MDJ

Effect of Water Cinnamon Extract on Mutans Streptococci , in Comparison to Chlorhexidine Gluconate and Zac *(In Vitro and In Vivo Study)*

Dr. Jinan Mohammed Rashad, B.D.S., M.Sc. in Preventive Dentistry

Abstract

Background: The purpose of this study was to evaluate the effect of different concentrations of water cinnamon extract on growth of mutans streptococci, in comparison to chlorhexidine gluconate (0.2%), Zac [0.12% chlorhexidine with 0.05% sodium fluoride (Maleh Chemical products, Syria)] and deionized water *in vitro* and viability counts of mutans streptococci among a number of volunteers.

Material and Methods: Stimulated saliva was collected from six healthy looking students aged (22-24) from which mutans streptococci were isolated. Sensitivities of mutans streptococci according to Agar Well Technique showed that mutans streptococci were more sensitive to chlorhexidine and Zac compared to water cinnamon extract. The effect of these agents on the viability counts of mutans streptococci in comparison to the control *in vitro* were studied, a significant reduction in the counts of bacteria at concentrations 20% of water cinnamon extract was illustrated ($P<0.05$), at higher concentrations water cinnamon extract showed a highly significant reduction in the counts of bacteria($P<0.001$), concerning chlorhexidine, and Zac there was a highly significant reduction in the counts of bacteria in comparison to the control after 24 hour($P<0.001$). The study involved one *in vivo* experiment to test the effect of water cinnamon extract (20%) against salivary mutans streptococci in comparison to 0.2% chlorhexidine, Zac and deionized water. Stimulated saliva was collected from 20 subjects (22-25), they were divided into four groups, each group rinse once with either chlorhexidine, Zac, cinnamon extract or deionized water. A non significant difference was found between cinnamon and chlorhexidine compared to deionized water in the counts of bacteria after thirty minute. There was a highly significant difference between Zac and deionized water, rinsing with Zac resulted in a highly significant reduction in the count of bacteria within this time followed by chlorhexidine which was not significantly difference from cinnamon, deionized water showed a slight reduction in the counts of bacteria. The same result was shown after one hour, within this time chlorhexidine resulted in a highly significant reduction in the counts of bacteria compared to deionized water. After two hours of rinsing there was no significant difference between cinnamon and deionized water in the counts of bacteria.

Conclusion: Cinnamon would be a useful compound for development of antibacterial agents against mutans streptococci although its effectiveness was less than chlorhexidine but it may has potential for use in mouthwash for preventing dental caries.

Keywords: water cinnamon extract, mutans streptococci, mouth rinse.

Introduction

Dental caries is a chronic, infectious disease caused by bacterial by-products that dissolve the enamel surface of teeth. The interaction of three factors allows this to happen: a susceptible tooth surface, specific bacteria particularly Streptococcus mutans and diet rich in substrates, particularly sugars [1]. Dental caries remain a public health problem for many developing countries and for underprivileged populations in developed countries [2, 3]. Prevention of dental caries is directed to the increase of resistance of teeth to acid attack by the multiple fluoride therapy, diet modification involving restriction of sugar intake, an ordinary oral hygiene measures involving mechanical plaque control in addition to chemical plaque control through the use of antimicrobial agents as a mouth wash [4, 5]. Currently, chlorhexidine is the most potent chemotherapeutic agent against mutans streptococci and dental caries, however, the incidence of side effects such as teeth discoloration, undesirable taste, dryness and burning sensation in the mouth discourage patients to use this mouth wash [6]. Furthermore the combination of chlorhexidine and fluoride has additional effect against plaque accumulation and cariogenic microorganisms by the presence of fluoride [7].

Natural products are now preferred by a large proportion of the population and have been reported to possess antimicrobial activity [8]. These products have recently been shown to be a good alternative to synthetic chemical substances for caries prevention [9]. Extracts from Siwak, green and black tea and garlic have proved to be effective against Streptococcus mutans. [10, 11, 12, 13] Cinnamon (Cinnamomum zeylanicum) is one of these herbs which have been used extensively for treatment of several conditions, it is a member of the Lauraceae family. Cinnamon is the inner bark of the cinnamon tree which has been used as a spice for thousands of years in dried form as sticks or in ground form as cinnamon powder. The characteristic flavor and aroma of cinnamon comes from a compound in the essential oil of the bark called cinnamonaldehyde. Besides using it in cooking, cinnamon is also thought to have health benefits [14]. In traditional medicine cinnamon is used for colds, flatulence, nausea and diarrhea. It's also believed to improve energy, vitality, and circulation [15]. Studies have found that cinnamon may have antibacterial and antifungal properties [16, 17]. Cinnamon is also useful as a food preservative to inhibit the growth of common food-borne bacteria such as Salmonella and E coli [18]. However, there is a lack of studies regarding its specificity against cariogenic bacteria especially mutans streptococci which may prove its potency against this bacteria.

Materials and method

Preparation of the Crude Aqueous Cinnamon Extract:

Cinnamon was purchased from the market, ground into fine powder in an electrical mixer. One hundred gram of finely powdered cinnamon mixed with one liter of sterile deionized water and kept in a water bath at 60 °C for five hours, then filtered through sterile filter paper (Whatman, UK). The extract then left to dry at 40°C in hot air oven for evaporation of water. The extract was preserved in a refrigerator until use [19].

Collection of Saliva:

Stimulated saliva was collected from six healthy looking students aged

(22-24) years in order to carry out *in vitro* experiments.

Isolation of Mutans streptococci:

Salivary samples were homogenized by vortex mixer for two minutes, and then ten- fold dilution was performed. From dilution 10^{-3} of salivary samples 0.1 was taken and spread in duplicate on the Mitis Salivarius Bacitracin agar media, the plates were incubated anaerobically for 48 hour at 37°C then aerobically for 24 hour at room temperature. The colonies were identified on the basis of morphology of the colonies, Gram's stain, and biochemical test [The ability of mutans streptococci to ferment sugar was tested by addition of selected types of sugar (sorbitol or manitol) in a concentration of 1% in Brain Heart Infusion Broth] [20].

Bacterial Activation:

This was achieved by addition of pure isolates of mutans streptococci about 0.1ml added to 10 ml of sterile Brain Heart Infusion broth (pH 7.0), incubated aerobically for 18 hour before each experiment.

Experiment One: Sensitivities of Mutans Streptococci to Different Concentrations of Water Cinnamon Extract, Chlorhexidine, Zac and Deionized Water, *In Vitro.*

Different concentrations of water cinnamon extract in addition to chlorhexidine gluconate (0.2%), Zac and deionized water were used in this experiment. A volume of 25 ml of Mueller Hinton Agar was poured into sterile glass petridishes, left at room temperature for 24 hour. To each plate 0.1 ml of mutans streptococci inoculum was spread, left for 20 minute at room temperature then wells of equal size and depth were prepared in each plate, each well was filled with 0.2 ml of the test agents. Plates were left at room temperature for one hour then incubated anaerobically for 24 hour at 37°C, zone of inhibition was measured across the diameter of each well.

Experiment Two: Effects of Water Cinnamon Extract, Chlorhexidine, Zac, and Deionized Water on Viability Counts of Mutans Streptococci, *In Vitro*:

Different concentrations of cinnamon extract were prepared. Brain Heart Infusion broth (pH 7.0) was distributed in test tubes by 8.9 ml in each one. One ml of the test agent was added to each tube, after that 0.1 ml of bacterial inoculum was added to both study and control tubes. From the control tube 0.1 ml was transferred to 0.9 ml of sterile phosphate buffer saline (pH 7.0) and a ten–fold dilution was performed. From dilutions 10^{-3}, 0.1ml was taken and spread in duplicate on Mitis Salivarius Bacitracin agar plates, the plates then incubated anaerobically at 37°C for 48 hour. Then colony forming unit per milliliter (CFU/ml) was counted, this value was considered as the initial count of bacteria. Study and control were incubated aerobically at 37°C for 24 hour. From each tube of the control and study 0.1 ml was transferred to 0.9 ml of phosphate buffer saline and a ten fold dilution was performed. From dilutions 10^{-3}, 0.1 ml was taken and spread in duplicate on Mitis Salivarius Bacitracin agar plates, the plates then incubated anaerobically at 37°C for 24 hour. The colony–forming unit per milliliter was counted (CFU/ ml) for all the plates.

Experiment Three: Effects of Water Cinnamon Extract, chlorhexidine, Zac, and Deionized Water on Salivary Counts of Mutans Streptococci, *In Vivo*:

The effects of these agents were tested on the saliva of a group of volunteers, the volunteers participated in this experiment were 20 subject, they were divided into four groups five in each one. The first group was the

experimental group that used water cinnamon extract (20%) as a mouth rinse, the second group rinsing with chlorhexidine (0.2%), the third one used Zac as mouth rinse, and the last group rinsed with deionized water. All the volunteer participated in this experiment were healthy looking with no medical history, did not receive any antimicrobial agents during the last two weeks prior to the study and not wearing any fixed or removable prosthesis or orthodontic appliance. The age range was (22-25) years. The volunteers were asked to suspend their usual oral hygiene practice at the day of experiment [21].

Procedure:

Each volunteer was given a piece of Arabic gum and asked to chew it for one minute only, then stimulated saliva was collected in sterilized screw capped bottles [22]. After one minute, each volunteer was asked to rinse with 10 ml of test agent for one minute then expectorate. Stimulated saliva was recollected in the following points: after 30 minute of rinsing, one hour, and, two hours. During this time, the volunteers were asked not to eat or drink any thing except water. Salivary samples were dispersed for two minutes by vortex mixer, then 0.1 ml of saliva transferred to 0.9 ml of sterile phosphate buffer saline (pH 7.0), and ten-fold dilutions were performed. From the dilution 10^{-3}, 0.1 ml was taken and spread in duplicate on Mitis Salivarius Bacitracin agar plates, these plates were incubated anaerobically for 48 hour at 37°C then aerobically for 24 hour at room temperature. The number of colonies was expressed as colony–forming units multiplied by the dilution factor per milliliter of saliva (CFU/ml).

Statistical Analysis:

Processing of the data was carried using SPSS package, which include the following:

1-Calculation of the statistical parameters, mean, standard deviation.
2-Student's t-test for calculating the significance of differences between means of different groups.

Results

Sensitivities of Mutans Streptococci to Different Concentration of Water Cinnamon Extract, and Antimicrobial Agents *In Vitro*.

Sensitivities of mutans streptococci to different concentrations of water cinnamon extract, Zac, chlorhexidine gluconate and deionized water are seen in Table (1). For cinnamon extract, diameter of inhibition zones were found to increase as the concentration of cinnamon extract increased. The highest zone of inhibition was found by Zac followed by chlorhexidine. No zone of inhibition was shown for deionized water. Using t-test to compare each concentration of water cinnamon extract with either chlorhexidine or Zac, a highly significant difference was shown between chlorhexidine and different concentrations of water cinnamon extract, a significant difference was found on comparing between concentration 50% water cinnamon extract and chlorhexidineTable (2), the same results was shown on comparing between Zac and different concentration of cinnamon extract Table (3).

Effect of Water Cinnamon Extract and Antimicrobial Agents on Viability Count of Mutans Streptococci, *In Vitro:*

According to this experiment the counts of mutans streptococci was recorded before the application of the tested agents, this was considered as the initial counts of bacteria, after 24 hour of incubation period, the number of mutans streptococci was counted. A

statistically highly significant increase in the number of bacteria was recorded after 24 hour (t-test =35.055, P<0.001, d.f=8). Table (4) shows mean, standard deviation for counts of bacteria before and after the application of the tested agents. The counts of bacteria after 24hour were compared with that after the application of different agents, results showed that there was no significant reduction in the counts of bacteria at concentration of 15% water cinnamon extract, the same result was shown for deionized water. A significant reduction in the counts of bacteria at concentrations 20% of water cinnamon extract was illustrated (P<0.05), at concentrations of 25%and 30% of water cinnamon extract a highly significant difference was found, concerning chlorhexidine, and Zac there was a highly significant reduction in the counts of bacteria in comparison to the control after 24hour (Table 5).

Effects of Water Cinnamon Extract, CHX and Deionized Water on the Viability Counts of Salivary Mutans Streptococci, *In Vivo*:

Mean and standard deviation of counts of bacteria estimated before and after rinsing with 20% cinnamon water extract, chlorhexidine gluconate, Zac and deionized water at each time interval is seen in (Table 6), For all agents tested a reduction in the counts of bacteria was observed after 30 minute, which continued after one hour for Zac and chlorhexidine then gradually increased but remained less than that of the baseline, rinsing with either water cinnamon extract or deionized water resulted in a reduction in the count of bacteria for after 30 minute then gradually decreased until reached the baseline value after two hours. (Table 7) shows statistical differences in counts of bacteria in the baseline and the other time points, for water cinnamon extract a significant difference in the counts of bacteria was reported after 30 minute and after one hour, concerning chlorhexidine a significant reduction in the count of bacteria was shown after half an hour, while a highly significant reduction was found after one hour, rinsing with Zac result in a highly significant reduction in the counts of bacteria after 30 minute and after one hour, for deionzed water, no significant reduction in the counts of bacteria was shown for the following time point after rinsing. All tested agents showed no significant difference after two hours of rinsing. Table (8) illustrates the statistical difference between agents in addition to control in counts of bacteria at different time points. After 30 minutes a non significant difference was found between cinnamon and chlorhexidine, rinsing with either chlorhexidine or cinnamon resulted in a significant reduction in the counts of bacteria compared to deionized water, there was a highly significant difference between Zac and deionized water, rinsing with Zac resulted in a highly significant reduction in the count of bacteria within this time followed by chlorhexidine which was not significantly difference from cinnamon, deionized water showed a slight reduction in the counts of bacteria. The same result was shown after one hour, within this time chlorhexidine resulted in a highly significant reduction in the counts of bacteria compared to deionized water. After two hours of rinsing there was no significant difference between cinnamon and deionized water in the counts of bacteria.

Discussion

Cinnamon is mainly used in foods mainly because it gives desirable flavors and aromas, in addition it

shows antimicrobial activity, the antimicrobial activity of cinnamon has been studied since the end of the last century and the active components were determined [23], however, there is a lack of information concerning its specificity against cariogenic bacteria in regard to its effect on growth. For this reason, cinnamon was selected to test its effect on mutans streptococci bacteria. Sensitivities of mutans streptococci to different concentrations of water cinnamon extract in comparison to chlorhexidine gluconate (0.2%), Zac and deionized water were tested using Agar Well Technique, cinnamon extract was found to inhibit the growth of these bacteria. A minimum concentration needed to produce inhibition zone was 10%. Several studies have shown that cinnamon had strong and consistent inhibitory effects against various pathogens [24, 25]. The antibacterial activity has been attributed to the presence of some active constituents, as will be discussed late. The zone of inhibition was found to increase as the concentration of cinnamon extract was increase, these findings were in coincidence with Nanasombat S and Lohasupthawee [26]. Sensitivities of mutans streptococci to chlorhexidine and Zac were tested and compared to water cinnamon extract. The highest zone of inhibition was shown by Zac followed by chlorhexidine both of them showed highly significant difference compared to water cinnamon extract. Chlorhexidine is considered as a safe and effective antiseptic agent for the reduction of mutans streptococci levels, both in plaque and saliva, the anti-bacterial action is due to a disturbance of the transport through the cell membrane and of the bacterial metabolism, and by causing leakage through the cell membrane [27]. Clinical and *in vitro* studies have demonstrated that its association with fluoride is particularly effective against *mutans* streptococci. According to Emilson [28], the small fluoride ion can reach mutans streptococci which survive in retention sites and in incipient enamel lesions, interfering with their metabolic activities and, thus, contributing to delay their reappearance. In the current study effects of water cinnamon extract, chlorhexidine gluconate, Zac and deionized water on the viability counts of mutans streptococci *in vitro* were tested, concerning water cinnamon extract a significant reduction in the counts of bacteria was shown at concentration of 20% compared to the control after 24 hour, while a highly significant difference was found at higher concentrations. Mechanisms where by cinnamon extract inhibit growth of bacteria especially against mutans streptococci are still unclear, most explanations concerning the antibacterial effect of cinnamon in general related the inhibitory effect of cinnamon to its essential oils. An important characteristic of essential oils and their components is their hydrophobicity, which enable them to partition the lipids of the bacterial cell membrane, disturbing the cell structures and rendering them more permeable. Extensive leakage from bacterial cells or the exit of critical molecules and ions will lead to death [29]. It has been shown that cinnamon antimicrobial properties are mainly related to its cinnamaldehyde content followed by eugenol and carvacrol contents [30]. The effect of deionized water, Zac and chlorhexidine on the viability counts of mutans streptococci were tested separately, results showed that there was no significant difference in the counts of bacteria for deionized water compared to the control after 24 hour, this could be explained by the complete resistant of these bacteria to

deionized water, where as chlorhexidine and Zac showed highly significant reduction in the counts of bacteria in comparison to the control after 24 hour, it seems that chlorhexidine gluconate weather used alone or in combination with sodium fluoride in form of zac is much more effective in the inhibition of bacterial growth. This observation added to the earlier studies carried out by Emilson [28] and Kulkarni and Damle [31]. Water cinnamon extract (20%) was tested for its effect on salivary mutans streptococci among a group of volunteers, in comparison to Zac, chlorhexidine and deionized water. A marked reduction in bacterial counts was noticed for all agents with the exception of deionized water which showed a slight reduction. The results of the present study showed the superiority of chlorhexidine mouth rinse especially when used in combination with sodium fluoride, the presence of fluoride may enhance remineralization of outer enamel surface and increase their resistance to acid dissolution, this was proved by numerous experimental and human studies [32, 33]. Concerning cinnamon, although its effectiveness in reducing the counts of mutans streptococci was less than chlorhexidine and Zac, but it can be use as anticaries agent, however, further studies are needed regarding the effect of cinnamon extract on other cariogenic determinants of mutans streptococci such as adherence, acidogenicity and ability to form extracellular polysaccharide.

References

1- Seminario A, Broukal Z, Ivancakova R. Mutans streptococci and the development of dental plaque. Prague Medical Report 2005; 106(4): 349-358.

2- Al-Azawi L. Oral health status and treatment needs among Iraqi five years old kindergarten children and fifteen years old students. A national survey. Ph.D. Thesis, College of Dentistry, University of Baghdad, Iraq, 2000.

3- Petersen P, Bourgeois D, Ogawa H, Estupinan-Day S, Ndiaye C. The global burden of oral diseases and risks to oral health. Bulletin of the WHO 2005; 83: 661-669.

4- Murray J, Nunn J, Steele J. The prevention of oral disease. 4th ed .Oxford University Press Inc., New York. 2003; p. 71.

5- Moynihan P, and Petersen P. Diet, nutrition and the prevention of dental diseases. Public Health Nutrition 2004; 7(1A): 201-226.

6- Sheen S and Addy M. An *in vitro* evaluation of the availability of cetylpyridinium chloride and chlorhexidine in some commercially available mouthrinse products. Br Dent J 2003; 194(4): 207-210.

7- Emilson C. Potential efficacy of chlorhexidine against mutans streptococci and human dental caries. J Dent Res 1994; 73(3): 682-691.

8- Ceylane A and Daniel Y. Antimicrobial activity of spices. Journal of Rapid Methods and Automation in Microbiology 2004; 12 (1) : 1-55.

9- Singh J, Kumar A, Budhiraja S, HoodaA. Ethnomedicine: use in dental caries. Braz J Oral Sci. 2007 ; 6 (21): 1308-1312.

10- El-Samarrai S, Al-Deen and Al-Azawi L. Comparative effects of Siwak and tooth-brush on plaque index and gingival index among groups of dental students. Iraq Dent J 1997; 19: 169-181.

11- Al-Nidawi A. Effect of Siwak extracts on Mutans streptococci, in comparison to selected antimicrobial agents (*in vitro* and *in vivo* study). M.Sc.Thesis, College of Dentistry, University of Baghdad, 2004.

12- Al-Izzy M. Antibacterial effects of black and green tea extract on Mutans streptococci and Lactobacilli (*in vitro* and *in vivo* study). M.Sc. Thesis, College of Dentistry, University of Baghdad, 2005.

13- Al-Alousi J. Effect of garlic extracts on streptococci and mutans streptococici, in comparison to chlorhexidine gluconate (a comparative *in vitro* and *in vivo* study). M.Sc.Thesis, College of Dentistry, University of Baghdad, 2007.

14- Ooi LS, Li Y, Kam SL, Wang H, Wong EY, Ooi VE. Antimicrobial activities of cinnamon oil and cinnamaldehyde from the Chinese medicinal herb Cinnamomum cassia Blume. Am J Chin Med 2006;34(3):511-22.

15- Pszczola DE. 2001: A Spice Odyssey. Food Technology. January, 2001; 55: 36-44.
16- Burt S. Essential oils: their antibacterial properties and potential applications in foods-a review. International Journal of Food Microbiology 2004 94 (3): 223-253.
17- Quale JM, Landman D, Zaman MM, Burney S, Sathe SS. In vitro activity of Cinnamomum zeylanicum against azole resistant and sensitive Candida species and a pilot study of cinnamon for oral candidiasis. Am J Chin Med. 1996;24(2):103-9.
18- Arora D, Kaur J. Antimicrobial activity of spices. Int J Antimicrob Agents 1999;12:257–62.
19- Kannappan S, Jayaraman T, Rajasekar P, Ravichandran M K, Anuradha C V. Cinnamon bark extract improves glucose metabolism and lipid profile in the fructose-fed rat. Singapore Med J 2006; 47(10) : 858-863.
20- Holbrook W and Beighton D. Streptococcus mutans levels in saliva and distribution of serotypes among 9-year-old Icelandic children. J of Scand Dent Res 1986; 95: 37-42.
21- Jenkins S, Addy M, Wade W, Newcombe R. The magnitude and duration of the effects of some mouth rinse products on salivary bacterial counts. J Clin Periodontol 1994; 21: 397-401.
22- Thylstrup A and Fejerscov O. Text book of cariology 1st ed. Munksgaard, Copenhagen, 1996.
23- Alexander O. Gill and Richard A. Holley* Mechanisms of Bactericidal Action of Cinnamaldehyde against Listeria monocytogenes and of Eugenol against L. monocytogenes and Lactobacillus sakei. Appl Environ Microbiol 2004; 70(10): 5750–5755.
24- Senhajio O, Faif M, Elyachioui M. Antibiosis by cinnamon extracts against antibio-resistant strains. International Journal of Agriculture and biology 2005;7 (5):724-728.
25- Yuste J, Fung D. Inactivation of Listeria monocytogenes Scott A 49594 in apple juice supplemented with cinnamon. J Food Prot 2002; 65:1663–6.
26- Nanasombat S and Lohasupthawee P. Antibacterial activity of crude ethanolic extracts and essential oils of spices against Salmonellae and other Enterobacteria. J KMITL Sci Tech 2005; 5 (3): 527-538.
27- Mandel I D. Chemotherapeutic agents for controlling plaque and gingivitis. J Periodontol 1988; 15: 488-98.
28- Emilson C. Potential efficacy of chlorhexidine against mutans streptococci and human dental caries. J Dent Res 1994; 73(3): 682-691.
29- Kwon, J, Yu A, and. Park H. Bactericidal effects and inhibition of cell separation of cinnamic aldehyde on Bacillus cereusBacillus cereus. Lett. Appl. Microbiol 2003; 37:61-65.
30- Moleyar, V., and P. Narasimham.. Antibacterial activity of essential oil components. Int. J. Food Microbiol 1992; 16:337-342.
31- Kulkarni V V and Damle S G. Comparative evaluation of efficacy of sodium fluoride, chlorhexidine and triclosan mouth rinses in reducing the mutans streptococci count in saliva: an *in vivo* study. J Indian Soc Pedo Prev Dent 2003; 21 (30): 98-104.
32- Luoma H. The effects of chlorhexidine and fluoride combinationon the potassium, sodium and phosphorus content and acid production of cariogenic streptococci. Arch Oral Biol 1972; 17:1431-1437.
33- Katz S. The use of fluoride and chlorhexidine for the prevention of radiation caries.JADA 1982; 104:164-170.

Table (1): Zones of Inhibition in Millimeter (Mean and Standard Deviation) of Water Cinnamon Extract, Zac, Chlorhexidine and Deionized Water, *In Vitro*.

Concentration	Water cinnamon Extract		Zac		Chlorhexidine 0.2%		Deionized water	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
5%	0.00	0.000	21.60	2.074	20.80	1.440	0.00	0.000
10%	10.00	0.612						
15%	10.90	1.341						
20%	11.80	1.605						
25%	12.70	1.204						
30%	13.00	1.173						
35%	14.30	0.758						
40%	15.00	0.791						
45%	15.80	0.570						
50%	17.00	0.707						

SD Standard Deviation.

Table (2): Statistical Differences between the Inhibition Zone of Chlorhexidine and Different Concentrations of Cinnamon Water Extract.

Concentration of Cinnamon Water Extract	Mean	t-test	P-Value	Description
5%	0.00	-	-	-
10%	10.00	15.429	0.000	HS
15%	10.90	11.246	0.000	HS
20%	11.80	9.333	0.000	HS
25%	12.70	9.647	0.000	HS
30%	13.00	9.390	0.000	HS
35%	14.30	8.928	0.000	HS
40%	15.00	7.893	0.000	HS
45%	15.80	7.217	0.000	HS
50%	17.00	5.295	0.001	S

The inhibition zone of chlorhexidine is (20.80 mm).

Table (3): Statistical Differences between the Inhibition Zone of Zac and Different Concentrations of Cinnamon Water Extract.

Concentration of Cinnamon Water Extract	Mean	t-test	P-Value	Description
5%	0.00	-	-	-
10%	10.00	11.996	0.000	HS
15%	10.90	9.687	0.000	HS
20%	11.80	8.357	0.000	HS
25%	12.70	8.299	0.000	HS
30%	13.00	8.072	0.000	HS
35%	14.30	7.393	0.000	HS
40%	15.00	6.650	0.000	HS
45%	15.80	6.031	0.000	HS
50%	17.00	4.695	0.002	S

The inhibition zone of Zac is (21.60 mm).
Significant P<0.05, ** Highly Significant P<0.001.

Table (4): Effect of Different Concentration of Water Cinnamon Extract, Zac, Chlorhexidine and Deionized Water on the Viability Counts of Mutans Streptococci *in Vitro*:

Items		Count of Mutans Streptococci $\times 10^3$	
		Mean	SD
Control (without test agents)	Initial **	65.60	9.072
	After 24 hour	271.20	9.471
Water cinnamon extract (after 24 hour)	15%	262.60	14.673
Water cinnamon extract (after 24 hour)	20%	238.40	17.644
Water cinnamon extract (after 24 hour)	25%	218.40	14.467
Water cinnamon extract (after 24 hour)	30%	195.60	12.012
Zac (after 24 hour)	0.12%	15.80	9.471
Chlorhexidine (after 24 hour)	0.2%	30.00	9.354
Deionized water (after 24 hour)	-	273.80	7.294

Table (5): Statistical Test between Different Concentration of Water Cinnamon Extract, Zac, Chlorhexidine, and Deionized Water in Comparison with the Counts of Mutans Streptococci(without test agents) after 24 hour.

Agents	Count of bacteria after 24 hour $\times 10^3$		Description
	t-test	p-value	
Water cinnamon extract 15%	1.101	0.303	NS
Water cinnamon extract 20%	3.663	0.006	S
Water cinnamon extract 25%	6.828	0.000	HS
water cinnamon extract 30%	11.051	0.000	HS
Zac 0.12%	51.832	0.000	HS
Chlorhexidine 0.2%	40.516	0.000	HS
Deionized water	0.486	0.640	NS

Table (6): Values of Mean and Standard Deviation of Count Mutans Streptococci by Time Points.

Time	Cinnamon Water Extract		Chlorhexidine		Zac		Deionized Water	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Baseline	428.60	60.293	408.80	81.223	368.80	70.942	457.40	80.164
30 minute	207.80	48.556	174.60	70.205	110.80	20.777	418.80	86.410
One hour	220.40	54.834	152.60	51.159	93.20	43.694	428.40	86.318
Two hours	425.00	39.006	319.00	69.911	250.60	41.344	459.00	61.583

Table (7): Statistical Difference between Counts of Salivary Mutans Streptococci in Baseline, and after Thirty Minute, One Hour and Two Hours.

Time / Agent	After 30 minute	After 1 hour	After 2 hour
Water cinnamon extract	t = 5.600* df = 8	t = 3.344* df=8	NS
Chlorhexidine	t = 4.878* df =8	t = 5.968** df =8	NS
Zac	t = 7.804** df = 8	t = 7.396** df	NS
Deionized water	NS	NS	NS

df Degree of freedom.

Table (8) Statistical Difference between Agents at Different Time Points.

After thirty minute

Between	Chlorhexidine	Deionized water	Cinnamon water extract
Zac	NS	t = 7.749** df = 8	t = 4.107* df= 8
Chlorhexidine		t = 4.905* df =8	NS
Deionized water			t = 4.760* df= 8

After One Hour

Between	Chlorhexidine	Deionized water	Cinnamon water extract
Zac	NS	t = 7.747** df = 8	t = 4.057* df =8
Chlorhexidine		t = 6.146** df= 8	NS
Deionized water			t = 4.548* df=8

After Two Hours

Between	Chlorhexidine	Deionized water	Cinnamon water extract
Zac	NS	t = 6.282** df =8	t = 6.861** df = 8
Chlorhexidine		t = 3.360* df = 8	NS
Deionized water			NS

P<0.001 High Significant, P<0.05 Significant, NS Non Significant,