ANTIADHERENCE AND ANTIBIOFILM OF *Lavandula angustifolia* ESSENTIAL OIL AGAINST PATHOGENIC ORAL BACTERIA

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DEDICATION

Specially dedicated to my parent, siblings, and friends for always being by my side throughout this meaningful journey. Thank you.

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ABSTRACT

There are many types of oral care products in the market that can be used to solve oral diseases. However, several oral care products could contain chemicals such as chlorhexidine or sodium lauryl sulphate (SLS) that might contribute to side effects in the form of mucosal desquamation, xerostomia (dry mouth) or burning sensation in oral cavity. In order to overcome the issues related to oral disease, a substitute of antibacterial agent that is more natural is required to reduce the risk of side effects caused by the use of chemicals from common oral care products. *Lavandula angustifolia* is known for its antibacterial properties and its use as an essential oil. Due to its medicinal properties, current research is aimed at evaluating the antibacterial activity of essential oil from *Lavandula angustifolia* on the growth of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus mutans.* The antibacterial activity was determined based on colour changes of minimum inhibitory concentration (MIC) reagent (3-(4, 5-dimethythiazol-2-yl)-2, 5-diphenyltetrazolium bromide) during the initiation of MIC experiment using microbroth dilution method. About 0.1% chlorhexidine (*w/v*) solution was used as a positive control while 10% of dimethyl sulfoxide (DMSO) (v/v) was used as a negative control. The MIC value of essential oil was determined at 0.78% for both *E. coli* and *S. aureus*, whereas the MIC values of the same were at 1.56% and 6.25% for *S. mutans* and *P. aeruginosa*, respectively. In regard to the value of MIC, each bacteria were subjected to further test on the antiadherence and antibiofilm activity. Accordingly, the anti-adherence activity for *E. coli* and *P. aeruginosa* were of 65.95 ± 6.83% and 74.07 ± 1.85%, which was slightly high compared to *S. aureus* and *S. mutans* that produced anti-adherence activities at 61.78 ± 1.27% and 48.52 ± 13.17%, respectively. *E. coli* and *P. aeruginosa* also exhibited a slight high in antibiofilm activity in a range of 85% to 90% after using essential oil from lavender as compared to 77% to 79% of anti-biofilm activity in *S. aureus* and *S. mutans*. The morphological analysis by FESEM showed that *S. mutans* encountered modifications to its cellular membrane. A much severe defect also had been observed on the coccus-shaped bacteria whenever *S. mutans* were exposed to essential oil from lavender at MIC and half the MIC values. As a conclusion, the essential oil from *Lavandula angustifolia* is capable to reduce the growth of oral bacteria which can be implemented as a potential substitute ingredient to common chemicals from oral care products.

Keywords: antiadherence, antibiofilm, essential oil, oral bacteria

ABSTRAK

Pelbagai jenis produk pembersih mulut di pasaran yang boleh digunakan untuk menyelesaikan masalah berkaitan penyakit oral. Namun, beberapa produk penjagaan mulut yang mengandungi bahan-bahan kimia seperti klorheksidin dan natrium lauret sulfat mungkin boleh menyebabkan kesan-kesan sampingan dalam bentuk pengelupasan lapisan mukosa, mulut kering atau kesakitan yang dirasakan dalam rongga mulut. Bagi menangani isu-isu berkaitan penyakit oral, gantian ke atas agen antibakteria yang lebih bersifat semula jadi diperlukan untuk mengurangi risiko kesan-kesan sampingan disebabkan penggunaan bahan-bahan kimia yang biasa didapati dari produk-produk penjagaan mulut. *Lavandula angustifolia* dikenali dengan sifat-sifat antibakteria dan penggunaannya sebagai minyak pati. Disebabkan sifat-sifat perubatan yang dimiliki, penyelidikan terkini tertumpu pada penilaian aktiviti antibakteria dari minyak pati *Lavandula angustifolia* ke atas pertumbuhan *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* dan *Streptococcus mutans.* Aktiviti antibakteria dinilai melalui perubahan warna reagen kepekatan rencatan minima 3-(4, 5-dimetilthiazol-2- yl)-2, 5-difeniltetrazolium bromida semasa melakukan eksperimen ke atas kepekatan rencatan minima dengan menggunakan kaedah mikropencairan. Sebanyak 0.1% sebatian klorheksidin digunakan sebagai kawalan positif sementara 10% dimetil sulfoksida (DMSO) digunakan sebagai kawalan negatif. Nilai kepekatan rencatan minima bagi minyak pati ditentukan pada 0.78% untuk kedua-dua *E. coli* dan *S. aureus*, manakala nilai kepekatan rencatan minima untuk *S. mutans* dan *P. aeruginosa* adalah 1.56% dan 6.25%. Berikutan dengan nilai kepekatan rencatan minima, setiap bakteria dijalankan ujian lanjutan ke atas aktiviti anti-pelekatan dan anti-biofilem. Selanjutnya, aktiviti anti-pelekatan bagi *E. coli* dan *P. aeruginosa* adalah 65.95 ± 6.83% and 74.07 ± 1.85% di mana ianya sedikit tinggi berbanding dengan *S. aureus* dan *S. mutans* yang menghasilkan aktiviti-aktiviti anti-pelekatan pada 61.78 ± 1.27% and 48.52 ± 13.17%. *E. coli* dan *P. aeruginosa* juga mempamerkan sedikit peningkatan dalam aktiviti anti-biofilem dalam julat dari 85% ke 90% selepas menggunakan minyak pati dari lavender berbanding dengan 77% ke 79% aktiviti anti-biofilem pada *S. aureus* and *S. mutans.* Analisa morfologi menerusi FESEM menunjukkan bahawa *S. mutans* mengalami perubahan ke atas sel membrannya. Kerosakan yang lebih teruk diperhatikan ke atas bakteria berbentuk kokus bilamana *S. mutans* didedahkan kepada minyak pati dari lavender pada nilai kepekatan rencatan minima dan separuh dari nilai kepekatan rencatan minima. Sebagai penutup, minyak pati dari *Lavandula angustifolia* berupaya untuk mengurangkan pertumbuhan bakteria oral di mana ianya berpotensi untuk digunapakai sebagai bahan pengganti ke atas bahan-bahan kimia yang biasa didapati dari produk penjagaan mulut.

Kata kunci: antipelekatan, antibiofilem, minyak pati, bakteria oral

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LIST OF ABBREVIATIONS

ATCC - American Type Culture Collection
B - Blank
BHI - Brain Heart Infusion
CHX - Chlorhexidine
dH_2O - Distilled water
DMSO - Dimethyl sulfoxide
EO - Essential Oil
FESEM - Field emission scanning electron microscope
MIC - Minimum Inhibitory Concentration
MTT - 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NA - Nutrient Agar
NaCl - Sodium chloride
NaOH - Sodium hydroxide
NB - Nutrient Broth
OD - Optical Density
PBS - Phosphate Buffered Saline
SEM - Standard Error of Mean
spp. - species
WHO - World Health Organization
ATCC - American Type Culture Collection
B - Blank

LIST OF SYMBOLS

°	-	Degree
°C	-	Degree Celsius
µL	-	Microlitre
mg	-	Milligram
mg / mL	-	Milligram per millilitre
µg	-	Microgram
g	-	gram
mL	-	Millilitre
mm	-	Millimetre
nm	-	Nanometre
h	-	Hour
k	-	kilo
min	-	Minute
rpm	-	Revolution per minute
v / v	-	Volume per volume
w / v	-	Weight per volume
%	-	Percent
pH	-	Potential of hydrogen
p	-	Probability
>	-	Greater than
<	-	Less than
+ve	-	Positive
-ve	-	Negative
±	-	Plus minus
Ωm	-	Ohm meter
<	-	Less than
>	-	More than

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Dental caries, plaques or cavities are example of oral diseases associated with pathogenic oral bacteria. Due to high prevalence of occurrence, oral disease is considered a global health problem once the condition became severe, difficult to manage and is not cost-effective (Freires *et al*., 2015). Oral pathogenic bacteria can lead to several severe dental problems such as pericoronitis, gingivitis, endodontitis and perimplantitis (Al-Terehi *et al*., 2018). In a process to encounter infection and to balance-out the load of oral bacteria, most people relied on the use of antiseptic mouth rinse that contained fluoride, povidoneiodine, chlorhexidine, and alcohol (Karbach *et al*., 2015). However, the use of antiseptic mouth rinse leads to health problem that includes tooth staining, alteration to taste, mucosal desquamation, xerostomia (dry mouth) or burning sensation (Freires *et al*., 2015; Karbach *et al*., 2015). Following the issues of side effect, it is imperative to have the antiseptic mouth rinse improved and developed to include natural active ingredients that can overcome the effects of harsh chemicals in dental products.

Essential oils are mainly used as an oil for therapeutic, aromatherapy, cosmetics, and other purposes (Djilani and Dicko, 2012). The aromatic properties of the essential oils are characterized based on their chemical constituents and functional groups which are relatively complex (Swamy *et al*., 2016). Accordingly, the bioactivity of essential oils is determined based on the types of chemical constituents comprising 20 – 60 bioactive compounds that are highly distinctive (Swamy *et al*., 2016). The chemical constituents and stereochemical properties of essential oils differ following the variation of geographical location, environment, and method of extraction. Essential oils can be extracted from different parts of plants' organ which includes stem, leaves, or flowers using various extraction techniques such as hydrodistillation, hydrodiffusion, effleurage, steam distillation, cold pressing, solvent extraction, microwave-assisted process and carbon dioxide extraction (Faleiro, 2011 ; Hamid *et al*., 2011). Subsequently after extraction, the essential oils would have to go through chemical characterization to measure its bioactivity efficiency.

Essential oils exhibit several mechanisms in inhibiting bacterial growth (Wijesundara and Rupasinghe, 2018). Among the reported mechanisms include destabilization of bacterial membranes, destruction of protein membrane, force depletion on proton motive, and release of cell contents (Nazzaro *et al.*, 2013 ; Lopez-Romero *et al*., 2015). In some cases, the use of essential oils might have lead to cell wall disturbance, cell permeability, protein synthesis and also changes to the pH (Faleiro, 2011). Other essential constituents were found to disrupt the bacterial motility or inhibit the toxin secretion to the outer environment (Swamy *et al*., 2016). According to Chouhan *et al*. (2017), nanoparticles (NPs) functionalized with essential oils have a significant antimicrobial properties against the proliferation of multidrug- resistant pathogens due to the increase of chemical stability and solubility, rapid decline of evaporation and minimal degradation of active essential oil components.

There is a growing trend in the number of research related to the bioactivity of the essential oils against various pathogenic microorganisms. Lavender essential oil, which was derived from *Lavandula* spp. had been used as a therapeutic agent and as a complementary medicine since long time ago. Despite of regular use of essential oil in daily life, the knowledge behind the bioactivity of essential oil is still preliminary and has not been extensively researched. Therefore, it is an objective of this study to evaluate the anti-adherence and anti-biofilm activity of essential oil from *Lavandula angustifolia* on selected oral pathogenic bacteria. This study also includes observation on bacteria that is structurally affected after being exposed to essential oil.

1.2 Problem Statement

In 2016, the Global Burden of Disease Study estimated 3.58 billion people equated to half of the world's population to be affected by oral diseases. Dental plaques and dental caries are examples of oral disease that happened due to high number of harmful microorganisms and low number of beneficial microbiota inside the mouth cavity. Therefore, toothpaste and oral rinses are used as a mean to reduce oral infections. However, dental products that contained synthetic chemicals such as sodium lauryl sulphate (SLS) or sodium lauryl sarcoside had reportedly causing oral desquamation in patient suffering from oral infection. Such isolated case of incompatible oral products was solved by advising the patient to switch conventional toothpaste to non-SLS toothpaste. Essential oils used in therapeutic and aromatherapy were known to exert bioactivity by acting as an antibacterial agent. Such potential use of essential oil has paved ways for discovery on substitutes of common antibacterial agent used in the production of healthcare products. Despite of the awareness, the mechanistic action of essential oils against bacteria remains debatable and the role of antibacterial was not clear to be use in overcoming side effects which warrant more investigation. Thus, the highlight of this research is to investigate the antibacterial properties of essential oil against the proliferations of pathogenic oral bacteria which could bring down the frequency of side effects.

1.3 Research Objectives

The objectives of this research were aimed to solve the above problem statements, in which the objectives include:

a) To evaluate antibacterial activity of essential oil from lavender on selected Gram-positive and Gram-negative oral bacteria.

b) To identify anti-adherence and anti-biofilm activities of essential oil from lavender on selected pathogenic oral bacteria.

c) To determine effects of essential oil from lavender on the structure of microbial cell membrane using field emission scan electron microscope (FESEM) analysis.

1.4 Significant of Study

Dental care products containing synthetic chemical have unknowingly introduced side effects to its' user which could be detected through but not limited to tooth staining, alteration to taste, mucosal desquamation and xerostomia (Freires *et al*., 2015; Karbach *et al*., 2015). Due to the occurrence of side effects, there is a need to replace such chemical that is known to cause side effects during and after use of conventional dental products. One of the approach was by adding plant-based compound such as essential oils to replace synthetic chemicals used in conventional dental products. Essential oils are eco-friendly, biodegradable, natural and renewable (Pandey *et al*., 2017). Compared to synthetic chemical in oral products, natural products are considered to be more stable due to the specific interactions of active compounds to the host cells, reducing side effects. Essential oils also contained various secondary metabolites that can be used to fight oral bacteria. Based on previous study, essential oil from lavender has high chemical composition of linalool and linalyl acetate (Anastasiou and Buchbauer, 2017) which contributed to antibacterial characteristics of essential oil (Sokovicx́ *et al.,* 2010). Blažeković *et al*. (2018) indicated that the antimicrobial property of essential oils from *Lavandula* displayed on a number of food-related pathogenic bacteria was due to the effect of linalool. Hence, essential oils of lavender could be used as alternative to antibiotics or antibacterial agents in conventional dental products. Thus, the significant of this study is to evaluate the biological properties of essential oil from lavender against the growth of known pathogenic bacteria that resides in oral cavity. In addition, this study aims to understand the protective mechanism exerted by essential oil and its' influence to bacterial growth which could determine the level of destruction introduced to the bacteria when the essential oils were applied. Such studies could be of useful for future references in view of using essential oil as derivatives for dental care product without affecting the colonization of good bacteria within mouth. Further identification on cell recognition was vital in order to prevent recurrence of side effects caused by specific pathogen which leads to better implementation of essential oil in dental care application system.

1.5 Scope of Study

Essential oil from lavender originated from doTERRA brand was used and tested for antibacterial activity against four types of cariogenic oral bacteria. The minimum inhibitory concentration (MIC) of essential oil against Gram positive *bacteria* (*S. aureus* and *S. mutans*) and Gram-negative bacteria (*E. coli* and *P. aeruginosa*) were determined through colour changes of MTT assay. The potential of anti-adherence and anti-biofilm activity of lavender essential oil on selected bacteria were evaluated based on the measurement of bacterial absorbance that adhered to the surface of universal bottle and capturing the absorbance of biofilm formation inside 96-well plates, respectively. Next, the structural alteration on microbial cell membranes of *S. mutans* was investigated by Field Emission Scanning Electron Microscopy (FESEM) in order to justify the antibacterial properties of essential oil from lavender.

REFERENCES

Abdullah, B. H., Hatem, S. F., & Jumaa, W. (2015). A comparative study of the antibacterial activity of clove and rosemary essential oils on multidrug resistant bacteria. *UK Journal of Pharmaceutical Biosciences*, *3*(1), 18–22.

Ademiluyi, A. O., Oyeleye, S. I., & Oboh, G. (2016). Biological activities, antioxidant properties and phytoconstituents of essential oil from sweet basil (Ocimum basilicum L.) leaves. *Comparative Clinical Pathology*, *25*, 169–176.

Al-Terehi, M., Shershab, S., Al-Rrubaei, H. A., & Al-Saadi, A. H. (2018). Some oral pathogenic bacteria, isolation and diagnosis. *Journal of Pure and Applied Microbiology*, *12*(3), 1495–1498.

Alves, S., Duarte, A., Sousa, S., & Domingues, F. C. (2016). Study of the major essential oil compounds of Coriandrum sativum against Acinetobacter baumannii and the effect of linalool on adhesion, biofilms and quorum sensing. *Biofouling*, *32*(2), 155–165.

Anastasiou, C., & Buchbauer, G. (2017). Essential oils as immunomodulators: Some examples. *Open Chemistry*, (15), 352–370.

Arshad, A. I., Ahmad, P., Dummer, P. M. H., Alam, M. K., Asif, J. A., Mahmood, Z., Mamat, N. (2020). Citation Classics on Dental Caries: A Systematic Review. *European Journal of Dentistry*, *14*(1), 128–143.

Asahi, Y., Miura, J., Tsuda, T., Kuwabata, S., Tsunashima, K., Noiri, Y., … Hayashi, M. (2015). Simple observation of Streptococcus mutans biofilm by scanning electron microscopy using ionic liquids. *AMB Express*, *5*(1), 0–8.

Azizan, N., Mohd Said, S., Abidin, Z. Z., & Jantan, I. (2017). Composition and antibacterial activity of the essential oils of Orthosiphon stamineus benth and Ficus deltoidea jack against pathogenic oral bacteria. *Molecules*, *22*(2135), 1–18.

B, S., & J, P. (2015). Evaluation of the Antibacterial Activity of a Sizable Set of Essential Oils. *Medicinal and Aromatic Plants*, *04*(02), 1–5.

Bagheri-Nesami, M., Shorofi, S. A., Nikkhah, A., Espahbodi, F., & Ghaderi Koolaee, F. S. (2016). The effects of aromatherapy with lavender essential oil on fatigue levels in haemodialysis patients: A randomized clinical trial. *Complementary Therapies in Clinical Practice*, *22*, 33–37.

Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, *6*, 71–79.

Bardají, D. K. R., Reis, E. B., Medeiros, T. C. T., Lucarini, R., Crotti, A. E. M., & Martins, C. H. G. (2015). Antibacterial activity of commercially available plant-derived essential oils against oral pathogenic bacteria. *Natural Product Research: Formerly Natural Product Letters*, 1178–1181.

Bikmoradi, A., Seifi, Z., Poorolajal, J., Araghchian, M., Safiaryan, R., & Oshvandi, K. (2015). Effect of inhalation aromatherapy with lavender essential oil on stress and vital signs in patients undergoing coronary artery bypass surgery: A single-blinded randomized clinical trial. *Complementary Therapies in Medicine*, *23*(3), 331–338. https://doi.org/10.1016/j.ctim.2014.12.001

Blažeković, B., Yang, W., Wang, Y., Li, C., Kindl, M., Pepeljnjak, S., & Vladimir-Knežević, S. (2018). Chemical composition, antimicrobial and antioxidant activities of essential oils of Lavandula × intermedia 'Budrovka' and L. angustifolia cultivated in Croatia. *Industrial Crops and Products*, *123*(February), 173–182.

Borges, L. P., Ferreira-Filho, J. C. C., Martins, J. M., Alves, C. V., Santiago, B. M., & Valença, A. M. G. (2016). In Vitro Adherence of Oral Bacteria to Different Types of Tongue Piercings. *Scientific World Journal*, *2016*.

Calo, J. R., Crandall, P. G., O'Bryan, C. A., & Ricke, S. C. (2015). Essential oils as antimicrobials in food systems - A review. *Food Control*, *54*, 111–119.

Chouhan, S., Sharma, K., & Guleria, S. (2017). Antimicrobial activity of some essential oils—present status and future perspectives. *Medicines*, *4*(58), 1–21.

Cui, H. Y., Zhou, H., Lin, L., Zhao, C. T., Zhang, X. J., Xiao, Z. H., & Li, C. Z. (2016). Antibacterial activity and mechanism of cinnamon essential oil and its application in milk. *Journal of Animal and Plant Sciences*, *26*(2), 532–541.

Devi, V. D., Kalpana, G., & Saranraj, P. (2017). Antibacterial activity of essential oils against human pathogenic bacteria. *Advances in Biological Research*, *11*(6), 357–364.

Diao, W. R., Hu, Q. P., Zhang, H., & Xu, J. G. (2014). Chemical composition, antibacterial activity and mechanism of action of essential oil from seeds of fennel (Foeniculum vulgare Mill.). *Food Control*, *35*(1), 109–116.

Djilani, A., & Dicko, A. (2012). The therapeutic benefits of essential oils. *Nutrition, Well-Being and Health*, 156–178.

Dye, B. A., Thornton-Evans, G., Li, X., & Iafolla, T. J. (2015). Dental caries and sealant prevalence in children and adolescents in the United States, 2011-2012. *NCHS Data Brief*, (191), 1–8.

Faleiro, M. L. (2011). The mode of antibacterial action of essential oils. *Science Against Microbial Pathoges: Communicating Current Research and Technological Advances*, 1143–1156.

Freires, I. A., Denny, C., Benso, B., Alencar, S. M. de, & Rosalen, P. L. (2015). Antibacterial activity of essential oils and their isolated constituents against cariogenic bacteria: A systematic review. *Molecules*, *20*(4), 7329–7358.

Furiga, A., Lonvaud-Funel, A., Dorignac, G., & Badet, C. (2008). In vitro anti-bacterial and anti-adherence effects of natural polyphenolic compounds on oral bacteria. *Journal of Applied Microbiology*, *105*(5), 1470–1476.

Gomes, L. C., & Mergulhão, F. J. (2017). SEM analysis of surface impact on biofilm antibiotic treatment. *Scanning*, 1–7.

Hamid, A. ., Aiyelaabge, O., & Usman, L. . (2011). Essential oils : Its medicinal and pharmacological uses. *International Journal of Current Research*, *3*(2), 86–98.

Heller, D., Helmerhorst, E. J., Gower, A. C., Siqueira, W. L., Paster, B. J., & Oppenheim, F. G. (2016). Microbial Diversity in the Early In Vivo -Formed Dental Biofilm . *Applied and Environmental Microbiology*, *82*(6), 1881–1888.

Huang, R., Li, M., & Gregory, R. L. (2011). Bacterial interactions in dental biofilm. *Virulence*, *2*(5), 435–444.

Hui, L., He, L., Huan, L., Xiaolan, L., & Aiguo, Z. (2010). Chemical composition of lavender essential oil and its antioxidant activity and inhibition against rhinitis-related bacteria. *African Journal of Microbiology Research*, *4*(4), 309–313.

Kang, M. S., Oh, J. S., Kang, I. C., Hong, S. J., & Choi, C. H. (2008). Inhibitory effect of methyl gallate and gallic acid on oral bacteria. *Journal of Microbiology*, *46*(6), 744–750.

Karbach, J., Ebenezer, S., Warnke, P. H., Behrens, E., & Al-Nawas, B. (2015). Antimicrobial effect of australian antibacterial essential oils as alternative to common antiseptic solutions against clinically relevant oral pathogens. *Clinical Laboratory*, *61*, 61–68.

Kouidhi, B., Al Qurashi, Y. M. A., & Chaieb, K. (2015). Drug resistance of bacterial dental biofilm and the potential use of natural compounds as alternative for prevention and treatment. *Microbial Pathogenesis*, *80*, 39–49.

Kwiatkowski, P., Łopusiewicz, Ł., Kostek, M., Drozłowska, E., Pruss, A., Wojciuk, B., Dołęgowska, B. (2020). The antibacterial activity of lavender essential oil alone and in combination with octenidine Dihydrochloride against MRSA strains. *Molecules*, *25*(95), 1–15.

Laheij, A. M. G. A., Kistler, J. O., Belibasakis, G. N., Välimaa, H., & de Soet, J. J. (2012). Healthcare-associated viral and bacterial infections in dentistry. *Journal of Oral Microbiology*, *4*(2012), 1–10.

Ledder, R. G., Timperley, A. S., Friswell, M. K., Macfarlane, S., & McBain, A. J. (2008). Coaggregation between and among human intestinal and oral bacteria. *FEMS Microbiology Ecology*, *66*(3), 630–636.

Lemes, R. S., Alves, C. C. F., Estevam, E. B. B., Santiago, M. B., Martins, C. H. G., Dos Santos, T. C. L., … Miranda, M. L. D. (2018). Chemical composition and antibacterial activity of essential oils from Citrus aurantifolia leaves and fruit peel against oral pathogenic bacteria. *Annals of the Brazilian Academy of Sciences*, *90*(2), 1285–1292.

Li, G., Wang, X., Xu, Y., Zhang, B., & Xia, X. (2014). Antimicrobial effect and mode of action of chlorogenic acid on Staphylococcus aureus. *European Food Research and Technology*, *238*(4), 589–596.

Liang, Y., Gao, H., Chen, J., Dong, Y., Wu, L., He, Z., Zhou, J. (2010). Pellicle formation in Shewanella oneidensis. *BMC Microbiology*, *10*(1), 291.

Lof, M., M. Janus, M., & P. Krom, B. (2017). Metabolic interactions between bacteria and fungi in commensal oral biofilms. *Journal of Fungi*, *3*(40), 1–13.

Lopez-Romero, J. C., González-Ríos, H., Borges, A., & Simões, M. (2015). Antibacterial effects and mode of action of selected essential oils components against Escherichia coli and Staphylococcus aureus. *Evidence-Based Complementary and Alternative Medicine*, *2015*, 1–9.

Lu, L., Hu, W., Tian, Z., Yuan, D., Yi, G., Zhou, Y., … Li, M. (2019). Developing natural products as potential anti-biofilm agents. *Chinese Medicine (United Kingdom)*, *14*(1), 1–17.

Lv, F., Liang, H., Yuan, Q., & Li, C. (2011). In vitro antimicrobial effects and mechanism of action of selected plant essential oil combinations against four food-related microorganisms. *Food Research International*, *44*(9), 3057–3064.

Markova, J. A., Anganova, E. V., Turskaya, A. L., Bybin, V. A., & Savilov, E. D. (2018). Regulation of Escherichia coli Biofilm Formation (Review). *Applied Biochemistry and Microbiology*, *54*(1), 1–11.

Moghimi, R., Ghaderi, L., Rafati, H., Aliahmadi, A., & Mcclements, D. J. (2016). Superior antibacterial activity of nanoemulsion of Thymus daenensis essential oil against E. coli. *Food Chemistry*, *194*, 410–415.

Najjar, T. (2017). Bacterial mouth infections: overview, bacterial endocarditis secondary to oral foci of infection, cardiovascular and cerebrovascular. *MedScape*, 1–11.

Nazzaro, F., Fratianni, F., De Martino, L., Coppola, R., & De Feo, V. (2013). Effect of essential oils on pathogenic bacteria. *Pharmaceuticals*, *6*(12), 1451–1474.

Palmer, R. J., Gordon, S. M., Cisar, J. O., & Kolenbrander, P. E. (2003). Coaggregation-mediated interactions of streptococci and actinomyces detected in initial human dental plaque. *Journal of Bacteriology*, *185*(11), 3400–3409.

Pandey, A. K., Kumar, P., Singh, P., Tripathi, N. N., & Bajpai, V. K. (2017). Essential oils: Sources of antimicrobials and food preservatives. *Frontiers in Microbiology*, *7*(2161), 1–14.

Prabuseenivasan, S., Jayakumar, M., & Ignacimuthu, S. (2006). In vitro antibacterial activity of some plant essential oils. *BMC Complementary and Alternative Medicine*, *6*(39), 1–8.

Rahman, M., Islam, M. N., Islam, M. N., & Hossain, M. S. (2015). Isolation and identification of oral bacteria and characterization for bacteriocin production and antimicrobial sensitivity. *Dhaka University Journal of Pharmaceutical Sciences*, *14*(1), 103–109.

Rodríguez-Tudela, J. L., Barchiesi, F., Bille, J., Chryssanthou, E., Cuenca-Estrella, M., Denning, D., … Verweij, P. E. (2003). Method for the determination of minimum inhibitory concentration (MIC) by broth dilution of fermentative yeasts. *Clinical Microbiology and Infection*, *9*(8), i–viii.

Sakkas, H., & Papadopoulou, C. (2017). Antimicrobial activity of basil, oregano, and thyme essential oils. *Journal of Microbiology and Biotechnology*, *27*(3), 429–438.

Santiago, K. B., Piana, G. M., Conti, B. J., Cardoso, E. de O., Andrade, B. F. M. T., Zanutto, M. R., … Sforcin, J. M. (2018). Microbiological control and antibacterial action of a propoliscontaining mouthwash and control of dental plaque in humans. *Natural Product Research*, *32*(12), 1441–1445.

Sharifi-Rad, J., Salehi, B., Varoni, E. M., Sharopov, F., Yousaf, Z., Ayatollahi, S. A.,Iriti, M. (2017). Plants of the Melaleuca genus as antimicrobial agents: From farm to pharmacy. *Phytotherapy Research*, 1475–1494.

Shi, C., Song, K., Zhang, X., Sun, Y., Sui, Y., Chen, Y., Xia, X. X. (2016). Antimicrobial activity and possible mechanism of action of citral against cronobacter sakazakii. *PLoS ONE*, *11*(7), 1–12.

Smigielski, K., Raj, A., Krosowiak, K., & Gruska, R. (2009). Chemical Composition of the Essential Oil of Lavandula angustifolia Cultivated in Poland. *Journal of Essential Oil-Bearing Plants*, *12*(3), 338–347.

Sokovicx́, M., Glamočlija, J., Marin, P. D., Brkić, D., & Van Griensven, L. J. L. D. (2010). Antibacterial effects of the essential oils of commonly consumed medicinal herbs using an in vitro model. *Molecules*, *15*, 7532–7546.

Swamy, M. K., Akhtar, M. S., & Sinniah, U. R. (2016). Antimicrobial properties of plant essential oils against human pathogens and their mode of action: An updated review. *Evidence-Based Complementary and Alternative Medicine*, 1–22.

Tada, A., & Hanada, N. (2010). Opportunistic respiratory pathogens in the oral cavity of the elderly. *FEMS Immunology and Medical Microbiology*, *60*(1), 1–17.

Thurnheer, T., & Belibasakis, G. N. (2014). Integration of non-oral bacteria into in vitro oral biofilms. *Virulence*, *6*(3), 258–264.

Van Houdt, R., & Michiels, C. W. (2005). Role of bacterial cell surface structures in Escherichia coli biofilm formation. *Research in Microbiology*, *156*(5–6), 626–633.

Vuotto, C., & Donelli, G. (2014). Field Emission Scanning Electron Microscopy of Biofi lm- Growing Bacteria Involved in Nosocomial Infections. *Microbial Biofi Lms: Methods and Protocols, Methods in Molecular Biology*, *1147*, 73–84.

Wang, C., van der Mei, H. C., Busscher, H. J., & Ren, Y. (2020). Streptococcus mutans adhesion force sensing in multi-species oral biofilms. *Npj Biofilms and Microbiomes, 6*(1), 1–9.

Wang, T. H., Hsia, S. M., Wu, C. H., Ko, S. Y., Chen, M. Y., Shih, Y. H., … Wu, C. Y. (2016). Evaluation of the antibacterial potential of liquid and vapor phase phenolic essential oil compounds against oral microorganisms. *PLoS ONE, 11*(9), 1–17.

Yamaji, K., Nakao, M., Hanada, N., Kaneko, K., Miyazaki, K., Ito, M., … Imai, S. (2015). Screening of probiotic candidates in human oral bacteria for the prevention of dental disease. *Plos One*, *10*(6), 1–20.