

## Research Report

# Inhibition of dental plaque formation by toothpaste containing propolis

Nurin Aisyiyah Listyasari and Oedijani-Santoso

Department of Oral and Dental Health  
Faculty of Medicine, Universitas Diponegoro  
Semarang – Indonesia

### ABSTRACT

**Background:** Plaque is the main cause of caries and periodontal disease. Caries and periodontal disease can be prevented by inhibiting dental plaque formation. To inhibit the formation of plaque, teeth must be brushed with toothpaste. According to previous studies, propolis contains apigenin and tt-farnesol classified as flavonoid that can inhibit the formation of dental plaque by inhibiting glucosyltransferase enzyme and membrane integrity of *Streptococcus mutans*. **Purpose:** The aim of this study was to determine the effect of toothpaste containing propolis on the formation of dental plaque. **Methods:** Post test with only control group design was used. The subjects of this study were 30 boarding school students of Hidayatullah, Yayasan Al-Burhan, Gedawang, Semarang, divided into two groups, randomized control group and treatment group. Control group was not treated with toothpaste containing propolis. Meanwhile, treatment group was treated with toothpaste containing propolis. Plaque then was measured by using plaque index of Sillness and Loe method after using toothpaste containing propolis for four hours. Afterwards, the data was analyzed by a computer program, Mann-Whitney test, with its significance  $p < 0.05$ . **Results:** The result of Mann-Whitney test showed a significant difference, 0.002 ( $p < 0.05$ ), between the control group and the treatment group. The median of the control group was about 3.41, while that of the treatment group was about 0.58. **Conclusion:** The use of toothpaste containing propolis can prevent dental plaque formation.

**Key words:** Propolis, tooth paste, dental plaque

### ABSTRAK

**Latar belakang:** Plak merupakan penyebab utama terjadinya karies dan penyakit periodontal. Karies dan penyakit periodontal dapat dicegah dengan menghambat pembentukan plak gigi. Untuk mencegah terbentuknya plak, gigi harus digosok menggunakan pasta gigi. Penelitian terdahulu menyebutkan bahwa propolis mengandung flavonoid apigenin dan tt-farnesol yang mampu menghambat aktivitas enzim glukosiltransferase dan menghambat pembentukan membran bakteri *Streptococcus mutans* yang berperan pada pembentukan plak gigi. **Tujuan:** Tujuan dari penelitian ini adalah untuk mengetahui pengaruh pasta gigi dengan kandungan propolis terhadap pembentukan plak gigi. **Metode:** Menggunakan rancangan post test only control group design. Sampel penelitian ini adalah santri Pondok Pesantren Hidayatullah Yayasan Al-Burhan, Gedawang, Semarang, sebanyak 30 santri dibagi dalam dua kelompok secara acak yaitu kelompok kontrol diberikan pasta gigi tanpa kandungan propolis dan kelompok perlakuan diberikan pasta gigi dengan kandungan propolis. Plak diukur dengan menggunakan indeks plak menurut Sillness and Loe sesudah menggunakan pasta gigi dalam jangka waktu kurang lebih empat jam. Data diolah menggunakan program komputer dengan analisis statistik non parametrik Mann-Whitney dan taraf signifikansi diterima bila  $p < 0,05$ . **Hasil:** Analisis statistik non parametrik Mann-Whitney menghasilkan perbedaan rerata bermakna ( $p < 0,05$ ) antara kelompok kontrol dan perlakuan sebesar 0,002. Nilai tengah skor plak pada kelompok kontrol sebesar 3,41 dan pada kelompok perlakuan sebesar 0,58. **Kesimpulan:** Penggunaan pasta gigi dengan kandungan propolis dapat menghambat pembentukan plak gigi.

**Kata kunci:** Propolis, pasta gigi, plak gigi

Correspondence: Oedijani-Santoso, c/o: Bagian Kesehatan Gigi dan Mulut, Fakultas Kedokteran Universitas Diponegoro. Jl. dr. Sutomo No. 18, Semarang 50321, Indonesia. E-mail: [oediyanisantoso@yahoo.com](mailto:oediyanisantoso@yahoo.com)

## INTRODUCTION

Dental plaque plays an important role in causing oral health problems. Dental plaque is a soft layer that consists of a collection of microorganisms breeding in a matrix. Dental plaque is firmly attached to the tooth surface that is not brushed.<sup>1</sup> Some studies showed that at the beginning of the formation of dental plaque, gram-positive cocci, such as *Streptococcus mutans* (*S. mutans*), *Streptococcus sanguis*, *Streptococcus mitis*, and *Streptococcus salivarius*, is mostly found.<sup>1-3</sup>

To prevent dental and oral health problems, brushing the teeth with toothpaste is needed since it can not only help to prevent oral disease, but also to make our permanent teeth strong.<sup>4, 5</sup> Fluoride contained in toothpaste is a chemical substance that can prevent our teeth from dental cavities, however, the use of fluoride in large amounts over certain period of time can cause enamel fluorosis, tooth enamel with speckled spots caused by fragile tooth enamel with an irreversible blackish brown color.<sup>6</sup>

The use of natural ingredients actually can reduce the side effects of chemicals on the body, so the addition of natural ingredients in toothpaste can support dental and oral health care program.<sup>7</sup> Propolis is a natural substance collected by honey bees from various types of plants, especially from buds and leaves. The benefit of propolis in oral health is as an anti-bacterial because of the flavonoid contained in it. Apigenin and tt-farnesol are classified as important flavonoids because they can prevent glucosyltransferase enzyme activity and subsequently inhibit the growth of *S. mutans* causing dental plaque formation.<sup>8, 9</sup>

The study was aimed to determine the effect of toothpaste containing propolis on the formation of dental plaque, assessed by plaque index measured with Sillness and Loe method. Finally, this research was expected to provide benefits and evidence that toothpaste containing propolis may reduce dental plaque scores, so it can provide an alternative option of effective toothpaste for society and medical personnel to prevent dental plaque formation.

## MATERIALS AND METHODS

This research was an experimental research with Post Test Only Control Group Design.<sup>10</sup> The population in this research were students of Pondok Pesantren Hidayatullah of Yayasan Al-Burhan in Gedawang, Semarang. The samples were obtained by consecutive sampling. Based on the calculation, the samples obtained were about 15 respondents in control group and treatment group.<sup>11, 12</sup> Allocation random was then conducted by using a two-sided currency coin in order to divide the samples into two groups, the control group and the treatment group.

Inclusion criteria for this research were that the age of the patients must be from 12 years old to 18 years old, with a complete permanent dentition, well aligned or has slightly

crowded, as well as that there must be no dental caries and no fixed orthodontic appliances. Meanwhile, the exclusion criteria were uncooperate patients, or that they consumed foods other than food provided by the researchers during the treatment.

The independent variable, moreover, was giving toothpaste containing with propolis, while dependent variable was dental plaque score. Possible confounding variable was the method of brushing teeth. Thus, to control this confounding variable, the information about the correct way of brushing teeth was given. The data collected, was primary data derived from plaque score measured in the control group and in the treatment group after the treatment. The research then was started after obtaining ethical clearance from KEPK in Faculty of Medicine, Diponegoro University/Kariadi Hospital, Semarang.

Patients in the control group were asked to brush their teeth without toothpaste containing with propolis, while the patients in the treatment group were asked to brush their teeth by using toothpaste containing with propolis. Then, they were also asked to eat food that had been provided by the researchers and to have activities as usual. The measurement of dental plaque scores in the two groups was conducted by using Sillness and Loe method for next 4 hours.<sup>4</sup>

The normality of the data obtained from the treatment group was analyzed with Shapiro Wilk test. Based on the results of the normality analysis, it was known that the distribution of the data was not normal, so the transformation of the data was needed. However, since the transformation of the data distribution was not normal, an alternative test, Mann-Whitney test, was conducted as a non-parametric statistical test with significant value of  $p < 0.05$  (95% confidence level).<sup>10</sup>

## RESULTS

The age and gender distribution of subject were shown in Table 1 and 2. The assessment of dental plaque score in the control group and in the treatment group (by using Sillness & Loe method) can be seen in Figure 1.

The results of the statistical analysis of the dental plaque score using a non-parametric test, Mann-Whitney, show  $p$  values about 0.002 with significant value  $p < 0.05$ . Thus, it can be indicated that there were significant differences between the plaque scores in the control group and those in the treatment group. It shows that the plaque scores in the treatment group were lower than those in the control group.

Furthermore, the results of the statistical analysis of the ages of the patients using a non-parametric test, Mann-Whitney, show  $p$  value about 0.593 with significant value  $p < 0.05$ . Therefore, it can be indicated that there was no significant difference between the ages of the subject with dental plaque in the control group and those in the treatment group.

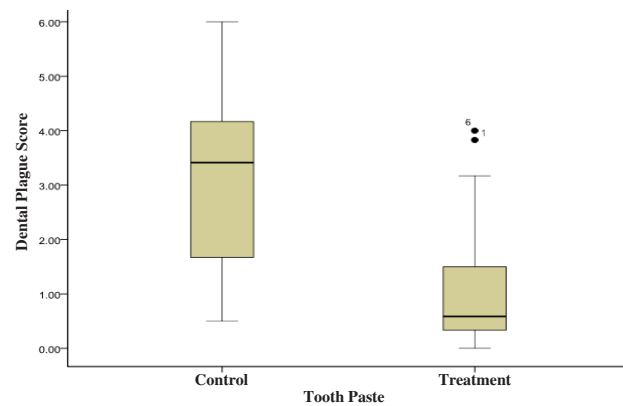
The results of the statistical analysis of gender of the patients using Chi-square test show p value about 1 with significant value  $p < 0.05$ . It can be indicated that there was no relationship between gender of the subject with dental plaque in the control group and those in the treatment group.

## DISCUSSION

Dental plaque formation is started with the formation of acquired pellicle, a thin layer, formed by deposition of salivary glycoprotein on dental enamel.<sup>2,4</sup> Microorganisms forming extracellular polysaccharide, *S. mutans*, adhere to the surface of acquired pellicle.<sup>4,13</sup> Glucosyltransferase enzyme contained in *S. mutans* then will change sucrose into extracellular polysaccharide. The polysaccharide will strengthen the adherence of bacteria's surface on dental pellicle components.<sup>2,4,14</sup>

The results of this study indicate that toothpaste containing propolis may reduce dental plaque scores, indicated by the results of non-parametric statistical analysis with Mann-Whitney, p value of 0.002 ( $p < 0.05$ ). It can be concluded that there was the significant difference of the dental plaque score in the control group and that in the treatment group. The dental plaque scores in the treatment group can indicate that patients who brushed their teeth by using toothpaste containing with propolis had lower dental plaque scores than those in the control group. This declining of dental plaque scores in the treatment group can indicate the inhibition of dental plaque formation.

Propolis is a resin compound produced by bees from various plants. Most of the biological activity of propolis



**Figure 1.** The assesment results of dental plaque score in the control group and in the treatment group.

comes from flavonoids contained in it.<sup>15</sup> The high number of flavonoids in propolis have a role as antibacterial, especially in inhibiting bacterial growth in oral cavity. Flavonoids contained in propolis of bee honey can inhibit the growth of *S. mutans* in vitro.<sup>7</sup> Propolis derived from honeybee have good potential as antibacterial.<sup>16</sup> The content of antibacterial propolis is beneficial for reducing oral bacteria in vitro and in vivo. In other worlds, propolis has antibacterial ability in vitro against oral *Streptococcus* and bacteria in saliva for clinical study.<sup>17</sup>

Mechanism of antibacterial activity of propolis is actually related with flavonoid contained in it. Flavonoids contained in propolis contain apigenin and tt-farnesol which can inhibit plaque formation process. Mechanism of apigenin activity in preventing plaque formation is through the inhibition of glucosyltransferase enzyme activity in *S. mutans* that later can inhibit the formation of extracellular polysaccharides caused by bacteria. Meanwhile, tt-farnesol has high antibacterial capability to inhibit the growth and metabolism of *S. mutans* in order to disrupt the formation of bacterial membrane.<sup>15,18</sup> Both components can inhibit the accumulation and composition of polysaccharide, the biofilm layer of *S. mutans*, without disrupting the bacterial survival. Apigenin and tt-farnesol have bacteriostatic capability so that they can overcome infection without killing normal oral microorganisms and can also not cause bacterial resistance.<sup>15</sup>

Several studies have shown that flavonoids can inhibit the formation of dental plaque. Catechin flavonoids contained in apples can inhibit the formation of dental plaque.<sup>12</sup> A similar study, strawberries contain catechin flavonoids can inhibit the formation of dental plaque.<sup>19</sup> Catechin flavonoids contained in grapes can inhibit the formation of dental plaque. The mechanism of catechins in inhibiting the formation of dental plaque is actually by inhibiting the activity of glucosyltransferase enzyme (Gtfs) and by killing the growth of bacteria causing dental plaque, such as *S. mutans*.<sup>12,19,20</sup> Finally, it can be concluded that the use of toothpaste containing with propolis can inhibit dental plaque formation.

**Table 1.** The distribution of the subjects of this research based on the age of the patients in the control group and in the treatment group

Age (year)	Control group		Treatment group	
	Number	%	Number	%
12	0	0	1	6.25
13	5	35.71	3	18.75
14	2	14.28	7	43.75
15	2	14.28	2	12.5
16	3	21.42	2	12.5
17	2	14.28	1	6.25
Σ	14	100	16	100

**Table 2.** The distribution of the samples based on gender

Sex	Control		Treatment	
	Number	%	Number	%
Male	7	50	8	50
Female	7	50	8	50
Number	14	100	16	100

## REFERENCES

1. Ariningrum. Beberapa cara menjaga kebersihan gigi dan mulut. Jakarta: Cermin Dunia Kedokteran; 2008; (45–51).
2. Marsh PD, Devine DA. How is the development of dental biofilms influenced by the host. *J Clin Periodontol* 2011; 38 (s11): 28–35.
3. Kolenbrander PE, Palmer Jr., RJ, Rickard AH, Jakubovics NS, Chalmers NI, Diaz PI. Bacterial interactions and successions during plaque development. *Periodontol* 2000, 2006; 42(1): 47–79.
4. Putri MH, Herijulianti E, Nurjannah N. Ilmu pencegahan penyakit jaringan keras dan jaringan pendukung gigi. Jakarta: EGC 2010. p. 54–64, 93–5, 111–2.
5. Attin T, Hornecker E. Tooth brushing and oral health: how frequently and when should tooth brushing be performed. *Oral Health Prev Dent J*. 2005; 3 (135–40)
6. Paine ML, Slots Jorgen, Rich SK. Fluoride use in periodontal therapy: a review of the literature. *JADA* 2001; 129(2): 69–7.
7. Sabir A. Aktivitas antibakteri flavonoid propolis *Trigona* sp terhadap bakteri *Streptococcus mutans* (in vitro). *Majalah Kedokteran Gigi (Dent J)* 2005; 38: 135–41.
8. Ahuja V, Ahuja A. Apitherapy: a sweet approach to dental diseases Part II: Propolis. *J Academy Adv Dent Res* 2011; 2(2): 1–8.
9. Mahmoud L. Biological activity of Bee propolis in health and disease. *Asian Pacific J Cancer Prev* 2006; 7: 22–31.
10. Dahlan MS. Statistik untuk Kedokteran dan Kesehatan. Edisi 4. Jakarta: Salemba Medika; 2009. p. 4–20.
11. Dahlan MS. Besar sampel dalam penelitian kedokteran dan kesehatan. Jakarta: PT. ARKANS; 2006. p. 14–15, 59–63.
12. Ayu R. Pengaruh pasta gigi dengan kandungan buah apel (*Pyrus malus*) terhadap pembentukan plak gigi. *Diponegoro University Institutional Repository* 2012; 37137(1): 1–15.
13. Zijng V, Van Leeuwen MBM, Degener JE, Abbas F, Thurnheer T, Gmur R. Oral biofilm architecture on natural teeth. *Plos One* 2010; 5(2): 1–9.
14. Marsh PD. Dental plaque as a biofilm and a microbial community-implications for health and disease. *BMC Oral Health* 2006; 6(1): 1–7.
15. Liberio SA, Pereira LA, Araujo MJ, Dutra RP, Nascimento FRF, Neto VM. The potential use of propolis as a cariostatic agent and its actions on mutans group streptococci. *J Ethnopharmacol* 2009; 125(1): 1–9.
16. Parolia A, Thomas MS, Kundabala M, Mohan M. Propolis and its potential uses in oral health. *Int J Medicine and Medical Sci* 2010; 2(7): 210–215.
17. Khalid A, Afaf D, Ameira M. Propolis as a natural remedy: An update. *Saudi Dent J* 2001; 13: 45–49.
18. Koo H, Hayacibara MF, Schobel BD, Cury JA, Rosalen PL, Park YK. Inhibition of *Streptococcus mutans* biofilm accumulation and polysaccharide production by apigenin and *tt*-farnesol. *J Antimicrobial Chemotherapy* 2001; 52(5): 782–89.
19. Kusumaningsih. Pengaruh pasta gigi dengan kandungan buah Stroberi (*Fragaria chiloensis* L.) terhadap pembentukan plak gigi. *Diponegoro University Institutional Repository* 2012; 188(1): 1–18.
20. Amiati RD. Pengaruh pasta gigi dengan kandungan buah Anggur (*Vitis vinifera*) terhadap pembentukan plak gigi. *Diponegoro University Institutional Repository* 2012; 129 (1): 1–17.