J. Appl. Environ. Biol. Sci., 8(10)12-18, 2018

ISSN: 2090-4274
Journal of Applied Environmental and Biological Sciences
www.textroad.com

Evaluations of Arak Extract Effects and Comparison with Different Toothpastes on Oral Pathogens

Mounir M. Salem- Bekhit[1], Fars K. Alanazy[2]

[1]King Saud University, Department of Pharmaceutics, College of Pharmacy, P. O. Box 2457, Riyadh, Saudi Arabia.
[3]King Saud University, Kayyali Chair for Pharmaceutical Industries, College of Pharmacy, P. O. Box 2457, Riyadh, Saudi Arabia.

Received: June 13, 2018
Accepted: September 9, 2018

ABSTRACT

Purpose: To evaluate the *in vitro* antimicrobial activity of the arak crude extracts (*Salvadora persica*) compared to different toothpastes containing active ingredients against some selected different oral hygiene microorganisms.
Methods: Different strains implicated in oral diseases were tested for their susceptibility to the aqueous extract of arak as well as examining toothpastes using the agar diffusion method. Crude extracts of arak and selected toothpastes were challenged using *E. faecalis* at time intervals of 1, 10, 20, 40 and 60 min.
Results: Results showed that the aqueous extract of arak exhibited antimicrobial activity which was similar to that of the commercially available tested toothpastes.
Conclusion: According to these findings, it was concluded that arak stick can be used for oral hygiene as a good alternative to the toothpaste.
KEY WORDS: Arak, *Salvadora persica*, cariogenic bacteria, antimicrobial activities.

INTRODUCTION

Periodontal pathogens are one of the common problems and leading causes for development of dental plaque and periodontal diseases. The obtainable methods for the oral health protection are mostly chemical and mechanical. Toothpastes and toothbrushes are commonly used for plaque control and teeth cleaning [1]. There is a variety of plants commonly used as conventional chewing sticks or toothbrushes. The "arak or miswak" is the largely used tree brush wood since early times, which was obtained from a stick of a plant called *Salvadora persica*. The root pieces are generally aromatic and become spongy after water soaking [2]. Though the World Health Organization (WHO) has encouraged the usage of chewing sticks and promoted advance study of their effectiveness [3], limited studies had assumed the prospective antimicrobial activities of chewing sticks [4].

Many reports recommended that the presence of pathogens in medicinal plants was strongly related to the previous handing, drying methods and type of storage used. Different chemical methods of decontamination have therefore been used. Irradiation offers an effective option to chemical fumigation. Several countries have approved irradiation of medicinal plants for microbial decontamination; amongst these countries USA, Brazil, Argentina, France, and South Africa [5].

Toothpastes or gel are used to clean and develop the aesthetic appearance and health of teeth. Fluoride is considered as one of the best and effective caries inhibiting agents. Approximately, wholly toothpastes contain fluoride in their formulation [6]. Usually the total fluoride amount is ranged between 0.1 – 0.15%. Antiseptic composites can be included in some toothpaste like triclosan which have distinct antibacterial effects. Also, essential oils, like menthol, eucalyptol, basil and thymol, have many valuable properties as natural antimicrobial actions [7]. Few previous studies have investigated the comparative antimicrobial activity of arak and toothpastes. The current study aimed to:1) investigate the *in vitro* antimicrobial effectiveness of arak extract and different formulas of toothpastes against oral hygiene microorganisms. 2) evaluate the gamma radiation effects and storage time on the microbial contamination of arak.

MATERIALS AND METHODS

Samples of Chewing sticks, arak powder and toothpastes:

This study was carried out on arak (siwak), the most commonly used chewing stick in Saudi Arabia. Its sticks and powder were purchased from the local market in Riyadh, Saudi Arabia. The samples were packed (25g each) in sealed polyethylene bags to prevent recontamination. As well as, three commercially available toothpastes identified as formulas 1, 2 and 3 were purchased from the local market of Riyadh. Each formula

Corresponding Author: Mounir M. Salem-Bekhit, PhD. Department of Pharmaceutics, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia. Tel: +966 501783166
e-mail: mounirmsalem@yahoo.com; mbekhet@ksu.edu.sa

contains different active ingredients that were described as sodium monofluoro-phosphate 0.76% and triclosan 0.10% (formula 1), fluoride 1450 ppm and calcium glycerophosphate (formula 2) and basil (Ocimum sanctum) and herbal extracts (formula 3) as described on the package.

Media:

The isolated organisms were grown in standard laboratory culture media prepared according to the specifications of the manufacturers. Media utilized included plate count agar, Czapeks-Dox yeast agar, Baird-Parker agar and Brain heart infusion agar (Difco Labs., Detroit, Michigan, USA), MacConkey agar and Tryptic soy broth (Basigstoke, Hants, UK).

Irradiation and storage:

The irradiated (1-10 kGY) and non-irradiated (control) arak sticks and powder were stored at ambient temperature for 6 months in sealed bags.

Microbiological quality of arak:

From both arak sticks and powder, twenty-five grams of each irradiated and non-irradiated samples were suspended in 225 ml of sterile saline and subjected to serial dilutions using normal saline solution (0.9% NaCl). Proper dilutions were used in enumeration of microbial counts. Total aerobic bacterial counts were enumerated on plate count agar [8] using pour plate technique then the plates were incubated at 30° C for 3 days. Total molds and yeasts were counted on Czapeks yeast extract agar medium according to the method described by [25] using pour plate technique then plates were incubated at 25° C for 5 days. The total numbers of thermophilic spore forming bacteria were determined according to the method described by [9] using plate count agar medium. The presence of coliforms was determined by cultivation of the tested samples on MacConkey agar plate according to [10]. *Staphylococcus aureus* were counted on laboratory prepared Baird-Parker medium according to [11] using surface plate technique. Suspected colonies were submitted to coagulase activity and biochemical reactions.

Antimicrobial spectrum of the arak extract and the toothpastes:

For determining the antimicrobial spectrum of the arak extract and the toothpastes, seven bacterial strains representing Gram negative and Gram positive bacteria and one yeast were used. They were grown on nutrient agar, Sabouraud agar and de Man, Rogosa, Sharpe (MRS) for *Lactobacillus acidophilus*. Out of them, six pathogenic strains were isolated from clinical samples; *E. coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* (as Gram negative); *Staphylococcus aureus*, *Enterococcus faecalis* (as Gram positive) and *Candida albicans* (as Yeast). Two other strains were used; *Bacillus cereus*, ATCC 11778 and *Lactobacillus acidophilus*, DSM 20079.

Preparation of arak extract:

For 10 days, the arak sticks were kept dried at room temperature before extraction process. The sticks were reduced to small pieces then crushed to fine powder. In sterile dry screw-capped bottles, ten grams of fine powder was kept separately and the bottles were kept in a cool dry place for seven days before extraction. In each bottle, 100ml of sterile water was added to the fine powder and left to soak at 4°C. After 48 hours, the extract was centrifuged for 10 minutes at 2000 rpm. Finally, the supernatant was sifted through a membrane filter (0.45 mm) and freeze-dried as described by [12].

Antimicrobial activity of arak using agar diffusion method:

According to [13], the antimicrobial activity of arak extract was determined using the hole-plate diffusion technique. The plates were moistened with a suspension of the selected pathogenic microorganism, which contained approximately 10^7CFU/ml using sterile cotton swab. Into the wells, 100µl from the arak extract was added and allowed to diffuse for 30 min. at 37°C, the plates were incubated for 24 hours and at 30°C for 48 h for bacteria and yeast growth respectively. The diameter of inhibition zones was measured and the experiment was repeated, in its entirety, twice more to ensure repeatability. On the other hand, 10 ml of each toothpaste formula were moved to a small flask containing 5 ml of sterile water and homogenized by vortex mixer. After minutes, 100µl of each formula were added into the wells made at the center of pre-inoculated blood agar plates and allowed to diffuse. Plates were incubated as previously mentioned then the inhibition zones diameter was measured as described by [14]. The bacterial strain which showed sensitivity to the arak extract and all the tested formulas were chosen for further studies.

Antimicrobial activity of arak using microbial death profile (challenge test):

The profile of the microbial death (log CFU/ml vs. time) was evaluated according to [14]. Microbial suspension of the chosen microorganism (approximately 10^7CFU/ml) was transferred under aseptic condition to

a tube with 10 ml of the tested arak extract or the other tested formulas. Using the pour plate method, the viable microorganism was counted. The series of decimal dilution were made employing 9ml of sterile saline. After 10, 20, 40 and 60 min, the identical procedures were used again and the viable counts were determined.

Statistical analysis

All experiments were done in triplicate as a minimum. Data from those experiments were saved in an EXCEL 5.0 program (Microsoft) and the statistical analyses were carried out using version 19.0 SPSS software (SPSS, Chicago, USA). Differences were considered significant at $p < 0.05$.

RESULTS

Microbiological quality of arak:

Arak sticks and powder were evaluated for their natural microbiological quality i.e. total aerobic bacterial count, total molds and yeasts and spore forming bacteria. They were also examined for the presence of coliforms and *Staphylococcus aureus* (table 1 and 2). The level of microbial contamination in Arak powder was higher than that in arak sticks. The total bacterial counts, total mold and yeast counts and the counts of spore forming bacteria in non- irradiated control arak sticks were $4.0x10^5$, $8.0x10^3$ and $1.7x10^5$cfu/g, respectively. The corresponding counts in non-irradiated (control) arak powder were $6.0x10^5$, $8.5x10^3$ and $5.2x10^5$cfu/g. The examined sticks and powder had coliforms and *Staphylococcus aureus* at values $5.6x10^3$, $6.3x10^2$cfu/g, respectively.

Table (1): Effect of different doses of gamma irradiation and storage time on the total bacterial and fungal count containing arak (sticks and powder).

Microbial isolates	Doses (kGy)	Number of Survivors							
		Sticks				Powder			
		Storage period (month)							
		0	2	4	6	0	2	4	6
Total bacterial count	0	$4x10^5$	$2.8x10^4$	$3.2x10^6$	$4x10^6$	$6x10^5$	$6.9x10^3$	$4.9x10^4$	$1.3x10^5$
	1	$2.5x10^3$	$2.5x10^3$	$1.5x10^6$	$2.5x10^6$	$4.1x10^4$	$4x10^3$	$4.6x10^3$	$1x10^4$
	2	$2x10^3$	$1x10^3$	$4x10^4$	$4.2x10^4$	$8x10^3$	$7.2x10^2$	$7.5x10^2$	$1x10^3$
	3	$7x10^2$	$1x10^2$	$3.7x10^2$	$7.7x10^2$	2.9x10	6.3x10	8x10	$2.9x10^2$
	4	$5.6x10^2$	<10	$5x10^1$	5.5x10	<10	<10	<10	<10
	5	<10	<10	<10	<10	<10	<10	<10	<10
	6	<10	<10	<10	<10	<10	<10	<10	<10
Total molds and yeasts	0	$8x10^3$	$2.5x10^3$	$4.5x10^3$	$4.7x10^3$	$8.5x10^3$	$5.1x10^3$	$6.4x10^4$	$1.2x10^5$
	1	$3.5x10^3$	$1.9x10^3$	$2.9x10^3$	$3.2x10^3$	$3.7x10^3$	$3.4x10^3$	$7.9x10^3$	$1x10^4$
	2	$1x10^3$	$1.7x10^2$	$3.4x10^2$	$1.6x10^3$	$2.1x10^2$	$1.8x10^2$	$2.7x10^2$	7.1x10
	3	$1x10^2$	$1.1x10^2$	8x10	<10	1.9x10	3.2x10	7.1x10	7.1x10
	4	<10	<10	<10	<10	<10	<10	<10	<10
	5	<10	<10	<10	<10	<10	<10	<10	<10
	6	<10	<10	<10	<10	<10	<10	<10	<10

Table (2): Effect of different doses of gamma irradiation and storage time on the isolated microorganism containing arak (sticks and powder).

Microbial isolates	Doses (kGy)	Number of Survivors							
		Sticks				Powder			
		Storage period (month)							
		0	2	4	6	0	2	4	6
Thermophilic spore former bacteria	0	$1.7x10^5$	$1.4x10^4$	$2.1x10^4$	$4.5x10^5$	$5.2x10^5$	$3.7x10^3$	$7.5x10^3$	$3.7x10^4$
	1	$1x10^5$	$5x10^2$	$7.1x10^2$	$3.9x10^3$	$5x10^3$	$9.8x10^2$	$9.7x10^2$	$7.6x10^3$
	2	$1.2x10^4$	$2x10^2$	2.8x10	$2.7x10^2$	$3.3x10^3$	2.3x10	2.3x10	3.2x10
	3	$7x10^3$	2.9x10	1.9x10	3.8x10	$2.2x10^3$	<10	<10	<10
	4	$5x10^3$	<10	<10	<10	<10	<10	<10	<10
	5	<10	<10	<10	<10	<10	<10	<10	<10
	6	<10	<10	<10	<10	<10	<10	<10	<10
Coliforms	0	$5.6x10^3$	$6x10^2$	$3.2x10^3$	$7.1x10^3$	$7.6x10^2$	$1.5x10^2$	$6x10^3$	$6.2x10^3$
	1	$4.2x10^3$	6x10	$1.4x10^3$	$3.6x10^3$	2.5x10	1.4x10	$4x10^2$	$3.9x10^2$
	2	$3x10^3$	<10	$3.7x10^2$	$4.1x10^2$	1.5x10	<10	$3.1x10^2$	$2.9x10^2$
	3	$7.1x10^2$	<10	1.4x10	1.5x10	1.2x10	<10	$1.9x10^2$	2x10
	4	<10	<10	<10	<10	<10	<10	1.5x10	<10
	5	<10	<10	<10	<10	<10	<10	<10	<10
	6	<10	<10	<10	<10	<10	<10	<10	<10
Staph. aureus	0	$4.7x10^3$	$1.1x10^3$	$4.1x10^3$	$4.5x10^3$	$6.3x10^2$	$1.7x10^2$	$7.2x10^2$	$7.4x10^2$
	1	$4.1x10^3$	$4.7x10^3$	$2.9x10^3$	$3x10^3$	2.5x10	2.5x10	$6.5x10^2$	$6.3x10^2$
	2	$2.9x10^3$	$2x10^2$	3.7x10	$2.7x10^2$	2x10	2x10	3.7x10	2.7x10
	3	7.2x10	<10	<10	$1.9x10^2$	2x10	2x10	1.9x10	1x10
	4	<10	<10	<10	<10	<10	<10	<10	<10
	5	<10	<10	<10	<10	<10	<10	<10	<10
	6	<10	<10	<10	<10	<10	<10	<10	<10

Antimicrobial activity of arak using agar diffusion method:

Antimicrobial activities of arak extract as well as three different toothpastes; formulas 1, 2 and 3, was determined by using agar diffusion method against 8 pathogenic microorganisms. Even though most of the tested organisms were not strictly oral pathogens, selection depended on the role that those organisms play in oral hygienic and probability for causing dental diseases, periapecal lesions, periodontal abscesses and possible gingivitis [12]. It is clear from the data in table (3) that arak extract and the other tested formulas exhibited different levels of antimicrobial activities against the tested organisms.

Table (3) Diameter of inhibition zone (mm) of arak extract and three different formulas of toothpastes on some oral pathogens.

Tested samples	Diameter of inhibition zone (mm)							
	E. faecalis	*K. pneumonia*	*Ps. Aeruginosa*	*E. coli*	*Staph. aureus*	*L. acidophilus*	*B. cereus*	*C. albicans*
Arak extract	15	--	30	--	--	--	--	--
Formula 1	20	--	22	22	30	35	20	--
Formula 2	25	--	22	40	40	--	24	--
Formula 3	24	--	--	30	20	--	23	--

(-) no activity

Antimicrobial activity of Arak using microbial death profile (challenge test):

The results indicated that among the 8 tested microorganisms, *E. faecalis* was generally the most liable microorganism to all the tested formulas, therefore, this strain was chosen for the challenge test. The obtained data in table (4) and the figure (1) showed that all tested formulas were active against *E. faecalis* when reduced to 3 logarithmic cycles after 1 min of contact, a condition that's similar to brushing teeth while arak extract showed reduction of 2 logarithmic cycles. The data also showed that the activities of all the tested formulas were increased significantly by increasing time of contact.

Table (4): Effect of time on the Antimicrobial activity of arak extract and three toothpastes formulas on the number of survivors of *E. faecalis* selected strain (challenge test).

Time (minutes)	Log number of survivors (cfu/ml)				
	Arak extract	Formula 1	Formula 2	Formula 3	Control
0	8.60	8.60	8.60	8.60	8.60
1	6.60	5.93	5.78	5.30	8.60
10	6.30	5.78	5.32	3.80	8.59
20	5.20	5.78	5.70	3.38	8.57
40	3.56	4.90	4.93	3.20	8.61
60	3.0	4.48	3.80	3.0	8.60

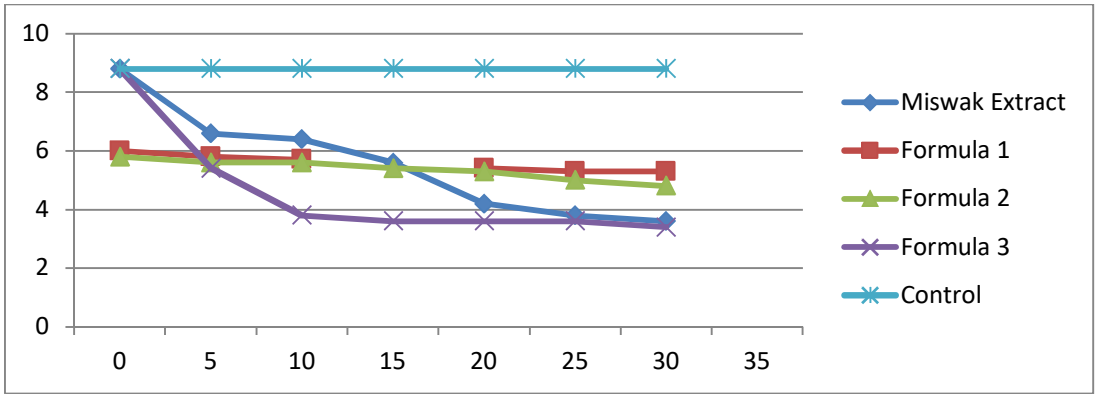

Figure (1): Lethality profile (log cfu/ml x minute) of *E. faecalis* for the arak extract and the other tested formulas.

DISCUSSION

The present study aimed to investigate the antimicrobial activity of arak extract in comparison with different formulas of toothpastes against oral hygiene microorganisms in addition to evaluation the effects gamma radiation on the microbial contamination of arak. Tables (1) and (2) indicated that both non-irradiated (control) arak sticks and powder had high microbial load. These results have been confirmed by [15] who found that the total bacterial and mold counts contaminating qarad were $8.2x10^6$ and $2.9x10^4$cfu/g, respectively. Generally, the results revealed that arak sticks and powder samples were of unsatisfactory from the view of

microbiological quality. It is clear from the same table that irradiation caused a marked decrease in all tested microbial counts and this decrease was proportional with irradiation dose. The irradiation dose of 5KGy was effective and sufficient to reduce all tested microflora contaminating both arak sticks and powder to non-detectable levels. [16] Studied the effects of ionizing energy and ozone treatments on the microbial decontamination of aloe powder. They found that gamma irradiation at 7.5-10 kGy reduced all bacterial count including coliforms and fungi to below detection levels. Treatment by ozone at up to 18 ppm for hours was not adequate to eliminate microorganis from the tested powder. The results showed that, the microbial weight of the control samples was enriched by storage time. It decreases in the number of survivors/g after 2 months of storage. On the other hand, increase in the total microbial load was noticed as storage progressed until 6 months but the rate of increase was much more pronounced in control samples (non-irradiated) in comparison with irradiated ones. All the microbial counts in arak sticks and powder samples exposed to 5kGy remained below detectable levels throughout the storage period (6 months). This indicated that irradiation treatment greatly reduced the initial counts and delayed the growth of microorganisms hence, extended the shelf-life of the tested samples. Long time storage boosted mold reduction in 5 kGy. However, [17] reported that by relying on the prevalent flora for complete fungal sterilization, the lethal dose required was reported to be not less than 5 kGy or not more than 7.5 kGy.

According to [18], the agar diffusion method has the ability to be used as a preliminary test for identifying antimicrobial activity in substances physical-chemical properties, as for instance, its diffusion coefficient as well as the medium where the diffusion occurs, is likely to obtain a qualitative sign of antimicrobial activity. The data from table (3) showed that *K. pneumoniae* was resistant to arak extract and all tested formulas are in agreement with [19] who reported that *K. pneumoniae* was resistant to the aqueous extracts of seven different kinds of chewing sticks including arak. The observed resistance may come from cell membrane permeability or due to further genetic factors as reported by [13]. The results tabulated in table (3) revealed that the arak extract was most effective on *Ps. aeruginosa* causing inhibition zone 30mm. This could attribute to the fact that arak extract inhibits the active transport oxidative phosphorylation and oxygen uptake by *Ps. aeruginosa* [20]. Among the tested microorganisms, *E. faecalis* was also susceptible to the arak extract and all the tested formulas. This may be due to the fact that the aqueous extract of arak contained some anionic components like nitrate (NO_3^-) which apply antimicrobial activities against various bacteria and has been reported to have an effect on the active transport of proline in *E. coli* [20]. The antimicrobial activity of formula 1 and 2 is a result of sodium monofluro-phosphate and triclosan which affects many essential enzymes of cell growth as reported by [21] and the cytoplasmic membrane causing lysis of the microorganisms [22]. The antimicrobial activity of formula 3 is attributed to the inhibitory properties of the herbal extracts where the essential oils kill microorganisms by distributing their cell walls, preventing their enzymatic activity, inhibit bacterial aggregation, release endotoxins and slow their multiplication as reported by [23]. On the other hand, arak extract had no antimicrobial effect on any other tested microorganism. These findings were consistent with the results obtained by [19] who found that *Staph. aureus* was not inhibited by the aqueous extract of the tested chewing sticks including arak. On the contrary, others found that *E. faecalis* was affected by aqueous extract of *Acacia arabica* (kikar). The tested toothpastes formula had antimicrobial effect on *E. coli, E. faecalis, Staph. aureus,* and *B. cereus* where they inhibited the growth of these strains and *L. acidophilus* was affected only by formula 1. In this study, the results showed that *K. pneumonia* and *C. albicans* were resistant to arak extract and to all of the tested formulas. These findings are similar to that obtained by other authors [19]. Although the tested formulas 1 and 2 showed remarkable bacteriostatic activity on *E. faecalis*, we must take into consideration that the FDA restricts the content of fluoride in toothpaste to 1150 ppm because of its harmfulness and therefore too much amount of fluoride can reproduce fluorosis, a common finding today. Also, ingested triclosan may affect the probiotic intestinal microflora of the human gastrointestinal tract which serves as defense system against pathogenic bacteria, hence, one become more susceptible to infectious diseases such as rotavirus, often resulting in diarrhea [20].

Based on our results, we concluded that, gamma radiation dose of 5 kGy was sufficient to decontaminate the tested arak sticks and powder from aerobic bacteria, mold, yeast, thermophilic arak sticks and main pathogens. Also, arak can be considered a good alternative to the toothpaste as it is cheap, easily available, doesn't require expertise or any more resources for manufacturing, has potential antimicrobial activity and safe for users, thus, it is advised as an important and operative tool for oral hygiene.

ACKNOWLEDGEMENT

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding this work through research group project No RGP-VPP-202.

REFERENCES

[1] Fischman, S. The history of oral hygiene products: How far have we come in 6000 years? *Periodontol*., 2000; 1997; 15: 7–14.

[2] Almas, K., Al-Zeid, Z. The immediate antimicrobial effect of a toothbrush and miswak on cariogenic bacteria: a clinical study. *Journal of contemporary dental practice*, 2004, 15;5(1):105-114

[3] Wu, C.D., Darout, I.A., Skaug, N. Chewing sticks: Timeless natural toothbrushes for oral cleansing. *J. Periodontal Res.,* 2001; 36: 275–84.

[4] Vuuren, S. F. and Viljoen, A.M, The in vitro antimicrobial activity of toothbrush sticks used in Ethiopia South *African Journal of Botany*, 2006; 72(4):646-648.

[5] FAO/IAEA: Food Irradiation Newsletter 19, No. 2, Food Preservation Section, FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, International Atomic Energy Agency, Vienna. 1995. pp 201–222.

[6] Fejerskov O, Ekstrand J, Burt BA, Fluoride in dentistry. 2nd. ed. Copenhagen: Munksgard; 1996. pp. 231-256.

[7] Saeki, Y., Ito, Y., Shibata, M., Sato, Y., Okuda, K. and Takazoe, I., Antimicrobial action of natural substances on oral bacteria. *Bull. Dent. Coll.*, 1998; 30 (3): 129-135.

[8] ICMSF, In: Microorganisms in Foods, 2nd edition, Blackwell Scientific Publications. 1986. Pp. 133-156.

[9] Harrigan, W.F., Laboratory Methods on Food Microbiology, 3rd edition, Academic Press, San Diego, CA, USA 1998. pp. 116-148.

[10] APHA, American Public Health Association: Diagnostic Procedures and Reagents, 4th ed., ADPH Inc., New York, 1963; pp 293–308.

[11] Baird-Parker, A.C. The Occurrence and Enumeration, According to a New Classification of Micrococci and Staphylococci in Bacon and on Human and Pig Skin. *J. Appl. Bact.*, 1962; 25: 352–361.

[12] Al-Lafi, T. and Ababneh, M. The effect of the extract of the miswak (chewing sticks) used in Jordan and the Middle East on oral bacteria. *Int. Dept.*, 1995; 45(3): 218-222.

[13] El-Astal, Z.Y., Aera, A. Aam, K. Antimicrobial Activity of Some Medicinal Plant Extracts in Palestine. *Park. J. Med. Sci*., 2005; 21: 187-193.

[14] Bou-Chacra, N.A., Gobi, S.S., Ohra, M.T. Pinto, T.J.A. Antimicrobial activity of four different dental gel formulas on cariogenic bacteria evaluated using the linear regression method. *Braz. J. Pharm. Sci.*, 2005; 41: 323-331.

[15] Swailam, H.M. and Abdullah, M.I. Radiation sterilization of some spices and medicinal plant. *Egypt. J. Microbiol.,* 2002; 2: 70-87.

[16] Yook, H.S., Chung, Y.J., Kim, J.O., Kwon, O.J., Kim, S., Byun, M.W. Effects of ionizing energy and ozone treatments on the microbial decontamination and physicochemical properties of aloe powders and bee pollen. *J. Food Sci. Nutr.,* 1997; 2:89-95.

[17] Onyenekwe, P.C. and Ogbado, G.H., Proc. 1st National conference on Nuclear Methods (NCNM-92)-, Zaria, 2-4 October, 1992. pp. 124-126.

[18] Barry, L. A., Thornsberry, C. Susceptibility test: diffusion test procedures. In: Ballows, A., Hausler, W.L., Isenberg, D. H., Shadomy, J. H., eds. Manual of Clinical Microbiology: ASM, Washington, 1991. p.1117-1125.

[19] Almas, K. The antimicrobial effects of seven different types of Asian chewing sticks. *Odonto-Stomatologie Tropicale.*, 2001; 96: 17-20.

[20] Darout, I.A., Christy, A.A., Skaug, N. and Egeberg P.K., Identification and Quantification of Some Potentially Antimicrobial Anionic Components in Miswak Extract. *Indian J. Pharmacology*, 2000; 32: 11-14.

[21] Hu, D., Zhang, Y.P., Petrone, M., Volpe, A.R., DeVizio. W., Giniger, M. Clinical effectiveness of a triclosan/copolymer/sodium fluoride dentifrice in controlling oral malodour: a 3 week clinical trial. *Oral Dis.*, 2005; 11: 51-53.

[22] Rolla, G., Kjaerheim, V. and Waler, S.M., Preceedings of the 2nd European Workshop on Periodontology, Chemicals in Periodentics, February 3-6, 1996, Ittingen, Thurgau, Switzerland, Quintessence Verlag, Berlin, 1996. Pp. 120-130.

[23] Nascimento, G.F., Locatelli, J., Freitas, P.C. and Silva, G.L., Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Braz. J. Microbiol*., 2000; 31: 247-256.