Effect of tooth immersion duration in Roselle extract solution on discoloration of enamel

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Abstract. Roselle contains anthocyanins, a group of color pigments. This study determined the effect of immersing 10 bovine teeth in roselle extract solution for 15, 30, 45, and 60 min on enamel discoloration. Color was measured using the VITA Easyshade and CIEL*a*b* color scales. Repeated analysis of variance testing showed significant differences in ΔL*, Δa*, Δb*, and ΔE color values between the control and treatment groups (P < 0.05). Therefore, roselle extract solution may cause enamel discoloration.

1. Introduction

Roselle (Hibiscus sabdariffa) extract exhibits many useful properties such as antihypertensive effects and provides various health benefits such as in the liver fever. It also exhibits antimicrobial effects toward a specific type of bacteria. According to Adebisi and Ojokoh [1], Roselle extract may inhibit growth of several types of bacteria. Suwandi concluded that H. sabdariffa extract exhibits antibacterial effects against Streptococcus sanguinis, which can cause gingivitis [2]. Because of these benefits, roselle is being consumed increasingly by many people, especially in the form of tea or brewing water. Roselle tea has an acid-like taste and red color due to anthocyanins, which is a natural coloring agent found in plants and can be used for food coloring [3,4,5].

Regular consumption of a colored substance can cause visible staining on the teeth, which can interfere with a person’s esthetic appearance [7]. Teeth discoloration has become one of the most common reasons for a patient to visit the dentist [6].

Some studies have been performed on enamel discoloration in dentistry. Joiner et al. evaluated pellicle adsorption of black tea and red wine components by measuring the discoloration (ΔE) using a Minolta Chroma Meter [8]. Hydroxyapatite pieces immersed in black tea for 60 min produced a ΔE increase from 0 to 1.25, which increased again to 1.75 after 2 h of immersion. When immersed in red wine, the level of discoloration after 60 min was higher than that obtained when immersed in black tea due to 10 times greater pellicle adsorption. Black tea contains a color substance called tannin, whereas red wine contains anthocyanin [8]. Talib et al. studied discoloration of resin acrylic after immersion in roselle petal extract for 5, 10, and 15 min. Discoloration increased from 1.30 to 1.56 and then to 2.04, although the discoloration was not significant [9].

Previous studies have shown that immersion in solutions containing a color substance for 60 min can cause discoloration, whereas immersion for less than 15 min did not cause significant discoloration.
Because anthocyanin in roselle may lead to enamel discoloration, we studied the effects of exposure to roselle for 15–60 min on teeth.

2. Methods
In this experimental laboratory study, 10 bovine anterior teeth were divided into 4 groups and immersed in roselle extract solution for 15, 30, 45, and 60 min. All bovine teeth were cleaned; the roots and pulps were cut; and the teeth were polished using a polishing machine. The bovine labial enamel surfaces were evaluated.

Roselle petals, in the form of a dry medicine (simplisia) obtained by maceration and polishing stages of dry roselle, were immersed in Aquadest, filtered, and evaporated to produce a thick extract. Then the extract was dissolved in Aquadest to form a 5% solution, pH 2.791.

Color (L*, a*, and b* values) was measured before immersion using the VITA Easyshade spectrophotometer. Then, the specimens were immersed in roselle extract and incubated at 37 °C for 15, 30, 45, and 60 minutes. After immersion, L*, a*, and b* values were measured with the VITA Easyshade spectrophotometer, and discoloration values (ΔE) were calculated using the CIEL*a*b* formula.

Differences in L* (ΔL*), a* (Δa*), b* (Δb*), and discoloration ΔE were analyzed using the Shapiro–Wilk test. Normal distribution was tested with repeated analysis of variance (ANOVA) and the post-hoc pairwise comparisons test with Bonferroni correction. Abnormal distribution was tested with Friedman’s test and post-hoc analysis with the Wilcoxon signed rank test. The significance level was set at P = 0.05.

3. Results
Differences in L* (ΔL*), a* (Δa*), b* (Δb*), and discoloration (ΔE) are shown in Tables 1. Repeated ANOVA showed a difference in Δa*, ΔL*, and ΔE (P < 0.05) after 15, 30, 45, and 60 min of immersion. Post-hoc pairwise comparisons with Bonferroni correction showed a difference in Δa*, ΔL*, and ΔE between all treatment groups (P < 0.05).

The Friedman test showed a difference in Δb* (P < 0.05) after immersion at all time periods. Post-hoc analysis with the Wilcoxon signed rank test showed a difference in Δb* values between all treatment groups (P < 0.05).

| Variable | Immersion Duration (min) | N  | Mean ± SD       |
|----------|-------------------------|----|----------------|
| ΔL       | 15 min                  | 10 | −15.5100 ± 4.43858 |
|          | 30 min                  | 10 | −20.2300 ± 5.18117 |
|          | 45 min                  | 10 | −24.0700 ± 5.17409 |
|          | 60 min                  | 10 | −26.5400 ± 5.17691 |
| Δa       | 15 min                  | 10 | 9.2600 ± 2.95191  |
|          | 30 min                  | 10 | 12.5900 ± 3.54916 |
|          | 45 min                  | 10 | 14.1800 ± 3.45762 |
|          | 60 min                  | 10 | 15.6800 ± 3.48419 |
| Δb       | 15 min                  | 10 | 3.3600 ± 2.17266  |
|          | 30 min                  | 10 | 0.5900 ± 4.18607  |
|          | 45 min                  | 10 | −0.9200 ± 3.71478 |
|          | 60 min                  | 10 | −1.7900 ± 3.53064 |
| ΔE       | 15 min                  | 10 | 18.50824507 ± 5.256093214 |
|          | 30 min                  | 10 | 24.24335085 ± 5.928925304 |
|          | 45 min                  | 10 | 28.22528900 ± 5.952596139 |
|          | 60 min                  | 10 | 31.09443788 ± 6.040505228 |
4. Discussion
Our results showed a significant difference in $\Delta a^*$, $\Delta L^*$, $\Delta b^*$, and $\Delta E$ ($P < 0.05$). The increase in discoloration ($\Delta E$) was directly proportional to the duration of immersion in roselle extract. The longer the immersion, the greater the $\Delta E$ value. All $\Delta E$ values were $>3.3$; hence, the discoloration that occurred could not be accepted clinically [10]. $\Delta L^*$ was decreased (negative value), which means that the color became darker after a longer exposure duration. The $\Delta a^*$ value was positive and increased, indicating that the intensity of the reddish enamel color increased with a longer immersion duration. The $\Delta b^*$ value was both positive and negative in this study. Initially at 30 min, the $\Delta b^*$ value was positive (yellowish color), but the intensity decreased, whereas after 45–60 min of exposure, the $\Delta b^*$ value became negative, indicating that the hue changed to bluish and the intensity increased.

Discoloration ($\Delta E$) not only depended on pigment color type but also was influenced by pH. Pigment ingredients with an acidic pH may cause significant extrinsic discoloration compared with neutral or basic materials. This is because acids not only cause discoloration but also dissolve the hard teeth tissue [11] due to pH being lower than the critical pH of enamel. When exposed to acid, hydroxyapatite (Ca$_{10}$[PO$_4$]$_6$[OH$_2$]) reacts with hydrogen to release Ca$^{2+}$, PO$_4^-$, and OH$^-$ ions. Ca$^{2+}$, which is a divalent cation, reacts with the anthocyanin and produces deposits [12]. The roselle extract solution ($\text{pH } 2.791$) contains color substances, specifically anthocyanin, which causes discoloration ($\Delta E$).

The darker color indicated by $\Delta L^*$ was suspected to be due to anthocyanin deposition on the enamel surface due to the dissolution of the hard teeth tissue [11]. The greater the amount of pigment, the more the light absorption and the less the light reflection; hence, the tooth color became darker [13].

The positive difference in red–green hue ($\Delta a^*$), which resulted in the change of color toward redness, was the result of the reaction of anthocyanin with delphinidin and cyanidin 3-sambubioside-3-sambubioside, which are components of roselle. The two types of anthocyanin have a red or purple color [14,15]. Anthocyanin deposition on the teeth was facilitated by tissue dissolution due to the acidic pH of the roselle extract solution.

Changes in $b^*$ ($\Delta b^*$) values were initially positive (yellowish hue) and then became negative (bluish hue). The yellowish changes in $\Delta b^*$ 30 min previously may be due to the tannin compounds. Tannin is found in almost all plants, and its color is yellow or brown [16]. Besides anthocyanin, roselle extract solution also contains as much as 1.76% tannin. This finding is supported by previous studies that also found tannin in roselle [17,18]. At 45–60 min of exposure, the $b^*$ ($\Delta b^*$) value changed to negative (bluish hue) due to the reaction between tannin and iron (Fe), which can occur even in an in vitro condition [19]. Because roselle also contains a high concentration of Fe [10], Fe will react with tannin and produce a bluish color [19].

5. Conclusion
It can be concluded that teeth immersed in a 5% roselle extract solution ($\text{pH } 2.791$) for 15, 30, 45, and 60 min will showed an increase in enamel discoloration.

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