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Original Article

Antibacterial activity against *Streptococcus mutans* and inhibition of bacterial induced enamel demineralization of propolis, miswak, and chitosan nanoparticles based dental varnishes

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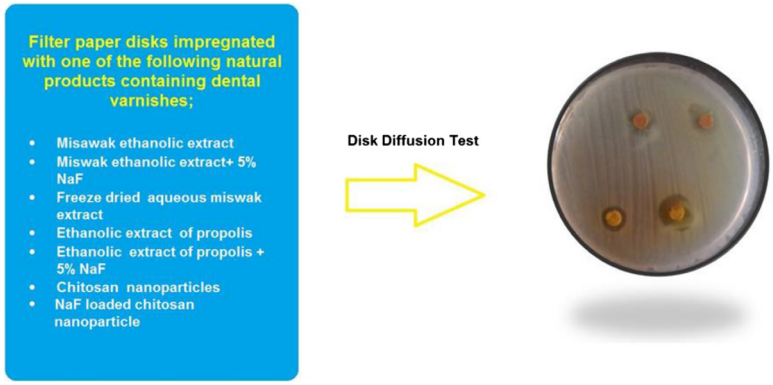
GRAPHICAL ABSTRACT



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ABSTRACT

Using natural products can be a cost-effective approach for caries prevention especially in low income countries where dental caries is highly prevalent and the resources are limited. Specially prepared dental varnishes containing propolis, miswak, and chitosan nanoparticles (CS-NPs) with or without sodium fluoride (NaF) were assessed for antibacterial effect against *Streptococcus mutans* (*S. mutans*) using disk diffusion test. In addition, the protective effect of a single pretreatment of primary teeth enamel specimens against *in vitro* bacterial induced enamel demineralization was assessed for 3 days. All natural products containing varnishes inhibited bacterial growth significantly better than 5% NaF varnish, with NaF loaded CS-NPs (CSF-NPs) showing the highest antibacterial effect, though it didn't significantly differ than those of other varnishes except miswak ethanolic extract (M) varnish. Greater inhibitory effect was noted with varnish containing freeze dried aqueous miswak extract compared to that containing ethanolic miswak extract, possibly due to concentration of antimicrobial substances by freeze drying. Adding natural products to NaF in a dental varnish showed an additive effect especially compared to fluoride containing varnish. 5% NaF varnish showed the best inhibition of demineralization effect. Fluoride containing miswak varnish (MF) and CSF-NPs varnish inhibited demineralization significantly better than all experimental varnishes, especially during the first 2 days, though CSF-NPs varnish had a low fluoride concentration, probably due to better availability of fluoride ions and the smaller size of nanoparticles. Incorporating

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natural products with fluoride into dental varnishes can be an effective approach for caries prevention, especially miswak and propolis when financial resources are limited.

Introduction

Dental caries is a biofilm-induced oral disease with *S. mutans* playing a key role in the development of virulent cariogenic biofilms [1]. Thus, decreasing the bacterial burden of the oral cavity is one of the fundamental biological goals in preventing dental caries.

Dental varnishes can be applied easily and quickly, and can deliver an active agent as fluoride or chlorhexidine to the teeth safely and in high concentration [2]. The most important anti-caries effect of fluoride results from its local action on the tooth/plaque interface, through promotion of remineralization and minimizing demineralization. It also prevents acid production by *S. mutans* [3]. However, fluoride by itself is not a potent antimicrobial agent. One study compared the effect of different fluoride varnishes on *S. mutans* and *S. sobrinus* biofilms formation *in vitro* and found that the greatest number of viable bacteria was found with the fluoride varnish that released the highest concentration of fluoride into the formed biofilms. In the same study, a combination of fluoride and chlorhexidine varnishes showed the lowest bacterial counts [4]. Although fluoride remains the mainstay for the prevention of dental caries, additional approaches are required to enhance its effectiveness. In this context, the combination of fluoride with antimicrobial agents such as xylitol and chlorhexidine was recommended by some guidelines for the prevention of dental caries especially in high risk individuals [5,6].

Due to the increase of antibiotic resistance and side effects of some antimicrobials on one hand, and the safety, availability, and relatively low costs of natural products on the other hand, a variety of natural products have been assessed for caries prevention as well as incorporated into dental products [1]. Propolis, a natural beehive product, is a complex resinous material that inhibits *S. mutans* growth and ability to adhere to tooth surfaces [7–10]. The minimum inhibitory concentration (MIC) of ethanolic extract of propolis (EEP) on *S. mutans* varies from 25 to 100 μg/mL [7,10,11]. A minimum bactericidal concentration (MBC) of more than 1600 μg/mL was reported [7,10]. Propolis also reduced human dental plaque accumulation and its insoluble external polysaccharide content [12]. It is a non-toxic material and its antimicrobial activity is attributed to the presence of flavonoids and terpenoids [1].

Miswak obtained from the roots or twigs of Arak (*Salvadora persica*) tree, which is found in many Asian and African countries, is one of many plants that have antimicrobial potential [1]. Antimicrobial, anti-tumor, anti-inflammatory, and wound healing properties of miswak extract have been linked to its content of tannic acid, alkaloids, eucalyptol, sulphur compounds, benzylisothiocynate, and benzyl nitrate. Its aqueous extract was also reported to have high calcium, but low fluoride content [13–15]. Its extracts possess plaque inhibiting and antimicrobial properties against cariogenic bacteria by inhibiting their growth and acid production [16–19]. The MIC for ethanolic and aqueous extracts of miswak against *S. mutans* was reported to be 50 mg/mL and 150 mg/mL, respectively [19].

Chitosan is a natural polymer obtained by alkaline hydrolysis of chitin, a natural compound that is found in arthropod extroskeletons, shells of crustaceans, and insects' cuticles. Because of its innate biocompatibility, biodegradability, and lack of toxicity; chitosan, and its nanoparticles received great attention in the pharmaceutical, food, agriculture, textile, and tissue engineering industries [20]. Chitosan has antitumor, wound-healing, mucoadhesive, and antimicrobial activities [20–22]. Its positive charge facilitates its adhesion to bacterial cell walls giving bacteriostatic or bacteriocidal activities to the material. Moreover, it is not known to cause antibacterial resistance [22]. The antibacterial mechanism of chitosan may include the interaction of cationic chitosan with the anionic cell surface, increasing membrane permeability and leakage of cellular material from the cell. Chitosan may also interfere with mRNA synthesis and imbedding protein synthesis [20,23]. An inhibitory effect against *S. mutans* was reported [22,24–30]. Chitosan interfered with *S. mutans* adhesion and primary biofilm formation [24,25] up to a week with little to no decrease in efficiency [24]. In addition, chitosan caused significant reductions in mature biofilm survival [24,25]. Chitosan-based mouthwash showed significantly higher antibacterial activity against *Streptococcus and Enterococcus* species than commercially available essential oils and chlorhexidine mouthwashes [25,26]. Moreover, CS-NPs have been developed for drug encapsulation. Drugs carried by CS-NPs can be released through degradation of chitosan, leading to a sustained-release effect. The nanosized structure allows permeation through cell membranes, which makes it an effective carrier of drugs in biological systems to achieve improved bioavailability of the drug [20,31,32]. Thus, the present study sought to assess the *in vitro S. mutans* susceptibility to specially formulated dental varnishes containing propolis, miswak, or chitosan nanoparticles, with or without NaF, as well as, to assess the protective effect of pretreating enamel of primary teeth with those varnishes against bacterial induced demineralization.

Material and methods

Miswak extracts preparation

Miswak aqueous extract

Freshly cut miswak chewing sticks were collected from the twigs of Arak (*Salvadora persica*) trees in Saudi Arabia (Mecca city) and identified by an agriculturist. Ten g of sundried and ground sticks were soaked in 100 mL sterile distilled water for 48 h at 4 °C. The extract was then centrifuged and the supernatant was filtered through a 0.45 mm filter paper [19]. The extract was then freeze dried for 7 days in a freeze drying machine (Martin Christ, Alpha 1-2 LD, Vacuubrand GMBH+ Co KG, Germany).

Miswak ethanolic extract

The extract was prepared according to Noumi et al. [33]. Ten g of miswak powder were added to 100 mL of 95% ethyl alcohol and soaked for 24 h at room temperature. Supernatant was filtered through a 0.45 mm filter paper and the extract was kept in tightly closed screw capped containers at 4 °C.

Propolis ethanolic extract preparation

EEP was prepared by mixing 50 g of propolis fine chips collected from the top of the combs of the hives of honey bees (*Apis mellifera carnica* L.) during autumn with 500 mL of 95% ethyl alcohol in a dark bottle at room temperature for 4 days with intermittent stirring. The mixture was filtered with a filter paper, and then left at room temperature until ethanol evaporated and the product obtained a honey-like consistency. The EEP was then stored at 4 °C [8].