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Effect of water Clove Extract on Streptococci and Mutans Streptococci, in Comparison to Chlorhexidine Gluconate *(A Comparative In Vitro and In Vivo Study)*

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Abstract

Recently, in many parts of the world there is a rich tradition in the use of natural products for the treatments of many infectious diseases; many herbal remedies have been used because of their antibacterial, anti- inflammatory, cytostatic, antifungal and antiviral. Stimulated saliva was collected from four healthy looking females aged (20-21) from which mutans streptococci were isolated. Sensitivities of mutans streptococci according to Agar Well Technique showed that water clove extract was effective in the inhibition of these bacteria, mutans streptococci were more sensitive to chlorhexidine compared to water extract as indicated by the wider zones of inhibition on the Mueller Hinton Agar. An *in vitro* experiment was conducted to evaluate the effect of these agents on acid formation by mutans streptococci, the result showed that chlorhexidine gluconate and water clove extract were effective in retardation of acid formation. The study involved one *in vivo* experiment to test the effect of water clove extract (10%) against salivary streptococci and mutans streptococci in comparison to 0.2% chlorhexidine and deionized water. Stimulated saliva was collected from 18 volunteers aged (24-27 years) they were divided into three groups each group rinsed once with either chlorhexidine, deionized water or clove water extract for one minute. The counts of bacteria were recorded at different time points (one minute prior to the rinse, 30 minutes after rinsing, one hour and two hours). No significant difference in the counts of streptococci was found between deionized water and water clove extract for all time points, rinsing with any one of these agents resulted in a slight decreased in the counts of these bacteria, chlorhexidine showed a sharp reduction in the counts of bacteria which was highly significant ($P<0.001$). For mutans streptococci, a highly significant differences were found between the three mouth rinses ($P<0.001$) in the counts of bacteria in the following time points (after thirty minute, after one hour). Within these times chlorhexidine was shown to be the most effective in reducing the counts of these bacteria followed by water clove extract. The result of the present study showed the effectiveness of clove especially against mutans streptococci, although it was less than chlorhexidine, but it can be use as effective anticaries agent. This study aimed to evaluate the effect of different concentrations of water clove extract in comparison to chlorhexidine gluconate 0.2% and deionized water on growth, acidogenicity of mutans streptococci *in vitro* and viability counts of streptococci and mutans streptococci among a number of volunteers.

Keywords: water clove extract, chlorhexidine, deionized water.

Introduction

Dental caries is one of the most prevalent infectious diseases of man; the principle causative agents are a group of streptococcal species referred to as the mutans streptococci [1, 2]. The caries inducing properties of mutans Streptococci depend on its acid producing and glucan synthesis activities. The acidogenicity is an important cariogenic potential of mutans streptococci, fermentation of carbohydrate results in acid production which may lead to demineralization of enamel surface and initiation of a carious process [3]. Dental caries is a dynamic process since periods of demineralization alternate with periods of remineralization through the action of fluoride, calcium and phosphorous contained in oral fluids [4]. The traditional preventive treatment approaches for dental caries include dietary counseling on reduced intake of refined sugars, oral hygiene instruction and topical application of fluoride [5]. Mouthwashes are very useful in reduction of mutans streptococcal counts, clinical applications for antimicrobial mouth rinses fall into two broad categories: preventative and therapeutic. The antimicrobial agents will ideally have the following properties: broad spectrum antimicrobial efficacy, retain antimicrobial efficacy at low concentrations, fast acting, non-toxic, non-irritant, pleasant/neutral odor and taste, good oral retention properties, not too disruptive to the oral microbial ecology, globally regulatory approved, chemically defined, chemically stable, physically stable and cost effective [6]. There is no antimicrobial agent, with the exception of fluoride has received as much experimental attention as the bisbiguanide chlorhexidine, this substance considered as the most effective and best documented agent against caries [7]. Phytochemicals have recently been shown to be a good alternative to synthetic chemical substances for caries prevention [8, 9]. Clove (Syzygium aromaticum) is one of these herbs which have been used for a variety of diseases and health conditions, cloves are the dried, unopened inflorescence of the clove tree, which is a member of Myrtaceae family. Cloves vary in length from about ½ to ¾ inch and contain 14 – 20% essential oil, its buds have been regarded as safe when taken orally for medicinal use. It have been used by humans for medicinal applications for over two thousand years, being chewed to alleviate the pain of toothache, and are also widely used to disinfect root canals in temporary fillings and as an oral anesthetic [10]. Cloves are strongly pungent due to their high content of eugenol, which is a chemical compound presents in clove, and is known to inhibit the growth of bacteria, and sometimes used to eliminate bad breath or to ameliorate the pain of a bad tooth [11]. Clove is a natural antibiotic with broad antimicrobial activities against Gram-positive, Gram – negative, as well as fungi [12, 13]. However, there is a lack of studies regarding its specificity against cariogenic bacteria especially streptococci which may prove its potency against this bacteria.

Materials and Methods

Preparation of the crude Aqueous Clove Extract: The clove was crushed to coarse powder and sieved through No. 20 mesh size. One hundred gram of clove powder was boiled in one liter of deionized water and allowed to boil to a final volume of one hundred ml. The concentrated mixture was filtered using Whatman

number one filter paper, the extract then left to dry at 40°C in hot air oven for evaporation of water. Then it was preserved in a refrigerator until use [14].

Collection of Saliva: This study was conducted at the College of Pharmacy/ University of Baghdad, stimulated saliva was collected from four healthy looking students aged (20-21) 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 [15]. The colonies were identified on the basis of morphology of the colonies, Gram's stain, and biochemical test.

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 Clove Extract, Chlorhexidine and Deionized Water, *In Vitro.*

Different concentrations of water clove extract in addition to chlorhexidine gluconate (0.2%) 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 Clove Extract, Chlorhexidine, and Deionized Water on the Acidogenicity of Mutans Streptococci In Vitro.

The procedure performed on seven isolates of mutans streptococci, stainless steel wire holding a tooth was used for the artificial plaque accumulation. The wire was threaded in one end in the root of a previously cleaned and polished sound first premolar, the other end of the wire was inserted in the center of a test tube. The test tube, the wire and the tooth were sterilized by autoclave. Each one of the sterilized wire holding a tooth was inserted in 10 ml of Brain Heart Infusion broth containing 5% sucrose (pH 7.0). Bottles were inculcated with 2% of mutans streptococci except for the control negative which contain (5% sucrose broth with wire holding a tooth). The study and control broths were incubated at 37°C aerobically, every 24 hour (for period of three days) each wire holding a tooth was transferred to a fresh 5% sucrose broth, incubated aerobically at 37°C this allowed for further accumulation of bacterial deposit. In the fourth day, coated teeth in addition to the control negative were immersed in 10 ml aqueous solution of clove extract, chlorhexidine, and deionized water. Wires and teeth were removed and washed with sterile deionized water

for one minute including control negative and control positive, teeth were left to dry at room temperature for five minutes then placed in a fresh 5% sucrose broth containing 1% bromocresol purple as an indicator (pH 7.0) test tubes are incubated for seven days. Positive reaction (acid formation) was indicated by the change in the color from purple to yellow [16]. Results were recorded as follow:

+	Yellow color	No effect
+/-	Orangecolor	Weak
-	Purple	Effective

Experiment Three: Effects of Water Clove Extract, Chlorhexidine and Deionized Water on Salivary Counts of Streptococci and 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 18 subject aged (24-27), they were divided into three groups six in each one. The first group was the experimental group that used (10%) water clove extract as a mouth rinse, while the second group was the control positive group rinsing with chlorhexidine (0.2%), the last group was the control negative rinsed with deionized water. All the volunteer participated in this experiment were healthy looking with no medical history, not receiving any antimicrobial agents during the last two weeks prior to the study, not wearing any fixed or removable prosthesis or orthodontic appliance, the volunteers were asked to suspend their usual oral hygiene practice at the day of experiment [17].

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 [18]. 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 for growth of mutans streptococci, and on Mitis Salivarius agar plates for growth of streptococci, 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 and standard deviation.

2-Student's t-test and analysis of variance (ANOVA) for calculating the significance of differences between means of different groups.

Results

Sensitivities of mutans streptococci to different concentrations of water clove extracts, chlorhexidine gluconate and deionized water are seen in Table (1), diameter of inhibition zones were

found to increase as the concentration of clove water extract increased. No zone of inhibition was shown for deionized water, the highest inhibition zone was shown by chlorhexidine. Using t-test to compare each concentration of water clove extract with chlorhexidine. A highly significant difference was shown between chlorhexidine and different concentrations of water clove extract, at concentration 60% there was a significant difference between water clove extract and chlorhexidine (Table 2), it seems that sensitivity of mutans streptococci was higher to chlorhexidine compared to different concentrations of water clove extract in which P-value<0.001.

Effect of Water Clove Extract on Acid Formation of Mutans Streptococci, *In Vitro*:

For all the tested agents with all different concentrations used change in the color of indicator was seen, the result showed that concentrations 10%, 15% and 25% of water clove extract were effective in the retardation of acid formation as detected by change in the color of indicator from deep purple to orange, the same result was shown for chlorhexidine (0.2%). Change in the color of the indicator from deep purple to yellow was seen at concentration of 5% water clove extract, the same result was shown for the control positive tubes, no one of tested agent was able to prevent the acid formation completely. For control negative tubes there were no change in the color along the seven days of the experiment. Concerning the time, there were no differences in the results noticed whether the teeth were treated for one or two minutes (Table 3).

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

Salivary Streptococci:

Mean and standard deviation of counts of bacteria estimated before and after rinsing with 10% clove water extract, chlorhexidine gluconate, and deionized water at each time interval is seen in (Table 4), after 30 minute a slight reduction in the counts of bacteria was shown by deionized water and water clove extract which tended to increase gradually after one hour until reached to the baseline after two hour. For chlorhexidine there was a marked decrease in the counts of bacteria after 30 minute which continued after one hour then the counts of bacteria were gradually increased but still lower than the baseline. The difference between the counts of streptococci for the three mouth rinses was examined by ANOVA test which is shown in Table (5), a highly significant difference was shown between groups after 30 minute and after one hour of rinsing. LSD test was performed to compare each two mouth rinses, a significant difference was shown between chlorhexidine and clove extract after 30 minute. After one hour, a highly significant difference was found between clove extract and chlorhexidine, for comparing chlorhexidine with deionized water, the result revealed a highly significant difference between chlorhexidine and deionized water after 30 minute and one hour of rinsing, chlorhexidine within these times showed a mark reduction in the counts of bacteria as compared to deionized water and clove extract which showed only a slight reduction in the counts of bacteria (Table 6).

Mutans Streptococci:

Mean and standard deviation of counts of bacteria estimated before and after rinsing with 10% clove water extract, chlorhexidine, and deionized water at each time interval is seen in (Table 7). For deionized water there

was a slight reduction in the counts of bacteria after 30 minute of rinsing, after that time the counts of bacteria was raised but remained less than that of the baseline, rinsing with water clove extract or chlorhexidine, resulted in a sharp reduction in the count of bacteria which continued for after one hour for chlorhexidine while for water clove extract it continued for after half an hour and then gradually increased after that time but still lower than the baseline. The difference between the counts of mutans streptococci for the three mouth rinses was examined by ANOVA test which is shown in Table (8), no significant differences were found between the three agents before rinsing in the counts of bacteria, while a highly significant difference was found in the following time points for all the three agents except after two hour within that time a significant reduction in the count of bacteria was shown. LSD test was performed to compare each two mouth rinses, a significant difference in the counts of mutans streptococci was shown between clove and deionized water at the following time points after rinsing. A highly significant difference was found between deionized water and chlorhexidine at the following point times of rinsing except after two hours within this time a significant difference was found, there was no significant difference in the count of bacteria between chlorhexidine and clove at different time points after rinsing except after one hour within that time a significant difference was shown (Table 9).

Discussion

Sensitivities of mutans streptococci to different concentrations of water clove extract in comparison to chlorhexidine gluconate (0.2%) and deionized water was tested using Agar Well Technique, clove extract was found to inhibit the growth of these bacteria. A minimum concentration needed to produce inhibition zone was 10%, many studies showed that clove extract contains a variety of chemical compounds for which many biological activities of clove attributed, Phytochemical studies indicate that the clove contains free eugenol, eugenol acetate, caryophyllene, sesquetrepene ester, phenyl propanoid, β caryophyllene, eugenol and acetyle eugenol, tannins, flavonoids, and myricetin [19, 12]. One of the important constituent of clove are the essential oils, most of the ingredients of essential oils belong to the terpenoids family, terpenoids are weakly to moderately soluble in water it is generally assumed that the antimicrobial activities of terpenoids are due to their ability to disrupt the lipid structure, thus causing a loss of membrane integrity, dissipation of the proton motive force, and impairment of intracellular pH homeostasis [20], however, further studies are needed to detect these antibacterial components. The zone of inhibition was found to increase as the concentration of clove extract was increase, it has been shown that the adsorption of spice on the bacterial cell depended on its concentration [21], this explanation can be applied to the present study. Sensitivities of mutans streptococci to chlorhexidine as determined by the diameter of the zone of inhibition in millimeter was tested and compared to water clove extract, these bacteria were more sensitive to chlohexidine gluconate compared to water clove extract. It is well known that chlorhexidine is an effective agent especially against mutans streptococci the mode of action of chlorhexidine on these bacteria varies with its concentration, and more specific effects have been observed with lower

(bacteriostatic) concentrations which are based on disturbance of bacterial cell functions, enzymes and cell receptors [22]. The present study tested the effect of water clove extract, chlorhexidine gluconate and deionized water on acid production by mutans streptococci, the result showed that water clove extract was effective in reducing acid production by bacteria. Suggestion for acid reduction by clove could not be found in the literature, it may be attributed to the ability of clove extract to penetrate the mass of bacteria and exert their effect either directly or by affecting the enzymes necessary for acid production. In this study the effect of chlorhexidine and deionized water on acidogenicity was also tested separately. Deionized water was completely ineffective in retardation of acid production, where as chlorhexidine was effective in reducing the acidogenicity of these bacteria. This was in agreement with other studies which showed that acid production by dental plaque decreases after chlorhexidine application [23], although the mechanism of action has not been elucidated, it has been claimed that chlorhexidine inhibit the glucose and sucrose phosphotransferase system (PTS) in membranes of Streptococcus mutans [24]. The effectiveness of water clove extract (10%) on salivary streptococci and mutans streptococci was tested among a group of volunteers, in comparison to chlorhexidine gluconate and deionized water. Bacterial counts were estimated following a single rinse of the agents at different time points (30 minute, one hour, and two hours). A slight reduction in the counts of streptococci and mutans streptococci was noticed for deionized water, the greatest reduction in the counts of these bacteria was shown by chlorhexidine. In the present study variation in the results for streptococci and mutans streptococci was found following rinsing with water clove extract, rinsing with clove resulted in a slight reduction in the counts of streptococci, while a sharp reduction in mutans streptococcal counts was noticed following rinsing with this agent. The presence of resistant strains of oral streptococci to this agent probably might explain its ineffectiveness against streptococcal bacteria The effect of clove mouth rinse on mutans streptococci in the present study was in coincidence with Haji and Rahim (2006) who showed that clove extract had antimicrobial activity against mutans streptococci, this may give an indication for specificity of clove extract on mutans streptococci, however further studies are needed regarding the effect of clove extracts on other cariogenic determinants of mutans streptococci such as adherence. Chemical constituents of clove especially those with antimicrobial action like eugenol on the growth and cariogenic determinants of mutans streptococci need to be studied with the possibility of using certain reagents for detection such constituents.

References

1- Marsh P D. Are dental diseases examples of ecological catastrophes? Microbiology 2003; 149(2): 279-294.
2- Kidd E and Fejerscov O. What constitutes dental caries? Histopathology of carious enamel and dentin related to the action of cariogenic biofilms. J Dent Res 2004; 83(Spec Iss C): C35-C38.
3- Kidd E and Bechal S. Essential of dental caries. The disease and its managements.2nd ed. Oxford University Press Inc., New York. 2002; p.1, 67-68, 143.
4- Gao X, Fan Y, Kent R, Van Houte J and Margolis H. Association of caries activity with the composition of dental plaque fluid. J Dent Res 2001; 80(9): 1834-1839.
5- Featherstone J. The science and practice of caries prevention. JADA 2000; 131: 887–899.

6- Fischman S and Yankell S. Dentifrices, mouth rinses, and tooth whiteners In: Harris N and Christen A. Primary Preventive Dentistry 4th ed. Appleton and Lange, Stamford, Connecticut. 1995; P: 607-609.
7- Addy M. Chlorhexidine compared with other locally delivered antimicrobials. J of Clin Periodontol 1986; 13: 957-964.
8- Karen W and Edzard E. Herbal medicines for treatment of bacterial infections: a review of controlled clinical trial. J of Antimicrobial Chemotherapy 2003; 51: 241–246.
9- Badrina FA, Zidan OA. Natural products for dental caries prevention. J Med Food 2004; 7:381-4.
10- Alqareer A, Alyahya A, Andersson L. The effect of clove and benzocaine versus placebo as topical Anesthetics. J of Dentistry 2006; 34(10):747-50.
11- Lee J, Shibamoto T. Antioxidant property of aroma extract isolated from clove buds [Syzygium aromaticum (L)]Merr. J Et Perry Food Chemistry 2001; 74:443-44.
12- Gislene G.; Juliana L, Paulo C , Giuliana L. Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria. Brazilian Jof Microbiology (2000) 31:247-256.
13- Nunez L; Miguel D, Jorge C. Antifungal properties of clove oil (Eugenia caryophylata) in sugar solution. Brazilian J of Microbiology 2001; 32: 123-126.
14- Haji Z, Begum H.Comparative studies on the effect of crude aqueous (CA) and solvent (CM) extracts of clove on the cariogenic properties of streptococcus mutans. J of OralScience 2006; 48(3):117-123.
15- 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.
16- Keyes P and McCabe R. The potential of various compounds to suppress microorganisms in plaque produced *in vitro* by a Streptococcus or an Actinomycete. JADA 1973; 86: 296-300.
17- 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.
18- Thylstrup A and Fejerscov O. Text book of cariology 1st ed. Munksgaard, Copenhagen, 1996.
19- Alma M, Murat E, Siegfrie N. Chemical composition and content of essential oil from the bud of cultivated Turkish clove (Syzygium aromaticum L.). BioResources 2007; 2(2), 265-269.
20- Ouattara B, Ronald E, Richard A., Gabriel J, Bégin A. Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms. International J of food Microbiology 1997; 37: 155-162.
21- 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.
22- Grönroos L, Mättö J, Luoma A R and Alaluusua S. Chlorhexidine susceptibility of mutans streptococcal serotypes and ribotypes. Antimicrobial agents and chemotherapy 1995; 39(4): 894-898.
23- Van Loveren C, Buijs J. F, J. M. ten Cate. The effect of triclosan toothpaste on enamel demineralization in a bacterial demineralization model. Journal of Antimicrobial Chemotherapy 2000; 45: 153-158.
24- Giertsen E, Scheie A and Rölla G. *In vivo* effects of zinc and chlorhexidine on dental plaque ureolysis and glycolysis. J Dent Res 1989; 68(6): 1132-1134.

Concentration	Water Clove Extract	Chlorhexidine 0.2%	Deionized water

	Mean	SD	Mean	SD	Mean	SD
10%	11.20	1.681				
20%	13.20	0.570				
30%	14.40	0.652				
40%	15.90	0.742				
50%	16.50	1.118				
60%	19.20	0.837				

Time	Clove Water Extract	Chlorhexidine		Deionized Water	
		22.00	1.275	0.00	0.000

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

Table (2): Statistical Test between Chlorhexidine and Each Concentration of Water Clove Extract.

Concentration of Clove Water Extract	t-test	P-Value	Description
10%	11.45	0.000	HS
20%	14.09	0.000	HS
30%	11.87	0.000	HS
40%	9.25	0.000	HS
50%	7.25	0.000	HS
60%	4.11	0.003	S

Table (3): Effects of Different Concentrations of Water Clove Extract, Chlorhexidine 0.2%, and Deionized Water on the Acidogenicity of Mutans Streptococci, *In Vitro*.

Agents	**Time/ min**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
Control positive	**1**	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+
Control negative	**1**	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-
Water clove extract 5%	**1**	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+
Water clove extract 10%	**1**	+/-	+/-	+/-	+/-	+/-	+/-	+/-
	2	+/-	+/-	+/-	+/-	+/-	+/-	+/-
Water clove extract 15%	**1**	+/-	+/-	+/-	+/-	+/-	+/-	+/-
	2	+/-	+/-	+/-	+/-	+/-	+/-	+/-
Water clove extract 25%	**1**	+/-	+/-	+/-	+/-	+/-	+/-	+/-
	2	+/-	+/-	+/-	+/-	+/-	+/-	+/-
Chlorhexidine 0.2%	**1**	+/-	+/-	+/-	+/-	+/-	+/-	+/-
	2	+/-	+/-	+/-	+/-	+/-	+/-	+/-
Deionized Water	**1**	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+

Table (4): Values of Mean and Standard Deviation of Count Streptococci by T

	Mean	SD	Mean	SD	Mean	SD
Baseline	380.50	78.207	445.50	43.657	402.33	67.805
30 minute	337.00	69.533	179.17	55.962	376.50	70.634
One hour	350.17	79.482	151.83	43.815	386.50	69.157
Two hour	376.17	55.668	358.51	76.154	403.67	19.947

Table (5): ANOVA Test between Water Clove Extract, Chlorhexidine and Deionized Water for Streptococci Count for the Five Time Intervals.

Time	F	P	Description
Baseline	1.561	0.242	NS
30 minute	15.147	0.000	HS
One hour	22.057	0.000	HS
Two hours	1.003	0.390	NS

Table (6): LSD Test between Each Two Groups for Salivary Streptococci.

Group 1	Deionized Water		Deionized Water		Chlorhexidine	
Group 2	Chlorhexidine		Clove		Clove	
	Mean difference	P	Mean difference	P	Mean difference	P
30 minute	197.3333**	0.000	39.5000	0.314	157.8333*	0.001
One hour	234.6667**	0.000	36.3333	0.355	198.333**	0.000

NS Non Significant, * Significant P<0.05, ** Highly Significant P<0.001

Table (7): Values of Mean Standard Deviation of Count Mutans Streptococci by Time Points.

Time	Clove water extract		Chlorhexidine		Deionized Water	
	Mean	SD	Mean	SD	Mean	SD
Baseline	433.17	73.706	521.83	56.432	474.33	46.740
30 minute	311.17	59.804	253.83	78.942	449.83	42.659
One hour	315.67	48.409	227.50	80.919	460.00	41.1339
Two hour	379.00	89.931	303.83	50.459	467.83	45.688

Table (8): ANOVA Test between Water Clove Extract, Chlorhexidine and Deionized Water for Mutans Streptococci Count for the Five Time Intervals.

Time	F	P	Description
Baseline	3.281	0.066	NS
30 min.	15.720	0.000	HS
One hour	23.432	0.000	HS
Two hour	9.536	0.002	S

Table (9): LSD Test between Each two Groups for Salivary Mutans Streptococci.

Group 1	Deionized Water		Deionized Water		Chlorhexidine	
Group 2	Chlorhexidine		Clove		Clove	
	Mean difference	P	Mean difference	P	Mean difference	P
30 minute	196.0000	0.000**	138.6667	0.002*	57.3333	0.132
One hour	232.5000	0.000**	144.3333	0.001*	88.1667	0.021*
Two hour	164.0000	0.001*	88.8333	0.032*	75.1667	0.064

* Significant P<0.05, ** Highly Significant P<0.001