Original Article

Synthesis, characterization, and antimicrobial activity of silver nanoparticles derived from *Mentha* X piperita + Ocimum tenuiflorum: An *in vitro* study

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Abstract

The objective of the study was to synthesize silver nanoparticles using *Mentha X Piperita* (Mint) + *Ocimum tenuiflorum* (tulsi) and to confirm its size and shape. 0.5 mg of tulsi and 0.5 mg mint were diluted in distilled water (100 ml). The dissolved formulation was heated for 15 min at 70°C and filtered. The filtrate was homogeneously combined with 0.9 mg of silver nitrate to prepare nanoparticles of silver (AgNPs). The characterization of the obtained nanoparticle was done using transmission electron microscopy. Using agar disc diffusion assay, the antibacterial property was evaluated against common oral microbes at different concentrations. Silver nanoparticles showed excellent antimicrobial activity against *Streptococcus mutans* at 100 μL concentration. At 25 and 50 μL, all microbes showed similar extent of antimicrobial activity when quantified. Tulsi and mint prove to be effective in synthesizing silver nanoparticles that have good antimicrobial activity against oral microbes.

Key words: Antimicrobial activity, mint, nanobiomedicine, oral microbes, tulsi

INTRODUCTION

Nanobiotechnology is a branch of biology that uses nanoscale concepts and methods to understand and manipulate nonliving and living bio systems, in order to create new products.[1] Nanotechnology is a cutting-edge interdisciplinary technology that combines material sciences, chemistry, and biology. The shape, dimension, and surface structure of nanoparticles influence chemical, physical, optical, chemical, and electronic properties.[2,3] This is especially true for silver (Ag) and gold (Au), owing to their increased surface plasmon resonance oscillations.[4,5]

To produce safe, environmentally friendly, nontoxic, and sustainable materials, there has been a growing need for ecofriendly aids for metallic nanoparticle synthesis.[6-9] Nanoparticles of silver have paved way owing to its inert antimicrobial property.[10-12] Because of excellent antibacterial and antiviral capabilities, they are employed in dermatological products, food industry, clothing, and cosmetics[9,12-19] and thus are being touted as next-generation antimicrobials.[20-30]

The same essential property could be used in the field of dentistry to develop and produce products that provide high antimicrobial effect. Hence, the present study aimed at biological synthesis of silver nanoparticle using tulsi and

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mint[31-33] and evaluated its antimicrobial activity against common oral microbes.[34-37]

METHODOLOGY

Plant extract preparation

0.5 mg of dried and powdered *Mentha X Piperita* and 0.5 mg of *Ocimum tenuiflorum* was homogeneously mixed with 100 ml distilled and heated in a heating mantle at 70°C [Figure 1].

A filter paper (Whatman no. 1) was used to filter the solution. Postfiltration, the supernatant was collected in a flask.

Nanoparticle preparation

AGNPs were obtained due to reduction of silver from silver nitrate solution. In the procedure of nanoparticle synthesis, 1 mmol silver nitrate was added to the herbal formulation. The mixture was processed overnight on an orbital shaker to obtain a homogenous solution. The process was continued until color changes were noticed. Ultraviolet–visible (UV-vis) spectroscopic analysis was used to monitor the synthesis of nanoparticles at hourly intervals. The substrate was then centrifuged for 20 min and the pellet was collected [Figure 2].

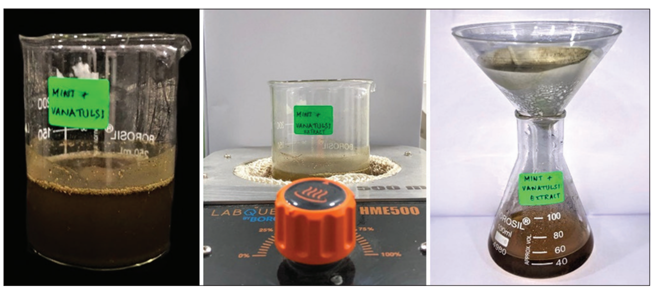

Figure 1: Extract of Mint and Tulsi

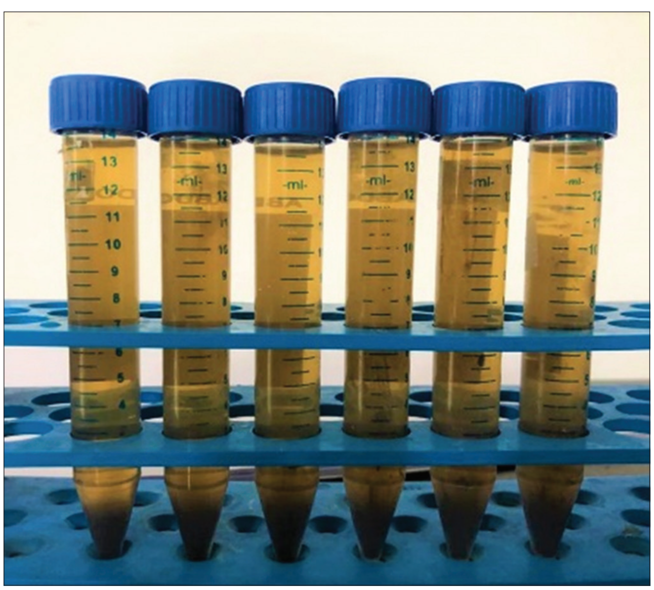

Figure 3: Characterization of silver nanoparticle

Ultraviolet–visible spectroscopy analysis of AgNPs

The peak of absorption of the AgNPs was recorded using UV-Vis spectroscopy. The scanning range of the samples was between 350 and 660 nm. All UV-Vis absorption spectra were read against distilled water [Figure 3].

Antimicrobial activity

The antimicrobial property of synthesized AgNPs was evaluated against common oral microbes, *Streptococcus mutans, Staphylococcus aureus, Candida Albicans,* and *Enterococcus faecalis.* Zone of inhibition was measured using the agar diffusion technique to evaluate the antibacterial activity. Varied concentrations of the AgNPs (25 µL, 50 µL, and 100 µL) were added into the wells made on nutrient agar plate. At 37°C, the agar plates were incubated for 24 h. Using a vernier caliper, the zone of inhibition was measured to quantify the antimicrobial effect [Figures 4 and 5].

RESULTS AND DISCUSSION

Ultraviolet-visible spectroscopy

A yellowish discoloration of AgNPs was observed due to surface plasmon vibrations. At 440 nm, the continuous intensity as a function of reaction time was observed [Figure 6].

Antimicrobial activity

The AgNPs that were synthesized showed strong antibacterial action against oral microorganisms [Figure 7]. The activity against *S. mutans* at 100 uL was more when compared to the activity against other oral pathogens at different

Figure 2: Nanoparticle preparation

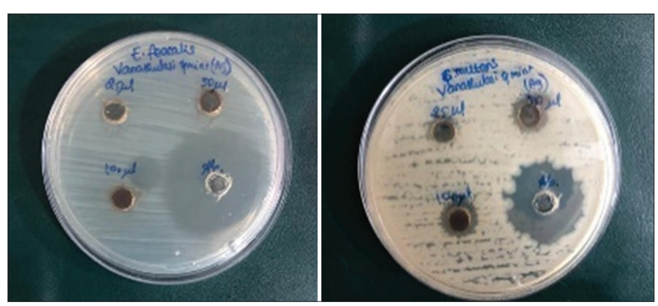

Figure 4: Antimicrobial activity against *Staphylococcus aureus* and *Streptococcus mutans*

Figure 5: Antimicrobial activity against *Candida albicans* and *Enterococcus faecalis*

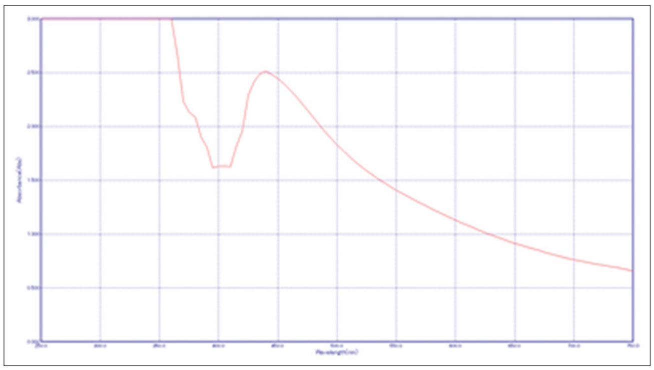

Figure 6: Ultraviolet visible spectroscopy graph of AgNPs

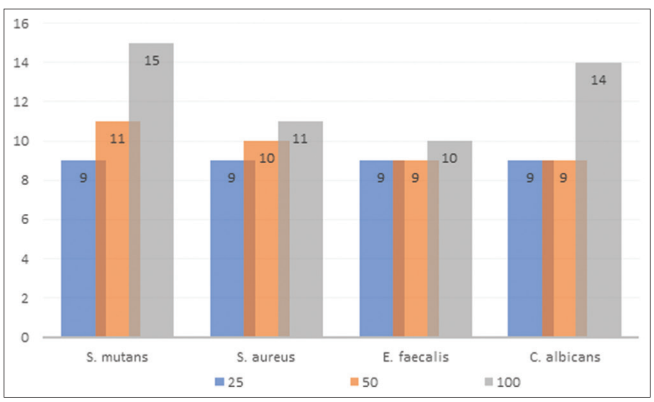

Figure 7: Antimicrobial activity of silver nanoparticles against different pathogens at different concentrations

concentrations. The activity of AgNPs was also increased against *C. albicans* at 100 uL concentration. Antimicrobial activity was also observed against *S. aureus* and *E. faecalis* but to a lesser extent than *S. mutans* and *C. albicans.*

Silver (Ag) is known to have a broad-spectrum antibacterial action and has been used in dentistry for many years.[38-40] Besides being an effective antimicrobial agent, Ag ions have advantages such as sustained ion release[41] and low bacterial resistance.[42] Silver nanoparticles have been created with the advent of nanotechnology and have demonstrated powerful antibacterial capabilities.[6,18] The integration of AgNPs seeks to eliminate or reduce microbial aggregation on the dentition, hence enhancing oral hygiene and quality of life. AgNPs' antibacterial action on Gram-positive and Gram-negative bacteria is not comparable.[42] There are conflicting findings about AgNPs' antibacterial effectiveness against Gram-negative and Gram-positive microorganisms. Ag nanoparticles are more susceptible toward Gram-negative bacteria than Gram-positive bacteria,[35,43-46] whereas few researchers have reported otherwise.[46-49] Bacterial cell membranes are −ve charged, but AgNPs are +ve charged; when +ve charged AgNPs act on −ve charged membranes, molecular changes occur, resulting in enhanced permeability of the bacterial cell membrane. As a result of uncontrolled trafficking through the cytoplasmic membrane, cells die.[48] By connecting with the genomic component in the bacterial cell, AgNPs can cause harm by inhibiting the transcription and translation processes.[50]

AgNPs' antibacterial activity may be divided into two, biocidal effect and inhibitory action. In the former, bacterial cellular division was halted, but in the latter, cell death occurred due to biocidal action of Ag nanoparticles.[51] The antibacterial activity of AgNPs is subject to variability by parameters such as pH, temperature, bacterium species, and AgNO3 concentration.[52] This owes to the smaller surface area of AgNPs that is accessible for bacterial cell contact and hence increased bactericidal activity than larger AgNPs.[48] The antimicrobial action of plant-mediated AgNPs can be due to the following: (i) the reactive oxygen species such as superoxide anions (O2) and hydroxyl radicals (OHC) formation, (ii) bacterial protein denaturation as Ag ions in Ag NP's bonds with sulfhydryl groups, and (iii) Ag released from AgNPs causes cell damage leading to cell death.[53] Mahendra *et al*. stated that the bacterial cell death occurs due to activity of AgNPs on the bacterial respiratory chain.[48] According to Amro *et al.*, increasing release of membrane proteins and lipopolysaccharides changes the membrane permeability resulting in cell death.[54] According to electron spin resonance spectroscopy research, free radicals generated by AgNPs on contact with bacteria makes the cell membrane porous leading to cell death.[55] Silver is an acid, which reacts with base. The bacterial cells are largely composed of bases such as sulfur and phosphorus.[37] Due to the presence of phosphorus and sulfur in DNA, AgNPs react with bases and damage the DNA, leading to cell death.[36,40] This might explain the acquired antibacterial action of AgNPs, as well as the activity of herbal extracts against oral microorganisms.

The economic and simple synthesis protocol is a highlight of this study. Being an environmentally friendly method, this protocol could be utilized for the development of antimicrobial agents for use in dentistry. This is substantiated with the results obtained from the antimicrobial assay conducted in this study where silver nanoparticles showed good activity against *S. mutans* at 100 µL, whereas all other microbes showed similar inhibition zones at 25 and 50 µL.

CONCLUSION

Chemical methods of synthesis of AgNPs pose biological and toxic hazards. Hence, a plant-mediated AgNPs production technique is essential as it is more environmentally accepted, economic, and does not require chemical agents and other mechanical aids. As a result, the application of green synthesis of AgNPs might have a significant influence in the future.

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Conflicts of interest

There are no conflicts of interest.

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