EFFECT OF SPINACH (AMARANTHUS HIBRIDUS L.) LEAVES EXTRACT SOLUTION AND MILK TO LEVEL OF TOOTH DISCOLORATION DUE TO COFFEE

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Abstract

Purpose: The present study aimed to analyze the effect of combination of spinach leaves extract solution and milk on the level of tooth discoloration caused by coffee.

Methods: In the present in vitro study, 24 teeth were color-measured with VITA Easyshade® and divided into four groups, which are control group and three other groups that were immersed in spinach leaves extract solution of 10%, 20%, and 30% plus milk for 60 minutes. Then, specimens were then immersed in coffee for 24 hours. Afterwards, the teeth color change was measured.

Results: Kruskal-Wallis test showed significant difference in ΔL* (brightness) among each group. Compared to control group, two-Tailed Independent T-Test and Wilcoxon Test showed significant difference in ΔL*of all extract group, Δa* (degree of green-red color) of group 10% and 20%, Δb* (degree of blue-yellow color) of none group, and ΔE (value of color change) of group 20%. Pearson Correlation Test did not show any significant correlation between extract concentration and ΔL*, Δa*, Δb*, or ΔE.

Conclusion: Within the limitations of the present study, it can be concluded that the combination of Spinach (Amaranthus hybridus L.) leaves extract solution and milk can reduce level of tooth discoloration caused by coffee. However, there is no significant correlation between the increasing concentrations of spinach leaves extract solution and level of tooth discoloration due to coffee.

Keywords: calcium oxalate, coffee, milk, spinach leaves extract, tooth discoloration

Introduction

Coffee is a very popular drink all over the world (1). It can cause teeth discoloration by changing the color of teeth biofilm (2). Coffee is rich with bioactive substances, such as nicotinic acid, trigonelline, quinolinic acid, tannic acid, pyrogallic acid and caffeine (3). Tannin or tannic acid is responsible for making the teeth become darker. Tannin also makes the pH of coffee become acidic (3,4). Acidic condition will weaken the enamel and make it more vulnerable to get infiltrated by stain (2). Caffeine makes people get addicted to coffee so it will become daily habit to consume coffee (5).

Teeth color change is a big esthetic problem. Bright teeth color makes individual feel more confident in public. Treatment options to fix discoloration of teeth are quite varied, such as porcelain or composite veneer, bleaching, and full veneer crown. However, all of the treatment options are relatively expensive and invasive (6).

Color is described in terms of hue, value and chroma (7). Hue is term to differentiate color into red, blue or green (7,8). Value is the brightness of a color from black to white (7). Chroma is saturation level, intensity of a color with same hue, like from pink to dark red (7,8). Tooth color is valued from three aspect described by Commission Internationale d'Eclairage (CIE), the L* (brightness) value where it shows the degree of black-white (0 for black and 100 for white), a* (degree of green-red) where a*<0 means greener and a*>0 is more reddish, and b* (degree of blue-yellow) where b*<0 means more blue and b*>0 is more yellowish (9). The value of color change is generally represented by ΔE, formula to calculate it (10).

There is a unique phenomenon happens when people eat spinach. Some people are wondering why their teeth feel weird, a bit gritty or chalky after they eat spinach salad. It is called spinach teeth sensation (11). This phenomenon is caused by the oxalic acid content in spinach that reacts with calcium ions from saliva and the spinach itself forming calcium oxalate crystal. Since calcium oxalate is insoluble, it deposits on teeth surface. Drinking milk will exaggerate spinach teeth sensation, because milk gives additional calcium (12). Calcium content in saliva is only1 mmol/L, while milk contains higher calcium which is 1200 mg/L (13,14). Milk contains the highest calcium compared to other foods containing calcium like sardines, almond, broccoli or cabbage (15).

Spinach (Amaranthus hybridus L.) contains carbohydrate, fat, vitamin, sodium, calcium, phosphor, zinc, potassium, thiamin, riboflavin, and niacin. Spinach is consisted of 91% water (16), and it contains high amount of oxalate acid content (17). Oxalate is soluble in water (18), and the highest oxalate content is in the leaves (19). Based on research conducted by Hartini on five different vegetables frequently consumed, calcium oxalate content in spinach leaves is the highest, which is 39% (20). Oxalate content varies depending on water content, age of the plant, soil type, climate, and genetic factor (17,19,21). Oxalate in spinach increases at initial growth, decreases as the plant grows older, and reduced by the heat (18,21). Spinach leaves also have high calcium content of 99 mg/100 g (22). The ratio of oxalate acid: calcium in spinach is about 4:1 (18).

In normal and alkalic condition, calcium and oxalate acid will form calcium oxalate crystal (23). Calcium oxalate reaction is acid and base reaction as seen below (18).

Calcium oxalate on tooth can cover dentinal tubules, forming a layer than can reduce dentine permeability. Phytocomplex of spinach 1.5% spray solution has been used topically to prevent dissolving smear layer and exposure of dentinal tubules after periodontal instrumentation. The similar solution can also be used to prevent dentine hypersensitivity due to scaling and root planing procedure (24).

The present study aimed to evaluate the effect of spinach leaves extract solution and milk on level of tooth discoloration caused by coffee. It was hypothesized that oxalate film will be formed on enamel surface, similar to the spinach teeth phenomenon, which will act as a protection from tannic acid of coffee that darken the teeth. Furthermore, a secondary aim of this study was to assess correlation between concentration of spinach leaves extract solution and level of tooth discoloration in order to know the optimal concentration for the application.

Materials and Methods

Spinach Leaves Maceration: 5 kg of fresh green spinach was cleaned and sorted only for its leaves. Spinach leaves were then dried into 500 g simplicia and ready to be macerated. Maceration was done by putting simplicia into a pot immersed in aquadest solvent with comparison of 1:4 (500 g of simplicia in 2 liters of solvent). The simplicia was then stirred for 3 hours and left for 24 hours. Filtrate was then filtered with lint and put into a glass pot. Afterwards, the residue was re-macerated with the same method once more. The filtrate was collected and filtered. The solvent was vapored with rotavapor until becoming the dense extract (54.1 g).

Phytochemical Screening Test: Spinach leaves dense extract sample was examined for its calcium, oxalate acid, calcium oxalate, tannin, and water content. Technique used to examine calcium content was AAS (Atomic Absorption Spectrophotometry), for oxalic acid and calcium oxalate was HPLC (High-Performance Liquid Chromatography), for tannin was spectrophotometry, and for water content was using gravimetry method.

Specimen Preparation: Freshly extracted human premolar teeth were collected and kept in NaCl solution 0.9%. All surfaces besides enamel were covered by nail paint so that they would not get any contact with the materials.

Spinach Leaves Extract Solution Making: Spinach leaves extract was made into varying concentration by weighing the extract and water using digital weighing-machine. 10% extract means 10 g of spinach leaves extract dissolved in 100 ml aquadest, 20% extract means 20 g of spinach leaves extract dissolved in 100 ml aquadest, 30% extract means 30 g of spinach leaves extract dissolved in 100 ml aquadest. Using the result of measuring water content in the spinach leaves dense extract, the weight of dense extract and aquadest needed to make different concentration were able to calculate.

Tooth Protection with Calcium Oxalate: Teeth specimens were immersed in spinach leaves' extract solution with concentration of 10%, 20%, and 30% which each was mixed with 1 ml of high calcium milk, left for 60 seconds. Calcium content of the milk (brand Ultra) is 20% AKG which means 20% of daily need. Calcium daily need is about 1 gram (15). It means that the milk contains 0.2 gram/125 ml or 1.6 mg/ml.

Coffee Staining: Specimens were immersed in coffee made from Nescafe Classic coffee powder and boiled drinking water (brand Aqua) with comparison of 1 gram of coffee powder in 10 ml of water. The coffee was stirred 50 times with spoon and cooled until room temperature. The pH was examined with litmus paper. Teeth were soaked in 4 ml coffee per vial and kept in 37°C incubator for 24 hours.

Tooth Color Change Test: Tooth color was examined twice with color description of CIE L*a*b* using VITA Easyshade®. First measurement was done before given any action, and the second measurement was performed after immersing in coffee. Both data were compared and analyzed. All changes of L*, a*, and b* (ΔL*, Δa*, and Δb*) values were obtained from tooth color difference after coffee contact and the initial color of the tooth (L0, a0, and b0). Color change (ΔE*) was calculated using the formula.

Statistical Analysis: To analyze difference between effects of each extract concentration (10%, 20%, and 30%), comparative tests towards color change value (ΔE*) was done using One-Way ANOVA or Kruskal-Wallis (depends on normality of data distribution). Comparative test was also done using Independent T-Test or Mann-Whitney/Wilcoxon (depends on normality of data distribution). To see the correlation between extract concentration and tooth discoloration, Two-Tailed Pearson correlation test was conducted. All analysis was done using Windows SPSS 17.0 program.

Results

1. Phytochemicaland PH test results

Pytochemical screening test results are presented in Table 1. PH test results are shown in Table 2.

2. Teeth Color Test Results

Teeth color test results are demonstrated in Figure 1 and Table 3.

3. Statistic Test Results

a. One Way ANOVA and Kruskal-Wallis:

Because the sample is less than 50, data distribution normality test was done using Saphiro-Wilk test. If the significance value<0.05, it means that the data is not normally distributed. In One Way ANOVA and Kruskal-Wallis, significance value <0.05 means significantly different (Table 4).

b. Independent T-Test and Mann-Whitney/Wilcoxon Test:

These tests were done to compare each color change in every group independently to control.

- Normality of Data Distribution (Saphiro-Wilk): Because the sample is less than 50, Saphiro-Wilk testwas used to know data distribution normality. Significance value <0.05 means the data is not normally distributed (Table 5).

- Independent T-Test and Mann-Whitney/Wilcoxon Test Results: Normallydistributeddatawastestedwith Independent T-Test, while abnormally distributed data was tested with non-parametric test that is Mann-Whitney/Wilcoxon. Significance value<0.05 means it is significantly different (Table 6).

c. Two-Tailed Pearson Correlation Test

Significance value of Two-Tailed Pearson correlation test<0.05 means it is significantly correlated. If there is a significant correlation, it can be tested for the correlation strength. Positive value means direct correlation, negative value means inverse correlation. Correlation value > 0.5 means both variables have a strong correlation, while value< 0.5 means weak correlation (Table 7).

Sample	Solvent	Analysis	Method	Result	Unit
Spinach Leaves Dense Extract	Water	Water content	Gravimetric	51.18	%
		Tannin	Spectro	2.57	%
		Ca	AAS	211.38	mg/100g
		Ca Oxalate	HPLC	2.43	%
		Oxalic Acid		3.46	

Table 1. Phytochemical Screening Test Results(private or public)

	pH
10% spinach leaves extract solution	7
20% spinach leaves extract solution	6
30% spinach leaves extract solution	5
Milk	7
Coffee (extrinsic stain soaking solution)	5

Table 2. PH Test Results

	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 ΔE*	7.05	4.8	2.47	3.35

**Minus sign shows the decreasing number from the initial value.

Table 3. Average Value of Color Change

	ΔL*	Δa*	Δb*	ΔE*
Data Normality (Saphiro-Wilk)	Sig. = 0.000 (abnormal)	Sig. = 0.001 (abnormal)	Sig. = 0.088 (normal)	Sig. = 0.003 (abnormal)
One Way ANOVA	-	-	Sig. = 0.108 (not significantly different)	-
Kruskal-Wallis	Asymp. Sig. = 0.007 (significantly different)	Asymp. Sig. = 0.086 (not significantly different)	-	Asymp. Sig. = 0.057 (not significantly different)

Table 4. Data Normality, One Way ANOVA, and Kruskal-Wallis Test Results

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

Table 5. Data Distribution Normality Test Results

Results:

Kruskal-Wallis test showed significant difference in ΔL* (brightness) among each group. Compared to control group, two-Tailed Independent T-Test and Wilcoxon Test showed significant difference in ΔL*of all extract group, Δa* (degree of green-red color) of group 10% and 20%, Δb* (degree of blue-yellow color) of none group, and ΔE (value of color change) of group 20%. Pearson Correlation Test did not show any significant correlation between extract concentration and ΔL*, Δa*, Δb*, or ΔE.

Discussion

Tannin is one of the factors that can cause teeth extrinsic discoloration (7). From tannin test, spinach leaves dense extract contains tannin with small percentage (2.57%) if compared to coffee which has 19.5% to 23.1% tannin (25). Moreover, in the present study, spinach leaves extract has been diluted to 10%, 20%, and 30% concentration. Therefore, tannin content in spinach leaves extract would not give any significant discoloration to teeth.

Calcium content in spinach leaves extract is very small, which is only 211.38 mg/100g or 0.0021138%. This shows that free calcium ion in spinach leaves extract had reacted with existing oxalic acid. Calcium oxalate content (2.43%) is the result of the reaction. Because the content of oxalic acid is higher than calcium ion in spinach leaves extract, some oxalic acid remains unreacted (3.46%). Thus, spinach leaves extract is still potential to form calcium oxalate deposition on tooth surface when it met calcium from milk.

From pH test, it was seen that 30% spinach leaves extract solution and coffee are acidic (pH=5). Critical pH of enamel hydroxiapatite to demineralize is 5.5 and for fluorapatite is 4.5 (26). If the soaking solution is acidic, it can demineralize enamel, tannin can infiltrate and causing stain. Even though the 30% spinach leaves extract solution is acidic, the pH will increase when mixed with milk which pH is 7. Calcium is alkalic, acid-base reaction of oxalic acid and calcium will produce calcium oxalate salt that is neutral which pH is around 6.65-6.75 (27,28). Calcium oxalate can be formed if the pH is neutral or alkali; therefore, it is very important to keep the pH not acidic (23).

When compared simultaneously, Kruskal-Wallis test shows significant difference at ΔL* (brightness) in each group. But when comparing one by one versus control, it can be seen that group immersed in 10% spinach leaves extract solution has significant difference at ΔL* and Δa*, group 20% has significant difference at ΔL*, Δa*, and ΔE*, while group 30% is only significantly different at ΔL*.It can be concluded that tooth brightness change (ΔL*) decreased significantly when the tooth was protected by the calcium oxalate before stained with coffee. Furthermore, spinach leaves extract of 10% and 20% concentration also decreased Δa* (less reddish) compared to control group. While the whole color change (ΔE*) is only significantly different when tooth immersed in 20% spinach leaves extract.

Coffee with its acidity can demineralize tooth enamel so that staining material like tannin can penetrate into deeper enamel or even into dentine, deposited there, and cause discoloration (5). Discoloration will become permanent and cannot be removed directly because when the oral pH is back to neutral, enamel is remineralized, and the stain is trapped inside. Nevertheless, with blocking from calcium oxalate crystal on tooth surface, tooth enamel does not directly contact with coffee. Thus, no demineralization happens, and tannin is just deposited on the surface of the calcium oxalate, which can be removed easily from tooth surface with mechanical action. Eventually, the discoloration does not happen permanently.

Calcium oxalate tends to deposite on the rough surface of tooth because the crystal needs mechanical retention to attach. Due to the different roughness of each tooth surface, calcium oxalate deposition was not evenly distributed. It makes agap for tannin to penetrate and has a direct contact to enamel surface; thus, the tooth still have slight color change. However, the presence of calcium oxalate has lowered the effect of coffee discoloration.

Pearson correlation test shows no significant correlation between spinach leaves extract concentration and level of tooth color change (ΔL*,Δa*, Δb*, and ΔE*). Increasing extract concentration does not significantly lowering the color change. Therefore, the ability of protecting the tooth from discoloration due to coffee is not far different among 10%, 20% or 30% extract.

Conclusion

Within the limitations of the present study, it can be concluded that the combination of Spinach (Amaranthus hybridus L.) leaves extract solution and milk can reduce level of tooth discoloration caused by coffee. This effect seems to be due to formation of calcium oxalate layer on tooth enamel surface. However, there is no significant correlation between the increasing concentrations of spinach leaves extract solution and level of tooth discoloration due to coffee. It seems that spinach leaves extract and milk have a great potency to be developed into preventive products of teeth discoloration due to extrinsic stain from foods and drinks.

	Control vs 10%	Control vs 20%	Control vs 30%
ΔL*	p = 0.004 (significantly different)	p = 0.013 (significantly different)	p = 0.030 (significantly different)
Δa*	p = 0.006 (significantly different)	p = 0.020 (significantly different)	p = 0.054 (not significantly different)
Δb*	p = 0.372 (not significantly different)	p = 0.147 (not significantly different)	p = 0.229 (not significantly different)
ΔE*	p = 0.137 (not significantly different)	p = 0.037 (significantly different)	p = 0.054 (not significantly different)

Table 6. Independent T-Test and Mann Whitney/Wilcoxon Test Results

	ΔL*	Δa*	Δb*	ΔE*
Significance	0.060 (insignificant)	0.420 (insignificant)	0.081 (insignificant)	0.187 (insignificant)
Two-Tailed Pearson Correlation	0,452	-0,203	-0,422	-0,326

Table 7. Two-Tailed Pearson Correlation Test Results

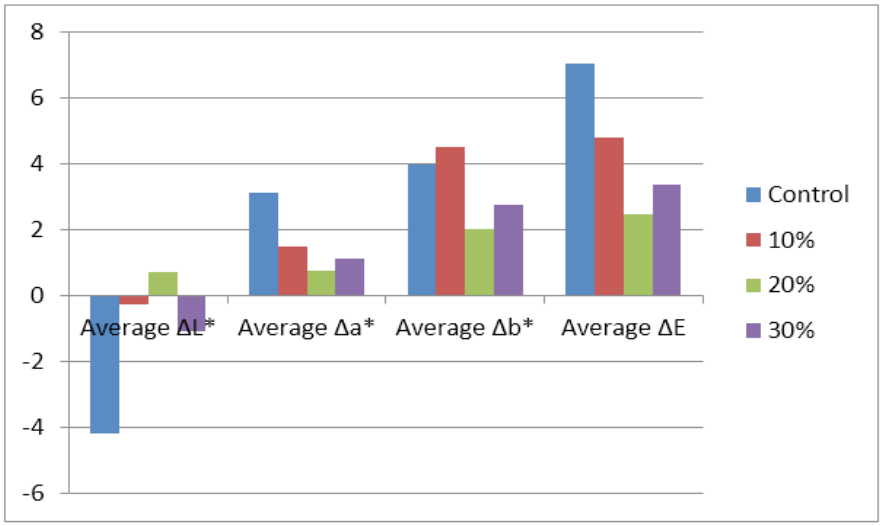

Figure 1. Diagram of Color Change Average

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