



Antibiofilm Activity of Ethanolic Extract of *Zingiber officinale* against *Aggregatibacter actinomycetemcomitans* ATCC 43718 (serotype B)

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ABSTRACT

Red ginger is used to treat bacterial infections, diarrhea, and cough. Therefore, this study aimed to evaluate the antibiofilm activity of an ethanolic extract of *Zingiber officinale* against *Aggregatibacter actinomycetemcomitans* ATCC 43718 (serotype B). The *Zingiber officinale* extract was obtained by maceration. An antibiofilm activity assay was used with the dilution method with the various formulas of 20;10;5;2,5;1,25 % w/v. The biofilm inhibition concentration (MBIC₅₀) was then calculated. SEM analysis was conducted to evaluate bacterial biofilm growth after treatment. The data were then analyzed using IBM SPSS Statistics version 21.0 with one-way Analysis of Variance (ANOVA). The results showed that the ethanolic extract of *Zingiber officinale* inhibited *A. actinomycetemcomitans* ATCC 43718 (serotype B). This proves that the chemical compounds of red ginger effectively inhibited biofilm bacterial growth. The extracellular polymeric substance (EPS) matrix was degraded after treating the cell biofilm. These findings suggest that the ethanolic extract of *Zingiber officinale* has potential against *A. actinomycetemcomitans*. Further research is needed to determine the safety and efficacy of *A. actinomycetemcomitans* ATCC 43718 (serotype B) can be developed as an alternative antibiofilm

Keywords: *Aggregatibacter actinomycetemcomitans*, antibiofilm, ethanolic extract, *Zingiber officinale*

INTRODUCTION

Periodontitis is a one of the periodontal diseases and induced the inflammatory disease that causes loss the tooth supporting tissue (Åberg *et al.* 2014; Nagasawa *et al.* 2014). Chronic oral infection such as periodontitis are a common source of in a risk factor for several disease, including insulin resistance, rheumatoid arthritis, osteoporosis, and the complication in pregnancy (Arigbede *et al.* 2012). The main microorganisms responsible for these conditions are *A. actinomycetemcomitans*. The long-term use of antibiotics use should be carefully and should be avoided (Dhillon & Varshney 2013). Thus, effective alternatives to antibiotics are needed to reduce the antibiotic resistance (Walsh *et al.* 2016).

Zingiber officinale is one of the most widely used plants in Asia, Australia, and other countries (Dhanik *et al.* 2017; Wang *et al.* 2017). Ginger (*Z. officinale* Rosc.) is a member of the Zingiberaceae family and is widely used as a spice and in traditional plant medicine. Based

on the shape, size, and color of the rhizome. These three varieties of ginger are known, such as the gajah ginger (*Zingiber officinale* var. *officinale*), emprit ginger (*Z. officinale* var. *amarum*), and red ginger (*Zingiber officinale* var. *rubrum*) (Setyawan 2002). Steam distillation of *Z. officinale* produces ginger oil which mainly contains sesquiterpene hydrocarbons. Gingerol is one of the main compound components found in *Z. officinale* (Syafitri *et al.* 2018). The ginger had the beneficial pharmacological effect such as diabetes militus, inflammation, cancer, bacterial infection, nausea (Syafitri *et al.* 2018).

Ginger contains secondary metabolites such as phenols, terpenoids, flavonoids, and essential oils. In general, the secondary metabolites can inhibit the growth of microorganism, including *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus* sp., *Candida albicans*, *C. glabrata* dan *C. krusei* (Aghazadeh *et al.* 2016; Tanghetti 2013) Alkaloid had benefit in medicinal agent such as diabetes, malaria, cardiac dysfunction, and cancer (Ain *et al.* 2016).

Based on the previous research, the concentration of squeezed red ginger 25% inhibited the bacteria *A. actinomycetemcomitans* was 11.2 mm (Saptiwi *et al.* 2018). Ginger extract inhibited the biofilm growth of *Pseudomonas aeruginosa* (Kim & Park 2013). Therefore, a study to determine the potency of ethanolic extract of *Z. officinale* against *A. actinomycetemcomitans* biofilm is needed. In this

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study, we aimed to determine the effectiveness of *Z. officinale* as an antibiofilm agent against *A. actinomycetemcomitans*.

METHODS

Sample Extraction

Samples of Rhizomes of *Zingiber officinale* were obtained from B2P2TOOT, Tawangmangu, Central Java. The dried rhizome of *Z. officinale* is mashed by blending. The dry powder of *Z. officinale* was extracted using the maceration method for 2 × 24 h, and the solvent was changed twice. The macerate was then concentrated using a rotary evaporator.

Phytochemical Assay of *Z. officinale* extract

Phytochemical assays (alkaloids, flavonoids, tannins, saponins, and beta-sitosterol) were performed using a quantitative spectrophotometric method.

Bacterial Inoculum Preparation

Bacterial inoculum preparation of *Aggregatibacter actinomycetemcomitans* ATCC 43718 (serotype B) was determined using a modified method according to Shanmugam *et al.* (2015). *A. actinomycetemcomitans* from the stock culture was taken, and as much as one sterile loop was placed into 5 ml of BHI medium. The bacterial cultures were incubated in an anaerobic atmosphere at 37°C for 24 hours. Furthermore, the culture of *A. actinomycetemcomitans* was diluted using 0,9% NaCl until the turbidity was equal to the Farland equivalent of 0.5 standard (1.5×10^8 CFU/mL).

Antibiofilm Activity Assay

The ethanolic extract of *Z. officinale* was diluted with dimethyl sulfoxide (DMSO) at different concentration, 20;10;5;2,5;1,25 %. The positive control used mouthwash containing 2% chlorhexidine, with the well plates anaerobically incubated at 37°C for 24 h. Furthermore, 100 µL of BHI containing 2% sucrose, bacterial suspension, and ethanolic extract of *Z. officinale* was added to the well plates and incubated at 37°C for 24 h. These biofilms were stained with 1 % (v/v) crystal violet, and 95% ethanol was added to the well plates. The well plates were also measured using a microplate reader at 595 nm, with three replicates being produced in this analysis (Utami *et al.* 2021).

Scanning Electron Microscopy Analysis

Coverslips were placed on 24-well microtiter plates with 100 µL of total volume containing BHI, sucrose, microbial suspension, and the test compound. The plate was incubated at 37°C for 24 hours in an anaerobic condition (Utami *et al.* 2020). Then, coverslips were fixed with glutaraldehyde and examined under SEM at magnifications from 3000x.

SEM was used to observe morphological changes in the biofilm following treatment with the test compounds.

Analysis Data

The OD value obtained from the biofilm inhibition assay was analyzed using one-way ANOVA with SPSS, with a confidence level of 95%.

RESULTS AND DISCUSSION

Phytochemical Content of *Z. officinale* extract

In this study, *Z. officinale* extract was prepared using the maceration method. Ethanol was used for extraction because it can easily penetrate cell walls. Furthermore, the ethanol is a good and effective solvent for extracting polyphenol compounds and has a polarity index of 5.2. (Zuraida *et al.* 2017).

The total alkaloid, flavonoid, tannin, and saponin content in the ethanolic extract of *Z. officinale* is presented in Table 1. Alkaloids, flavonoids, tannins, and saponins are known to exhibit antibacterial and antibiofilm activities (Alvita *et al.* 2017; Kováč *et al.* 2023; Neumann *et al.* 2022). The activity plays an important role in the underlying structure, interfering with the permeability of the bacterial cell membrane, denaturing proteins, and damaging the bacterial cell wall (Kurniawan & Aryana 2015; Othman *et al.* 2019). The previous study has shown that the phytochemical content of ginger rhizome contains flavonoids, alkaloids, terpenoids, steroids, and saponins (Ridwan *et al.* 2022).

Antibiofilm Activity Test

In this study, the antibiofilm activity of *Z. officinale* extract against *A. actinomycetemcomitans* was observed due to its phytochemical content. The phytochemical content of the plants, including alkaloids, flavonoids, tannins, and saponins, differed.

The results of the antibiofilm activity of the extract *Z. officinale* indicated the highest effect of 56,03 % (Figure 1) in 20% b/v concentration of the extract *Z. officinale*, while the 10% dan 5 % w/v concentration of the extract *Z. officinale* were inhibition effect of 55,71 % and 52,3 %, respectively. The 5% w/v concentration of *Z. officinale* extract showed MBIC₅₀. The inhibitory effect of the *Z. officinale* extract on the growth of *A. actinomycetemcomitans* biofilm showed that the extract can inhibit the growth of biofilm bacteria. The

Table 1 The phytochemical content of the ethanolic extract of *Zingiber officinale*

	Average± SD
Alkaloid	3,5±0,04
Flavonoid	0,32±0,02
Tannin	9,8±0,05
Saponin	2,32±0,03

phenolic compound degraded the EPS of the biofilm (Utami *et al.* 2022).

Scanning Electron Microscopy Analysis

SEM confirmed that 20% v/v *Z. officinale* extract effectively degraded EPS in *A. actinomycetemcomitans* biofilms (Figure 2). The extract of *Z. officinale* could be a good choice for preventing *A. actinomycetemcomitans* biofilms.

This study provides evidence that *Z. officinale* extract affects biofilm formation. but also induces morphological changes. The ethanolic extract of *Z. officinale* contains alkaloids, tannins, saponins, and flavonoids. Alkaloids play an important role in inhibiting biofilm formation by inhibiting quorum-sensing activity or cell-to-cell communication (Park *et al.* 2008). Tannins can reduce metabolic activity in biofilms. Tannins can form complexes with metal ions, making them toxic to microbial membranes (Akiyama *et al.* 2001; Cobrado *et al.* 2012). Tannins and flavonoids inhibit the expression of *icaA* and *icaD*, which are regulators of biofilm formation. The *icaA* and *icaD* genes can synthesize Polysaccharide Intercellular

Adhesion (PIA), which has an essential role in the formation of biofilms (Rohde *et al.* 2010).

This study provides evidence relating to the extract of *Z. officinale*. The extract of *Z. officinale* inhibit *A. actinomycetemcomitans*. Although we demonstrated that the extract of *Z. officinale* has an effect on *A. actinomycetemcomitans*, there were some limitations to this study that need to be considered. The degradation effect was also investigated. However, our findings can serve as a foundation for further research on the antibiofilm potential of *Z. officinale* extracts.

This study confirmed that the effectiveness of *Z. officinale* extract on biofilm formation and morphological changes is influenced by its concentration. The 20 % concentration of *Z. officinale* extract showed the strongest ability to inhibit biofilm formation and morphological changes. This finding highlights the potential of *Z. officinale* extract as an antibiofilm candidate for treatment. Further research on red ginger is needed to reveal the drug delivery system of red ginger extract.

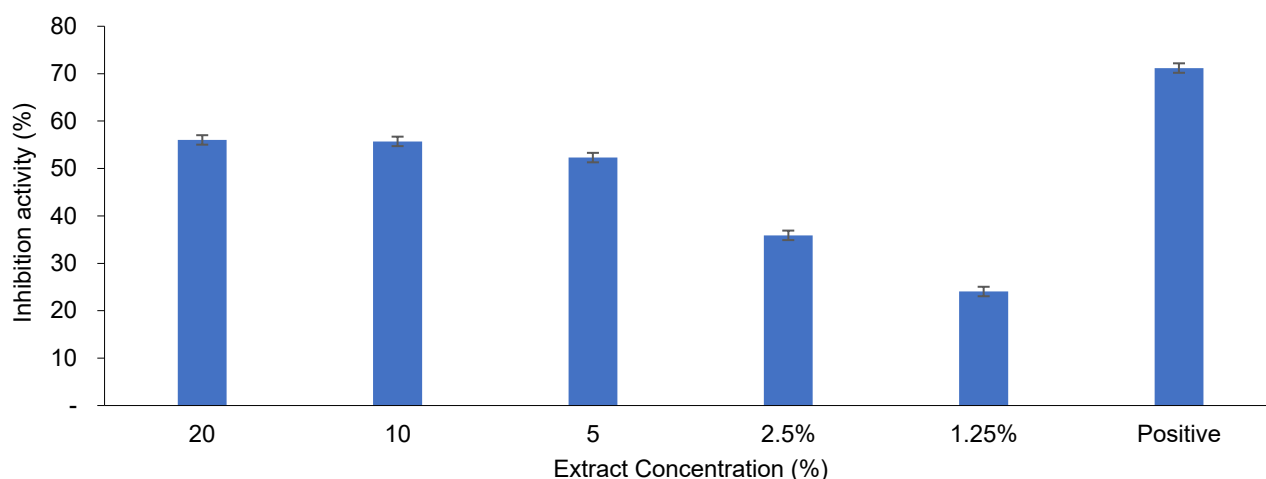


Figure 1 The results of the percentage of inhibition anti-biofilm of red ginger extract against *A. actinomycetemcomitans*.

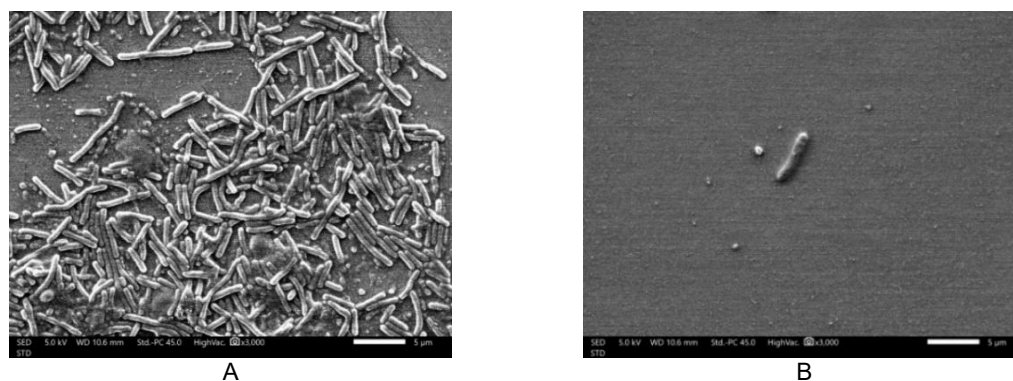


Figure 2 Biofilm formation of *A. actinomycetemcomitans* ATCC 25175 on coverslips was monitored by SEM at a: control (untreated) cell, b: cells pre-treated with *Z. officinale* at 20 % b/v (magnification 3000x).

CONCLUSION

The extract of *Z. officinale* can inhibit *A. actinomycetemcomitans*, which causes gingivitis, in an in vitro study. The active compounds in this extract include flavonoids, saponins, alkaloids, and tannins. The extract *Z. officinale* can be used as a new alternative against oral biofilm

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CONFLICT OF INTEREST

The authors declare no conflicts of interest relevant to this article.

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