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RESEARCH ARTICLE

Synthesis, Characterization, Antimicrobial Evaluation, and Cytotoxic Effects of Miswak Synthesized Silver Nanoparticle Mouth Rinse

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ABSTRACT

Aim: The aim of this study is to formulate miswak-synthesized silver nanoparticles and, its characterization and incorporate this into mouthwash along with xylitol, to test for its antimicrobial activity and cytotoxicity.

Materials and methods: Silver nanoparticle was synthesized using miswak. The formed nanoparticle was characterized using transmission electron microscope. Mouthwash was formulated by incorporating it along with xylitol. Antimicrobial activity was assessed against *Streptococcus mutans, Lactobacillus sp, Staphylococcus aureus, and Candida albicans*. Cytotoxic testing was assessed on nauplli stage of developing shrimp.

Results: The mouthwash showed efficient zone of diffusion against *Staphylococcus aureus* and *Streptococcus mutans* and a very strong antifungal activity. Silver nanoparticles were formed of size 5–25 nm and the particles were evenly scattered and was of spherical, angular, and rod shaped. The mouth rinse at higher concentrations showed mild toxicity.

Conclusion: Silver nanoparticle formulated using miswak is strong antibacterial and antifungal agent. Xylitol is an efficient anticariogenic agent and it will reduce the bacterial load in the plaque samples. Thus, miswak-mediated silver nanoparticles incorporated into mouthwash along with xylitol can be an effective alternative to commercial mouthwash.

KEYWORDS:
cytotoxicity; miswak; silver nanoparticle; transmission electron microscopy; xylitol

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INTRODUCTION

Mouthwash is widely used as an adjuvant to oral hygiene. Patients undergoing orthodontic treatment specifically have difficulty in maintaining oral hygiene due to the interference of brackets and wire with accessibility in using a tooth brush. These brackets and wire also tend to act as a nidus for plaque accumulation.

Miswak (*Salvadora persica*) is widely used as a component in oral hygiene and it is also a compound in many commercially available tooth pastes and mouth washes. Miswak contains fluoride nearly 1.0 (μg/g) and also releases calcium and phosphor into water.[1] Extract of miswak prepared from water extraction method and cold infusion method have shown significant activity against *Streptococcus mutans*.[2] Higher level concentrations of these extract have also shown some antifungal activity when experimentally used to test for its activity against *Candida albicans*.[3] Whole unextracted

Salvadora persica was examined by immersing it in agar plate and it has shown antibacterial effects in periodontitis and caries causing bacteria.[4] The methanolic extract of miswak has shown to be resisted by *Lactobacillus acidophilus* and *Pseudomonas aeruginosa*.[5] Being a plant extract, there are almost no chances of toxicity and from previous studies, there is no toxic effect to mice either as an aqueous or as an ethanolic extract up to doses of 1200 mg/kg.[6]

Nanoparticles are widely used in dentistry and it has been incorporated among various fields like for polishing paste, dental implant coatings, fillings, and in prevention of caries.[7] Nanoparticle can, in fact, be considered as nano antibiotics due to their antimicrobial activity.[8] In recent times, the diseases and pathology associated with antibiotic resistant bacteria are on the rise and the incorporation of nanoparticles in the currently available medicinal agents would be a strategy to deal with the resistant variety of the bacteria.[9] Silver is one of the oldest metals that has been used in medicine for its efficiency against infections. The benefits of silver are definitely more than its risk factors.[10] It has been incorporated into products that are available commercially and it is being used an as antimicrobial agent.[11] Silver nanoparticles can exist as substances that can be spherical, tubular, or irregularly shaped and can exist as fused, aggregated, or agglomerated in form.[12] Chitosan–silver nanoparticles has shown very strong antibacterial activity against Staphylococcus aureus, Lactobacillus in previous studies.[13] Silver nanoparticles release silver ions with positive charges and these will have a tendency to get attracted to the DNA and the proteins which contain the negative charge, as well as cling on to the bacterial cell wall causing the disruption of its cell wall.[14]

Xylitol is known as an agent to reduce caries by its action of inhibiting *S. mutans*. Many studies in the past has compared this sweetener with sugars, and the mean values of viable S. *mutans* in plaque were lower in the xylitol group rather than in the sucrose or fructose groups.[15] Xylitol can be used on a day-to-day basis for the purpose of replacing other sugars and for caries prevention.[16] Plaque samples of individuals who consume xylitol on a regular basis has shown a significant decrease in the levels of *S. mutans* and a consumption of frequency 5–6 g, three or more times per day.[17]

The acceptance of using nanotechnology in the products of oral hygiene are now widely emerging and in future, it will immensely contribute to the oral care and it will expand the field of application in pastes, dentifrices, toothpastes, and mouthwashes. Thus, the aim of this study is to synthesize silver nanoparticles using miswak and to formulate a mouthwash by incorporating the formed silver nanoparticle and xylitol and to test for its antimicrobial property. To also test for potential toxicity of the formulated mouthwash.

MATERIALS AND METHODS

PART A: SYNTHESIS OF NANOPARTICLE-BASED MOUTH RINSE

The procedure followed in the formulation of this mouthwash is explained here. Miswak stems were taken and shredded into small pieces and the bark shavings were boiled in order to obtain an extract from them; 5 g of miswak was taken along with 100 mL distilled water. To the extract, 60 ml of silver nitrate solution that was prepared using 0.016 mM of silver nitrate was added. The preparation was kept along with a magnetic stirrer and for boiling to promote dissolution and this was continuously observed. The solution was taken after 48 h and centrifuged in 6 test tubes of 16 mm each and the pellets were collected. The collected pellet particle is to be assessed by transmission electron microscope (TEM) to observe the characteristics of the formed nanoparticles.

The next step is the formulation of mouthwash is the addition of 0.3 gm of xylitol which was taken in a test tube along with 0.001 gm sodium benzoate and 0.1 gm sodium lauryl sulphate. To this, 10 ml of water and 60 ml of nanoparticles were added to make the mouthwash. Silver nanoparticles that were synthesized using miswak was added along with a commonly available flavoring agent – peppermint oil and addition of sodium benzoate in 0.001 gm to act as an acid buffer. Focusing on the sweetening agents, we have used xylitol as an alternative to sucrose that is a known anti-cariogenic agent and a remineralizing agent and the quantity added was 0.3 gm. Figure 1 is a pictorial representation of the formulation of mouthwash.

PART B: ANTIMICROBIAL ACTIVITY OF MOUTHWASH

The formed mouthwash was tested for antimicrobial activity against *Streptococcus mutans, Lactobacillus sp, Staphylococcus aureus, and Candida albicans* (Figure 3). The prepared mouthwash was compared against the known antibiotic: the antibiotic used for bacteria were amoxicillin and the antifungal agent used was fluconazole. Table 1 shows the results against the antimicrobial activity. The zone of inhibition formed by 100-μl mouthwash was as effective against the antibiotic against *Staphylococcus aureus* and *Streptococcus*

Figure 1 Preparation of miswak synthesized silver nanoparticles mouthwash with xylitol.

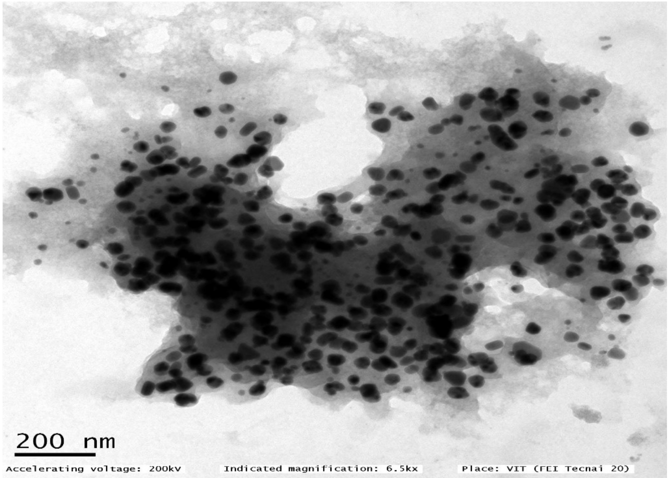

Figure 2 Transmission electron microscope results.

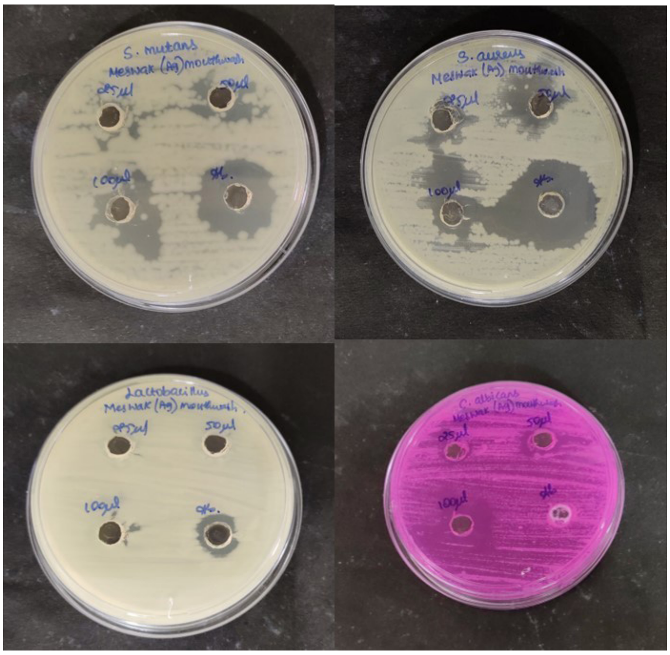

Figure 3 Antimicrobial study.

Table 1 Zone of inhibition.

Microorganisms	20 μL	50 μL	100 μL	Antibiotic
Staphylococcus aureus	14	24	28	30
Streptococcus mutans	9	18	25	25
Lactobacillus sp	9	9	9	14
Candida albicans	11	18	25	11

mutans. The activity against *Lactobacillus* was not as strong as that of against the antibiotic. Strong antifungal activity of the mouthwash was noted and the zone of inhibition formed was higher than the inhibition zone formed by the comparison of antibiotic fluconazole.

PART C: TOXICITY TESTING

Shrimp eggs were cultured using a culture containing iodine-free salt in water and sodium bicarbonate added to this as a source of nutrition. Nauplii of the shrimp that were formed in 24 h were collected and added 10 in one well to a six well ELISA plate (Figure 4). Silver nanoparticles in varying concentrations of 5, 10, 15, 20, and 25 μl were added to each well and one well was used as a control. The survival of the nauplii was assessed after 24 h.

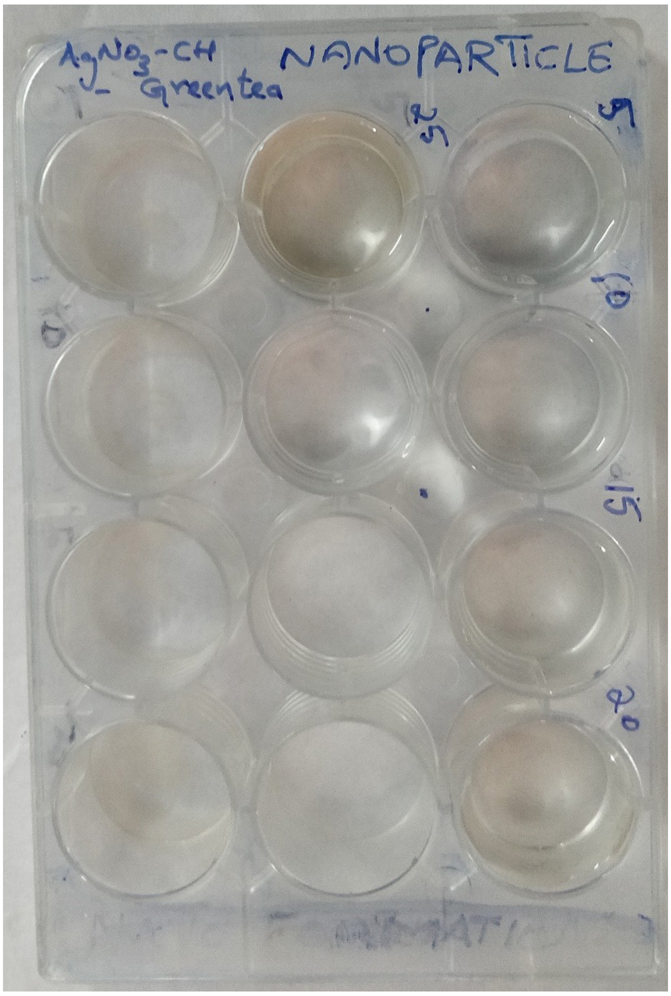

Figure 4 Cytotoxic effect.

RESULTS

The results of the antimicrobial activity showed that the mouthwash prepared was almost equally efficient to the control in forming the zone of diffusion against *Staphylococcus aureus* and *Streptococcus mutans*. These significant zones of inhibition were seen at a concentration of 100 μl. The activity of the mouthwash against *Lactobacillus* was not as strong as that of the antibiotic, where a 14-mm zone of inhibition as observed in Table 1. The zone of inhibition against Lactobacillus did not improve even at higher concentrations. Strong antifungal activity of the mouthwash was noted and the zone of inhibition formed was higher than the inhibition zone formed by the comparison antibiotic fluconazole.

The surface morphology was assessed using TEM and silver nanoparticles was formed of size 5–25 nm and the particles were evenly scattered and were spherical, angular, and rod shaped (Figure 2).

Table 2 shows the results of cytotoxicity when assessing the number of live napulli after 24 h when different concentrations of the mouthwash was added. There were eight organisms

Table 2 Cytotoxicity – assessing the number of live napulli after 24 h.

Concentration of miswak mediated silver nanoparticles mouthwash (in microlitres)	No. of live organisms
5	8
10	8
20	8
40	7
80	8
Control in saline	10

active in all the concentrations except in 40 μl; there were only seven organisms active.

DISCUSSION

The indigenous mouthwash prepared from miswak-plant-mediated silver nanoparticles could be used as an alternative to commercially available mouthwashes for reducing plaque load as an adjunct to tooth brushing in patients. The silver nano particles have been synthesized using green synthesis from miswak stem. Green synthesis is very affordable, environment-friendly, and nontoxic as it uses phytochemicals as reducing agents to reduce the silver ions. Mouthwash can be hence prepared in a simple way which will be eco-friendly and nontoxic. 600 μL of silver nanoparticles were used to prepare 10 ml of mouthwash with all the other ingredients essential for a complete preparation. We can say that it could be used as an alternative to commercial mouthwash as the antimicrobial effect was evaluated by measuring the zone of inhibition around different concentrations of mouthwash in the nutrient agar petri dish plates against most common oral pathogens responsible for producing plaque like *S. aureus, S. mutans*, *Lactobacillus*, *and C. Albicans* (Table 1). From the results of our study we evaluated that the mouthwash thus formulated has strong antimicrobial activity against *S.mutans* and *S.aureus* and antifungal activity was comparable with the commercial antifungal agent when tested for *Candida albicans*. The nanoparticles formed were of the size 5-25nm. The mouthwash when tested for cytotoxicity against shrimp nauplii showed that the mouthwash even in higher concentrations did not affect the developing nauplii and seven–eight live organisms were present active after 24 h.

Numerous studies in literature have attempted to test the efficacy of silver nanoparticles activity on various microbial organisms. Output water when passed through a silver nanoparticle coated polyurethane foam for *E.coli*, the output results were zero bacterial load for all the dilutions of silver nanoparticles.[18] The role of graphene oxide nanocomposite had displayed a dose dependent manner and has shown excellent activity against *E.coli* and *S. aureus*.[14] Silver nanoparticles based antibacterial coatings have shown to be very effective against pathogenic species such as *S. epidermidis, S. aureus, P. aeruginosa*, by inhibiting the biofilm growth on the surface and also tested using diffusion assays.[19] In dentistry, silver nanoparticles have been incorporated in various regions; and in resin, substantially more bacteria were eradicated from the AgNPs incorporated hybrid dental resin rather than pure dental resin.[20] Silver chitosan nanoparticle has shown to have an inhibiting effect on the blue color production of X gal and thus it is an indication of QS effect and it reduces the production of *S. mutans and P. gingivalis*.[21] Green synthesis from stem extract of Cissus quadrangularis has shown strong antimicrobial activity by disc diffusion against *Klebsiella planticola* and *Bacillus subtilis*.[22] In literature, various plants have been used over the time in order for green synthesis of silver nanoparticles like *Vitis vinifera* , algae extract, *Piper nigrum*, *Garcinia mangostana,* and innumerable other other plants.[23–26]. In the present study, we have chosen S. persica as our plant base for the preparation of the nanoparticles since there are various previous studies indicating that there is *change in the proportions of salivary bacteria especially S. mutans* in favor of species with a lower risk of inducing caries.[27] There are various commercial tooth pastes available and in many previous studies, they have evaluated *S. persica* and it has shown a good efficacy in bacterial plaque removal.[28–30]

In the present study, the cytotoxicity was assessed by testing the mouthwash in brine solution of developing shrimp napulli and we found that in increased concentration also, the mouthwash did not affect its development and seven to eight organisms were active after 12 h. Cytotoxicity experiments previously conducted to determine the effects have shown that there is an increase in the cytotoxicity of silver nanoparticles as there is a decrease in the size and attributes to the size-related differences in toxicity seen in various studies.[31] In a study where there was the effect of silver nanoparticles, studied on the drosophila eggs, the increase in silver concentration in Drosophila tissue exposed to 20–30 nm silver particles compared with both the 100-nm silver particles and the 500–1200-nm silver particles could make the smaller particles more toxic to higher trophic levels.[32] Silver nanoparticle toxicity when tested with *Daphnia magna* has shown that the toxicity was attributed to the function of dissolved Ag concentration.[33]

An advantage of this mouthwash is it is devoid of alcohol, hence it can be safely used in gingivitis, mucositis, and so on which are encountered in orthodontic patients and also in immunocompromised patients. We have used xylitol as a sweetener as it is a known anti-cariogenic agent and it will be beneficial in the prevention of white spot lesions. Miswak is a known indigenous material that is widely used in commercial mouthwashes and toothpaste and is a strong antibacterial and antifungal agent. Thus, from the study we carried out, we propose that the miswak silver nanoparticles mouthwash with xylitol can be an effective alternative to the commercial mouthwash to be used as an adjuvant in oral hygiene.

CONCLUSION

Silver nanoparticle formulated using miswak is a strong antibacterial and antifungal agent. Xylitol is an efficient anticariogenic agent and it reduces the bacterial load in the plaque samples. Thus, miswak-mediated silver nanoparticles incorporated into mouthwash along with xylitol can be an effective alternative to commercial mouthwash.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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