



## Comparative Efficacy of Pineapple Extract and Lemon-Salt Solution as Natural Tooth Bleaching Agents - In Vitro Comparative Study

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### ABSTRACT:

**Introduction:** People are often driven by various motivations to whiten their teeth, with the primary goal being cosmetic enhancement. Many individuals aim to stand out by flaunting a brilliant smile and often opt for professional teeth bleaching, as it offers proven and pleasing aesthetic results through conservative methods.

**Objectives:** The principal objective of the present in vitro comparative study is to evaluate how effectively the pineapple extract and lemon-salt solution work as natural bleaching agents for teeth.

**Methods:** Thirty-six extracted permanent teeth were obtained, and after ultrasonic cleaning, the teeth were artificially stained by immersing them in coffee decoction for 48 hours. Readings of tooth color were evaluated using a camera RGB color picker application on an Android mobile device with a 64 MP camera. Then, the teeth were divided into three groups, each comprising 12 samples. Group 1 teeth were immersed in distilled water, Group 2 in pineapple extract, and Group 3 in lemon-salt solution for three days. The teeth color assessment test was repeated. Then, the difference was assessed by statistical tests, one-way ANOVA, and Tukey post hoc tests for intergroup comparisons. The Wilcoxon test was employed to measure the pre and post-test lightness of the teeth.

**Results:** The Wilcoxon test revealed significant differences ( $P < 0.05$ ) in brightness values before and after immersion in Group 2 and Group 3. The Tukey HSD test exposed a significant difference between Group 1 and Group 3, indicating that the lemon-salt solution resulted in a significantly ( $P < 0.05$ ) higher whitening degree than the other groups' comparisons.

**Conclusions:** In the present study, we emphasize the effectiveness of organic teeth-whitening agents like pineapple extract and lemon-salt solution. As a result, once these substances are standardized, they have the potential to become essential elements in regular products like toothpaste and other oral care items.

### 1. Introduction

There are various motivations behind people's desire to whiten their teeth, with cosmetic enhancement being the primary driver. Like individuals seeking a lighter skin complexion, many also aspire to showcase a dazzling smile to distinguish themselves from others [1]. In this context, professional teeth bleaching is preferred for those seeking whiter teeth, delivering proven and pleasing aesthetic results through conservative methods.

The effectiveness of bleaching procedures hinges mainly on identifying the source of color alteration, typically categorized as either extrinsic or intrinsic [2].

Consuming specific foods and beverages like coffee, red wine, or tobacco products causes extrinsic teeth staining. Inadequate oral hygiene practices can also contribute to extrinsic staining. Intrinsic staining is caused by pulp bleeding, which allows blood to enter the dentinal tubules and decompose, which can lead to the deposition



of chromogenic blood degradation products such as hemosiderin and hemin. Other potential causes of intrinsic staining include the systemic use of tetracycline antibiotics and pulp necrosis [3].

After pinpointing the cause of the staining, dentists can customize a therapeutic plan for each patient, considering their expectations, dental condition, and oral health. Typically, tooth bleaching occurs either in the dental office, utilizing more potent gels and shorter application times (in-office technique), or at home, with the dentist's oversight, using milder gels and more extended application periods (at-home technique), or sometimes a combination of both approaches. In these treatment protocols, gels containing hydrogen or carbamide peroxides represent the most common choice [2].

Throughout the bleaching process, precise control over the degree of whitening is challenging for both the patient and the dentist, leading to the potential for both excessive and insufficient bleaching. Moreover, there exists a risk of harming the soft tissues due to the corrosive properties of the highly concentrated peroxide used. Additionally, postoperative tooth sensitivity poses a higher risk with this technique. Tooth sensitivity has been recognized as a significant side effect of tooth-bleaching agents, alongside other potential side effects such as cervical root resorption, crown fracture, mucosal reactions, and alterations to the enamel surface [4].

Efforts to reduce the potential risks and expenses associated with teeth-whitening agents have led to the exploration of readily available natural products for brightening teeth. Nevertheless, these products are utilized without established scientific evidence to support their safety or effectiveness. Natural remedies include activated charcoal, apple cider vinegar, baking soda, banana peels, coconut oil, lemon and strawberry purees [5]. Our current research aims to evaluate how effective pineapple extract and lemon salt solution can be used as natural tooth-bleaching agents.

## 2. Objectives

The principal aim of the present in vitro comparative study is to evaluate how effectively the pineapple extract and lemon-salt solution work as natural bleaching agents for teeth.

## 3. Methods

The present in vitro comparative study was approved by the Institutional Scientific Research Committee on March 1, 2023. The study was extended six months from March 1 till August 31, 2023.

### Specimen collection

In this research, 36 permanent teeth were cleaned using an ultrasonic scaler to eliminate calculus, debris, stains, and any remaining soft tissue.

### Inclusion Criteria

Teeth free from caries and cracks and extracted due to periodontal issues were considered for inclusion in the study.

### Exclusion Criteria

Teeth affected by enamel hypoplasia, fluorosis, caries, fracture or affected with drug-induced extrinsic or intrinsic stains were excluded to eliminate the confounding factors.

### Specimen staining

The collected natural teeth were stored in distilled water for future use. The teeth underwent artificial staining through the use of a coffee decoction. The decoction was prepared by adding 200 mg of robusta coffee powder into 100 ml of deionized boiling water at 100 °C. After the mixture had cooled to room temperature, it was filtered and placed in a 100 mL glass beaker. All 36 teeth chosen for the study were submerged in this prepared staining solution for 48 hours. Subsequently, they were thoroughly rinsed with water and stored at room temperature under 100% humidity conditions for future use.

### Color Assessment

The color assessment was performed using an RGB camera color picker application on an Android mobile device with a 64 MP camera from a 12 cm distance, and the investigator was unaware of the treatment solutions or groups to avoid bias. RGB values were recorded at three points on the facial surface (incisal/occlusal third, middle third, cervical third). The mean value of the three points was calculated and then converted to CIE lab values using the Colorizer website.



## Experimental groups

A total of 36 Extracted permanent teeth were collected and divided into three groups, each comprising 12 samples (Table 1). Pre-immersion tooth color readings were assessed and recorded. Group 1 teeth were immersed in distilled water, Group 2 in pineapple extract, and Group 3 in lemon-salt solution for three days. Post-immersion tooth color readings were evaluated and compared with pre-immersion readings.

### Preparation of Pineapple Extract

A single ripe pineapple was peeled and cut into small cubes. Subsequently, 250 grams of the pineapple, without any additional water, were placed in a blender and blended until a consistent liquid mixture was obtained. This resulting mixture was centrifuged at a rate of 2000 revolutions per minute for 4 minutes while maintaining a temperature of 4 °C. Then the clarified liquid was separated by filtration and carefully preserved in an air-tight container.

### Preparation of Lemon and Salt Solution

Three fresh lemons were cut into halves and the juice (30 ml) was squeezed into a container to which one teaspoon of sea salt was added and stirred thoroughly till the salt was completely dissolved. This solution was diluted by adding a small amount of distilled water and filtered using a strainer to remove undissolved salt particles. The final solution was stored in an air-tight container at room temperature.

### Bleaching

Teeth were immersed in pineapple extract and lemon-salt solution for three days, with the solution changed every 24 hours. Color measurements were taken similar to the stained teeth.

### Whitening Measurement

The color assessment was expressed as E values. The difference between initial (E1) and final (E2) color assessments represented the  $\Delta E$  value, expressed in terms of lab values (L for brightness, a for redness to greenness, b for yellowness to blueness).  $\Delta E$  was calculated through the formulation:  $\Delta E = ((\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2)^{1/2}$ .

## Data Collection

The thirty-six collected teeth samples were subjected to whitening procedures, and measurements were taken before (L1) and after (L2) immersion in the test and control groups. The whitening degree ( $\Delta E$ ) was calculated based on these measurements.

## 4. Results

The one-way ANOVA test evaluated the significance of color change between the groups (Table 2).

The one-way ANOVA test presented a significant difference among the groups ( $P < 0.05$ ), indicating that there were variations in the whitening degree ( $\Delta E$ ) among the test and control. Since the P-value is smaller, it strongly supports the alternate hypothesis.

### Tukey HSD Test

To assess and further compare the statistical differences between pairs of means of whitening degree, the Tukey Honest Significant Difference test was performed (Table 3).

The Tukey HSD test exposed a significant difference between Group 1 and Group 3, indicating that the lemon-salt solution (Group 3) resulted in a significantly ( $P < 0.05$ ) higher whitening degree compared to distilled water (Group 1).

### Wilcoxon Test for Brightness

Wilcoxon test was applied to compare the pre and post-immersion lightness values in test and control groups (Table 4).

The Wilcoxon test results revealed significant differences ( $P < 0.05$ ) in brightness values before and after immersion in Group 2 and Group 3, indicating that pineapple extract (Group 2) and lemon-salt solution (Group 3) significantly affected brightness. There was no significant difference in (Group 1) the teeth immersed in distilled water.

In summary, the study revealed that Group 3 (lemon-salt solution) resulted in the highest whitening degree ( $\Delta E$ ) among the test groups (Table 5&6). Group 2 (pineapple extract) also showed a significant difference. Group 1 (distilled water) had the least whitening degree with a significant difference compared to groups 2 and 3.

**Table 1: Experimental Groups:**

Experimental Groups of Teeth	Test solutions
Group 1	Distilled Water
Group 2	Pineapple Extract
Group 3	Lemon-Salt Solution

**Table 2: Results of One-Way ANOVA**

Source	DOF	SOS	MS	F Stat	P-Value
Groups	2	462.45	231.22	3.73	0.03

DOF - Degrees of Freedom; SOS - Sum of Squares; MS - Mean Square

**Table 3: Tukey HSD Test for Post-Hoc Comparison**

Pairwise Comparisons	Means of $\Delta E$ Difference	P-Value
Group 1 vs. Group 2	6.45	0.24460
Group 1 vs. Group 3	8.72	0.02775
Group 2 vs. Group 3	3.45	0.53659

Whitening degree  $\Delta E$  value - the difference between initial (E1) and final (E2) color assessments

**Table 4: Wilcoxon Test Results for Brightness**

Test Groups (N)	L1 (Pre-test) Mean $\pm$ SD	L2 (Post-test) Mean $\pm$ SD	P-Value
1 (12)	58.3 $\pm$ 6.05	59.1 $\pm$ 4.32	0.68
2 (12)	66.8 $\pm$ 5.18	74.6 $\pm$ 4.87	0.001
3 (12)	68.4 $\pm$ 4.47	77.7 $\pm$ 5.90	0.009

SD - Standard Deviation

## 5. Discussion

Teeth whitening is achieved by an oxidation process in which the staining molecules are broken down through a chemical reaction with oxygen radicals produced by hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) [6]. While hydrogen peroxide,

at concentrations ranging from 10% to 38% [7], can be applied at a dental office or home to attain impressive results, it is essential to note that specific clinical side effects are associated with its use. These include tooth sensitivity [8], irritation of the gums [9], mucosal hyperplasia/irritation in the gastrointestinal tract [10], alterations in dentin and enamel surface structure, texture, composition, and microhardness, as well as various effects on existing dental restorations such as composites [11], amalgams [10], and IRM in the patient's mouth. These effects are unavoidable [12,13] while using hydrogen peroxide.

**Table 5: Summary of whitening degree**

Test Groups	Pre-test Color ( $\Delta E1$ ) Mean $\pm$ SD	Post-test Color ( $\Delta E2$ ) Mean $\pm$ SD	Effect ( $\Delta E$ ) Mean $\pm$ SD
Group 1	58.33 $\pm$ 6.05	59.05 $\pm$ 4.32	2.83 $\pm$ 1.86
Group 2	66.75 $\pm$ 5.18	74.62 $\pm$ 4.87	9.31 $\pm$ 1.32
Group 3	68. $\pm$ 4.468	77.69 $\pm$ 5.90	13.9 $\pm$ 1.86

Whitening degree  $\Delta E$  value - the difference between initial (E1) and final (E2) color assessments

**Table 6: Summary of results**

Statistical Analysis	Results
One-way ANOVA	Significant difference among groups (P<0.05)
Tukey HSD test	Group 1 vs Group 3 (P<0.05)
Wilcoxon test	Significant differences in brightness values (P<0.05)

While professional teeth whitening products have demonstrated their superior effectiveness in tooth bleaching over the years, there remains uncertainty regarding their biological safety and the assurance of using them without adverse effects. To address the safety concerns, herbal teeth whitening products have recently increased in popularity. These herbal alternatives are effective, biologically safe, have fewer adverse effects, and are cost-effective [14].

The staining of teeth, a common concern among individuals seeking a brighter smile, was induced using



the coffee solution. Tannic acid, found in roasted coffee, possesses a potent denaturing capability that affects the pellicle protein, ultimately enhancing its susceptibility to staining. Furthermore, Robusta coffee is enriched with chromogenic compounds. When the pellicle forms, saliva proteins form selective bonds with the enamel surface through calcium bridges, and subsequently, chromogens engage with the pellicle through hydrogen bridges. These chromogens become incorporated into the pellicle, and the inherent hue of the chromogen itself determines the resulting color [15].

In this study, we examined the efficacy of natural bleaching agents, specifically pineapple extract and lemon-salt solution, compared to distilled water as a response to the increasing demands for non-invasive and natural teeth-whitening methods.

Bromelain, the enzyme present in pineapple, shows the following functions: anti-inflammatory, anti-edematous, antibiotic, fibrinolytic, anticoagulative, anticancer, antithrombotic, assists potential postoperative healing and decreases post-surgical discomfort and swelling. In addition to the above functions, several studies have demonstrated the tooth-whitening properties of bromelain when added as an additive to hydrogen peroxide [6,14].

Lemons contain essential nutrients such as vitamin C, magnesium, potassium, and calcium. They also possess teeth-whitening properties due to their malic acid content. Malic acid, a dicarboxylic acid found in lemons, can brighten teeth by oxidizing the tooth enamel's surface. Lemon's acidity is relatively high, with a pH ranging from 2 to 3, which is considerably lower than the critical pH of enamel (pH 5.5). This acidic nature can lead to demineralization, making teeth appear whiter [16]. Calcium carbonate in marine salt is responsible for its whitening effect, as it works to eliminate extrinsic stains through a mechanical abrasive action [17]. Research has confirmed that when used consistently during regular brushing routines, sea salt and lemon-based toothpaste can demonstrate teeth-whitening benefits [18,19].

Our experimental design involved immersing stained tooth samples into distilled water (control group), pineapple extract, and a lemon-salt solution. The immersion period was extended for three days, with daily replacement of the solution to maintain the freshness and

effectiveness of the treatment. The significant results observed in both the pineapple extract and lemon-salt solution groups, when compared to the control group, suggest the potential of these natural bleaching agents in tooth whitening. These findings align with previous research on the components of these solutions.

While our study provides promising results, it is essential to acknowledge some limitations. Firstly, this study was conducted *in vitro*, and the findings may not directly translate to *in vivo* situations. Further research involving clinical trials is essential to validate these results and assess their safety and efficacy in real-world settings. Additionally, the potential long-term effects and tooth sensitivity associated with these natural bleaching agents should be considered in future investigations.

## 6. Conclusion

The present research underscores the effectiveness of natural teeth-whitening agents such as pineapple extract and lemon-salt solution. Consequently, once their standardization is achieved, these natural substances can serve as crucial components in everyday products like toothpaste and other dental hygiene products. This study catalyzes advocating using natural food-based ingredients for teeth whitening, contributing to a more environmentally friendly approach. It also opens the door for future investigations involving diverse biological agents.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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