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Evaluation of Antibacterial Activity of *Cinnamomum Zeylanicum* Extract on Cariogenic Microorganisms of the Dental Plaque In-Vitro Study and Scanning Electron Microscopic Assessment

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Abstract

The present study was conducted to evaluate the antibacterial activity of *Cinnamomum zeylanicum* aqueous extracts and ethanol extracts on different type of dental plaque microorganisms. Screening study was performed to detect the potential antibacterial activity against *S. aureus, E. coli, S. mutans, Lactobacillus* and dental plaque pool samples. From the screening test, Minimum Inhibitory Concentration (MIC) values were determined. The lowest MIC value was 25 mg/ml of aqueous and 12.5 mg/ml of ethanol extract for *S. aureus*. The highest MIC values were seen in dental plaque anaerobic pool samples with 300 mg/ml of aqueous extract and 150 mg/ml of ethanol extract. The MIC values for aqueous extracts ranged from 25 to 300 mg/ml where as ethanol extract ranged from 12.5 to 150 mg/ml. The high concentration of ethanol extract, 100 mg/ml in the fixed plant concentration test showed the most inhibition effect for all the organisms tested. Generally, the *Cinnamomum zeylanicum* ethanol extracts demonstrated a stronger antibacterial activity compared to the aqueous extract. This study also compares the antibacterial activity of chlorhexidine with the plant extracts but chlorhexidine showed a higher antibacterial effect on the microorganisms where almost all organisms inhibited The morphological structures of *S. mutans Lactobacillus* were observed under SEM before and after treatment with *Cinnamomum zeylanicum* extracts and chlorhexidine digluconate mouthwash. This study did not detect any physical changes occurring in shapes and structures of the bacteria after treatment with *Cinnamomum zeylanicum* extracts. The scanning electron micrograph of bacteria after treatment with chlorhexidine digluconate mouthwash showed visible changes in the shapes and structures of the bacteria. C*innamomum zeylanicum* present promising antibacterial properties which could be used to inhibit dental plaque formation

Key words: C*innamomum zeylanicum extract*, Cariogenic bacteria, dental plaque, Chlorhexidine digluconate mouth wash

Introduction

Dental plaque is a thin whitish or pale yellow biofilm that builds up on the teeth. These biofilm are complex aggregation of diverse microorganisms. Overgrowth or imbalance of the dental plaque microbial communities will lead to the growth of more pathological organisms

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deeper inside the bacterial matrix of plaque biofilm [1]. Different degrees of pathological bacterial that colonizes the tooth and gingiva at different site will results in different types of oral diseases. The oral environment enhancing the growth of acidogenic and aciduric microorganism's results tooth decay and dental carries which is characterized by irreversible solubilization of tooth mineral by acid produced by these organisms [2].

Apart from dissoluting minerals from teeth, the microbial community also affects the gums adjacent to the teeth causing periodontal diseases. This disease occurred when the dental plaque organisms induces inflammatory responses due to various compounds elicited such as toxins in the tissue [3].

For as long as the science of dentistry has existed, there has been theorizing about the cause of dental caries. Today, all experts on dental caries generally agree that it is an infectious and communicable disease and that multiple factors influence the initiation and progression of the disease. The disease is recognized to require a host (tooth in the oral environment), a dietary substrate, and aciduric bacteria.' The saliva (also considered a host component), the substrate, and the bacteria form a biofilm (plaque) that adheres to the tooth surface. Treating the oral infection by reducing the number of cariogenic microorganisms and establishing a favorable oral environment to promote predominantly remineralization of tooth structure over time will stop the caries process and cure the disease. Curing the disease currently requires modifications by the patient and/or caretaker and relies on their compliance in making the necessary modifications. Research efforts are on-going to find a feasible method of achieving caries immunity that would be far less dependent on patient compliance [4].

Studies by Orland' and by Fitzgerald, Jordan, and Achard" demonstrated that dental caries will not occur in the absence of microorganisms. Animals maintained in a germ-free environment did not develop caries even when fed a high-carbohydrate diet. However, dental caries did develop in these animals when they were inoculated with microorganisms from caries-active animals and then fed cariogenic diets [5].

A number of microorganisms can produce enough acid to decalcify tooth structure, particularly aciduric streptococci, lactobacilli, diphtheroids, yeasts, staphylococci, and certain strains of sarcinae. *Streptococcus mutans* has been implicated as one of the major and most virulent of the caries-producing organisms. Consequently *S. mutans* has been targeted in a large share of research [3]. conducted an extensive review of the literature regarding the etiology of caries. He concluded that the evidence suggests that S. *mutans,* possibly *Streptococcus sobrinus,* and lactobacilli are human odontopathogens. He stated that aciduricity appears to be the most consistent attribute of S. *mutans* and is associated with its cariogenicity. He also observed that other aciduric species such as *S. sobrinus* may be more important in smooth-surface decay and are perhaps associated with rampant caries. Loesche concluded the review with the suggestion that treatment strategies that interfere with the colonization of S. *mutans* may have a profound effect on the incidence of caries in humans.' As caries research proceeds, there seems to be increasing evidence that disease may result from a group of microbial species in the tooth-adhering biofilm. Currently, which

combinations of organisms are most blameworthy is not clear. The acids that initially decalcify the enamel have a pH of 5.5 to 5.2 or less and are formed in the plaque material, which has been described as an organic nitrogenous mass of microorganisms firmly attached to the tooth structure. This film, which exists primarily in the susceptible areas of the teeth, has received a great deal of attention. Considerable emphasis is currently being given to plaque and its relationship to oral disease. Supragingival plaque control is largely the responsibility of the individual, using tooth brushes and interdental cleaning devices, however; mechanical plaque eradication is considered for most as time consuming, requires above average of motivation, skill and is more difficult for handicapped people [6].

This supports the concept of employing agents to control plaque and which require minimal cooperation and skill in their use this is the concept, which under lies, chemical plaque control.

Methods of chemical plaque control are being investigated. The method that has received the most attention during the past decade is the use of antimicrobial agents whose action is selective against certain types of microorganisms, including S. *mutans.*

A number of chemical agents which have antiseptic or antimicrobial action have been used, with variable success, to inhibit dental plaque formation. Among these are; phenolic compounds, Bis-biguanidaes, Pyrimidines, Quaternary ammonium compounds, Oxygenating agents, halogens, heavy metal salts [7]..And among these agents, chlorhexidine is, thus far, the most studied and effective antiseptic for plaque inhibition [8,9]. But in oral use as a mouth rinse chlorhexidine has been reported to have a number of side effects including: brown discoloration of the teeth, some restorative materials and mucosa, bitter taste, and some time sloughing of oral mucosa which restricts its general use. Other chemical antiplaque agents have been tested, none has shown equal or better results than chlorhexidine without eliciting unfavourable side effects,[10,11,12] In order to overcome such side effects the world health organization (WHO) advice researchers to investigate the possible use of natural products such as herb and plant extracts. Herb and plant extract have been used in oral hygiene products for many years if not centuries [7]. A number of clinical studies have shown effect of using mouth washes extracted from herbs such as Sanguinarina [13], Myrtus communis [14] Qureucus infectoria [15], Capparis spinosa [16], in the prevention of dental plaque accumulation.

Avoid many serious side effects, beside that this drug has a good therapeutic benefit [17] Cinnamomum zeylanicum, also known as cinnamon is a small ever-green tree with the height of 10-15 meters tall. It is one of the world's oldest spices belonging to the *Lauraceae* family with the genus *Cinnamomum.* There is hundreds of cinnamon species where most of the species are aromatic, comprises from this genus and *Cinnamomum zeylanicum,* with the synonym *Cinnamomum verum* is among them. This plant originated from Sri Lanka and Southern India [18].where the best cinnamon was grown and produced but it was also commercially grown in India, Indonesia, Vietnam, Madagascar and Egypt. The most used part of the Cinnamomum zeylanicum tree is the stem bark, which is widely used as spice. Each part of the *Cinnamomum zeylanicum* plant has a significant chemical composition that varies from

one another. The major constituents of the stem bark is cinnamaldehyde, which make up 75% of the total bark constituents [16] Other chemical components present in the bark are cinnamyl acetate, eugenol, cryophyllene and benzyl benzoate. *Cinnamomum zeylanicum* extracts have the widest spectrum of bacterial inhibition activity where its antibacterial properties can inhibit the growth of many types of bacteria compared to other plants [19, 20]. In previous studies, *Cinnamomum zeylanicum* extracts can inhibit gram positive and gram negative bacteria with low concentrations [21, 22]. This promising antibacterial activity of cinnamon causes cinnamon to be studied constantly with a wider range of bacteria. This study was driven based on a few main objectives where evaluating the antibacterial activity was the main goal. The aims were to evaluate the antibacterial activity of *Cinnamomum zeylanicum* aqueous extract and ethanol extracts on different cariogenic microorganisms of the dental plaque and to compare the overall effects of *Cinnamomum zeylanicum* extracts against a potent antibacterial mouthwash, Chlorhexidine digluconate.To evaluate the antibacterial effects of *Cinnamomum zeylanicum* aqueous extract and ethanol extracts on different on dental plaque pool sample (supragingival and subgingival plaque).To compare the morphology of *Streptococcus mutans* and *Lactobacillus* before and after treatment with *Cinnamomum zeylanicum* aqueous extract , ethanol extracts and Chlorhexidine digluconate mouthwash respectively by examination under Scanning Electron Microscope

Material and Methods

Preparation of Plant Extracts

Cinnamomum zeylanicum dried stem bark was purchased from a local market and extracted using two types of solvents which is water **(**Aqueous Extract**)** and ethanol (Ethanol Extract). Both extractions require different procedures. Both extracts were submitted to lyophilization by a freeze-dryer to produce powdered forms of the extracts. Lyophilization removes the solvents from the solutes and stabilizes the formulation so that it can retain satisfactory pharmacological activity during long-term storage. The freeze-dried products were stored in sterile universal bottles and refrigerated (-4°C) until time of use.

Dental Plaque Microorganisms

Four types of microorganisms used in this study where 2 microorganisms were used as internal control whereas 2 microorganisms represent cariogenic dental plaque bacteria. The internal control organisms were *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922).The dental plaque bacteria' which was *Streptococcus mutans* and *Lactobacillus*. These microorganisms were standardized to a fix amount of $1x10^8$ cells/ml after an overnight incubation with the help of hemocytometer, before these organisms were subjected to the antibacterial test later on. Apart from these microorganisms, a dental plaque pool sample was also obtained, where dental plaques were extracted randomly from the mouth and cultured in Brain Heart Infusion (BHI) broth and THIO Glycolate broth. These broths were chosen to grow aerobic, anaerobic and facultative bacteria respectively.

Experimental Controls

The positive control used throughout this study was Chlorhexidine digluconate antibacterial

mouthwash. On the other hand, sterile distilled water was used as the negative control.

Experimental Procedures

Disk Diffusion Test:

The Diameter of Inhibitory Zone (DIZ) for all the disks (positive and negative control, two aqueous extracts and two ethanol extracts) was observed where clear zones (complete inhibition) were seen on the agar and the diameters were measured using a ruler to the nearest millimeter (mm) readings. This test was done in duplicates.

Minimum Inhibitory Concentration (MIC) Test

The minimum inhibition concentration values were also studied for these organisms in order to determine the lowest concentration of plant extracts that can inhibit visible growth of these tested organisms after an overnight incubation. The lowest plant concentration that managed to inhibit the visible growth of the tested organisms was defined as the Minimum Inhibitory Concentration values. This test was also done in duplicates at different time.

Fix Plant Concentration Test

The procedures involved in this test are the opposite of the procedures involved in MIC where the bacterial dilution was done first instead of plant extracts concentration. The results will defined the lowest bacterial concentration and the number of bacteria that can be inhibited by the fixed plant concentration used.

Examination under Scanning Electron Microscope

The bacteria samples were observed under scanning electron microscope (JEOL-JSM 6400) at 10 KV. General pattern of bacterial structures was examined under magnification of x3500 and further being magnified up to x10000 and x20000 for specific morphological features. This procedure were carried out to evaluate the changes occur in the bacteria in terms of shape and structure before and after treatment with *Cinnamomum zeylanicum* extracts and Chlorhexidine digluconate mouthwash respectively

Results

1-Disk Diffusion Test

Table 1 shows the diameter of inhibition zones seen on agar according to each of the organisms tested. For the *S. aureus, S. mutans* plates, there are zones seen around the tested disks except the negative control disk. Higher concentration of plant extract showed a larger diameter of inhibition created compared to the lower concentration of plant extract and the ethanol extracts showed a greater inhibition in comparisons to the aqueous extracts .*E. coli* and *Lactobacillus* showed inhibition zones for the entire plant extracts disk tested except for the 100 mg/ml of aqueous extract disk where there was no zone seen around the disk. On the other hand, the dental plaque pool samples showed no inhibition zones for the entire plant extracts disk except for the higher concentration of ethanol extract which is 200 mg/ml. For the positive control disk, inhibition zones can be seen for all the organisms tested. The largest inhibition zone which is 22.5 mm. The negative control disk, inoculated with sterile distilled water doesn't inhibit the tested organisms; therefore no inhibition zones can be seen around every negative control disks used.

2- Minimum Inhibitory Concentration (MIC) Test Minimum Inhibitory Concentration (MIC) Value.

The graph showed the lowest plant concentration needed to inhibit the growth of the microorganisms. The values are the same for the two independent tests done and these values were based on the bacterial growth seen on the agar plates after an overnight incubation in the confirmation step (spot inoculation). The last plant concentration that inhibits the growth of these organisms is regarded as the MIC values. From the graph, the MIC value for S. aureus was 25 mg/ml of aqueous extract and 12.5 mg/ml of ethanol extract. These values are the lowest MIC values obtained in this study. The MIC values for *E. coli, S. mutans* and *Lactobacillus* were 100 mg/ml, 50 mg/ml and 200 mg/ml for the aqueous extract respectively. On the other hand, 25 mg/ml of ethanol extract is the MIC value for both *E. coli* and *S. mutans* whereas 50 mg/ml ethanol extract inhibits *Lactobacillus.*

This MIC values were the same for the anaerobic dental plaque pool samples. Higher aqueous extract was needed which is 300 mg/ml to inhibit both aerobic and facultative dental plaque pool samples but only 75 mg/ml of ethanol extract required to inhibit both of these samples. There was no inhibition seen by the negative controls therefore bacteria were seen growing on the plates. The positive control showed inhibition for all the tested organisms and dental plaque pool samples.

3- Fix Plant Concentration Test

The results for this test were determined by the highest concentration of bacteria that can be inhibited by the plant extract concentration chosen. Therefore, the readings starts from the lowest bacterial concentration which is 1x103 cells/ml up to the maximum concentration of $1x10^8$ cells/ml. Table 2 shows the bacterial concentration inhibited by 100 mg/ml of aqueous cinnamon extract, which is the low aqueous extract concentration. *S. aureus* and *S. mutans* were the only organisms to be completely inhibited whereas *E. coli* was inhibited up till $1x10^6$ cells/ml only. No inhibition was seen for *aerobic* and anaerobic dental plaque pool samples even at the lowest bacterial concentration,$1x10^3$ cells/ml. For the facultative pool sample, $1x10^3$ cells/ml of bacteria can be inhibited by 100 mg/ml of aqueous extract.

Table 3 represents the results for the inhibition by the high concentration of aqueous extract (200 mg/ml). The numbers of organisms inhibited were better compared to the low concentration where *S. aureus, E. coli and S. mutans* were completely inhibited and no sign of growth seen on the agar. The numbers of bacteria inhibited were increased in dental plaque pool samples with the high aqueous extract given where $1x10^6$ cells/ml of aerobic dental plaque pool sample is being inhibited. The results for the ethanol extract were shown in Table 4 and Table 5 where in Table 4, the lower ethanol extract was tested. Based on the results, 100 mg/ml of ethanol extract can inhibit more organisms with *S. aureus, E. coli* and *S. mutans* being completely inhibited. High concentration of *Lactobacillus* $1x10^6$ cells/ml was inhibited whereas significant reductions of dental plaque pool samples were seen. The facultative pool sample bacteria were inhibited with the highest number, $1x10^6$ cells/ml. In comparison, aerobic dental plaque pool samples were inhibited slightly higher compared to the anaerobic pool samples.

The results presented in Table 5 showed the best inhibition of bacteria in which all the organisms tested were completely inhibited up to the highest number of bacteria, $1x10^8$ cells/ml

except anaerobic dental plaque pool sample. The *Cinnamomum zeylanicum* ethanol extract concentration that was chosen to represent the higher plant concentration was 100 mg/ml. With this concentration, $1x10^7$ cells/ml of anaerobic dental plaque pool samples were managed to be inhibited. This showed that with 100 mg/ml of the ethanol extract, low concentration of these bacteria can be completely killed and inhibited. These results were confirmed based on the growth of the organisms on the agar plates after an overnight incubation. The results for the second run were the same as the initial test done with no differences.

4. Assessment of the bacterial morphology by examination under Scanning Electron Microscope (SEM)

Dental plaque cariogenic bacteria then were assessed for their morphological changes after treatment with *Cinnamomum zeylanicum* extracr and chlorhixidine digluconate mouthwash under Scanning Electron Microscope (SEM). Two bacteria species were chosen *S. mutuns* and *Lactobacillus.nucleatum*. All of the bacteria were examined under general view of magnification (x3500) and specific view of magnifications (x10000 and x20000) to get better morphological details. Scanning electron micrographs obtained before *Cinnamomum zeylanicum* extract and chlorhixidine digluconate mouthwash treatment showed that all bacteria species were in normal shapes which means that *S. mutuns* was present in shape of cocci meanwhile *Lactobacillus.nucleatum* was in shape of nice bacilli. After treatment with *Cinnamomum zeylanicum* extract and chlorhixidine digluconate mouthwash, these two bacterial samples were then viewed under SEM to look for their morphological changes. There were no physical changes was observed on all of the bacteria after they have been treated with *Cinnamomum zeylanicum* extract The bacterial cells were still viable with the shapes and structures remained the same as they were before the treatment. However, there were obvious morphological changes seen on the bacterial shapes and structures after undergoing Chlorhexidine digluconate mouthwash treatment. *S. mutans* shrank and formed irregular shape with no longer in shape of cocci **(Figure 1).** Viable bacterial cells do have smooth cell surface but death cells no longer retain the smooth surface. That is what happened to the *Lactobacillus* after treatment with Chlorhexidine. *Lactobacillus* shrank the cells surface wrinkled and the cell wall ruptured **(Figure 2).**

Discussion

In the present study, the antibacterial activities of *Cinnamomum zeylanicum* were evaluated against various dental plaque organisms including the dental plaque pool sample. The disk diffusion test was done to screen the antibacterial activity of the plant extract. The concentration chosen was based on literature review of previous studies where the disk diffusion test results gave a hint of the resistance or susceptibility of the tested organisms against the plant extracts. The more susceptible organisms required a lower starting value of plant extract for the Minimum Inhibitory Concentration (MIC) test whereas the resistance organisms needed a higher concentration of extract. The MIC test was used as the main antibacterial test to evaluate the antibacterial activity because this test can determine the lowest plant concentration needed to inhibit visible (99%) bacterial growth. From the MIC test, the Minimum Bactericidal Concentration (MBC) can

also be identified but MBC was not emphasized in this study. After the MIC value of each organisms determined, a random low and high plant extract concentration was chosen to be used in the fixed plant concentration test. The values chosen for the later test is within the MIC value of the highest and lowest MIC range for all the tested organisms. The motive of this test was to evaluate the number of bacteria that can be inhibited by the chosen plant concentration and to see the effects of *Cinnamomum zeylanicum* extracts if used at fix concentration as a mouthwash or tooth paste. This study uses chlorhexidine digluconate as the positive control because it's the recommended antibacterial mouthwash to control dental plaques as well as treating gingivitis [23]. The antibacterial effects of *Cinnamomum zeylanicum* was compared with the antibacterial effects of Chlorhexidine digluconate.

Based on the antibacterial tests done, the ethanol extract required at least half of the aqueous plant extract concentration to inhibit the same organisms tested. This was well expected because the major chemical constituents in the cinnamon bark was cinnamaldehyde which dissolved in solvents like 95% ethanol. Therefore, limited amount of this compound dissolved in aqueous extracts making the antibacterial activity of this particular extract lesser compared to the ethanol extract. But the results obtained in this present study were not consistent with the previous studies which proved low concentration of *Cinnamomum zeylanicum* extracts and MIC values can inhibit the relatively same organisms tested in this study where the MIC values from this study was very higher. Cinnamaldehyde is more soluble in pentene solvents compared to ethanol which explains the need of a higher concentration of the ethanol extract to exhibit the similar antibacterial effects towards the microorganisms [24].

The first organism to be inhibited at the lowest plant concentration was *S. aureus* followed by *S. mutans.* It showed that gram-positive cocci bacteria were the most susceptible to the cinnamon extract. Between *S. aureus* and E. *coli,* higher concentration of extracts needed to kill *E. coli* because *E. coli* is a gram-negative bacilli organism. But *Lactobacillus* which is anaerobe gram-positive bacterium required a higher plant extract perhaps due to its environmental conditions where it grows that causes *Lactobacillus* to be more resistance. For the dental plaque pool samples, the anaerobic cultures were the most resistance leading to a higher MIC values. Microscopic observation showed more gram-negative and gram-positive bacilli organisms seen in the culture compared to the aerobic broth which comprised more of gram-positive cocci bacteria.

Generally, the gram-positive organisms were less resistance towards the antibacterial properties of *Cinnamomum zeylanicum* extracts compared to the gram-negative organisms. The type of bacterial cell wall plays a crucial role. Gram-positive bacteria's cell wall has a thick layer of peptidoglycan, including teichoic and teichuronic acids. The peptidoglycan layer provides rigidity and protects the cell contents [2]. But the peptidoglycan layer is easily penetrated by antibacterial agents that managed to kill the bacteria and provide less protection due to its ineffective permeability barrier. In comparison, the gram-negative cell wall is more complex where it has an outer phospholipidic membrane that carries the structural lipopolysaccharide components. This characteristic makes

the cell impermeable to lipophilic solutes, while porins constitutes a selective barrier to the hydrophilic solutes. Therefore, with these special features, the gram-negative bacteria were more resistance to certain antibacterial agents including low concentration of cinnamon extracts.Cinnamaldeyhde interacts with the cell membrane which disrupts and leaks out small ions from the inner cell components. This interaction affected the integrity of the cell which induces the depletion of intracellular ATP (adenosine triphosphate) molecules. The interaction also caused more cell constituents to be release to the environment and the alteration of intracellular pH to a lower pH.

The high resolving power of Scanning Electron Microscope (SEM) has enabled the three dimensional images of the bacterial structure to be observed. The morphological structures of *S. mutans Lactobacillus* were observed under SEM before and after treatment with *Cinnamomum zeylanicum* extracts and chlorhexidine digluconate mouthwash. This study did not detect any physical changes occurring in shapes and structures of the bacteria after treatment with *Cinnamomum zeylanicum* extracts. Therefore, it explains the results of MIC test done earlier in which *Cinnamomum zeylanicum* extracts showed antimicrobial effects less than chlorhexidine digluconate mouthwash. The scanning electron micrograph of bacteria after treatment with chlorhexidine digluconate mouthwash showed visible changes in the shapes and structures of the bacteria. Chlorhexidine digluconate mouthwash caused most of the bacteria to shrink and to form wrinkled surfaces. In some bacteria such as *Lactobacillus*, there was ruptured in cell wall, which then makes the cell content to outburst during observation.

In comparison, chlorhexidine, a cationic substance that binds to bacterial cell wall and kills it [25]. The mechanisms of chlorhexidine is more efficient in disrupting the organisms' cell wall therefore, the antibacterial properties of chlorhexidine is better compared to cinnamon extracts. This explanation was side by side with the present study results which showed that chlorhexidine mouthwash could inhibit all the organisms tested including the dental plaque pool samples. This study showed that the ethanol extract of C*innamomum zeylanicum* has a better antibacterial properties compared to the aqueous extract in the entire antibacterial test done.

Conclusion

Cinnamomum zeylanicum ethanol extracts demonstrated a stronger antibacterial activity compared to the aqueous extract, It could be used as mouth wash to inhibit dental plaque formation. In a much wider scope, research is also needed to determine how the active compound in *Cinnamomum zeylanicum* inactivate and kill bacteria.

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Figure 1: Flow chart demonstrating the procedures involved in this study

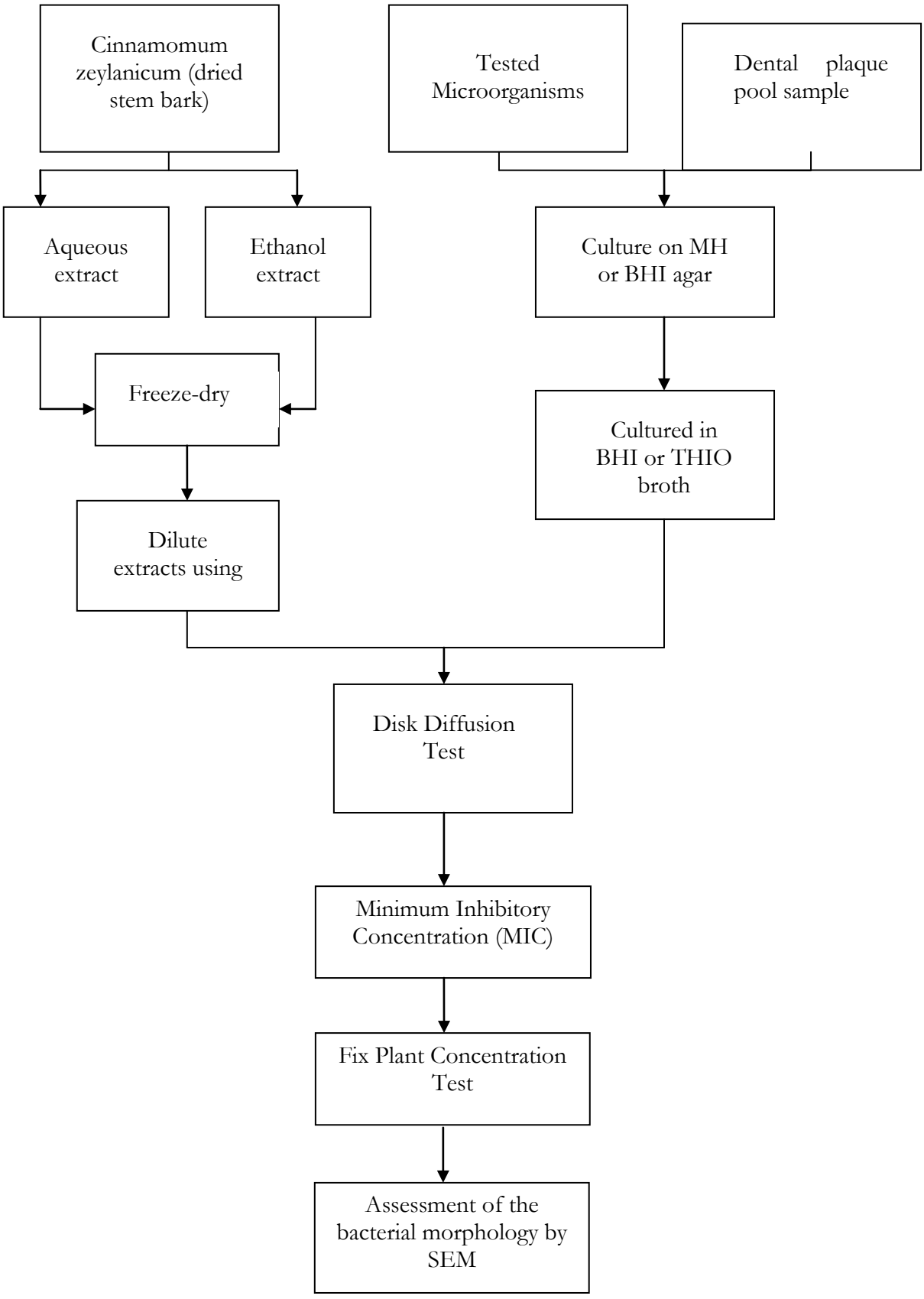


Table 1: The average Diameter of Inhibition Zone (DIZ) of various disk for the two independent Disk Diffusion tests seen on agar plate for each type of organisms tested.

DIZ (mm) / Organism	Aqueous Extract (mg/ml)		Ethanol Extract (mg/ml)		Chlorhexidine Mouthwash (+)control	Distilled water (-) control
	200	100	200	100		
S. aureus	10	9	16	14	22.5	-
E. coli	11	-	14	11.5	20	-
S. mutans	12	8.5	13.5	10	15	-
Lactobacillus	9	-	11	6.5	14	-
Dental plaque (Aerobic)	-	-	11	-	13.5	-
Dental plaque (Facultative)	-	-	8	-	11	-
Dental plaque (Anaerobic)	-	-	9	-	13	-

(Diameter of disk used = 6 mm)

Table 2: The number of bacterial concentration (cells/ml) for each organism inhibited by the low aqueous extract (100 mg/ml)

Organisms	Fixed plant concentration (Low concentration:100 mg/ml)						CHX (+) control	Distilled water (-) control
	Bacteria concentration (cells/ ml)							
	$1x10^8$	$1x10^7$	$1x10^6$	$1x10^5$	$1x10^4$	$1x10^3$	$1x10^8$	
S. aureus	-	-	-	-	-	-	-	+
E. coli	+	+	-	-	-	-	-	+
S. mutans	-	-	-	-	-	-	-	+
Lactobacillus	+	+	+	+	+	+	-	+
Dental plaque (Aerobic)	+	+	+	+	+	+	-	+
Dental plaque (Facultative)	+	+	+	+	+	-	-	+
Dental plaque (Anaerobic)	+	+	+	+	+	+	-	+

+ = Bacteria growth **-** = No bacteria growth

Table 3: The number of bacterial concentration (cells/ml) for each organism inhibited by the high aqueous extract (200 mg/ml)

Organisms	Fixed plant concentration (High concentration: 200 mg/ml)						CHX (+) control)	Distilled water(-) control
	Bacteria concentration (cells/ ml)							
	$1x10^8$	$1x10^7$	$1x10^6$	$1x10^5$	$1x10^4$	$1x10^3$	$1x10^8$	
S. aureus	-	-	-	-	-	-	-	+
E. coli	-	-	-	-	-	-	-	+
S. mutans	-	-	-	-	-	-	-	+
Lactbacillus	+	-	-	-	-	-	-	+
Dental plaque (Aerobic)	+	+	-	-	-	-	-	+
Dental plaque (Facultative)	+	+	+	-	-	-	-	+
Dental plaque (Anaerobic)	+	+	+	+	-	-	-	+

+ = Bacteria growth **-** = No bacteria growth

Table 4: The number of bacterial concentration (cells/ml) for each organism inhibited by the Low Ethanol Extract (50 mg/ml)

Organisms	Fixed plant concentration (Low concentration: 50 mg/ml)						CHX (+) control)	Distilled water (-) control
	Bacteria concentration (cells/ ml)							
	$1x10^8$	$1x10^7$	$1x10^6$	$1x10^5$	$1x10^4$	$1x10^3$	$1x10^8$	
S. aureus	-	-	-	-	-	-	-	+
E. coli	-	-	-	-	-	-	-	+
S. mutans	-	-	-	-	-	-	-	+
Lactobacillus	+	+	-	-	-	-	-	+
Dental plaque (Aerobic)	+	+	+	+	+	-	-	+
Dental plaque (Facultative)	+	+	-	-	-	-	-	+
Dental plaque (Anaerobic)	+	+	+	+	+	+	-	+

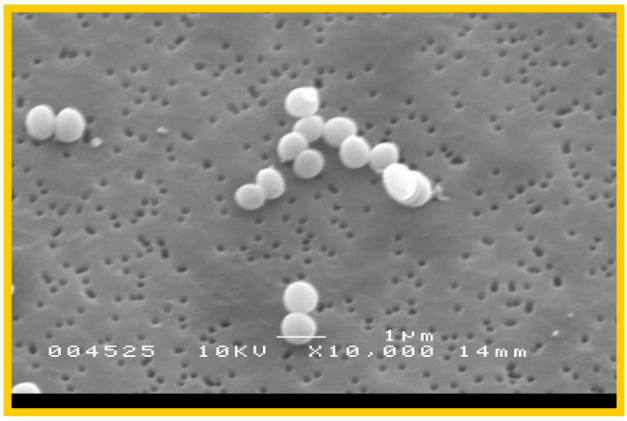
+ = Bacteria growth **-** = No bacteria growth

Table 5: The number of bacterial concentration (cells/ml) for each organism inhibited by the High Ethanol Extract (100 mg/ml)

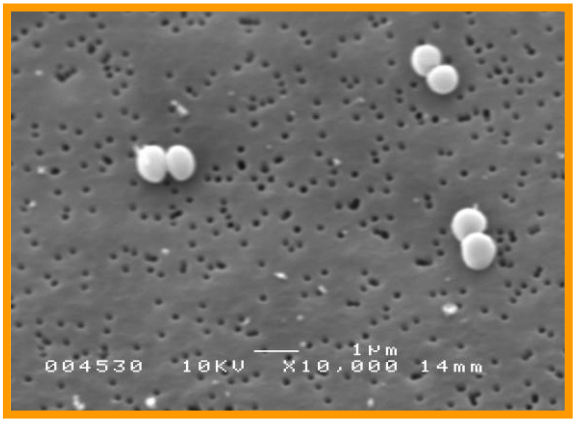
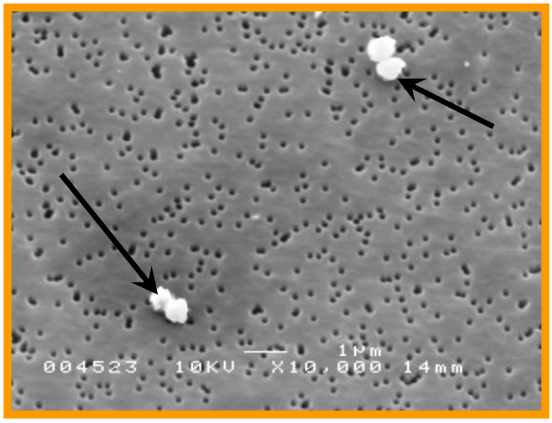
Organisms	Fixed plant concentration (High concentration: 100 mg/ml)						CHX (+) control)	Distilled water (-) control
	Bacteria concentration (cells/ ml)							
	$1x10^8$	$1x10^7$	$1x10^6$	$1x10^5$	$1x10^4$	$1x10^3$	$1x10^8$	
S. aureus	-	-	-	-	-	-	-	+
E. coli	-	-	-	-	-	-	-	+
S. mutans	-	-	-	-	-	-	-	+
Lactobacillus	-	-	-	-	-	-	-	+
Dental plaque (Aerobic)	-	-	-	-	-	-	-	+
Dental plaque (Facultative)	-	-	-	-	-	-	-	+
Dental plaque (Anaerobic)	+	-	-	-	-	-	-	+

+ = Bacteria growth **-** = No bacteria growth

Figure 1: Scanning electron micrographs of *S. mutans* before and after treatment with *Cinnamomum zeylanicum* and Chlorhexidine digluconate mouthwash.



S. mutans before treatment with *Cinnamomum zeylanicum* and chlorhixidine digluconate mouthwash.

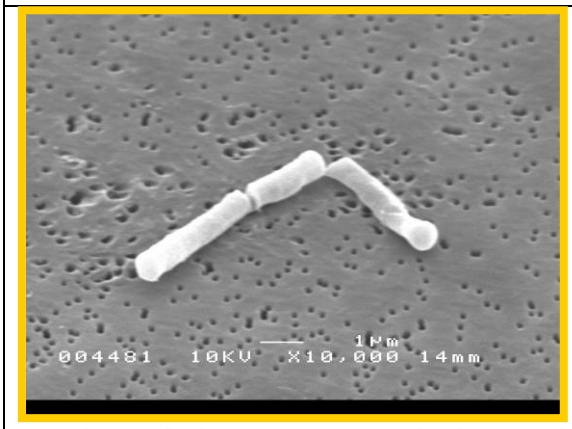
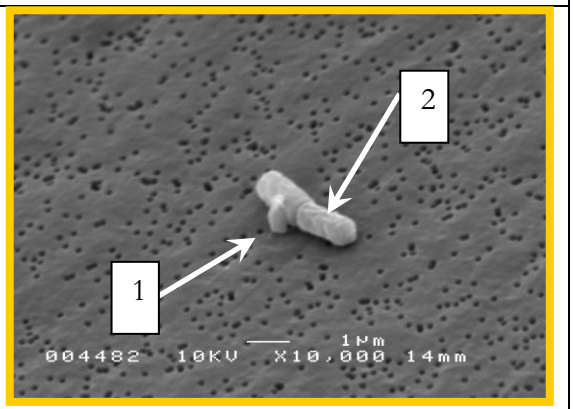
After treatment with	
Cinnamomum zeylanicum	Chlorhexidine Digluconate mouthwash.
	
No physical changes seen on the bacterial cells.	The bacterial cells shrank and formed irregular shapes.

(Magnification: x1000010KV)

*****The black arrows show the irregular shapes of bacteria after treatment with Chlorhexidine digluconate mouthwash.

Figure 2: Scanning electron micrographs of *Lactobacillus* before and after treatment with *Cinnamomum zeylanicum* and Chlorhexidine digluconate mouthwash.

Lactobacillus before treatment with *Cinnamomum zeylanicum* and Chlorhexidine digluconate mouthwash.

After treatment with	
Cinnamomum zeylanicum	Chlorhexidine digluconate mouthwash.
 No physical changes seen on the bacterial cells.	 The bacterial cell wall ruptured and the cell surface wrinkled.

(Magnification: x1000010KV)

*****The first arrow shows the bacterial cell wall ruptured and the second arrow shows wrinkled surface of bacterial cells