

A Novel Biosynthesis, Characterization and Antimicrobial Activity of Silver Nanoparticles Using Leaves Extract of *Aloe vera* Plant

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Abstract: *The area of nanotechnology is one of the most important fields of studies in recent materials science. Novel applications of nanoparticles and nanomaterials are rapidly emerging day by day. Different plant materials have the potential in synthesizing metallic nanoparticles traditionally by wet chemical methods, thereby providing an alternative to the conventional toxic chemical methods. The present was aimed at producing eco-friendly silver nanoparticle from 1mM aqueous solution of AgNO₃ using the leaf extract of Aloe vera as reducing as well as capping agent in a cost effective way. The green synthesized silver nanoparticles were confirmed by visual observation with the appearance of yellowish brown solution in the reaction mixture and by UV-Vis spectroscopy. The silver nanoparticles were found to be spherical in shape with variable size ranging from 2.53 to 31.03 nm, as obtained by TEM analysis. The anti-microbial activity of green synthesized silver nanoparticles was affirmed against Staphylococcus aureus and Escherichia coli.*

Keywords: *Aloe vera*, green synthesis, silver nanoparticles, antimicrobial activity, *Staphylococcus aureus*, *Escherichia coli*.

1. Introduction

Nanoparticles (nps) especially metallic ones are of significance interest because of the modification of properties observed due to size effects, catalytic modification, electronic, and optical properties of the monometallic nps (Elizondo *et al.* 2012). In recent years biosynthesis of metallic nanoparticles is receiving significant attention due to the increasing demand to develop clean, nontoxic chemicals, environmentally non maligen solvents and renewable materials (Gericke and Pinches, 2006; Harris and Bali, 2008). As a result, scientist in the area of nanoparticle synthesis and assembly have turned towards the using of biological system such as yeast, fungi, bacteria and plant extracts for the production of biocompatible metal and semiconductor nps through the process of control nucleation and growth of inorganic nanoparticles (Kasthuri *et al.* 2009; Shankar *et al.* 2003) in place of synthetic chemicals such as NaBH₄, citrate, or ascorbate. These chemicals are toxic to human and pose a threat to the environment (Chen *et al.* 2006; Kuo *et al.* 2003).

Plant-mediated biological synthesis of nanoparticles is gaining importance due to its simplicity, cost effectiveness and eco-friendliness and as possible alternative to toxic chemical method. Plant assisted reduction of metal nanoparticles and the role of phytochemicals has been studied and investigated in the light of IR spectroscopic studies that the main phytochemicals responsible are terpenoids, flavonoids, ketones, aldehydes, amides and carboxylic acids. Flavonoids, organic acids and quinones which are responsible for immediate reduction are the main water soluble phytochemicals. Extracts of *Magnolia kobus* and *Diopryros kaki* leaf extracts have been used to synthesize gold nanoparticles. Temperature is investigated as an important factor affecting nanoparticle formation and reported that polydisperse particles with a size range of 5-300nm was obtained at lower temperature while the

formation of smaller and spherical particles is supported at higher temperature (Song *et al.* 2009).

Studies have shown that numbers of metallic nanoparticles especially those of silver and gold had been synthesized by using various plants such as alfalfa (Gardea *et al.* 2003), *Cinnamomum camphora* (Huang *et al.* 2007), neem (Shankar *et al.* 2004a), *Embllica officianalis* (Ankamwar *et al.* 2005a), lemongrass (Shankar *et al.* 2003), and tamarind (Ankamwar *et al.* 2005b). However, the potential of the plants as biological materials for the synthesis of nanoparticles is still under study.

Silver has been widely used as an antimicrobial agent for centuries; the recent develop in interest for this element particularly depends on the increasing danger of antibiotic resistance, caused by the indiscriminate use of antibiotics (Panaek *et al.* 2006, Sambhy *et al.* 2006). It is generally known that silver nanoparticles may attach to the cell wall, thus disturbing cell-wall permeability and cellular respiration. The nanoparticles may also penetrate inside the cell causing damage by interacting with phosphorus- and sulfur- containing compounds such as DNA and protein. Another possible contribution to the bactericidal properties of silver nanoparticles is the release of silver ions from particles (Sambhy *et al.* 2006).

Aloe vera is an essential and traditional medicinal plant belonging to the family Liliaceae. It is indigenous to Africa and Mediterranean countries. It is reported to grow wild in the islands of Cyprus, Malta, Sicily, Canary cape, Cape Verde and arid tracts of India. The plant is a hardy perennial tropical plant which can be cultivated in drought prone areas and is one of the crops whose potential is yet to be exploited, despite being identified as a new plant resource with the most promising prospects in the world. This plant contains two main classes of Aloins; nataloins, which produce picric and oxalic acids with nitric acid, and fail to give a red coloration with nitric acid; and barbaloins, which produce aloetic acid (C₇H₂N₃O₅),

chrysammic acid ($C_7H_2N_2O_6$), picric and oxalic acids with nitric acid, being reddened by the acid. It has been extensively used in cosmetics, as anti-microbial agent such as fungi and bacteria (Jamir *et al.* 1999). In this study silver nanoparticles was synthesized using the leaf extract of aloe vera and the anti-bacterial activities of the nanoparticles was investigated after its characterization using UV-vis Spectroscopy and Transmission electron microscope.

2. Material and Methods

2.1 Preparation of plant extract

Fresh leaves of *Aloe vera* were collected from the field and washed thoroughly with sterile distilled water before chopped into small pieces. Afterwards 10g of clean chopped leaves were taken into a flask of 100 ml sterile double distilled water and boiled for 5 minute at 80°C. The extract was decanted and then filtered using Whatman filter paper and used as reducing as well as capping agents in the synthesis of AgNPs.

2.2 Preparation of silver nanoparticles

1mM aqueous solution of silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. 10 ml of *Aloe vera* extract was taken and added into 90 ml of aqueous solution of 1 mM Silver nitrate and incubated in the dark, overnight at room temperature. Brownish yellow solution was formed, indicating the successful formation of silver nanoparticles.

2.3 UV-visible spectrum analysis

Equal amount of sample aliquot and distilled water (1ml each) were mixed in a 10 mm-optical-path-length quartz cuvettes, and the UV-vis spectrum analysis of the reaction mixture was carried out to detect the reduction of pure Ag^+ ions. The concentration of AgNPs produced was measured using a Systronics UV double beam spectrophotometer, at a resolution of 1 nm, between 200 and 800 nm (Fig. 1).

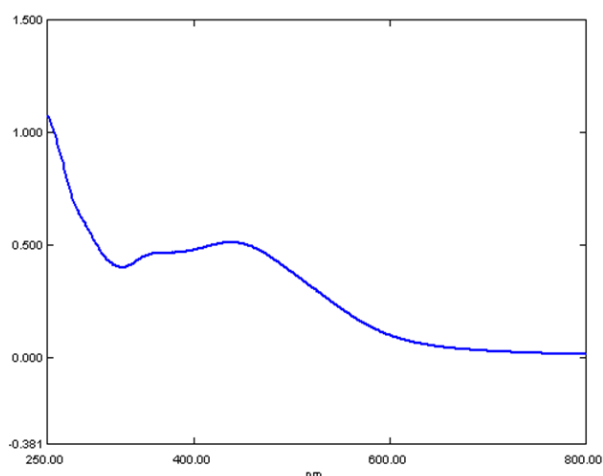


Figure 1: UV-vis absorption spectrum of clear yellowish brown colloidal AgNPs prepared by leaf extract of *Aloe vera*

2.4 Transmission Electron Microscopy

Samples for transmission electron microscopy (TEM) analysis were prepared at the ratio of 50: 50 of AgNPs solution to that of double distilled water. 50 μ L of the prepared sample was dropped onto carbon-coated copper TEM grids, and then allowed to dry. Excess solution was removed and the grids were further dried prior to viewing with TEM microscope which was operating at an accelerating voltage at 120 kV.

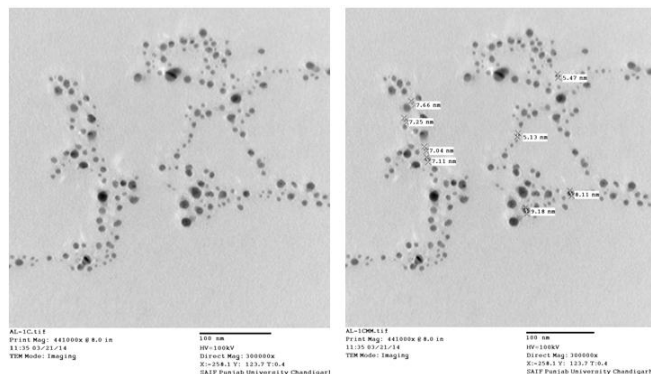


Figure 2: TEM image of silver AgNPs prepared by green synthesis using leaf extract of *Aloe vera*

2.5 Antimicrobial Sensitivity Assay

A modified protocol of Kirby-Bauer as described by Sham (2010) was adapted for the *in vitro* antimicrobial activity of the synthesized silver nanoparticles against *Escherichia coli* and *Staphylococcus aureus* by disc diffusion using Nutrient Agar (NA). The plates were prepared by pouring 15ml of molten media into sterile Petri-plates, allowed to solidify for 5minutes. 0.1ml of inoculums suspension was swabbed uniformly on the surface and left to dry for 5minutes. 0.01ml of (1mM) concentrations of the AgNPs solution was loaded on 5mm sterile individual discs formed. The loaded discs were then placed on the surface of medium and the compound was allowed to diffuse for 5minutes before incubating at 37°C for 24hours. Streptomycin disc was used as positive control whereas for negative control, aqueous solution of $AgNO_3$ was used. At the end of incubation, inhibition zones formed around the disc were measured with a transparent ruler in millimeter. All assays were performed in triplicates and the mean value recorded for each of two organisms under study.

3. Result and Discussion

It is well recognized that the structure and size distribution of metallic nanoparticles synthesized by the reduction of metallic salts in solution largely depends on different reaction conditions which includes temperature, time, concentration, molar ratio of metallic salt/reducing agent, mode and manner of addition of reagents, presence and type of protective agents, frequency and nature of perturbation, and whether nucleation is homogeneous or heterogeneous (Sanguesa *et al.* 1992). It is evident that reduction of silver ion into silver particles during the reaction with plant extracts could be followed by characteristic color change (Elumalai *et al.* 2010). The result of the successful synthesis of silver nanoparticles

obtained from this study is confirmed by earlier finding which reported that the green synthesized silver nanoparticles showed a yellowish – brown color in aqueous solution due to the surface Plasmon resonance phenomenon (Elumalai *et al.* 2010). Characterization of biosynthesized nano silver particles was carried using UV-Vis Spectroscopy and TEM. UV-Vis spectrograph of the colloidal solution of silver nanoparticles has been determined as a function of time. Absorption spectra of Nano silver particles formed in the reaction media has absorbance peak at 413 nm, widening of peak indicated that the particles are polydispersed (Fig.1).

The structure and size of the silver nanoparticles analyzed using transmission electron microscope (Fig.2 A & B) indicates the micrographs images of TEM analysis of the green synthesized silver nanoparticles at different magnifications. It is evident from the TEM analysis that silver nanoparticles joined to formed nano-clusters was view at lower magnification and stable, small, spherical with size range of 2.53-31.03nm were characterized at higher magnification level. TEM analysis also indicated that the green synthesized silver nanoparticles were not only spherical in shape but also crystalline in structure.

Similar phenomenon was also reported by Malik and Sehwat (2013).

The anti-microbial activities of biosynthesized silver nanoparticles against *E. coli* and *S. aureus* were also investigated. Both silver ions and silver nanoparticles were known to have excellent anti-microbial activities (Furno *et al.* 2004). The result obtained showed the silver nanoparticle to be effective against the two tested bacterial isolates at 1mM concentration (Table 1). The highest toxicity was observed against *S. aureus* (13mm) this could be attributed to its cell wall nature which allow easy passage of the particles as compared to *E. coli* (9.5mm). This result is in line with those found by (Mostafa *et al.* 2013). In another study *S. aureus* was found to be less affected by silver nanoparticles at 1mM concentration compare with *E. coli* at similar concentration and this could be due to the characteristics of some bacterial strains (Kim *et al.* 2007). The inhibition zone produced by positive control was more (22mm), however, due to the rapid emergence of resistance strains the silver nanoparticles will be the best alternative.

Table1: The antimicrobial activity of silver nanoparticle synthesis using leaf extract of *Aloe vera*

Microorganisms	Zone of inhibition in millimeter		
	Silver nanoparticle	Positive control	Negative control
<i>E. coli</i>	9.5±0.67	22±0.53	6.5±0.43
<i>S. aureus</i>	13±0.57	21.5±0.64	7.5±0.44

Key: Positive control – Streptomycin & Negative control – Silver nitrate

4. Conclusions

The green synthesis of silver nanoparticles was achieved using the leaf broth of *Aloe vera*, the particles were found to be spherical in shape having an average size of 9.12nm following characterization by TEM. Further study confirmed the antibacterial activities of the synthesized nano silver particles against both Gram positive and negative bacteria with the Gram positive (*S. aureus*) showing highest sensitivity. Thus present study ascertains the use of medicinal plant for biosynthesis of silver nanoparticles and its potential use against microbes.

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