Bio Synthesis of Silver Nanoparticles and Their Antibacterial Activities

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Abstract

Silver nanoparticles (NPs) were rapidly synthesized by treating silver ions with Tridax procumbens extract. Plant extract is very cost effective and eco friendly and thus can be economic and effective alternative for the large scale synthesis of silver nanoparticles. Characterization of newly synthesized silver nanoparticles was made using UV-vis spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, X-ray diffraction (XRD) and Scanning electron microscopy (SEM).The results indicated that the plant extract, which have aldehyde groups, played a reducing and controlling role during the formation of silver NPs in the solutions. The anti-bacterial activity of AgNPs was investigated by using one Gram-negative and one Gram-positive bacteria .The bacterial strains are showing good antibacterial activity.

***Key words**: Tridax procumbens extract, Silver Nitrate Solution, Silver Nanoparticles, Antibacterial activity*

1. Introduction

Metal nanoparticles have been used in a wide-ranging application in various fields. Specifically, as shapes, sizes, and compositions of metallic nanomaterials are significantly linked to their physical, chemical, and optical properties, technologies based on nanoscale materials have been exploited in a variety of fields from chemistry to medicine [1-3]. Recently, silver nanoparticles (AgNPs) have been investigated extensively due to their superior physical, chemical, and biological characteristics, and their superiority stems mainly from the size, shape, composition, crystallinity, and structure of AgNPs compared to their bulk forms [4-8].

Many researchers have developed a keen interest in the synthesis of silver nanoparticles due to their enhanced antimicrobial activity and their use as anticancer agents [9-17]. Silver in pure form has the highest electrical and thermal conductivity among all metals and has lowest contact resistance [18]. There are studies and reports that nano-silver can evidently have adverse effects on humans as well as on the environment [19]. However, the green approach offers toxic chemical free and eco-friendly synthesis of AgNPs. There are various reports of using green, environment friendly reducing and capping agents for nanomaterial synthesis [20-21]. Leaves of different plants such as Azadirachta indica (neem), Ocimum tenuiflorum (black Tulsi), Ficus benghalensis (Banyan tree), Psidium guajava, Candida albicans, Fusarium graminaerum, Trichoderma viride etc. have been used for the synthesis of Ag nanoparticles [22-28]

The use of plants for synthesis of nanoparticles is a comparatively new and under-researched technique. Synthesis of metal nanoparticles using plant extracts is very cost effective, so it can be used as an economic and valid alternative for the large-scale production of metal nanoparticles. The advantage of using plants for the synthesis of nanoparticles is that they are easily available, safe to handle and possess a broad variability of metabolites that may aid in reduction.

The *Tridax procumbens* L.extract contains a lot of biomolecules, such as polysaccharides, amino acids and proteins [29-30], which could be used as reductants to react with silver ions and as scaffolds to direct the formation of silver NPs in solution. To the best of our knowledge, the use of this plant extracts at room temperature for the green synthesis of silver NPs, has not been reported.

2. Experimental details

2.1 Preparation of *Tridax procumbens* leaf extract

About 20g of freshly, taxonomically authenticated healthy leaves of *Tridax procumbens* were collected, washed thoroughly with double distilled water, cut into fine pieces and boiled with100mL double distilled water in Erlenmeyer flask for 8-10 min. The extract was cooled to room temperature and filtered through whattman filter paper (no.42).

2.2 Preparation of Silver Nanoparticles

In a typical experiment, 10mL of the *Tridax procumbens* leaf extract was added to 10 ml of a silver nitrate solution. After 10 minutes, the colour of the solution changed indicating the formation of silver nanoparticles. The product was washed with distilled water and then dried at room temperature.

2.3 UV-Vis spectral analysis

UV-VIS spectra were measured using a TU-1901 model UV- Visible double beam spectrophotometer (Beijing Purkinje General Instrument Co., Lt, China).

2.4 FTIR spectral analysis

FTIR spectra were performed and recorded with a Fourier-transform infrared spectrophotometer Nicolet 870 between 4000 and 400 cm^{-1}, with a resolution of 4 cm^{-1}.

2.5 SEM analysis

The morphologies and compositions of the Cu_2O nanoparticles were examined by Scanning Electron Microscopy (SEM), using a LEO 1455 VP equipped with energy dispersive.

2.6 X-ray diffraction studies

X-Ray Diffraction (XRD) patterns were recorded with a Philips analytical X-ray diffractometer Using CuKα radiation (λ= 1.5406 Å).

2.7 Antibacterial assay

The silver nanoparticles synthesized using *Tridax procumbens extracts* were tested for antibacterial activity by agar well-diffusion method against Gram positive (*Staphylococcus aureus*) and Gram negative strains *(Pseudomonas aueroginos*). The pure bacterial culture was sub cultured on nutrient agar and Potato Dextrose Agar (PDA) respectively. Wells of 10 mm diameter were made on nutrient agar and PDA plates using gel puncture. Each strain was swabbed uniformly onto the individual plates using sterile cotton swabs. Using a micropipette, different concentrations of the sample of nanoparticles solution (10 µl, 20 µl, 30 µl and 40 µl) was poured onto each well on the plates. After incubation at 37°C for 24 hours, the different level of zone of inhibition of bacteria was measured using the Hi antibiotic zone scale.

3. Results and Discussion

The addition of silver nitrate solution to the plant extract containing carbohydrates as a major constituent having aldehyde groups may cause the reduction of Ag^+ to form silver nanoparticles. The possible reduction mechanism leading to the formation of silver nanoparticles was proposed in the following equation.

$$\mathbf{RCHO + Ag^+ + H_2O \longrightarrow RCOOH + Ag + 2H^+}$$

(From leaf extract)

Therefore, we took advantage of the ready reactivity of solution with reducing sugars to innovate a facile method for the synthesis of silver nanoparticle.

The silver nanoparticles were characterized by UV-Vis spectroscopy, one of the most widely used techniques for structural characterization. Figure 1 represented the UV-Vis spectra of dark-brown silver nanoparticles synthesized by *Tridax procumbens* leaf extract which showed surface plasmon absorption and at 463 nm, indicated the presence of Ag nanoparticles [31].

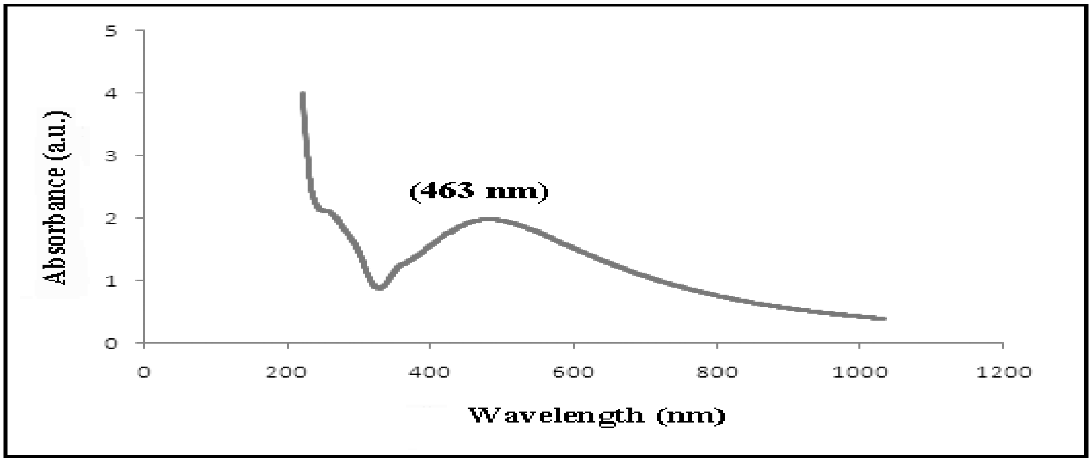

Figure.1 UV-Vis spectrum of Silver nanoparticles

The FTIR spectra of silver nanoparticles showed peaks at 3456, 1586 and 1386 cm^{-1} in the region 4000 - 400 cm^{-1} as shown in Figure 2. The peaks at 1586 cm^{-1} (asymmetric) and 1386 cm^{-1} (symmetric) indicated the presence of (-COO-) carboxylate ions, responsible for formation of the silver nanoparticles [32]. The peak at 3456 cm^{-1} is the characteristic band of hydrogen bonded OH group that may be due to the formation of nanoparticles from the aqueous phase.

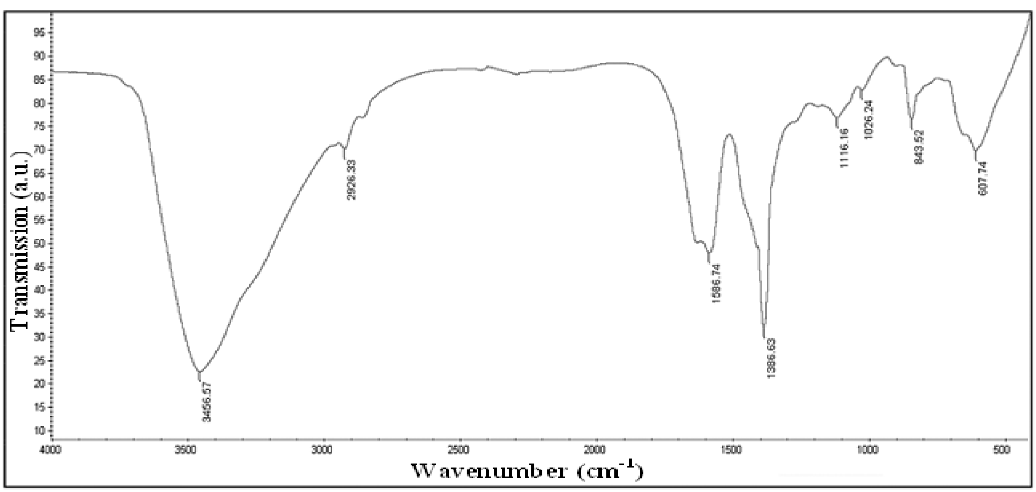

Figure.2 FTIR spectrum of silver nanoparticles

The X- ray diffraction patterns obtained for the Ag nanoparticles synthesized using *Tridax procumbens* leaf extract was shown in Figure 3. The XRD spectrum had three distinct diffraction peaks at 38.2°, 46.7° and 64.4° which were indexed as (111), (200) and (220) of the cubic face-centered silver. The obtained data were matched with the Joint Committee on Powder Diffraction Standards (JCPDS) file no. 03-0921. The crystallite sizes could be estimated using Debye-Scherrer equation $D = K\lambda/\beta\cos\theta$ where the constant K is taken to be 0.94, λ is the wavelength of X-ray, and β and θ are the half width of the peak and the Bragg angle respectively and it was calculated in the range of 40-50 nm.

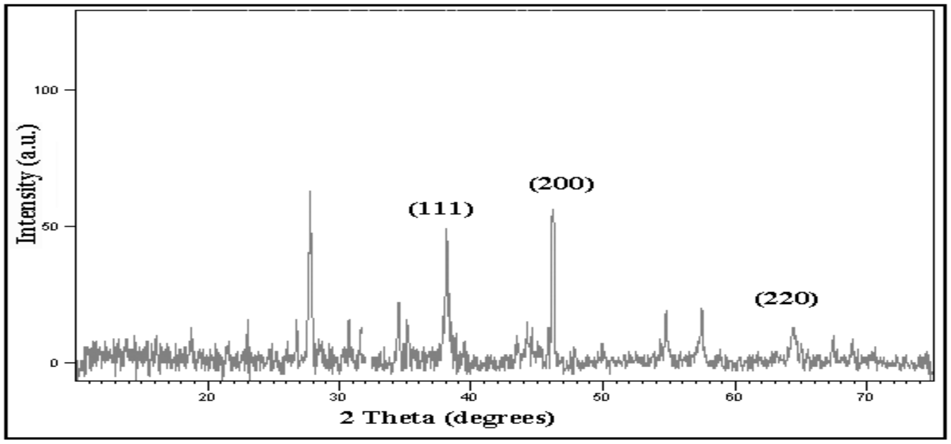

Figure.3 XRD pattern of Silver nanoparticles

Scanning Electron Microscopy (SEM) was used to identify the morphology of the synthesized silver nanoparticles as shown in Figure 4. It was seen that shapes of the silver nanoparticles appeared like agglomerated spheres with rough surfaces. Also it was appeared that these particles have rough surfaces and may be composed of smaller nanoparticles.

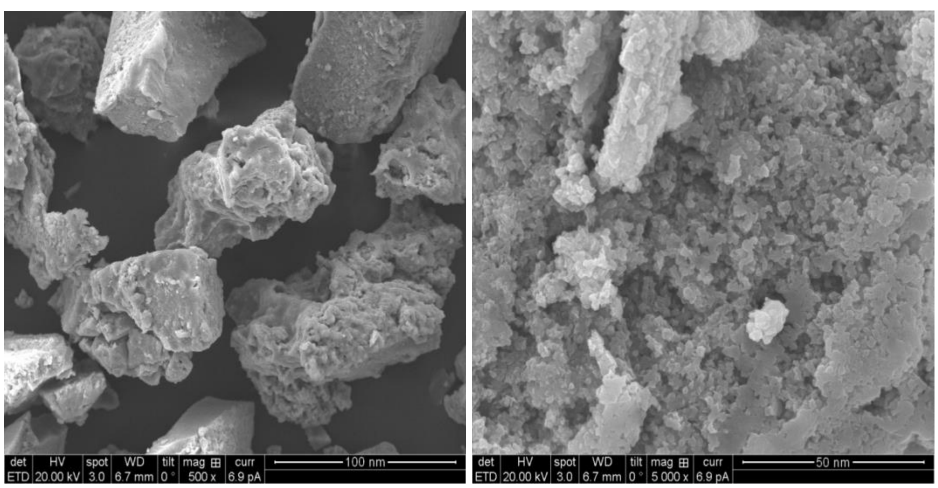

Figure.4 SEM images of Silver nanoparticles at different magnification levels

The antibacterial activity of silver nanoparticles were investigated against Gram positive (Staphylococcus aureus) and Gram negative strains (Pseudomonas aueroginosa) using well diffusion technique are shown in Figure 5**.**

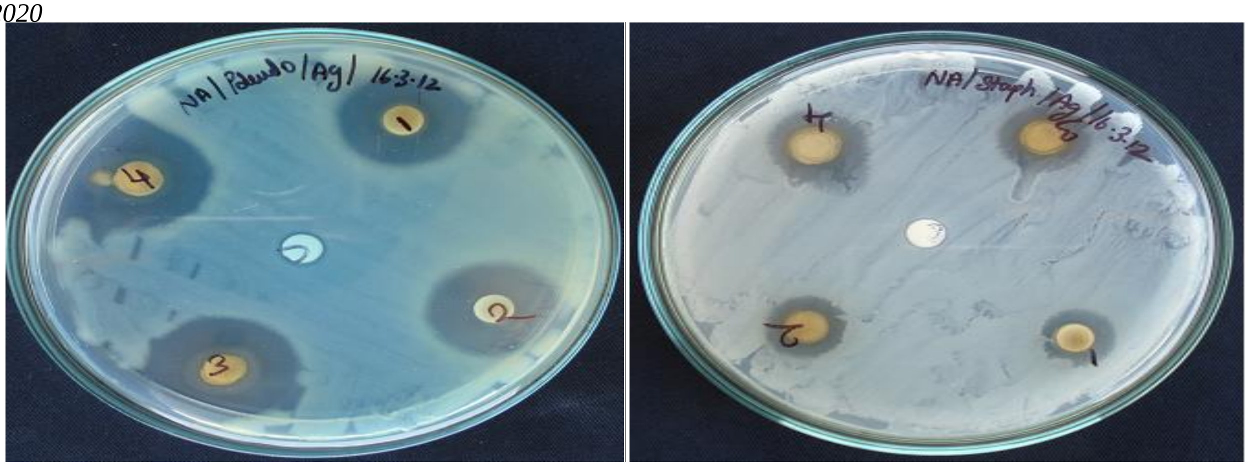

Figure5. Antibacterial activity of Ag Nanoparticle against bacterial pathogens.

The diameter of inhibition zones around each well with Ag NPs is represented in table-1.

Table 1. Zone of inhibition (mm) of *Tridax procumbens* mediated silver nanoparticles (µl)

Test organism	Concentration of nanoparticles(µl)			
	10 µl	20 µl	30 µl	40 µl
Pseudomonas aeruginosa	14	15	16	18
Staphylococcus aureus	8	9	11	12

The number of bacterial colonies grown on agar plates as a function of the different concentration of silver nanopar ticles when gradually declined when the concentration of nanoparticles increased. Results clearly demonstrate that newly synthesized silver nanoparticles are promising antimicrobial agent against the pathogens employed.

4. Conclusion

The waste leaves of *Tridax procumbens* plant were utilized in the synthesis of Ag nanoparticles. The reduction of silver ions and stabilization of the silver NPs was thought to occur through the participation of reducing sugars. The bioreduced silver nanoparticles were characterized using UV-Vis, XRD, SEM and FTIR spectroscopic techniques. Most importantly, the reaction was simple and convenient to handle, and it is believed that it has advantages over other biological syntheses. The antimicrobial potential of synthesized Ag nanoparticles was tested against bacterial pathogens by agar well diffusion assay. The AgNPs was showing strong antimicrobial potential against the tested bacteria.

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