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Original Article

Antimicrobial Evaluation, Degree of Solubility, and Water Sorption of Universal Dental Adhesive Incorporated with Epigallocatechin-3-Gallate: An *In-vitro* Study

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Abstract

Background: Several strategies have been developed to reduce collagen degradation in the adhesive interface. Epigallocatechin3gallate (EGCG) has the ability to stabilize collagen, and it is effective in microbial reduction. **Aims and Objectives:** The objective of the present study was to evaluate the antimicrobial potential, water sorption (WS), and solubility of a universal adhesive incorporated with EGCG at concentrations of 0.02%, 0.1%, and 0.5%, respectively. **Materials and Methods:** Atotal of 40 specimens of composite resin disks, to which the adhesives were applied, were divided into four groups: control (without EGCG), 0.02%, 0.1%, and 0.5%. The specimens were submitted to an *in vitro* cariogenic challenge, inoculated with *Streptococcus mutans* for 3 days for biofilm formation. The generated biofilm was collected, and the colonyforming units were established. For the solubility test, the specimens using the adhesive were divided into the same groups mentioned previously ($n = 10$). Adhesive models were made following an ISO standard for sorption and solubility tests. Microbiological data were submitted to the analysis of variance (ANOVA), followed by the Tukey test ($P < 0.05$). ANOVA was used to evaluate WS and solubility, and comparisons were made by *post hoc* analysis by the Student–Newman–Keuls method ($P < 0.05$). **Results:** A statistical difference was observed regarding the antimicrobial potential between the groups without and with EGCG at 0.5% ($P = 0.03$). EGCG 0.5% presented the highest values of solubility and WS ($P < 0.01$ and $P = 0.009$, respectively). **Conclusion:** The addition of 0.5% EGCG was capable of inhibiting biofilm formation; however, it caused significant alteration of the solubility and sorption of the adhesive.

Keywords: Antimicrobials, dental adhesives, flavonoids, *Streptococcus mutans*, water solubility

Introduction

The evolution and improvement of adhesive systems still present some limitations, especially regarding their adhesion to dentin, which is composed of high contents of water and organic substances, namely type-I collagen. Furthermore, the presence of dentinal tubules confers their own characteristics to the tissue. These tubules change conformation according to the proximity to the pulp and are involved by hypermineralized dentin, known as peritubular dentine and filled with less mineralized intertubular dentin.[1,2] The greater the proximity to the pulp and the larger the concentration and diameter of the tubules, the greater the amount of peritubular dentin. The adhesion process is achieved primarily through the intertubular dentin on account of its less mineralized content. Thus, in regions closer to the pulp, adhesion is more complicated. Another factor that hinders dentin adhesion is the presence of the smear layer.[3]

In the long run, adhesion to dentin undergoes significant reduction[4] since the hybrid layer sustains degradation,[5] which is caused by several factors, including the hydrophilic properties of the resinous monomers[6] and the residual solvent residues, due to their incomplete evaporation during the adhesive technique.[7] In addition to the adhesive interface, the dentin is subjected to degradation due to the activation of matrix metalloproteinases (MMPs) and proteolytic enzymes

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capable of hydrolyzing the organic matrix of demineralized dentin.[3]

In self-etching adhesives, 10%–30%[8] by weight of water is added to hydrophilic formulations to ionize methacrylates and solubilize the liberated calcium and phosphate ions from dentin. If this water does not evaporate, it will dilute the comonomers and may interfere with bonding[9] and decrease the mechanical properties of the resin.[10] Besides that due to the presence of acids, highly polar functional groups, and the presence of water and ethanol solvents, all-in-one adhesives and self-etching primers are inherently hydrophilic. They rapidly absorb water, which results in the weakening of the polymer network and polymer swelling.[11]

All of these factors can lead to failure of the tooth/restoration interface. Therefore, researchers have sought to incorporate agents that can improve the properties of adhesive systems to increase their longevity, enhancing clinical performance.

Several substances have already been incorporated into these systems, including glutaraldehyde, propolis extract and even some antibiotics, such as metronidazole. However, when associated with the adhesive, these compounds have impaired its mechanical performance.[12] Epigallocatechin-3-gallate (EGCG) is a polyphenol present in green tea, which has antioxidant, antimicrobial, anti-diabetic, and anti-inflammatory properties, as well as preventing cancer. This substance has been analyzed using different strategies in restorative dentistry, either in denture pretreatment or incorporation in adhesive systems.[13] In addition, it has the ability to inhibit MMP2, MMP9, and MT1-MMP, although the mechanism by which this occurs remains controversial. EGCG is believed to irreversibly degrade matrix metalloproteinases, binding to collagenases by hydrogen bonds and hydrophobic interactions, causing conformational changes in structure or catalytic site.[14-16]

Some studies have already demonstrated the anti-bacterial effect and increased binding stability provided by EGCG on intraradicular dentin-adhesive interfaces,[17] as well as the compound's low toxicity and anti-inflammatory properties when in contact with pulp cells.[18,19] Yoo *et al*. reported the significant antimicrobial activity of EGCG against Gram-positive and Gram-negative bacteria and fungal. It is believed that the substance suppresses the specific virulence factors associated with their cariogenicity (gtfB, gtfC, and gtfD).[20]

Thus, EGCG presents properties whose perspectives may be positive, aiming at the improvement of adhesive systems. However, the use of the compound in self-etching dental adhesives targeting antimicrobial action and its influence on physical properties has been poorly investigated.

Self-etching adhesives contain relatively more hydrophilic components since functional monomers and water are present and necessary for a viable bond. Hydrophilic monomers undergo a greater tendency to interact with water,[21] and there may be a different response regarding the EGCG incorporated into these adhesives.

The objective of this study was to evaluate the antimicrobial potential, water sorption (WS), and solubility of a universal dental adhesive *in vitro* using it as a self-etching agent in dentin incorporated with EGCG at concentrations of 0.02%, 0.1%, and 0.5%, respectively.

Subjects and Methods

Antimicrobial evaluation

Manipulation of the experimental adhesive system

EGCG (Sigma Aldrich, St. Louis, MO, USA) was incorporated directly into the Universal Single Bond commercial adhesive (3M ESPE, St. Paul, MN, USA). Initially, the bulk ratio of both products was measured on a precision scale (AUX-220; Shimadzu, Tokyo, Japan) at 23°C and relative humidity of 60%. Afterward, manual manipulation was carried out for 5 min, and subsequent total homogenization was performed using a tube agitator (QL-901; Biomixer, São Paulo, SP, Brazil) in the dark for 1 min. Homogenization was carefully checked, and the blend was only used when no crystal was visualized. After the incorporation of EGCG into the adhesive, the experimental adhesives were divided into four groups with different concentrations of the compound: Group 1 (without EGCG), Group 2 (0.02%), Group 3 (0.1%), and Group 4 (0.5%).[13]

Specimen preparation

Three specimens were utilized in each group, and the experiment was repeated three times. Composite resin (Filtek Z350, 3M ESPE, St. Paul, MN, USA) was applied in silicone molds (5 mm diameter x 2 mm thickness) and photopolymerized (Poly Wireless Photopolymerizer, KaVo, Joinville, SC, Brazil) with an irradiance of 1100 mW/cm^2 for 20 s. Next, an adhesive layer of each group was applied onto the specimens and photopolymerized for 20 s, after which all of them were immersed into 10 mL of sterile distilled water at 37°C, shaken for 2 h to remove excess unpolymerized monomers, and dried at room temperature before being sterilized in hydrogen peroxide plasma.

Microbiological model of *Streptococcus mutans* biofilm formation *in vitro*

Streptococcus mutans UA 159 was cultured overnight at 37°C in a sterile brain–heart infusion broth (BHI CM0225; Oxoid LTD, SP, Brazil) in a partial atmosphere (5% CO2). After 18 h, the Gram test was conducted to verify the existence of *S. mutans* exclusively. The obtained bacterial suspension was adjusted to a specific optical density of 10^8 through the McFarland Scale. For the experiment, a 24-well plate (Cell Culture Plate-24 wells, Prolab, São Paulo, SP, Brazil) was utilized, in which each well contained 2.0 mL of tryptone-soybean broth and 1.0% of previously filtered sucrose and was inoculated with 0.1 mL (2×10^8 colony-forming units [CFU] mL^{-1}) of the *S. mutans* UA159 culture. Finally, the specimens were inserted

into each well. Bacterial inoculation was performed only on the 1st day, and the culture medium was replaced daily during 3 consecutive days. The 24 well plates were incubated at 37°C in 5% CO2 throughout the entire experimental period. At each transfer, the culture samples were cultured on BHI agar plates and incubated at 37°C in a 5% CO2 atmosphere to verify purity.[22]

Collection of formed biofilm

A 0.9% saline solution (NaCl) was prepared at a ratio of 0.9 g/100 mL and preautoclaved (121°C, 15 min). On the 3rd experimental day, the biofilm formed on the specimens was removed and inserted into 5.0 mL Eppendorf® tubes (Eppendorf® AG, Alto da Lapa, SP, Brazil) containing 1.0 mL of the previously prepared saline solution. The tubes containing the collected biofilm and the saline solution were vortexed (Vortex TS-2000A VDRL Shaker-Biomixer, Curitiba, Brazil) to disperse bacterial cells.[22]

Microbiological analysis

The suspension obtained in the Eppendorf® tubes was diluted in decimal series (1:10–1:100,000) with 0.9% saline solution (NaCl). The samples were plated in triplicate on BHI agar and incubated for 48 h at 37°C in a 5% CO_2 atmosphere.

The representative colonies with typical *S. mutans* morphology were counted after 48 h, and the results were expressed in CFU.[22]

Water sorption/solubility

Specimen preparation

WS and solubility were determined following ISO 4049:200037.[23] The specimens (n = 10) were prepared by dispensing the adhesive systems using micropipettes in a Teflon matrix (6 mm diameter × 1 mm thick). A Mylar strip and a glass slide were placed on the discs before the light activation for 40 s using a photopolymerizer (Poly Wireless Photopolymerizer, KaVo, Joinville, SC, Brazil) with an irradiance of 1100 mW/cm^2 (irradiance at both sides of each specimen). After polymerization, they were ground and slowly polished to a 0.5-mm thickness using 600 grit SiC polishing papers and stored in a silica-containing desiccator at 37°C.

The specimens were repeatedly weighed in 24 h intervals until a constant mass (m1) was obtained (i.e., variation was <0.2 mg in any 24 h period) on an analytical scale (AUX-220; Shimadzu, Tokyo, Japan). The volume of each specimen was calculated with a 0.001 mm precision digital caliper (Absolute Digimatic; Mitutoyo, Tokyo, Japan) to measure the diameter and thickness, and the volume (V) was expressed in mm^3. Next, the specimens were individually stored in sealed glass vials with 10 mL of distilled water at 37°C for 7 days. Afterward, the specimens were weighed after gently wiped on absorbent papers to obtain a constant mass (m2), and then they were returned to the desiccator. The specimens were finally weighed as aforementioned up to the stabilization of mass (m3). Water sorption (WS) and solubility (SL) (μg/mm^3) were calculated using the following formula:

WS = (m2–m3)/VSL = (m1–m3)/V.[24]

Statistical analysis

The microbiological experiment was performed in triplicate, and the data were transformed into a logarithm. Unidirectional analysis of variance (ANOVA) was also conducted, followed by the Tukey test.

In the solubility test, statistical procedures were performed with the Sigmastat 3.5 (Systat Software Inc., San Jose, CA, USA) for Windows statistical program software. A Shapiro–Wilk test was applied to all groups to analyze the normal distribution of errors and the Barlett test for homoscedasticity. For WS and solubility was used one-way ANOVA and comparisons *post hoc* was analyzed by Student–Newman–Keuls Method. The level of statistical significance was set at $P < 0.05$.

Results

A statistical difference was observed regarding the antimicrobial potential between the groups without and with EGCG at 0.5% ($P = 0.03$), as shown in Figure 1.

Table 1 showed WS and solubility mean values and standard deviation for adhesive systems.

WS was significantly influenced by adhesives systems ($P = 0.009$; $F = 5.475$). The incorporation of EGCG 0.1% and EGCG 0.5% significantly increased the WS ($P < 0.05$). There was no statistically significant difference between Single Bond Universal (SBU) and EGCG 0.02% ($P > 0.05$).

Solubility was significantly influenced by adhesives systems ($P < 0.001$; F = 18.416). There was no significant difference between SBU and EGCG 0.02% ($P > 0.05$). There was a statistically significant difference between SBU, EGCG 0.1% ($P = 0.02$), and EGCG 0.5% ($P < 0.01$). On the other hand, among the EGCG groups, EGCG 0.5% presented the highest values of solubility ($P < 0.05$).

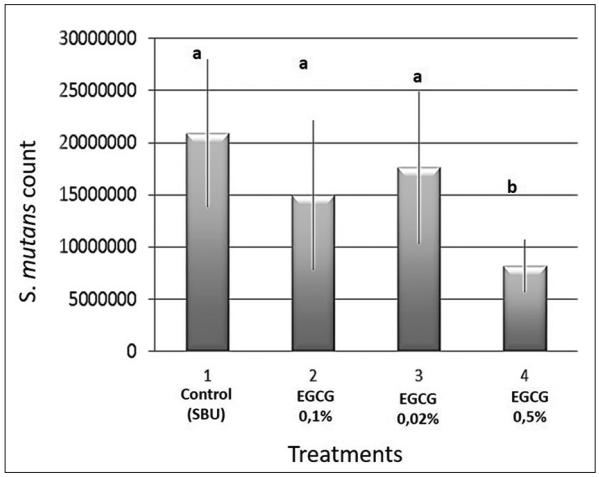

Figure 1: Data expressed in logarithm. Vertical lines represent standard deviations. Different lower case letters represent statistical difference ($P < 0.05$)

Table 1: Mean (standard deviation) of water sorption and solubility of adhesives

Groups ($n=5$)	Water Sorption (μg/mm³)	Solubility (μg/mm³)
SBU (control)	224.5 (46.7)^{A}	96.0 (17.4)^{A}
EGCG 0.02%	295.0 (54.2)^{AB}	135.9 (25.5)^{A}
EGCG 0.1%	407.3 (125.7)^{B}	237.3 (67.0)^{B}
EGCG 0.5%	444.0 (128.1)^{B}	321.1 (75.9)^{C}

*Distinct superscript letters indicate a statistical difference in the same columns ($P<0.05$). EGCG – Epigallocatechin-3-gallate, SBU – Single Bond Universal

Discussion

EGCG exhibits antimicrobial activity against *S. mutans*, suppressing specific virulence factors, such as inhibition of expression of genes gtfB and gtfC, which are responsible for the adherence of the microorganism to the dental structure.[25] Xu *et al*.'s study showed that concentrations below the minimum inhibitory concentration of EGCG (78.1 μg/mL) were able to inhibit *S. mutans* biofilm formation *in vitro*.[26] According to Du *et al*.'s study, when analyzing EGCG incorporation, the antibacterial activity (50% inhibition of biofilm formation) of the dental adhesives increased after incorporating the substance at 200 μg/mL and 300 μg/mL, whereas the addition of 100 μg/mL of EGCG to the adhesive revealed no inhibition.[13] Yu *et al*.(2017a) verified that epigallocatechin-3-O-(3-Omethyl)-gallate (EGCG-3Me), at a concentration of 400 μg/mL alone, was able to inhibit the biofilm formation of a model day *S. mutans* microbiological.[17]

In another study, de Assis *et al*. analyzed the effect of EGCG on carious dentin at concentrations of 0.5%, 1.0%, and 2.0%. The authors reported not being able to reduce *S. mutans* levels at the studied concentrations.[27]

The present study revealed a decrease in *S. mutans* biofilm formation with the addition of 0.5% EGCG to the adhesive. Several studies corroborate the antimicrobial activity of the compound on *S. mutans*. However, standardized assessments are required to regulate the ideal minimum concentration for EGCG to obtain antimicrobial activity without altering other physical-chemical properties.

When altered, properties such as sorption and solubility can structurally modify adhesive systems, negatively affecting their performance and function.[28] Self-etching adhesives generally exhibit sorption and solubility in high water due to the incorporation of acid monomers into their composition.[11]

Fonseca *et al*. (2019) show that ECGC incorporation at a concentration of 0.5 wt% results in lower solubility of the template adhesive. They related such findings to the possibility that the substance reduces the hydrophilicity of the adhesive. Neri, *et al*., in 2014, demonstrated that the incorporation of EGCG to self-etching adhesive at concentrations of 0.1% and 0.01%, presented a reduction of solubility compared to the control group, relating the result to the fact that it is difficult to obtain a homogeneous mixture between the branched catechin molecule (EGCG) and the polymer network. The bonds between the HEMA and Bis-GMA monomers present in the adhesive and the hydroxyls present in EGCG have great potential, allowing to suppose that it would be more difficult to release the components, leading to a reduction of solubility.[29]

On the other hand, the present study demonstrated that incorporation of EGCG at 0.1% and 0.5% concentrations significantly increased the sorption and solubility of the adhesive, corroborating the study by Pallan *et al*., 2012 which found that incorporation of EGCG (1% and 2% by weight) increased solubility when added to self-etching adhesive. Possibly, the increase due to the combination of EGCG with residual monomers and oligonomonomers that are more easily released. The polar characteristics of EGCG, due to the various hydroxyl groups present in its composition,[30,31] may also be a factor that contributed to the increased solubility of the experimental adhesive.

Sorption phenomena and solubility of adhesive systems can cause detrimental changes in structure, interfering with the function of adhesives. Therefore, it is desired that the adhesives have low solubility and sorption in water to achieve the characteristics closer to an ideal adhesive.[32]

The present study showed a significant difference in the sorption and solubility of the 0.5% EGCG-incorporated adhesive in relation to the control group without EGCG, demonstrating that the addition of the substance caused the solubility of the adhesive interface to increase. However, the 0.5% EGCG concentration showed the highest antimicrobial activity.

In this regard, it can be noted that there are numerous studies on the antimicrobial potential of EGCG, WS, and solubility in adhesive interference. However, there is a significant variability in the results found in the literature and regarding the applied methodology, rendering it difficult to attribute the findings to a specific factor or a set of elements. Therefore, more studies on the standardization of concentration, methodology, and substrate are necessary, as well as the development of clinical studies to obtain consistent conclusions to establish an ideal concentration capable of obtaining antimicrobial activity without altering or impairing physicochemical characterization and resin-dentin-bonding stability of adhesive systems.

Conclusion

In the present study, EGCG was able to reduce the formation of dental biofilm when incorporated into the self-etching universal adhesive at 0.5% of concentration. However, it caused a significant alteration of the solubility and sorption of the adhesive.

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Conflicts of interest

There are no conflicts of interest.

References

1. Perdigão J. New developments in dental adhesion. Dent Clin North Am 2007;51:333-57, viii.
2. Van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M, Vijay P, *et al*. Adhesion to enamel and dentin: Current status and future challenges. Oper Dent 2003;28:215-35.
3. Khamverdi Z, Rezaei-Soufi L, Rostamzadeh T. The effect of epigallocatechin gallate on the dentin bond durability of two self-etch adhesives. J Dent (Shiraz) 2015;16:68-74.
4. García-Godoy F, Tay FR, Pashley DH, Feilzer A, Tjäderhane L, Pashley EL. Degradation of resin-bonded human dentin after 3 years of storage. Am J Dent 2007;20:109-13.
5. Shono Y, Terashita M, Shimada J, Kozono Y, Carvalho RM, Russell CM, *et al*. Durability of resin-dentin bonds. J Adhes Dent 1999;1:211-8.
6. Pashley DH, Carvalho RM. Dentine permeability and dentine adhesion. J Dent 1997;25:355-72.
7. Tay FR, Pashley DH, Garcìa-Godoy F, Yiu CK. Single-step, self-etch adhesives behave as permeable membranes after polymerization. Part II. Silver tracer penetration evidence. Am J Dent 2004;17:315-22.
8. Salz U, Zimmermann J, Zeuner F, Moszner N. Hydrolytic stability of self-etching adhesive systems. J Adhes Dent 2005;7:107-16.
9. Jacobsen T, Söderholm KJ. Some effects of water on dentin bonding. Dent Mater 1995;11:132-6.
10. Ikeda T, De Munck J, Shirai K, Hikita K, Inoue S, Sano H, *et al*. Effect of evaporation of primer components on ultimate tensile strengths of primer-adhesive mixture. Dent Mater 2005;21:1051-8.
11. Ito S, Hashimoto M, Wadgaonkar B, Svizero N, Carvalho RM, Yiu C, *et al*. Effects of resin hydrophilicity on water sorption and changes in modulus of elasticity. Biomaterials 2005;26:6449-59.
12. Martins DO, Vasconcelos MR, Portela AI. Antimicrobial agents in adhesive systems. Rev Bras Odontol 2014;71:130-4.
13. Du X, Huang X, Huang C, Wang Y, Zhang Y. Epigallocatechin-3-gallate (EGCG) enhances the therapeutic activity of a dental adhesive. J Dent 2012;40:485-92.
14. Cheng XW, Kuzuya M, Kanda S, Maeda K, Sasaki T, Wang QL, *et al*. Epigallocatechin-3-gallate binding to MMP-2 inhibits gelatinolytic activity without influencing the attachment to extracellular matrix proteins but enhances MMP-2 binding to TIMP-2. Arch Biochem Biophys 2003;415:126-32.
15. Martin-De Las Heras S, Valenzuela A, Overall CM. The matrix metalloproteinase gelatinase A in human dentine. Arch Oral Biol 2000;45:757-65.
16. Hannas AR, Pereira JC, Granjeiro JM, Tjäderhane L. The role of matrix metalloproteinases in the oral environment. Acta Odontol Scand 2007;65:1-3.
17. Yu HH, Zhang L, Yu F, Li F, Liu ZY, Chen JH. Epigallocatechin-3-gallate and Epigallocatechin-3-O-(3-O-methyl)-gallate Enhance the Bonding Stability of an Etch-and-Rinse Adhesive to Dentin. Materials (Basel) 2017;10:183.
18. Demeule M, Brossard M, Pagé M, Gingras D, Béliveau R. Matrix metalloproteinase inhibition by green tea catechins. Biochim Biophys Acta 2000;1478:51-60.
19. Dell'Aica I, Caniato R, Biggin S, Garbisa S. Matrix proteases, green tea, and St. John's wort: Biomedical research catches up with folk medicine. Clin Chim Acta 2007;381:69-77.
20. Yoo S, Murata RM, Duarte S. Antimicrobial traits of tea- and cranberry-derived polyphenols against *Streptococcus mutans*. Caries Res 2011;45:327-35.
21. Malacarne J, Carvalho RM, de Goes MF, Svizero N, Pashley DH, Tay FR, *et al*. Water sorption/solubility of dental adhesive resins. Dent Mater 2006;22:973-80.
22. Melo MA, Rolim JP, Zanin IC, Barros EB, da-Costa EF, Rodrigues LK. Characterization of antimicrobial photodynamic therapy-treated Streptococci mutans: An atomic force microscopy study. Photomed Laser Surg 2013;31:105-9.
23. Ito S, Hoshino T, Iijima M, Tsukamoto N, Pashley DH, Saito T. Water sorption/solubility of self-etching dentin bonding agents. Dent Mater 2010;26:617-26.
24. Neri JR, Yamauti M, Feitosa VP, Pires AP, Araújo Rdos S, Santiago SL. Physicochemical properties of a methacrylate-based dental adhesive incorporated with epigallocatechin-3-gallate. Braz Dent J 2014;25:528-31.
25. Xu X, Zhou XD, Wu CD. The tea catechin epigallocatechin gallate suppresses cariogenic virulence factors of *Streptococcus mutans*. Antimicrob Agents Chemother 2011;55:1229-36.
26. Xu X, Zhou XD, Wu CD. Tea catechin epigallocatechin gallate inhibits *Streptococcus mutans* biofilm formation by suppressing gtf genes. Arch Oral Biol 2012;57:678-83.
27. de Assis JS, Lima RA, Marques Lima JP, Azevedo Rodrigues LK, Santiago SL. Effect of epigallocatechin-3-gallate application for remaining carious dentin disinfection. J Conserv Dent 2015;18:51-5.
28. Fonseca BM, Barcellos DC, Silva TM, Borges AL, Cavalcanti BD, Prakki A, *et al*. Mechanical-physicochemical properties and biocompatibility of catechin-incorporated adhesive resins. J Appl Oral Sci 2019;27:e20180111.
29. Pallan S, Furtado Araujo MV, Cilli R, Prakki A. Mechanical properties and characteristics of developmental copolymers incorporating catechin or chlorhexidine. Dent Mater 2012;28:687-94.
30. Lambert JD, Sang S, Hong J, Kwon SJ, Lee MJ, Ho CT, *et al*. Peracetylation as a means of enhancing *in vitro* bioactivity and bioavailability of epigallocatechin-3-gallate. Drug Metab Dispos 2006;34:2111-6.
31. Kim WJ, Kim JD, Kim J, Oh SG, Lew YW. Selective caffeine removal from green tea using supercritical carbon dioxide extraction. J Food Eng 2008;89:303-9.
32. Porto IC, de Aguiar FH, Brandt WC, Liporoni PC. Mechanical and physical properties of silorane and methacrylate-based composites. J Dent 2013;41:732-9.