Int J Pharm Bio Sci 2017 Apr ; 8(2): (P) 156-162

Original Research Article Nanotechnology

International Journal of Pharma and Bio Sciences

ISSN
0975-6299

SYNTHESIS OF SILVER NANOPARTICLES VIA GREEN APPROACH USING FRUIT EXTRACT OF*CUPRESSUS SEMPERVIRENS*L. AND THEIR ANTIMICROBIAL EVALUATION

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ABSTRACT

In this study, silver nanoparticles have been synthesized using extract of *Cupressus sempervirens* fruit in aqueous solution at room temperature. The fruit extract was able to reduce Ag^+ to Ag^0 and stabilized the nanoparticles. Different physico-chemical techniques including UV-Vis spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM) and transmission electron microscopy (TEM) with EDX were used for the characterization of biosynthesized silver nanoparticles obtained. The surface resonance band appeared at 450 nm is an evidence for formation of AgNPs. The XRD pattern showed the characteristic Bragg peaks of (111), (200), (220) and (311) facets of the face centre cubic (fcc) silver nanoparticles and confirmed that these nanoparticles are crystalline in nature. SEM micrograph showed that the synthesized Ag-NPs have spherical shape. HRTEM analysis showed particles with an average size of 29 nm. The silver nanoparticles possess significant antimicrobial potential against *B.cereus* and *K. pneumoniae.*

KEY WORDS: ***Cupressus sempervirens*, silver nanoparticles, XRD, SEM, TEM, antimicrobial activity**.

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Received on: 30-08-2016
Revised and Accepted on: 16-03-2017
DOI: http://dx.doi.org/10.22376/ijpbs.2017.8.2.p156-162

INTRODUCTION

Nowadays, nanotechnology has grown to be an important research field in all areas including medicinal chemistry[1]. Nanotechnology deals with the nanoparticles having a size of 1-100 nm. The nano particles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology[2,3]. Particles of this size scale (1-100 nm)show different and novel chemical, physical and biological properties and activities in comparison to the bulk materials[4].Metallic nanoparticles are one of the most promising antibacterial nanomaterials[5]. Silver is efficient antimicrobial agent compared to other salts due to their extremely large surface area, which provides better contact with microorganism[6]. Silver nanoparticles (Ag-NPs) are proved to have high potential antimicrobial, antiplasmodial, and antifungal properties, so recently, they have been used for controlling harmful microorganismssuch as bacteria, molds, yeasts and viruses[7,8].Different routes have been developed to synthesize Ag-NPs while striving to consume less energy, increase efficiency and more importantly to develop ecofriendly procedures[9,10,11,12]. One of such methods is the production of Ag-NPs using biological systems, such as bacteria[9,13], fungi[14], algae[15,16]and plants[17,18].The use of plant extractor plant biomass for the production of nanoparticles could be an alternative to chemical and physical methods in an eco-friendly manner[19].Plants extracts/products having a wide range of metabolites, silver nanoparticles produced by plant extracts are more stable and rate of synthesis is faster in comparison to microorganism.Metabolites and other constituents as reducing agents present in the plants acts as stabilizing and capping agents, so there is no need of adding capping and stabilizing agents from out side[20].*Cupressussempervirens*, the Mediterranean cypress (also known as Italian cypress)belongs to *Cupressaceae* family. It was native to the Mediterranean basin. However, it is distributed in North America and subtropical Asia at high altitude. *C. sempervirens* L. were reported to have antiseptic, aromatherapeutic, astringent, balsamic, and anti-inflammatory activities[21]. Its dried fruits are used for the inflammation treatment, toothache, and laryngitis and also as a contraceptive as well asastringent. *Cupressus sempervirens* L. revealed that it contains active constituents such as flavonoids (rutin, quercetin, and myricitrin), phenolic compounds (anthocyanidin, flavones, flavonols and tannins), and essential oils. The present study reports green synthesis of Ag-NPs using fruit extract of *C. sempervirens* and to evaluate antimicrobial properties of synthesized Ag-NPs. To the best of our knowledge, this study is the first time report of Ag-NPs synthesis using aqueous fruit extract of *C. sempervirens.*

MATERIALS AND METHODS

Materials

All analytical reagents and media components were purchased from Merck Chemicals. The deionized water was used throughout the experiment. All glassware's were properly washed with distilled water and oven dried before use. Fresh fruit of *C. sempervirens* were collected from Almora, Uttarakhand and identified by Botany Division, Central Drug Research Institute, Lucknow.The voucher specimen (No.24421) is stored in the herbarium of the institute.

Preparation of Plant extract

Prior to the experiment, *C.sempervirens* fruit was rinsed thoroughlyby deionized water. The plant material shade dried at room temperature. The dried plant material was powdered and 10 g of powder mixed with 100 mL of deionized water in a 250 mL Erlenmeyer flask and mixture was boiled for 15 min. The solution was cooled at room temperature and filtered by Whatman filter paper No. 1. The filtrate was collected and stored at 4°C for further experiment.

Preparation of silver nanoparticles

Silver nanoparticles were synthesized by reducing the freshly prepared 3 mM silver nitrate ($AgNO_3$) and stored under dark conditions with aqueous extract of the plant. The reaction mixture was prepared in ratio of 9:1 (V/V) comprised of freshly prepared silvernitrate solution and plant extract respectively. After 15 min the color change of the solution from pale yellow to dark brown was observed. The solution was stored at room temperature for the complete settlement of nanoparticles. After 24 hours the reaction mixture was centrifuged at 4000 rpm for 20 min and pellets were collected followed by washing with deionized water and dried in water glass.

UV-VIS spectra analysis

The UV-Visible spectrum of synthesized silver nanoparticles was analysed by spectrophotometer (UV-Visible Perkin Elmer Lambda 25). Absorption wavelength was studied range between 200 and 800 nm. The silver nanoparticles show the surface Plasmon resonance at 400 to 500 nm in the UV-Visible spectrum.

XRD analysis of silver Nanoparticles

The particle size and nature of the Ag-NPs were determined with X-ray diffraction using Smartlab (Rigaku), operating at40 kV, 40 mA with Cu K α radiation at 2θ angle ranging from 20˚ to 80˚. The crystallite size of the silver nanoparticles was estimated using Debye Sherrer's equation. $D = 0.94\ \lambda / \beta \cos \theta$ where D is the average crystallite size, λ is the wavelength of X-ray source (0.15406 nm), β is the full width at half maximum (FWHM), and θ is the diffraction angle.

SEM analysis of silver nanoparticles

To observe the morphology of the synthesized Ag-NPs, images were obtained by a FESEM (Sigma,Carl Zeiss) instrument. Thin films of the sample were prepared on a gold coated copper grid by just dropping a very small amount of the sample on the grid. The details regarding applied voltage, magnification used and size of the contents of the images were implanted on the images itself.

TEM analysis of silver nanoparticles

The particle size shape and surface morphology was confirmed using Transmission electron microscopy

(TEM). High Resolution Transmission Electron Microscopy (HRTEM) was performed by Tecnai G2 F30 S-Twin(FEI) machine, operated at an accelerated voltage of 300 kV. These images were taken by drop coating Ag-NPs on a carbon-coated copper grid. Additionally, addition presence of metals in the sample was analysed by Energy Dispersive Spectroscopy (EDS). EDS photograph of Ag-NPs were carried out by the HRTEM instrument equipped with EDX detector as mentioned above.

Antibacterial Property of Ag-NPs

The antibacterial activity of the silver nanoparticles was determined against the human pathogenic bacteria *viz. Bacillus cereus* and *Klebsiella pneumoniae* by disk diffusion method sing a Mueller-Hinton agar culture medium (Hi-media). The standard cultures were inoculated 10^8 CFU/ml in petri dishes with MH agar medium and then paper disks of 5mm diameter were laid on the inoculated standard culture, which was instilled with nanoparticles neat solution in DMSO. Petri dishes were incubated at 37^0C for 24 hrs. and antimicrobial activity was determined by measuring the zone of inhibition around the disk. The test was done in triplicate.

RESULTS AND DISCUSSION

UV-Vis spectra Analysis

The silver nanoparticles were characterized by UV-Vis spectroscopy, one of the most widely used techniques for structural characterization of silver nanoparticles. Silver nanoparticles (AgNPs) appear pale yellowish to dark brown in colour in aqueous medium as a result of surface Plasmon vibrations[22]. The green synthesis of silver nanoparticles using *C. sempervirens* extract was successfully carried out. The UV-vis spectra recorded after time intervals of 15 min, 30 min, 60 min and 24 h from the initiation of reaction are shown in Figure 1. Absorption spectra of Ag-NPs formed in the reaction media has absorption maxima in the range of 441 to 450 nm due to surface Plasmon resonance of Ag-NPs. In the spectrum, broadening of peak indicated that the particles are polydispersed.

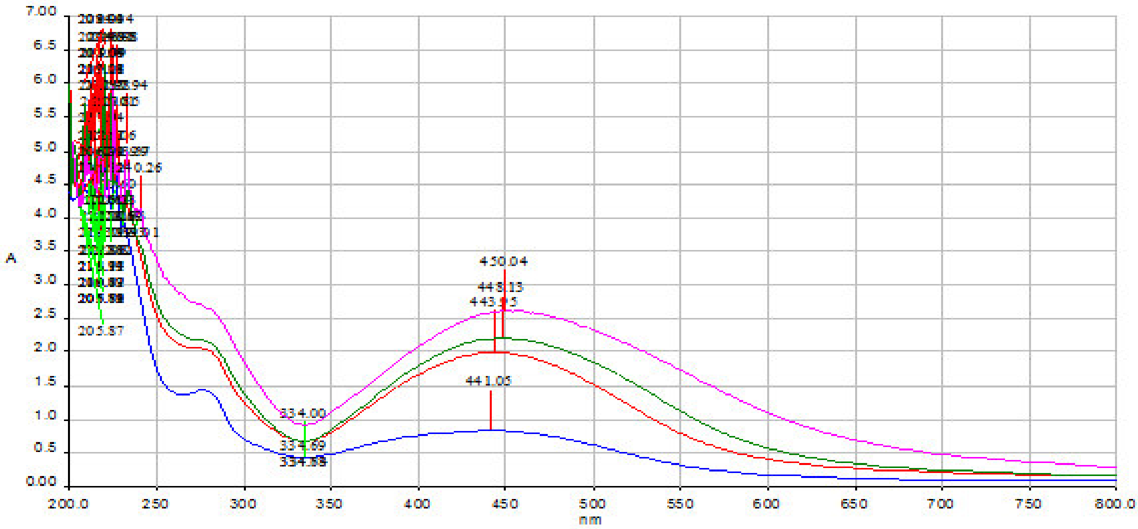


Figure 1
UV-Vis absorption spectra of Ag-NPs

XRD analysis

The XRD pattern of synthesized silver nanoparticles using *C. sempervirens* extract were recorded and XRD pattern is shown in Figure 2. The XRD pattern confirms the crystalline structure of the Ag-NPs. In the spectrum, peaks observed at 38.17^0, 44.26^0, 64.54^0 and 77.41^0 are corresponded to (111), (200), (220) and (311) planes of pure face centered crystalline (fcc) structure of silver. Crystallite size of Ag-NPs as estimated from the FWHM of different peaks using the Scherrer's formula and diffraction lines observed at 2θ angle are given below (Table.1).

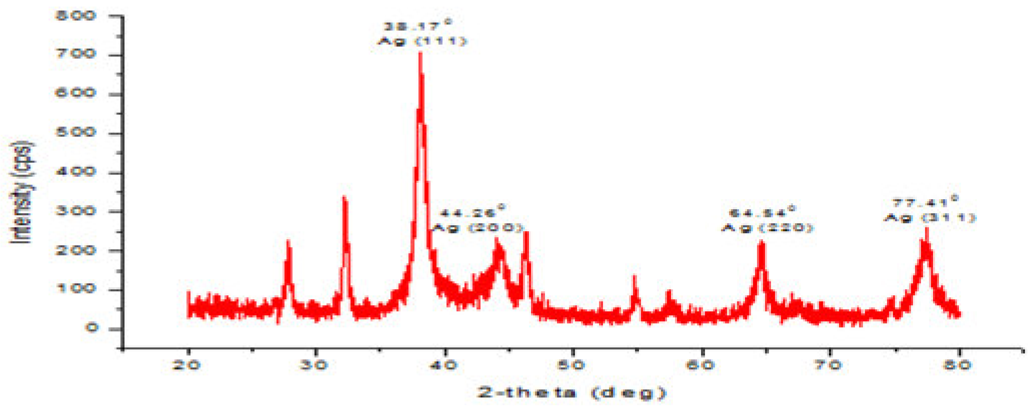


Figure 2
XRD pattern of silver nanoparticles of C. sempervirens

Table 1
Crystalline size of Ag-NPs synthesized using C. sempervirens

No.	2-Theta (deg)	D (ang)	FWHM (deg)	Plane	Average size Nm
1.	38.17^0	2.3540	0.79	100	11.1
2.	44.26^0	2.045	1.71	200	5.2
3.	64.54^0	1.4395	0.74	220	13.2
4.	77.41^0	1.2291	1.30	311	8.2

SEM analysis
The SEM micrograph showing the high density Ag-NPs synthesized by the *C.sempervirens*further confirmed the development of silver nanoparticles. The SEM micrographs of nanoparticles showed that Ag-NPs are spherical shaped, well distributed without aggregation in solution. (Figure 3)

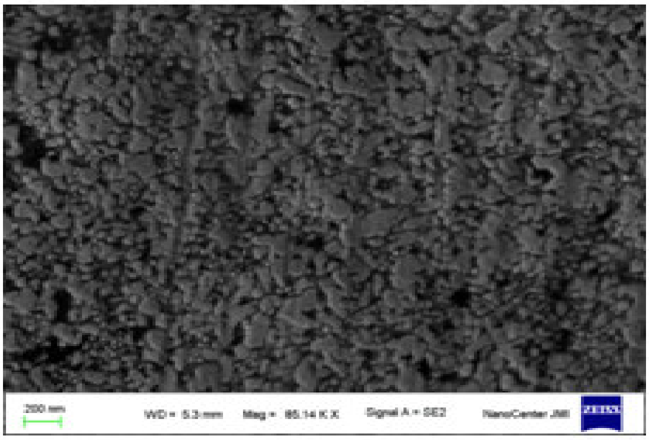


Figure 3
SEM micrograph of AgNPs

TEM analysis
The bioreduced Ag-NPs were elucidated with the help of TEM to determine the shape and average size of Ag-NPs. HRTEM images of Ag-NPs derived from the extract of *C. sempervirens* are shown in Figure 4. TEM images of Ag-NPs at various magnifications confirmed spherical shapes of silver nanoparticles with most clearly apparent average size of 29 nm and approximately the synthesized Ag-NPs are homogeneous in nature. Further analysis of the silver particles by energy dispersive spectroscopy confirmed the presence of the signal characteristic of elemental silver. The EDS profile shows a strong silver signal along with weak oxygen, chlorine and aluminium peaks, which may have originated from the biomolecules bound to the surface of the silver nanoparticles Figure 5.Figure 6shows selected area electron diffraction pattern (SAED) of the silver nanoparticles. The silver particles are crystalline, as can be seen from the selected area diffraction pattern recorded from one of the nanoparticles in the aggregate. SAED spots that corresponded to the different crystallographic planes of face-centered cubic (fcc) structure of elemental silver.

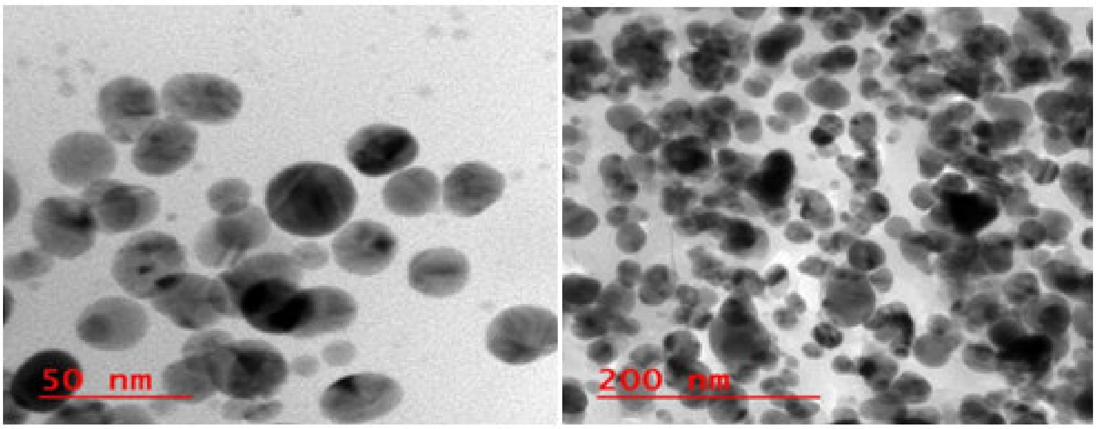


Figure 4
HRTEM images ofAg-NPs of C. sempervirens

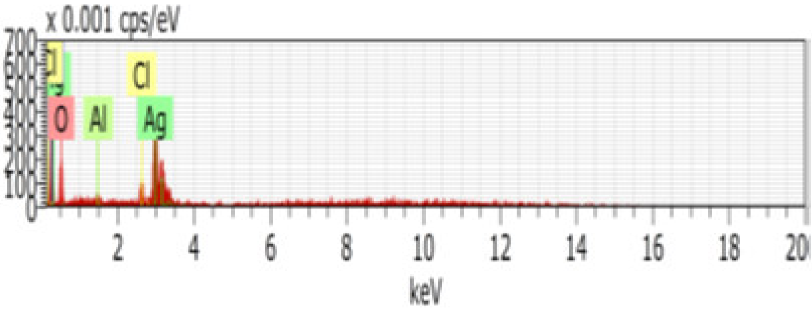


Figure 5
EDX Spectra of Ag-NPs

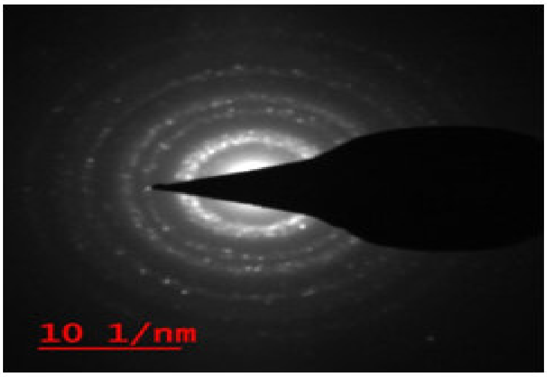
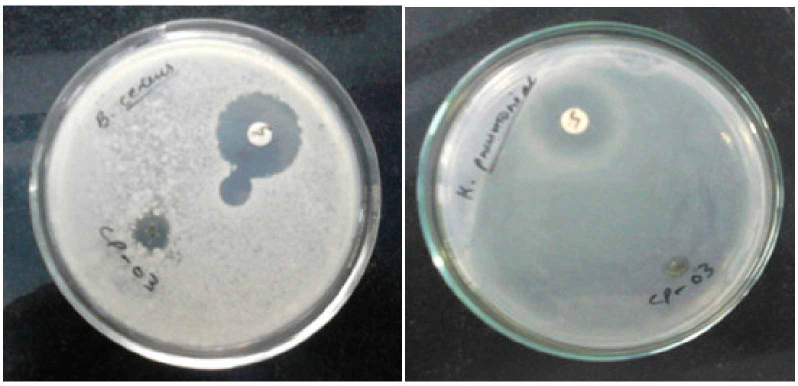


Figure 6
Selected area electron diffraction showing the characteristic crystal planes of elemental silver

Antibacterial activity of Ag-NPs

The antimicrobial activity of biosynthesized silver nanoparticles was examined carried out against both gram positive *B. cereus* and gram negative *K. pneumoniae* bacteria (Figure 7). Biosynthesized silver nanoparticle showed clear zone of inhibition as indicated in Table-2.Streptomycin 25µg/ml was used as positive Control. Synthesized silver nanoparticles showed significant antibacterial activity against both bacteria.



(A) **(B)**

Figure 7
Antibacterial studies of synthesized silver nanoparticles of C. sempervirens

Table 2
Showing zoneof inhibition against bacteria pathogens

S.NO	Organism	Zone of inhibition (mm)	Positive control (Streptomycin-25µg/ml)
1	*B. cereus*	12.3	22.6
2	*K. pneumoniae*	11.6	18

CONCLUSION

The biogenic silver nanoparticles synthesis from the fruit extract of *C. sempervirens* was studied in this communication. This is a simple, green, ecofriendly and efficient method to synthesize silver nanoparticles at room temperature without using any harmful reducing agents. Silver nanoparticles have been appropriately characterized using UV-vis spectroscopy, XRD, SEM, TEM and EDS analysis. These analysis showed that nanoparticles are crystalline, monodispersed and spherical with average size of 29 nm. The synthesize silver nanoparticles exhibit good significant antimicrobial activity against both bacteria. Therefore, the silver nanoparticles synthesis by this green method will be priving of potential use in medical applications.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the financial support provided for the research work by the HNB (A Central University) Srinagar Garhwal Uttarakhand Dept. of chemistry, and also JMI New Delhi for technical support.

CONFLICT OF INTEREST

Conflict of interest declared none.

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We sincerely thank the above reviewers for peer reviewing the manuscript