# Inhibitory Effects of Lemongrass Oil on Multispecies Subgingival Biofilm Formation *in Vitro*

*Pinsuwan W* Suwannarong W** Khunkitti W*** Taweechaisupapong S**


## *Abstract*

*Cymbopogon citratus (lemongrass) oil is a volatile oil obtained from the lemongrass leaves. It was reported to exhibit antibacterial activity towards periodontal pathogens, such as Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis. However, there is no report on inhibitory effects of lemongrass oil (LG) against multispecies subgingival biofilm formation. The purpose of this study was to investigate the inhibitory effects of LG on subgingival biofilm formation using an in vitro model. Subgingival plaque samples from 5 periodontitis patients were cultivated in saliva-coated 96-well microtiter plates in the presence of LG at concentrations 1 - 6 µL/mL under anaerobic atmosphere at 37°C for 2, 4 and 8 days by replacing every 2 days with fresh medium alone or LG-containing medium. Quantification of biofilm formation was determined by staining with crystal violet. TaqMan-based real-time polymerase chain reaction was used to quantify A. actinomycetemcomitans and total bacteria in subgingival biofilm. The results showed that LG exhibited more than 80% anti-biofilm activity at 6 µL/mL. Moreover, LG at concentration 6 µL/mL was able to kill all A. actinomycetemcomitans in subgingival biofilm on day 2, 4, and 8. These results revealed that LG is able to inhibit in vitro subgingival biofilm formation and reduce the numbers of A. actinomycetemcomitans in multispecies subgingival biofilm.*


***Key words**: Aggregatibacter actinomycetemcomitans/ Subgingival biofilm/ Lemongrass oil/ Periodontitis*

***Received:** August 27, 2019*
***Revised:** February 08, 2020*
***Accepted:** March 04, 2020*

## *Introduction*

According to the World Health Organization, 10-15% of the world populations suffer from severe periodontitis.[1] Most common infections of the oral cavity, e.g. caries, gingivitis and periodontitis, are resulted from the accumulation of biofilms. It is well documented that bacteria growing in a biofilm, as is the case of periodontitis, are more recalcitrant to the action of antibiotics than bacteria growing in a planktonic state.[2] The bacterial species within subgingival biofilms were grouped into six bacterial complexes as described by Socransky.[3,4] Socransky identified a red complex harboring *Porphyromonas gingivalis, Tannerella forsythia*, and *Treponema denticola*. Further complexes were orange, purple, green, yellow, and blue complex. The orange complex is consisted of *Fusobacterium nucleatum/ periodonticum* subspecies, *Prevotella intermedia, Prevotella nigrescens* and *Peptostreptococcus micros*.

The purple complex is comprised of *Veillonella parvula, Actinomyces odontolyticus, Aggregatibacter actinomycetem comitans* serotype b, *Selenomonas noxia* and *Actinomyces-naeslundii* genospecies 2 (*A. viscosus*). The green complex is consisted of *Capnocytophaga* species, *Camphylobacter concisus, Eikenella corrodens* and *Aggregatibacter actino-mycetemcomitans* serotype a. The yellow and blue complex are dominated by *Streptococcus* and *Actinomyces* species, respectively

Periodontitis is a polymicrobial infection caused by a group of specific microorganisms such as *A. actinomycetem comitans*, *P. gingivalis*, *T. denticola, P. intermedia* and *T. forsythia*[5-7] that are cohabitating in subgingival plaque. *A. actinomycetemcomitans* is the primary etiologic agent of localized aggressive periodontitis that causes rapid attachment and bone loss and has shown to adversely affect

** Biofilm Research Group, Faculty of Dentistry, Khon Kaen University, Amphur Muang, Khon Kaen.*
*** Department of Oral Biomedical Sciences, Faculty of Dentistry, Khon Kaen University, Amphur Muang, Khon Kaen.*
**** Faculty of Pharmaceutical Sciences, Khon Kaen University, Amphur Muang, Khon Kaen.*

the psychology, function and aesthetic of an individual.[8-10] A study in a Thai population reported that the presence of *P. gingivalis,* and high colonization by *A. actinomycetem comitans, T. denticola*, and *P. intermedia* play an important role in severe periodontitis.[11] A combination of *A. actinomycetemcomitans, P. gingivalis*, and *P. intermedia* also showed the strongest association with the disease in a Finnish population.[12] Another Finnish study reported that the presence of *A. actinomycetemcomitans* with *P. gingivalis* and *T. denticola* in saliva has been shown to contribute to deepened pockets.[13] However, the presence of *A. actinomycetemcomitans* in mild chronic periodontitis in a Thai population was also reported.[7]

The complexity of the subgingival microbiota of periodontal plaque and interplay of host-immune response makes the disease difficult to treat. Previous studies reported resistance of periodontopathic bacteria isolated in patients with chronic periodontitis to several antimicrobial agents commonly used in anti-infective periodontal therapy.[14,15] With the rise in bacterial resistance to antibiotics, there is considerable interest to search for other classes of antimicrobials for the control of subgingival plaque.

Our previous studies reported that *Cymbopogon citratus* (lemongrass) oil exhibit effective killing activity against several different bacteria, i.e. *A.actinomycetemcomitans* ATCC 43718, *P. gingivalis* W50, *Streptococcus mutans* ATCC 25175, *Staphylococcus aureus, Streptococcus agalactiae, Bacillus cereus* and *Escherichia coli* and possessed a strong inhibitory effect on Candida biofilms.[16-21] The minimum growth inhibitory and minimum bactericidal concentration values of lemongrass oil (LG) against *A. actinomycetemcomitans* ATCC43718 were 0.0313 and 0.0625 µL/mL, respectively.[17] The killing and anti-biofilm activity of LG can be attributed to the presence of various chemical components in the oil such as citral and geraniol[16,17,19] which lead to a decrease in the bacterial load and inhibit plaque biofilm formation. Several studies have demonstrated that terpenes (i.e. citral, geraniol, linalool, menthol, and thymol), which are the major components of essential oils, alter cell permeability by penetrating between the fatty acyl chains making up the membrane lipid bilayers, disrupting lipid packing and changing membrane fluidity.[22,23] Braga and Dal Sasso (2005) suggested that these phenomena led to major surface alterations and morphological modifications.[23] However, no report about the spectrum of action of LG against multispecies subgingival biofilm formation was found. In order to provide more information about LG for its potential development as a new potential therapeutic agent which may help in the treatment of periodontitis, we evaluated the inhibitory effects of LG on biofilm formation *in vitro* using subgingival biofilm model.

## Materials and Methods

**Preparation of lemongrass oil** LG was purchased from Thai China Flavours & Fragrances Industry Co. (Thailand). They were dissolved in 95% ethanol to an initial concentration of 900 µL/mL and further diluted with the solution contained 5% ethanol and 5% Tween 80 to a concentration of 64 µL/mL before used.

**Subjects and sampling of subgingival plaque** Subjects were healthy Thai adults who sought periodontal treatment at the Faculty of Dentistry, Khon Kaen University, Khon Kaen, Thailand. The criteria used for selection included: subjects between the ages of 35 and 45 years with no known systemic diseases, who had not received antibiotics within the previous 3 months, and who were not taking medications that might influence the subgingival microbiota. The study protocol was approved by the Khon Kaen University ethical review committee. Informed consent was obtained from all volunteers. Then subgingival plaque samples were collected from five individuals with chronic periodontitis. The sampling site had pocket probing depths ranging from 4 to 5 mm and clinical attachment loss ≥ 3 mm. The subgingival plaque was collected by inserting a sterile absorbent paper point to the sulcus depth and moving it laterally along the tooth surface and the sulcular epithelial lining. The paper point sample was immediately placed into 1-ml thioglycollate medium and gently sonicated to disperse the bacterial cells.

**Saliva collection and processing** Unstimulated saliva was obtained in 5-mL samples from the same subjects who had donated subgingival plaque and processed as previously described.[24-26] In brief, each saliva sample was diluted (1:10) with pre-reduced, anaerobically-sterilized Ringer solution, containing 0.05% cysteine (Sigma Chemical Co, St Louis, MO) as a reducing agent, and centrifuged at 2,000 x g for 10 min to remove any particulate matter. The supernatant was passed through 0.45 µm sterile syringe filter.

**PCR detection of *A. actinomycetemcomitans* in subgingival plaque** Subgingival plaque samples were suspended in 1 mL sterile double distilled water, pelleted, and resuspended in 200 µL of DNA isolation reagent (InstaGene Matrix, Bio-Rad Lab, Hercules, CA, USA). Total DNA was extracted according to the manufacturer's instructions. The suspension was centrifuged and 5 µL of resulting supernatant was used for PCR. The PCR reactions (Taq PCR Core Kit, Qiagen, Valencia, CA, USA) were carried out as previously described using oligonucleotide primers specific for *A. actinomycetemcomitans*, sense 5' AAA CCC ATC TCT GAG TTC TTC TTC 3', antisense 5' ATG CCA ACT TGA CGT TAA AT 3'.[27] The PCR product was analyzed by 1% agarose gel electrophoresis. DNA of *A. Actinomycetem comitans* ATCC 43718 was used as positive control.

**Inhibitory effects of lemongrass oil on subgingival biofilm formation** To determine the effects of LG in inhibition of biofilm formation in test groups, subgingival plaque sample from each periodontitis patient was cultivated in saliva-coated 96-well microtiter plate in the presence of LG at concentrations 1-6 µL/mL under anaerobic atmosphere at 37°C for 2, 4 and 8 days.[26,28] A bacterial suspension in the wells contained only medium without LG served as a control group. The initial count of total bacteria, which were inserted into each well, was $1x10^6$ CFU/mL. For model 1, the supernatant was removed at 48-h intervals and the wells were added with fresh medium without LG. For model 2, the supernatant was removed and the wells were added with medium containing 1-6 µL/mL LG at 48-h intervals. At the indicated times (2,4 and 8 days), the supernatant in each well was removed and the wells were washed with 200 µL of sterile phosphate buffered saline. The attached bacteria were stained for 15 min with 150 µL of 1% crystal violet. Excess stain was removed with running tap water. The plates were air dried and the dye bound to the adherent cells was solubilized with 150 µL of 33% (v/v) glacial acetic acid per well. After transferring the dye solution to a new plate, the optical density (OD of each well was measured at 595 nm using microplate reader (Varioskan Flash, Thermo Fisher Scientific Inc., USA). Percentage of biofilm formation inhibition of LG was calculated using the formula [1-($OD_{595}$sample/$OD_{595}$control)x100. Concentrations of LG exhibiting 50% inhibition ($IC_{50}$) of subgingival biofilm formation in each model were determined. All experiments were repeated on three separate occasions, with triplicate determinations in each experiment.

**Real-time PCR** The bacterial DNAs from biofilm in 96-well microtiter plate were extracted by using Insta Gene Matrix (Bio-Rad Lab., CA, USA) according to the manufacturer's instructions. Dilutions of known amounts of reference strains DNA (10-$10^8$ cells) were used to determine the standard curve for real-time quantification.

Primers and probes[29,30] used in this study are shown in Table 1. Taq Man probes were labeled with the reporter dye 6-carboxyfluorescein (FAM) at the 5' end and with non-fluorescent quencher (NFQ) at the 3' end. Amplification and detection of bacterial DNA by real-time PCR were performed using Applied Biosystem 7500 Fast Instrument (Applied Biosystems, USA). Samples were assayed in a 20 µL reaction mixture containing 2 µL of template DNA, 10 µL of 2X TaqMan® Universal PCR Master Mix (Applied Biosystem), 1 µL of 20X assay mix containing probe, forward and reverse primers (Applied Biosystem). The cycling conditions used were as follows: 50°C for 2 min, 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min each. All data were analyzed using the Applied Biosystem 7500 Fast software.

**Table 1** Real-time PCR primers and probes used in this study

| Primers and Probes | Product size (bp) | Reference |
|---|---|---|
| ***A. actinomycetemcomitans*** | 77 | Morillo et al. 2004 |
| Forward 5'-ACGCAGACGATTGACTGAATTTAA-3' | | |
| Reverse 5'- GATCTTCACAGCTATATGGCAGCTA-3' | | |
| Probe 5'- FAM-TCACCCTTCTACCGTTGCC- NFQ-3' | | |
| **Total bacteria** | 466 | Nadkarni et al. 2002 |
| Forward 5'-TCCTACGGGAGGCAGCAGT-3' | | |
| Reverse 5'-GGACTACCAGGGTATCTAATCCTGTT-3' | | |
| Probe 5'-FAM- CGTATTACCGCGGCTGCTGGCAC-TAMRA-3' | | |

To determine the validity of using the universal probe and primers set to estimate the total number of bacteria in a mixed culture, three bacteria, *P. gingivalis*, *A. actinomycetemcomitans* and *Streptococcus mutans*, were grown separately *in vitro* and equal volumes of the three cultures mixed together. The number of *P. gingivalis*, *A. actinomycetemcomitans* and *S.mutans* in CFU was determined by serial dilution on agar plates and compared with the relative bacterial load determined by real-time PCR using the universal probe and primers set.[29]

**Statistical analyses** Kruskal-Wallis analysis and Dunn's multiple comparison tests were carried out for comparison of biofilm formation and bacterial number between the test and control groups in both models. P values of <0.05 was considered statistically significant.

## Results

*A. actinomycetemcomitans* in the subgingival plaque samples from all periodontitis patients in this study was verified by PCR. The results showed that all subgingival plaque samples harboring *A. actinomycetemcomitans* (Figure 1). The anti-biofilm activity of LG depended on the dose and more than 80% anti- biofilm activity was observed at concentration 6 µL/mL on day 2, 4, and 8 of both models (Figure 2). The 50% inhibition concentrations ($IC_{50}$) of LG in each model were showed in Table 2. It was clear that older biofilm showed less susceptible to LG. However, LG at concentration 6 µl/mL was able to kill all *A. actinomycetemcomitans* in subgingival biofilm on day 2, 4, and 8 of both models (Figure 3A). Although lower total bacterial numbers in subgingival biofilm of test groups were found at concentration 6 µL/mL on day 2, 4, and 8 of both models, they were not significantly different compared with those of untreated control in both models (Figure 3B).


**Figure 1** Detection of *A. actinomycetemcomitans* DNA using agarose gel electrophoresis. The gel shows staining for *A. actinomycetemcomitans* DNA amplified by conventional PCR[27] from five subjects. Lane 1: negative control; Lane 2: *A. actinomycetemcomitans* ATCC 43718; Lane 3-7: subgingival plaque samples from subjects No. 1-5, respectively; Lane 8: a 100 bp DNA ladder as a molecular weight marker

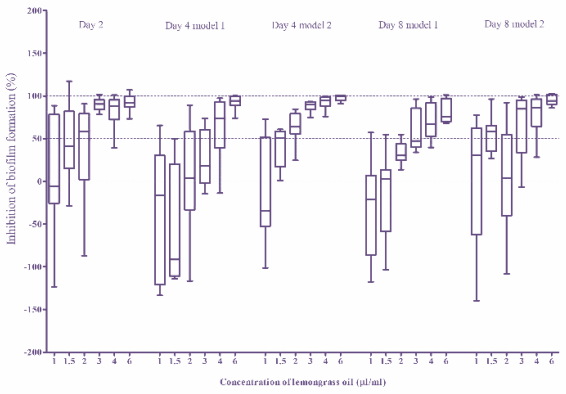


**Figure 2** Effects of different concentrations of lemongrass oil on biofilm formation. The presented percentages of inhibition were calculated from the formula [1-($OD_{595}$ sample/$OD_{595}$ control)]x100 %. Results are from 5 periodontitis patients (N= 5). The upper and lower box margins represent 75$^{th}$ percentile and 25$^{th}$ percentile, respectively. The horizontal line inside each box indicates the median (50$^{th}$ percentile).

**Table 2** Concentrations of lemongrass oil exhibiting 50% inhibition ($IC_{50}$) of subgingival biofilm formation in each model

| Day | 2 | 4 model 1 | 4 model 2 | 8 model 1 | 8 model 2 |
|---|---|---|---|---|---|
| $IC_{50}$ (µl/ml) | 1.7 | 3.6 | 1.8 | 3.1 | 2.9 |
| 95% Confidence Intervals | 1.5 – 1.9 | 2.9 – 4.4 | 1.6 – 2.0 | 2.2 – 4.3 | 1.7 – 4.8 |

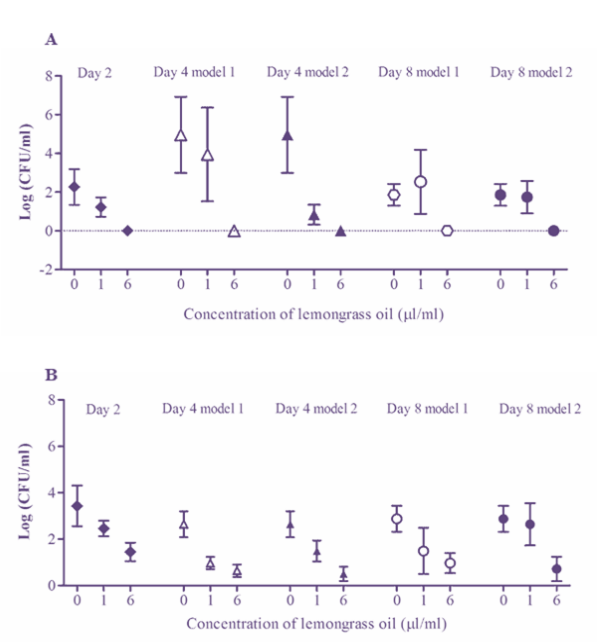


**Figure 3** Effects of lemongrass oil on cell numbers of *A. actinomycetemcomitans* (A), and total bacteria (B) in subgingival biofilm of tested and untreated control groups.

## Discussion

Essential oils are well known for having antibacterial effect. From our previous studies, we found that LG possessed antibacterial activity towards periodontal pathogens growing in a planktonic state.[17] Inhibitory effect of LG on monospecies biofilm formation was also reported.[16] In addition, LG exhibited the strong inhibitory effects against several *Candida* spp. in both planktonic and biofilms form.[19,20] In the present study, we further investigated the effects of LG on multispecies biofilm formation and biofilm-grown bacteria. Our data demonstrated that LG is able to inhibit *in vitro* subgingival multispecies biofilm formation and reduce the numbers of *A. actinomycetemcomitans* and total bacteria. These results support the antimicrobial properties of LG against *A. actinomycetemcomitans* that were reported from our previous study.[17] However, killing activity of LG against *A. actinomycetemcomitans* in multispecies biofilm was observed at concentration of 6 µL/mL which was much higher than the minimum bactericidal concentration of LG against *A. actinomycetemcomitans* growing in a planktonic state (0.0625 µL/mL) found in our previous report.[17] These findings are consistent with several studies which reported that biofilm bacteria can be up to 1,000 times more resistant to antimicrobial agents than their planktonic counterpart.[31,32] In addition, multispecies biofilms are more resistant to antimicrobial agents than monospecies biofilm.[33-35]

*A. actinomycetemcomitans* is one of the most common bacteria involved in periodontal disease. It is associated with the localized aggressive form of periodontitis that causes rapid attachment and bone loss.[9,10] In this study, we found that all subgingival plaque samples harboring *A. actinomycetemcomitans* (Figure 1). Although LG at concentration 6 µL/mL was able to kill all *A. actinomycetemcomitans* in subgingival biofilm, total numbers of bacteria in test groups of both models were not significantly different compared with those of untreated control. A probable explanation for this may be that the concentration of LG used in the present study does not reach minimum bactericidal concentration towards other bacteria in subgingival biofilm. These findings indicated an advantage of using LG for periodontal disease treatment since it can kill *A. actinomycetemcomitans* without changing the subgingival ecology in an *in vitro* model. However, further clinical tests will be necessary to determine whether the results *in vivo* will be validated.

The age of the biofilm also affects its susceptibility to LG as shown in Table 2. We found that older (4- or 8-day-old) biofilms were more resistant to LG than were younger (2-day-old) biofilm. These results are in accordance with those

reported by several other groups of investigators.[28,32,36,37] Since the compositions of bacterial species and biofilm matrix, i.e. polysaccharides, proteins, nucleic acids and lipids, between older and younger biofilm are different, these can be attributed to the higher antimicrobial resistance of older biofilms. One of the reasons for antimicrobial resistance of biofilms is that the antimicrobial agents must diffuse through the biofilm matrix to contact and inactivate the organisms within the biofilm. The extracellular polymeric substances retard diffusion either by chemically reacting with the antimicrobial molecules or by limiting their rate of transport. However, the mechanisms behind the increased resistance of older biofilms to antimicrobial agents are still the subject of much research and debate.

In conclusion, the results obtained in this study demonstrated that LG possesses inhibitory effects on multispecies subgingival biofilm formation and is able to reduce the numbers of *A. actinomycetemcomitans* and total bacteria *in vitro*. From our previous studies, LG possessed antibacterial activity towards caries and periodontal pathogens and was able to reduce oral malodour.[17] Moreover, LG possessed antifungal and antibiofilm activities and could modulate candidal colonization.[19,20] Altogether, the results obtained indicate the multiple advantages of LG and suggest potential for developing LG as a natural oral hygiene product against oral infection in people.

## Acknowledgement

This work was supported by grants from Faculty of Dentistry, Khon Kaen University.

## References

1. Petersen PE, Ogawa H. Strengthening the prevention of periodontal disease: the WHO approach. J Periodontol 2005;76(12):2187-93.
2. Hoiby N, Bjarnsholt T, Givskov M, Molin S, Ciofu O. Antibiotic resistance of bacterial biofilms. Int J Antimicrob Agents 2010;35(4):322-32.
3. Socransky SS, Haffajee AD. Periodontal microbial ecology. Periodontol 2000 2005;38:135-87.
4. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL, Jr. Microbial complexes in subgingival plaque. J Clin Periodontol 1998;25(2):134-44.
5. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL, Jr. Microbial complexes in subgingival plaque. J Clin Periodontol 1998;25(2):134-44.
6. Pradhan-Palikhe P, Mantyla P, Paju S, Buhlin K, Persson GR, Nieminen MS, et al. Subgingival bacterial burden in relation to clinical and radiographic periodontal parameters. J Periodontol 2013;84(12):1809-17.
7. Wara-aswapati N, Pitiphat W, Chanchaimongkon L, Taweechaisupapong S, Boch JA, Ishikawa I. Red bacterial complex is associated with the severity of chronic periodontitis in a Thai population. Oral Dis 2009;15(5):354-9.
8. Fine DH, Markowitz K, Furgang D, Fairlie K, Ferrandiz J, Nasri C, et al. *Aggregatibacter actinomycetemcomitans* and its relationship to initiation of localized aggressive periodontitis: longitudinal cohort study of initially healthy adolescents. J Clin Microbiol 2007;45(12):3859-69.
9. Zambon JJ. Periodontal diseases: microbial factors. Ann Periodontol 1996;1(1):879-925.
10. Slots J, Ting M. *Actinobacillus actinomy cetemcomitans* and *Porphyromonas gingivalis* in human periodontal disease: occurrence and treatment. Periodontol 2000 1999;20:82-121.
11. Torrungruang K, Jitpakdeebordin S, Charatkulangkun O, Gleebbua Y. *Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans*, and *Treponema denticola*/*Prevotella intermedia* Co-Infection Are Associated with Severe Periodontitis in a Thai Population. Plos One 2015;10(8).
12. Hyvarinen K, Laitinen S, Paju S, Hakala A, Suominen-Taipale L, Skurnik M, et al. Detection and quantification of five major periodontal pathogens by single copy gene-based real-time PCR. Innate Immun 2009;15(4):195-204.

13. Paju S, Pussinen PJ, Suominen-Taipale L, Hyvonen M, Knuuttila M, Kononen E. Detection of Multiple Pathogenic Species in Saliva Is Associated with Periodontal Infection in Adults. J Clin Microbiol 2009;47(1):235-8.
14. Ardila CM, Granada MI, Guzman IC. Antibiotic resistance of subgingival species in chronic periodontitis patients. J Periodontal Res 2010;45(4):557-63.
15. Rams TE, Degener JE, van Winkelhoff AJ. Antibiotic resistance in human chronic periodontitis microbiota. J Periodontol 2014;85(1):160-9.
16. Aiemsaard J, Aiumlamai S, Aromdee C, Taweechaisupapong S, Khunkitti W. The effect of lemongrass oil and its major components on clinical isolate mastitis pathogens and their mechanisms of action on *Staphylococcus aureus* DMST 4745. Res Vet Sci 2011;91(3):e31-7.
17. Satthanakul P, Taweechaisupapong S, Paphangkorakit J, Pesee M, Timabut P, Khunkitti W. Antimicrobial effect of lemongrass oil against oral malodour micro-organisms and the pilot study of safety and efficacy of lemongrass mouthrinse on oral malodour. J Appl Microbiol 2014; 118(1):11-7.
18. Lertsatitthanakorn P, Taweechaisupapong S, Aromdee C, Khunkitti W. *In vitro* bioactivities of essential oils for acne control. Int J Aromather 2006;16(1):43-9.
19. Taweechaisupapong S, Aieamsaard J, Chitropas P, Khunkitti W. Inhibitory effect of lemongrass oil and its major constituents on Candida biofilm and germ tube formation. S Afr J Bot 2012;81:95-102.
20. Taweechaisupapong S, Ngaonee P, Patsuk P, Pitiphat W, Khunkitti W. Antibiofilm activity and post antifungal effect of lemongrass oil on clinical *Candida dubliniensis* isolate. S Afr J Bot 2012;78:37-43.
21. Aiemsaard J, Aiumlamai S, Taweechaisupapong S, Aromdee C, Khunkitti W. Chemical composition, antioxidant activity and antibacterial action of eight essential oils against clinical isolates of mastitis pathogens. Int J Essential oil Ther 2010;4:37-43.
22. Bard M, Albrecht MR, Gupta N, Guynn CJ, Stillwell W. Geraniol interferes with membrane functions in strains of Candida and Saccharomyces. Lipids 1988;23(6):534-8.
*23*. Braga PC, Dal Sasso M. Morphostructural alterations of *Candida albicans* induced by thymol. A scanning electron microscopy study. J Chemother 2005;17:109.
24. Walker C, Sedlacek MJ. An in vitro biofilm model of subgingival plaque.Oral Microbiol Immunol 2007;22(3): 152-61.
25. Ruangcharoen S, Suwannarong W, Lachica M, Bolscher JGM, Nazmi K, Khunkitti W, et al. Killing activity of LFchimera on periodontopathic bacteria and multispecies oral biofilm formation in vitro. World J Microbiol Biotechnol 2017;33(9):167.
26. Taweechaisupapong S, Pinsuwan W, Suwannarong W, Kukhetpitakwong R, Luengpailin S. Effects of *Streblus asper* leaf extract on the biofilm formation of subgingival pathogens. . S Afr J Bot 2014;94:1-5.
27. Ashimoto A, Chen C, Bakker I, Slots J. Polymerase chain reaction detection of 8 putative periodontal pathogens in subgingival plaque of gingivitis and advanced periodontitis lesions. Oral Microbiol Immunol 1996; 11(4):266-73.
28. Sedlacek MJ, Walker C. Antibiotic resistance in an in vitro subgingival biofilm model. Oral Microbiol Immunol 2007;22(5):333-9.
29. Nadkarni MA, Martin FE, Jacques NA, Hunter N. Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set. Microbiology 2002;148(1):257-66.
30. Morillo JM, Lau L, Sanz M, Herrera D, Martin C, Silva A. Quantitative real-time polymerase chain reaction based on single copy gene sequence for detection of periodontal pathogens.J Clin Periodontol 2004;31(12):1054-60.
31. Lewis K. Riddle of biofilm resistance. Antimicrob Agents Chemother 2001;45(4):999-1007.
32. Donlan RM, Costerton JW. Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev 2002;15(2):167-93.

33. Schwering M, Song J, Louie M, Turner RJ, Ceri H. Multi-species biofilms defined from drinking water microorganisms provide increased protection against chlorine disinfection. Biofouling 2013;29(8):917–28.
34. van der Veen S, Abee T. Mixed- species biofilms of *Listeria monocytogenes* and *Lactobacillus plantarum* show enhanced resistance to benzalkonium chloride and peracetic acid. Intl J Food Microbiol 2011;144(3):421–31.
35. Wang R, Kalchayanand N, Schmidt JW, Harhay DM. Mixed biofilm formation by Shiga toxin- producing *Escherichia coli* and *Salmonella enterica* serovar Typhimurium enhanced bacterial resistance to sanitization due to extracellular polymeric substances. J Food Prot 2013;76(9):1513–22.
36. Anwar H, Strap JL, Chen K, Costerton JW. Dynamic interactions of biofilms of mucoid *Pseudomonas aeruginosa* with tobramycin and piperacillin. Antimicrob Agents Chemother 1992;36(6):1208-14.
37. Amorena B, Gracia E, Monzon M, Leiva J, Oteiza C, Perez M, et al. Antibiotic susceptibility assay for *Staphylococcus aureus* in biofilms developed in vitro. J Antimicrob Chemother 1999;44(1):43-55.

***Corresponding Author***

*Suwimol Taweechaisupapong*
*Department of Oral Biomedical Sciences*
*Faculty of Dentistry, Khon Kaen University,*
*Amphur Muang, Khon Kaen 40002.*
*Tel.: +66 4320 2405 #45125*
*Fax: +66 4320 2862*
*E-mail: suvi_taw@kku.ac.th*

# ผลของน้ำมันหอมระเหยตะไคร้ต่อการยับยั้งการสร้างไบโอฟิล์มใต้เหงือกที่เกิดจากเชื้อก่อโรคหลายชนิด

*วชิราพร ปิ่นสุวรรณ* วราภรณ์ สุวรรณรงค์** วัชรี คุณกิตติ*** สุวิมล ทวีชัยศุภพงษ์**


## บทคัดย่อ

*น้ำมันตะไคร้ เป็นน้ำมันหอมระเหยที่ได้จากใบตะไคร้ มีรายงานการศึกษาพบว่าน้ำมันตะไคร้มีฤทธิ์ยับยั้งเชื้อก่อโรคปริทันต์ เช่น เชื้อแอกกริเกทิแบกเทอร์ แอคทิโนมัยซิเทมโคมิแทนส์ และพอร์ไฟโรโมแนส จิงจิวาลิส แต่ยังไม่มีการศึกษาถึงประสิทธิภาพของน้ำมันตะไคร้ต่อการยับยั้งการสร้างไบโอฟิล์มใต้เหงือกที่เกิดจากเชื้อก่อโรคหลายชนิด การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาผลของน้ำมันตะไคร้ต่อการยับยั้งการสร้างไบโอฟิล์มใต้เหงือกในห้องปฏิบัติการ โดยทำการเก็บคราบจุลินทรีย์ใต้เหงือกจากผู้ป่วยโรคปริทันต์อักเสบจำนวน 5 คน มาเลี้ยงในเพลทชนิด 96 หลุม ที่เคลือบด้วยน้ำลายและใส่น้ำมันตะไคร้ที่ความเข้มข้น 1-6 ไมโครลิตรต่อมิลลิลิตร จากนั้นนำไปบ่มเพาะในสภาวะปราศจากออกซิเจน ที่อุณหภูมิ 37 องศาเซลเซียส เป็นเวลา 2, 4 และ 8 วัน โดยทำการเปลี่ยนอาหารเลี้ยงเชื้อทั้งที่มีและไม่มีน้ำมันตะไคร้ทุก 2 วัน จากนั้นทำการวัดปริมาณไบโอฟิล์มด้วยการย้อมคริสตัล ไวโอเลต และศึกษาปริมาณเชื้อแอกกริเกทิแบกเทอร์ แอคทิโนมัยซิเทมโคมิแทนส์ และเชื้อแบคทีเรียโดยรวมในไบโอฟิล์มใต้เหงือกด้วยวิธีเรียลไทม์ พีซีอาร์ ผลการศึกษา พบว่า น้ำมันตะไคร้ที่ความเข้มข้น 6 ไมโครลิตรต่อมิลลิลิตร สามารถยับยั้งการสร้างไบโอฟิล์มได้มากกว่าร้อยละ 80 นอกจากนี้ยังพบว่าน้ำมันตะไคร้ที่ความเข้มข้น 6 ไมโครลิตรต่อมิลลิลิตร มีประสิทธิภาพในการทำลายเชื้อแอกกริเกทิแบกเทอร์ แอคทิโนมัยซิเทมโคมิแทนส์ ในไบโอฟิล์มใต้เหงือกได้หมด ณ เวลา 2, 4 และ 8 วัน ผลการศึกษานี้ บ่งชี้ว่าน้ำมันตะไคร้มีประสิทธิภาพยับยั้งการสร้างไบโอฟิล์มใต้เหงือก และลดเชื้อแอกกริเกทิแบกเทอร์ แอคทิโนมัยซิเทมโคมิแทนส์ ในไบโอฟิล์มใต้เหงือก ที่เกิดจากเชื้อก่อโรคหลายชนิดได้*


***คำไขรหัส:*** *แอกกริเกทิแบกเทอร์ แอคทิโนมัยซิเทมโคมิแทนส์/ ไบโอฟิล์มใต้เหงือก/ น้ำมันตะไคร้/ โรคปริทันต์อักเสบ*


## ผู้รับผิดชอบบทความ

*สุวิมล ทวีชัยศุภพงษ์*

*สาขาวิชาชีวเวชศาสตร์ช่องปาก*

*คณะทันตแพทยศาสตร์ มหาวิทยาลัยขอนแก่น*

*อำเภอเมือง จังหวัดขอนแก่น 40002*

*โทรศัพท์: 0 4320 2405 #45125*

*โทรสาร: 0 4320 2862*

*จดหมายอิเล็กทรอนิกส์: suvi_taw@kku.ac.th*

** กลุ่มวิจัยไบโอฟิล์ม คณะทันตแพทยศาสตร์ มหาวิทยาลัยขอนแก่น อำเภอเมือง จังหวัดขอนแก่น*

*** สาขาวิชาชีวเวชศาสตร์ช่องปาก คณะทันตแพทยศาสตร์ มหาวิทยาลัยขอนแก่น อำเภอเมือง จังหวัดขอนแก่น*

**** คณะเภสัชศาสตร์ มหาวิทยาลัยขอนแก่น อำเภอเมือง จังหวัดขอนแก่น*