Antibacterial potential of strawberries and basil extract combination against *Streptococcus sanguinis* (ATCC 10556)

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ABSTRACT

Introduction: *Streptococcus sanguinis* is a commensal microorganism as well as a pioneer colony in forming dental plaque. Oral biofilm formation can be prevented by a mechanical cleaning procedure followed by the use of mouthwash. The current gold standard for mouthwash is chlorhexidine. Nevertheless, it has side effects that are not recommended for long-term use. Previous studies had proven that herbal-based mouthwashes such as basil leaves (*Ocimum basilicum*) and strawberry fruit (*Fragaria x ananassa*) have been shown to have antibacterial properties. The effectivity of antibacterial activity phenomenon in combined extracts has been reported in other studies. This research aims to observe the antibacterial potential of the *F. x ananassa* and *O. basilicum* extract combinations against *S. sanguinis* (ATCC 10556). **Methods:** The sample of this study was a combination of *F. x ananassa* and *O. basilicum* extract, which initially screened for their antibacterial activities. Antibacterial activities of *F. x ananassa* and *O. basilicum* extracts against *S. sanguinis* were observed using Kirby Bauer method, while Minimum Inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) by serial microdilution method. The 2% concentration from each extract was combined in 1:1, 1:2, and 2:1 volume ratio variations then tested for inhibitory zones, MIC, and MBC. **Results:** *F. x ananassa extract* had 0.125% and 0.25% for MIC and MBC respectively, while *O. basilicum* extract showed the value of MIC and MBC as 0.031% and 0.063% against *S. sanguinis* (ATCC 10556). The extract combinations in 1:1, 1:2, and 2:1 volume ratio variations showed 0.016% for MIC and 0.031% for MBC. **Conclusions:** It was concluded that combining extracts of 2 *% F. x ananassa* and 2% *O. basilicum* in various ratios were observably to have the antibacterial potential against *S. sanguinis* (ATCC 10556).

Keywords: Antibacterial activity; Strawberry; *Fragaria x ananassa* extract; Basil; *Ocimum basilicum* extract; *Streptococcus sanguinis*.

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INTRODUCTION

Caries is a disease in the oral cavity that is multifactorial, infectious, chronic, and infectious, caused by the complex interaction between cariogenic bacteria of the oral cavity and fermented carbohydrates on the tooth surface over time which leads to demineralization of the hard tissues of the teeth. Dental caries are common and widely spread throughout the world population and are affecting the quality of life. It is caused by an imbalance of the normal flora of the oral cavity. Many studies have shown that although commensal microorganisms appear to support oral health and fight pathogens if they are not present in balance they are more pathogenic than commensal.

Streptococcus sanguinis is a Gram-positive bacterium, non-sporing, immobile, facultative anaerobic, commensal, and widely distributed in the oral cavity. *S. sanguinis* is known as a pioneer colony in the formation of dental plaque biofilms by assisting the attachment of other organisms in the biofilm.[1,2,3] Based on data from National Institute of Health Research and Development (NIHRD). Indonesia Basic Health Research (RISKESDAS) 2018, the prevalence of dental caries in the Indonesian population is 53.2%, which is approximately 93,998,727 people.[4] Plaque control is one of the caries prevention efforts that can be done mechanically by brushing teeth or with a combination of mouthwash. The basic ingredients contained in mouthwash consist of two types, namely chemical and herbal. Chemical mouthwashes generally contain active ingredients such as hydrogen peroxide, chlorhexidine, and alcohol.[5]

Beyond the proven efficacies of chemical-based mouthwash in controlling plaque formation, drawbacks were also observed and being reported in previous studies . Chlorhexidine has been shown to be effective in controlling plaque formation, but it cannot be used in the long term because it contains alcohol, leaves an unpleasant taste on the tongue, and has side effects that can cause staining of teeth and restorations.[5] Dry mouth, toxicity to oral connective tissue, and soreness of the mouth are some of other side effects caused by chemical-based mouthwash. Study reported the incidence of oral desquamations following the use of chemical-based mouthwash in children.[3,6] Chemical-based side effect findings lead to searches for natural product potentials in dentistry as alternatives.

A review has discussed the efficacy of various herbal-based extracts and active compounds as a comparable antibacterial agent to chlorhexidine and sodium fluoride. Phytochemical properties of natural products are potential for their antibacterial and antioxidant activities, and being widely evaluated in the discovery of drugs.[7] Natural products own a large range of chemical structures with uniques mechanism of actions that yields promising drug discoverings.[8,9]

The advantages of plant-based are still compared to chemical products. Herbal mouthwashes are greatly in-demand due to their ability to fight oral pathogens and possesses fewer side effects compared to chemical products.[5,10,11,12] Herbal-based mouthwash can be obtained from vegetables and fruits, including strawberries and basil leaves. Strawberries (*Fragaria x ananassa*) have been shown to have many nutrients, such as potassium, magnesium, phosphorus, calcium, iron, vitamins A, E, K, and C.

Several studies have shown that *F. x ananassa* contains flavonoid compounds that have antibacterial and antioxidant activities.[13,14,15] Another active ingredient, xylitol, has been shown to prevent dental plaque formation, bacterial attachment, and inhibit enamel demineralization by reducing acid production. This plant has active compounds such as essential oils, alkaloids, saponins, flavonoids, triterpenoids, steroids, tannins, and phenols.[16,17,18,19,20]

Empirically, the potential of herbal products has been utilized as remedies for many ailments. It has been consumed in the form of a single extract or mixture of several herbal plants, but until now there is little information about the interaction of combinations between herbal ingredients.

The combination of two drugs can cause pharmacodynamics interactions, which is a phenomenon where the effect of one drug changes due to the presence of the other drug. The combination can provide synergistic or antagonistic effects. A synergistic effect is a goal that is pursued in the development of drug combinations in order to increase the efficacy and therapeutic effect of a drug.[21,22,23]

A study by Chiedozie explained that the combination of herbal extracts which when used singly showed antibacterial and anti-inflammatory activities, will lead to synergistic properties and increase the activity better compared to a single usage.[24] This study also revealed that herbal-based mouthwashes can replace chemical-based mouthwashes to maintain oral hygiene and prevent caries.[24,25] The purpose of this study was to observe and determine the antibacterial potential of the combined extracts of *F. x ananassa* and *O. basilicum* against *S. sanguinis* (ATCC 10556).

METHODS

This research was a descriptive study on antimicrobial activities of *F. x ananassa* and *O. basilicum*) extract combinations on the growth of *S. sanguinis* ATCC 10556 by determining the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the combination of the extract through a microdilution method.

Both plants have been identified by Plant Taxonomy Laboratory, Herbarium Jatinangor, Department of Biology, Faculty of Mathematics and Natural Science, Universitas Padjadjaran, Jatinangor, Sumedang as *F. x ananassa* (Duchesne ex Weston) Duchesne ex RozierF and *Ocimum basilicum L.* The research was conducted at the Laboratory of Natural Products, Faculty of Mathematics and Natural Science, Universitas Padjadjaran, Jatinangor, Sumedang.

Materials and Instruments

F. x ananassa was cultivated from Ciwidey District, Bandung Regency and *O. basilicum* was obtained from Cicalengka District, Bandung Regency, Indonesia in August 2018–*Streptococcus sanguinis* ATCC 10556 was prepared in Mueller Hinton Broth and Mueller Hinton Agar media for antibacterial test, and an anaerobic jar was used for the assay. Microdilution method for the antibacterial assay was using a 96-well microplate, micropipettes, microtubes, incubators, and Biochrom reader.

Extraction of F. x ananassa and O. basilicum

Both samples of fresh *F. x ananassa and O. basilicum* were individually cut into small pieces and extracted using a maceration method by immersing the samples in methanol solvent for 3 x 24 hours at room temperature. The immersions were then filtered to obtain filtrates. These filtrates were evaporated using a rotary evaporator at a temperature of 40°C on low pressure to obtain concentrated methanol extracts of *F. x ananassa and O. basilicum*.

Antibacterial assay of methanolic extracts F. x ananassa and O.basilicum

Antibacterial screening of methanolic extracts of *F. x ananassa and O. basilicum* was initially observed for their inhibition zone using Kirby-Bauer disk diffusion in serial concentrations of 1%, 2%, 3%, 4%, and 5% against *S. sanguinis*. Bacteria solutions were prepared by growing 1 inoculating loops of bacteria in 5mL broth medium, incubated for 24 hours at 37°C. Following incubation, the turbidity of the solution was measured using a microplate reader at 620 nm and diluted to make a 0.5 Mc Farland bacteria culture solution.

Later, 100 µL of the culture solution was swabbed on the Muller Hinton agar medium, and 6mm diameter paper discs containing 20 µL samples were impregnated on the agar surface. Incubation of the samples was set for 48 h at 37°C and the whole process was repeated twice. Following the incubation, the inhibition zone was measured. The least concentrations of each sample that showed an inhibition zone was then set as the concentration to be used for the MIC and MBC assay.

Combination of extracts with ratios of 1:1, 1:2, and 2:1 of active extract of *F. x ananassa* and *O. basilicum* were evaluated for their MIC and MBC values. This antibacterial assay was using 96-well microplates. Pre-cultured bacteria were prepared in Mueller Hinton broth at 37°C under an anaerobic condition. Together with the extract combinations were then serially diluted two-fold at 37°C and incubated for 24 h.

The turbidity was measured using a microplate reader at 620 nm. Estimation of MIC value can be determined by the least concentration where the bacteria cells can be observed through the OD value and a confirmation procedure were done by swabbing the MIC in-well solution and incubated for 24 hours at 37°C, whereas the MBC

is the least concentration that no bacteria growth observed through media colony counting.

RESULTS

To estimate the active antibacterial concentration of both *F. x ananassa* and *O. basilicum* extracts, each of the samples with 1%, 2%, 3%, 4%, and 5% concentrations were observed and measured for their inhibition zones against *S. sanguinis* (ATCC 10556). Methanol extract of *F. x ananassa* showed an inhibition zone at a concentration of 2% (Table 1), while the antibacterial activity of methanol extract of *O. basilicum* was observed in a concentration of 2% as well (Tabel 1).

Table 1. Antibacterial screening of methanolic extract of *F. x ananassa and O. basilicum* against *S. sanguinis*

	Inhibition Zones (mm)				
	1%	2%	3%	4%	5%
F. x ananassa	0	7.8	8.6	9.4	11.4
O. basilicum	0	7.4	10.5	10.5	11.4

Table 2. Data of MIC and MBC of Methanol extract of *F. x ananassa* 2% and *O. basilicum* 2% against *S. sanguinis*

Extract	Concentration (%)	
	MIC	MBC
F. x ananassa (2%)	0.125	0.25
O. basilicum (2%)	0.312	0.63

These concentrations of both samples were further used for determining MIC and MBC of each and the combination of the extracts.

The MIC for 2% *F. x anana*ssa was 0.125%. This is confirmed by the turbidity value read at a concentration of 0.125%, and further readings showed that no bacteria growth at a concentration of 0.25%. Therefore, the MBC of 2% extract of *F. x ananassa* against *S. sanguinis* is 0.25% (Table 2). For the extract of *O. basilicum*, the readings showed that the MIC is 0.031%, and the MBC is at a concentration of 0.63%. Each of the extracts were then combined with ratios (volume to volume) of 1:1, 1:2, and 2:1, and each combination was tested for its MIC and MBC. Referring to Table 3, all volume to volume ratios of *F. x ananassa* to *O. basilicum* extract combinations tested (1:1, 1:2, 2:1) showed similar values for their MICs and MBCs. The MICs were 0.0156% and the MBCs were 0.0312% (Table 3).

Table 3. Data of MIC and MBC of F. x ananassa to O. basilicum extract combinations ratio (vol:vol) against *S. sanguinis*

Ratio	Concentration (%)	
	MIC	MBC
1 : 1	0.0156	0.0312
1 : 2	0.0156	0.0312
2 : 1	0.0156	0.0312

DISCUSSION

The minimum concentration for *F. x ananassa* and O. *basilicum* to inhibit *S. sanguinis* were 2% for both the extracts. Even though the inhibition zone diameters were slightly observed visually, indeed, they have shown the antibacterial activities and were confirmed with the lower concentration (1%) that did not show any inhibition zone. This finding was proven to have a contradictory result with the study on *O. basilicum* conducted by Anggriani et al.[26] that stated the minimum inhibitory zone is at 5,000 ppm which is equal to 0.5%. Meanwhile, the result was found to be similar with the study conducted by Rikmasari et al.[27] Similar result for strawberry sensitivity test were also contradictory, Phillip et al[28] definitely stated that despite their high polyphenol content, strawberry did not show any antibacterial activity.

The studies mentioned previously were using similar method, however, the current study had different result. This phenomenon would probably be due to the purity and sensibility of the bacteria used. Impurity and prolonged storing time of bacteria , will alter bacteria properties, whereas source of the natural products will yield result inconsistencies as well.[29] Furthermore, as the concentration increased, the inhibition zone appeared to correlate linearly. Therefore, the concentration of 2% of the samples was estimated as the least concentration owing to the antibacterial effect of S. *sanguinis*, and is further used in the study.

The MIC and MBC values for the combination of *F. x ananassa* and *O. basilicum* extracts with a ratio of 1:1, 1:2, and 2:1 against *S. sanguinis were* shown to be lower than the MIC values and MBC each extract of *F. x ananassa* and *O. basilicum* respectively, MIC 0,125% and 0.016 % with an MBC value of 0,25% and 0.031 %. The ratio variations

for the combination of the two single extracts did not perform any different towards *S. sanguinis* and gave the same MIC and MBC values. This phenomenon could be due to the relatively wide concentration ratio being tested that results in the same values. It also showed that the combination of the two single extracts could reduce MIC and MBC against *S. sanguinis* bacteria (ATCC 10556).

Previous data on the combination of *F. x ananassa* and *O. basilicum* were not available, thus this result can give a new perspective on the combinations studied in this research. In general, the effects that arise from an herbal ingredient, especially herbal ingredients that are combined are often unpredictable. Che et al.[24] stated, when herbal ingredients are used in combination, interactions can occur between the components contained in each ingredient. The most desirable interactions are those that can yield additional therapeutic benefits.[30,31]

A combination of *F. x ananassa* and *O. basilicum* extracts which both had antibacterial activity against *S. sanguinis* show lower MIC and MBC compared to the MICs and MBCs of each of the extracts. Pharmacological activity of a single herbal ingredient can prolong the effect, increase potency, and/or reduce the reverse effect to be synergistic or antagonistic when combined with other herbal ingredients. In this study, the combination of the two single extracts showed synergistic results. A condition is said to be synergistic when the combination of two antimicrobial agents produces an antibacterial effect that is greater than the power shown by the individual components.[22,24,25,30,31]

A synergistic effect can be achieved if the added extract affects other targets or its components interact with each other to increase solubility thereby increasing the antibacterial activity of one or more compounds of the main extract.[22,24,25,30,31] This synergistic effect is related to the active substance contained in every single compound which when combined will increase the effectiveness of the active substance compared if works alone. *F. x ananassa* and *O. basilicum* both had been phytochemically screened for their phenolic compounds, flavonoids, and tannins. This active substance has antiseptic and antibacterial effects that work by damaging the bacterial cell membrane as well as being an antioxidant.[15,16,19,22,30] Alkaloid, polyphenol, flavonoids, tannins, and quinon derived from *F. x ananassa* and terpenes and caffeic acid of *O. basilicum* play role in effective antibacterial activity against *S. sanguinis*.[5,32]This study combines the extracts of *F. x ananassa* and *O. basilicum*, a combination that is expected to increase the therapeutic effect and can be developed as a potential and more effective mouthwash against *S.sanguinis*.

CONCLUSION

The combined extracts of *F. x ananassa* 2% and *O. basilicum* in were observably have the antibacterial potential against *S. sanguinis* (ATCC 10556).

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