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Strawberry Extract's Effects on *Enterococcus faecalis* and *Porphyromonas gingivalis* Biofilms *in vitro*

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ABSTRACT

Background: *Enterococcus faecalis* (*E. faecalis*) and *Porphyromonas gingivalis* (*P. gingivalis*) are oral bacteria related to root canal infection and periodontal disease pathogenesis. Strawberries (*Fragaria x ananassa*) fruit are rich in vitamins and minerals, have antibacterial and antioxidant effects. **Objective**: This study investigated the inhibition effect of strawberry extract on monospecies and multispecies *E. faecalis* and *P. gingivalis* bacteria grown as biofilms *in vitro*. **Methods:** This study used *E. faecalis* ATCC 29212 *and P. gingivalis* ATCC 33277. It analyzed the effect of strawberry extract on bacteria biofilm formation using a biofilm assay on microplate wells. Five concentrations of strawberry extracts were used (100%, 50%, 25%, 12.5%, and 6.25%), and the inhibition effect was observed after a 1h, 3h, 6h, and 24h incubation period. Biofilms without the strawberry extract were used as the negative controls, and crystal violet and safranin ($0.5\%^{w}/_{v}$) were used to count the biofilm mass. The biofilms grown on microplates were counted using an ELISA reader at 450 nm after 200 mL of 90% ethanol was added to attract the absorbed stain. The strawberry extract inhibition effectiveness on the biofilm formation of each bacterium tested was analyzed using one-way Anova, where $p<0.05$ was defined as a significant difference. **Result**: The strawberry extract inhibited the tested monospecies and multispecies bacteria biofilm formation. The optimal strawberry extract concentration for the inhibition of either monospecies biofilms was 100%. However, the optimal incubation time for the strawberry extract to inhibit the multispecies biofilm formation was 24h, which was the study's biofilm maturity phase. **Conclusions:** The 100% strawberry extract concentration inhibited the formation of both the monospecies and multispecies *E. faecalis* and *P. gingivalis* biofilms. Future studies are needed to evaluate the potential of strawberry extract as an alternative dental therapy.

Keywords : biofilms, *Enterococcus faecalis, Porphyromonas gingivalis,* strawberry

Background

The most prevalent oral infectious diseases are dental caries and periodontal diseases. Dental caries is the dissolution of teeth by acid produced in the oral bacteria metabolism of dietary carbohydrates.[1] Plaque bacteria ferment carbohydrates to produce acid and reduce the plaque pH to 5.0–4.5.

Caries is considered an endogenous disease due to the major bacterial species that induce the demineralization.[2,3] *E. faecalis* is a catalase negative, Gram-positive coccus that generally occurs in pairs and short chains. This species is commonly found in the oral cavity as a persistent bacterium in infected root canal teeth, especially in endodontic cases with persistent periapical lessions.[4] *P. gingivalis* is an opportunistic, Gram-negative, oral anaerobe bacterium that is frequently isolated as a pathogen that causes periodontal disease.[5]

Several primary methods are used to prevent endodontic-related infection and periodontal disease. Primary prevention procedures are performed before clinical symptoms of a disease arise.[6] However, in recent years, people have begun to prefer natural remedies over chemical remedies.[7]

The popularity of strawberry as a fruit crop is due primarily to its unique aroma, sweet taste, bright color, and high concentration of nutrients. Strawberries contain a high concentration of flavonoids, which have potential human health benefits, including acting as an antioxidant. Strawberries, which are rich in vitamins and minerals, have antibacterial and antioxidant effects,[8] contain proanthocyanidin,[8-11] catechin,[9,11-12] ellagic acid,[9,13-14] and anthocyanin.[10,12,15-16] Previous studies state that the ingredients in strawberry juice showed antibacterial effects on *Streptococcus mutans*, which can help inhibit biofilm formation.[8] Therefore, this study evaluated the inhibition effect of strawberry extract on monospecies and multispecies *E. faecalis* and *P. gingivalis* biofilms *in vitro*.

Materials and Methods

The effects of strawberry extract on *E. faecalis* and *P. gingivalis* biofilms were analyzed using a biofilm assay. One colony of cultured stock (-80°C *E. faecalis* ATCC 29212 or *P. gingivalis* ATCC 33277) was inoculated in Brain Heart Infusion (BHI) broth and incubated at 37°C under anaerobe conditions. After a 24h incubation period. The suspension was diluted to achieve the optical density (OD_{600}) of 0.250–0.300 and obtain a 10^8 CFU/mL bacteria concentration. The 200 µL *E. faecalis* and 200 µL *P. gingivalis* suspensions were distributed into 96-microplate wells and incubated for 24h to grow the bacterial biofilm. The strawberry extract at different concentrations (100%, 50%, 25%, 12.5%, and 6.25%) was added to the wells, and the biofilm inhibition effect was observed after 1h, 3h, 6h, and 24h. Biofilms without the strawberry extract were used as a negative control. Crystal violet ($0.5\%^{w}/_{v}$) was used to count the biofilm mass of the *E. faecalis* biofilm, and safranin ($0.5\%^{w}/_{v}$) was used to count the biofilm mass of the *P. gingivalis* biofilm. The biofilm formation was counted using an ELISA reader at 450 nm after 200 µL of 90% ethanol was added.

Statistical Analysis

The differences between the experimental groups were analyzed using one-way ANOVA. A *p*-value less than 0.05 ($p < 0.05$) was considered statistically significant.

Results

Strawberry extract inhibits the growth of monospecies *E. faecalis* biofilm and multispecies *E. faecalis* and *P. gingivalis* biofilms. Inhibiting *E. faecalis* required a 100% strawberry extract concentration and a 6h incubation period (OD = 0.860 ± 0.028) (Fig.1). Inhibiting the *P. gingivalis* biofilm required a 100% strawberry extract concentration and a 6h incubation period (OD = 0.187 ± 0.029) (Fig.2). Inhibiting multispecies biofilm required a 100% strawberry extract concentration and a 24h incubation period (OD = 0.138 ± 0.014) (Fig.3). The one-way Anova test results showed a significant difference ($p < 0.05$) between several concentrations and incubation times.

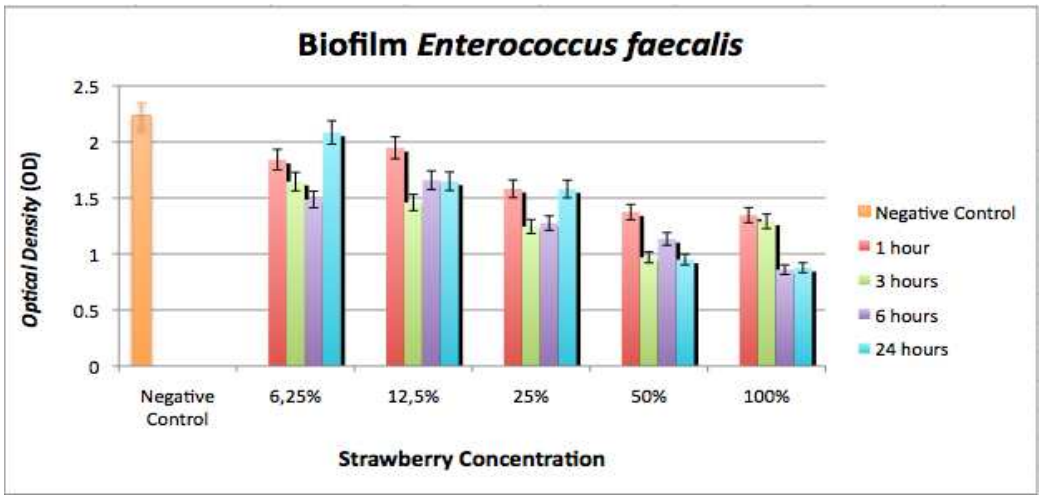

Figure 1. Strawberry potency to inhibit *Enterococcus faecalis* biofilm formation.

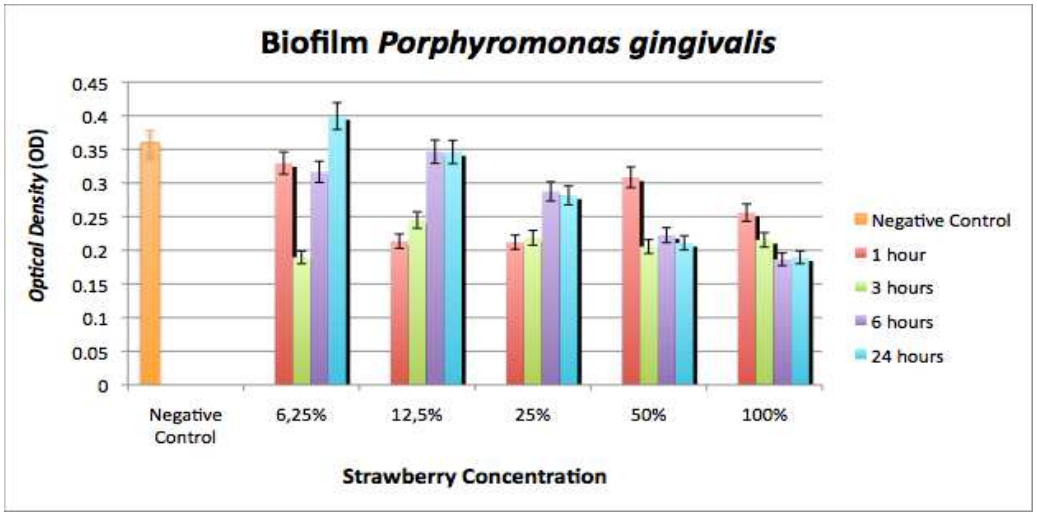

Figure 2. Strawberry potency to inhibit *Porphyromonas gingivalis* biofilm formation.

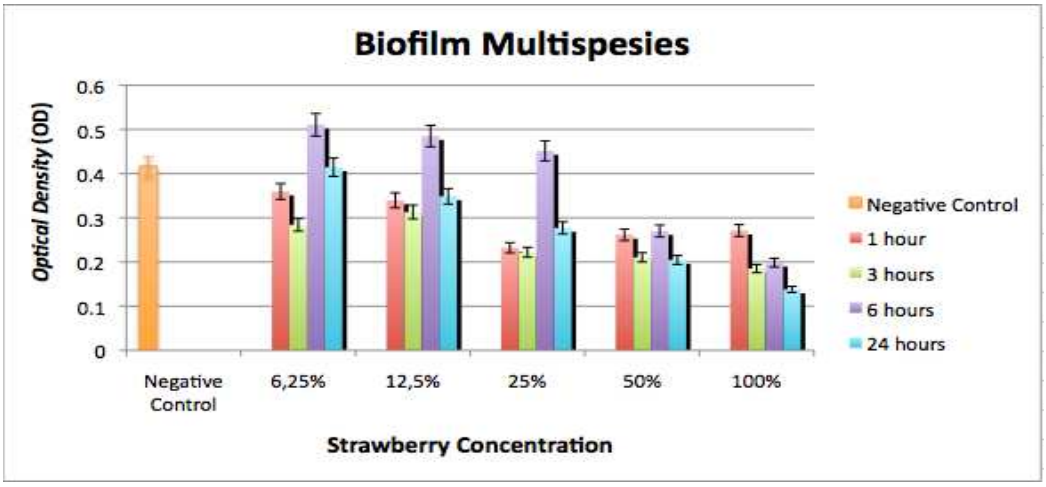

Figure 3. Strawberry potency to inhibit multispecies (*E. faecalis* and *P. gingivalis*) biofilm formation.

Discussion

This study evaluated the effects of strawberry (*Fragaria* x *ananassa*) extracts on the biofilm formation of monospecies and multispecies *E. faecalis* and *P. gingivalis in vitro*. The extract concentration level was adjusted by serial dilution of extracted strawberry in several concentrations using BHI broth as the medium. The extracts were made using fresh strawberries (*Fragaria* x *ananassa*). Based on Balitro laboratory result, it has been shown that our strawberry extract contained high levels of flavonoids (proanthocyanidin, anthocyanin, ellagic acid and catechin) 50.52 ppm and for catechins 0.69% by using spectrophotometry as the testing method. Strawberry (*Fragaria* x *ananassa*) contains proanthocyanidin, catechin, ellagic acid, dan anthocyanin.

Present studies demonstrated that pro-anthocyanidin had an inhibiting effect on *Staphylococcus aureus* biofilms[17], catechins in cacao fruit (*Theobroma caccao*) inhibits *Streptococcus mutans*[18], ellagic acid on pomegranate seeds (*Punica granatum L.*) inhibits *Candida albicans*[19], and anthocyanins in grape skin (*Vitis vinifera L. var. Alphonse Lavallee*) inhibit *Bacillus cereus*, *Micrococcus luteus* and *Escherichia coli*[20]. It has been reported in previous studies that strawberry juice has an antibacterial effect against *Streptococcus mutans* that can help to inhibit biofilm formation[21].

Based on the group of concentration, this study showed that strawberry extract 100% is the optimal concentration to inhibit biofilm in each bacterium tested thus that it showed the most effective in inhibiting the biofilm targeted. Normality test with Shapiro-Wilk method obtained in this study for *E. faecalis*, *P. gingivalis* and multispecies have normal distributed data group. Whereas One Way ANOVA test were obtained in this study for *E. faecalis*, *P. gingivalis*, and multispecies is 0,000 which means significantly different. Then proceeded with the Post Hoc test with Tukey Honestly Significant Difference (Tukey HSD) to detect significant differences based on data from the study. Based on Tukey HSD test, each incubation time and concentration showed significant differences if compared with the negative control (BHI). Overall based on data of *E. faecalis*, *P. gingivalis*, and multispecies biofilm, it has been proven that the strawberry extract was effective to inhibit the growth of biofilm formation.

Conclusion

Strawberry extract is able to inhibit *E. faecalis* and *P. gingivalis*, either as mono or multispecies biofilms. Thus it could be used an alternative therapy in preventing endodontic-related infection and periodontal disease. However, future studies are still needed to evaluate its *in vivo* effect.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

References

1. Decker RT, Loveren CV. Sugars and Dental Caries 1,2,3,4. The Am J of Clin Nutr 2003; 78: 881-920.
2. Soesilo D, Santoso RE, Diyatri I. Peranan Sorbitol Dalam Mempertahankan Kestabilan pH Saliva Pada Proses Pencegahan Karies. Maj. Ked. Gigi. (Dent. J.) 2005; 38(1): 25-28.
3. Sahebi S, Khosravifar N, SedighShamsi M, Motamedifar M. Comparison of the Antibacterial Effect of Sodium Hypochlorite and Aloe Vera Solutions as Root Canal Irrigants in Human Extracted Teeth Contaminated with Enterococcus faecalis.J Dent Shiraz Univ Med Sci 2014; 15(1): 39-43.
4. Veras HN, Rodrigues FF, Botelho MA, Menezes IR, Coutinho HD, Costa GM. Antimicrobial Effect of Lippia sidoides and Thymol on Enterococcus faecalis Biofilm of the Bacterium Isolated from

Root Canals. Sci World J. 2014; 1-2.
5. Sang SG, Rong H, Wang JB, Xie YQ. Effects of Porphyromonas gingivalis Extracellular Vesicles on Human Periodontal Ligament Fibroblasts. Int J Clin Exp Med 2014; 7(2): 379-383.
6. Angela A. Pencegahan Primer Pada Anak yang Beresiko Karies Tinggi. Maj. Ked. Gigi. (Dent. J.) 2005; 38(3): 130-134
7. Djamil MS. Pengembangan dan Pemberdayaan Bahan Alam di Bidang Kedokteran Gigi: Jakarta, 2009.
8. Schaart JG, Dubos C, Fuente IR, Houwelingen AM, Vos RC, Jonker HH et al. Identification and Characterization of MYB-bHLH-WD40 Regulatory Complexes Controlling Proanthocyanidin Biosynthesis in Strawberry (Fragaria x ananassa) Fruits. New Phytol. 2013; 197: 454-467.
9. Huang WY, Zhang HC, Lue WX, Li CY. Survey of Antioxidant Capacity and Phenolic Composition of Blueberry, Blackberry, and Strawberry in Nanjing. J Zheijang Univ Sci B 2012; 13(2): 94-102.
10. Hanhineva K, Karenalampi S, Aharoni A. Recent Advances in Strawberry Metabolomics. Gene Genome Genomics.2011;5(1):65-7
11. Ruiz FA, Prortales RB, Blanco JM, Caballero JL. The Strawberry Plant Defense Mechanism: A Molecular Review. Plant and Cell Physicology.2011;52(11): 1873-1903.
12. Serrano ML, Barcelo AR. Histochemical Localization and Developmental Expression of Peroxidase and Polyphenol Oxidase in Strawberries. J. Amer. Soc. Hort. Sci 2001; 126(1): 27-32.
13. Lovric SK, Novak I. Determination of Ellagic Acid in Strawberries, Raspberries and Blackberries by Square-Wave Voltammetry. Int J Electrochem Sci 2011; 6: 4638-4647
14. Espin JC, Larrosa M, Conesa MTG, Barberan FT. Biological Significance of Urolithins, the Gut Microbial Ellagic Acid-Derived Metabolites: The Evidence So Far. Heindawi Publishing Coorporation 2013: 1-15.
15. Manach C, Scalbert A, Morand C, Jimenez L. Polyphenols: Food Sources and Bioavailability. The Am J Clin Nutr. 2004; 79: 727-747.
16. Zheng Y, Wang SY, Wang CY, Zheng W. Changes in Strawberry Phenolics, Anthocyanins, and Antioxidant Capacity in Response to High Oxygen Treatments. LWT. 2007; 40: 49-57.
17. Chelius C, Duffty C. Effects of Cranberries on Biofilms.[unpublished Bachelor Thesis on the Internet]. Woscester Polytechnic Institute; 2013. Avaliable from : https://web.wpi.edu/Pubs/E.../Cranberries_Effect_on_Biofilms_Chelius_Duffty.pdf
18. Marsaban. Perbandingan Efek Antibakterial Ekstrak Buah Cacao (Theobroma caccao) Pada Berbagai Konsentrasi Terhadap Streptococcus mutans. Universitas Diponegoro: Semarang, 2007.
19. Santoso S, Rahayu ID, Chandra HK. Efek Ekstrak Etanol Biji Buah Delima (Punica Granatum L.) Terhadap Penghambatan Jamur Candida Albicans Secara in vitro. Thesis; Faculty of Medicine. Brawijaya University 2012 . Available from : http:/repository.ub.ac.id/id/eprint /26188
20. Winarsih S, Asmaningsih E, Nina A. Efektivitas Ekstrak Etanol Kulit Anggur Bali (vitis vinifera L. var. Alphonse lavallee) Sebagai Antibakteri Terhadap Escherichia coli Secara in vitro. Thesis; Faculty of Medicine. Brawijaya University 2014 . Available from : http:/repository.ub.ac.id/id/eprint/26994
21. Erycesar G. Perbandingan Efek Antibakteri Jus Stroberi (Fragaria vesca L.) Pada Berbagai Konsentrasi Terhadap Streptococcus mutans. S1, Universitas Diponegoro, Semarang, 2007.