

Antibacterial and Antifungal Activities of the Leaf Extracts of *Mentha piperita* L.

Jeffrey,¹ Dewi Lidya Ichwana Nasution,² Myrna Nurlatifah Zakaria,³ Anisa Fitriani,⁴ Aurorin Silvia Junir⁴

¹Department of Pediatric Dentistry, Faculty of Dentistry, Jenderal Achmad Yani University, Cimahi, Indonesia

²Department of Periodontology, Faculty of Dentistry, Jenderal Achmad Yani University, Cimahi, Indonesia

³Department of Restorative Dentistry, Faculty of Dentistry, University of Malaya, Kuala Lumpur, Malaysia

⁵Faculty of Dentistry, Jenderal Achmad Yani University, Cimahi, Indonesia

Abstract

Herbal medicines are increasingly used due to the presence of bioactive compounds with antibacterial and antifungal properties. This study aimed to evaluate the antibacterial and antifungal effects of *Mentha piperita* L. extract against *Porphyromonas gingivalis* and *Candida albicans*. A laboratory-based experimental study with a post-test only control group design was conducted from September 2023 to January 2024. The test organisms were exposed to *M. piperita* L. extract at concentrations of 3.125%, 6.25%, 12.5%, 25%, 50%, and 100%. The antibacterial and antifungal activities were evaluated using the agar well diffusion method to determine inhibition zones, followed by broth dilution tests to identify the Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), and Minimum Fungicidal Concentration (MFC). Statistical analysis was performed using one-way ANOVA with a significance level of $p < 0.05$. Results showed that all concentrations inhibited the growth of both microorganisms, with MIC values of 12.5% and 25% for *P. gingivalis* and *C. albicans*, respectively. The MBC for *P. gingivalis* and MFC for *C. albicans* were both observed at 100%. There were significant differences ($p < 0.05$) in the inhibition zones across the concentrations tested. In conclusion, *Mentha piperita* L. extract demonstrates significant antibacterial and antifungal activities against *Porphyromonas gingivalis* and *Candida albicans*, suggesting its potential as a natural antimicrobial agent for oral infections.

Keywords: Antibacterial, Antifungal, *Candida albicans*, *Mentha piperita* L., *Porphyromonas gingivalis*

Introduction

The term oral microbiota, oral microbiome, and oral microflora refer to the microorganisms that inhabit the oral cavity.¹ The oral cavity and associated nasopharyngeal regions provide favorable conditions for microbial growth. The relatively stable temperature of approximately 37°C and the salivary pH range of 6.5–7 create an environment suitable for the proliferation of many microorganisms. In addition, saliva functions as a medium that supplies nutrients to microorganisms while maintaining adequate hydration for bacterial survival.²

Candida albicans is a dimorphic fungus capable of growing in two morphological forms: budding yeast cells that develop into blastospores

and pseudohyphae. Although *C. albicans* is part of the normal flora of the oral cavity, it can proliferate under certain conditions, increasing its virulence and transforming into a pathogenic organism that causes infection,³ called oral candidiasis. On the other hand, papaya leaves (*Carica papaya* L.) lead to the destruction of the supporting structures of teeth and eventual tooth loss in adults.⁴ One of the main bacteria associated with periodontitis are *Porphyromonas gingivalis*.⁵

This anaerobic, gram-negative rod-shaped bacterium is commonly detected in patients with periodontitis, gingivitis, and oral inflammatory conditions.⁶ *P. gingivalis* express several virulence factors that contribute to inflammation and tissue destruction, including lipopolysaccharide (LPS), gingipain, collagenase, lectins, capsules, proteases, superoxide dismutase, and fimbriae.⁷ LPS can trigger innate immune response by activating Toll-like receptors (TLRs), while fimbriae facilitate bacterial adhesion to host cells and other microorganisms, promoting

Corresponding Author:

Jeffrey
Department of Pediatric Dentistry, Faculty of Dentistry,
Jenderal Achmad Yani University, Cimahi, Indonesia
Email: jeffrey_dent2000@yahoo.com

This is an Open Access article licensed under the Creative Commons Attribution-NonCommercial 4.0 International License (<http://creativecommons.org/licenses/by-nc/4.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original author and source are properly cited.

biofilm formation and bacterial invasion.⁸ The *P. gingivalis* strain ATCC 33277 is widely used in research to study the pathophysiology of periodontal infections and is known for its strong biofilm-forming capacity.^{9,10}

Chlorhexidine is a broad-spectrum antiseptic commonly used in dentistry to prevent and treat oral infections, including periodontitis. It is available in various formulations such as mouth rinses, gels, and varnishes, and is considered the gold standard for chemical plaque control. Chlorhexidine acts by binding to bacterial cell walls, disrupting membrane integrity, and inhibiting essential enzymatic activities, ultimately leading to bacterial cell death. Despite its effectiveness, prolonged use may cause side effects such as tooth staining, altered taste perception, and mucosal irritation.^{11,12}

For fungal infections caused by *Candida albicans*, antifungal agents from azole groups such as ketoconazole are commonly used. Ketoconazole acts by binding to ergosterol in the fungal cell membrane, disrupting membrane structure and function. However, its use may cause side effects including headaches, diarrhea, nausea, and vomiting.¹³

Traditional herbal therapies are increasingly explored as alternative treatments because they are believed to provide therapeutic benefits with fewer side effects. Mint leaves (*Mentha piperita* L.) are widely known herbal plants used in many parts of the world. Mint is commonly incorporated into oral care products such as mouthwash, candy, toothpaste, and chewing gum. In addition to enhancing flavor and aroma, mint possesses antibacterial and antifungal properties and may support oral health. Mint leaf extracts have also been reported to exhibit antioxidant, anti-inflammatory, anti-allergic, anticarcinogenic, and antispasmodic activities.¹⁴

In laboratory studies, *Mentha piperita* extract has demonstrated antifungal activity against *Candida albicans*.¹⁵ Previous research by Wenji et al.¹⁴ reported that chloroform and methanol extracts of *Mentha piperita* leaves showed antifungal activity against *C. albicans*, with the strongest effect observed at an 80% concentration. Similarly, Audreylia et al.¹⁵ found that mint leaf extracts at concentrations of 25% and 75% inhibited germ tube formation in *C. albicans*, with a significant reduction observed at 75% compared with the control group. However, many of these studies focused primarily on antifungal activity using non-ethanolic extracts and did not evaluate antibacterial activity against oral pathogens relevant to periodontal disease.

Previous studies have also demonstrated that *Mentha piperita* exhibits antibacterial activity against several oral pathogens, including *Aggregatibacter actinomycetemcomitans*, *Staphylococcus aureus*, *Streptococcus mutans*, and *Streptococcus sanguinis*. The antimicrobial activity of mint leaves is attributed to several bioactive compounds such as alkaloids, saponins, flavonoids, tannins, and terpenoids. Alkaloids exhibit antibacterial activity by depolarizing bacterial cell membranes, intercalating with bacterial DNA, and inhibiting messenger RNA (mRNA).¹⁶ Saponins contribute to antimicrobial activity by disrupting microbial cell membranes, while flavonoids are known for their antimicrobial, antioxidant, anti-inflammatory, and antifungal properties.^{17,18} The antibacterial effect of plant extracts often increases with higher concentrations, resulting in stronger inhibition of bacterial growth.¹⁹

This study provides new insights by testing a broad range of *Mentha piperita* L. extract concentrations against both *P. gingivalis* and *C. albicans*, two clinically relevant oral pathogens. The simultaneous evaluation of antibacterial and antifungal activities, particularly at low concentrations, contributes to the growing search for natural antimicrobial alternatives. Given the increasing interest in natural antimicrobial agents, this study aimed to evaluate the antibacterial and antifungal activities of *Mentha piperita* L. leaf extract at concentrations of 3.125%, 6.25%, 12.5%, 25%, 50%, and 100% against *Porphyromonas gingivalis* and *Candida albicans*, two clinically relevant oral pathogens.

Methods

This laboratory experimental study aimed to evaluate the antibacterial and antifungal effects of mint leaves (*Mentha piperita* L.) extract on the growth of *Porphyromonas gingivalis* (ATCC 33277) and *Candida albicans* (ATCC 10231). The study used post-test only control group design and was conducted from September 2023 to January 2024. The culture media used included Potato Dextrose Agar (PDA) and Potato Dextrose Broth (PDB) media, and Mueller-Hinton agar (MHA) and (Mueller Hinton Broth) for bacterial culture media with 8 different concentration groups, including control positive with ketoconazole, chlorhexidine, control negative with PDA, PDB, MHA, and MHB, and treatment group. The subject of this research was mint leaves (*Mentha piperita* L.) and the object of this research was *Candida*

albicans ATCC 10231 and *Porphyromonas gingivalis* ATCC 33277. The inclusion criteria in this study were colonization of pure *Candida albicans* ATCC 10231 colonies grown on PDA and PDB media, pure *Porphyromonas gingivalis* ATCC 33277, which grows on Mueller–Hinton Broth and Mueller–Hinton agar media, and mint leaves (*Mentha piperita* L), which are ready to harvest at the age of 3–4 months and are not accompanied by pest contamination. The mint leaves (*Mentha piperita* L.) extraction method used the maceration technique. The exclusion criteria in this study were colonization of *Candida albicans* and *Porphyromonas gingivalis* ATCC 33277 which was contaminated with other microorganisms, young mint leaves, and mint leaves contaminated with plant pests before use.²⁰

The sample size was determined using Federer’s formula, resulting in 24 samples divided into 8 treatment groups, each with three repetitions. The independent variable was *Mentha piperita* L. leaf extract, while the dependent variables were *Candida albicans* ATCC 10231 and *Porphyromonas gingivalis* ATCC 33277.

Operational definitions and measurement tools were clearly defined and standardized. Microbial density was adjusted using the McFarland Standard, weighing was performed using an analytical balance, and colony growth was quantified with a digital colony counter.

Data analysis was conducted using SPSS software. The normality of the data distribution was assessed using the Shapiro-Wilk test, while homogeneity of variance was evaluated using Levene’s test. Differences between groups were analyzed using one-way analysis of variance (ANOVA). Data were considered normally distributed and homogeneous when $p \geq 0.05$. Post

hoc testing using Tukey’s Honestly Significant Difference (HSD) test was performed to evaluate differences in MIC and MBC values for *Candida albicans*. If the data were not homogeneous ($p < 0.05$), Dunnett’s T3 test was applied to compare the inhibitory zones among treatment groups.

The experimental procedures included preparation of mint leaf extract, phytochemical screening, antibacterial and antifungal activity testing, and determination of minimum inhibitory concentration (MIC), Minimum Fungal Concentration (MFC), and minimum bactericidal concentration (MBC) tests. All laboratory procedures were conducted in accordance with safety and ethical standards. This study received ethical approval from the Health Research Ethics Committee of Universitas Padjadjaran (NO. 1276/UN6.KEP/EC/2023).

Results

This study evaluated the phytochemical content and antimicrobial activity of mint leaf (*Mentha piperita* L.) extract against *Candida albicans* and *Porphyromonas gingivalis*. Phytochemical screening demonstrated that the mint leaf extract contained flavonoids, saponins, phenols, tannins, triterpenoids, terpenoids, and alkaloids in varying concentrations.

Among these compounds, phenols, terpenoids, and alkaloids were present in the highest amounts, followed by flavonoids and triterpenoids, which were detected at high levels. Saponins were found in moderate amounts, whereas tannins were present at relatively low levels. (Table 1)

The inhibitory test of mint leaf extract against *Candida albicans* was evaluated using

Table 1 Results of Phytochemical Screening

Phytochemical Screening	Results of Extract Analysis	
	Results of Observation	Conclusion
Flavonoids	Yellow coloration formed in the amyl alcohol layer	+++
Saponins	Stable foam formation	++
Phenols	Black coloration formed	++++
Tannins	Orange coloration formed	+
Steroids	Red–purple coloration	-
Triterpenoids	Red–purple coloration	+++
Terpenoids	Purple coloration	++++

Notes: ++++: very high content; +++ : high content; +: moderate content; -: low content; -: negative / no content

Table 2 Measurement of the Diameter of The Inhibition Zone of Mint Leaf Extract Against *Candida albicans* and *Porphyromonas gingivalis*

Treatment	Inhibition Zone Diameter (mm)			Mean	Standard Deviation (SD)	Relative Standard Deviation (RSD) (%)
	1	2	3			
<i>Candida albicans</i>						
100% concentration	8.0	7.9	8.1	8.0	0.1	1.3
50% concentration	6.0	5.1	4.0	5.0	1.0	19.3
25% concentration	4.0	3.9	4.1	4.0	0.1	3.0
12.50% concentration	3.4	3.3	2.8	3.1	0.3	10.4
6.25% concentration	0	0	0	0.0	0.0	0.0
3.13% concentration	0	0	0	0.0	0.0	0.0
Negative control	0.0	0.0	0.00	0.0	0.0	0.0
Positive control (ketokonazole 10%)	10.1	8.8	8.0	9.0	1.1	11.8
<i>Porphyromonas gingivalis</i>						
100% concentration	7.5	8.1	7.3	7.6	0.4	5.6
50% concentration	4.9	5.0	5.0	5.0	0.1	1.5
25% concentration	3.7	2.7	3.0	3.1	0.5	16.2
12.50% concentration	2.3	2.7	2.6	2.6	0.2	8.0
6.25% concentration	0.0	0.0	0.0	0.0	0.0	0.0
3.125% concentration	0.0	0.0	0.0	0.0	0.0	0.0
Negative control	0.0	0.0	0.0	0.0	0.0	0.0
Positive control (chlorhexidine 0.2%)	11.0	11.0	11.0	11.0	0.1	0.6

eight treatment groups consisting of six extract concentrations (3.125%, 6.25%, 12.5%, 25%, 50%, and 100%), a positive control (ketoconazole 10%), and a negative control. The results showed that the inhibitory activity

increased with increasing extract concentration. (Table 2.)

The highest inhibition zone against *Candida albicans* was observed at a concentration of 100% with an average diameter of 8.02

Table 3 Absorbance Value of Each Treatment

Sample	Absorbance			Mean OD	Standard Deviation (SD)	Relative Standard Deviation (RSD) (%)
	1	2	3			
100% Concentration	0.0119	0.0099	0.0109	0.0109	0.0010	9.17
50% Concentration	0.0310	0.0289	0.0300	0.0300	0.0011	3.51
25% Concentration	0.0454	0.0449	0.0459	0.0454	0.0005	1.10
12.5% Concentration	0.0672	0.0491	0.0596	0.0586	0.0091	15.50
6.25% Concentration	0.0989	0.1022	0.0897	0.0969	0.0065	6.68
3.125% Concentration	0.1185	0.1240	0.1437	0.1287	0.0133	10.29
Negative Control	0.1306	0.1139	0.1191	0.1212	0.0085	7.05
Positive Control	0.0056	0.0048	0.0062	0.0055	0.0007	12.69

Table 4 MIC and MBC Test Results of Mint Leaf Extract against *P. gingivalis*

Treatment	Inhibition (%)			Mean (%)
	1	2	3	
100%	90.2	91.8	91.0	91.0 ^a
50%	74.4	76.2	75.3	75.3
25%	62.5	63.0	62.1	62.5
12.5%	44.6	59.5	50.8	51.6 ^b
6.25%	18.4	15.7	17.7	17.3
3.125%	2.2	-2.3	-2.1	-0.7
Negative control	-7.8	6.0	1.7	0.0
Positive control	95.4	96.0	94.9	95.4

^a:Minimum Bactericidal Concentration (MBC); ^b: Minimum Inhibitory Concentration (MIC)

mm. Inhibition began to appear at a concentration of 12.5%, with an average diameter of 3.1 mm. As expected, the negative control produced no inhibition zone, whereas the positive control showed a mean inhibition zone of 9.0 mm.

The mint leaf extract demonstrated antibacterial activity against *Porphyromonas gingivalis*. The largest inhibition zone was observed at the concentration (100%) with an average diameter of 7.6 mm. No inhibition was observed at concentrations of 3.125% and 6.25%. The positive control (chlorhexidine 0.2%) showed the highest inhibitory effect with an average inhibition zone of 11.0 mm, whereas the negative control showed no antibacterial activity.

Statistical analysis of the inhibition zone data was performed using SPSS version 25. The Shapiro–Wilk test indicated that the data were normally distributed ($p > 0.05$), while Levene’s test showed that the data were not homogeneous

($p < 0.05$). Therefore, a Dunnett T3 post hoc test was applied following one-way ANOVA. The analysis revealed significant differences between treatment groups ($p < 0.001$).

The minimum inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MBC) of mint leaf extract against *Porphyromonas gingivalis* were determined by observing turbidity in broth dilution assays. The MIC was identified at a concentration of 12.5%, whereas the MBC was observed at a concentration of 100%. Bacterial growth was further evaluated by measuring optical density (OD) values using spectrophotometry, as shown in Table 3.

The highest absorbance value was observed at a concentration of 3.125% (0.1287), indicating greater bacterial growth, while the lowest absorbance was observed in the positive control (0.0055). Increasing concentrations of mint leaf extract were associated with a reduction in bacterial growth. The percentage of bacterial

Table 5 Viability and Inhibition of *Candida albicans* After Treatment

Treatment	Viability (%)	Inhibition (%)
100% concentration	11.1±1.3 ^a	88.9±1.3 ^e
50% concentration	26.4±3.5 ^b	73.6±3.5 ^d
25% concentration	32.9±3.3 ^b	67.1±3.3 ^d
12.5% concentration	66.3±1.9 ^c	33.7±1.9 ^c
6.5% concentration	93.4±0.5 ^d	6.6±0.5 ^b
3.13% concentration	136.0±0.1 ^e	-36.0±0.1 ^a
Negative control	100.0±7.5 ^d	0.0±7.5 ^b
Positive control	7.6±0.2 ^a	92.4±0.2 ^e

*data are presented as mean ± standard deviation. Different superscript letters (a–e) indicate statistically significant differences based on the post hoc test ($p < 0.05$)

Table 6 MIC and MFC Test Results of Mint Leaf Extract Against *Candida albicans*

Sample	Inhibition 1 (%)	Inhibition 2 (%)	Inhibition 3 (%)	Mean (%)
100% concentration	87.6	90.2	89.1	88.9 ^a
50% concentration	77.3	70.2	73.2	73.6
25% concentration	70.4	63.8	67.2	67.1 ^b
12.50% concentration	33.5	35.7	31.9	33.7
6.25% concentration	6.0	7.0	6.7	6.6
3.125% concentration	-35.9	-36.0	-36.1	-36.0
Negative control	7.5	-7.5	0.0	0.0
Positive control	92.3	92.7	92.4	92.4

^a:Minimum Fungicidal Concentration (MFC); ^b:Minimum Inhibitory Concentration (MIC)

inhibition was calculated using the following formula:

$$\text{Inhibition (\%)} = (\text{Control absorbance} - \text{Sample absorbance}) / \text{Control absorbance} \times 100$$

These results indicate that mint leaf extract inhibit the growth of *Porphyromonas gingivalis* at a concentration of 12.5%, while the bactericidal effect was observed at 100%.

Following the normality and homogeneity tests, an One-Way ANOVA was performed to determine differences among treatment groups. The analysis showed a significant difference among groups ($p < 0.05$). Therefore, a post hoc tukey's honestly significant difference (HSD) test was conducted to identify specific differences between treatment groups. The results are presented in Table 5.

The results presented in Table 5 indicate statistically significant differences between treatment concentrations ($p < 0.05$). Statistical analysis of MIC and MBC measurements was also performed. The normality test indicated that the data were normally distributed ($p > 0.05$), while the homogeneity test showed that the data were not homogeneous ($p < 0.05$). Therefore, a Dunnett T3 post hoc test was applied following one-way ANOVA. Statistical analysis was conducted using IBM SPSS Statistics version 25. The results showed significant differences among treatment groups ($p < 0.001$). The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of mint leaf extract against *Candida albicans* were determined using broth dilution assays. Eight treatment groups were evaluated using extract concentrations of 3.125%, 6.25%, 12.5%, 25%, 50%, and 100%.

The MIC value was observed at a concentration of 25%, while the MFC value was identified at a concentration of 100%, based on turbidity observations in the reaction tubes (Table 6).

Based on the results shown in Table 6, mint leaf extract began to inhibit the growth of *Candida albicans* at a concentration of 25%, where the inhibition rate exceeded 50%. The highest antifungal activity was observed at a concentration of 100%, which produced inhibition levels comparable to the positive control. The negative control showed no antifungal activity.

Discussion

Phytochemical analysis of the mint leaf (*Mentha piperita* L.) extract revealed the presence of several bioactive compounds, including flavonoids, saponins, phenols, tannins, terpenoids, and alkaloids were detected in the highest concentrations. The composition and quality of the mint leaf extract is influenced by plant origin, environmental factors, genetics, nutrition, plant chemotype, harvest time, harvest yield, and post-harvest handling.²¹ Previous research conducted by Rajinder Singh et al. reported that mint leaves originating from the Benghazi region of Libya exhibited antifungal activity against gram-positive and gram-negative microorganisms and demonstrated antiviral properties.²² In addition, *Mentha piperita* L. has been widely recognized for its antibacterial and antimicrobial properties.²³ Madhavan et al. reported that mint leaves from inhibited the growth of *Streptococcus mutans*, *Aggregatibacter actinomycetecomitans*, and *Candida albicans*.¹⁶

In the present study, mint leaf extract demonstrated antifungal activity against *Candida albicans*. The formation of an inhibition zone began at a concentration of 12.5% with an average diameter of 3.12 mm, while the highest diameter of the inhibition zone is a concentration of 100% with an average diameter of 8.02mm. This can be compared with previous research conducted by Wenji, which showed that the antifungal activity of (*Mentha piperita* L.) against *Candida albicans* showed that the highest inhibitory zone diameter was at the highest concentration, namely 80% with a diameter of 10 mm.¹⁴

The results of the research that has been conducted show that mint leaf extract (*Mentha piperita* L.) 12,5%, 25%, 50%, 100%, and Chlorhexidine 0.2% have an inhibitory effect on the growth of *P. gingivalis*, which is characterized by the presence of a light zone around the paper disc. Based on the research results, it was found that the inhibitory power of mint leaf extract on the growth of *P. gingivalis* had the highest average at a concentration of 100%, namely 7.6 mm and lowest average at concentrations of 3,125% and 6,25%, namely of 0.0 mm, while the positive control had an average of 10.96 mm and the negative control had an average of 0.00 mm. this research is similar to the statement from Saravani et al.²⁴ who carried out inhibitory power tests on *Mentha piperita* that the greatest antibacterial power was obtained from the highest concentration. The greater the concentration, the greater the diameter of the inhibition zone.

The results of this research show that the minimum inhibitory concentration (MIC) value in this study was a concentration of 25% with an average inhibition of 3 repetitions of 67.1%. The Minimum fungicidal concentration (MFC) value in this study was 100%, with an average inhibition value for 3 repetitions being 89.0%. The ability of mint leaf extract as an antifungal agent was still lower than that of the positive control Ketoconazole, but the difference between the 100% concentration and ketoconazole showed that the results were not significantly different. Ketoconazole can inhibit fungal growth in a broad spectrum of superficial and systemic infections. The negative control group (distilled water) contained neutral compounds, so the percentage of inhibition was negligible. In contrast to earlier studies by Wenji et al.,¹⁴ the antifungal activity of mint leaf extract (*Mentha piperita* L.) in methanol and chloroform revealed that the inhibition zone diameter of the positive

control, ketoconazole, was smaller than that of the extract, indicating that the extracts contained more potent antifungal activity.

In this study, the MIC was defined as the lowest concentration producing more than 50% inhibition (MIC₅₀), which was observed at a concentration of 12.5% for *Porphyromonas gingivalis*. The minimum bactericidal concentration (MBC) was observed at a concentration of 100%, where the inhibition rate approached 99%. Previous studies have also reported antimicrobial activity of *Mentha piperita* against various microorganisms. Madhavan et al. demonstrated inhibitory effects of mint extract against *Streptococcus mutans*, *Aggregatibacter actinomycetemcomitans*, and *Candida albicans*.¹⁶ Furthermore, Al-Mijalli et al.²³ reported that the MIC values of *M. piperita* against several bacterial species—including *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enterica*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Listeria monocytogenes*—ranged from 0.005% to 0.625%, with MBC values ranging from 0.005% to 2.5%, indicating strong bactericidal activity.

The antimicrobial activity observed in this study may be attributed to several bioactive compounds present in mint leaves, including flavonoids, saponins, tannins, terpenoids, and alkaloids. Saponins can disrupt microbial cell membranes by interacting with transmembrane proteins, reducing cell permeability and leading to impaired cellular metabolism. Tannins can interact with microbial enzymes and proteins through hydrogen bonding, resulting in cellular damage. Alkaloids exert antimicrobial effects by disrupting bacterial cell walls, intercalating with microbial DNA, and inhibiting messenger RNA (mRNA) transcription.^{17,24}

Although mint leaf extract demonstrated antibacterial activity, its inhibitory effect against *Porphyromonas gingivalis* was still lower than that of the positive control, chlorhexidine. Chlorhexidine exerts strong antibacterial activity by altering bacterial cell membrane permeability, leading to cytoplasmic leakage and cell death. In contrast, the negative control containing dimethyl sulfoxide (DMSO) did not exhibit antibacterial activity. These findings are consistent with studies by D'Ercole et al. and Tsou et al., which reported that chlorhexidine demonstrates stronger inhibitory effects against *P. gingivalis* compared with natural extracts.^{25,26}

This study was conducted under controlled laboratory condition, which may not fully represent the effectiveness of *Mentha piperita* L. under clinical conditions. Furthermore, the

study focused only on two microorganisms (*Porphyromonas gingivalis* and *Candida albicans*) which may limit the generalizability of the findings to other oral microorganisms. In addition, the use of crude ethanol extracts may result in variability in the concentration of active compounds. Future studies should aim to isolate and characterized the specific bioactive components responsible for the antimicrobial activity. Investigating the effects of these compounds on a broader range of oral microorganisms and exploring potential synergistic effects with existing antimicrobial agents may further support their application in oral healthcare.

In conclusion, *Mentha piperita* L. extract demonstrated inhibitory effects against the growth of *Porphyromonas gingivalis* and *Candida albicans*, indicating its potential as a natural antibacterial and antifungal agent for oral health applications.

References

- Gao L, Xu T, Huang G, Jiang S, Gu Y, Chen F. Oral microbiomes: increasingly important in the oral cavity and whole body. *Protein Cell*. 2018;9(5):488–500. doi:10.1007/s13238-018-0548-1
- Deo P, Deshmukh R. Oral microbiome: Unveiling the fundamentals. *J Oral Maxillofac Pathol*. 2019;23(1):122. doi:10.4103/jomfp.JOMFP_304_18
- Medawati A, Andriani I, Driana AR, Hidayati N. Activity of active compounds of papaya leaf (*carica papaya* l.) In inhibiting the growth of fungus *candida albicans* in the oral cavity. *Formosa J Sustain Res*. 2023;2(7):1717–28.
- Kang S, Dai A, Wang H, Ding PH. Interaction between autophagy and *porphyromonas gingivalis*-induced inflammation. *Front Cell Infect Microbiol*. 2022;12:1–15. doi: 10.3389/fcimb.2022.892610
- Ding Y, Ren J, Yu H, Yu W, Zhou Y. *Porphyromonas gingivalis*, a periodontitis causing bacteria, induces memory impairment and age-dependent neuroinflammation in mice. *Immun Aging*. 2018;15(1):1–8. doi: 10.1186/s12979-017-0110-7
- Fiorillo L, Cervino G, Laino L, D'Amico C, Mauceri R, Tozum TF, et al. *Porphyromonas gingivalis*, periodontal, and systemic implications: a systematic review. *Dent J*. 2019;7(4):1–15. doi: 10.3390/dj7040114.
- J. Jia, N. Han, J. Du, L. Guo, Z. Luo, and Y. Liu. Pathogenesis of important viral factors of *Porphyromonas gingivalis* via toll-like receptors. *Front Cell Infect Microbiol*. 2019;9:1–14. Doi: 10.3389/fcimb.2019.00262
- Mei F, Xie M, Huang X, Long Y, Lu X, Wang X et al. *Porphyromonas gingivalis* and its systemic impact: current status. *Pathogens*. 2020;9(11):944. doi: 10.3390/pathogens9110944
- Romero-Lastra P, Sánchez MC, Llama-Palacios A, Figuero E, Herrera D, Sanz M. Gene expression of *Porphyromonas gingivalis* ATCC 33277 in an in vitro multispecies biofilm. *Roop RM, editor. PLoS One*. 2019;14(8):1–18. doi: 10.1371/journal.pone.0221234
- Bengtsson T, Khalaf A, Khalaf H. Secreted gingipain from *Porphyromonas gingivalis* colonies exert potent immunomodulatory effects on human gingival fibroblasts. *Microbiol Res*. 2015;178:18–26. doi: 10.1016/j.micres.2015.05.008
- Bescos R, Ashworth A, Cutler C, Brookes ZL, Belfield L, Rodiles, A et al. Effects of Chlorhexidine mouthwash on the oral microbiome. *Sci Rep*. 2020;10(1):1–8. doi: 10.1038/s41598-020-61912-4
- Dumitriu AS, Păunică S, Nicolae XA, Bodnar DC, Albu Ștefan D, Suciuc I, et al. Effectiveness of Chlorhexidine in the Mechanical Treatment of Peri-Implant Mucositis. *Healthcare*. 2023;11(13):1918. doi: 10.3390/healthcare11131918
- Gow NAR, Yadav B. Microbe profile: *Candida albicans*: A shape-changing, opportunistic pathogenic fungus of humans. *Microbiol (United Kingdom)*. 2017;163(8):1145–7. doi: 10.1099/mic.0.000499
- Wenji KI, Rukmi I, Suprihadi A. In vitro antifungal activity of methanolic and chloroform mint leaves (*mentha piperita* l.) extracts against *candida albicans*. *J Phys Conf Ser*. 2019;1217(1):012136. doi: 10.1088/1742-6596/1217/1/012136
- Audreylia E. *Mentha piperita* extract is a potential antifungal agent against *Candida albicans* and *Candida krusei*. *Curr Res Environ Appl Mycol*. 2020;10(1):236–41. doi: 10.5943/CREAM/10/1/
- Jafaar HJ, Isbilen O, Volkan E, Sariyar G. Alkaloid profiling and antimicrobial activities of *Papaver glaucum* and *P. decaisnei*. *BMC Res Notes*. 2021;14(1):348. doi: 10.1186/s13104-021-05762-x
- Sharma K, Kaur R, Kumar S, Saini RK, Sharma S, Pawde SV, et al. Saponins: a

- concise review on food-related aspects, applications and health implications. *Food Chem Adv.* 2023;2:100191. doi:10.1016/j.focha.2023.100191
18. Masyita A, Sari RM, Astuti AD, Yasir B, Rumata RN, Emran TB, et al. Terpenes and terpenoids are the main bioactive compounds of essential oils; their roles in human health and potential application as natural food preservatives. *Food Chem X.* 2022;13:1–14. doi:10.1016/j.fochx.2022.100217
 19. Ekalu A, Habila JD. Flavonoids: isolation, characterization, and health benefits. *Beni-Suef Univ J Basic Appl Sci.* 2020;9(1):1–14. doi: 10.1186/s43088-020-00065-9
 20. Sugiaman VK, Jeffrey J, Widowati W, Ferdiansyah R, Novianto A. Antioxidant and Antimicrobial Potential of Sappan Wood Extract against *Porphyromonas gingivalis*. *Majalah Kedokteran Bandung.* 2024;56(2):80–7. doi: 10.15395/mkb.v56.3323.
 21. Hudz N, Kobylinska L, Pokajewicz K, Horčínová Sedláčková V, Fedin R, Voloshyn M, et al. *Mentha piperita*: essential oils and extracts, their biological activities, and perspectives on the development of new medicinal and cosmetics products. *Molecules.* 2023;28(21):7444. doi:10.3390/molecules28217444 org/10.3390/molecules28217444.
 22. Singh R, Shushni MAM, Belkheir A. Antibacterial and antioxidant activities of *Mentha piperita* L. *Arab J Chem.* 2015;8(3):322–8. doi:10.1088/1742-6596/1217/1/012136
 23. Brookes ZLS, Bescos R, Belfield LA, Ali K, Roberts A. The current use of chlorhexidine for the management of oral disease: a narrative review. *J Dent.* 2020;103:1–9. doi: 10.1016/j.jdent.2020.103497
 24. Saravani S, Ghaffari M, Valizadeh M, Ali-Malayeri F, Biabangard A. Antimicrobial activity of *Mentha piperita*, *rosmarinus officinalis*, and *Withania somnifera* Prepared by Ultrasound Against *Escherichia coli* Isolated from Poultry Stool. *Gene, Cell Tissue.* 2021;9(3)1–7. doi: 10.5812/gct.109104
 25. D'Ercole S, D'Addazio G, Di Lodovico S, Traini T, Di Giulio M, Sinjari B. The *porphyromonas gingivalis* load is balanced by 0.20% chlorhexidine gel. a randomized, double-blind, controlled, microbiological, and immunohistochemical human study. *J Clin Med.* 2020;9(1):1–16. doi: 10.3390/jcm9010284
 26. Tsou Hu, Yang Yan, and Lin. Potential oral health care agent from coffee against virulence factor of periodontitis. *Nutrients.* 2019;11(9):2235. doi: 10.3390/nu11092235