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Effect of spinach leaf (*Amaranthus hybridus* L.) extract solution and milk on the level of dental discoloration due to coffee

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Abstract. Coffee is a popular drink that can cause teeth discoloration. Milk is high in calcium. Oxalic acid from spinach can react with calcium to form calcium oxalate crystals. We analyzed the level of tooth discoloration due to coffee. Specimens of 24 teeth were divided into a control and three test groups. The test groups were immersed in 10%, 20%, and 30% spinach leaf extract solution plus milk for 60 min and then in coffee for 24 h. The teeth-color change was measured using the VITA Easyshade® device. A significant difference in ΔL* color value was noted in all extract groups, in Δa* of the 10% and 20% groups, and ΔE in the 20% group compared with controls. No group showed a difference in Δb*. There was no significant correlation between extract concentration and ΔL*, Δa*, Δb*, or ΔE. Spinach leaf extract solution and milk can decrease the level of tooth discoloration due to coffee, but there is no significant correlation between extract concentration and the level of tooth discoloration.

1. Introduction

Coffee beverages contain tannin (tannic acid), which is responsible for the brownish discoloration that occurs in the teeth. Tannic acid also decreases the pH of the coffee drink, making it acidic [1]. Acidic conditions will soften the enamel so that it is more susceptible to infiltration of the stain [2].

The color of the tooth is a combination of intrinsic color and extrinsic stains on the tooth surface. Extrinsic stains can come from cigarette smoke, foods, beverages containing tannins (such as coffee, tea, and wine), use of chlorhexidine, or metals, such as lead and iron [3]. Dental discoloration is a considerable aesthetic problem. Maintenance options to treat discoloration are certainly numerous, such as ceramic or composite veneers, bleaching, and artificial crowns (full veneer crown). However, all treatment options are relatively expensive and invasive [4].

A unique phenomenon occurs when people eat spinach (*Amaranthus hybridus* L.). Some have wondered why their teeth feel weird, slightly gritty, or chalky after they eat a fresh spinach salad ("spinach teeth" sensation) [5]. According to Jennifer Moltoni, administrative coordinator of the Department of Mouth, Infection and Immune Disease at the Harvard School of Dental Medicine, this sensation is due to the oxalic acid content of spinach, which reacts with calcium ions from saliva and spinach itself and forms calcium oxalate crystals. Because calcium oxalate is insoluble, it is deposited on the tooth surface.

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The sensation of spinach teeth will be felt more when spinach is chewed while drinking milk, because milk provides additional calcium [6]. Calcium content in saliva is only 1 mmol/L, whereas milk is very high in calcium (approximately 1200 mg/L) [7,8]. Spinach has a very high oxalic acid content [9], with the highest concentration of calcium oxalate occurring in the leaves [10]. Hartini [11] studied five leafy vegetables and found that spinach leaves contained the highest calcium oxalate content (39%). In addition, spinach leaves also have a fairly high-calcium content (99 mg calcium per 100 g spinach) [12].

Under normal and alkaline pH conditions, calcium and oxalic acid bind to form calcium oxalate crystals [13]. When applied to the calcium-containing tooth surface, calcium oxalate closes the dentinal tubule, forming a layer that reduces dentine permeability. A phytocomplex extract of spinach in the form of a 1.5% spray solution has been used topically to prevent solubility of the smear layer and opening of the dentine tubule after periodontal instrumentation. Similar solutions also can be used to prevent dentine hypersensitivity caused by scaling and root planning procedures [14].

We examined the effect of spinach leaf extract and milk on tooth discoloration levels in the hope that a layer of calcium oxalate will form on the enamel surface, such as with the "spinach teeth" phenomenon that will protect the teeth from color change due to tannic acid in coffee. In addition, we also tested the relationship between the increased concentration of spinach leaf extract and discoloration level of the tooth.

2. Methods

2.1. Spinach leaf maceration

Green fresh spinach (5 kg) was washed, spinach leaves were selected, and the trunk was removed. The leaves were then dried to form a simplicia (as much as 500 g) that was ready to be macerated. The simplicia was soaked in Aquadest solvent at a ratio of 1:4 (i.e., 500 g simplicia soaked in 2 L of solvent), stirred for 3 h and then stored for 24 h. The filtrate was removed, filtered with gauze, and placed into a glass bottle. The residue was macerated in the same way again, and the resulting filtrate was accommodated into one and then filtered. The filtrate was evaporated with a rotary evaporator to obtain a viscous extract weighing 54.1 g.

2.2. Phytochemical screening test

Leafy spinach extract samples were tested for calcium, oxalic acid, calcium oxalate, tannin, and water content. Atomic absorption spectrophotometry (AAS) was used to test for calcium, and high-performance liquid chromatography (HPLC) was used for oxalic acid and calcium oxalate. Tannin was tested using spectrophotometry, and water content was tested with gravimetry.

2.3. Specimen preparation

Human premolar teeth that were collected and stored in 0.9% NaCl solution were obtained for study. The other side of the enamel surface (i.e., cementum from the cementoenamel junction to the apical foramen) was coated with clear nail polish to avoid exposure to the experimental material.

2.4. Preparation of spinach leaf extract

Spinach leaf extract solutions were prepared in several concentrations: 10%, 20%, and 30% solutions were prepared by dissolving 10, 20, and 30 g, respectively, of spinach leaf extract in 100 mL of Aquadest. On the basis of the results of the water content test on condensed spinach leaf extract, the weight of viscous extract and Aquadest needed to make the spinach leaf extract solution could be calculated in various concentrations.

2.5. Dental protection with calcium oxalate

Dental specimens were immersed in the three spinach leaf extract solutions, mixed with Ultra brand high-calcium milk (each 1 mL), shaken until homogeneous, and stored for 60 min. The calcium content in Ultra milk was 20% RDA (Recommended Dietary Allowance) (20% daily calcium requirement).

Human daily calcium requirement is approximately 1 g [9]. Mean calcium content in Ultra milk is 0.2 g/125 mL or 1.6 mg/mL.

2.6. Coffee exposur

The specimens were immersed in a coffee solution made from Nescafé Classic coffee (Nestle), Panjang, Lampung) and Aqua (Tirta Investama, DANONE-AQUA, Indonesia) drinking water packed with a ratio of 1 g coffee powder in 10 mL water. The coffee solution was stirred as much as 50 times with a spoon, cooled to room temperature, and pH tested with litmus paper. Teeth were soaked in the coffee solution (up to 4 mL per vial) and incubated at 37 °C for 24 h.

2.7. Dental color change test

Tooth color was tested by the CIE L*a*b* color description using the VITA Easyshade® tool before treatment (L0, a0, and b0) and then after exposure to the coffee solution. All changes in L*, a*, and b* (ΔL*, Δa*, and Δb*) from before and after coffee exposure were recorded. The color change (ΔE*) was calculated by the formula.

2.8. Analysis data method

To analyze the differences in the effect of each concentration of the extract solution (10%, 20%, and 30%), we conducted a comparative test of the color change scores (AE*) using one-way analysis of variance (ANOVA) or the Kruskal–Wallis test (depending on the normality of distribution data). A comparative test was also done one by one to control group using the independent *t*-test or Mann–Whitney/Wilcoxon tests (depending on the normality of the data distribution). The two-tailed Pearson correlation was used to correlate increasing concentrations of the extract used with tooth discoloration. All analyses were performed with the SPSS 17.0 Windows program (SPSS, Inc., Chicago, IL, USA).

3. Results

3.1. Phytochemical screening test results (Table 1)

Table 1. Phytochemical screening test results

Samples	Solvent	Analysis Type	Method	Result	Unit
Spinach Leaf Extract Condensed	Water	Water content	Gravimetry	51.18	%
		Tanin	Spectrophotometer	2.57	%
		Ca	AAS	211.38	mg/100 g
		Ca Oxalate	HPLC	2.43	%
		Oxalic acid		3.46	

3.2. pH test results

pH was tested with litmus paper, and the following results were obtained (Table 2):

- spinach leaf extract 10%, pH 7
- spinach leaf extract 20%, pH 6
- spinach leaf extract 30%, pH 5
- milk, pH 7
- Coffee solution, pH 5 for exposure of extrinsic stain on the teeth

3.3. Teeth-color test results

Table 2. The mean score of color changes

	Control	10%	20%	30%
Average ΔL*	−4.2**	−0.28**	0.71	−1.08**
Average Δa*	3.12	1.48	0.75	1.1
Average Δb*	3.98	4.5	2.03	2.77
Average AE*	7,05	4.8	2.47	3.35

** Negative figures indicate impairment of the initial score.

3.4. Test results statistics

3.4.1. One-way ANOVA and Kruskal–Wallis tests

Because the sample size was <50, the Shapiro–Wilk distribution normality test was used. A significance score of <0.05 indicated that the data were not normally distributed. In the one-way ANOVA and Kruskal–Wallis tests, a significance score <0.05 indicated a significant difference. A summary of the test results with SPSS 17 is shown in Table 3.

Table 3. One-way ANOVA and Kruskal–Wallis normality test results

	ΔL*	Δa*	Δb*	AE*
Normality (Shapiro–Wilk)	Sig. = 0.000 (abnormal)	Sig. = 0.001 (Abnormal)	Sig. = 0.088 (normal)	Sig. = 0.003 (abnormal)
1-Way ANOVA	-	-	Sig. = 0.108 (Not significant)	-
Kruskal–Wallis	Asymp. Sig. = 0.007 (Different meaningful)	Asymp. Sig. = 0.086 (Not unlike meaningful)	-	Asymp. Sig. = 0.057 (Not unlike meaningful)

3.4.2. Independent t-test and Mann–Whitney/Wilcoxon tests

These tests were done to compare one-by-one changes in color independently of each treatment group when compared with controls.

Because the sample size was <50, the Shapiro–Wilk distribution normality test was used. A significance score of <0.05 indicated that the data were not distributed normally. A summary of the test results with SPSS 17 is shown in Table 4.

Table 4. Normality test result data distribution.

	Control vs. 10%	Control vs. 20%	Control vs. 30%
ΔL*	Sig. = 0.000 (Abnormal)	Sig. = 0.000 (Abnormal)	Sig. = 0.000 (Abnormal)
Δa*	Sig. = 0.053 (Normal)	Sig. = 0.019 (Abnormal)	Sig. = 0.020 (Abnormal)

Table 4. *Continue*

Δb*	Sig. = 0.235 (Normal)	Sig. = 0.563 (Normal)	Sig. = 0.033 (Abnormal)
AE*	Sig. = 0.087 (Normal)	Sig. = 0.040 (Abnormal)	Sig. = 0.025 (Abnormal)

For normal distribution data, the independent *t*-test was used. For abnormal distribution data, the Mann–Whitney/Wilcoxon nonparametric tests were used. A significance score of <0.05 indicated a significant difference (Table 5).

Table 5. Results of independent *t*-test and Mann–Whitney/Wilcoxon tests.

	Control vs. 10%	Control vs. 20%	Control vs. 30%
ΔL*	*P* = 0.004 (Significant difference)	*P* = 0.013 (Significant difference)	*P* = 0.030 (Significant difference)
Δa*	*P* = 0.006 (Significant difference)	*P* = 0.020 (Significant difference)	*P* = 0.054 (Not significant)
Δb*	*P* = 0.372 (Not significant)	*P* = 0.147 (Not significant)	*P* = 0.229 (Not significant)
AE*	*P* = 0.137 (Not significant)	*P* = 0.037 (Significant difference)	*P* = 0.054 (Not significant)

3.4.3. Two-tailed Pearson correlation test

A significance score of <0.05 for the two-tailed Pearson correlation test indicated a significant correlation, and the strength of the correlation was checked. A positive score indicated that the variable correlated in the same direction, whereas a negative score indicated that the variables correlated in opposite directions (>0.5 indicated a close correlation, whereas <0.5 indicated a weak correlation; Table 6).

Table 6. Results of two-tailed Pearson correlation test.

	ΔL*	Δa*	Δb*	AE*
Significance	0.060 (not significantly different)	0.420 (not significantly different)	0.081 (not significantly different)	0.187 (not significantly different)
Two- tailed Pearson correlation	0.452	−0.203	−0.422	−0.326

4. Discussion

Tannins (tannic acid) cause extrinsic staining on teeth [15]. Our results indicated that the viscous spinach leaf extract solution also contained tannins, but with a small percentage (2.57%) compared with coffee (range, 19.5%–23.1%) [16]. Thus, tannin content in spinach leaf extract should not have a significant effect on teeth staining, especially when diluted to concentrations of 10%, 20%, and 30%.

Calcium content in the extract was small (only 211.38 mg/100 g, or 0.0021138%), which indicated that the free calcium ions in spinach leaf extract reacted with oxalic acid. Calcium oxalate formed (2.43%) as a result of a reaction of calcium with oxalic acid. Because the oxalic acid content in spinach

leaves was higher than that of calcium ions, there remained free oxalic acid that had not reacted (3.46%). Thus, extracts of spinach leaves still have the potential to form calcium oxalate deposits on the tooth surface when in contact with calcium from milk.

pH testing demonstrated that a 30% spinach leaf extract plus coffee solution was acidic (pH 5). The critical pH for demineralizing enamel hydroxyapatite was 5.5, whereas that for fluoroapatite was 4.5 [17]. An acidic soaking solution can demineralize enamel and allow tannin substances to enter and cause staining. However, even though a 30% spinach leaf extract solution was acidic, its pH rose because it was mixed with milk (pH 7). Calcium is alkaline, and the acid–base reaction of oxalic acid and calcium oxalate resulted in calcium salts, which are neutral (i.e., approximately 6.65–6.75) [18,19]. Calcium oxalate can form in neutral or alkaline pH conditions [13]. Therefore, it was very important that the pH was not acidic.

Tooth discoloration was observed from three aspects. The L* score (brightness level) indicated the degree of black-and-white (0 for black and 100 for white); the a* score indicated degrees of green–red ($a^* < 0$, more green and $a^* > 0$, reddish); and the b* score indicated the blue–yellow degree ($b^* < 0$, more bluish and $b^* > 0$, more yellowish). The squared root sum of each difference in brightness, degree of red–green, and blue–yellow degree was the color change score.

A nonparametric Kruskal–Wallis test showed significant differences in ΔL^* (brightness level) for each treatment group. However, when tested individually against a control, the 10% solution group had a significant difference in ΔL^* and Δa^*, the 20% solution group had a significant difference in ΔL^*, Δa^*, and AE^*, whereas the 30% solution group only had a significant difference in ΔL^*. From this, we concluded that the decrease in tooth brightness (ΔL^*) will be reduced significantly if, before exposure to coffee, the tooth is protected by calcium oxalate on the surface. Otherwise, with 10% and 20% solutions, the degree of redness was also reduced compared with the control group, which was directly exposed to coffee. The overall color change (AE^*) showed a significant difference only with the 20% solution.

Teeth soaked in 20% spinach leaf extract initially showed increased levels of brightness (L*), but with no significant scores (mean $\Delta L^* = 0.71$). However, these results cannot be considered because the score was quite small and insignificant. Despite the slightly increased L* score, the a* and b* scores also increased, which means that the teeth became more reddish and yellowish.

From analysis of the mean discoloration graph, the protection capabilities of calcium oxalate were reduced in the 30% solution compared with the 20% solution, but it was still better than the 10% solution. The greatest tooth discoloration due to extrinsic coffee stains was in the control group, followed in order by the 10%, 30%, and 20% solution groups. Thus, the most optimal concentration to reduce the discoloration effect of coffee staining was soaking with a 20% spinach leaf extract solution.

When the spinach leaf extract solution was mixed with milk and then stored, precipitation of material appeared in the bottom of the vial. The amount of sediment formed was directly proportional to the concentration of the extract; the higher the concentration, the more calcium oxalate precipitates formed. This shows that the calcium content of milk was fairly high (1.6 mg/mL) and the oxalic acid in spinach leaf extract had completely reacted with the calcium ions. The precipitated calcium oxalate that could be seen visually from the vial bottle was transparent, colored slightly whitish. Salt deposits calcium oxalate crystals with a brownish color and was easily cleaned by mechanical means.

Coffee with an acidic pH can demineralize tooth enamel so that materials, such as tannins, can enter into deeper layers of enamel or even to dentine and become deposited there, resulting in a color change [20]. The color change will be permanent and cannot be immediately removed because, when the oral pH becomes neutral again, the enamel will experience remineralization, thus trapping the stain in the tooth enamel. However, when the calcium oxalate crystals are blocked on the tooth surface, the enamel is not exposed directly to the acid of coffee, so it is not demineralized and stains, such as tannins, are simply deposited on the surface of the calcium oxalate and then can be cleaned easily from the tooth surface. The tooth discoloration is not permanent.

Deposition of calcium oxalate on the tooth surface tends to be uneven because calcium oxalate salt crystals must be attached by mechanical retention. Therefore, each surface roughness of tooth enamel

is different, so that deposition of calcium oxalate is uneven. This causes persistence of the gap for coffee to penetrate and contact the enamel surface so that the teeth still experience discoloration. However, in our study, the presence of calcium oxalate due to immersion in spinach leaf extract solution plus milk was able to reduce the effects of coffee discoloration.

Data from Pearson correlation test results showed no significant correlation between the concentration of the extract and the degree of brightness change (ΔL*), changes in the red–green (Δa*) or blue–yellow (Δb*) degrees, or discoloration of the teeth (AE*). Increasing concentrations of the extract did not affect color changes significantly. The 10% spinach leaf extract solution provided a protective effect, but it was not much different from that of the 20% and 30% solutions.

Spinach leaf extract and milk have great potential for preventing tooth discoloration due to extrinsic stains from food and drinks. Calcium oxalate can attach to the tooth surface so that the reaction between oxalic acid and calcium ions should occur on the tooth surface. Minimum incubation time necessary for calcium oxalate was 2 to 17 min. However, crystal size could potentially increase with time. A soaking time of 60 min (adapted to the duration of chewing gum) also ensures that formation of the calcium oxalate reaction was complete. But if less than it was supposed to it should be protected if a reaction has occurred on the enamel surface.

5. Conclusion

Spinach leaf extract solution and milk can form a layer of calcium oxalate on the enamel surface, thereby reducing the level of discoloration of teeth due to coffee. However, there was no significant relationship between increases in spinach leaf extract concentration and level of tooth discoloration due to coffee.

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