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Effect of mouth wash extracted from *Salvadora persica* (Miswak) on dental plaque formation: A clinical trail

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Chewing sticks or Miswak are used for teeth cleaning in many parts of the world, these Miswaks are believed to contain chemical substances which inhibit plaque formation and gingivitis. In the present study, *Salvadora persica* (Miswak) was extracted with 60% ethanol and was examined for its toxic effect, assessed its antibacterial activity and evaluated clinically for its effect on dental plaque formation. A 4 day plaque regrowth, double - blind, crossover design was used in which 10 dental students volunteers were rendered plaque free (0.3), ceased tooth cleaning, then, asked to rinse twice daily for 1.5 min each time with 10 ml of chlorhexidine 0.2% mouth rinse and three times daily for 1.5 min each time with 10 ml of *S. persica* 10% solution and placebo mouth rinse. On day five, plaque was scored by the plaque index system (PLI). A wash out period of 2 days was allowed in which the volunteers returned to self- performed plaque control, then a new test period was initiated. Statistical analysis showed that the mean PLI score were 1.48 for *S. persica* mouth rinse, 0.48 for chlorhexidine and 2.07 for placebo mouth rinse. Acute toxicity test revealed no mortality among the experimental animals which is an indication that *S. persica* crude extract solution is well tolerated, disk diffusion test showed a marked antibacterial effect *in vitro* and this effect is concentration dependent, had an effect in *in vivo*, but this effect cannot be considered absolute.

Key words: Mouthwash, Miswak, dental plaque.

INTRODUCTION

Supragingival plaque control is largely the responsibility of the individual, using tooth brushes and interdental cleaning devices, however; mechanical plaque eradication is considered for most as time consuming, requires above average of motivation, skill and is more difficult for handicapped people (Lindhe, 2003). Also, the prevalence of gingivitis, from young age in all population, and the occurrence or recurrence of periodontal disease is high (Brown et al., 1996). These observations suggest that mechanical cleaning alone by a considerable proportion of individuals is insufficiently good to maintain gingival health and, in the susceptible individual, to prevent

periodontal disease occurrence and progression or recurrence.

This supports the concept of employing agents to control plaque and require minimal cooperation and skill in their use This is the concept, which underlies, chemical supra gingival plaque control.

A number of chemical agents which have antiseptic or antimicrobial action have been used, with variable success, to inhibit supragingival plaque formation and the development of gingivitis. Among these are; phenolic compounds, Bis-biguanidaes, Pyrimidines, Quaternary ammonium compounds, Oxygenating agents, halogens, heavy metal salts (Mandel, 1988). And among these agents, chlorhexidine is, thus far, the most studied and effective antiseptic for plaque inhibition and prevention of gingivitis when used twice daily as mouth rinse (Ribeiro et al., 2007). But in oral use as a mouth rinse chlorhexidine

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has been reported to have a number of side effects including: brown discoloration of the teeth, some restorative materials and mucosa, bitter taste, and sometime sloughing of oral mucosa which restricts its general use. Other chemical antiplaque agents have been tested, none has shown equal or better results than chlorhexidine without eliciting unfavourable side effects (Hellden et al., 1981; Addy et al., 1995).

In order to overcome such side effects the world health organization (WHO) advice researchers to investigate the possible use of natural products such as herb and plant extracts. Herb and plant extract have been used in oral hygiene products for many years if not centuries (Mandel, 1988). A number of clinical studies have shown the effects of using mouth washes extracted from herbs such as *Sanguinarina* (Abbas et al., 1985), *Myrtus communis* (Walid and Fouad, 2001), *Qureucus infectoria* (Walid and Fouad, 2000), *Capparis spinosa* (Fouad and Wassan, 2001) and *Cinnamon* (Fouad et al., 2000) in the prevention of dental plaque accumulation and subsequent gingival inflammation.

Salvadora persica is a medicinal plant whose roots have been used by many people in Africa, South America, Middle East and Asia. The precise method for the use of the Miswak was recorded by Babylonian 5000 B.C. and the fashion ultimately spread throughout the Greek and Romanian empire.

In fact, the chewing stick which is known as Miswak or siwak among Moslems had fallen into general use by the time the holly prophet Mohammed started his mission in about 543 A.D. Prophet Mohammed said that the Miswak is an implement for the cleaning of teeth and pleases Allah (Lewis, 1980).

One of Mohammed's biographers wrote: Even the approach of death did not keep the prophet from demanding the siwak because it is the most elegant thing that one can use, and the most fitting to be found beautiful. For it makes the teeth white, clarifies the understanding, makes the breath fragrant, extinguishes the gall, dries up the phlegm, strengthens the gums around the teeth, makes the glance clear, sharpens the power of the vision, opens the bowels and whets the appetite (Almas, 2001).

S. persica, a very popular plant in the middle east, contains a number of identified antimicrobial and other prophylactic components including fluoride (Al-Lafi and Ababneh, 1995), alkaloids (Khalessi et al., 2004), sulphur compounds (Ezmirly et al., 1979), glucosinolates (Darmani et al., 2006) and volatile oils such as benzyl isothiocyanate (Khalessi et al., 2004).

It has been demonstrated that extracts of *S. Persica* improved gingival health and inhibited growth of cariogenic bacteria (Khalessi et al., 2004). Another study showed that *Streptococcus mutans* was more susceptible to Miswaks antimicrobial activity than *Lactobacilli* (Almas and Al-Zeid, 2004). In this regards, Almas et al. (2005) compared antimicrobial activity of eight commercially available mouth rinses and 50% Miswaks extract against seven microorganisms. They found that mouth rinses

containing chlorhexidine had the maximum antibacterial activity, while Miswak extract had low antibacterial activity. Additionally, Sofrata et al. (2007) showed that mouth rinsing with *S. persica* extract, compared with water rinsing, resulted in protracted elevation of plaque pH and the difference between two groups was statistically significant at 30 min.

The World Health Organization (WHO) has recommended and encouraged the use of these sticks as an effective tool for oral hygiene (WHO 1987), it cleans the dental structures and prevent problems in two ways: by mechanical action of the soft wood fibres, and by therapeutic action of a chemical constituent of the chewing stick itself (Almas, 2001).

From the above it is not clear if the plant has any chemical anti dental plaque property. For this reason the present study addresses the problem with the following objectives: to determine antibacterial activity of *S. Persica* alcoholic extract *in vitro*, to evaluate acute toxicity profile of its ethanol extract and to determine clinically the effect of the *S. persica* extract on dental plaque formation when used as mouthwash.

MATERIALS AND METHODS

Alcoholic extraction of *S. persica* chewing sticks

Two hundred grams of *Salvadora persica* chewing sticks were cut using a sharp knife, then ground to powder using a food blender.

The powder was extracted with one litre of 60% ethanol; the mixture was left for 24 h. Then filtered through Whatman No.1 filter paper. The extract was autoclaved at 40°C until it becomes dry (Al-Jebbory, 1994).

Assessment of antibacterial activity

The antibacterial activity of the *S. persica* extract was evaluated by using the disk diffusion test technique (British Society for Antimicrobial Chemotherapy, 2005) sterile paper disks were moistened for 15 min in 1, 3, 5 and 10% solution of plant extract and 0.2% Chlorhexidine solution as positive control and distilled water as a negative control. Supra gingival plaque was collected by using sterilized periodontal probes from 10 patients, the plaque samples were pooled, streaked on blood agar plate, incubated at 37°C for 48 h. The cultured dental plaque was transferred into nutrient broth and incubated for 24 h at 37°C. Blood agar plate streaked by previously cultured broth with supragingival plaque using loop wire then, these disks were placed on the streaked blood agar plate; the plate was incubated at 37° for 24 h and examined for zone of inhibition. This procedure was repeated four times (Christofilogianis, 2001).

Evaluation of the toxicological profile of the extract

The acute toxicity test was studied in group of 32 male albino mice which were injected intra peritoneally per body weight with varying concentration of plant extract (10, 30, 50 and 100 mg/ml). Then the concentration of the injected extract was increased to 2 g/kg. The acute toxicity LD₅₀ was calculated as the geometric mean of the dose that resulted in 100% lethality and that which caused no lethality at all (Goth, 1978).

Measurement of the elimination of *S. persica* extract from the oral cavity

Quantitative assessments of the release of *S. persica* extract was evaluated in saliva using Electronic spectrophotometer. Saliva sample were collected from five dental students volunteer who was instructed to rinse their mouth with 10 ml of 10% *S. persica* solution for 2 min. following the morning meal. They were also instructed not to change their eating and drinking habits. Following the rinse, 3 ml of unstimulated saliva was collected in small test tubes 16 times according to fixed time schedule. The first sample was taken 5 min after expectorating the mouth wash and the last one 24 h later. Then 1 ml of saliva mixed with 2 ml 2 M NaOH and 20 ml of chloroform in glass stoppered flask, 15 ml of the organic phase is aspirated and transferred to a test tube, 4 ml 0.1 M glycine buffer were added with PH adjusted to 2.10 ± 0.05 . 3 ml of the buffer transferred to a 1 cm cavette and the extinction at 360 nm was measured against buffer as reference. The extinction of the unknown (Ex) and the mean value of the extinction of the standard (Est) and the blanks (Ebl) were used for calculating the amount of *S. persica* extracts in the unknown sample using the following equation.

$$\text{Mg in the sample} = \frac{\text{Ex} - \text{Ebl} \times (\text{mg in the standard})}{\text{Est} - \text{Ebl}}$$

Clinical trial

Mouth wash preparation

Three different mouthwash preparations were tested in the study:

- 1) Placebo mouthwash: Distilled water as negative control.
- 2) *Salvadora persica* mouth wash: Alcoholic extract, at a concentration of 10 mg/ml
- 3) Chlorhexidine mouth wash: 0.2% chlorhexidine digluconate as a positive control.

The rinsing preparations were filled in identical but coded bottles. An instruction of usage was written on it.

Subjects selection criteria

The subjects enrolled in this study had to satisfy the following criteria:

- 1) Male dental student volunteers who should have at least 20 teeth with good alignment (wisdom teeth were excluded).
- 2) None of the volunteers were wearing fixed or removable orthodontic appliances or partial dentures.

Subjects were excluded from the study if they had any of the following:

- 1) A history of epilepsy, convulsion or fainting spells.
- 2) Hepatitis or other liver disease within the last 12 months.
- 3) Systemic diseases such as hypertension, blood diseases and heart diseases.
- 4) Allergy or hyper sensitivity to chlorhexidine.
- 5) Use of antibiotics within seven days of the baseline.
- 6) Presence of periodontal tissue breakdown or pathological lesion (Siegrist et al., 1986).

Preparation of volunteers

The nature of the trial was explained to each volunteer verbally by the examining clinician. During a preparatory period of 2 weeks, the participants were exposed to a professional plaque control measure (Axelsson and Lindhe, 1981a and b). The oral hygiene session were conducted by the examining clinician and required at least 15 - 20 min each. At the end of this preparatory period, each participant received a professional scaling and polishing.

Study design

The study was a double blind, crossover design. A group of 10 male dental students, 22 years of age volunteered to participate in this clinical trial from the College of Dentistry, University of Baghdad. They had total of 275 teeth and 1100 teeth surfaces.

These volunteers participated in three test periods; each period lasted five days from Saturday - Wednesday, in which each volunteer was instructed to rinse with each of the following mouth rinses:

- 1) The test solution: *S. persica* alcoholic extract at 10% concentration. The instruction was to rinse three times daily with 10 ml of the solution at duration of 1.5 min.
- 2) The positive control: 0.2% chlorhexidine digluconate solution. 10 ml, 2 times, each time 1.5 min duration.
- 3) The negative control: Distilled water 10 ml, 3 times daily each times 1.5 min duration. And during which, all mechanical oral hygiene measures were withdrawn, they were also instructed not to change their eating and drinking habits, at the end of each test period a washout period of 2 days was allowed during which the volunteers were instructed to resume the previous mechanical plaque control measures (Abbas et al., 1985; Axelsson and Lindhe, 1981a). The volunteers were asked to fill out a questionnaire concerning their opinion about the taste and other side effects of the rinsing solution like ulceration, change in the test and burning sensation in the mouth.

Clinical examination

A single clinician carried out all examinations, the presence and amount of plaque was examined and scored on day 5 using the plaque index system PLI (Silness and Loe, 1964).

Statistical analysis

The statistical analysis was carried out using the student's t-test with 5% significance level. Separate analysis was made for the individual mean, for the mean for group of teeth and tooth surfaces.

RESULTS

Extraction product

200 g of *S. persica* was yield to alcoholic extract of approximately 15 g.

Antibacterial activity test

The inhibition zone of the various concentrations, which were recorded after 24 h incubation at 37°C are shown in Table 1. The best antibacterial activity *in vitro* (12 mm)

Table 1. Inhibition zone of types of solution.

Types of mouth wash	Inhibition zone (mm)			
1 mg/ml <i>S. persica</i>	0	0	3	3
3 mg/ml <i>S. persica</i>	0	0	2	4
5 mg/ml <i>S. persica</i>	7	6	7	8
10 mg/ml <i>S. persica</i>	8	7	8	12
0.2% Chlorhexidine	12	10	12	16
Distilled water	0	0	0	0

Table 2. Concentration of alcoholic extract of *Salvadora persica* in saliva.

Time	<i>S. Persia</i> 10 mg/ml solution (mg/ml)
After 5 min	6.7
After 1 h	4.54
After 2 h	3.79
After 3 h	3.04
After 4 h	2.29
After 5 h	1.03
After 6 h	0.5
After 8 h	0
After 10 h	0

was obtained with 10 mg/ml concentration, 5 mg/ml concentration shows significant but, however, less than that of 10 mg/ml whereas 1 and 3 mg/ml failed to show significant activity.

Acute toxicity

The procedure of acute toxicity test revealed no mortality among the experimental animals, which, in turn, indicates that this substance is well tolerated.

Measurement of the elimination of *S. persica* extract from the oral cavity

The concentration of the 10 mg/ml *S. persica* solution in saliva at various time points are shown in Table 2.

Results from electronic spectrophotometer revealed traces of extract after 6 h, then vanishes at 8 h.

Clinical trial

Table 3 demonstrated that the mean plaque index scores for the maxillary and mandibular teeth after 5 days of daily rinsing with *S. persica*, CHX and placebo mouth rinses were 1.48, 0.48 and 2.07, respectively.

Table 4, 5 and 6 represent a summary of a comparative significance between different mouth washes regarding their effect on dental plaque formation. The statistical analysis of data showed that:

- 1) A very highly significant difference ($p < 0.001$) between *Salvadora persica* and placebo mouth rinse for plaque index scores in all tooth surfaces.
- 2) *Salvadora persica* mouth rinse resulted in a very highly significant ($p < 0.001$) higher plaque index scores than the 0.2% Chlorhexidine mouth rinse in all groups of teeth and tooth surfaces.

Figure 1 illustrates the percentage distribution of (PLI) scores for the three mouthwashes in all teeth surfaces. For score (0) Chlorhexidine was 54%, *S. persica* was 8.6% and placebo was 0.5% for score (1) Chlorhexidine was 43.4% *S. persica* was 35% and placebo was 22.5% for score (2), Chlorhexidine was 2.6% *S. persica* was 55.9% and placebo was 45.7%.

For score (3), Chlorhexidine was 0%, *S. persica* was 0.5% and placebo was 31.2%.

Figures 2 and 3 show the percentage distribution of PLI scores for mandibular and maxillary teeth respectively.

Figures 4 and 5 show the percentage distribution of PLI scores for buccal, lingual teeth surfaces respectively.

DISCUSSION

There is a continued interest in identifying efficient antiplaque agents that could be used daily without side effects (Prabuseenivasan et al., 2006). Folk medicine is a potential source of medicaments and has recently become a focus of dental research (Fouad et al., 2000; Walid and Fouad, 2000 and 2001; Fouad and Wassan, 2001). Chewing sticks or Miswaks are still popular compared with the use of modern tooth brushes, the type of chewing sticks used in the Middle East is derived from the plant *S. persica* (Almas, 2001).

Alcoholic extraction of *S. persica* yielded 15 gm for each 200 gm of sticks; this is considered as a commercially valued (Almas and Al-Zeid, 2004).

The *in vitro* activity of *S. persica*, revealed that its action is a concentration dependent, where 10 mg/ml solution produced the greatest zone of inhibition around each paper disc in the agar diffusion assay (Table 1) when compared with other solutions. Yet the inhibition zones were less pronounced than that of Chlorhexidine solution at all concentrations this result was in agreement with that presented by Almas et al. (2005).

The acute toxicity test (single dose toxicity) is still of considerable importance for the assessment of risk posed by new chemical substances, and for better control of natural and synthetic agents in the human environment (Zbinden and Flury-Roversi, 1981). Evaluation of toxicological profile of the extract by intra peritoneal

Table 3. The mean plaque index scores for all surfaces: All teeth, maxillary teeth and mandibular teeth.

Mouth wash	All surfaces					
	All teeth		Maxillary teeth		Mandibular teeth	
	Mean	S.D. E	Mean	S.D. E	Mean	S.D. E
Water	2.075	0.7442	2.1685	0.6612	1.9857	0.8068
<i>S. persica</i>	1.4818	0.6572	1.4167	0.7476	1.5446	0.5497
Chx	0.4868	0.5503	0.4769	0.5426	0.4964	0.557

Table 4. A summary of a comparative significance between different mouth washes. Regarding their effect on dental plaque formation.

All surfaces	Mouth wash	
	CHX versus <i>S. persica</i>	<i>S. persica</i> versus water
All teeth	VHS	VHS
Maxillary teeth	VHS	VHS
Mandibular teeth	VHS	VHS

CHX: Chlorhexidine; VHS: Very highly significant. $P < 0.001$.

Table 5. A summary of a comparative significance between different mouth washes. Regarding their effect on dental plaque formation.

All surfaces	Mouth wash	
	CHX versus <i>S. persica</i>	<i>S. persica</i> versus water
Incisors and canines	VHS	VHS
Premolars	VHS	VHS
Molars	VHS	VHS

CHX: Chlorhexidine; VHS: Very highly significant. $P < 0.001$.

Table 6. A summary of a comparative significance between different mouth washes. Regarding their effect on dental plaque formation.

All surfaces	Mouth wash	
	CHX versus <i>S. persica</i>	<i>S. persica</i> versus water
Approximal surfaces	VHS	VHS
Buccal surfaces	VHS	VHS
lingual surfaces	VHS	VHS

CHX: Chlorhexidine; VHS: Very highly significant. $P < 0.001$.

injection did not cause death among the experimental animals even with a very high does which was 2.04 g/kg. Therefore; the extract is considered as a well-tolerated substance and so the LD50-test is without practical or theoretical relevance. This is according to Zbinden and Flury-Roversi (1981) who stated that "substances that are

well tolerated by small rodents at oral doses of 5 g/kg and parenteral doses 2 g/kg should not be subject to a LD50-test". This result is in accordance with that presented by Abo Al-Samh and Al-Nazhan (1997). The spectrophotometer assessments were carried out using the Electronic spectrophotometer in the present study to measure the

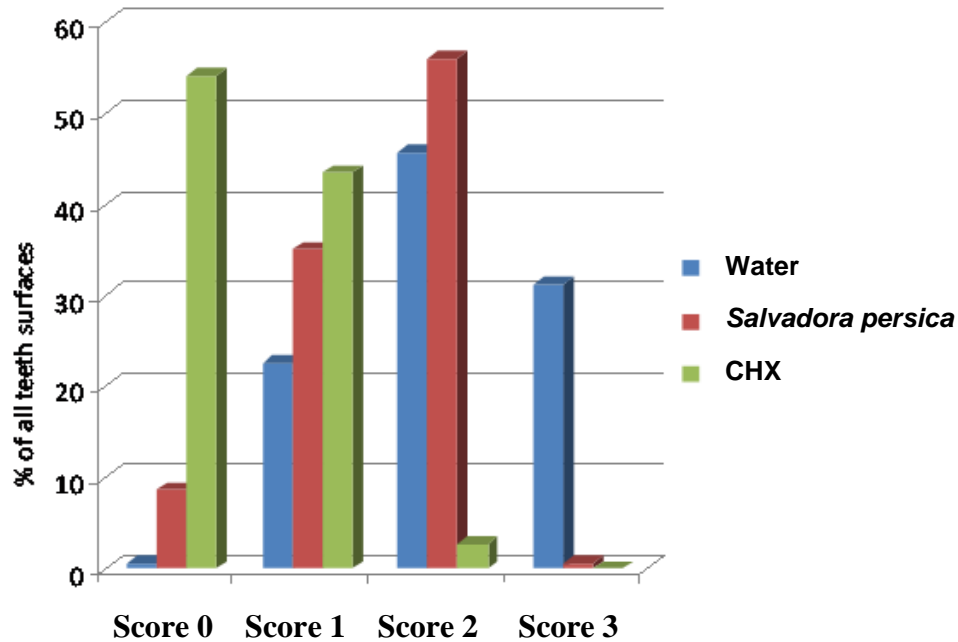


Figure 1. The percentage distribution of all surfaces according to plaque index scores.

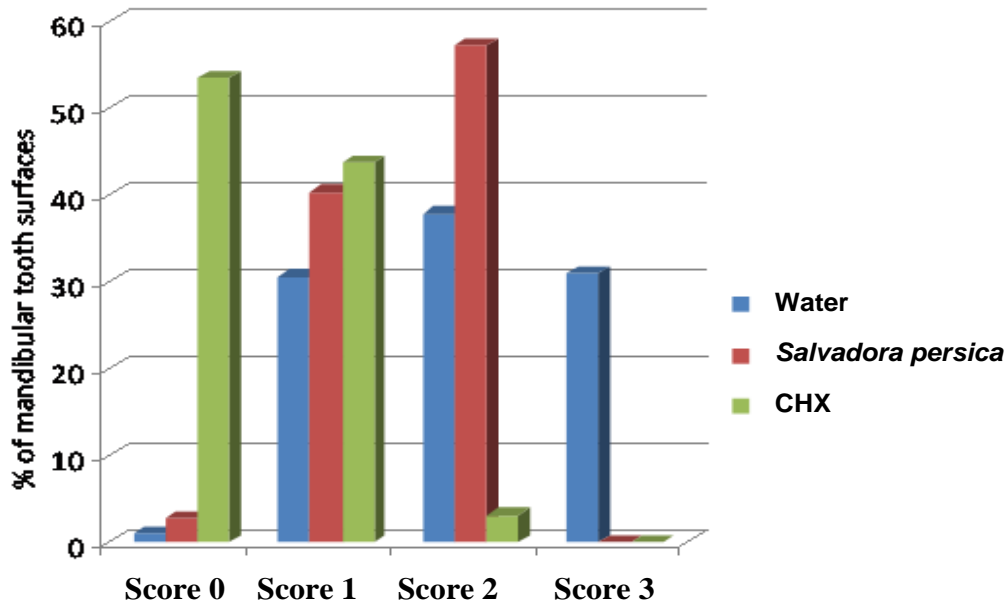


Figure 2. The percentage distribution of mandibular tooth surfaces according to plaque index scores.

releases of *Salvadora persica* solution from oral cavity has certain limitation and does not give the accurate picture. But rather an approximate one of the substantivity when compared with the radioisotopes technique, which was used for Chlorhexidine by Bonesvoll et al. (1974).

Traces of *S. persica* 10 mg/ml solution were detected up to 6 h after expectoration; this substantivity in approxi-

mate but it gives a comparable gross picture. Therefore; volunteers were instructed to rinse their mouth with the solution three times daily in order to make a relatively accurate comparison.

Five-day experimental period length was chosen because plaque accumulation reaches measurable volumes after 4 - 5 days of no oral hygiene (Theilade et al., 1966).

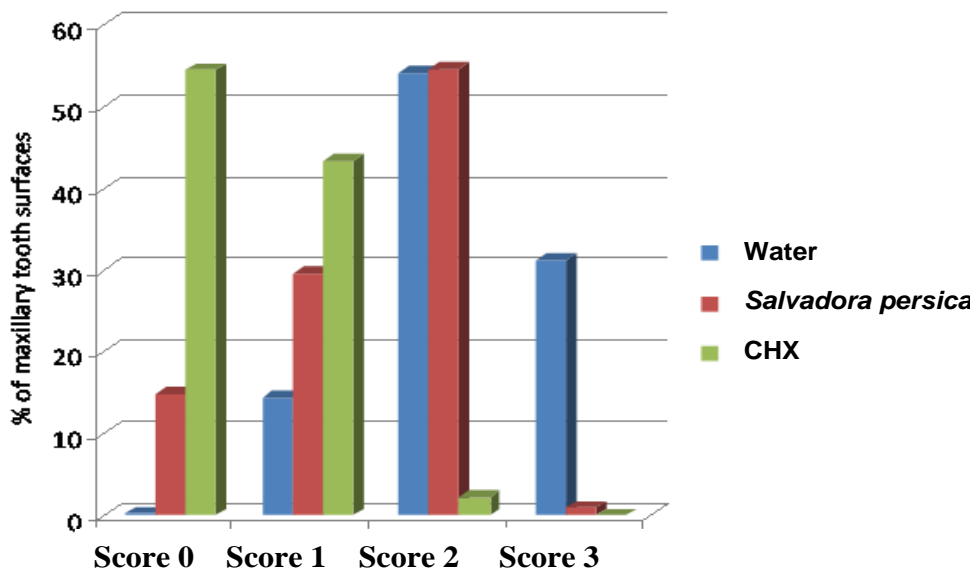


Figure 3. The percentage distribution of maxillary tooth surfaces according to plaque index scores.

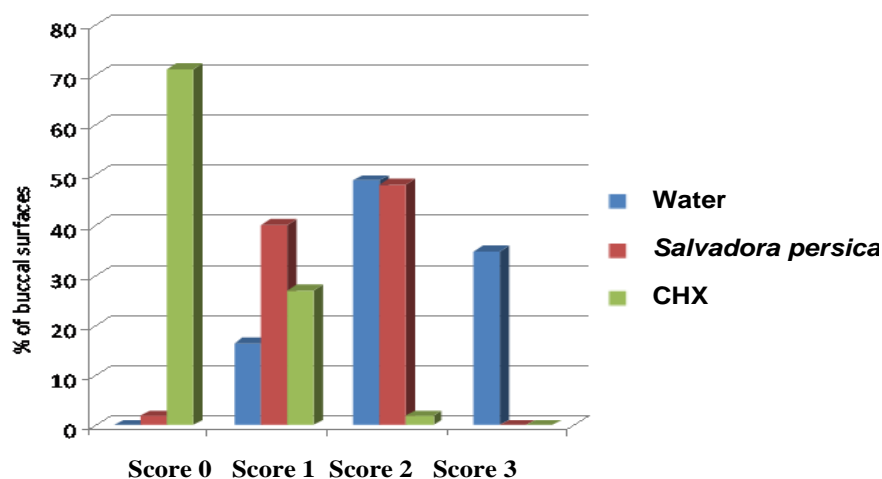


Figure 4. The percentage distribution of buccal surfaces according to plaque index scores.

S. persica mouthwash inhibited plaque formation and yielded to mean PLI score lower than that of placebo, but also higher than that recorded for Chlorhexidine (Table 7) in most part of dentition. Therefore; *S. persica* had a very highly significant ($p < 0.001$) effect on plaque accumulation in comparison with placebo this finding was in accordance with that presented by Sforata et al. (2007). Nonetheless, Chlorhexidine showed a very highly significant reduction in the overall bacterial plaque accumulation when compared with *S. persica*.

Irrespective of the agents that have been used, plaque tends to be predominant on mandibular surfaces than on

maxillary surfaces (Table 3, Figures 2 and 3), on buccal surfaces than on lingual surfaces (Table 8, Figures 4 and 5). This is in agreement with Moran et al. (1995). This is probably attributable to the masticatory and tongue actions. More on posterior teeth than on anterior (Table 4A), this is consistent with Axelsson and Lindhe (1987). Also plaque accumulations tend to be predominant on interproximal teeth surfaces (Table 8, Figures 4, and 5). This is in agreement with Lang et al. (1982). Eight of ten volunteers complain from the taste of the plant extract solution, two of ten showed a reversible staining of teeth. Other than these, they expressed on

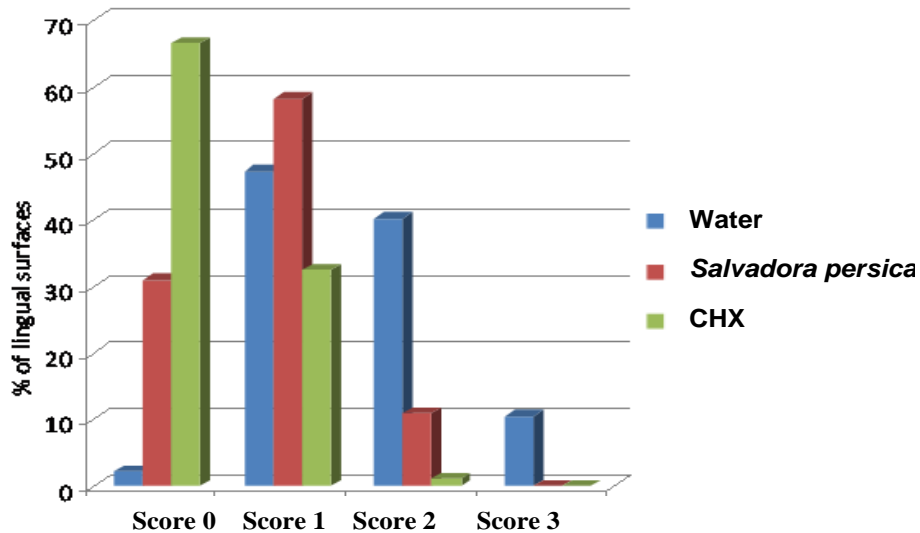


Figure 5. The percentage distribution of lingual surfaces according to plaque index scores.

Table 7. The mean plaque index scores for group of teeth.

Mouth wash	All surfaces					
	Incisors and canines		Premolars		Molars	
	Mean	S.D. E	Mean	S.D. E	Mean	S.D. E
Water	2.0313	0.7431	2.0906	0.7308	2.13	0.7583
Salvadora persica	1.4063	0.7163	1.4531	0.6115	1.6333	0.5774
CHX	0.4667	0.5357	0.4937	0.5485	0.5116	0.5751

Table 8. The mean plaque index scores for group of teeth.

Mouth wash	Approximal surfaces		Buccal surfaces		Lingual surfaces	
	Mean	S.D. E	S.D. E	S.D. E	Mean	S.D. E
Water	2.2669	0.6781	2.1848	0.6917	1.5884	0.7048
Salvadora persica	1.7818	0.4553	1.5636	0.5323	0.8	0.6161
CHX	0.6514	0.552	0.3058	0.4991	0.3453	0.4985

major side effects.

Overall observations indicate that *S. persica* crude extract solution is well tolerated showed a marked antibacterial effect *in vitro* and this effect is concentration dependent, has an effect in *in vivo*, but this effect cannot be considered absolute.

It is note worthy to mention that the chewing sticks, which have been tested in the present study, were cut from the tree one month earlier, kept in a plastic package, closed tightly and kept in the freezer at -5°C in order to keep them fresh. The fresher the stick, it seems the more effective it will be.

In summary, the rational explanation of the attractiveness of *S. persica* chewing stick as a tool for teeth

cleaning is:

- Cheapness, safeness, its shape is like brush, contains chemical constituents with variable actions.
- It seems to be two in one, which means it, gathers the tooth paste and tooth brush in one implement named "Miswak".

Conclusions

S. persica alcoholic extract produced remarkable antibacterial activity *in vitro* at 10 mg/ml concentration, is well tolerated and safe. As a mouth rinse is less effective

than chlorhexidine in preventing plaque accumulation and more effective than placebo on dental plaque accumulation.

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