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The effect of fractionated ethanol red ginger (*Zingiber officinale var. rubrum*) extract on the pH level of *Streptococcus gordonii* metabolism (*In-vitro Laboratory Experimental Research*)

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ABSTRACT

Introduction: Oral health is important for the quality of human life, so maintaining oral health is necessary. However, many people have dental caries as one of the main diseases in the oral cavity. Dental caries can occur due to pH falling in the oral cavity by acid-producing bacteria, such as *Streptococcus gordonii,* which metabolizes sucrose as an energy source and produces lactic acid. Then, the environmental pH decreases. Chemicals are widely used as antibacterial agents, but long-term use can cause negative effects, hence antibacterial agents with minimal side effects are needed. Red ginger is one of the options that appears as a promising antibacterial agent. Even though a lot of research has been investigated regarding red ginger, there is limited information on the effect of fractionated red ginger extract on pH in bacterial metabolism, especially *Streptococcus gordonii*. This study aims to analyze the effect of the other components in red ginger besides its essential oil on the pH level of *Streptococcus gordonii* metabolism.
Methods: The groups in this study are control group 1 (bacteria, BHIB), control 2 (bacteria, BHIB, 5% sucrose), and treatment groups (bacteria, BHIB, 5% sucrose, extract red ginger 1.56% and 0.78%). Data were analyzed with Duncan's One-Way ANOVA and Post-Hoc Multiple Comparison.
Results: The pH level of control group 2 was lower than group control 1 and treatment groups ($p<0.05$).
Conclusion: Fractionated ethanol extract of red ginger inhibits the decreasing pH level in the *Streptococcus gordonii.*

Keywords: red ginger, sucrose, pH, *Streptococcus gordonii,* Human & Health.
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INTRODUCTION

Oral health is important for the quality of life because the oral cavity is the entrance of the substrate.[1] Moreover, an oral cavity is an ideal place for all bacteria to grow, including pathogenic bacteria that cause disturbances in the masticatory system, aesthetics, pain, and discomfort in speaking, hence it can reduce the quality of human life as a consequence.[2]

The main disease in the oral cavity is caries. Caries is a multifactorial disease characterized by the demineralization of enamel due to organic acid produced by acid-producing bacteria.[3] WHO (World Health Organization) in 2022 reports that oral diseases affect nearly 3.5 billion people worldwide. Generally, 2 billion people have caries in permanent teeth, and 514 million children have caries in primary teeth. Therefore, promotive, preventive, curative, and rehabilitative efforts are still needed to improve dental and oral health.[4]

Streptococcus gordonii is an oral commensal bacteria, gram-positive, anaerobic, facultative which is located in the human oral cavity as a primary colonizer with *Streptococcus intermedius, Streptococcus mitis, Streptococcus oralis*, or other primary colonizers in the process of plaque formation on the tooth surface and aggregate with other microorganisms that contribute to oral infections such as dental caries and periodontitis.[5,6] *Streptococcus gordonii* and dental caries are closely related to pH level. The pH in the oral cavity can decrease through metabolizing the glucose, and carbohydrate intake by acid-producing bacteria such as *Streptococcus gordonii* to produce lactic acid. If the acidic conditions in the oral cavity cannot be neutralized, then the caries process will continue.[7] Currently, various chemicals have been widely used as antibacterial agents. However, long-term use can increase bacterial resistance and kill the normal flora of the oral cavity. Thus, other antibacterial agent options that are effective and have minimal side effects are needed.[8]

Ginger (*Zingiber officinale*) has been known for a long time and it is widely used as herbal medicine by Indonesians, as much

as 50.36%. One of its types is red ginger (*Zingiber officinale var. rubrum*) which has many advantages over other varieties. Red ginger comprises monoterpenes, sesquiterpenes, diterpenes, vanilloids (gingerol, shogaol, paradol, zingerone, gingerdione, gingerdiol), flavonoids, proteolytic enzymes (zingibain), et cetera.[9,10] Red ginger is known to exhibits immunomodulatory, antioxidant, anti-inflammatory, and antimicrobial activity.[11]

Essential oil as the main component in red ginger, which is often researched, has antibacterial activity. However, red ginger has other important antibacterial components, including gingerols, shogaols, saponins, tannins, alkaloids, et cetera.[10] Therefore, the red ginger used in this study was fractionated by ethanol (essential oil was fractionated). The aim of this study is to analyze the effect of the other components in red ginger besides its essential oil on the pH level of *Streptococcus gordonii* metabolism.

MATERIALS AND METHODS

This research is a laboratory analytical research with a posttest-only control group design. It was conducted at the Research Center of the Faculty of Dental Medicine, Universitas Airlangga. This research has received approval from Universitas Airlangga Faculty of Dental Medicine Health Research Ethical Clearance Commission (Number: 348/ HRECC.FODM/ VI/ 2022).

The laboratory equipment used was an autoclave (Hirayama, Saitama, Japan), beaker glass, micropipette (Dumo 200-1000µl Variable, India) and tips, vortex mixer (Velp Scientifica Wizard Advance IR, Usmate Velate, Italy), 15ml centrifuge tube and rack, tube (OneMed, Sidoarjo, Indonesia), centrifuge (Thermo Fisher Scientific, Oxoid, Waltham, MA USA), incubator (Espec Model BNA-111, Serial 1311003165, Osaka, Japan), pH meter (Mettler Toledo, Columbus, Ohio, USA), digital analytical scale (Mettler Toledo, Columbus, Ohio, USA), stopwatch. The materials used were *Streptococcus gordonii* ATCC 51656, fractionated red ginger extract, *Brain Heart Infusion Broth* medium (Thermo Fisher Scientific, Oxoid, Waltham, MA USA), distilled water, KCl and MgCl2 salt solutions, KOH 0.2M and HCL 1M, 5% sucrose (TCI S0111, Tokyo, Japan).

Preparation of Bacterial Sample

Streptococcus gordonii ATCC 51656 is used in this research. Bacteria were cultured by inoculating one use of *Streptococcus gordonii* bacteria into Brain Heart Infusion Broth (BHIB) medium and incubating at 37^{0}C for 24 hours.[11]

Preparation of Red Ginger Fractionated Ethanol Extract

The red ginger (*Zingiber officinale var. rubrum*) used in this study was obtained from UPT Materia Medica Batu, East Java Health Department. The red ginger was washed thoroughly, cut into thin pieces, then put into the boiler and fractionated using the steam distillation method for 6 hours. Then, a liebig condenser is displayed in the boiler, collecting the steam and essential oils while condensing the steam into water. 700 ml of water from the liebig condenser was put into a separatory funnel. The essential oil was put into a rotary evaporator at 50^{o}C and a vacuum pump to produce red ginger essential oil extract (separating the essential oil).

Then, the sliced ginger was dried in an oven at 40-50^{o}C for 1-2 days and grinded to obtain ginger simplicia powder. Extraction using the maceration method by immersion in 96% ethanol for 24 hours and filtering using a Buchner funnel, vacuum pump, and filter paper for up to three times immersion. After three times of immersion, the liquid extract was put into a rotary evaporator to separate the ethanol, and a concentrated red ginger extract was obtained. Fractionated red ginger extract was diluted using distilled water to get 0.78% and 1.56% concentrations.[12]

pH Level Test on *Streptococcus gordonii* Metabolism

The pH level test procedure refers to the modified method of Hasan et al., 2015.[11] *Streptococcus gordonii* ATCC 51656 in BHIB media, 0.78% and 1.56% fractionated red ginger extract, 5% sucrose solution was vortexed until the suspension was homogeneous. The centrifuge tube was prepared and labeled A (control group 1 consists of bacteria and BHIB), B (control group 2 consists of bacteria, BHIB, and 5% sucrose), C (treatment group consists of bacteria, BHIB, 5% sucrose, and 0.78% fractionated red ginger extract), and D (the treatment group consists of bacteria, BHIB, 5% sucrose, and 1.56% fractionated red ginger extract). *Streptococcus gordonii* ATCC 51656 in BHIB media was harvested by using centrifugation at 3500rpm for 10 minutes and washed twice by using washing buffer (50Mm KCl and 1Mm $MgCl_2$). Then, the harvested bacteria were suspended in a washing buffer with or without fractionated ginger extract as labeled tube appropriately. All groups were titrated until the pH reached 7.2-7.4 with 0.2M KOH or HCL 1M solution. 5% sucrose was added to labeled groups appropriately. Then all groups were incubated for 60 minutes. pH level in each group was measured using a Mettler Toledo pH meter. The data obtained were analyzed using the *One-Way ANOVA* test.

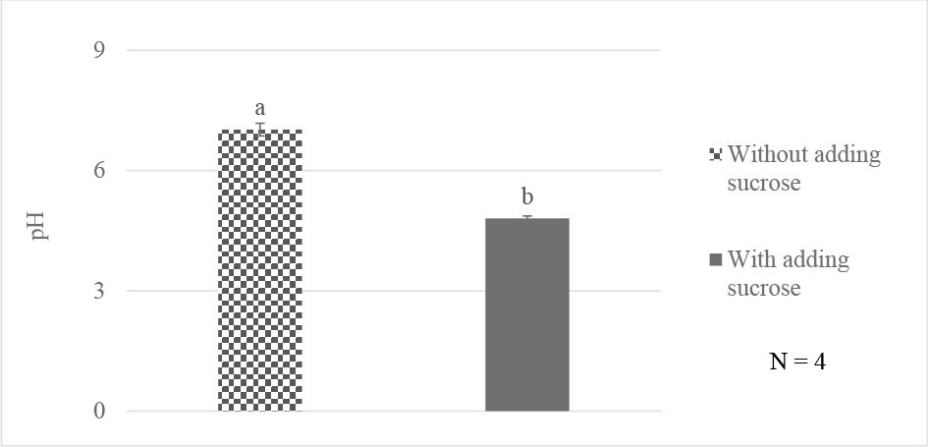
RESULTS

In this study, 0.78% and 1.56% of fractionated ginger extract were used to analyze the effect of fractionated red ginger on the pH level of *Streptococcus gordonii.* In Figure 1, control group 1 consisting of *Streptococcus gordonii* in BHIB showed a higher pH level (7.02±0.157) than control group 2 consisting of *Streptococcus gordonii* with adding 5% sucrose in BHIB (4.81±0.059). There is a significant difference between those groups ($p<0.05$).

In Figure 2, the treatment groups with 0.78% (6.42 ± 0.078) and 1.56% (6.28 ± 0.181) showed higher pH levels than control group 2 (4.81 ± 0.059). There are significant differences between the treatment groups and the control group ($p<0.05$). Moreover, there is not a significant difference between the treatment groups ($p>0.05$). The fractionated red ginger extract can inhibit the lowering pH level of *Streptococcus gordonii* metabolism.

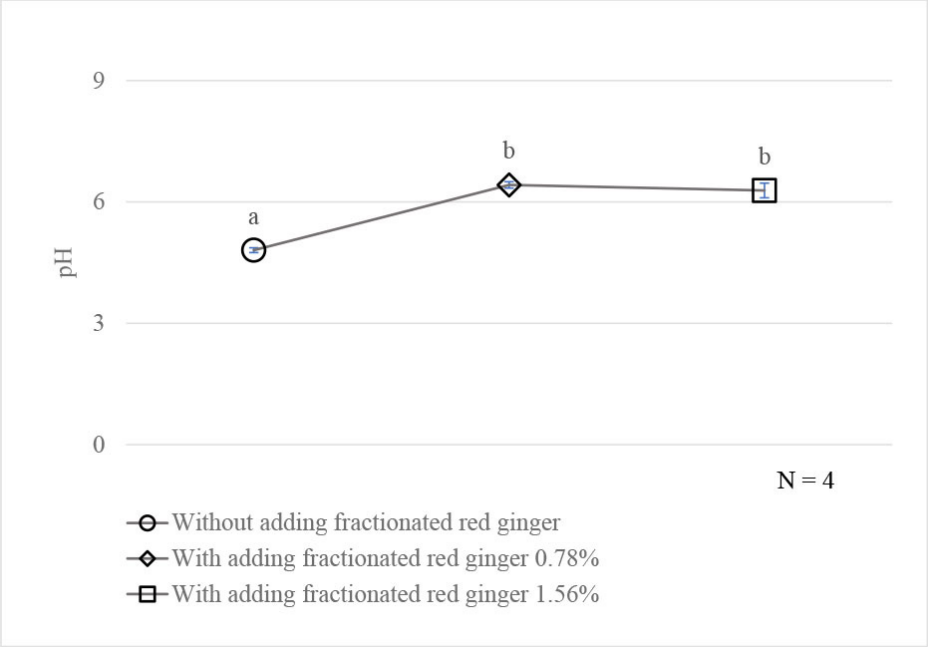
DISCUSSION

Streptococcus gordonii is a gram-positive commensal bacterium in the oral cavity that acts as a primary colonizer in plaque formation and contributes to the development of dental caries.[6] This bacterium can create acidic conditions



*a, b = notation (different letters indicate a significant difference between groups; p<0.05)

Figure 1. pH level of the control group on *Streptococcus gordonii* metabolism with/without 5% Sucrose in BHIB.



*a, b = notation (different letters indicate significant differences between groups); p<0.05

Figure 2. pH level on *Streptococcus gordonii* with/without fractionated red ginger in BHIB with 5% sucrose.

through glucose metabolism, then facilitate more acid resistance and produce bacteria to grow, such as *Actinomyces, Bifidobacterium, Lactobacillus, and Streptococcus mutans (Ecological shifting)*. If acid production occurs continuously without any action to neutralize the acid, then the demineralization on the enamel surface as an initial stage of dental caries will occur.[7]

Red ginger has various benefits, including antibacterial, analgesic, anti-diabetic, anti-inflammatory, antioxidant, anti-biofilm, and anti-hypertensive properties. In previous studies, red ginger was identified as an antibacterial agent on gram-positive and gram-negative bacteria such as *Pseudomonas aeruginosa, Staphylococcus aureus*, and *Bacillus subtilis*.[10]

Much information regarding red ginger, especially the essential oil in red ginger as antibacterial agents has been investigated, such as β-caryophyllene, ar-curcumin, β-sesquiphellandrene, and zingiberene.[13,14] However, red ginger has other important components besides the essential oil, that have antibacterial activity, such as gingerols, shogaols, saponins, tannins, and alkaloids.[10] In this research using fractionated red ginger means the essential oil was removed. Then the other components besides the essential oil are investigated.

From the research results, adding sucrose 5% to the control group decreased the pH level (Figure 1). This is because sucrose is a source for bacteria to produce energy and organic acid through metabolic processes. Sucrose is a disaccharide that can be simplified into glucose and fructose. Glucose will be metabolized by bacteria as an energy source, with organic acids as the final product through the Embden-Meyerhof-Parnas pathway causing a pH fall. In addition, sucrose can also stimulate glucosyltransferase (GTF) enzyme activity, which synthesizes sucrose into glucan. Glucan plays a role in the attachment process of *Streptococcus gordonii* bacteria to the tooth surface, leading other aciduric bacteria to aggregate and form biofilms which can lower the pH of the oral cavity.[7]

Bacteria metabolize glucose through a glycolysis process involving various enzymes, such as hexokinase, phosphoglucoisomerase, phosphofructokinase, aldolase, triose phosphate isomerase, glyceraldehyde 3-phosphate dehydrogenase, phosphoglycerate kinase, phosphoglycerate mutase, enolase, and pyruvate kinase. Some products of the glycolysis process are pyruvate, ATP, and NADH. Pyruvate can be converted into lactate, acetate, ethanol, and formate through various pathways that play a role in pH falling.[15]

From the research results, the treatment group had a higher pH level than control group 2. The results showed that 0.78% and 1.56% fractionated red ginger effectively inhibited the pH from falling in Streptococcus gordonii's metabolism. Red ginger components besides essential oils such as gingerols, shogaols, saponins, tannins, and alkaloids can inhibit pH falling by reducing the glucose metabolism activity and the number of living bacteria that make acid production decrease. The chemical compounds in red ginger can interfere with the bacterial cell wall components, damaging the cell wall and cell membrane and causing bacterial cell

death. Therefore, it reduces the number of bacteria and decreases glucose metabolism through glycolysis by bacteria, hence inhibiting the pH falling.[10,16,17]

Red ginger contains various chemical compounds that inhibit bacterial growth by interfering with bacterial cell wall components, such as cell wall proteins, teichoic acid, and peptidoglycan. Damaged cell walls and cell membranes can cause cytoplasmic leakage, release intracellular compounds from the cell, cause bacterial cell lysis, and cause death as a consequence. Bacterial cell death and disruption of glycolysis can affect the decrease of organic acid products produced by bacteria. Thus, the pH falling is inhibited.[7,10] Hence, it can be concluded that fractionated red ginger extract inhibits the pH falling on the *Streptococcus gordonii* metabolism, with 0.78% as an effective concentration.

This study has limitations. This study looked at the ability of fractionated red ginger extract against *Streptococcus gordonii* bacteria in vitro; therefore, more research is needed to see how ginger extract interacts with various types of bacteria in the oral cavity involved in the caries process, such as *Streptococcus mutans*, *Lactobacillus acidophilus, Bifidobacterium*, and so on. Furthermore, there is a need for in vivo research.

CONCLUSION

In conclusion, our research findings indicate a significant effect of the fractionated ethanol extract of red ginger (*Zingiber officinale var. rubrum*) inhibits the decreasing pH level in the *Streptococcus gordonii* metabolism.

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AUTHOR'S CONTRIBUTION

All authors made substantial contributions to the conception and design of the study, the collection of the data, the analysis and interpretation of data, the drafting of the article, the critical revision of the article for important intellectual content, and the final approval of the version to be published.

ETHICAL CLEARANCE

This research has received approval from Universitas Airlangga Faculty of Dental Medicine Health Research Ethical Clearance Commission (Number: 348/ HRECC.FODM/ VI/ 2022).

CONFLICT OF INTEREST

The authors affirm that the mentioned conflicts of interest did not compromise the objectivity, integrity, or validity of the research findings presented in this paper.

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