Effect of cinnamon extract solution on tooth enamel color

J Anggono¹, M Damiyanti¹ and Y K Eriwati*¹

¹Department of Dental Material, Faculty of Dentistry, Universitas Indonesia, Jakarta, 10430, Indonesia

*E-mail: yosiarianto@gmail.com

Abstract. Cinnamon extract solutions can be used as alternatives to standard mouthwash. Indeed, various compounds present in cinnamon, including cinnamaldehyde, eugenol, and tannin, may have antibacterial effects. However, tannin can cause extrinsic tooth discoloration. Therefore, the aim of this study was to identify the effect of cinnamon extract solutions on tooth enamel color. To this end, 12 premolar teeth were immersed in a 4% or 12% cinnamon extract solution (each n = 6) for 60 min, 120 min, and 180 min. Then, the enamel color was evaluated using Vita Easy Shade, and the results were tested with repeated ANOVA and Friedman tests, with post-hoc Wilcoxon tests, to compare enamel color changes between the exposure time groups. Unpaired t-tests and Mann Whitney tests were, then, used to compare enamel color changes between the concentration groups. Greater changes were observed with longer times of immersion and greater cinnamon extract concentration. Regardless, all the samples demonstrated clinically unacceptable color changes (ΔE > 3.5). In addition, the assessment of exposure time groups showed significant changes (p < 0.05), while no significant changes were observed between the concentration groups (p > 0.05). In summary, while the antibacterial compounds found in cinnamon may have benefits for oral health, our findings indicate that cinnamon extract solutions cause clinically unacceptable color changes to tooth enamel.

1. Introduction

Public awareness of the many benefits of maintaining proper oral health is increasing. Accordingly, there has been a rapid development and distribution of numerous oral health products. Commonly used, among these products, is mouthwash, which has many benefits, including refreshing breath, killing bacteria, and preventing gingivitis and plaque formation. Mouthwash is available as a wide selection of basic materials that may be considered primarily chemical or natural in nature. However, as natural-based products typically offer both lower toxicity and alcohol content than chemical-based products, the preference for more natural-based products is increasing.

Indonesia is the second largest exporter of cinnamon after Sri Lanka, based on the data from the Food and Agriculture Organization (2005). Cinnamon (Cinnamomum burmanii Bl.) is a plant widely used in spices, drinks, beauty products, perfumes, as an additional ingredient in betel chewing, and in medicinal herbs to treat canker sores [1,2]. In addition, cinnamon is a natural ingredient that can be used in mouthwash. Cinnamon bark oil contains cinnamaldehyde and eugenol, which have been proven to be effective against fungi and bacteria that cause dental caries and periodontal diseases such as C.albicans, S.mutans, S.salivarius, S.sanguis, S.aureus, A.comitans, P.intermedia, and P.gingivalis [3,4]. In this regard, it has been shown that a 3.12% cinnamon oil solution can inhibit the growth of S. mutans [5].

Although the compounds that make up cinnamon have beneficial effects on oral health, they also have the potential to discolor tooth enamel, which is primarily due to the presence of tannin (Wallis, 1951). Indeed, tannin, which is found in a number of foods and beverages, can lead to external tooth...
discoloration [6]. In this study, we analyzed the effect of cinnamon extract mouthwash on tooth enamel color changes further by immersing tooth samples in different cinnamon solution concentrations for varying exposure times.

2. Methods
This study was a laboratory experimental study performed on tooth specimens immersed in 4% or 12.5% cinnamon extract solutions for 60 min, 120 min, or 180 min. The 4% cinnamon extract solution was chosen for this study since it has been previously reported that a 3.12% cinnamon oil solution was effective at inhibiting the growth of \textit{S}.\textit{mutans} [7]. In addition, a 12.5% cinnamon oil solution has been shown to inhibit the growth of some bacteria and fungi that cause oral infections, including \textit{S}.\textit{mutans}, \textit{S}.\textit{aureus}, \textit{C}.\textit{albicans}, and \textit{C}.\textit{galbrata} [5]. Sample immersion times were adapted from previous \textit{in vivo} studies showing a significant reduction in the number of \textit{S}.\textit{mutans} with certain exposure times. In a previous study, subjects were instructed to gargle with the cinnamon extract solution for 1 min. Our study used 60 min, 120 min, and 180 min exposure times, which were representative of gargling regularly for two times a day for 30 days, 60 days, or 90 days, respectively, to definitively observe any significant changes in enamel color caused by the cinnamon solution.

The Federer formula was used to calculate the sample size of this study. There were two groups of concentrations (4% and 12.5%) and four groups of exposure times (before treatment, 60 min, 120 min, and 180 min). Thus, there were 8 groups in this experimental study. The control group represented the same specimen stored in a saline solution before treatment. Based on the Federer formula, four samples were needed for each concentration group; however, to ensure the validity of our results, we used six samples for each concentration. The samples used in this study were 12 newly extracted human premolar teeth without abnormalities, including discoloration and dental caries. Samples were stored in a saline solution before the study began.

To prevent the penetration of the cinnamon extract solution into the specimen dentin tubules, the apical foramen of the samples was smeared with colored varnish before treatment. Each specimen was stored in a plastic tube and was numbered according to the treatment group.

Initial data was taken using the Vita Easy Shade scale, which includes the lightness value (brightness), \(a^*\) (red-green range), \(b^*\) (blue-yellow range), and the value of the Classical Shade Guide and 3D Tooth Master. The data collection was performed three times for each specimen and then was averaged.

Cinnamon extract solutions were prepared by extracting the cinnamon using the maceration method and modifying it into a solid form at Balai Penelitian Tanaman Rempah dan Obat (BALITTRO). A pH meter was used to measure the solution degree of acidity (pH) for each concentration. The solutions were stored in dark glass bottles at room temperature with the concentration information labeled on each bottle.

The samples were immersed in the 4% \((n = 6)\) or 12.5% \((n = 6)\) cinnamon extract solution, and then stored in an incubator at 37°C. The data collection was performed after 60 min. Then, the samples were immersed again in the cinnamon extract solution and put back in the incubator for 60 min to obtain 120 min total immersion time. At this time, the color change was measured again. Then, the samples were immersed again in the cinnamon extract solution and put back in the incubator for 60 min to obtain 180 min total immersion time, after which the color change was measured again.

Repeated dependent ANOVA tests were used to compare color changes between the groups, and the non-parametric Friedman and Post-Hoc Wilcoxon tests were used when the data were not normally distributed. Then, the data were analyzed with the unpaired \(t\)-test to compare the color changes between the concentration groups. The non-parametric Mann Whitney test was used when the data were not normally distributed.
3. Results
The study results included changes of $L^*$, $a^*$, $b^*$, the $\Delta E^*$ value, specimen clinical color, and the cinnamon extract solution pH test. Table 1 shows the average color change of the specimens after immersion.

Table 1. The mean color change before and after immersion in cinnamon extract solution.

| Concentration | Before treatment | 60 minutes | 120 minutes | 180 minutes |
|---------------|-----------------|------------|------------|------------|
| $\Delta L^*$  | $\Delta a^*$    | $\Delta b^*$ | $\Delta E^*$ |
| 4% Solution   | 83.45           | 82.43      | 81.77      | 79.8       |
| 12.5% Solution| 81.43           | 81.43      | 76.76      | 77.4       |

Table 2. Significance value of $\Delta L^*$, $\Delta a^*$, $\Delta b^*$ between exposure time groups with the repeated ANOVA test

| Duration | Value of $\Delta L^*$ | Value of $\Delta a^*$ | Value of $\Delta b^*$ | Value of $\Delta E^*$ |
|----------|-----------------------|-----------------------|-----------------------|-----------------------|
| 60 min   | 0.46 0.513 0.121 0.252 | 0.04 0.248 0.045 0.177 |
| 120 min  | 0.149 0.114 0.021 0.051 | 0.03 0.028 0.028 0.016 |
| 180 min  | 0.164 0.251 0.014 0.017 | 0.085 0.022 0.042 0.076 |

Table 3. Significance value of $\Delta L^*$, $\Delta a^*$, $\Delta b^*$ dan $\Delta E^*$ between the concentration groups with the unpaired t-test

| Concentration | 60 min | 120 min | 180 min |
|---------------|--------|---------|---------|
| 4% dan 12.5% solutions* | 0.584 0.69 0.849 |
| 4% dan 12.5% solution** | 0.937 0.86 0.606 |
| 4% dan 12.5% solution*** | 0.378 0.338 0.314 |
| 4% dan 12.5% solution**** | 0.538 0.664 0.649 |

The result demonstrated an increase in the $L^*$ value after samples were immersed in the cinnamon extract solutions. No significant differences were found between the exposure time groups or the concentration groups from the samples immersed in either the 4% or the 12.5% cinnamon extract solutions ($p > 0.05$) (Table 3).
Table 4. The mean change of $a^*$ and $\Delta a^*$ values before and after immersion in the cinnamon extract solution.

| Treatment                                      | Before Treatment | $\Delta a^*$ 60 min | $\Delta a^*$ 120 min | $\Delta a^*$ 180 min |
|------------------------------------------------|------------------|----------------------|----------------------|----------------------|
| 4% Cinnamon extract solution                   | 0.62             | 1.77                 | 1.15                 | 2.30                 |
| 12.5% Cinnamon extract solution                | $-0.03$          | 1.15                 | 1.18                 | 1.43                 |

$\Delta a^*$ denotes the change in value of $a^*$

Table 5. Mean change of $b^*$ and $\Delta b^*$ values before and after immersion in cinnamon extract solutions.

| Treatment                                      | Before Treatment | $\Delta b^*$ 60 min | $\Delta b^*$ 120 min | $\Delta b^*$ 180 min |
|------------------------------------------------|------------------|----------------------|----------------------|----------------------|
| 4% Cinnamon extract solution                   | 30.38            | 2.17                 | 3.03                 | 3.98                 |
| 12.5% Cinnamon extract solution                | 28.12            | 4.05                 | 4.53                 | 5.72                 |

$\Delta b^*$ denotes the change in the value of $b^*$

Table 6. Average change in $\Delta E^*$ value before and after immersion in the cinnamon extract solution.

| Treatment                                      | $\Delta E^*$ 60 Min | $\Delta E^*$ 120 Min | $\Delta E^*$ 180 Min |
|------------------------------------------------|----------------------|----------------------|----------------------|
| 4% Cinnamon extract solution                   | 3.88                 | 5.01                 | 6.82                 |
| 12.5% Cinnamon extract solution                | 4.73                 | 5.62                 | 7.59                 |

$E^*$ Value (Total Color Change)
Figure 1. The average change in the ΔE* value before and after immersion in the 4% and 12.5% cinnamon extract solutions for 60 min, 120 min, and 180 min

There was an increase in the ΔE* value with the length of exposure for the 4% and the 12.5% cinnamon extract solutions based on the average ΔE* value change before and after immersion. Changes in the ΔE* value when samples were immersed in the 12.5% concentration group represented the highest value in the whole of the exposure group compared with ΔE* value samples immersed in the 4% concentration group. The highest ΔE* value change was 7.59, which was obtained from samples immersed in the 12.5% cinnamon extract solution for 180 min. The smallest ΔE* value was 3.88, which was obtained from specimens immersed in the 4% cinnamon extract solution for 60 min. All of the ΔE values obtained from the two concentrations after immersion for 60 min, 120 min, and 180 min were > 3.5. Thus, we concluded that cinnamon extract solutions result in unacceptable color changes in tooth enamel.

Our data above showed a significant change in the ΔE* value for specimens immersed in the 4% cinnamon extract solution for each exposure time group. The significant difference was seen from the ΔE* value increase. In addition, a significant change in the ΔE* value was shown for specimens immersed in the 12.5% cinnamon extract solution for 60 min and 180 min. However, our results of the unpaired t-test show no significant differences between the 4% and 12.5% cinnamon extract solutions in the 60 min, 120 min, and 180 min exposure time groups (p > 0.05).

The color changes of the tooth enamel in this study were clinically measured using the Vita Classical Shade Guide A1-D4 and the 3D Toothguide Master. Table 7 presents our results from these evaluations.

| Group | No. | Before | Classical Guide | Before | Tooth 3D Master |
|-------|-----|--------|-----------------|--------|-----------------|
|       |     | 60 Min | 120 Min | 180 Min | 60 Min | 120 Min | 180 Min |
| 1.    | A3  | B4     | A3,5         | A3,5   | 2.5 m3         | 3M3    | 3M3    | 3M3    |
| 2.    | A3  | A3,5   | A3,5         | A4 (A3,5)| 2.5 m3        | 3M3    | 3,5M3  | 4M3    |
| 3.    | B3  | B3     | B3           | B3     | 2M3            | 2.5 m3 | 2.5 m3 | 2.5 m3 |
| 4.    | B4  | A3,5   | A3,5         | A3,5 (A4)| 3M3           | 3M3    | 3,5M3  | 3,5M3  |
The pH value of each cinnamon extract solution was measured with a pH meter. The pH of 4% and 12.5% cinnamon extract solution were 5.38 and 5.45 respectively.

4. Discussion

The results of our study indicated that there was a decline in the L* value (lightness), along with an increase in the a* value (red-green) and b* value (blue-yellow) with increased exposure times after samples were immersed in the 4% and 12.5% cinnamon extract solutions for 60 min, 120 min, or 180 min. The decreased L* value showed a darker color change; however, it clinically looks white still because the L* value was still above 50 and approaching 100. The increased a* value reached a positive value or a > 0, indicating there was a color change toward redness. The increased b* value reached a positive value or b > 0, indicating there was a color change toward yellow. The increased a* and b* values were also seen along with an increasing ΔE* value in the longer exposure time group (i.e., 180 min) and the greater concentration group (i.e., 12.5% cinnamon extract solution).

The decreasing L* value shows that the specimen underwent a color change to a darker color, which is consistent with previous research results by Nordbo (1977) stating that specimen immersion in 0.2% tannic acid causes a brownish discoloration of teeth [8]. Cinnamon contains a large amount of tannins (around 10%). As determined from tests performed on BALITRO the cinnamon extract solution used in this study contains approximately 10.4% tannin content. This tannin content can cause a darker discoloration of the tooth samples after treatment. Additionally, Firdausni, Failisnur, and Diza (2011) also stated that the cinnamon color intensity comes from tannins and will increase with immersion time length and temperature during the maceration process to produce a denser color [7]. This is consistent with the decreasing L* value we observed, which makes specimens darker with longer immersion times.

The greater change in the a* value serves to turn the specimen a more reddish color than the initial value. The red color comes from the reddish-brown color of the cinnamon extract solution. In addition, the 24 hour maceration process of the cinnamon extract performed in this study proved to produce a slightly red color that increases with the duration of the maceration process [7]. The greater change in the b* value we observed served to turn the specimen into a more yellowish color than the initial value. This may be due to the physical color properties of tannin, which range from clear yellowish to light brown [9]. In addition, cinnamon (Cinnamomum burmannii Bl.) also contains 55–65% cinnamaldehyde, which has a physical yellowish color. Thus, the content of cinnamaldehyde likely also affects the color of tooth enamel [10].
The changes observed in the L*, a*, and b* values were seen entirely from the calculation of the \( \Delta E^* \) value. In Figure 1 of the \( \Delta E^* \) value, which generally represents the color change, an increased value of \( \Delta E^* \) was observed, along with increasing exposure time and concentration. The statistical test between exposure time groups showed a significant difference for the 4% concentration in all the treatment groups and for the 12.5% concentration between 60 min and 180 min exposure time groups. In addition, the \( \Delta E^* \) value increased along with prolonged exposure times for each concentration. The minimal \( \Delta E^* \) value (3.88) was observed in samples immersed in the 4% concentration for 60 min.

Based on the American Dental Association classification, the color change values (\( \Delta E^* \)) of < 3.5 are clinically acceptable [11]. Therefore, it can be concluded from the results of our study that gargling with a 4% cinnamon extract solution for 1 month provides the most minimal discoloration effect; however, the color change is not clinically acceptable because it has a value above 3.5. Collectively, our findings indicate that cinnamon extract solutions can cause clinically unacceptable enamel discoloration; thus, it is necessary to consider the long-term use of cinnamon extract as an active ingredient in mouthwash.

Clinical color measurements using the Classical Guide and the 3D Toothmaster were used, along with instrumental color measurement. The specimens tended to change color and became darker, as determined from measurements with the Classical Guide and the 3D Toothmaster; however, a significant change was seen in the instrumental color measurement.

The 4% cinnamon extract solution has a pH value of 5.38, while at a concentration of 12.5%, the pH was 5.45. Both concentrations of the cinnamon extract solution have pH values below the enamel critical pH. Cinnamon extract solutions with a pH below the critical pH of enamel can make the chemical components of hydroxyapatite dissolve because of the acidic environment. These changes in the tooth enamel structure can make the enamel surface porous, which can lead to increased deposits of tannins from the cinnamon extract solution. Tannin deposits found from the cinnamon extract solution sediment were seen at both concentrations of samples after immersion for 120 min.

5. Conclusion
The immersion of tooth samples in 4% and 12.5% cinnamon extract solutions caused enamel discoloration, which was observed as decreased L* values that changed the color to become darker, and increased a* and b* values, which changed the color to become more reddish and yellowish, respectively. The overall color change was not clinically acceptable for either solution concentration after immersion for 60 min, and significantly increased along with the exposure time.

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