, the 10% carbamide peroxide gel and gel base have a p-value of 0.000 and 0.002 ($p < 0.05$), respectively. There was a significant difference in teeth hardness value after application among the groups that received 75% extract treatment, positive and negative control ($p < 0.05$). However, no significant difference was observed in the groups that received 50% and 25% extract treatment ($p > 0.05$). All groups exhibited an increase in teeth hardness value after application. In addition, there was no significant difference in the increase of tooth hardness among the groups (Table 2).

Table 1. Tooth hardness value before and after application of apple extract gel

| Groups | Before (VHN) | After (VHN) | ΔVHN | p-value |
|---|---|---|---|---|
| Extract 75% | 367.78±16.18 | 421.59±17.65 | 53.81 ± 30.39 | 0.017* |
| Extract 50% | 381.47 ± 17.7 | 418.41± 19.31 | 36.94 ± 34.08 | 0.073 |
| Extract 25% | 384.93±11.27 | 403.29±14.63 | 18.37 ± 23.10 | 0.150 |
| Carbamide peroxide 10% | 381.29 ±5.7 | 419.84±4.93 | 38.54 ± 1.55 | 0.000* |
| Base gel | 372.04±14.28 | 424.49 ± 7.08 | 52.44 ± 15.75 | 0.002* |

Asterisk (*) shows a significant difference

Table 2. The difference of tooth hardness values in all groups

| Groups | ΔVHN | p-value |
|---|---|---|
| Extract 75% | 53.81 ± 30.39 | |
| Extract 50% | 36.94 ± 34.08 | |
| Extract 25% | 18.37 ± 23.10 | .169 |
| Carbamide peroxide 10% | 38.54 ± 1.55 | |
| Base gel | 52.44 ± 15.75 | |

## 4. Discussion

This study found that gel application of apple extract-based gel concentrations of 75%, 50%, and 25% showed change in teeth colour. This is consistent with a study by Hardini et al., showing that 50% apple extract significantly improved teeth whiteness.[22] The previous study used only one concentration of extract and set the untreated teeth as a control, while this study showed that extract of 25% exhibited a significant increase colour change compared with the base gel. The greatest colour alteration occurred in the 75% extract group, while the lowest was shown in gel base group. The modest colour change observed in gel-base treatment groups can be associated with the absence of teeth-whitening agents in gel base. There was a significant difference between application of 75% apple extract gel and the 10% carbamide peroxide group, where the 75% extract gel experienced a higher increase in colour change compared to the positive control. This suggests that gel with 75% extract is more effective in altering teeth colour than the 10% carbamide peroxide-based gel, which is the most widely used home bleaching material today.

Colour changes in the treatment group can be attributed to the content of malic acid in apples. Malic acid, a group of carboxylic acids, possesses the capability to whiten teeth by neutralizing and oxidizing the surface of teeth enamel, thereby promoting a whitening effect.[22,23] This is consistent with previous study that found gel-based malic acids effectively whitened teeth.[19] The other study also showed that the immersion of teeth in apple water extract resulted in teeth becoming whiter.[24] The 75% apple extract gel application group has the highest colour change value since the 75% concentration contains the most malic acid compared to the other concentrations.

The higher the percentage of extract, the greater the active component contained in gel,[25] leading to an increased degree of colour change as the concentration of apple extract gel rises. Teeth hardness values in all groups improved after applying gel extract, with significant results in the 75% apple extract gel, 10% carbamide peroxide, and gel base groups. The greatest change in hardness value was found in the 75% apple extract gel group, while the lowest value occurred in the 25% apple extract gel group. Malic acid is known to play a role in the process of erosion, as acids bind calcium in teeth enamel, causing porosity and contributing to dental erosion and reduced enamel hardness.[26] However, this study showed an increase in enamel hardness following apple extract gel treatment. This effect could be due to the adjusted pH of apple extract to 6.5, preventing the demineralization process on tooth enamel. Moreover, the elevated teeth hardness value in all groups including in base gel treated samples may come from artificial saliva used as the storage medium after application. Immersion of the samples in artificial saliva is to provide a condition that resembles the oral cavity and replaces some of the functions of natural saliva. The artificial saliva solution contains calcium ions and phosphate ions that support tooth remineralization. The remineralization process occurs when calcium, phosphate, and fluorine ions come into contact with tooth enamel.[27] The longer the immersion, the greater the increase in tooth hardness value, proportional to the duration of application of the remineralization material.[28] Although the base gel was not expected to impact tooth hardness, soaking the samples in artificial saliva after applying the base gel might have influenced the enamel structure.

This study involved an 8 hours gel application time, with samples stored in artificial salivary media for 16 hours. During this period, demineralization occurs, but the remineralization process takes longer. This result is consistent with the other study showing that the calcium and phosphorus content has a significant effect on increasing the surface hardness value of teeth enamel, that is the higher the frequency of exposure of teeth enamel to solutions containing calcium and phosphorus, the greater the increase in teeth enamel hardness.[29] No significant difference was found in change in teeth hardness between the groups after applying apple extract gel. This is likely due to all experimental groups experiencing an increase in teeth hardness as a result of being stored in an artificial saliva solution. According to Anita et al, immersion of teeth in artificial saliva will increase hardness.[30] Although the use of 10% carbamide peroxide did not diminish enamel hardness, apple extract gel performed greater results. Besides the artificial saliva as a storage medium that could augment the hardness, another possible reason might be the distinct pH levels of these two agents. Gel from apple extract was set to a pH of 6.5 the 10% carbamide peroxide had a pH of 5.5. However, the acidic pH (less than 5.5) interferes with enamel structure by loss of minerals through demineralization.[31] In addition, an in vitro study reported a carbamide peroxide-based teeth whitening gel reduced fibroblast viability.[21] While another investigation showed this peroxide-containing agent caused teeth sensitivity or even gingival irritation [32,33], hence an alternative bleaching agent derived from natural plants is a promising approach to reducing the side effects associated with peroxide-based whitening agents.

This study showed the significant variations in teeth discolouration and hardness values following application of apple extract gel at 75%, 50%, and 25% concentrations. Specifically, teeth hardness increased more significantly with the 75% concentration of apple extract gel than with the 10% carbamide peroxide gel. Therefore, the 75% concentration of apple extract-based gel is more appropriate to use because it has a greater hardness value and colour change value than 10% carbamide peroxide gel. However, this study lacks several important evaluations, such as measurements of surface roughness, the toxicity of extract on tooth tissue, and the effects of various gel application times. In this study, the limited teeth samples are one of the rationales to perform all those evaluations. Further study is required to investigate the influence of apple extract-based gel on cell viability, particularly fibroblast from gingiva, to ensure this substance is sufficiently safe for the oral tissue. Other investigations including enamel roughness value and the different time intervals of gel application are also necessary to carry out in the future.

## 5. Conclusion

In conclusion, apple extract-based gel could improve teeth colour change, which was similar to or even greater alteration compared to 10% carbamide peroxide. Moreover, this extract apple did not attenuate enamel hardness value in all concentrations. These results suggested that gel from apple extract can be potentially used as an alternative teeth whitening agent, due to its positive effect on the improvement of teeth colour as well as hardness.

## 6. Acknowledgements

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## 7. Conflict of Interest

The authors declare that there are no conflicts of interest to disclose concerning this study.

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