Journal of Research in Medical and Dental Science
2021, Volume 9, Issue 2, Page No: 93-99

Available Online at: www.jrmds.in
eISSN No. 2347-2367: pISSN No. 2347-2545

Antibacterial Activity of *Salvadora persica L.* Ethanol Extracts against Root Canal Pathogens from Clinical Isolates

Abdulwahab Abuderman[1], Ponmurugan Karuppiah[2,3], Muhammad Musthafa Poyil[1]*, K.P Shamna[4]

[1]Department of Basic Medical Sciences, College of Medicine, Prince Sattam bin Abdulaziz University, Al-Kharj, 11942, Saudi Arabia.

[2]Department of Microbiology, K.S. Rangasamy College of Arts and Science, Tiruchengode - 637 215, Namakkal, Tamil Nadu, India

[3]Department of Botany and Microbiology, College of Science, King Saud University, P. Box: 2455, Riyadh 11451, Saudi Arabia

[4]Department of Iatrochemistry and Ayurvedic Pharmaceutics, M.V.R Ayurveda Medical College, Parassinikadavu – 670563, Kannur, India

ABSTRACT

The maintenance of oral health and hygiene is of prime importance in all the cultures. But the use of synthetic chemicals and antibiotics brings about many adverse effects, and therefore, the science has been searching in nature for potential natural remedies. Salvadora persica is widely used as chewing sticks, and its regular users have been observed to have a high degree of oral health and hygiene. In the present study, with standard protocols, we analyzed various phytochemical constituents of the Salvadora persica root and its ethanol extract's combined activity against the most common types of root canal bacteria including Streptococcus mutants, Lactobacillus acidophilus, Staphylococcus aureus, Actinomyces sp. etc. The extract presented significant activity against all the tested bacteria. Salvadora persica displayed the highest zone of inhibition of 17.6 mm against Streptococcus mutants Strain1 and the lowest zone of inhibition towards Actinomyces sp. (8.3 mm). The MIC value of crude ethanolic extracts was 31.25 µg/ml against Streptococcus mutants. Based on this study and other similar studies, we concluded that the Salvadora persica L. "miswak" could be used as a tool in overall oral hygiene.

Key words: *Salvadora persica, Root canal*, Ethanol extracts, Phytochemical compounds

HOW TO CITE THIS ARTICLE: Abdulwahab Abuderman, Ponmurugan Karuppiah, Muhammad Musthafa Poyil, K.P Shamna, Antibacterial Activity of Salvadora persica L. Ethanol Extracts against Root Canal Pathogens from Clinical Isolates, J Res Med Dent Sci, 2021, 9 (2): 93-99.

Corresponding author: Muhammad Musthafa Poyil

e-mail✉: pmusthu@gmail.com

Received: 18/01/2021

Accepted: 05/02/2021

INTRODUCTION

Decaying of teeth has been listed as a chronic infection that occurs commonly around the globe. The situation is severe in developing countries as 60–90% of school going children and most of the elders are affected by this disease [1]. The odonto-periopathic bacteria present in the plaque are the primary cause of these dental infections. Reducing the number of bacteria or preventing them completely is vital in tackling these infections [2,3]. Despite the effectiveness of commonly, used antibiotics like erythromycin, penicillin etc. in preventing dental caries in animals and humans [4], their side effects including supra-infections, teeth staining and hypersensitivity reactions make them less prevalent in the treatment [5,6]. Also, the emergence and spread of multidrug-resistant (MDR) bacteria due to the uncontrolled use of antibiotics, the need for the search of new antibacterial agents from the nature is highly demanded [7]. This is how the researchers have begun to concentrate on plant-based bioactive compounds which are effective and at the same time economic with no remarkable side effects.

Since time unknown, the use of plants and other natural substances in dental care and in the overall cleanliness of the oral cavity has been practiced worldwide and recorded [8]. Researchers have concluded that the toothbrushes used in the modern period should have been developed

from the plant sticks chewing and 'tooth picking' habit practiced by Babylonians since 3500 BC as mentioned in Roman and Greek records [9]. Even today, the herbal sticks with a high degree of antimicrobial potentials are being used by many of the communities in oral hygiene and as a remedy for many of the dental diseases [10]. Various species of plants are being used to prepare chewing sticks in the Middle East, Africa, Asia and South America [11].

It has been estimated that there are 182 plants which can be used in brushing the tooth and the stick widely known as the "miswak," taken from Salvadora persica is considered as the most commonly used one [12]. *Salvadora persica* grow as a shrubs or small trees with trunks less than one foot in diameter. The sticks have an attractive fragrance with a pungent taste. The bark of this plant is cracked and the extremities are pendulous and whitish in color [13]. The plant is seen in different parts of the world such as Nepal, India, Pakistan, Malaysia, Iran, Iraq, Saudi Arabia and in African countries [14]. Most parts of the plant including the stem and roots are used in oral hygiene [11] including the toothpicks made by smaller sticks [15]. Several studies using the aqueous extract from the plant have detected the presence of components with antibacterial activities against periodontal and cariogenic pathogens including *Staphylococcus aureus*, *Streptococcus mutans, Streptococcus salivarius and* anaerobic *Streptococci* [16,17]. Underlining its importance in oral health, the World Health Organization (WHO) has encouraged and recommended the Miswak-*Salvadora persica* sticks chewing in the different parts of the globe where it is traditionally used for the dental care and overall buccal hygiene [16].

Chemically, the *Salvadora persica* has various potential organic and inorganic compounds assisting in oral health maintenance like antibacterials, antiviral, tooth strengthening agents, anti-carcinogens, anti-genotoxins, abrasives, coating agents, etc. [18,19]. The primary dental caries causing bacteria, dominating in most of the cases have been identified as *Streptococcus mutans* and *Lactobacillus* sp. and having acidogenic and uricabilitic properties which are the determining factors in dental caries formation they ensure an acidic environment along with some other acidophiles [18,20-23]. The biofilm formation by these bacteria and other species thereby developing into a matrix in dental plaques is another major issue. The lactic acid production as a result of carbohydrate fermentation by many agents including *Streptococcus mutans, Candida albicans* and *Lactobacillus* sp. deteriorate the scenario resulting in total destruction of the tooth surfaces [24,25]. Dental caries may lead to further complications as the buccal bacterial flora even can promote carcinogens. Culture-independent methods have elucidated a much greater complexity of oral microbial flora promoting carcinogens by the cumulative activities against members of the community [24]. The present study examines the chemical components present in the *Salvadora persica* extracts and their effect on selected, clinically isolated root canal bacterial pathogens using different methods.

EXPERIMENTAL

Collection and extraction of plant materials

The healthy plant root samples of *Salvadora persica* were collected from the medicinal plant markets in India and were identified by K. Rajarathinam, Department of Botany, V.H.N.S.N. College, Virudhunagar, Tamil Nadu, and India. Surface washed roots were cut into small pieces with the help of a sterile knife. Then it was powdered by a blender. This powder was subjected to extraction. The ethanol extraction of *Salvadora persica* root samples was done using the Soxhlet apparatus with standard procedure suggested by Anees et al. [26].

Test microorganisms

The most common root canal disease causing bacteria were collected from Sun Micro Laboratory, Erode, Tamil Nadu, India. Also, reference strains Streptococcus mutants (MTCC 890), *Staphylococcus aureus*, *Lactobacillus acidophilus* (MTCC 447), *Actinomyces* sp. were obtained from MTCC, Chandigarh, India.

Screening of antibacterial properties of S*alvadora persica* ethanol extracts

Agar well diffusion

At a cell density of 1 X 108 CFU/ml (in accordance with the 0.5 McFarland standards), the broth culture of the selected bacteria was prepared. Using a sterilized cotton swab, the aliquot was spread onto Muller Hinton agar. The plates

were dried at room temperature by keeping for 30 minutes [27]. Using a sterile cork borer, wells with a diameter of 6 mm were made on the plates keeping a 2 mm from the plate edge. The leaf extracts at a volume of 50 µl were added aseptically into agar well. The positive controls were 30 µg/ml of chloramphenicol. The negative controls were the extraction solvents, *viz* ethanol, ethyl acetate and hexane. The agar plates were kept for about 40 minutes on the working table for solidification. The plates were then incubated at 37°C for 24 hours. The tests were conducted in triplicates. A zone of inhibition with ≥ 7 mm diameters formed surrounding the wells was considered as the sensitivity of the organisms to the extract [28].

Minimum inhibitory concentration (MIC) determination

The extracts which produced an inhibition zone with ≥ 7 mm diameter were selected to find the MICs by agar well diffusion methods. For this, the serial dilutions of the extracts (500 µg/ml) were prepared as 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, and 1:256 to bring 250 µg/mL, 125 µg/mL, 62.5 µg/mL, 31.25 µg/mL, 15.63 µg/mL, 7.81 µg/mL, 3.95 µg/mL and 1.95 µg/mL concentrations respectively. The wells were filled with the extracts aseptically. After incubating at 37°C for 24 hours, the zones inhibition was measured. The lowest concentration at which the growth inhibited was recorded as the MIC value for the sample.

Phytochemical screening of the extracts

A preliminary screening test for certain phytochemical components like carbohydrates, glycosides, alkaloids, fixed oil and fats, phenolic compounds, phytosterols, flavonoids, proteins and amino acids, saponins, lignin, gum and mucilage etc. was performed using standard protocols.

RESULTS

The findings of this investigation proved that *Salvadora persica* root ethanol extract is an effective tool for inhibiting the tested root canal bacteria such as *Staphylococcus aureus, Streptococcus mutants, Lactobacillus acidophilus* and *Actinomyces* sp. The inhibition response of *Salvadora persica* in the agar well diffusion method on the selected root canal bacteria is displayed in Table 1 and Figure 1. The ethanol extracts showed inhibitory activity with greater effectiveness against most of the eight microorganisms used in this investigation, followed by acetone and hexane extracts. Among the root canal clinical pathogens *Staphylococcus aureus* and *Streptococcus mutants* were the most susceptible, followed by *Lactobacillus acidophilus* and *Actinomyces* sp., which were the

Table 1: Antibacterial activity of *Salvadora persica* extract against bacterial strains.

Bacterial strains	Zone of inhibition (mm)/Solvent extracts		
	Hexane	Acetone	Ethanol
Streptococcus mutants (MTCC497)	10.5	11.8	20.3
Lactobacillus acidophilus (MTCC10307)	8.6	10.2	12.6
Actinomyces sp. (MTCC 9775)	8.4	10.8	12.4
Staphylococcus aureus (MTCC 3160)	11.2	13.5	16.7

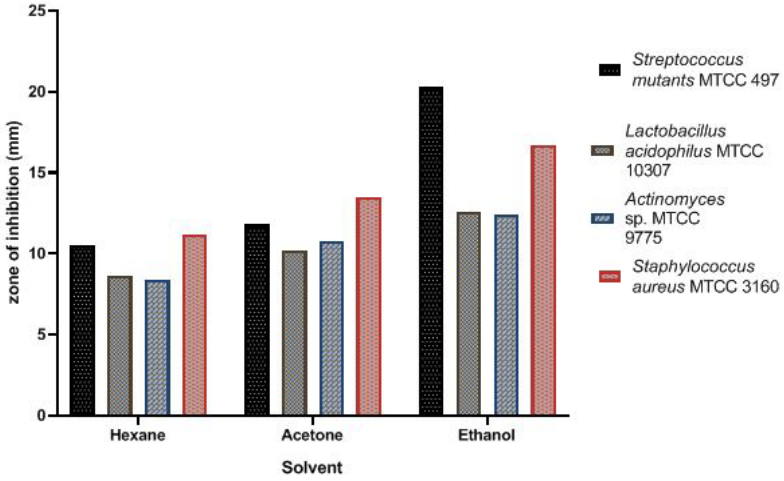

Figure 1: Antibacterial activity of extract of the *Salvadora persica* against bacterial strains.

least inhibited by the *Salvadora persica* ethanol extracts. The diameter of zone inhibition varied between 8.5 mm and 17.6 mm (Table 2 and Figure 2). The ethanolic extract of *Salvadora persica* showed the highest zone of inhibition of 17.6 mm against *Streptococcus mutants Strain 1*, followed by the *Staphylococcus aureus Strain* 2 (16.7 mm); the lowest inhibition zone exhibited against *Actinomyces* sp. (8.3 mm).

The MIC values of crude ethanolic extracts were 31.25, 62.50, 125, 250, 500 µg/ml against *S. mutants* Strain 1 and 3, *S. aureus* Strain 2, *S. mutants* Strain 4, *L. acidophilus* and *Actinomyces* sp. respectively (Table 3 and Figure 3). The MICs were as low as 31.25 µg/ml of extracts against root canal bacteria and underline the presence of potential bioactive compounds in the ethanolic extracts. Hence, it can be suggested that the ethanolic extracts of *Salvadora persica* may have molecules with potential activities for treating dental caries caused by pathogenic bacteria.

Thus, as shown above, the preliminary investigation for the presence of potential phytochemical compounds in the root extracts of *Salvadora persica* showed the activity of various chemical constituents, the majority of which are effective antimicrobial compounds. This work confirmed the presence of glycosides, carbohydrates, phytosterols, alkaloids, saponins, lignin, phenolic compounds, flavonoids, tannins, proteins and amino acids, fixed oils and gum and mucilage.

DISCUSSION

The findings of this investigation are underlined by a couple of studies conducted by various researchers. For example, Almas et al. [29] reported that the extract of *Salvadora persica* was effective against *Streptococcus mutans* and *Enterococcus faecalis*. In another study, Almas et al. [30] could find that the immediate antimicrobial effects of *Salvadora persica* high in the case of *Streptococcus mutans* whereas it was not that effective in the case of *Lactobacillus* sp. But the present *in vitro* study shows that the effect of the *Salvadora persica* miswak extract is almost the same on both *S. mutans* (8 mm inhibition zone) and *L. acidophilus* (7 mm inhibition zone). Another investigation by Sofrata et al. [31] showed that the *Salvadora*

Table 2: Antibacterial activity of the *Salvadora persica* extract against dental caries causing bacterial strains.

Clinical isolates	Zone of inhibition (mm)						
	Standard antibiotics*				Solvent extracts		
	M (5 mcg)	V (30 mcg)	S (10 mcg)	C (30 mcg)	Hexane	Acetone	Ethanol
S. mutants Strain 1	10.6	16	11	18	12.3	14.2	17.6
S. mutants Strain 2	11	15.2	13.2	17.2	10.4	10.6	15
S. mutants Strain 3	12.5	16.3	17.3	19.3	9.7	13	16.2
S. mutants Strain 4	10.6	17	19	15	9.5	10.9	14.5
L. acidophilus	8.7	18.6	15.6	18.6	8.7	9.1	12.2
Actinomyces sp.	9	10.2	11.2	14	8.3	9	10.9
S. aureus Strain 1	15.3	14.5	17.5	12.5	9	10.6	15
S. aureus Strain 2	12	16.3	15.3	19.3	9.5	10.8	16.7

*M–Methicillin; V-Vancomycin; S-*Streptomycin*; C-Chloramphenicol

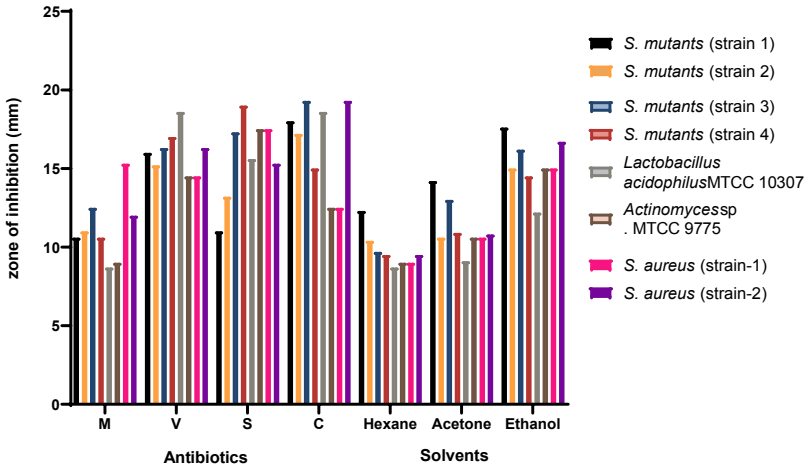

Figure 2: Antibacterial activity of the *Salvadora persica* extract against dental caries causing bacterial strains.

Table 3: MIC value of ethanolic extracts of *Salvadora persica*.

Clinical isolates	MIC (µg /ml)
S. mutants Strain 1	31.25
S. mutants Strain 2	500
S. mutants Strain 3	31.25
S. mutants Strain 4	62.5
L. acidophilus	250
Actinomyces sp.	500
S. aureus Strain 1	125
S. aureus Strain 2	31.25

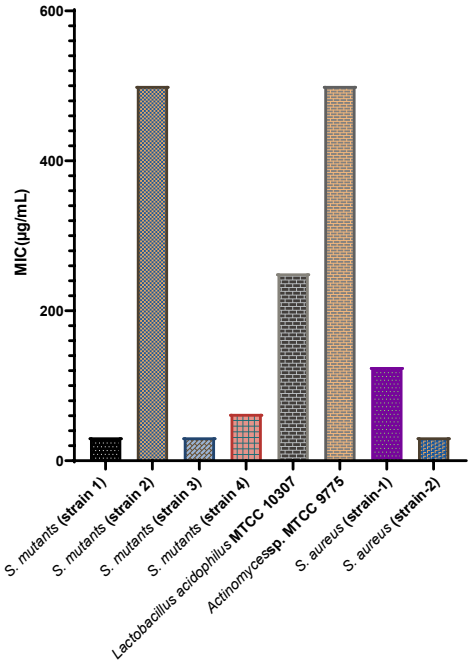

Figure 3: MIC value of ethanolic extracts of *Salvadora persica*.

persica pieces have strong activity against the microorganisms related with the periodontitis and dental caries when the pieces are suspended above the agar plate or embedded in agar.

A study by Al-Bayati et al. [32] on the effect of *Salvadora persica* aqueous and ethanol extracts on the seven dental pathogens viz. *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus mutans, Lactobacillus acidophilus, Enterococcus faecalis, Actinomyces* sp., *and Candida albicans* showed an impressive result in finding that the aqueous extract inhibited the growth of all of the selected pathogens whereas the ethanolic was ineffective against *Lactobacillus acidophilus* and *Actinomyces* sp. The aqueous extract showed the most potential activity against *Enterococcus faecalis*. The antifungal activity was almost equal in the case of both the extracts against *Candida albicans* when analyzed by turbidity tests [32]. Another study by Chelli-Chentouf et al. [33] on the activity of *Salvadora persica* extract on selected bacteria showed the highest inhibition against *E. coli*. In this case, the more potential activity by the ethanolic extract was against Gram-negative (6.5-12 mm) bacteria when compared with the Gram-positive (1–8 mm) bacteria. This could be explained by the variation in the cell wall composition, including the amount of lipopolysaccharides (LPS) layer [34]. Also, this LPS layer makes Gram-negative bacteria comparatively more resistant to antimicrobial compounds. The efficiency of the *Salvadora persica* ethanolic extract on aerobic bacteria of the oral cavity was also affirmed by Al Sadhan et al. [35]. They could find that *Streptococcus mutans* was suppressed in a greater extent than the *Lactobacillus* sp. The phytochemical analysis result of this investigation is also supported by the findings of many other studies. Several other investigations suggested the presence of alkaloid-alvadorine, trimethylamine, chlorides, terpenes, vitamin C, carbohydrates, sulfur, glycosides, large amounts of fluoride and silica, small amounts of tannins, saponins, flavonoids, and sterols. The roots and bark of the *Salvadora persica* tree are composed of 27% ash; a high ratio of alkaloids, such as salvadorine and trimethyl amine; chlorides and fluorides; moderate concentrations of silica, sulfur, vitamin C; and small quantities of tannins, flavonoids, saponins and sterols [36-39].

The antibacterial activity of *Salvadora persica* is supposed to be due to the combined activity of a number of compounds present. According to Vahabi et al. [40] it is due to a combined effect of tannins, chlorides, salvadorine, trimetylamine, thiocynate, nitrates and sulphor. The alkaline compound '*salvadorine*' present in *Salvadora persica* is thought to have an antimicrobial effect apart from gingival stimulation [37]. Al-Bayati et al. [32] reported that the antibacterial effect can also be compounds like tannins, alkaloids, saponins and terpenoids. Also, the clinically detectable gingivitis may be reduced by the astringent effect of tannins by their glucosyl transferase activity, thereby reducing plaque and gingivitis [41]. According to Al-Bagieh et al. [42] the high sulfate content of *Salvadora persica* had an inhibitory effect on the growth of *Candida albicans*. The low, acidic 5.5 pH of *Salvadora persica* extract can also disturb the formation of anitrophile flora. According to Guiraud et al. [43] *Staphylococcus* sp., *Enterobacteriaceae* and *Enterococci* were found it difficult to grow

in a medium with acidic pH. Nevertheless *Streptococcus* sp. and *Staphylococcus* sp. can grow at this pH. In an interesting study, Lemos et al. [44] could understand that the virulence of *Streptococcus mutans* resides in three significant attributes: tooth surface biofilms farming ability, ability to produce organic acids and the ability to tolerate environmental stresses, including low pH. The promoter effect of components in the extracts of *Salvadora persica* was hypothesized by Darout et al. [45] which lead to possible antimicrobial activities. Also, eugenol exerts antibacterial, anti-inflammatory and local anaesthetic effects on the dental pulp [46]. Sofrata et al. [47] identified a volatile compound, the isothiocyanate. Root extracts of *Salvadora persica* as well as commercial synthetic benzyl isothiocyanate exhibited rapid and strong bactericidal effects against oral pathogens involved in periodontal diseases, such as *Porphyromonas gingivalis* (ATCC 33277) and *Aggregatibacter actinomycetemcomitans* HK 1519, as well as against other Gram-negative bacteria (*Escherichia coli)* laboratory strain MC4100 and *Escherichia coli* K12 LPS-mutant strain D21f2). On the other hand, Gram-positive bacteria, such as *Lactobacillus acidophilus* (NCTC 1723) and *Streptococcus mutans* (CCUG 27624) mainly displayed growth inhibition or remained unaffected. Many studies have been carried out on different types of chewing sticks and focused primarily on the antimicrobial activity of those sticks.

CONCLUSION

Our present study, which analyzed the activity of ethanol extract of *Salvadora persica* root on selected root canal bacteria finds that *Salvadora persica* the most widely used chewing stick, has the potential to have an antibacterial effect on root canal bacteria and its repeated use can provide good oral health. Several descriptive studies conducted by various researchers worldwide also confirm the results of the investigation. The detailed studies on phytochemical constituents responsible for this activity of these potential compounds are required. In any case, the *Salvadora persica* miswak can be recommended for complete oral health care in the form of toothbrush, toothpick, or mouth wash because of its effectiveness, neutrality, availability, simplicity and inexpensive nature.

ACKNOWLEDGEMENT

The authors are grateful to the Deanship of Scientific Research, Prince Sattam bin Abdulaziz University, Al-Kharj, Saudi Arabia for its support and encouragement in conducting the research and publishing this report.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

REFERENCES

1. Eid SA, Khattab NM, Elheeny AA. Untreated dental caries prevalence and impact on the quality of life among 11 to14-year-old Egyptian schoolchildren: A cross-sectional study. BMC Oral Health 2020; 20:1-1.
2. Duque AD, Malheiros Z, Stewart B, et al. Strategies for the prevention of periodontal disease and its impact on general health in Latin America. Section III: Prevention. Br Oral Rese 2020; 34.
3. Chelli-Chentouf N, Meddah AT, Mullié C, et al. In vitro and in vivo antimicrobial activity of Algerian Hoggar *Salvadora persica L*. extracts against microbial strains from children's oral cavity. J Ethnopharmacol 2012; 144:57-66.
4. Kubo I, Muroi H, Himejima M. Antimicrobial activity of green tea flavor components and their combination effects. J Agr Food Chem 1992; 40:245-248.
5. Qiu W, Zhou Y, Li Z, et al. Application of antibiotics/ antimicrobial agents on dental caries. BioMed Res Int 2020; 2020.
6. Tredwin CJ, Scully C, Bagan-Sebastian JV. Drug-induced disorders of teeth. J Dent Res 2005; 84:596-602.
7. An TD, Pothiraj C, Gopinath RM, et al. Effect of oil-pulling on dental caries causing bacteria. African J Microbiol Res 2008; 2:63-66.
8. Halawany HS. A review on miswak (*Salvadora persica*) and its effect on various aspects of oral health. Saudi Dent J 2012; 24:63-69.
9. Wu CD, Darout IA, Skaug N. Chewing sticks: Timeless natural toothbrushes for oral cleansing. J Periodont Res 2001; 36:275-284.
10. http://worldental.org/oralhygiene/importance-of-herbs-for-dental-health/121
11. Elvinlewis M, Hall JB, Adututu M, et al. The dental-health of chewing-stick users of Southern Ghana-Preliminary findings. J Preventive Dent 1980; 6:151-159.
12. Elvin-Lewis M. The therapeutic potential of plants used in dental folk medicine. Tropical Dent J 1982; 5:107-117.
13. Mirkamandar E, Shakibaie MR, Adeli S, et al. In vitro antimicrobial activity of *Salvadora persica* extract on *Helicobacter pylori* strains isolated from duodenal ulcer biopsies. Microbiol Res 2012; 3:38-41.
14. Khoory T. The use of chewing sticks in preventive oral

hygiene. Clinical preventive dentistry. 1983; 5:11-14.

15. Ezoddini-Ardakani F. Efficacy of Miswak (*salvadora persica*) in preventing dental caries. Health 2010; 2:499.
16. Amoian B, Moghadamnia AA, Barzi S, et al. *Salvadora persica* extract chewing gum and gingival health: Improvement of gingival and probe-bleeding index. Complementary Therapies Clin Practice 2010; 16:121-123.
17. Khan M, Alkhathlan HZ, Khan ST. Antibiotic and antibiofilm activities of *Salvadora persica L*. essential oils against *Streptococcus mutans*: A detailed comparative study with chlorhexidine digluconate. Pathogens 2020; 9:66.
18. Jassoma E, Baeesa L, Sabbagh H. The antiplaque/ anticariogenic efficacy of *Salvadora persica* (Miswak) mouth rinse in comparison to that of chlorhexidine: A systematic review and meta-analysis. BMC Oral Health 2019; 19:1-4.
19. Lai G, Li M. The possible role of *Candida albicans* in the progression of dental caries. Int Res J Microbiol 2011; 2:504-506.
20. Munson MA, Banerjee A, Watson TF, et al. Molecular analysis of the microflora associated with dental caries. J Clin Microbiol 2004; 42:3023-3029.
21. Chhour KL, Nadkarni MA, Byun R, et al. Molecular analysis of microbial diversity in advanced caries. J Clin Microbiol 2005; 43:843-849.
22. Corby PM, Lyons-Weiler J, Bretz WA, et al. Microbial risk indicators of early childhood caries. J Clin Microbiol 2005; 43:5753-5759.
23. Aas JA, Griffen AL, Dardis SR, et al. Bacteria of dental caries in primary and permanent teeth in children and young adults. J Clin Microbiol 2008; 46:1407-14017.
24. Salehi B, Kregiel D, Mahady G, et al. Management of *Streptococcus mutans-Candida spp*. Oral biofilms Infections: Paving the way for effective clinical interventions. J Clin Med 2020; 9:517.
25. Peters BM, Jabra-Rizk MA, Graeme AO, et al. Polymicrobial interactions: Impact on pathogenesis and human disease. Clin Microbiol Reviews 2012; 25:193-213.
26. Ahmad A, Alkarkhi AF, Hena S, et al. Extraction, separation and identification of chemical ingredients of *Elephantopus scaber L*. using factorial design of experiment. Int J Chem 2009; 1:36.
27. Bauer AT. Antibiotic susceptibility testing by a standardized single disc method. Am J Clin Pathol 1966; 45:149-158.
28. Kloucek P, Polesny Z, Svobodova B, et al. Antibacterial screening of some Peruvian medicinal plants used in Calleria District. J Ethnopharmacol 2005; 99:309-312.
29. Almas K. The antimicrobial effects of extracts of *Azadirachta indica (Neem)* and *Salvadora persica (Arak)* chewing sticks. Indian J Dent Res 1999; 10:23-26.
30. Almas K, Al-Zeid Z. The immediate antimicrobial effect of a toothbrush and miswak on cariogenic bacteria: A clinical study. J Contemporary Dent Practice 2004; 5:105-114.
31. Sofrata AH, Claesson RL, Lingström PK, et al. Strong antibacterial effect of miswak against oral microorganisms associated with periodontitis and caries. J Periodontol 2008; 79:1474-1479.
32. Al-Bayati FA, Sulaiman KD. In vitro antimicrobial activity of *Salvadora persica L*. extracts against some isolated oral pathogens in Iraq. Turkish J Biol 2008; 32:57-62.
33. Chelli-Chentouf N, Meddah AT, Mullié C, et al. In vitro and in vivo antimicrobial activity of *Algerian Hoggar Salvadora persica L*. extracts against microbial strains from children's oral cavity. J Ethnopharmacol 2012; 144:57-66.
34. Leclerc D, Asselin A. Detection of bacterial cell wall hydrolases after denaturing polyacrylamide gel electrophoresis. Can J Microbiol 1989; 35:749-753.
35. Al-Sadhan R, Almas K. Miswak (chewing stick): A cultural and scientific heritage. Saudi Dent J, 1999; 11:80-87.
36. Al-Lafi T, Ababneh H. The effect of the extract of the miswak (chewing sticks) used in Jordan and the Middle East on oral bacteria. Int Dent J 1995; 45:218-222.
37. Almas K. Miswak (chewing stick) and its role in oral health. Post Graduate Dent 1993; 3:214-218.
38. Akhtar M, Ajmal M. Significance of chewing sticks (miswaks) in oral hygiene from a pharmacological view point. J Pak Med Assoc 1981; 31:89-95.
39. Farooqi MI, Srivastava JG. The tooth-brush tree (*Salvadora persica*). Quarterly J Crude Drug Res 1968; 8:1297-1299.
40. Vahabi S, Najafi E, Alizadeh S. In vitro antimicrobial effects of some herbal essences against oral pathogens. J Med Plants Res 2011; 5:4870-4878.
41. Gazi M, Davies T, Al-Bagieh N, et al. The immediate and medium-term effects of Meswak on the composition of mixed saliva. J Clin Periodontol 1992; 19:113-117.
42. Al-Bagieh NH, Idowu A, Salako NO. Effect of aqueous extract of miswak on the in vitro growth of *Candida albicans*. Microbios 1994; 80:107-113.
43. Guiraud JP. Microbiologie Alimentaire: Paris Dunod 1998; 652.
44. Lemos JA, Burne RA. A model of efficiency: Stress tolerance by *Streptococcus mutans*. Microbiol 2008; 154:3247-3255.
45. Darout IA, Albandar JM, Skaug N, et al. Salivary microbiota levels in relation to periodontal status, experience of caries and miswak use in Sudanese adults. J Clin Periodontol 2002; 29:411-420.
46. Goyal M, Sasmal D, Nagori BP. *Salvadora persica L*. (miswak) chewing stick for complete oral care. Int J Pharmacol 2011; 7:440-445.
47. Sofrata A, Santangelo EM, Azeem M, et al. Benzyl isothiocyanate, a major component from the roots of *Salvadora persica* is highly active against gram-negative bacteria. PLoS ONE 2011; 6:1-10.