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Comparative Analysis of Herbal Products for Color Change in Artificially Stained Teeth Using Spectrophotometer - An *In Vitro* Study

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Abstract

Aim: The study aims to compare the herbal products for color change in artificially stained teeth using a spectrophotometer.

Materials and Method: Baseline color values of 40 randomly selected artificially stained premolars were obtained. The specimens were randomly divided into four groups of 10 teeth each. Group 1 (n-10)- *Ananas comosus*, group 2- *Fragaria* x *ananassa*, group 3- Apple cider vinegar, control group 4% to 30% hydrogen peroxide. The color difference was examined under spectrophotometer.

Result: All the agents that have been tested showed a significant color change of which ananas comosus gives better result than *Fragaria* x *ananassa*.

Conclusion: Within the limitations of the study. *Ananas comosus* whitens the teeth better than *Fragaria* x *ananassa* and apple cider vinegar but lesser than hydrogen peroxide.

Keywords: Bleaching; Hydrogen peroxide; Spectrophotometer; *Ananas comosus*

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Introduction

Esthetic smile makeovers have become a common entity these days. Delivering a bright smile becomes a part of treatment protocol of such procedures. Many abnormalities of the oral cavity have been identified, one of which is discoloration and color changes of the teeth. Indeed, tooth discoloration is a major concern of dentists as it has a high prevalence in society. Consumers and patients have always had a strong desire for white teeth which has given rise to a growing trend in the increased use of 'over-the-counter' tooth whitening products. Teeth color is a combination of intrinsic color of the teeth and the presence of extrinsic stains that might accumulate on the tooth surface [1,2]. Extrinsic stains are linked with the adsorption of materials into the acquired pellicle on the enamel surface [3]. Factors influencing extrinsic stain formation include poor tooth brushing technique, smoking, areca nut chewing, dietary intake of colored foods (e.g., red wine, coffee and tea consumption), subject's age and the use of certain cationic agents such as chlorhexidine or metal salts like tin and iron [4-6].

Several corrective measures were introduced in the field of esthetic dentistry to treat discolored teeth. Laminate veneers, direct resin restorations, crowns or fixed prostheses were included in those corrective measures. A conservative treatment option i.e., dental bleaching is useful when restorative procedures are performed to correct color abnormalities. Teeth whitening products can be roughly classified into two main categories that include peroxide-containing bleaching agents and dentifrices. Bleaching agents can be used at home or done by a dentist. Typically, they are associated with tooth sensitivity and mild irritation of the soft tissue. On the other hand, dentifrices operate either by polishing, chemical chelation, or some other non-bleaching actions, so they are considered much milder but less effective [7-9].

Aim

The objective of this *in vitro* study is Compare and Analyze the Color change in herbal products in artificially stained teeth using spectrophotometer.

Materials and Method

Specimen collection

Forty maxillary and mandibular premolars extracted for orthodontic reasons were collected

from oral and maxillofacial surgery department of Rajasthan Dental College and Hospital. The teeth were later examined for visible cracks, caries defects, decalcifications, and any other developmental anomalies. The defective teeth were discarded. The selected teeth were cleaned of calculus using an ultrasonic scaler.

Staining of specimens

Artificial staining protocol used for the study was strictly followed as recommended by the Alofi et al. [10], the specimens were immersed in a Tea solution. The Tea solution was prepared with (TATA Tea) mixed in 500 ml water at boiling temperature. After waiting for 10 min to achieve drinking temperature, the specimens were immersed into the coffee for 24 h. Then, the teeth were thoroughly washed and stored at 37°C, 100% humidity. Baseline color of the tooth was analyzed using spectrophotometric analysis. The teeth were randomly divided into four groups of 10 teeth each, as follows:

Group 1: (n=10)-35% Hydrogen peroxide (Control Group)

Group 2: (n=10)-Apple cider vinegar

Group 3: (n=10)-Pineapple

Group 4: (n=10)-Strawberry

Preparation of strawberry juice and pineapple juice

The juice was prepared by scraping the pulp of strawberry and pine apple, followed by grinding. It was then weighed to 20 ml for bleaching. Apple cider vinegar (used as a food preservative, in cooking purpose) and Hydrogen Peroxide (used for dental purpose) were available commercially.

Bleaching protocol

Twenty mL of solution is taken from each group, and then the specimens are then engrossed in the extracts for 6 h in all the four groups, and the post color change was analyzed [11] (Figures 1-9).

Testing method

In this study UV-Vis reflectance spectrophotometer is used to test the whitening effect of stained teeth. After immersion of 6 h in the solution the teeth were fixed in a small plate of spectrophotometer one by one. Plate containing Barium Sulphate (Barium sulphate is a soft crystalline solid made by reacting barium hydroxide and other barium source with sulfuric acid and has a long history as a translucent white pigment. It has a high light scattering property) kept in spectrophotometer to check the post color change on the screen [12].

The testing was done with reflectance spectrophotometer over a white background, which recorded color variables L*, a*, b* in accordance to CIEL*a*b color system using a formula:

$$\Delta E = ([\Delta L^*]\ 2+\ [\Delta a^*]\ 2+\ [\Delta b^*]\ 2)^{½}$$

Results

Post hoc analysis

The mean color change in the strawberry juice group was 1.365 ± 0.627, in the Apple cider-4-Abs was 0.920 ± 0.935, in the hydrogen peroxide group was 0.091 ± 0.052 and in the pineapple group it was 0.462 ± 0.366. The difference between the three groups was statistically significant when analyzed using the One Way ANOVA. The pair wise intergroup comparison was done using the independent T test and post hoc analysis the intergroup comparison was significant between strawberry *vs.* apple cider, strawberry *vs.* hydrogen peroxide, apple cider *vs.* pineapple and pineapple *vs.* hydrogen peroxide. The intergroup comparison of strawberry *vs.* pineapple, apple cider *vs.* hydrogen peroxide, apple cider *vs.* pineapple was statistically non-significant (p=0.001).

Figure 1: Sample for study.

Figure 2: Tea solution for study.

Figure 3: Stained sample.

Figure 4: Hydrogen Peroxide.

Statistical analysis

The data for the present study was entered in the Microsoft Excel 2007 and analyzed using the SPSS statistical software 23.0 Version. The descriptive statistics included mean, standard deviation. The level

Figure 5: Apple cider vinegar.

Figure 6: Pineapple juice.

of the significance for the present study was fixed at 5%.

The intergroup comparison for the difference of mean scores between independent groups was done using the One Way ANOVA and post hoc analysis. The intergroup comparison was done using the independent t test.

The Shapiro-Wilk test was used to investigate the distribution of the data and Levene's test to explore the homogeneity of the variables. The data were found to be homogeneous and normally distributed. Mean and Standard Deviation (SD) were computed for each variable.

Mean

$$\overline{X} = \frac{\Sigma X}{N}$$

Where:

$\overline{X}$ = the data set mean

Σ = the sum of

X = the scores in the distribution

N = the number of scores in the distribution

Range

$$Range = X_{highest} - X_{lowest}$$

Where:

$X_{highest}$ = largest score

X_{lowest} = smallest score

Variance

$$SD^2 = \frac{\Sigma\left(X - \overline{X}\right)^2}{N}$$

The simplified variance formula

Figure 7: Strawberry juice.

Figure 8: Sample for study.

Figure 9: Spectrophotometer.

$$SD^2 = \frac{\Sigma X^2 - \frac{\left(\Sigma X\right)^2}{N}}{N}$$

Where:

SD^2 = the variance

Σ = the sum of

X = the obtained score

$\overline{X}$ = the mean score of the data

N = the number of scores

Standard Deviation (N)

$$SD = \sqrt{\frac{\Sigma\left(X - \overline{X}\right)^2}{N}}$$

The simplified standard deviation formula

$$SD = \sqrt{\frac{\Sigma X^2 - \frac{\left(\Sigma X\right)^2}{N}}{N}}$$

Where:

SD = the standard deviation

Σ = the sum of

X = the obtained score

$\overline{X}$ = the mean score of the data

N = the number of scores

One way ANOVA

The formula for the one-way **ANOVA** F-test statistic is

$$F = \frac{\text{between-group variability}}{\text{within-group variability}}$$

The between-group variability" is

$$\sum_{i=1}^{K} n_i \left(\overline{Y}_{i\cdot} - \overline{Y}\right)^2 / (K-1)$$

where Y_i denotes the sample mean in the i^{th} group, n_i is the number of observations in the i^{th} group, $\overline{Y}$ denotes the overall mean of the data, and K denotes the number of groups.

The "within-group variability" is

$$\sum_{i=1}^{K}\sum_{j=1}^{n_i}\left(Y_{ij} - \overline{Y}_{i\cdot}\right)^2 / (N-K),$$

where Y_{ij} is the j^{th} observation in the i^{th} out of K groups and N is the overall sample size.

Discussion

Beauty and perfect appearance have a great value in the society. The “perfect smile” is important to many people including straight and light-colored teeth.

The lightning of the color of a tooth through an application of chemical agent to oxidize the organic pigmentation in the tooth is Bleaching. A great variety of etiologies are responsible for tooth discoloration. Discoloration is any change in the hue, color and translucency of a tooth due to any cause.

According to Jorge Rodriguez-Martinez tooth discoloration strongly depends on how the light is absorbed or reflected by its surface. In general tooth stains are classified according to their location in intrinsic or extrinsic discoloration [1].

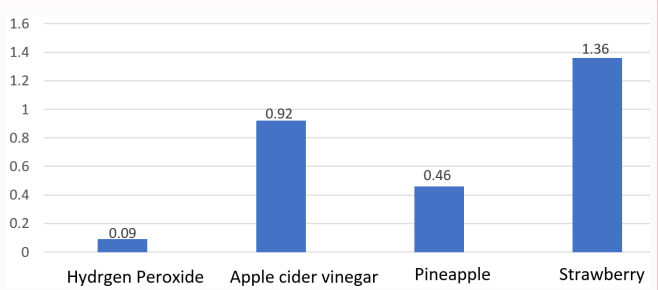
A study done by Li and Greenwell has proved various negative effects of hydrogen peroxide like tooth sensitivity, gingival irritation, potential adverse effects on enamel and restorations [13,14] because of ionic dissociation of HP releases free radicals namely nascent oxygen, per-hydroxyl, hydroxyl radical and superoxide anions [15,16]. The consequent shift of the visible absorption spectrum of compound from a longer to a shorter wavelength (a colorless compound) forms the basis of whitening/bleaching action on the substrate. However, the permeability of enamel to small ions (Atkinson HF 1947), results

Table 1: Intergroup comparison between the four groups using one way ANOVA.

Group	N	Mean ± Std. Deviation
Group 1 (Hydrogen Peroxide)	10	0.09 ± 0.05
Group 2 (Apple cider vinegar)	10	0.92 ± 0.36
Group 3 (Pineapple)	10	0.46 ± 0.93
Group 4 (Strawberry)	10	1.36 ± 0.62

Table 2: Post Hoc analysis between inter and intra groups.

	Mean Square	df	F	P-value
Between Groups	3.05	3	8.68	0.00 (Sig)
Within Groups	0.35	36		
Total		39		



Graph 1: Obtained results were collected and analyzed statistically using ONE WAY ANOVA for multiple group comparison.

in the formation of peroxide apatite which shows hydrogen peroxide is strong bleaching agent [17,18].

The purpose of our study was to compare the efficacy of three herbal products (20 ml of Strawberry juice, Pine apple juice, Apple cider vinegar) with 35% Hydrogen Peroxide to analyze the color change in artificially stained teeth using spectrophotometer, so as to overcome the drawbacks of previously used bleaching agents [19].

In the present study, 40 premolars (maxillary and mandibular) extracted for orthodontic reasons were collected and subjected to study as explained (in detail) in materials and method section [20,21].

Obtained results were collected and analyzed statistically using ONE WAY ANOVA for multiple group comparison followed by Post hoc analysis between inter and intra groups (Tables 1-3, Graph 1).

The mean color change in the control Group-1 (Hydrogen Peroxide) was 0.09 ± 0.05, Group 2 (Apple cider vinegar) was 0.92 ± 0.93, Group-3 (Pineapple) was 0.46 ± 0.36, Group-4 Strawberry juice was 1.36 ± 0.62.

The difference between the three study groups was statistically significant when analyzed using the One Way ANOVA (Table 1).

There was statistically significant (P=0.001) difference between control Group-1 (Hydrogen Peroxide) and Group-2 (Apple Cider vinegar). Whereas there was not statistically significant difference between Group-3 (Pineapple Juice) P=0.102.

It’s statistically proven that Group-3 (Pineapple) gives a non-significant result with Group-1 (Hydrogen Peroxide) P=0.00 whereas it was statistically significant with Group-2 (Apple Cider Vinegar) P=0.09 and Group-4 (Strawberry Juice) P=0.00.

So, the result defines no significant difference between Control Group (Hydrogen Peroxide) and Pineapple juice and also shows the significant difference between Group-3 (Pineapple juice) with Group-2 (Apple cider vinegar) and Group-4 (Strawberry juice).

The result of present study has demonstrated that among all the four tested groups, the mean color change for control group (35% Hydrogen Peroxide) is highest followed by pineapple juice and Strawberry juice was the least [22].

In this study, vegetative enzyme extracted from pineapple was used. Pineapple contains bromelain, catalase, and polyphenol peroxidase. At this pH, the extract obtained from pineapple causes disruption of adhered proteins causing stains. Bromelain present in this extract acts as a predominant oxidizing agent [23].

Our results are in agreement with the study of Chitra Janardhanan, Vejai Vekaash to evaluate the color change in human enamel

Table 3: Post hoc analysis.

Inter Group Comparison		Mean Difference	Std. Error	P value
H_2O_2-3-Abs	Strawberry	1.27	0.265	0.001 (sig)
	Apple cider-4-Abs	0.82	0.265	0.004 (Sig)
	Pineapple	0.37	0.265	0.17 (non-Sig)
Apple cider vinegar-Abs	Strawberry	0.90	0.265	0.10 (non-Sig)
	H_2O_2-3-Abs	0.37	0.265	0.00 (Sig)
	Pineapple	0.45	0.265	0.09 (Sig)
Pineapple	Strawberry	0.44	0.265	0.00 (Sig)
	Apple cider-4-Abs	0.45	0.265	0.09 (Sig)
	H_2O_2-3-Abs	0.37	0.265	0.17 (non-Sig)
Strawberry	Apple cider-874-Abs	0.90	0.265	0.10 (non-Sig)
	H_2O_2-3-Abs	1.27	0.265	0.00 (Sig)
	Pineapple	0.44	0.265	0.00 (Sig)

bleached with three different concentrations of hydrogen peroxide, containing pineapple extract as an additive in two different timings, using reflectance spectrophotometer, which results that all the groups and subgroups evaluated in the study, the mean values obtained with the use of pineapple extract along with hydrogen peroxide showed statistically significant whitening when compared to the specimens that were bleached only with hydrogen peroxide ($P<0.05$). The results obtained in the study can be directly attributed to the role of enzyme extracts obtained from pineapple. The proteolytic enzyme bromelain has played a major role in bleaching process, by removing or disrupting the protein portion of the pellicle layer adhered to the tooth surface.

Conclusion

The aim of this *in vitro* study was to compare and analyze the color change in herbal products in artificially stained teeth using spectrophotometer. Within the limitation of this study all the tested solution in the current study has shown a significant color change. Amongst this pineapple juice can be used as a substitute for conventional bleaching agents which has shown maximum variances when compared to other herbal products. Although the effect of bleaching pineapple juice was inferior to hydrogen peroxide, the caustic effect was very less or no effect. The presence of proteolytic enzyme bromelain has played a major role in bleaching process, by removing or disrupting the protein portion of the pellicle layer adhered to the tooth surface and helps to maintain the health without compromising it.

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