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Research Article

PLANT-MEDIATED SYNTHESIS OF SILVER NANOPARTICLES WITH DIVERSE APPLICATIONS

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ABSTRACT

Objective: Green synthesis of multifunctional silver nanoparticles (SNPs) using leaf extracts of *Simarauba glauca* (Sg) and *Artocarpus altilis* (Aa), a good alternative to the electrochemical methods were exploited in this study for potential use as a biomedical agent in the medical field.

Methods: Synthesis of SNPs using leaf extracts of Sg and Aa was carried out. The SNPs were characterized by UV-visible spectrophotometry, scanning electron microscopy, and Fourier transform - infrared spectroscopy analysis and evaluated for their multifunctionality.

Results: The SNPs were synthesized from the leaf extracts of Sg and Aa using silver nitrate with the reaction time of 2 minutes and 10 minutes, respectively. UV-visible absorption scan revealed a characteristic peak at 420 nm indicative of the surface plasmon resonance for SNP. The synthesized SNPs were of size ranges from 33 to 50 nm and 37.9 nm synthesized from the leaf extracts of Sg and Aa, respectively. Furthermore, the synthesized SNPs have shown excellent antibacterial activity against test pathogens, applicability in sewage water treatment, biofilm degradation and having perfect hemocompatibility.

Conclusion: The biologically synthesized SNPs from the plant extracts could be of immense use in the medical field for their efficient antimicrobial function. The other valuable applications make it a potential agent in the relative biomedical area.

Keywords: Biofilm degradation, Biomedical agent, Hemocompatibility, Silver nanoparticles.

INTRODUCTION

Nanotechnology is a rapidly growing field with its application in science and technology for the purpose of manufacturing new materials at the nanoscale level. Recently, biosynthetic methods employing either microorganisms or plants extract have emerged as an alternative to more complex chemical synthetic procedures to obtain nanomaterials. Biological synthesis of nanoparticles (NPs) has upsurge in the field of nanobiotechnology to create novel materials that are eco-friendly, cost effective, stable NPs with great importance for wider applications especially in the field of medicine.

Different types of nanomaterials, such as copper, zinc, titanium magnesium, iron oxide, and gold, have been synthesized. Of these, silver NPs (SNPs) are playing a major role in the field of nanotechnology and nanomedicine due to its effective antimicrobial potential [1].

The major advantage of using plant extracts for SNP synthesis is that they are easily available, safe, and nontoxic in most cases, have a broad variety of metabolites that can aid in the reduction of silver ions, and are quicker than microbes in the synthesis. Though the exact mechanism involved in each plant varies as the phytochemical involved varies, the major mechanism involved is the reduction of the ions [2].

With the huge plant diversity much more plant species are in the way to be exploited and reported in future era toward rapid and single step protocol with green principle [3]. With the above back ground, the present study reports for the first time, the synthesis of SNPs with multi-applications from leaf extracts of *Simarauba glauca* (Sg) (Lakshmi taru) and *Artocarpus altilis* (Aa) (Breadfruit tree).

METHODS

All chemicals used were of analytical grade and obtained from Sigma Chemical Co. (USA) or Hi-Media/Merck/Qualigens (India). In all experiments, the measurements were carried out with duplicated parallel sets.

Synthesis of SNPs

Fresh and healthy leaves of Aa and Sg were collected and thoroughly washed with distilled water. 5 g of leaf sample was weighed, ground with mortar and pestle, and boiled with 100ml of distilled water for 15 minutes. The suspension was cooled and filtered.

SNP synthesis was carried out by mixing 90 ml of the filtrate with 10 ml of aqueous solution of 1mM $AgNO_3$. The mixture was incubated until a color change was obtained. Then, the reaction mixture was centrifuged at 10000 rpm for 20 minutes. The supernatant was discarded and the pellet obtained was washed repeatedly with sterile distilled water, dried and finely powdered for characterization [4]. The effect of various concentrations of substrate $AgNO_3$ (0.5-25 mM) on the synthesis of SNPs was also studied.

Characterization of SNP

UV-vis spectral analysis was done using Spectrophotometer (Chemito UV2300) by measuring the absorbance from 350 nm to 600 nm. The scanning electron microscope (SEM) analysis was carried out to characterize particle shape, distribution, and approximate size. The dry powdered sample was coated with 80% gold and 20% palladium with quorum SC7620 sputter coater to make them conductive and analyzed with Zeiss Evo 18, SEM. Fourier transform-infrared spectroscopy (FTIR) analysis of the dried SNP was done using Shimadzu FTIR, and spectrum was recorded in the range of 500-4000/cm at a resolution of 4/cm.

Applications of SNP

Evaluation of antibacterial activity

The antibacterial activity of SNPs was carried out using the well diffusion assay on Mueller-Hinton agar plates against *Bacillus subtilis*, *Staphylococcus aureus, Escherichia coli,* and *Proteus vulgaris* [5]. Different concentrations (2, 4, 6, 8 10, and 12 µg/mL) of SNP solutions synthesized from the leaf extracts of Sg and Aa were added to wells. The plates were incubated at 37°C for 24 hrs, and the zone of clearance around the well was measured.

Determination of minimum inhibitory concentration (MIC)

The MIC of SNP was calculated by broth dilution method in 96 well microtitre plates as per Sarker *et al.* [6] with slight modifications using resazurin indicator solution. *B. subtilis*, *S. aureus, E. coli,* and *P. vulgaris* were inoculated in nutrient broth and incubated at 37°C for 24 hrs. 10 µL of the culture, appropriate volume SNP solution along with resazurin indicator was added to respective wells to obtain a final concentration of 10-50 µg/mL. Appropriate controls were maintained. The plate was incubated overnight at 37°C. Blue of indicator (resazurin) is reduced in the presence of living bacteria. In the absence of living bacteria, the color of the indicator remains blue. The color change from blue to pink, if any was observed. The lowest concentration at which no color change occurred was recorded as the MIC against the respective clinical pathogens.

Biofilm inhibition

B. subtilis was inoculated in freshly prepared sterile LB broth incubated at 37°C until an OD of 0.5 was attained at 600 nm. 100 µL of the culture was added to a sterile 96 well microtitre plate and incubated at 37°C for 24 hrs to allow the formation of biofilm. After 24 hrs, 10 µg of SNP solution was added in the respective well and sterile LB broth was added in the control well and incubated for 24 hrs at 37°C. Then, the liquid from the wells were replaced with 100 µL of fresh sterile LB broth and kept overnight. Next day, the wells were washed with 200 µL of 0.9% sterile saline three times and dried at 50°C for 1 hrs followed by the addition of 0.3% crystal violet to the dried wells for 15 minutes and washed gently under running tap water. The dye incorporated by the adherent cells was solubilized with 100 µL 70% ethanol:10% isopropanol. The absorbance of each well was measured at 590 nm using BMG labtech - microplate reader. The absorbance difference between treated and control wells was considered as an index of bacterial adherence to the surface and thus the activity of biofilm. Experiments were performed in triplicate. The data are expressed as means ± standard deviation. The percentage inhibition of biofilm activity was calculated using the following equation [7].

$$\text{Percentage inhibition} = \frac{(\text{OD of sample} - \text{OD of control})}{\text{OD of control}} \times 100$$

Water treatment using SNP coated stones

The solutions of SNP were coated onto stones to check their efficacy in water treatment. Sterile stones of approximately 12 g in weight were coated with SNP (1 µg/g) and dried. These stones were then dipped in 100 mL domestic sewage water sample with CFU more than 10^{14} in a 250 ml conical flask. The flask was incubated at 37°C overnight, and 100 µL was spread plated on nutrient agar plates, incubated at 37°C overnight and observed for the reduction in bacterial growth. Untreated sewage water sample was used as control [8].

Hemocompatibility activity

A simple procedure was followed to check the hemolytic activity of the synthesized SNP by the well diffusion method using blood agar plate. Wells were made on blood agar plate, and 10 µg of SNP solutions were added. 3% H_20_2 was used as a positive control. The plate was incubated at room temperature for 24 hrs and observed for the zone of clearance [9].

Statistical analysis

Values are presented as the mean ± standard error of the three triplicates of the experiments.

RESULTS AND DISCUSSION

Synthesis of SNPs

The aqueous silver ions were reduced to SNPs when added to natural leaf extract of Sg and Aa. It was observed that the color of the solution turned from pale yellow to dark brown within 2 minutes for Sg SNP's while the color change for Aa SNP's was from pale yellow to brown at the end of 10 minutes, which indicated the formation of SNPs (Fig. 1a and b). Plants tender a superior option for the synthesis of the nanoparticle, as the methods are free from toxic chemicals; furthermore, natural capping agents are readily supplied by the plants [2].

The formation and stability of the reduced SNPs in the colloidal solution was monitored by UV-vis spectrophotometer analysis. The UV-vis spectra showed maximum absorbance at 420 nm, which varied with different concentrations of silver nitrate (Fig. 2a and b). The optimum concentration of $AgNO_3$ for the synthesis was found to be 3 mM and 4 mM for Sg SNP's, and Aa SNP's, respectively. A report by Gurunathan *et al.* [10] revealed that by controlling the environment of nanoparticle synthesis, SNPs of various sizes and shapes could be synthesized.

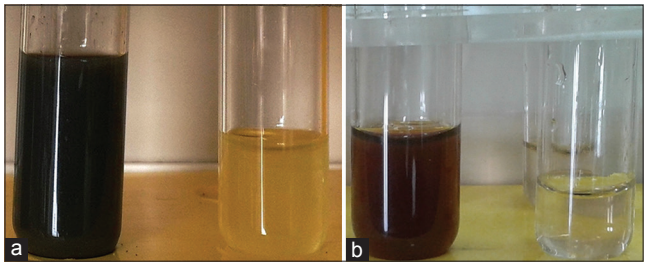

Fig. 1: (a) The color change. (i) Biosynthesized silver nanoparticles (SNPs) in a colloidal dispersion using *Simarauba glauca* (Sg) leaf extract. Color change from pale yellow to dark brown observed in 1 mM silver nitrate within two min of reaction time. (ii) Aqueous leaf extract of Sg. (b) The color change. (i) Biosynthesized SNP's in a colloidal dispersion using *Artocarpus altilis* (Aa) leaf extract. Color change from transparent to brown observed in 1 mM silver nitrate after 10 minutes of reaction time. (ii) Aqueous leaf extract of Aa

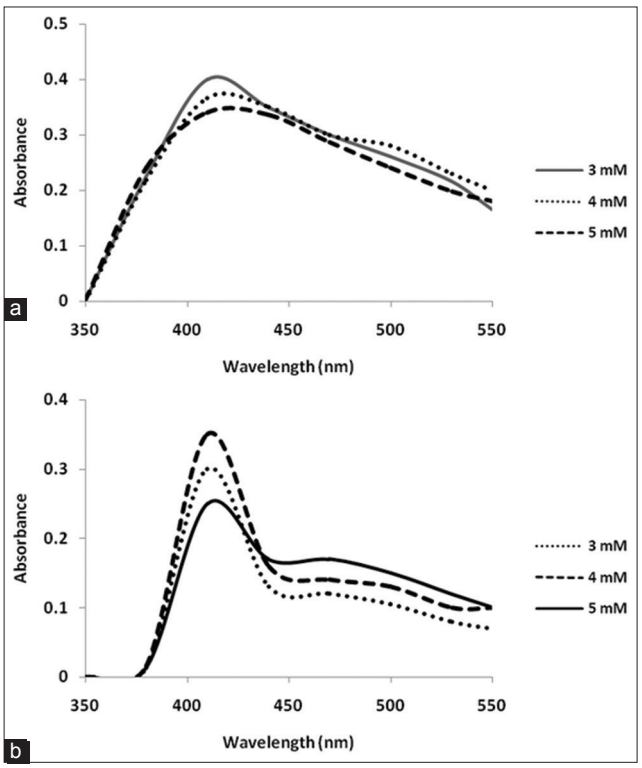

Fig. 2: (a) UV-vis spectra of silver nanoparticles (SNPs) synthesized from *Simarauba glauca* leaf extract recorded as a function of the different concentration of silver nitrate. (b) UV-Vis spectra of SNP's synthesized from *Artocarpus altilis* leaf extract recorded as a function of the different concentration of silver nitrate.

Characterization of SNP

The absorption spectrum of the synthesized SNP (Fig. 2a and b) showed a well-defined plasmon band at 420 nm, characteristic of nanosized silver. Seshadri *et al.* [11] reported that the observation of a broad surface plasmon peak in the 400-450 nm range is a characteristic of SNPs, confirming the synthesis of a nanosized silver particle in the present investigation. It is well-known that the shape of the spectrum and the wavelength at which the maximum absorbance is occurred is strongly related to the particle size and relative dimensions.

To gain further insight into the features of the SNPs, analysis of the sample was performed using SEM. SEM analysis clearly shows the presence of the synthesized SNPs of size ranges from 33 nm to 50 nm for Sg SNP's; 37.9 nm for Aa SNP's as seen in Fig. 3a and b. It was observed that smaller sized particles were almost spherical in shape and were in agreement with the shape of surface plasma resonance band in UV-visible spectra [12].

FTIR measurements were carried out to identify the biomolecules responsible for capping and efficient stabilization of the synthesized NPs. The FTIR spectra of SNP (Fig. 4a and b) showed strong absorption peaks at 3,250/cm for Sg SNP's which have resulted from stretching of the N-H band of amino groups or is an indicative of bonded O-H hydroxyl group which are mainly involved in the reduction of silver to SNPs. The results also confirmed that the proteins present in the leaf extracts act as reducing agents and stabilizers for NPs and prevent agglomeration. This suggests that the biological molecules could possibly perform dual functions of formation and stabilization of SNPs in the aqueous medium [13].

Applications of SNP

Antibacterial activity

The significant feature of silver is its broad spectrum antimicrobial property. Accordingly, several researchers have studied the antimicrobial activity of SNP. The dose-dependent antibacterial activity of SNPs synthesized by green route was investigated against various pathogenic organisms, and the result is shown in Fig. 5 a and b. Several mechanisms have been proposed to explain the inhibitory effect of SNPs on bacteria. It is assumed that the high affinity of silver towards sulfur and phosphorus is the key element of the antimicrobial effect. Due to the abundance of sulfur containing proteins on the bacterial cell membrane, SNPs can react with sulfur-containing amino acids inside or outside the cell membrane, which in turn affects bacterial cell viability. It was also suggested that silver ions from SNPs can interact with phosphorus moieties in DNA, resulting in inactivation of DNA replication and eventually resulting in cell death [14].

MIC

A simple and reliable method has been used to determine MIC of the synthesized nanoparticle using a dye. Resazurin is a blue non-fluorescent and non-toxic dye that turns pink and fluorescent when reduced to resorufin by oxidoreductases within viable cells while dye color remains unchanged in case of non-viable cells [6]. The MIC of Sg SNP's and Aa SNP's for *E. coli* is 10 µg/mL; *S. aureus* is 12 µg/mL; *B. subtilis* is 10 µg/mL; and *P. vulgaris* is 11 µg/ml. The results suggest that NPs exhibited excellent bactericidal effects against test cultures. A report by Ansari *et al.* [15] showed the MIC of SNPs to be 12.5 µg/mL against *S. aureus* which is consistent with the present report.

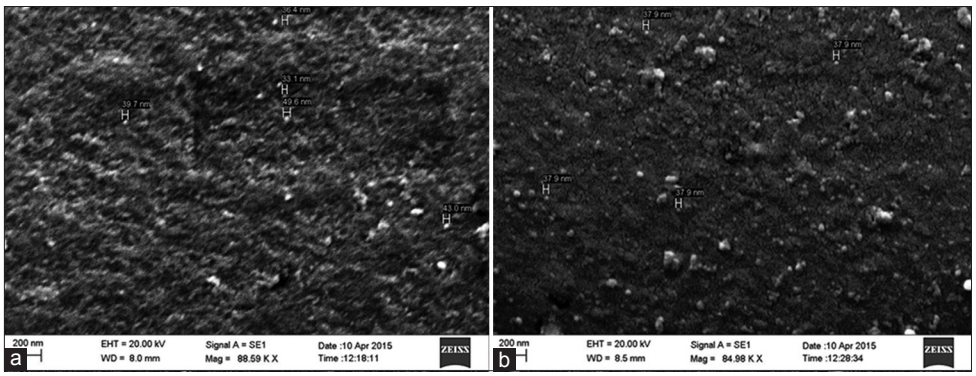

Fig. 3: (a) Scanning electron microscope (SEM) image of silver nanoparticles (SNP's) synthesized from *Simarauba glauca* leaf extract. (b) SEM image of SNP's synthesized from *Artocarpus altilis* leaf extract

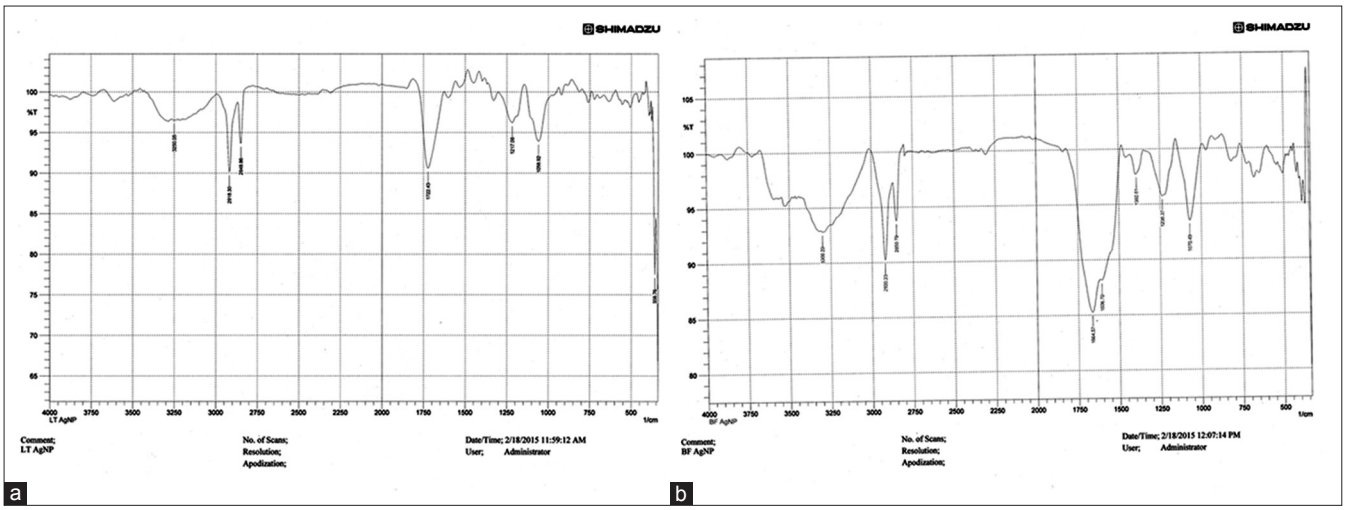

Fig. 4: (a) Fourier transform-infrared spectroscopy (FTIR) spectra of silver nanoparticles (SNP's) synthesized from *Simarauba glauca* leaf extract. (b) FTIR spectra of SNP's synthesized from *Artocarpus altilis* leaf extract

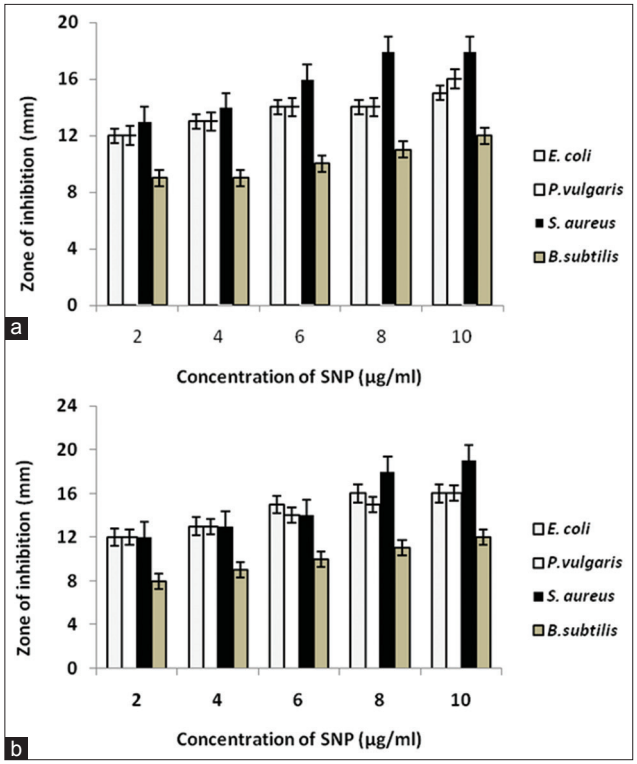

Fig. 5: (a) Dose-dependent antimicrobial activity of silver nanoparticles (SNP's) synthesized from *Simarauba glauca* leaf extract. Error bars are mean ± standard error (SE) (n=3). (b) Dose-dependent antimicrobial activity of SNP's synthesized from *Artocarpus altilis* leaf extract. Error bars are mean ± SE (n=3)

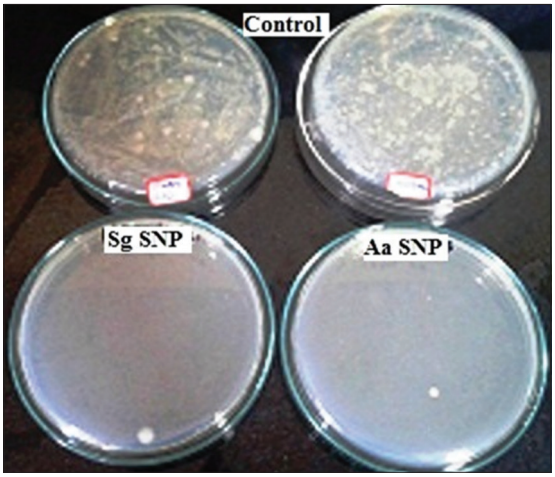

Fig. 6: Results of sewage water treatment with stones coated with silver nanoparticles (SNP's) synthesized from *Simarauba glauca* leaf extract and *Artocarpus altilis* leaf extract. Control - untreated sewage water showing mat growth of bacteria

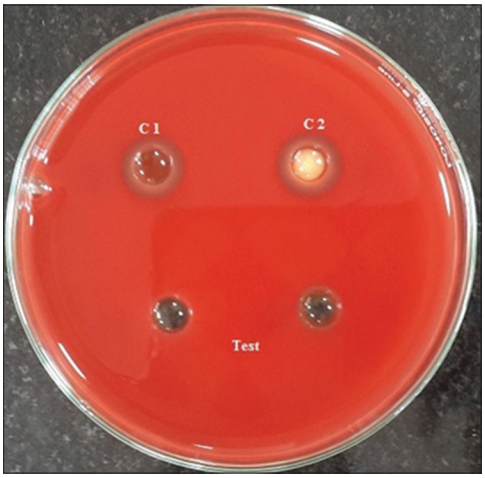

Fig. 7: Hemolytic activity silver nanoparticles (SNP's) synthesized from *Simarauba glauca* leaf extract and *Artocarpus altilis* leaf extract on blood agar plate. (C1 [control]: 3 mM $AgNO_3$; C2 [control]: 5 mM $AgNO_3$)

Water treatment using SNP coated stones

In this study, the application of SNPs as an antimicrobial agent for effective sewage water treatment was investigated. The result (Fig. 6) clearly shows the no growth/reduction in bacterial growth on the plate spread plated with water sample treated with the stones coated with SNP as compared to the control plate proving that the green synthesized SNPs can be effectively used in water treatment. The present result is in agreement with the report by Saklani *et al.* [16] who proposed the use of SNP coated stones in the form of a filter for water purification.

Biofilm inhibition

Biofilm infections are extremely challenging to treat because antimicrobials are less effective. The presence of biofilms causes numerous problems in the field of medicine as well as marine biofouling, it interferes with the clinical therapy of chronic and wound-related infections as well as persistent infections of various medical devices. Although numerous strategies have been established and are currently in use to control biofilms, the search for novel, natural, and effective antibiofilm agents still continues.

In this study, the use of NPs as alternatives to control biofilms has been explored. SNPs have been shown to modify the surface properties of bacterial cells and reduce their adhesive properties [7]. Compared to the control, preformed *B. subtilis* biofilms treated with SNP (10 µg/mL) for 24 hrs showed inhibition of 75.25±0.21% and 64.52±0.27% for Sg and Aa extract, respectively.

Therefore, the present results reveal that biologically synthesized SNPs not only inhibited the growth of the bacteria but also removed the biofilm formed by it. This inhibitory effect of SNPs on the existing biofilm may due to be the presence of water channels throughout the biofilm. Since in all biofilms water channels are present for nutrient transportation, SNPs may directly diffuse through the exopolysaccharide layer through the pores and may impart antimicrobial function. This study thus demonstrates the futuristic application of SNPs based on its potential anti-biofilm activity. Kalishwaralal *et al.* [17] while studying the effect of SNPs on the biofilm formed by *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* reported the elimination of the pre-biofilm besides the inhibition of biofilm formation.

Hemolytic activity

Evaluation of hemocompatibility of NPs should be considered as one of the factors of assessing systemic toxicity. Incubation of silver (Ag) NPs with human RBC demonstrated that SNPs are non-hemolytic (Fig. 7) substantiating its biocompatibility. While Kutwin *et al.* [18] reported that Pt and Au NPs were hemocompatible and did not show the agglutination of erythrocyte and also no precipitation properties.

CONCLUSION

It has been demonstrated that the leaf extract of Sg and Aa are capable of producing SNPs. The biosynthesized SNPs showed excellent biocompatibility, antimicrobial activity, and biofilm inhibition property. Current achievements in this field, although promising, need further work to fully harness the potential of NPs for biomedical applications and to enable their incorporation into clinical practice. Thus, the

biologically synthesized SNPs could be of immense use in medical field. Combining distinctive novel properties of NPs provides a unique opportunity for physicists, chemists, biologists, and material scientists to mould the new area of nanobiotechnology.

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