Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Antibiofilm Activity of Eugenol Against *Aggregatibacter actinomycetemcomitans* ATCC 43718 (serotype B)

Diyah Tri Utami [1,2 *], Triana Hertiani [3,4], Sylvia Utami Tunjung Pratiwi [3,4], Ahmad Randy [2], Anif Nur Artanti [1], Dian Eka Ermawati [1], Sholicah Rahmani [1], Heru Sasongko [1], Wisnu Kundarto [1], M Fiqri Zulpadly [1]

[1)]Department of Pharmacy, Universitas Sebelas Maret, Jl. Ir. Sutami 36A, Surakarta, 57126, Indonesia

[2)]Research center for Pharmaceutical Ingredients and Traditional Medicine, National Research, and Innovation Agency (BRIN), Kawasan PUSPIPTEK, Serpong, South Tangerang, Banten 15314, Indonesia

[3)]Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Sekip Utara, Yogyakarta 55281, Indonesia.

[4)] Pusat Kolaborasi Riset Biofilm (Biofilm Research Collaboration Centre, UGM-BRIN), Jl. Farmako Sekip Utara, Yogyakarta 55281, Indonesia

Abstract

Eugenol is the major constituent [70% to 90%] in the aromatic oil extract from *Syzygium aromaticum* (cloves) and *Cinnamomum zeylanicuma*, which is widely used as a flavouring for meats, stews, cakes, and teas. This plays a major role in the microbial activity against oral bacteria, specifically *Aggregatibacter actinomycetemcomitans* ATCC 43718 (serotype B*)*, which causes periodontitis in humans. Therefore, this study aims to evaluate eugenol for its antibiofilm activity against *Aggregatibacter actinomycetemcomitans* ATCC 43718 (serotype B). An inhibition growth assay was used with the microdilution method, which often utilized a 96-microplate with an anaerobic technique. Absorbance was also measured by a microplate reader on λ of 595 nm, with the optical density (OD) value used to determine the inhibition percentage. To evaluate biofilm bacterial growth after the treatment, SEM analysis was adopted. The results showed that eugenol had an inhibitory effect on *A. actinomycetemcomitans* at 0.125% v/v, according to the $MBIC_{50}$ value. Eugenol showed significant antibiofilm activity ($P < 0.05$) against *A. Actinomycetemcomitans.* This proved that the biofilm bacterial growth was effectively inhibited by the chemical compound. In this case, the extracellular polymeric substance (EPS) matrix degraded after the treatment of the cell biofilm. Based on these results, eugenol was observed to have a great potential in periodontitis, as an inhibitor against the biofilm growth of *A. actinomycetemcomitans* ATCC 43718 (serotype B).

Keywords: Biofilm, Eugenol, *Aggregatibacter actinomycetemcomitans* ATCC 43718 (serotype B)

1. Introduction

A complex microbial community is found to inhabit the oral cavity, where biofilm oral may cause periodontitis and caries [1,2]. Furthermore, periodontitis is a one of the periodontal diseases [3], and dental plaque is reported to cause the presence of a biofilm of anaerobic bacteria [4]. The initial chronic form of gingivitis is also related to periodontitis, as several previous reports showed its association with some systematic diseases. These types of chronic oral infections are often observed as risk factors for several health disorders, including rheumatoid arthritis, insulin resistance, osteoporosis, and pregnancy complications [5]. For biofilms, the use of antibiotics is often infective due to the presence of an extracellular matrix [6–10]. This indicates that bacterial growth on teeth is prevented by regular brushing and administration of antibiotics, including chlorhexidine, fluoride, and cetylpyridinium chloride. However, these drugs have some side effects, such as digestive tract irritation and tooth colour transformation [11].

Eugenol is a chemical compound with antibiofilm and antibacterial activities. It is reported to

*Corresponding author e-mail: diyahtriutami12@staff.uns.ac.id.; (Diyah Tri Utami).

Receive Date: 10 June 2022, **Revise Date:** 27 July 2022, **Accept Date:** 02 August 2022, **First Publish Date:** 02 August 2022
DOI: 10.21608/EJCHEM.2022.143899.6278

have polymicrobial inhibition biofilms of *Streptococcus mutans, Actinomyces viscosus, Streptococcus sanguinis,* and *Lactobacillus acidophilus* [12]. This was in line with Utami et al. (2021), where eugenol degraded the extracellular matrix of polymicrobial biofilms. It also had the inhibition biofilm activity of *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli,* and *Candida albicans* [13]. With the increasing threat of periodontitis, the effects of this chemical compound against *A. actinomycetemcomitans* ATCC 43718 (serotype B) need to be highly considered. Therefore, this study aims to determine the effectiveness of eugenol, as an antibiofilm agent in *A. actinomycetemcomitans* ATCC 43718 (serotype B).

Material and Methods

Materials

The materials used in this study included eugenol (Sigma Aldrich, Germany), crystal violet (Himedia, India), 96 flatbottom polystyrene microplate (Iwaki, Japan), sucrose (Oxoid, UK), Brain Heart Infusion (BHI) (Oxoid, UK), coverslip (Thermo Scientific, US), MHA (Muller Hinton agar; Oxoid, UK), 95% ethanol MRS (Merck, Germany), and anaerogen gas pack (Oxoid, UK).

Equipment

The utilized equipment included a microtiter plate reader (optic Ivymen System 2100-C, Spain), Laminar air flow, incubator, multichannel micropipette (Socorex, Swiss), autoclave (Sakura, Japan), micropipette of 2-10 µL, 20-200 µL, and 100-1000 µL (Socorex, Swiss), micropipette pipetman (Gilson, France), spectrophotometry (Geneys 10 UV Scanning, 335903; Thermo Scientific, USA), and flat bottom polystyrene 96 well (Iwaki, Japan).

Test Organisms: Bacterial Strains

The *A. actinomycetemcomitans* ATCC 43718 (serotype B) bacterial strains were used in this study. These organisms were cultured on Brain Heart Infusion (BHI) and anaerobically incubated at 37°C for 18-24 h.

Biofilm Formation Inhibition Assay In Vitro

Eugenol was diluted with dimethyl sulfoxide (DMSO), using different concentration levels, i.e., 0.125% v/v, 0.25% v/v, 0.5% v/v and 1% v/v. The positive control also used a Listerine® mouthwash of 1% v/v, with the well plates anaerobically incubated at 37°C for 24 h. Furthermore, a 100 µL BHI containing 2% sucrose, bacterial suspension, and eugenol, was added to the well plates and incubated at 37°C for 24 h. These biofilms were stained with a crystal violet of 1% v/v, as a total of 95% ethanol was then added to the well plates. The well plates were also measured by a microplate reader at 595 nm, with three replicates being produced in this analysis [14].

Scanning Electron Microscope Analysis

Based on the scanning electron microscope analysis, the bacterial strains were grown on the coverslip in an anaerobic condition, using various eugenol concentrations for 24 h at 37°C. In this case, Listerine® was used for positive control, as the biofilms growing without test compounds functioned as control sources. Based on previous study, biofilms used an anaerobic condition for incubation [12]. Coverslips were also opened and washed with sterile aquadest, accompanied by the cleansing with 1% glutaraldehyde. These were coated using carbon tape, placed into an auto fine coater, and analyzed by SEM 6400 [14].

Statistical Analysis

Data were analyzed using the windows 16.0 version of the Statistical Package for Social Sciences (SPSS Inc., Chicago, USA), while significance was determined through a one-way ANOVA method. This was accompanied by posthoc Bonferroni tests, and differences were considered significant with $P < 0.05$

Results

Determination of $MBIC_{50}$ of Eugenol for *A. actinomycetemcomitans ATCC 43718 (serotype B)*

Biofilm Growth Inhibition

The MIC_{50} was determined to analyze the activity of eugenol against *A. actinomycetemcomitans.* Based on the results, the biofilms were highly inhibited by the chemical compound, indicating a significant effect with $P < 0.05$. The data also showed that the application of 1% and 0.125% v/v of eugenol had the highest and lowest antibiofilm effects on *A. actinomycetemcomitans*, respectively. This proved that the increasing concentration of the chemical compound caused a decrease in the growth of biofilms (Figure 1).

A. actinomycetemcomitans is an anaerobic facultative bacteria, which adult population often causes chronic oral inflammation [15]. From the results, eugenol showed oral biofilm inhibition at an $MBIC_{50}$ value of 0.125% v/v. However, a 1% v/v concentration was highly effective on *A. actinomycetemcomitans.*

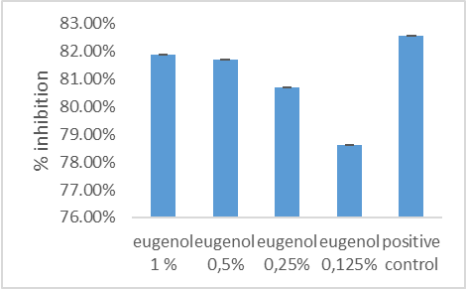

Figure 1. Eugenol biofilm inhibition against *A. actinomycetemcomitans*

Scanning Electron Microscope Analysis

SEM was used to display the morphological changes of *A. actinomycetemcomitans* biofilms. After the cell treatment with eugenol, the extracellular polymeric substance (EPS) matrix was degraded and inhibition was observed for biofilm growths.

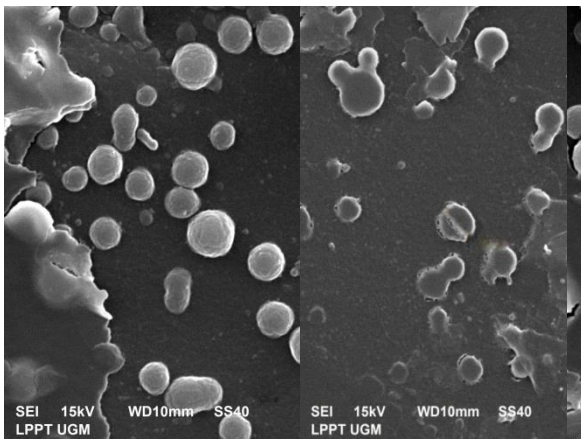

Figure 2. (a) The biofilm formation of A. *Actinomycetemcomitans* ATCC 25175 on coverslips was monitored by SEM, at a: control (untreated) cell, (b) cells pre-treated with eugenol at 0.125 % v/v

Discussion

Oral biofilms cause periodontal disease and caries, as the infection is consistent with some systematic disorders, such as rheumatoid, cardiovascular, and neurodegenerative disparities [9,16,17]. In this study, eugenol was evaluated as an antibiofilm agent, regarding the biological effects in inhibiting the growth and formation of *A. actinomycetemcomitans*, which causes periodontitis. The high biofilm formation of this bacterial strain also leads to great persistence. Based on Figure 1, eugenol inhibited the growth of *A. actinomycetemcomitans* at various concentrations, with 0.125% v/v being the minimum effective value. This indicated that the lowest concentration exhibited optimal inhibition performances, irrespective of its analytical quantity. Therefore, eugenol was observed as a potent inhibition agent against *A. actinomycetemcomitans* at various concentrations. This was in line with a previous study, where the chemical compound had an antibacterial effect against *A. actinomycetemcomitans* and *S. mutans.* Clove oil contains eugenol as a major component, which active mechanism causes protein synthesis denaturation and cell membrane disruption in various microorganisms [18]. The mechanism also caused the quorum sensing suppression of *S. mutans* [19,20].

According to previous reviews, eugenol showed an inhibition effect against the formation of monomicrobial and polymicrobial biofilms, such as *Streptococcus mutans, Streptococcus sanguinis, Actinomyces viscosus,* and *Lactobacillus acidophilus.* It was also confirmed to have the antimicrobial activity as well as was higher and greater than thymol. In addition, the mechanism degraded the EPS matrix [12,21] and suppress the quorum sensing of *S. mutans* [19].

The results showed that eugenol was capable of degrading the EPS of *A. actinomycetemcomitans* biofilms. In this present study, 1% v/v of Listerine was used as the positive control, due to containing menthol, thymol, sodium fluoride, *Camelia sinensis* (Green tea leave extract), and methyl salicylate. A shown in Figure 2, the damaging of the cell membrane disruption of *A. actinomycetemcomitans* biofilms was observed. This confirmed that eugenol disrupted the bacterial process for nutrient transport and lysis.

One limitation of this study was that only one bacterial strain was evaluated while periodontitis infections are polymicrobial. In further research, eugenol should be studied as a toothpaste formulation to determine if it enhances the elimination of bacteria in a shorter time.

Conclusion

Eugenol showed an antibiofilm effect against *A. actinomycetemcomitans*, subsequently promoting its development as a new alternative against oral biofilms.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

The authors are grateful to Penelitian Hibah Grup Riset (Penelitian HGR-UNS) A (Research group grant, HGR-UNS) A, which has supported this study through Universitas Sebelas Maret research Project 2022, No. 254/UN27.22/PT.01.03/2022.

References

1. Szafrański SP, Deng ZL, Tomasch J, Jarek M, Bhuju S, Rohde M, et al. Quorum sensing of Streptococcus mutans is activated by Aggregatibacter actinomycetemcomitans and by the periodontal microbiome. BMC Genomics. 2017 Mar 20;18(1):238.
2. Utami DT, Pratiwi SUT, Haniastuti T, Hertiani T. Cinnamaldehyde's Potential Inhibitory Effect towards Planktonic and Biofilm of Oral Bacteria. IJPR [Internet]. 2020 Dec 2 [cited 2022 Jan 24];13(01). Available from: http://www.ijpronline.com/ViewArticleDetail.aspx?ID=18536
3. Nagasawa T, Shimizu S, Kato S, Nakatsuka Y, Kado T, Hidaka T. Host-microbial co-evolution in periodontitis associated with27 Aggregatibacter actinomycetemcomitans

infection. Journal of Oral Biosciences. Journal of Oral Biosciences. 2014;56:11–7.
4. Kesić L, Petrović M, Obradović R, Pejčić A. The Importance of Aggregatibacter Actinomycetemcomitans in Etiology of Periodontal Disease. Acta Medica Medianae. 2009;48(35–37).
5. Arigbede A, Babatope BO, Bamidele MK. Periodontitis and systemic diseases: A literature review. J Indian Soc Periodontol. 2012;16(4):487–91.
6. Hamzah H, Hertiani T, Utami S, Nuryastuti T. Efficacy of Quercetin against Polymicrobial Biofilm on Catheters. Research Journal of Pharmacy and Technology. 2020 Nov 1;13:5277–82.
7. Hamzah H, Hertiani T, Utami S, Nuryastuti T, Puspitasari A. Antibiofilm studies of zerumbone against polymicrobial biofilms of Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Candida albicans. International Journal of Pharmaceutical Research. 2020 Aug 11;12:1307–14.
8. Hertiani T, Pratiwi S, Hamzah H. The Inhibition Activity of Tannin on the Formation of Mono-Species and Polymicrobial Biofilm Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans. Majalah Obat Tradisional. 2019 Sep 12;24:2019.
9. Utami DT, Tunjung Pratiwi SU, Spaink HP, Haniastuti T, Hertiani T. Antibiofilm effect of C-10 massoia lactone toward polymicrobial oral biofilms. J Adv Pharm Technol Res. 2021 Mar;12(1):89–93.
10. Yang L, Liu Y, Wu H, Høiby N, Molin S, Song Z. Current understanding of multi-species biofilms. International Journal of Oral Science. 2011;3(2):74–81.
11. Gursoy UK, Gursoy M, Gursoy OV, Cakmakci L, Könönen E, Uitto VJ. Anti-biofilm properties of Satureja hortensis L. essential oil against periodontal pathogens. Anaerobe. 2009 Aug;15(4):164–7.
12. Utami D, Pratiwi ST, Haniastuti T, Hertiani T. Eugenol and thymol as potential inhibitors for polymicrobial oral biofilms: An in vitro study. J Int Oral Health. 2021;13(1):45.
13. Hamzah H, Pratiwi SUT, Hertiani T. Efficacy of Thymol and Eugenol Against Polymicrobial Biofilm. Indonesian Journal of Pharmacy. 2018 Dec 17;29(4):214.
14. Ahmed H, Salih R, Salih A. Evaluation of local Origanum vulgare aqueous extract for eradication of biofilm production bacteria. Egypt J Chem. 2021 Aug 18;65(2):413–9.
15. Utami DT, Pratiwi SUT, Haniastuti T, Hertiani T. Degradation of Oral Biofilms by Zerumbone from Zingiber zerumbet (L.). Research Journal of Pharmacy and Technology. 2020 Aug 12;13(8):3559–64.
16. Jin L, Armitage G, Klinge B, Lang N, Tonetti M, Williams R. Global oral health inequalities: task group--periodontal disease. Advances in dental research [Internet]. 2011 [cited 2022 Jun 7];23(2). Available from: https://pubmed.ncbi.nlm.nih.gov/21490234/
17. Fiorillo L, Cervino G, Laino L, D'Amico C, Mauceri R, Tozum TF, et al. Porphyromonas gingivalis, Periodontal and Systemic Implications: A Systematic Review. Dent J (Basel). 2019 Dec 11;7(4):114.
18. Utami DT, Pratiwi SUT, Haniastuti T, Hertiani T. Efficacy of Quercetin on Degradation of Streptococcus sanguinis and Streptococcus mutans Biofilms. International Medical Journal. 2020;25(04):8.
19. Nuñez L, Aquino MD. Microbicide activity of clove essential oil (Eugenia caryophyllata). Braz J Microbiol. 2012 Oct;43(4):1255–60.
20. Adil M, Singh K, Verma PK, Khan AU. Eugenol-induced suppression of biofilm-forming genes in Streptococcus mutans: An approach to inhibit biofilms. J Glob Antimicrob Resist. 2014 Dec;2(4):286–92.
21. Karicheri R, Antony B. Antibacterial Activity of Essential Oil of Syzygium aromaticum (L.) Merr. Perry (Cloves) against Clinical Isolates of Aggregatibacter actinomycetemcomitans. International Journal of Applied Biology Pharmacetuical Technology. 2015;6(2):157–61.
22. Jabbar A, Hamzah H, Nandini E, Nurwijayanto A, Setyowati E, Syakri S, et al. The Effectiveness of Begonia Multangula Blume Leaf Ethanol Extract as Polymicrobial Antibiofilm on Catheters. Egyptian Journal of Chemistry [Internet]. 2022 Apr 6 [cited 2022 Jul 20];0. Available from: https://ejchem.journals.ekb.eg/article_229425.html