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Studies on the green synthesis of silver nanoparticles and their characterization using leaf extracts of *Terminalia chebula*

KEYWORDS: Silver nanoparticles, Nanoscale, FTIR, AFM, Surface Plasmon Resonance (SPR).

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ABSTRACT *The nanotechnology springs from advancements in material science- the ability to fabricate nanoscale materials in an uniform and reliable manner. Silver had been used since time immemorial in different chemical form to treat burns, wounds and several infections caused by pathogenic bacteria. Advancement of biological process of nanoparticles synthesis is evolving into a key area of nanotechnology. A green rapid biogenic synthesis of silver nanoparticles (Ag NPs) using ethyl acetate leaf extract of was Terminalia chebula demonstrated in the present study. The formation of silver nanoparticles was confirmed by Surface Plasmon Resonance (SPR) at 420nm using UV–visible spectrophotometer. The reduction of silver ions to silver nanoparticles by Terminalia chebula leaf extract was evidenced by metal-plant interactions. Plants responded to heavy metal stress by metal complexation process like production of phytochelations or by other metal chelating peptides. Synthesized nanoparticles were characterized using UV–visible spectroscopy, Fourier Transformed Infra Red spectroscopy (FT-IR), powder X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Elemental Data Analysis (EDAX) and Transmission Electron Microscopy (TEM). The stability of silver nanoparticles was checked and found to be stable at 250C. The approach of green synthesis seemed to be cost efficient, eco-friendly and easy alternative to conventional methods of silver nanoparticles synthesis.*

INTRODUCTION:

Nanotechnology is the most captivating area of research in the field of material science. Nanoparticles, generally considered as particles with the size of up to 100nm, exhibited completely new or enhanced properties as compared to the larger particles of the bulk material that they were composed of based on explicit characteristics such as size, distribution and morphology (Williams and Wildrenbery, 2005). Nanoparticles of noble metals, such as silver were also broadly applied in products that directly comes in contact with the human body, such as shampoos, soaps, detergents, shoes, cosmetic products and toothpastes, besides medical and pharmaceutical applications such as optical receptors, polarizing filters, catalysts in chemical reaction, biolabelling and as antimicrobial agents (Balaprasad, 2010). Therefore, there is a growing necessitate to develop the environment friendly processes for nanoparticle synthesis without using lethal chemicals. Synthesis using biological-organisms, especially medicinal tree extracts that secrete the functional molecules for the reaction, is compatible with the green chemistry principles: the bio-organism is (i) eco- friendly as are (ii) the reducing agent employed, and (iii) the capping agent in the reaction (Gardea-Torresday, *et al.*, 2003).

Use of silver nanoparticle is relatively new because of their high reactivity and large surface area to volume ratio. The basic mechanism in all cases involved the accumulation of nanoparticles after the reduction of metal ions. This reduction process was mediated by some reducing agents or enzymes bound to the cell wall or proteins (Chandran, et al., 2006). The choice of the *Terminalia chebula* leaf extract was based on the presence of aromatic compounds. The plant contained a variety of phytochemical compounds such as phenols, amino acids, flavones etc and exhibited antioxidant activity. The plant extract was reported to have activities of scavenging superoxide anion radicals, 1,1-Diphenyl-2-Picrylhydrazyl radicals (DPPH), hydroxyl radicals, hydrogen peroxide, chelating ferrous iron, and ferric ion reducing potential. These molecules were expected to self assemble and cap the metal nanoparticles formed in their presence and thereby induced some shape control during metal ion reduction (Ahmad *et al.*, 2010).

METHODOLOGY:

Collection and preparation of leaf extracts:

Fresh leaves of *Terminalia chebula* were collected and washed with mercuric chloride and dried with water absorbent paper. Then they were cut into small pieces. Finally dispensed in 100ml of sterile distilled water and boiled for one hour at 80^{o}C. The extract was collected in separate conical flasks by typical filtration process.

Biosynthesis of silver nanoparticles:

1mM aqueous solution of silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. 5ml of extract from each sample was added with 100ml of silver nitrate solution. The colour change from pale green to dark brown was checked frequently. They were incubated at room temperature for 24 hours. The colour change indicated the synthesis of silver nanoparticles.

Ultra Violet-Visible Spectroscopic analysis:

The reduction of pure silver ions were monitored by measuring the UV-Vis Spectrophotometer at wavelength range (200-800nm) of the reaction medium for $AgNO_3$ along with leaf extract, after diluting small aliquots (10ml) of the sample with distilled water. UV-Vis spectral analysis was done by using UV-Vis spectrometer (HELIOS λ, Thermo Electron Corporation). Metal-plant interaction was observed for 3 days (24, 48, 72 hours) and measured at wavelength ranged from 200-800nm.

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Fourier Transform Infra Red (FTIR) Spectroscopic Analysis:
Fourier Transform Infra Red Spectroscopy dimensions were conceded to make out the biomolecules for synthesis of silver nanoparticles. To remove any unconventional biomass residue or composite that was not the capping ligand of the nanoparticles, the remaining solution of 100ml after reaction were centrifuged at 5000 rpm for 10 min and the ensuing suspension was redispersed in 10ml sterile distilled water. Three times centrifuging and redispersing procedures were repeated. Then, the purified pellets were air dried and analyzed by Schimadzu Japan at a resolution of 1cm/1(1cm prefix la minus 1).

X-Ray Diffraction (XRD) measurements:
The silver nanoparticle solution thus obtained were purified by frequent centrifugation at 5000rpm for 20 minutes followed by redispersion of the pellet of (Ag) nanoparticles into 10ml of deionized water. After air drying the purified nanoparticles, XRD measurement was carried out on films of the dehydrated pellet powder drop coated onto glass substrates on a Schimadzu XRD 6000 instrument operating at a voltage of 20 kV and a current of 30mA with Cu k α 1 radiation. The crystallite domain size was calculated from the width of the XRD peaks, assuming that they were free from non-uniform strains, using the Scherrer formula.

$$D= 0.94 \lambda / \beta \cos \theta$$

where D is the average crystallite domain size perpendicular to the reflecting planes, λ is the X-ray wavelength, β is the full width at half maximum (FWHM), and θ is the diffraction angle. To eliminate additional instrumental broadening the FWHM was corrected, using the FWHM from a large grained Si sample.

β corrected = (FWHM2sample- FWHM2si)1/2

Scanning Electron Microscopic (SEM) analysis:
Scanning Electron Microscopic (SEM) analysis of the particles were made using JOEL-JSM 6390 SEM machine. Thin films of the sample were arranged on a carbon coated copper grid by just dipping a very small amount of the sample on the grid, extra solution was detached using blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

Energy-Dispersive X-Ray Analysis (EDAX):
In order to take out EDAX analysis, the leaf extracts reduced to silver nanoparticles were dried out and drops coated on to carbon film and performed on JOEL-JSF 6000 SEM instrument outfitted with a Thermo EDAX measurement.

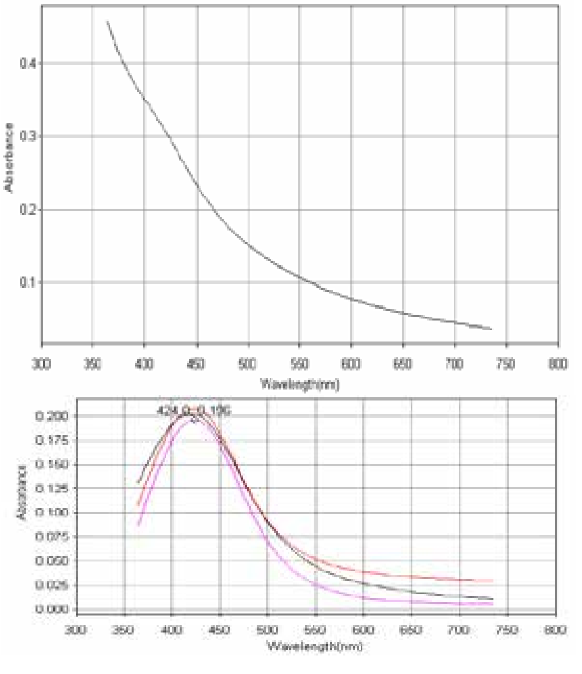
Transmission Electron Microscopic (TEM) analysis:
Transmission Electron Microscopy (TEM) (HITACHI, H-7500) was a microscopic technique whereby a beam of electrons was transmitted through an ultra-thin specimen, interacting with the specimen as it passed through. Silver nanoparticle image was formed from the interaction of the electrons transmitted through the specimen; the images of silver nanoparticles were magnified and focused onto an imaging device.

RESULTS AND DISCUSSION:
The formation of silver nanoparticles was preliminarily confirmed by the change in the colour of the solution. The colour change of the ethyl acetate leaf extract of *Terminalia chebula* from green to brown colour was due to the excitation of the Surface Plasmon Resonance and SPR band, both of which played an important role in the confirmation of formation of silver nanoparticles (Figure: 1.). It also indicated that the reduction of silver ion occurred in the aqueous solution by the ethyl acetate leaf extract of *Terminalia chebula*. UV spectra obtained from the reaction of reduction of silver ions which polydispersed nanoparticles had showed broadening peak in the absorbance band at the wavelength of 424 nm (Figure: 2). 1mM concentration of silver nitrate showed SPR wavelength at 420nm. Similar results were obtained using the fruit extract of *Terminalia chebula* with different concentrations of silver nitrate. It was because of the bioavailability of functional groups in the 10 ml of fruit extract of *Terminalia chebula* involved in the reduction of silver ion (Bar *et al.*, 2009).

Figure: 1. Biosynthesis of silver nanoparticles ($AgNO_3$ + ethyl acetate leaf extract of Terminalia chebula)

Whereas: PC-Positive control (Silver nitrate), NC-Negative control (Plant extract), E10-ethylacetate (Biosynthesized silver nanoparticles-$AgNO_3$ + Leaf extract of Terminalia chebula

Figure: 2. Comparative study of synthesized silver nanoparticles under UV-VIS spectroscopy:



Control (Silver nitrate) Synthesized silver nanoparticle (24hours-black, 48hours-red, and 72hours-rose)

FTIR analysis revealed the functional groups present in the silver nanoparticles synthesized using the leaf extract of *Terminalia chebula.* FT-IR analysis showed IR bands at

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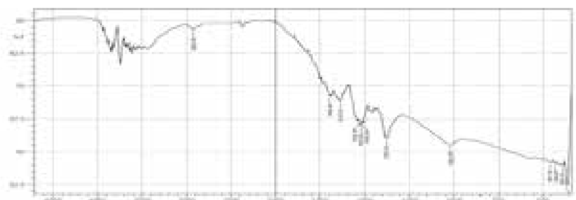
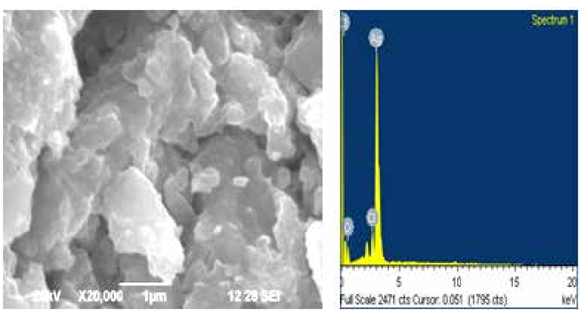
1020.38, 1508.38, 1523.82, 1539.25, 1637.62, 1695.99 cm−1. The band at 1020.38 cm−1 corresponded to -C-O-stretching; the peak at 1508.38 corresponded to aromatic amine –C-N-stretching. The bands appeared at 1523.82 and 1539.25 cm−1 were due to Carboxylate ion –COO-and-C-O-stretching respectively. The bands appeared at 1637.62 and 1695.99 cm−1 could be assigned to Carboxylate ion –COO- and -C=C-, -C=O-stretching respectively (Figure: 3).

Figure: 3. Synthesized silver nanoparticles under Fourier Transform InfraRed (FTIR) spectroscopy:



Scanning Electron Microscopy (SEM) image showed the morphological character of silver nanoparticles synthesized using leaf extract of *Terminalia chebula*. This image showed that the sizes were around 10 to 35 nm with formation of anisotropic nanostructures of pentagons, spherical and triangular shaped nanoparticles with size less than 100 nm. This SEM image also showed the aggregation of the silver nanoparticles. The EDAX spectrum showed the signal for silver and no other signals were observed which suggested that silver nanoparticles synthesized were very pure (Figure: 4).

Figure: 4. Scanning Electron Microscope (SEM) and EDAX image of synthesized Silver Nanoparticles

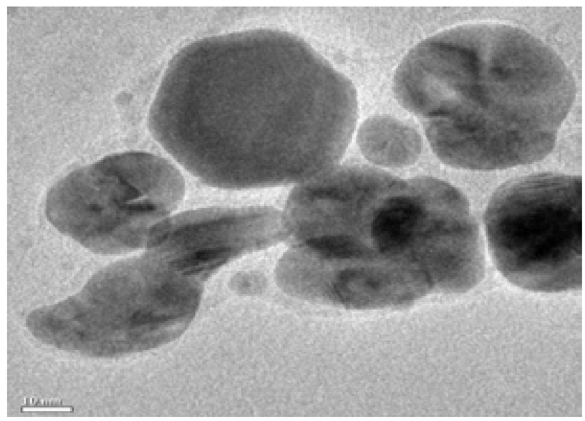


Element	App Conc.	Intensity Corrn.	Weight%	Weight% Sigma	Atomic%
O K	5.46	0.3301	20.63	1.51	62.17
Cl K	1.97	0.9503	2.58	0.23	3.51
Ag L	57.60	0.9356	76.79	1.48	34.32
Totals			100.00		

Silver nanoparticles were basically free from other impurities. Crystal behaviour of the purified solid silver nanoparticles was evaluated using Powder XRD. Powder XRD pattern of the synthesized silver nanoparticles showed four distinct diffraction peaks at 38.26, 44.25, 64.53 and 77.52 which could be assigned to (1 1 1), (2 0 0), (2 2 0) and (3 1 1) of face centred cubic (fcc) Ag NPs respectively. The lattice constant was in agreement with the database of Joint Committee on Powder Diffraction Standards (JCPDS. No. 01-087-0597).

Morphology and particle size of Ag NPs were characterized using HR-TEM. Typical HR-TEM micrographs at different magnifications (5, 10, 20, 50nm) were shown in (Figure: 5). It was clear from the TEM images that the formation of anisotropic nanostructures of pentagons, spherical and triangular shaped nanoparticles with size less than 100 nm. This anisotropy was due to lack of protective bio-molecules for lateral formed nascent nanocrystals. To attain thermodynamic stability, these fresh nanocrystals under lack of protective bio-molecules developed triangles and hexagon crystals (Narayanan and Sakthivel, 2011).

Figure: 5. Transmission Electron Microscope (TEM) image of synthesized silver nanoparticles:



Stability check of biosynthesized silver nanoparticles using different incubation temperature was done by using UV-Visible spectroscopy, which showed a good stability at 25^{0}C.

CONCLUSION:

A facile rapid green eco-friendly method to synthesize silver nanoparticles using the ethyl acetate leaf extract of *Terminalia chebula* had been developed. Polyphenols present in the form of hydrolysable tannins served both as reducing and capping agents. This method could be a promising alternative to the traditional reduction routes to avoid usage of toxic chemicals. This green method might find various medicinal as well as technological applications in demands.

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