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Antibacterial Effectiveness Test of Mouth Spray Extract Preparations Mint Leaves (*Mentha piperita L.*) Concentrations of 5%, 10%, 25%, 50% against Lactobacillus acidophilus bacteria

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Abstract: *Lactobacillus acidophilus* one of the bacteria that causes caries among other *Lactobacillus* species. Mint leaves (*Mentha piperita L.*) contain active compounds as antibacterials. The aim of this research was to determine the effectiveness of mint leaf extract (*Mentha piperita L.*) mouthspray in concentrations of 5%, 10%, 25%, 50% against *Lactobacillus acidophilus*. Type of this research is true experimental with post test only control group design. The sample is pure culture of *Lactobacillus* acidophilus and 24 students Dentist Study Program, Prima Indonesia University. Antibacterial testing used the disc diffusion method and the solution was made with pure extract and distilled water. Organoleptic data was collected by questionnaire then analyzed using the Kruskal-Wallis and Mann-Whitney. The results is showed the majority of panelists stated did not like the color, aroma and taste of the mouthspray of mint leaf extract (*Mentha piperita L.*). In the antibacterial test, the inhibition zone was obtained with concentrations of 10%, 25%, 50% and positive control was 3.70 ± 4.273 mm; 8.88±1,250 mm; 11.33 ± 0.126 mm; 11.34 ± 0.126 mm, while the 5% and negative control had no obstacles. This research shows that mouthspray solution has a safe formula as an oral preparation and has antibacterial activity.

Keywords: Antibacterial; Lactobacillus acidophilus; Mint leaves; Mouth spray

Introduction

Periodontal disease is conventionally defined as an inflammatory disorder involving soft and hard periodontal structures (Nocini R, et al., 2020). Periodontal disease is an oral cavity disease that affects almost all humans in the world and reaches 50% of the adult population (Newman, et al., 2012). The most common periodontal diseases are gingivitis and periodontitis.

Based on WHO's 2022 global oral health status report, severe periodontal disease is estimated at approximately 19% of the global adult population, representing more than 1 billion cases worldwide. Estimates of the prevalence and cases of severe periodontal disease per region throughout the world show that Southeast Asia is one of the regions with the highest number of cases. According to 2018 RISKESDAS data, the prevalence of periodontitis cases in Indonesia reached 74.1%.

Periodontal disease can be classified into gingivitis and periodontitis (Hasan, 2014). Gingivitis is inflammation around the gingival edges caused by chronic plaque retention (Carranza, 2012). Periodontium infection, known as periodontitis, is a chronic peripheral inflammatory disease initiated by microbes residing in the oral cavity. Clinically, chronic periodontitis is characterized by gingival erythema, edema, periodontal pockets and damage to the tooth supporting tissue (Ding et al., 2018).

The main cause of periodontal disease is the accumulation of bacteria in plaque on the tooth surface

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which interacts with pathogenic bacteria accompanied by inadequate host immunity (Agnes, 2013). Supragingival plaque is dominated by gram-positive bacteria such as *Streptococcus mutans* and *Lactobacillus* (Maulana, et al., 2022). *Lactobacillus acidophilus* is the most dominant bacteria that causes caries among other *Lactobacillus* species (Utami DSA, 2017).

Periodontal disease can be prevented by reducing the appearance of plaque on the teeth (Rahmawan BA, et al., 2018.). To prevent inflammatory changes in periodontal tissue, effective measures in the form of plaque control can be carried out during every periodontal treatment (Diah, 2012). According to (Greenstain, 2000) there are various treatment methods for periodontal disease, one of which is mechanical instrumentation, ultrasonic debridement, supragingival and subgingival irrigation, local drug administration, systemic antibiotics and modulation of the host response.

Apart from the treatment methods above, mouthwash is also a method to help relieve the symptoms of gingivitis, inflammation and can also be relied on to destroy pathogenic bacteria (Banu & Gayathri, 2016). The active ingredients in mouthwash formulas can come from chemicals or natural ingredients (Anastasia A, et al., 2017). However, chemical mouthwashes that contain alcohol can trigger undesirable effects, such as a burning sensation in the oral cavity, xerostomia, and even the risk of oral cancer (Oktanauli et al., 2017).

Many mouthwashes that use natural extracts from herbal plants have been developed because they have antibacterial properties with minimal side effects (Oktanauli et al., 2020). Herbal extract solutions packaged in sprayer form are more environmentally friendly, alcohol-free and use less harmful chemicals compared to commercial mouthwash or other mouth odor removers (RS Resmisari et al., 2021).

Mint leaves (*Mentha piperita L.*) are a plant with antibacterial power. The chemical compounds contained in mint leaves (*Mentha piperita L.*) such as flavonoids, polyphenols, tannins and menthol have the potential to be antibacterial. Mint leaf extract (*Mentha piperita L.)* has antibacterial and antioxidant activity on gram-positive and gram-negative bacteria (Singh, et al., 2011). The content of mint leaf extract (*Mentha piperita L.*) can provide quite large free radical scavenging activity and also shows quite large antibacterial and antifungal activity against selected bacteria and fungi (Pramila, DM, et al., 2011). In line with research (Heryawan, et al., 2021) which concluded that mint leaves (*Mentha piperita L.*) have an antibacterial effect, if the concentration of an antibacterial compound is higher, the effectiveness will increase.

Based on the statements above, researchers are interested in conducting antibacterial tests on mint leaf extract (*Mentha piperita L.*) oral spray preparations in concentrations of 5%, 10%, 25% and 50% against *Lactobacillus acidophilus* bacteria.

Method

Research Design

This study employs a post-test only control group design in a true experimental design, meaning that measurements and observations are made following treatment.

Time and Place Research

Research procedures were carried out at the Integrated Laboratory of Prima Indonesia University. The research was conducted from August to October 2023.

Research Sample

This research population used pure cultures of *Lactobacillus acidophilus* bacteria and students of the 6th semester class of 2020, Dentist Education Study Program, Prima Indonesia University. The research sample size was determined by using the Federer formula to determine the number of repetitions so that valid data could be obtained. The number of repetitions for each sample is 4 times to avoid bias. The questionnaire research sample was carried out directly on 24 panelists who were randomly selected in general.

Tools and materials used

The equipment used in this research is a maceration vessel, stirring rod, plastic container, 60 mesh sieve, funnel, volume pipette, analytical balance, cotton swab, paper disc, measuring cup, blender, Laminar Air Flow (LAF), rotary evaporator, micropipette, petri dish, incubator, round tube, autoclave, bunsen, spectrophotometer, vortex mixer, sterile cotton, sterile gauze, tube rack, magnetic stirrer and vernier caliper. The materials used in this research consisted of mint leaves (*Mentha piperita L.*) ± 2.5 kg, *sulfuric acid* (H2SO4), *acetic acid* (CH3COOH), pure isolates of *Lactobacillus acidophilus* bacteria, Brain Heart Infusion Broth (BHIB) media, Nutrient Agar (NA), Mc Farland standard 0.5 (1% sulfuric acid solution 9.95 ml and 1.175% barium chloride solution 0.05 ml), 70% ethanol, DMSO, 0.2% *Chlorhexidine gluconate* and sterile distilled water.

Making Extracts

± 2.5 kg of dried mint (*Mentha piperita L.*) leaves are made into flour. Then the mint leaves (*Mentha piperita L.*) were sieved using a 60 mesh sieve. Finely chopped mint (*Mentha piperita L.*) leaves were weighed to determine the amount of solvent needed. Mint leaf powder (*Mentha piperita L.*) is put into a plastic container then macerated using a solvent in the form of 70% ethanol or sterile

distilled water for 24 hours. The ratio between mint leaf powder (*Mentha piperita L.*) and solvent is 1:5. Make sure the powder is submerged in the solvent while stirring occasionally. The maceration process is carried out at LAF to avoid contamination. In this maceration process, two layers are formed, namely the solvent at the top, and mint leaf powder (*Mentha piperita L.*) at the bottom. Next, the solution is filtered to separate it from the dregs, then evaporated using a rotary evaporator until a thick extract is obtained. (RS Resmisari et al., 2021).

Organoleptic Test

Mint (*Mentha piperita L.*) mouth spray testing was carried out on 24 panelists who were selected by dividing samples of mint (*Mentha piperita L.*) mouth spray at concentrations of 5%, 10%, 25% and 50%. Then it was explained to the panelists how to fill out the questionnaire that had been provided and collect the questionnaires after all the panelists had finished filling in the questionnaires for analysis (Zulistina M, 2019).

Media Preparation

Preparation of the test bacteria, namely a pure isolate of *Lactobacillus acidophilus* bacteria, was planted in NA media for making bacterial culture, then the NA media was put into an incubator to be incubated for 1×24 hours at a temperature of 37ºC. After that, a suspension was made by taking Lactobacillus acidophilus from the culture medium using a loop, then putting it in a test tube containing 1 ml of sterile BHIB, then putting it in an incubator and incubating for 1×24 hours at a temperature of 37ºC. Next, dilution was carried out by adding sterile distilled water and homogenized until the turbidity was comparable to the Mc Farland standard 0.5 (1.5×108) (Munier NF, et al., 2021).

Antibacterial Testing

The inhibitory power test used agar diffusion using the Kirby Bauer method using disc paper. The rejuvenated pure culture of *Lactobacillus acidophilus* is taken and cultured in sterile distilled water then homogenized in a vortex machine. The NA medium on the petri dish was smeared with the culture of *Lactobacillus acidophilus* bacteria with a sterile cotton swab on the surface of the hardened agar medium. The media that has been smeared is placed on paper discs that have been soaked in a solution of mint leaf extract (*Mentha piperita L.*) with 4 different concentrations, namely 5%, 10%, 25% and 50%, DMSO served as the negative control and 0.2% Chlorhexidine gluconate as the positive control. After marking each treatment, it was incubated at 37°C for 24 hours. The inhibition zone or clear zone formed from each media using disc paper was measured using a caliper in mm (Magvirah T., et al. 2019).

Data Collection and Analysis Methods

The data collected is qualitative data and quantitative data. Qualitative data in the form of questionnaire results based on the preferences of 24 panelists for mouth spray preparations of mint leaves (*Mentha piperita L.*) in organoleptic tests. Quantitative data was obtained from antibacterial testing. The data that has been collected will be presented in the form of tables and graphs. Quantitative data in the form of antibacterial test results with the SPSS 20 (Statistical Product and Service Solution) program using the Kruskal-Wallis and Mann-Whitney tests.

Result and Discussion

Organoleptic Color Test

The mouth spray's color and the organoleptic test results for mint leaf extract (*Mentha piperita L.*) at concentrations of 5%, 10%, 25%, 50% are shown (Table 1). Based on the research results, the majority of panelists stated that they were neutral towards the color of mouth spray of mint leaf extract (*Mentha piperita L.*) at all concentrations.

Table 1. Organoleptic Color Test

Color	Concentration			
	5%	10%	25%	50%
	n (%)	n (%)	n (%)	n (%)
Strongly dislike	1 (4,2%)	2 (8,3%)	2 (8,3%)	2 (8,3%)
Disilike	6 (25%)	3 (12,5%)	6 (25%)	7 (29,2%)
Neutral	13 (54,2%)	18 (75%)	10 (41,7%)	11 (45,8%)
Like	4 (16,7%)	1 (4,2%)	6 (25%)	4 (16,7%)
Really like		0	0	0

Organoleptic Aroma Test

The organoleptic test results for the mouth spray aroma of mint leaf extract (*Mentha piperita L.*) at concentrations of 5%, 10%, 25%, 50% are listed (Table 2). Based on the results, the majority of panelists stated that they liked the aroma of the mouth spray of mint leaf extract (*Mentha piperita L.*) concentrations of 10%, 25% and 50%, but most did not like the aroma of the 5% concentration.

Table 2. Aroma Organoleptic Test

Aroma	Concentration			
	5%	10%	25%	50%
	n (%)	n (%)	n (%)	n (%)
Strongly dislike	0	3 (12,5%)	5 (20,8%)	3 (12,5%)
Disilike	12 (50%)	6 (25%)	8 (33,3%)	9 (37,5%)
Neutral	7 (29,2%)	12 (50%)	10 (41,7%)	11 (45,8%)
Like	5 (20,8%)	3 (12,5%)	1 (4,2%)	1 (4,2%)
Really like	0	0	0	0

Organoleptic Taste Test

The organoleptic test results for the mouth spray taste of mint leaf extract (*Mentha piperita L.*) at concentrations of 5%, 10%, 25%, 50% are listed (Table 3). Based on the results, the majority of panelists stated that they were neutral towards the taste of the mint leaf extract (*Mentha piperita L.*) mouth spray with a concentration of 5%, but most did not like the aroma of the mouth spray with concentrations of 10%, 25% and 50%.

Table 3. Organoleptic Taste Test

Taste	Concentration			
	5%	10%	25%	50%
	n (%)	n (%)	n (%)	n (%)
Strongly dislike	3 (12,5%)	4 (16,7%)	4 (16,7%)	8 (33,3%)
Disilike	7 (29,2%)	9 (37,5%)	14 (58,3%)	9 (37,5%)
Neutral	10 (41,7%)	7 (29,2%)	3 (12,5%)	5 (20,8%)
Like	4 (16,7%)	4 (16,7%)	3 (12,5%)	2 (8,3%)
Really like	0	0	0	0

Mean Inhibition Zone Diameter

The effectiveness of the antibacterial test for mouth spray preparations of mint leaf extract (*Mentha piperita L.*) at concentrations of 5%, 10%, 25%, 50% against *Lactobacillus acidophilus* bacteria is shown (Table 4). Based on the results, the mean ± SD diameter of the inhibition zone in the mouth spray group of mint leaf extract (*Mentha piperita L.*) concentration 10%, 25%, 50%, K (+) was 3.70 ± 4.273 mm; 8.88±1,250 mm; 11.33 ± 0.126 mm; 11.34 ± 0.126 mm, while the group with a concentration of 5% and K (-) found no inhibition.

Table 4. Effectiveness of antibacterial test of mouth spray preparations of mint leaf extract (*Mentha piperita L.*) concentrations of 5%, 10%, 25% and 50% against *Lactobacillus acidophilus* bacteria

Group	Replication				*Mean*±SD
	1	2	3	4	
5%	0	0	0	0	0
10%	7.5	7.3	0	0	3,70±4,273
25%	10.5	9	8.5	7.5	8,88±1,250
50%	13.5	13.5	11.5	10.5	11,33±0,126
K(+)	11.3	11.5	11.2	11.3	11,34±0,126
K(-)	0	0	0	0	0

Antibacterial Test

The effectiveness of the antibacterial test for mouth spray preparations of mint leaf extract (*Mentha piperita L.*) at concentrations of 5%, 10%, 25%, 50% against *Lactobacillus acidophilus* bacteria is shown (Table 5).

Table 5. Effectiveness of antibacterial test of mouth spray preparations of mint leaf extract (*Mentha piperita L.*) concentrations of 5%, 10%, 25% and 50% against *Lactobacillus acidophilus* bacteria.

Group	*Mean*±SD	p
5%	0	0,001*
10%	3,70±4,273	
25%	8,88±1,250	
50%	11,33±0,126	
K (+)	11,34±0,126	
K (-)	0	

Note: Kruskal-Wallis *Significant

Based on the results, the p value = 0.001 (p≤0.05) this indicates a notable difference. in the mean diameter of the inhibition zone for mouth spray of mint leaf extract (*Mentha piperita L.*) at concentrations of 5%, 10%, 25%, 50% against *Lactobacillus acidophilus*. From the results it can be stated that there is an effectiveness of the antibacterial test of the mouth spray preparation of mint leaf extract (*Mentha piperita L.*) at concentrations of 5%, 10%, 25%, 50% against *Lactobacillus acidophilus* bacteria.

Antibacterial Test Between Two Different Group

The effectiveness of the antibacterial test for mouth spray preparations of mint leaf extract (*Mentha piperita L.*) at concentrations of 5%, 10%, 25%, 50% against *Lactobacillus acidophilus* bacteria between two different groups is shown (Table 6).

Table 6. Effectiveness of antibacterial test of mouth spray preparations of mint leaf extract (*Mentha piperita L.*) concentrations of 5%, 10%, 25% and 50% against *Lactobacillus acidophilus* bacteria between two different groups

Group	5%	10%	25%	50%	K (+)	K (-)
5%	-	0,131	0,014*	0,013*	0,013*	1,000
10%	0,131	-	0,028*	0,019*	0,019*	0,131
25%	0,014*	0,028*	-	0,028*	0,020*	0,014*
50%	0,013*	0,019*	0,028*	-	0,304	0,013*
K (+)	0,013*	0,019*	0,020*	0,304	-	0,013*
K (-)	1,000	0,131	0,014*	0,013*	0,013*	-

Note: Mann-Whitney *Significant

Based on the results, there was a significant difference in the effectiveness of the antibacterial test of mouth spray of mint leaf extract (*Mentha piperita L.*) at concentrations of 5%, 10%, 25%, 50% against *Lactobacillus acidophilus* between two different groups (p≤0.05), except for the 5% and 10% concentration groups and the negative control, between the 10% concentration group and the negative control, and between the 50% concentration and the positive control.

Discussion

Lactobacillus acidophilus is a gram-positive bacteria that is non-motile or non-spore, producing lactic acid

which can be identified in the saliva of caries sufferers. These bacteria are considered as initiator microorganisms in the process of caries formation, especially in the dentin area (Gumilar et al., 2022; Nurhalisa et al., 2020). The aim of this research was to determine the effectiveness of the antibacterial test of a mint leaf extract (*Mentha piperita L*) mouth spray against *Lactobacillus acidophilus* bacteria. Mouth spray from mint leaf extract (*Mentha piperita L.*) is available in four concentrations, namely 5%, 10%, 25%, 50% to determine the differences in the antibacterial abilities contained in the mouth spray preparations.

Antibacterial activity can be seen using several methods, one of which is the disc diffusion method. This method is most often used to analyze antibacterial activity (Nurhayati et al., 2020). Based on the results of observations of the antibacterial test of *Lactobacillus acidophilus* with a mouth spray preparation of mint leaf extract (*Mentha piperita L.*), it showed that there were several inhibitory zone diameters produced with different concentrations after incubation at 37^{o}C for 24 hours. The mean ± SD diameter of the inhibition zone in the 10%, 25%, 50% concentration preparation group was 3.70 ± 4.273 mm; 8.88±1,250 mm; 11.33 ± 0.126 mm; and the average diameter of the inhibition zone of the positive control was 11.34 ± 0.126 mm, while the concentration of 5% and the negative control found no inhibition.

In general, many factors influence the inhibitory diameter for bacterial growth, including the concentration of the test material. There is a differentiation in the diameter of the inhibitory area due to the content at that concentration. The greater the concentration, the larger the active component will be and the resulting inhibition zone will also be larger (Dzulasfi et al., 2023). In this study, it was seen that the higher the concentration of mint leaf extract (*Mentha piperita L.*) in the mouth spray preparation, the greater the diameter of the inhibition zone against *Lactobacillus acidophilus* bacteria. Previous research results from (Hasanuddin & Salnus, 2020) also stated that the higher the concentration in an extract, the larger the inhibition zone formed. However, this is contrary to research (Zeniusa et al., 2019) that extracts with a concentration of 20% are more effective than concentrations of 40%, 60%, 80% and 100%, which have higher antibacterial activity.

Testing for significant differences in the mean diameter of the inhibition zone in mouth spray preparations of mint leaf extract (*Mentha piperita L.*) at concentrations of 5%, 10%, 25%, 50% against *Lactobacillus acidophilus* bacteria using the Kruskal-Wallis statistical test because the research data was not normally distributed and not homogeneous, the results can be declared significant (p≤0.05). Mouth spray preparations of mint leaf extract (*Mentha piperita L.*) concentrations of 10%, 25%, 50% are effective as an antibacterial against *Lactobacillus acidophilus*. Research results (Utami et al., 2023) also state that mouth spray contains safe ingredients for oral preparations and is antibacterial. Likewise, research by Singh et al (2015) showed that mint leaves (*Mentha piperita L.*) were effective as an antibacterial on gram-negative and gram-positive bacteria and as an antioxidant.

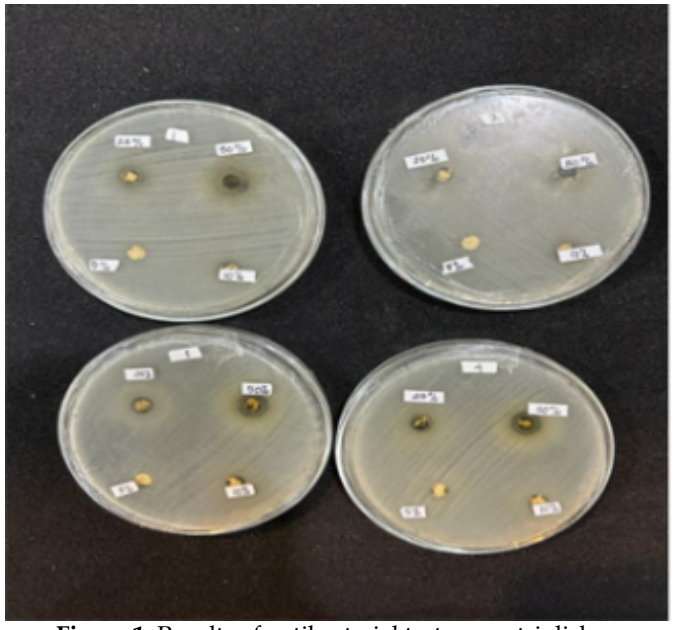

Figure 1. Results of antibacterial tests on petri dishes

Apart from the concentration of the test material, the active compound components contained also influence the diameter of the inhibition zone formed. Tuntun (2016) in (Magvirah et al., 2019) states that the mechanism of action of active compounds is useful as an antibacterial by destroying and entering the bacterial cell wall, bacterial cell proteins can be deposited and poison the protoplasm. According to the results of phytochemical screening conducted by Singh et al (2015), mint leaves (Men*tha piperita L.*) contain terpenoids, steroids, phenols, flavonoids and tannins. Other literature sources state that most mint leaves (*Mentha piperita L.*) are a source of phenolic acids, such as gallic, chlorogenic, p-coumaric, neochlorogenic, ferulic and rosmarinic acids, epicatechin, quercetin and quercetin-3-rutinoside.

Based on the results of the Mann-Whiney follow-up test, it was found that a mouth spray of mint leaf extract (*Mentha piperita L.*) with a concentration of 50% showed a difference in effect but was not significant compared to the positive control. From these results it means that a concentration of 50% is the most effective concentration because its antibacterial ability is almost equivalent to Chlorhexidine gluconate 0.2%. Mouthwash is considered the gold standard for oral hygiene due to its broad spectrum of antibacterial properties that prevent

plaque accumulation (Mandalas et al., 2022). Even though the antibacterial ability of the 50% concentration of mint leaf extract (*Mentha piperita L.*) mouth spray is almost equivalent to 0.2% Chlorhexidine gluconate, the diameter of the inhibition zone of this mouthwash is the largest compared to other groups when compared. This is in line with research (Ariyani et al., 2021) that the inhibition zone in the positive control is wider than the inhibition zone in the negative control or treatment group.

Figure 2. Mouth spray of mint leaves (*Mentha piperita L.*)

This study used a mouth spray solution only with a pure mixture of mint leaf extract (*Mentha piperita L.*) and sterile distilled water in concentrations of 5%, 10%, 25%, 50%. Organoleptic tests are carried out by paying attention to the physical appearance of the mouth spray preparation including color, aroma and taste (Sitti Zubaydah, 2022). Based on research results from 24 young adult panelists, 4 men and 20 women with an age range of 20-22 years, it was found that the majority of panelists stated that they were neutral towards the color of all concentrations of mint leaf extract mouth spray (*Mentha piperita L.*). For aroma, most of the panelists were neutral towards the aroma of mint leaf extract mouth spray (*Mentha piperita L.*) concentrations of 10%, 25% and 50% rather than the 5% concentration which many stated they did not like. Judging from the taste, on average the panelists were neutral with the taste of the mouth spray preparation of mint leaf extract (*Mentha piperita L.*) at a concentration of 5%, while at other concentrations the panelists said they did not like it.

The results of previous organoleptic mouth spray research from (Utami et al., 2023) showed that mouthwash spray made from a combination of noni leaf extract and ginger rhizome in liquid form has a distinctive odor and is yellow or even dark green and homogeneous. The ideal requirements for an organoleptic test are the results of the mouth spray being clear or transparent, not cloudy, and the absence of air bubbles (Sitti Zubaydah, 2022).

Conclusion

The average diameter of the inhibition zone for mouth spray preparations of mint leaf extract (*Mentha piperita L.*) concentrations of 10%, 25%, 50% and the positive control for Lactobacillus acidophilus bacteria was 3.70 ± 4.273; 8.88±1.250; 11.33 ± 0.126; 11.34 ± 0.126. Mint leaf extract (*Mentha piperita L.*) mouth spray preparation at a concentration of 5% and the negative control found no inhibition against *Lactobacillus acidophilus* bacteria. There is an antibacterial test for the effectiveness of a mouth spray preparation of a pure mixture of mint leaf extract (*Mentha piperita L.*) with sterile distilled water at concentrations of 5%, 10%, 25%, 50% against *Lactobacillus acidophilus*.

The majority of panelists stated that they were neutral regarding the color of mint leaf extract (*Mentha piperita L.*) mouth spray preparations at concentrations of 5%, 10%, 25%, 50%. The majority of panelists stated that they liked the aroma of mint leaf extract mouth spray (*Mentha piperita L.*) at concentrations of 10%, 25%, 50%. The majority of panelists stated that they were neutral towards the taste of the mouth spray preparation of mint leaf extract (*Mentha piperita L.*) at a concentration of 5%.

This research can be used as a reference for future research in making mouth spray formulas from plant extracts with other tests such as pH tests, stability tests and viscosity tests. Follow-up testing with a hedonic test to test the level of preference for mouth spray preparations of mint leaf extract (*Mentha piperita L.*) concentrations of 10%, 25%, 50%.

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Conceptualization and methodology by Firdha Muharraran; software and validation by Susiani Tarigan; the rest of this research by Rizqina Delfira. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest
The authors declare no conflict of interest.

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