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Inhibitory Effect of Essential Oil Against Oral Pathogens

Research Article

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Abstract

Oral diseases are reflectedas the most common noncommunicable diseases and are associated with serious local and systemic disorders. Oral pathogens can grow and spread in the oral mucosae and frequently in biomaterials leading to several disorders such as dental caries and periodontal disease. However essential oils have shown interest in medicine because of their antibacterial, antifungal, and antioxidant properties.

Methodology: *Lavendula officianalis, Mentha piperita, Syzygium aromaticum, Cinnamomum zeylanicum Blume and Salvia officinalis* essential oils were investigated against antibiotic-resistant *Staphylococcus aureus, Escherichia coli and Streptococcus mutans*. Essential oils with high inhibition zone during bioassay method were further investigated using microdilution essay to investigate the minimum inhibition concentration. Essential oils at their minimum inhibition concentration were further tested for morphological changes using scanning electron microscope.

Results: *Salvia officinalis and Cinnamomum zeylanicum Blume* essential oils out of five selected essential oils exhibited great inhibition diameters. Cinnamomum zeylanicum Blume essential oil had MIC of 10 µg/mlfor treated *S. aureus,* 20 µg/ml for treated *E. coli* and showed 5µg/ml for S. mutans cells treated by *Salvia officinalis* essential oil. The SEM results have shown drastic morphological alterations when treated with Salvia officinalis and Cinnamomum zeylanicum Blume essential oil at their minimum inhibition concentrations.

Conclusion: *Salvia officinalis and Cinnamomum zeylanicum Blume* essential oil have revealed promising results for the treatment oral pathogens as discussed here.

Keywords: Essential Oils; Antimicrobial Activity; Oral Pathogens; Tooth Decay; Bacterial Infections.

Abbreviations: Minimum Inhibition Concentration (MIC); Scanning Electron Microscope (SEM).

Introduction

Oral diseases are global public health challenge affecting over 3•5 billion people and have negative economic and health impacts particularly in low income countries described by the World Bank [1-3]. In many low-income countries such as Central African Republic, Benin, Burundi, Burkina Faso, Ethiopia, Lesotho, Mali Malawi, Mozambique, Uganda oral diseases remain largely untreated because treatment costs exceed available resources [2, 4, 5]. Oral diseases continue to be a major public health problem in South Africa that needs to be addressed especially in disadvantaged communities [6, 7].

The most prevalent and noteworthy oral diseases globally are dental caries commonly known as tooth decay, periodontal disease, tooth loss, cancers of the lips [8]. The World Health Organization (WHO) highlights that oral diseases affect about 60–90% of school children and majority of adult's loss natural teeth globally [9-11], also believe that tooth decay is one of the most prevalent diseases and affects about 50% of children across the globe. The major cause of tooth decay is the bacterial biofilm that covers a tooth surface [8, 12, 14]. The biofilm formation is mostly sugar driven and results in the phasic demineralization and remineralisation of dental hard tissues [14]. Tooth decay occurs mainly on the tooth crown and later the root surfaces if it is not treated [14].

The bacterial growth in the mouth causes individuals to lose a tooth and therefore, dental implantology was introduced and dental implants are used to replace the missing tooth [15]. A missing

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tooth is substituted with dental implants to which a crown, bridge or denture can be attached. However, endosseous implants can fail either early or later stage of insertion due to implant infection [16, 17]. Periodontitis and peri-implantitis are inflammatory diseases caused by periodontal pathogenic bacteria destroying the supporting peri-implant tissue. As the bacteria migrate down the surface of the tooth or the implant, the inflammation spreads along with it [17, 18].

Oral pathogens route of transmissions such as staphylococcus aureus and *escherichia coli* can be through ingestion of contaminated food or water [19]. Escherichia coli is a nosocomial pathogen commonly known to cause urinary tract infections [20]. According to Ardila and Villa-Correa [21]. escherichia coli is associated with early implant failure and this bacterium is resistant to antibiotics such as doxycycline, amoxicillin, metronidazole, and aminoglycosides [21]. Furthermore, early implant failure is also associated with certain strains of bacteria such as streptococci, anaerobic gram positive cocci, and anaerobic gram negative rods [22]. A major health problem is that bacteria introduced during the placement of implants can lead to infection and a worst-case scenario is an implant failure just after the peri-implantation process [16, 17, 23]. Infections around biomaterials such as dental implants are difficult to treat and almost all infected implants must be removed, which is why it is so important to prevent infection of implanted dental implants [16, 17].

The primary bacterial colonizers on the surface of dental implants are Streptococci (Streptococcus viridans, Streptococcus mitis, Streptococcus oralis). Furthermore, Porphyromonas gingivalis, Prevotella intermedia are the causative mutans, Streptococcus sobrinus [24, 25]. According toSridhar et al., [23] bacteria colonization on the surface of dental implants are major contributors to rising number of dental implant failures and thus preventative and treatment options are needed Oral health preventative and treatment techniques.

Treatment options such as prophylactic systemic antibiotic regimens have been recommended to minimise infections after dental implant placement [16]. However Dhir [25], reported that bacteria have become antibiotic resistant due to the over prescription of antibiotics for individuals.

Fortunately, essential oils have demonstrated their potency to inhibit the growth of drug-resistant microbial strains which are even difficult to be treated by conventional antibiotics [26-27]. Essential oils are used as antimicrobial, anti-diabetic, antioxidants, treatment for cancer and cardiovascular diseases [26-27]. However, there is still limited information regarding the effects of the essential oils on the health and well-being of the oral cavity [28]. This has encouraged the search for new types of effective and nontoxic microbial agents among natural compounds which are found in aromatic plants and which have been used in folk medicine, in cosmetics and aromatherapy. Therefore, the current study would investigate the potency of selected essential oils (*Lavendula officianalis, Mentha piperita, Syzygium aromaticum, Cinnamomum zeylanicum Blume and Salvia officinalis*) against oral pathogens in the quest of preventing oral pathogens from affecting dental implants.

Materials and Methods

Essential oils

Essential oils have been known to exhibit important biological activities such as antimicrobial activity against several pathogens, however there is still limited information regarding the effects of the essential oils on the health and well-being of the oral cavity. Five essential oils namely *Lavendula officianalis, Mentha piperita, Syzygium aromaticum, Cinnamomum zeylanicum Blume and Salvia officinalis* were selected based on literature. These essential oils were obtained from the local suppliers. A preliminary investigation was conducted to determine the efficacy of the five selected essentials against the selected strains. Thus, essential oils that exhibit greater inhibition against oral pathogens were further investigated.

Microorganisms

Microorganisms used in this study were obtained from Laboratory Specialities (Pty) Ltd, South Africa. The strains used were *Staphylococcus aureus* (ATCC 25923), *Streptococcus mutans* (ATCC 25175) and *Escherichia coli* (ATCC 25922) which are known to be major causes of tooth decay.

Bioassay preparation

Quantitative microbial bioassay method was used to test the biological activity of essential oils inhibiting bacteria growth [29]. Bacterial strainsused in bioassay were regrown on petri dishes and incubated for 24 hours. Next, each bacterium was suspended in sterilized distilled water (dH20) and 0.2 ml spread out on PCA (0.5% m.v-1 agar). This produced a homogenous lawn across the surface of the agar Next, a well (0.5 cm in diameter and depth) was constructed at the center of each petri dish and 46 µl of the concentration of essential oils were added with ethanol. Ethanol (96%) was added alone to the wells as a control. All plates were incubated at 37°C for 24 hours until different textured growth zones were observed and their inhibition zone diameters (mm) were measured. Furthermore, minimum inhibitory concentrations (MIC) of essential oils with greater inhibition zones (cinnamon and sage) were determined using the microdilution assay.

Microdilution assay

Cinnamomum zeylanicum Blume and Salvia officinalis essential oils were selected for microdilution assay because of their significant inhibitory effect on *Staphylococcus aureus, Streptococcus mutans and Escherichia coli.* For microdilution essay, the inoculate of the bacterial strains were prepared in Mueller-Hinton Broth (MHB) for 24 hours, then suspensions of the 24 hours cultured bacteria in MHB broths were adjusted to 0.5 McFarland turbidity standard (approximately 1.5 x108 cfu/mL) [30]. Each bacterial suspension was dispensed into the 96-well sterile microtiter plate [31]. Each essential oil namely Cinnamomum zeylanicum Blume and Salvia officinalis were added to the first row of wells and serial dilutions were performed to achieve final concentrations of 40, 20,10, 5, 2.5 and 1.25µg/ml. The plate was then covered with a sterile plate sealer and incubated at 37°C overnight. To indicate growth after 24 hours; 20 µl of p-iodonitrotetrazolium violet (INT) was

added to each well. The plate was then incubated at 37°C for 20 minutes. Growth was indicated by colour change ranging from pink to violet. The experiment was run twice in duplicate for each concentration of incubation.

Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) was used to assess morphological changes that occur due to activity of *Cinnamomum zeylanicum Blume and Salvia officinalis* essential oils on the bacteria *S. aureus, E. coli and S. mutans*. Scanning electron microscopy (SEM) was used. Treated and untreated bacteria cells (from microdilution assay method) were primary fixed using 3% v/v of a sodium phosphate buffered glutaraldehyde solution at pH 7.0 for three hours and then samples were centrifuged and then further buffered in solution (1% m/v) of osmium tetroxide for an hour. Then the sample was dehydrated in a graded series of ethanol solution (30%, 50%, 70%, 90%, and 100% for 20 min per solution and the 100% dehydration was done twice for an hour). The samples were dehydrated with ethanol, critical dried and put on a filter paper and coated with uranium to make it electrically conductive. The coated sample was then examined using a SEM. Images were taken to investigate the morphological changes induced by essential oils on the bacteria cells [32, 33].

Results and Discussion

The antimicrobial activities of essential oils were qualitatively assessed by obtaining MIC values as shown in (Table 1- Table 3) for all tested microorganisms, particularly antimicrobial activity (MIC 5 µg/mL) of *Salvia officinalis* sage essential oil was noteworthy against *S. mutans*. This shows that *Salvia officinalis* essential oil has a very strong antibacterial activity. Furthermore, out of the five essential oils, *Cinnamomum zeylanicum Blume and Salvia officinalis* exhibited significant inhibitory effect (Figure 1) with an inhibition diameter of 40 mm for *Salvia officinalis* against *S. mutans* and 45 mm inhibition diameter of cinnamon oil against *E. coli and S. aureus* (40 mm). The results of different inhibition zones in (Figure 1) indicate that different essential oils have different efficacy this may be due to the modes of action of each essential oil against bacterial species. According to Goni et al., [34] enrichment of fresh products with Cinnamomum zeylanicum Blume known as cinnamon oil has been effective in reducing subsequent colonies of specific pathogens, especially fungi. In this study *Cinnamomum zeylanicum Blume* essential oil has shown antimicrobial activity against *S. aureus, E. coli and S. mutans* with an inhibition zone of(40mm) for *S. aureus*, (45mm) for *E. coli* and (10 mm) for *S. mutans*.

Different oil components may be active against different microorganisms therefore it is recommended that Gas chromatography-mass spectrometry (GC-MS) method be performed to investigate the chemical components responsible for inhibiting microbial growth. Mentha piperita (Peppermint oil) essential oil is known to be remarkably powerful for fighting oral pathogens and slaying common bacteria that can lead to cavities and gum disease [35]. Peppermint in the literature is also known to be capable of altering membrane permeability causing leakage of the cytoplasm and death by breaking cell membrane of *E. coli* [35, 36]. However, in this study peppermint essential oil shown the least antimicrobial activity against *S. aureus, E. coli and S. mutans* with an inhibition zone of *S. aureus* (6mm), *E. coli* (30mm) and *S. mutans* (10 mm) in (Figure 1).

Table 1. The minimum inhibitory concentration of *Cinnamomum zeylanicum Blume* against *S. aureus*.

Dilution of cinnamon oil and control(µg/ml)	≥40	≥20	≥10	≥ 5	≥2.5	≥1.25
Diluted cinnamon	-	-	-	+	+	++
Undiluted cinnamon	-	-	-	-	-	-
control	+++	+++	+++	+++	+++	+++

Table 2. The minimum inhibitory concentration of *Cinnamomum zeylanicum Blume* against *E.coli*.

Dilution of cinnamon oil and control(µg/ml)	≥40	≥20	≥10	≥ 5	≥2.5	≥1.25
Diluted cinnamon	-	-	+	+	++	++
Undiluted cinnamon	-	-	-	-	-	-
control	+++	+++	+++	+++	+++	+++

Table 3. The minimum inhibitory concentration of *Salvia officinalis* against *S. mutans*.

Dilution of sage oil and control(µg/ml)	≥40	≥20	≥10	≥ 5	≥2.5	≥1.25
Diluted sage	-	-	-	-	+	++
Undiluted sage	-	-	-	-	-	-
control	+++	+++	+++	+++	+++	+++

Data reported as' +++ strong growth of bacteria '++ medium growth '+' growth n '- 'inhibition growth of bacteria (sensitive to essential oils)

Syzygium aromaticum (Clove oil) is known to be effective against oral bacteria and fungi such as *C. albicans, S. aureus and S. mutans* associated with dental caries and periodontal disease [35, 37-38]. Syzygium aromaticum essential oil has shown antimicrobial activity against *S. aureus, E. coli and S. mutans* with an inhibition zone of *S. aureus* (7mm), *E. coli* (16mm) and *S. mutans* (20 mm) and this oil is one of the oils with least antimicrobial activity against *S. aureus*.

Out of the five essential oils, *Cinnamomum zeylanicum Blume* exhibited significant inhibitory effect with inhibition diameter of 45 mm for *E. coli* and 40 mm inhibition diameter against *S. aureus*.

Lavendula officianalis (lavender) is known to be responsible for destroying the cell wall and cytoplasmic membrane of the bacteria and further inhibiting its growth [39]. In literature lavender essential oil is known to have antimicrobial activity against enterococcus faecalis, staphylococcus aureus, candida albicans and Escherichia [39, 40]. In this study lavender essential oil, it has shown antimicrobial activity against *S aureus, E. coli and S. mutans* with least inhibition zone of *S. aureus* (5mm), *E. coli* (20mm) and *S. mutans* (20mm).

Morphological changes induced by essential oils on bacterial cell

Antibiotic resistance is a major public health problem because microorganisms can survive and multiply even in the presence of antimicrobial agents such as antibiotics [41]. Microorganisms prevent antimicrobial agents from reaching its target which is mainly the bacteria cell wall by reducing their ability to penetrate the cell. The bacteria cell also uses efflux pumps to pump out the antimicrobial agent preventing it to penetrate or gain entry into the cell [41]. Therefore essential oils are considered as most effective antimicrobial agents because of their mechanism of action which involves degrading of the bacterial cell wall, damaging the cytoplasmic membrane and cytoplasm coagulation [23, 42].

Alteration on the morphology of *Staphylococcus aureus, Streptococcus mutans and Escherichia coli* after being treated with *Cinnamomum zeylanicum Blume and Salvia officinalis* essential oils was observed under the scanning electron microscope (SEM).

The oral bacteria were treated with the oils at their respective MIC values in (Table 1-Table 3) and the SEM results are shown in (Figure 2 -Figure 4). There were noteworthy alterations on the bacterial cells with regards to its shape and size observed when treated with essential oils. Tariq et al., [26], believes that essential oils can easily penetrate through the bacterial cell membranes. The SEM results have shown that *Cinnamomum zeylanicum Blume and Salvia officinalis* essential oil has increased bacterial cell membrane permeability of *Staphylococcus aureus, Streptococcus mutans and Escherichia coli* leading to leakage of cellular content. *Cinnamomum zeylanicum Blume and Salvia officinalis* essential oil managed to pass through the bacterial cell wall of *Staphylococcus aureus, Streptococcus mutans and Escherichia coli*.

In Figure 2 (B) the morphology of the bacterial cells was slightly altered to loss of cellular content, bacteria cell damage, cell wall division and roughage when compared to the controls (untreated bacteria). Figure 2 (B) shows that few cells appeared collapsed and lysed, causing possibly leakage of the intracellular contents. These observations suggest that the mechanism of antibacterial activity of *Salvia officinalis* essential oil may be related to the disturbance of membrane structure or cell wall of the bacteria upon exposure.

Cinnamomum zeylanicum Blume essential oil targets the synthesis of cell walls and the cell membrane and similar results were observed in Figure 2 (B) where the cell wall of the *S. aureus* cell damaged leading to loss of cellular content. In Figure 3 (B) drastic morphological alterations upon the treatment of *S. aureus* with *Cinnamomum zeylanicum Blume* were observed as compared to the controls (untreated bacteria) in (A). It is possible that the inhibition and interference with synthesis of the cell wall may have caused the tested *S. aureus* bacterial cells to lose control over its shape and size and further disruption in homeostasis leads to shrinkage and eventually death of those cells. Furthermore, Cinnamomum zeylanicum Blume essential oil has caused roughage and high loss of cellular content. Husain et al., [43] believes that chemical compound of cinnamon oil such as cynammyldehyde, eugenol, especially the compound of cynammyldehyde are responsible for inhibiting growth of *S. aureus*.

In Figure 4 (B) shows drastic morphological alterations upon treatment of *E. coli* with *Cinnamomum zeylanicum Blume* as compared to the controls (untreated bacteria) in Figure 4 (A). In Comparison with normal bacterial cells, the treated bacteria cells showed that the cells became pleomorphic, irregular in size and some ruptured. The bacterial cells also presented irregular shape in Figure 4 (B); the *E. coli* cells became elongated after being treated. Similar results were observed in a study conducted by Zhang et al., [44]where *Cinnamomum zeylanicu*m Blume oil destroyed the bacteria cell wall leading to leakage of cellular content.

Figure 1. A graph of bacteria inhibition zones.

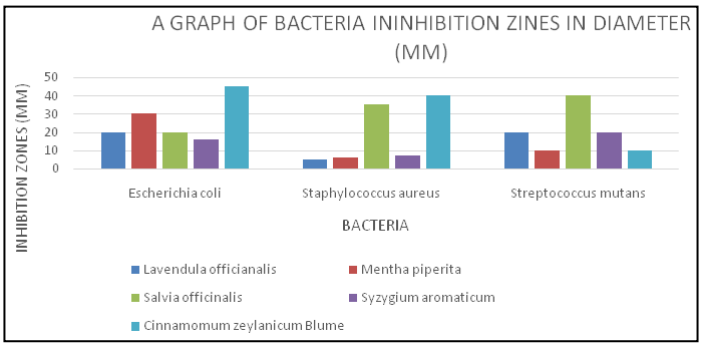


Figure 2. *S. mutans*(A) control.
B: *S. mutans* cells treated with *Salvia officinalis* oil showing loss of cell content (LCC), cell division (CD), damaged cell wall (DCW).

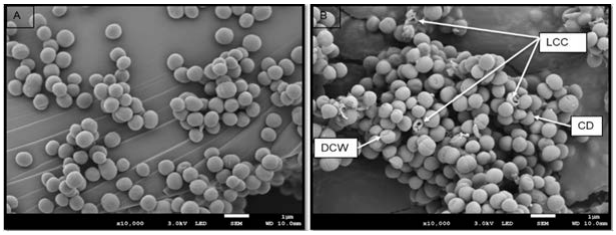


Figure 3. *S. aureus* cell (A) control.
B: *S. aureus* cells treated with *Cinnamomum zeylanicum Blume* showing swollen bacteria cells (SW), shrinkage of bacteria cells (SH), loss of cellular content (LCC).

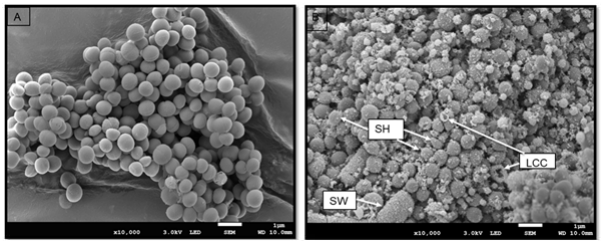
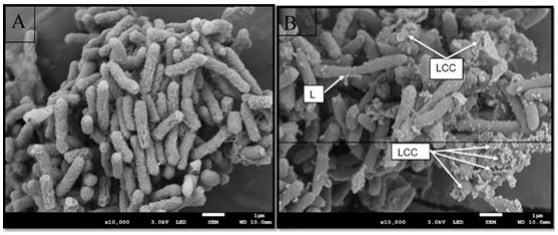


Figure 4. *E. coli cells* (A) control.
B: *E. coli* cells treated with *Cinnamomum zeylanicum Blume* oil), loss of cell content (LCC), elongated cell (L).



Conclusion

Antimicrobial agents such as essential oils need to be considered as potential future antimicrobials because of their mechanism of action against a bacterial cell. *Cinnamomum zeylanicum Blume and Salvia officinalis* essential oil pass through the bacterial cell wall gain entrance into bacteria cell causing disruption and therefore essential oils have a potential to be used as antimicrobial agents against *Staphylococcus aureus, Streptococcus mutans and Escherichia coli-bacteria*. The SEM results have also shown drastic morphological alterations such as leakage of bacteria cell contents, cells became pleomorphic, irregular in size and some ruptured, when treated with *Salvia officinalis and Cinnamomum zeylanicum Blume* essential oil at their minimum inhibition concentrations .The bioassay results showed that L*avendula officianalis, Mentha piperita, Syzygium aromaticum, Cinnamomum zeylanicum Blume and Salvia officinali*s essential oil can act as an effective antimicrobial agents against *Staphylococcus aureus, Streptococcus mutans and Escherichia coli* bacteria. Interestingly, *Salvia officinalis and Cinnamomum zeylanicum Blume* essential essential oil showed the most significant inhibitory effect on oral pathogens in the present work.

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