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Volatile compounds of *Salvadora persica* inhibit the growth of oral *Candida* species

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ABSTRACT

Objective: The antibacterial effect of *Salvadora persica* has been demonstrated both *in vitro* and *in vivo*. However, data on its possible antifungal effect is scarce. Therefore, the aim of the present study was to investigate the antifungal effect of solid or pulverized *S. persica* on clinically important oral *Candida* species *in vitro*.

Design: The antifungal activity of *S. persica* was examined against reference strains and clinical isolates of oral *Candida* species by two different methods. In an agar diffusion test, solid as well as pulverized pieces of *S. persica* were tested. Mounting the *S. persica* test specimens inside the lid tested growth inhibition by volatile compounds.

Results: *S. persica* exhibited antifungal activity against all *Candida* species tested. In particular, the volatile compounds of solid test specimens demonstrated strong growth inhibition, whereas pulverized *S. persica* revealed no antifungal activity. Parameters such as storage and incubation time as well as the diameter of the sticks influenced the growth inhibition.

Conclusions: Volatile compounds of *S. persica* have antifungal activity against oral *Candida* species. Storage time after harvesting may play an important role for the strength of this antifungal activity.

1. Introduction

Rapidly increasing oropharyngeal candidiasis of an opportunistic nature is an alarming clinical problem in elderly and critically ill patients. However, also individuals with general and local predisposing factors, *e.g.* immunosuppressive or wide-spectrum antibiotic therapy, nutritional deficiencies, diabetes, dental caries, periodontitis or ill-fitting dentures, may suffer from oropharyngeal candidiasis.[1,2] The majority of oral candidiases are caused by the opportunistic pathogen *Candida albicans*, followed by non-albicans *Candida* species, such as *Candida glabrata*, *Candida krusei*, *Candida tropicalis*, *Candida dubliniensis*, and *Candida guilliermondii*. An alarming development of resistance against antifungal agents has been widely recognized in the international medical community.[3–6]

Mouthrinses containing chlorhexidine are often used for the prevention and treatment of oral infections, but there are mixed results on its clinical effect as it is the case for a variety of other oral hygiene products containing chemical antifungal agents or essential oils.[7] Therefore, to prevent and control oropharyngeal *Candida* infections, alternative strate-

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gies may be of importance, particularly in developing countries.[8]

Natural chewing sticks made of *Salvadora persica* may be considered to be 'the first toothbrush of mankind'. Its use has been documented by Babylonians, Greeks, Romans, Jews, and Egyptians.[9] In addition, it has been recommended by the Islamic tradition (Sunnah). Commonly known as 'miswak', it is still used as aid for the oral hygiene in many parts of the Islamic world and beyond.[10] The use of chewing sticks for oral health especially in regions where it is established, may play an important role and its use has been encouraged by the World Health Organization.[11]

Many plants, such as *Acacia arabica*, *Anogeissus leiocarpus*, *Azadirachta indica*, *Diospyros lycioides*, *Distemonanthus benthamianus*, *Fagara zanthoxyloides*, *Garcinia kola*, *Glyphea brevis*, *Massularia acuminata*, *Sorindeia warneckei*, *Terminalia glaucescens*, *Vernonia amygdalina*, and *Vitex doniana*, have been used for the preparation of chewing sticks.[12] The most common plant for this purpose is, however, *S. persica*.[13] In addition to its mechanical properties on plaque removal[14], antibacterial and antifungal effects have been demonstrated.[15,16]

Yet, in all studies investigating the antifungal activity of *S. persica* so far, plant extracts were used and variables such as dose-dependency, incubation time, structural characteristics of the test specimens, and time after harvesting have not been systematically tested. Hence, the aim of the present study was to investigate the antifungal effect of solid or pulverized *S. persica* on clinically important oral *Candida* species *in vitro*. Based on previous studies, an antifungal effect was expected. It was also hypothesized that the proximity/extremity of the test specimen from the stem and age after harvesting affected the antifungal efficacy.

2. Materials and methods

2.1. Harvesting and preparation of the plant material

S. persica sticks, originating from Al-Gizan, Saudi Arabia, were packed airtight and transported to the University of Basel within 7 days after harvesting. After arrival, the sticks were stored at room temperature.

The sticks were cut under sterile conditions in pieces with a length between 4 and 6 mm. The resulting test specimen had a diameter of 5–10 mm and weights of 0.10–0.25 g. The tree bark was not removed.

Additionally, *S. persica* pieces of analogous size and weight were ground in a ball mill (Pulver Muechle Fritsch Pulverisette 501, Totabànya, Hungary).

Test specimens of similarly treated *Glycyrrhiza glabra* root, grown in Turkey, served as negative controls. In preliminary tests, these specimens did not show any antifungal activity. Test specimens with a diameter of 5 or 10 mm and weights of 0.1 or 0.2 g, respectively, were used.

2.2. Candida species and growth conditions

The *Candida* strains used in this study are: *C. albicans* ATCC 32032, *C. albicans* ATCC 90028; nine clinical *C. albicans* isolates (University of Helsinki, Finland), namely *C. albicans* 914, *C. albicans* 806, *C. albicans* 808, *C. albicans* 841, *C. albicans* 663, *C. albicans* 667, *C. albicans* 816, *C. albicans* 918, *C. albicans* 822; *C. tropicalis* ATCC 750T, *C. krusei* ATCC 6258, *C. guilliermondii* ATCC 6260, *C. dubliniensis* ATCC MYA-646, and *C. glabrata* CCUG 32725 (Culture Collection University of Gothenburg, Sweden). All clinical isolates are of oral origin.

They were preserved in glycerin milk at −70 °C. Prior to the experiments, the strains were inoculated on Columbia blood agar plates (Columbia Agar Base [BBL Becton Dickinson, Allschwil, Switzerland] supplemented with 5 mg/l hemin, 0.5 mg/l menadione, and 50 ml/l human blood) and incubated under aerobic conditions at 37 °C for 48 h. The purity of cultures was controlled by their colony morphology and cellular appearance.

2.3. Antifungal testing

Three colonies each were suspended in 3 ml Sabouraud Dextrose Broth (Becton Dickinson, Allschwil, Switzerland) and incubated aerobically at 37 °C for 24 h. Sabouraud Dextrose Agar plates (Becton Dickinson) were evenly inoculated with this suspension using a cotton swab.

The antifungal testing was modified after Sofrata et al.[17] Briefly, for the agar diffusion test, *S. persica* test specimen of known weight, diameter, and age were gently placed on the agar surface.

To test for the antifungal activity of volatile compounds, *S. persica* test specimens were mounted inside the lid (lid-mounted test). This was done by attaching one of the cut surfaces of the test specimens to the lid using double-sided adhesive tape (Selbstklebebänder, Migros-Genossenschafts-Bund, Zürich, Switzerland). The other surface was facing the agar plate inoculated with the *Candida* suspensions. The tip of the test specimen was approximately 5 mm above the agar surface. The plates were incubated aerobically at 37 °C, examined after either 48 h or 96 h for zones of inhibition (area of no growth) and photographed.

The antifungal activity of pulverized *S. persica* was tested similarly in an ascending weight-dose against *C. albicans* ATCC 90028 and *C. tropicalis* ATCC 750T, respectively. For the agar diffusion test, pulverized *S. persica* was placed in the middle of the agar surface. To test for volatile antifungal compounds, the agar plate inoculated with the respective *Candida* suspension was placed upside down the lid containing pulverized *S. persica*. The plates were incubated aerobically at 37 °C and examined after 48 h.

G. glabra test specimen of analogous size and weight and the adhesive tape alone served as controls.

Each experiment was done three times.

2.4. SEM and EDS

S. persica test specimens of varying diameter and age were dehydrated, critical-point-dried, sputter-coated with 40 nm gold (Baltec MED O20, Liechtenstein), and examined morphologically in a scanning electron microscope (SEM) with 5 kV (Philips XL30, The Netherlands). Energy-dispersive X-ray spectroscopy (EDS) was used for element analysis.