

RESEARCH ARTICLE

Spectrophotometric Analysis of Color Changes in Enamel Following Exposure to Herbal Bleaching Agents: An *In Vitro* Study

¹Jyothi Lekshmi, ²Saranya S Nair, ³Rajesh Pillai, ⁴Nettiyath O Varghese, ⁵Afzal A Salim, ⁶Nikhil Murali

ABSTRACT

Aim: This study aims to evaluate the color change in enamel bleached with herbal bleaching agents using a spectrophotometer.

Materials and methods: 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)-Musa sapientum, group 2-citrus sinensis, group 3-citrus limon, control group 4 to 35% hydrogen peroxide. The color difference was examined using spectrophotometer

Results: All the agents that have been tested showed a significant color change since ΔE is more than 3.3, of which Musa sapientum gives the best result as compared to citrus groups.

Statistical analysis: The results were statistically analyzed using the Mann-Whitney test.

Conclusion: Within the limitations of this study, Musa sapientum whitens the teeth better than citrus sinensis and citrus limon but lesser than hydrogen peroxide.

Keywords: Citrus limon, Citrus sinensis, Hydrogen peroxide, Musa sapientum, Spectrophotometer, Tooth bleaching.

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INTRODUCTION

Tooth enamel is composed of minerals (96%) and organic matters and water (4%). Mineral crystals are opaque with organic proteins present between them. Consequently, pigments may soak into this space.¹ Tooth whitening has

been consistently implemented since its introduction. Dental bleachers currently used in dental clinics contains carbamide peroxide, sodium perborate and hydrogen peroxide that might cause side effects such as root resorption, dentin sensitivity and stomatitis.²

Tooth whitening (tooth bleaching), is either the restoration of a natural tooth shade or whitening beyond the natural shade of tooth. It is the lightening of the color of a tooth through the application of a chemical agent to oxidize the organic pigmentation in the tooth. Restoration of the underlying natural tooth shade is possible by simply removing surface stains caused by extrinsic factors or stainers such as tea, coffee, tobacco and other carbonated drinks.

Bleaching is a suggested treatment modality in cosmetic dentistry. Various agents and techniques for vital bleaching have entered the market; the primary ingredient of these agents revolve around the chemistry of hydrogen peroxide. In the present study, hydrogen peroxide is taken as the control group. Common side effects associated with bleaching include increased sensitivity of the teeth and irritation of the gums, which could be avoided with the use of natural/herbal bleaching agents.

Hydrogen peroxide and its precursor carbamide peroxide have been used in various concentrations ranging from 10 to 40% to achieve the desired results. Although hydrogen peroxide provides outstanding results, the related clinical side effects remain inevitable. Change in surface texture, composition, and microhardness of enamel are reported when bleaching is performed with hydrogen peroxide.^{3,4}

Recently, over-the-counter (OTC) bleachers have been popularized. Whitening toothpaste and patches containing hydroxyapatite and peroxide compounds are generally used. However, chemically synthesized dental bleachers might have side effects if are wrongfully used. There has been mounting interest in alternative whitening using vegetables and fruits as sources of natural dental bleachers.⁵ In the present study, bleaching efficiency of musa sapientum (ripe banana) is compared with the citrus group and hydrogen peroxide. The whitening effect of citron has been substantiated in previous studies.

^{1,2}Postgraduate Student, ³HOD, ⁴Principal and Professor, ⁵Professor, ⁶Senior Lecturer

¹⁻⁶Department of Conservative Dentistry and Endodontics, PMS College of Dental Science and Research, Trivandrum, Kerala, India

Corresponding Author: Jyothi Lekshmi, Postgraduate Student, Department of Conservative Dentistry and Endodontics, PMS College of Dental Science and Research, Trivandrum, Kerala, India, e-mail: drbjyothilekshmi@gmail.com

Citrus groups contain many compounds like Tange-raxanthin, Tangeritin, Terpinen-4-ol, Terpinolene, Tetra-decanal, Threonine, Thymol, Thymyl-methyl-ether, Tryptophan, Tyrosine, Cis-3-hexanol, Cis- carveol, Citric-acid, Citronellal, Citronellic-acid, Citronellyl-acetate, Cystine, Decanal, Decanoic- acid, Decanol, Nobiletin which is attributed to the whitening action of citrus.⁶

Presence of high amounts of potassium, magnesium, and manganese in the ripe banana which indirectly potentiates the effect on bleaching of teeth but there is no scientifically validated evidence to prove the above, which we have corroborated in this study.

AIM

This study aims to evaluate the color change in enamel bleached with herbal bleaching agents using a spectro-photometer.

MATERIALS AND METHODS

Specimen Collection

Forty maxillary and mandibular premolars extracted for orthodontic reasons were collected. The teeth were later examined for visible cracks, caries defects, decalcifica-tions, and any other developmental anomalies (Fig. 1) The defective teeth were discarded. The selected teeth were cleaned of calculus using an ultrasonic scaler and were stored in 0.2% thymol, refrigerated at 4°C until use.

Staining of Specimens

Artificial staining protocol used for the study was strictly followed as recommended by Sulieman et al.⁷ A 2 g tea bag (Tetley, Bengaluru, India) was suspended in 100 mL of boiling water. After 5 minutes, the solution was cooled to room temperature. The tooth was stained by immersing it for 24 hours in the solution (Fig. 2). Then, the

teeth were thoroughly washed and stored at 37°C, 100% humidity.

Baseline color of the tooth was analyzed using spec-trophotometric analysis.

The teeth were randomly divided into four groups of 10 teeth each, as follows:

- Group 1: (n = 10)–Musa sapientum
- Group 2: (n = 10)–Citrus sinensis
- Group 3: (n = 10)–Citrus limon
- Group 4: (n = 10)–35% hydrogen peroxide (control group)

Preparation of Musa Sapientum Extract

The extract was prepared by scraping the inner surface of the peel, followed by grinding. It was then weighed to 5 gm and finally diluting it with 100 mL of distilled water (Fig. 3).

Extraction of Orange and Lemon Peels

Orange and lemon peel were separated from the fruit and were amended into fine pieces. Then these portions were placed on a preheated oven at 50°C and paused until it develops dryness. They were then ground, the solutions were then primed by taking 5 g of all these extracts and are diluted with 100 mL of distilled water (Figs 4 and 5).

This was done in order to standardize the concentra-tion of the extracts prepared as 5%.

The pH of the solution was then measured using pH meter (Fig. 6). The pH of Musa sapientum is 6.4, citrus sinensis is 6.6, and citrus limon is 6.2, whereas the pH of hydrogen peroxide is 2.3.

Bleaching Protocol

Twenty mL of solution is taken from each group, and then the specimens are then engrossed in the extracts for 6 hours in all the four groups, and the post color change was analyzed.



Fig. 1: Samples used for study



Fig. 2: Stained samples

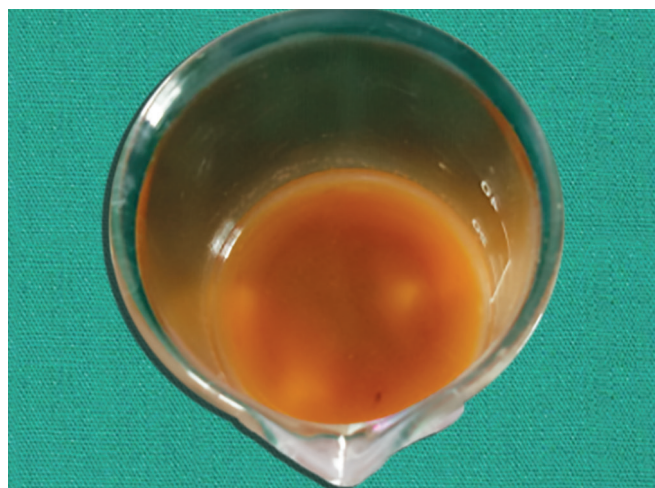


Fig. 3: Extract of musa sapientum

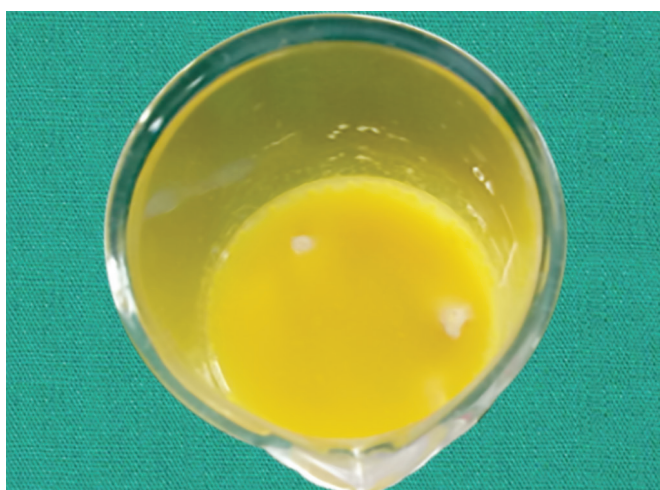


Fig. 4: Extract of citrus sinensis



Fig. 5: Extract of citrus limon



Fig. 6: PH meter



Fig. 7: Spectrophotometer

Testing Methodology

The testing was done with spectrophotometric analysis (Fig. 7), which recorded color variables L*, a*, b* in accordance to CIEL*a*b color system using a formula:

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

RESULTS

The results of this *in vitro* study are given in Table 1 and were plotted graphically (Graph 1).

Statistical Analysis

Mann-Whitney test was performed to effectively verify the pre-post color difference within and amongst the samples.

DISCUSSION

The yearning to have white teeth and thus a more pleasant smile has become an important esthetic need of patients today. Management protocol depends on the etiology, preliminary from the most conservative procedure bleaching to extensive partial coverage veneer.^{8,9}

Although a wide arena of esthetic restorative materials is available for the management of discolored anterior teeth, bleaching still remains a viable option in assured cases. Many factors affect the treatment outcome including patient and clinician-related factors. The patient-related factors involve the patient's needs, age, expectations, and affordability. The clinician-related factors are the availability of bleaching materials and a thorough knowledge of the material science, including methodologies and techniques involved.^{10,11}

Commercially offered bleaching agents contain a wide spectrum of the concentration of hydrogen peroxide or its precursor carbamide peroxide to suit individual clinical needs. It is obvious that higher concentration of hydrogen peroxide is always be tagged with its own side effects.

Commonly known local risks associated with tooth bleaching include primarily tooth sensitivity, gingival irritation, as well as potential adverse effects on enamel and restorative materials.¹² Hydrogen peroxide at a microscopic level, causes surfaces roughness. On prolonged exposure, it destroys the organic component by disrupting the intraprismatic and interprismatic enamel.¹³ It also penetrates deep into the dentine surface thus causing postoperative sensitivity. In addition, it causes

irritation or burns to the soft tissues in the oral cavity.¹³ Hence, there is always a quest to find alternatives to hydrogen peroxide.

Hence, this *in vitro* investigation was taken up to evaluate the efficacy of use of fruit extracts for bleaching human enamel.

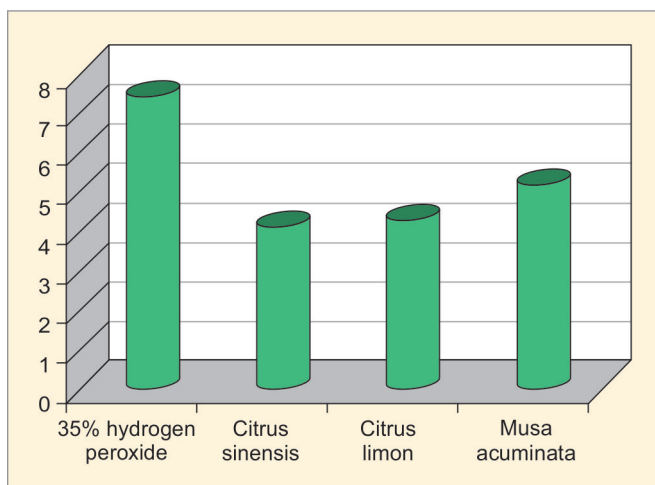
The CIELAB¹⁴ system was used to measure of the color difference in bleached teeth. This system is a 3D uniform color space with equal distances corresponding to equally perceived color differences. This system has three axes: The L* axis represents lightness and extends from 0 (black) to 100 (white); and a* and b* represent the redness-greenness and yellowness-blueness axis, respectively. When a* and b* coordinates approach zero, the colors become neutral.

Even though colorimeter is another alternative mode to evaluate the color change, it has its inherent drawback of not being able to detect color in the curved surface like tooth structure. Also, it has been established that spectrophotometer demonstrates a high level of accuracy and reproducibility. The color change values obtained from the spectrophotometer were analyzed on the basis of CIE values. The use of L*a*b values were recommended by CIE. The reason being these values were used in the study because they are more close to human perception.¹⁵

In this study, all the agents tested showed a significant color change since ΔE is more than 3.3, of which Musa sapientum gives the best result as compared to citrous groups. Amazing minerals along with the whitening capabilities of astringent salicylic acid in musa peels effectively brightens teeth without wearing down the enamel. Mineral content of musa peel is given below¹⁶ (Table 2).

High amounts of potassium present in the extract decreases tooth sensitivity and manganese helps to form a hard tooth enamel and fights off tooth decay.

The previous study by Lee⁵ has shown that citrus limon and citrus reticulata also showed significant color change whose results are in accordance with our study.



Graph 1: Color change following bleaching

Table 1: Mean delta E value

Solution	Mean ΔE value
35% Hydrogen peroxide	7.4
Citrus sinensis	4.1
Citrus limon	4.3
Musa acuminata	5.2

Table 2: Mineral content of banana

Element	Concentration (mg/g)
Potassium	78.10 \pm 6.58
Calcium	19.20 \pm 0.00
Sodium	24.30 \pm 0.12
Iron	0.61 \pm 0.22
Manganese	76.20 \pm 0.00
Bromine	0.04 \pm 0.00
Rubidium	0.21 \pm 0.05
Strontium	0.03 \pm 0.01
Zirconium	0.02 \pm 0.00
Niobium	0.02 \pm 0.00

Citric acid compound in citrus was known for its ability in whitening the discolored teeth. It also contains OH group on the chemical structure. Citrus sinensis is a great source of vitamin C, pectin, limonene, glucarate and soluble fiber which act as natural teeth whiteners.

According to Pratiw et al.,⁶ orange peel has several active compounds, like Hexadecanoic acid 1-(hydroxymethyl)-1,2-ethanediyl ester and Androstan which have OH cluster that is known for whitening the teeth.

A study published in a 2010 issue of the "American Journal of Dentistry" found that toothpaste containing d-limonene was significantly effective in reducing smoking stains on teeth and d-limonene alone inhibited the development of further smoking stains.

In this study, we have used peel instead of pulp since orange and lemon peel is not acidic like the fruits and hence will cause only a minimal erosion to enamel.

This study found that natural fruit extracts of citron and *musa sapientum* had a teeth-whitening effect. This may have important implication for the development of natural dental bleachers (over the counter products). Further studies should be done to evaluate the surface characteristics of the tooth following bleaching with herbal agents. Bleaching time of the specimens is the major limitations of the study. Nonetheless, this study provides basic data for the development of natural dental bleachers in follow-up studies in the future.

CONCLUSION

All the bleaching agents that have been tested in the current study has shown a significant color change. Amongst this *musa sapientum* can be used as a substitute for conventional bleaching agents which has shown maximum variances when compared to the other herbal bleaching agents. Although the effect of bleaching of *musa sapientum* was found to be inferior to hydrogen peroxide, the caustic effect was very minimal. The presence of potassium, manganese, magnesium and various other minerals present in the peel of *musa sapientum* will recharge the tooth and helps to maintain the health without compromising on it.

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