



ANTIBACTERIAL EFFECT OF AQUEOUS AND ALCOHOLIC GINGER EXTRACTS ON PERIODONTAL PATHOGEN *PORPHYROMONUS GINGIVALIS* [IN VITRO STUDY]

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ABSTRACT

In present study *Porphyromonas gingivalis* (*P. gingivalis*) strains were isolated by collecting of sub gingival plaque from 50 patients with chronic periodontitis of pocket depth at least 5 mm to be cultured under anaerobic conditions for 48 hours in suitable culture media using anaerobic gas pack and anaerobic jar in the incubator. Presence of the target microorganism is confirmed using morphological characteristics, Gram's stain, biochemical tests (Indole test, oxidase test, catalase test, coagulase test, urease test and Analytical profile index test), hemolytic ability and antibiotic sensitivity. Ginger was extracted by using water and alcohol. The minimum inhibitory concentration (MIC) of alcoholic ginger extract that inhibits *P. gingivalis* growth was 30% (0.3 g/ml) concentration, while MIC of aqueous ginger extract was 50% (0.5 g/ml) concentration. The minimum bactericidal concentration (MBC) of alcoholic ginger extract that kills *P. gingivalis* was 60% (0.6 g/ml) concentration while, the MBC of aqueous ginger extract was 80% (0.8 g/ml) concentration. Both ginger extracts were effective against *Porphyromonas gingivalis* with bacteriostatic and bactericidal actions, nonetheless, alcoholic extract was more active than aqueous extract and *Porphyromonas gingivalis* was more sensitive than *Aggregatibacter actinomycetem comitans* to both extracts.

KEYWORDS: Ginger, Periodontitis, *Porphyromonas gingivalis*.

INTRODUCTION

Periodontal diseases importances are coming from its consideration among the most prevalent oral infection affecting mankind worldwide. The main cause of periodontal disease is the multiplication of opportunistic pathogens within the gingival crevice (Topcuoglu and Kulekci, 2015). Periodontitis is the inflammatory diseases caused by a certain bacterial complex in dental plaque biofilm (Nategh *et al.*, 2015). *Porphyromonas gingivalis* which is a member of the highly investigated black pigmented bacteroids, it comprises a high percentage of the sub gingival microbiota in periodontal pockets. It has been recognized as key causative bacteria in the pathogenesis of destructive chronic periodontitis (Ready *et al.*, 2008). In the last years, attention on the effect of plants and its medicament product development has increase significantly (Thuille *et al.*, 2003; Alanis *et al.*, 2005) and because of the most manufacturing antimicrobial agent have many side effects also probability of development of antimicrobial resistance, therefore the plants and there natural products consider a good alternate to these agents (Olila *et al.*, 2001; Pawar and Nabar, 2010). Ginger belongs to *Zingiberaceae* family (Sharma *et al.*, 2010). This plant has powerful aromatic and medicinal properties and is characterized by their tuberous or non- tuberous rhizomes (Chen *et al.*, 2008). Ginger has direct anti-microbial activity so it can be applied in the treatment of bacterial infections (Tan and Vanitha,

2004). Beside the antibacterial activity, the plant is reported to have, anti-oxidant, antiprotozoal, anti-fungal, anti-emetic, anti- rhinoviral and anti-inflammatory activity (Ficker *et al.*, 2003). As ginger demonstrates these properties, and as there were no previous studies that evaluate the effect of ginger extracts (aqueous and alcoholic) against *Porphyromonas gingivalis* and compare the results with *Aggregatibacter actinomycetem comitans*, with 0.2% CHX and D.W., this research was conducted.

MATERIALS & METHODS

The present study involved two experiments *in vitro*. It was conducted at Microbiological laboratory for the postgraduate students in The Basic Science Department/College of Dentistry/Baghdad University. The colonies of *P. gingivalis* were identified on the basis of the morphology of the colonies (Socransky and Haffajee, 1992), Gram stain and several biochemical tests (indole, oxidase, catalase, coagulase, urease and Analytical profile index API test) (Sue Katz, 2013 (a); Karen Reiner. 2013; Sue Katz, 2013 (b); Benita Brink, 2013), hemolytic ability (Ray *et al.*, 2004) and antibiotic sensitivity test.

Preparation of selective culture media for *P. gingivalis*: 40 gm of trypticase soy agar was dissolved in 1000 ml D.W., heating till boiling, sterilized by autoclaving, and left to cool down to about 40-45°C, after that, 50 ml sterile blood were added to 30 ml hemin and 5 mg/ml of vitamin K and mixing

well then the media poured into sterile Petri dishes (Brian *et al.*, 2001; Forbes *et al.*, 2007).

Preparation of a selective liquid broth media for *P. gingivalis*: 30 gm of trypticase soy broth powder dissolved in 1000 ml D.W. and heating till boiling and sterilized by autoclaving, later left to cool down to about 40-45°C in a water bath, then aseptically adding 30 ml of hemin to 5 mg/ml of vitamin K, mixing well and the media dispensed into sterile glass tube, later left to cool down to about 25°C, then stored at the refrigerator until used (Brian *et al.*, 2001).

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 purposes of the study and patient's consents and agreements were obtained before collecting the samples.

The sub gingival plaque samples were collected from fifty systematically healthy patients (males and females) age range was (40-60) years old, suffering from chronic periodontitis, which required the presence of at least 4 sites with probing pocket depth 4mm and clinical attachment loss of (1-2) mm or more (Lang *et al.*, 1999). Samples were taken from one pocket for each patient, and from the deepest part of the periodontal pocket using Gracey curette and put on a swab that is immediately inserted into a transport media 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.

Extraction of aqueous and alcoholic extracts of ginger

Aqueous extract: 40 gm of ginger powder were placed in 160 ml of sterile distilled water and left at room temperature for 24 hrs. with continuous mixing using magnetic stirrer. Then mixture was filtered and dried using incubator at 40°C. The liquid evaporated, and the precipitated extract was left at the base of the baker (Al - Joboory and Al - Rawi, 1994).

Alcoholic extract: 40gm 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 firmly closed with cotton and foil to prevent loss 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 40°C for 24 hrs (Nweze and Okafor, 2010).

First experiment: Sensitivity of *P. gingivalis*: The sensitivity of *P. gingivalis* to various concentrations of ginger extracts (20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100%), 0.2% chlorohexidine gluconate as a positive control and (D.W.) as a negative control have been done by using agar well diffusion method.

The *P. gingivalis* was spread on the surface of the selective agar 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. Plates were incubated anaerobically for 72 hrs., zone of inhibition was measured by using ruler.

Second experiment: Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of alcoholic and aqueous ginger

extracts against *P. gingivalis*: **a.** Serial dilution was performed: 9 ml of *P. gingivalis* broth was dispersed into test tube, then 1ml of bacterial suspension was added to obtain 10 ml. Then from the first tube we took 1 ml and dispersed to the second test tube and complete with 9 ml of *P. gingivalis* broth to achieve the first dilution, the procedure was repeated for 4 times in 4 sequential tubes to reach 5 folds dilutions (Benson, 2002).

b. The MIC "is the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism" (Andrews, 2001).

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 extract were added to its designated tube. After that the tubes anaerobically incubated for 48 hrs., next, the tubes were inspected to see if there was any turbidity (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 (Mims and Playfair, 1993). Swab was taken from each tube and spread on the selective agar media plates and incubated anaerobically for 48 hrs., the plates that showed 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: $0.05 > P > 0.01$ and HS: $P < 0.01$.

The same procedures method and experiments were done on *Aggregatibacter actinomycetem comitans* for both ginger extracts, and the results presented in (part 1 study) (Sana'a M Awad and Maha A Ahmed, 2016).

RESULTS

Isolation and identification of *P. gingivalis*: According to their morphological characteristics, *P. gingivalis* colonies appeared as round spherical on selective agar media plates, with raised or convex surface, microscopic examination showed that *P. gingivalis* cells were Gram negative.

Biochemical tests revealed that *P. gingivalis* was indole test positive, while, oxidase, catalase, coagulase and urease tests were negative and regarding to API test of *P. gingivalis*, it was recorded as (*Porphyromonas asaccharolytica*), also it showed positive hemolytic ability and it was sensitive to both Kanamycin and Vancomycin antibiotics.

Sensitivity of *P. gingivalis*: The diameter of inhibition zones were found to be increased as the concentrations of both extracts increased, aqueous ginger extract demonstrated that all concentrations showed mean values of inhibition zones less than CHX except 100% concentration which revealed higher mean value of inhibition zone (15.67 mm) than CHX (15.48 mm). Alcoholic ginger extract, 20%, 30%, 40%, 50%, 60%, and 70% concentrations exhibited mean values less than CHX, while, 80%, 90% and 100% concentrations showed higher mean values of inhibition zones (16.14 mm),

(18.08 mm) and (18.84 mm) respectively than CHX (15.71) but, D.W. revealed no inhibition zone. One way ANOVA test demonstrated highly significant differences among

different concentrations of each of aqueous and alcoholic ginger extracts, CHX and D.W., Table (1).

TABLE 1: The statistical analysis of *P. gingivalis* inhibition zones by different concentrations of aqueous and alcoholic ginger extracts, CHX and D.W.

Agents	Conc.	No.	*Mean	±SD	ANOVA test	
CHX	0.2%	8	15.48	1.06	F= 221.741 P= 0.000 HS *d.f.= 87	
	20%	8	8.59	0.82		
	30%	8	8.19	0.86		
	40%	8	10.83	0.73		
	Aqueous	50%	8	11.20		0.51
	ginger	60%	8	12.32		0.82
	extract	70%	8	12.94		0.70
		80%	8	13.47		1.06
		90%	8	14.71		1.07
		100%	8	15.67		1.10
D.W.		8	0	0		
CHX	0.2%	8	15.71	0.89	F= 366.518 P= 0.000 HS d.f.=87	
	20%	8	9.12	1.26		
	30%	8	10.01	0.68		
	40%	8	12.01	0.70		
	Alcoholic	50%	8	12.22		1.02
	ginger	60%	8	13.92		0.76
	extract	70%	8	14.98		0.70
		80%	8	16.14		0.54
		90%	8	18.08		0.66
		100%	8	18.84		0.67
D.W.		8	0	0		

*Mean: Mean in mm, *d.f.: degree of freedom

Comparisons between each pair of different concentrations of each ginger extracts, the aqueous extract revealed almost highly significant differences with all concentrations, except the non-significant differences between 20% with 30%, 40% with 50% as well as 70% with 60% and 80% .

Alcoholic extract illustrated almost highly significant differences with all concentrations, except, 40% showed non significant difference with 50%, the same result between 90% with 100%, Table (2).

TABLE 2: Comparisons of mean values of *P. gingivalis* 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%	0.41	0.341	NS	-0.89	0.024	S	
	40%	-2.23	0.000	HS	-2.89	0.000	HS	
	50%	-2.60	0.000	HS	-3.11	0.000	HS	
	60%	-3.72	0.000	HS	-4.80	0.000	HS	
	70%	-4.35	0.000	HS	-5.86	0.000	HS	
	80%	-4.88	0.000	HS	-7.02	0.000	HS	
	90%	-6.12	0.000	HS	-8.96	0.000	HS	
	100%	-7.08	0.000	HS	-9.72	0.000	HS	
	30%	40%	-2.64	0.000	HS	-2.00	0.000	HS
		50%	-3.01	0.000	HS	-2.21	0.000	HS
60%		-4.13	0.000	HS	-3.91	0.000	HS	
70%		-4.76	0.000	HS	-4.97	0.000	HS	
80%		-5.29	0.000	HS	-6.13	0.000	HS	
90%		-6.53	0.000	HS	-8.07	0.000	HS	
100%		-7.49	0.000	HS	-8.83	0.000	HS	
40%		50%	-0.37	0.390	NS	-0.21	0.583	NS
		60%	-1.49	0.001	HS	-1.91	0.000	HS
		70%	-2.12	0.000	HS	-2.97	0.000	HS
	80%	-2.65	0.000	HS	-4.13	0.000	HS	
	90%	-3.88	0.000	HS	-6.07	0.000	HS	
	100%	-4.84	0.000	HS	-6.83	0.000	HS	
	50%	60%	-1.12	0.010	HS	-1.70	0.000	HS
		70%	-1.75	0.000	HS	-2.76	0.000	HS
		80%	-2.28	0.000	HS	-3.92	0.000	HS
		90%	-3.52	0.000	HS	-5.86	0.000	HS
100%		-4.48	0.000	HS	-6.61	0.000	HS	
60%		70%	-0.62	0.148	NS	-1.06	0.008	HS

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	80%	-1.15	0.008	HS	-2.22	0.000	HS
	90%	-2.39	0.000	HS	-4.16	0.000	HS
	100%	-3.35	0.000	HS	-4.92	0.000	HS
	80%	-0.53	0.218	NS	-1.16	0.004	HS
70%	90%	-1.77	0.000	HS	-3.10	0.000	HS
	100%	-2.73	0.000	HS	-3.85	0.000	HS
	90%	-1.24	0.005	HS	-1.94	0.000	HS
80%	100%	-2.20	0.000	HS	-2.70	0.000	HS
90%	100%	-0.96	0.027	S	-0.75	0.056	NS

TABLE 3: Comparisons of mean values of *P. gingivalis* 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%	6.89	0.000	HS	8.59	0.000	HS
	30%	7.29	0.000	HS	8.19	0.000	HS
	40%	4.65	0.000	HS	10.83	0.000	HS
	50%	4.28	0.000	HS	11.20	0.000	HS
	60%	3.16	0.000	HS	12.32	0.000	HS
	70%	2.54	0.000	HS	12.94	0.000	HS
	80%	2.01	0.000	HS	13.47	0.000	HS
	90%	0.77	0.075	NS	14.71	0.000	HS
	100%	-0.19	0.655	NS	15.67	0.000	HS
	Alcoholic ginger extract	20%	6.59	0.000	HS	9.12	0.000
30%		5.70	0.000	HS	10.01	0.000	HS
40%		3.70	0.000	HS	12.01	0.000	HS
50%		3.49	0.000	HS	12.22	0.000	HS
60%		1.79	0.000	HS	13.92	0.000	HS
70%		0.73	0.064	NS	14.98	0.000	HS
80%		-0.43	0.273	NS	16.14	0.000	HS
90%		-2.37	0.000	HS	18.08	0.000	HS
100%		-3.12	0.000	HS	18.84	0.000	HS

TABLE 4: Statistical analysis and comparisons between mean values of *P. gingivalis* inhibition zones for the same concentrations of aqueous and alcoholic ginger extracts

Conc.	Descriptive statistics				Mean difference		
	Aqueous		Alcoholic		d.f.=14		
	Mean	±S.D.	Mean	±S.D.	t-test	p-value	Desc.
20%	8.59	0.82	9.12	1.26	-0.98	0.342	NS
30%	8.19	0.86	10.01	0.68	-4.69	0.000	HS
40%	10.83	0.73	12.01	0.70	-3.30	0.005	HS
50%	11.20	0.51	12.22	1.02	-2.55	0.023	S
60%	12.32	0.82	13.92	0.76	-4.07	0.001	HS
70%	12.94	0.70	14.98	0.70	-5.82	0.000	HS
80%	13.47	1.06	16.14	0.54	-6.31	0.000	HS
90%	14.71	1.07	18.08	0.66	-7.58	0.000	HS
100%	15.67	1.10	18.84	0.67	-6.97	0.000	HS

From Table(3), both aqueous and alcoholic ginger extracts showed highly significant differences between CHX and D.W. with all extracts concentrations, except, between CHX with 90% and 100% concentrations of aqueous extract, and between CHX with 70% and 80% alcoholic extract concentrations they were non significant differences .Table (4), demonstrated that the mean values of inhibition zones of aqueous ginger extract were less than that of alcoholic extract with almost highly significant differences between both types of ginger extracts at all concentrations but, there was non significant difference with 20% .

It was clear from Table (5) that the mean values of inhibition zones of both ginger extracts and CHX of *P. gingivalis* were higher than for *A.a*, hence, highly significant differences

were almost found between both types of bacteria *P. gingivalis* and *A. a* (results from part 1 study) with all ginger extracts concentrations, except the non significant differences with 30% aqueous ginger extract concentration, as well as, for 20%, 30% and 50% alcoholic extract concentrations, Table (6).

The MIC of alcoholic ginger extract that inhibits *P. gingivalis* growth was 30% (0.3 g/ml) , while, MIC of aqueous ginger extract was 50% (0.5 g/ml) concentration also, Chlorohexidine gluconate 0.2% showed bacteriostatic effect against *P. gingivalis*

The MBC of alcoholic ginger extract that kills *P. gingivalis* was 60% (0.6 g/ml), while, the MBC for aqueous ginger extract was 80% (0.8 g/ml).

TABLE 5: Statistical analysis and comparisons between mean values of *A.a* and *P. gingivalis* inhibition zones for all concentrations of aqueous ginger extract, CHX and D.W.

Aqueous ginger extract conc.	Descriptive statistics				Mean difference		
	<i>A.a</i>		<i>P. gingivalis</i>		d.f.=14		
	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	8.19	0.86	-1.59	0.135	NS
40%	9.12	0.76	10.83	0.73	-4.58	0.000	HS
50%	9.86	0.94	11.20	0.51	-3.53	0.003	HS
60%	10.40	1.02	12.32	0.82	-4.15	0.001	HS
70%	10.53	1.08	12.94	0.70	-5.32	0.000	HS
80%	11.03	1.55	13.47	1.06	-3.69	0.002	HS
90%	11.76	1.13	14.71	1.07	-5.36	0.000	HS
100%	12.77	1.14	15.67	1.10	-5.19	0.000	HS
CHX 0.2%	14.19	0.76	15.48	1.06	-2.79	0.014	S
D.W.	0	0	0	0	-	-	-

TABLE 6: Statistical analysis and comparisons between mean values of *A.a* and *P. gingivalis* inhibition zones for all concentrations of alcoholic ginger extract, CHX and D.W.

Alcoholic ginger extract conc.	Descriptive statistics				Mean difference		
	<i>A.a</i>		<i>P. gingivalis</i>		d.f.=14		
	Mean	±S.D.	Mean	±S.D.	t-test	p-value	Desc.
20%	8.59	0.82	9.12	1.26	-0.98	0.342	NS
30%	9.63	1.08	10.01	0.68	-0.85	0.408	NS
40%	10.06	0.72	12.01	0.70	-5.48	0.000	HS
50%	11.81	0.48	12.22	1.02	-1.05	0.312	NS
60%	13.05	0.72	13.92	0.76	-2.37	0.033	S
70%	13.11	0.74	14.98	0.70	-5.21	0.000	HS
80%	14.48	0.60	16.14	0.54	-5.77	0.000	HS
90%	15.12	0.70	18.08	0.66	-8.70	0.000	HS
100%	15.38	0.77	18.84	0.67	-9.58	0.000	HS
CHX 0.2%	14.39	0.62	15.71	0.89	-3.45	0.004	HS
D.W.	0	0	0	0	-	-	-

DISCUSSION

Ginger has strong antibacterial activity and to some extent antifungal properties (Nielsen and Rios, 2000). Fresh ginger was reported to contain protein, fat, minerals, fibers, carbohydrates, lipids, vitamins and protease (Indian Council of Medical Research (ICMR), 2003; Ibrahim *et al.*, 2010). The existence of oxygenated mono- and sesquiterpenes, phenolic compounds (shogaol, gingerol) is responsible for the antimicrobial activity of ginger (Singh *et al.*, 2008; Michielin *et al.*, 2009), that are lipid-soluble phenol compounds extracted from the ginger root (Wang *et al.*, 2009; Liu, 2011).

Most of the phenols are protein denaturing agents; they can change the cell permeability, which cause swelling and rupture of the bacterial cells, most of them are metal chelators that attach to the active site of metabolic enzymes, reducing enzyme activities and lead to decelerating bacterial metabolism and reproduction (Fisher, 1992). Generally, results showed that alcoholic and aqueous ginger extracts were able to inhibit the growth of *P. gingivalis*, and the antimicrobial activity of both extracts on *P. gingivalis* increase when the concentration of the ginger extracts increased, so the diameters of inhibition zones were found to be increased this may be attributed to the amount of the dissolved active constituents of the extract will be more abundant as the concentration increased (Mahasneh *et al.*, 1996).

The *A. a* was less sensitive than *P. gingivalis* to both ginger extracts and CHX since by using t-test there were almost highly significant differences between *P. gingivalis* and *A.a*

for both extracts, this could be due to the hereditary contents of isolates which may alter the susceptibility of the organisms by modifying the target to be attacked by the active constituents of the extract like lipids of the microbial membrane, inhibiting the constituents, or modifying the structures of these constituents by some enzymes rendering them less effective compounds (Qanbar and Al-Mizraqchi, 2009).

Alcoholic extract concentrations had more antibacterial activity by displaying higher mean values of inhibition zones than aqueous extract concentrations and by using t-test, there were almost highly significant differences between the same concentration of alcoholic and aqueous extracts, this could be due to the amount of active component in the alcoholic ginger extract (10-gingerol) and polarity of the solvent (ethanol alcohol) which has great ability to dissolve the biologically active component of ginger (Mahasneh *et al.*, 1996).

There were no previous study to compare the results that obtained from this study with but, Miri-Park *et al.*, 2008 studied the effect of ginger active constituents (10- gingerol and 12- gingerol) on some kinds of periodontal anaerobic pathogens *in vitro* (*P. gingivalis*, *Porphyromonas endodontalis* and *Prevotella intermedia*) that successfully inhibited the growth pattern of these periodontal pathogens.

The MIC needed to inhibit *P. gingivalis* growth in broth media was 30% (0.3 g/ml) concentration of alcoholic ginger extract, which represented the bacteriostatic effect of the extract against *P. gingivalis*, while, MIC of aqueous ginger extract was 50% (0.5 g/ml).

Hence, gingerol and shogaol not only attack cell walls and cell membranes *i.e.*, disturbing their permeability and releasing of their intracellular constituents (*e.g.* ribose sodium glutamate), these compounds have different routes of action, since they interfere with (membrane functions, electron transport, nutrient uptake, protein and nucleic acid synthesis and enzyme activity). Consequently, these ingredients might possess numerous invasive targets which could cause the inhibition of bacterial pathogens (Bajpai *et al.*, 2009). The positive control agent used in this experiment, Chlorohexidine gluconate 0.2% revealed bacteriostatic effect against *P. gingivalis*.

The MBC of alcoholic ginger extract which kills *P. gingivalis* was 60% (0.6g/ml) concentration that mean the agent displayed bactericidal effect against *P. gingivalis* while, the MBC of aqueous ginger extract was 80% (0.8 g/ml). Hence, 0.2% CHX did not revealed bactericidal effect because, CHX in low concentration (0.2%) did not demonstrate bacteriostatic effect against periodontal pathogens (Frentzen *et al.*, 2002).

There were no other studies to compare the results with but, a study by Park *et al.*, 2008 revealed that 10-gingerol and 12-gingerol (ginger active ingredients) showed bacteriostatic activity at MIC range of 6-74 µg / ml and MBC range of 4-14µg/ml against (*P. gingivalis*, *Porphyromonas endodontalis* and *Prevotella intermedia*)

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