

Original Article

Effect of Green Tea, Black Tea and Moringa Oleifera on Remineralization of Artificially Demineralized Enamel and Dentin: An In-vitro Microhardness Analysis

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Abstract

Objectives: The present in vitro study aimed to investigate and compare the effect of green tea, black tea and Moringa Oleifera on artificially demineralized enamel and dentin.

Methods: Forty intact maxillary molar teeth were sectioned mesiodistally. The demineralized samples of enamel and dentin were randomly divided into four subgroups according to the treatment utilized. Group 1: Green tea extract; Group 2: Black tea extract; Group 3: Moringa Oleifera extract and Group 4: Artificial saliva (control). The samples were subsequently evaluated using microhardness tester. Data were tabulated and statistically analyzed using ANOVA and t-test.

Results: A statistically significant difference was found between baselines followed by after treatment, while the lowest mean value was found in after demineralization. A statistically non-significant difference was found between the four tested groups in enamel samples. In dentin, the highest mean value was found in Moringa Oleifera while the lowest mean value was found in black tea. The highest percentage of change was found in Dentin showed higher percentage of change after treatment compared to enamel.

Conclusion: Black tea, green tea and Moringa Oleifera enhanced the remineralization process of demineralized enamel and dentin, and thus, might be considered as an effective natural remineralizing agents.

Keywords: Black Tea, Demineralization, Green tea, Microhardness, Moringa Oleifera, Remineralization.

1. Introduction

The principle of non-invasive remineralizing treatment is more acceptable during the early stages of dental caries disease

(Christensen, 2005). Adequate levels of calcium, phosphate and fluoride ions can produce sub-spatial remineralization of enamel (Li *et al.*, 2014). In addition, dentin matrix serves as

scaffold for mineral crystallites deposition (Kinney *et al.*, 2003). The true functional remineralization involves the stabilization of both organic and inorganic components through the use of anti-collagenolytic agents during the remineralization process (Jose, Sanjeev and Sekar, 2016).

It has been well documented that fluoride treatment remains the best remineralizing method for early enamel caries (Ten Cate, 2008). Unfortunately, fluoride lack the ability to guide the formation of mineral crystals and fails to form oriented and ordered mineral crystals on the surface of enamel (Fan, Sun and Moradian-Oldak, 2009). Chronic ingestion of high doses of fluoride leads to dental fluorosis, skeletal fluorosis in severe cases, where fractures and calcification of ligaments and tendons can occur, leading to reduced joint mobility (Aoun *et al.*, 2018).

In recent years, attention has been focused on the use of natural products as they have both advantages of minimal side effects and being alcohol and/or sugar-free, which are the two most common ingredients found in over the counter products (Shekar *et al.*, 2015).

Black and green tea are the most researched upon among the other types due to their high content of polyphenols (catechins) like epigallocatechin gallate (EGCG) that exhibits profound inhibitory effect on both collagenase and elastase. In addition, it has antibacterial properties due to presence of bioactive chemicals including polyphenols, mineral, and volatile oil (Thring, Hili and Naughton, 2009). The high fluoride content supports its remineralizing effect (Campanella, Bonanni and Tomassetti, 2003). Although green tea has traditionally been considered safe, some studies reported liver injury, thus consumption of green tea should be cautioned in patients with liver diseases (Patel *et al.*, 2013). The high dose of black tea during pregnancy induced

nephrotoxicity of experimental pregnant rats at the end of postnatal period and retarded the normal growth of their embryos (Ramachandran *et al.*, 2016)

Moringa Oleifera has more calcium than milk, resulting in high remineralization effect of enamel and dentin (Gandji *et al.*, 2018). The leaves and stems are known to have a large amount of their calcium bound in calcium oxalate crystals. It is rich in potassium, iron, Vitamin C and A (Farooq *et al.*, 2012).

Moreover, occlusion of dentinal tubules by formation of different sized mineral particles was detected with application of *Moringa Oleifera* (Khalaf *et al.*, 2016). However, *Moringa* extracts (weekly doses greater than 46 mg/kg/day) produced hepatotoxicity and nephrotoxicity. Toxicity of *Moringa Oleifera* depends on concentration, part of the plant used, and manner of preparation and routes of administration (Stohs and Hartman, 2015). Various animal studies have assessed the safety of *Moringa Oleifera* extracts, and have demonstrated a high degree of safety (Moodley, 2017).

No studies till now have been conducted comparing the remineralizing effect of *Moringa Oleifera* extract with different herbal extracts on enamel and dentin remineralization. Therefore, the purpose of this study to verify the hypothesis that black tea, green tea and *Moringa Oleifera* can positively affect the remineralization potential of demineralized enamel and dentin. The null hypothesis was that the application of tested herbal extracts did not have any effect on remineralization potential of artificially demineralized enamel and dentin.

2. Material & methods

2.1. Experimental design

Microhardness of enamel and dentin samples before immersion in demineralized solution

(baseline), after demineralization and after immersion in water extracts were evaluated. Group 1: Green tea; Group 2: Black tea; Group 3: *Moringa Oleifera* and Group 4: Artificial saliva (control). The quantitative outcome variable was Vickers microhardness values.

2.2. Preparation of demineralizing solutions

Demineralizing solution for enamel was adjusted to pH4.8 and it consisted of 50mM acetic acid 2.2 mM CaCl₂ with 2.2 mM NaH₂PO₄. While for dentin, 0.0476 mM sodium fluoride (NaF), 2.2 mM calcium chloride dihydrate (CaCl₂·2H₂O), 2.2 mM potassium dihydrogen phosphate (KH₂PO₄), 50 mM acetic acid (CH₃COOH), was adjusted with 10 mM potassium hydroxide (KOH) to a pH 5.0 (Tschoppe, Meyer-Lueckel and Kielbassa, 2008).

2.3. Preparation of artificial saliva:

The composition of artificial saliva used was Na₃ PO₄ (3.90 mM), NaCl (4.29 mM), KCL (17.98 mM), CaCl₂ (1.10 mM), MgCl₂ (0.08 Mm), H₂SO₄ (0.50mM), NaHCO₃ (3.27 mM) and distilled water with the pH adjusted to 7.2.

2.4. Preparation of the extracts:

Samples of black tea, green tea (*Lipton tea, imported and packed in Egypt by Unilever Mashreq Co.*) and *Moringa Oleifera* leaves (*National research centre, Giza, Egypt*) were dried naturally for one month and grounded in a blender before extraction. For water extracts, each of air-dried powdered leaves (20 gm.) were topped with 100 ml boiled distilled water and incubated for 2 h at room temperature then filtered using Whatman (No.1) filter paper (El-Kholy et al., 2017).

2.4. Tooth selection

Forty freshly extracted sound human maxillary molar teeth, extracted for periodontal reasons after getting the patients' approval (age

of 20-40 years), were used in this *in-vitro* study. Immediately after extraction, they were washed, scrubbed and scaled carefully using hand scaler (DENTSPLY Ash instruments, Surrey, UK) to remove any remnants of periodontal ligaments, plaque and blood. Teeth were decontaminated in 10% formalin for 7 days as it is suitable for research purposes, and effectively destroys all kinds of microorganisms (Sandhu et al., 2012). Then they were examined using magnifying lens to exclude fractures, cracks, caries, enamel malformations or other defects. After cleaning and examination, the selected molars were stored at room temperature in distilled water where they could be used in this study within two weeks of extraction.

2.5. Specimen preparation:

Radicular part of each tooth was removed, and the coronal part was then longitudinally sectioned in the mesio-distal direction into two sections resulting into 80 specimens using a high-speed diamond tipped disc under water coolant. The specimens were then randomly divided equally into two groups to prepare enamel and dentin specimens having color as a criterion to differentiate enamel and dentine. For enamel group, samples were cut at level of enamel to expose fresh layer of enamel (6 X 4 X 4m³) using a diamond tipped disc under high speed and water coolant. The dentin sample was cut at level of dentine, the enamel was removed to expose the dentin (6 X 4 X 4m³), using a coarse grit diamond under water coolant and ultrasonicated in a deionized water bath to remove any debris. Specimens were embedded in self cure acrylic resin and allowed to set to create blocks. The surface was flattened and polished using 400, 800, 1000, 1200 grit abrasive. A 3 mm × 3 mm window was created in the middle of the sample surface using adhesive tape and coating the rest of the surface with nail varnish. Once the samples were adequately dried, the adhesive tape was removed

from the tooth surface using an explorer, exhibiting a rectangular area on the surface.

2.6. Demineralization of samples:

The samples were inserted into tubes containing 20ml of demineralizing solution at room temperature for 14 days for enamel samples and 5 days for dentin samples to create lesions. The demineralized solution was refreshed daily and not stirred during the demineralization period (Tschoppe *et al.*, 2011). Subsequently, the samples were rinsed thoroughly with deionized water to eliminate the demineralized gel from the sample surface.

2.7. Sample treatment:

Samples were randomly divided into 4 subgroups and were immersed in 20ml of tested solution for 8 days (Rubel *et al.*, 2016). Tested solutions were refreshed daily.

2.8. Microhardness assessment

Vicker's micro hardness tester (Wilson Tukon 1102 micro hardness tester Buehler Germany) at load of 100 gm. was used. The load was applied smooth for 10 seconds and after load removing, two impression diagonals are measured, usually to the nearest 0.1- μm with a filar micrometer, and averaged. The Vickers hardness (HV) was calculated using:

$$\text{MHV} = 1854.4L/d^2 \text{ (Where the load L is in gf and the average diagonal d is in } \mu\text{m.)}$$

The examiner took three indentations Vickers hardness number at spacing of 100 microns and the mean value was calculated.

2.9. Statistical analysis

The mean and standard deviation values were calculated and data were explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests. Repeated measure ANOVA test was used to compare between more than two groups in related samples. Paired sample t-test test was

used to compare between two groups in related samples. One-way ANOVA followed by Tukey post hoc test was used to compare between more than two groups in non-related samples. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

3. Results

The mean and standard deviation values of enamel and dentin groups are presented in table (1,2) and figure (1,2) respectively. A statistically significant difference was found between baseline, demineralized and after treatment with all tested solutions ($P < 0.05$). The highest mean value was found in baseline followed by after treatment, while the lowest was found in after demineralization in enamel and dentin groups in the four tested groups.

A statistically non-significant difference between the four tested groups in enamel samples ($P > 0.05$). The highest mean value of enamel microhardness was found in *Moringa Oleifera* (219) followed by saliva (196.59) and green tea (192.33) while the lowest mean value was found in black tea (168). Regarding dentin samples, the highest mean value was found in *Moringa Oleifera* (43.25) followed by green tea (38) and saliva (35), while the lowest mean value was found in black tea (29).

The mean, standard deviation of percentage of change in both enamel and dentin are presented in table (3) and figure (3). A statistically significant difference was found between enamel and dentin after treatment with black tea (42.83%, 58.13% respectively), saliva (29.51%, 52.36%) and *Moringa Oleifera* (28.40%, 44.12%). The highest percentage of change was found in dentin (58.13%) while the lowest percentage of change was found in enamel (28.40%).

Table (1): The mean, standard deviation (SD) of Enamel in different groups

Variables	Enamel						p-value
	Baseline		Demineralized		After treatment		
	Mean	SD	Mean	SD	Mean	SD	
Black tea	294.48 ^{aA}	14.78	125.50 ^{aC}	9.84	168.00 ^{aB}	6.19	0.012*
Green tea	292.67 ^{aA}	27.39	125.00 ^{aC}	34.96	192.33 ^{aB}	36.13	0.003*
Saliva	279.67 ^{aA}	12.82	138.50 ^{aC}	10.31	196.59 ^{aB}	19.67	0.005*
Moringa	305.25 ^{aA}	16.65	136.75 ^{aC}	24.46	219.00 ^{aB}	23.55	0.001*
p-value	0.336ns		0.750ns		0.069ns		

Means with different small letters in the same column indicate statistically significance difference. Means with different capital letters in the same row indicate statistically significance difference *; significant (p<0.05) ns; non-significant (p>0.05)

Table (2): The mean, standard deviation (SD) of Dentin in different groups.

Variables	Dentin						p-value
	Baseline		Demineralized		After treatment		
	Mean	SD	Mean	SD	Mean	SD	
Black tea	70.17 ^{aA}	6.02	22.17 ^{aC}	4.29	29.50 ^{bB}	4.85	0.001*
Green tea	74.17 ^{aA}	11.12	24.34 ^{aC}	5.66	38.00 ^{abB}	4.88	0.003*
Saliva	75.45 ^{aA}	3.98	21.67 ^{aC}	3.53	35.89 ^{abB}	3.91	0.017*
Moringa	77.42 ^{aA}	7.53	23.84 ^{aC}	5.11	43.25 ^{aB}	5.83	0.006*
p-value	0.630ns		0.854ns		0.018*		

Means with different small letters in the same column indicate statistically significance difference. Means with different capital letters in the same row indicate statistically significance difference *; significant (p<0.05) ns; non-significant (p>0.05)

Table (3): The mean, standard deviation (SD) of percentage of change in both enamel and dentin in different groups.

Variables	Percentage of change							
	Black tea		Green tea		Saliva		Moringa	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Enamel	42.83% ^b	3.84	34.48% ^a	9.43	29.51% ^b	8.75	28.40% ^b	4.14
Dentin	58.13% ^a	4.24	48.25% ^a	7.96	52.36% ^a	5.37	44.12% ^a	6
p-value	0.002*		0.067ns		0.011*		0.005*	

Means with different small letters in the same column indicate statistically significance difference. *; significant (p<0.05) ns; non-significant (p>0.05)

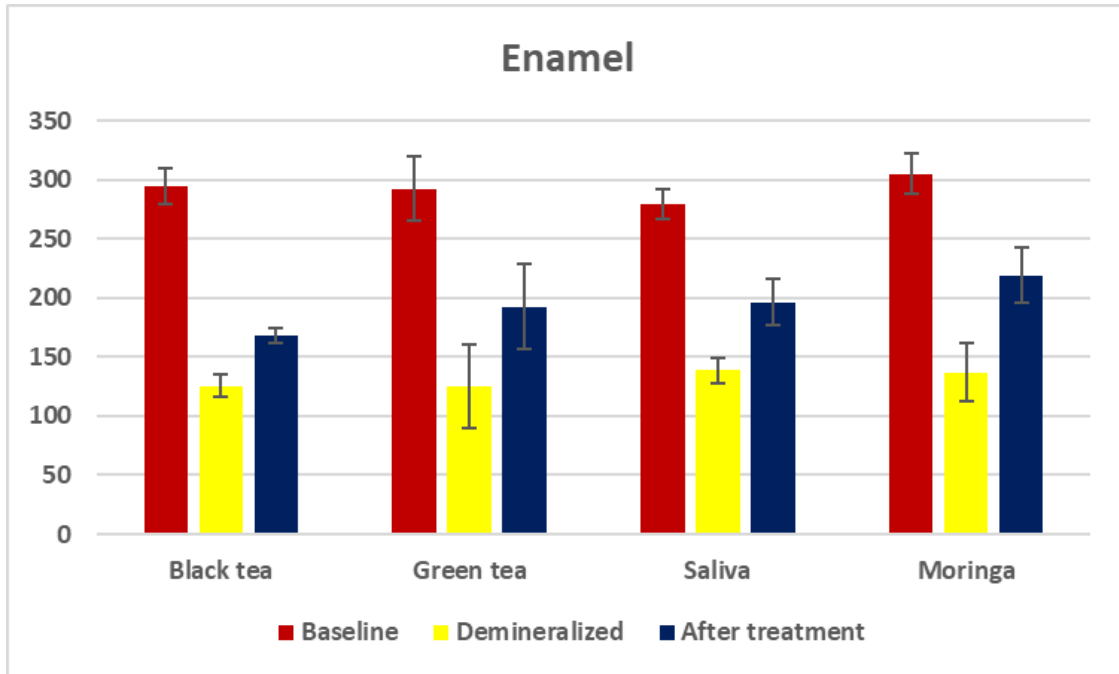


Figure (1): Bar chart representing Enamel results for each group

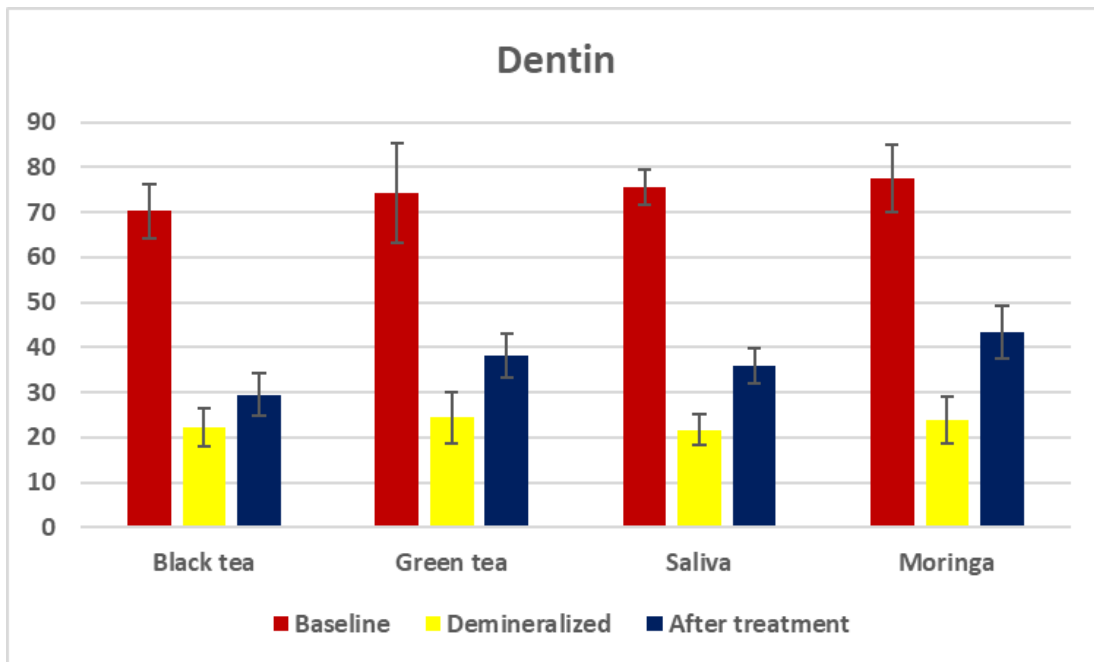


Figure (2): Bar chart representing Dentin results for each group

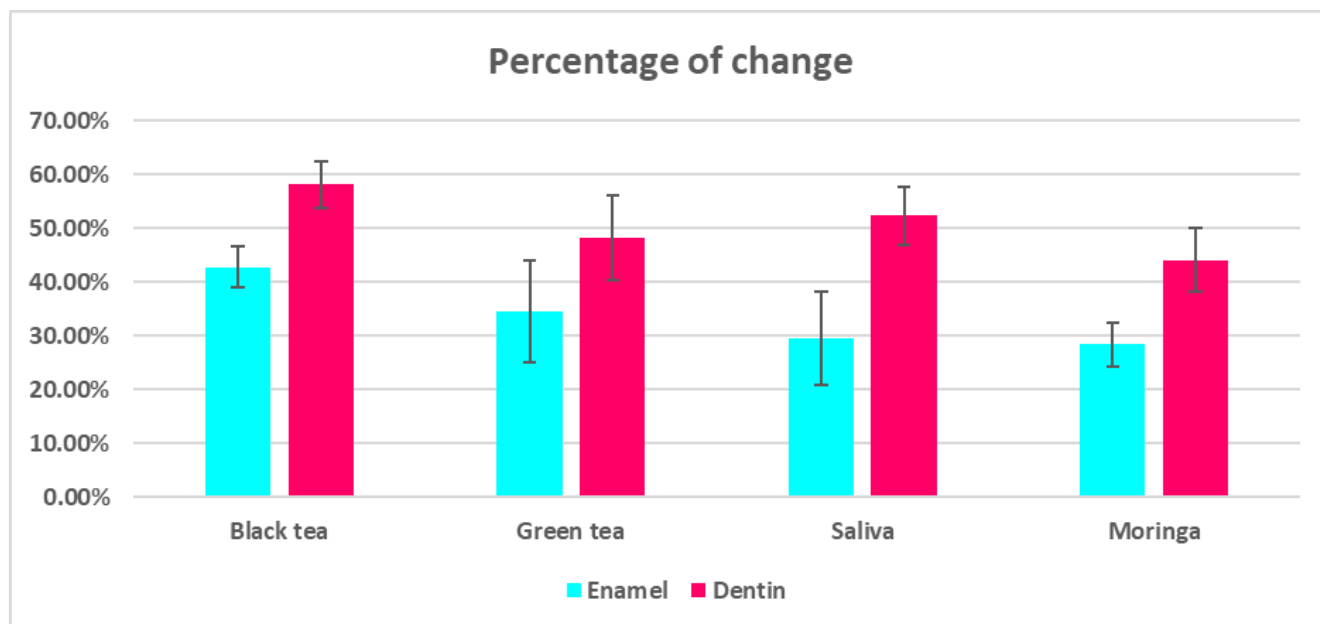


Figure (3): Bar chart representing relation between Enamel and Dentin results for different groups

4. Discussion

Green tea, black tea and *Moringa Oleifera* were comparatively evaluated in this study for their effect on remineralization of artificially demineralized enamel and dentin using microhardness test.

Tea is the world's most widely consumed beverage. Black tea exhibited strong antimicrobial effect against *streptococcus mutans* and *lactobacillus* bacteria as it contains phenolic compounds that degrade the cell wall leading to cell death (Allah, Ibrahim and Al-Atrouny, 2011).

Various *Moringa Oleifera* extracts have been previously evaluated on the basis of their relative polyphenol, flavonoid contents, and it was found that water extract of leaves exhibit the greatest activities of these indicators

(Vongsak, Sithisarn and Gritsanapan, 2014). Thus, water extract of *Moringa Oleifera* leaves was selected in the present study.

Measuring the surface microhardness is a simple, rapid, and nondestructive method (Palaniswamy *et al.*, 2016). The specimens were stored in 20 ml of tested solutions 8h storage period as the average time for consumption of one cup of hot drinks is 15 min, and the average consumption is 3.2 cups per day. Therefore, 8h storage time simulated consumption of tea over 10 days period (Guler *et al.*, 2009) .

Enamel results showed a statistically significant difference between demineralized samples and after treatment indicating that all tested solutions are capable of remineralizing enamel tissues equally. Similar results were stated by (Babu *et al.*, 2017) who revealed that

green tea, black tea and sodium fluoride showed increase of enamel fluorescence indicating remineralization of demineralization samples. This could be attributed to the action of tea on collagen network which stabilized the collagen and maintain it in expanded state thus the interfibrillar spaces are kept open for remineralization. Moreover, *Moringa Oleifera* medicinal properties derives from secondary metabolites, such as alkaloids, tannins, flavonoids, steroids, saponins, coumarins, quinones and resins (Farooq *et al.*, 2012). These results were in agreement with (Khalaf *et al.*, 2016) who revealed that the SEM analysis of enamel specimens' after application of *Moringa* showed blockage of enamel prisms with appearance of mineralized deposits along the porous defects. (Hasan and Bds, 2011) examined microscopical changes of different types of tea in preventing tooth decay and revealed that black tea produced enamel remineralization.

Regarding dentin samples, a statistically significant difference was found between black tea, green tea, saliva and *Moringa Oleifera*. The highest mean value was found in *Moringa Oleifera* (43.25) followed by green tea (38) and saliva (35), while the lowest mean value was found in black tea (29). This may be due to *Moringa Oleifera* leaves have been reported to be a rich source of β -carotene, protein, vitamin C, calcium and potassium and act as a good source of natural antioxidants (Farooq *et al.*, 2012). The higher mean values of green tea (38) in comparison to black tea (29); could be due to the small amount of fluoride in black tea which only help the remineralization to start (Hasan and Bds, 2011). The main chemical difference between green and black tea is that the former contains simple catechins whereas in the later many of these have been oxidized and condensed, during a manufacturing process known as 'fermentation' to larger dark-colored molecules including theaflavins and thearubigins. Black tea contains other elements

such as Al, K, Mn and Mg which may substitute calcium ion of hydroxyapatite crystals thus forming other crystals (Campanella, Bonanni and Tomassetti, 2003).

The results of the current study demonstrated higher percentage change of microhardness in dentin compared to enamel. The initiation of caries-like lesion in the present study was conducted in 14 days for enamel and 5 days for dentin that demonstrated high cariogenic challenges; however considerable increase in surface microhardness percentage of change were observed for both enamel and dentin. Additionally, all-natural leaves containing polyphenols has been classified as Proanthocyanidine-based components which has an extremely high affinity to bind to Proline-rich proteins such as collagen, forming a proline-PA complex. Although traditionally mature dental enamel is considered to be free of collagen, (Açil *et al.*, 2005) reported that Type I collagen is found in enamel; however, the concentration of collagen in enamel was considerably lower as compared to that in dentin explaining lower results of enamel than dentin.

Further researches are needed to study the exact chemical analysis of each plant with the amounts of active ingredients that would affect their influence on enamel and dentin remineralization.

5. Conclusion

Green tea, black tea and *Moringa Oleifera* enhanced the remineralization process of demineralized enamel and dentin, and thus, might be considered as an effective natural remineralizing agents for daily intake.

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