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Novel synthesis of nanosilver particles using plant active principle aloin and evaluation of their cytotoxic effect against *Staphylococcus aureus*

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PEER REVIEW

Peer reviewer

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Comments

The paper was well written with good English. The results were well presented. The results pointed to the commercialization of aloin coated silver nanoparticles.
Details on Page S95

ABSTRACT

Objective: To develop a reliable, eco-friendly and easy process for the synthesis of silver nanoparticles using aloin, the active principle of medicinal plant '*Aloe vera*' and to evaluate antimicrobial activity against *Staphylococcus aureus* (*S. aureus*), a causative organism of most of the diseases in livestock and to standardize the level of safety of synthesized silver nanoparticles.

Methods: Characterization using UV-vis spectrophotometry, DLS technique, FT-IR and SEM. Tube dilution method was carried out to evaluate the MIC of the compound against *S. aureus*. MTT assay was performed to evaluate the level of safety of nanoparticles.

Results: UV-vis absorption spectrum showed a maximum absorption around 200 nm for aloin mediated silver nanoparticles (ANS). The size of the particles as measured by DLS technique was 67.8 nm. The results of FT-IR analysis indicated the involvement of hydroxyl, carboxyl, amine and nitrile groups in the synthesis and stabilization of aloin mediated silver nanoparticles. SEM images showed that ANS with cubical, rectangular, triangular and spherical morphology and measured sizes of the agglomerated nanoparticles are in a range of 287.5 to 293.2 nm, however the average size of an individual particle is estimated to be approximately 70 nm. The compound (ANS) showed a MIC of 21.8 ng/mL against *S. aureus* and showed an *in vitro* spleenocyte viability of more than 80% at the highest concentration of 87.5 mg/L per well.

Conclusions: Aloin consists of functional groups which reduced Ag⁺ ions to Ag⁰ ions and helped in synthesis of silver nanoparticles. The synthesis process has further enhanced the antimicrobial activity of nanosilver. The compound is also proved to be safe at the level many times higher than the MIC.

KEYWORDS

Aloin, Silver, Nanoparticles, Drug, Encapsulation

1. Introduction

Silver nanoparticles owing to the characteristics like reduced size and increased surface area[1] provide enormous hope for use in biological systems by virtue of broad spectrum antimicrobial activity[2-4] which includes bactericidal action against both Gram positive and Gram negative bacteria including multidrug resistant strains[5-9].

Further, the development of bacterial resistance has not been reported against silver nanoparticles[10,11] making it a good choice for therapeutic consideration in most of the livestock diseases. The additional advantage with silver nanoparticles resides in the fact that they are relatively non-toxic and safe antibacterial agents[12] requiring much smaller concentrations for antimicrobial effect[6,13]. Further, the biological synthesis of nanoparticles using plant

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sources (phytogenic) is advantageous over other methods of synthesis in being cost effective, practically non-toxic and eco friendly^[14,15].

Aloin, the major constituent of *Aloe vera* species was reported to have anti-microbial^[16]. It is also reported to have anti-inflammatory^[17,18], anticancer and anti-tumor activity^[19]. *Staphylococcus aureus* (*S. aureus*) was reported to be the leading cause of mastitis worldwide^[20] and also for noscomial and community acquired infections. The annual economical loss caused by staphylococcal bovine mastitis globally was estimated to be USD 2 billion^[21]. Further, *S. aureus* was reported to be the main reason for the use of antibiotics in dairy cows^[22].

In the present study, synthesis and characterization of nanoparticles phytogenically derived by the reduction of silver nitrate using aloin were carried out and the antimicrobial activity of the compound was investigated on *S. aureus* which is a major cause of most of the livestock diseases of economic importance. Further, the safety level of the compound is determined.

2. Materials and methods

2.1. Synthesis of aloin mediated silver nanoparticles (ANS)

The aloin (Sigma aldrich) was purchased and was stored in the refrigerator at -4°C . Two percent aloin solution was prepared with the dissolution of appropriate quantities in the distilled water and made up to 100 mL. About 0.1 mol/l silver nitrate solution was prepared and 90 mL of which was added to 10 mL of 2 percent aloin solution at 95°C with vigorous stirring. Then change in colour of the solution was observed from pale yellow to brown which indicates the formation of ANS. The prepared solution was cooled to the room temperature normally and finally particles were allowed to settle for 24 h. The solution was then changed to a plastic container for further characterization.

2.2. Characterisation of silver nanoparticles

2.2.1. UV-visible spectra analysis

The reduction of pure Ag^+ ions was monitored by measuring the UV-visible spectrum of the reaction medium at 5 h after diluting a small aliquot of the sample into distilled water. UV-visible spectral analysis was done by using UV-vis spectrophotometer UV-2450 (Shimadzu).

2.2.2. SEM analysis of silver nanoparticles

SEM (Scanning electron microscope) analysis was done using Hitachi S-4500 SEM machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid was allowed to dry by putting it under a mercury lamp for 5 min.

2.2.3. DLS technique–particle size measurement

DLS (Dynamic light scattering) analyzes the velocity distribution of particle movement by measuring dynamic fluctuations of light-scattering intensity caused by the brownian motion of the particle. This technique yields a hydrodynamic radius or diameter, which is calculated using the Stokes–Einstein equation from the above mentioned measurements. The measurements were carried out using the instrument Nanopartica SZ-100 (HORIBA).

2.2.4. Fourier transform infrared spectroscopy (FT-IR analysis) measurements

FT-IR analysis was carried out on TENSOR-27 (BRUCKER) in the diffuse reflectance mode operated at a resolution of 4 cm^{-1} in the range of $400\text{ to }4000\text{ cm}^{-1}$ to evaluate the functional groups that might be involved in nanoparticle formation.

2.3. Anti-bacterial activity

Tube dilution method was adopted to determine the minimum inhibitory concentration (MIC) of the test compound against *S. aureus*. *S. aureus* isolated from cases of bovine mastitis which was subjected to cultural, biochemical and molecular characterization at Department of Veterinary Microbiology, N.T.R. College of Veterinary Science, Gannavaram was used for the study. A total of 10 sterile test tubes were taken. About 1 mL of the test compound was added to first and second tubes and to the rest of tubes 1 mL of sterile normal saline was added. Two fold serial dilution of the test compound was made from the second tube and 1 mL was discarded from the last tube. About 5 mL sterile nutrient broth was added to all the test tubes. Then 50 μL of standardized (0.5 McFarland unit) overnight broth culture was added to all the tubes. The tubes were incubated for 18 h at 37°C . The end point was defined as the lowest concentration of the test compound at which there was no visible growth. The lowest concentration of the test compound inhibiting the growth of the organisms is recorded as MIC.

2.4. Cytotoxicity study (MTT assay)

MTT is water soluble, yellow coloured tetrazolium salt that enters the cells and passes into mitochondria, where it is reduced to an insoluble purple formazan. The cells are then solubilized with an organic solvent and the released formazan is measured spectrophotometrically. Since the reduction of MTT occurs in metabolically active cells, the level of activity is a measure of cell viability.

3. Results

3.1. UV-visible spectra analysis

UV-visible absorption spectrum of ANS is shown in Figure 1. The spectra of ANS showed absorption maximum at 200 nm

corresponds to the surface plasmon resonance of the formed silver nanoparticles.

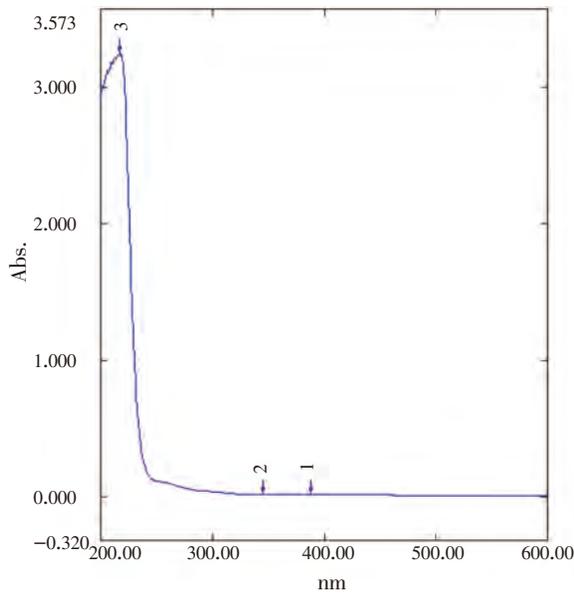


Figure 1. UV-visible spectrum of aloin mediated silver nanoparticles.

3.2. SEM analysis of silver nanoparticles

The SEM image analysis of ANS is depicted in Figure 2. It showed that the particles are cubical, rectangular, triangular and spherical in shape with uniform distribution. However, in most of the occasions agglomeration of the particles was observed. The measured sizes of the agglomerated nanoparticles are in the range of 287.5 to 293.2 nm; however the average size of an individual particle is estimated to be 70 nm.

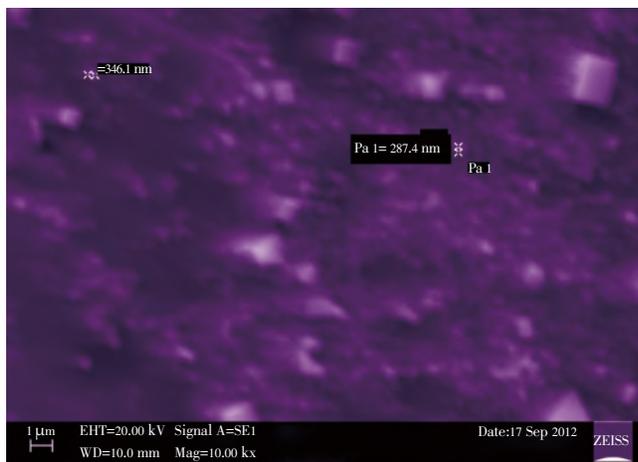


Figure 2. SEM image of aloin mediated nanosilver.

3.3. DLS technique–particle size measurement

The size of the aloin mediated silver nanoparticles was measured using DLS technique and the size was recorded as 67.8 nm which is in good agreement with the SEM analysis (70 nm). DLS particle size measurement of ANS is shown in Figure 3.

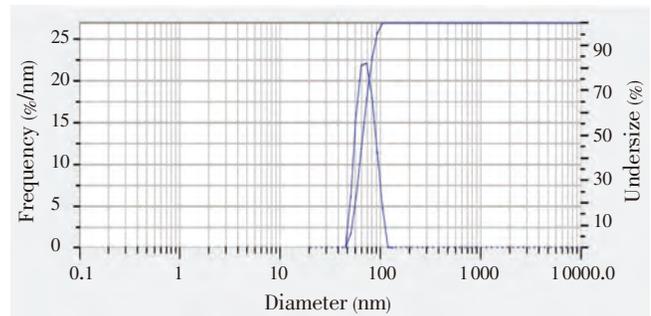


Figure 3. DLS technique–particle size measurement of ANS.

3.4. FT-IR analysis

The result of FT-IR analysis for ANS is depicted in Figure 4. Spectra of ANS showed transmission peaks at 3355, 1636 and 1507 cm^{-1} . The peak at 1636 cm^{-1} indicates primary amines, the peak at 3355 cm^{-1} corresponds to O-H, as also H-bonded phenols and alcohols in ANS while the peak at 1507 cm^{-1} in ANS corresponds to involvement of nitriles ($-\text{C}=\text{N}$) groups.

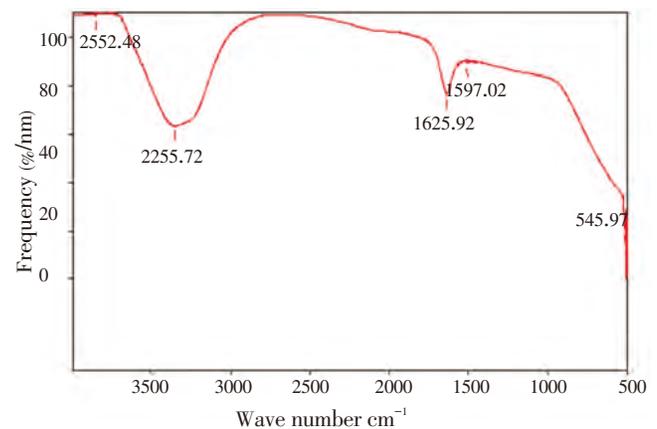


Figure 4. FT-IR spectrum of aloin mediated silver nanoparticles.

3.5. Antibacterial activity

The MIC of the test compounds against *S. aureus* was depicted in Figure 5. The MIC obtained by tube dilution method was 21.8 ng of ANS/mL.

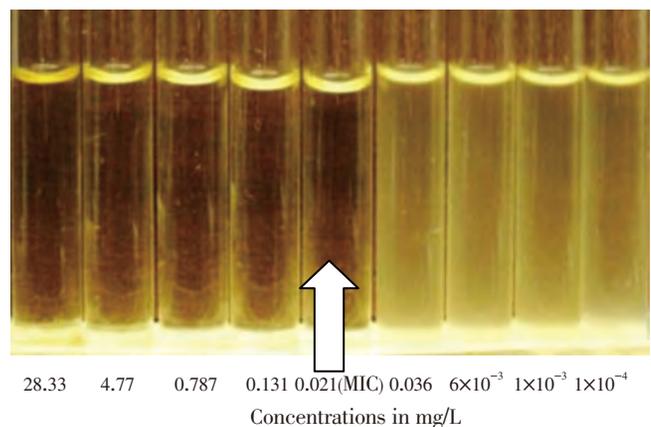


Figure 5. Determination of MIC: Tube dilution assay.

3.6. Cytotoxicity study (MTT assay)

The MTT assay results were represented graphically in Figure 6. In the MTT assay, the spleenocytes maintained a survivability of above 80% at a concentration of 87.5 mg/L of the compound and was above 80% at all lower concentrations.

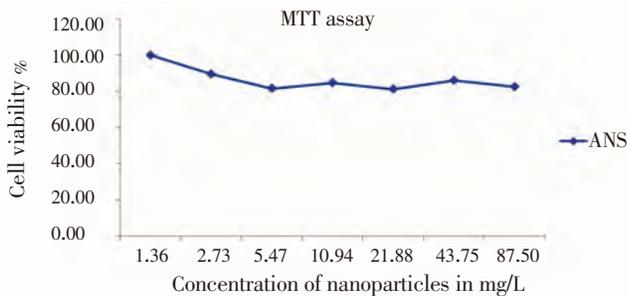


Figure 6. Cytotoxicity of nanoparticles.

4. Discussion

Green synthesis of nanosilver using plant extracts was reported to be superior to chemical synthesis in that, the former compounds offer better advantages as they are widely distributed, safe to handle, easily available with a range of metabolites[23]. In the present study, silver nanoparticles were synthesised using phyto-compound aloin and the formed silver nanoparticles were characterized using UV-visible spectroscopy, SEM, DLS technique and FT-IR analysis. The production of silver nanoparticles is demonstrated by the sharp peak around 200 nm for aloin mediated silver nanoparticles in UV-vis spectrum, which indicates the availability of reducing biomolecules in aloin. Analysis of SEM image shows the formation of silver nanoparticles and indicates the agglomerated appearance with cubical, rectangular, triangular shape and size varying from 287.5 to 293.2 nm. The average size of an individual particle is estimated to be approximately 70 nm. The results of DLS technique used for the measurement of size of ANS in solution form showed a size of 67.8 nm which is in good agreement with the SEM analysis (70 nm). The results of the FT-IR studies indicated the involvement of hydroxyl, carboxyl and primary amine functional groups of aloin in the synthesis of silver nanoparticles. ANS showed better antibacterial properties against *S. aureus* as evidenced by MIC value 21.8 ng/mL when compared to the phyto compound aloin alone which showed a MIC value of 62.5 µg/mL[16]. The enhanced effect of ANS might be due to the bactericidal properties of silver nanoparticles[24]. Cytotoxicity studies revealed that ANS showed no toxicity at the doses studied *i.e.*,

between 1.36–87.5 mg/L in mouse spleenocytes. Synthesis of nanosilver using phyto-genic compound aloin made it more safer when compared to chemical synthesized nanosilver which is reported to be toxic by Sommayeh *et al.*[25] who conducted toxicity study of chemical mediated nanosilver on osteoblast cancer cell line and demonstrated concentration-dependent toxicity and IC₅₀ value of 3.42 µg/mL.

In conclusion, ANS were found to possess powerful antibacterial effect and were also found to be safe. Hence, keeping in view of the economics of production, safety and efficacy of the compound, ANS could provide a promising alternative to the use of traditional antibiotics.

Conflict of interest statement

We declare that we have no conflict of interest.

Comments

Background

Plant mediated synthesized nanoparticles, silver in particular has gaining lot of importance in biological applications. Novel antimicrobial properties of silver nanoparticles have attracting many researchers to search new avenues to production of the same. Aloin is known from the ancient times, as an antimicrobial and anti-inflammatory agent. If these properties of aloin are coupled with the silver nanoparticles, then a conjunctive effect of both may be observed and this is the background of the work proposed in the present manuscript.

Research frontiers

Production of phyto-genic silver nanoparticles through easy and cost-effective methods is the order of the day in recent scientific research. Novel coating of aloin on the silver nanoparticles has many advantages interms of enhanced antimicrobial properties and thereafter, for commercialization.

Related reports

There are a number of reports available on the synthesis and application of phyto-genic silver nanoparticles. But the reports on coating the silver nanoparticles with the novel compounds like aloin are scarce.

Innovations & breakthroughs

Aloin coated silver nanoparticles were prepared and were

tested for their novel antimicrobial properties. This may be the first report on this kind of preparation of silver nanoparticles with aloin coating.

Applications

This is the first report of its kind and promising lot of scope for commercialization to apply them in animal science for disease control.

Peer review

The paper was well written with good English. The results were well presented. The results pointed to the commercialization of aloin coated silver nanoparticles.

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