ISSN (Print) : 2580–6548
ISSN (Online): 2541–321X

Volume 4 • Issue 3 • September-December 2020

SCIENTIFIC DENTAL JOURNAL

Official Journal of Faculty of Dentistry, Trisakti University, Jakarta, Indonesia

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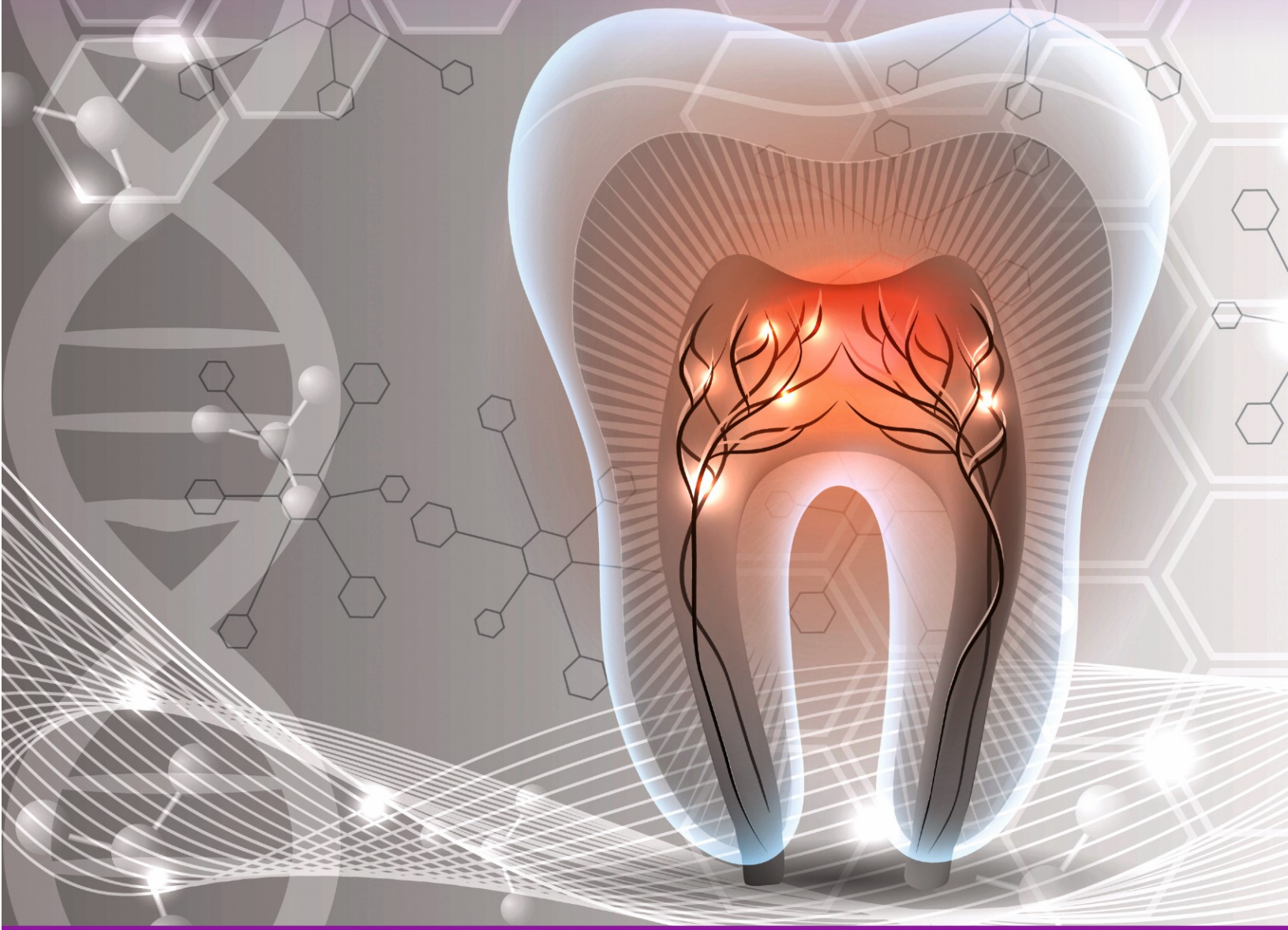

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Scientific Dental Journal

Volume 4 | Issue 3 | September - December 2020

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CASE REPORTS

Original Article

Effectiveness of Brewed Green Tea and Mouthwash Containing Green Tea Extract against *Streptococcus mutans* and *Porphyromonas gingivalis* in Saliva

Mita Juliawati, Marta Juslily, Abdul Gani Soulissa[1], Armelia Sari Widyarman[2], Elly Munadziroh[3]

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Abstract

Background: Green tea is known to exert an antibacterial effect against cariogenic pathogens. **Objectives:** This study aimed to determine the effect of brewed green tea as mouth rinse against *Streptococcus mutans* and *Porphyromonas gingivalis* in saliva and compare it to the effect of a commercial mouthwash containing green tea extract. **Methods:** Saliva of 30 healthy individuals aged 19–40 years was collected before treatment, 1 min after gargling, and 1 week after daily treatment with brewed green tea as a mouth rinse or commercial mouthwash containing green tea. Bacterial DNA was extracted from salivary samples and evaluated using quantitative polymerase chain reaction. The total number of DNA targets was analyzed using SYBR Green and 16S ribosomal RNA gene-specific primers for *S. mutans* and *P. gingivalis*. The data were statistically analyzed using a paired *t*-test. The level of significance was set to $P < 0.05$. **Results:** Green tea mouth rinse and mouthwash containing green tea extract significantly reduced the number of *S. mutans* and *P. gingivalis* in the participants' saliva after 1 week of use ($P < 0.05$). There was no significant difference between the effects of brewed green tea mouth rinse and commercial mouthwash containing green tea. **Conclusion:** The use of mouthwash containing green tea and brewed green tea mouth rinse reduces the number of *S. mutans* and *P. gingivalis* in saliva. Brewed green tea can be used as a mouth rinse with effects comparable to those of commercial mouthwash containing green tea. Further studies are warranted to explore its effects on other oral pathogens.

Keywords: *Brewed green tea, green tea, mouth rinse, Porphyromonas gingivalis, Streptococcus mutans*

Received : 07-08-20
Revised : 24-08-20
Accepted : 15-09-20
Published Online: 17-10-20

Background

Green tea is produced from the leaves of *Camellia sinensis*, a species of plant that belongs to the genus *Camellia*, a genus of flowering plants in the family Theaceae. Green tea is known to exert antibacterial effects against cariogenic pathogens.[1] Among the most common pathogenic microbes are *Streptococcus mutans*, which causes dental caries, and *Porphyromonas gingivalis*, which causes periodontal disease and is commonly found in high levels in chronic periodontitis.[2]

Mouthwash is a liquid used for several purposes. It eliminates bacteria, acts as an astringent, and exerts healing effects by treating infections or preventing dental caries. Mouthwashes contain antibacterial compounds that help treat infection by inhibiting bacterial growth and decrease the bacterial levels in dental plaque.[3] The ideal mouthwash is nontoxic and effectively reduces or eliminates plaque accumulation.[4] Previous study showed that there is no evidence indicating toxic effects

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DOI: 10.4103/SDJ.SDJ_39_20

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How to cite this article: Juliawati M, Juslily M, Soulissa AG, Widyarman AS, Munadziroh E. Effectiveness of brewed green tea and mouthwash containing green tea extract against *Streptococcus mutans* and *Porphyromonas gingivalis* in Saliva. Sci Dent J 2020;4:101-4.

such as irritation, burn, vesicle, or mucous disturbance was reported on green tea extract for mouthwash.[5] This indicates that both brewed green tea and mouthwash containing green tea are safe and nontoxic. Akande *et al*. distinguish between two generations of mouthwash based on pharmacological characteristics. First-generation agents, containing compounds such as cetylpyridinium chloride and sanguinarine, eliminate bacteria on contact but have a limited effect against oral pathogens. Second-generation agents, such as those containing chlorhexidine, have long-term effects against oral microflora.[5,6]

The use of antimicrobial agents in gingivitis patients has been proven to decrease the number of periodontal pathogens, reduce the periodontal pocket depth, and provide optimal treatment.[7] A number of products designed to assist individuals in achieving and maintaining good oral hygiene are currently available on the market. Previous studies have demonstrated the biochemical and microbiological effects of mouthwash against plaque accumulation.[3,8,9]

Mouthwashes containing herbal ingredients have been proven to inhibit the activity of several bacteria, including *S. mutans*. The zone of inhibition of some herbal mouthwashes is larger than that of some commercial mouthwashes.[10] Experimental and epidemiologic studies have shown that green tea consumption prevents alveolar bone resorption by inhibiting osteoclast survival through caspase-mediated apoptosis and is thus beneficial to periodontal health.[11,12]

No previous studies have investigated the effects of brewed green tea as a mouth rinse compared to commercial mouthwash containing green tea on oral pathogens in saliva. Therefore, this study aimed to determine the effect of brewed green tea as a mouth rinse against *S. mutans* and *P. gingivalis* in saliva and compare it to the effect of a commercial mouthwash containing green tea extract.

Materials and Methods

Study design

This study included 30 patients in the Dental Hospital, Faculty of Dentistry of Trisakti University, Jakarta, Indonesia, randomly chosen according to the following inclusion criteria: males or females aged 19–40 years. The exclusion criteria were tobacco, alcohol, and drug use and also systemic diseases. All patients signed informed consent forms. The study was approved by the Ethics and Biomedicine Research Committee of the Faculty of Dentistry of Trisakti University with number 313/KE/FKG/04/2016.

Sample collection

Saliva was collected from the patients before treatment and after 1 week of gargling twice daily (in the morning and at night) with brewed green tea (n = 15) or mouthwash containing green tea extract (Listerine; n = 15) after toothbrushing. The saliva was collected using the spitting method.[13] Mouthwash containing green tea extract was used in this study because it exerts antibacterial effects, contains natural ingredients, such as essential oils, and does not contain alcohol. The main ingredients of mouthwash containing green tea are 0.6% methyl salicylate, thymol, menthol, eucalyptol, green tea extract, and 220 ppm fluoride.[14]

Bacterial DNA extraction from saliva

Bacterial DNA was extracted from the saliva samples, and the bacterial number was evaluated using quantitative polymerase chain reaction (qPCR). DNA samples were extracted using the heat shock method. The samples were centrifuged at × 4500 g for 15 min and washed with phosphate-buffered saline. An aliquot of 100 µL of cell suspension containing 10^8 cells/mL was transferred to microtubes and centrifuged at × 10,000 g for 10 min at 4°C. It was subsequently incubated at 100°C for 20 min, after which the tubes were immediately frozen in ice (0°C) for 10 min. Centrifugation at × 10,000 g was then performed for 2 min, and the supernatant was moved into new 1.5 mL microcentrifuge tubes. The suspension containing the DNA sample was stored at −20°C.

Quantitative polymerase chain reaction

The total amount of DNA target was quantified using qPCR with SYBR Green (Applied Biosystems, USA) and 16S ribosomal RNA (rRNA) gene-specific primers for *S. mutans* and *P. gingivalis*.[15] The primer sequences are shown in Table 1. The qPCR procedure was as follows: initial denaturation at 95°C for 10 min (1 cycle), followed by 40 cycles at 94°C for 15 s and annealing at 60°C for 1 min and 95°C for 15 s. All procedures were performed in triplicate. Quantitation was performed using standard curves from known concentrations of DNA containing the respective amplicon for each set of primers.

Statistical analysis

The normality of the data was assessed with the Shapiro–Wilk test.[13] Differences between pre- and posttreatment

Table 1: The primer sequences[15]

16s rRNA gene	Sequence (5'-3')
S. mutans (forward)	GCCTACAGCTCAGAGATGCTATTCT
S. mutans (reverse)	GCCATACACCACTCATGAATTGA
P. gingivalis (forward)	TGCAACTTGCCTTACAGAGGG
P. gingivalis (reverse)	ACTCGTATCGCCCGTTATTC

S. mutans: Streptococcus mutans, P. gingivalis: Porphyromonas gingivalis

values were analyzed using a paired-samples *t*-test. Differences between the two experimental groups were analyzed using an independent samples *t*-test. The level of statistical significance was set to $P < 0.05$. The statistical analysis was performed using IBM SPSS Statistics version 20 (IBM, Armonk, NY, USA).

Results

The standard curve formula used in this study was $y = 0{,}047 \times ^{2} - 40{,}116x + 46{,}092$ with $R^2 = 1$ and $y = -0{,}2862 \times 13{,}766$ with $R^2 = 0{,}9888$ for *S. mutans* and *P. gingivalis,* respectively. The result showed that gargling with brewed green tea and mouthwash containing green tea for 1 week resulted in a reduction in the number of *S. mutans* [Figure 1a and b] and *P. gingivalis* [Figure 2a and b] in the participants' saliva. The reduction of *P. gingivalis* was statistically significant after 1-week gargling with brewed green tea and mouthwash containing green tea ($P < 0.05$). There was no significant difference between the effects of brewed green tea and the commercial mouthwash ($P > 0.05$) [Figures 1 and 2].

Discussion

In this study, we examined the effectiveness of mouthwash containing green tea extract against *S. mutans* and *P. gingivalis* using qPCR. The results showed a statistically significant reduction in the number of *S. mutans* and *P. gingivalis* after using mouthwash containing green tea extract for 1 week ($P < 0.05$). PCR technology (conventional and real-time PCR [qPCR]) is the most commonly used in the pathogen detection because of its high sensitivity and specificity. However, a major drawback of PCR is its inability to differentiate the DNA from dead and viable cells.[16]

Daily use of mouthwash containing green tea for 1 week resulted in a reduction of more than 25% in *S. mutans* compared to the pretreatment levels in some participants but an increase in others. This can be attributed to the patients' diets and oral hygiene. Sugar can increase undissolved glucan biosynthesis and cause strong bacterial adhesion to the tooth surface.[17,18]

Dental caries can be prevented using antimicrobial agents to suppress the growth of cariogenic microorganisms.[19] The same is true of using mouthwash containing green tea extract against periodontal disease. Catechins contained in green tea can inhibit *P. gingivalis*, *Prevotella intermedia*, and *Prevotella nigrescens* adhesion to buccal epithelial cells. [11] Toxin production of *P. gingivalis* metabolites is inhibited by green tea catechins with a 3-galloyl moiety radial stearic structure, epigallocatechin gallate, and gallocatechin gallate, which are the main polyphenols contained in tea.[20] These catechins have been shown to exert bactericidal effects against black-pigmented anaerobic rod-shaped Gram-negative bacteria, such as *P. gingivalis* and *Prevotella* species. A combination of green tea catechins using a local slow-release distribution and mechanical treatments can improve periodontal health. Study showed peptidase activity in gingival fluid can

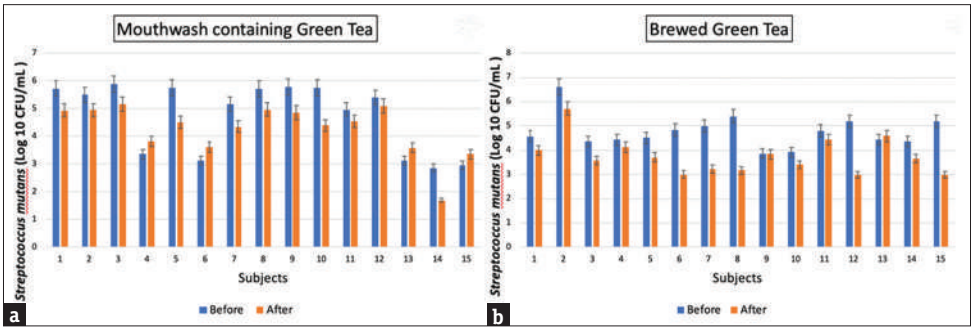

Figure 1: Comparison between the effects of mouthwash containing green tea extract (a) and brewed green tea (b) against *Streptococcus mutans* in saliva

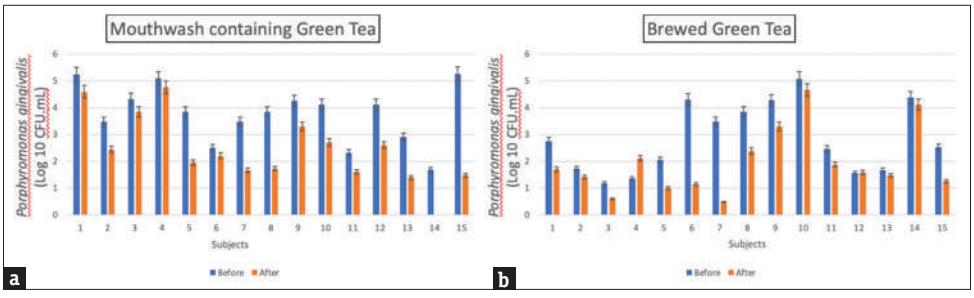

Figure 2: Comparison between the effects of mouthwash containing green tea extract (a) and brewed green tea (b) against Porphyromonas gingivalis in saliva

be maintained at low levels but reach 70% of placebo levels.[21] Green tea can reduce the severity of periodontal disease by mediating the host's inflammatory response against periodontal pathogens.[11,22] The next suggestion is that green tea with the above significant results can be an alternative to natural mouthwash since the brewed green tea is safer and more cost-effective.[23]

Conclusion

This study shows that the use of brewed green tea mouth rinse and mouthwash containing green tea extract can reduce the number of *S. mutans* and *P. gingivalis* in saliva. This mouth rinse might be effective in preventing dental caries and periodontal disease. Moreover, brewed green tea used as a mouth rinse has effects comparable to those of commercial mouthwash containing green tea extract. Further studies on their effects against other oral pathogens are warranted.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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by Abdul Gani Soulissa

Submission date: 07-Apr-2022 11:42AM (UTC+0700)
Submission ID: 1804030680
File name: Streptococcus_mutans_and_Porphyromonas_gingivalis_in_Saliva.pdf (484.93K)
Word count: 2855
Character count: 15522

Original Article

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Mita Juliawati, Marta Juslily, Abdul Gani Soulissa[1], Armelia Sari Widyarman[2], Elly Munadziroh[3]

Departments of Public Health, [1]Periodontic and [2]Microbiology, Faculty of Dentistry, Trisakti University, West Jakarta, [3]Department of Dental Material, Faculty of Dental Medicine, Airlangga University, Surabaya, Indonesia

Abstract

Background: Green tea is known to exert an antibacterial effect against cariogenic pathogens. **Objectives:** This study aimed to determine the effect of brewed green tea as mouth rinse against *Streptococcus mutans* and *Porphyromonas gingivalis* in saliva and compare it to the effect of a commercial mouthwash containing green tea extract. **Methods:** Saliva of 30 healthy individuals aged 19–40 years was collected before treatment, 1 min after gargling, and 1 week after daily treatment with brewed green tea as a mouth rinse or commercial mouthwash containing green tea. Bacterial DNA was extracted from salivary samples and evaluated using quantitative polymerase chain reaction. The total number of DNA targets was analyzed using SYBR Green and 16S ribosomal RNA gene-specific primers for *S. mutans* and *P. gingivalis*. The data were statistically analyzed using a paired *t*-test. The level of significance was set to $P < 0.05$. **Results:** Green tea mouth rinse and mouthwash containing green tea extract significantly reduced the number of *S. mutans* and *P. gingivalis* in the participants' saliva after 1 week of use ($P < 0.05$). There was no significant difference between the effects of brewed green tea mouth rinse and commercial mouthwash containing green tea. **Conclusion:** The use of mouthwash containing green tea and brewed green tea mouth rinse reduces the number of *S. mutans* and *P. gingivalis* in saliva. Brewed green tea can be used as a mouth rinse with effects comparable to those of commercial mouthwash containing green tea. Further studies are warranted to explore its effects on other oral pathogens.

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How to cite this article: Juliawati M, Juslily M, Soulissa AG, Widyarman AS, Munadziroh E. Effectiveness of brewed green tea and mouthwash containing green tea extract against *Streptococcus mutans* and *Porphyromonas gingivalis* in Saliva. Sci Dent J 2020;4:101-4.

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Sample collection

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Bacterial DNA extraction from saliva

Bacterial DNA was extracted from the saliva samples, and the bacterial number was evaluated using quantitative polymerase chain reaction (qPCR). DNA samples were extracted using the heat shock method. The samples were centrifuged at × 4500 g for 15 min and washed with phosphate-buffered saline. An aliquot of 100 µL of cell suspension containing 10^8 cells/mL was transferred to microtubes and centrifuged at × 10,000 g for 10 min at 4°C. It was subsequently incubated at 100°C for 20 min, after which the tubes were immediately frozen in ice (0°C) for 10 min. Centrifugation at × 10,000 g was then performed for 2 min, and the supernatant was moved into new 1.5 mL microcentrifuge tubes. The suspension containing the DNA sample was stored at −20°C.

Quantitative polymerase chain reaction

The total amount of DNA target was quantified using qPCR with SYBR Green (Applied Biosystems, USA) and 16S ribosomal RNA (rRNA) gene-specific primers for *S. mutans* and *P. gingivalis*.[15] The primer sequences are shown in Table 1. The qPCR procedure was as follows: initial denaturation at 95°C for 10 min (1 cycle), followed by 40 cycles at 94°C for 15 s and annealing at 60°C for 1 min and 95°C for 15 s. All procedures were performed in triplicate. Quantitation was performed using standard curves from known concentrations of DNA containing the respective amplicon for each set of primers.

Statistical analysis

The normality of the data was assessed with the Shapiro–Wilk test.[13] Differences between pre- and posttreatment

Table 1: The primer sequences[15]

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S. mutans: Streptococcus mutans, P. gingivalis: Porphyromonas gingivalis

values were analyzed using a paired-samples *t*-test. Differences between the two experimental groups were analyzed using an independent samples *t*-test. The level of statistical significance was set to $P < 0.05$. The statistical analysis was performed using IBM SPSS Statistics version 20 (IBM, Armonk, NY, USA).

Results

The standard curve formula used in this study was $y = 0{,}047 \times ^{2} - 40{,}116x + 46{,}092$ with $R^2 = 1$ and $y = -0{,}2862 \times 13{,}766$ with $R^2 = 0{,}9888$ for *S. mutans* and *P. gingivalis,* respectively. The result showed that gargling with brewed green tea and mouthwash containing green tea for 1 week resulted in a reduction in the number of *S. mutans* [Figure 1a and b] and *P. gingivalis* [Figure 2a and b] in the participants' saliva. The reduction of *P. gingivalis* was statistically significant after 1-week gargling with brewed green tea and mouthwash containing green tea ($P < 0.05$). There was no significant difference between the effects of brewed green tea and the commercial mouthwash ($P > 0.05$) [Figures 1 and 2].

Discussion

In this study, we examined the effectiveness of mouthwash containing green tea extract against *S. mutans* and *P. gingivalis* using qPCR. The results showed a statistically significant reduction in the number of *S. mutans* and *P. gingivalis* after using mouthwash containing green tea extract for 1 week ($P < 0.05$). PCR technology (conventional and real-time PCR [qPCR]) is the most commonly used in the pathogen detection because of its high sensitivity and specificity. However, a major drawback of PCR is its inability to differentiate the DNA from dead and viable cells.[16]

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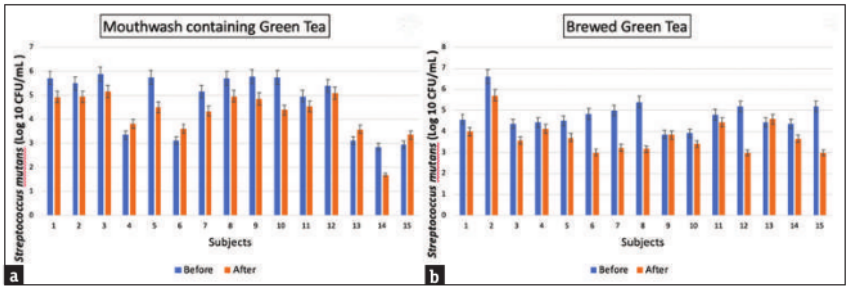

Figure 1: Comparison between the effects of mouthwash containing green tea extract (a) and brewed green tea (b) against *Streptococcus mutans* in saliva

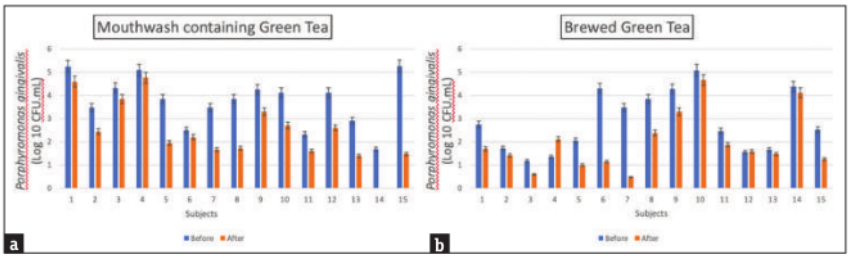

Figure 2: Comparison between the effects of mouthwash containing green tea extract (a) and brewed green tea (b) against Porphyromonas gingivalis in saliva

be maintained at low levels but reach 70% of placebo levels.[21] Green tea can reduce the severity of periodontal disease by mediating the host's inflammatory response against periodontal pathogens.[11,22] The next suggestion is that green tea with the above significant results can be an alternative to natural mouthwash since the brewed green tea is safer and more cost-effective.[23]

Conclusion

This study shows that the use of brewed green tea mouth rinse and mouthwash containing green tea extract can reduce the number of *S. mutans* and *P. gingivalis* in saliva. This mouth rinse might be effective in preventing dental caries and periodontal disease. Moreover, brewed green tea used as a mouth rinse has effects comparable to those of commercial mouthwash containing green tea extract. Further studies on their effects against other oral pathogens are warranted.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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