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RESEARCH ARTICLE

Novel Approach to Synthesis Silver Nanoparticles using Leaf Extract of *Trichosanthes cucumerina* L. and its Antibacterial Activity

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ABSTRACT

Green synthesis of nanoparticles is evolving into an important branch of nanotechnology, because biological methods are considered safe and ecologically sound for the nanomaterial fabrication as an alternative to conventional physical and chemical methods. In this work, we have investigated a simple and eco-friendly biosynthesis of silver nanoparticles using *Trichosanthes cucumerina* L. leaf extract as reducing agent. The optical properties of silver nanoparticles have been measured using UV-VIS Spectroscopy. The absorption peak due to the Surface Plasmon Resonance of silver nanoparticles at wavelength of about 420nm was observed. Functional groups of the silver nanoparticles were confirmed through Fourier Transform Infrared Spectroscopy (FTIR). Scanning Electron Microscopy (SEM) analysis showed the morphology of the silver nanoparticles and the Energy Dispersive Spectroscopy (EDS) spectrum of the solution containing silver nanoparticles confirmed the presence of elemental silver signals. Structural characterization of synthesized silver nanoparticles carried out by X-ray diffractometer (XRD) shows that, the synthesized silver nanoparticles are face centered cubic crystalline. Further, these biologically synthesized nanoparticles were found to be highly toxic against different multi drug resistant human pathogens. Plant extract is very cost effective and ecofriendly and thus can be economic and effective alternative for the large scale synthesis of silver nanoparticles.

KEYWORDS

Trichosanthes Cucumerina L, Silver Nanoparticles, Antibacterial Activity

INTRODUCTION

Nanoparticles are of great scientific interest as they are effectively a bridge between bulk materials and atomic or molecular structure; its research is currently an area of intense scientific interest due to a wide variety of potential applications in biomedical, optical and electronic fields. Especially silver nanoparticles have been extensively used in catalysis, biomedicine, antibacterial activity, imaging and nanophotonics, as well as Surface-Enhanced Raman Scattering (SERS) detection[11]. Based on its antimicrobial activity, silver has become one of the most prominent nanomaterial, silver nanoparticles (AgNPs) being present in many consumer applications, such as disinfectants, deodorants and room spray, bedding, washing machines, humidifiers, shampoo, kitchen tools, toys and fabrics. Use of biological organisms such as microorganisms, plant extract of plant biomass could be an alternative to chemical and physical methods for the production of nanoparticles in an eco-friendly manner[3, 7, 9]. Among the various

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bioreactants, *Trichosanthes cucumerina* L. plant (Fig.1 (a)) was chosen for the present study. Since, the plant is a rich source of nutrition. It is constituted with proteins, fat, fibre, carbohydrates, vitamin A and E. The total phenolics and flavonoids contents are 46.8% and 78.0% respectively[1]. The triterpenoids found are 23, 24-dihydrocucurbitacin D, 23, 24-dihydrocucurbitacin B and cucurbitacin B. Recently, cucurbitacins are also known to possess a number of potent pharmacological effects, deriving largely from their cytotoxic, anti-cancer and anti-inflammatory properties. In this work, we explore the potential use of shade dried *Trichosanthes cucumerina* L. leaves in the synthesis of silver nanoparticles and its antibacterial activity.

MATERIALS AND METHOD

Preparation of Leaf Extract

Trichosanthes cucumerina L. leaves were shade dried and ground into fine powder. For the synthesis of silver nanoparticles, 100 µl of 1mM silver nitrate ($AgNO_3$- Sigma Aldrich) aqueous solution was added to the leaf extract (200 mg leaf powder+ 200 ml) and gently heated until the colour of the solution changed from pale yellow to yellowish brown colour indicating the formation of silver nanoparticles. The bio-reduced silver nanoparticles solution was collected and monitored by periodic sampling of aliquots (5 ml) of aqueous component and measuring UV-Visible spectra of the solution. The remaining silver nanoparticles solution was centrifuged thrice at 10,000 rpm for 20 minutes.

The products were collected and characterized by X-ray diffraction (XRD) with a RIGAKKU X-ray diffractometer (using CuKα = 1.5408 Å radiation). The morphology, size, and structure of the products were analyzed by Field Emission Scanning Electron Microscopy (FE-SEM). The FT- IR spectra of silver nanoparticles were recorded by using Perklin Elmer model-Spectrum Rx1 FT-IR Spectrophotometer instrument. UV-Visible Spectrum analysis was carried out on Lambda 35 UV-Visible Absorption Spectroscopy.

Bacterial cultures such as *Escherichia coli* (MTCC 724), *Klebsiella pneumonia* (MTCC 432), *Proteus vulgaris* (MTCC 425), *Pseudomonas aeruginosa* (MTCC 741) and *Salmonella typhi* (MTCC 531) were obtained from Microbial Type Culture Collection (MTCC), Indian Institute of Microbial Technology, Chandigarh, India. Disc diffusion method was adopted to evaluate the antibacterial activity of silver nanoparticles synthesized from leaf extract of *Trichosanthes cucumerina* L. Luria Bertani (LB) broth/ agar medium was used to cultivate bacteria. Standard antibiotic disc Rifampicin was used as control and it was obtained from Himedia.

RESULTS AND DISCUSSION

UV-Visible Absorption Spectroscopy

In this study, the formation of silver nanoparticles by leaf extract of *Trichosanthes cucumerina* L. was investigated. The appearance of yellowish brown colour in the reaction flasks indicates the formation of silver nanoparticles and no colour change was observed in control[2]. The progress of the reaction between metal ions and the leaf extracts were monitored by UV-Visible Spectra of silver nanoparticles in aqueous solution with different reaction times. It was observed that the peak centered at 420 nm with increasing reaction time from 15 min to 60 min samples and with no absorption peak in control i.e. without reductant (Fig.1). This clearly indicates the interaction between silver ions and biomolecules present in the aqueous leaf extract. The reduction of silver ions and the formation of stable nanoparticles occurred quickly within an hour of reaction, making it one of the fastest bio-reduction methods to produce silver nanoparticles.

Fourier Transform Infrared Spectroscopy

The silver nanoparticles solution was centrifuged at 10,000 rpm for 20 min. The pellet was washed three times with 20 ml of de-ionized water to get rid of the free proteins/enzymes that are not capping the silver nanoparticles[10].

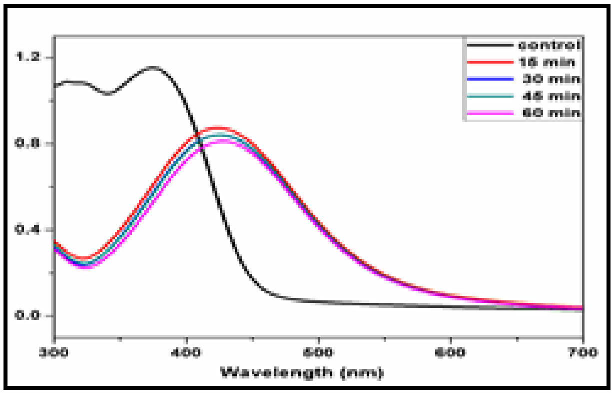

Figure 1: UV–Visible absorption spectrum

Fig.2 IR spectra of silver nanoparticles indicate the bands at 3430 cm^{-1} are attributed to the stretching vibration absorption of –OH of adsorbed water. The bands at 1625.51cm^{-1} is due to stretching vibrations of the –C=O. 1103.51cm^{-1} are the C-N asymmetric stretching vibrations. The bands at 621.06 cm^{-1} are attributed to the bending vibrations of the –C=O. This suggests that, the biological molecules could possibly perform dual functions of reduction and stabilization of silver nanoparticles in the aqueous medium.

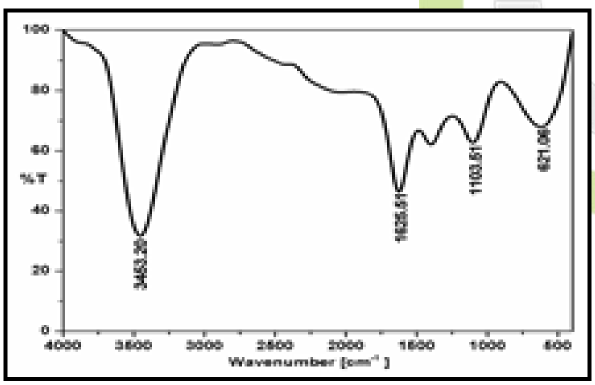

Figure 2: Fourier Transform Infrared Spectrum

FE-SEM and EDS

The SEM micrograph of the silver nanoparticles synthesized using *Trichosanthes cucumerina* L. leaf extract is shown in Fig 3. A thin film of the sample was prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid. Then the film on the SEM grid was allowed to dry and the images of nanoparticles were taken. It was found that, the silver nanoparticles were spherical and uniformly distributed over the entire surface of the sample. Energy Dispersive X-ray (EDX) Spectroscopy analysis was performed to check the chemical composition of the film. The spectrum showed the presence of high silver signals. The silver nanocrystallites display an optical absorption band peaking at 3KeV which is typical of the absorption of metallic silver nanocrystallites[5] (Fig.3)

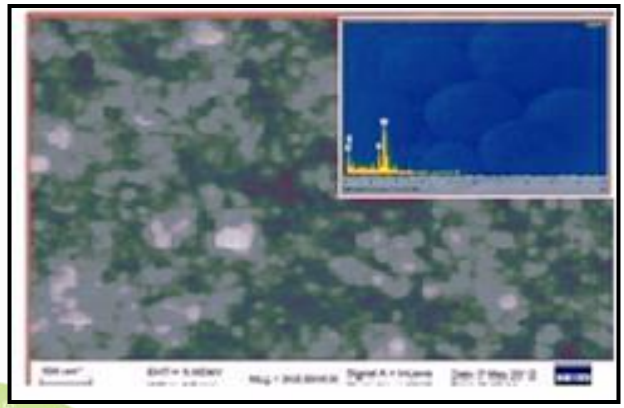

Figure 3: Field Emission Scanning Electron Microscopy & EDS

X-RAY Diffraction Studies

The crystalline nature of the silver nanoparticles was confirmed from the X-ray diffraction analysis. The XRD patterns of *Trichosanthes cucumerina* L. leaf extract are shown in Fig.4. The XRD pattern with the diffraction peaks at 38.20, 44.50, 52.20 and 64.10 corresponding to the 111, 200, 211 and 222 facets of the face centered cubic crystal structure.

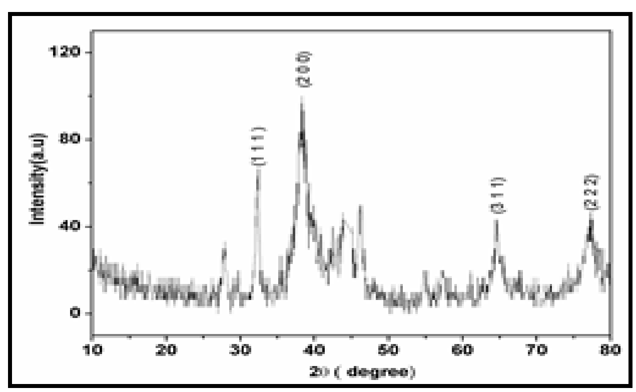

Figure 4: XRD spectrum

The average crystallite size of the silver nanoparticles is calculated following the Debye-Scherrer formula

$$D = 0.94\lambda/\beta \cos\theta \quad \ldots\ldots\ldots\ldots\ldots (1)$$

Where D is the average crystallite size, λ is the wavelength of the X-ray beam (1.5406 Å), θ is the scattering angle in degrees, β and is the full width at half maximum in radians. The estimated average crystallite size is found to be 62 nm.

Antibacterial Activity

Antibacterial activity was carried out using five different strains-*Escherichia coli, Klebsiella pneumonia, Proteus vulgaris, Pseudomonas aeruginosa* and *Salmonella typhi* by standard disc diffusion method. Luria Bertani (LB) broth/agar medium was used to cultivate bacteria. Zone of inhibition was measured and compared with standard antibiotic disc Rifampicin (control).

The synthesized silver nanoparticles showed inhibition zone against all the studied bacterial species (Fig.5). Maximum zone of inhibition was found to be 17 mm in *E.coli* and minimum 10 mm in *Proteus vulgaris* (Table-1). *Klebsiella pneumonia* showed better zone of inhibition than the standard antibiotic disc-Rifampicin. These nanoparticles can directly interact with bacterial cells causing oxidative stress that result in deadly damage[6].

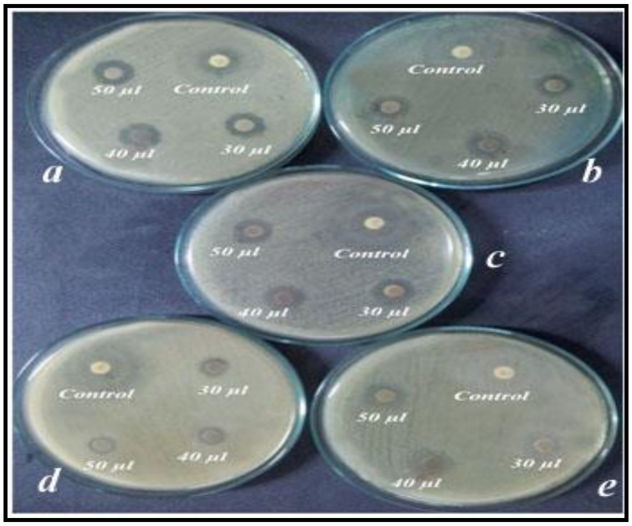

Figure 5: Antimicrobial activity of silver nanoparticles synthesized from leaf extract of *Trichosanthes cucumerina* L. *against various* pathogenic bacterial strains *Klebsiella pneumonia* b) *Salmonella typhi* c) *Pseudomonas aeruginosa* d) *Proteus vulgaris* e) *Escherichia coli*

The anti-bacterial activity of silver nanoparticles depends upon the bioavailability of silver ions[4] and due to the presence of reducing agents[8]. It is well understood that silver nanoparticles possess elevated surface area that hinder microbial growth at least concentration which revealed silver nanoparticles as novel antibacterial agent.

Table 1: Antibacterial activity of silver nanoparticles synthesized from leaf extract of *Trichosanthes cucumerina* L. against some human pathogenic organisms

S.No	Name of the Bacterial Strains	Antibiotic disc (Rifampicin)	Silver nanoparticles synthesized using Leaf extract of *Trichosanthes cucumerina* L. Zone of Inhibition (mm)		
			30µl	40µl	50µl
1	*Escherichia coli*	25	13	14	17
2	*Klebsiella pneumonia*	14	14	14	15
3	*Proteus vulgaris*	20	10	11	15
4	*Pseudomonas eruginosa*	15	14	14	15
5	*Salmonella typhi*	27	12	13	14

CONCLUSION

The biological synthesis of silver nanoparticles using aqueous leaf extract of *Trichosanthes cucumerina* L. provides an ecofriendly, simple, fast and much cost efficient route for the synthesis of silver nanoparticles. We characterized these nanoparticles using UV-Visible, SEM, XRD and FTIR spectroscopic techniques. The results proved that silver nanoparticles synthesized from leaf extract of *Trichosanthes cucumerina* L. showed maximum activity at a least concentration which exposed silver nanoparticles as an excellent antibacterial agent.

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