Effect of Garlic Extracts on Streptococci and Mutans Streptococci, in Comparison to Chlorhexidine Gluconate (A comparative in vitro and in vivo study)

Jinan M. Rashad
B.D.S., M.Sc., Lecturer,Dental preventive department,Technical medical institute,Baghdad.
Sulafa K. El-Samarrai
B.D.S., M.Sc., Ph.D. - Prof.- Department of Pedodontic and preventive dentistry.College of Baghdad.

ABSTRACT

Background: Garlic is a medicinal plant with anti-inflammatory, antimicrobial and immune-boosting properties. The aim of the present study was to assess and compare the effect of garlic extracts with those of chlorhexidine gluconate and de-ionized water against viability counts of Streptococci and *Mutans Streptococci*.

Materials and Methods: The effect of different concentrations of garlic extracts (water and ethanol) on growth and viability counts of *Mutans Streptococci* in comparison to 0.2% chlorhexidine gluconate and de-ionized water were evaluated through a series of in vitro experiments. Water garlic extract (10%), were tested in comparison to chlorhexidine (0.2 %) and de-ionized water, regarding the effect on viability counts of salivary *Mutans Streptococci* and Streptococci, among a group of volunteers.

Results In vitro experiments; Statistically non significant reduction in the viable counts was recorded when garlic extracts (water and ethanol) were used at concentration 5%, while at higher concentrations both types of garlic extracts showed statistically significant reduction in the counts of *Mutans Streptococci* ($P<0.05$).

Rinsing with any one of these agents resulted in a slight decrease in the salivary counts of bacteria, while 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 30 minute, after one hour and after two hours of rinsing). Within these times chlorhexidine was shown to be the most effective in reducing the counts of these bacteria followed by water garlic extract.

Conclusion: Garlic extract was effective against *Mutans Streptococci* when tested both in vitro and in vivo.

Key words: Garlic extracts, chlorhexidine, Streptococci, *Mutans Streptococci*.

INTRODUCTION

Dental caries can be reduced by eliminating established *Mutans Streptococci* population from the oral cavity through mechanical plaque control (tooth brushing and inter dental cleaning), increasing the acid–resistance of teeth through effective use of fluorides and control of the carbohydrate composition of the diet [1- 3].

Recently, there has been a dramatic increase in the use of plant products and herbs like Siwak, green and black tea, and Myrtus communis [4 - 7]. Garlic (Allium Sativum) is one of these herbs which have been used for a wide variety of diseases and health conditions like periodontal diseases and dental caries [8, 9]. However, there is a lack of studies regarding its specificity against cariogenic bacteria especially Streptococci which may prove its potency against this bacteria. The aim of the present study was to assess and compare the effect of garlic extracts with those of chlorhexidine gluconate and de-ionized water against the viability counts of Streptococci and *Mutans Streptococci*.

MATERIALS AND METHODS

This study was conducted in the laboratory of College of Dentistry/ University of Baghdad. Garlic was extracted by two methods which were:

1. *Water Garlic Extract:* This was prepared according to the method described by Tsao *et al*,[10]. A 200 gm was taken and sliced then homogenized in 200 ml of sterile de-ionized water in a blender at high speed for two minutes; garlic was kept in a large bottle which was sealed with aluminum foil for two hours at room temperature. The extract was filtrated using Whatman No. one filter paper, then left to dry at 40˚C in hot air oven for evaporation of water. A thick paste was obtained this was kept in small bottles in refrigerated conditions until use.
2. *Ethanol Garlic Extract:* It was prepared according to the method described by Onyeagba *et al*, [11], about 200 gm of garlic was taken and soaked in one liter of 99% ethanol in a large bottle sealed with foil and allowed to stand for 72 hour then this was filtrated by Whatman filter paper No. one. The extract then left to dry at 40˚C in hot air oven, then the extracted paste was kept in a small bottle in the refrigerator until use.

Isolation of Mutans Streptococci: stimulated salivary samples were collected from five healthy looking dental student aged 22- 23 years for isolation of

bacteria, collection of saliva from voluntaries were performed under standardized condition. Samples were homogenized by vortex mixer for two minutes, and then ten- fold dilution was performed by transferring 0.1 ml of saliva to 0.9 ml of phosphate buffer saline (pH 7.0). 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 an aerobically using a gas pack or candle for 48 hour at 37˚C then aerobically for 24 hr. at room temperature [12].

Experiment One:

Sensitivities of *Mutans Streptococci* to Different Concentrations of Garlic Extracts, Chlorhexidine and Deionized Water, *In Vitro.*

Different concentrations of water and ethanol garlic extracts in addition to chlorhexidine gluconate (0.2%) were used in this experiment, they were as follow:

Water garlic extract 5%,10%,15%,20%,25%,30%,35%,40%,45%,50%.

Ethanol garlic extract 5%,10%,15%,20%,25%,30%,35%,40%,45%,50%.

A volume of 25 ml of Mueller Hinton Agar was poured into sterile glass petri dishes, left at room temperature for 24 hour. To each plate 0.1 ml of *Mutans Streptococci* inoculums was spread, left for 20 minute at room temperature then wells of equal size and depth were prepared about, each well was filled with 0.2 ml of the test agent. Plates were left at room temperature for one hour then incubated an aerobically for 24 hr. at 37˚C; zone of inhibition was measured across the diameter of each well.

Experiment Two:

Effects of Garlic Extracts, Chlorhexidine, and Deionized Water on Viability Counts of *Mutans Streptococci*, *In Vitro*.

Different concentrations of garlic extracts were prepared. Brain Heart Infusion broth (pH 7.0) were prepared and 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 inoculums 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 MSB agar plates, the plates then incubated an aerobically at 37˚C for 48 hr. Then colony forming unit per milliliter (colony forming unit/ml) was counted, this value was considered as the initial count of bacteria. Study and control were incubated aerobically at 37˚C for 24 hr. From each tube of the control and study 0.1 ml was transferred to 0.9 ml of PBS 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 hr. The colony–forming unit per milliliter was counted (colony forming unit/ ml) for all the plates.

Experiment Three:

Effects of Water Garlic Extract, Chlorhexidine and De-ionized 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, they were divided into three groups six in each one. The first group was the experimental group that used water garlic extract as a mouth rinse (10%), while the second group was the control positive group rinsing with chlorhexidine gluconate (0.2%), the last group which was the control negative rinsed with de-ionized 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, not wearing any fixed or removable prosthesis or orthodontic appliance, the age range was 25-30 years. The procedure was conducted at 9.00 a.m. which was at least two hours following their breakfast, the volunteers were asked to suspend their usual oral hygiene practice at the day of experiment [13].

Procedure: Each volunteer was given a piece of Arabic gum (0.5gm) and asked to chew it for one minute only, then stimulated saliva was collected in sterilized screw capped bottles [14]. After one minute, each volunteer was asked to rinse with 10 ml of test agent for one minute then expectorate. Stimulated saliva were recollected in the following points: after one minute of rinsing, 30 min, one hour, and, two hours during this time, the volunteers were asked not to eat or drink anything except water, pH and flow rate for each salivary sample was tested. 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 an aerobically for 48 hr. at 37˚C then aerobically for 24 hr. at room temperature, colonies were counted by colony counter. The number of colonies was expressed as colony–forming units multiplied by the dilution factor per

milliliter of saliva (colony forming unit/ml).

Statistical analysis performed using SPSS for calculation of the statistical parameters, mean and standard deviation. Student's t–test applied for calculating the significance of differences between the different variables, accepted at 0.05.

RESULTS

Sensitivities of *Mutans Streptococci* to Different Concentration of Garlic Extract (Water and Ethanol), Chlorhexidine gluconate and De-ionized Water, *In Vitro.*

A highly significant difference was shown between Chlorhexidine gluconate and different concentrations of water garlic extract (Table1), as the sensitivity of *Mutans Streptococci* was higher to chlorhexidine compared to different concentrations of water garlic extract ($P < 0.001$). On comparison of chlorhexidine to different concentrations of ethanol garlic extract, a highly significant difference was shown at concentrations 5%, 10%, 15% at ($P < 0.001$), at these concentrations sensitivities of *Mutans Streptococci* to ethanol garlic extract was lower than that of chlorhexidine, while at concentrations 25%, and 30% no significant difference in sensitivities between the two agents was found. As the concentration of ethanol garlic extract was increased the sensitivity of bacteria to this agent became more compared to chlorhexidine and at concentrations 35%, 40%, 45%, 50% the difference was highly significant, (Table 2).

Effect of Garlic Extracts (Water and Ethanol), Chlorhexidine gluconate and De-ionized Water on Viability Count of *Mutans Streptococci*, *In Vitro*

The counts of bacteria after 24hr. 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 5% water garlic extract and 5% ethanol garlic extract, the same result was shown for de-ionized water. A significant reduction in the counts of bacteria at concentrations 10% and 15% of water garlic extract ($P<0.05$) was also seen, the same result was illustrated for ethanol garlic extract at conc. 10% and 15%, concerning chlorhexidine gluconate there was a highly significant reduction in the counts of bacteria in comparison to the control after 24hr. (Table 3).

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

Salivary Streptococci:

Mean counts of bacteria estimated before and after rinsing with 10% garlic water extract, chlorhexidine gluconate, and de-ionized water at each time interval is seen in (Figure 1), there was a slight reduction in the mean counts of bacteria after one minute of rinsing with water garlic extract or chlorhexidine, in contrast de-ionized water showed a slight increased in the counts of bacteria. After 30 min. a slight reduction in the counts of bacteria was illustrated by de-ionized water and water garlic extract which tended to increase gradually after one hour, until reached to the baseline after two hours. For chlorhexidine there was a marked decrease in the counts of bacteria after 30 min. which continued after one hr. then the counts of bacteria were gradually increased but still lower than the baseline. (Table 4) illustrates values of standard deviations of the mean counts of bacteria.

Mutans Streptococci: Mean counts of bacteria estimated before and after rinsing with 10% garlic water extract, chlorhexidine gluconate, and de-ionized water at each time interval is seen in (Figure 2). For de-ionized water there was a slight reduction in the counts of bacteria immediately after rinsing, which continued after 30 minute, then the counts of bacteria raised after one hour, then showed a slight reduction which continued for two hours and remained less than that of the baseline. Immediately after rinsing with water garlic extract or chlorhexidine gluconate a slight reduction in the counts of bacteria was seen followed by sharp reduction after 30 minutes, then the count was gradually increased for the following time points but remained less than that of the baseline. The greatest reduction was shown by chlorhexidine gluconate followed by garlic extract; de-ionized water showed the highest bacterial counts. (Table 5) illustrates values of standard deviations of the mean counts of bacteria.

DISCUSSION

Sensitivities of *Mutans Streptococci* to different concentrations of garlic extracts in comparison to chlorhexidine gluconate (0.2%) and de-ionized water was tested using Agar Well Technique. For water garlic extract a minimum concentration needed to produce inhibition zone was 10%, while for ethanol garlic extract a lower concentration was needed to inhibit the growth of bacteria which was (5%). Processed garlic contains a variety of oil and water soluble sulfur compounds for which many biological activities of garlic attributed [15]; in this study variation in the amount and types of these antimicrobial compounds present in the two types of garlic extracts may explain the variation in the sensitivity of bacteria. The zone

of inhibition was found to increase as the concentration of garlic extracts was increase for both types with highly significant differences between most of these concentration, for ethanol garlic extract, zones of inhibition were much higher than that of water garlic extract of the same concentration, this may be related to that the active chemical constituents that might have the ability to dissolve better in ethanol than in water, as will be discussed later.

Sensitivities of *Mutans Streptococci* to chlorhexidine was tested and compared to garlic extracts (water and ethanol), these bacteria were more sensitive to chlorhexidine gluconate compared to both types of garlic extracts. 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, more specific effects have been observed with lower (bacteriostatic) concentrations which is based on disturbance of bacterial cell functions, enzymes and cell receptors [16]. As the concentration increased, ethanol garlic extract was much more effective than chlorhexidine in this study, this may be difficult to explain, and one can assume that *Mutans Streptococci* are more sensitive to ethanol garlic extract at high concentrations than chlorhexidine or the permeability of bacterial cell wall by ethanol garlic extract with these concentrations might be better than chlorhexidine.

The effect of garlic extracts (water and ethanol), chlorhexidine gluconate, and de-ionized water on the viability counts of *Mutans Streptococci in vitro* were tested, a significant reduction in the counts of these bacteria was shown at concentration of 10% and 15% for both types of garlic extracts compared to the control after 24 hour. Mechanisms where by garlic extract inhibit growth of bacteria especially against *Mutans Streptococci* are still unclear, most explanations concerning the antibacterial effect of garlic in general related the inhibitory effect of the main antimicrobial constituent of garlic which has been identified as the oxygenated sulfur compound, thio-2-propene -1-sulfinic acid S–allyl ester referred to as allicin, it was shown that this compound react very rapidly with free thiol groups via thiol-disulphide exchange, therefore, it is thought that the main mechanism of action of allicin against bacteria is through interaction with thiol containing enzymes including cysteine proteases and alcohol dehydrogenises [17, 18].

The effect of de-ionized water 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 de-ionized water compared to the control after 24 hour, this could be explained by the complete resistant of these bacteria to de-ionized water, where as chlorhexidine showed highly significant reduction in the counts of bacteria in comparison to the control after 24 hour and garlic extracts. It is well known that chlorhexidine is a potent antibacterial agent particularly against *Mutans Streptococci* [19, 20].

Table1: Statistical Test between Chlorhexidine and Concentrations of Water Garlic Extract.

Concentration of Garlic Water Extract	*t-test*	*P-Value*	*Description*
5%	-	-	-
10%	24.42	0.000	HS
15%	21.08	0.000	HS
20%	18.58	0.000	HS
25%	20.01	0.000	HS
30%	18.22	0.000	HS
35%	16.98	0.000	HS
40%	14.03	0.000	HS
45%	8.00	0.000	HS
50%	7.28	0.000	HS

Table 2: Statistical Test between Chlorhexidine and Concentration of Ethanol Garlic Extract.

Concentration of Garlic Ethanol Extract	*t-test*	*P-Value*	*Description*
5%	24.36	0.000	HS
10%	13.40	0.000	HS
15%	8.68	0.000	HS

Concentration of Garlic Ethanol Extract	t-test	P-Value	Description
20%	3.38	0.003	S
25%	1.58	0.133	NS
30%	0.70	0.494	NS
35%	4.91	0.000	HS
40%	5.52	0.000	HS
45%	8.91	0.000	HS
50%	10.68	0.000	HS

Considering *in vitro* data obtained in the present garlic extract has been shown to be effective against Streptococci and *Mutans Streptococci*. Further studies are needed regarding the effect of garlic extracts on other cariogenic determinants of *Mutans Streptococci* such as adherence and ability to form extracellular polysaccharide, also future studies are needed concerning the effect of garlic extracts on other types of cariogenic bacteria as lactobacilli and Actinomyces.

The effectiveness of water garlic extract (10%) on salivary Streptococci and *Mutans Streptococci* was tested among a group of volunteers, in comparison to chlorhexidine gluconate and de-ionized water. A slight reduction in the counts of Streptococci recorded for garlic extract, while a sharp reduction in *mutans streptococcal* counts was noticed, this was in coincidence with Groppo *et al* [21] who showed that garlic mouth rinse had antimicrobial activity against *Mutans Streptococci,* but not against other oral microorganisms, this may give an indication for specificity of garlic extract on *Mutans Streptococci*. The presence of resistant strains of oral Streptococci to this agent probably might explain its ineffectiveness against Streptococcal bacteria. The result of the present study showed the effectiveness of garlic especially against *Mutans Streptococci*, although it was less than chlorhexidine gluconate, but it can be use as effective anti caries agent, however, many problems should be solved before such strategy can be accomplished which associated with reducing the strong odor of garlic and eliminating of burning sensation that could occur during or following rinsing.

Table 3: Statistical Test between Different Concentration of Garlic Extracts (Water, Ethanol), Chlorhexidine, and De-ionized Water in Comparison with the Counts of *Mutans Streptococci* after 24 hour.

Agents	Count of bacteria after 24 hr. $\times 10^3$		Description
Water garlic extract 5%	0.113	0.913	NS
Water garlic extract 10%	2.355	0.046	S
Water garlic extract 15%	3.927	0.004	S
Ethanol garlic extract 5%	2.304	0.05	NS
Ethanol garlic extract 10%	4.047	0.004	S
Ethanol garlic extract 15%	5.293	0.001	S
Chlorhexidine 0.2%	19.45	0.000	HS
De-ionized water	0.257	0.804	NS

Table 4: Values of Standard Deviation of Mean Count Streptococci by Time Points.

Time	Garlic water extract	CHX	DW
	SD	SD	SD
Baseline	45.806	76.033	83.250
One min.	72.644	76.904	85.854
30 min.	77.017	67.036	77.787

Time	Garlic water extract	CHX	DW
	SD	SD	SD
One hr.	91.821	59.642	91.934
Two hr.	56.266	64.272	106.432

Table 5: Values of Standard Deviation of Mean Counts *Mutans Streptococci* by Time Points.

Time	Garlic water extract	CHX	DW
	SD	SD	SD
Baseline	71.306	54.302	72.496
One min.	65.879	79.792	55.029
30 min.	54.160	47.744	82.250
One hr.	56.337	16.363	87.113
Two hr.	85.490	46.908	87.618

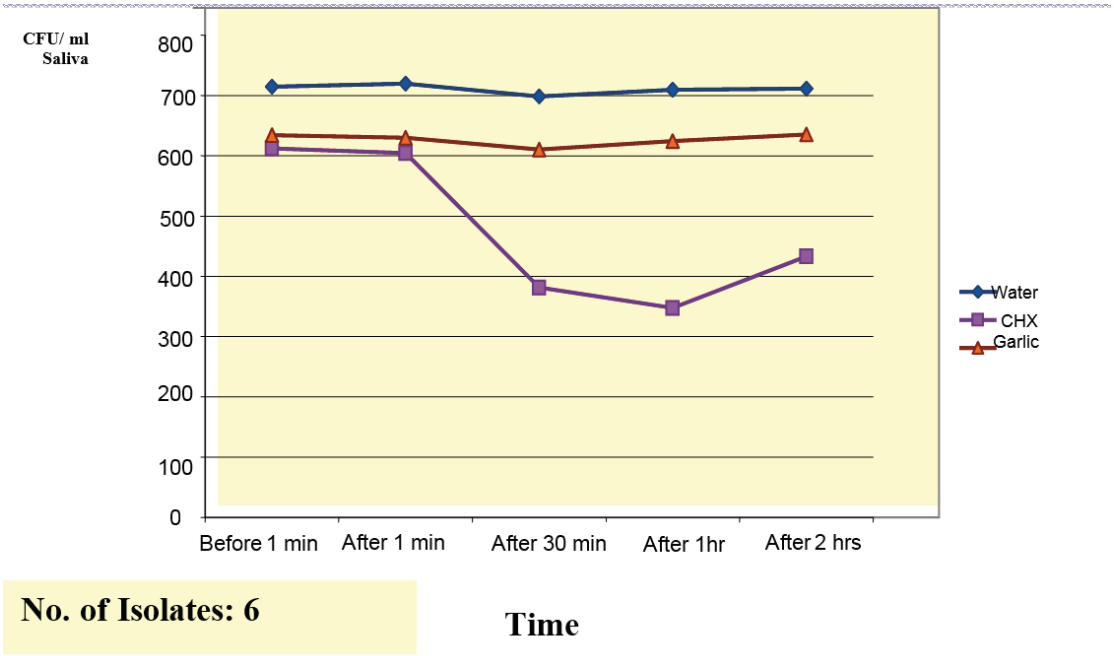

Figure 1: Mean Counts of Salivary Streptococci $\times 10^3$ of Volunteers Before and After Rinsing With 10% Garlic Water Extract, Chlorhexidine Gluconate, and De-ionized Water at Different Time Points.

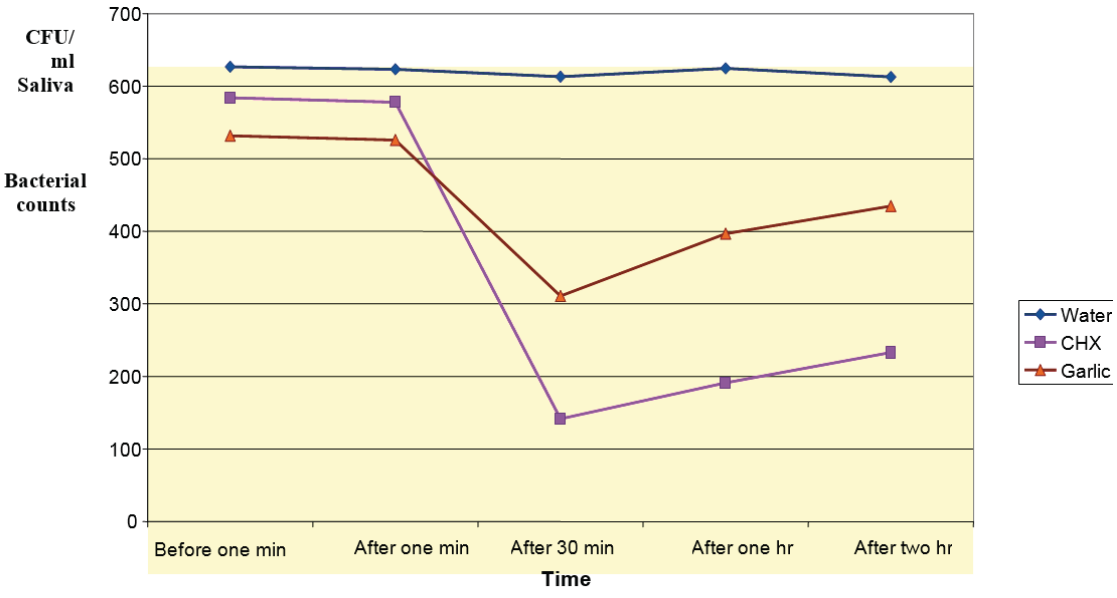

Figure 2: Mean Counts of Salivary *Mutans Streptococci* $\times 10^3$ of Volunteers Before and After Rinsing With Garlic Water Extract, Chlorhexidine Gluconate, and Deionized Water at Different Time Points.

REFERENCES

1. Moynihan P, Petersen P. Diet, nutrition and prevention of dental diseases. Public health nutrition 2004; 7:201-26.
2. Weyant R. Seven systematic reviews confirm topical fluoride therapy is effective in preventing dental caries. J Evid Dent Base Pract 2004; 3:129–135.
3. Palmer C, Wolfe SH. Position of the American Dietetic Association: the impact of fluoride on health. J Am Diet Assoc 2006; 105:1620–1628.
4. 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.
5. 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.
6. 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.
7. Al-Anbori D. Effect 3 of Myrtus communis extracts on *Mutans Streptococci* (*in vitro and in vivo* study). M.Sc. Thesis, College of Dentistry, University of Baghdad, 2006.
8. Bakri I and Douglas C. Inhibitory effect of garlic extract on oral bacteria. Archives of Oral Biology 2005; 50: 645-651.
9. Groppo F, Ramacciato J, Motta R, Ferraresi P, Sartoratto A. Antimicrobial activity of garlic against oral streptococci. Int J Dent Hyg 2007;5(2):109-15.
10. Tsao S, Hsu C, Yin M. Garlic extract and two diallyl sulphides inhibit methicillin–resistant Staphylococcus aureus infection in BALB/Ca mice. J of Antimicrobial Chemotherapy 2003; 52: 974-980.
11. Onyeagba R, Ugbogu O, Okeke C, Iroakasi O. Studies on the antimicrobial effects of garlic (Allium sativum Linn.), ginger (Zingiber officinale Roscoe) and lime (Citrus aurantifolia Linn). J of African Biotechnology 2004; 3(10): 552-554.
12. Holbrook W and Beighton D. Streptococcus mutans levels in saliva and distribution of serotypes among 9-year-old Icelandic children. J of Scan Dent Res 1986; 95: 37-42.
13. 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.
14. Thylstrup A and Fejerscov O. Text book of cariology 1st ed. Munksgaard, Copenhagen, 1996.
15. Amagase H, Petesch B L, Matsuura H, Kasuga S, Itakura Y. Intake of garlic and its bioactive components. J Nutrition 2001; 131:955S-962S.
16. 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.
17. Ankri S, Miron T, Rabinkov A, Wilechek M, Mirelman D. Allicin from garlic strongly inhibits cysteine proteinases and cytophatic effects of Entamoeba histolytica. Antimicrob Agents Chemother 1997; 41:2286-8.
18. Bachrach G, Jamil A, Naor R, Tal G, Ludmer Z, Steinberg D. Garlic Allicin as a Potential Agent for Controlling Oral Pathogens. J Med Food. 2011; 6.
19. Emilson C. Potential efficacy of chlorhexidine against *Mutans Streptococci* and human dental caries. J Dent Res 1994; 73(3): 682-691.
20. Kulkarni V, 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.
21. Groppo F C, Ramacciato J C, Simoes R P, Florio F M, Sartoratto A. Antimicrobial activity of garlic, tea tree oil, and chlorhexidine against oral microorganisms. J Int Dent 2002; 52(6): 433-7.