

Evaluation of Various Concentrations of *Punica granatum* Extract Gel's Potency in Teeth Whitening: An *In Vitro* Study

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Received on: 16 February 2024; Accepted on: 30 May 2024; Published on: 27 September 2024

ABSTRACT

Teeth whitening is a conservative procedure utilized to overcome tooth discoloration. Malic acid and ellagic acid obtained from white pomegranate can be used to whiten teeth.

Aim: The purpose of this research was to evaluate the efficacy of white pomegranate extract gel as a bleaching agent against tooth discoloration and its effects on the enamel surface.

Materials and methods: Forty-eight mandibular incisors were utilized as samples. All teeth were submerged for 12 days in the coffee solution. Utilizing spectrophotometry, tooth discoloration was measured. After being soaked in a coffee solution and then in a white pomegranate extract gel, the item was dried.

Group I: Ten teeth soaked in white pomegranate extract gel with a concentration of 70%.

Group II: Ten teeth soaked in white pomegranate extract gel with a concentration of 80%.

Group III: Ten teeth soaked in white pomegranate extract gel with a concentration of 90%.

Group IV: Around 10% of carbamide peroxide gel as a positive control for 10 teeth. Each specimen was immersed for 4 hours per day for 7 days. Using a spectrophotometer, tooth discoloration was assessed following immersion in white pomegranate gel extract and carbamide peroxide gel, and with a stylus profilometer, the surface roughness of the enamel was assessed.

Results: The Delta E value of 90% white pomegranate extract gel was higher than the positive control. The average surface roughness value of white pomegranate extract gel increased with an increase in its concentration; hence, 70% of white pomegranate extract gel had the least enamel surface roughness.

Conclusion: Based on the results, group III (90% white pomegranate extract gel) was determined to be the most effective as a teeth-whitening agent, and group I (70% white pomegranate extract gel) showed the least surface roughness. White pomegranate extract gel has an influence on tooth discoloration and teeth whitening attempts.

Keywords: Bleaching agent, CIE color lab, Natural remedy, Surface roughness, Teeth bleaching, White pomegranate.

Journal of Operative Dentistry and Endodontics (2023): 10.5005/jp-journals-10047-0133

INTRODUCTION

Punica granatum L. (Pomegranate) is a plant with a long lifespan and tolerance for drought. Pomegranate juice has high levels of fructose, sucrose, and glucose. It also contains simple organic acids such as ascorbic acid, citric acid, fumaric acid, ellagic acid, and malic acid. Furthermore, it includes modest levels of all amino acids, including proline, methionine, and valine.¹ Both the juice and peel are rich in polyphenols, tannins, and flavonoids that show the therapeutic value of pomegranate owing to its antioxidative, anti-inflammatory, and high vitamin and mineral content capabilities. Ellagitannin is a form of tannin that generates hydroxybenzoic acid, which is also known as ellagic acid. Punicalagin and punicalin are two more ellagitannins discovered in pomegranate juice and peel. Pomegranate contains several types of compounds, including anthocyanins, luteolin, catechins, terenoids, flavan-3-ols, and flavonols.² The main pigments responsible for the color of pomegranate fruit are anthocyanins, which are part of the flavonoid family. Pomegranate fruit comprises six primary anthocyanin chemicals, including the mono- and diglucosides of cyanidin (red), pelargonidin (orange), and delphinidin (purple).³ White pomegranate is an alteration produced by *PgLDOX*, a structural gene implicated in the anthocyanin production pathway in pomegranate.⁴ The contents of ellagic acid and malic acid in

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How to cite this article: Narayan SA, Elangovan SK, K Madhuram, *et al.* Evaluation of Various Concentrations of *Punica granatum* Extract Gel's Potency in Teeth Whitening: An *In Vitro* Study. *J Oper Dent Endod* 2023;8(2):25-31.

Source of support: Nil

Conflict of interest: None

white pomegranate are potent oxidizers that can function as a natural teeth-whitening solution. Dental whitening is one of the conventional techniques that may be applied to overcome tooth discoloration. Tooth color is important to most people, and the discoloration or staining can negatively affect their quality of life. Tooth discoloration can be caused by both internal and external influences, including smoking, eating tannin-rich foods, and using chlorhexidine.⁵ The most popular teeth-bleaching chemicals are hydrogen peroxide or carbamide peroxide in gels or toothpaste with various concentrations. The bleaching agent hydrogen

peroxide has a negative effect on tooth enamel because it causes demineralization. Demineralization is the process of eliminating mineral ions from dental enamel. Enamel consists mostly of hydroxyapatite. Demineralization occurs when calcium bonds from hydroxyapatite dental enamel are released owing to changes in its microstructure. The negative effects of bleaching with hydrogen peroxide or carbamide peroxide have led to a search for natural bleaching chemicals that can provide the same whitening impact as hydrogen peroxide while minimizing unwanted effects.⁶

Pomegranate (*Punica granatum L.*) contains ellagic acid, making it a suitable natural component. Due to their increased flavonoid content compared to other varieties white pomegranates are commonly used in herbal medicine. Organic bleaching agents are a more secure, affordable, and readily available solution.

The purpose of this *in vitro* investigation was to assess the efficiency of various concentrations of *Punica granatum L* extract gel as a whitening agent against external tooth discoloration through a Commission Internationale de l'Eclairage (CIE) color lab spectrophotometer for color analysis and a profilometer for surface roughness for a week for 2 hours per day.

MATERIALS AND METHODS

The sample size calculation was done using G Power software 3.1. Study samples included 48 human mandibular lower incisors, which were extracted for surgical reasons and obtained from the Karpaga Vinayaga Institute of Dental Sciences under a protocol set by the Institutional Ethics Committee of the Karpaga Vinayaga Institute of Dental Sciences before the commencement of the study (KIDS/IEC/2022/II/007). The samples were kept at 37°C in distilled water mixed with thymol until used.

All the samples were then immersed for 12 days in the coffee solution to cause discoloration in the samples. Baseline value analysis for color analysis and surface roughness for all samples was done after 7 days post immersion of samples in coffee solution (Fig. 1). Forty-eight human mandibular lower incisor samples were divided into four groups ($n = 12$), including group I: 70% white pomegranate extract gel; group II: 80% white pomegranate extract gel; group III: 90% white pomegranate extract gel (Fig. 2); and group IV: 16% carbamide peroxide gel, the positive control.

PREPARATION OF EXTRACT

Punica granatum L extract was made by separating the fruit skin. The arils were mashed with 96% ethanol solvent, and it was stirred. Post 3 days' storage, the sample was aggregated using a filter paper and a Buchner funnel. Filtered extract was heated gently. The extract was first supplemented with Carbopol 940, followed by distilled water to form a pellucid solution. Subsequently, 10% sodium hydroxide (NaOH) was added to form a gel mass. Subsequently, 0.3 gm of Nigapin was dissolved in 96% ethanol. Finally, propylene glycol was mixed to form a homogeneous mass of 100% pure *P. granatum* extract (Fig. 3).⁷ From which we will procure 70% white pomegranate gel by mixing 70 gm of the acquired extract with 100 ml of double-distilled water; similarly, 80% white pomegranate gel by mixing 80 gm of the acquired extract with 100 ml of double-distilled water; and 90% white pomegranate gel by mixing 90 gm of the acquired extract with 100 ml of double-distilled water.

Prior to the application of the extracts, the pH of all the groups was analyzed with a pH meter, and samples were immersed in the respective concentrated extracts for 2 hours every day for 1 week. Post-application, the extract was washed with distilled water and

immersed in artificial saliva. Color analysis was done after 1 week through the CIE LAB spectrophotometer, and surface roughness analysis was done through the Stylus profilometer.

Statistical Analysis

The data were compiled in a Microsoft Excel sheet. The statistical analysis work was performed using SPSS version 25 for Windows. The normality of the data was checked through the Shapiro-Wilks test. The data were normally distributed; therefore, parametric analysis was done. A one-way ANOVA and *post hoc* Tukey test were performed. All *p*-values were set at 0.05.

RESULTS

The baseline color coordinates (pre-bleach values) (L^* , a^* , b^*) (ΔE) were documented. (71.19 ± 0.97). All the post-bleach samples showed considerably greater mean ΔE values (Table 1) compared to the respective pre-bleach sample (baseline values) with *p*-value 0.05. In order to ascertain if the white pomegranate extract gel can whiten the surface of enamel, the mean Delta E value of a 16% carbamide peroxide gel was employed as a positive control. The Delta E value of 90% white pomegranate extract gel was slightly higher than the positive control, and it exhibited the ability to whiten the tooth enamel surface.

The average surface roughness value of white pomegranate extract gel increased with an increase in its concentration (Table 2). On comparison with the 16% carbamide peroxide - control group, the average surface roughness value of the white pomegranate extract gel of 70, 80, and 90% was comparatively minimal but not lesser than the baseline value of 1.75 ± 0.37 .

The Delta E value is highly statistically significant in the 80 and 90% white pomegranate extract gel groups in post-bleached specimens (Table 3).

The average surface roughness value of white pomegranate extract gel is highly statistically significant for all groups except for the 70% white pomegranate extract gel group vs 80% white pomegranate extract gel group and the 80% white pomegranate extract gel group versus 90% white pomegranate extract gel group (Tables 4 and 5).

In pairwise comparison (Table 6), baseline values and post-intervention values in each of the four groups show a statistically significant change.

Between the control group and baseline vs post-intervention surface roughness, there is no statistically significant difference. The 70, 80, and 90% white pomegranate extract gel groups show statistically significant differences when compared with baseline and post-intervention (Table 7).

DISCUSSION

The use of white pomegranate extract gel lightened the color of the teeth. Ellagic acid and malic acid are natural ingredients currently used to whiten decolorized teeth. Due to the presence of a powerful oxidizing agent, ellagic acid, in the white pomegranate extract, the yellowing of the teeth lightens. When the OH and H⁺ radicals are liberated from the ellagic acid, they interact with the organic molecules to break up the electron conjugation. This results in a change in the energy absorption process because smaller, lighter-colored organic molecules are formed. When OH clusters in ellagic acid react with the organic molecules in tooth enamel, they break readily and have a greater electronegativity.⁸ Ellagic acid is



Fig. 1: Post immersion of samples for seven days in coffee solution

a naturally occurring polyphenol antioxidant found in a variety of fruits and vegetables, such as almonds and walnuts, strawberries, peaches, pears, pomegranates, persimmons, raspberries, and black raspberry.⁸ Strawberries contain 0.43–4.64 mg/gm dry weight ellagic acid.⁹ Pomegranate contains comparatively more ellagic acid than other fruits and vegetables.¹⁰ The bleaching process is more successful in fruits with a higher ellagic acid content.¹¹

The presence of ascorbic acid in strawberries along with maleic and citric acid, tends to increase the surface energy, remove surface stains, and increase the surface roughness when compared with white pomegranate.^{12,13} Maleic acid, which is present in the white pomegranate extract, binds to calcium in teeth to produce enamel crystal porosity, which in turn leads to dental erosion and

a reduction in enamel hardness; hence, different concentrations of white pomegranate gel's efficacy were evaluated in this study over a specific time interval.

Samples were stored in distilled water mixed with thymol at 37°C until use to prevent dehydration and microbial growth on them. All the samples were immersed and stored for 12 days^{14,15} in freshly prepared coffee solution as they displayed clinically unfavorable staining due to the presence of chlorogenic acid and tannins.

To avoid bias in selecting contrary variables and to correlate with the ideal extract gel, concentrates of 90, 80, and 70% of white pomegranate gel were prepared to evaluate the efficacy of white pomegranate gel on teeth whitening.

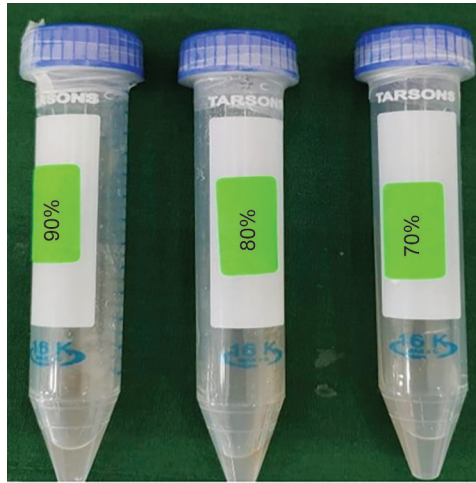


Fig. 2: Around 70, 80, and 90% white pomegranate extract gel



Fig. 3: Preparation of extract

Table 1: Average and standard deviation of absorbance values

Group	Mean	Standard deviation	p-value
70% white pomegranate extract gel	85.93	1.32	0.000
80% white pomegranate extract gel	86.34	1.33	0.000
90% white pomegranate extract gel	97.57	1.46	0.000
16% carbamide peroxide gel	96.82	1.41	0.000

p-value < 0.005

Table 2: Average and standard deviation values of surface roughness

Group	Mean	Standard deviation	p-value
70% white pomegranate extract gel	1.93	1.4	0.000
80% white pomegranate extract gel	1.81	0.45	0.000
90% white pomegranate extract gel	1.69	0.16	0.000
16% carbamide peroxide gel	2.24	1.58	0.000

The 90% white pomegranate extract gel had a greater Delta E value than the positive control and other groups. The

average surface roughness value of white pomegranate extract gel increased as its concentration increased, and 70% of white pomegranate extract gel had the least enamel surface roughness.

Generally, 10 and 16% concentrations of carbamide peroxide are used for home bleaching for a period of 2 hours per day for 3 weeks. The higher the concentration, the better the whitening efficacy and the greater the discomfort after application. Its effect on the oral cavity is gingival irritation and tooth sensitivity, which decrease the enamel surface hardness and cause enamel demineralization.⁷

The pH value of strawberry was pH = 3.45–5.32,¹³ and the pH values of 70, 80, and 90% white pomegranate were approximately 7 when tested with a pH meter. Therefore, a lower pH, as in carbamide peroxide and strawberry, plays a damaging role because it leads to the dissolution of tooth enamel, which in turn leads to dental erosion.¹⁶

Variations in the progression of tooth discoloration from the mean baseline absorbance value are correlated with tooth enamel thickness and age (older teeth have more secondary dentin, which will affect the enamel color translucency). Since the current study was performed in an *in vitro* model, the teeth of various patients were used as specimens for the purpose of the study, resulting in variations in enamel layers.

Table 3: One-way ANOVA for Delta E values

Parameters	Sum of squares	df	Mean square	f	Sig.
Between groups	1025.487	3	341.829	27.776	0.000
Within groups	541.493	44	12.307		
Total	1566.980	47			

(I) Group	(J) Group	Mean difference (I-J)	p-value	95% Confidence interval	
				Lower bound	Upper bound
1	2	-1.72083	0.629	-5.5447	2.1031
	3	-6.46750*	0.000	-10.2914	-2.6436
	4	-11.89583*	0.000	-15.7197	-8.0719
2	1	1.72083	0.629	-2.1031	5.5447
	3	-4.74667*	0.010	-8.5706	-0.9228
	4	-10.17500*	0.000	-13.9989	-6.3511
3	1	6.46750*	0.000	2.6436	10.2914
	2	4.74667*	0.010	0.9228	8.5706
	4	-5.42833*	0.002	-9.2522	-1.6044
4	1	11.89583*	0.000	8.0719	15.7197
	2	10.17500*	0.000	6.3511	13.9989
	3	5.42833*	0.002	1.6044	9.2522

Table 4: One-way ANOVA done for surface roughness

Parameters	Sum of squares	df	Mean square	f	Sig.
Between groups	3.870	3	1.290	22.034	0.000
Within groups	2.576	44	0.059		
Total	6.447	47			

Table 5: Post hoc test for Delta E

(I) Group	(J) Group	Mean difference (I-J)	Std. error	Sig.	95% Confidence interval	
					Lower bound	Upper bound
1	2	0.77750*	0.09879	0.000	0.5137	1.0413
	3	0.55667*	0.09879	0.000	0.2929	0.8204
	4	0.48917*	0.09879	0.000	0.2254	0.7529
2	1	-0.77750*	0.09879	0.000	-1.0413	-0.5137
	3	-0.22083	0.09879	0.130	-0.4846	0.0429
	4	-0.28833*	0.09879	0.027	-0.5521	-0.0246
3	1	-0.55667*	0.09879	0.000	-0.8204	-0.2929
	2	0.22083	0.09879	0.130	-0.0429	0.4846
	4	-0.06750	0.09879	0.903	-0.3313	0.1963
4	1	-0.48917*	0.09879	0.000	-0.7529	-0.2254
	2	0.28833*	0.09879	0.027	0.0246	0.5521
	3	0.06750	0.09879	0.903	-0.1963	0.3313

*The mean difference is significant at the 0.05 level

As far as current research indicates, this is the first investigation to analyze the effectiveness of different concentrations of white pomegranate extract gel as a bleaching agent against enamel

surface alterations and tooth discoloration. The results of Muga IFR et al.¹⁶ have correlated with our study in proving white pomegranate gel as a potent alternative for whitening teeth. The evaluation was

Table 6: Pairwise comparison for Delta E values paired samples test

Group	Paired differences								
	Mean	Standard deviation	Std. error mean	95% Confidence interval of the difference		t	df	Sig. (2-tailed)	
				Lower	Upper				
1 Pair 1 Post - stain - baseline stain	4.80917	4.84769	1.39941	1.72909	7.88924	3.437	11	0.006	
2 Pair 1 Post - stain - baseline stain	6.14333	4.05083	1.16937	3.56956	8.71711	5.254	11	0.000	
3 Pair 1 Post - stain - baseline stain	11.75750	4.82075	1.39163	8.69454	14.82046	8.449	11	0.000	
4 Pair 1 Post - stain - baseline stain	17.14667	5.83174	1.68348	13.44135	20.85198	10.185	11	0.000	

Table 7: Pairwise comparison for surface roughness paired sample test

Group	Paired differences								
	Mean	Std. deviation	Std. error mean	95% Confidence interval of the difference		t	df	Sig. (2-tailed)	
				Lower	Upper				
1 Pair 1 Post SR - Baseline SR	-0.17167	0.37097	0.10709	-0.40737	0.06403	-1.603	11	0.137	
2 Pair 1 Post SR - Baseline SR	-0.82333	0.43935	0.12683	-1.10248	-0.54419	-6.492	11	0.000	
3 Pair 1 Post SR - Baseline SR	-0.67333	0.34445	0.09943	-0.89218	-0.45448	-6.772	11	0.000	
4 Pair 1 Post SR - Baseline SR	-0.46250	0.39306	0.11347	-0.71224	-0.21276	-4.076	11	0.002	

Group I - 70% white pomegranate extract gel, Group II - 80% white pomegranate extract gel, Group III - 90% white pomegranate extract gel, Group IV - 10% carbamide peroxide gel

carried out for up to 1 week because, in a clinical situation, the patients will be given a weekly review appointment to monitor their progress.¹⁷

CONCLUSION

Natural bleaching products are safer, more affordable, and easier to use when compared to synthetic options. An alternative for whitening the surface of teeth can be done with the use of white pomegranate extract gel with further clinical research. The present study was designed in such a way that the samples were subjected to different treatments to simulate the oral environment.

Ethical Approval

Institutional Ethics Committee of the Karpaga Vinayaga Institute of Dental Sciences (KIDS/IEC/2022/II/007).

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