Research Article

Facile Green Synthesis of Silver Nanoparticles by *Artemisia pallens* Leaves Extract and Evaluation of Antimicrobial Activity

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Abstract

The present work deals with the use of aqueous extract of *Artemisia pallanes* (Linn.) leaves for synthesis of silver nanoparticles from aqueous solution of silver nitrate at room temperature. Synthesized silver nanoparticles showed the surface plasmon resonance band at 422 nm whereas FT-IR analysis confirmed that, the biomolecules are responsible for the reduction of silver ions to silver nanoparticles. The crystal symmetry and unit cell dimensions were confirmed by XRD pattern. X-ray diffraction analysis indicated that the crystalline silver nanoparticles were cubic in nature. Scanning electron microscopy technique was used to examine the morphology of the sample and showed the formation of spherical structure with an average grain size about 28 nm where as Energy Dispersive X-ray analysis gave strong signal of silver at 3Kev. The biosynthesized silver nanoparticles showed effective antimicrobial activity against human pathogens.

Keywords: antimicrobial activity; *Artemisia pallens*; biosynthetic method; crystalline silver nanoparticles; phytochemical analysis.

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Introduction

The development of dependable, environmentally benign processes for the synthesis of nanoscale materials is an important aspect of nanotechnology. As compared to their bulk materials, nanostructures have attracted great attention due to their striking and incredible properties. Synthesis of metal nanoparticles with different size range and their self-assembly is considered important due to their potential applications in optical devices [1], catalytic [2], electronic [3], sensor technology [4], biological labeling [5], treatment of some cancers [6], etc. Among heavy metal nanoparticles, silver nanoparticles (AgNPs) have received major attention due to unique and tunable surface plasmon resonance [7]. Synthesis of silver nanoparticles with different size range and their self-assembly is considered important due to their potential applications such as antiparasitic [8], pediculocidal [9], larvicidal [10], acaricidal [11], antifungal [12], bactericidal [13], etc.

Various techniques including chemical and physical means have been developed to synthesize metal nanoparticles, such as chemical reduction [14], heat evaporation [15], electrochemical reduction [16], photochemical reduction [17], sonochemical [18], microwave assisted process [19] and recently via green chemistry route [20]. Unfortunately many of the reported nanoparticle synthesis involved the use of hazardous chemicals, low material conversions, high energy requirements, difficult and wasteful purifications. Hence there is a growing need to develop environmentally friendly process for nanoparticles synthesis without using toxic chemicals and methods. Biosynthetic methods employing either microorganisms or plant extracts have emerged a simple and viable alternative to existing synthetic procedures.

In this paper, we report biosynthesis of AgNPs by using aqueous extract of *Artemisia pallens* leaves at room temperature. Further it was extensively characterized by different analytical techniques and their antimicrobial activity was evaluated against various pathogenic bacteria.

Materials and Methods:

Materials

All the chemicals used were of analytical grade and obtained from Sigma-Aldrich. Fresh, green and mature leaves of *Artemisia pallens* crop were collected from "Western ghat region" of Maharashtra (India) and the taxonomic identification was made in Department of Botany, Shivaji University, Kolhapur (MS, India). The voucher specimen was numbered and kept in our research laboratory for further reference. All solutions were freshly prepared using double distilled water and kept in dark at room temperature for reduction of silver ions.

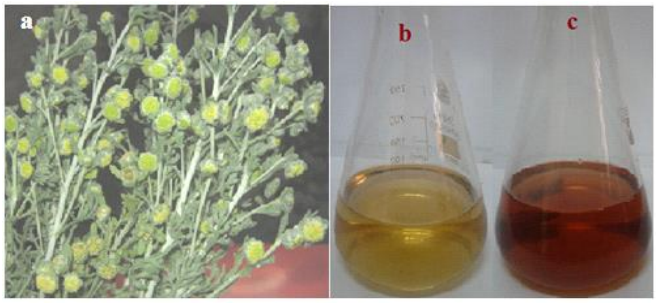

Figure 1 a) *Artemisia pallens* b) *Artemisia pallens* leaves extract c) AgNPs aqueous suspension

Preparation of Aqueous Extract of *Artemisia pallens* Leaves

Fresh, green clean leaves of *Artemisia pallens* were air dried and kept in the hot air oven at 60 °C for 24 h. The dried leaves were ground to a fine powder and 2 g of powder was taken in 500 mL Erlenmeyer flask containing 50 mL double distilled water. The resulting mixture was then boiled for 30 minutes and filtered through Whatman filter paper no. 41. This concentrated aqueous extract (30-35 mL) was kept in refrigerator for further use.

General procedure for AgNPs synthesis

For the synthesis of AgNPs, 5 mL aqueous extract of *Artemisia pallens* leaves was taken and diluted to 25 mL by double distilled water. This diluted extract then added drop wise in 25 mL of silver nitrate solution (1 mM) at room temperature with constant stirring for 3 hours. The resulting brown solution was centrifuged at 15,000 rpm for 20 minutes and then washed with distilled water (3x10 mL). The solid obtained was dried under vacuum and used for further study.

Characterization methods and instruments

Synthesized AgNPs were characterized by UV-visible spectroscopy, Fourier Transform - Infrared (FT-IR), X-ray diffraction (XRD), Scanning electron microscopy (SEM) and energy dispersive X-ray spectrometry (EDS). The UV-visible spectra were recorded over 200-800 nm range with UV-3600 PC UV-VIS NIR Spectrophotometer (Shimadzu). IR were recorded on Thermo Scientific Neconet 6700 FTIR, in the range 4000–400 cm^{-1}, while XRD patterns were recorded on Bruker AXS model D-8, (10 to 70°range, scan rate = 1° min^{-1}) equipped with a monochromator and Ni-filtered Cu Kα radiation. SEM was performed using a HITACHI S-4800 instrument to study the morphology of silver nanoparticles. The EDS was carried out on a DX-700HS spectrometer.

Results and discussion

The formation of AgNPs was visually observed by gradual change in color from colorless to dark brown after adding aqueous leaves extract of *Artemisia pallens* in aqueous $AgNO_3$ solution. The color change arises because of the excitation of surface Plasmon vibrations in the AgNPs [21]. The strong surface Plasmon resonance band appeared at 422 nm in UV-Visible spectrophotometer (**figure 2**) and broadening of peak indicated that the particles are polydispersed.

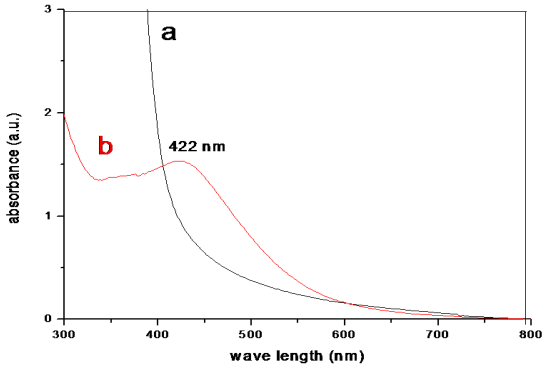

Figure 2 UV-visible absorption spectra of a) aqueous extract of *Artemisia pallens* leaves b) AgNPs suspension

FT-IR (figure 3) measurements were carried out to identify the possible biomolecules responsible for the reduction and stabilization of AgNPs synthesized by *Artemisia pallens* leaves extract. The broad peak at 1384 cm^{-1} shows the presence of nitro group in $AgNO_3$. The peak at 1569 cm^{-1} was assigned to C=C stretching vibration in aliphatic compounds, which may be characterized by the presence of high content of terpenoids and flavonoids. The peak 1379 cm^{-1} was due to deformation of CH_2 and CH_3 groups in aliphatic compounds [22]. The peaks at 1214 cm^{-1} arises most probably from the C-O groups of polyols such as hydroxyflavones and catechins [23]. The peak near 669 cm^{-1} is assigned for CH out of plane bending vibrations of substituted ethylene system C=C (cis) [24]. The total disappearance of the bands at 1569, 1214 and 669 cm^{-1} after bioreduction may be due to the polyols are mainly responsible for the reduction of Ag^+ ions.

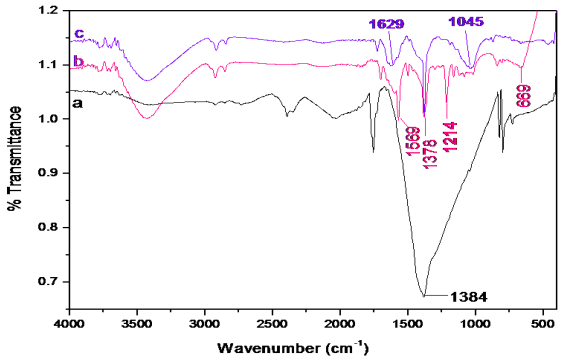

Figure 3 FT-IR spectrum of (a) $AgNO_3$ (b) *Artemisia pallens* leaves extract (c) AgNPs aqueous suspension

Figure 4 shows XRD pattern of biosynthesized AgNPs. All the high-intensity reflections in this sample at 38.31°, 44.27° and 64.49° are corresponding to 111, 200 and 220 planes. These are in good agreement with earlier reported values [JCPDS file no. 04-783]. By the comparison of obtained XRD spectrum with the standard, it was confirmed that the AgNPs formed are in the form of cubic nanocrystals. The full width at half maximum (FWHM) values measured for 220 plane of reflection was used with the Debye–Scherrer's equation $d = 0.9\ \lambda/\beta\cos\theta$. The average size of the nanoparticles was calculated as 28nm.

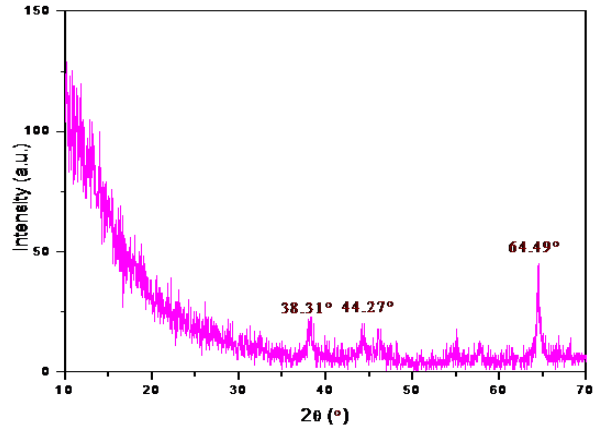

Figure 4 X-ray diffraction patterns of AgNPs synthesized by aqueous extract of *Artemisia pallens* leaves

Biosynthesized AgNPs by *Artemisia pallens* were scanned using SEM (**figure 5**) and showed less aggregated particles with spherical shape. It was shown that relatively spherical and uniform AgNPs were formed with diameter of 25 to 35 nm in range. AgNPs were assembled on the surface due to the interactions such as hydrogen bonding, Vandar-waals force of attraction and electrostatic interactions between the bio-organic capping molecules bound to the AgNPs.

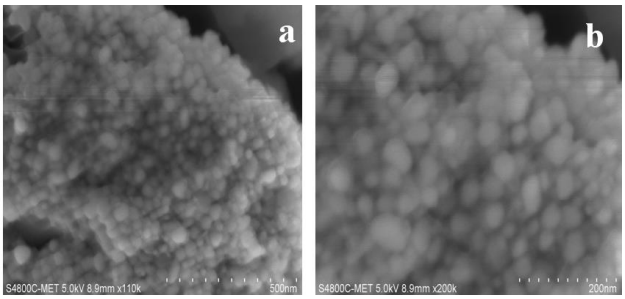

Figure 5 SEM images of AgNPs synthesized by aqueous extract of *Artemisia pallens* leaves

The presence of elemental silver was confirmed by EDS (**figure 6**) analysis. The silver nanocrystallites display an optical absorption peak at 3 KeV, which is typical of the absorption of metallic silver nanocrystallites [25].

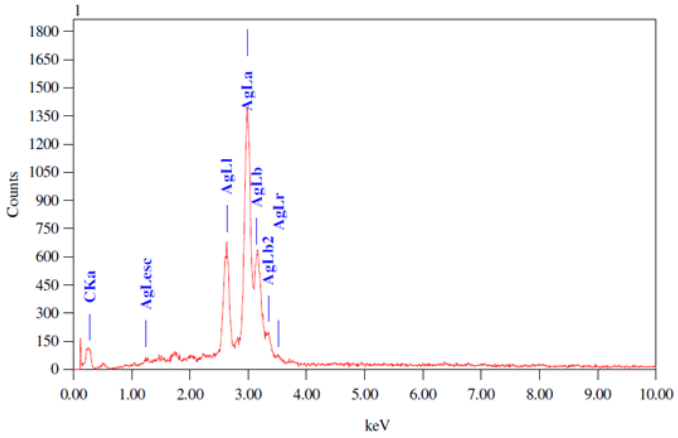

Figure 6 EDS spectrum of AgNPs synthesized by aqueous extract of *Artemisia pallens* leaves

Antimicrobial Studies

Biosynthesized AgNPs was screened for antimicrobial activity against pathogenic bacteria by using antibiotic sensitive assay of paper disc method with a well size of 5mm diameter and 0.05 mg/mL of samples (**Figure 7**). The synthesized AgNPs were highly effective in their antimicrobial activity against *Staphylococcus aureus, Bacillus cereus* and *Escherichia coli* than antibiotics. Chloromphenical of 0.5 mg/mL concentration was used as a control antimicrobial agent. The synthesized AgNPs showed inhibition zone against all the studied bacteria and maximum zone of inhibition was found to be 18 mm in *Bacillus cereus* and minimum of 12 mm in *Pseudomonas aeruginosa* as shown in **Table 1**.

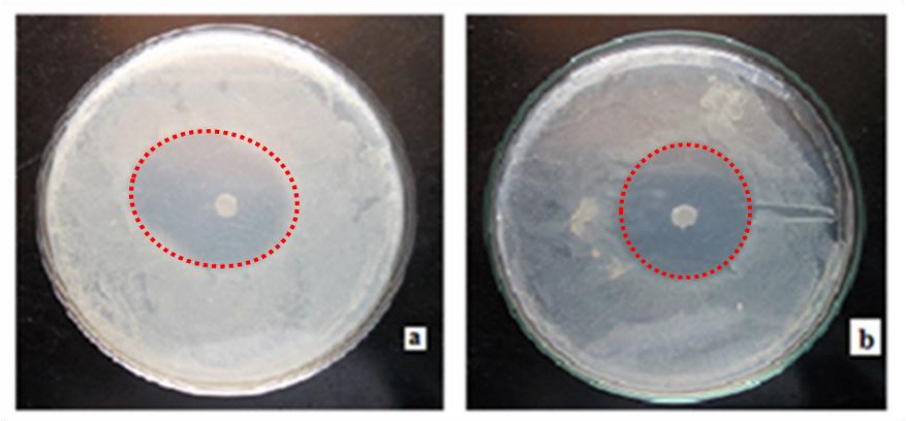

Figure 7 Antimicrobial activities of a) Chloromphenical b) AgNPs against *Bacillus cereus*

Table 1 The antimicrobial activity of biosynthesized AgNPs

Name of the bacterial species	Zone of Inhibition (mm)	
	Ag nanoparticle (0.05 mg/mL)	Reference drug (0.5 mg/mL)
Staphylococcus aureus	15	18
Escherichia coli	16	20
Klebsiella pneumonia	14	22
Bacillus cereus	18	19
Pseudomonas aeruginosa	12	22

Conclusion

In conclusion we have demonstrated the use of a natural, renewable, non-toxic and low-cost aqueous extract of *Artemisia pallens* leaves as a bioreducing agent for effective synthesis of AgNPs at room temperature. The UV–visible, FT-IR, XRD, SEM and EDS analysis strongly suggested the formation of elemental AgNPs instead of silver oxide. The average particle size is found to be 28 nm with spherical shape. The biosynthesized AgNPs showed effective antimicrobial activity against selected human pathogens than routine antibiotic drug.

Acknowledgements

We gratefully acknowledge the financial support from the University Grants Commission (UGC) and Department of Science and Technology (DST) for FIST and SAP respectively, New Delhi, India.

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Publication History
Received 28th June 2014
Revised 10th July 2014
Accepted 15th July 2014
Online 14th Aug 2014