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Research Article

Effectiveness of *Matricaria chamomilla* Essential Oil on *Aggregatibacter actinomycetemcomitans* and *Treponema denticola* Biofilms

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KEYWORDS

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Treponema denticola

ABSTRACT

Introduction: *Matricaria chamomilla* (chamomile) is known to possess antimicrobial, anti-inflammatory, and antioxidant properties. **Objectives:** The purpose of this study was to determine how effectively *Matricaria chamomilla* essential oil acts against *Aggregatibacter actinomycetemcomitans* and *Treponema denticola* biofilms in vitro. **Methods:** *Aggregatibacter actinomycetemcomitans* ATCC-29522 and *T. denticola* ATCC-35405 were separately cultured in brain heart infusion (BHI) broth at 37°C for 2 4h in anaerobic conditions. Each bacterial suspension (200 uL, 10^7 CFU/mL) was cultured in 96-well plates for 48 h to form a biofilm. Thereafter, biofilms were treated with chamomile essential oil at concentrations of 3.12%, 6.25%, 12.5%, 25%, 50%, and 100% in a time-dependent experiment. Readings were taken at 1 h, 3 h, 6 h, and 24 h. Biofilm mass was evaluated using crystal violet staining (for *A. actinomycetemcomitans*) and safranin staining (for *T. denticola*). Biofilms treated with chlorhexidine (0.2%) and untreated biofilms were used as positive and negative controls, respectively. Data were statistically analyzed using one-way analysis of variance (ANOVA), with the significance level set to $p<0.05$. **Results:** Chamomile essential oil significantly reduced the biomass of the biofilms ($p<0.05$). The most effective chamomile oil concentrations for inhibiting *A. actinomycetemcomitans* and *T. denticola* biofilms were 100% and 50%, respectively, with 24 h incubation periods. The results of ANOVA and the post hoc Least Significant Difference (LSD) test showed a significant reduction ($p<0.05$) in biofilm mass for all concentrations of chamomile essential oil compared to the negative control across all incubation times. **Conclusion:** The data suggest that chamomile essential oil can inhibit the biofilm formation of *A. actinomycetemcomitans* and *T. denticola* biofilms. It could, therefore, be useful as an alternative treatment to inhibit the biofilms composes of the bacteria tested in periodontal disease cases. However, continued researches are necessary to further explore the mechanisms of this effect.

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INTRODUCTION

Oral diseases are common health problems, which can affect quality of life and general health.[1] Periodontal diseases are prevalent both in developed and developing countries, and they affect approximately 20–50% of the global population.[2] More than 700 different species of bacteria, which cause oral diseases, exist in the oral cavity. One such bacterial disease is periodontal disease.[3]

Periodontal disease affects the tissues supporting teeth, reduces periodontal attachment, and can lead to tooth loss.[2] It is an inflammatory disease caused by bacterial colonization, which forms plaque biofilms. Biofilms are aggregations of microorganisms, which attach to a closed surface in extracellular polymeric substances.[4] Bacteria that can cause periodontal disease include *Aggregatibacter actinomycetemcomitans* and *Treponema denticola*, both of which have a large number of virulence factors with various activities that can damage periodontal tissue.[5] *A. actinomycetemcomitans* is a Gram-negative bacterium.[6] Its high virulence is related to oral cavity colonization and periodontal tissue damage.[7] *T. denticola* as Gram-negative bacterium has also been proven to attach to the fibroblast and epithelial cells of periodontal tissue, inducing the virulence factor.[8]

With research showing the increasing prevalence of oral and tooth disease, awareness concerning these diseases, including periodontitis, is also increasing. Because the etiology of periodontitis is bacterial colonization, which leads to plaque biofilms, preventing biofilm formation can prevent periodontitis.[9] In dentistry, periodontitis can be treated by removing the gingival plaque by scaling and root planning. These treatments can also be combined with antibiotics to reduce gingival inflammation and pocket depth while increasing clinical attachment.[10]

Antibiotic administration in periodontitis treatment has many advantages, but it also has several disadvantages for human health such as bacterial resistance to antibiotic.[11] Thus, alternative medicine should be explored for treating oral disease effectively and economically.[12] Herbal medicines largely have no side effects, so they can be effective alternative treatments.[13] Many studies have proven that herbal treatments can be very useful for inhibiting oral pathogen biofilms.[14–17]According to the World Health Organization (WHO), more than 80% of the global population relies on traditional medicines (including herbs), most of which are grown for primary healthcare purposes.[18] The antimicrobial activities of herbs and herbal products, such as essential oils, have already been reviewed, and essential oils have been shown to be capable of inhibiting the formation of biofilms, which cause periodontitis.[12]

Matricaria chamomilla (chamomile), which originated in Asia and Europe, is a member of the Asteraceae family.[19] It has antioxidant, antimicrobial, antidepressant, and anti-inflammatory properties and is made up of apigenin, caffeic acid, luteolin, flavonoid, and coumarin compounds.[20,21] A previous study has stated that chamomile essential oil could inhibit the growth of *Porphyromonas gingivalis*[22] and *Staphylococcus aureus*.[23] The present research, therefore, is to analyze the effectiveness of chamomile essential oil in inhibiting the biomass developed by *A. actinomycetemcomitans* or *T. denticola* in monospecies biofilm format.

MATERIALS AND METHODS

Chamomile Essential Oil Preparation and Phytochemical Test

Chamomile leaves were obtained from Indonesian Spice and Medicinal Crops Research Institute (ISMCRI), Bogor, West Java, Indonesia . Thirty-five kg of these leaves were dried and subsequently distilled until 100 mL of chamomile essential oil was obtained. This essential oil was diluted with dimethyl sulfoxide (DMSO 5%) and 0.5% tween 20 (v/v) into concentrations of 50%, 25%, 12.5%, 6.25%, and 3.12%. Gradual dilution for each concentration were used to perceive the efficacy. Then, a phytochemical qualitative test was conducted with these samples.

Bacterial Culture

Aggregatibacter actinomycetemcomitans ATCC 29522 and *T. denticola* ATCC 35405 were separately cultured in brain heart infusion (BHI) (Oxoid, Hampshire) agar via the streaking method and were inserted into anaerobic jars containing O_2 (20%), N_2 (30%) and CO_2 (5%) at 37°C and incubated for 24 h. Then, the colonies were re-cultured in tubes with 15 ml of BHI broth. The tubes were closed and incubated for 2 × 24h at 37°C in anaerobic conditions. Each bacterium concentration was measured using an enzyme-linked immunosorbent assay (ELISA) reader (SAFAS MP96, SAFAS, Monaco) at a wavelength of 490 nm until the cultures reached optical density of 0.25–0.3 ($1x10^7$ CFU/mL).[17]

Biofilm Assay

As much as 200 µL ($1x10^7$ CFU/mL) of *A. actinomycetemcomitans* and *T. denticola* were separately distributed into 96-well, flat-bottom microplates (Merck, Darmstadt, Germany) and were incubated for 48 h at 37°C in an anaerobic atmosphere as mention above to form biofilms. Subsequently, the chamomile essential oil was added to the wells at concentrations of 100%, 50%,

25%, 12.5%, and 6.25%, and the inhibition effect was observed after 1 h, 3 h, 6 h, and 24 h. The following sequence of hours was used according to biofilm formation stage. The supernatant was removed by inversion and vibrated, and the plate was rinsed twice with phosphate-buffered saline (PBS). The biomass was measured using crystal violet (0.5% w/v) and safranin (0.5% w/v) sating for *A. actinomycetemcomitans* and *T. denticola*, respectively. The optical density was measured with an ELISA reader (SAFAS MP96, SAFAS, Monaco) at a wavelength of 490 nm by adding 200 µL of 90% ethanol to the wells.[17] The biofilm well without adding chamomile essential oil was used as a negative control, while the biofilm to which 0.2% of chlorhexidine was added served as a positive control.

Data Analysis

The data were analyzed via the Shapiro–Wilk normality test and the homogeneity test. If the data were normally distributed and homogenous ($p>0.05$), then one-way analysis of variance (ANOVA) was applied. If there were significant differences ($p<0.05$), then the post hoc Least Significant Difference (LSD) test was applied, with the significant difference value set to $p<0.05$. (SPSS, IBM, Armonk, NY, USA)

RESULTS

The phytochemical test results showed that chamomile contains alkaloid, saponin, tannin, phenolic, flavonoid, triterpenoid, and glycoside compounds (Table 1). The biofilm assay results showed that chamomile essential oil has an inhibitory effect on *A. actinomycetemcomitans* (Fig. 1) and *T. denticola* (Fig. 2) biofilms, significantly reducing both types of biofilms ($p<0.05$). The most effective chamomile oil concentrations for inhibiting *A. actinomycetemcomitans* and *T. denticola* biofilms were 100% (OD:0,205) and 50% (OD:0,017), respectively compared to negative control (OD Aa: 1,649 and OD Td; 1,930). Result also showed the most significant reduction of biofilms after 24 h incubation periods ($p<0.05$). The results of ANOVA and the post hoc Least Significant Difference (LSD) test showed a significant reduction ($p<0.05$) in biofilm mass for all concentrations of chamomile essential oil compared to the negative control across all incubation times. All treatment was done in triplicate.

Tabel 1. Phytochemical Qualitative Assay

Phytochemical Qualitative Assay	Examination	Examination Result
Chamomile Essential Oil	Phytochemical Examination:	
	Alkaloids	+
	Saponin	+
	Tannin	+
	Phenolic	+
	Flavonoid	+
	Triterpenoid	+
	Steroid	-
	Glycoside	+

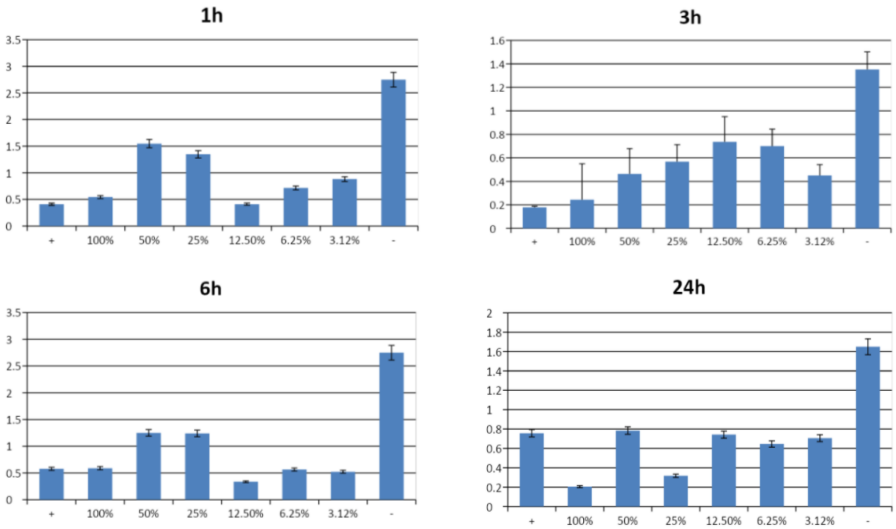

Figure 1. Graphs showing the inhibitory effect of chamomile essential oil against *A. actinomycetemcomitans* biofilm after 1 h, 3 h, 6 h and 24 h. The Y axis is the optical density of the biofilms and the X axis is the chamomile essential oil concentrations (100%, 50%, 25%, 12.5%, 6.25% and 3.12%). Chlorhexidine (0.2%) was used as a positive control and biofilm well without treatment was used as a negative control.

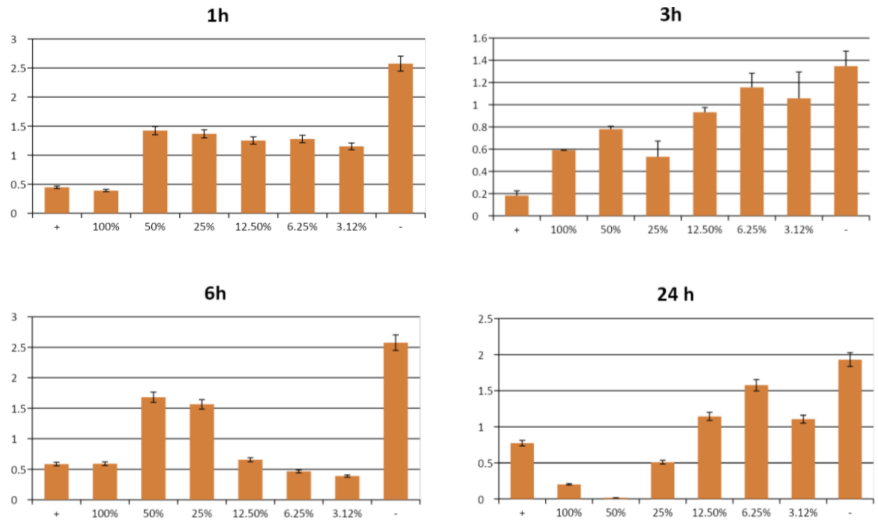

Figure 2. Graphs showing the inhibitory effect of chamomile essential oil against *T. denticola itans* biofilm after 1 h, 3 h, 6 hand 24 h. The Y axis is the optical density of the biofilms and the X axis is the chamomile essential oil concentrations (100%, 50%, 25%, 12.5%, 6.25% and 3.12%). Chlorhexidine (0.2%) was used as a positive control and biofilm well without treatment was used as a negative control.

DISCUSSION

This study suggest that chamomile essential oil can inhibit the biofilm formation of *A. actinomycetem-comitans* and *T. denticola* biofilms. This result is in accordance with studies done by Hans and Chung, et al. They proved that chamomile essential oil effectively inhibits the growth of *S. aureus* and *P. gingivalis.* Chamomile exerts an antibacterial influence by disturbing membrane integrity and inhibiting cell respiration; that is, α-bisabolol promotes the disruption of bacterial cell membranes, allowing them to be permeated by exogenous solutes.[23,24] Therefore, we assumed the same biology mechanism had occurred when the herbal test solution was added into wells containing *A. actinomycetemcomitans* or *T. denticola.* The biofilms developed by each bacterium was found to be decreased after the addition of chamomile essential oil.[25]

The phytochemical test results in this study indicated that chamomile contains alkaloid, saponin, tannin, phenolic, flavonoid, triterpenoid, and glycoside compounds, all of which are known to have an antibacterial effect.[26] Various concentrations of chamomile essential oil were employed in this research to analyze the effectiveness of the oil in small concentrations. Five precents of DMSO and 0.5% Tween 20 (v/v) were used to dilute this essential oil. DMSO is a colorless liquid, which is used as a solvent in water and which dissolves polar and nonpolar compounds.[27] Tween 20 is used as an emulsion agent in some medicines and foods to dissolve essential oil into water-based mixtures.[28] These compound does not have effect on biofilm growth. Essential oils are insoluble in water; thus, the DSMO and Tween 20 were added to create the necessary emulsions.[29]

The following sequence of 1 h, 3 h, 6 h and 24 h incubation period was used according to biofilm formation stage. First stage: the pellicle formation formed on the tooth surface caused by planktonic and free-floating bacteria, second stage: the early colonization of the reversible bacteria and secondary colonization by the irreversible bacteria, and last stage are maturation stage and the dispersion phase where the virulent bacteria disperse and can readily colonize other surfaces.[30] Our data showed that Chamomile essential oil has been proven to inhibit the biofilm formation of *A. actinomycetemcomitans* and *T. denticola* in all incubation period. We also observed that the effective concentrations of chamomile essential oil for inhibiting *A. actinomycetemcomitans* and *T. denticola* biofilms were 100% with and 50%. Recent study has shown that chamomile essential oil has antibacterial, antiviral, antioxidant, and anti-inflammatory properties. Of chamomile's components, α-bisabolol has proven to be a major part of its antibacterial activity.[31] α-bisabolol can inhibit bacterial pathogens by damaging their cell membranes and thereby inhibiting the growth of a biofilm.[32]

CONCLUSION

Chamomile essential oil effectively inhibits *A. actinomycetemcomitans* and *T. denticola* biofilm growth. Chamomile essential oil might be useful as an alternative

treatment of periodontal diseases. However, future studies are needed to further explore to ascertain its efficacy in vivo.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest related to this study.

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