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EFFECT OF KECOMBRANG ETHANOL EXTRACT (*ETLINGERA ELATIOR*) ON *FUSOBACTERIUM NUCLEATUM* BIOFILM DEGRAGATION

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ABSTRACT

Fusobacterium nucleatum is a Gram-negative anaerobic bacterium that plays a crucial role in periodontal disease progression through biofilm formation. Kecombrang (*Etlingera elatior*) is a traditional medicinal plant containing bioactive compounds with antimicrobial properties. This study aimed to evaluate the effect of kecombrang extract on *F. nucleatum* biofilm degradation. This experimental study used ethanolic extracts from three parts of kecombrang (leaves, stems, and flowers) at concentrations of 1.56, 3.12, 6.25, 12.50, 25, and 50 mg/mL. *F. nucleatum* biofilm was formed and treated with various extract concentrations. Chlorhexidine 0.2% was used as positive control and DMSO 1% as negative control. Biofilm degradation percentage was measured using crystal violet assay at 490 nm wavelength. Data were analyzed using Two-Way ANOVA to evaluate the main effects of plant parts and extract concentrations, followed by Tukey's HSD post-hoc test. All kecombrang extracts showed dose-dependent biofilm degradation activity. Flower extract demonstrated the highest efficacy with degradation percentages ranging from 36.37% to 86.22% at concentrations 1.56-25 mg/mL. Leaf extract showed degradation of 38.2—82.08%, while the stem extract achieved 34.00-80.22% degradation at the same concentration range. The $MBEC_{50}$ values were 2.62 mg/mL for flower extract, 3.68 mg/mL for leaf extract, and 4.99 mg/mL for stem extract. Kecombrang extracts possess significant biofilm degradation activity against *F. nucleatum*, with flower extract showing the most promising results comparable to chlorhexidine positive control.

KEYWORDS:

Biofilm Degradation, Kecombrang, *Etlingera elatior*, *Fusobacterium nucleatum*, Periodontal Disease

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INTRODUCTION

Periodontal disease represents one of the most prevalent oral health conditions globally, affecting approximately 20-50% of the global population[1]. The pathogenesis of periodontal disease is primarily attributed to the formation of complex microbial biofilms on tooth surfaces and subgingival environment. Among the key pathogenic bacteria involved in periodontal disease progression, *Fusobacterium nucleatum* stands out as a critical bridging organism that facilitates the colonization and aggregation of other periodontal pathogens[2-4].

Fusobacterium nucleatum is a Gram-negative, obligate anaerobic bacterium that possesses unique coaggregation properties, enabling it to interact with both early and late colonizers in the periodontal biofilm. The bacterium produces various virulence factors, including lipopolysaccharides, outer membrane

proteins, and metabolic byproducts that contribute to tissue destruction and inflammatory responses in periodontal tissues. The biofilm structure protects antimicrobial agents and host immune responses, making conventional treatment approaches less effective [4,5].

Current periodontal therapy relies heavily on mechanical debridement supplemented with chemical agents such as chlorhexidine. However, concerns regarding bacterial resistance, side effects, and limited penetration into biofilms have prompted researchers to explore alternative antimicrobial strategies from natural sources. Traditional medicinal plants offer a rich reservoir of bioactive compounds with proven antimicrobial properties and potentially fewer adverse effects [6,7].

Kecombrang (*Etlingera elatior*), a member of the Zingiberaceae family, is widely distributed across Southeast Asia and has been traditionally used for various medicinal purposes. Previous phytochemical studies have identified numerous bioactive compounds in kecombrang, including flavonoids, phenolic compounds, and terpenoids, which exhibit antimicrobial, anti-inflammatory, and antioxidant activities. However, limited research has been conducted on its specific effects against periodontal pathogens and biofilm structures [8,9].

The development of effective anti-biofilm agents from natural sources represents a promising approach to managing periodontal disease[7,10]. The advantages of utilizing kecombrang extracts include their natural origin, potential for reduced side effects, multi-target antimicrobial mechanisms, and sustainable availability as a traditional medicinal plant [11–13]. This study aimed to evaluate the antibiofilm activity of ethanolic extracts from different parts of kecombrang (leaves, stems, and flowers) against *F. nucleatum* biofilms and to determine the most effective plant part and concentration for biofilm degradation, ultimately contributing to the development of evidence-based natural therapeutics for periodontal disease management.

METHODS

Materials and Equipment

The research utilized various laboratory instruments including a rotary evaporator (Heidolph), flat-bottom 96-well microplates (Iwaki), a microplate reader (Diatex®), a CO_2

incubatur (ESCO), an autoclave (All American), micropipettes (OneMed), an analiytical balance (Mettler Toledo), and standard laboratory glassware (Pyrex). The bacterial strain *Fusobacterium nucleatum* ATCC 25586 was sourced from the Health Laboratory Center in Yogyakarta. Fresh kecombrang plant materials were procured from PT. TF Indonature at the Faculty of Agriculture, Jenderal Soedirman University. Culture media and reagents comprised Brain Heart Infusion Broth/BHI-B (Himedia), Mueller Hinton Agar/MHA (Himedia), Phosphate Bufferes Saline/PBS (Hyclone), 1% Dimethyl sulfoxide/DMSO (Merck), 0.2% chlorhexidine gluconate/CHX (Minosep), sterile distilled water, ethanol (70-96% concentration), and 1% crystal violet solution.

Kecombrang Plant Extraction

Kecombrang plants were collected, selected, and separated into flower petals, leaves, and stems. The selected plant materials were thoroughly washed with water, then dried in an oven at 50°C dried until they reached a moisture content of 8-10%, requiring approximately 20 hours of drying time. The dried materials were then ground into a homogeneous simplicia powder. Each dried simplicia was extracted using the maceration method with 70% ethanol solvent for 48 hours. The concentrated crude extracts were then diluted to obtain a range of concentrations: 50, 25, 12.5, 6.25, 3.125, and 1.56 mg/mL, utilizing 1% DMSO as the diluent solvent[14–17]. Phytochemical screening was conducted to identify the presence of bioactive compounds, including alkaloids, anthraquinones, phenolic compounds, flavonoids, saponins, steroids/terpenoids, and tannins using standard tube-based qualitative methods[13].

Biofilm Degradation Test

The biofilm degradation test was performed using a microtiter plate assay utilizing a 1% crystal violet staining technique. *Fusobacterium nucleatum* bacteria were revitalized in BHI-B medium anaerobically. Bacterial turbidity was adjusted to match 0.5 McFarland (bacterial count $1x10^8$ cells/mL) in sterile saline solution. Following this standardization, 100 µL of BHI-B medium was added to 96-well flat-bottom microplates. The 100 µL bacterial suspension was inoculated and incubated at 37°C for 48 hours under anaerobic conditions. After incubation, planktonic cells were removed,

followed by triple washing with PBS to eliminate residual unattached cells [14,15,18].

A total of 100 µL BHI-B medium and 100 µL extract of various concentrations were added. A 1% DMSO solution was used as a negative control, and 0.2% CHX was used as a positive control. Growth control with BHI-B medium was used to determine the degradation percentage. Incubation was perfomed anaerobically for 60 minutes at 37°C. The suspension in the well plate was discarded and washed with PBS three times to remove treatment residues[14,15,19].

A total of 200 µL of 1% crystal violet solution was added to each well to quantify the biofilm degradation test results and incubated for 15 minutes at room temperature. The well plate was then washed again using PBS three times and allowed to dry. Subsequently, 200 µL of 96% ethanol was added to each well plate to dissolve the formed biofilm and incubation was performed for 15 minutes at room temperature. Spectrophotometric analysis was conducted using a microplate reader at 490 nm wavelength to determine optical density values [20,21]. The percentage of biofilm degradation was calculated using the following formula:

Percentage of degradation = [(C-T)/C] x 100

Where *C* is the average absorbance per well for untreated biofilm and *T* is the average absorbance per well for the treated biofilm with extract treatment[22].

Statistical Analysis

Data in the form of degradation percentage results were analyzed with Two-Way ANOVA at 95% confidence level ($p<0.05$) to evaluate the main effects of plant parts (flower, leaf, stem) and extract concentrations (1.56, 3.125, 6.25, 12.5, 25, and 50 mg/mL) as well as their interaction effects on biofilm degradation. Post-hoc analysis was performed using Tukey's HSD test to determine significant differences between treatment groups. Subsequently, the $MBEC_{50}$ (Minimum Biofilm Eradication Concentration) value was determined using linear regression analysis by plotting loq concentration versus percentage degradation to determine the extract concentration capable of degrading at least 50% of bacterial biofilm formation. All statistical analyses were performed using SPSS version 25.0 software.

RESULT AND DISCUSSION

Phytochemical Screening Results

Preliminary phytochemical screening of kecombrang extracts revealed the presence of

several bioactive compounds across all plant parts tested. The results are summarized in Table 1.

Table 1. Phytochemical screening results of kecombrang extracts

Bioative Compound	Extract		
	Flower	Leaf	Stem
Alkaloids	+	+	+
Anthraquinones	-	-	-
Phenolic compounds	+	+	+
Flavonoids	+	+	+
Saponins	+	+	+
Steroid/Terpenoids	+	+	+
Tannins	+	+	+

Note: + = present, - = absent

All three extracts (flower, leaf, and stem) contained alkaloids, phenolic compounds, flavonoids, saponins, steroids/ terpenoids, and tannins. However, anthraquinone compounds were not detected in any of the extracts. The consistent presence of these bioactive compounds across different plant parts suggests their potential contributions to the observed biofilm degradation activity, which aligns with previous phytochemical studies on *Etlingera elatior.*

Our phytochemical screening results are consistent with several previous studies on *Etlingera elatior*. Effendi *et al.* (2019) reported similar findings in their analysis of kecombrang stem and leaves, confirming the presence of alkaloids, flavonoids, saponins, and terpenoids [16]. Similarly, Salman & Indriana (2021) identified alkaloids, flavonoids, and saponins in kecombrang flower extracts, supporting our current findings [17].

Notably, Ernilasari *et al.* (2021) reported the presence of phenolic compounds and flavonoids in kecombrang leaves, flowers, and fruits, with quantitative differences between plant parts[9]. Their study demonstrated that flower extracts contained higher concentrations of total phenolic compounds compared to leaves and stems, which aligns with our observations of superior biofilm degradation activity in flower extracts. This correlation between phytochemical content and biological activity suggests that the concentration of bioactive compounds may be a critical factor determining therapeutic efficacy. The absence of anthraquinone compounds in our study is consistent with most previous reports on *Etlingera elatior*, indicating that this class of compounds is not typically found in significant quantities in this species.

The synergistic effects of these bioactive compounds likely contribute to the observed biofilm degradation activity[16,17]. Flavonoids such as quercetin and kaemferol possess well-documented antimicrobial properties and can

disrupt bacterial cell walls and biofilm matrices through interference with energy metabolism and reduction of cell membrane permeability by disrupting phospholipase and ATPase enzyme activities[25].

Biofilm Degradation Activity

The biofilm degradation activity of kecombrang extracts against *F. nucleatum* is shown in Figure 1.

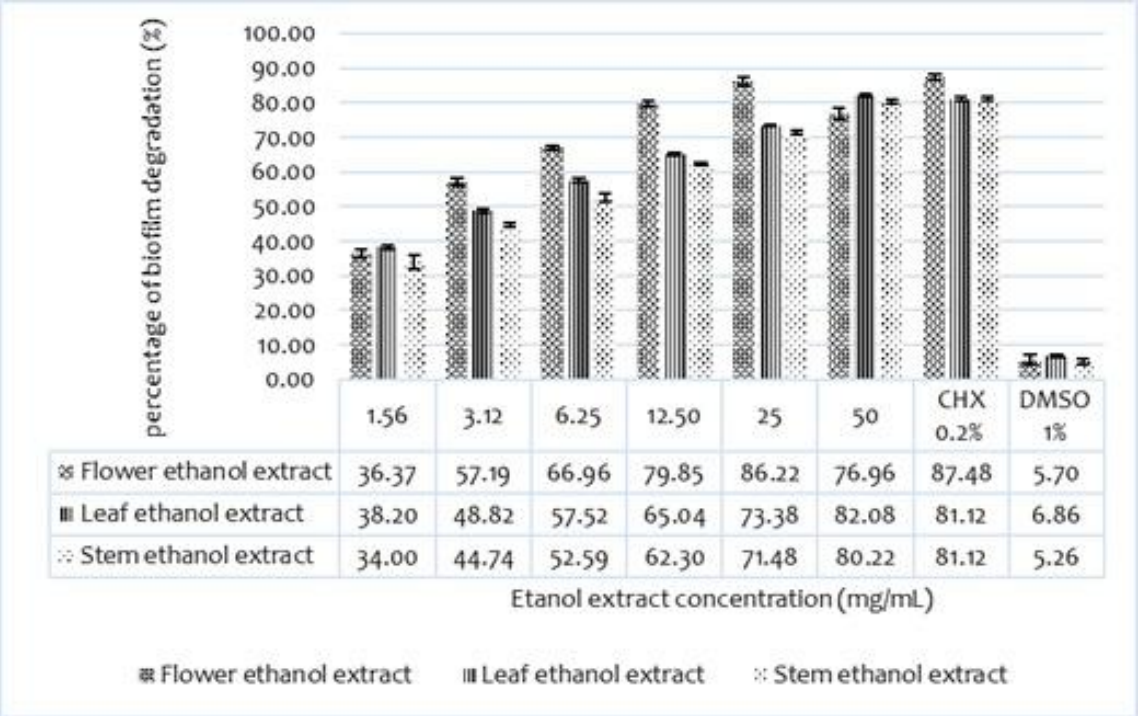

FIGURE 1. The biofilm degradation activity of kecombrang extracts against *F. nucleatum* (n=3 for each treatment group)

Figure 1 indicates that the flower extract demonstrated superior degradation activity in comparison to the leaf and stem extracts, with all extracts exhibiting concentration-dependent effects. The flower extract achieved optimal degradation of 86.22% at 25 mg/ mL, which was comparable to the positive control 0.2% CHX (87.48%), and significantly higher than the negative control 1% DMSO (5.70%).

All three kecombrang ethanol extracts demonstrated concentration-dependent biofilm degradation activity against *F. nucleatum*, with the flower extract exhibiting superior efficacy. The flower extracts achieved the highest degradation activity, ranging from 36.37% (1.56 mg/ mL) to a peak of 86.22% (25 mg/mL), though activity decreased to 76.96% at 50 mg/mL. The leaf extracts showed moderate activity with a consistent dose-response relationship ranging from 38.20% to 82.08% across all tested concentrations. The stem extracts displayed the lowest activity, with

the percentage of degradation increasing from 34.00% to 80.22%.

All extracts showed significant activity compared to the negative control (1% DMSO) which exhibited only 5.70% degradation.

Flower extract at 25 mg/mL (86.22%) demonstrated efficacy comparable to chlorhexidine (87.48%) with no significant difference ($p>0.05$). All extracts exhibited dose-dependent activity within the concentration range of 25-50 mg/mL. $MBEC_{50}$ values were determined using linear regression analysis: the $MBEC_{50}$ of flower extract was 2.62% mg/mL, leaf extract was 3.68 mg/mL, and stem extract was 4.99 mg/mL.

Two-way ANOVA showed significant main effects for both, plant part ($p<0.001$) and extract concentration ($p<0.001$) on biofilm degradation. The interaction effect between plant part and concentration was also statistically significant ($p<0.001$), indicating that the efficacy of different concentrations varied depending on the plant part used. Post-hoc Tukey HSD test showed that flower extract was significantly different from leaf extract and stem extract ($p<0.05$). All extract concentrations showed significant differences from the negative control (p,0.001), with higher concentrations generally showing greater efficacy up to the optimum point.

The present study demonstrates significant biofilm degradation activity of kecombrang extract against *F. nucleatum*, with flower extract showing superior efficacy compared to leaf and stem extracts. These findings offer valuable insights into the potential therapeutic applications of kecombrang as a natural biofilm degradation agent for periodontal disease treatment. The superior biofilm degradation activity of flower extract can be attributed to the higher concentration of bioactive compounds typically found in the reproductive organs of plants[23,24]. $MBEC_{50}$ values below 10 mg/mL suggest potential for development as alternative adjuvant agents in the treatment of periodontal disease.

Our results align with previous studies by Ichsyani *et al.* (2021) who reported similar concentration-dependent effects of kecombrang extracts against *Aggregatibacter actinomycetemcomitans* biofilm, achieving degradation rates of 85.54% for flower extract, 84.43% for leaf extract, and 72.10% for stem extract at optimal concentrations. The

comparable efficacy of kecombrang flower extract (86.22%) to chlorhexidine (87.48%) in our study against *F. nucleatum* suggests its potential as an alternative antimicrobial agent, possibly with fewer side effects than synthetic compounds[14].

The observed decrease in activity at 50 mg/mL concentration for the flower extract may indicate potential cytotoxic effects or precipitation of bioactive compounds at high concentrations. This phenomenon has been reported in other natural product studies and emphasizes the importance of determining optimal therapeutic concentrations. The biphasic response pattern suggests that 25 mg/mL represents the optimal concentration for flower extract, providing maximum biofilm degradation activity without adverse effects[26–28].

Biofilm Degradation Mechanisms

The mechanism of biofilm degradation by kecombrang extracts may involves multiple pathways, potentially similar to those proposed for chlorhexidine, facilitated by the diverse bioactive compounds identified in the phytochemical screening. Based on the literature, alkaloid compounds, being polar, may potentially interfere with peptidoglycan formation and penetrate the extracellular polymeric substances (EPS) layer surrounding bacteria, which could contribute biofilm damage[29,30].

Saponin compounds have been suggested to cause damage to bacterial cytoplasmic membranes, potentially leading to cell lysis. Tannin compounds may precipitate with various types of proteins and polysaccharides, possibly allowing them to pass through cell membranes and potentially suppress enzymes such as glucosyltransferase. The terpenoid content in the extract may bind to transmembrane proteins on the outermost layer, potentially damaging porins and cell membranes, which could reduce cell wall permeability and inhibit bacterial growth[31–33].

The bioactive compounds may potentially disrupt the extracellular polymeric substances (EPS) matrix that provides structural integrity to biofilms. Additionally, the compounds might interfere with bacterial adhesion, quorum sensing communication, and metabolic processes essential for biofilm maintenance. The proposed multi-target approach of natural compounds often results in reduced likelihood

of bacterial resistance development compared to single-target synthetic agents[34]. However, further mechanistic studies are needed to confirm these proposed pathways and elucidate the exact molecular mechanisms responsible for the observed biofilm degradation activity.

Clinical Implications

From a clinical perspective, the biofilm degradation activity of kecombrang extracts presents promising opportunities for developing novel periodontal therapeutic agents. The natural origin of these compounds may offer advantages in terms of biocompatibility and reduced side effects compared to conventional antimicrobials[10,34].

Several limitations of this study should be acknowledged. The in vitro nature of the experiments may not fully represent the complex oral environment where multiple bacterial species interact in polymicrobial biofilms. Future studies should investigate the effects of kecombrang extracts on multispecies biofilms and in vivo models. Additionally, the identification and isolation of specific bioactive compounds responsible for antibiofilm activity would provide valuable insights for developing standardized therapeutic formulations.

The findings also suggest the need for optimization studies to enhance the stability and bioavailability of kecombrang extracts. Formulation approaches such as nanoencapsulation or combination with penetration enhancers could improve the clinical effectiveness of these natural compounds in biofilm degradation applications. However, further research is needed to evaluate the safety profile, optimal formulation, and clinical efficacy of kecombrang-based products.

CONCLUSION

This study demonstrates that ethanolic extracts of kecombrang (*Etlingera elatior*) possess significant biofilm degradation activity against *Fusobacterium nucleatum*. Among the three plant parts tested, flower extract exhibited the highest efficacy, achieving 86.22% biofilm degradation at 25 mg/mL concentration, which is comparable to 0.2% chlorhexidine. The concentration-dependent activity observed across all extracts suggests a potential for developing natural biofilm degradation agents for periodontal disease management. These findings support the traditional use of kecombrang and warrant further investigation for clinical application.

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