

Comparative Study of *In Vitro* Antibacterial Activity of Miswak Extracts and Different Toothpastes

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ABSTRACT

This study determines the antimicrobial efficacy of miswak (aqueous and alcoholic) extracts comparing with five different toothpastes, which are available in Iraqi market, against different kinds of bacteria. The antibacterial efficacy of aqueous and alcoholic extracts of *Salvadora persica* and different kinds of toothpastes was studied against different cariogenic and pathogenic bacteria including *Streptococcus mutans*, *Lactobacillus acidophilus*, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* by well diffusion method. *Salvadora persica* extracts and toothpastes exhibited the greatest antimicrobial activities towards *Staphylococcus aureus*, *Streptococcus mutans*, *Lactobacillus acidophilus*, *E. coli* and *Pseudomonas aeruginosa* respectively. Alcoholic extract was dominant on aqueous extract in inhibiting the tested microorganisms. Toothpastes A were dominant comparing with the other toothpastes in inhibiting the tested microorganisms. The impact of toothpastes was almost more than *Salvadora persica* extracts on tested microorganisms. According to the good efficacy of *Salvadora persica* extracts on the tested microorganisms, it has antibacterial effects and could be used as a therapeutic agent and therefore, it appears to be a potent antimicrobial agents that could be considered as a medicinal plant.

Keywords: Miswak, *Salvadora Persica*, Chewing Sticks, Toothpastes *Streptococcus Mutans*, Antimicrobial Activity

1. INTRODUCTION

Plants are more important in human's life and fulfill his every day's needs. They are used as cosmetic, food, flavors, ornamental and medicine (Syam *et al.*, 2008). Medicinal plants have become part of complementary medicine worldwide, because of their potential health benefits (Gomez-Flores *et al.*, 2006). The use of medicinal plant as the first medicines is a universal phenomenon (Seyydneyad *et al.*, 2010).

Various plant extracts have been widely used in the past to cure infectious diseases and healing of wounds. Plants have great potential against infectious agents and can be used for therapeutic purposes (Upadhyay *et al.*, 2010).

Miswak (*Salvadora persica*) is one of the most commonly used medicinal plants for oral hygiene among global Muslim community (Sher *et al.*, 2010).

The name Miswak, also called miswaak, siwak, siwaki depending on the Arabic dialect and the country, is known in English as the natural toothbrush (Elvin-Lewis, 1980; Hattab, 1997; Al-Sadhan and Almas, 1999).

The Miswak (miswaak, siwak) is a natural toothbrush made from the twigs of the *Salvadora persica* tree (Arak). Miswak was used by the Babylonians some 7000 years ago; they were later used throughout the Greek and Roman empires and have been used by Jews, Egyptians and in the Islamic empires. It is believed that this

precursor to the modern day toothbrush was used in Europe until about 300 years ago. Today, Miswak is being used in Africa, South America, Asia, the Middle East including Saudi Arabia and throughout the Islamic countries (Kirtikar and Basu, 1996; Bhandari, 1990).

Extracts from *Salvadora persica* showed antibacterial activity against *Streptococcus mutans* and plaque control (Al-Lafi and Ababneh, 1995; Khalessi *et al.*, 2004; Al-Otaibi *et al.*, 2004; Almas *et al.*, 2005).

The use of this stick as an effective tool for oral hygiene has been recommended and encouraged by the World Health Organization (WHO, 1987).

Many of the world populations including India, Pakistan, several African countries, the Arab countries and most of the Muslim world still use miswak (Corbet *et al.*, 2000).

Various components of *Salvadora persica* have been reported to have beneficial biological properties, including significant antibacterial and antifungal activity (Al-Bagieh *et al.*, 1994; Al-Lafi and Ababneh, 1995; Almas *et al.*, 1997). Furthermore, extracts from miswak are reported to be effective against some periodontal pathogens and other bacteria that are important during development of dental plaque (Rotimi and Mosadomi, 1987; Almas, 2001; Poureslami *et al.*, 2007; Sofrata *et al.*, 2007).

Chemically, *Salvadora Persica* through chemical studies showed that it is composed of trimethyl amine, salvadorine, chlorides, high amounts of fluoride and silica, sulphur, vitamin C, small amounts of tannins, saponins, flavonoids and sterols (Darout *et al.*, 2000; Alali and Al-Lafi, 2003)

Although the wide use of miswak in many countries, in Iraq it has not received any attention. Therefore, the aim of this study is to compare the antimicrobial activity of miswak extracts with five toothpastes against different species of bacteria using well diffusion assay.

2. MATERIALS AND METHODS

2.1. Plant Material

Collection of plant materials: *Salvadora persica* chewing sticks were purchased from the local market of Mecca-Kingdom of Saudi Arabia.

2.2. Toothpastes

Five brands of toothpastes were purchased from local market of Basra city-Iraq. Their ingredients are shown in **Table 1**.

2.3. Preparation of *Salvadora Persica* Extracts

Preparation of alcoholic extract of miswak was carried out by taking 800 g of *Salvadora Persica* chewing sticks and cutting them with a sharp knife. The resulting pieces of *Salvadora persica* were ground to a powder with a commercially available food blender. 120 mL of 60% ethanol was added to 40 g of powder in a sterile well capped flask, left for 3 days at room temperature and then filtered using number 1 filter paper. The extract then evaporated in a rotary evaporator at 40°C until ethanol removing. The extract stored in sterile screw-capped vials in the refrigerator until needed (Al-Koubaisi, 2001; Darmani *et al.*, 2003).

Table 1. Ingredients of various toothpastes tested for its antimicrobial activity

Toothpastes	Ingredients as listed on packages
A	Aqua, Sorbitol, Hydrated Silica, PEG-6, Sodium Lauryl Sulfate, Tetrapotassium Pyrophosphate, Disodium Pyrophosphate, Tetrasodium Pyrophosphate, Aroma, Cellulose Gum, Xanthan Gum, Sodium Fluoride, Carbomer, Sodium Saccharin, Triclosan, CI 77891, Glycerin, Limonene, CI 74160 Contains sodium fluoride (0.321%)
B	Precipitated calcium carbonate, sorbitol, silica, aluminum hydroxide, carrageenan, CMC, fluoride, saccharine, sodium lauryl sulphate, sodium monofluorophosphate, flavor, water.
C	Sorbitol, Hydrated Silica, PEG-8, Sodium Lauryl Sulfate, Cellulose Gum, Dicalcium phosphate dehydrate, flavor, CI 77891, Sodium Fluoride, Sodium Saccharin, Propyl Paraben Sodium.
E	Calcium Carbonate, Aqua, Sorbitol, Hydrate Silica, Sodium Lauryl Sulfate, Sodium Monofluorophosphate, Aroma, Cellulose Gum, Potassium Citrate, Trisodium Phosphate, Sodium Saccharine, Calcium Glycerophosphate, Phenylcarbinol, Glycerin, Limonene, CI 12490
D	Sorbitol, Hydrated Silica, PEG-12, Sodium Lauryl Sulfate, Tetrasodium Pyrophosphate, Cellulose Gum, Sodium Lauryl Sulfate, Flavor, Sodium Saccharine, Sodium Fluoride, limonene, CI 16035, CI 17200.

To prepare the aqueous extract, *Salvadora persica* chewing sticks were cut into small pieces and ground to powder form in a ball mill. The powder was weighed into 10 gm portions and placed in a sterile screw capped bottle to which 100 mL of sterile deionized distilled water was added. The extract was allowed to soak for 48 h at 4°C before the mixture was centrifuged at 2000 rpm for 10 min (Al-Lafi and Abadneh, 1995). The supernatant was passed through a 0.45 mm membrane filter. The extract stored in sterile screw-capped vials in the refrigerator until needed.

2.4. Microorganisms

Streptococcus mutans and *Lactobacillus acidophilus* were clinical isolates, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* were obtained from the Department of Biology, College of Science, University of Basrah, Iraq.

2.5. Antimicrobial Assay

The antimicrobial activity of *Salvadora persica* extracts and toothpastes was determined according to Chrkraborty (1996). Nutrient agar poured in Petri dish. 0.1 mL (containing approximately 10^5 bacteria) of *Streptococcus mutans*, *Lactobacillus acidophilus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* separately spreaded and a cork borer was used to make bore (5×5 mm in diameter) on the surface. After surface solidification, 0.1 mL of *Salvadora persica* extracts and toothpastes was added to each bore. Plates were incubated in anaerobic jar at 37°C for 18-24 h. The antimicrobial activity of *Salvadora persica* extracts and toothpastes against *Streptococcus mutans*, *Lactobacillus acidophilus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* was determined by measuring the inhibition zones around each bore.

2.6. Phytochemical Analysis

Salvadora persica extracts were subjected to preliminary phytochemical analysis to find the presence of the following constituents: flavonoids, sterols,

saponins, tannins, basic alkaloids, Reducing components (Edeoga *et al.*, 2005; Egwaikhide and Gimba, 2007).

3. RESULTS

Antibacterial activity test showed that alcoholic extract of miswak had growth inhibitory effects on several tested microorganisms more than aqueous extract. Inhibition zone was wide against *Staphylococcus aureus* followed by *Streptococcus mutans*, *Lactobacillus acidophilus*, *E. coli* and *Pseudomonas aeruginosa* respectively for both extracts as shown in **Table 2**. For aqueous extract the inhibition zone ranged between 0 to 4.70 cm, while alcoholic extract zone of inhibition ranged between 3.49 to 6.06 cm.

For toothpastes antibacterial activity, toothpaste A had maximum zones of inhibition against the tested microorganisms compared to all other toothpaste formulations followed by D, C, E and B toothpaste formulations respectively as shown in **Table 3**. Inhibition zone was wide against *Staphylococcus aureus* followed by *Streptococcus mutans*, *E. coli* and *Lactobacillus acidophilus* for most toothpastes. Toothpastes A and E had inhibition zone against *Pseudomonas aeruginosa* while toothpastes B, C and D had no inhibition zone against *Pseudomonas aeruginosa*.

Among all the investigated toothpastes, toothpaste A emerged as the most effective, based on the mean diameter of the zone of microbial inhibition produced by the toothpastes in agar well diffusion method, against all the five microorganisms tested. Its inhibition zones ranged between 4.00 to 7.00 cm.

Table 2. Mean zone of inhibition (cm) of Aqueous and alcoholic extracts of Miswak

Microorganisms	Aqueous extract	Alcoholic extract
<i>Streptococcus mutans</i>	4.45±0.22	4.57±0.33
<i>Lactobacillus acidophilus</i>	4.23±0.13	4.31±0.20
<i>E. coli</i>	3.11±0.55	4.00±0.00
<i>Staphylococcus aureus</i>	4.70±0.67	6.06±0.46
<i>Pseudomonas aeruginosa</i>	0	3.49±0.66

Table 3. Mean zone of inhibition (cm) of toothpastes

Toothpastes	<i>Streptococcus mutans</i>	<i>Lactobacillus acidophilus</i>	<i>E. coli</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>
A	5.50±0.50	5.00±0.60	4.00±0.56	7.00 ±0.00	5.60±0.50
B	3.00±0.00	2.00±0.25	0	4.80±0.22	0
C	5.00±0.34	5.00±0.00	4.00±0.20	5.25±0.30	0
D	5.20±0.10	4.50±0.30	4.30±0.44	5.40±0.00	0
E	4.50±0.35	3.00±0.00	0	5.00±0.00	2.50±0.00

Table 4. Phytochemical constituents of *salvadora persica*

Phytochemicals	Aqueous extract	Alcoholic extract
Flavonoids	-	+
Sterols	-	++
Saponins	+	++
Tannins	+	++
Basic Alkaloids	-	+
Reducing components	+	+

++ = Moderate presence, + = Slight presence, - = Absence

Phytochemical screening results of *Salvadora persica* extracts are shown in **Table 4**. For aqueous extract, results revealed absence of flavonoids, sterols and basic alkaloids, Slight presence of saponins, tannins and reducing components.

For alcoholic extract, results revealed slight presence of flavonoids basic alkaloids and reducing components. Moderate presence of sterols, saponins and tannins.

4. DISCUSSION

Chewing sticks selection in many countries based on many factors. The use of miswak is most common in the Middle East region, it is cheap, readily available in urban and rural areas of the countries. Its taste is agreeable and not unpleasant and reported to have anti-plaque and many other pharmacological properties (Lewis, 1980).

In the present study a variety of gram positive and gram negative bacteria were used in screening antimicrobial activity of aqueous and alcoholic extracts of *Salvadora persica* and different kinds of toothpastes.

The results of the current study clearly demonstrated that aqueous and alcoholic extracts of *Salvadora persica* as well as toothpastes could inhibit the growth of several cariogenic and pathogenic bacteria, however, the effectiveness varied against the different tested microorganisms.

Study results in agreement with (Almas *et al.*, 2005; Darmani *et al.*, 2006) who examined the effects of miswak extracts on the growth of the various cariogenic microorganisms including *Streptococcus mutans*. The result showed inhibition in growth of *Streptococcus mutans*. Al-Bayati and Sulaiman (2008) investigated the aqueous and methanol extracts of *Salvadora persica* for its antimicrobial activities against seven isolated oral pathogens. The aqueous extract inhibited all isolated microorganisms. Sofrata *et al.* (2008) reported antibacterial effect of *Salvadora persica* against oral microorganisms such as *Streptococcus mutans*, *Lactobacillus acidophilus*.

Al-Bayaty *et al.* (2010); Shingare and Chaugule (2011) and Masoumeh *et al.* (2012) had also found the miswak extract as an effective antimicrobial agent which is comparable to study results.

Salvadora persica extracts and toothpastes exhibited the greatest antimicrobial activities (as determined by the diameters of the inhibition zones) towards *Staphylococcus aureus*, *Streptococcus mutans*, *Lactobacillus acidophilus*, *E. coli* and *Pseudomonas aeruginosa* respectively, this result in agree with Al-Lafi and Ababneh (1995) who tested the antibacterial activity of *Salvadora persica* against some oral aerobic and anaerobic bacteria and reported that the extract of these sticks had a drastic effect on the growth of *Staphylococcus aureus* and a variable effect on other bacterial species.

Alcoholic extract was dominant on aqueous extract in inhibiting the tested microorganisms maybe because of its phytochemical constituents comparing with aqueous extract (**Table 4**). This result in agree with (Moustafa *et al.*, 1987; Al-Bagieh and Almas, 1997) who found that ethanol extract of miswak has been shown to have a stronger microbial inhibitory effect on different microorganisms than the aqueous extract and in disagree with Al-Bayati and Sulaiman (2008) who found that aqueous extract of *Salvadora persica* was more effective than alcoholic extract in inhibiting tested bacteria. While toothpastes A was dominant comparing with the other toothpastes in inhibiting of tested microorganisms maybe because it has different components which may inhibited or reduced microorganisms growth.

Phytochemical constituents such as alkaloids, flavonoids, tannins, phenols, saponins and several other aromatic compounds are secondary metabolites of plants that serve a defense mechanism against predation by many microorganisms, insects and other herbivores (Bonjar *et al.*, 2004).

The phytochemical constituents of the selected plants investigated are summarized in **Table 4**. Analysis of *Salvadora persica* extracts revealed the presence of flavonoids, sterols, saponins, tannins, basic alkaloids and reducing components in alcoholic extract and saponins, tannins and reducing components in aqueous extract which could be responsible for the observed antimicrobial property of alcoholic extract in compare with aqueous extract. This result in agree with (Wolinsky and Sote, 1984; Ohtani *et al.*, 1992; Darout *et al.*, 2000; AbdELRahman *et al.*, 2003) who attributed the antimicrobial property of *Salvadora persica* extracts to its different phytochemical constituents.

These bioactive compounds are known to act by different mechanism and exert antimicrobial action.

Flavonoids are hydroxylated phenolic substance known to be synthesized by plants in response to microbial infection and it should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls (Cowan, 1999). Sterols have been reported to have antibacterial properties, the correlation between membrane lipids and sensitivity for steroidal compound indicates the mechanism in which steroids specifically associate with membrane lipid and exerts its action by causing leakages from liposomes (Epanand *et al.*, 2007). Saponin antimicrobial property is due to its ability to cause leakage of proteins and certain enzymes from the cell (Zablotowicz *et al.*, 1996).

Tannins bind to proline rich proteins and interfere with the protein synthesis (Shimada, 2006).

In this study, *Staphylococcus aureus* was found to be sensitive to alcoholic and aqueous extracts. The highest sensitivity of *Staphylococcus aureus* may be due to its cell wall structure and outer membrane (Zaika, 1988).

Study results suggest that gram-positive bacteria are generally more sensitive to the *Salvadora persica* extracts than gram-negative maybe because of the structure of membrane that the gram-positive bacteria are simpler than gram-negative ones. This was consistent with the previous studies on other spices and herbs (Ceylan and Fung, 2004).

Pseudomonas aeruginosa was resistant to aqueous extract and toothpastes B, C and D while *E. coli* was resistant to toothpastes B and E. That probably could be due to cell membrane permeability or due to other genetic factors and this result is supported by (Nazif, 2002; Motamedi *et al.*, 2009).

Finally, the impact of toothpastes was almost more than *Salvadora persica* extracts on tested microorganisms maybe because toothpastes have different components with sufficient concentration to be more effective comparing with *Salvadora persica* extracts.

5. CONCLUSION

The findings suggest that miswak extracts as well as toothpastes have an inhibitory effect on cariogenic and pathogenic bacteria. Toothpastes were almost more effective in inhibiting cariogenic and pathogenic bacteria compared to *Salvadora persica* extracts. Among toothpastes formulations, toothpaste a was found to be more effective than other toothpastes

against the tested microorganisms. Miswak can be a good alternative to the toothpastes since it is inexpensive and readily available. It is available in most rural areas of the poor countries. It does not need expertise or any extra resources to manufacture it. Thus it appears to be a potent antimicrobial agent that could be considered as a medicinal plant.

6. REFERENCES

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