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## Stain removal efficacy of a novel dentifrice containing papain and Bromelain extracts – an *in vitro* study

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**Abstract:** *Objective:* To assess the *in vitro* stain removal efficacy of a novel commercially available dentifrice. *Materials and methods:* Twenty four human molar teeth (12 in test and 12 in control group) were cut to obtain enamel specimens approximately 9 mm<sup>2</sup> and placed on a microscopic slide using mounting adhesive. They were cycled in human saliva, staining model and dried for brushing regimen using a customized brushing apparatus. Lightness values were recorded at baseline, after staining and after brushing regimen using Adobe Photoshop software. Stain removal efficacy was tested using the either of the two dentifrice slurries for 2 one minute brushing cycles. *Results:* The mean lightness increment for test and control after brushing regimen was  $13.7 \pm 6.35$  and  $3.16 \pm 1.29$  respectively. There was statistically significant difference in percentage removal of stains for test and control groups ( $66.99 \pm 9.57$  and  $25.89 \pm 16.2$   $P < 0.001$ ). *Conclusion:* There was significant stain removal with new whitening dentifrice when compared with control. Further clinical trials are recommended to evaluate this significant difference.

**Key words:** Bromelain, image analysis, papain, stains

## Introduction

The appearance and colour of teeth are of concern to a large number of people seeking dental treatment. There has been a recent increase in 'over-the-counter' tooth whitening products in the market for the treatment of tooth staining and discoloration that have no professional involvement in their application (1).

The agents causing extrinsic staining of teeth can be divided into those compounds which get incorporated into the pellicle that produce stains as a result of their basic colours (tobacco smoking and chewing, tea and coffee), and those compounds that lead to staining by chemical interaction at the tooth surface (cationic antiseptics and metal salts) (1). These extrinsic stains can be removed by professional prophylaxis. However, the patient's use of a dentifrice can also contribute to dental stain removal and prevention of recurrent discolouration. The dentifrice's stain-removal property is to a certain extent related to the abrasives present in its composition (2). It is important to point out that if, on one hand, an increased dentifrice abrasiveness leads to improved stain removal efficacy, on the other hand, it causes increased tooth wear. Therefore, whitening dentifrices have been introduced in the market. These dentifrices contain

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active ingredients specifically utilized for tooth stain removal. It is generally believed that these ingredients act by disrupting and/or removing the protein portion of the pellicle/plaque layer that forms on the surface of teeth over time, thus removing the stains that are bound to these proteins (3). Other factors related to stain removal are bacterial plaque control and the detergent present (sodium lauryl sulphate) in the dentifrice (4).

Clinical indices for measurement of stain are quick and easy to use, but they raise reliability problems because of their subjective nature. Reflectance spectrophotometry for the colour assessment of extracted teeth has been shown to give reproducible results, but can lead to systematic errors because teeth have curved surfaces and they are translucent (5). As an alternative objective approach to spectrophotometry, customized digital image analysis system to assess stain build-up and removal on acrylic blocks and extracted teeth has been found to be a reliable measurement method (6).

A variety of *in vitro* models are described in the literature for evaluating the efficacy of oral care products on extrinsic stains. They used a range of substrates and model stains, for example, perspex and black tea/chlorhexidine (7), hydroxyapatite discs and ferric-tannate (8). The classic Stookey *et al.* (1982) (9) method used bovine enamel and coffee/tea/gastric mucin/soy broth/sarcina lutea turtox mixture. However, these models lacked either the appropriate substrate i.e. human enamel or the stains encountered in routine lifestyle.

The present study used an *in vitro* model using human saliva with tea, instant coffee, chlorhexidine, areca nut and tobacco extracts on human enamel slabs to simulate stain accretion. The efficacy of a novel commercially available dentifrice (Glodont tooth paste, Group Pharmaceuticals Ltd, Mumbai, India) was tested and compared with a control tooth paste (Colgate Regular, Colgate Palmolive Pvt Ltd, India) for *in vitro* stain removal.

## Materials and methods

### Enamel slab preparation

Twenty four human molar teeth were selected for this study in accordance with the colour similarity that most of them presented. Very dark or light teeth that stood out against the others were excluded by the visual method as appeared to the eye of the examiner. Buccal surfaces of these teeth were cut to obtain enamel specimens approximately  $3 \times 3 \text{ mm}^2$ . The enamel specimens were mounted on a microscopic slide using DPX mounting adhesive (Merck Specialties Pvt Ltd, Mumbai, India) in such a manner that only the enamel surfaces were exposed. DPX mounting adhesive is a colourless, synthetic resin which helps in rapid mounting of the specimen. The enamel surfaces were smoothed and polished using pumice slurry with the help of a polishing cup. They were then examined under a light microscope (4x) to exclude those with cracks. The specimens were etched with 37% phosphoric acid for 15 s and rinsed to expedite stain accumulation and adherence. The study was approved by Institutional Review Board of Manipal University.

### Scoring and setup

The amount of *in vitro* stain was graded digitally using only the Luminosity or Lightness (L value of Adobe Photoshop version 8.0. L that represents the lightness of the stain before and after brushing, with L = 255 being white, and L = 0 being black). Digital images of each enamel slab were captured using a high-resolution digital camera (Nikon® Japan, D300 36 mm Extension tube, Micro-Nikkor 60 mm lens, 11 aperture, 1/8th shutter speed and 400 ISO film speed) fixed to customized stand under standard polarized lighting conditions (Fig. 1). The camera was positioned at  $45^\circ/0^\circ$  geometry with respect to the lights. Images of all the enamel slabs were taken in a dry state before and after incubation. Once the images were obtained, they were then transferred to a computer. The images were opened in Adobe Photoshop and whole enamel slab was outlined using lasso tool in the toolbar option of Adobe Photoshop software. A histogram was then provided that gave the mean L-value of all the pixels within the area of interest. This procedure was repeated for five specimens randomly to check the reliability in obtaining the L values. The coefficient of reliability of the image analysis system was found to be 0.98. The pre-stain values were used as baseline to match Lightness values of the enamel slabs between test and control groups.

### Collection of saliva

Stimulated human saliva was collected from 25 donors using paraffin wax into sterile disposable containers. All the saliva collected was poured and mixed in a large container. The enamel slabs were then immersed and incubated at  $37^\circ\text{C}$  in the mixed human saliva for 24 h.

### Staining model

The staining solution was prepared using tea, coffee, arecanut with tobacco and chlorhexidine in the following manner: 1 g of tea leaves was taken and brewed in 100 ml of water for 3 min.



Fig. 1. The digital image analysis system showing specimen position, standardised lighting and camera position.

The tea was then filtered through a fine mesh and allowed to cool down gradually to room temperature. Similar procedure was followed for instant coffee. One serving of locally available arecanut, betel leaf, lime, tobacco leaf and one packet of commercially available smokeless tobacco were also boiled separately for 3 min in 100 ml water, filtered and cooled. All these solutions were then mixed in a container. To this mixture, 30 ml of chlorhexidine (0.2%) was added.

### Stain cycling

The enamel slabs were immersed in freshly prepared staining solution and incubated for 24 h until visible stains were seen. Once visible stains formed on these enamel slabs, digital images of all the enamel slabs were obtained under the same conditions as described previously. Twenty four enamel slabs were equally divided into two groups according to the baseline Lightness values. The enamel slabs of one group were brushed with test dentifrice and other group with control dentifrice. Brushing was done using a custom made brushing machine for two cycles of 1 min each with slurry of respective dentifrice.

### Customized brushing apparatus

A custom made brushing apparatus was constructed using a commercially available powered toothbrush (289.78 g). The powered toothbrush was mounted using a specially designed acrylic handle. The handle was fixed to an acrylic holder using a nut and bolt assembly. An acrylic stand was made to place the slides of enamel slabs. All these parts were mounted on a stable platform made of plywood using screws. A closed coil Ni-Ti spring was ligated to the brush head and a hook on the plywood base to apply a static load. (Fig. 2) Using dontrix gauze, the tension of the spring was adjusted to  $150 \pm 10$  g. The tension was calibrated and re-checked periodically between the brushing cycles.

### Dentifrice slurry

The dentifrices were used as slurries prepared by mixing 15 g of test or control dentifrice with 45 ml of deionized water. Fresh slurries were made for both test (Papain, Bromelain,



Fig. 2. Customised brushing apparatus used in the study.

Miswak, Neem and 1000 ppm fluoride) and control (calcium carbonate, sorbitol, titanium dioxide, sodium silicate, sodium saccharin and 1000 ppm fluoride) dentifrice. The Relative Dentine Abrasive value for the test and control toothpastes was 67 and 70, respectively as per the data provided by the manufacturers.

### Brushing regimen

Brushing regimen consisted of brushing each specimen for two consecutive cycles. Following each brushing regimen, the specimen was rinsed, blot dried and scored again for stain. Digital images of enamel slabs at baseline, after staining and post brushing were recorded and analysed for Lightness values.

### Statistical analysis

The mean scores of the Lightness values were entered in SPSS version 14 (SPSS Inc., Chicago, IL, USA). Independent sample t-test was performed for baseline Lightness value matching. Independent sample t-test was performed to compare the percent removal stains. Confidence interval of 95% was considered. Percent removal stains was calculated as follows:

$$\text{Removal (\%)} = \frac{L * (\text{post-brushed}) - L * (\text{stained})}{L * (\text{baseline}) - L * (\text{stained})} \times 100$$

## Results

The mean Lightness values of specimens were found to be similar before and after incubation with saliva ( $149.4 \pm 11.4$ ,  $149.2 \pm 11.5$   $P = 0.06$ ). The pellicle layer did not alter the Lightness value as the pellicle formed over 24 h declined to impart any stain. Therefore, Lightness value prior to pellicle formation was considered as baseline value. There was no significant difference between the mean Lightness values for test and control groups at baseline that is before incubation (Table 1). Similarly, there was no significant difference between the mean Lightness values for test and control after staining ( $P = 0.07$ ). The mean lightness increment for test and control groups after brushing regimen was  $13.7 \pm 6.4$  and  $3.2 \pm 1.3$  Adobe L values, respectively. The difference in percentage removal of stains for test was more when compared with control group and the difference was statistically significant ( $P < 0.001$ ) (Table 2, Fig. 3).

Table 1. Comparison of mean baseline lightness value for test and control group

	Group	Mean $\pm$ SD	P-value
Baseline lightness value	Test	149.3 $\pm$ 11.3	0.99
	Control	149.4 $\pm$ 12.1	

Significance level:  $P < 0.05$ .

Group	Stained lightness value	Post-brushing lightness value	Lightness increment	Percentage removal of stains	P-value
Test	128.5 ± 7.5	142.3 ± 8.1	13.7 ± 6.4	67 ± 9.6	<b>&lt;0.001</b>
Control	135.2 ± 9.6	138.3 ± 9.9	3.2 ± 1.3	25.9 ± 16.2	

Significance level:  $P < 0.05$ . Bold values signify statistically significant results.

**Table 2. Comparison of mean stained value for stained and post-brushing lightness, lightness increment and percentage increment of lightness between test and control groups**

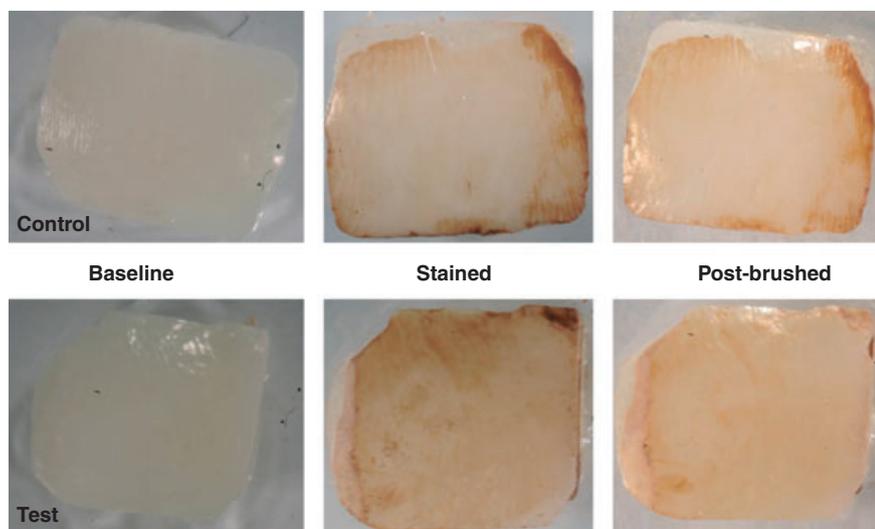


Fig. 3. Baseline, stained and post-brushed specimens for control and test groups.

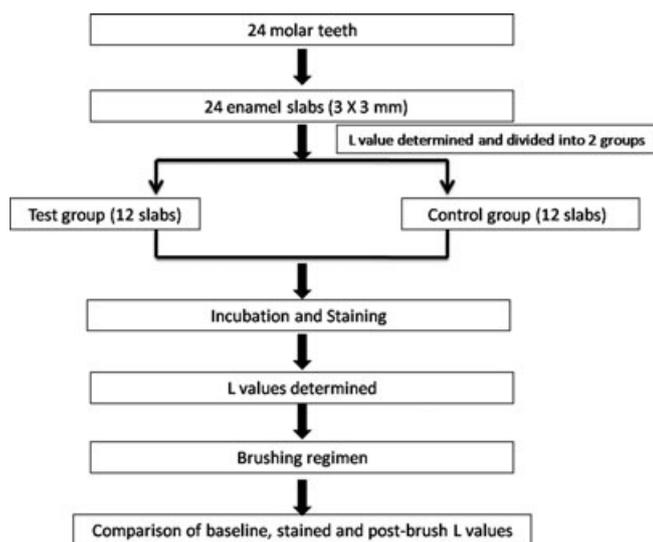


Fig. 4. Flow chart showing the study design.

## Discussion

The present study evaluated the stain removal efficacy of a novel dentifrice in comparison with a control dentifrice. In this study, a staining model was developed using human enamel and human saliva. Stains were allowed to form using a mixture of tea, coffee, chlorhexidine, tobacco and areca nut extracts to mimic the real life stain accretion (Fig. 4). Tobacco and areca

nut chewing is widely practised in India and South East Asia. They are the most common aetiological agents for extrinsic tooth stains in these areas. By incorporating tobacco and areca nut extracts in the staining solution, this model was made to reflect a proper substrate as well as an economical and realistic way of inducing stains on the enamel. The enamel slabs were incubated in non-sterile saliva because the bacteria present in the saliva can get deposited on the enamel surface and take up more stains.

A number of *in vitro* studies have reported the use of commercially available brushing machines (10, 11). The huge cost of these machines makes them limited to only developed countries and large scale pharmaceutical testing companies. Hence, in the present study, a customized brushing apparatus was designed using a commercially available powered toothbrush that allowed brushing at consistent brush stroke speed and brush head pressure.

The custom-made frame for the digital camera was designed as described previously to allow standardized, accurate and reproducible lightness measurements of enamel slabs (10). The standardized lighting included in the image analysis system closely reflected standard daylight conditions and provided a consistent lighting source throughout the period of the study. The images obtained in dry state, however, showed no significant difference in the Lightness values between the two groups at baseline as well as post-staining. Thus, desiccation of enamel slabs would not significantly alter the outcome.

In this study, there were no significant differences in the Lightness value between test and control groups at baseline.

The Lightness value did not differ before and after pellicle formation. So the L values following subsequent staining and brushing was compared with the pre-incubation values. The present study showed that the mean Lightness value of test group after brushing was significantly higher than that of the control group. Similar results were reported by Lyon Jr (1991) (12) with Citroxain (mixture of papaya, alumina and sodium citrate) containing dentifrice (Rembrandt) using a customized shade guide. The test dentifrice in the present study contained extracts of papain and bromelain, which are proteolytic enzymes. They disrupt and/or remove the protein portion of the pellicle/plaque layer that forms on the surface of teeth over time, thus removing the stains that are bound to these proteins. Hence, statistically significant increase in the mean Lightness values of the test dentifrice could partly be attributed to these enzymes along with the abrasive systems. Since both the dentifrices tested had similar amount of fluoride concentrations (1000 ppm), the effect of fluoride uptake after etching would have been the same. These types of *in vitro* studies help in evaluating the efficacy of dentifrices in removing stains before planning expensive and time consuming clinical trials. Further clinical trials are recommended with different concentrations of papain and bromelain to determine dose-response relationship *in vivo*.

## References

- 1 Watts A, Addy M. Tooth discolouration and staining: a review of the literature. *Br Dent J* 2001; **190**: 309–316.
- 2 Meyers IA, McQueen MJ, Harbrow D, Seymour GJ. The surface effect of dentifrices. *Aust Dent J* 2000; **45**: 118–124.
- 3 Oral compositions providing enhanced tooth stain removal Available at: <http://www.freepatentsonline.com/6379654.html> (accessed on 25-04-2010).
- 4 Pontefract H, Sheen S, Moran J. The benefits of toothpaste –real or imagined? Review of its role in tooth whitening. *Dent Update* 2001; **28**: 67–70.
- 5 Van der Burght TP, ten Bosch JJ, Borsboom PCF, Kortsmit WJPM. A comparison of new and conventional methods for quantification of tooth colour. *J Prosthet Dent* 1990; **63**: 155–162.
- 6 Lath DL, Johnson C, Smith RN, Brook AH. Measurement of stain removal *in vitro*: a comparison of two instrumental methods. *Int J Dent Hygiene* 2006; **4**: 129–132.
- 7 Smith RG, Moran J, Addy M, Doherty F, Newcombe RG. Comparative staining *in-vitro* and plaque inhibitory properties *in-vivo* of 0.12% and 0.2% chlorhexidine mouthrinses. *J Clin Periodontol* 1995; **22**: 613–617.
- 8 Dawson PL, Walsh JE, Morrison T, Grigor J. Dental stain prevention by abrasive toothpastes: a new *in vitro* test and its correlation with clinical observations. *J Cosmet Sci* 1998; **49**: 275–283.
- 9 Stookey GA, Burkhard TA, Schemehorn BR. *In vitro* removal of stain with dentifrices. *J Dent Res* 1982; **61**: 1236–1239.
- 10 Hunt SA. The relative cleaning effectiveness of manual and powered toothbrushes. *J Clin Dent* 2002; **13**: 211–214.
- 11 Lima DANL, Silva ALF, Aguiar FHB *et al.* *In vitro* assessment of the effectiveness of whitening dentifrices for the removal of extrinsic tooth stains. *Braz Oral Res* 2008; **22**: 106–111.
- 12 Lyon TC Jr, Parker WA, Barnes GP. Evaluation of the effects of application of a citroxain containing dentifrice. *J Esthet Dent* 1991; **3**: 51–53.