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THE EFFECT OF GINGER FOR GARGLING ON SALIVARY PROFILE AND IN-VIVO ANTIMICROBIAL ACTIVITY

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ABSTRACT

The Effect of Ginger for Gargling on Salivary Profile and In-Vivo Antimicrobial Activity. Infectious diseases have a high incidence rate, especially during the Covid-19 pandemic, caused various efforts to prevent and treatment the infection. One of the effort is use of traditional herbs, Ginger. Ginger is a thibbun-nabawi herb that known to have antimicrobial activity. This study aims to determine the effect of gargling activity with ginger decoction on salivary profile and in-vivo antimicrobial tests. The study was conducted in University of Darussalam Gontor with respondents who experienced mouth ulcer and dental caries. The treatment was divided into 5 groups, positive control, negative control, ginger decoction in 3 concentrations, 10%, 20%, and 30%. The sample used was respondent's saliva obtained by the spitting method. Samples were tested for pH, and volume and incubated in a microbial growth medium to calculate the number of colonies and observations in a microscope for pathogen identification. Influence of salivary profile and colony count before and after treatment tested with T-Test analysis. The results of the pH test showed an increase in the pH value to be more alkaline after being given a solution of gargling, although the pH increase was the smallest in the negative control group and the highest in the ginger group at 30% ($p>0.05$). Measurements of saliva volume showed a decrease in saliva volume ($p>0.05$). Differences in the number of colonies before and after treatment in the control group (+) showed significant differences compared to other groups. The minimum decrease in the number of colonies was showed by the Ginger group of 30% with insignificant differences before and after treatment. The antimicrobial activity test showed ginger decoction had a minimum antimicrobial activity due to ginger decoction was not good at diluting secondary metabolites of ginger that have antimicrobial activity

Keywords: ginger, gargling, salivary profile, antimicrobial

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1. INTRODUCTION

Disease of the oral cavity such as sore throat, toothache, and inflammation usually occur due to an infection in the oral cavity. Infectious diseases are diseases that are caused by pathogens such as bacteria, viruses, fungi, and protozoa. Nearly half of the deaths are caused by infectious diseases, so various prevention and treatment efforts have been made to reduce morbidity and mortality.

The mouth is an organ system that is most prone to infection because it is the outermost part of the body's defense system. Infections of the mouth and throat are common infections in the community, such as flu, cough, sore throat, bad breath, and dental plaque, where the most common pathogens are *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus.*

Traditional herbs are natural ingredients that are used for generations by community to treat health problems. Traditional herbs have secondary metabolites that have a potential antimicrobial activity such as alkaloids, tannins, and flavonoids.[1] One of the traditional herbs that have antimicrobial potential is ginger. Ginger contains gingerol, paradol, zingerone, and zerumbone which have antimicrobial activity. Ginger exhibits antimicrobial activity against *E coli, Salmonella typhi* and *Bacilus subtilis*.[2] Ginger is also one of the plants included in Thibbun Nabawi and is mentioned in the Qur'an Surah Al Insan verse 17 which states that ginger is heaven's beverage.

Evaluation of antimicrobial activity in herbs is generally carried out in-vitro, so the novelty of this study is that the research was carried out in-vivo by considering the salivary profile as an indicator of oral and throat health. In addition, this research can also be used as one of the innovations in the development of herbal medicines that support the role of pharmacists.

2. METHOD

Tools and Materials

The tools used in this study were antimicrobial test preparation tools including hot plates, Bunsen, autoclaves, incubator, ovens, petridishes, triangles, ose needles, microscopes, glass preparations, colony counters, and Laminar Air Flow (LAF).

The materials used in this study were ginger, agar media for the antimicrobial test including blood agar plates, potato dextrose agar, aquades, and povidone iodine 1 % as positive controls.

Preparation of Ginger Decoction

The ginger sample was washed clean, then drained until the water content was reduced. After that, 1000 grams of chopped ginger was taken and roughly ground using a mortar and pestle to increase the surface area and volume of the ginger content. Ginger was boiled to get a 100% solution, then diluted to a concentration of 10%, 20%, and 30%

Population and Sample

The study population was female students at UNIDA Gontor Female Campus with inclusion criteria: 1) Respondents who have experienced dental caries and/or mouth sores, 2) Respondents who have experienced problems with swallowing and rinsing, and 3) Respondents who wanted to collect their saliva as samples. The exclusion criteria in this study included: 1)

Respondents who have experienced an uncomfortable sensation of gargling during treatment, and 2) Respondents who have experienced tooth pain.

The study was conducted with 5 test groups, positive control, negative control, 10% ginger decoction, 20% ginger decoction, and 30% ginger decoction. Determination of the number of samples was determined by the Federer Formula:

$$(n-1)(t-1) \geq 15$$

$$(n-1)(5-1) \geq 15$$

$$n \geq 4{,}75$$

The number of samples for each group was 4,75 so it was rounded up to 5 respondents.

Research Procedure. The study was conducted with 5 test groups: positive control, negative control, 10% ginger decoction, 20% ginger decoction, and 30% ginger decoction. The positive control group was treated with a mouthwash containing 1% povidone-iodine, while the negative control group was treated with a mouth rinse containing mineral water.

Respondents were asked to rinse their mouths with the treatment 2 times a day for 3 consecutive days. Saliva sampling was carried out on the first day before treatment and the last day after treatment. A sampling of saliva using the spitting method.

Measurement of pH and Saliva Volume

The volume of saliva was measured with a measuring cup. Salivary pH was measured objectively using a calibrated Ohaus digital pH meter.

Antimicrobial Activity

Evaluation of Antimicrobial Activity included counting the number of colonies, gram staining, and identification of pathogens with a microscope. The saliva sample that has been obtained was poured into the agar media. The media used in this study was rejuvenation media, namely Blood Agar Plate (BAP) as a growth media for *Streptococcus mutans* and *Staphylococcus aureus*. Potato Dextrose Agar (PDA) as a growth media for the fungus *Candida albicans*. Petri dish that contained the pathogen in the media then had been incubated at 37^{o}C for 2x24 hours. Formed colonies were observed and counted using a colony count. The gram staining test was used to differentiate the pathogen from gram-positive bacteria or gram-negative bacteria. Identification of pathogens was done by observing under the microscope.

Data Analysis.

The data obtained were analyzed using the SPSS statistical application to examine the effect of ginger decoction on the salivary profile and antimicrobial activity. The Normality test was carried out to see the distribution of the data obtained. The analysis used the T-test to test the influence of the salivary profile and the number of bacterial colonies before and after being given treatment. Antimicrobial activity of different concentrations was analyzed using One-Way Anova.

3. RESULT

The study was conducted with 5 test groups, namely 2 control groups and 3 test groups with 10%, 20% and 30% ginger decoction, where each group had 5 respondents. Saliva profile that included salivary pH and salivary volume can be seen in the following table.

Table 1. Salivary pH Test

Groups	Mean		Difference average	Sig.
	Pre	Post		
Control (+)	7,34	7,44	-0,1	0,643

Control (-)	7,40	7,46	-0,06	0,891
Ginger 10%	7,00	7,14	-0,14	0,108
Ginger 20%	7,02	7,20	-0,18	0,367
Ginger 30%	7,16	7,46	-0,3	0,169

All treatment groups showed an increase in pH values to become more alkaline after being given a gargling decoction, although the increase in pH was the smallest in the negative control group and the highest in the 30% ginger group. The significance test showed that there was no significant difference between salivary pH before and after treatment.

Saliva volume measurements between treatment groups were shown in Table 2 below

Table 2. Salivary Volume Test

Groups	Mean		Difference average	Sig.
	Pre	Post		
Control (+)	2,52	2,02	0,50	0,214
Control (-)	2,80	2,34	0,46	0,189
Ginger 10%	2,66	1,80	0,86	0,030*
Ginger 20%	2,64	1,86	0,78	0,164
Ginger 30%	2,84	2,56	0,28	0,593

*p < 0,05 (signifikan)

Salivary volume between before and after treatment showed a decrease in volume, where the smallest decrease was in the 30% Ginger group and the largest in the 10% Ginger group, where the difference in salivary volume before and after administration showed a significant difference.

The antimicrobial activity test in this study was by looking at the differences in the number of colonies before and after treatment as shown in the following table.

Tabel 3. Number of Colonies

Groups	Mean		Difference average	Sig.
	Pre	Post		
Control (+)	308,80	69	239,8	0,043*
Control (-)	265,80	132	133,8	0,080
Ginger 10%	242,00	140,6	101,4	0,465
Ginger 20%	262,60	226,2	36,4	0,617
Ginger 30%	208,80	187	21,8	0,852

*p < 0,05 (signifikan)

The difference in the number of colonies before and after treatment in the control group (+) showed a significant difference, with the largest decrease in the number of colonies compared to the other groups. The smallest decrease in the number of colonies was shown by the 30% Ginger group with an insignificant difference before and after treatment.

The results of the analysis between groups to see changes in the salivary profile and the number of colonies after treatment showed a p value >0.05 so that neither the control group nor the test group showed significant differences in the number of colonies, salivary pH and salivary volume after treatment.

Identification of pathogens from respondents with dental caries and mouth ulcers showed fungi and bacteria were found in each group. Identification of the fungus was carried out on respondents with mouth ulcers where the culture used selective media of fungi, namely PDA. Observations under a microscope with a magnification of 40 x 10 showed the presence of Candida albicans. Candida albicans was a normal flora in the human oral cavity and generally becomes a pathogen when a person's immune system decreases.[3]

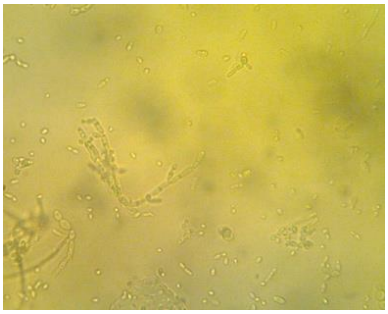

Figure 1. *Candida Albicans* in respondents with mouth ulcer (magnification 40x10)

Identification of pathogens in respondents with dental caries used the gram staining technique, which showed colonized bacteria were purple in color indicating gram-positive bacteria. Observations with a microscope showed long-chain coccus-shaped bacteria, namely Streptococcus mutans. Streptococcus mutans was a gram-positive coccus bacterium that is commonly found in the oral cavity and was the main cause of dental caries.[4]

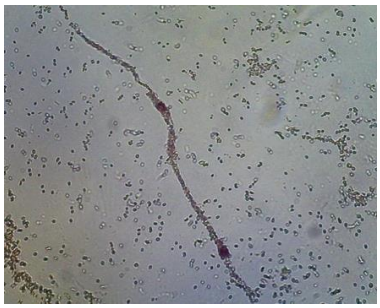

Figure 2. *Streptococcus mutans* in respondents with dental caries (magnification 40x10)

Pathogen identification was carried out to find the cause of oral thrush and dental caries, and to see the antimicrobial activity of ginger to inhibit or eliminate these pathogens. In addition to the discovery of the main pathogen causing mouth ulcers and dental caries, the researchers also found the growth of other pathogens from the samples taken, namely the fungus Aspergillus. Aspergillus was a common fungus in fertile environments. Although Aspergillus was found in agar media, the researchers could not conclude that Aspergillus had a role in causing the respondent's mouth disorders.

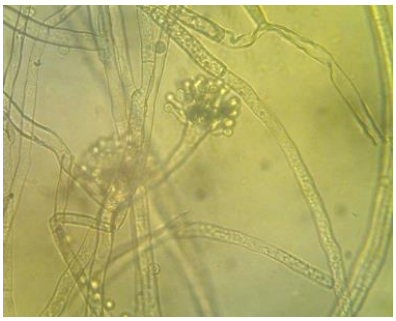

Figure 3. Aspergillus in respondents saliva (magnification 40x10)

5. DISCUSSION

Saliva has an important role in maintaining the health of the oral cavity, among its functions as lubrication and protection, solvent and cleaning of food in the oral cavity, as a buffer system to prevent the colonization of pathogens, and maintain the health of tooth enamel.[5] Stimulated saliva production in normal adults ranges from 1 – 3 mL/minute, while unstimulated saliva production ranges from 0.25 – 0.35 mL/minute. The results of studies that tested the volume of saliva did not show an increase in volume after giving ginger decoction. This was different from many previous studies which show ginger can increase the rate of salivary secretion. The increase in the rate of salivary secretion in ginger was influenced by several things, such as the oleoresin which gives a spicy taste, the sharp aroma of ginger essential oil, and the activity of rinsing which was a mechanical stimulus for salivary secretion.[6] Other studies have shown that ginger can overcome xerostomia (dry mouth) in complications of diabetes mellitus because

it stimulates the salivary secretion.[7] The decrease in salivary secretion in this study could be caused by several things, including unstimulated saliva sampling, which at 6 am with 1 hour without eating or drinking. The amount of unstimulated saliva tends to be less than when it was stimulated.

The salivary buffer system was measured by salivary pH. Normal salivary pH was 6 to 7, which was slightly acidic. Saliva as a buffer has a function to prevent bacterial colonization, and salivary pH neutralizes acids produced by acidogenic microorganisms, to prevent enamel demineralization. The results of post-treatment pH measurements showed an increase in pH, although not significant. The greatest increase in pH was shown in the 30% ginger decoction treatment group.

Ginger is an herb that has been known to have various benefits, including antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, and anti-tumor activity.[2] Ginger contains metabolites that have antibacterial and antifungal activity, such as gingerol and shogaol. Previous studies have shown that ginger ethanol extract has inhibitory activity against various fungi, including Candida albicans.[8] Many researchers have studied the antibacterial activity of ginger was related to the active components of ginger (zingiberen, α-farnesen, 6-gingerol, and α-curcumene) which increased the permeability of the bacterial cell membrane, causing the release of bacterial intracellular components, including nucleic acids which are very important macromolecules for bacterial survival. The release of intracellular components caused bacteria to lose their basic structural functions so at certain concentrations, it caused bacterial cell death. Ginger's active metabolites also interfere with bacterial metabolism by affecting mitochondria and bacterial enzyme activity.[9,10] The activity of ginger in inhibiting bacteria was greater in gram-positive bacteria. This was because gram-negative bacteria have a lipid bilayer which provides more protection to the antimicrobial components of ginger.[9,11] This study showed a decrease in the number of Streptococcus mutans bacterial colonies in both the control and treatment groups. The positive control group with povidone-iodine showed the greatest reduction in the number of colonies. In the treatment group, the greatest decrease was shown in the 10% ginger decoction group ($p>0.05$). The antimicrobial activity of ginger decoction was low and not statistically significant. This was supported by previous research which showed that ginger water extract exhibited lower antimicrobial activity compared to methanolic and ethanolic extracts because the flavonoids and folate components dissolve better in organic solvents.[12]

The antifungal activity of ginger decoction in this study showed no decrease in the number of colonies before and after treatment. This is because ginger decoction cannot maximally attract active metabolites that play a role in antifungal activity. The antifungal activity of ginger has been extensively studied and its activity was optimal in the form of essential oils compared to the form of aqueous/boiled extracts and organic solvents. The essential oil of ginger was rich in zingiberene, zingerone, and trans-[6]-shogaol which have a strong antifungal activity where the hydrophobic of the essential oil can damage the fungal cell wall.[9]

Gargling was an activity that has been known to maintain oral health because it has the benefit of cleaning the oral cavity and stimulating salivary secretion which contains compounds and enzymes to inhibit microbial growth. Giving ginger decoction for gargling was one of the innovations in using ginger that was easily applied by the community, although the results of this study showed that ginger decoction was not

good enough to show antimicrobial activity. Therefore further research can be carried out to formulate mouth rinses with ginger extract and essential oil as well as in vivo testing to see the effectiveness of these mouth rinses.

6. CONCLUSION

Ginger decoction as mouthwash showed an increase in respondent's salivary pH although the change was not statistically significant. The volume of saliva after treatment showed a decrease in volume, which was not in line with previous studies. This was expected because the saliva collection method was not stimulated, so the post-treatment volume was small. The potential antimicrobial activity showed a decrease in the number of microbial colonies although not significantly different.

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