Antibacterial effectiveness test of broccoli extract (*Brassica oleracea var. italica*) against the growth of *Porphyromonas gingivalis* bacteria: laboratory experiment

Muhammad Harits Nasri[1]
Edrizal Edrizal[2*]
Kornialia Kornialia[3]

[1]**Department of Orthodontics, Faculty of Dentistry, Universitas Baiturrahmah, Indonesia**
[2]**Department of Orthodontics, Faculty of Dentistry, Universitas Baiturrahmah, Indonesia**
[3]**Undergraduate Program in Dentistry, Universitas Baiturrahmah, Indonesia**

***Corresponding Author**
Email | Edrizalburhan@yahoo.com

Submitted | 18 February 2026
Revised | 11 March 2026
Accepted | 25 April 2026
Published | 30 April 2026
DOI: 10.24198/jkg.v38i1

p-ISSN 0854-6002
e-ISSN 2549-6514

Sitasi | Edrizal E, Kornialia K, Nasri MH. Antibacterial effectiveness test of broccoli extract (Brassica oleracea var. italica) against the growth of Porphyromonas gingivalis bacteria: laboratory experiment. J. Kedokt. Gigi Univ. Padjadjaran. 2026;38(1):85-93. **DOI:** 10.24198/jkg.v38i1.

ABSTRACT

Introduction: Broccoli may serve as a potential antibacterial agent due to its bioactive compounds, including flavonoids, saponins, quinones, tannins, terpenoids, polyphenols, and glucosinolates. These compounds may contribute to antibacterial activity and could provide a natural alternative to conventional antibacterial agents. This study aimed to determine the inhibitory effect of broccoli (*Brassica oleracea var. italica*) extract on the growth of Porphyromonas gingivalis. **Methods:** This study employed a laboratory experimental design. Broccoli was extracted using the maceration method with ethanol as the solvent. Antibacterial activity was evaluated using Mueller–Hinton Agar (MHA) medium and the disk diffusion method. The treatment groups consisted of 10%, 30%, and 60% broccoli extract concentrations, with metronidazole as the positive control and dimethyl sulfoxide (DMSO) as the negative control. Antibacterial activity was determined based on the diameter of the inhibition zones. The data were analyzed descriptively and presented in tables. **Results:** The 10% broccoli extract exhibited weak antibacterial activity, with an inhibition zone of 3.8 mm. The 30% extract showed moderate antibacterial activity, with an inhibition zone of 4.75 mm, while the 60% extract demonstrated strong antibacterial activity, with an inhibition zone of 7.6 mm. The positive control, metronidazole, produced strong inhibition zones at all tested concentrations. **Conclusion:** All tested concentrations of ethanolic broccoli extract exhibited antibacterial activity against Porphyromonas gingivalis, ranging from weak to strong inhibition. However, the antibacterial activity of the broccoli extract was lower than that of metronidazole.

Keywords

Antibacterial, broccoli, Porphyromonas gingivalis

Uji daya hambat ekstrak brokoli (Brassica oleracea var. Italica) terhadap pertumbuhan Porphyromonas gingivalis: studi eksperimen

ABSTRAK

Pendahuluan: *Brokoli dapat menjadi alternatif antibakteri sebab kandungan di senyawa seperti flavonoid, saponin, kuinon, tanin, terpenoid, polifenol, serta glukosinolat. Brokoli ini berfungsi sebagai antibakteri alami tanpa menyebabkan resistensi apabila dikonsumsi secara terus-menerus. Tujuan dari penelitian untuk mengetahui daya hambat ekstrak brokoli (Brassica oleracea var. italica) terhadap pertumbuhan Porphyromonas gingivalis.* ***Metode:*** *Penelitian yang digunakan yakni eksperimental laboratoris. Teknik ekstraksi yang digunakan yaitu maserasi dengan zat pelarut etanol. Aktivitas antibakteri dengan media Mueller Hinton Agar (MHA) dievaluasi berdasarkan zona hambat memakai metode difusi cakram bersama kelompok perlakuan ekstraksi brokoli 10%, 30%, 60%, kontrol positif metronidazol, dan kontrol negatif DMSO. Uji analisis data dilakukan dengan* ***Rumus perhitungan diameter zona hambat.*** ***Hasil:*** *Zona hambat antibakteri ekstrak brokoli kategori lemah pada konsentrasi 10% (3,8 mm), konsentrasi 30% (4,75 mm), dan kategori sedang pada konsentrasi 60% (7,6 mm). Seluruh konsentrasi pada kontrol positif didapatkan zona hambat dalam kategori kuat.* ***Simpulan****: Semua konsentrasi ekstrak etanol brokoli yang diuji memiliki efek antibakteri terhadap Porphyromonas gingivalis dengan kategori ringan sampai sedang, namun efeknya belum setara dengan metronidazol.*

Kata kunci

Antibakteri, brokoli, Porphyromonas gingivalis

INTRODUCTION

According to the 2018 Basic Health Research (Riskesdas) survey, periodontal disease is the second most common oral disease in Indonesia after dental caries, accounting for 42.8% of cases.[1] Periodontal disease involves the destruction of the tissues that support the teeth, primarily due to the accumulation of bacterial plaque over time.[2] Risk factors that may increase the severity of periodontal disease include age, sex, level of knowledge, behavior, smoking habits, coffee consumption, stress levels, and systemic conditions. Periodontal disease is classified into two main types: gingivitis and periodontitis.[3]

Gingivitis is a bacterial infection confined to the gingival tissues that causes reversible damage. It is characterized by clinical signs such as gingival redness, swelling, and bleeding. If left untreated, gingivitis can progress to a more severe condition, causing damage to the tissues supporting the teeth and eventually leading to periodontitis.[4] In Indonesia, the incidence of periodontitis, a form of periodontal disease, is reported to be 74.1%. The prevalence of periodontal disease in Indonesia, particularly periodontitis, continues to increase annually and has become highly prevalent.[1]

Periodontitis is an inflammatory condition of the periodontal tissues caused by plaque accumulation at the dentogingival junction, the interface between the teeth and gingiva, where various microorganisms adhere to the surface. This condition can lead to alveolar bone destruction and progressive loss of tooth attachment. Periodontal tissue damage occurs as a result of products produced by oral microorganisms, such as endotoxins, collagenase, and hyaluronidase, which can damage the gingival epithelium and connective tissue.[1] Periodontitis is generally caused by Gram-negative bacteria associated with subgingival plaque, including *Porphyromonas gingivalis*, *Prevotella intermedia*, *Actinobacillus* (*Aggregatibacter*) *actinomycetemcomitans*, and *Fusobacterium nucleatum*.[6]

Porphyromonas gingivalis is an anaerobic Gram-negative bacterium that is highly prevalent in the gingival sulcus, subgingival plaque, tongue, and tonsils, accounting for approximately 75% compared with other bacterial species.[7] *Porphyromonas gingivalis* is considered one of the major etiological agents involved in the development and progression of periodontitis, particularly chronic periodontitis.[8] In invading periodontal tissues, *Porphyromonas gingivalis* utilizes various virulence factors to dysregulate innate immune and inflammatory responses. The virulence factors produced by *Porphyromonas gingivalis* include fimbriae, capsules, polysaccharides, lipopolysaccharides, and hemolysins, which contribute to its pathogenicity in the oral cavity.[9] Research by Zhang indicates that *Porphyromonas gingivalis* can also invade osteoblasts in the alveolar bone, resulting in a reduction in osteoblast numbers, an increase in osteoclast numbers, and subsequent bone resorption.[10]

Standard treatment for periodontal disease involves an initial therapy phase that includes oral hygiene education, plaque control through procedures such as scaling and root planing, and antibiotic administration.[11,12] Metronidazole is commonly used in the treatment of periodontitis because of its effectiveness against the bacterial pathogens responsible for the disease.[13] However, prolonged antibiotic use can contribute to bacterial resistance; therefore, herbal medicine therapy represents a potential alternative approach. Herbal therapy using medicinal plants may provide pharmacological benefits with relatively few side effects.[14]

As a tropical country rich in biodiversity, Indonesia is home to a wide variety of medicinal plants, including more than 2,039 species identified, such as broccoli.[15] Broccoli, scientifically known as *Brassica oleracea* var. *italica*, is a vegetable belonging to the family *Brassicaceae*. It is a variety of cauliflower characterized by green flower heads and tender stalks. The plant originated in Europe, with historical evidence of its presence

in Cyprus, southern Italy, and the Mediterranean region dating back approximately 2,000 years. Broccoli was introduced to Indonesia in the 1970s and can thrive in subtropical environments. It adapts well to highland areas with relatively low humidity.[16] The optimal temperature for broccoli cultivation is below 23°C; however, broccoli quality may decline when temperatures are suboptimal during the flowering stage.[17] West Sumatra Province contains highland areas with suitable temperatures for broccoli cultivation, such as Nagari Padang Laweh in Agam Regency, where temperatures range from approximately 20°C to 30°C. These environmental conditions have encouraged local communities to cultivate subtropical crops such as broccoli.[15]

Broccoli is a vegetable that can be consumed either cooked or raw.[18] It offers various health benefits due to its antioxidant, anticancer, antimicrobial, anti-inflammatory, and antihypertensive properties.[19] These beneficial properties may help address various health conditions, including cardiovascular disease, hypercholesterolemia, and diabetes.[20] Broccoli also exhibits antibacterial activity, which is attributed to the presence of bioactive compounds such as flavonoids, saponins, quinones, tannins, terpenoids, polyphenols, and glucosinolates.[15,21,22]

Broccoli extract has been tested against various bacterial species. A 2019 study by Auliya demonstrated that broccoli extract was effective against *Streptococcus mutans* at concentrations of 10%, 30%, 50%, 70%, and 90%, producing mean inhibition zones of 1.42 mm, 2.37 mm, 5.83 mm, 8.92 mm, and 11.07 mm, respectively.[15] A study conducted by Jaiswal et al. in 2011 showed that broccoli extract at concentrations of 0.09%, 0.17%, 0.35%, 0.7%, 1.4%, and 2.8% exhibited inhibitory activity against *L. monocytogenes*, *S. abony*, *E. faecalis*, and *P. aeruginosa*.[23] Other research has also indicated that fermented broccoli residue at a concentration of 25% can inhibit the growth of *Candida albicans* and various other pathogenic bacteria.[24]

However, most previous studies have focused on evaluating the antibacterial activity of broccoli against bacteria associated with dental caries, such as *Streptococcus mutans*, leaving a research gap regarding its effectiveness against periodontal pathogens. To address this gap, the novelty of the present study lies in evaluating the specific inhibitory effect of broccoli extract on the growth of the Gram-negative bacterium *Porphyromonas gingivalis*.[24] The foregoing discussion indicates that broccoli offers various health benefits. Accordingly, this study aimed to determine the inhibitory effect of broccoli (*Brassica oleracea* var. *italica*) extract on the growth of *Porphyromonas gingivalis.*

METHODS

This study was an *in vitro* laboratory experiment conducted using *Porphyromonas gingivalis* ATCC 33277, obtained from the Microbiology Laboratory Unit (UPT) at Andalas University. The test material was an ethanolic extract of broccoli cultivated in Nagari Padang Laweh, Agam Regency. A post-test-only control group design was employed, with the concentration of broccoli extract serving as the manipulated factor. To ensure the reliability and internal validity of the results, independent replications were performed for each treatment and control group, with five repetitions per group. Based on Federer's formula, the study consisted of 25 experimental units. The experimental treatments were randomly assigned to the treatment and control groups to minimize potential bias.

The study used broccoli florets. Botanical identification conducted at the Andalas University Herbarium confirmed the plant species as *Brassica oleracea* var. *italica*, belonging to the family Brassicaceae. A total of 20 kg of broccoli florets was sorted, thoroughly washed, dried, and re-sorted. The dried material was then finely ground and weighed, yielding 2 kg of powdered simplicia (dried plant material).

The extraction was performed using the maceration method. A total of 2 kg of the powdered *simplisia* was mixed with ten parts of distilled ethanol as the solvent in dark-colored glass containers. The mixture was stirred occasionally during the first 6 hours and then left to stand for 2 days before being filtered. The maceration process was repeated twice using the same powder-to-solvent ratio. All resulting macerates were collected and concentrated using a rotary evaporator until a viscous broccoli extract was obtained.

The samples in this study were divided into three treatment groups, one positive control group, and one negative control group. The three treatment groups received ethanolic broccoli extract at concentrations of 10%, 30%, and 60%. The positive control group received metronidazole, while the negative control group received dimethyl sulfoxide (DMSO). The positive control used metronidazole at a dose of 250 mg.

All instruments used in the study were prepared in clean and dry conditions. Heat-resistant glassware was wrapped and sterilized in an autoclave at 121°C and 1 atm pressure for 15 minutes. Metal instruments, such as inoculating loops and tweezers, were sterilized by flaming over a spirit lamp. The laminar airflow (LAF) cabinet was sterilized by spraying it with 70% alcohol and exposing it to ultraviolet (UV) light for 1 hour.

Porphyromonas gingivalis colonies grown on the culture medium were collected using an inoculating loop and transferred to Mueller-Hinton Agar (MHA) medium. The MHA medium was prepared by dissolving the required amount of MHA powder in 100 mL of distilled water in an Erlenmeyer flask. The medium was heated and stirred aseptically near a Bunsen burner until the MHA powder was completely dissolved and no visible particles remained. The prepared medium was then covered with paper and sterilized by autoclaving before being used for bacterial cultivation. The bacterial culture was incubated at 37°C for 24 hours.

The bacterial culture was incubated under anaerobic conditions by placing the culture medium in an airtight container or anaerobic chamber to create an oxygen-free environment suitable for the growth of anaerobic bacteria. After rejuvenation, 2–3 loops of the bacterial culture were transferred into a tube containing 5 mL of sterile 0.9% NaCl solution. The suspension was mixed thoroughly until homogeneous, and its turbidity was compared with a 0.5 McFarland standard.[25]

Antibacterial activity was evaluated using the disk diffusion method. A total of 0.1 mL of the bacterial suspension was inoculated onto the surface of MHA medium and spread evenly over the entire agar surface using a sterile cotton swab. The tested ethanolic broccoli extract was prepared at concentrations of 10%, 30%, and 60% using dimethyl sulfoxide (DMSO) as the solvent. The prepared extract was applied to sterile paper disks and placed on the inoculated MHA surface. The plates were then incubated at 37°C for 24 hours under appropriate anaerobic conditions.

The positive control consisted of metronidazole at a dose of 250 mg, while the negative control consisted of dimethyl sulfoxide (DMSO). After incubation, the Petri dishes were examined for the formation of clear zones around the paper disks. The presence of a clear zone surrounding the disk indicated antibacterial activity. The diameter of each inhibition zone was measured using a caliper.[26] Data were analyzed manually using a descriptive method and presented in tabular form. The results of the inhibition-zone measurements were documented and tabulated using Microsoft Word. The formula used to calculate the inhibition zone is presented in Figure 1.[26]

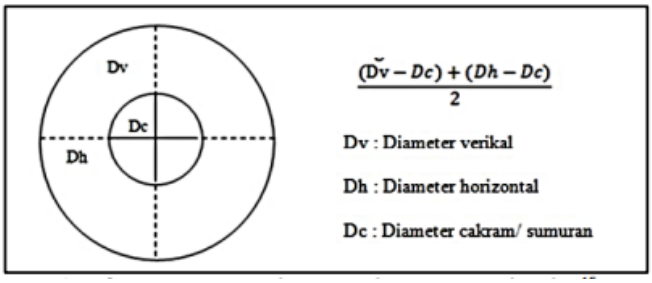


Figure 1. Formula for calculating the inhibition zone diameter [26]

RESULTS

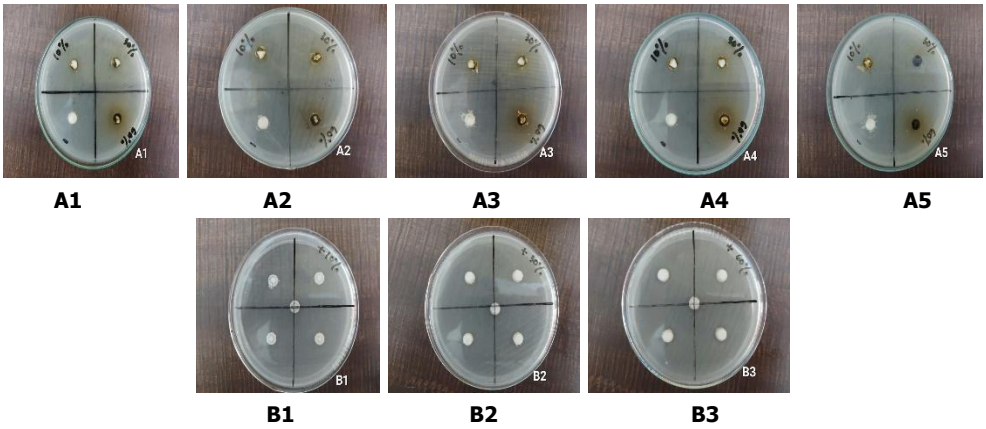
The study was conducted using 5 samples and 25 treatment groups. The results are presented in Table 1 and Figure 1.

Table 1. Mean inhibition zone (mm) for each study group

Treatment	Replication					Mean (mm)	Category
	1 (mm)		2 (mm)	3 (mm)	4 (mm)	5 (mm)	
10%	4	3.75	3.75	4	3.5	3.8	Weak
30%	4.75	4.75	4.5	4.75	5	4.75	Weak
60%	8	7.5	7	7.5	8	7.6	Moderate
Metronidazole (Positive Control + 10%)	11	11	11.75	11.5	11.75	11.4	Strong
Metronidazole (Positive Control + 30%)	12.25	12	12	11.75	12	12	Strong
Metronidazole (Positive Control + 60%)	14.75	15	14.75	15	12.55	14.41	Strong
DMSO (Negative Control −)	0	0	0	0	0	0	None

Table 1 presents the mean inhibition zone diameters against *Porphyromonas gingivalis* ATCC 33277 following treatment with broccoli (*Brassica oleracea* var. *italica*) extract. The 60% concentration produced the largest mean inhibition zone, measuring 7.6 mm, which was categorized as moderate, whereas the smallest inhibition zone, measuring 3.8 mm, was observed at the 10% concentration. For the positive control (metronidazole), the largest inhibition zone diameter was 14.41 mm, categorized as strong, at the 60% concentration. In comparison, the smallest inhibition zone diameter was 11.4 mm, also categorized as strong, at the 10% concentration. In contrast, the negative control (DMSO) showed no antibacterial activity against the tested bacteria.

In this study, seven experimental units were randomly assigned to five treatment groups: a negative control (DMSO), a positive control (metronidazole), and three broccoli extract concentrations (10%, 30%, and 60%). Following randomization and treatment application, three units from the positive control group (metronidazole) were excluded because no inhibition zones were observed, although inhibition was expected. This indicated a possible problem with the disks or a technical error during inoculation. Therefore, the analysis of these three units was repeated. Data analysis was performed by measuring the diameter of the inhibition zones.



Legend: (A) Inhibition zones at concentrations of 10%, 30%, and 60%, including the negative control: Figure A1, antibacterial test sample, replicate 1; Figure A2, antibacterial test sample, replicate 2; Figure A3, antibacterial test sample, replicate 3; Figure A4, antibacterial test sample, replicate 4; Figure A5, antibacterial test sample, replicate 5. (B) Inhibition zones for the positive control: Figure B1, positive control test sample at a 10% concentration; Figure B2, positive control test sample at a 30% concentration; Figure B3, positive control test sample at a 60% concentration.

Figure 1. Results of the Antibacterial Effectiveness Test

DISCUSSION

This study evaluated the antibacterial efficacy of broccoli extract (*Brassica oleracea* var. *italica*) against the growth of *Porphyromonas gingivalis* using extract concentrations of 10%, 30%, and 60%, with metronidazole as the positive control and DMSO as the negative control. The results showed that the 60% broccoli extract concentration produced a greater inhibitory effect against *P. gingivalis* than the 10% and 30% concentrations, with an inhibition zone diameter of 7.6 mm. These findings were consistent with those of a previous study by Auliya et al.,[15] which investigated the antibacterial activity of broccoli extract against *Streptococcus mutans* at concentrations of 10%, 30%, 50%, 70%, and 90%. Inhibition was indicated by the formation of a clear zone surrounding the paper disk impregnated with broccoli extract. The diameter of the resulting inhibition zone was measured in millimeters (mm) using a caliper, and the measurements were used to determine the inhibition-zone diameter.

The mean inhibition zone diameters produced by the different concentrations of broccoli extract were compared with those of the positive control (metronidazole), which produced the largest mean inhibition zone of 14.41 mm. These findings differed from those reported by Auliya et al.,[15] who reported a larger mean inhibition zone of 25.07 mm for the antibiotic control. Although the comparison indicated that the broccoli extract concentrations were less effective than metronidazole, the present findings demonstrated that all three concentrations of broccoli extract exhibited antibacterial activity against *Porphyromonas gingivalis*.

The inhibition of *Porphyromonas gingivalis* growth observed in this study was particularly important given the bacterium's role as a major pathogen in the development of periodontal disease. Previous research by Auliya et al.,[15] on the antibacterial activity of broccoli extract (*Brassica oleracea* var. *italica*) against *Streptococcus mutans* at concentrations of 10%, 30%, 50%, 70%, and 90% reported mean inhibition zones of 1.42 mm, 2.37 mm, 5.83 mm, 8.92 mm, and 11.07 mm, respectively.[15] Another study conducted by Sari and Basyarahil evaluated the inhibition zones produced by broccoli extract (*Brassica oleracea* var. *italica*) against *Staphylococcus aureus* at concentrations of 10%, 30%, and 50%, and reported mean inhibition zones of 2.8 mm, 6.61 mm, and 10.41 mm, respectively. These findings further support the

antibacterial potential of broccoli extract and suggest that higher extract concentrations may produce greater inhibitory effects against bacterial growth.[15]

These results indicated that increasing the extract concentration was directly associated with an increase in the mean diameter of the inhibition zone against *Porphyromonas gingivalis*. Conversely, decreasing the extract concentration resulted in a decrease in the mean diameter of the inhibition zone against the same bacterium. Based on the findings of this study, the researchers considered potential sources of bias that could have limited the external validity of the study, including the characteristics of the broccoli samples. Some samples had been exposed to excessive sunlight, which may have affected their quality and rendered them less suitable for use in antibacterial testing.

The antibacterial activity of broccoli was attributed to its bioactive compounds, including flavonoids, quinones, tannins, saponins, terpenoids, polyphenols, and glucosinolates. Flavonoids are antibacterial compounds that can cause protein denaturation and enzyme inactivation in bacterial cells. Quinones are organic compounds that can bind to proteins and form complexes with amino acids, thereby interfering with bacterial metabolism.[15,21] Tannins act as growth-inhibitory compounds by inhibiting extracellular enzymes in bacteria, resulting in enzyme inactivation.[7] Saponins exert antibacterial activity by disrupting bacterial cell membranes, increasing membrane permeability, and causing leakage of intracellular components.[28]

The positive control consisted of 250 mg of metronidazole, a nitroimidazole compound with bactericidal activity against anaerobic organisms. Metronidazole disrupts bacterial DNA synthesis under conditions of low redox potential. It is effective against anaerobic bacteria, including *Porphyromonas gingivalis* and *Prevotella intermedia*. Clinically, metronidazole has been used to treat gingivitis, acute necrotizing ulcerative gingivitis, chronic periodontitis, and aggressive periodontitis. It has been administered as a stand-alone therapy or in combination with root planing, periodontal surgery, or other antibiotics.[29]

The negative control consisted of dimethyl sulfoxide (DMSO). No inhibition zone was observed around the paper disk containing DMSO, indicating that DMSO did not exhibit antibacterial activity under the conditions of this study. DMSO is an organic solvent commonly used to dissolve both polar and nonpolar compounds and was selected as the negative control because it was not expected to interfere with the bacterial activity assay. The absence of an inhibition zone around the DMSO disk confirmed that the antibacterial activity observed in the treatment groups was attributable to the tested broccoli extract rather than to the solvent.[26]

This study was conducted entirely under controlled laboratory conditions (*in vitro*). Therefore, the findings cannot be directly extrapolated to *in vivo* conditions, in which the extract would be administered within a living organism. The *in vivo* environment is considerably more complex. It involves interactions with the immune system and normal microbiota, as well as variations in pH, nutrient availability, and other physiological factors that may influence the extract's antibacterial activity. Concentrations that are effective *in vitro* may not be achieved or maintained *in vivo*, and the extract may interact with other biological components, potentially altering its antibacterial effects.

Although the experimental treatments were randomized and aseptic procedures were implemented, several potential sources of bias and measurement error remained. These included variations in the quality and composition of the broccoli extract, which could have affected the consistency of its antibacterial activity, as well as potential variability in the manual measurement of inhibition zone diameters. The latter may have introduced inter-observer or intra-observer variability because the measurements relied on visual assessment. Furthermore, microenvironmental conditions within the Petri dishes, including temperature and atmospheric conditions, as well as subtle differences

in the agar medium and bacterial inoculum distribution, may have contributed to minor variations among replicates and consequently introduced random experimental error.

CONCLUSION

Broccoli extract (*Brassica oleracea* var. *italica*) at concentrations of 10%, 30%, and 60% exhibited antibacterial activity against *Porphyromonas gingivalis*. However, its antibacterial effectiveness remained lower than that of the positive control, metronidazole. The 60% concentration produced the largest inhibition zone, with a diameter of 7.6 mm. These findings demonstrate the potential of broccoli extract as a natural antibacterial agent against *Porphyromonas gingivalis. They may* contribute to the development of alternative approaches for the prevention and management of periodontal disease. The results also indicated a positive association between broccoli extract concentration and inhibition zone diameter, with higher concentrations producing greater antibacterial activity against *Porphyromonas gingivalis*. Therefore, broccoli extract shows potential for further development as a natural antibacterial agent for preventing periodontitis; however, further research and formulation optimization are required to achieve antibacterial efficacy comparable to that of metronidazole.

Author Contributions: Conceptualization, E.E.; M.H.N.; and K.K.; methodology, E.E.; M.H.N.; and K.K.; software, E.E.; M.H.N.; and K.K.; validation, E.E.; M.H.N.; and K.K.; formal analysis, E.E.; M.H.N.; and K.K.; investigation, E.E.; M.H.N.; and K.K.; resources, E.E.; M.H.N.; and K.K.; data curation, E.E.; M.H.N.; and K.K.; writing-original draft preparation, E.E.; M.H.N.; and K.K.; writing-review and editing, E.E.; M.H.N.; and K.K.; visualization, E.E.; M.H.N.; and K.K.; supervision, E.E.; M.H.N.; and K.K.; project administration, E.E.; M.H.N.; and K.K.; funding acquisition, E.E.; M.H.N.; and K.K. All authors have read and agreed to the published version of the manuscript.
Funding: This research received no external funding.
Ethical Approval: This study was an in vitro study and did not require ethical approval**.**
Informed Consent Statement: Informed consent was not applicable because this study was conducted entirely in vitro and did not involve human participants.
Data Availability Statement: The data obtained from the in vitro laboratory research were used in this study.
Conflicts of Interest: The authors declare no conflict of interest.

REFERENCES

1. Kementerian Kesehatan Republik Indonesia. Laporan nasional Riset Kesehatan Dasar (Riskesdas) 2018. Jakarta: Badan Penelitian dan Pengembangan Kesehatan; 2019. P. 154–165. Available from: http://www.yankes.kemkes.go.id/assets/downloads/PMK%20No.%2057%20Tahun%202013%20tentang%20PTRM.pdf
2. Surya LS, Setiawan, Besral. Hubungan faktor lokal, faktor sistemik dan faktor perilaku terhadap kejadian penyakit periodontal di Indonesia (analisis Riskesdas). Makassar Dent J. 2019;8(2):57–66.
3. Rohmawati N, Santik YDP. Status penyakit periodontal pada pria perokok dewasa. HIGEIA. 2019;3(2). https://doi.org/10.15294/higeia.v3i2.25497
4. Sari DR, Lestari C, Yandi S. Pengaruh pemberian asam usnat terhadap jumlah sel osteoblas pada tikus periodontitis. J B-Dent. 2018;5(2):124–34.
5. Marcano R, Rojo MÁ, Córdoba-Díaz D, Garrosa M. Pathological and therapeutic approach to endotoxin-secreting bacteria involved in periodontal disease. Toxins (Basel). 2021;13(8):533. https://doi.org/10.3390/toxins13080533
6. Carranza FA, Newman MG, Takei HH, Klokkevold PR. Carranza's clinical periodontology. 12th ed. St. Louis: Elsevier; 2015. P. 142.
7. Septiyani RI. Efektivitas ekstrak daun kelor (Moringa oleifera L.) dalam menghambat pertumbuhan bakteri Porphyromonas gingivalis [skripsi]. Semarang: Universitas Negeri Semarang; 2019.
8. Pujiastuti P, Lestari S. Perbedaan efektifitas antibakteri ekstrak daun sirih merah (Piper crocatum) pada Porphyromonas gingivalis dan Streptococcus viridans. Stomatognatic J Kedokt Gigi. 2015;12(1).
9. How KY, Song KP, Chan KG. Porphyromonas gingivalis: an overview of periodontopathic pathogen below the gum line. Front Microbiol. 2016;7:53. https://doi.org/10.3389/fmicb.2016.00053
10. Marcano R, Rojo MÁ, Córdoba-Díaz D, Garrosa M. Pathological and therapeutic approach to endotoxin-secreting bacteria involvedv in periodontal disease. Toxins (Basel). 2021;13(8):533. https://doi.org/10.3390/toxins13080533
11. Tedjasulaksana R. Metronidazol sebagai salah satu obat pilihan untuk periodontitis marginalis. J Kesehat Gigi. 2016;4(1):19–23.
12. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: a review. J Pharm Anal. 2016;6(2):71–79.

https://doi.org/10.1016/j.jpha.2015.11.005
13. Adha N, Ervina I, Agusnar H. The effectiveness of metronidazole gel based chitosan inhibits the growth of bacteria Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Fusobacterium nucleatum (in vitro). Int J Appl Dent Sci. 2017;3:33–37.
14. Abdelmagyd HA, Shetty DS, Al-Ahmari DMM. Herbal medicine as adjunct in periodontal therapies: a review of clinical trials in past decade. J Oral Biol Craniofac Res. 2019;9(3):212–217. https://doi.org/10.1016/j.jobcr.2019.05.001
15. Auliya S, Elianora D, Kornialia. Uji aktivitas antibakteri ekstrak brokoli (Brassica oleracea var. italica) terhadap Streptococcus mutans. Padjadjaran J Dent Res Stud. 2019;3(2):92–99.
16. Sedayu OL. Budidaya brokoli (Brassica oleracea var. botrytis L.) secara organik di Soga Farm Jawa Tengah [diploma thesis]. Lampung: Politeknik Negeri Lampung; 2022. Available from: http://repository.polinela.ac.id/3476/
17. Raleni N, Defiani M, Astarini I. Pertumbuhan vegetatif dan produktivitas berbagai kultivar brokoli (Brassica oleracea L. var. italica Plenck.). Metamorfosa. 2015;2(2):90–97.
18. Saputri GAR, Afrila AP. Penetapan kadar kalsium pada brokoli (Brassica oleracea L.) segar, kukus, dan rebus secara spektrofotometri serapan atom. J Analis Farmasi. 2017;2(4):251–257.
19. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: a review. J Pharm Anal. 2016;6(2):71–79. https://doi.org/10.1016/j.jpha.2015.11.005
20. Gudiño I, Martín A, Casquete R, Prieto MH, Ayuso MC, Córdoba MG. Evaluation of broccoli (Brassica oleracea var. italica) crop by-products as sources of bioactive compounds. Sci Hortic. 2022;304:111284. https://doi.org/10.1016/j.scienta.2022.111284
21. Egra S, et al. Aktivitas antimikroba ekstrak bakau (Rhizophora mucronata) terhadap Ralstonia solanacearum. Agrovigor. 2019;12(1). https://doi.org/10.21107/agrovigor.v12i1.5143
22. Hanani E. Analisis fitokimia. Jakarta: EGC; 2016.
23. Putri DA, Widodo HB, Ichsyani M, Naufalin R, Oedjijono. The Activities of Torch Ginger Flower (*Etlingera elatior*) Ethanol Extract on Degradation of *Porphyromonas gingivalis* Biofilm as Periodontal Pathogen. In: *Journal of Indonesian Dental Association*, 2023;6(1), S. 31–38. https://doi.org/10.32793/jida.v6i1.882
24. Liu N, Deng XJ, Liang CY, Cai HY. Fermented broccoli residue reduced harmful bacterial loads. J Appl Poult Res. 2018;27:590–596. https://doi.org/10.3382/japr/pfy032
25. Mutia SM, Annisa SE. Anti-bacterial activity of ethanol extract of Indian borage leaves. Healthy Tadulako J. 2021;7(1):30–34.
26. Tani PG, Womor PM, Khoman JA. Uji daya hambat daging buah sirsak terhadap Porphyromonas gingivalis. Farmakon. 2017;6(3). https://doi.org/10.35799/pha.6.2017.16597
27. Jannah M. Uji toksisitas antibakteri ekstrak Hydrilla verticillata [skripsi]. Malang: UIN Maliki; 2020.
28. Masyita A, Sari RM, Astuti AD, Yasir B, Rumata N, Emran TB, et al. Terpenes and terpenoids as main bioactive compounds of essential oils, their roles in human health and potential application as natural food preservatives. Food Chem X. 2022 Jan 19;13:100217. https://doi.org/10.1016/j.fochx.2022.100217
29. Tedjasulaksana R. Metronidazol sebagai salah satu obat pilihan untuk periodontitis marginalis. J Kesehat Gigi. 2016;4(1):19–23.
30. Santoso B, Imaduddin F, Sukanto H, Triyono J, Hidayat RLLG, Widodo PJ, et al. Procurement and operation technical for meniran extraction equipment. Mekanika. 2021;20(1). https://doi.org/10.20961/mekanika.v20i1.45487
31. Mariscal FMG, Arenas-de Larriva AP, Limia-Perez L, et al. Coenzyme Q10 supplementation for oxidative stress reduction. Int J Mol Sci. 2020;21(21):7870. https://doi.org/10.3390/ijms21217870