

# Antioxidant potency of mangosteen peel extract topical application in reversing reduced orthodontic brackets tensile strength after bleaching

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## ABSTRACT

**Background:** Nowadays, cosmetic dentistry has become an ever-increasing requirement with interest in it growing over time. Bleaching is one of the popular cosmetic treatments that has been proven to diminish the tensile bond strength (TBS) of orthodontic brackets attached to bleached teeth. Mangosteen peel (MP) extract contains antioxidants that may potentially reverse the reduction in TBS. **Purpose:** The purpose of this study was to evaluate the effect of MP extract on the post-bleaching TBS of brackets. **Methods:** The reported research constitutes an experimental in vitro study conducted on a total of 120 maxillary first premolar teeth randomly divided into six groups ( $n = 20$ ) as follows: negative-control (NC: no bleaching), positive-control (PC: bleaching + no treatment), and the treatment groups (bleaching + 10% sodium ascorbate (SA), 10% (MP10), 20% (MP20) and 40% (MP40) MP extract gel). Post-treatment, the brackets were bonded using Transbond XT and TBS testing was performed using a Universal Testing Machine. The ARI was examined by means of a stereoscopic microscope, while enamel morphological changes were observed through a Scanning Electron Microscope. The TBS-generated data was analyzed by means of Anova and Tukey tests. For the Adhesive Remnant Index, a Kruskal-Wallis analysis test was performed. **Results:** There was a significant TBS difference ( $P = 0.001$ ) between the various groups. The PC group showed the significantly highest TBS compared to the others ( $8.33 \pm 3.92$  MPa), whereas NC demonstrated the lowest ( $4.15 \pm 2.27$  MPa). The TBS value of the MP40 group was considerably higher than other groups treated with antioxidants ( $7.87 \pm 3.26$  MPa). The failure of orthodontic brackets using MP extract mostly occurred at the adhesive-bracket interfaces. **Conclusion:** Topical application of 40% mangosteen peel (MP) extract as an antioxidant after bleaching was effective in reversing the reduced post-bleaching tensile bond strength (TBS) of orthodontic brackets.

**Keywords:** brackets; bonding; bleaching; Mangosteen peel extract; tensile bond strength

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## INTRODUCTION

In contemporary society, popular interest in cosmetic dentistry has increased to the extent that it has become regarded as a necessity.<sup>1</sup> Tooth whitening or bleaching has become one of the most common procedures in this branch of dentistry, adopted by numerous dentists and their patients as the method of choice to improve tooth appearance due to its being minimally-invasive, easy, efficient and effective.<sup>2,3</sup> The number of patients seeking orthodontic

treatment who might have a history of tooth bleaching is increasing because, following previous procedures, they usually tend to be conscious of orthodontic problems and desire treatment.<sup>4</sup> Unfortunately, bleaching may lead to a reduction in orthodontic bracket bond strength.<sup>5–7</sup> In-office bleaching can produce a direct result including residual peroxide on the tooth surface which might inhibit the polymerization of the adhesive.<sup>8</sup> On the other hand, treating bleached teeth with antioxidants helps in the elimination of free oxygen radicals from the tooth surface

before bonding and, as a consequence, will reverse reduced bond strength.<sup>9</sup>

In vitro study has demonstrated the application of synthetic antioxidants such as sodium ascorbate to be effective in reversing the reduced bond strength of brackets after bleaching.<sup>10</sup> The limited routine use of synthetic antioxidants has increased because the efficiency of synthetic antioxidants is lower than that of natural antioxidants.<sup>11</sup> Moreover, the side-effects of synthetic antioxidants include the presence of residual electrons that are difficult for human tissues to recycle.<sup>12</sup> The interest in natural antioxidants of plant origin has developed considerably in recent years. Mangosteen is a tropical fruit whose peel is a source of powerful, natural antioxidants. Previous in vitro studies demonstrated that mangosteen peel (MP) extract is more efficient at controlling the oxidative reaction of free radical molecules compared to certain commercial antioxidants.<sup>13</sup> Therefore, the present study was carried out to evaluate the effect of MP extract topical application on the tensile bond strength of orthodontic brackets bonded with resin materials to bleached teeth.

## MATERIALS AND METHODS

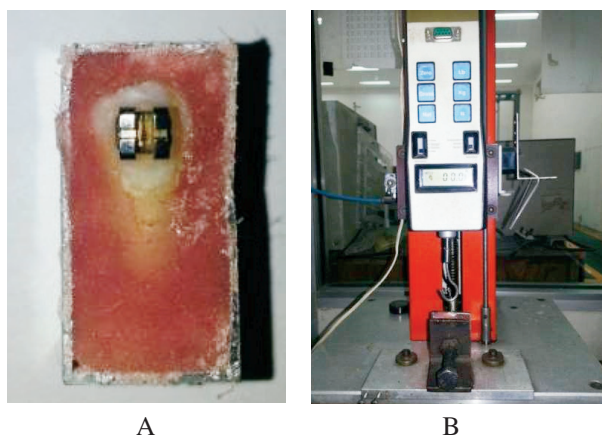
The research reported here represents an experimental laboratory study. Ethical approval was granted in 2016 by the Research Ethics Committee, Faculty of Dentistry, Universitas Gadjah Mada. A total of 120 human maxillary first premolars extracted for orthodontic purposes were collected. The inclusion criteria of the teeth comprised the following: healthy, intact enamel surface, caries-free, devoid of defects, cracks, or restorations and the absence of chemical-agent pre-treatment. The teeth were exposed to scaling using an Ultrasonic Scaler (Woodpecker UDS-A w LED, China) to remove organic debris, before being cleaned and decontaminated through a week-long immersion in 0.5% chlorine at 25°C, renewed once every two days to limit bacterial contamination. The teeth were mounted

in acrylic resin (Figure 1a). Samples were then randomly distributed between six groups ( $n = 20$ ) as follows: group 1 consisted of unbleached teeth (negative-control = NC), group 2 consisted of bleached teeth untreated before bracket bonding (positive-control = PC), group 3 were bleached before being treated with 10% sodium ascorbate and undergoing bracket bonding (SA), and groups 4, 5 and 6 were bleached and then topically treated with 10%, 20% and 40% MP (MP-10, MP-20, MP-40) extract gel before bracket bonding.

All of the samples were subjected to 40% hydrogen peroxide (Opalescence® boost, Ultradent, USA) on the enamel surfaces as a bleaching agent in accordance with the manufacturer's protocol. A uniform thickness of bleaching agent (0.5–1 mm) was applied to the enamel surface of each sample. After 20 minutes, the surfaces were washed with distilled water and gently dried with an air jet for 30 seconds. The bleaching procedure was re-applied twice as per the manufacturer's instruction to obtain optimal results. Groups 3, 4, 5, and 6 were then treated with an antioxidant agent in the following manner: 10% sodium ascorbate gel (manufactured by LPPT, Indonesia) and 10%, 20%, and 40% MP gel (manufactured from a sample containing MP extract, CMC-Na 2%, glycerin, propylene glycol, propylparaben and methylparaben by LPPT, Indonesia). 0.5–1 mm of antioxidant was attached to the enamel surfaces of the teeth with a sterile brush on conclusion of the bleaching process. After ten minutes, they were washed in distilled water and gently dried with an air syringe. Following completion of this exercise, the samples were submerged in artificial saliva solution for 24 hours prior to the initiation of the bonding procedure.

120 stainless steel edgewise premolar brackets (American orthodontics, USA) with a 0.022-inch slot and a bracket base surface area of 10.64 mm<sup>2</sup> were used in the study. The brackets were then bonded with Transbond XT (3M Unitek, USA) before being placed on the mid-buccal surfaces of the teeth using a bracket positioning gauge. Furthermore, each bracket was light-cured for 40 seconds in accordance with the manufacturer's recommendation (10 seconds per side: occlusal, cervical, mesial, and distal) using a light-curing unit (Litex 680A, Dentamerica, USA).

A tensile bond strength (TBS) test was performed using a Universal Testing Machine (Pearson Panke Equipment, London). A mounting jig aligned the bracket base parallel to the bottom of the mould and perpendicular to the force during the tensile strength test. TBS was measured at a crosshead speed of 0.5 mm/min (Figure 1b). The results obtained were converted to Mega-Pascal (MPa) by dividing the debonding force (N) by the bracket base surface area (10.64 mm<sup>2</sup>). Immediately after bracket debonding, the enamel surface of each specimen was examined at 10× magnification with a stereoscopic microscope (SMZ-2T, Nikon, Japan) to determine the amount of residual adhesive. Adhesive remnant index (ARI) scores at the failure sites were noted according to the classification applied by Artun and Bergland as follows: score 0 - no adhesive remained



**Figure 1.** (A) Sample mounted in self-curing acrylic resin, (B) Illustrations of the test construction for determining tensile bond strength.

on the tooth, 1 - less than half of the enamel bonding area was covered with adhesive, 2 - more than half of the enamel bonding area was covered with adhesive, and 3 - the enamel bonding area was fully covered with adhesive.<sup>14</sup> Measurement was conducted by three qualified examiners who were unsighted as to the composition of the samples. The examiners' analysis showed a high level of intra-examiner and inter-examiner agreement and reliability - *Kappa* index value (0.81). The structure of enamel was then observed using a scanning electron microscope (JEOL, JSM-6510 series, Japan) under 5000× magnification.

Statistical analysis was performed with statistical package for the social sciences (SPSS) software (version 22.0, Chicago, Ill). All data is presented as mean ± standard deviation. The data of the groups was subjected to a normality and homogeneity test. With respect to this, one-way Anova was used to determine the significance between the groups, while a Tukey honestly significant difference post-hoc test was applied to evaluate individual differences (Table 1). The ARI scores were examined by means of a Kruskal–Wallis analysis. To determine the differences between the groups, a Mann–Whitney U test was also performed. The significance for all statistical tests was set at a *P*-value less than 0.05.

**RESULTS**

The tensile bond strength of samples in each of the six study groups are shown in Table 1 in MPa (Mean ± standard deviation). These descriptive statistics clearly indicate the variation in TBS between the six groups with the maximum TBS value being found in the NC group (8.33 ± 3.92 MPa) and the minimum in the PC group (4.15 ± 2.27 MPa). Groups SA, MP10, MP20, and MP40, all of which were subjected to post-bleaching antioxidant treatment, show an improvement in TBS compared with the PC group, while the group treated with MP40 shows the highest TBS compared to other groups treated with antioxidants (7.87 ± 3.26 MPa). The results of the Anova indicated statistically significant differences between the tested groups (*P* < 0.05). The Tukey test showed that the TBS of the NC group was by far the highest compared to that of other groups. Furthermore, no statistical significant difference in TBS value existed between group NC and groups treated with SA and 40% MP extract (*P* > 0.05).

The ARI scores for all the tested groups are listed in Table 2. The results of the Kruskal-Wallis test showed significant differences between the groups (*P* < 0.05). ARI scores of 0 and 1 occurred with high frequency, while ARI

**Table 1.** Results of the ANOVA and Tukey tests comparing the TBS in the 6 groups tested

Group	n	TBS (MPa)	Min.	Max.	Sig*	p-value				
						PC	SA	MP-10	MP-20	MP-40
NC	20	8.33±3.92	6.48	12.97	<i>p</i> = 0.001*	0.000*	0.163	0.013*	0.048*	0.798
PC	20	4.15±2.27	2.11	6.02			0.048*	0.012*	0.000*	0.000*
SA	20	7.12±3.04	5.83	10.19				0.027*	0.037*	0.048*
MP-10	20	6.39±2.86	4.94	7.71					0.082	0.021*
MP-20	20	6.41±2.94	5.19	10.03						0.014*
MP-40	20	7.87±3.26	5.88	11.38						

Values are presented as mean ± standard deviation. \*Significant differences between groups (*p* < 0.05). ANOVA, Analysis of variance; TBS, tensile bond strength.

NC: negative-control, PC: positive-control. SA: sodium ascorbate, MP-10: 10% mangosteen peel extract, MP-20: 20% mangosteen peel extract, MP-40: 40% mangosteen peel extract.

**Table 2.** Distribution of the ARI scores of 6 groups tested and results of the Kruskal-Wallis test

Group	ARI scores								Kruskal Wallis	
	0		1		2		3		Chi-square	P
	N	%	N	%	N	%	N	%		
N	3	(15)	4	(20)	5	(25)	8	(40)	8.162	0,031*
P	7	(35)	13	(65)	0	(0)	0	(0)		
SA	5	(25)	4	(20)	7	(35)	4	(20)		
MP10	7	(35)	10	(50)	2	(10)	1	(5)		
MP20	5	(25)	8	(40)	4	(20)	3	(15)		
MP40	3	(15)	4	(20)	6	(30)	7	(35)		

Values are presented as number (%). \*Significant differences between groups (*P* < 0.05). ARI, Adhesive remnant index.

\*ARI scores: 0, no adhesive left on the tooth; 1 = less than half of the adhesive left on the tooth surface; 2 = half of the adhesive or more left on tooth surface; 3 = all adhesive left on tooth surface



scores of 2 and 3 were seen with less frequency in groups PC, MP10, and MP20. In contrast, the highest frequency of ARI scores of 3 were recorded by groups NC and MP40.

## DISCUSSION

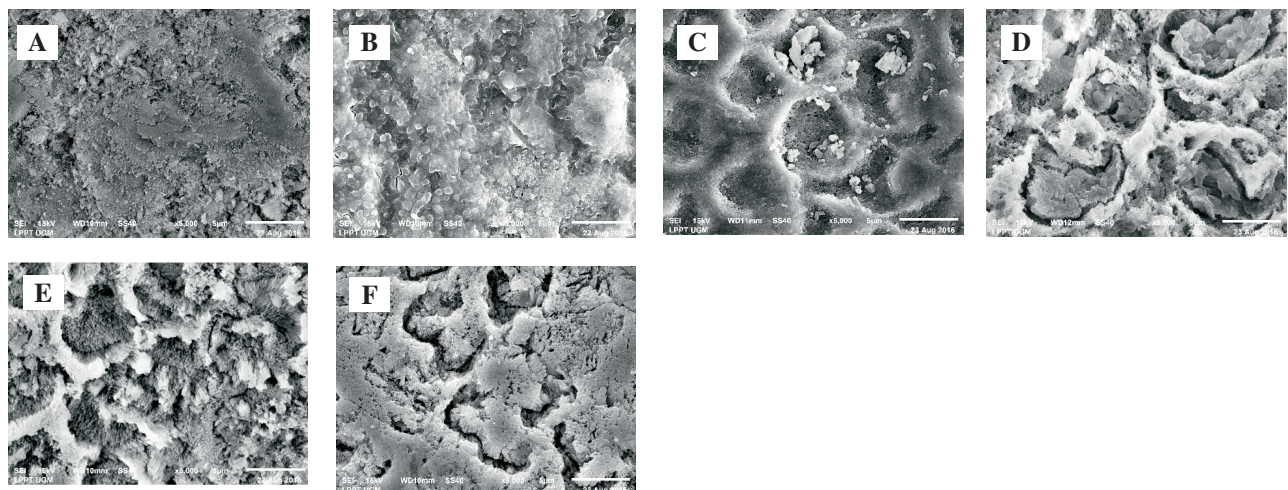
The results confirmed that there were increases in TBS in groups receiving antioxidant treatment. The NC group showed the highest values of TBS, while the PC group showed the lowest. The reduced TBS in the PC group compared to that of other groups may have been due to the remaining oxygen layer left by the bleaching process. This could have interfered the resin polymerization by free-radical mechanism.<sup>15</sup> Marković *et al.*,<sup>16</sup> recommended that clinically acceptable mean bond strength values range between 7.10 and 17.84 Mpa with no enamel fractures. However, Al Shamsi *et al.*,<sup>17</sup> state that an increased number of enamel fractures occurred when the bond strength passed 13.5 Mpa. These findings correspond favourably to the results here that the group treated with MP-40 showed average values within Marković's adequate range ( $7.87 \pm 3.26$  Mpa). This confirmed that samples subjected to peroxide agents could still resist the stresses generated by orthodontic forces without suffering enamel fractures.

Groups receiving antioxidant treatments showed significantly higher bond strength than that of the PC group. These findings were in accordance with the fact that the post-bleaching procedure use of antioxidants was effective in reversing compromised bond strength on completion of the elimination of residual oxygen.<sup>18</sup> It has been reported that sodium ascorbate (SA) is a potent antioxidant with the potential to relieve the reactive free radicals and neutralize their negative effect. SA is a derivative of ascorbic acid with

a neutral pH. It neutralizes the effect of the residual oxygen layer, while enabling free radical polymerization of resin-based materials to proceed without premature termination by restoring the modified redox potential of the oxidized bonding substrate, thus reversing the bond strength.<sup>19</sup> In the process following the application of SA, TBS values reached a level almost similar to that of the MP40 groups. However, MP40 groups showed significantly higher bond strength value than that of a group receiving SA treatments which could be associated with the fact that antioxidant present in MP extract is more potent than in the SA.

The NC group showed significantly higher bond strength than the other groups. This correlates with the fact that there are no changes in the unbleached teeth surface. Previous studies have shown that there is a change in the structure of enamel and the bond strength when the teeth are exposed to bleaching agents.<sup>2,3</sup> The reduction in bond strength has been related to morphological changes in mineralized tissues. Bleaching agents also affect the collagen network of dentin, resulting in denaturing and relative instability of the dentin organic matrix, thereby decreasing the bond strength.<sup>4</sup> In addition, TBS values of a group undergoing 40% MP extract treatment reached a level almost equal to those of the NC group. It is suggested that treatment with MP extract could approximate the bond strength values of teeth which had not experienced the bleaching procedure.

The results indicated that application of MP extract on bleached enamel surfaces could neutralize and overcome the negative effect of residual oxygen molecules and significantly increase the TBS of orthodontic brackets. MP also demonstrated potential antioxidant properties stronger than those of its pericarp and leaves.<sup>20</sup> Antioxidant properties of MP extract can inhibit or delay oxidation by scavenging free radicals (i.e. reactive oxygen species such



**Figure 2.** Photomicrographs of enamel surface in six groups tested using SEM at a magnification of 5.000X; A) NC group: negative-control, B) PC group: positive-control, C) SA group: sodium ascorbate, D) MP-10 group: 10% mangosteen peel extract, E) MP-20 group: 20% mangosteen peel extract, and F) MP-40 group: 40% mangosteen peel extract. Thick arrows indicate bubble like structures.

as hydroxyl, superoxide, nitric oxide, thiol and peroxy) by donating a hydrogen atom or electron which convert their radicals to more stable products.<sup>11</sup> MP extract contains alpha-mangostin has been reported as antioxidant by disintegrating oxygen molecule.<sup>21,22</sup> Furthermore, MP extract also contains a potent free radical scavenger, in the form of a flavonoid, called epicatechin.<sup>23</sup> Flavonoids can perform scavenging action on free radicals such as superoxide, hydroxyl, and 1,1-diphenyl 1-2-picrylhydrazyl (DPPH) and have metal chelating properties. The presence of the functional group OH in the structure and its position on the ring of the flavonoid molecule determines its antioxidant capacity.<sup>24</sup>

The morphology of the surface enamel in six groups was observed using an SEM as shown in Figure 2. Figure 2-A illustrates the lack of change in the morphology of the enamel in group NC with the highest TBS value. Conversely, the NC group had the lowest TBS value, showing changes to enamel surfaces, including: depression, cracks, porosity, and erosion (Figure 2-B). Meanwhile, Figure 2-F, demonstrates the morphology of the enamels in the MP40 group with the highest TBS value compared to that of other groups treated with antioxidants, presenting a minimal bubble-like structure formed in the enamel surfaces compared with SA, MP10, and MP20 groups. This finding is supported by the theory stating that the enamel surface of bleached teeth exhibited more extensive nano-leakage in the form of additional bubble-like structures observed there. This, in turn, indicates that the oxygen released by the hydrogen peroxide could be trapped within the adhesive during light-activation, inhibiting its polymerization, consequently decreasing bond strength.<sup>25</sup>

The ARI scores indicated significant differences between the various groups, although ARI scores of 0 and 1 were seen with high frequency. In groups PC, MP10, and MP20, there was a higher frequency of ARI scores of 1. This means that failures occurred at the enamel-adhesive interfaces. This could be clinically advantageous since, when brackets there fail, the less residual adhesive remains and tooth clean-up is likely to be easier and faster.<sup>26,27</sup> In this study, the NC, SA and MP40 groups demonstrated a higher frequency of ARI scores of 3 (all the adhesive remained on the enamel surface), implying a relatively stronger bond between the adhesive and the enamel surface. It can be concluded that the application of 40% mangosteen peel extract as an antioxidant was capable of reversing the reduced tensile bond strength of orthodontic brackets in bleached teeth.

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