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Role of *Salvadora persica* in eradication of *Enterococcus faecalis* isolated from infected dental pulp /in vitro study.

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ABSTRACT

Salvadora persica, also known as the toothbrush tree (Miswak), has been used since ancient times as a chewing stick for oral hygiene, it traditionally used for the treatment of oral infections. To investigate the antibacterial effect of a Miswak on *Enterococcus faecalis* in deep root canal dentin. One hundred extracted single rooted teeth were selected for this study. The root canals were prepared and shaped with sterile reamers (#15-35) and Miswak extract was injected into the canal and microbiological samples were collected from each tooth using sterile paper points before and after the treatment with diode laser and inoculated into (brain heart infusion agar and blood agar) for the isolation and identification of *Enterococcus faecalis*. Eleven (11%) isolates of *Enterococcus faecalis* were isolated from 100 single rooted canals, after injection with Miswak 10 (91%) isolates were eradicated, whereas only 1 (9%) isolate was resistant to Miswak activation. Miswak was very effective in the elimination of *Enterococcus faecalis* from infected single rooted canals.

Keywords: Dental pulp, *Enterococcus faecalis*, Eradication, Miswak, *Salvadora persica*.

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INTRODUCTION

Oral hygiene is one of the most important daily routine practices and keeps the mouth and teeth clean and prevent many health problems (Halawany, 2012).

People have been using different tools and chemicals to maintain their oral health, such as chewing sticks, tooth brushes, gum, mouth wash, and toothpaste (Rigges, et al., 2012); chewing-sticks were used by the Babylonians more than 7000 years ago (Dutta, S., & Shaikh, A., 2012).

Currently, 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) and his companions to achieve daily dental care, and the prophet emphasized the importance of using Miswak for oral hygiene (Abhary, M., and Al-Hazmi, A., 2016). Ahmed, H., and Rajagopal, K., 2013, were succeeded to evaluate the chemical composition of

S. persica such as flavonoids, salvadorine, cyanogenic glycosides, lignans, saponins, alkaloids, tannins, linoleic acid, stearic acid, salvadorea, vitamin C, silica and different salts are also known to possess significant antimicrobial activity.

Bacterial infection plays an important role in the development of necrosis in the dental pulp and the formation of periapical lesions (Monawer, A.T., and Abdukahar, I. M., 2016).

Enterococcus faecalis has been the micro-organism most commonly found (zoletti, G. O., et al., 2006), due to the specific characteristics such as: ability to colonize the dentin and tubules, which makes its removal by chemical and mechanical means difficult (Duggan, j. M., and Sedgley, C. M., 2007), and may even resist the medication (Saleh, R., et al., 2008) and irrigating solutions (oliveira, P.A., et al., 2007).

Monawer, A.T., and Abdukahar, I. M., 2016, showed that *E. faecalis* has proved to be a potentially important microorganism to the colonization or overgrowth in endodontic infections, being the dominant microorganism in post treatment apical periodontitis, and has often been isolated from the root canal in pure culture.

The aim of this study is to investigate the antibacterial effect of a Miswak on isolated *E. faecalis* from deep root canal dentin.

MATERIAL AND METHOD

The work was carried out in Microbiology Department, Nursing College, Duhok University; the period of this study was from November 2017 to March 2018.

Plant material

Dried plant samples were used in this study. Dry stems of *S. persica* from a local markets of Duhok city.

Plant extract

Miswak samples were used. Extraction was made with three different solvent: a mixture of acetone, water, and ethanol; then the extracts of the plant species were prepared by adding 4 g of small particle fresh plant material powder with a commercially available coffee blender to 40 ml solvent and allowing the mixtures to stand overnight at room temperature, after which the supernatants were filtered and dried under a controlled temperature (40°C) and then antibacterial activity was evaluated (Noumi, E., et al., 2011).

Bacterial collection & Identification:

One hundred extracted teeth with single rooted canal were collected randomly from different private dental clinics in Duhok city, Kurdistan province, Iraq.

Reamers of suitable sizes (#15-30) were used to enlarge the canal and remove the pulp tissues. Instrumentation was followed by irrigation with normal saline. The initial microbiological samples were obtained by inserting sterile paper point into the prepared canal (Monawer, A.T., and Abdukahar, I. M., 2016).

The *S. persica* (Miswak) extract was injected into the canal, which should be reaching the apex, and then the second sample was taken by inserting sterile paper point into the canal.

The sterile paper points were cultured into brain heart infusion broth. After incubation at 37°C for 24 hours, each sample was sub-cultured on brain heart infusion agar, and blood agar, After incubation at 37°C for 24 hours, colony morphology were noted and diagnosed by catalase test and gram stain. The suspected colonies were sub-cultured onto bile esculin agar and incubated at 37°C for 24 hours (Monawer, A.T., and Abdukahar, I. M., 2016).

RESULTS

Out of a total of 100 samples, 11 (11%) isolates of *E. faecalis* were isolated and 89(89%) showed negative cultures.

Fig. 1. Gram stain of *E. faecalis* using light microscope.

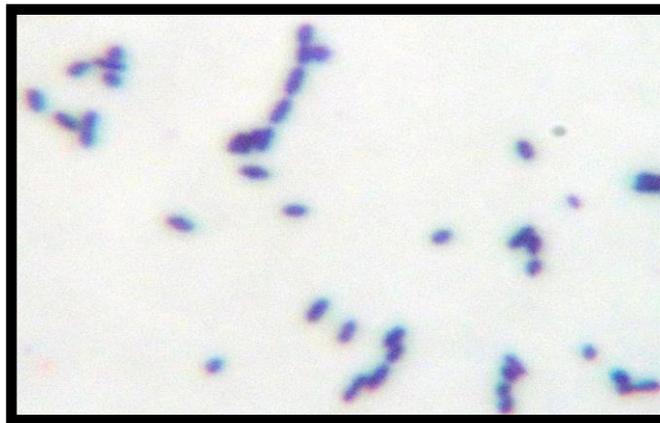


Fig. 2. *E. faecalis* tiny colonies cultured on Brain Heart Infusion agar.

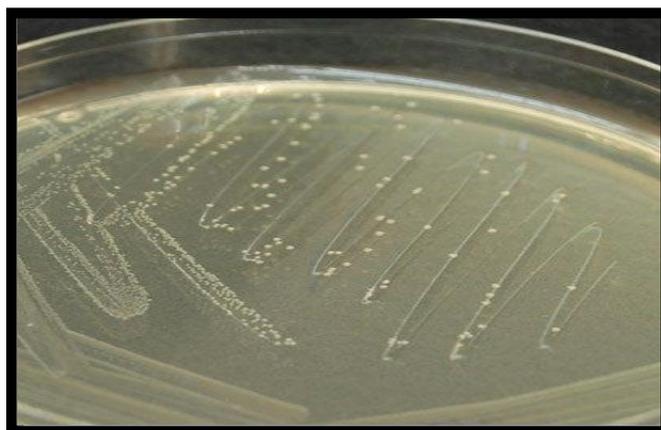


Fig. 3. *E. faecalis* white colonies on blood agar.



Fig. 4. Bile Esculin agar showing positive *E. faecalis* growth with blacking area.



TABLE I: NUMBER AND PERCENTAGE OF *E. FAECALIS* RESPONSE BEFORE AND AFTER TREATMENT WITH MISWAK EXTRACT

No. of samples	positive isolates of <i>E. faecalis</i>	negative isolates of <i>E. faecalis</i>	Before treatment with Miswak extract	After treatment with Miswak extract	
				Eradicated	Non eradicated
100	11 (11 %)	89(89%)	11 (100%)	10 (91%)	1 (9%)

Table [1] illustrated that Miswak extract eradicated 10 (91%) isolates of *E. faecalis*, while 1 (9%) was resistant.

DISCUSSION

In several studies, medicinal plant extracts, plant products and isolated phytochemical constituents showed highly significant antimicrobial activity, On the other hand, the observed bacterial resistance and undesirable side effect of certain antibiotics led scientific community to find new antibacterial compounds from medicinal plants (Ahmed, H., and Rajagopal, K., 2013), Miswak is a common name for *S. persica*, which is commonly used in Saudi Arabia and the Arab world. Miswak wicks clean between the teeth and do not break, regardless of the amount of pressure applied, as they are flexible and strong (Al-bayati, F.A., and Sulaiman, K. D., 2008).

S. persica has a wide geographic distribution ranging from India, Nepal, Malaysia, Pakistan, Iran, Iraq, Saudi Arabia, Egypt, and Africa (Kshirsagar, J.T., and Jareen, A.J., 2017).

In the present study, out of 100 sample only 11 (11%) cases of *E. faecalis* were isolated and 89(89%) were negative for *Enterococcus faecalis*, and after treatment with Miswak extract 10 (91%) cases of *Enterococcus faecalis* were eradicated , while only 1(9%) was resistant.

Previous studies have reported that *S. persica* extracts were effective against *Streptococcus mutans* and *Streptococcus faecalis*, even using low extract concentrations (Salehi, P., and Danaie, M. S.H., 2006).

A study by Al-bayati, F.A., and Sulaiman, K. D., 2008, revealed the aqueous extract of *S. persica* was active against all oral pathogens and *Streptococcus* species were the most sensitive; the highest inhibitory activity was seen against *Strep. Faecalis* (zone of inhibition: 22.3 mm) using the extract concentration of 200 mg/ml.

The data from a Saudi Arabia's study done by Researchers from the College of Medicine at Saudi Arabia's Najran University tested Penicillin-resistant *Enterococcus faecalis* against extracts of Miswak twigs suggested that Miswak successfully killed the bacteria, also the same study reported that the Miswak twigs not only fight bacteria that infect the mouth: They also fight the most lethal strains of antibiotic resistant bacteria (Al-Ayed, M.S.Z., et al., 2016).

CONCLUSION

Present study showed new natural antibacterial agent that can replace the synthetic ones to be used in oral hygiene; thus, the effective source of *S. persica* could be employed in all medicinal preparations to combat a wide variety of bacteria.

Miswak strongly recommended to be used as daily teeth cleaner. Further study should be done on the same subject using more samples.

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