Evaluation of Antibacterial Effects of Ginger Extract When Used as One Component of the Root Canal Sealers; (An *in vitro* Study)

***Wafaa Mohammed Ali, **Nahla Othman Mohammed Tawfiq, ***Rajaa Taher Sulieman**

***Faculty of Pharmacy, University of Mosul, Mosul, Iraq**
****,***Faculty of Dentistry University of Mosul, Mosul, Iraq**

Received 9/10/2011 Accepted 20/5/2012

Abstract

The present study aimed to evaluate the antimicrobial properties of aqueous ginger extract as endodontic sealer, against *Staphylococcus aureus, Enterococcus faecalis, Streptococcus sanguis, Candida albicans,* anaerobic bacteria and normal flora of oral cavity, and to evaluate the most effective dilution of ginger extract against *Enterococcus faecalis* in dentinal tubules in order to use it as one component of root canal sealer. Disc diffusion test and direct contact method were used to evaluate the antimicrobial properties of the aqueous ginger extract. The antimicrobial activity was tested 1, 3 and 30 days after dentinal tubules manipulation using different dentin sealer. The Results showed that the highest antimicrobial activity was exhibited by the 20% (w/v) aqueous ginger extract. Moreover, this ginger extract (20%) showed a remarkable antibacterial activity against *Enterococcus faecalis* in infected dentinal tubules when examined *in vitro*; the study indicates that ginger extract might have promising effect to be use as one component of root canal sealer.

Key Words: ginger, antimicrobial activity, root canal sealers, Enterococcus faecalis, endodontics.

Introduction

Root canal therapy was mainly used to prevent and treat periradicular inflammation by the elimination of microorganisms from the root canal system, which includes instrumentation, antimicrobial irrigation, intracanal dressing, and adequate filling with coronal restoration. [1, 2, 3] The success of endodontic treatments depend on elimination of bacteria and their substrate from the root canal system so the antimicrobial activity plays an important role in the efficacy of an endodontic sealer used during root canal filling, and for this reason many studies have dealt with the antibacterial activity of endodontic sealers [3-7]. Facultative microorganisms such as *Enterococcus faecalis* and *Staphylococcus aureus* and even *Candida albicans* have been considered to be the most resistant species in the oral cavity and possible cause of root canal treatment failure [8,9], especially *Enterococcus faecalis* which had been a common isolated from infected root canals, its well recognized as a pathogen associated with persistent apical – periodontitis in endodontically treated teeth and highly prevalent in failed root filled teeth[9] .The safety and lower side effects of many herbal extracts have suggested them as sources of new pharmaceutical preparations. [10-13] History of ginger and its applications were well documented, ginger (Zingiber officinale, F. Zingiberaceae) has been listed as "Generally Recognized as Safe"(GRAS) document in FDA. A dose of 0.5 – 1.0 gram of ginger powder ingested 2 – 3 time for periods, ranging from 3 months to 2,5 years did not cause any adverse effect[7]. Many studies found that rhizom of ginger has strong antibacterial effects and to some extent antifungal properties. [14, 15, 16] The ginger extract has antimicrobial action at levels equivalent to 2000 mg/ ml of the spices. [17] The active constituents of ginger inhibit multiplication of bacterial colonies like *Escherchia coli*, *Proteus* spp., *Staphylococci*, *Streptococci* and *Salamonella*. [19 - 20] Ginger extract inhibits *Aspergillus*, a fungus known for production of Aflatoxin (carcinogen).[6,21]Also fresh ginger juice showed inhibitory action against *A. Niger, S. Cerevisia, Mycoderma spp*. and *L. Acidophilus* at 4, 10, 12 and 14 % respectively at ambient temperature[7]. *Enterococcus faecalis* is an opportunistic, facultative anaerobic. It is well recognized as pathogen associated with persistent apical periodontitis in endodonticaly treatment teeth and is highly prevalent in failed root filled teeth. [1, 5, 9] Many studies have been directed towards finding an effective way to eradicate and/or prevent *E. faecalis from* gaining access to the root canal space. *E. faecalis* can gain entry into the root canal system during treatment, between appointments, or even after the treatment has been completed [7]. Therefore, it is important to consider treatment regimens aimed at eliminating or preventing the infection of *E. faecalis* during each of these phases. The need for root canal sealer with antibacterial action is required to maximize the disinfection, especially in those cases where an infection is resistant to regular treatment and therapy can't be successfully completed due to the presence of pain or continuing exudates [2, 5] . Thus, the aim of this study was to analyze *in vitro* antimicrobial activity of aqueous ginger extract on different microorganisms at different time intervals after manipulation in infected dentinal tubules.

Materials & Methods

This study was conducted in College of Dentistry, University of Mosul in two parts. The first part an in *vitro* evaluation of the antimicrobial properties of different concentrations of aqueous ginger extracts. The most effective concentration was choosing to be implemented in the second

part, which includes the evaluation the antimicrobial activity of this concentrations against *E. faecalis* in dentinal tubules in an *ex vivo* samples.

Preparation of aqueous ginger extracts

Two types of ginger powder were used; First one was purchased from the local market and the second was a commercially available powder (Ginger/ UAE). The aqueous extract was prepared using cold extraction techniques. Forty grams 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 after filtration it was dried using incubator at 40°C. The liquid has evaporated, and the precipitated extract was left at the base of the baker. [(22)] Five ml of distill water (D.W.) was added to 1 gram of this extract powder to produce 20% (w/v) as standard stock solution. Serial dilutions were prepared from this stock solution (10%, 5%, 2.5%, 1.25%, 0.625%, and 0.313% (w/v)). [(23)]

Antimicrobial susceptibility test

Disc diffusion method were used filter to study the antimicrobial activity of different concentrations of aqueous ginger extract on *Staphylococcus aureus*, *Streptococcus sanguis*, *Escherchia coli*, anaerobic bacteria, normal flora of mouth and *Candida albicans*. After the incubation period, the diameters of the zones of inhibition were measured and all the data were expressed in mm.[(24)]

Preparation of pastes

Depending on the results of the antimicrobial inhibition zone, the 20% ginger solution gives the widest inhibition zone and this concentration was selected to be used in this part of the study. The pastes were prepared daily by mixing 10 ml of aqueous ginger extracts (20%) with 0.2 gm of orabase. Orabase gel and Zinc Oxide Eugenol (ZOE) to be used as a control group.[(25,26)]

Assessment of antibacterial efficacy of ginger paste in dental tubules:

One hundred twenty freshly extracted teeth with single canal extracted (for different reasons) obtained from Department of Surgery, College of Dentistry, University of Mosul and private clinics to be used in this part of the study. The crowns were removed at the cement-enamel junction. The root canal of the teeth were instrumented. Same procedures were done to the apical one third of the canal section. In the coronal and apical section of the root specimens, small cavities were prepared (2.5 mm diameter and 1.5 mm in the depth) in order to leave some space for composite filling and temporary filling. Apical cavities closed by means of composite resin to prevent bacterial leakage, the root specimens then were sterilized by autoclave for 30 min at 121°C. Each tooth was transferred to brain heart infusion broth (BHI) and incubated for 24 hrs at 37°C as a test for sterility. These teeth were transferred to 2 ml sterile physiological saline (SPS) (Physio – Denta/ Syria) in individual tubes for wash out BHI and to avoid dehydration and contamination. They were then incubated for another 24 hrs at 37°C, following incubation in SPS. Each tooth was removed from SPS and root canals were carefully dried with sterile paper points under a septic condition. The root specimens glued upright in Petri dishes using a quick setting steel epoxy resin (Eaglestar/ USA), then inoculated with a standard volume of 10 µl (10 cfu) of *Enterococcus faecalis* suspension and incubated at 37°C for 24 hrs. All specimens were divided randomly into 3 groups (n =40), for evaluation of antibacterial efficacy of each material in a time dependent method

(1, 3 and 30 days)[2,3,25,26]. Then each group where subdivided in to four subgroups with ten teeth in each subgroup. The first subgroup teeth were filled completely with ginger paste (20%), while the second and the third subgroups were treated with ZOE and orabase respectively and the last subgroup were left untreated. In the first group, the materials were left in the canal for one day by sealing the orifice with temporary filling material, the treated and untreated teeth were stored in SPS for 24 hrs at 37°C after treatment, the cotton pledgets and paste of ginger and orabase were removed from the canals. Ssubsequently, root canals were dried with sterile absorbents paper points. The dentin chips were obtained using a special penetration drill (Co 213/208 Dentsply Suiss/France) they were collected on to separated sterile Petri dishes. The dentin chips of the teeth were diluted in 5 ml of BHI, 10 ml of this pipette out and poured onto blood agar plates. They were incubated for 24 hrs at 37°C and colony forming unit were enumerated. [2, 3, 25, 26] For the second and third groups (II, III): the teeth were treated in the same manner as previously described. All teeth were stored in SPS for three and thirty days respectively and the second bacteriologic samples were taken in the same manner as mention before [22, 23] .

The mean and standard deviation for bacterial counts of each group at different time intervals were calculated. The analysis of variance at level of significance (0.05 – 0.01) was performed through utilizing one way analysis of variance.

Results

The results in (Table 1) showed that the ginger extracts were effective against all types of microorganisms used in this study, and the (20%) concentration had the best antimicrobial effect among the different dilutions of aqueous ginger extract of with the zone of inhibition range from 25- 35 mm and 20- 30 mm for UAE and Iraqi powder respectively (figure 1and 2).

Table (1):- Antimicrobial activity of aqueous ginger extract using disc diffusion test.

Types of Solution	Concentration %	Inhibition zone (mm)					
		Staph.	Strep.	*E. coli*	N. flora	Anaerobic bact.	Candida
Iraqi Ginger extract	20	20	26	30	30	20	22
	10	18	24	25	18	18	20
	5	15	22	20	------	16	18
	2.5	14	22	------	-------	------	15
	1.25	------	------	------	------	-------	------
	0.625	-------	-------	-------	-------	------	-------
	0.3125	------	------	------	------	------	------
UAE Ginger extract	20	35	26	25	25	35	35
	10	30	20	22	22	30	30
	5	28	18	20	20	30	20
	2.5	25	------	------	20	25	18
	1.25	------	-------	-------	14	------	17
	0.625	-------	------	------	------	-------	12
	0.3125 %	------	------	------	-------	------	------

* Staph*: Staphylococcus aureus,* Strep: *Streptococcus sanguis, E. coli: Escherichia coli,* N. flora: normal flora of mouth, Anaerobic bact: anaerobic bacteria and *Candida albicans*.

*Data are mean of two replication.

*---No inhibition was observed.

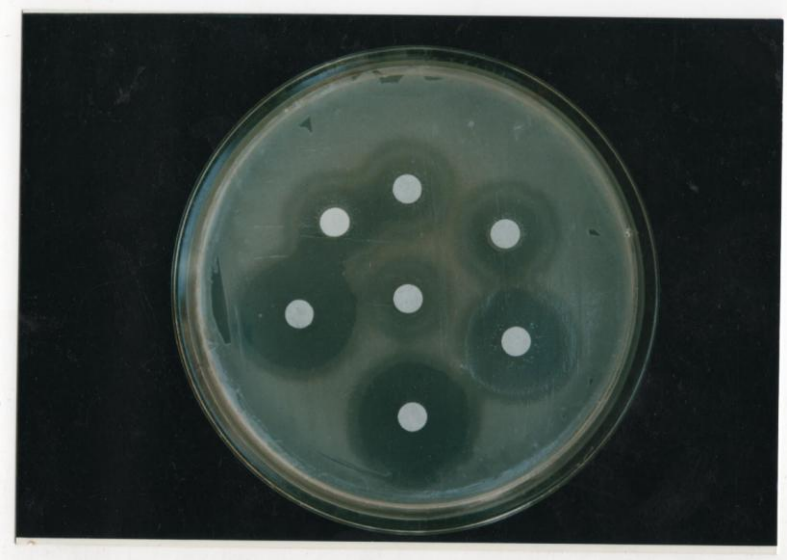

Figure (1):- Zones of bacterial inhibition formed around the socked disc with aqueous ginger extract (UAE) on normal flora of the mouth.

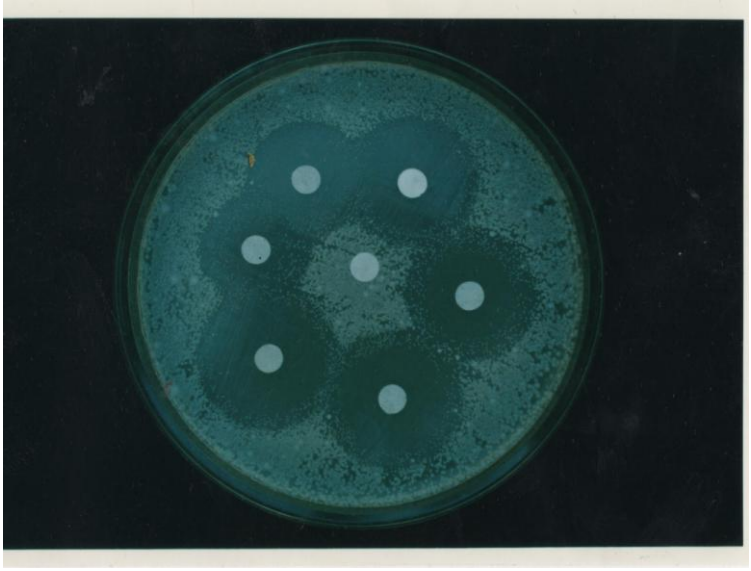

Figure (2):- Zones of bacterial inhibition formed around the socked disc with aqueous ginger extract (UAE) on *Candida albicans* of the mouth.

The antibacterial effect of the ZOE and ginger sealer against *E. faecalis* was significantly higher than orabase or untreated groups at different time intervals, ginger extract and ZOE sealer showed the highest inhibition at 30 days which was statistically significant ($P< 0.05$) but there were no significant differences in between them at these time intervals as shown in Tables (2 and 3) and Figure (3). Ginger paste give negative growth in 8 specimens and positive growth in 2 specimens only while ZOE (sealer) show negative growth in 7 specimens and positive growth in 3

specimens. Also the result reveal that the bacterial counts at each time interval for untreated specimens were significantly not different from the specimens that treated with orabase, which indicates that orabase use in this study have no antibacterial effect at these time intervals.

Table (2):- Comparison for the antibacterial effect of 20% ginger and ZOE sealer against *Enterococcus faecalis* at different time intervals:

Groups	Time interval		
	1 day	3 days	30 days
Untreated	109.2 ± 14.6 (A)	158.0 ± 25.7 (B)	198.0 ± 8.1 (C)
Orabase	110.0 ± 13.3 (A)	146.8± 28.5 (B)	190.5 ± 13.9 (C)
ZOE sealer	64.6 ± 34.5 (B)	6.70 ± 2.75 (A)	3.0 ± 5.1 (A)
20% Ginger	66.6 ± 37.8 (B)	5.90 ± 2.7 (A)	2.0 ± 0.4 (A)

*Data represents mean ± SD of bacterial count

*The different letters horizontally mean significant difference exist P<0.05.

Table (3):- Comparison between the antibacterial effect of different sealer against *Enterococcus faecalis* during study periods.

Time interval	untreated	Orabase	ZOE sealer	20% Ginger
1day	109.2 ± 14.6 (C)	110.0 ± 13.3 (C)	64.6 ± 34.5 (A)	66.6 ± 37.8 (A)
3days	158.0 ± 25.7 (B)	146.8± 28.5 (B)	6.70 ± 2.75 (A)	5.90 ± 2.7 (A)
30days	198.0 ± 8.1 (C)	190.5 ± 13.9 (C)	3.0 ± 5.1 (A)	2.0 ± 0.4 (A)

*Data represents mean ± SD of bacterial count.

*The different letters horizontally mean significant difference exist P<0.05.

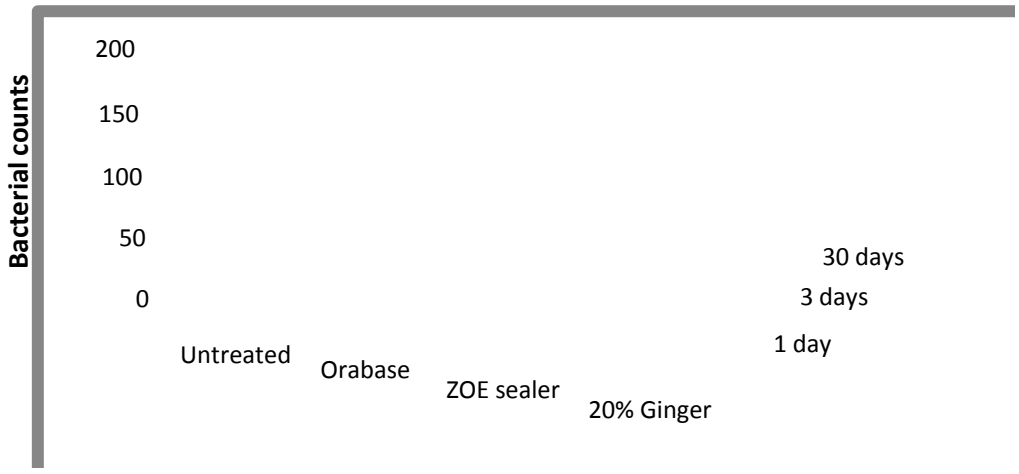

Figure (3):- Effect of Orabase, ZOE sealer and 20% Ginger on the bacterial counts after 1 day, 3 days and 30 days.

Discussion

The main goal of successful root canal therapy is to eliminate bacteria and microorganisms from root canal system and to prevent subsequent reinfection.[1,2,9] Three – dimensional sealing of the root canal system is another goals of endodontic treatment and is essential for preventing of canal re-infection and the maintenance of healthy periapical tissue[7, 8]. The agar diffusion method has been widely used to test the antimicrobial activity of dental materials and medications. [3,5] The advantage of this method is that it allows direct comparisons of root canal sealers against the test microorganisms, indicating which compound has the potential to eliminate bacteria in the local microenvironment of the root canal system. According to our results, 20% ginger extract produced the largest inhibitory zones of the microbial growth against all microorganisms studied in all times after manipulation. All sealers tested demonstrated a higher antimicrobial value in the first 24 h after manipulation, while the antimicrobials effect were prolonged up to 30 days, especially knowing that microorganisms can remain in the ramifications of the root canal system after chemo mechanical preparation and intracanal dressing.[1, 7, 9] This is in agreement with many studies which found that ZOE based sealer, has been shown to exhibit the greatest antimicrobial activity against *E. faecalis* when compared to other sealers.[1,2,5] *E. faecalis* possesses certain virulence factors including lytic enzymes, cytolysin, aggregation substance, pheromones, and lipoteichoic acid [7]. It has been shown to adhere to host cells, express proteins that allow it to compete with other bacterial cells, and alter host responses [7, 9]. Based on the statically differences among the root canal filling materials evaluated in this study, 20% ginger extract produced the largest inhibitory zones of the microbial growth against all microorganisms studied in all times after manipulation which was equal to the ZOE antimicrobial activity, and this is in agreement with many studies which found that ginger has strong antibacterial and to some extent antifungal properties. [14, 15, 22, 23] Roder (2004) results indicate that ginger had antibacterial effect on *E. faecalis* but not on *E. coli*. In vitro studies have shown that active constituents of ginger inhibit multiplication of bacteria. [11, 12] Ginger rhizome contain, the pungent substances namely gingerol, shogaol, zingerone, paradol and volatile oil The volatile oil consists of mainly mono and sesquiterpenes; camphene, beta-phellandrene, curcumene, cineole, geranyl acetate, terphineol, terpenes, borneol, geraniol, limonene, linalool, alpha-zingiberene (30-70%), beta-sesquiphellandrene (15-20%), beta-bisabolene (10- 15%) and alpha-farmesene,in addition to the oleoresin zingiberol, the principal aroma contributing component as well as zingiberene, gingediol, diarylheptanoids and phytosterols.[17, 18] The most effective antimicrobial constituent was found to be citral. In another advance, it was shown that ethanol extracts of ginger were able to inhibit growth of both gram-negative and gram-positive bacteria [12, 27], although the inhibitory effect was more pronounced for gram-positive bacteria. [28] Bactericidal activity against the highly resistant gram-negative bacteria *Pseudomonas aeruginosa* was notable also [29] . lthough research is still needed, our preliminary study showed that ginger preparation has good antimicrobial against *E. faecalis*. A well-

sealed coronal restoration and root canal filling are important steps in preventing bacteria from entering the canal space and provides steps that can be used to eliminate *E. faecalis* during endodontic retreatment. From the results of the present study we can conclude that 20% aqueous ginger extract can be used as one component of endodontic sealer to inhibit bacterial growth as effective antibacterial agent.

References

1. Yamash FMT. In vivo micobiological evaluation of the effect of biomechanical preparation of root canal using different irrigating solutions. End J. 2006; 14(1): 105 – 110.
2. Ajitha P and Roacrn T. Time dependent inhibitory effect of dentin on various calcium hydroxide medicaments (canine vitro study). Endo J. 2003; 15(7): 11 – 17.
3. Al-Khatib ZZ, Baum RH, Morse DR, Yesilsoy C, Bhambhani S, Furst ML. The antimicrobial effect of various endodontic sealers. Oral Surg 1990; 70:784-790.
4. Pumarola J, Berastegui E, Brau E, Canalda C, Jimenez de Anta MT. Antimicrobial activity of seven root canal sealers. Oral Surg 1992; 74:216-220.
5. Siqueira Jr JF, Gonçalves RB. Antibacterial activities of root canal sealers against selected anaerobic bacteria. J Endod 1996; 22:79-80.
6. Kaplan AE, Picca M, Gonzalez MI, Macchi RL, Molgatini SL. Antimicrobial effect of six endodontic sealers: an in vitro evaluation. Endod Dental Traumatol 1999;15:42-45.
7. Leonardo MR, da Silva LA, Tanomaru Filho M, Bonifácio KC, Ito IY. *In vitro* evaluation of antimicrobial activity of sealers and pastes used in endodontics. J Endod 2000;26:391-394.
8. Gomes BPFA, Ferraz CCR, Vianna ME, Rosalen PL, Zaia AA, Teixeira FB, Souza-Filho FJ. In vitro antimicrobial activity of calcium hydroxide pastes and their vehicles against selected microorganisms. Braz Dent J 2002;13:155-161.
9. Nageshwar RR, Kidyoorn HK, Hegde C. Efficacy of chlorhexidene paste against *Enterococcus faecalis*- An *in vitro* study. Endo J. 2004; 16(1): 61 – 64.
10. Tyler, V.E., Brady, L.R. and Robbers, J.E. Pharmacognosy. (8th Edition). Lea and Febiger, Philadelphia, p.156, 1981.
11. Meena MR. Studies on antimicrobial activity of various species and their oils. M.Sc. Thesis, Indian Agricultural Research Institite New Relhil. 1992, 31: 45 – 50.
12. Al – Nouman AY. Effect of some plants extracts on the growth and metabolism of gram positive and gram negative bacteria. Ph.D. Thesis, College of Science, Mosul University. 1998.
13. Al – Sandook TAA, Taqa AA, Hassan SA. Olive leafs and it is extract (oleuropein) in treatment of aphthus ulceration. Jordan J. 2000; 15: 24 – 26.
14. Gugnani, H.C. and Ezenwanze, E.C. Antibacterial activity of extracts of ginger (Zingiber officinale) and African oil bean seed (Pentaclethora macrophylla). J Commun Dis; 17: 233, 1985.
15. Akoachere JF, Ndip RN, Chenwi EB et al. Antibacterial effect of Zingiber officinale and Garcinia kola on respiratory tract pathogens. East Afr Med J. 2002 Nov; 79(11):588-92 2002.
16. Atai Z, Atapour M , Mohseni M. Inhibitory Effect of Ginger Extract on *Candida albicans*, American Journal of

Applied Sciences; 6 (6): 1067-1069, 2009

17. Govindarajan, V.S. Ginger: Chemistry, technology and quality evaluation (Part I). Crit Rev Food Sci Nutr; 17: 1, 1982.
18. James ME, Nanapane R. Identification and characterization of two bacteriocin producing bacteria isolated from ginger root. J Food Prod. 1999; 6(2): 899 – 911.
19. Gugnani, H.C. and Ezenwanze, E.C. Antibacterial activity of extracts of ginger (Zingiber officinale) and African oil bean seed (Pentaclethora macrophylla). J Commun Dis 17: 233, 1985.
20. Akoachere JF, Ndip RN, Chenwi EB et al. Antibacterial effect of Zingiber officinale and Garcinia kola on respiratory tract pathogens. East Afr Med J. 2002; 79(11):588-92 2002.
21. Zahra Atai, 2Manijeh Atapour and 3Maryam Mohseni. Inhibitory Effect of Ginger Extract on *Candida albicans*, American Journal of Applied Sciences 6 (6): 1067-1069, 2009
22. Al – Joboory A and Al – Rawi M. Natural pharmacology. 1st edition, Baghdad, dar Al – Huriah. 1994.
23. Al – Nouman AY. Effect of some plants extracts on the growth and metabolism of gram positive and gram negative bacteria. Ph.D. Thesis, College of Science, Mosul University. 1998.
24. Naudepitte J, Enghack K, Poit P, Henk C. Basic laboratory procedures in clinical bacteriology. 1991.
25. Brugger W, Hofer V, Stadter N. Antibacterial effect of endodontic dressing on Enterococcus faecalis in human root dentin. Acta Stomatol Croat. 2007; 41(3): 326 – 336.
26. Torabineijad M, Shabahaug S, Aprecio RM. The antibacterial effect of EDTA an in vitro investigation. J Endo. 2003; 29: 6 – 9.
27. Roder E. The synergistic and individual antimicrobial impacts of green tea and ginger on common gasterointestinal bacteria. Current Med Ch. 2004; 11(11): 34 – 38.
28. Onyeagba R, Ugbogu O, Oke K. Studies on the antimicrobial effect of garlic allium (stivumlinn), ginger (zingeiber officinol). African J Biotechnology. 2004; 3(10): 552 – 554.
29. Veena A, Ramtej J. Aqueous ginger extract ameliorates paraben induced cytotoxicity. Am J Clin Nut. 2006; 63(2): 117 – 119.