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Green Synthesis, Characterization and Antibacterial Activity of Silver Nanoparticles Using *Chenopodium Album* Leaf Extract

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The present study reports an eco-friendly and rapid green synthesis method of silver nanoparticles (Ag-NPs). The silver nanoparticles were successfully synthesized using *Chenopodium album* as a reducing as well as capping agent simultaneously. The silver nanoparticles were characterized by UV-Visible absorption spectroscopy, Fourier transmission infrared (FT-IR) spectroscopy, X-ray diffraction (XRD) and scanning electron microscopy (SEM). The typical surface plasmon resonance of the Ag NPs was observed at ~ 428 nm. The X-ray diffraction analysis confirmed the silver nanoparticles had the face centered cubic (fcc) structure and the crystalline size was observed in the range of 10-30 nm. The SEM image illustrated that the particles were spherical in shape. In addition, the antibacterial activity of the silver nanoparticles was observed.

Keywords: Green synthesis, silver nanoparticles, *Chenopodium album*, XRD, FT-IR, antibacterial activity.

1 Introduction

Recent developments in nanotechnology has engineered nanomaterials, which are potentially safe in the direction of human welfare. Usage of plants in the synthesis of nanoparticles is quite novel leading to truly green chemistry technology. The application of nanotechnology is endless with a multidisciplinary facet including molecular diagnostics, catalysis, electronics, drug delivery and sensing[1]. Nanotechnology manipulates matter at the atomic, molecular and macromolecular level to create and control objects on the nanometer scale with the goal of fabricating novel materials, devices and systems that have new properties and functions because of their small size. Many of the materials synthesized by plants in nature can definitely be synthesized using them in laboratories even on a large scale. This considered being a very large possibility, so as to have eco-friendly. Nanotechnology is a broad interdisciplinary area of research, development and industrial activity, which has been growing rapidly universal from the past decade. Metallic nanoparticles of specific sizes and morphologies can be readily synthesized using chemical and physical methods[2–6]. The literature is complete with the investigations of the use of plant extracts [7], fungi[8], algae[9] proteins and enzymes[10] as the reductant for carrying out the synthesis of nanoparticles with a multiplicity of shapes and morphologies in high yields, including multi-branched sophisticated silver and/or gold nanomaterials[11]. Biosynthetic processes have received much attention as a viable alternative for the development of metal nanoparticles, where plant extract is used for the synthesis of nanoparticles without any chemical ingredients. Mostly, biosyntheses of Ag NPs using plant extracts are carried out at room temperature, resulting in a low reaction rate and conversion of silver ions. Rapid biosynthesis of metal nanoparticles by plant extract has till now received less reportage.

Many biological approaches of green synthesis have been reported till date using plant leaf extract preparation of Ag-NPs such as *Phoma glomerata*[12], *aloe*[13], *Plectranthus amboinicus* [14], *Dalbergia spinosa*[15], *Ananas comosus*[16], tea[17], *Myrmecodia pendan*[18]. In this work, *Chenopodium album* plant was used to prepare Ag NPs. This plant has important role in the medicinal value, which cures epilepsy, cough, abdominal pain, leukoderma and fever[19]. The species are cultivating with grain and vegetable crop as well as used in animal feed in Asia. The following phytochemicals has been identified in the *Chenopodium album* plant are flavanoids, phenolic amides, cinnamic acid amides, saponin, aopcarotinoid, chinoalbicin, phenols, xyloside

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and lignans. Abdel Aziz *et al.* reported that the antimicrobial effect of Ag NPs against S. aureus (G positive bacteria). Moreover the prepared Ag NPs using the plant extract *Chenopodium album* showed greater microbial activity against the tested micro organism[20]. Awwad *et al.* synthesized Ag NPs using Carob leaf extract and studied its antibacterial activity against E. coli pathogen[21]. Dwivedi *et al*. reported the synthesis of silver and gold NPs using *Chenopodium album* leaf extract. They characterized the silver and gold NPs through FTIR, XRD, UV-Visible spectroscopy and TEM studies. They could not study antibacterial activity of the silver and gold NPs[22]. With the incidence and raise of microorganisms resistant to multiple antibiotics and the continuing prominence on health care expenses, researchers have try to develop new efficient antimicrobial agents for free of confrontation and cost effect. Such problems and needs have led to the renaissance in the use of metal linked antiseptics, which may hold a broad spectrum of activities and lower tendency to persuade microbial resistance than antibiotics[23]. In the present study, an attempt has been made to synthesis of Ag-NPs using leaf extract of *Chenopodium album via* a simple and rapid green method. In addition, the anti-bacterial activity of the Ag NPs is studied and reported.

2 Materials and Methods

2.1 Plants and chemicals

AR grade silver nitrate ($AgNO_3$) was purchased from sigma Aldrich chemicals and fresh *Chenopodium album* leaves were collected from the Alakkudi village, Nagappattinam District, Tamil Nadu, India. The plants were identified in the Department of Botany, Annamalai University.

2.2 Preparation of the leaf extract

Fresh leaf of *Chenopodium album* was cleaned with running tap water followed by distilled water. 10 g of leaves in 100 ml of distilled water was boiled at 80 °C for 10 min and filtered with Whatman No: 1 filter paper. The filtrate was collected and kept at 4 °C and was further used for synthesis of silver nanoparticles.

2.3. Synthesis of silver nanoparticle

Different concentrations (0.5-2.5 ml) of aqueous extract of *Chenopodium album* was added to 0.01mM aqueous $AgNO_3$ solution at room temperature. The mixture was stirred and kept in the dark at room temperature. The solution was continuously observed by means of colour change by naked eye as well as UV-Visible spectrophotometer. The obtained nanoparticles solution was purified by repeated centrifugation at 10,000 rpm for 20 min followed by redispersion of the pellet in deionized water.

2.4 Organisms

The assessment of antibacterial activity was carried out using two different stains. The used microorganisms are namely, Bacillus subtilis and Escherichia coli. These Microorganisms are collected from National Chemical Laboratory, Pune, India. The microbial cultures were maintained at the Department of Pharmacy, Annamalai University, Annamalai Nagar, Tamil Nadu and India.

2.5 Characterization techniques

The UV-Visible absorption spectrums were recorded using a Shimedzu-UV 1800 spectro–photometer. The absorbance spectrums of colloidal samples were recorded from 200 to 800 nm. The X-ray diffraction (XRD) analysis was carried out by XPERT-PRO X-ray diffractometer using monochromatic CuKα radiation (λ=1.5406A°) running 40 kV and 30 mA at 2θ angle configuration. The scanning was done in the angle of 20° - 80°. The diffractograms were compared with the Joint Committee on Powder Diffraction Standards (JCPDS) library to account for the crystalline structure. The Fourier transform infrared (FT-IR) spectra of *Chenopodium album* leaf powder and the silver nanoparticles were obtained in the range 4000 to 400 cm^{-1} with an RX1-Perkin Elmer FT-IR spectrophotometer by KBr pellet method. The Scanning electron microscopy (SEM) analysis of synthesized silver nanoparticles was done using a JEOL-JSM-5610LV model.

2.6 Antibacterial activity

The antibacterial activity of the synthesised Ag-NPs was studied by the standard disc diffusion method. The overnight grown bacterial suspensions of *Escherichia coli* (ATCC 8739), *Bacillus subtilis* (ATCC 6633) were swabbed on separate nutrient agar (NA) plates using L-rod. The prepared silver nanoparticles (Ag-NPs) were tested to evaluate the minimum Inhibitory concentration (MIC) required to inhibit the growth of the seven tested pathogens selected in this study. For these different dilutions of biosynthesized Ag-NPs varying from 5, 10 and 15 mg/ml were prepared with two fold symmetry. Two petri-plates were taken and 20 ml of Agar media was poured into these petri-plates and inoculated with

each bacterial pathogen. When the agar gets solidified, four discs (5 mm diameter) were made in each petri plate with a cork borer and each disc was filled with different dilutions of Ag NPs (5 mg/ml, 10 mg/ml and 15 mg/ml) solutions. The disc loaded with 2 mg/ml of antibiotic ofluxin which was used as positive control. The plates containing the bacteria and Ag-NPs were incubated at 37 °C and then examined for confirmation appears as a clear area around the disc. The diameter of such zones of inhibition was deliberate using a metre ruler, and the mean value for each organism was recorded and expressed in millimeters.

4 Results and discussion

4.1 UV-Vis spectra analysis

The UV-Visible spectroscopy is a valuable tool for optical and structural classification of Ag-NPs. The UV-Visible spectrums (Fig. 1) were recorded at different concentrations of *Chenopodium album* leaf extract. The $AgNO_3$ (0.01mM) solution added leaf extract concentration is fixed from 0.5 ml to 2.5 ml. Due to the reaction of $AgNO_3$ and leaf extract, the colour of the solution is changed from yellow to yellowish brown indicates the formation of Ag nanoparticles. The formation of silver nanoparticles was observed to change colour from yellow to yellowish brown[24]. The maximum absorption wavelength and band gap energy are presented in the Fig. 1.

In the present study, it could be observed that the intensity absorption peak is directly related to the concentration of the leaf extract. The absorption spectra show a gradual decrease of the absorbance, accompanied by a blue shift in the wavelength from 458 -428 nm. The higher concentration peak is shifted to the lower wavelength side might be due to the Ag-NPs exhibits a blue shift by 30 nm. Free electron in the Ag NPs evoked by absorbing visible light and transmitted to a higher energy level, but the electrons are unstable in an excited state and returns to the base energy level and simultaneously a photon is emitted[25, 26]. Moreover, the resonance frequency of surface plasmon in the metallic nanoparticles depends on shape, size and environment that NPs are in it[27, 28]. As the concentration of the *Chenopodium album* extract increases, more number of biomolecules are available to reduce silver ions and forms a large number of very small nanoparticles, which gives rise to sharp and intense surface plasmon resonsnce (SPR)[29, 30]. As the size of the particle decreases, the absorption peak usually shifts toward the blue wavelength, higher frequency and energies [31]. So, the higher concentration of the sample is used for further analysis.

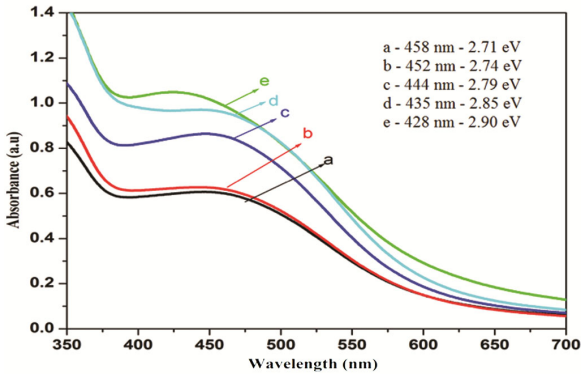

Fig. 1 — UV-Visible absorbance spectrum of Ag NPs in different concentration of *Chenopodium album leaf* extract (a) 0.5 ml (b) 1 ml (c) 1.5 ml (d) 2 ml *(e)* 2.5 ml with $AgNO_3$ solution.

4.2. FT-IR analysis of Ag-NPs

Figure 2(a-b) show the FT-IR spectra of aqueous *Chenopodium album* leaf extract and synthesized silver nanoparticles respectively. The aqueous *Chenopodium album* leaf extract has shown the peaks at 684, 1006, 1629, 2924 and 3412 cm^{-1} corresponding to the C-H bending vibration of alkynes, C-O stretching vibration of carboxylic acid,

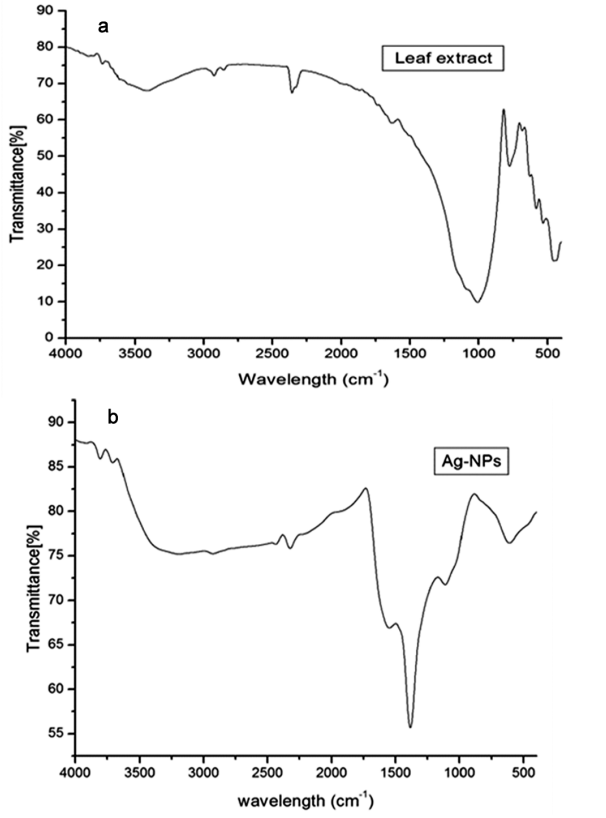

Fig. 2 — FT-IR spectra of (a) *Chenopodium album leaf* extract and (b) synthesized AgNPs from *Chenopodium album* leaf extract

N-H bending vibration of amines, C-H stretching vibration of alkanes and O-H stretching vibration of carboxylic acids respectively. After the reaction with $AgNO_3$, the peaks are shifted to 611, 1111, 1382, 1546, 2926 and 3190 cm^{-1}. The absorbance band at 611 cm^{-1} is due to the C-H bending alkynes group. The medium band 1111 cm^{-1} is raised of C-N stretching in aliphatic amines. The strong absorbance band 1382 cm^{-1} corresponds to C-N stretching vibrations of aromatic amines[32]. The weaker band at 1546 cm^{-1} was identified as the N-H bending vibration of amide the medium band at 2926 cm^{-1} is the C-H stretching vibration of alkanes. The weak band at 3190 cm^{-1} is O-H stretching carboxylic acids. From the present results, it can be concluded that some of the bioorganic compounds from *Chenopodium album* leaf extract formed as a strong reducing and capping agent on the nanoparticles.

4.3 XRD analysis

From the XRD pattern (Fig. 3), the structure and particle size of Ag nanoparticles were determined. The diffraction angles at 2θ are 38.19, 44.37, 64.56 and 77.47 degrees were assigned to face centered cubic (fcc) structure. The XRD pattern of Ag-NPs shows four intense peaks at (111), (200), (220) and (311) planes (JCPDS file no: 893722). Average crystallite size of Ag nanoparticles is 28 nm determined by Sherrer's equation. One is able to calculate the values of average crystallite size (D) and micro strain (ε) from XRD spectrum using the following Equations,

$$D = K\lambda/\beta \cos\theta \quad \ldots (1)$$

$$\varepsilon = \beta/4 \tan\theta \quad \ldots (2)$$

where, K denotes the Scherrer's constant (K = 0.94), λ is the X-ray wavelength, β the full-width at half-maximum of diffraction line in radian and θ is half diffraction angle. Some of the other peaks are also presented. This observation conforms the crystallization of bio-organic stage occurs on the surface of the Ag nanoparticles. The average crystalline size, lattice constant and cell volume micro strain are tabulated in the Table 1[1]. The calculated lattice constant was in good agreement with the reported value and exhibit a smaller cell volumes that of bulk. Earlier reports confirm our results in the characterization of Ag nanoparticles[33-35].

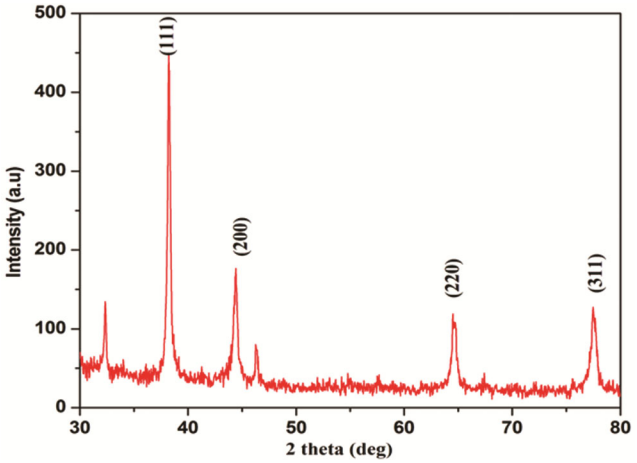

Fig. 3 — X-ray diffraction pattern of Ag-NPs synthesized using *Chenopodium album* leaf extract

4.4 Scanning electron microscope study

The synthesized silver nanoparticles were further characterized by SEM analysis. The SEM determines the surface morphology and size of the particles. It was noted that the particles were predominantly cluster of spherical bead like structure. The particles other than the spherical shaped were also presented. The SEM image in Fig. 4 reveals the formation of cluster of spherical bead like structure of silver nanoparticles with non-uniform distribution[36].

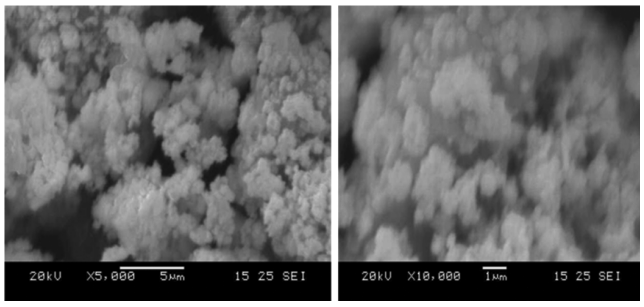

Fig. 4 — SEM image of bio synthesized silver nanoparticles.

Table 1 — The crystalline size, lattice parameter, cell volume and micro strain value of bio-synthesized nanoparticles

2θ	Orientation	FWHM	Crystalline size (nm)	Lattice parameter (nm)	Cell volume (nm)	Micro strain
38.197	(111)	0.246	33.72	4.0799	67.9123	0.00314
44.370	(200)	0.295	27.75	4.0810	67.9672	0.00330
64.560	(220)	0.246	24.80	4.0799	67.9123	0.00261
77.470	(311)	0.246	29.18	4.0836	68.0972	0.00189

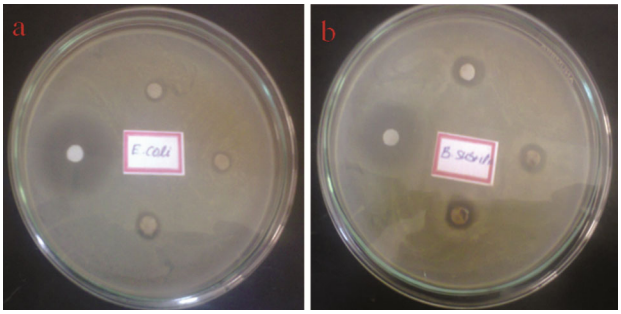

Fig. 5 — Antibacterial activity of Ag-NPs synthesised from *Chenopodium album* leaf extract

4.5 Antibacterial activity

Biosynthesized silver nanoparticles were analyzed for their antibacterial activity against *E. coli* and B.subtilis by disc diffusion method. Fig. 5 shows the antibacterial activity of silver nanoparticles. The vulnerability of *E.coli* and *B.subtilis* showed that the silver nanoparticles from *Chenopodium album* leaf extract controlled antibacterial properties kept gram negative and gram positive pathogen.

The silver nanoparticles (15 mg/ml) reveals that the test zone of inhibition of about 9.5 and 9.0 mm present for *E.coli* and *B.subtilis* as shown in Fig. 5. Similar results are also observed by Shakeel et al (2016)[37]. The *E. coli* depicted the highest sensitivity to nanoparticles compared to *B.subtilis*[14]. The changes in the membrane structure of bacteria as a result of the interaction with silver cations lead to the increased membrane permeability of the bacteria[38]. Ag-NPs with larger surface area provide a better contact with microorganisms[39]. Thus, these Ag-NPs are ability to penetrate the cell membrane or attach to the bacterial surface based on their size, smaller particles having the larger surface area available for interaction will give more bactericidal effect than the larger particles[40, 41]. Cui *et al.* (2016) suggested that the mechanism of antimicrobial activity is due to by free radical generation, where the Ag-NPs was interacted with bacteria, consequently release the silver ions inside of the cell and the contents of the cells leaked out, ultimately, it leads to the protein denaturation[42]. The resulting in antibacterial activity increased covering permeability and consequently death of the bacteria.

In the present work, bio-synthesized Ag NPs was analyzed for their ant-bacterial activity against *E.coli* and *B.subtilis*. But, the previous studies showed the ant-bacterial activity of Ag NPs against S. aureus and E.coli. In my work, I have observed the better antibacterial activity for higher concentration (15 mg/ml) of E.coli. Also, in this work, the particle size is confirmed by XRD and SEM image.

5 Conclusions

The silver nanoparticles has been synthesised by green synthesis method using the *Chenopodium album* plant extract. The synthesized Ag-NPs using *Chenopodium album* provides natural, cost effective and has antibacterial application. The silver nanoparticles has the size of 28 nm were synthesized using *Chenopodium album* extract by varying the concentration of the leaf extract. The nanoparticles were characterized by UV–Visible, FT-IR, SEM and XRD measurements. The X-ray diffraction study confirms that the silver nanoparticle has the fcc structure. The good antibacterial activity of green synthesized silver nanoparticles was evaluated against *E.coli* and *B. subtilis* pathogens.

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