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Antibacterial Effect of Aqueous and Alcoholic Ginger Extracts on Periodontal Pathogen Aggregatibacter Actinomycetem Comitans [An in Vitro Study] (Part 1)

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Ginger, Periodontitis, Aggregatibacter actinomycetem comitans

Abstract

Among natural food sources with antimicrobial activities, ginger rhizome has been used as widely grown food spices and medicinal crops for centuries. Furthermore, it possess antifungal and antioxidant properties due to the phenols – related constituents (gingerols) that constrain the growth of many Gram positive and Gram negative bacteria including some periodontal bacteria. The Actinomycetem comitans is a portion of the normal microbiota in numerous healthy individuals but is also a major etiological agent in some aggressive and chronic types of periodontitis.

The present study was conducted to test the effect of aqueous and alcoholic ginger extracts on the growth of Aggregatibacter actinomycetem comitans in comparision to 0.2% chlorohexidine gluconate mouth wash and distilled water in vitro, determination of ginger extracts minimum inhibitory concentration and minimum bactericidal concentration and detection of active ingredients of ginger extracts by using the high-performance liquid chromatography as well as chemical elements.

Introduction:

The increased antibiotics application lead to development of antimicrobial resistance of the microorganisms that became a serious problem[(1)]. Consequently there is a wide need to find the substitute of chemotherapeutic medications for treatment of diseases especially those derived from plants that are easily obtainable and have less side effects[(2)]. Ginger (Zingiber Officinale) is a medicinal plant that has been used widely all over the world, for thousands of years and for treatment of a wide range of unrelated ailments including arthritis, cramps, sore throats, infectious diseases, pains, constipation, hypertension and fever[(3)]. Also alcoholic ginger extract exhibited antimicrobial effect agains Mutans streptococcus and Candida albicans[(4-7)], as well as against some Gram negative periodontal pathogenic bacteria (Porphyromonas gingivalis, Porphyromonas endodontalis and Prevotella intermedia) [(8)]. Periodontitis "is an inflammatory disease of tooth-supporting tissues, characterized by loss of connective tissue attachment and alveolar bone". The sub gingival plaque bacteria is the primary etiological agent of periodontitis. It is mostly believed that periodontitis is a polymicrobial disease, and more relevant to complex interactions between specific pathogens than are individual species [(9)]. Three of these bacteria, Aggregatibacter actinomycetem comitans, Tannerella forsythia, and Porphyromonas gingivalis, were formally designated as etiological agents of periodontitis [(10)]. Numerous studies recommended that the consequence of periodontal treatment is superior if the

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detection of certain pathogens especially Aggrecatibacter actinomycetem comitans and Porphyromonas gingivalis can no longer present after therapy [11,12]. As ginger possesses antibacterial and antioxidant properties, and as there were no previous studies that evaluate the effect of ginger extracts (aqueous and alcoholic) against Aggrecatibacter actinomycetem comitans and compare the effect with 0.2% chlorohexidine and distilled water, this study was conducted.

Materials and method:

The present study involved two experiments in vitro and it was conducted at Microbiological laboratory for the postgraduate students in The Basic Science department/College of Dentistry/ Baghdad University**.**

*Extraction of aqueous and alcoholic extracts of ginger

Aqueous extract: 40 gm of ginger powder were placed in 160 ml of sterile distilled water (D.W.) and left at room temperature for 24 hrs. with continuous mixing by using magnetic stirrer. Then the mixture was filtered and dried using incubator at 40°C. The liquid evaporated, and the concentrated extract was left at the base of the baker[13]. Alcoholic extract: 40 gm of ginger powder were put in a glass jar then 500 ml of 99.9 ethanol alcohol was added and mixed well. The container was sealed with cotton and foil to prevent evaporation of alcohol and left at room temperature for 24 hours. Then, the contents were filtered after that concentrated by evaporating the solvent alcohol in a hot air oven at 40ºC for 24 hrs. [14].

*The determination of ginger active ingredients by using the high-performance liquid chromatography (HPLC), was done in the Ministry of Science and Technology, Department of material research .Which, is a chromatographic technique applied to discrete the active ingredients of ginger essential phenols that depend on the retention time of each ingredient. Spectrophotometer was used for the quantitative determination of chemical elements of alcoholic and aqueous ginger extracts.

*Preparation of a selective agar media for A.a : 40 gm of trypticase soy agar was dissolved in 1000 ml D.W., then heating till boiling, sterilization by autoclaving and leftward till cool down to about 40-45º C in a water bath, after that, 50 ml of sterile blood were added to 128 µg/ml bacitracin and 8 µg/ml malachite green and mixed well then the media was poured into sterile Petri dishes [15].

***** Preparation of a selective liquid broth media for A. a: 30 gm of trypticase soy broth powder dissolved in 1000 ml of D.W., then heating till boiling, and sterilized using autoclave and left to cool down to about 40- 45ºC in a water bath, next, aseptically adding 128 µg /ml of bacitracin and 8 µg /ml of malachite green mixed well then the media was poured into sterile glass tube,then left to cool down to about 25ºC and stored at the refrigerator till used[15].

*The plaque samples were obtained from patients attending the clinic at the department of Periodontics in the teaching hospital of Dentistry College / Baghdad University. The patients were informed about the goals of the study and patient's agreements and approvals were gained before samples collection.

The sub gingival plaque samples were collected from 50 systematically healthy patients (males and females) with age range of (40-60) years old, suffering from chronic periodontitis, which required the existence of at least 4 sites with probing pocket depth ≥ 4mm and clinical attachment loss of (1-2) mm or more[16]. Samples were collected from one pocket for each patient, and from the deepest part of the periodontal pocket applying Gracey curette and the sample placed on a swab which is introduced into a transfer media immediately and transferred to the lab to be inoculated on the selective agar media under anaerobic conditions using anaerobic gas pack and anaerobic jar at 37°C for 48 hrs.

*Isolation and identification of A.a colonies was made depending on the morphology of the colonies, Gram stain and several biochemical tests (Indole, oxidase, catalase, coagulase, urease and API tests), hemolytic ability and antibiotic sensitivity test.

* First experiment: Sensitivity of A.a: Agar well diffusion method was done to test the sensitivity of A.a to different concentrations of ginger extracts (20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100%), 0.2% chlorohexidine gluconate (CHX) as a positive control and distilled water as a negative control. The A.a was spread on the selective media, several wells (4-6) of equal size and depth were prepared in each agar plate, each well was filled with 0.1 ml of the agent (ginger extracts) being tested and other wells filled with CHX and D.W. agents. Plates were incubated anaerobically for 72 hrs., a ruler was used for the measurement of inhibition zone.

*Second experiment: Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of alcoholic and aqueous ginger extracts against A.a:

a. Serial dilution was performed: 9 ml of A.a broth is dispersed into test tube, and then 1ml of bacterial suspension was added to achieve 10 ml. Then from the first tube we took 1 ml and dispersed to the second test tube and complete with 9 ml of A.a broth to have the first dilution, then we repeat the procedure for 4 times in 4 sequential tubes to reach 5 dilutions .

b. The MIC is the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism [17].

Test tubes were labeled by the No. of the different concentrations of the ginger extracts (aqueous and alcoholic), after that 1 ml of bacterial suspension were added to each tube then 0.5 ml of the ginger extracts were added to its designated tube. Then the tubes anaerobically incubated for 48 hrs., after that, the tubes were examined to notice if any turbidity was present (turbidity indicates bacterial growth), the tubes that lack the turbidity were identified as the MIC.

c. The MBC is the lowest concentration of an antimicrobial agent required to kill a particular bacterium [18]. A swab was taken from each tube that showed absence of turbidity and spread on the selective agar media plates and incubated anaerobically for 48 hrs., the plates that displayed no growth were identified as MBC. Statistical analysis was done using mean, standard deviation S.D., One-way Analysis of variance test (ANOVA) test, least significant difference (LSD) and Independent sample t-test. In the statistical evaluation, the following levels of significance were used, NS: $P > 0.05$, S: $0.05 \geq P > 0.01$ and HS: $P \leq 0.01$.

Results:

*Isolation and identification of A.a : According to their morphological characteristics, A.a colonies were white to grayish in color, about 1 mm in diameter and they adhered well to the agar, microscopic examination revealed that A.a were gram negative rods, arranged in short or medium length chains.

Biochemical tests revealed that A.a was oxidase and catalase positive but, it was indole, coagulase and urease negative,and according to API test, A.a was listed as (Haemophilus actinomycetem comitans), also it displayed positive hemolytic ability and it was resistant to both Kanamycin and Vancomycin antibiotics.

*Sensitivity of A.a to different concentrations of ginger extracts,0.2% CHX and D.W. : The diameter of inhibition zones were found to increase as the concentrations of both ginger extracts increased. Hence, all aqueous ginger extract concentrations revealed mean values of inhibition zones less than the CHX, 100% concentration showed maximum mean value of inhibition zone was (12.77 mm). Alcoholic ginger extract concentrations 80%, 90% and 100% displayed mean values of inhibition zones higher than 0.2% CHX, whereas, D.W. showed no inhibition zone. The mean values of inhibition zones of alcoholic extract started at 20% concentration which was (8.59 mm) while, it became (15.38 mm) at 100% concentration. One way ANOVA test showed highly significant differences amongst different concentrations of each ginger extract**,** 0.2% CHX and D.W., Table (1). From Table (2), the concentrations of aqueous ginger extract revealed highly significant differences, but, the differences were significant between 50% with 80% and 90% with 100%. While non-significant

differences between 20% with 30%, and 60% with 70% as well as, 50% with 40%, 60% and 70%, in addition, 80% with 90%, 70% and 60%. Alcoholic ginger extract demonstrated highly significant differences, except for concentrations 30% with 40%, 60% with 70% and 90% with both 80% and 100%,since there were non-significant differences, however there was significant difference between 80% with 100%. Table (3), demonstrated highly significance differences between CHX, D.W. and each concentration of aqueous and alcoholic ginger extracts, except, the concentrations 80% and 90% of alcoholic extract that revealed non-significant and significant differences respectively with CHX. Table (4), revealed that the mean values of inhibition zones of alcoholic extract in all concentrations higher than that of aqueous, with highly significant differences between mean values of A.a inhibition zones for the same concentration of both ginger extracts, but, there was significant difference at 40% concentration. The HPLC analysis, Table (5), of active ingredients displayed that alcoholic extract had higher contents of 10-gingerol and 6-shogoal than the aqueous ginger extract, while aqueous extract had higher contents of 6-gingerol and 8-gingerol as compared with alcoholic extract. While the detection of chemical elements of ginger extracts showed that Potassium present in highest concentration from the other elements, followed by Magnesium. The least element concentration in both ginger extracts was Copper (Cu) element. However, alcoholic ginger extract had higher concentrations of all elements except, Copper, whereas Calcium (Ca) found only in the alcoholic extract, Table (6). The MIC of aqueous ginger extract was 80% concentration ,while of alcoholic ginger extract was 50%, CHX also showed bacteriostatic effect on A.a. The MBC of aqueous ginger extract was 100%, while of alcoholic ginger extract was 80%.

Discussion

The fresh ginger was described to contain fibers, protein, carbohydrates fat, lipids (including glycerides, phosphatidic acid, lecithins, and fatty acids), protease, minerals e.g. (iron, calcium, magnesium, potassium, and phosphorous), as well as it contains vitamins such as(thiamine, riboflavin, niacin and vitamin C) [19, 20]. Ginger has strong antibacterial effect and to some extent antifungal activities [21].

Generally, numerous studies revealed that ginger antimicrobial effects primarily was due to the existence of oxygenated mono and sesquiterpenes phenolic constituents (shogaol, gingerol) [22], which are lipid-soluble phenol compounds mostly isolated from the root of ginger [23,24] . Most of the phenols can change the cell permeability because they are protein denaturing agents, which may lead to swelling and rupture of the bacterial cells, also most of them are metal cheaters that bind to the active site of metabolic enzymes, decreasing the enzyme activities and therefore decelerating bacterial metabolism and reproduction [25].

*Results of A.a sensitivity to ginger extracts revealed that both alcoholic and aqueous extracts had the ability to inhibit the growth of A.a and the antimicrobial activity of both extracts increase when the ginger extracts concentration increased, so the diameters of inhibition zones was also increased, this may be due to the amount of the dissolved active ingredients of the extracts will be more abundant as the concentration increased. It is clear from this study that the concentrations of alcoholic extract had more antibacterial activity by displaying higher mean values of inhibition zones for A.a than the concentrations of aqueous extract, the comparisons between these two extracts showed almost highly significant differences between the same concentrations of the alcoholic and aqueous extracts that could be because of the amount of active

component in the alcoholic ginger extract 10-gingerol (hence, its concentration was higher in alcoholic extract than the aqueous in this study) which is the primary active ginger phenol against periodontal anaerobic pathogenic bacteria, [8]. Indeed the polarity of the solvent (ethanol alcohol) which has great ability to dissolve ginger biologically active constituents [26] .

*Detection of MIC and MBC of aqueous and alcoholic ginger extracts against A.a : The MIC required to inhibit A.a growth in broth media was 50% (0.5 g/ml) concentration of alcoholic ginger extract, while, MIC of aqueous ginger extract was 80% (0.8 g/ml) concentration and this represented the bacteriostatic activity of the extracts against A.a . The 0.2% Chlorohexidine gluconate applied in this experiment as a positive control displayed also bacteriostatic effect against A.a .The MBC of alcoholic ginger extract that kills A.a was 80%(0.8 g/ml) concentration while, the MBC of aqueous ginger extract was 100% (1g/ml) concentration which represented the bactericidal effect of the extracts against A.a . Hence, gingerol and shogaol, the lipid-soluble phenols that are mainly isolated from the ginger root[27], have various routes of action which means that these compounds not only attack cell walls and cell membranes i.e., affecting their permeability and release of intracellular constituents (e.g. ribose sodium glutamate), but they also interfere with (electron transport, membrane functions, nutrient uptake, protein and nucleic acid synthesis and enzyme activity). Thus, these compounds might have numerous invasive targets that lead to the inhibition of pathogenic bacteria [27]. There were no other studies to compare the results with.

* Detection of the active constituents of ginger extracts and chemical elements: The HPLC analysis results of alcoholic and aqueous ginger extracts revealed that alcoholic extract had higher content of 6-shogoal and 10-gingerol that are the main active ginger phenol with powerful antibacterial effect against periodontal anaerobic bacteria, [8]. Since, several studies showed that the antimicrobial activity of ginger mainly caused by the presence of phenolic compounds (shogaol,gingerol)[24],while aqueous extract contained higher content of 6-gingerol and 8-gingerol than alcoholic extract that are of less effect against periodontal pathogens [8].A study done by Park et al.,2007[8],which signified that the ethanol and n-hexane ginger extracts exhibited antibacterial effect against three of the anaerobic Gram-negative bacteria, Porphyromonas endodontalis, Porphyromonas gingivalis and Prevotella intermedia, that are responsible of periodontal diseases, they found that [10] -gingerol and [12] - gingerol effectively inhibited the growth pattern of these periodontal pathogens.

*Detection of ginger extracts chemical elements: Similar elements were detected in both extracts but varied in their concentrations. Since alcoholic ginger extract exhibited higher concentrations of (K), (Mg), (Mn), (Fe) and (Zn) than the aqueous extract whereas, the other elements are almost equal and (Ca) found only in alcoholic extract, this could be due to the polarity of ethanol alcohol that has the capacity to dissolve ginger biological active component [28].Since, (K), the third most copious mineral in human body, and it is a powerful element in general health improvement ; from other side, (Mg) revealed its significance in many biological and detoxification procedures, this reflect the powerful antioxidant effect of ginger.

Table (1): The statistical analysis *of A.a* inhibition zones by different concentrations of aqueous and alcoholic ginger extracts, CHX and D.W.

Agents	**Conc.**	**No.**	***Mean**	**±S.D.**	**ANOVA test**
CHX	0.2%	8	14.19	0.76	
Aqueous ginger extract	20%	8	7.34	0.39	**F= 130.017** **P= 0.000** **HS** ***d.f.= 87**
	30%	8	7.65	0.41	
	40%	8	9.12	0.76	
	50%	8	9.86	0.94	
	60%	8	10.40	1.02	
	70%	8	10.53	1.08	
	80%	8	11.03	1.55	
	90%	8	11.76	1.13	
	100%	8	12.77	1.14	
D.W.		8	0	0	
CHX	0.2%	8	14.39	0.62	
Alcoholic ginger extract	20%	8	8.59	0.82	**F= 314.939** **P= 0.000** **HS** **d.f.=87**
	30%	8	9.63	1.08	
	40%	8	10.06	0.72	
	50%	8	11.81	0.48	
	60%	8	13.05	0.72	
	70%	8	13.11	0.74	
	80%	8	14.48	0.60	
	90%	8	15.12	0.70	
	100%	8	15.38	0.77	
D.W.		8	0	0	

*****in mm, *d.f=degree of freedom

Table (2): Comparisons of mean values of *A.a* inhibition zones between each pair of different concentrations of aqueous and alcoholic ginger extracts by LSD test

Conc.		**Aqueous ginger extract**			**Alcoholic ginger extract**		
		Mean difference	**p-value**	***Desc.**	**Mean difference**	**p-value**	**Desc.**
20%	30%	-0.31	0.504	NS	-1.03	0.005	HS
	40%	-1.79	0.000	HS	-1.47	0.000	HS
	50%	-2.52	0.000	HS	-3.21	0.000	HS
	60%	-3.06	0.000	HS	-4.45	0.000	HS
	70%	-3.19	0.000	HS	-4.51	0.000	HS
	80%	-3.69	0.000	HS	-5.89	0.000	HS
	90%	-4.42	0.000	HS	-6.52	0.000	HS
	100%	-5.43	0.000	HS	-6.79	0.000	HS
30%	40%	-1.47	0.002	HS	-0.44	0.220	NS
	50%	-2.21	0.000	HS	-2.18	0.000	HS
	60%	-2.75	0.000	HS	-3.42	0.000	HS
	70%	-2.88	0.000	HS	-3.48	0.000	HS
	80%	-3.38	0.000	HS	-4.86	0.000	HS
	90%	-4.11	0.000	HS	-5.49	0.000	HS
	100%	-5.12	0.000	HS	-5.76	0.000	HS
40%	50%	-0.74	0.117	NS	-1.74	0.000	HS
	60%	-1.28	0.007	HS	-2.99	0.000	HS
	70%	-1.40	0.003	HS	-3.04	0.000	HS
	80%	-1.90	0.000	HS	-4.42	0.000	HS
	90%	-2.63	0.000	HS	-5.06	0.000	HS
	100%	-3.65	0.000	HS	-5.32	0.000	HS
50%	60%	-0.54	0.249	NS	-1.24	0.001	HS
	70%	-0.67	0.157	NS	-1.30	0.000	HS
	80%	-1.17	0.014	S	-2.68	0.000	HS
	90%	-1.90	0.000	HS	-3.31	0.000	HS
	100%	-2.91	0.000	HS	-3.58	0.000	HS
60%	70%	-0.13	0.789	NS	-0.06	0.868	NS
	80%	-0.63	0.183	NS	-1.44	0.000	HS
	90%	-1.36	0.005	HS	-2.07	0.000	HS
	100%	-2.37	0.000	HS	-2.34	0.000	HS
70%	80%	-0.50	0.286	NS	-1.38	0.000	HS
	90%	-1.23	0.010	HS	-2.01	0.000	HS
	100%	-2.24	0.000	HS	-2.28	0.000	HS
80%	90%	-0.73	0.120	NS	-0.63	0.076	NS
	100%	-1.74	0.000	HS	-0.90	0.013	S
90%	100%	-1.01	0.032	S	-0.27	0.454	NS

* Desc.= Description

Table (3): Comparisons of mean values of *A.a* inhibition zones between each concentration of aqueous and alcoholic ginger extracts with CHX and D.W. by LSD test

Ginger extracts	Conc.	CHX 0.2%			D.W.		
		Mean difference	P-value	Desc.	Mean difference	P-value	Desc.
Aqueous ginger extract	20%	6.85	0.000	HS	7.34	0.000	HS
	30%	6.54	0.000	HS	7.65	0.000	HS
	40%	5.06	0.000	HS	9.12	0.000	HS
	50%	4.33	0.000	HS	9.86	0.000	HS
	60%	3.79	0.000	HS	10.40	0.000	HS
	70%	3.66	0.000	HS	10.53	0.000	HS
	80%	3.16	0.000	HS	11.03	0.000	HS
	90%	2.43	0.000	HS	11.76	0.000	HS
	100%	1.42	0.003	HS	12.77	0.000	HS
Alcoholic ginger extract	20%	5.79	0.000	HS	8.59	0.000	HS
	30%	4.76	0.000	HS	9.63	0.000	HS
	40%	4.33	0.000	HS	10.06	0.000	HS
	50%	2.58	0.000	HS	11.81	0.000	HS
	60%	1.34	0.000	HS	13.05	0.000	HS
	70%	1.28	0.001	HS	13.11	0.000	HS
	80%	-0.10	0.789	NS	14.48	0.000	HS
	90%	-0.73	0.042	S	15.12	0.000	HS
	100%	-1.00	0.006	HS	15.38	0.000	HS

Table (4): Statistical analysis and comparisons between mean values of *A.a* inhibition zones for the same concentrations of aqueous and alcoholic ginger extracts

Conc.	Descriptive statistics				Mean difference d.f.=14		
	Aqueous		Alcoholic				
	Mean	±S.D.	Mean	±S.D.	t-test	p-value	Desc.
20%	7.34	0.39	8.59	0.82	-3.90	0.002	HS
30%	7.65	0.41	9.63	1.08	-4.85	0.000	HS
40%	9.12	0.76	10.06	0.72	-2.55	0.023	S
50%	9.86	0.94	11.81	0.48	-5.21	0.000	HS
60%	10.40	1.02	13.05	0.72	-6.01	0.000	HS
70%	10.53	1.08	13.11	0.74	-5.59	0.000	HS
80%	11.03	1.55	14.48	0.60	-5.89	0.000	HS
90%	11.76	1.13	15.12	0.70	-7.14	0.000	HS
100%	12.77	1.14	15.38	0.77	-5.39	0.000	HS

Table (5): Concentrations of each constituent of aqueous and alcoholic ginger extracts

Subjects	Conc. of standard	Conc. of constituents of aqueous ginger extract	Conc. of constituents of alcoholic ginger extract
6-gingerol	25	217.26	118.5
8-gingerol	25	60.30	48.80
6-shogoal	25	73.05	105.20
10-gingerol	25	6.96	102.90

Table (6): Chemical elements concentrations in alcoholic and aqueous ginger extracts

Elements	Alcoholic ginger extract conc. µg/ml	Aqueous ginger extract conc. µg/ml
Potassium (K)	60000	7045.9
Magnesium (Mg)	6747	587.3
Phosphorous (p)	223.42	219.87
Iron (Fe)	221.3	23.54
Manganese (Mn)	205	30.37
Zinc (Zn)	27.8	11.35
Copper (Cu)	2.393	3.184
Calcium (Ca)	49.81	Nil

References

1-Abimbola KA, Obi CL, Alabi SA, Olukoya DK, Ndip RN. Current Status on biotyping antibiogram and plasmid profiles of E. coli isolates. J East Afr. Med;1993; 70:207-210.

2- Khulbe K,Sati SC.. Antibacterial Activity of Boenninghausenia albiflora Reichb. (Rutaceae). J. Afr. Biotechnol;2009; 8(22):6346-6348.

3- Ali BH, Blunden G, Tanira MO, Nemmar A. .Some phytochemical, pharmacological and toxicological properties of ginger (Zingiber Officinale Roscoe): A review of recent research. Food Chem Toxicol;2008; 46(2): 409-420.

4- Akihiro O, Fumie S, Tsugio S.. Screening of Antibacterial activities of Edible Plants against Streptococcus mutans. Food Sci. Techno;2008;14(2):190 – 193.

5- Atai Z, Atapour M, Mohseni M.. Inhibitory Effect of Ginger extract on Candida albicans. American Journal of Applied Sciences;2009; 6(6): 1067- 69.

6- Joe M, Blegay W, Coniglio K.. Antimicrobial activity of some common spices against certain human pathogens. J Medicinal Plants Res.;2009; 3(11): 1134-36.

7-Tuqa Akram Wel, Ahlam Taha Mohammed. Effect of ginger extract on Mutans streptococci in comparison to chlorhexidine gluconate. Journal of Baghdad College of Dentistry; 2013; :25, P: 179-184.

8-Park M, Bae J, Lee D.S. . Antibacterial activity of [10]- gingerol and [12]-gingerol isolated from ginger rhizome against periodontal bacteria. Phytother. Res.;2008; 22(11):1446-9.

9-Lourenco TG, Heller D, Silva-Boghossian CM, Cotton SL, Paster BJ, Colombo AP.. Microbial signature profiles of periodontally healthy and diseased patients. J Clin Periodontol ;2014; 41: 1027–1036.

10-American Academy of Periodontology.. Consensus report: section on epidemiology. Ann.Periodontol.1996; 1:216-218.

11- Haffajee AD, Dzink JL, Socransky SS.. Effects of modified Widman flap surgery and systemic tetracycline on the subgingival microbiota of periodontal lesions. J Clin periodontal;1988; 15:255-62.

12-Rodenburg JP, van Winkilhoff AJ, Winkel EG,Goene RJ,Abbas F, de Graff J. . Occurence of Bacteroids gingivalis,

Bacteroids intermedius and Actinobacillus actinomycetem comitans in severe periodontitis in relation to age and treatment history. J Clin periodontal; 1990; 17(6):392-9.

13- Al – Joboory A, Al – Rawi M.. Natural pharmacology. 1st edition, Baghdad, dar Al – Huriah1994.

14- Nweze E, Okafor J. .Antifungal activities of wide range of medical plants extracts and essential oils against Scedosporium apiospermum isolates.America Eurasian J of Sc Res;2010;5(3):16169.

15-Mythireyi D, and Krishnababa MG. . Aggregatibacter Actinomycetem comitans, an Aggressive Oral Bacteria - A Review. International Journal of Health Sciences & Research; 2012; 2; Issue: 5; (www.ijhsr.org).

16-Lang NP, Bartold PM,Cullinam M. . International classification workshop. Consensus report: chronic periodontitis. Annals of periodontology;1999; 4:53.

17- Andrews JM. "Determination of minimum inhibitory concentrations". Journal of Antimicrobial Chemotherapy;2001; 48 (suppl1):5-16.

18-Mims, Playfair. . Medical microbiology, Mosby Europe,1993; 35.31.

19-ICMR Bulletin. .Ginger: Its role in xenobiotic metabolism, 2003;33(6), 57-63.

20-Ibrahim TA, Dada IBO, Adejare RA. . Comparative phytochemical properties of crude ethanolic extracts and physicochemical characteristics of essential oils of Myristical fragrans (nutmeg) seeds and Zingiber officinale (ginger) roots. EJEAFChe; 2010;9 (6), 1110-1116.

21-Nielsen PV, Rios R. . Inhibition of fungal growth on bread by volatile compounds from spices and herbs and mustard essential oil. Inter J Food Microbiol;2000; 60: 219-229.

22-Singh G, Kapoor IP, Singh P, de Heluani CS, de Lampasona MP, et al.,. Chemistry, antioxidant and antimicrobial investigations on essential oil and oleoresins of Zingiber officinale. Food and Chem Toxicol ;2008; 46: 3295–3302.

23-Michielin EM, Salvador AA, Riehl CA, Smânia A Jr, Smânia EF, et al. . Chemical composition and antibacterial activity of Cordia verbenacea extracts obtained by different methods. Bioresource Tech 2009; 100: 6615–6623.

24-Wang W, Li CY, Wen XD, Li P, Qi LW. . Simultaneous determination of 6-gingerol, 8-gingerol, 10-gingerol and 6-shogaol in rat plasma by liquid chromatography–mass spectrometry: Application to pharmacokinetics. J Chromatogr B Analyt Technol Biomed Life Sci B;2009; 877: 671–679.

25-Fisher C..Phenolic Compounds in Food and their effects on Health, ACS Symp Ser; 1992; 506.

26-Mahasneh AM, Abbas JA, El-Oqilah AA. . Antimicrobial activity of extracts of herbal plants used in the traditional medicine of Bahrain. J Plytotherapy Res;1996;10(3):257-253.

27-Liu Z. . Diterpene Glycosides as Natural Solubilizers. United States Patent Application Publication. Patent Department Taylor, Porter, Brooks & Philips.2011.

28-Bajpai VK, Al-Reza SM, Choi UK, Lee JH, Kang SC. . Chemical composition, antibacterial and antioxidant activities of leaf essential oil and extracts of Metasequioa glyptostroboides. Miki ex Hu. Food Chem Toxicol ;2009; 47: 1876–1883.