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Effectiveness of *Salvadora persica* extracts against common oral pathogens

Hanan Balto [a,*], **Ibrahim Al-Sanie** [b], **Sultan Al-Beshri** [c], **Abdullah Aldrees** [d]

[a] *Division of Endodontics, Department of Restorative Dental Science, Dental Caries Research Chair, College of Dentistry, King Saud University, Riyadh, Saudi Arabia*
[b] *College of Dentistry, King Saud University, Riyadh, Saudi Arabia*
[c] *Department of Periodontics and Community Dentistry, College of Dentistry, King Saud University, Saudi Arabia*
[d] *Division of Orthodontics, Department of Pediatric Dentistry and Orthodontics, College of Dentistry, King Saud University, Riyadh, Saudi Arabia*

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Abstract *Objective:* The purpose of this study was to evaluate the antibacterial activity of ethanol and hexane extracts of *Salvadora persica* against common oral pathogens.

Materials and methods: Well diffusion, Minimum Inhibition Concentration (MIC), Minimum Bactericidal Concentration (MBC), and Broth microdilution tests were used to determine the optimum antimicrobial concentrations of *S. persica* extracts against *Streptococcus mutans* (*S. mutans*), *Streptococcus sanguis* (*S. sanguis*), and *Streptococcus salivarius* (*S. salivarius*) over 1, 3, 6, 12, and 24 h. Chlorhexidine (CHX) 0.2% was used as a positive control.

Results: The findings showed that the microbial activity of both extracts was concentration-dependent. Ethanol extract of *S. persica* at 25, 50, and 100 mg/ml had more growth inhibitory effect against all isolates compared to hexane extract. In addition, ethanol extract at 8 mg/ml (MBC value) was able to eradicate the growth of all isolates. *S. sanguis* and *S. salivarius* were very sensitive to hexane extract and required 4 mg/ml (MBC value) for their eradication while *S. mutans* was the most resistant (MBC = 8 mg/ml).The statistical findings of CFU counts showed no significant difference ($p = 1.000$) in antibacterial effectiveness between the two extracts against all isolates. A significant decline overtime in CFU counts was noted, except at 12 h and 24 h where no significant difference ($p = 0.793$) was observed and was comparable to CHX.

Conclusion: Ethanol and hexane extracts of *S. persica* were found to exhibit maximum antimicrobial activity against *S. mutans*, *S. sanguis* and *S. salivarius* at high concentrations.

* Corresponding author at: P.O. Box 62645, Riyadh 11595, Saudi Arabia. Fax: +966 14679016.
E-mail addresses: h_balto@yahoo.com (H. Balto), ibrahim0505@gmail.com (I. Al-Sanie), albeshri.su@gmail.com (S. Al-Beshri), amaldrees@ksu.edu.sa (A. Aldrees).

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1. Introduction

Chemical treatment besides mechanical cleaning is needed to maintain gingival health, control plaque and prevent periodontal disease occurrence and progression (Al-Bayaty et al., 2010). However, with the increasing incidence of oral diseases, the global need for alternative prevention and treatment methods that are safe and effective has expanded (Halawany, 2012). Herbal medicine has been used for a long time for dental plaque, microorganism control, and maintenance of oral health (Fine, 1995; Mandel, 1988). The toothbrush tree, *Salvadora persica* (*S. persica*), known locally as "Miswak," is a member of the Salvadoraceae family. It is a small tree with soft, whitish, yellow wood, and has been used in Africa, South America, the Middle East, and Asia as a traditional oral hygiene tool (Noumi et al., 2010; Sofrata et al., 2008). 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 (Batwa et al., 2006).

It has been reported that extracts of *S. persica* possess various biological properties, including significant antimicrobial (Al lafi and Ababneh, 1995; Al-Sohaibani and Murugan, 2012; Masood et al., 2010; Sofrata et al., 2008) and anti-inflammatory (Ibrahim et al., 2011) properties, and lack of toxicity (Balto et al., 2014; Darmani et al., 2006). The antimicrobial and cleaning effects of *S. persica* may be attributed to various chemicals contained in its extracts such as trimethyamin, salvadorine, chloride, fluoride in large amounts, silica, sulfur, mustard, vitamin C, saponins, tannins, cyanogenic glycoside, and benzylisothiocyanate (Akhtar and Ajmal, 1981; Darout et al., 2000a,b). *S. persica* has demonstrated cleansing efficacy, ability to remove the plaque, and decrease ginigival bleeding (Batwa et al., 2006; Darout et al., 2000a,b) when used as a chewing stick. As a mouth wash, *S. persica* has improve periodontal health, reduce microbial plaque accumulation and lower carriage rate of cariogenic bacteria (Al-Otaibi et al., 2004; Khalessi et al., 2004).

Various methods for obtaining *S. persica* extract have been used, mainly aqueous and alcohol extracts (Al-Sabawi et al., 2007; Al-Bayati and Sulaiman, 2008), while others have used *S. persica* pieces without extraction (Sofrata et al., 2008). The antimicrobial effects of *S. persica* against a range of pathogens have been evaluated (Al-Bayati and Sulaiman, 2008; Khalessi et al., 2004; Poureslami et al., 2007). The results of these experiments are variable and sometimes contradictory as to the most effective *S. persica* extract preparation method, its concentrations, and which of the bacterial species are affected by *S. persica* extract.

Balto et al. (2013) have screened the antimicrobial activities of seven *S. persica* extracts against *Enterococcus faecalis* and *Candida albicans*. They have demonstrated that ethanol and hexane extracts exhibit the maximum antimicrobial activity against both microbes. Further study (Balto et al., 2014) has shown that both extracts (ethanol and hexane) were non-cytotoxic on human gingival fibroblast cells. Hexane extract has never been tested against common oral pathogens and in light of the previous promising findings (Balto et al., 2013, 2014), the aim of the current study was to assess the antibacterial activity of ethanol and hexane extracts of *S. persica* against *Streptococcus mutans*, *Streptococcus sanguis*, and *Streptococcus salivarius*.

2. Materials and methods

The study was carried out at the Laboratory of Microbiology, College of Medicine, King Saud University.

2.1. Extracts preparation

The roots of *S. persica* were collected from Al-Makhwah, which is located in the southern region of the Kingdom of Saudi Arabia, in March 2010. The plant was identified by a taxonomist and a voucher specimen (#1745) was deposited at the herbarium, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia for future reference. The stock solution was prepared by extracting the fresh ground roots three times with the following solvents: hexane and 10% ethanol. All extracts were prepared by percolating 100 g of dried powder in each solvent three times every 24 h, with fresh solvent used each time. The extracts were freeze-dried to ensure that the remaining solvent was completely removed. All *S. persica* extracts were suspended in dimethyl sulfoxide (DMSO) at a concentration of 100 mg/ml. The stock solution was sterilized using 10 KG of gamma radiation and kept in a freezer at −20 °C.

2.2. Test organisms

Three Gram-positive strains were used in this study, *Streptococcus mutans* (ATCC25175), *Streptococcus sanguis* (ATCC10556) and *Streptococcus salivarius* (ATCC13419) were taken from frozen stock culture (Dental Caries Research Chair, College of Dentistry, King Saud University), inoculated on a sheep blood agar plate (Oxoid Ltd, Basingstoke) and grown overnight at 37 °C. Cells were collected by centrifugation (900×*g* for 10 min) and the pellets were re-suspended in brain heart infusion broth (BHIB).

2.3. Tests for antimicrobial activities

2.3.1. Well diffusion method

It is based on the diffusion of the antibacterial substance in the agar. All test isolates were mixed with normal saline to achieve a turbidity equivalent to a 0.5 McFarland standard (approximately 10^8 colony-forming units per milliliter [CFU/ml]). This was further diluted by 1:100 to give a final concentration of 10^6 CFU/ml. Three Muller-Hinton (MH) agar plates with 5% blood (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) were inoculated with microbial suspensions (one plate/ bacteria/ extract). Four small wells were created by indenting the agar with a clean pipette. Each resulting well was approximately 6 mm in diameter and accommodated approximately 90–95 microliters (μl) of extract. Each well was then 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, respectively. All experiments were performed in duplicate for each herbal extract. Following incubation at 35 °C for 48 h anaerobically, the zone of herbal diffusion from the well into the agar was measured in millimeters. The shortest distance (mm) from the outer margin of the well to the initial point of microbial growth was considered as the inhibitory zone. Results were recorded as the average of the

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