

Research Article

## Antimicrobial Activity of Indigenous Chewing Stick *Salvadora persica* against Oral Pathogens

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### Abstract

The study investigated the antimicrobial potency of *Salvadora persica* extracts against selected oral pathogens. Root and stem parts of the plant with ethanol and methanol solvents were used for extraction. Three pure bacterial isolates *Streptococcus pyogenes*, *Streptococcus mutans* and *Staphylococcus aureus* were used to test the resistance against the extract. Disk diffusion method using antibacterial susceptibility disks was applied to note the zone of inhibition. The results showed that both root and stem extracts were effective against all tested bacterial pathogens. As the concentration of the extracts increased from 0.2 g/L to 0.8 g/L and the time of incubation from 24 to 48 h, the potency of the extract becomes effective for both extracts. The largest mean zone of inhibition (29.17 mm) was recorded by methanolic root extract against *Streptococcus mutans* at 48 h of incubation period on the highest concentration. Similarly, the least mean zone of inhibition (11.67 mm) and (12.66 mm) were recorded by methanol root extracts and ethanol stem extracts against *Streptococcus pyogenes* within 24 h respectively. Thus, both root and stem plant parts are effective in curing oral bacterial pathogens and it can be used as a mouth rinse and preventive measure against tooth decay.

**Keywords:** *Salvadora persica*, oral pathogens, susceptibility test, tooth decay.

### Introduction

For thousands of years, human beings have been using plant source to treat illnesses since plants contain novel chemical compounds which have a potential use in medicine and other applications. Plants contain many active compounds such as phenols and flavonoids, which are deposited in their specific parts like leaves, flowers, bark, seeds, fruits, roots, etc. The medicinal effects of plant materials come from the combination of these secondary products (Tonhubthithong *et al.*, 2001). Different researchers showed that phytochemicals are used as antioxidants, antibacterial, antifungal, antidiabetic, antiinflammatory, antiarthritics and radioprotective activity (Kumar *et al.*, 2008a,b; Wong *et al.*, 2009). Tooth decay is one of the most common chronic infections in the world and major problem of oral health conditions in developing countries, affecting 60-90% of the school children (Patro *et al.*, 2008). Thus, different kinds of plants have played an important role in oral hygiene for many decades. Among these plants, *Salvadora persica* belongs to the Salvadoraceae family which grows in a different area of the world such as the Middle East and Africa for keeping oral hygiene (Sher *et al.*, 2011). It contains important phytoconstituents such as vitamin C, salvadorine, salvadorurea, alkaloids, trimethylamine, cyanogenic glucosides, tannins, saponins and salts mostly as chlorides (Alai *et al.*, 2004).

The stick of this plant is used against fungal and bacterial infections (Almas and Al-Zeid, 2004). The traditional use of *Salvadora persica* as an antimicrobial stick toothbrush for oral hygiene and to treat gum inflammation is a century old practice and a part of the Greek Arab system of medicine (Sher *et al.*, 2011). Similarly, many rural communities in most African countries including Ethiopia still depend mainly on locally available plants for oral hygiene. *Salvadora persica* is the main plant species used as chewing sticks for the mechanical removal of plaque from the surface of the teeth in Ethiopia particularly Tigray and Afar region. However, many chewing stick users in different parts of Tigray believed that these sticks are known to clean decay teeth and making the teeth whiter than promoting oral health and hygiene. This is because less scientific evidence about *S. persica* in promoting oral health, hygiene in the area. Therefore, it is possible to use the plant as an alternative to antibiotics for treating oral pathogens by extracting phytochemicals and active elements from this plant. In this regard, the study was focused on examining the antimicrobial potential of *S. persica* against selected oral infection-causing bacterial strains.

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## Materials and methods

**Collection of plant materials:** Plant materials (*Salvadora persica*) were collected from different locations of Northeast Tigray (Kolla Temben district), Ethiopia. It was taken to botany laboratory, Biology department of Mekelle University for identification, preparation, and separation of each plant parts (roots, sticks, and leaves).

**Preparation and extraction of plant materials:** Each part of the plant was separated into roots, leaf and stem. The root and stem of the plant were then cut and chopped into small pieces using scissors and hammer separately. The prepared plant materials were then transferred to the drying cabinet set at 75°C for 20-30 min for drying before being reduced to fine powder with the aid of a mechanical grinder. Each dried plant materials were grinded into fine powders. The fine powders were separated from the larger by sieving using a fine sieve having 0.5 mm pore size and stored in polyethylene bags. For each plant material, two different solvents (ethanol and methanol) were used for the extraction of the essential oils. About 30 g of each plant powder was dissolved and soaked in 300 mL of each solvent and kept for 72 h with repeated stirring, after which the resulting solutions were filtered using Whatman No. 1 filter paper. The extracts were finally rotary dried using vacuum evaporator to obtain the concentrate. A 3 g/L solution of each extract was prepared and fractionated into 0.2, 0.3, 0.7 and 0.8 g/L concentrations for the bioassay.

**Bacterial strains:** A total of three pure bacterial strains, *Streptococcus pyogenes* and *Staphylococcus aureus* were obtained from Mekelle University, Ayder Referral Hospital (ARH), School of Dentistry, Department of Medical Microbiology isolated from patients with dental disease and a *Streptococcus mutans* was obtained from veterinary medicine college, Department of microbiology. The collected pure isolates were maintained in slants of nutrient broth and stored in a refrigerator at 4°C for further use.

**Antimicrobial assay and Susceptibility test:** Antimicrobial activity was evaluated by noting the zone of inhibition against the test organisms. The colonies of a 24 h plate culture of each organism were transferred aseptically into sterile normal saline in a test tube and mixed thoroughly for uniform distribution in the nutrient broth. Nutrient agar was prepared for bacterial susceptibility tests of each extract. From each bacterial inoculums, 1 mL of each bacterial samples were transferred into nutrient agar medium using disk diffusion method and spread using a sterile cotton swab on the surface of nutrient agar (Eloff, 1998). Finally, antibacterial susceptibility disks were used to note the zone of inhibition and zone of inhibition for each extract measured within 24 and 48 h interval.

**Data analysis:** The data were analyzed using SPSS, version 19.0 and ANOVA test were also employed to determine the significant difference between the extracts.

## Results

The antimicrobial effect of various concentrations of *Salvadora persica* against *Streptococcus pyogenes*, *S. mutans* and *Staphylococcus aureus* was examined. The findings indicated that different concentrations of both methanol and ethanol extracts of the chewing sticks exhibited growth inhibition against all the tested bacterial strains (Table 1-4). It showed highly significant action ( $P < 0.05$ ) in all concentrations except methanol extracts of stem at 0.2 g/L concentration which showed non-significant value ( $P > 0.05$ ). The highest antimicrobial activity in all organisms was recorded from root extracts than in the stem extract in all concentrations. Similarly, the effect of the extracts also increased as the incubation period increased from 24 to 48 h for both extracts against all the tested bacterial strains. The statistical analysis also showed the inhibitory action of methanol extracts of the chewing sticks was more pronounced on *Streptococcus mutans* than *Staphylococcus aureus* and *Streptococcus pyogenes*.

## Discussion

According to a study by Abdul et al. (2010), chewing sticks are commonly used in counties like Jordan, Saudi Arabia and Emirates in particular and the Middle East, Asia, and Africa in general, in addition to many other areas for oral hygiene, religious and social purposes. Thereby, the use of chewing sticks has been recommended by the world health organization (WHO) as an effective tool for oral hygiene (Cai et al., 2000) or dried and kept for later use. Hence, the present research finding showed that *Salvadora persica* stem and root plant extracts were effective against selected oral pathogens. Similar findings were reported by Alireza et al. (2014), Mohammed, (2013) and Chelli-Chentouf et al. (2012). The findings of the study revealed that methanol and ethanol extracts showed antibacterial potency for all tested bacterial strains and the inhibition zone increased when the concentration of the extracts increased from 0.2 g/L to 0.8 g/L (Table 1-4). This conformed to the work by Abdul et al. (2010) and Mohammed et al. (2016). The highest zone of inhibition (29.17 mm) was recorded on *Streptococcus mutans* by root extract within 48 h of incubation period and 0.8 g/L concentration. This result was agreed with the finding of Abdul et al. (2010) and Almas and Al-Zeid, (2004). The different reactions and strength of each strain to various extracts were likely due to each solvent extracted different chemical components of *S. persica*. *Streptococcus mutans* for root extracts than *Staphylococcus aureus* and *Streptococcus pyogenes*. This finding was also indicated in the work of Almas and Al-Zeid, (2004); Abdul et al. (2010) and Howalda et al. (2002).

Table 1. Zone of Inhibition (Mean  $\pm$  SD) of the test organism to methanolic extracts of *Salvadora persica* root.

Test organisms	Incubation period (h)	0.2 g/L	0.3 g/L	0.7 g/L	0.8 g/L
<i>S. pyogenes</i>	24	15.17 $\pm$ 0.70	16.33 $\pm$ 0.49	17.50 $\pm$ 0.42	21.17 $\pm$ 0.30
	48	17.50 $\pm$ 0.42	19.00 $\pm$ 0.25	20.17 $\pm$ 0.30	20.17 $\pm$ 0.40
<i>S. Mutans</i>	24	19.5 $\pm$ 0.92	21.33 $\pm$ 0.98	23.00 $\pm$ 1.09	25.17 $\pm$ 1.16
	48	25.17 $\pm$ 1.22	25.17 $\pm$ 1.40	26.83 $\pm$ 1.30	29.17 $\pm$ 1.81
<i>S. aureus</i>	24	11.50 $\pm$ 0.88	13.83 $\pm$ 0.79	15.00 $\pm$ 1.03	16.17 $\pm$ 0.79
	48	14.50 $\pm$ 0.76	16.00 $\pm$ 0.63	16.83 $\pm$ 0.60	17.33 $\pm$ 0.49

Table 2. Zone of Inhibition (Mean  $\pm$  SD) of the test organism to ethanolic extracts of *Salvadora persica* root.

Test organisms	Incubation period (h)	0.2 g/L	0.3 g/L	0.7 g/L	0.8 g/L
<i>S. pyogenes</i>	24	11.67 $\pm$ 1.22	13.17 $\pm$ 0.87	15.67 $\pm$ 0.49	17.50 $\pm$ 0.42
	48	14 $\pm$ 0.57	15.17 $\pm$ 0.47	17.00 $\pm$ 0.44	18.00 $\pm$ 0.96
<i>S. Mutans</i>	24	15.67 $\pm$ 1.14	17.33 $\pm$ 1.3	19.5 $\pm$ 0.67	21.33 $\pm$ 0.49
	48	20.33 $\pm$ 0.49	22.17 $\pm$ 0.47	25.17 $\pm$ 1.22	26.5 $\pm$ 1.38
<i>S. aureus</i>	24	10.00 $\pm$ 0.57	11.33 $\pm$ 0.42	13.33 $\pm$ 0.42	14.00 $\pm$ 0.36
	48	13.33 $\pm$ 0.42	14.67 $\pm$ 0.33	16.17 $\pm$ 0.47	17.17 $\pm$ 0.40

Table 3. Zone of Inhibition (Mean  $\pm$  SD) of the test organism to methanolic extracts of *Salvadora persica* stem.

Test organisms	Incubation period (h)	0.2 g/L	0.3 g/L	0.7 g/L	0.8 g/L
<i>S. pyogenes</i>	24	14.67 $\pm$ 0.06	15.33 $\pm$ 0.06	17.33 $\pm$ 0.06	18.33 $\pm$ 0.06
	48	17.00 $\pm$ 0.1	17.67 $\pm$ 0.15	19.33 $\pm$ 0.15	20.33 $\pm$ 0.06
<i>S. Mutans</i>	24	17.00 $\pm$ 0.1	18.33 $\pm$ 0.06	20.00 $\pm$ 0.1	20.66 $\pm$ 0.15
	48	17.00 $\pm$ 0.1	18.33 $\pm$ 0.06	19.00 $\pm$ 0.1	19.66 $\pm$ 0.12
<i>S. aureus</i>	24	16.00 $\pm$ 0.1	18.33 $\pm$ 0.12	19.33 $\pm$ 0.05	20.33 $\pm$ 0.15
	48	17.00 $\pm$ 0.1	19.00 $\pm$ 0.1	20.00 $\pm$ 0.1	20.33 $\pm$ 0.06

Table 4. Zone of Inhibition (Mean  $\pm$  SD) of the test organism to ethanolic extracts of *Salvadora persica* stem.

Test organisms	Incubation period (h)	0.2 g/L	0.3 g/L	0.7 g/L	0.8 g/L
<i>S. pyogenes</i>	24	12.66 $\pm$ 0.15	13.67 $\pm$ 0.21	15.00 $\pm$ 0.17	15.33 $\pm$ 0.21
	48	14.33 $\pm$ 0.15	15.67 $\pm$ 0.05	17.00 $\pm$ 0.17	18.00 $\pm$ 0.1
<i>S. Mutans</i>	24	15.33 $\pm$ 0.12	17.00 $\pm$ 0.10	18.33 $\pm$ 0.12	19.00 $\pm$ 0.10
	48	14.66 $\pm$ 0.21	16.00 $\pm$ 0.30	16.67 $\pm$ 0.25	17.67 $\pm$ 0.23
<i>S. aureus</i>	24	14.66 $\pm$ 0.06	15.67 $\pm$ 0.12	16.67 $\pm$ 0.15	17.33 $\pm$ 0.15
	48	16.33 $\pm$ 0.12	18.00 $\pm$ 0.10	19.67 $\pm$ 0.12	20.00 $\pm$ 0.1

As the data revealed, at all concentrations of the methanol extracts, closely related results and effect on *S. mutans* within 48 h incubation period was recorded. These results were in line with many studies that showed the antimicrobial activity of *S. persica* extract against different bacteria (Claesson *et al.*, 2008) and (Lika *et al.*, 2014). However, for both extracts, the minimum zone of inhibition was obtained at the lowest concentration *S. persica* extracts (0.2 g/L and 0.3 g/L) within 24 h incubation period. These results were in agreement with previous findings by Abdul *et al.* (2010), Lika *et al.* (2014), Mohammed *et al.* (2016) and Adel and Slalh (2012).

On the other hand, the least difference in the effect of incubation period was also recorded for the *S. pyogenes*, however, the concentration of the extract showed a marked inhibition difference from low to a high concentration. This was also true for *S. aureus* only for methanol extracts for both incubation periods, while concentration 0.7 g/L and 0.8 g/L ethanol extract showed similar inhibition response with methanol extract at 48 h incubation period. Moreover, the least mean of the zone of inhibition (11.67 mm) and (12.66 mm) were also measured at methanolic root extracts and ethanol stem extracts against *S. pyogenes* within 24 h of incubation period at 0.2 g/L concentration respectively. The highest antimicrobial activity in all organisms was recorded by root extracts than stems in all concentrations.

## Conclusion

The results of the study showed both root and stem extracts of *Salvadora persica* using methanol and ethanol solvents were effective against the tested bacterial strains. The best antibacterial performance was observed from the root extracts than the stem both at the low and highest concentration of the extract and its inhibitory effect was also increased when the time of incubation is increased from 24 to 48 h. On the other hand, the extracts were more effective against *Streptococcus mutans* than the other tested bacterial strains.

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