In-vitro Inhibitory Effect of *Cinnamomum zeylanicum* and *Eugenia caryophyllata* Oils on Multispecies Anaerobic Oral Biofilm

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Abstract

The study aimed to determine the in-vitro inhibitory effect of *Cinnamomum zeylanicum* Blume (cinnamon) and *Eugenia caryophyllata* (clove) oils on combined *Porphyromonas gingivalis* and *Fusobacterium nucleatum* biofilm. Following a steam distillation extraction technique, oils from cinnamon bark and clove buds were tested for their antibacterial anti-adhesion and anti-biofilm activities on mixed biofilm containing *P.gingivalis* and *F.nucleatum*. The quantification of viable and adhered bacterial cells on 96-well plates using the crystal violet test was analysed and morphology of the bacteria in biofilm was observed under the scanning electron microscope (SEM).

Our study demonstrated *C.zeylanicum* oil inhibited satisfactory anti-adhesion effect and significantly disrupted the biofilm formation at 1.25-5.0 mg/mL whereas *E.caryophyllata* oil suppressed the adhesion of bacteria and disrupted the formation of biofilm at 0.63-5.0 mg/mL. The SEM study showed prominent changes including ruptures and flatten bacterial wall in samples with *E.caryophyllata* oil while pitted and flatten cells were seen in *C.zeylanicum* samples compared to the smooth and distinguished cell wall seen in untreated samples or even more damaged cells in Ampicillin samples.

We conclude that *C.zeylanicum* and *E.caryophyllata* oils showed significant in vitro anti-biofilm properties against mixed *P.gingivalis* and *F.nucleatum* biofilm and prominently altered the bacterial wall morphology. This finding may suggest potential therapeutic properties of these oils in controlling biofilm-related oral diseases.

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Introduction

As the oral cavity is the complex ecosystem with diverse microorganisms, preventing opportunistic invasion of pathogenic microorganisms is a crucial component of oral disease management[1]. In recent decades, the healing and therapeutic qualities of herbal essential oil and its use in dentistry have spurred development of many herbal-based oral care products. Clove (*Eugenia caryophyllata)* and cinnamon (*Cinnamomum zeylanicum)* oils are some of the most widely used essential oils in contemporary alternative medicine (CAM) for dental remedies and oral care products generally due to their antibacterial, antiseptic and biofilm-inhibiting properties. The therapeutic benefit of herbal oils is focused on the ability to prevent and control biofilm-related diseases such as caries, gingivitis and periodontitis[2-5].

It has been well-accepted that dental biofilm inflicts vast challenges to dental practitioners and health care providers due to its potential to develop many bacterial-induced diseases and resistance towards antibiotic and chemotherapeutic agents[6-9]. The presence and interaction of various group of microorganisms adhering to each other within complex extracellular polymeric substances creates resistant environment against other invading pathogens, antimicrobials and chemotherapeutic agents[10-13]. This is further complicated by the

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host's immunological defence and susceptibility to antimicrobial agents[14-16]. Consequently, controlling dental biofilms becomes a challenge for oral health care professionals. Hence, understanding the oral biofilm characteristics and its communities in addition to ensure proper control measures may enable oral diseases prevention.

Having been accepted as naturally occurring, highly concentrated with pleasant fragrant and in addition to their reported medicinal benefits, *E. caryophyllata* and *C. zeylanicum* oils extracted through steam distillation are being considered as possible source of new antimicrobials agent with broad spectrum of actions[17,18]. However, not much has been reported on their activities on mixed biofilm and their possible mechanism of action[19-20]. Thus, the present study aimed to evaluate the inhibitory effect of both oils on the formation of mixed *Porphyromonas gingivalis* and *Fusobacterium nucleatum* biofilm and their disruptive activity towards pre-formed biofilm.

Materials and methods

Herbal plants and oil preparations

Oils of *C. zeylanicum* bark and *E. caryophyllata* flower buds were extracted using hydrodistillation method with Clevenger apparatus for 8h at the Drug and Herbal Research Centre, Faculty of Pharmacy, Universiti Kebangsaan Malaysia. Oils obtained from the extraction process were later separated and dried using anhydrous sodium sulphate powder, sealed in glass container and kept in 4^{o}C until further use[21].

Bacterial culture and growth maintenance

Oral Gram-negative obligate anaerobes *P. gingivalis* ATCC 53978 (strain W50) and *F. nucleatum* ATCC 25586 used in this study were obtained from the Melbourne Dental School, University of Melbourne (courtesy of Professor Dr. Stuart G. Dashper). Blood agar supplemented with hemin (Calbiochem, Netherland), vitamin K (menadione) (Merck KGaA, Germany) and cysteine (Merck KGaA, Germany) was used to isolate and maintain the growth of the bacteria. In addition, brain heart infusion (Oxoid Ltd, England) and typticase soy broth (Oxoid Ltd, England) supplemented with hemin, vitamin K (menadione) and cysteine was used for the growth of biofilm. The bacteria were cultured and incubated at 37^{o}C under anaerobic condition. These microbiological procedures were in accordance to standard procedure recommended by guidelines from the Clinical and Laboratory Standards Institute 2004 (CLSI) with a few modifications[22].

Anti-adhesion assay

The purpose of this assay was to determine the effect of both herbal oils on adhesion ability of *P. gingivalis* and *F. nucleatum* in oral biofilm formation. An aliquot of bacterial suspension of 105 cfu/mL were dispensed in a 96-well microtiter plate containing respective oils at 1:1 ratio (oil solution: bacterial suspension). Untreated bacterial cells in suspension medium acted as negative control whereas Ampicillin-treated cells (5.0 mg/mL) acted as positive control. Following a 72h anaerobic incubation at 37°C, wells were washed with sterile distilled water to remove non-adhered bacteria and excess broth. The remaining adhered bacteria were quantitated using crystal violet staining procedure. Each well was stained with 0.1% crystal violet dye (Labchem, New Zealand) (50-100 µL) and incubated at room temperature for 15min before being washed for three times with sterile distilled water. Then, the remaining adhered cells were fixed with 100 µL of 95% ethanol (95% ethanol in water) for 10 min. Stained cells were extracted and their optical density (OD) were measured at 595 nm wavelength using a microplate reader. All assays were carried out in triplicates and repeated in three independent experiments.

Anti-biofilm assay

The disruptive effect of both herbal oils on pre-formed biofilm was investigated and quantitated using the colorimetric assay by crystal violet staining. A 72h preformed biofilm of *P. gingivalis* and *F. nucleatum* was developed in a 96-well microtitre plate incubated anaerobically at 37°C. The oils were then added into the pre-formed biofilm and the plates were further incubated anaerobically for 48h. Each plate contained a positive control of Ampicillin-treated biofilm (5.0 mg/mL) and a negative control of untreated pre-formed biofilm in a suspension medium. Then, 0.1% crystal violet staining procedure as described above was used to

measure the optical density of untreated and treated biofilms[23-25]. All assays were carried out in triplicates and repeated in three independent experiments.

Morphological changes of bacterial wall

The changes on the bacterial wall after exposure to the oils and controls were observed under the electron microscope (SEM). The methods were modified from Yin-Hua et al.[26] and da Silva Trentin et al.[27] Mixed bacteria culture was prepared similar to the method in anti-biofilm assay and then dispensed into a 24-well plate containing 5 mg/mL *C. zeylanicum* and *E. caryophyllata* oils, broth and 5.0 mg/mL Ampicillin respectively. In each well, there was a cut glass slide placed horizontally at the bottom of the well. Then, the plate was incubated for 72h in an anaerobic condition. Following this, the glass slides were collected and fixed with vapour fixation technique using 2% glutaraldehyde with 0.1 M phosphate buffered saline (PBS) for a minimum of 10min. Subsequently, a sample dehydration technique was carried out using 50% then 70% ethanol for 10min each. All samples were then attached to aluminium stubs, sputter coated with gold palladium and finally viewed under scanning electron microscope at 1000x, 2000x and 5000x magnification. The morphology of the bacteria on each groups of samples were recorded for comparison.

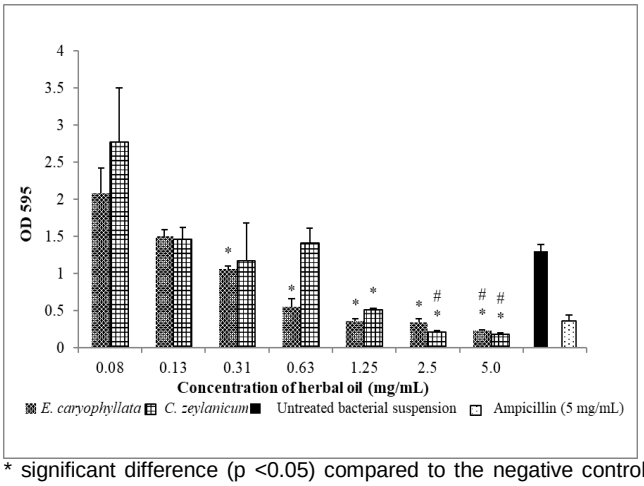
Statistical analysis

The mean and the standard deviation of the absorbance readings were obtained, and t-test was carried out to compare the mean values between the tested essential oils and the controls.

Results

Anti-adhesion assay

The activity of *C. zeylanicum* and *E. caryophyllata* oils against the attachment of *P. gingivalis* and *F. nucleatum* in the formation of oral biofilm are shown in Figure 1. Generally, our study indicated that the oils showed positive inhibition on adhesion of both Gram-negative anaerobes compared to control. The *E. caryophyllata* oil demonstrated significant inhibition of bacterial cell attachment at concentration ranging from 0.31–5.0 mg/mL whereas *C. zeylanicum* oil exhibited its inhibitory activity at higher concentrations ranging from 1.25–5.0 mg/mL in comparison to negative control. *E. caryophyllata* oil at 5.0mg/mL showed significant inhibition on adhesion of *P. gingivalis* and *F. nucleatum* compared to Ampicillin 5 mg/mL, while *C. zeylanicum* oil was at concentrations of 2.5 mg/mL and 5.0 mg/mL respectively.



* significant difference (p <0.05) compared to the negative control (untreated bacterial suspension).
\# significant difference (p <0.05) compared to the positive control (Ampicillin at 5mg/mL).

Figure 1. Anti-adhesion activity of *C.zeylanicum* and *E.caryophyllata* oils against *P.gingivalis* and *F.nucleatum* biofilm.

Development of the pre-formed biofilm

The features of pre-formed mixed biolfilm of *P. gingivalis* and *F. nucleatum* is illustrated in Table 1. The presence of mixed biofilm was detected by observation of turbidity in wells and then crystal violet stain compared to well without bacteria inoculum (clear wells). When stained with Gram stain and observed under the light microscope, presence of pink small rods and motile pink fusiform or spindle rod-shaped, parallel wall and round to tapered ends indicated presence of *P. gingivalis* and *F. nucleatum* respectively.

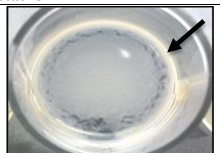
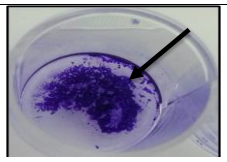
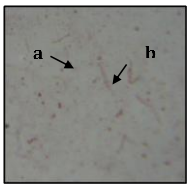
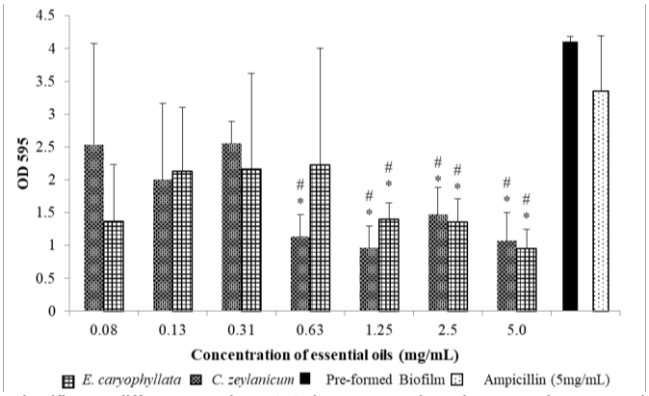
Description	Feature
Biofilm consists of *P. gingivalis* and *F. nucleatum* observed as whitish layer (arrow) in a well of a 96-microtitre plate.	
Crystal violet-stained biofilm (arrow) after crystal violet staining procedure done in a microtitre plate.	
Gram stain of biofilm showing presence of mixed *P. gingivalis* (arrow **a**, singular small rod,) and *F. nucleatum* (arrow **b**, fusiform rod-shaped, parallel wall, round to tapered ends).	

Table 1. Features of pre-formed *P.gingivalis* and *F.nucleatum* biofilm.

Anti-biofilm assay

The biofilm disruption activity of *C. zeylanicum* and *E. caryophyllata* oils is presented in Figure 2. Comparing the significant disruptive effect on those biofilm-grown bacterial cells of both oils to the positive (Ampicillin) and the negative control used in this study, the most prominent effect was observed at the concentration ranging from 0.63 – 5.0 mg/mL and also at the concentration ranging from 1.25 – 5.0 mg/mL with *E. caryophyllata* and *C. zeylanicum* oils, respectively. The obtained qualitative and quantitative results from this study leads to the conclusion that both herbal oils are able to efficiently eradicate oral biofilms of *P. gingivalis* and *F. nucleatum*.



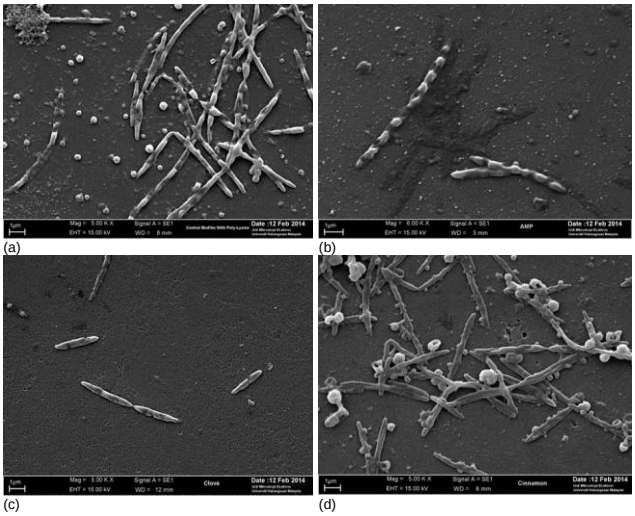
*significant differences ($p < 0.05$) compared to the negative control (untreated bacterial suspension).
#significant differences ($p < 0.05$) compared to the positive control (ampicillin 5 mg/mL).

Figure 2. Anti-biofilm activity of *C.zeylanicum* and *E.caryophyllata* oil on pre-formed *P.gingivalis* and *F.nucleatum* biofilm.

Morphological changes of bacteria

Figure 3 shows the SEM photos of the bacterial wall changes following exposure to different samples. Mixed biofilm in untreated well (Figure 3(a) - negative control) were observed to be unaffected (normal) with plenty of *P. gingivalis* cells seen as small rods and smooth wall while *F. nucleatum* as smooth surface and spindle shaped. Meanwhile, for the biofilm exposed to ampicillin (Figure 3(b)) - positive control), *P. gingivalis* were absent/scarce while *F. nucleatum* are scarce and ruptured. There were dark shadows in the shape of cell present at the base of slide, which may indicate presence of intracellular ground substance released following rupture of cell wall.[36]

There was also absence of *P. gingivalis* in the samples exposed to the *E. caryophyllata* oil while F.nucleatum was very few and either ruptured or separated and slightly flattened (Figure 3(d)). Finally, mixed biofilm exposed to *C. zeylanicum* had both *P. gingivalis* and *F. nucleatum* present as sample in Figure 3(a), but cells were more enlarged. In addition, *P. gingivalis* cells were pitted while *F. nucleatum* was observed to be more flattened.



(a) Mixed biofilm in untreated well (negative control); *P.gingivalis* seen as small rods with smooth wall while *F.nucleatum* as spindle shaped with smooth surface,
(b) Mixed biofilm exposed to ampicillin (positive control); absence of *P.gingivalis* while *F.nucleatum* are scarce and seen as ruptured, dark shadow present in the base of slide,
(c) Mixed biofilm exposed to *E.caryophyllata* oil; absence of *P.gingivalis* while isolated *F.nucleatum* ruptured, separated and slightly flattened,
(d) Mixed biofilm exposed to *C.zeylanicum*; *P.gingivalis* present but enlarged and pitted while *F.nucleatum* appeared enlarged and flattened.
All views at SEM magnification 5000x.

Figure 3. Bacterial cell changes following exposure to oils and controls.

Discussion

Some scientific studies have demonstrated that the multifactorial nature and evolving composition of oral biofilm which consist of the densely packed bacteria and the matrix hinder the penetration and diffusion of the antimicrobial agents contributing to their great resistance mechanism[28,29]. In recent years, there is an increase trend of urge in the development of strategies to treat oral biofilm and its causative agents. To date, many herbal plants and their essential oils have been widely investigated in order to overcome the oral microbial drug resistance and patient management problems and therefore confirming the antibacterial and anti-biofilm activities against a wide range of pathogenic Gram positive and Gram negative bacteria[30-33]. Thus, the use of antimicrobials from natural sources to prevent the attachment and disturb the oral biofilm formation has been considered as another option in the management of oral diseases involving dental plaque aside from the efficient mechanical removal of the oral biofilm.

Numerous studies have shown that the chemical composition which may vary depending on the species of plant used (parts of the plants, genetic variation and harvesting time), the environmental condition of the plants used (season and geography) and also the method of preparation could affect the antibacterial activity of herbal plants and their essential oils. The ease of its extraction and little to nontoxic effect to overall biological system of human body with positive beneficial effects to health may increase the medicinal value of herbal plants and their essential oils. Inhibition on the resistance mechanism, action on multiple target sites, exceptional ability to penetrate human tissue and disruption of the physicochemical interactions among the bacterial cells or between bacterial cells and the host are some of the mode of antibacterial activities that have been proposed to explain the synergism between the chemical components within the essential oils[34-36].

Although the mechanism of action of the oils on bacteria cells was not studied in this research, we found consistent evidence to support possible disruptive action of these oils on Gram negative bacteria. It was suggested that the permeability of bacterial cells could significantly be altered by various potent antibacterial agents[37,38]. Several others studies suggested that *C. zeylanicum* and *E. caryophyllata* oils may disrupt cell membrane integrity as well as interfere with the organisation of quorum-sensing between cells to inhibit biofilm establishment.[39-41] Also, the anti-biofilm effect may be resulted from the synergistic action of the herbal oils constituents such as Cinnamaldehyde (an aromatic aldehyde) and eugenol (phenylpropanoid) as major components of *C. zeylanicum* and *E. caryophyllata* respectively. In essence, these studies showed possible specific features of components within the *C. zeylanicum* and *E. caryophyllata* oils that may lead to: i) disorders on the morphological framework and physiological function of bacterial cell membrane, which reduce the permeability of the bacteria wall and cause leakage of intracellular component and ultimately death of cell, and ii) inhibiting growth of bacteria by interfering with the natural process inside bacterial cell[42-45]. Thus, it is noteworthy to emphasize these findings and ours which advocate the potential anti-biofilm effect of both herbal oils to act against Gram negative bacteria comparable to Ampicillin, as commonly used systemic antibiotics[46].

Conclusions

The *C. zeylanicum* and *E. caryophyllata* oils showed significant in vitro anti-adhesion and disruptive anti-biofilm properties against mixed *P. gingivalis* and *F. nucleatum* biofilm. Both oils also prominently altered the bacterial wall morphology comparable to Ampicillin activity. As oral biofilm critically influences the development and progression of oral infectious diseases, the results from this study may provide an option of using herbal oils from clove and cinnamon as future therapy for biofilm-related oral diseases.

Declaration of Interest

The authors report no conflict of interest.

References

1. Aas, J.A., Paster, B.J., Stokes, L.N., Olsen, I., Dewhirst, F.E. Defining the normal bacterial flora of the oral cavity. Journal of Clinical Microbiology 2005;43(11):5721-5732.
2. Moon, S-E., Kim, H-Y., Cha, J.D. Synergistic effect between clove oil and its major compounds and antibiotics against oral bacteria. Archives of Oral Biology 2011;56(9):907-916.
3. Zainol, S.N., Said, S.M., Abidin, Z.Z., Azizan, N., Majid, F.A.A., Jantan, I. Synergistic benefit of Eugenia caryophyllata L. and Cinnamomum zeylanicum Blume essential oils against oral pathogenic bacteria. Chemical Engineering Transactions 2017;56:1429-1434.

4. Zainal-Abidin, Z., Mohd-Said, S., Abdul Majid, F.A., Wan Mustapha, W.A., Jantan, I. Anti-bacterial activity of cinnamon oil on oral pathogens. The Open Conference Proceedings Journal 2013;4(1):12–16.
5. Mohd Ali, N.A., Mohtar, M., Shaari, K., Rahmanii, M., Al,i A.M., Jantan, I. Chemical composition and antimicrobial activities of the essential oils of Cinnamomum aureofulvum Gamb. Journal of Essential Oil Research 2002;14(2):135-138.
6. Valen, H., Scheie, A.A. Biofilms and their properties. European Journal of Oral Sciences 2018;126(Suppl 1):13–18.
7. Stoodley, P., Sauer, K., Davies, D.G., Costerton, J.W. Biofilms as complex differentiated communities. Annual Reviews in Microbiology 2002;56(1):187-209.
8. Parsek, M.R., Singh, P.K. Bacterial biofilms: an emerging link to disease pathogenesis. Annual Reviews in Microbiology 2003;57(1):677-701.
9. Dunne Jr., W.M. Bacterial adhesion: seen any good biofilms lately? Clinical Microbiology Reviews 2002;15(2):155–166.
10. Anggraini, M., Djais, A.A., Soekanto, S.A. Candida albicans biofilm profiles on various denture base materials. Journal of International Dental and Medical Research 2018;11(1):191-196.
11. Hojo, K., Nagaoka, S., Ohshima, T., Maeda, N. Bacterial interactions in dental biofilm development. Journal of Dental Research 2009;88(11):982-990.
12. Huang, R., Li, M., Gregory, R.L. Bacterial interactions in dental biofilm. Virulence 2011;2(5):435-444.
13. Marsh, P.D. Dental plaque: biological significance of a biofilm and community life-style. Journal of Clinical Periodontology 2005;32(Suppl 6):7-15.
14. Høiby, N., Ciofu, O., Johansen, H.K., Song, Z.J., Moser, C., Jensen, P.Ø., Molin, S., Givskov, M., Tolker-Nielsen, T. and Bjarnsholt, T. The clinical impact of bacterial biofilms. International Journal of Oral Sciences 2011;3(2):55-65.
15. Socransky, S.S., Haffajee, A.D. Dental biofilms: difficult therapeutic targets. Periodontology 2000 2002;28(1):12–55.
16. Mah, T., O'Toole, G. Mechanisms of biofilm resistance to antimicrobial agents. Trends in Microbiology 2001;9(1):34-39.
17. Saviuc, C.M., Drumea, V., Olariu, L, Chifiriuc, M.C., Bezirtzoglou, E., Lazăr, V. Essential oils with microbicidal and antibiofilm activity. Current Pharmaceutical Biotechnology 2015;16(2):137–151.
18. Edris, A.E. Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: A review. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives 2007:21(4):308-323.
19. Shen, S., Zhang, T., Yuan, Y., Lin, S., Xu, J., Ye, H. Effects of cinnamaldehyde on Escherichia coli and Staphylococcus aureus membrane. Food Control 2015;47:196–202.
20. Unlu, M., Ergene, E., Unlu, G.V., Zeytinoglu, H.S., Vural, N. Composition, antimicrobial activity and in vitro cytotoxicity of essential oil from Cinnamomum zeylanicum Blume (Lauraceae). Food and Chemical Toxicology 2010;48(11):3274-3280.
21. Azizan, N., Mohd-Said, S., Zainal-Abidin, Z., Jantan, I. Composition and Antibacterial Activity of the Essential Oils of Orthosiphon stamineus Benth and Ficus deltoidea Jack against Pathogenic Oral Bacteria. Molecules 2017;22(12):2135.
22. Clinical and Laboratory Standards Institute. M11-A6 Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria, Approved Standard - Sixth Edition 2004. Clinical and Laboratory Standards Institute: Wayne, PA, USA.
23. Adukwu, E.C., Allen, S.C.H., Phillips, C.A. The anti-biofilm activity of lemongrass (Cymbopogon flexuosus) and grapefruit (Citrus paradisi) essential oils against five strains of Staphylococcus aureus. Journal of Applied Microbiology 2012;113(5):1217–1227.
24. Gursoy, U.K., Gursoy, M., Gursoy, O.V., Cakmakci, L., Könönen, E. and Uitto, V.J. Anti-biofilm properties of Satureja hortensis L. essential oil against periodontal pathogens. Anaerobe 2009;15(4):164–167.
25. Zainal-Abidin, Z., Abdul-Wahab, N.A., Ghazi-Ahmad, M.K., Mohd-Said, S. In vitro Antibacterial Activity of Zingiber officinale and Orthosiphon stamineus on Enterococcus faecalis. Journal of Agricultural Science 2017;9(13):112-121.
26. Shih, Y.H., Chang, K.W., Hsia, S.M., Yu, C.C., Fuh, L.J., Chi, T.Y. and Shieh, T.M. In vitro antimicrobial and anticancer potential of hinokitiol against oral pathogens and oral cancer cell lines. Microbiological Research 2013;168(5):254–262.
27. da Silva Trentin, D., Giordani, R.B., Zimmer, K.R., Da Silva, A.G., Da Silva, M.V., dos Santos Correia, M.T., Baumvol, I.J.R. and Macedo, A.J. Potential of medicinal plants from the Brazilian semi-arid region (Caatinga) against Staphylococcus epidermidis planktonic and biofilm lifestyles. Journal of Ethnopharmacology 2011;137(1):327–335.
28. Kim, S.M., Kim, H.C., Lee, S.W. Characterization of antibiotic resistance determinants in oral biofilms. Journal of Microbiology 2011;49(4):595-602.
29. Stewart, P.S. Mechanisms of antibiotic resistance in bacterial biofilms. International Journal of Medical Microbiology 2002;292(2):107-113.
30. Gyawali, R., Ibrahim, S.A. Natural products as antimicrobial agents. Food Control 2014;46:412–429.
31. Thosar, N., Basak, S., Bahadure, R.N., Rajurkar, M. Antimicrobial efficacy of five essential oils against oral pathogens: An in vitro study. European Journal of Dentistry 2013;7(Suppl 1), S71.
32. Swamy, M.K., Akhtar, M.S., Sinniah, U.R. Antimicrobial properties of plant essential oils against human pathogens and their mode of action: an updated review. Evidence Based Complementary Alternative Medicine 2016.
33. Nazzaro, F., Fratianni, F., De Martino, L., Coppola, R., De Feo, V. Effect of essential oils on pathogenic bacteria. Pharmaceuticals 2013;6(12):1451–1474.
34. Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M. Biological effects of essential oils - a review. Food Chemical Toxicology 2008;46(2):446–475.
35. Burt, S. Essential oils: their antibacterial properties and potential application in foods - a review. International Journal Food Microbiology 2004;94(3):223–253.
36. Amalaradjou, M.A.R., Narayanan, A., Venkitanarayanan, K. Trans-cinnamaldehyde decreases attachment and invasion of uropathogenic Escherichia coli in urinary tract epithelial cells by modulating virulence gene expression. Journal of Urology 2011;185(4):1526–1531.
37. Kim, Y.G., Lee, J.H., Kim, S.I., Baek, K.H., Lee, J. Cinnamon bark oil and its components inhibit biofilm formation and toxin production. International Journal of Food Microbiology 2015;195:30–39.
38. Yadav, M.K., Chae, S.W., Im, G.J., Chung, J.W., Song, J.J. Eugenol: a phyto-compound effective against methicillin-resistant and methicillin-sensitive Staphylococcus aureus clinical strain biofilms. PLoS One 10 2015;10(3):e0119564.
39. Amalaradjou, M.A.R., Narayanan, A., Baskaran, S.A., Venkitanarayanan, K. Antibiofilm effect of transcinnamaldehyde on uropathogenic Escherichia coli. Journal of Urology 2010;184(1):358–363.
40. Adil, M., Singh, K., Verma, P.K., Khan, A.U. Eugenol-induced suppression of biofilm-forming genes in Streptococcus mutans: An approach to inhibit biofilms. Journal of Global Antimicrobial Resistance 2014;2(4):286–292.
41. Domadia, P., Swarup, S., Bhunia, A., Sivaraman, J., Dasgupta, D. Inhibition of bacterial cell division protein FtsZ by cinnamaldehyde. Biochemical Pharmacology 2007;74(6):831–840.
42. Stoeken, J.E., Paraskevas, S., van der Weijden, G.A. The long-term effect of a mouthrinse containing essential oils on dental plaque and gingivitis: a systematic review. Journal of Periodontology 2007;78(7):1218–1228.
43. Claffey, N. Essential oil mouthwashes: a key component in oral health management. Journal of Clinical Periodontology 2003;30: 22–24.

44. Carson, C.F., Mee, B.J., Riley, T.V. Mechanism of action of Melaleuca alternifolia (Tea tree) oil on Staphylococcus aureus determined by time-killed, lysis, leakage and salt tolerance assays and electron microscopy. Antimicrobial Agents Chemotherapy 2002;46(6):1914-1920.
45. Zengin, H., Baysal, A.H. Antibacterial and antioxidant activity of essential oil terpenes against pathogenic and spoilage-forming bacteria and cell structure-activity relationship evaluated by SEM microscopy. Molecules 2014;19(11):17773-17798.
46. Kaur, S.P., Rao, R., Nanda, S. Amoxicillin: A broad spectrum antibiotics. International Journal of Pharmacy and Pharmaceutical Sciences 2011;3(3):30-37.