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Biosynthesis, Characterization and Antibacterial Capability of Silver and Copper Nanoparticles Using Aqueous Leaf Extract of *Salacia chinensis* L

Jaykumar J Chavan[1,2]* and Dhanaji M Ghadage[1]

[1]Department of Botany, Yashavantrao Chavan Institute of Science, Satara, India
[2]Department of Biotechnology, Yashavantrao Chavan Institute of Science, Satara, India

Abstract

Salacia chinensis L. is highly valued taxa having several medicinal implications. The study aimed to synthesize and characterize the silver (AgNPs) and copper nanoparticles (CuNPs) using the aqueous leaf extract of *S. chinensis* and their test against pathogenic bacteria. The silver and copper nanoparticles were synthesized using different concentrations of aqueous $AgNO_3$ and $CuNO_3$ solutions; however 4 mM was found optimal concentration. The synthesized nanoparticles were characterized using UV-Vis spectroscopy; scanning electron microscopy and the antibacterial capability of nanoparticles were evaluated using agar well diffusion method. The AgNPs and CuNPs were formed within an hour of the reaction and showed maximum UV-Vis absorption at 445 nm and 570 nm respectively. SEM analysis revealed the size (50-200 nm) and diverse shape (spherical, road) of the synthesized nanoparticles. The efficiency of synthesized AgNPs and CuNPs against the bacterial species viz. *Escherichia coli, Bacillus subtilis, Staphylococcus aureus* and *Pseudomonas averogenosa* validated the action of these nanoparticles against these infectious microbes. The study illustrated the capacity of leaves of *S. chinensis* towards the synthesis of silver and copper nanomaterial and AgNPs were found with enhanced antibacterial capability against test microbes.

Keywords: Antibacterial capability; Copper nanoparticles; Leaf extract; *Salacia chinensis*; SEM; Silver nanoparticles

Introduction

Saptarangi (*Salacia chinensis* L.) is highly valued medicinal plant of family Hippocrateaceae which is commonly known as Saptarangi and Saptachakra in the Ayurvedic system of traditional medicines [1]. Different plant parts of *S. chinensis* are compelling source of active phytoconstituents like phenolics, flavonoids and good natural source of antioxidants [2-4]. The roots also showed the presence of antidiabetic and anticancerous principles like salacinol, kotalanol, and mangiferin [5-8]. The plant also functions as astringent, abortifacient carminative, blood tonic, cardio-tonic, in amenorrhea and dysmenorrhoea, [1,5,9] and used in treatments of various ailments and diseases [10]. Various extracts of *S. chinensis* also described to possess several pharmacological properties including antimicrobial, antihyperglycemic, anti-inflammatory, immuno-modulatory, anti-mutagenic activities antitumor, anti-HIV and immunomodulatory activities [2,5,11-13].

In recent years, synthesis of metal nanoparticles is a subject of focused research interest due to its wide potential applications in biomedical, optical and electronic fields [14]. The enhanced pharmacological and biomedicinal performance of *S. chinensis* can be achieved with metal nanomaterials. Literature reveals that, the stem of *Salacia chinensis* has the capability towards synthesis of silver nanoparticles [15]. However, leaves of *S. chinensis* are chief source of many bioactive constituents. Hence in the present study, reducing potential of *S. chinensis* aqueous leaf extract have been exploited for the eco-friendly and bio-utilizable synthesis of silver (AgNPs) and copper nanoparticles (CuNPs). These nanoparticles were characterized by UV-VIS spectroscopy, SEM and the antibacterial properties were also evaluated.

Material and Methods

Chemicals and materials

Silver nitrate ($AgNO_3$), copper nitrate ($CuNO_3$), Nutrient Agar and MacConkeys agar were procured from Hi-Media, India. *S. chinensis* leaves were collected from Amboli locality of the Northern Western Ghats. Bacterial species viz; *Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Pseudomonas averogenosa* were obtained from the Department of Microbiology and Biotechnology, Yashavantrao Chavan Institute of Science, Satara.

Preparations of *Salacia chinensis* leaf extract (SCLE)

Freshly collected leaves of *S. chinensis* were washed thoroughly under running tap water to remove traces of impurities and were blotted to dry. Shade dried leaves were then powdered using grinder. Ten gram powder was suspended for extraction in 100 ml boiling distilled water for 2 hours on water bath. Initially, the suspension was filtered through muslin cloth and then with Whatmann filter paper 1 to get the crude extract. Final volume of *Salacia chinensis* leaf extract (SCLE) was adjusted to 100 ml by distilled water and the same was used for synthesis of AgNPs and CuNPs.

Synthesis of silver nanoparticles (AgNPs)

SCLE extract (10% w/v) were added to 4 mM aqueous $AgNO_3$ solution in 1:4 concentrations. The reaction was carried out at room temperature ranging from 25 to 30°C for 60 minutes. Synthesis of AgNPs was established by colour change and UV-Visible spectrophometric analysis. AgNPs were separated out by centrifugation and washed with sterile distilled water. Purified AgNP pellets were kept for oven drying at 60°C for 24 h which were then scrapped out and stored.

Synthesis of copper nanoparticles (CuNPs)

CuNPs were synthesized by using 10% SCLE and the 4 mM

***Corresponding author:** Jaykumar J Chavan, Department of Botany, Yashavantrao Chavan Institute of Science, Satara 415001, India, Tel: 9102162234392; Fax: 9102162234392; E-mail: jaychavansu@gmail.com

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aqueous $CuNO_3$ solution in a 1:4 ratio at room temperature (25 to 30°C) for 60 min. Change in colour indicated synthesis of CuNPs. The reaction mixture was centrifuged and CuNPs were separated out. After washing with sterile distilled water, purified pellets were transferred to petri plate and were kept for oven drying at 60°C for 24 h. The dried CuNPs were scrapped out for the further study.

Characterization of Nanoparticles

The UV-Vis spectrometric scan was carried out on Systronics-119 to monitor the bioreduction of AgNPs and CuNPs at room temperature at the resolution of 1 nm between 300 to 800 nm ranges as a function of time. Reaction mixture (2 ml) was analyzed every time from initiation of reaction to 60 minutes at time intervals of 10 minutes. The surface morphology and size range was recorded by scanning electron microscopy (JEOL JSM 6360 with an accelerating voltage 20 kV). SEM analysis was performed by the previously described procedure [15]. A drop of AgNPs and CUNPs colloidal suspension was deposited on carbon grid and dried under low vacuum (10-130 pa). The samples were scanned at magnification of 3000x and the spot was fixed and was analyzed by EDX system to confirm the presence of silver and copper.

The antimicrobial activity of AgNPs and CuNPs were evaluated by using the agar well diffusion method against the pathogenic bacteria including gram positive *viz. Bacillus subtilis, Staphylococcus aureus* and gram negative *viz. Escherichia coli*, *Pseudomonas averogenosa* [16]. The bacterial cultures were maintained on Nutrient Agar and MacConkeys agar slants. The microorganism was activated by inoculating a loopful of the strain in the nutrient broth (25 ml) and incubated at room temperature. Inoculum (0.5 ml) was added to the agar plates. For the agar well diffusion, a well was made in the seeded plates with the help of a cup-borer (8.5 mm) in the medium. Freshly prepared AgNPs samples (100 µL) and CuNPs (100 µL) were added into the wells. The SCLE extract (100 µL) used as a negative control while streptomycin was used as positive control. The samples were kept for incubation for 24 h at 37°C. A zone of inhibition was observed around the well after the incubation period and the observations were recorded.

Results and Discussion

Synthesis of silver (AgNPs) and copper (CuNPs) nanoparticles

Silver and copper nanoparticles were synthesized by addition of *S. chinensis* leaf extract to the solutions of silver nitrate and copper nitrate. The leaves are capable to synthesize the silver and copper nanoparticle. The synthesis of AgNPs were confirmed based on visual observation of colour change from colourless to brown and synthesis of CuNPs was confirmed as of colourless to reddish brown (Figure 1A-1E). The synthesized nanoparticles were reduced easily and were found stable. This might me due to the presence of bioactive constituents including kotalanol, salacinol and mangiferin in the leaves of *S. chinensis*. Similarly, stable metal nanoparticles *viz.* silver, copper and gold nanoparticles were synthesized from leaves of *Artemisia nilagirica* [17] and *Terminalia arjuna* [18]. In the present study, the AgNPs and CuNPs were successfully separated and purified by repeated centrifugation. The dispersal in to the deionized water was also confirmed the purity of synthesized nanoparticles.

Characterization of AgNPs and CuNPs

In the present study, along with of SCLE, $AgNO_3$ extract showed absorbance peak at 445 nm which confirmed the synthesis of AgNPs. However, an individual aqueous extracts of SCLE, $AgNO_3$ and $CuNO_3$ did not showe any peak (Figure 2A). Synthesis of AgNPs confirmed by absorbance peak at 445 nm (Figure 2B) are in line with previous report as the λmax values of AgNPs were found in the visible range of 400-500 nm [19]. However, the larger particle size might shift in the plasmon band for higher wavelength [20]. Formation of CuNPs was confirmed by UV spectroscopy which showed a characteristic peak at 630 nm (Figure 2C).

Scanning electron microscopy provided the platform for studying the size and shape of the metal nanoparticles. SEM microphotographs of the AgNPs and CuNPs obtained by the reduction of aqueous leaf extract of *S. chinensis* revealed the nanoparticles differed in size as well as shape (Figure 3A and 3B). The resulting silver nanoparticles were in the size range of 50-200 nm. Morphologically polymorphic AgNPs are triangular, hexagonal, deformed spherical and rod shaped, however the CuNPs were tubular and rod shaped. Both the nanoparticles showed the aggregation and nanocluster might be due to the dryness stimulated by evaporation of solvent during the reaction period. Synthesis in group or bunch may lead to decrease in their biological activities [21].

Antibacterial activity of AgNPs and CuNPs

AgNPs and CuNPs are recognized as the broad spectrum antimicrobial agents with its wide application potential in medical and agricultural field [22,23]. The antibacterial results obtained for *S. chinensis* leaf derived AgNPs and CuNPs against test organisms are represented in Figure 4A and 4B. The zone of inhibition observed after 16-18 hours of activity. The present study revealed that, *Escherichia coli, Bacillus subtilis* are more susceptible to AgNPs while *Pseudomonas averogenosa* and *Streptococcus aureus* showed minimal level of susceptibility (Figure 5). In the present study, AgNPs have the superior antibacterial capability against tested bacterial strains as compared to CuNPs. This might be the synthesis of CuNPs in the clusters or in

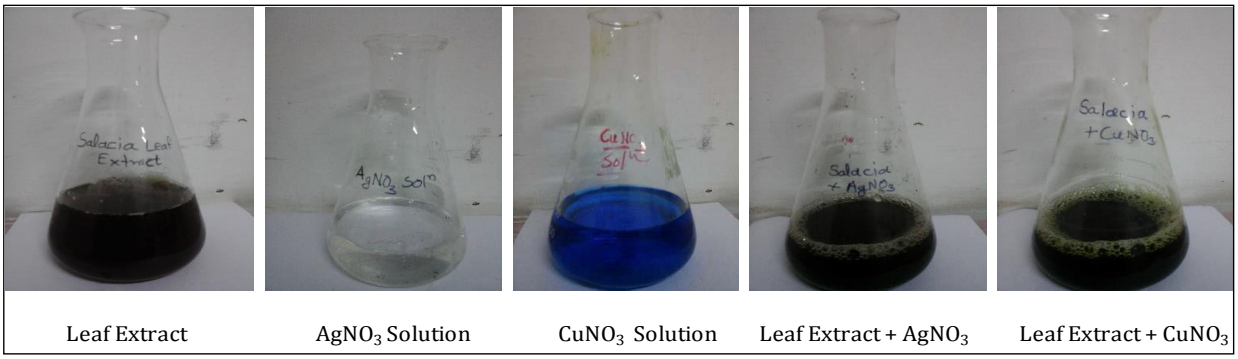


Figure 1: Biosynthesis of AgNPs and CuNPs from aqueous leaf extracts of *S. chinensis*.

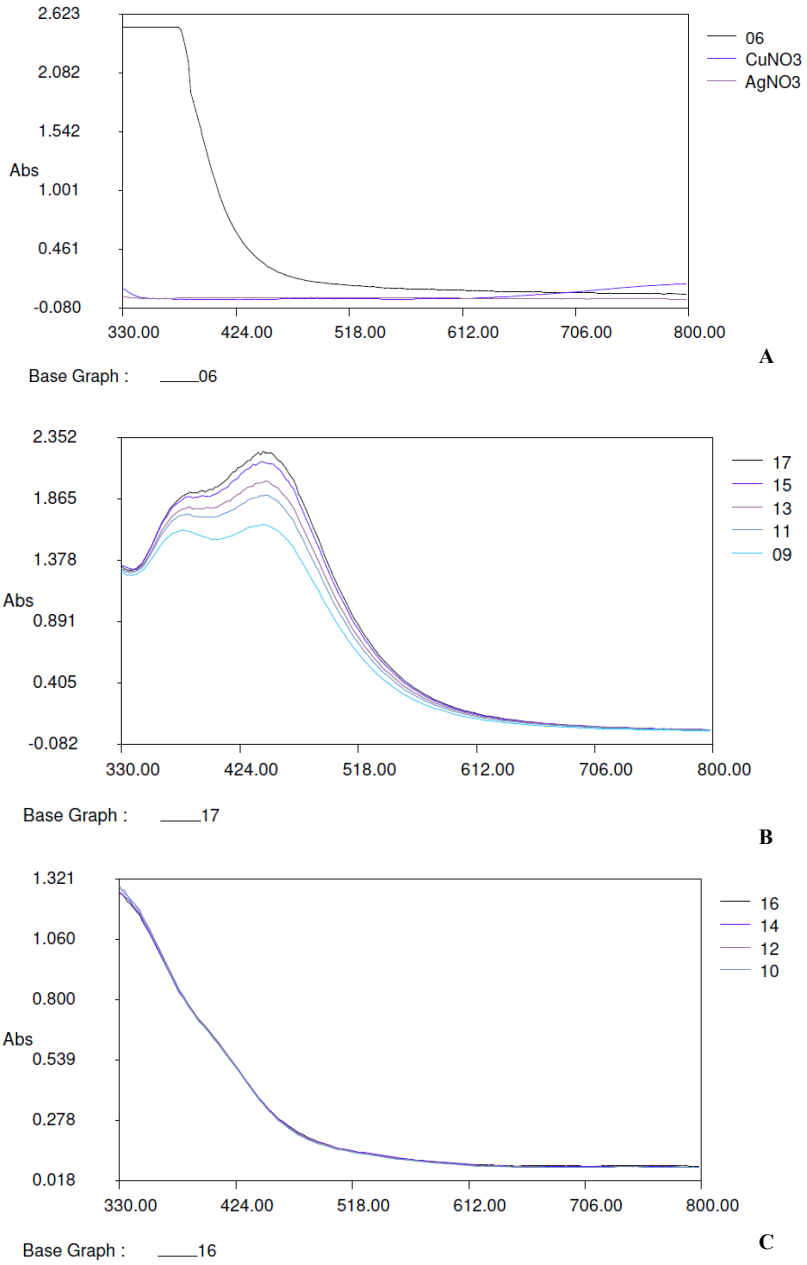


Figure 2: UV-visible profiles. A: Aqueous leaf extract, $AgNO_3$ and $CuNO_3$; B: Synthesized AgNPs and C: Synthesized CuNPs.

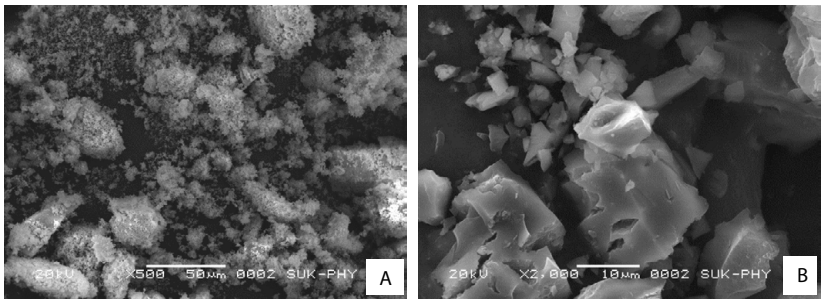
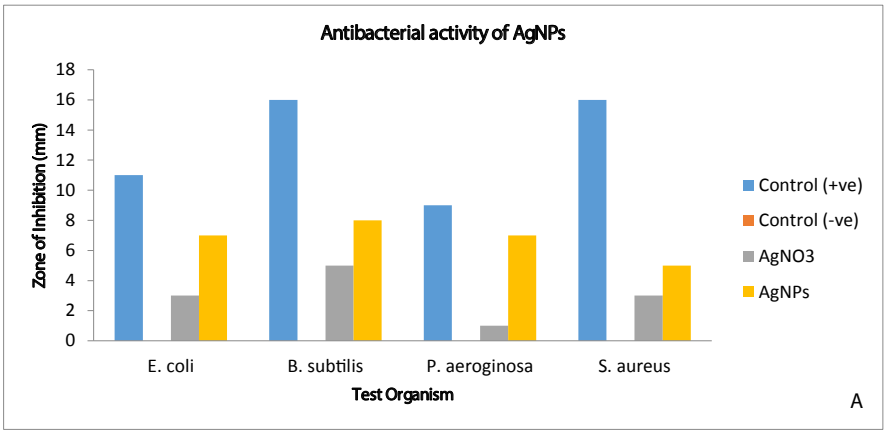


Figure 3: Scanning electron micrograph of synthesized nanoparticles. A: AgNPs and B: CuNPs.



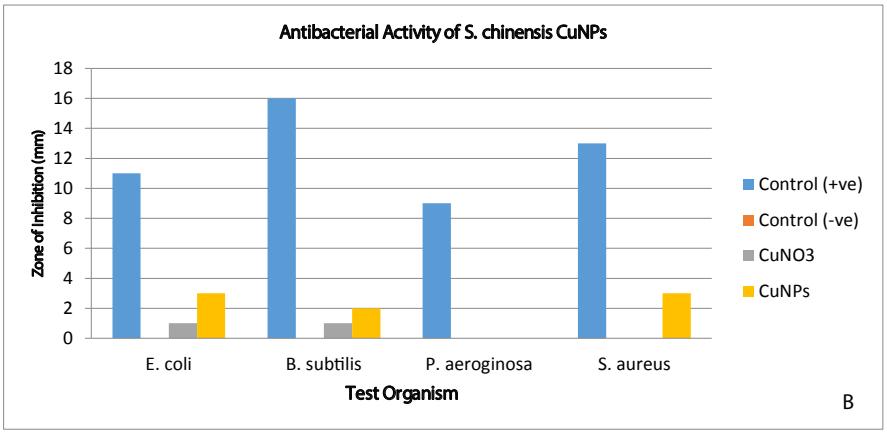
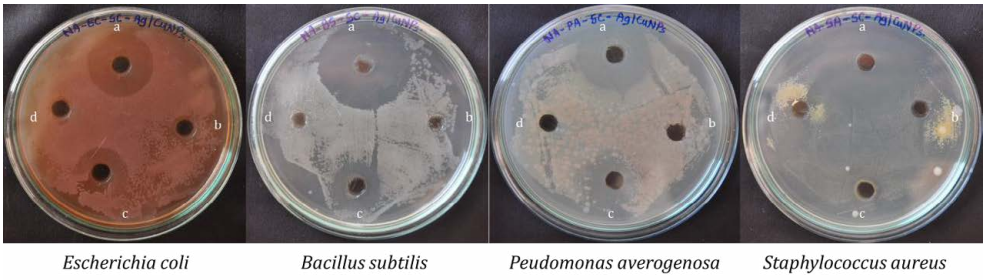


Figure 4: Effect of nanoparticles on pathogenic bacteria. A: AgNPs and B: CuNPs.



a: +ve control (Streptomycin), b: S. chinensis aq. leaf extract , c: AgNPs, d: CuNPs

Figure 5: Antibacterial activity assay against Gram +ve and Gram -ve bacteria depicting zone of inhibitions.

aggregates instead of singular synthesis. The findings are competent with the previous study which confirmed that silver nanoparticles are effective against human pathogens and as antimicrobial agents [15,24,25]. The metal nanoparticles attaches to surfaces of cell membranes as granules [26] troubling the permeability of membranes and respiratory functions, with dysfunction of metabolic pathways. It also inhibits the cell division by damaging inner cell organelles and DNA [27].

Conclusion

Eco-friendly and cost-effective procedure is developed for biosynthesis of silver and copper nanoparticles from aqueous leaf extract of *S. chinensis*. The leaves showed the bioreduction potential towards $AgNO_3$ and $CuNO_3$ to produce AgNPs and CuNPs. Owing to its antibacterial properties, the AgNPs showed its efficiency towards different gram positive and gram-negative bacterial strains which suggest that silver metal in nanoparticles range could be effectively used as an antibacterial agent. Comparatively, AgNPs were more efficient against tested microbes than the CuNPs. The bio-utilizable size of AgNPs makes it useful against development of antimicrobial drugs, in medical devices and also in agricultural field.

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Conflict of Interest

The authors declared no conflicts of interest.

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