

## In vitro antimicrobial activity of Miswak extracts against some oral pathogenic isolates

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Abdul-Ghany Omer \*

Sozan Muhsin Qarani \*\*

Amera Kamal Khalil \*\*\*

### ABSTRACT

**Background and Objectives:** Siwak, Miswak, or Arak (*Salvadora persica*) belongs to Salvadoraceae and is considered as the most widely used twigs since early times by Babylonians some 7000 years ago, it was later used throughout the Greek and Roman empires, and has also been used by ancient Egyptians and Muslims. The aim of this study was to determine the antibacterial activities of different extracts of Siwak on the growth of different oral isolates of *Streptococcus mutans*, *Staphylococcus aerus* and *Candida albicans*.

**Methods:** Experiments were conducted in the laboratories of the College of Science, University of Salahaddin and College of Dentistry, Hawler Medical University for a period from Jun. 2008 to Feb. 2009, to determine the effects of different Siwak (*Salvadora persica*) extracts at concentrations of 500, 1000, 5000 and 10000 µg/ml on the growth of different oral isolates of *Streptococcus mutans*, *Staphylococcus aureus*, and *Candida albicans*.

**Results:** The results showed that all Siwak extracts (especially Siwak aqueous extract) were effective against *Streptococcus mutans* and *Staphylococcus aureus*. The strongest antibacterial activity was observed using the concentrations of 10000 µg/ml. While Siwak extracts were ineffective against *Candida albicans*.

**Conclusions:** Siwak extracts had significant antibacterial effect against *Streptococcus mutans* and *Staphylococcus aureus*, and ineffective against *Candida albicans*.

**Key words:** Miswak, chewing stick, *Salvadora persica*, cariogenic bacteria.

### INTRODUCTION:

The scientific name of Miswak is *Salvadora Persica*, this plant belongs to the Salvadoraceae family, chemicals such as sodium chloride, calcium oxalate, silica, fluoride, sulfated compounds, vitamin C and tannic acid have been found in this plant. More over, this plant contains saponin, flavonoid, an alkaloid name salvadorini, trimethylamine, an herbal steroid named beta-sitosterol and benzyle isothiocyanate<sup>1</sup>. It is claimed that the vitamin C and sitosterol contents of this plant have great roles in strengthening the gum capillaries and preventing gum inflammation<sup>2</sup>, sulfated compounds and isothiocyanate are known to be responsible for Antibacterial effects

of the plant, while fluoride and calcium salts are effective in preventing dental caries<sup>3</sup>. Trimethylamine is known to be effective in decreasing plaque accumulation and the tannins, tannic acid and benzyl isothiocyanate, are reported to have antimicrobial effect and help the healing of the gum inflammation<sup>4</sup>. It has been reported that extracts of miswak possess various biological properties, including significant antibacterial, antifungal, and anti-plasmodial effects<sup>5-7</sup>. In recent years, human pathogenic microorganism have developed resistance in response to the use of commercial antimicrobial drugs commonly used, in addition they are expensive and undesirable side effects of certain antibiotics are present, this forced scientists to look for new

\*B.Sc. (Agonomy)/ M.Sc. (Pesticider and weed control)/ Ph..D. (Physiology of weeds and medical plants) Assistant Professor, College of Science/ University of Salahaddin.

\*\* B.Sc. (Microbiology) /M.Sc. (Medical Microbiology-Microbial Genentics) Assistant Lecturer / College of Dentistry / Hawler Medical University

\*\*\* B.Ds. /M.Sc. (Oral Pathology) Assistant Professor / College of Dentistry / Hawler Medical University.

antimicrobial substances, such as medical plants, for this reason this study was conducted to evaluate the antimicrobial activity of aqueous, chloroform, ethanol, and ethyl acetate extracts of Miswak against some oral pathogenic microorganism like *Streptococcus mutans*, *staphylococcus aureus*, and *Candida albicans*, that cause most predominant infections in oral cavity and to determine the required concentration of the plant extract for exerting the antimicrobial effect.

#### **MATERIAL AND METHOD :**

##### **A. Collection of plant samples and preparation of extracts:**

The fresh dried stems of *Salvadora* tree were brought from the local markets in Erbil city, Iraq, and they were shade dried and crushed into powder using electrical grinder (Phila.PA.USA Arther Co). To 100gm of raw powder, 200 ml of distilled water, 95% chloroform, 96% ethanol, or 95%ethyl acetate separately were added in a volumetric flask, lifted for 24 hours on an electric shaker. Filtration was done by Buchner system apparatus using a filter paper (Whatman No.1). The extracts were concentrated with Rotary Vacuum Evaporator at 40-45° C until a gummy product was obtained, and freeze-dried until use<sup>1,8,9</sup>. Stock solution at concentrations of 100000 µg/ml was prepared by taking 2gm of crude extract into a flask containing 15 ml of distilled water and 5 ml of dimethyl sulfoxide (flask No.1). After that 0.5 or 1 ml were taken from the stock solution, each were poured into flask 2 and flask 3 respectively each containing 10 ml of distilled water, 0.1 ml from the flasks No.1, 2 and 3 were poured on to disks using micropipettes as concentrations of 10000, 500 and 1000 µg/ ml respectively and 0.5 ml from the flask No.3 as 5000 µg/ ml<sup>9,10</sup>.

**B. Microbial cultures and inoculums preparation:** The following oral microorganisms: *Streptococcus mutans*, *Staphylococcus aureus* and *Candida albicans* (four isolates of each microorganisms) were isolated from patients attending the

dental clinics of Collage of Dentistry/ Hawler Medical University. The isolates were used to test the activity of the extracts: the tested microorganisms were isolated from excavated soft caries of a badly carious teeth, from gingival pockets of partially erupted wisdom tooth with pericoronitis or root canals of a dead infected tooth associated with periapical abscess, and from the palate and tissue surfaces of upper old complete dentures used at night by the patients for longer periods of time. All the microorganisms were carefully identified using standard microbiological methods<sup>11</sup>. Nutrient broth and Sabouraud dextrose agar were used for growing and diluting the microorganism suspensions. Bacterial strains were grown to exponential phase in nutrient broth at 37°C for 18 hour, while concentration was evaluated by measuring the diameter of inhibition zone. *Candida albicans* was inoculated on petri dishes containing Sabouraud dextrose agar media, and incubated at 31° C for 48 hours<sup>19</sup>. In order to test the ability of Miswak extract in the inhibiting bacterial and fungal growth which compared with the standard antibiotic disc, the disc diffusion method was used by preparing sterile filter paper disc (6 mm) in diameter which impregnated with a known volume (20 µl) and appropriate concentration of the extract (10000, 5000,1000 and 500 µg/ml), and placed on a plate of sensitivity testing agar (Nutrient agar for bacteria and Sabouraud dextrose agar for fungus, inoculated with isolated bacteria and fungus respectively). Standard 6 mm discs containing amoxicillin (25 µg/disc), penicillin, and amphotericin B (10 µg/disc) were used as positive controls, paper discs loaded with 20 µl of sterile water or different solvents used in the study, were used as a negative control. At the end of the incubation period, the antimicrobial activity of each concentration was evaluated by measuring the diameter of inhibition zone. The experiments were conducted in the Laboratories of the Colleges of Science, University of Salahaddin / Erbil, and

College of Dentistry, Hawler Medical University/ Erbil during the period from 2008-2009.

**C. Statistical analysis:** All data were Expressed as means  $\pm$  standard error (SE) and statistical analysis was carried out using statistical software (SPSS version 11.5). Data analysis was made using one-way analysis of variance (ANOVA). The comparisons between groups were done using Duncan test.  $P < 0.05$  was considered as statistical significant.

#### RESULT:

The antimicrobial effect of various concentrations of Siwak against *Streptococcus mutans* and *Staphylococcus aureus* was examined. Results in Figure (1 & 2) indicated that, different concentrations of Siwak aqueous extract have inhibitory effects against *Streptococcus mutans* and *Staphylococcus aureus*. It showed highly significant action ( $P < 0.05$ ) on these bacteria at concentration (10000  $\mu\text{g/ml}$ ) which is parallel to the effects of Amoxicillin in some isolated bacteria. The extract at concentration of (5000  $\mu\text{g/ml}$ ) also showed antibacterial activity on most isolated bacteria but at lower rate when compared to the concentration (10000  $\mu\text{g/ml}$ ). On the other hand, the aqueous extract of Siwak at concentration (500 & 1000  $\mu\text{g/ml}$ ) exhibited less effect. The effect of Siwak aqueous extract on *Streptococcus mutans* was higher than *Staphylococcus aureus* at different concentrations. The data in Figure (3 & 4) revealed that, the Siwak chloroform extract showed a marked inhibition in the growth of most isolated *Streptococcus mutans* at concentrations (1000 & 5000  $\mu\text{g/ml}$ ), while their effect at concentration (10000  $\mu\text{g/ml}$ ) on the same group of bacteria was approximately, similar to the inhibition zone of amoxicillin and statistically there were no significant differences between them ( $P > 0.05$ ). The results of chloroform extract on *Staphylococcus aureus* revealed significant inhibitory action ( $P < 0.05$ ) at different concentrations, especially at (10000  $\mu\text{g/ml}$ ) which is near to the

effect of Amoxicillin. The statistical analysis showed that, the inhibitory action of chloroform was more pronounced against *Streptococcus mutans* when compared to the action on *Staphylococcus aureus*, while their effect at concentration (10000  $\mu\text{g/ml}$ ) were near to the effect of Amoxicillin but was lower than revealed in other extractions. Whereas, the results of the effect of ethanol extract on the growth of *Staphylococcus aureus*, showed that their effect at concentration (5000  $\mu\text{g/ml}$ ) was the same as in the concentration (10000  $\mu\text{g/ml}$ ) and less than Amoxicillin effect. The results of Siwak ethyl acetate (as shown in Figure 7 & 8) indicate that, the different concentration had significant effect ( $P < 0.05$ ) against most tested bacteria (*Streptococcus mutans* and *Staphylococcus aureus*). Their effects on *Streptococcus mutans* was approximately similar concentrations (500, 1000 & 5000  $\mu\text{g/ml}$ ). While higher reduction was pronounced at concentration (10000  $\mu\text{g/ml}$ ) which is parallel to the effect of Amoxicillin. As it shown in Figure-8, there was a marked inhibitory effect of ethyl acetate on *Staphylococcus aureus* at different concentration but was lower than pronounced against *Streptococcus mutans*. In general, ANOVA test showed that, the rate of inhibition action of Siwak by using different solvents and concentrations among *Streptococcus mutans* were higher than *Staphylococcus aureus* and the most effective inhibitory action was marked by using Siwak aqueous extract. The effect of different Siwak extract and their concentration displayed in Table(1). The antifungal effect of Siwak extracts on *Candida albicans* isolated from oral cavity was tested, and the results showed less inhibitory effect on the growth of most of the tested microorganisms and the ethyl acetate extract gave stronger activities than all other solvents extraction as shown in Table(2).

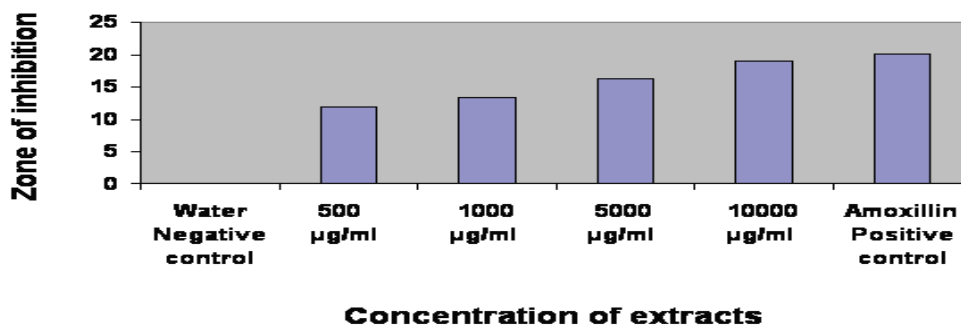


Figure 1: The effect of Siwak aqueous extraction the growth of *Streptococcus mutans* isolates

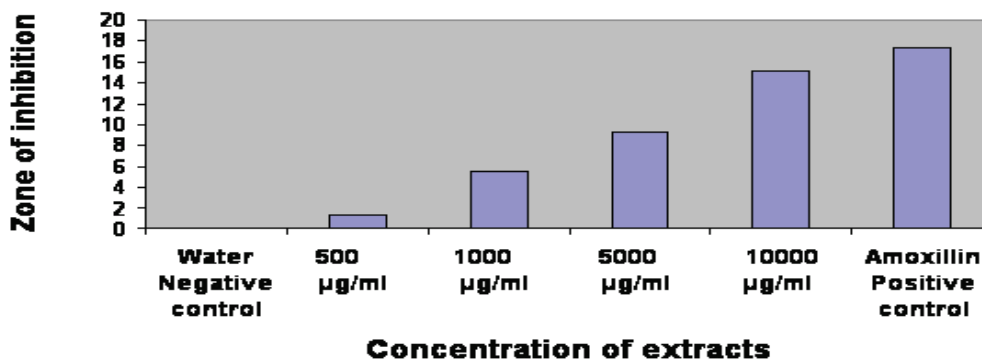


Figure 2: The effect of Siwak aqueous extract on the growth of *Staphylococcus mutans* isolates

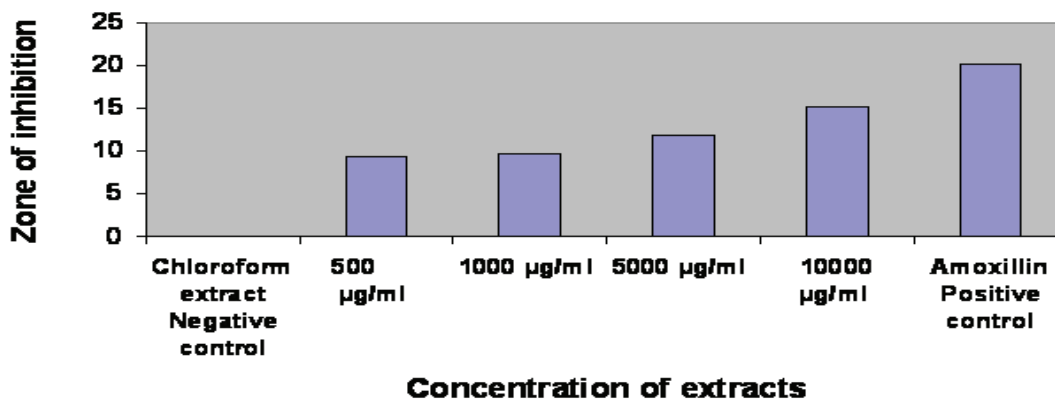


Figure 3: The effect of Siwak chloroform extract on the growth of *Streptococcus mutans* isolates

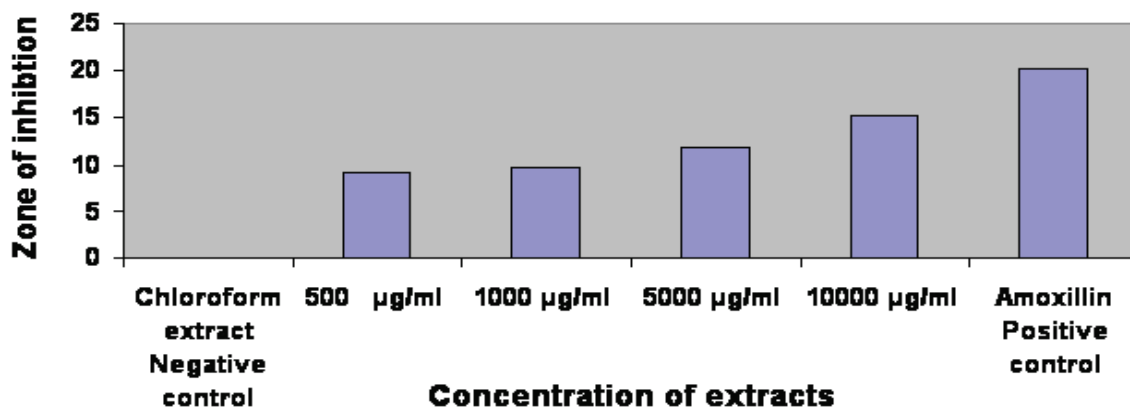


Figure 4: The effect of Siwak chloroform extract on the growth of *Staphylococcus aureus* isolates

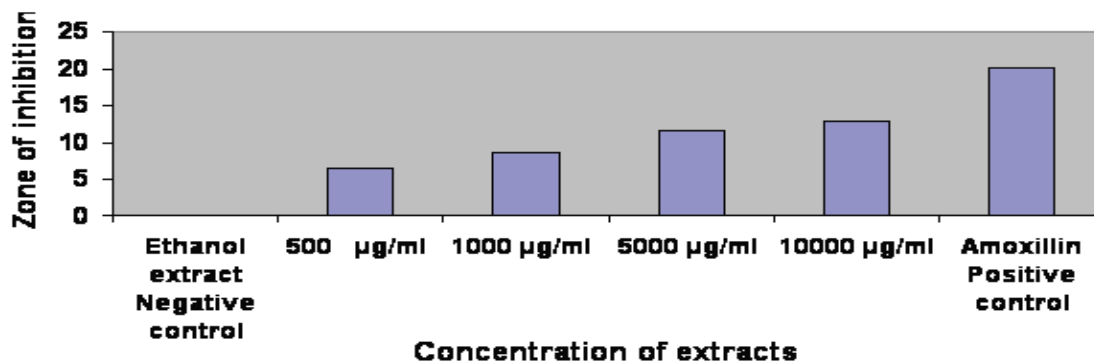


Figure 5: The effect of Siwak ethanol extract on the growth of *Streptococcus mutans* isolates

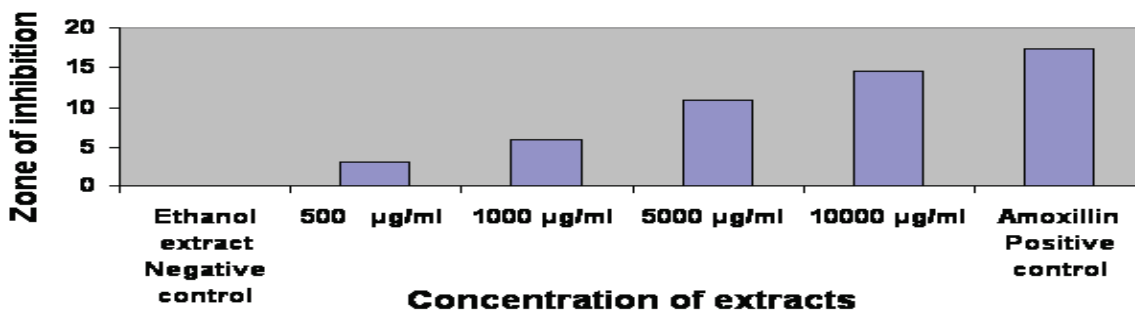
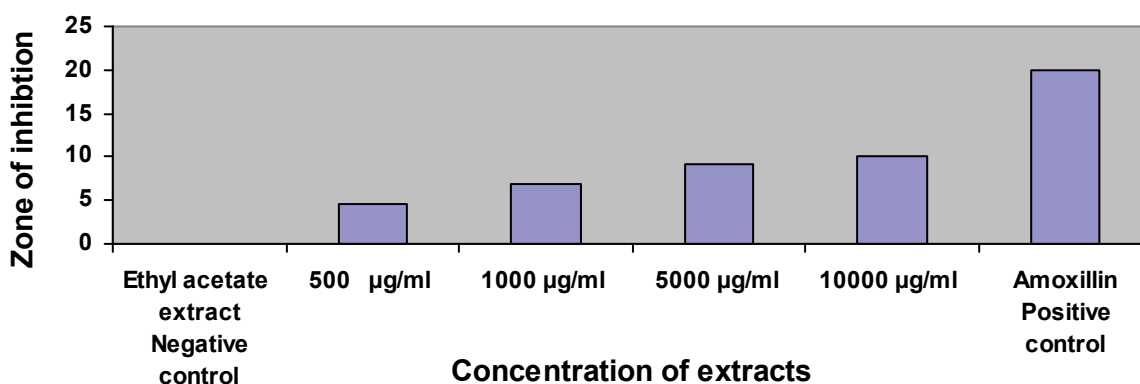
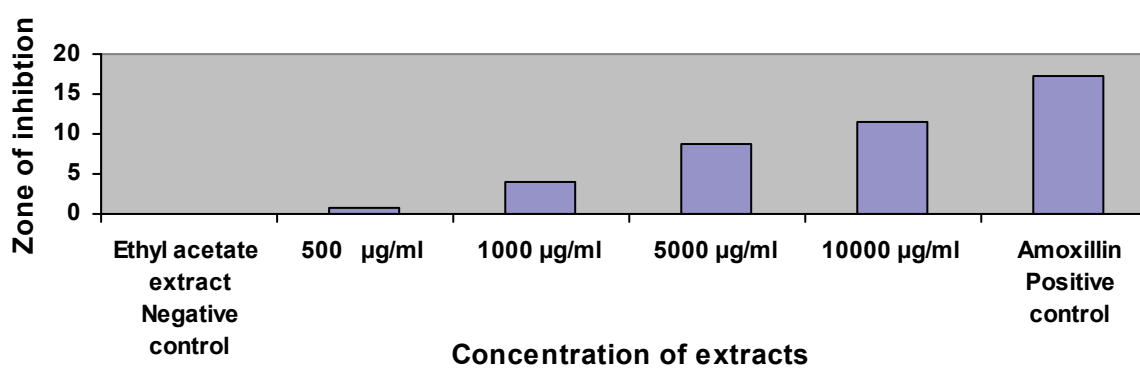


Figure 6: The effect of Siwak ethanol extract on the growth of *Staphylococcus aureus* isolates



**Figure 7:** The effect of Siwak ethyl acetate extract on the growth of *Streptococcus mutans* isolates



**Figure 8:** The effect of Siwak ethyl acetate extract on the growth of *Staphylococcus aureus* isolates

**Table 1:** Diameter of the inhibition zone (mm) of *Streptococcus mutans* and *Staphylococcus aureus* isolates treated with Siwak using different solvent and concentrations

Concentrations	Treatment	Mean	Treatment	Mean
Control	Aqueous extracts	0	Ethanol extracts	0
500µg/ ml		0		0.1
1000µg/ ml		0		0.095
5000µg/ ml		0.08		0.095
10000µg/ ml		0.137		0.162
Amphotericin B		18.4		18.9
Control	Chloroform extract	0	Ethyl acetate extracts	0
500µg/ ml		0.125		0.107
1000µg/ ml		0.213		0.1
5000µg/ ml		0.226		0.126
10000µg/ ml		0.233		0.357
Amphotericin B		14.6		17.6

**Table 2:** The effects of different types of extracts on the growth of *Candida albicans* isolates

Concentrations	Treatment	Mean	Mean	Treatment	Mean	Mean
		<i>Strep. mutans</i>	<i>Staph. aureus</i>		<i>Strep. mutans</i>	<i>Staph. aureus</i>
Control	Aqueous extracts	0.000	0.000	Ethanol extracts	0.000	0.000
500µg/ ml		11.9500	6.5425		9.3000	4.5000
1000µg/ ml		13.4250	8.6500		9.6875	6.8500
5000µg/ ml		16.3500	11.6125		11.8425	9.1000
10000µg/ ml		19.0250	13.0075		15.1875	10.1500
Amoxillin		20.1500	20.1500		20.1500	19.9000
Control	Chloroform extract	0.000	0.000	Ethyl acetate extracts	0.000	0.000
500µg/ ml		1.2500	3.0000		1.7750	8.000
1000µg/ ml		5.6000	5.9500		6.5250	3.8250
5000µg/ ml		9.3500	10.9000		9.2000	8.7000
10000µg/ ml		15.1000	14.6250		14.0750	11.4750
Amoxillin		17.3000	17.3000		17.300	17.3000

### DISCUSSION:

Chewing sticks are commonly used in 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. The World Health Organization has recommended and encouraged the use of chewing sticks as an effective tool for oral hygiene in areas where such customary (WHO, 1987)<sup>12</sup>. The present study was conducted to investigate the antimicrobial activity of Miswak extract against some oral microorganisms. According to the findings, the *streptococcus mutans* was the most susceptible strain, followed by the *Staphylococcus aureus*. This results agree with that of Almas and Al-Zeid 2004<sup>3</sup>, and disagree with other report<sup>13</sup>, and this may be due to the use of different solvents with different concentrations, in addition to the differences in the origin of *Salvadora persica*. Their effects on the growth were most likely due to release of chemicals from the extract to the medium when they were mixed. The different reactions of each strain to various extracts indicated that each solvent extracted different chemical components of Miswak. The strength of antimicrobial activity may also depend on the pH of the extract since the highest pH

was demonstrated in the water extract, while the lower pH were associated with the other solvents. This assumption comes in agreement with the study of Almas in 1999<sup>14</sup>. Howaida et al, 2002 study the effect of miswak crude extracts using different solvents on the in vitro growth of some selected oral microbes. Strains of *Streptococcus mutans*, *Lactobacillus acidophilus*, *Actinobacillus Actinomycetemcomitans*, *Prevotella intermedia*, and *Candida albicans* were tested for susceptibility to the antimicrobial effects of crude extracts. Ethanol extract was the most potent, and the most susceptible was *Streptococcus mutans*<sup>8</sup>. The antibacterial effect of Miswak was studied by Claesson et al 2008, on bacteria implicated in the etiology of dental caries they found that the inhibitory effect was less on *Streptococcus mutans*<sup>15</sup>. Poureslami et al, 2007 also found that miswak extract can be used in mouth rinses and tooth pastes because of the antibacterial effects<sup>4</sup>. A study found that Miswak extract was fungistatic at concentration of 0.05, 0.1, 0.5µg/ ml and fungicidal at 1.0µg/ ml<sup>16</sup>. The differences in the results may be due to the use of extract obtained from the roots of *Salvadora persica*. Al-Bagieh et al 1994<sup>17</sup>, Abo Al Samh and Al-Bagieh 1996<sup>18</sup>, Al-Bayati and Suliman 2008<sup>19</sup> and Deborah et al 2006<sup>2)</sup> found



that the aqueous and methanolic extracts of miswak root were found to inhibit the growth of *Candida albicans*. This may be due to the use of methanol extract, or the roots instead of the stems, the different concentrations of extract in the study, different type of yeast strains, solution area, and different assay methods used.

### CONCLUSIONS :

It was concluded that extracts of *Salvadora persica* (Miswak) inhibited most *Streptococcus mutans* and *Staphylococcus aureus* bacterial growth significantly, but their effectiveness varied according to the type and concentration of the extract used, but there was no significant effect on *Candida albicans* growth, so it is a good oral hygiene agent, and can be a great help in developing countries for preventing and controlling dental caries.

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