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Green Synthesized Silver Nanoparticles From The Medicinal Plant Wrightia Tinctoria And Its Antimicrobial Potential

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Abstract: In recent years a number of physical, chemical and biological techniques were applied for the development of metal nanoparticles (NPs). In present study, we synthesized silver nanoparticles from leaf extract of *Wrightia tinctoria*. This plant belongs to family Apocynaceae and the extract of this plant have shows potential medicinal properties for extensive range of human being diseases. The resulting silver nanoparticle were characterized by using UV – Visible Absorption spectra of Ag nanoparticles formed in the reaction media has absorbance maxima at 515 nm, X- ray diffraction (XRD) intensities were recorded from 10 º to 70 º at 2 theta angles. To study the crystalline nature of the silver nanoparticles and Fourier transform infrared spectroscopy (FTIR) spectra revealed the presence of different functional groups like Alcohol (O-H stretching, H-bonded), Alkane (C-H stretching), Alkene (C=C stretching), Aromatic (C=C stretching), Amine (C-N stretching), Ether (C-O stretching). Further, the silver nanoparticles were isolated from plant leaves and tested for antimicrobial activity of antibiotics (Tetracycline), silver nanoparticles and the combined effect of both antibiotics and nanoparticles against *Staphylococcus aureus*, *Vibrio cholerae*, *Micrococcus luteus* and *Klebsiella pneumoniae*.
Keywords: silver nanoparticles; X-ray diffraction; spectroscopy; Tetracycline; antimicrobial.

Introduction

The history of nanomaterials is quite long; nevertheless, major developments within nanoscience have taken place during the last two decades. Research in nanomaterials is a multidisciplinary effort that involves interaction between researchers in the field of Physics, Chemistry, Mechanics, Material science, and even Biology and Medicine. The term nanoparticle, which represents another form of nanometerials, came in to frequent use in the early 1990s by the material science community to represent particles that are composed of up to tens of thousands of atoms but confined to size less than 100 nm, until then more general terms like submicron and ultra fine particles were in use. Reportedly, the first nanoparticles based technology, which is heterogeneous catalysis, was developed in the early nineteenth century, followed by the use of silver nanoparticles in photography [1].

One of the fields in which nanotechnology finds extensive applications is nanomedicine, an emerging new field which is an outcome of fusion of nanotechnology and medicine. Medicine is no more physician job exclusively; the materials and devices designed at the level of nanoscale are for diagnosis, treatment, preventing diseases and traumatic injury, relieving pain and also in the overall preservation and improvement of health[2].

Nanotechnology can improve our understanding of living cells and of molecular level interactions. A number of nanoparticles based therapeutics has been approved clinically for infections, vaccines and renal diseases[3].

Silver nanoparticles are widely used for its unique properties in catalysis, chemical sensing, biosensing, photonics, electronic and pharmaceuticals[4] and in biomedicine especially for antibacterial agents [5] and antiviral agents [6]. Silver nanoparticles have a great potential for use in biological including antimicrobial activity [7]. Antimicrobial capability of silver nanoparticles allows them to be suitably employed in numerous household products such as textiles, food storage containers, home appliances and in medical devices [8]. Silver nanoparticles have been widely used for development of biological and pharmaceutical processes, products, and applications such as coating material for medical devices, orthopedic or dental graft materials, topical aids for wound repair, clothing, underwear and socks, textile products, and even washing machines [9].

The antimicrobial property of silver is connected to the amount of silver and rate of silver released. Silver in its metallic state is inert but it reacts with the moisture in the skin and the fluid of the wound and gets ionized. The ionized silver is highly reactive, as its binds to tissue proteins and brings structural changes in the bacterial cell wall and nuclear membrane and nuclear membrane important to cell distortion and death. Silver also binds to bacterial DNA and RNA by denaturing and inhibits bacterial replication [10]. Silver have some antifungal and antiviral activities. Silver metal and silver dressings, when used in reasonable amounts, has no negative effects on the human body towards many pathogens such as bacteria, viruses, fungi, yeast etc [11].

Materials and Method

Preparation of *Wrightia tinctoria* leaf broth

The AR grade silver nitrate ($AgNO_3$) was purchased from Sigma-Aldrich chemicals and fresh *Wrightia tinctoria* plant were collected from surroundings of Govt. Arts College, Thiruvannamalai, Tamil Nadu, India. The *W. tinctoria* dried plant extract used for the reduction of Ag^+ ions to Ag° was prepared by taking 20g of thoroughly washed finely cut leaves in 500 ml Erlenmeyer flask along with 100 ml of distilled water and then boiling the mixture for 5 min. before decanting it. Further, the extract was filtered with Whatman No. 1 filter paper and stored at 4°C and used for further experiments.

Test organisms

The bacterial strains *Staphylococcus aureus*, *Vibrio cholerae*, *Micrococcus luteus* and *Klebsiella pneumoniae* were collected from the Microbial Type Culture Collection (MTCC), The Institute of microbial Technology, Chandigarh, India.

Thirty Six grams of Muller Hindon Media (Hi-Media) was mixed with distilled water and then sterilized in autoclave at 15lb pressure for 15 minutes. The sterilized media were poured into petridishes. The solidified plates were bored with 6mm dia cork porer. The plates with wells were used for the antibacterial studies. The antibacterial assays were done by well diffusion method [12].

Synthesis of silver nanoparticles

In a representative experiment, the leaf extract (0.5 ml) was added to 10 ml of 1 mM $AgNO_3$ aqueous solution. The bioreduced aqueous component (0.5 ml) was used to measuring UV-Vis spectra of the solution. The particle suspension was diluted 10 times with distilled water to avoid the errors due to high optical density of the solution.

UV-Vis spectral analysis

The colour change in reaction mixture (metal ion solution + plant extract) was recorded through visual observation. Synthesized silver nanoparticles was confirmed by sampling the aqueous component of two hour after reaction and the absorption maxima was scanned by UV-Vis spectrophotometer at the wavelength of 325 – 825 nm on Beckman Du-50 Spectrophotometer.

X-ray diffraction studies

The formation and quality of compounds were checked by X-ray diffraction (XRD) spectrum. The mixture was centrifuged at 10000 rpm for 10 minutes in a refrigerated centrifuge, followed by redispersion of the pellet in acetone. The dispersed pellets were dried in an incubator at 37°C for 1 week. The size of the purified Ag nanoparticles was analyzed by X-ray powder diffraction crystallography SEIFERT JSO-

DEBYEREX-2002 (Germany) diffractometer with Cu-Kα radiation (λ=1.540nm). A scan rate of 0.04^o per second and a scan range between 10 -70^o, 2 theta in flat plate geometry with Cu radiation.

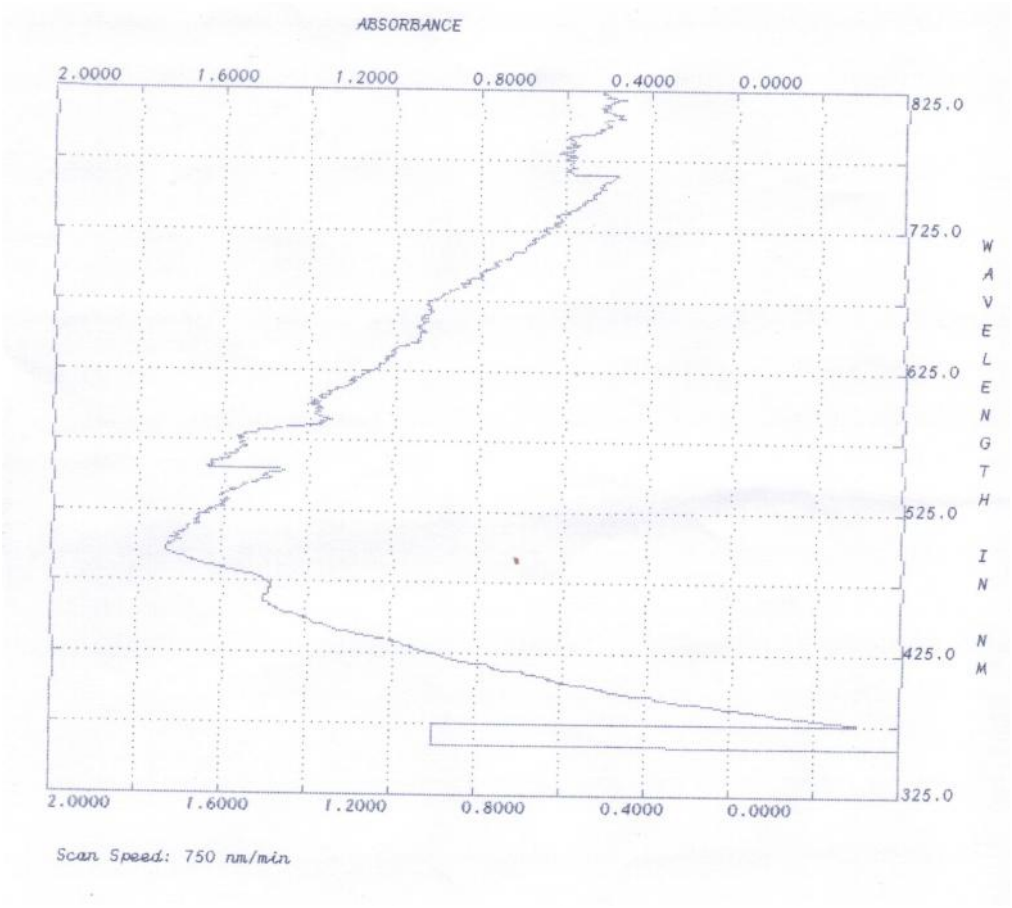
FTIR analysis of silver nanoparticles

To remove any free biomass residue or compound that is not the capping ligand of the nanoparticles, the residual solution of 100 ml after reaction was centrifuged at 5000 rpm for 10 min. The supernatant was again centrifuged at 10000 rpm for 60 min and the pellet was obtained. This is followed by redispersion of the pellet of Ag-NPs into 1 ml of deionized water. Thereafter, the purified suspension was freeze dried to obtain dried powder. Finally, the dried nanoparticles were analyzed by ALPHA FT-IR Spectrometer (from Bruker, Germany).

Results and Discussion

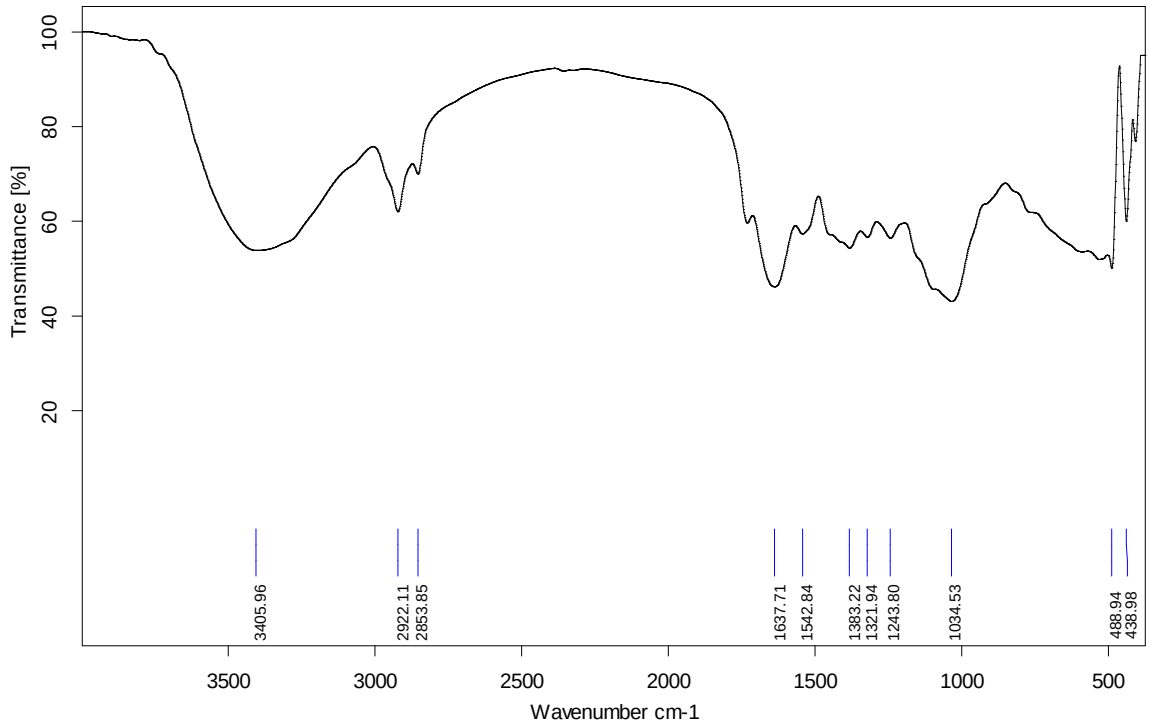
The time of addition of extract into the metal ion solution was measured as the start of the reaction. It is well known that silver nanoparticles exhibit yellowish brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles [13]. The leaf extracts was used for the synthesis of silver nanoparticles. The reaction started within first hour of the incubation with silver nitrate. This was confirmed by the appearance of brown colour in the reaction mixture. Figure 1 show the UV-vis spectra which are recorded after the completion of the reaction. In order to validate the synthesis of silver nanoparticles were subjected to the UV – Vis spectrophotometer analysis after five hour incubation. A peak specific for the synthesis of silver nanoparticles was obtained at 490 nm. The frequency and width of the surface plasmon absorption depend on the size and shape of the metal nanoparticles as well as on the dielectric invariable of the metal itself and the surrounding medium [14]. Supposing the same particle shape, medium dielectric invariable and temperature, the mean diameter of the nanoparticles strongly influence the SPR band in aqueous solution [15].

Figure 1: UV-Vis Absorption Spectrum of nanoparticle synthesized from *Wrightia tinctoria* extract



The FT-IR measurements were carried out to identify the possible biomolecules responsible for the reduction of the Ag^+ ions and capping of the bioreduced silver nanoparticles synthesized by *Wrightia tinctoria* leaf extract. Figure 2 represents the FTIR spectrum of the leaf extract and shows peaks situated at about 3405.96 cm^{-1}, 2922.11 cm^{-1}, 2853.85 cm^{-1}, 1637.71 cm^{-1}, 1542.84 cm^{-1}, 1383.22 cm^{-1}, 1321.94 cm^{-1}, 1243.80 cm^{-1}, 1034.53 cm^{-1} in the region of 4000 cm^{-1} to 500 cm^{-1}. The FTIR spectra exposed the presence of different functional groups like Alcohol (O-H stretching, H-bonded), Alkane (C-H stretching), Alkene (C=C stretching), Aromatic (C=C stretching), Amine (C-N stretching), Ether (C-O stretching), respectively. The peak at 1624 cm-1 is associated with stretch vibration of –C=C- [16] and is assigned to the amide 1 bonds of proteins. The absorption at about 1384 cm-1 is notably enhanced indicating residual amount of NO3 in the solution [17]. The peak at 1539 cm-1 may be assigned to symmetric stretching vibrations of –COO- (carboxyl ate ion) groups of amino acid residues with free carboxyl ate groups in the protein [18]. The peak at 3427 cm-1 indicates polyphenolic OH group along with the peak of 882 cm-1 which represents the aromatic ring C-H vibrations, indicate the involvement of free catechin [19]. The peaks at 1000-1200 cm-1 indicate C-O single bond and peaks at 1620-1636 cm-1 represent carbonyl groups (C=O) from polyphenols such as catechin gallate, epicatechin gallate and theaflavin [20].

Figure 2: FTIR spectra of silver nanoparticles synthesized by *Wrightia tinctoria* plant extract



Debye-Scherrer's equation

$D = K\lambda / \beta . \cos\theta$

Where,

$\beta = \pi / 180 * FWHM$

(FWHM= Full Width Half Maximum)

$K = 0.94$

$\lambda = 1.540598\ A^o$

$K\lambda = 0.94 * 1.540598\ A^o$

$= 1.4482$

To study the crystalline nature of the silver nanoparticles of *Wrightia tinctoria*, the XRD analysis was undertaken, Figure 3 revealing only eight peaks at degree (2θ) 10.00, 11.90, 12.88, 15.89, 17.90, 22.05, 27.80

and 32.16 corresponding to eight diffraction component of silver. The broadening of X-ray peaks observed is primarily due to the small particle size. The spectra were recorded in Seifert -Jso-Debyeflex 2002 X-ray diffractometer. The mean size of silver nanoparticles was calculated using the Debye-Scherrer's equation. An average size of the AgNPs synthesized by *Wrightia tinctoria* was 10.0 nm with size ranging from 5.0 nm and 20.5 nm (Table 1).

Figure 3: XRD pattern of silver nanoparticles formed after reaction of *Wrightia tinctoria* extract

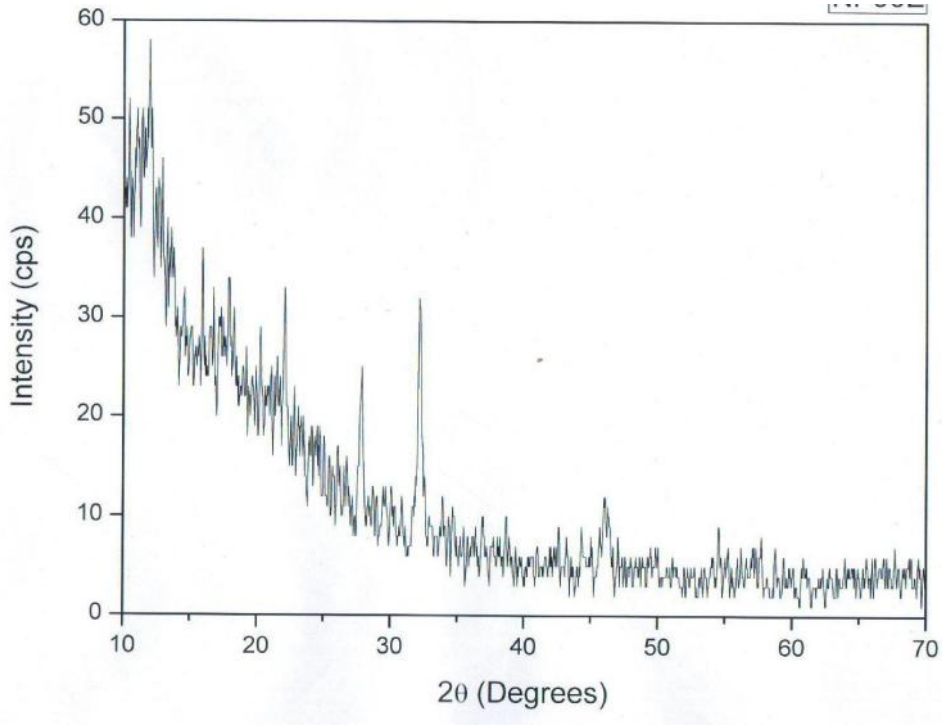


Table 1: Measurement of the size of AgNPs of *Wrightia tinctoria* by using Debye-Scherrer's equation

S.NO	2θ	FWHM	β= π / 180 * FWHM	Cosθ	D = Kλ / ß Cosθ
1	10.0000	0.1000	0.001746	0.9962	8.3 nm
2	11.9040	0.1600	0.002794	0.9946	5.2 nm
3	12.8792	0.0800	0.001397	0.9937	10.3 nm
4	15.8920	0.0400	0.000698	0.9904	20.5 nm
5	17.9032	0.0400	0.000698	0.9878	20.5 nm
6	22.0533	0.1600	0.002794	0.9815	5.1 nm
7	27.8008	0.1600	0.002794	0.9707	5.0 nm
8	32.1585	0.1600	0.002794	0.9609	5.0 nm

Average = 8.3nm+5.2 nm + 10.3 nm + 20.5 nm + 20.5 nm + 5.1 nm + 5.0 nm + 5.0 nm / 8 = **10.0nm**

Table 2: Inhibition zone of *Wrightia tinctoria* against bacterial pathogens

Sl. No.	Name of the organisms	Zone of inhibition (in mm)			
		Control	Tetracycline	Ag Np	Tetracycline + AgNp
1	***Staphylococcus aureus***	-	15.0±1.0	-	8.0±1.4
2	***Vibrio cholerae***	-	25.5±0.8	13.0±2.4	21.0±3.7
3	***Micrococcus luteus***	-	21.0±1.5	8.0±2.8	20.0±1.3
4	***Klebsiella pneumoniae***	-	20.5±1.2	-	20.0±3.7

Ag Np - silver nanoparticles synthesized from *Wrightia tinctoria*

Silver is well known as one of the most widespread antimicrobial substances. The individual and collective effects of the bioreduced silver nanoparticles with antibiotics (Tetracycline) were investigated against four bacterial strains using the well diffusion method. The diameter of the silver nanoparticle inhibition zones against different pathogenic cultures was observed (Table 2). Silver nanoparticles exhibited maximum inhibition zone against *Vibrio cholerae* while moderate activity was found for *Micrococcus luteus*. Silver nanoparticles were found to be comparatively no active in killing *Staphylococcus aureus* and *Klebsiella pneumoniae*. Combined with the silver nanoparticles, the antibacterial activity of the antibiotics showed wide variation. When combined with Tetracycline was found to be superior against *Vibrio cholerae* and followed by *Micrococcus luteus* and *Klebsiella pneumoniae*. The smaller particles lead to the greater antimicrobial effects [21]. The effect of antibacterial activity is higher in the case of silver nanoparticles synthesized at 60◦C compared to 25◦C because of being smaller in size [22, 23].

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