ORIGINAL ARTICLE

Antibacterial Effect of Halia Bentong Ethanolic Extract on *Porphyromonas gingivalis*: A Preliminary Investigation

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ABSTRACT

Introduction: Plant constituents offer therapeutic benefits with fewer adverse effects than conventional drugs. Halia Bentong (*Zingiber officinale* Roscoe), a rhizomatous herb, is commonly used as a spice. Its medicinal properties help treat various ailments and produce oral hygiene products. While previous studies have shown Halia Bentong's antibacterial properties, its effectiveness against *Porphyromonas gingivalis* has yet to be explored. This preliminary study evaluates the antibacterial activity of Halia Bentong ethanolic extract against *P. gingivalis*. **Materials and Methods:** Halia Bentong was oven-dried, extracted with absolute ethanol, and prepared at concentrations ranging from 100 to 0.8 mg/mL. Moisture content, extraction yield, and total phenolic content were measured. Growth inhibition, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) were determined via broth microdilution. Anti-adhesion activity was assessed by co-culturing the extract with *P. gingivalis* for 48 hours, followed by crystal violet staining. Scanning electron microscopy (SEM) evaluated the effects on *P. gingivalis* structure. **Results:** Halia Bentong recorded a moisture content of 84.5% and an extraction yield of 10%. The total phenolic content was 42.33 ± 2.41 mg GAE/g. The MIC and MBC of Halia Bentong ethanolic extract were 1.625 mg/mL. *P. gingivalis* growth was inhibited by the extract in a concentration-dependent manner, while anti-adhesion activity was inversely dependent on concentration. SEM showed surface changes and reduced cell numbers in treated compared to untreated samples. **Conclusion**: These preliminary findings suggest that Halia Bentong ethanolic extract has antibacterial activity against *P. gingivalis*, warranting further investigation into its potential against periodontopathogens.

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Keywords: Halia Bentong, *Zingiber officinale*, Ethanolic extract, *Porphyromonas gingivalis*, Antibacterial

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INTRODUCTION

Periodontal disease is an oral disease that induces inflammation of the periodontium, which consists of gingiva, alveolar bone, periodontal ligament, and cementum. The National Oral Health Survey of Adults 2020 (NOHSA 2020) reported that the prevalence of periodontitis among adult Malaysians was 38.2% (1). It is a significant public health problem due to its high prevalence and ability to result in tooth loss. It can negatively affect chewing function and aesthetics, impairing quality of life. There is growing evidence that periodontal disease is a potential risk factor for systemic conditions, such as cardiovascular diseases, diabetes mellitus (2), Alzheimer's disease (3), rheumatoid arthritis (4), and preterm/low birth weight (5). The potential mechanisms may be attributed to the existence of periodontal pathogens and their metabolic byproducts, which affect the immune response outside the oral cavity, thus contributing to the development of comorbidities (6).

Periodontal disease is initiated by a synergistic polymicrobial community in which different microbial members fulfil distinct roles that culminate in oral microbial dysbiosis (7). Dysbiosis or imbalance in the microbial

ecosystem within the sub-gingival plaque will disrupt the host's innate, inflammatory, and adaptive immune responses. These responses increase the production of cytokines and chemokines by the gingival epithelium, leading to the expression of adhesion molecules. The molecules increase the permeability of gingival capillaries and the chemotaxis of polymorphonuclear neutrophils via the junctional epithelium into the gingival sulcus. Eventually, a periodontal pocket will be formed due to the loss of supporting connective tissue, alveolar bone, and subsequent tooth mobility and tooth loss. The pathogenic microorganisms commonly associated with periodontal disease are Gram-negative anaerobes. These include *Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum, Tannerella forsythia, Treponema denticola, Eikenella corrodens, and Porphyromonas gingivalis* (8).

In the formation of a dysbiotic oral microbial ecosystem, *Porphyromonas gingivalis* has been described as the keystone pathogen of periodontal disease (9), and its presence is associated with the clinical presentation of chronic periodontitis (10). *P. gingivalis* possesses virulence factors such as gingipains, lipopolysaccharide, capsule, and fimbriae that assist in the colonisation of bacteria, invasion of the host cells, evasion of the host immune system, and extraction of nutrients from the host (11). *P. gingivalis* clinical isolates have demonstrated in vitro resistance to clindamycin, metronidazole, amoxicillin, and azithromycin (12, 13). Since antibiotic resistance by pathogenic bacteria remains a grave concern in today's medicine, there is an urgent need to find a novel anti-infective drug to ensure effective therapy.

For decades, the medical and dental fields have widely accepted herbal medicines as alternative treatments. They comprise plant constituents that have therapeutic benefits with fewer side effects, natural activity, advanced safety margin, and low cost as compared to conventional drugs (14). The growing resistance of pathogens to widely used therapeutic substances, including antibiotics and antiviral drugs, has sparked a renewed interest in finding new anti-infective compounds. Previous studies have reported the use of several plants in dentistry, such as clove (*Syzygium aromaticum*) (15), cinnamon (*Cinnamomum zeylanicum*) (16), and miswak (*Salvadora persica*) (17). Several herbal formulations containing clove, cinnamon, and miswak are already available in the market for oral use due to their antibacterial and anti-inflammatory properties (15-17). Ongoing research continues to support the therapeutic potential of plant-derived compounds in dentistry, reinforcing their emerging role in modern oral healthcare.

Ginger, or its scientific name, *Zingiber officinale* Roscoe, is a well-known herbaceous plant that belongs to the Zingiberaceae family. It is a perennial plant with thick aromatic rhizomes. Ginger has been a culinary spice and a medicinal herb for centuries, attributed to its arsenal of reported biological activities. The bioactive constituents of ginger include phenolic compounds such as gingerols, shogaols, and zingerone, along with volatile terpenes like zingiberene, as well as various vitamins and minerals (18).

Several varieties of ginger are planted in Malaysia, such as Halia Bentong, Halia Taiwan, Halia Tanjung Sepat, and Halia Bara. Halia Bentong is characterised by its large, dull yellowish rhizomes that are less fibrous compared to other ginger varieties. The Halia Bentong has a moist and viscous texture, which can produce more juice that is rich in fibre. This variety is noted for its stronger aroma and spicier flavour (19). It is cultivated in the highland areas in Bentong, Pahang. It is well-known as high-quality ginger and is in high demand among consumers. Halia Bentong is registered in the ASEAN Intellectual Property Rights as a geographical indication in the ASEAN region for its supreme quality (20).

Many studies have been carried out on ginger's antibacterial properties against pathogens of oral disease. Among them are *Porphyromonas gingivalis, Porphyromonas endodontalis, Fusobacterium nucleatum* (21), *Streptococcus sanguinis, Streptococcus mutans* (22), *Enterococcus faecalis* (23), and *Candida albicans* (24). Previous research on Halia Bentong was more comprehensive regarding its chemical constituents, antioxidant, anticancer, and proteolytic activities (25¬–27). Studies on the antibacterial activity of Halia Bentong are scarce and reported in selected Gram-negative and Gram-positive bacteria (28). There are yet studies to report on the antibacterial activity of Halia Bentong against pathogenic oral bacteria. Thus, this preliminary study aims to determine the antibacterial effects of the ethanolic extract of Halia Bentong on *P. gingivalis*, a major periodontopathogen. The scanning electron microscopy (SEM) findings on the structural changes of *P. gingivalis* after treatment with the Halia Bentong ethanolic extract were also reported. The preliminary findings from this study will pave the way for further investigations into the therapeutic potential of Halia Bentong in the management of periodontal disease.

MATERIALS AND METHODS

Ethanol extraction of Halia Bentong

Halia Bentong ethanolic extract (HBEE) was prepared according to (29), with modifications. Fresh Halia Bentong rhizomes were obtained from a local store in Bentong, Pahang, Malaysia, and a voucher specimen (ID030/2022) was deposited at Universiti Kebangsaan Malaysia's Herbarium, Faculty of Science and Technology, Bangi, Selangor, Malaysia. The rhizomes were washed, peeled, sliced, and oven-dried (Shel Lab, Cornelius, United States) at 60 °C for 48 hours until a constant weight was achieved. Moisture content was

calculated based on weight differences before and after drying. The dried slices were ground into powder, and 10 g of powdered ginger was extracted with 500 mL absolute ethanol (Emsure, Merck, Germany) for 24 hours with continuous shaking at room temperature. Absolute ethanol was selected for crude extraction due to its amphiphilic properties, which allow efficient solubilisation of both polar and moderately non-polar phytochemicals. The extract was filtered twice, first through a muslin cloth and then using Whatman No. 1 filter paper. Ethanol was removed via rotary evaporation (Buchi, MD, United States), and the resulting concentrate was stored at 4°C until further use. The extraction yield (%) was determined as the ratio of extract weight to dried rhizome weight.

Total phenolic content

The total phenolic content of the HBEE was determined using the Folin-Ciocalteu method with modifications (30). Two hundred microlitres of HBEE with a concentration of 1 mg/mL were mixed with 800 µL of deionised water and 100 µL of Folin-Ciocalteu reagent (Merck, Germany), followed by incubation at room temperature for 3 minutes. After adding 300 µL of Na_2CO_3 (Sigma-Aldrich, United States) (20% w/v), the mixture was further incubated at room temperature for 120 minutes in the dark. The absorbance of the mixture was measured against a blank at a wavelength of 765 nm using a spectrophotometer (Jenway, Cole-Parmer, United Kingdom) . Total phenolic content was expressed in mg gallic acid equivalent (GAE)/g extract, calculated from a standard curve prepared with 0 to 100 mg/L gallic acid (Tokyo Chemical Industry, Japan).

Antibacterial effects of HBEE on *P. gingivalis*

a. Bacteria culture and growth maintenance

P. gingivalis ATCC 33277 reference strain was used in this study. The bacteria were grown and maintained on defibrinated horse blood agar and brain heart infusion–trypticase soy broth (BHI-T), prepared using brain heart infusion (BHI) (Becton Dickinson, USA) and trypticase soy broth (Oxoid, UK). The broth was supplemented with haemin (5 mg/mL) (Sigma-Aldrich, USA), vitamin K (5 mg/mL) (Sigma-Aldrich, USA), and cysteine (0.5 mg/mL) (Bio Basic, Canada), modified from (31). The bacteria were cultured and incubated for 48 hours at 37°C in a container with an anaerobic sachet (Oxoid AnaeroGen, Thermo Scientific, United Kingdom).

b. Broth microdilution assays

The HBEE paste was dissolved into 100% dimethyl sulfoxide (Acros Organics, Thermo Scientific) and centrifuged at 10,000 x g, 20 °C for 10 minutes to remove insoluble impurities and sediment prior to serial dilution. The clear supernatant of the centrifuged extract was then serially diluted (two-fold) in BHI-T broth in microcentrifuge tubes to yield eight concentrations (100, 50, 25, 12.5, 6.25, 3.125, 1.625, and 0.08 mg/mL), following the broth microdilution technique. The concentration range (100 to 0.08 mg/mL) was selected based on solubility and feasibility, using a two-fold serial dilution similar to our previous study on *Orthosiphon stamineus extract* (23), to ensure the MIC and MBC values could be accurately captured.

The prepared dilutions of HBEE were then transferred to a 96-well microtitre plate (Sorfa, China), with 50 µL of each dilution mixed with 50 µL of a standardised suspension of *P. gingivalis* (10^6 CFU/mL), resulting in a final volume of 100 µL per well for the MIC test. Untreated bacterial cells served as the negative control, while BHI-T broth was used as the growth control. Amoxicillin (0.05 mg/mL) (Bio Basic, Canada), a clinically used antibiotic for periodontal infections, was the positive control. Following 48 hours of anaerobic incubation at 37°C, the turbidity of the suspension was measured as optical density (OD) using a plate reader (Varioskan® Flash Microplate Reader, Thermo Scientific, USA) at a 590 nm wavelength. The percentage of bacterial growth inhibition was then determined using the formula below (23):

$$\text{Growth inhibition (\%)} = \frac{OD_{control} - OD_{test}}{OD_{control}} \times 100$$

Minimum inhibitory concentration (MIC) was determined as the lowest concentration that exhibits 100% growth inhibition on *P. gingivalis*. To determine the minimum bactericidal concentration (MBC), 10 µL of bacterial suspension from wells that showed no turbidity was inoculated onto the BHI-T agar plate. The plate was then anaerobically incubated for 48 hours at 37°C. The MBC was determined to be the lowest HBEE concentration, showing no bacterial growth on the agar.

c. Anti-adhesion assay

The anti-adhesion effect of the HBEE was assessed using the crystal violet staining method (32), with modifications. One hundred microlitres of *P. gingivalis* suspension (10^6 CFU/mL) were co-incubated with 100 µL of each diluted HBEE concentration in BHI-T broth (100, 50, 25, 12.5, 6.25, 3.125, 1.625, and 0.08 mg/mL) in 96-well microtitre plates, resulting the final volume of 200 µL per well. The plates were then placed in an anaerobic jar (Mitsubishi AnaeroPack, Thermo Scientific, United Kingdom) with an anaerobic sachet (Oxoid AnaeroGen, Thermo Scientific, United Kingdom) and incubated for 48 hours at 37°C. The untreated bacterial cells were the negative control, while amoxicillin (0.05 mg/mL)-treated cells were the positive control. Following incubation, the broth containing unattached cells was pipetted out of the wells. The remaining attached cells were stained with 0.1% crystal violet (Sigma-Aldrich, USA) and incubated for 15 minutes at room temperature. The

crystal violet solution was pipetted out, and the wells were rinsed three times with distilled water. Next, 200 µL of 80% ethanol/20% acetone was added into the wells to extract the crystal violet solution absorbed by the cells. The absorbance was measured using a plate reader at a wavelength of 590 nm.

d. Scanning electron microscopy (SEM) analysis

The SEM analysis was carried out according to (23), with modifications. The HBEE (1 x MIC) was added to the *P. gingivalis* culture and dispensed into a 12-well plate containing sterile cut glass slides. After 24 hours of incubation, the slides bearing the attached *P. gingivalis* cells were taken out and fixed with the vapour of 2% glutaraldehyde for 12 hours at room temperature. After the fixation, the cells were rinsed several times with 0.1 M phosphate-buffered saline (pH 7) (Sigma-Aldrich, USA) for a minimum of 10 minutes. The cells underwent dehydration with ethanol rinses at 25%, 50%, and 70% concentrations, each for 10 minutes. The glass slides were mounted on aluminium mounting stubs and sputter-coated with gold. Morphological evaluation of treated and untreated *P. gingivalis* cells was conducted using a field emission scanning electron microscope (FESEM) (Zeiss Supra 55VP, Germany) at various magnifications. Samples from two independent experiments were analysed. Multiple areas of each sample were examined, and representative fields of view were selected to ensure that the images captured were consistent with the overall morphological observations.

e. Statistical analysis

The experiments were conducted three times independently and analysed in triplicate. Statistical analysis was performed using SPSS version 26 (IBM, USA). The Shapiro–Wilk test was used to assess the normality of the data. The total phenolic concentration data were normally distributed and are presented as mean ± standard deviation (SD). As the growth inhibition and anti-adhesion data did not meet the assumption of normality, they were analysed using the Kruskal–Wallis H test. Statistical significance was set at $p < 0.05$.

RESULTS

Moisture Content, Extraction Yield, and Total Phenolic Content of Halia Bentong

In this study, the moisture content of the Halia Bentong rhizomes was measured at 84.5%. The extraction yield recorded for the prepared Halia Bentong paste was 10%. The total phenolic content in the ethanolic extract of Halia Bentong rhizomes was 42.33 ± 2.41 mg GAE/g.

Growth inhibition, MIC, and MBC of HBEE against *P. gingivalis*

HBEE's inhibition of *P. gingivalis* growth was concentration-dependent, with significant inhibition observed at 1.625 mg/mL and above. The extract exhibited growth inhibition comparable to amoxicillin, the positive control, at this concentration. However, statistical analysis showed that while some extract concentrations produced inhibition levels similar to amoxicillin, others did not exhibit a significant difference (Figure 1). The MIC and MBC of HBEE were determined to be 1.625 mg/mL, indicating a bactericidal effect rather than a bacteriostatic one.

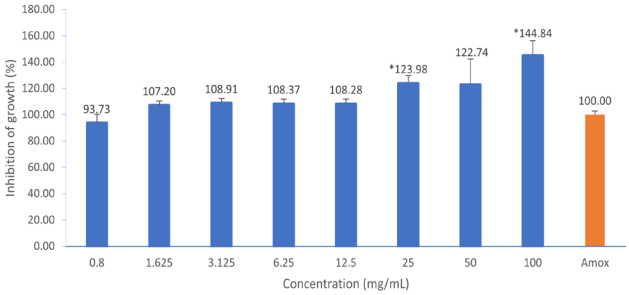

Figure 1: Growth inhibition of *P. gingivalis* grown in suspension following 48 hours of HBEE exposure. The growth inhibition of all tested concentrations was expressed relative to 100% inhibition by the positive control, 0.05 mg/mL amoxicillin (Amox). Error bars represent the mean ± SD of the experiments performed in triplicate. *Significant differences ($p<0.05$) between HBEE and 0.05 mg/mL amoxicillin.

Anti-adhesion activity

The anti-adhesion effect of HBEE against *P. gingivalis* exhibits inverse concentration-dependent behaviour, which is the opposite of growth inhibition. There was a significant difference in the absorbance reading between the lower HBEE concentrations (0.8-12.5 mg/mL) and 50 mg/mL HBEE as compared to the negative control, untreated bacterial cells ($p<0.05$) (Fig. 2). These results suggest that treatment with lower concentrations of HBEE disrupted the adhesion activity of *P. gingivalis*.

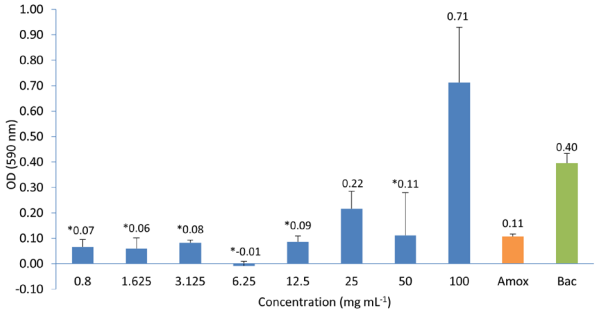

Figure 2: Anti-adhesion activities of HBEE towards *P. gingivalis*. The concentration of the positive control, amoxicillin (Amox), was 0.05 mg/mL. The negative control was the untreated *P. gingivalis* (Bac). Error bars represent the mean ± SD of the experiments performed in triplicate. *Significant differences ($p<0.05$) between HBEE and untreated bacterial cells.

SEM analysis

Figure 3A shows the morphology of untreated bacterial cells, which are abundant and round. Fig. 3B shows the morphology of *P. gingivalis* treated with amoxicillin, demonstrating the irregular shape of bacterial cells and the presence of depression on the bacterial cell surface. *P. gingivalis* treated with HBEE is shown in Fig. 3C, in which the cells were distributed sparsely, less abundant, and had a more elongated shape than the untreated bacteria.

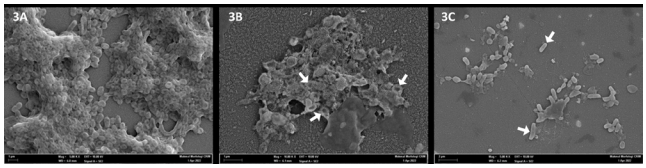

Figure 3: SEM images of the treated *P. gingivalis* with HBEE (C) and amoxicillin (B), compared to untreated *P. gingivalis* (A). Fig. B and C were viewed at 5,000x magnification; Fig. A was viewed at 10,000xmagnification. The tested concentration of HBEE was 1 x MIC, and the amoxicillin concentration was 0.05 mg/mL.

DISCUSSION

Porphyromonas gingivalis is a keystone pathogen in periodontitis and has been associated with systemic diseases (33), prompting the search for alternative antimicrobial agents. As antibiotic resistance becomes an increasing concern, there is growing interest in plant-derived therapeutics such as ginger. This study examined the antibacterial and anti-adhesion effects of Halia Bentong ethanolic extract (HBEE) against *P. gingivalis*, a target that has not previously been studied with this particular ginger variety.

Like other ginger varieties, HBEE may enhance antibiotic efficacy, allowing for lower doses and reducing resistance risk. Its phytochemical diversity further suggests a lower likelihood of bacterial adaptation compared to synthetic antibiotics. Although studies on Halia Bentong are limited, its classification as a *Zingiber officinale* variety implies similar antimicrobial potential. Importantly, *Zingiber officinale* var. *rubrum* (Halia Bara) is rich in terpenoids (e.g., monoterpenes, sesquiterpenes), which contribute to its broad-spectrum antibacterial activity against *S. aureus, E. coli*, and *K. pneumoniae* (34). In contrast, Halia Bentong's ethanolic extract has been shown to be phenolic-rich, containing gingerols and shogaols, which have been linked to bacterial membrane disruption, biofilm inhibition, and quorum-sensing suppression (35). Given *P. gingivalis* is a Gram-negative anaerobe with distinct pathogenic mechanisms, the phenolic-rich profile of Halia Bentong may offer a more targeted antimicrobial mechanism compared to terpenoid-dominant varieties. These compositional differences highlight the potential of Halia Bentong as a targeted antibacterial agent against periodontal pathogens and provide the basis for the following investigation into its physicochemical characteristics and antibacterial effects against *P. gingivalis*.

The Halia Bentong moisture content in this study was measured at 84.5%, which falls within the moisture content range for fresh ginger (85-90%) (36). Removal of moisture will prevent the rhizomes from being susceptible to microbial growth or enzymatic actions that may compromise the bioactivity of their phytochemicals (37). The extraction yield of a plant product is influenced by several factors, such as the raw materials used, extraction solvent, extraction temperature, and extraction time (37). In this study, the Halia Bentong yield was 10% when the rhizomes were oven-dried, followed by ethanol extraction. In previous studies, ethanol extraction has been employed for other plants, with various concentrations or combinations with different solvents (38, 39). The selection of ethanol as the extraction solvent was based on its amphiphilic nature, which allows it to solubilise a wide spectrum of phytochemicals. Since this study focused on the biological activity of a crude extract rather than isolated compounds, absolute ethanol was chosen to maximise the range of extractable constituents. A study by (40) demonstrated that absolute ethanol effectively extracts gingerols and shogaols from *Zingiber officinale*, supporting its use for preparing phytochemical-rich ginger extracts.

The total phenolic content for Halia Bentong in this study is 42.33 ± 2.41 mg GAE/g, which is higher compared to 15.63 ± 0.50 mg GAE/g in a previous study that also employed oven drying and absolute ethanol extraction (40). This difference may be attributed to the ginger powder: ethanol mixture ratio, where (40) used a lower ratio (1:10) than the one in this study (1:50). A study has compared the total phenolic contents between different plant material: solvent ratios and reported that the higher the solvent ratio was, the higher the total phenolic content measured (41).

In this study using HBEE, the recorded MIC was 1.625 mg/mL. This study evaluated antibacterial potency through broth microdilution assays (MIC and MBC values) rather than agar well diffusion assays, which measure zones of inhibition. While zones of inhibition provide a qualitative assessment of antibacterial activity, MIC and MBC determinations offer a quantitative measure of the minimum concentration required to inhibit or kill bacterial cells in a liquid medium. The results demonstrate that at 1.625 mg/mL, HBEE exhibits bactericidal activity comparable to amoxicillin, supporting its potential as an antimicrobial agent. In two similar studies using ginger ethanolic extracts, one study reported a higher MIC of 300 mg/mL against *P. gingivalis* (42), while another reported a lower MIC of 50 µg/mL (21). The differences in these reported MICs could be attributed to the differences in the method used to prepare the ginger extract and the botanical origin of the studied plant. In this study, the MIC value of HBEE is similar to its MBC value, suggesting that HBEE is bactericidal rather than bacteriostatic. The extract

inhibits *P. gingivalis* growth at a concentration of 1.625 mg/mL and exerts a bactericidal effect on the bacteria at the same concentration. This is an encouraging result, indicating that the ethanolic extract of Halia Bentong has the potential to be an effective antimicrobial agent.

The broth microdilution assay determined the growth inhibition of *P. gingivalis* by HBEE. Amoxicillin was used as the positive control since it is a common antibiotic clinically used for periodontal infections. In this study, *P. gingivalis* growth was inhibited by the extract in a generally direct concentration-dependent manner, where the highest concentration showed the highest inhibition and the lowest concentration showed the lowest activity. At a concentration of 1.625 mg/mL and above, HBEE showed growth inhibition comparable to amoxicillin (100%), indicating that the *P. gingivalis* growth inhibition can already be achieved at a relatively lower concentration. This suggests that HBEE has strong antibacterial properties against *P. gingivalis*.

The initiation of oral diseases by microorganisms requires the pathogen to adhere to the surfaces in the oral cavity (43). The teeth and oral mucosa are covered with saliva in the oral cavity, making the surfaces more susceptible to pathogen adhesion. Substances with bacterial anti-adhesion properties are especially beneficial in preventing bacteria from adhering to a surface, a crucial first step for a pathogen to initiate infection. This study observed a concentration-dependent anti-adhesion effect of HBEE on *P. gingivalis*, which was opposite to its growth inhibition pattern. Significant anti-adhesion activity was observed at lower HBEE concentrations, suggesting a potential mechanism distinct from its bactericidal effect. A previous study on Indonesian ginger (*Zingiber purpureum*, commonly known as Bangle) reported its antibiofilm activity against *P. gingivalis*, demonstrating that c- and t-banglene reduced biofilm formation by 40–47% at sub-MIC concentrations (44). However, Bangle lacks key bioactive compounds such as gingerol and shogaol, which are abundant in Halia Bentong and have been linked to antimicrobial activity in other studies (21, 22). Given these compositional differences, this study is the first to report the anti-adhesion effect of a crude ethanolic extract of Halia Bentong ginger on *P. gingivalis*. While the presence of gingerols and shogaols in Halia Bentong has been reported in previous studies, their specific contribution to the observed activity in this crude extract warrants further investigation. Additionally, at higher extract concentrations, *P. gingivalis* adhesion was not significantly inhibited, suggesting that certain unknown compounds in HBEE may modulate bacterial attachment in a concentration-dependent manner. Future studies are needed to identify the specific phytochemicals responsible for this effect. A similar phenomenon has been reported in a previous study involving ginger oil and *Orthosiphon stamineus* extract, where higher concentrations unexpectedly promoted *Enterococcus faecalis* adhesion (23). It was suggested that certain unidentified compounds in crude plant extracts may exert contrasting effects depending on concentration, potentially masking anti-adhesive actions at higher doses. This could also apply to HBEE, considering its complex phytochemical composition. Further studies are warranted to isolate and characterise the specific components involved in this modulatory behaviour.

In the SEM analysis, the cut glass slides served as a substratum for the *P. gingivalis*, demonstrating the pathogen's adhesion ability. *P. gingivalis* cells treated with HBEE exhibited sparser distribution, lower abundance, and an elongated morphology compared to untreated cells. These findings suggest that the extract may alter the *P. gingivalis* cell surface properties, potentially reducing its ability to adhere. Ginger phenolic compounds, mainly gingerols and shogaols, have been linked to antimicrobial activity, with 6-gingerol and 6-shogaol previously reported to disrupt the bacterial membrane of *P. aeruginosa* (45). Although *P. gingivalis* is also a Gram-negative bacterium, its outer membrane composition differs, which may influence the extent of membrane disruption caused by these compounds. The observed changes in bacterial morphology in this study suggest that similar mechanisms may be at play. However, further investigations, such as membrane integrity assays and transmission electron microscopy imaging, are needed to confirm the exact mode of action in *P. gingivalis*.

FimA is a major fimbrial protein in *P. gingivalis* that facilitates bacterial adhesion to host surfaces (46). The disrupted surface structures observed in SEM images of HBEE-treated bacteria may reflect alterations in fimbrial integrity, which could potentially affect FimA function or expression. Although not directly assessed in this study, this hypothesis warrants further investigation.

One limitation of this study is the use of a crude extract without standardisation for specific active compounds. Although previous studies have identified phenolic constituents such as gingerols and shogaols in Halia Bentong, their exact concentrations in this extract were not quantified. Future studies should include phytochemical profiling and standardisation to correlate compound levels with observed bioactivities better.

CONCLUSION

This study is the first to demonstrate that Halia Bentong ethanolic extract (HBEE) exhibits both antibacterial and anti-adhesion activity against *P. gingivalis*, a key periodontal pathogen. The MIC and MBC values indicate a bactericidal effect, likely attributed to phenolic compounds (e.g. gingerols, shogaols), which are known to disrupt bacterial membranes and interfere with biofilm formation, and may also affect quorum sensing pathways, as reported in previous studies. SEM

analysis revealed structural changes in *P. gingivalis* following treatment, supporting its effect on bacterial surface integrity. Compared to other ginger varieties, Halia Bentong is phenolic-rich rather than terpenoid-dominant, which may underlie its specific antimicrobial action against anaerobic periodontal bacteria.

These findings offer novel insight into the potential of Halia Bentong as a natural oral antimicrobial. The observed anti-adhesion effect represents a unique contribution to the field, supporting further investigation into its phytochemical profile, molecular mechanisms, and possible application as an adjunctive agent in periodontal therapy.

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