

Article

An In Vitro Study Of Antibacterial Efficacy Of *Salvadora Persica* (Miswak) Extract Against *Enterococcus Faecalis*

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Abstract— *Salvadora persica* is widely used in dental field to improve dental health and promote dental hygiene. Although sodium hypochlorite is highly recommended and regarded as the most effective irrigant in root canal treatment to eliminate bacteria, it can cause harm to patients manifested by severe pain, rapid swelling and also obstruct breathing airway. However, it is believed that the chemical composition of *Salvadora persica* helps to treat and act as an antibacterial agent in root canal therapy. This study aims to obtain the crude compound from *Salvadora persica* by using the freeze drying method and to compare the antibacterial activity of the *Salvadora persica* and sodium hypochlorite against *Enterococcus faecalis* in aerobic condition. Antibacterial activity results showed that 150 mg/ml of *Salvadora persica* give comparable effect with sodium hypochlorite against *Enterococcus faecalis*. It is believed that 60% of alcoholic *Salvadora persica* extraction is an effective antimicrobial agent to be utilized as an irrigant in root canal treatment.

Keywords— root canal; *Enterococcus faecalis*; *Salvadora persica*; sodium hypochlorite; intracanal medication.

I. INTRODUCTION

It is generally accepted in endodontic practice that sodium hypochlorite (NaOCl) is the most suitable solution for irrigation of the root canal system. Sodium hypochlorite is selected because it is a strong base that acts as an organic solvent which give the effect on degradation on amino acid and hydrolysis through the production of chloramine molecules in root canal treatment [40]. In case of an endodontic infection, a highly effective irrigant needs to be utilised to ensure a clean root canal area and reduction of the microbial load. Opportunistic microbes such as *Enterococcus faecalis* at the surface area of

crown will invade into the root canal system and reside in pulp. The main problems affecting pulp are cracked tooth, deep cavity, repeated dental treatment and also trauma the patient. Souto and Colombo, (2008) stated that, *Enterococcus faecalis* have been found to associate to the chronic endodontitis, inflammation of the dental pulp by forming colonization in oral cavity.

In 1987, in order to overcome the side effects, the World Health Organization (WHO) encouraged the researchers to investigate the possible use of natural product such as herbs and plants extract [49]. Herb and plant extract have been used in oral hygiene products for many years if not centuries [28]. It is

believe that, the chemical composition of the *Salvadora persica* can help treat and act as antibacterial properties. This is supported by Daurot et al., (2002) that *Salvadora persica* may have inhibitory effect on selective bacteria [16]. It is also proven that *Salvadora persica* have antibacterial properties against oral pathogen where chemical compounds such as sulphate gave inhibitory effects on growth of *Candida albicans* [6], *Streptococcus sp.* and *Staphylococcus aureus* [7]. In addition, the growth of the most sensitive microorganism in the oral cavity, *Enterococcus faecalis* was affected by the use of *Salvadora persica* [9]. Different methods have been used to obtain the crude of *Salvadora persica* for antimicrobial activities against the oral microorganism. Al-Bayati and Sulaiman (2008) claimed that, the extraction of *Salvadora persica* by using aqueous and methanol solution gave different strengths of inhibition to oral pathogens [5]. Complementary to this, aqueous efficiently inhibited the activities of all selected oral pathogens as compared to methanol extract which was resisted by *Lactobacillus acidophilus* and *Pseudomonas aeruginosa*. Moreover, the strongest antibacterial activity against *Enterococcus faecalis* was shown by the aqueous extraction assay [5]. In addition, to reduced antibacterial activity in oral cavity, it was suggested by Mansour *et al.*, (1996) that alcoholic extract is more effective than aqueous extract [29].

In this study, we obtained the crude compound from *Salvadora persica* using ethanol extraction method and compared the antibacterial activity of *Salvadora persica* and sodium hypochlorite. We expected a certain amount of concentration *Salvadora persica* that could give a comparable effect to the sodium hypochlorite in root canal treatment.

II. MATERIAL AND METHOD

The family name of this specimen is Salvadoraceae. It is scientifically called as a *Salvadora persica*. It is most commonly known as Mustard tree and Tooth brush tree by local name. Fresh *Salvadora persica* stems were used in this study. The barks were removed to eliminate the unwanted debris and fungus that could affect the experiment. It was sent to Kuliyyah of Pharmacy (IIUM) and undergone herbarium vouchering purposes. The registered number for vouchering specimen was PIIUM 0286.

A. Sample Preparation

Firstly, the stems of the *Salvadora persica* were cleaned and washed with running tap water to remove dirt and soil. The barks were removed by using sharp knife.

The stems of the *Salvadora persica* were weighed in order to prepare for plant extraction. The method of oven drying was used following the method from Chan et al., (2012) with slight modification by using freeze drying to get the plant's crude. Three hundred and fifteen gram of *Salvadora persica* stems were weighed by using analytical balance. Oven drying method process was used based on the principle that dry air will absorb the moisture released from samples due to high temperature and carried away by the circulation. The temperature was set up at 55°C for 3 days according the recommendation of previous researcher. Temperatures between 50°C to 60°C are recommended for oven drying process in order to avoid destruction of phytochemical contained in the plant. The stems

were dried until a constant was achieved. Next, the samples were cut into discs before being grounded into fine powder by using food blender. The fine powder of *Salvadora persica* helped increase the surface area during the extraction process.

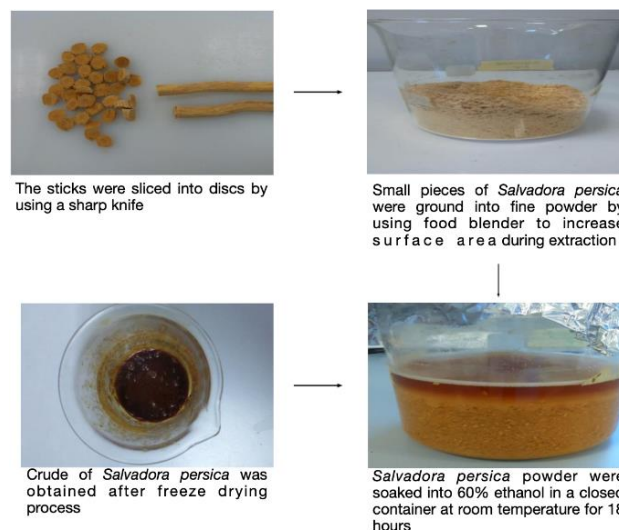


Fig. 1 Diagram of preparation and extraction of *Salvadora Persica*

B. Media Preparation

Thirty seven grams of BHI powder was dissolved in 1L of distilled water. The mixture was then sterilized by autoclave at 121°C for 15 minutes. The sterilized solution was poured into sterile agar plates and allowed to solidify for a few minutes. The plates were covered with Para film before kept in the refrigerator at 4°C. Forty seven grams of BHI powder was dissolved in 1L of distilled water. The mixture was then sterilized by autoclave at 121 °C for 15 minutes. The sterilized broth was poured into 6 falcon tubes separately with 10 ml volume each. The falcon tubes and remaining broth in the Schott bottles were kept in the refrigerator at 4 °C.

The preparation of 5.0 ml culture was done in falcon tube. Sterile pipet tips and eppendorf tubes were used in this process. The eppendorf tubes were labeled with the information of bacteria and the prepared date. Then, 0.5 ml out of 5ml culture prepared previously was taken and added into 1.5ml sterile eppendorf tube. Then, 0.50 ml of sterile glycerol solution was added into it and mixed well. The bacteria stocks were prepared in many tubes and quickly placed into -80 C. The Gram staining was done in order to confirm the growth of bacteria and to differentiate between gram - positive and gram - negative bacteria. First, the culture from BHI broth was plated onto BHI agar plate to grow the colony. After the incubation for 18 hours in 37°C in incubator, the plate was taken out and a single colony was spread onto thin film over slide and diluted with one drop of distilled water. The slide with the specimen was heat-fixed by passing it over a flame quickly. Then, the fixed smear slide was flooded with crystal violet for one minute where the slide was appeared in purple color. After that, the slide was rinsed off under running water and was flooded with iodine solution for one minute. Then, the slide was immersed in 95% alcohol for 20 seconds. Lastly, the slide was counterstained with safranin solution for one minute. The slide was left to dry before viewing under light microscope. The slide was microscopically observed under a 100x objective with

one drop of immersion oil. Gram – positive bacteria stained with deep violet was observed during microscopically examination.

C. Sample Extraction

Ethanol solution was used to extract the phytochemical component of *Salvadora persica*. *Salvadora persica* powder were soaked into 60% of ethanol and wrapped up in aluminum foil. The alcoholic extract is more effective for antibacterial activity against the oral pathogen as compared to aqueous extract [29]. *Salvadora persica* extraction was placed in room temperature for 18 hours. The solution was stirred every 4 hours to get the equivalent extract from the powder. Then, the extract was filtered through Whatman No.1 filter paper in order to separate the sedimentation. About 500ml of fresh solution was obtained during the process of filtration. The extract of *Salvadora persica* solution was sent to Natural Product Laboratory at Kulliyah of Sciences to evaporate the solvent of alcohol by using Rotary evaporator. Freeze drying process took place after the evaporation of the solution. It is a special form of drying that removes all the moisture from a sample. The methanol extract was subjected to complete freezing at -85°C for a few hours, resulting in a rigid product without denaturation of enzyme and any chemical changes. The extract was then evaporated overnight until the final crude of *Salvadora persica* was obtained at 16.55 grams.

D. Freeze Drying

Freeze drying method was used in this experiment in order to get the high yield of the crude of *Salvadora persica*. The process of freeze drying includes the process of sublimation and removal of bounded water molecule in the solution via desorption process. By using this method, it keeps the product at low temperature along the process to avoid any changes from occurring in terms of product appearance and characteristic. From 315 grams of *Salvadora persica* at the earlier weight, the final crude obtained after freeze drying process was 16.55 grams. The crude was stored in a beaker at room temperature and covered with aluminum foil to prevent evaporation and exposure to excessive light.

E. Preparation of Different Concentrations of *Salvadora Persica*

The different concentrations of the *Salvadora persica* were prepared in a falcon tube. The working solution that has been prepared was 200 mg/ml. Two hundred milligram per milliliter of *Salvadora persica* was prepared by weighing 3 grams of the crude of *Salvadora persica* by using analytical balance and diluted in 15 ml of ultra-pure water in falcon tube. Then, the concentration was diluted to 150mg/ml, 125 mg/ml, 100 mg/ml, 50 mg/ml and 25 mg/ml. The volume concentration of *Salvadora persica* was based on previous research with slightly modification. The preparation of different concentrations of *Salvadora persica* were calculated by using the formula $M1V1 = M2V2$. It is the process of decreasing the concentration of solute in solution, usually by mixing with more solvent such as pure distilled water in this experiment. M1V1 is the moles before dilution and M2V2 is the moles after dilution. The remaining crude was stored in a dark place at room temperature for further use.

F. Bacteria Culture Activation

Enterococcus faecalis (ATCC ®19433™) was purchased in May 2016. A little amount of the bacteria from the primary vial was inserted into the BHI broth and the tube was placed in an incubator for 18 hours at 37°C . The Minimal Inhibitory Concentration of the *Salvadora persica* extracts were determine based on a micro broth dilution method in 96 multi- well microtitter plate. The dissolved extracts were prepared from lowest concentration to highest concentration of *Salvadora persica*. 150 mg/ml, 125 mg/ml, 100 mg/ml, 75 mg/ml of concentration of *Salvadora persica* were prepared in a falcon tube. Hundred microliter of BHI broth were added to each well from the first well to the sixth well. Then, different concentrations were added into the ir respective wells (150 mg/ml, 125 mg/ml, 100 mg/ml, and 50 mg/ml concentration of *Salvadora persica*). Ten microliter of bacteria was added into each well. Sodium hypochlorite served as a positive control in the sixth well while negative control in well seven contained broth and bacteria only. The microtitter plate was prepared for aerobic condition. After pipetting the prepared solution, the plates for aerobic condition were incubated at 37°C for 18 hours. The lowest concentration at which colour change occurred was taken as the MIC value [5][14]. The determination was carried out in triplicate and repeated three times to ensure the results obtained were reliable.

G. MIC and MBC

The Minimal inhibition concentration (MIC) was done first before proceeding with the Minimum Bactericidal Concentration (MBC). MIC is the lowest concentration of an antimicrobial agent that can inhibit the visible growth of the microorganism after an incubation of 18 hours. It was used to evaluate the efficacy of antibacterial used in this experiment. The multi-plate 96 – well was placed in the multi- plate reader to obtain the results. The lowest value of the MIC was selected and plated onto the agar plate. The MBC was carried after the lowest MIC was determined. Several serial dilutions were done before plating the sample onto the agar plate. Ten microliter from serial dilution was transferred from the tube to the BHI agar and incubated for 18 hours at 37°C . The appeared colonies on the BHI agar plate were counted and the CFU/ml was calculated.



Fig. 2 MIC was done in 96 -well multiplate

H. Statistical Analysis

The statistical analysis of MIC value was carried out using ANOVA. Colonies of the bacteria formed on the agar plate after 18 hours of incubation were counted using the CFU/ml formula.

III. RESULTS AND DISCUSSION

A. Overview of general finding

There is a continued effort in identifying the *Salvadora Persica*'s efficiency in terms of antibacterial activity towards *Enterococcus faecalis*. Eventhough, there were studies on *Salvadora persica* previously, but it was performed on different types of bacteria which were Gram- negative and fungus and a few Gram-positive bacteria. Despite the fact that sodium hypochlorite is regarded as the most effective solution and the most widely used root canal irrigant, there is a chance that natural products could take part in root canal treatment. Moreover, the results of previous studies revealed that the ethanol extract of Miswak showed stronger anti-bacterial action than an aqueous extract [14].

B. Comparison of Antibacterial Activity of Salvador Persica and Sodium Hypochlorite

Normality tests was carried out for Week 4 until Week 6. Shapiro- Wilk was computed only when the sample size is less than 50 and the resultant p value was selected to determine the normality of distributed data. In the normality test, a large p - value was selected to conclude that the data is normally distributed. The data was normally distributed, with p- value > 0.005 when normality tests were conducted for Week 4, 5 and 6.

ANOVA test of antibacterial activity was carried out to test the equality of the means in three or more independent groups. The results obtained were statistically significant at 0.001 which was less than the p value at 0.05. This was continued with Post Hoc test to discover the significance between the groups. Previous in vitro studies revealed that the action of the bacterial activity was dependant on certain concentration that has been tested. Almas et al. (2005) shows that 10 mg/ml of *Salvadora persica* produced greater zone of inhibition in agar diffusion assay when compared to other concentrations. The following readings of the absorbance were taken using a micro plate reader. It is a method to measure the cells that contained in the sample solution by measuring the intensity of light as a beam of light passes through at certain ranges of wavelength. The optical density of the bacterial suspension was standardized using the micro plate reader at 600nm wavelength as suggested by Mehmet and Ayce (2016).

In this study, the results show the lowest MIC among the concentration was at 50mg/ml 0.844 ± 0.038 when compare to the positive control 0.094 ± 0.015 . Meanwhile at the concentration of 150 mg/ml, shows the highest absorbance at 1.498 ± 0.0674 among the tested concentration. The highest concentration may suggest that the absorbance reading of dead bacteria were included after 18 hours of incubation period as it was not an effective concentration that inhibits the bacteria growth. In addition, due to some technical error, the absorbance reading could be due to concentrations of *Salvadora persica*. The controls for each concentrations of *Salvadora persica* were not taken before the incubation procedure. Only the standard blank which contained broth was set up in this study. As the concentration of *Salvadora persica* increases, the higher the reading of absorbance were obtained. The reading of growth absorbance of bacteria treated with *Salvadora persica* showed increasing trend as compared to negative control which only

contained broth and nutrient supply. It may suggest that the higher concentration does not inhibit the growth of bacteria.

Regarding the effectiveness inhibition of bacteria, Colony forming unit (CFU/ml) was used to estimate the number of viable of *Enterococcus faecalis* in the sample. Counting the colony of the bacteria required a cell culture and only viable cells on the agar plate were counted. The serial dilution 10^{-4} and 10^{-6} plates were selected, and streaked on BHI agar. The inhibition growth of *Enterococcus faecalis*, positive sample that tested with sodium hypochlorite shows 252.149 CFU/ml. It acts as a baseline to compare the effectiveness of different concentrations of *Salvadora persica* with positive control. 150mg/ml of *Salvadora persica* represented the lowest inhibition of bacteria at 123.905 CFU/ml while 50 mg/ml of the concentration gave the highest CFU at 3502.475CFU/ml after 18 hours of incubation at 37°C . The result of MIC in week 4, 5, 6 were significant at 0.001 which was less than $p < 0.05$. The test was continued with Post hoc test to discover the reasons of the significance.

C. Future Work

Studies on root canal irrigation are quite limited. There are a few scientific journals or papers published about the plant related to root canal irrigation and endodontics. Thus, there are insufficient data to be compared with the present findings. Secondly, there were budget constrains during the laboratory work. Regarding to the protocol and procedure, there was one missing step that was not done in this research which was the reading absorbance of control crude *Salvadora persica*. The only control that was set up in this research was the negative control which contained broth and bacteria and blank. In addition, laboratory work in performing the inhibition study by plating the bacteria onto the agar plate and plate counting procedure required extra effort.

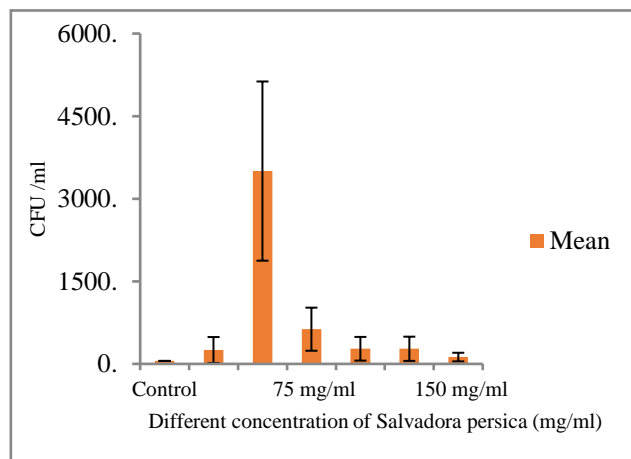


Fig. 3 Growth inhibition of *Enterococcus Faecalis*

TABLE I
 CONCENTRATION OF *ENTEROCOCCUS FAECALIS* GROWTH ABSORBANCE WITH DIFFERENT CONCENTRATIONS OF *SALVADORA PERSICA* AFTER 18 HOURS INCUBATION (N=9)

<i>Sample (mg/ml)</i>	<i>Mean</i>	<i>SD</i>	<i>p – value</i>	
			<i>Negative</i>	<i>Positive</i>
50	0.84	0.04	0.001	0.001
75	0.88	0.07	0.001	0.001
100	1.10	0.08	0.001	0.001
125	1.31	0.10	0.001	0.001
150	1.50	0.07	0.001	0.001
Control				
Positive	0.09	0.01	0.001	
Negative	0.64	0.22		0.001

TABLE 2
 VIABILITY OF *ENTEROCOCCUS FAECALIS* ENUMERATED BY PLATE COUNT AFTER 18 HOURS OF INCUBATION (N=6)

	Control	Positive	50mg/ml	75mg/ml	100mg/ml	125mg/ml	150mg/ml
	54	0.666	23.3	1.33	0	0	0
	TMTC	1.33	.	15.5	4.67	2	3.66
	TMTC	14.3	.	.	47.3	18.3	6.77
	TMTC	33.3	86.6	533.3	0	233	0
	TMTC	1430	6700	2300	1330	1360	300
Mean	TMTC	252.15	3502.48	630.03	274.83	274.43	123.91
SD	#	577.21	3986.17	959.16	527.12	539.16	192.58
N	6	6	6	6	6	6	6

TABLE 3
GROWTH ABSORBANCE OF MICROBROTH DILUTION ASSAY

No	Control	150mg/ml	125mg/ml	100mg/ml	75mg/ml	50mg/ml	Positive
1	0.5845	1.599	1.431	1.097	0.892	0.895	0.087
2	0.4539	1.582	1.436	1.153	0.948	0.862	0.103
3	0.4131	1.559	1.394	1.153	0.938	0.878	0.115
4	0.7921	1.411	1.173	0.986	0.768	0.803	0.092
5	0.9366	1.45	1.363	1.182	0.834	0.818	0.066
6	1.0334	1.478	1.195	1.22	0.777	0.836	0.081
7	0.4883	1.436	1.266	1.017	0.918	0.789	0.098
8	0.5787	1.463	1.268	1.042	0.944	0.831	0.108
9	0.5107	1.504	1.251	1.087	0.94	0.885	0.095
Mean	0.6435	1.498	1.3086	1.1041	0.8843	0.8441	0.0939
SD	0.2232	0.0674	0.0997	0.0791	0.0728	0.0377	0.0148
N	9	9	9	9	9	9	9
SEM	0.074	0.022	0.033	0.026	0.024	0.013	0.005
OD	100	132.8	103.4	71.58	37.43	31.18	-85.41

IV. CONCLUSIONS

From this study, it can be concluded that low yield crude of *Salvadora persica* were obtained by using freeze drying method. Antibacterial activity of *Salvadora persica* is comparable with sodium hypochlorite at the concentration 150mg/ml based on growth of inhibition of *Enterococcus faecalis*. It is believed that 60% of alcoholic *Salvadora persica* extraction is an effective antimicrobial agent for utilize as an irrigant in root canal treatment to extract out the polar and non-polar molecule.

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REFERENCES

- [1] Abo Al – Samh D. Al- Bagieh N (1996) . A study of Antibacterial activity of the *Salvadora persica* extract in vitro. *Biomedical Letters*53: 225-238 doi: 10.1155/2016/7083964
- [2] Akhilanand Chaurasia Ranjit Patil, Amit Nagar (2013). A Review Article, *Salvadora persica* in Oral Cavity - An Update, *Journal of Oral Biology and Craniofacial Research* 3 pp. 98-101 doi: 10.1016/j.jobcr.2012.09.004
- [3] Article, *Salvadora persica* in Oral Cavity - An Update, *Journal of Oral Biology and Craniofacial Research* 3 pp. 98-101 doi: 10.1016/j.jobcr.2012.09.004
- [4] Akhtar MS, Ajmal M. Significance of Chewing Sticks (*Salvadora Persica*) in oral hygiene from pharmacological viewpoint. *Journal of Pakistan Medical Association*. 2000;4-84-95 doi: 10.4103/1305-7456.178297
- [5] Al-Bayati, F., Sulaiman, K., (2008). *In vitro* Antimicrobial Activity of *Salvadora Persica* Extracts Against Isolated Oral Pathogens in Iraq, *Turk Journal Biology*, 32, pp.57-62. doi: 10.1155/2016/7083964
- [6] Al-Bageih, N., Idowu, A., Salako, N.O., (1994). Effect of Aquours Extract of *Salvadora persica* on the *in vitro* Growth of *Candida albicans* Microbios, 80(323), pp. 107-113.
- [7] Al-Lafi T., Ababneh H., (1995), The Effect of the Extract of the *Salvadora persica* (chewing sticks) used in Jordan and the Middle East on oral bacteria. *International Dental Journal*, 45, pp. 218-222.
- [8] Al-Mas, Khalid, (2001). The Antimicrobial Effects of Seven Different Types of Asia Chewing Sticks. *Odonto-Stomalogie Tropicale Journal*, 24(96), pp. 17-20.
- [9] Almas K., Al-BAGieh, N. Akpata, E., (1997). *In vitro* Antimicrobial Effects of Freshly Cut and 1 month old *Salvadora persica* (chewing stick). *Biomedical. Lett*, 56, pp.145-149. doi: 10.12669/pjms.302.4284
- [10] Amir Moeintaghavi, Hamidreza Arab, Mehrangiz khajekaramodini, Rohollah Hosseini Hossein Danasteh,

- Hamed Niknami (2012). *In vitro* Antibacterial Comparison of Chlorhexidine, Persica Mouthwash and *Salvadora persica* Extract. *Journal of Contemporary Dental Practice*, 13(2), pp. 147-152. doi: 10.5005/jp-journals-10024-1111
- [11] Assadi SG, Asadi ZG. Chewing sticks and oral hygiene habits of the adult Pakistani population. *Int Dent J*. 1997; 47:275-8 doi: 10.1002/j.1875-595X.1997.tb00789.x
- [12] A.T.Khalil, Benzylamides from *Salvadora persica*, *Archives Pharmacol Research*, 29, pp 952-956.
- [13] Azhal Iqbal (2012). International Journal of Health Sciences, Antimicrobial Irrigants in Endodontic Therapy, *International Journal Health Sciences (Qassim)*, 6(2), pp.182-192
- [14] Bayaty F., Abdulla M, Hassan M, Roslan S, Hussain S, Said H (2010). Effect of mouthwash extracted from Miswak (*Salvadora persica*) on periodontal pathogenic bacteria in vitro study. *Science and Social Research*, 4:p178-810. doi: 10.5897/JMPR10.198
- [15] Bystrom G, Sundaqvist G, The Antibacterial Action on Sodium Hypochlorite and EDTA in 60 cases of Endodontic Therapy. *International Endodontic*, 18, pp.35-40. doi: 10.1111/j.1365-2591.1985.tb00416.x
- [16] Daurot I, Albandar, J., Skaung N., Ali R., (2002). Salivary Microbiota Level in Relation to Periodontal Status, Experience of Caries and *Salvadora persica* use in Sundanese Adults. *Journal Clinical Periodontal*, 29(5), pp.411-420. doi: 10.1034/j.1600-051X.2002.290505.x
- [17] Dominic Peter Laverty (2014), Sodium Hypochlorite Accident During Endodontic Treatment, *Journal of Restorative Dentistry* vol 2 pp 94-100 doi: 10.16966/2378-7090.114
- [18] Estrela C, Silva JA de Alencer AH, Leles CR, Decurcio DA (2008), Efficacy of sodium hypochlorite and Chlorhexidine Against *Enterococcus faecalis*: A systemic review. *Journal Applicato Oral Science*, 16:364 doi: 10.1590/s1678-77572008000600002
- [19] Fouad Hussein Al-Bayaty, Aiman Hamad Al-Koubaisi, Nidhal Abdul Wahid Ali, Mahmood Ameen Abdulla (2010). Effect of Mouthwash extracted from *Salvadora persica* on dental plaque formation: A clinical trial. *Journal of Medical Plants Research*, 4(14) pp 1446-1454. doi: 10.5897/JMPR10.198
- [20] Gatot A, Arbelles J, Leiberman A, Yanai-Inbar I (1991) Effect of Sodium Hypochlorite on soft tissues after inadvertent injection beyond root apex. *Journal of Endodontics*:7-573-4 doi: 10.1016/S0099-2399(06)81725-5
- [21] Gerhardt CR, Eppendorf K, Kozlowski A, Brandt M (2004), Toxicity of Concentrated Sodium Hypochlorite used as an Endodontic Irrigant. *International Endodontic Journal* 37:272-280 doi: 10.1111/j.0143-2885.2004.00804.x
- [22] Guile EE, Al-Shammery, AR, El-Backly MN. Oral health survey of Saudi Arabia: Oral Health Knowledge Attitude and Practice Among Adults. *Journal Dentistry Research* 1996:75 doi: 10.4236/health.2010.25074
- [23] Hassan Sulaiman Hallway (2012). A Review on *Salvadora persica* and Its Effect on Various Aspect of Oral Health. *The Saudi Dental Journal*, 24, pp.63-69. doi: 10.1016/j.sdentj.2011.12.004
- [24] Hales JJ, Jackson CR, Everett AP, Moorre SH (2001). Treatment Protocol for the Management of a Sodium Hypochlorite Accident During Endodontic Therapy, *General Dentistry* 49:278-281
- [25] Hulsmann M, Hahn W., (2000). Complications during Root Canal Irrigation - Literature Review and Case Report. *International Endodontic Journal*, 33(3), pp 186-193. doi: 10.1046/j.1365-2591.2000.00303.x
- [26] Khalessi AM, Pack AR, Thomson WM, Tompkins GR. Extracts of *Salvadora persica*. *Int Dent J*. 2004;54:279-283
- [27] Maisa o Al-Sebaei, Omar A Halabi, Ibrahim E El-Hakim (2015). Sodium Hypochlorite Accident resulting in life threatening airway obstruction during root canal treatment: a case report. *Clinical Cosmetic and Investigational Dentistry* 7, pp.41-44. doi: 10.2147/CCIDE.S79436
- [28] Mandel ID (1988). Chemotherapeutic Agents for Controlling Plaque and Gingivitis. *Journal Clinic Periodontal* 15, pp. 488-498. doi: 10.1111/j.1600-051X.1988.tb01020.x
- [29] Mansour MI, Al-Khateeb TL and Al-Mazraoo AA., (1996). The Analgesic Effect of *Salvadora persica*. *Saudi Dental Journal*, 8, pp.87-91.
- [30] Mark D Essner, Amjad Javed, Paul D Eleazer (2011), Oral Surgical Oral Med Oral Radiol Endod. Effect of Sodium Hypochlorite on human pulp cells : an vitro study, 112(5):662-666 doi: 10.1016/j.tripleo.2011.04.030
- [31] Mumghamba EG, Manji KP, Michael J. Oral hygiene practices, periodontal conditions, dentition status and self-reported bad mouth breathe among young mothers, Tanzania. *Int J Dent Hyg*. 2006;4:166-173. doi: 10.1111/j.1601-5037.2006.00186.x
- [32] Nordin Fatin - Majdina, Haji Abd Razak Monica @ MUNirah, Mohd Bakri MARina (2014), Sains Malaysiana, Effect of *Salvadora persica* Extract on the bacterial population in single-species Biofilm, 43(12): 1889-1893. doi: 10.17576/jsm-2014-4312-10
- [33] Orstaik D, Haapasalo M, (1990). Disinfection by endodontic, irritants and dressing of experimentally infected dentinal tubules. *Endodontic Dental Traumatol*, 6 p.p. 142-149 doi: 10.1111/j.1600-9657.1990.tb00409.x
- [34] Qian-Qian Wang, Cheng - Fei Zhang, Chun - Hung Chu, Xiao - Fei Zhu (2012). Prevalence of *Enterococcus faecalis* in saliva and filled root canals Of teeth associated with apical periodontitis, *International Journal of Oral Science*, 4, pp. 19-20. doi: 10.1038/ijos.2012.17
- [35] Raed I, Al-Sadhan, Khalid Almas, (1999). *Salvadora persica* (Chewing stick): A cultural and Scientific Heritage. *The Saudi Dental Journal*, 11, 2.
- [36] Shamat A. M: Babiker I.A: Mukhtar M.S: Ahmed F.A (2010). A study of the seasonal and regional variations in nutritive value of some important plant species selected by camels in arid and semi-arid lands (ASAL) of Sudan. *Journal Applied Science. Restoration*, 6(8) 1265-1272.
- [37] Shabahang S, Torabinejad M (2003), In vitro antibacterial on *Enterococcus faecalis* contaminated root canals of extracted human teeth. *Journal Endodontic*, 29, pp.576-579.
- [38] Talal H A-l-Salman, Moataz Gh Al-Shaekh Ali, Osama M Al-Nu'aimey (2005). The antimicrobial effect of water extraction of *Salvadora persica* as root canal irrigant. *Al-Rafidain Dental Journal*, 5, 1, pp. 33-36. doi: 10.33899/rden.2005.45508
- [39] Walid H.Foud H (2001). The Effect of Myrtus Communes Extract mouth washing newly dental plaque. *Journal of American College Dentistry*, 8 pp. 41-49.
- [40] Zhang W, Torabinejad M, Liy (2003). Evaluation of cytotoxicity of MTAD using the MTT-tetraolium method. *Journal endodontic* 29, pp.654-657. doi: 10.1097/00004770-200310000-00010
- [41] British Dental Association (December, 2014), BDA Evidence Summary, Irrigation in Endodontics. Pp 2-8. doi: 10.1038/sj.bdj.2014.204
- [42] B. Gomes, E.T.Pinheiro, (2004). Microbiological Examination of Infected Dental Root Canals *Oral Microbiology Immunology*, 19, pp. 71-76. doi: 10.1046/j.0902-0055.2003.00116.x
- [43] Ercan E, Dalli M, Yavuz, Ozekinci T. (2006), Investigation of Microorganism In Infected Dental Root Canals,

Biotechnol & Biotechnol pp166-172. doi:
10.1080/13102818.2006.10817361

- [44] Lewis WH, Elvin-Lewis (1997) MPF. Oral Hygiene Medical Botany, John Wiley & Sons, New York, 226-270.
- [45] Nannin, E. C., & Murray, B.E. (2006). *Enterococcus* spp. In S.H. Gillespie, & P.M. Hawkey (Eds.), Principles and Practice of Clinical Bacteriology (2nd ed., pp. 59-71). West Sussex UK: John Wiley & Sons, Ltd.
- [46] Orwa C, A Mutua, Kindt R, Jamnadass R, S Anthony., (2009). Agroforestry Database: a tree reference and selection guide version 4.0
- [47] Public Health Agency of Canada, (2010). Enterococcus faecalis: Pathogen safetydatasheet - Infectious substances.
- [48] Teixeira, L.M., Carvalho, Maria da Gloria Siqueira & Facklam, R.R.(2007). Enterococcus In P.R.Murray (Ed.), *Manual of Clinical Microbiology*, 9, p.p. 430-442.
- [49] World Health Organization (1987) Prevention of Oral Disease. Geneva:WHO