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ANTIMICROBIAL EFFECT OF ALMISWAK (SALVADORA PERSICA LINN) EXTRACTS, AGAINST SELECTED PATHOGENIC MICROBES

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ABSTRACT

Introduction: Oral hygiene is one of the most important daily routine practices and keeps the mouth and teeth clean. In the Muslim world, the use of Miswak as a chewing stick is highly recommended as a Sunnah practiced by the prophet Mohammad (peace be upon him).This study was designed to scientifically establish antimicrobial effect of Miswak in vitro against some pathogenic microorganisms. **Methodology:** An experimental study design method was conducted in Qassim University Microbiology Laboratory. The twigs of S.persica were collected and alcoholic and hexane extracts were prepared using standard techniques. The antimicrobial properties of the extracts against Staphylococcus aureus, Psuedomonas aeruginosa, Candida albicans were tested by agar well diffusion method. **Result:** Different concentrations of Alcoholic extract 100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml the tested bacteria showed no zone of inhibition. When the same organisms were subjected to Hexan extracts of similar concentrations, significant zone of inhibition was noted particularly with 100 mg/ml concentration. Comparing the inhibition zone of *S. aureus*, *P.aeruginosa* and *C.albicans*, the 100 mg/ml concentration of hexan extract was more effective against *P. aeruginosa* producing 62.5mm inhibition zone as compared of 60mm inhibition zone against *C.albicans* and 40mm inhibition zone against *S.aureus*. **Conclusion:** Hexane extracts of *S. persica* was found to exhibit antimicrobial activity against *S.aurus*, *P.aeruginosa* and *C.albicans* at high concentration and lower concentration while alcoholic extract of miswak show no inhibitory effect against the same microorganisms.

KEYWORDS: Almiswak, Salvadora persica linn, Antimicrobial activity, S.aureus, P.aeruginosa, C.albicans.

1-INTRODUCTION

Oral hygiene is one of the most important daily routine practices and keeps the mouth and teeth clean and prevents many health problems. Modern dental care tools are designed to provide both a mechanical and chemical means of removing plaque and food residues from the surface and spaces between the teeth. Throughout history, people hae been using different tools and chemicals to maintain their oral health, such as chewing sticks, tooth brushes, gum, mouth wash, toothpaste and floss, which are all believed to evolve from botanical origins. Chewing sticks are considered the most popular among all of the dental care tools for their simplicity, availability, low cost and their traditional and/or religious value. Chewing-sticks were used by the Babylonians more than 7000 years ago. Currently, in the Muslim world, including Saudi Arabia the use of Miswak (*Salvadora persica* Linn) as a chewing stick is highly recommended as a Sunnah practiced by the prophet Mohammad (peace be upon him) and his companions to achieve daily dental care, and the prophet emphasized the importance of using Miswak for oral hygiene.

The scientific name of Salvadora persica Linn was given to the tree, in 1598 by the Spanish botanist, Dr. Laurent Garcin. The toothbrush tree (S. persica), known locally as “Miswak,” is a member of the Salvadoraceae family. It is a small evergreen tree with soft, whitish, yellow wood. The S. persica tree. (Miswak) which is 3 m in height and 30 cm in diameter and has thick succulent small leaves new stem branches are green to greyish in color while old branches are dark brown. It has aromatic roots, as well as warm and pungent taste. The tree is globally known as the toothbrush tree or chewing-sticks; it has many local names in different geographical regions such as Miswak or Arak in the Arab world, Koyoji in Japan, Qesam in Hebrew, Mastic in Latin and it is often known by the name of tooth brushing tree in European countries.The most common type of Miswak is derived

from the Arak tree that grows mainly in Saudi Arabia and in other parts of the Middle East.

S. persica is considered to be a medicinal herbal plant. It contains salvadorine and trimethylamine, which are shown to exhibit anti-bacterial effects on cariogenic bacteria such as *Streptococcus mutans with zone pf inhibition (8mm).* It has been shown that these active principles support periodontal health, (AlBayaty FH, AI-Koubaisi AH and Ali NAW et al, 2010) reduces the accumulation of biofilm-like dental plaque formation and exhibits fungi static activity against *Candida albicans.* (Noumi E et al, 2010).

Another study, investigated the presence of antimicrobial agents in Miswak extracts based on their polarity in different solvents. The results showed that Miswak contains more than one type of antimicrobial agent that inhibits the growth of both gram positive and negative bacteria. The zone of inhibition for Hexane and Ethanolic extracts (500ug) measured against *Escherichia coli (9mm Vs 10mm), Staphylococcus aureus (9mm Vs 26mm), Lactobacillus acidophilus (9mm Vs 9mm), Streptococcus mutans (19mm Vs 35mm) and Pseudomonas aeruginosa (0mm Vs 16mm).* The results showed a strong antimicrobial activity in the aqueous extract and less activity in alcoholic and nonpolar extracts. (Abdul-AzizAl-Hazmi and Mohammad Abhary, 2016).

AL-miswaks were tested against three different types of microorganisms isolated from oral swabs: against *Staphylococcus aureus (25mm), Streptococcus mutans (20mm) & Candida albicans (26mm)* by agar diffusion method. Inhibition zone was measured after 24 hrs of incubation at 37^{o}C. *S. persica* is considered to be a medicinal herbal plant. It exhibit strong antimicrobial effect against all three tested microorganisms.(Fasih Fatima et al, 2014).

This study was conducted to scientifically establish antimicrobial effect of miswak in vitro against selected microorganisms: Gram positive bacteria *(Staphylococcus aureus),* Gram-negative bacteria (*Pseudomonas aeruginosa)* and Fungus *(Candida albicans).*

2.0 -METHODOLGY

This was a Experimental study design method. The study included purchase of roots of *Salvadora persica* from a local market at Makkah al-Mukarramah and testing them in Qassim University Microbiology Laboratory by using agar well diffusion method. The exposure were antimicrobial resistance, type of microorganism, type of media, temperature and the outcome was antimicrobial effect of Miswak *(Salvadora persica)* against selected pathogens. The study was conducted during the period of September 2017 to January 2018.

2.1 Hexane and Alchoholic Extracts

This study was conducted on roots of *Salvadora persica* was purchased from a local market at Makkah al-Mukarramah, which is located in western Saudi Arabia. The sticks was dried in a 55 °C oven for three days and then ground into a fine powder using a coffee grinder. The Miswak extracts was prepared by adding 400 g of the Miswak powder to 200 ml of hexane and alcohol seperately, in a closed container and soaked at room temperature for 48 h. The solvents were filtrated through a Whatman No.1 filter paper and allowed to evaporate in a 40 °C oven for 72 h.

2.2 Extracts Dilutions & Disc Diffusion tests

Bacterial and fungal quality control strains (*Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*) used in this study, were available in the microbiology laboratory at AL-Qassim University. Antimicrobial testing of Almiswak was done by agar well diffusion method. Antibacterial and antifungal activities of Al-Miswak extracts were tested using agar well diffusion method by lawn culture. Microbial suspension was prepared in a sterile physiological saline equal to 0.5 McFarland standard, Muller-Hinton (MH) agar was inoculated with microbial suspensions (one plate/ microbes/ extract). Four small wells was created by indenting the agar with a clean pipette. Each resulting well was approximately 6mm in diameter and accommodated approximately 90–95 microliters (µl) of extract was, filled with neat, 1/2, 1/4, and 1/8 dilutions of S. persica extracts corresponding to 100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml, by Serial double dilution method. Same protocol was followed with both herbal extracts respectively. Following incubation *(Staphylococcus aureus*, *Pseudomonas aeruginosa*) at 37 °C for 24 and (*Candida albicans*) at 35 °C for 48h. Clotrimazole was used as a positive antifungal control for *Candida albicans*, Polymyxin was used as a positive antibacterial control for *Pseudomonas aeruginosa* and Vancomycin was used as a positive antibacterial control for *Staphylococcus aureus* while sterile saline was used as a negative control (Abdullah Aldrees et al, 2017).

2.3 Sensitivity tests of Sandard antibiotics Disc

A set of two antibiotics discs and one antifungal dics were used to compare with S. persica extracts activity, Vancomycin(VA30), Polymixin (Pb) Clotrimazole (CLT 10). Sensitivity of antibiotics against test strains was assessed by agar disc diffusion method. Sensitivity was predicted by degree of clear zone surrounding the disc after 24 hrs. measured as (mm diameter of zone of inhibition). The results of sensitivity tests were expressed as (0) = no sensitivity, (+) = (below 12) low sensitivity, (++) = (12-29) moderate sensitivity, (+++) = (30-45) high sensitivity and (++++) = (Above 45mm) higher sensitivity (Ali et al).

2.4 Statistical Analysis

Each experiment was repeated in triplicate sets and **the means** from the absolute data has been mentioned. The

comparison of antibacterial activity of the medicinal extracts, with standard antibiotics was evaluated by activity index (AI) (Sekhawat and Vijayvergia 2010, Ali et al 2016). The data was entered and analyzed using Microsoft excel software. The data was presented by using tables and graphs**.** Ethical approval for the this study was obtained from Qassim university, college of Applied Medical Sciences, Departmental Research Review Committee.

3.0 -RESULTS

The antibacterial and antifungal activities of Al-Miswak extracts with hexane and alcohol as tested on bacterial samples. The zone of extracts diffusion from the well into the agar was measured in millimeters. (Images No.1 &2).

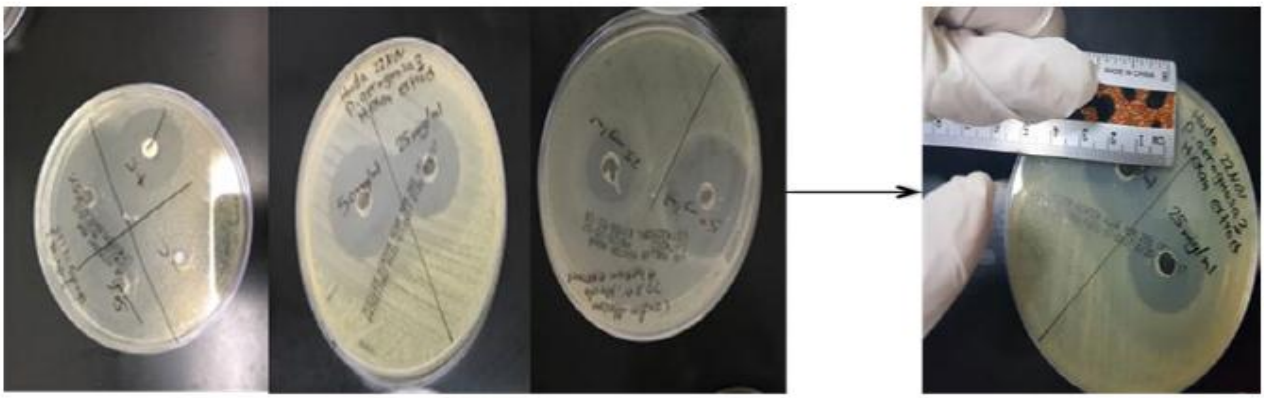

Image No.1 : Results of Hexane Extracts.

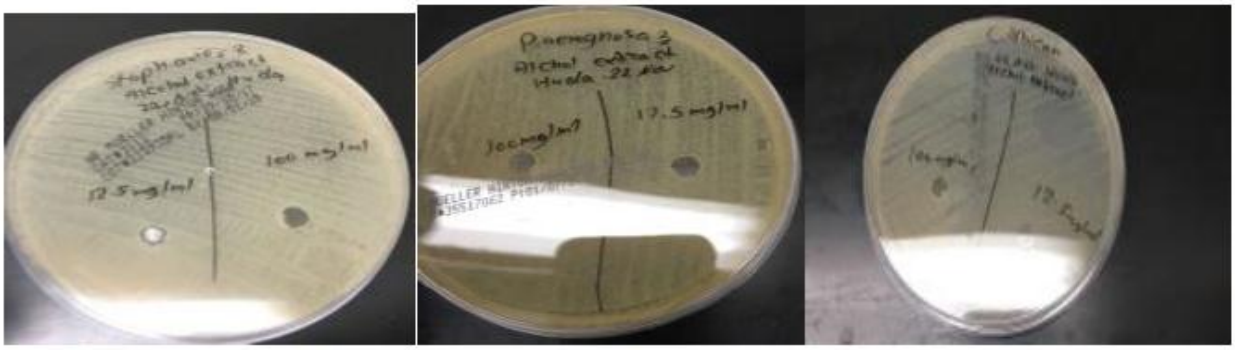

Image No.2. Results of Alchoholic Extracts.

With different concentrations of Alcoholic extract 100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml *Staphylococcus aureus*, *Pseudomonas aeruginosa and Candida albicans* showed no zone of inhibition ***(Image no;2, Table no: 1).*** While same organisms when subjected to Hexane extracts of same concentrations, significant zone of inhibition was noted particularly with 100 mg/ml concentration. Comparing the inhibition zone of *S. aureus, P.aeruginosa and C.albicans*, at 100 mg/ml concentrations, hexane extract was more effective exhibited, mean zone of inhibition of 40mmVs 62.5mm Vs 60mm, with maximum effect, against *P. aeruginosa* producing 62.5mm inhibition zone as compared of 60mm inhibition zone against *C.albicans* and 40mm inhibition zone against *S.aureus****.(Image no:1, Table no:1).***

Similarly Hexan extracts showed a significantly higher inhibitory with other concentrations also compared to Alcoholic extract. *S.aureus* was inbited Haxane extracts, as inhibitory zone of 40mm at 100 mg/ml, 20mm at 50 mg/ml, 18mm at 25 mg/ml and 18 at 12.5 mg/ml.

P. aeruginosa, inhibited by an inhibitory zone of 62.5mm at 100 mg/ml, 59mm at 50 mg/ml, 46mm at 25 mg/ml and 35 at 12.5 mg/ml. *C.albicans* also inhibited by an inhibitory zone of 62.5mm at 100 mg/ml, 59mm at 50 mg/ml, 46mm at 25 mg/ml and 35 at 12.5 mg/ml. ***(Image no:1, Table no:1).***

Table No. 1: Zone of Inhibitions with Hexane and Alchoholic Extracts.

S. persica extracts.	Microrganisms	Zone of Inhibition of Extracts at different Concentrations,			
		100 mg/ ml	50mg/ml	25mg/ml	12.5mg/ml
Hexane	S. aureus	40mm.(+++)	20mm. (++)	18mm.(++)	15mm(++).
	P. aeruginosa	62.5mm(++++)	59mm(++++)	46mm(+++)	35mm(+++)
	C. albicans	60mm(++++)	49mm(++++)	41mm(+++)	38mm(+++)
Alchoholic	S. aureus	0mm	0mm	0mm	0mm
	P. aeruginosa	0mm	0mm	0mm	0mm
	C. albicans	0mm	0mm	0mm	0mm

(0) = no sensitivity, (+) = (below 12) low sensitivity, (++) = (12-29) moderate sensitivity, (+++) = (30-45) high sensitivity and (++++) = (Above 45mm) higher sensitivity

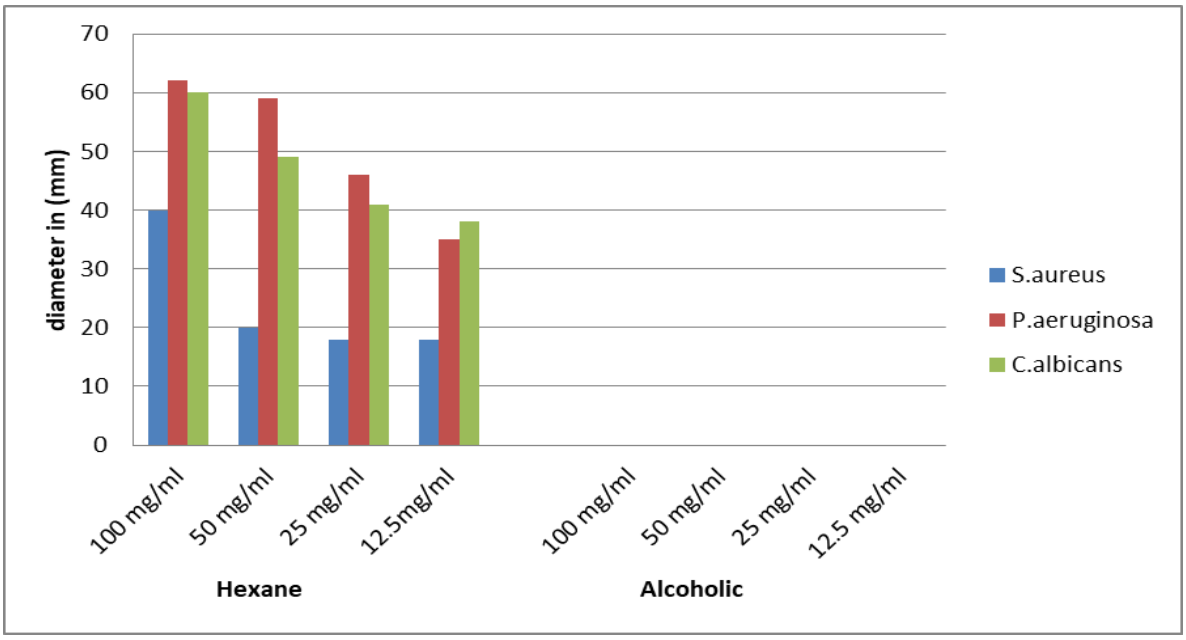

Figure No.1. Zone of Inhibitions with Hexane and Alchoholic Extracts.

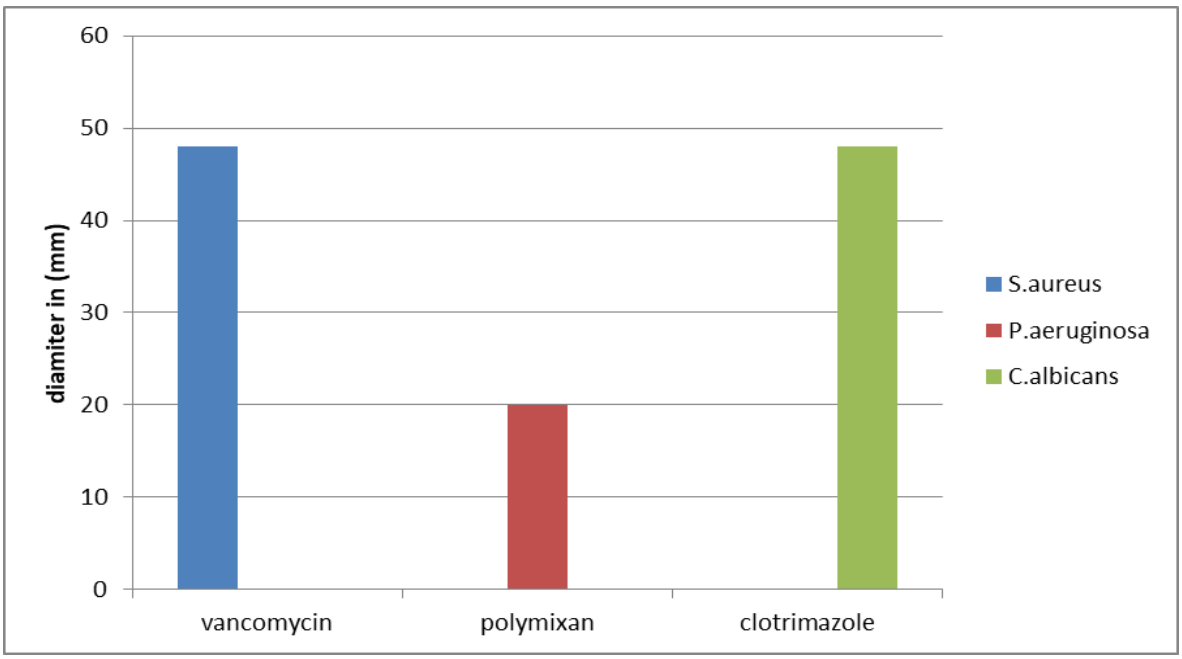

Figure No. 2: Zone of Inhibitions with Standard Antibiotic Discs.(Positive Controls).

The significant use of the S.persica extract, with standard antibiotic discs, was calculated through Activity Index **(table no.2).** More than 1 Activity Index (A.I) value indicated a significant role of S. persica extracts, while a value below zero, showed a stronger effect of standard antibiotic discs against tested pathogens. More (A.I) values showed more significant results by S. persica hexane extracts were observed.

Table No. 2: Activity index of S. persica Extracts compared to Standard dics.

S. persica extracts.	Microrganisms	Activity index at different concentration. A.I= Zone of Inhibition of Extracts/ Zone of Inhibition of Antibiotics.				Standard Antibiotic Dics.
		100 mg/ ml	50mg/ml	25mg/ml	12.5mg/ml	
Hexane	S. aureus	A.I=0.83	A.I=0.42	A.I=0.36	A.I=0.31	Vancomycin
	P. aeruginosa	A.I=3.13	A.I=2.95	A.I=2.3	A.I=1.75	Polymixin
	C. albicans	A.I=1.25	A.I=1.02	A.I=0.85	A.I=0.79	Clotrimazole
Alchoholic	S. aureus	A.I=0	A.I=0	A.I=0	A.I=0	Vancomycin
	P. aeruginosa	A.I=0	A.I=0	A.I=0	A.I=0	Polymixin
	C. albicans	A.I=0	A.I=0	A.I=0	A.I=0	Clotrimazole

4.0 -DISCUSSION

The antibacterial and antifungal activities of Al-Miswak extracts with hexane and alcohol as tested on bacterial samples. Antimicrobial activity was determined by agar disc diffusion method, which exhibited the given results. Hexan extracts showed a significantly higher inhibitory with other concentrations also compared to Alcoholic extract. *S.aureus* was inbited Haxane extracts, as inhibitory zone of 40mm at 100 mg/ml, 20mm at 50 mg/ml, 18mm at 25 mg/ml and 18 at 12.5 mg/ml. *P. aeruginosa*, inhibited by an inhibitory zone of 62.5mm at 100 mg/ml, 59mm at 50 mg/ml, 46mm at 25 mg/ml and 35 at 12.5 mg/ml. *C.albicans* also inhibited by an inhibitory zone of 62.5mm at 100 mg/ml, 59mm at 50 mg/ml, 46mm at 25 mg/ml and 35 at 12.5 mg/ml. of Alcoholic extract 100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml *Staphylococcus aureus, Pseudomonas aeruginosa and Candida albicans* showed no zone of inhibition.

The sensitivity pattern of each extract varied. These results are in variance with the results of the study done by (Abdul-AzizAl-Hazmi and Mohammad Abhary, 2016. The zone of inhibition for Hexane and Ethanolic extracts (500ug) measured against *Escherichia coli (9mm Vs 10mm), Staphylococcus aureus (9mm Vs 26mm), Lactobacillus acidophilus (9mm Vs 9mm), Streptococcus mutans (19mm Vs 35mm) and Pseudomonas aeruginosa (0mm Vs 16mm)* and also with the results of (Fasih Fatima et al, 2014) in which the results showed *S. persica* exhibited strong antimicrobial effect against *Staphylococcus aureus (25mm), Streptococcus mutans (20mm) & Candida albicans (26mm)* by agar diffusion method.

The present study, showed that the hexane extracts of *S.persica* were more effective against the gram negative bacteria *(P. aeruginosa)* than the gram positive bacteria *(S.aureus).*These results are consistent with. the results of the study done by (Sofrata et al, 2008) in which the result exhibited stronger antibacterial activity against the Gram-negative bacteria tested than the Gram-positive bacteria evaluated, as evidenced by the pronounced differences in inhibition zones associated with the Gram-negative species *A.actinomycetemcomitans, P. gingivalis, H. influenzae, and the Gram-positive species S. mutans and L. acidophilus*.

While comparing the efficacy of different extracts, Hexane extract of *S. persica* was found to exhibit stronger antimicrobial activity against Gram positive bacteria *(stapyloccus.aureus; 40mm)*, Gram-negative bacteria (*Pseudomonas aeruginosa: 62.5mm*) and Fungus (*Candida albicans: 60mm*). This observation is in accordance with the findings of Al-Sowygh et al, who reported a Hexane extract of *Salvadora Persica*, showed better inhibitory zones compared with chloroform, ethyl acetate, methanol-soluble, methanol-insoluble, ethanol, and water. The hexane extract of S. persica was found to exhibit maximum antimicrobial activity against *E. faecalis and C.albicans*. The differences in the activities of different extraction methods may be due to varying degrees of solubility of the active constituents in these two solvents.

The mean inhibition zones produced by the hexan extract used in this study against all three types of microorganism were quite large. For example, mean inhibition zone of 100 mg/ml and 50 mg/ml hexan extract against *Candida albicans* was 60mm and 49mm respectively, mean inhibition zone of 100 mg/ml and 50 mg/ml hexan extract against *P. aeruginosa* was 62.5mm and 59mm respectively and and mean inhibition zone of 100 mg/ml and 50 mg/ml hexan extract against *S.aureus* was 40mm and 20mm respectively. Antimicrobial activity of the miswak was the stronger at concentrations of hexan extract 100 mg/ml and the weaker at concentrations of hexan extract 12.5mg/ml. For example

P. aeruginosa also showed an inhibitory zone of 62.5mm at 100 mg/ml, 59mm at 50 mg/ml, 46mm at 25 mg/ml and35mm at 12.5 mg/ml while *Candida albicans* also showed an inhibitory zone of 60mm at 100 mg/ml, 49mm at 50 mg/ml, 41mm at 25 mg/ml and 38mm at 12.5 mg/ml and *S.aureus* also showed an inhibitory zone of 40mm at 100 mg/ml, 20mm at 50 mg/ml, 18mm at 25 mg/ml and18mm at 12.5 mg/ml. These results were similar to the results of Abdullah et al (2017) who reported Hexane as a solvent has been used for preparation of different extracts, in which the result exhibited stronger antibacterial activity at concentrations 100 mg/ml and the weaker at concentrations 12 mg/ml against *S. mutans(13mm Vs 6mm), S. salivarius(16mm Vs 6mm)* and *S. sanguis(14mm Vs 6mm)*. In contrast the alchoholic extracts showed stronger antimicrobial activity, compared to the hexane extracts.

5.0 -CONCLUSION

Antimicrobial activity of miswak is as good as any antibiotic or antifungal drug. Hexane extracts of *S. persica* exhibited profound antimicrobial activity against Gram positive bacteria (stapyloccus.aurus), Gram-negative bacteria (Pseudomonas aeruginosa) and Fungus (Candida albicans) at high concentration. while alcoholic extract of miswak show no significant inhibitory effect against the same microorganisms. Difference of standardized extraction protocol, might result in contradictory results with different microorganisms. Advanced techniques are required to obtain the chemicals responsible for the antimicrobial properties of the plant extract.

6.0 -REFERENCE LIST

1. Abdullah Aldrees, Ibrahim Al-Sanie, Hanan Balto, Sultan Al-Beshri. (2017) Effectiveness of Salvadora persica extracts against common oral pathogens: The Saudi Dental Journal, 29(1): 1-6.
2. Abdulbaqi, H., Himratul-Aznita, W. and Baharuddin, N. (2016). Evaluation of Salvadora persica L. and green tea anti-plaque effect: a randomized controlled crossover clinical trial. BMC Complementary and Alternative Medicine, 16(1): 493.
3. Al-Sowygh, H. Balto, T. Al-Howiriny, A. Al-Somaily, Y. Siddiqui, Z. (2013) Screening for the antimicrobial activity of Salvadora persica extracts against Enterococcus faecalis and Candida albicans, Int. J. Phytomed, 5(4): 486-492.
4. Al-Bayaty FH, AI-Koubaisi AH, Ali NAW, Abdulla MA. (2010) Effect of mouth wash extracted from *Salvadora persica* (Miswak) on dental plaque formation: A clinical trial. J Med Plant Res, 4: 1446–54.
5. Abd El-LatifHesham.(2016). Antibacterial activity of MiswakSalvadorapersica extracts against isolated and genetically identified oral cavity pathogens: Technology and Health Care, 24(s2): S841-S848.
6. Abdul-AzizAl-Hazmi, MohammadAbhary (2016). Antibacterial activity of Miswak (Salvadorapersica L.) extracts on oral hygiene: Journal of Taibah University for Science, 10(4): 513-520.
7. Ahmad H and Ahamed N. (2012) Therapeutic properties of meswak chewing sticks: A review. Afr J Biotechnol, 11: 14850–14857.
8. Amit Kumar, Padma K Bhat, Soumik Sarkar. (2012) Assessment of immediate antimicrobial effect of miswak extract and toothbrush on cariogenic bacteria- A clinical study: Journal of Advanced Oral Research, 3(1): 25-29.
9. Al-Otaibi M, (2004) The miswak (chewing stick) and oral health. Studies on oral hygiene practices of urban Saudi Arabians.Swed Dent J Suppl. 167: 2-75.
10. Dahiya, P., Kamal, R., Luthra, R., Mishra, R. and Saini, G. (2012).Miswak:A periodontist′s perspective.*Journal of Ayurveda and Integrative Medicine*, 3(4): 184-187.
11. Fasih Fatima, Khursheed Hashmi, Rafiq Khanani, Saima Naseem, and Shaheen Sharafat.(2014). In vitro evaluation of antimicrobial effect of miswak against common oral pathogens: Pakistan Journal of Medical Sciences, 30(2): 398-403.
12. Haque, M. and Alsareii, S. (2015). A review of the therapeutic effects of using miswak (Salvadora Persica) on oral health. Saudi Medical Journal, 36(5): 530-543.
13. Ibrahim M., AL Sahli A., Alaraidh, I, Al-Homaidan, A., Mostafa, E. and ELGaaly, G. (2015). Assessment of antioxidant activities in roots of Miswak (Salvadora persica) plants grown at two different locations in Saudi Arabia. Saudi Journal of Biological Sciences, 22(2): 168-175.
14. Khoirulzariah Ismail.(2016). The Use of Miswak as Toothbrush for Orthodontic Patient: Case Reports in Dentistry, 2016; 1: 1-3.
15. L.Wijaya, H. Sher, M. Nasser, (2011) Ethnobotanical and antibacterial potential of Salvadora persica L.: a well-known medicinal plant in Arab and Unani system of medicine J. Med. Plants Res, 5: 1224-1229.
16. Moodi Helal Al-Mutairi, Sarah Ali, Salah Mesalhy Aly and YousefAldebasi. Antibacterial activity of sider (ziziphus spina- christi), leaves extract against selected pathogenic bacteria. *ejpmr, 2016; 3(5): 138-144.*
17. Noumi E, Snoussi M, Hajlaoui H, Valentin E, Bakhrouf A.(2010) Antifungal properties of *Salvadora persica and Juglans regia* L. extracts against oral Candida strains. Eur J Clin Microbiol Infect Dis, 29: 81–8.
18. Salman Siddeeqh, Amrita Parida, Maji Jose and Vidya Pai (2016) Estimation of Antimicrobial Properties of Aqueous and Alcoholic Extracts of Salvadora Persica (Miswak) on Oral Microbial Pathogens - An In vitro Study, Journal of clinical and diagnostic research, 10(9): 13-16.
19. Shekhawat N and Vijayvergia R. Evaluation of Antimicrobial Potential of Some Medicinal Plants Against Plants and Human Pathogen. J. Pharma. Res, 2010; 3(4): 700–702.

20. Sofrata, A., Claesson, R., Lingström, P. and Gustafsson, A. (2008). Strong Antibacterial Effect of Miswak Against Oral Microorganisms Associated With Periodontitis and Caries. Journal of Periodontology, 79(8): 1474-1479.
21. Sofrata, A., Santangelo, E., Azeem, M., Borg-Karlson, A., Gustafsson, A. and Pütsep, K. (2011). Benzyl Isothiocyanate, a Major Component from the Roots of Salvadora Persica Is Highly Active against Gram-Negative Bacteria. *PLoS ONE*, 6(8): e23045.
22. S.G. Mohammed, (2013) Comparative study of in vitro antibacterial activity of miswak extracts and different toothpastes, " American Journal of Agricultural and Biological Science, 8(1): 82–88.
23. Zafar, M., Niazi, F., Naseem, M., Khurshid, Z. and Almas, K. (2016). Role of Salvadora persica chewing stick (miswak): A natural toothbrush for holistic oral health. *European Journal of Dentistry*, 10(2): 301.