



RESEARCH ARTICLE

REVISED The effect of the addition of propolis to resin-modified glass ionomer cement bracket adhesive materials on the growth inhibition zone of *Streptococcus mutans* [version 2; peer review: 2 approved]

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Abstract

Background: Orthodontic treatments progress alongside the development of adhesive materials. The present study aimed to determine the antibacterial properties of propolis, a natural product, in a mixture of resin-modified glass ionomer cement by observing the growth inhibition zone of *Streptococcus mutans*.

Methods: This was an *in vitro* study conducted on 45 samples of adhesive material, which were divided into control group (resin-modified glass ionomer cement bracket adhesive material), two groups of propolis concentrations (15%, and 25%) and duration (0, 15, and 30 days). The antibacterial effect of each sample was evaluated against *S. mutans* using an agar plate diffusion test. Measurement of the diameter of the growth inhibition zone of *S. mutans* was carried out. The data obtained were analyzed statistically. The significance value of the Kruskal Wallis and Mann-Whitney test was set at $p < 0.05$, while the significance value of the normality and homogeneity test was set at $p > 0.05$. Datas in this study that were not normally distributed ($p = 0.012$) but homogeneous ($p = 0.110$) were analyzed by the Kruskal-Wallis test ($p = 0.003$) and then the Mann-Whitney test was performed to determine differences in significance between groups.

Results: There was a relationship between concentration and duration of propolis to the growth inhibition zone of *S. mutans* ($p=0.003$). The addition of 25% propolis concentration inhibited the growth of *S. mutans* more than the addition of 15% and without propolis (control group). The addition of propolis to resin-modified glass ionomer cement for 15 days was more effective in inhibiting the growth of *S. mutans*.

Conclusion: The addition of propolis to adhesive materials provides an inhibitory effect on the growth of *S. mutans*, which may be effective in the world of preventive dentistry.

Keywords

Propolis, Resin Modified Glass Ionomer Cement, Inhibition Zone, Streptococcus mutans.

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REVISED Amendments from Version 1

This new version includes some minor changes in the background, discussion, detail of the method sections, and updated figures and tables (font, replacement of "resin modified" into "resin-modified", and "0% propolis" into "control group" as the reviewer's request).

Any further responses from the reviewers can be found at the end of the article

Introduction

Fixed orthodontic treatment can be a risk factor for plaque accumulation¹, which is significantly affected by the presence of bracket attachments and archwires². A large number of areas for microbial colonization during orthodontic treatment can cause plaque accumulation, especially around the bracket and cervical edge of the band³. As many as 60% of fixed orthodontic patients also show poor oral health, which is shown by the high plaque index value during orthodontic treatment and the presence of white spot lesions in ~50% of orthodontic patients^{4,5}. A study also reported that the incidence of fixed orthodontic patients with one new white spot lesion during treatment was 72.9% and the incidence of cavity lesions was 2.3%⁶.

The presence of fixed orthodontic appliances in the oral cavity increases microbial population. Observational studies have shown that there is a positive relationship between the use of fixed orthodontic appliances and the number of bacteria, such as *Streptococcus mutans*, on plaques, which is known as bacteria in early or initial caries⁷⁻⁹. These bacteria can attach to all surface locations in the oral cavity including the surface of the bracket and the area adjacent to the bracket¹⁰. Efforts to protect areas that are vulnerable to bacterial colonization needs to be done, especially the area around the bracket.

Fixed orthodontic treatment develops fast along with the development of adhesive materials used to attach brackets to the tooth surface¹¹. Brackets are attached to the teeth using acid etching or cemented using glass ionomer cement⁸. Resin-modified glass ionomer cement is developed by adding hydrophilic resins such as hydroxydimethacrylate and BIS-GMA to conventional glass ionomer cement. This material is not only known to be attached to the surface of the tooth, but is also able to release fluoride, has better physical properties, shorter setting time, and is more effective against humidity¹². At present, new research has been performed to develop a dental material with antibacterial activity, which will play an important role in preventing caries. Glass ionomer cement as bracket adhesive material can release fluoride but the desired antibacterial effect needs to be enhanced¹³.

Over the past few decades, the use of natural products for pharmacological purposes has increased in the world¹⁴. Propolis is a sticky resin substance collected by honey bees from the sap of plants, leaves, and buds, which are mixed with the sap and saliva of bees in the nest. There are more than 180 chemical substances contained in propolis and are influenced by the type of bee, climate, plants and trees, and the time of collection.

Bees use propolis to strengthen the nest wall and protect it from infection, and the human population uses this product for many purposes¹⁵, for example, propolis is known to provide protection against cariogenic bacteria and oral pathogens¹⁶. Hasan *et al.*¹⁷, stated that there are few studies about propolis activity from Indonesia.

Propolis, as a natural product, can be used in a mixture of glass ionomer cement in order to increase antibacterial activity^{13,18}. Megawati¹⁹ investigated the shear bond strength of metal brackets bonding to resin-modified glass ionomer cement adhesive materials combined with 25% and 50% propolis. The addition of 50% propolis concentration had better shear bond strength and was able to withstand shear bond strength of 6–8 MPa according to the standard strength of clinically acceptable adhesive materials. The antibacterial potential of propolis added to resin-modified glass ionomer cement needs to be further investigated because orthodontic treatment takes place over a long period of time. Hatunoglu *et al.*¹³ has also investigated that the addition of 25% and 50% Turkish propolis to glass ionomer cements increased antibacterial activity without modifying the mechanical properties of glass ionomer cements¹³ but they have not conducted time-related research. Further research on the effect of the addition of 15%, and 25% Indonesian propolis for 0, 15 and 30 days on resin-modified glass ionomer cement to the growth inhibition zone of *S. mutans* has never been done. Therefore, our research was the first study to investigate the relationship between concentration and duration of propolis to the growth inhibition zone of *S. mutans*.

The present study was an *in vitro* experimental study done in order to determine the antibacterial activity of resin-modified glass ionomer cement combined with Indonesian propolis on *S. mutans*. The hypothesis was there was a relationship between concentration and duration of propolis to the growth inhibition zone of *S. mutans*. Adding propolis to this orthodontic bracket adhesive will increase the antibacterial properties of the adhesive.

Methods

Ethical clearance for the study was obtained from Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta, Indonesia (No.001621/KKEP/FKG-UGM/EC/2018).

Preparation of propolis extract

Pure propolis was produced by honeybees (*Apis mellifera*) in Indonesia (Figure 1). The propolis (970g) was purchased from Klinik Apitheraphy Kusuma (Moyudan, Sleman, Daerah Istimewa Yogyakarta) and the production of the extract was done in LPPT Unit 1, Universitas Gadjah Mada, Yogyakarta, Indonesia. Propolis samples were chopped into small pieces and ground using a blender. Then, each 250 g sample of propolis was dissolved in 2500 mL of ethanol 80%, stirred at 800 rpm for 30 minutes using an electric stirrer (RW 20 digital; IKA, Germany) and left for 24 hours at room temperature. Rough particles were removed from the propolis extract using rough filter paper (58 cm × 58 cm) and the propolis was stirred once again for 30 minutes using an electric stirrer, left for 24 hours, and filtered. The filtrate was concentrated by a vacuum rotary



Figure 1. Propolis produced by *Apis mellifera*.

evaporator. Next, the extract is poured in a porcelain cup and heated with a waterbath (70 °C) to produce propolis extract. Samples were kept in a dry and dark place until they were used¹⁹.

Preparation of resin-modified glass ionomer cement containing propolis

Conventional adhesive resin-modified glass ionomer cement (Fuji Ortho LC, GC, Japan), which is made up of powder and liquid, was used in this study. Samples were prepared containing the conventional resin-modified glass ionomer cement liquid and the two concentrations of propolis (15% and 25%) :

(i) Resin-modified glass ionomer cement without propolis (Powder^{RMGIC}:Liquid^{RMGIC} ratio = 1:1) (n=15); (ii) resin-modified glass ionomer cement with 15% propolis (Powder^{RMGIC}:Liquid^{RMGIC}:Propolis Extract ratio = 1:0.85:0.15) (n=15); (iii) resin-modified glass ionomer cement with 25% propolis (Powder^{RMGIC}:Liquid^{RMGIC}:Propolis Extract ratio = 1:0.75:0.25) (n=15). Each group of samples was incubated for 0, 15, and 30 days (n=5/time duration). The adhesive materials were mixed according to the manufacturer's instructions. After mixing the powder and liquid of each cement, samples were put into cylindrical molds (5 mm in diameter and 0.64 mm thickness), and the upper surface was flattened by pressing down and exposed by light-curing unit (LY-B200, Liang Ya, China) for 10 seconds each surface (Figure 2)^{19,20}.

Agar disk diffusion test

Agar disk diffusion test was performed at the Microbiology Laboratory, Faculty of Veterinary, Universitas Gadjah Mada, Indonesia. *S. mutans* ATCC 25175 type strain was used throughout the study. It was pure culture from the isolated laboratory of the Microbiology, Faculty of Veterinary, UGM, Yogyakarta, Indonesia. Bacterial strain from stock cultures was cultivated in Brain Heart Infusion broth at 37°C for 24 hours, corresponding

to 10⁸ CFU/mL using the McFarland scale. *S. mutans* was spread uniformly on the surface of Mueller Hinton Agar plates to produce a lawn. Adhesive samples were inserted in the plates. After a 24h incubation period in an incubator at 37°C, the plates were taken out of the incubator and the antibacterial activity was evaluated using a digital caliper to measure the diameter of halos of growth inhibition of the strain at three different points. The inhibitory zone was considered the distance (mm) from the outside margin of the samples to the initial point of the microbial growth (Figure 3). The mean was calculated for each sample and all measurements were performed by the same blinded operator²¹.

Statistical analysis

Data were analyzed using SPSS IBM for Windows (Version 22.0). The normality and homogeneity of variance in each group was confirmed before analyzing the data. Saphiro-Wilk normality test is conducted to determine the distribution of data and the Levene homogeneity test is carried out to determine the homogeneity of data variance. Datas of resin-modified

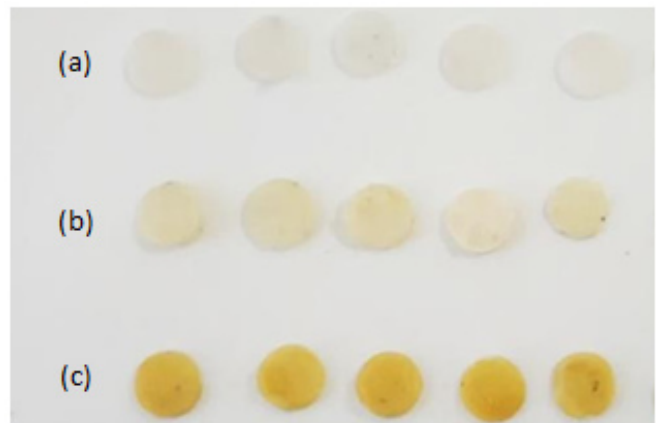


Figure 2. Adhesive materials ready to be tested. Resin-modified glass ionomer cement (a) control, (b) 15% propolis, and (c) 25% propolis.

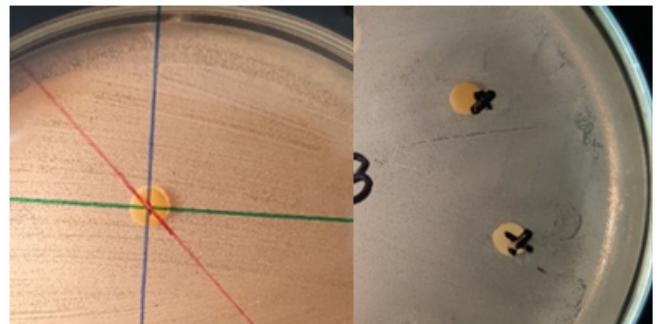


Figure 3. Measurement of growth inhibition zone diameter. Left panel, measurement guidance; right panel, growth inhibition zone of *S. mutans*.

glass ionomer cement without propolis, 15%, and 25% propolis groups for 0, 15, and 30 days group were not normal ($p = 0.012$) but homogenous ($p = 0.110$), so they were analyzed using Kruskal Wallis test and then Mann-Whitney test to determine the significance differences between groups. The significance level was set at 5%. The significance value of the normality and homogeneity test was ($p > 0.05$) while the significance value of the Kruskal Wallis and Mann-Whitney test was ($p < 0.05$).

Results

Propolis, as a natural product, was combined with resin-modified glass ionomer cement in order to assess antibacterial activity against *S. mutans*. The mean diameters of bacterial

growth inhibited by different concentrations and duration of propolis combination in the adhesive are shown in Table 1 and Figure 4. The growth inhibition zone in the present study was shown as a transparent or clear area around the adhesive materials.

Table 1 and Figure 4 provides summarized data regarding the agar diffusion method and show the mean and standard deviation values of the diameter of growth inhibition zone for each sample in the groups against the *S. mutans* strain. Clear inhibition zones were shown showing that the propolis enhanced the antibacterial effect of the resin-modified glass ionomer cement. Among the concentrations (control, 15%, and 25% propolis), control group still showed inhibitory effect reflecting

Table 1. Means and standard deviation of growth inhibition zone of *Streptococcus mutans* among groups. Percentages are propolis concentration in resin-modified glass ionomer cement.

Adhesive materials	Duration (days)	n	Mean (mm)	Standard deviation
Resin-modified glass ionomer cement (control)	0	5	0.940	0.370
	15	5	11.730	0.720
	30	5	8.75	0.359
Resin-modified glass ionomer cement with 15% propolis	0	5	2.132	0.293
	15	5	12.754	1.053
	30	5	12.360	0.359
Resin-modified glass ionomer cement with 25% propolis	0	5	2.412	0.328
	15	5	17.658	0.928
	30	5	13.604	0.892

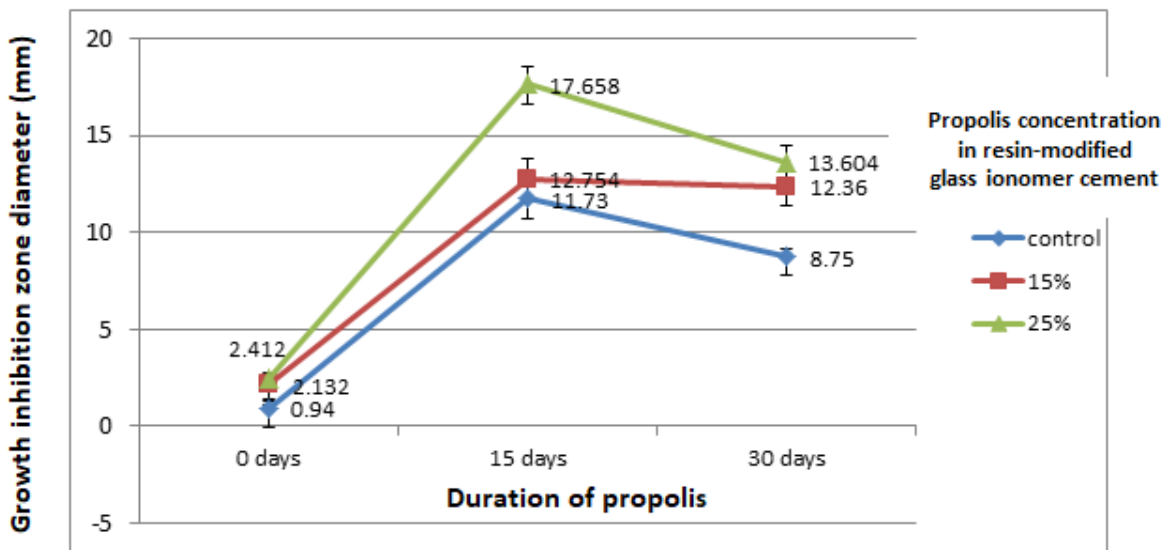


Figure 4. Means and standard deviation of growth inhibition zone of *Streptococcus mutans* among groups.

that the resin-modified glass ionomer cement has its own antibacterial activity. The addition of 25% propolis revealed higher antibacterial activity than 15% and control (Table 1 and Figure 4). As can be seen in Table 1 and Figure 4, the mean diameter of growth inhibition zone for 0 days was lower than 30 and 15 days. In general, the duration of propolis after 15 days resulted in a greater inhibition zones compared with 30 and 0 days.

Looking at the data together (concentration and duration, it was observed that there was an interaction between concentration and duration of propolis to the growth inhibition zone against *S.mutans* (Table 2 and Figure 5). The antibacterial activity of

resin-modified glass ionomer cement with 25% propolis for 15 days was the highest among all other concentrations for all tested days ($p < 0.05$).

Discussion

An increase in the number of bacteria and plaques in the oral cavity of fixed orthodontic patients is one challenge for orthodontists. *Streptococcus mutans* is the first bacteria that play an important role in the etiology of caries because this species form a colony and be the initiator of other bacteria to do the same thing²². In the present study, a growth inhibition zone as a high concentration of propolis increased the antibacterial activity of the adhesive material to *S.mutans*, which was shown by an

Table 2. Mann-Whitney test results between adhesive materials.

*Statistically significant ($p < 0.05$)

Group	IA	IB	IC	IIA	IIB	IIC	IIIA	IIIB	IIIC
IA		0.009*	0.009*	0.009*	0.009*	0.009*	0.009*	0.009*	0.009*
IB			0.009*	0.009*	0.175	0.117	0.009*	0.009*	0.009*
IC				0.009*	0.009*	0.009*	0.009*	0.009*	0.009*
IIA					0.009*	0.009*	0.347	0.009*	0.009*
IIB						0.917	0.009*	0.009*	0.076
IIC							0.009*	0.009*	0.016*
IIIA								0.009*	0.009*
IIIB									0.009*
IIIC									

I: Resin-modified glass ionomer cement; II: Resin-modified glass ionomer cement with 15% propolis ; III: Resin-modified glass ionomer cement with 25% propolis; A: 0 days; B: 15 days; C: 30 days

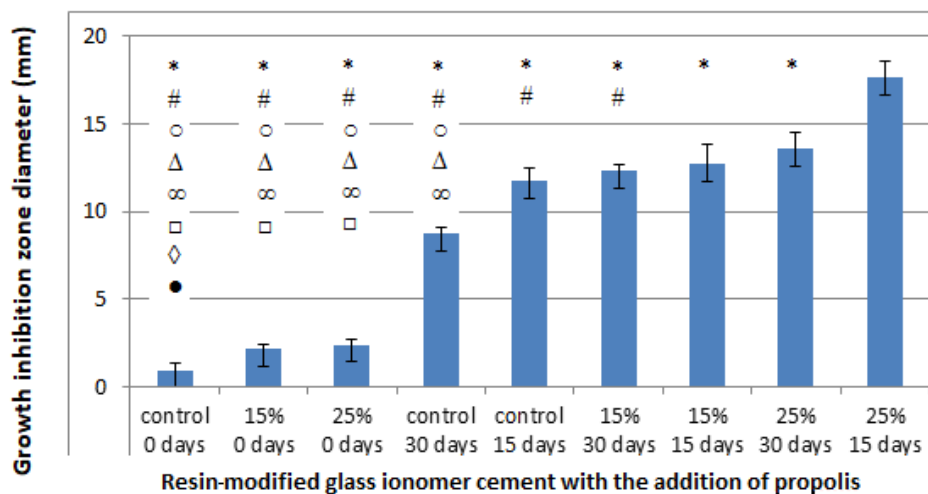


Figure 5. The graph of Mann-Whitney test result between adhesive materials. Note : Statistically significant ($p < 0.05$). * = < 25% 15 days, # = < 25% 30 days, o = < 15% 15 days, Δ = < 15% 30 days, ∞ = < control 15 days, □ = < control 30 days, ◇ = < 25% 0 days, ● = < 15% 0 days.

increase in the diameter of the growth inhibition zone. Resin-modified glass ionomer cement with the addition of 25% propolis showed the largest diameter of the inhibition zone in all periods, indicating the highest antibacterial activity compared to other treatment groups (15% propolis and control group). Therefore, the addition of propolis to orthodontic adhesive materials as an antibacterial agent to inhibit the growth of *Streptococcus mutans* can be considered. This is in accordance with Asdar *et al.*²², who stated that propolis could inhibit the growth of *S. mutans* and it appeared in diameter changes of the inhibition zone. The greater the concentration of propolis, the greater the effect of inhibition produced.

The results agree with many researchers who have demonstrated the antibacterial activity of propolis. The mechanism of propolis against microorganisms is complex. Propolis works by inhibiting bacterial mobility and enzyme activity, as well as affecting the cytoplasmic membrane, which changed membrane permeability¹⁵. The functional and structural damage is suspected to occur due to components in propolis extract, such as flavonoids as the main constituent (quercetin, galangin, and pinocembrin), caffeic acid, benzoic acid, and cinnamic acid^{17,23,24}. Koo *et al.*²⁵, revealed that flavonoids, the largest component of propolis *Apis mellifera*, works by inhibiting glycosyltransferase activity. Extracellular polysaccharides, generally glucans, are produced by glucosyltransferase and play a role in the cariogenicity of dental biofilms²⁶. Therefore, inhibition of glucosyltransferase interferes with cell metabolism through biochemical reactions and has the potential to prevent caries, especially in fixed orthodontic patients. Flavonoids interact with bacterial cell walls, forming complex compounds with extracellular proteins through hydrogen bonds so that they inhibit the activity of microorganisms, including bacterial mobility²⁷. In addition, Pelczar and Chan²⁸ showed that flavonoids denature and coagulate bacterial cell proteins so that cell damage cannot be repaired. Flavonoids could penetrate bacterial cell peptidoglycans so that the cell layer is not intact. The instability of cell walls causes the permeability of the cell and the control of the protein composition to be disrupted so that bacterial cells lose their shape and are lysed²⁸. Pelczar and Chan²⁸ also revealed that the higher the concentration of an antibacterial agent the stronger the antibacterial activity. An increased concentration of propolis added to the resin-modified glass ionomer cement in the present study resulted in a darker color. The antibacterial ability of the resin-modified glass ionomer cement increased with a higher propolis concentration. The results of the study were also in line with research conducted by Woo²⁹, who concluded that the addition of antibacterial agents to glass ionomer cement, such as propolis, which had a darker color of propolis indicated more flavonoid as an active substance; therefore, the antibacterial activity increased with higher flavonoid content.

The present results showed that the duration of propolis treatment significantly affected the growth inhibition zone of *S. mutans* on resin-modified glass ionomer cement. There were smaller diameter values of the growth inhibition zone on all types of adhesive materials with the addition of

propolis for 30 days compared with 15 days which indicated lower antibacterial activity of the adhesive material. This is similar to research that examined the antibacterial effect of resin-modified glass ionomer cement and concluded that there was an increase in the average diameter of inhibition zones over time, especially in the first week, namely days 1, 3 and 7³⁰.

Higher antibacterial activity on day 15 than day 0 in the present study could be caused by several things, such as changes in pH and release of fluoride. An inhibition zone was suspected to be caused by the production of a low pH around the test material. The resin-modified glass ionomer cement liquid component contained hydroxyethyl methacrylate (HEMA), which may have facilitated low pH and contributed to antibacterial properties³¹. Prasad and Maradia³² also added that the initial value of pH after mixing was also acid, where most bacterial growth would be suppressed then the pH value began to increase to a neutral level where it was not enough to inhibit bacterial growth. Kavita *et al.*³³, mentions that an increase in pH and a decrease in the release of fluoride ions explains the decrease in antibacterial activity.

Fluoride inhibits the acid production and glucans by *S. mutans*, which has been stated by Wiegand *et al.*³⁴, who reviewed that the release of fluoride could reduce demineralization, increase remineralization, and inhibit bacterial growth so that glass ionomer cement was cariostatic. Additionally, Featherstone³⁵ demonstrated that fluoride worked by inhibiting bacterial metabolism through changes in hydroxyapatite on enamel to fluorapatite so that the enamel was more resistant to acid and increased remineralization. The high release of fluoride in resin-modified glass ionomer cement occurred because the acid-base reaction was slowed down by the resin component, causing the ionized matrix to be less mature and capable of releasing more fluoride when compared to other materials, such as composite resins; greater pore size and porosity in resin-modified glass ionomer cement; lower solubility and higher proportion of liquid powder with high viscosity³⁶. Material with a resin content that is slightly like resin-modified glass ionomer cement has a higher porosity, which facilitates the diffusion of fluoride³⁷. This is also in line with research of Fucio *et al.*³⁸, who found that resin-modified glass ionomer cement changed in fluoride ion release over time. In that study, the release of fluoride at the beginning of the study occurred because the glass particles reacted with polyalkenoic acid, while continuous fluoride release could be caused by the ability of fluoride to diffuse through the cement pore. According to Toba *et al.*³⁹, the hydrophilic property of HEMA from resin-modified glass ionomer cement is required for the water absorption process and helps the diffusion of fluoride, which causes an increase in the release of fluoride ions.

In the present study, a smaller diameter of the inhibition zone on day 30 than day 15 indicated that the antibacterial effect of the material decreased over time. The result of this study was in accordance with Matalon *et al.*⁴⁰, who showed that high antibacterial potency of resin-modified glass ionomer cement at the beginning of their study decreased for the next 3 weeks

even though the antibacterial material was durable. Therefore, fluoride release of resin-modified glass ionomer cement could decrease significantly with long-term use⁴¹. The antibacterial activity of resin-modified glass ionomer cement in the present study was lower on the 30th day.

Statistical analysis showed that there was an interaction between concentration and duration of propolis to the growth inhibition zone of *S. mutans* ($p < 0.05$). This means that our hypothesis was accepted. The results of the study were following the study by Dastjerdie *et al.*⁵, where the antibacterial activity of adhesive materials to the growth of *S. mutans* depended on the type of cement and time.

Diameter of the inhibition zone in the present study was smaller on day 30 compared with day 15 with the addition of propolis; however, this is classified as a strong response (11–20 mm) compared to the response of resin-modified glass ionomer cement without addition of propolis, classified as a medium response (5–10 mm)⁴². A survey of 500 patients at a public health center in Jakarta and dental hospital at Universitas Indonesia conducted by Maringka and Herda⁴³ showed that 90% of respondents had experienced bracket detachment and around 60% of respondents experienced this event before their next appointment (three weeks after placement). Therefore, a strong response from the results of the day 30 treatment would be enough in the first 3 weeks. Although resin-modified glass ionomer cement as an orthodontic adhesive material that releases fluoride has been used, the addition of propolis to

orthodontic adhesive materials also provides an additional effect on the growth of *S. mutans* and was quite effective in this study. Propolis, as an antibacterial additive to resin-modified glass ionomer cement, still needs further research aimed at getting a combination of physical properties, propolis concentration, shear strength, and tensile strength that meet the optimal standard of orthodontic adhesive; increasing stability and adequating propolis resistance.

Conclusion

The addition of propolis to adhesive materials gives an inhibitory effect on the growth of *Streptococcus mutans*. There was an interaction between concentration and duration of propolis and antibacterial effect against *Streptococcus mutans*.

Data availability

Underlying data

Figshare: Growth Inhibition Zone Around Resin-modified Glass Ionomer Cement Bracket Adhesive, <https://doi.org/10.6084/m9.figshare.10263275.v4>⁴⁴.

This project contains the following underlying data:

- Growth inhibition zone around resin-modified glass ionomer cement bracket adhesive

Data are available under the terms of the [Creative Commons Zero “No rights reserved” data waiver](#) (CC0 1.0 Public domain dedication).

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Version 2

Reviewer Report 28 July 2020

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The authors have revised the manuscript successfully as per our suggestion. In addition, we strongly recommend this paper.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Orthodontics

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 08 April 2020

<https://doi.org/10.5256/f1000research.22785.r61777>

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Lidia Audrey Rocha Valadas 

Postgraduate Program in Development and Technological Innovation in Drugs, School of Pharmacy, Federal University of Ceara, Fortaleza, Brazil

Introduction:

- You should justify the reason for studying just *S.mutans*, why not more species related to orthodontics fixed appliances?

Materials and methods:

- You should cite the georeferencing of the extract and the chemical analysis performed to identify the main constituents.
- I don't agree with the statement about 3 groups with different concentrations - I suggest 2 groups and one control. 0% is not a propolis concentration group.

Discussion:

- Add the limitations of the study.
- Please check reference 34, it is a review, in your discussion you cited it as a research article.

References

1. Furtado Júnior JHC, Valadas LAR, Fonseca SGDC, Lobo PLD, et al.: Clinical and Microbiological Evaluation of Brazilian Red Propolis Containing-Dentifrice in Orthodontic Patients: A Randomized Clinical Trial. *Evid Based Complement Alternat Med*. 2020; **2020**: 8532701 [PubMed Abstract](#) | [Publisher Full Text](#)

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Cariology; Natural Products

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 14 Apr 2020

Stefani Kristanti Saputra, Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta, Indonesia

Dear Dr Lidia Valadas,

Thank you for your kind assistance in reviewing our manuscript and providing us with valuable advice for the manuscript and our future work. Please allow us to comment as follows:

Introduction:

You should justify the reason for studying just *S.mutans*, why not more species related to orthodontics fixed appliances?

The presence of fixed appliances in the oral cavity of orthodontic patients increases the microbial population, such as *Streptococcus mutans* known as bacteria that plays an important role in the etiology of caries. *S. mutans* is the first bacteria to form a colony and be the initiator of other bacteria to do the same thing.²²

Material and Methods:

1. You should cite the georeferencing of the extract and the chemical analysis performed to identify the main constituents.

The propolis was purchased from Klinik Apitheraphy Kusuma (Moyudan, Sleman, Daerah Istimewa Yogyakarta, Indonesia) and the production of the extract was done in LPPT Unit 1, Universitas Gadjah Mada, Yogyakarta, Indonesia. The pure propolis was produced by honeybees (*Apis mellifera*) and the main constituent of propolis *Apis mellifera* is flavonoid.

2. I don't agree with the statement about 3 groups with different concentrations - I suggest 2 groups and one control. 0% is not a propolis concentration group.

We thank you for this valuable insight and it will be considered in our revision.

Discussion:

1. Add the limitations of the study.

Propolis is sufficient to be used as an antibacterial additive to resin modified glass ionomer cement, but it still needs further research aimed at the following:

- a. get a combination of physical properties, propolis concentration, shear strength, and tensile strength that meet the optimal standard of orthodontic adhesive; and
- b. increase stability and adequate propolis resistance.

2. Please check reference 34, it is a review, in your discussion you cited it as a research article.

Yes, it is a literature review. The purpose of the article was to review the fluoride release and recharge capabilities, and antibacterial properties, of fluoride-releasing dental restoratives, and discuss the current status concerning the prevention or inhibition of caries development and progression. We will revise and update the discussion section.

We thank you again for the valuable insight.

Competing Interests: No competing interests were disclosed.

Reviewer Report 23 March 2020

<https://doi.org/10.5256/f1000research.22785.r60534>

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First of all, please allow me to congratulate the authors for attempting to undertake this study which I found quite interesting and useful as a reference for further study in the orthodontics material field. The manuscript itself is well-written and well-structured and I have to also commend the authors for this matter.

However, I may require some clarifications on the following issues:

1. The paper is well organized and easy to follow.
2. To improve the readability, it is recommended that the text is checked by a native English speaking person as many of the sentences might be misunderstood. I suggest a revision of the grammar structures by an expert editor in revising manuscripts.

Abstract:

1. Please add the p-value used for the Kruskal Wallis test ($p < 0.05$ or $p < 0.01$) - add homogeneity and normality test p-value used ($p > 0.05$) in the abstract section.
2. Please add the exact p-value obtained after the statistic analysis ($p = \dots$).
3. Please check the keywords according to the MESH NCBI data base and sort alphabetically.

Introduction:

1. Please add your hypothesis.
2. Please add a statement or sentence about whether there are any similar studies that have been done before or not? Or is your study the first study to investigate this topic?
3. Please add the critical or important issue within this study.
4. Please add the reason why only *S. mutans* was examined in this study.

Material and Methods:

1. It would better if you add the detailed protocol or cite the protocol reference.
2. It would better if you add the confirmation of *S. mutans* colony or the detail of ATCC.

3. It would be better if you add more specific methods than agar disk diffusion test to examine the combination of propolis and GIC inhibit the *S. mutans*.
4. In this study, the authors performed the Kruskal wallis test. It would be better if the authors also described the Levene's and Shapiro-Wilk test.

Results:

1. It would be better to understand and attract the reader if you present the data in the diagrams.
2. Please add the results of the Levene's and Shapiro-Wilk tests
3. Please indicate whether the difference is significant or not in your graph/diagrams as well, using some symbols (asterisk).

Discussion:

1. Discussion of the results is quite comprehensive. In analyzing the results, the authors also show citations from the previous study to support the explanation of these results.
2. The answer to the hypothesis of this study has been included at the beginning of the discussion section.
3. Please mention the limitation(s) of this study in the discussion section.

References:

- The supporting references are inadequate, please add the newest references that support this study.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Herbal in Orthodontics

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

Author Response 02 Apr 2020

Stefani Kristanti Saputra, Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta, Indonesia

Dear Prof Ida Bagus Narmada & Dr Alexander Patera Nugraha,

Thank you for your kind assistance in reviewing our manuscript and providing us with valuable advices. Please allow us to comment as follows:

Abstract:

1. Please add the p-value used for the Kruskal Wallis test ($p < 0.05$ or $p < 0.01$) - add homogeneity and normality test p-value used ($p > 0.05$) in the abstract section.

2. Please add the exact p-value obtained after the statistic analysis ($p = \dots$).

The significance value of the normality and homogeneity test was ($p > 0.05$) while the significance value of the Kruskal Wallis test was ($p < 0.05$).

Datas in this study that were not normally distributed ($p = 0.012$) but homogeneous ($p = 0.110$) were analyzed by the Kruskal-Wallis test ($p = 0.003$) and then the Mann-Whitney test was performed to determine differences in significance between treatment groups. The significance value of the Mann-Whitney test was ($p < 0.05$).

3. Please check the keywords according to the MESH NCBI data base and sort alphabetically.

Disk Diffusion Antimicrobial Tests. Glass Ionomer Cement. Propolis. *Streptococcus mutans*.

Introduction:

1. Please add your hypothesis.

There is an relationship between concentration and duration of propolis to the growth inhibition zone of *S. mutans*. Adding propolis to orthodontic bracket adhesive will increase the antibacterial properties of the adhesive.

2. Please add a statement or sentence about whether there are any similar studies that have been done before or not? Or is your study the first study to investigate this topic?

Hatunoglu et al. (2014) has investigated that the addition of 25% and 50% Turkish propolis to glass ionomer cements increased antibacterial activity without modifying the mechanical properties of glass ionomer cements¹³ but Hatunoglu et al. (2014) have not conducted time-related research. Research conducted by Megawati (2017)¹⁹ shows that the addition of 25% concentration of propolis to resin modified glass ionomer cement does not affect shear strength¹⁹. Further research on the effect of adding propolis concentrations of 0%, 15%, and 25% for 0, 15, and 30 days in resin modified glass ionomer cements on *Streptococcus mutans* growth inhibition zones has never been

done. Therefore, our research was the first study to investigate the relationship between concentration and duration of propolis to the growth inhibition zone of *S. mutans*.

3. Please add the critical or important issue within this study.

The presence of fixed appliances in the oral cavity of orthodontic patients increases the microbial population, such as *Streptococcus mutans* known as bacteria in early or initial caries. Glass ionomer cement is known to release fluoride but the desired antibacterial effect is still lacking.¹³ The antibacterial potential of propolis added to resin modified glass ionomer cement needs to be further investigated because orthodontic treatment takes place over a long period of time.

4. Please add the reason why only *S. mutans* was examined in this study.

S. mutans plays an important role in the etiology of caries. *S. mutans* is the first bacteria to form a colony and be the initiator of other bacteria to do the same thing.²²

Material and Methods:

1. It would be better if you add the detailed protocol or cite the protocol reference.

For the preparation of propolis extract, it refers to the research conducted by Megawati (2017)¹⁹. For the preparation of resin modified glass ionomer cement containing propolis, it refers to the research conducted by Megawati (2017)¹⁹ and Sodagar et al. (2017)²⁰. For the agar disk diffusion test, it refers to the research conducted by Yusuf (2016)²¹.

2. It would be better if you add the confirmation of *S. mutans* colony or the detail of ATCC.

The *Streptococcus mutans* used in our study was pure culture from the isolate laboratory of the Microbiology Faculty of Veterinary Medicine UGM, Yogyakarta, Indonesia.

3. It would be better if you add more specific methods than agar disk diffusion test to examine the combination of propolis and GIC inhibit the *S. mutans*.

The diffusion method is most widely used to determine the sensitivity of bacteria to material because it is relatively simple and inexpensive. Unfortunately, it cannot determine the bactericidal and bacteriostatic properties of the drug. Hatunoglu (2014) has used a dilution method to determine the value of Minimum Inhibitory Concentration (MIC) of antibacterial material as the antibacterial capacity of glass ionomer cement with propolis¹³. This has not been included in this project but it will surely be considered for our future work.

4. In this study, the authors performed the Kruskal wallis test. It would be better if the authors also described the Levene's and Shapiro-Wilk test.

Saphiro-Wilk normality test is conducted to determine the distribution of data and the Levene homogeneity test is carried out to determine the homogeneity of data variance. Data that is not normally distributed or not homogeneous are analyzed by the Kruskal-Wallis test and then the Mann-Whitney test was performed to determine differences in significance between treatment groups. The significance value of the normality and homogeneity test was ($p > 0.05$) while the significance value of the Kruskal Wallis and Mann-Whitney test was ($p < 0.05$).

Results:

1. It would be better to understand and attract the reader if you present the data in the diagrams. Please indicate whether the difference is significant or not in your graph/diagrams as well, using some symbols (asterisk).

We will provide the data in the diagram with the new version of the article.

2. Please add the results of the Levene's and Shapiro-Wilk tests

The results of the Shapiro-Wilk test was data in this study that were not normally distributed ($p = 0.012 < 0.05$) and the results of the Levene test was the datas were homogeneous ($p = 0.110 > 0.05$).

Discussion:

Please mention the limitation(s) of this study in the discussion section.

Propolis is sufficient to be used as an antibacterial additive to resin modified glass ionomer cement, but it still needs further research aimed at the following:

1. get a combination of physical properties, propolis concentration, shear strength, and tensile strength that meet the optimal standard of orthodontic adhesive; and
2. increase stability and adequate propolis resistance.

We thank you again for this valuable insight.

Competing Interests: No competing interests were disclosed.

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