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Biosynthesis of Silver Nanoparticles Using whole plant extract of the *Azadirachta indica* and Evaluation of Their Antimicrobial Activities

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Abstract:

Biomediated methods are considered to be a safer alternative to conventional physicochemical methods for the fabrication of nanomaterials due to their eco-friendly nature. In the present study, silver nanoparticles (AgNPs) were synthesized by using aqueous whole plant extract of the medicinal plant *Azadirachta indica*. The nanoparticles were also synthesized under ambient condition without any mechanical and chemical treatment. The silver nanoparticles were characterized by UV-vis., SEM, XRD, and TEM analysis. UV-vis. spectroscopic studies provided ample evidences for the formation of nanoparticles. The FTIR spectrum confirmed the presence of plant phytochemicals as stabilizing agent around the AgNPs. XRD and HR-TEM analyses clearly proved the crystalline nature of the nanoparticles. From the TEM images, the nanoparticles were found to be roughly spherical in shape. The AgNPs were also evaluated for their antimicrobial activity by well diffusion method against *B. Subtilis, E. coli, P. aeruginosa, S. aureus, A. niger*. They were found to be highly toxic against all the tested pathogenic strains.

1. Introduction:

The field of nanotechnology has generated great interest in recent years because of its impact on different fields like chemicals, electronics, agriculture, and medicine and space industry [1–6]. Nanoparticles are clusters of atoms in the size range of 1–100 nm. These nanoparticles possess defined chemical, optical and mechanical properties [7, 8]. Silver has been in use for centuries for treatment of different diseases. However, its use declined with the emergence of metallic silver [9, 10]. Metallic silver in the form of silver nanoparticles has made a remarkable comeback as silver nanoparticles, with enhanced chemical and physical properties [11, 12].

Biosynthesis provides advancement over chemical and physical method as it is cost effective, environment friendly and easily scaled up for large-scale synthesis and obviates the need for high pressure, energy, temperature, and toxic chemicals [13, 14]. Large-scale production by chemical and physical methods usually results in particles larger than several micrometers while the biological synthesis can be successfully used for production of small nanoparticles in large-scale operations [15].

Azadirachta indica commonly known as Neem belongs to Meliaceae family, and is well known in India and its neighboring countries for more than 200 years as one of the most versatile medicinal plant having a wide spectrum of biological activity. Every part of the tree has been used as a traditional medicine for household remedy against various human ailments, from antiquity.

2. Materials and Methods:

Azadirachta indica plant was collected in botanical garden at Dr N R S Ayurvedic Medical College, Bandar Road, Vijayawada. The plant parts were separated cleaned with sterile distilled water and then they were air dried for 7 days. The plants were ground to a fine powder. The powder obtained was extracted with distilled water. To 5g of powdered sample, 100ml of distilled water was added and boiled to 60-70^{o}C for about 10mins. Then the resulting crude extracts filtered through 0.25µ filter and stored in refrigerator. 10 ml of plant extract was added into 90 ml of prepared aqueous solution of 1mM silver nitrate for reduction into Ag+ ions and kept in magnetic stirrer for 1 hour at room temperature.

2.1 Characterization of synthesized nanoparticles:

2.1.1 UV-VIS Spectra analysis:

The reduction of pure Ag+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 3hours after diluting a 1ml of the sample into 4ml of distilled water. UV-Vis spectral analysis was done by using UV-VIS spectrophotometer.

2.1.2 SEM analysis of silver nanoparticles:

Scanning Electron Microscopic (SEM) analysis was done using instrument LEO 1420 VP Scanning Electron Microscopic. 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 were allowed to dry by putting it under a mercury lamp for 5min.

2.1.3 TEM Analysis:

The size and shape of the synthesized silver nanoparticles were examined using transmission electron microscopy (TEM) analysis. The sample was first sonicated (Vibronics VS 80) for 15 min. A drop of this solution was loaded on carbon-coated copper grids, and solvent was allowed to evaporate under Infrared light for 30 min. the sample was analyzed using TEM.

2.1.4 XRD analysis of silver nanoparticles:

The particle size and nature of the silver nanoparticle were determined using XRD. This was carried out using Bruker- D4 ENDEAVOR XRD-6000/6100 model with 30kv, 30mA with Cu kα radians at 2Θangle. X-ray powder diffraction is a rapid analytical technique primarily used for phase identification of a crystalline material and can

provide information on unit cell dimensions. The analyzed material is finely ground, and average bulk composition is determined. The particle or grain size of the particles on the silver nanoparticles was determined using Debye Sherrer's equation.

$$D = \frac{0.94\lambda}{B\cos\Theta}$$

2.1.5 EDX analysis of silver nanoparticles:

To gain further insight in to the features of the silver nanoparticles, analysis of the sample was performed using Energy Dispersive microanalysis techniques. EDX analysis was carried to confirm the presence of silver in the particles as well as to detect other elementary compositions of the particles.

2.2 Antimicrobial activity:

The antimicrobial activity of the synthesized nanoparticles was tested against four different pathogenic organism *B. Subtilis, E.coli, P. aeruginosa, S. aureus, A.niger*. Bacterial sensitivity to nanopaticles was commonly tested using a disc diffusion assay, utilizing antibiotics or NPs impregnated disks. After the incubation period, the growth inhibition zone was measured and the results of the inhibition were measured in milli meters.

3. Results and discussion:

The reduction of silver nitrate into silver nanoparticles during exposure to plant extracts is followed by a gradual increase in colour development from clear to yellowish brown, as a result of the surface plasmon resonance phenomenon (Figure 2). UV-visible spectrophotometric analysis is used to follow and confirm the formation of silver nanoparticles. The UV-visible spectrum of *A. indica* extract does not show any peak in the range from 250 to 700 nm. The first evidence for the formation of AgNP is obtained from the change in colour of the reaction mixture. The addition of extract to silver nitrate solution cause appreciable change in the colour of the solution. A peak was observed at about 421nm [figure 3] for the synthesized nanoparticles. This wavelength will be the suitable for the biologically synthesized nanoparticles. Figure 4 shows representative SEM images recorded at different magnifications from drop-coated films of the AgNPs synthesized by treating $AgNO_3$ solution with *A. indica* . The SEM images show a high density of AgNPs synthesized by *A. indica* plant extracts, which was further confirmed by EDX. From the EDX spectrum, it is clear that *A. indica* has a recorded weight percent (18%) of the AgNPs (Figure 5). The size and shape of the synthesized silver nanoparticles were examined using transmission electron microscopy (TEM) analysis. The TEM images of AgNPs synthesized by microwave method are given in Figure 6. It is clear that the nanoparticles are almost spherical in shape. The particle size distribution histogram drawn after ignoring any tiny particle (Figure 6(b)) shows that the size of the particles comes between 15 and 27 nm and the particle size is found to be 169, 400 and 83. The HR-TEM image shows clear lattice fringes which indicates that the growth of silver nanoparticles takes place preferentially on the (111) plane. The selected area electron diffraction (SAED) pattern AgNP-Alpinia shows circular rings which can be attributed to the face centered cubic structure of silver nanoparticles. The more intense circular ring closer to the centre is due to (111) reflections. The second ring is indexed to the (200) reflections. The third and fourth rings belong to (220) and (311) reflections, respectively. The clear circular rings also suggest that the synthesized silver nanoparticles are polycrystalline in nature. The antibiotic activity of AgNPs was investigated against various pathogenic organisms such as *B. Subtilis, E. coli, P. aeruginosa, S. aureus, A. niger* using well-disc method (Figure 7 and Table 1). The results of the antimicrobial inhibition showed that the synthesized nanoparticles show prominent growth inhibition on the growth of the pathogenic in the study. The particles show high growth inhibition for *A. niger* and it has no effect on the growth reduction of *B. Subtilis*. Except *B. Subtilis* the synthesized nano particles shows growth inhibition on all the pathogenic in the study.

4. Conclusion:

A green method to synthesize silver nanoparticles using the *A. indica* plant extract has been developed. The properties were characterised by UV, XRD, SEM, TEM and EDX. This characterization is of use for large scale silver nanoparticle production, and could result in economic viability, as well as being eco-friendly with many

applications. Further anti microbial activity of ionic or nanoparticle silver has a great potential for use in controlling pathogens. Silver may be less toxic to humans and animals than synthetic fungicides. The particles show potent antimicrobial activity against pathogenic microbes. The zones of inhibition obtained in the antimicrobial test suggest that the nanoparticles produced by this method have efficient antimicrobial activity. This green chemistry approach for nanoparticle synthesis has several promising attractions and can be used for the large scale production of silver nanoparticles which find application as nanocatalysts and antimicrobial agents.

6. Figures and tables:

Figure 1: Areal parts of the plant *A. indica*

Figure 2: bio-reduction of silver nitrate using *A. indica* extract

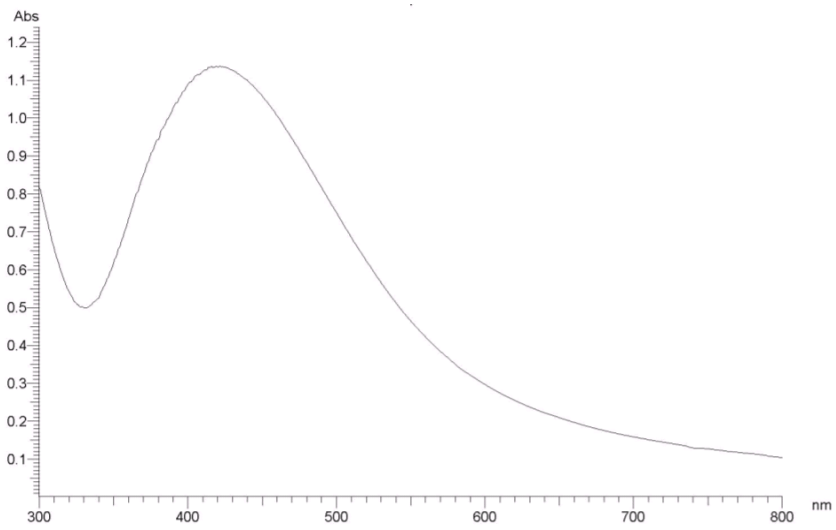
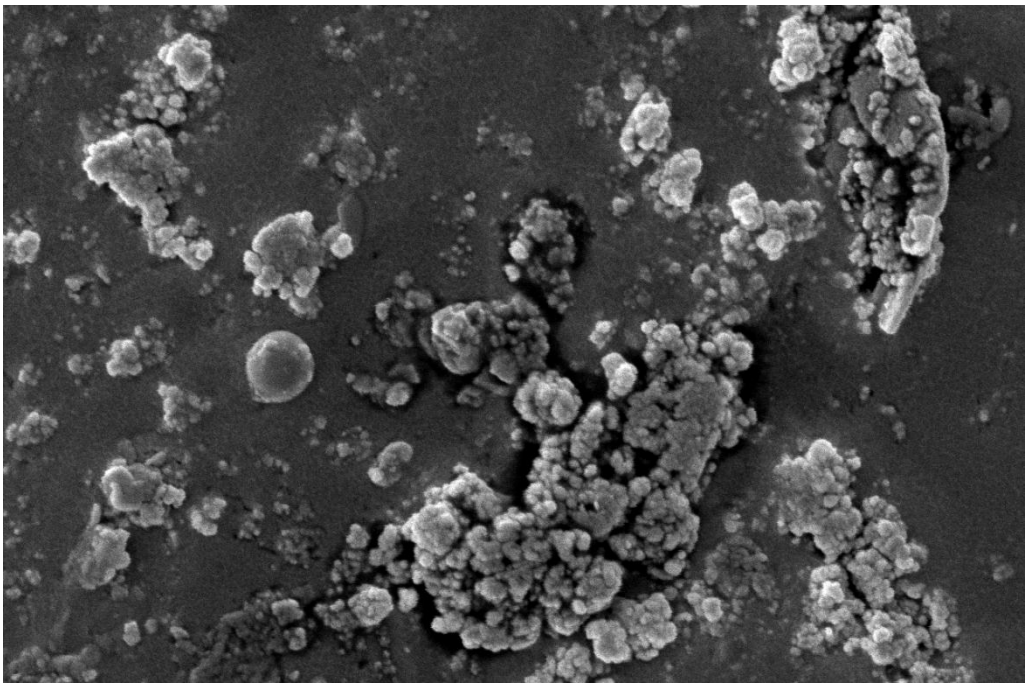
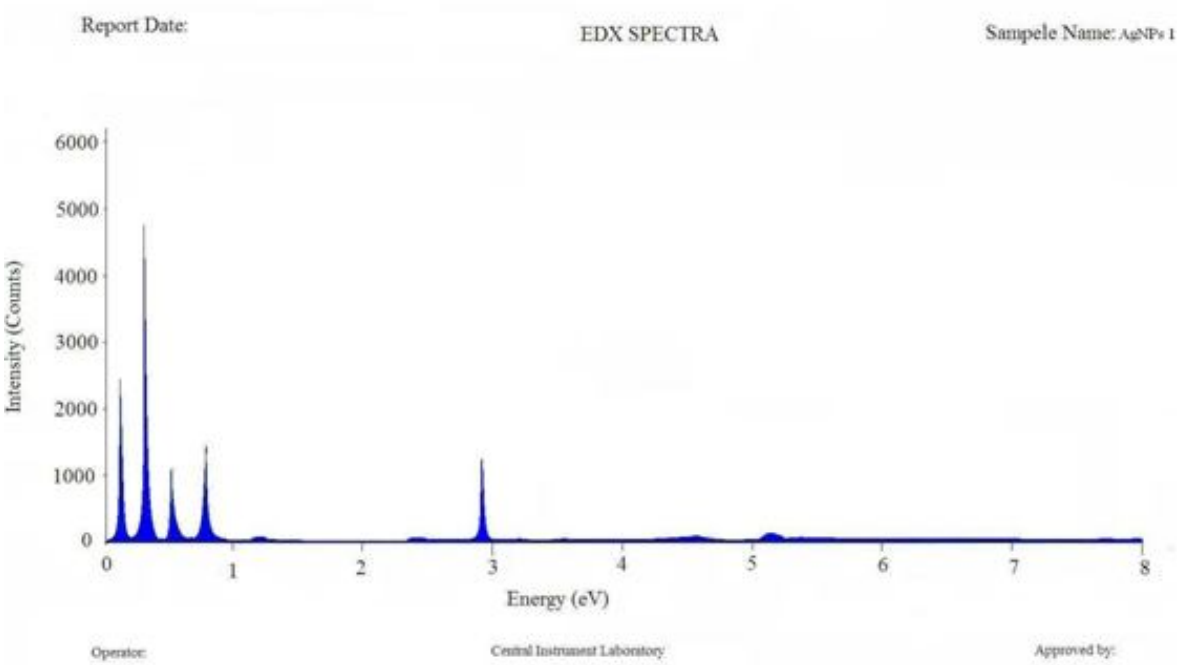


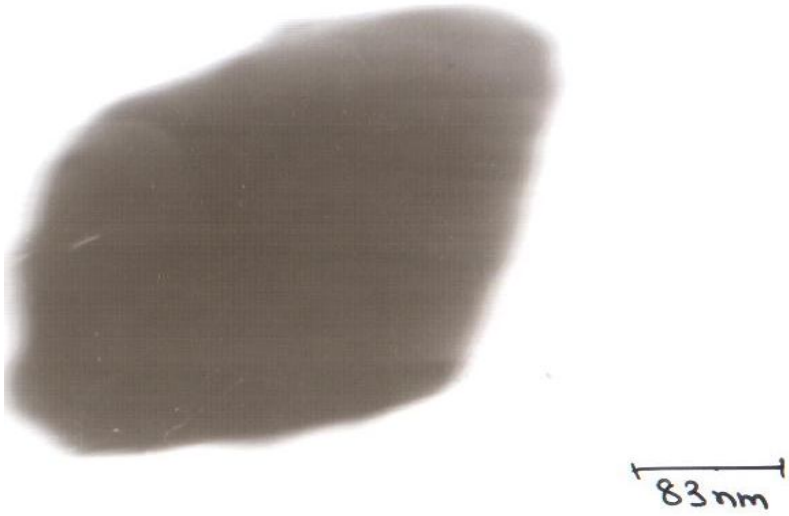
Figure 3: UV scanning spectra for *A. indica*



Figures 4: SEM images for the synthesized nano-particles of *A. indica.*



Figures 5: EDX images for the synthesized nano-particles of *A. indica*



Figures 6: TEM images for the synthesized nano-particles of *A. indica*

Figure 7: antimicrobial activity of silver nano particles

Nano particles	Concentration (µg/ml)	B. Subtilis (le24)	E. coli (ts22)	P. aeruginosa (GF)	S. aureus (SSB1)	A. niger
A. indica	100	-ve	2mm	1mm	1mm	2mm
	500	-ve	4mm	3mm	3mm	4mm
	1000	-ve	9mm	7mm	6mm	12mm

Table 1: Anti microbial activity of synthesise nano particles

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