ORIGINAL ARTICLE

Antibacterial and antibiofilm activity of mint leaves (*Mentha piperita L*) extracts against *Streptococcus mutans* UA159: a laboratory experiment

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ABSTRACT

Introduction: Oral health is a major concern in healthcare worldwide, with dental caries being a prevalent issue among children and adults. *Streptococcus mutans* is a primary bacterium implicated in the development of dental caries due to its acidogenic nature. Mint leaf (*Mentha piperita L.*) is a unique herbal plant that has antibacterial and antibiofilm properties against *S. mutans* and minimal side effects. The purpose of this study was to analyze the antibacterial effectiveness of mint leaf against the growth of *S. mutans* UA159 and its antibiofilm effects. **Methods:** This study was conducted experimentally with the posttest-only control group design, using the broth microdilution method in 6 test groups, namely mint leaf extract with a concentration of 3.125, 6.25, 12.5, 25, 50, and 100% and also two control groups, namely the negative control using aquadest and the positive control using 0.2% chlorhexidine. Biofilm growth is determined by comparing Optical Density (OD) values and then calculating the percentage of eradication of *S. mutans* biofilm formation. **Results:** The results indicated that the mint leaf extract exhibited antibacterial effects against *S. mutans*, with the largest inhibition zone diameter observed at a 100% concentration. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration $(MBC)_{90}$ values using the broth microdilution method in this study were 12.5% and 100% concentrations, respectively. Mint leaf extract has been shown to significantly inhibit the growth of *S. mutans* ($p<0.05$). The results of the inhibition test on biofilm formation at a concentration of 100% averaged 87.39%. **Conclusion:** Mint leaf extract has an antibacterial and antibiofilm effect on the growth of *S. mutans*. The level of inhibition of mint leaf extract on the growth of *S. mutans* is proportional to its concentration; the higher the concentration of the extract, the stronger the inhibitory ability.

KEYWORDS

Anti-Bacterial agents, biofilms, *Mentha piperita*, oral health, *Streptococcus mutans*.

INTRODUCTION

Oral health is a major concern in healthcare worldwide. Dental caries is a common oral health problem encountered in both children and adults. The 2018 Basic Health Research (Riskesdas) reported that the largest proportion of dental problems in Indonesia is dental caries (45.3%).[1] Dental caries can occur due to the presence of acid produced from the fermentation process of carbohydrate which is attached to the surface of the teeth by bacteria in the mouth.[2] Bacteria play a crucial role in the development of dental caries.

S. mutans is the most common bacterium responsible for dental caries.[3] *S. mutans* is a normal flora in the oral cavity, shaped like cocci, and is a gram-positive facultative anaerobic bacterium. This bacterium has the ability to produce acid by

fermenting carbohydrates, which can cause a decrease in the pH of the oral cavity and accelerate the demineralization process that leads to caries.[4] S. mutans UA159 is a serotype c chain consisting of 2,030,936 base pairs that have been completely sequenced. UA159 is naturally competent and contains all the essential genes for competence and quorum sensing.[5]

Biofilm is a collection of microorganism cells attached to a hard surface containing organic material and covered by an extracellular polymer matrix secreted by bacteria. The biofilm formation process begins with the adhesion of bacteria to the substrate, followed by bacterial growth and cell division, leading to colonization and biofilm formation.[6]

Maintaining Oral health hygiene to minimize the occurrence of dental caries can be done by reducing plaque accumulation using toothbrushes and mouthwash. Mouthwash contains antibacterial and antiseptic properties that function to reduce bacterial formation. A commonly used mouthwash is 0.2% chlorhexidine, known as the gold standard due to its proven antimicrobial properties, although it may cause side effects such as discoloration of teeth, dentures, and restorations.[7,8]

The use of natural materials for maintaining oral health is increasingly being used by many researchers as an alternative to synthetic materials with antibacterial properties.[9] The contents of natural materials have been proven to have many benefits for the body with minimal side effects. One such natural material that can be used is mint leaves with the scientific name *Mentha piperita L.*, known for their benefits, especially for oral health.[10]

The components found in mint leaves, such as flavonoids, polyphenols, tannins, saponins, alkaloids, steroids, and menthol, have antibacterial capabilities. Flavonoids work by denaturing cell proteins, rendering them nonfunctional and damaging the permeability of bacterial cell walls; polyphenols can disrupt the stability of bacterial cell membranes; tannins bind with proteins, reducing protein synthesis in both gram-negative and gram-positive bacterial cells and the synthesis of bacterial cell walls; alkaloids can lyse bacterial cell walls; steroids damage the plasma membranes of bacterial cells; and terpenoids can disrupt membranes through lipophilic compounds.[10-13]

These properties of mint leaves can interfere with the metabolism of microbes such as *S. mutans*. Previous research showed that combination of red betel leaves and mint leaves have the ability to inhibit the growth of *S. mutans*.[14] This study was an experimental laboratory aimed at evaluating the antibacterial and antibiofilm effects of mint leaf extract (*Mentha piperita L.*) against *S. mutans* UA159. Another study conducted by Raghavan et al. (2018) indicated that mint leaves have antimicrobial capabilities against oral microorganisms, including *Streptococcus mutans, Aggregatibacter actinomycetem-comitans,* and *Candida albicans*.[15]

Based on this background, the author was interested in conducting research on the antibacterial and antibiofilm effectiveness of mint leaf extract (*Mentha piperita L.*) against the growth of *S. mutans* using the broth microdilution method. The purpose of this study was to analyze the antibacterial effectiveness of mint leaf againts the growth of S. mutans UA159 and its antibiofilm effects.

METHODS

Employing a posttest-only control group design, this research was conducted in a laboratory using Mueller Hinton Broth (MHB) and Mueller Hinton Agar (MHA) media. The primary subject of the study was the mint leaf extract, while the research object was the *S. mutans* UA159 bacterium. Inclusion criteria included the use of pure *S. mutans* UA159 colonies grown on Mueller Hinton Broth (MHB) and Mueller Hinton Agar (MHA) media, and mint leaves aged 3-4 months without pest contamination with characteristic of green leaves measuring 2-5 cm, pointed

leaf tips, hairy upper leaf surface and smooth lower surface. Conversely, the exclusion criteria encompassed *S. mutans* colonies contaminated with other microorganisms and damaged or young mint leaves. Phytochemical test of the ethanolic mint leaf extract (*Mentha piperita L.*) was carried out using the Harborne procedure to determine the content of flavonoids, saponins, phenols, tannins, steroids, terpenoids, and alkaloids.[14-15]

Following Federer's formula for sample size calculation, the study included a total of 24 samples divided into 8 treatment groups 100, 50, 25, 12.5, 6.25, 3.125% concentrations, negative control (Aquadest), and positive control (Chlorhexidine 0.2%), each undergoing three repetitions. A wide range of equipment and materials were used, including standard laboratory equipment and materials such as mint leaf extract and bacterial growth media. The research process involved the preparation of mint leaf extract, phytochemical screening, antibacterial activity testing, and Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) tests.[14-16] The inhibition percentage was calculated using the following formula: Percentage of inhibition = (Negative control colonies − (Total treatment colonies/Negative control OD)) × 100%.

The method used for determining the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Mint Leaf Extract against *Streptococcus mutans* was the broth microdilution method. The number of colonies formed after 24 hours was counted using a spectrophotometer. The mint leaves MBC_{90} was defined as the concentration of extract required to achieve the killing of 90% of *S. mutans* bacteria contained in the mint leaves sample.

Biofilm growth was determined by comparing the Optical Density (OD) value of the treatment with the OD of the respective blank as measured by a microplate reader (Thermo Scientific™ Multiskan™, UK) with a wavelength of 490 nm and then calculating the percentage of eradication of *S. mutans* biofilm formation with the following formula:[14] Percentage of eradication=1-((OD sample–OD sample blank)/(OD solvent – OD solvent blank)) x 100%. The OD sample was optical density of mint leaves active compound + solvent + bacterial suspension; OD sample blank was optical density of mint leaves active compound + solvent; OD solvent was optical density of control solvent + bacterial suspension, and OD solvent blank was optical density of control solvent.

Ethical aspects of the research were emphasized, with laboratory procedures meeting safety and ethical standards. Data analysis was conducted using SPSS, which included tests for normality, homogeneity, and One Way ANOVA. The data distribution was not normal, namely the p value≤0.05 and was not homogeneous, so it was continued with the Dunnett T3 test to compare between treatment groups. The research was carried out at the Aretha Medika Utama Laboratory in Bandung, from October to December 2023.[15]

RESULTS

Phytochemical screening of mint leaf extract was conducted to identify the active compounds present in mint leaves (*Mentha piperita L.*). The groups of active compounds and secondary metabolites tested include flavonoids, saponins, phenols, tannins, steroids, terpenoids, and alkaloids.

Table 1. Results of phytochemical screening of mint leaf extract.

No	Phytochemical examination	Reference test method	Observation	Conclusion
1	Flavonoids	Color change observed from dark yellow to orange indicates polyphenols presence.	Change to yellow color on the amyl alcohol layer.	(+++)
2	Saponins	Formation of stable foam of 1–10 cm height for at least 10 minutes indicates saponins presence. Addition of 1 N HCl does not dissipate foam.	Foam formed for a significant duration.	(++)
3	Phenols	Formation of green, red, purple, blue, or black-green colors indicates phenolic compounds presence.	Black color formation.	(++++)
4	Tannins	Orange/red color in the amyl alcohol layer.	Orange color appeared.	(+)
5	Steroids/Triterpenoids	Red or purple color indicates triterpenoids, while green indicates steroids.	Red-purple color indicated absence of steroids and presence of triterpenoids.	Steroid (-), Triterpenoid (+++)
6	Terpenoids	Purple color formation indicates terpenoid compounds presence.	Purple color formed.	(++++)
7	Alkaloids	Orange color development after Dragendorff's reagent addition indicates alkaloids presence.	Orange color appeared.	(++++)

Note: ++++: Very high content, +++: High content, ++: Moderate content, +: Low content, -: Negative content/no presence

The testing of the inhibitory effect of mint leaf extract against *S. mutans* resulted in findings from 8 treatment groups using mint leaf extract at concentrations of 3.125, 6.25, 12.5, 25, 50, and 100%. It was observed that the largest zone of inhibition diameter was at the concentration of 12.5%, indicating the microbial sensitivity level to the antimicrobial compound. The larger the diameter of the inhibition zone formed, the higher the sensitivity of the microbe to the tested antimicrobial compound.

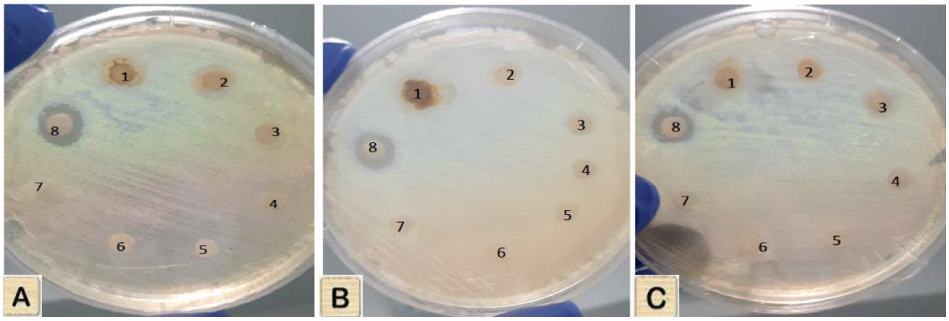

Figure 1 Observation Results of the Inhibition Zones of Mint Leaf Extract against *S. mutans* Note: Disk paper label key: (1) Mint Leaf Extract 100%; (2) Mint Leaf Extract 50%; (3)Mint Leaf Extract 25%; (4) Mint Leaf Extract 12.5%; (5) Mint Leaf Extract 6.25%; (6) Mint Leaf Extract 3.125%; (7) Growth Control; (8) Positive Control (Chlorhexidine 0.2%). This research was replicated in triplo: Fig 1A. first measurement, Fig 1B. second measurement, Fig 1C. third measurement.

Table 2. Inhibition Zone Diameters of Mint Leaf Extract Against *S. mutans*

Treatment	Inhibition Zone Diameter 1 (mm)	Inhibition Zone Diameter 2 (mm)	Inhibition Zone Diameter 3 (mm)	Average	Std Dev
100% Concentration	7.20	7.69	8.33	7.74	0.57
50% Concentration	4.87	4.12	4.96	4.65	0.46
25% Concentration	3.67	3.30	3.23	3.40	0.24
12.50% Concentration	2.35	2.17	2.75	2.42	0.30
6.25% Concentration	0.00	0.00	0.00	0.00	0.00
3.125% Concentration	0.00	0.00	0.00	0.00	0.00
Negative Control	0.00	0.00	0.00	0.00	0.00
Positive Control	10.98	10.95	11.02	10.98	0.04

Based on Table 2, it is indicated that the mint leaf extract exhibits the highest average clear zone at a 100% concentration, measuring 7.74 mm, with a minimum value of 7.20 mm and a maximum value of 8.33 mm. The smallest clear zones are observed at the concentrations of 3.125 and 6.25%, both measuring 0.00 mm, with both the minimum and maximum values being 0.00 mm. Meanwhile, the positive control of 0.2% Chlorhexidine has an average of 10.98 mm, and the negative control with aquades has an average of 0.00 mm.

The testing of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of mint leaf extract on the growth of *S. mutans* revealed that among the eight treatment groups using mint leaf extract at concentrations of 3.125, 6.25, 12.5, 25, 50, and 100%, the MIC value of the mint leaf extract against *S. mutans* was at a concentration of 12.5%, while the MBC_{90} value was at a concentration of 100%. These results were obtained by observing the turbidity levels in each test tube across the various concentrations

Table 3. Results of MIC and MBC_{90} Tests of Mint Leaf Extract Against *S. mutans*

Sample	Inhibition 1 (%)	Inhibition 2 (%)	Inhibition 3 (%)	Average
100% Concentration	94.81	94.32	93.44	94.19 (MBC_{90})
50% Concentration	81.99	79.45	80.72	80.72
25% Concentration	70.73	70.34	69.85	70.31
12.50% Concentration	57.42	60.36	46.66	54.81 (MIC)
6.25% Concentration	28.84	29.43	25.81	28.03
3.13% Concentration	4.18	5.73	4.47	4.79
Negative Control	-0.42	0.26	0.16	0.00
Positive Control	96.28	95.50	95.99	95.92

Based on Table 3, mint leaf extract can inhibit the growth of *S. mutans* starting from a concentration of 12.5%. At a concentration of 100%, it shows the highest values, almost equivalent to those of the positive control group. Meanwhile, the negative control demonstrates a lack of antibacterial effectiveness.

The data obtained from the measurement of the inhibition zone diameters were then subjected to statistical testing. In this study, the statistical tests employed were the Normality Test, Homogeneity Test, and Dunnett T3 Test. The Dunnet T3 test was used to compare between treatment groups if the data distribution was not normal, namely the p value<0.05 and was not homogeneous. Statistical analyses were performed using the IBM Statistics SPSS 25 software.

Table 4. Results of Inhibition Zone Measurements of Mint Leaf Extract Against *S. mutans*.

Treatment	Average Diameter (mm)	SD
100% Concentration	7.74 ± 0.57^{d}	7.32
50% Concentration	4.65 ± 0.46^{c}	9.92
25% Concentration	3.40 ± 0.24^{bc}	6.95
12.50% Concentration	2.42 ± 0.30^{b}	12.25
6.25% Concentration	0.00^{a}	0.00
3.125% Concentration	0.00^{a}	0.00
Negative Control	0.00^{a}	0.00
Positive Control (Chlorhexidine 0.2%)	10.98 ± 0.04^{e}	0.32

Table 5. ANOVA Test Results on Inhibition Zone

	Inhibition Zone			
	Sum of Squares	**df**	**Mean Square**	**F**
Between Groups	339.122	7	48.446	570.736
Within Groups	1.358	16	0.085	
Total	340.480	23		

Based on Table 4 and 5, the data analysis results show that all treatment groups have statistically significant differences as the p-values obtained are less than 0.05. The data presented are means ± standard deviation. The letters (a, b, bc, c, d, e) indicate significant differences according to Dunnett T3($p<0.05$). The data obtained from the measurements of percentage viability and inhibition were then subjected to statistical analysis. In this study, the statistical tests utilized were the Normality Test, Homogeneity Test, and Dunnett T3 Test. The statistical analysis was conducted using the IBM Statistics SPSS 25 software.

Table 6. Viability and inhibition of treatments against *S. mutans*.

Sample	Viability (%)	Inhibition (%)	Notes
100% Concentration	5.81 ± 0.69	94.19 ± 0.69	MBC_{90}
50% Concentration	19.28 ± 1.27	80.72 ± 1.27	
25% Concentration	29.69 ± 0.44	70.31 ± 0.44	
12.50% Concentration	45.19 ± 7.21	54.81 ± 7.21	MIC
6.25% Concentration	71.97 ± 1.94	28.03 ± 1.94	
3.13% Concentration	95.21 ± 0.83	4.79 ± 0.83	
Negative Control	100.00 ± 0.37	0.00 ± 0.37	
Positive Control (Chlorhexidine 0.2%)	4.08 ± 0.40	95.92 ± 0.40	

Table 7. ANOVA test results on viability and inhibition

		Sum of Squares	df	Mean Square	F	Sig.
Viability	Between Groups	31091.814	7	4441.688	601.760	0.001
	Within Groups	118.099	16	7.381		
	Total	**31209.912**	**23**			

Based on Table 6 and 7, the results of the data analysis indicate that all treatment groups show a statistically significant difference as the p-values obtained are less than 0.05. The data presented are mean±standard deviation. The letters (a, b, c, d, e, f) indicate a significant difference based on the Dunnett T3 ($p<0.05$.)

Table 8. Biofilm inhibitory activity of mint leaf extract

Treatment	Inhibition (%)
100% Concentration	87.39 ± 0.23 [g]
50% Concentration	70.08 ± 0.19 [f]
25% Concentration	64.34 ± 0.01 [e]
12.50% Concentration	28.21 ± 0.20 [d]
6.25% Concentration	6.60 ± 0.53 [c]
3.13% Concentration	-4.51 ± 0.29 [a]
Negative Control	0.00 ± 0.26 [b]
Positive Control (Chlorhexidine 0.2%)	91.25 ± 0.32 [g]
DMSO Control	1.09 ± 0.04 [b]

Table 9. ANOVA test results on inhibition of biofilm formation

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	38078.933	8	4759.867	64075 .583	.000
Within Groups	1.337	18	.074		
Total	**38080.270**	**26**			

Based on Table 8 and 9, the highest inhibitory test results on biofilm formation at a concentration of 100% averaged 87.39%, approaching positive control capabilities. The data presented are means ± standard deviation. The letters (a, b, c, d, e, f, g) indicate a significant difference based on the Dunnet T3 ($p<0.05$).

DISCUSSION

Phytochemical screening of mint leaf extract was conducted to identify the presence of secondary metabolites through phytochemical tests using reagents as compound detectors. This study found that the extract contained flavonoids, saponins, phenols, tannins, steroids/triterpenoids, terpenoids, and alkaloids (Table 1). This is consistent with previous research which stated that mint leaves contain flavonoids, saponins, tannins, and alkaloids.[12] However, in previous research there was no content of phenols, steroids/triterpenoids, and terpenoids. This may be caused by several aspects, namely: genetics, plant validity, growing environment, addition of growth supporting materials, harvest time, post-harvest handling, extraction technology, extract storage, extract thickening and drying technology.[17]

These active compounds disrupt the activity of the bacterium *S. mutans*. Flavonoids exert an inhibitory effect on *S. mutans* by reducing Extracellular polymeric substances (EPS) formation and stimulating *S. mutans* to release lactate dehydrogenase (LDH). Flavonoids in the mint leaf extract act as antibacterials by damaging the permeability of the cell walls of *S. mutans* and irreversibly denaturing cell proteins. Flavonoids will damage the cytoplasmic membrane, which plays a role in regulating the intake of nutrients or food substances. Bacterial metabolites will exit when the cytoplasmic membrane is damaged. Nutrients during the energy formation process cannot enter, causing the *S. mutans* cell walls not be able to grow back and leading to bacterial cell death.[15,18]

Another active compound acting as an antibacterial agent is polyphenols, which work by forming complexes with hydrolytic enzymes to disrupt cell membranes non-specifically. Polyphenols form hydrogen bonds that denature cell proteins, affecting the permeability of cell walls and cytoplasmic membranes.[19] Tannins in the mint leaf extract disrupt protein transport by activating adhesins and microbial cell enzymes, degrading bacterial cell walls by breaking down cell wall polypeptides, causing osmotic and physical pressure leading to *S. mutans* cell death.[20]

Saponins work as antibacterials by causing leakage of enzymes and proteins from within the cell, leading to cytoplasmic exit and cell death. Triterpenoids can cause damage to the plasma membrane (perforation and/or decrease in

membrane fluidity), inhibition of nucleic acid synthesis (topoisomerase inhibition), and inhibition of energy metabolism (caused by inhibition of NADH-c reductase and cytochrome) of *S. mutans*.[21]

The results of the research on the inhibitory power of mint leaf extract against *S. mutans* showed that the mint leaf extract could inhibit *S. mutans* with the largest inhibition zone diameter at a 100% concentration of 7.74 mm. The higher the extract concentration used, the larger the diameter of the inhibition zone produced. This occurred because the content of compounds in the 100% mint leaf extract was more abundant, thus greater in inhibiting the growth of *S. mutans*.... This is in line with research by Zahari et al., which stated that the larger the extract concentration, the larger the inhibition zone formed.[22-23]

The testing of the inhibitory effect of mint leaf extract against Streptococcus mutans resulted in the largest zone of inhibition diameter at the concentration of 12.5% (Figure 1) and the highest average clear zone at a 100% concentration (Table 2 and 4), indicating the microbial sensitivity level to the antimicrobial compound. A microbe's sensitivity to the antimicrobial compound under test increases with its inhibition zone's diameter. This is in line with the previous research in that the efficiency of an antibacterial compound increases with its concentration.[2,10,11]

Based on Table 3 and 5, it is shown that the Minimum Inhibitory Concentration (MIC) in this study was at a concentration of 12.5% with an average inhibition value from three repetitions of 54.81%. The Minimum Bactericidal Concentration $(MBC)_{90}$ in this study was at a concentration of 100% with an average inhibition value from three repetitions of 94.19%. Previous research by Desam N et al. stated that mint leaves can also inhibit the growth of several other bacteria besides *S. mutans*, including *Staphylococcus aureus*, *Micrococcus flavus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, and *Salmonella enteritides*.[22]

Several studies also showed that several plant extracts have MIC and MBC values against *S. mutans* with different MIC values at a concentration of 6.25% and an MBC concentration of 12.5%.[24] In the research conducted by Silva et al. using mint leaf extract in inhibiting the growth of *Staphylococcus aureus* and *Listeria monocytogenes*, it was also shown that the most effective concentration in inhibiting the growth of *S. mutans* was the highest concentration with MIC and MBC values of 3.7 and 7.43 µg/ml.[25]

The antibacterial capability of mint leaf extract is still lower compared to the positive control using chlorhexidine, but the difference between the 100% concentration and chlorhexidine as a positive control shows not significantly different results. It is also caused by mint leaf extract containing several active compounds and their activities of non sinergitic, while chlorhexidine is a single active compound.[26]

Chlorhexidine shows a high percentage of inhibition because it has the ability as antimicrobial and antifungal; chlorhexidine can also affect both aerobic and anaerobic bacteria. The negative control group using sterile water showed a very small percentage of inhibition, occurring because sterile water contains neutral compounds. The MHB medium that became clear was estimated to occur due to the presence of active compounds that act as antibacterials.[26]

Phytochemical screening on mint leaf extract was conducted to determine the presence of secondary metabolites, utilizing phytochemical tests with specific reagents for compound detection. The screening revealed flavonoids, saponins, phenols, tannins, steroids/triterpenoids, terpenoids, and alkaloids within the extract. This study corroborates previous research stating that mint leaves contain flavonoids, saponins, tannins, and alkaloids.[12] These active compounds interfere with the activities of *S. mutans* bacteria.

Flavonoids in the mint leaf extract serve as antibacterial agents by impairing bacterial cell wall permeability and irreversibly denaturing cell proteins. Flavonoids damage the bacteria's cytoplasmic membrane responsible for nutrient intake regulation. Bacterial metabolites are released when the cytoplasmic membrane is

compromised. During energy formation, nutrients cannot enter, preventing bacterial growth and resulting in bacterial cell death.[13,18]

Polyphenols, another active antibacterial agent, work by forming complexes with hydrolytic enzymes, nonspecifically disrupting cell membranes. Polyphenols form hydrogen bonds that denature cell proteins, impacting cell wall and cytoplasmic membrane permeability.[19] Tannins in mint leaf extract disrupt protein transport by activating microbial cell adhesins and enzymes, degrading bacterial cell walls and causing bacterial cell death due to osmotic and physical pressure.[20] Saponins act as antibacterials by causing enzyme and protein leakage from the cell, leading to cytoplasm exudation and cell death.[21]

The study's results on the inhibitory effects of mint leaf extract against *S. mutans* indicated that the extract can inhibit *S. mutans* with the largest inhibition zone diameter at a 100% concentration measuring 7.74 mm. The greater the extract concentration used, the larger the inhibition zone produced, due to the higher content of compounds in the 100% mint leaf extract, enhancing its growth-inhibitory capability against *S. mutans*. This is consistent with research by Zahari et al., which found that higher extract concentrations yield larger inhibition zones.[23]

While the antibacterial effectiveness of mint leaf extract is lower than that of the positive control, chlorhexidine, the difference between the 100% concentration and chlorhexidine as a positive control is not significant. Chlorhexidine exhibits a high inhibition percentage due to its antimicrobial and antifungal properties, affecting both aerobic and anaerobic bacteria. The negative control group using distilled water showed a very low percentage of inhibition because distilled water is a neutral compound. The clearing of the MHB medium is presumed to be due to the presence of antibacterial active compounds such as alkaloids, anthocyanins, tannins, saponins, and terpenoids.[26]

Antibiofilm agents with unique mechanisms to prevent biofilm formation may be considered potential strategies to address the biofilm problem. Preventing cell attachment to surfaces is the most important strategy to prevent biofilm development. From the results of this study, the highest inhibitory test results on biofilm formation at a concentration of 100% averaged 87.39%, approaching positive control capabilities (Table 6).[27]

One of the virulence factors of *S. mutans* is its ability to stick to tooth surfaces and create biofilms. Its capacity to endure in an acidic environment is accompanied by a rise in these virulence factors. Acid tolerance and production, adhesin attachment to cell surface proteins, glucosyltransferase (GTF) synthesis, which is implicated in glucan biosynthesis, and intracellular polysaccharides are among S. mutans' virulence factors.[13] The limitation of this research was that it only examined parts of the mint leaves. It is recommended to study other parts of the mint plant in future research.

CONCLUSION

Mint leaf (*Mentha piperita L.*) extract has an antibacterial and antibiofilm effect on the growth of *S. mutans*. The level of inhibition of mint leaf extract on the growth of *S. mutans* is directly proportional to its concentration. Implication of research of mint leaf (*Mentha piperita L.*) is that it is one of the best potential sources of biologically active substances to help improve oral health.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of Komisi Etik Penelitian Universitas Padjadjaran (KEP Unpad) with code No. 1275/UN6.KEP/EC/2023 for studies not involving humans or animals.

Informed Consent Statement: The research was "Not applicable" for studies not involving humans and animals.

Data Availability Statement: Data supporting reported results can be requested.

Conflicts of Interest: The authors declare no conflict of interest.

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