

Research Article



Extracellular Bio-Inspired Synthesis of Silver Nanoparticles Using Raspberry Leaf Extract against Human Pathogens

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ABSTRACT

Development of a biologically inspired experimental process for synthesis of nanoparticles is evolving into the area of nanotechnology research. Green synthesis of nanoparticles is very simple, non-toxic, cost effective and eco-friendly. The main aim of the investigation is to synthesize and characterize the silver nanoparticles using wound healing potent aqueous extract of raspberry leaf as a reducing agent and to evaluate their antibacterial activity. The formation of silver nanoparticles has been confirmed using UV-vis, XRD, SEM, EDX and FTIR analysis and its antibacterial activity was evaluated against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*. Silver nanoparticles showed a clear well defined inhibition zone compared to control plant extract. The results suggest the stabilized and reduced molecules of silver nanoparticles may act as an effective antibacterial agent for wound healing.

Keywords: Green synthesis, Raspberry leaf extract, Silver nanoparticles, Wound healing, Antibacterial activity.

INTRODUCTION

The field of nanotechnology is one of the most active areas of research in modern material sciences. Nanotechnology is a multi-disciplinary field of research with physics, biology and chemistry and material science.¹ Nanoparticles, generally considered as particles which are controlled or manipulated materials with a size of up to 100 nm, exhibit completely new novel properties and functions because of their specific characteristics such as size, distribution and morphology as compared to the larger particles.² Among the noble metal nanoparticles, silver nanoparticles have been considered as an important area of research due to their unique and tunable Surface Plasmon Resonance (SPR) and their applications in biomedical science including drug delivery, tissue / tumor imaging and extensively used as anti-bacterial agents in the health industry, food storage, textile coatings and a number of environmental applications.³ It has been shown that extracellularly produced silver nanoparticles can be incorporated in several kinds of wound dressing materials such as silver nanoparticles impregnated cloth⁴, hydrogels^{5,6}, films^{7,8} etc. Silver nanoparticles containing wound dressing materials are sterile and can be used to cure wounds and minimize the infection from the pathogenic bacteria.⁹

Chemical synthesis methods lead to presence of toxic chemicals that may have adverse effect in medical applications.¹⁰ Consequently, green synthesis of nanoparticles has received more attention to fabricate low cost, environmentally benign technology in nanoparticles synthesis.¹¹ Several biological systems including bacteria¹², fungi¹³, yeast¹⁴ enzymes¹⁵ and plants have been used for the synthesis of silver nanoparticles. It offers numerous benefits of eco-friendliness and

compatibility for pharmaceutical and other biomedical applications as they do not use any toxic chemicals as reducing agents like sodium borohydride¹⁶, trisodium citrate¹⁷, and sodium hydroxide¹⁸. Thus, it is essential need to develop an extracellular plant extract mediated silver nanoparticles is to minimize the hazardous substances to the human health and environment. Recently green synthesis of novel silver nanoparticles with antibacterial nature and new functional attributes using plant extracts like *Panicum virgatum*¹⁹, *Bryophyllum pinnatum*²⁰, *Ceratonia siliqua*²¹, *Ocimum sanctum*²² and *Mangifera indica*²³ etc.

Medicinal plants represent a rich source of antimicrobial agents. These plants have a potent source for the synthesis of most powerful drugs. Red raspberry (*Rubus idaeus* L.) belongs to the family of *Rosaceae*. It is woody, rounded shrub with perennial roots, pinnate leaves with pale green and biennial canes. The leaves and fruits are rich in citric acid, malic acid, tartaric acid, tannin, minerals and vitamins.²⁴ Raspberry plant has been used traditionally as an anti-septic, anti-abortient, anti-gonorrhoeal, anti-leucorrhoeal and anti-malarial. Externally, the leaves and roots are used for inflammations, wounds, burns and ulcers.^{25,26} In the present investigation, biosynthetic preparation and characterization of silver nanoparticles using aqueous extract of raspberry leaf was carried out and evaluated for their antibacterial activity.

MATERIALS AND METHODS

Raspberry leaves were collected from the herbal garden at Mother Teresa Women's University, Kodaikanal. AgNO₃ were purchased from Sigma Aldrich, Mumbai. *Pseudomonas aeruginosa* (MTCC-1034), *Staphylococcus aureus* (MTCC-7443) and *Escherichia coli* (MTCC-739)



were purchased from Microbial culture collection, Chandigarh.

Preparation of plant extract:

The collected fresh leaves of raspberry were weighed (4g) and washed double distilled water and incised into small pieces. Plant leaf samples were boiled in 40 ml of deionized water for 10 minutes. The boiled leaf extract of raspberry was filtered using Whatman No. 1 filter paper. From this extract, 20 ml of the sample was used for further analysis.

Synthesis of silver nanoparticles using plant extract:

1mM silver nitrate solution was taken in a conical flask 20 ml of plant extract was added by drop wise to this solution. The solution was kept under dark condition until colour changed to brown colour.

Characterization:

The bioreduction of silver ions in solutions was monitored by measuring the UV-Vis spectrum of the reaction medium. The UV-Vis spectral analysis of the sample was done by using Shimadzu UV-2600 spectrophotometer at room temperature operated at a resolution of 200 to 800 nm ranges. The silver nanoparticles solution was centrifuged at 10,000 rpm for 30 mins and the pellet was dried for further analysis. The reduction of pure X-ray diffraction (XRD) pattern of dried silver nanoparticles powder was recorded on a XPERT-PRO X-Ray Diffractometer equipped with Ni-filtered CuK α 1 radiation, Goniometer PW3050/60 (Theta/Theta) and the data was collected in the 2 θ range. The mean particle diameter of silver nanoparticles was calculated from the XRD pattern according to the line width of the plane, reflection peak using Scherrer formula. For FTIR measurement the dried pellet was grinded with potassium bromide crystals analyzed on a Perkinelmer, (Model- Frontier) FTIR spectroscopy. The FTIR spectrum was obtained in the mid IR region of 400 - 4000 cm⁻¹ and recorded using ATR (Attenuated Reflectance Technique). The morphology of the silver nanoparticles was analyzed using scanning electron microscope (SEM). Elemental composition of silver nanoparticles was analysed using energy dispersive X-ray spectroscopy (EDS).

Antibacterial assay

The efficiency of silver nanoparticles as an antibacterial compound was evaluated against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*. The disc diffusion method was used to assess the antibacterial activity of the synthesized silver nanoparticles.²⁷ Muller-Hinton Agar plates were inoculated with different bacterial strains such as, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*. Sterile Whatman filter paper discs (3mm) were containing different concentration of silver nanoparticles (50 μ l, 75 μ l and 100 μ l) and 100 μ l of raspberry leaf extract as a control. Sterile discs were placed on the medium and the plates were incubated at

37°C for 24 hrs in an incubator and observe zone of inhibition.

RESULTS AND DISCUSSION

In this study deals with the synthesis and characterization of silver nanoparticles using raspberry leaf extract. Silver nanoparticles were synthesized by the addition of raspberry leaf extract with 1mM concentration of silver nitrate solution, which resulted in reduction of silver ions into silver particles was taken place with the colour change. The formation of silver nanoparticles was occurred rapidly with the colour change from light green to brown within 20 minutes of incubation due to excitation of surface plasmon vibrations with the silver nanoparticles (Fig. 1A).

UV-Vis Spectrophotometric analysis

UV-Visible spectroscopy is a significant technique to ascertain the formation and stability of metal nanoparticles in aqueous solution. Noble metals are known to exhibit unique optical properties due to the property of Surface Plasmon Resonance (SPR) which is the collective oscillation of the conduction electrons in resonance with the wavelength of irradiated light.²⁸ The UV-Visible absorption of silver nanoparticles are exhibit maximum in the range of 400–500nm due to this property. The size and shape of metal nanoparticles determine the spectral position of plasmon band absorption as well as its width.²⁹ The mixture of raspberry leaf extract and silver nitrate solution was subjected to UV-vis spectra, based on the colour change and the absorbance of the reaction medium was noted 45minutes of time interval (Fig.1A) which is evident from visual observation of yellowish brown colour within 45minutes and it became light brown (1.30hrs) to dark brown within 3hrs of incubation period Figure 1B showed SPR bands of the colloidal silver nanoparticles were centred at 416 nm for different time of incubation period and raise in absorbance which explains the increased production of silver nanoparticles.

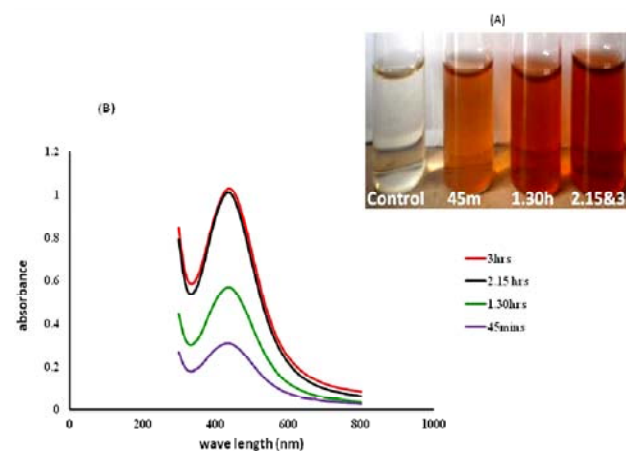


Figure 1: (A) Colour change of synthesized silver nanoparticles shows time interval of incubation (B) UV-vis absorption spectrum of silver nanoparticles shows respective time interval of incubation

X-ray diffraction analysis

The crystalline nature of silver nanoparticles was confirmed from the analysis of X-ray diffraction (XRD) pattern of control leaf sample of raspberry and synthesized silver nanoparticles. Figure 2 shows XRD pattern of control leaf sample without silver nitrate treatment which resulted in no peak corresponding to silver nanoparticles. Figure 3 depicts result of XRD pattern of leaf extract treated with silver nitrate solution. A comparison of XRD spectrum with the standard confirmed that the silver particles were formed in the form of nanocrystals. The four diffraction peaks at 2θ values of 38.17° , 44.60° , 64.46° and 77.40° were showed numbers of Bragg reflections that may be indexed as (1 1 1), (2 0 0), (2 2 0) and (3 1 1) planes of face centred cubic (fcc) structure of silver (JCPDS, file no. 89- 3722). The appearance of this sharp peak could have resulted from crystallization of silver nanoparticles which was due to reducing and stabilizing agent present in the raspberry leaf extract. The mean size of nanoparticles was calculated using Debye–Scherrer's equation ($D = K\lambda / \beta \cos \theta$, where D is the size of the particles, K is the shape dependent Scherrer's constant, λ is the X-Ray wavelength, β is the Full Width at Half Maximum (FWHM) and θ is the diffraction angle) by determining the width of the peaks and it was found to be 10-15 nm. This feature indicates that the nanocrystals are highly anisotropic.³⁰

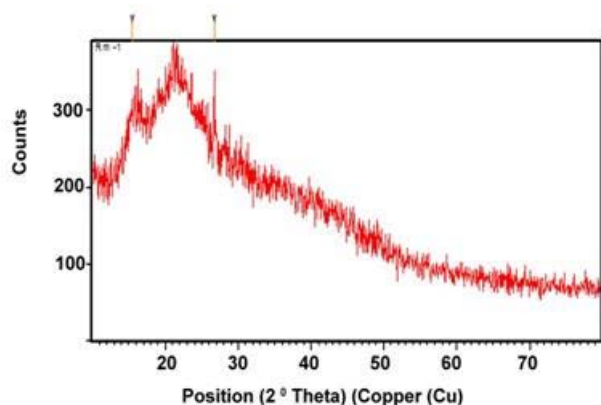


Figure 2: XRD pattern of control dried leaf powder of raspberry

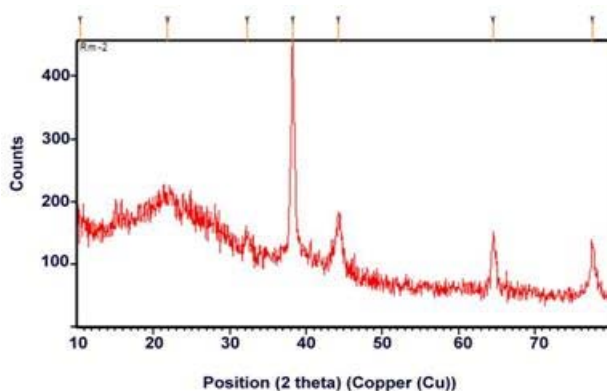


Figure 3: XRD pattern of silver nanoparticles synthesized from raspberry leaf extract with 1mM silver nitrate solution

Scanning Electron Microscopy (SEM)

Morphology of the silver nanoparticles was confirmed using SEM analysis, which indicates that the metal particles presence in nano-size.^{31,32} Figure 4 shows aggregation of spherical and square shaped silver nanoparticles was observed below 100nm with the magnification of 20,000X.

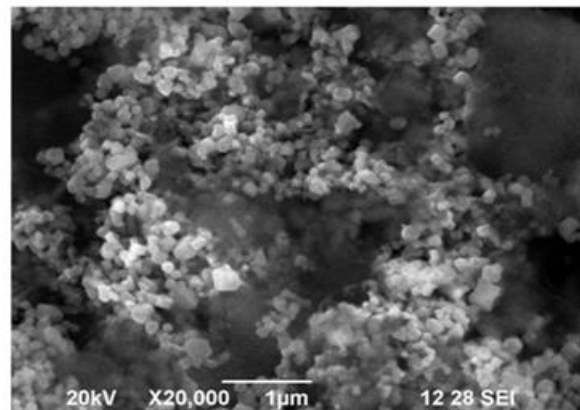


Figure 4: SEM micrograph of silver nanoparticles with the magnification of 20,000X

Energy Dispersive X-ray spectroscopic analysis (EDX)

The energy dispersive X-ray analysis reveals strong signal in the silver region and confirms the formation of silver nanoparticles (Fig. 5). EDX spectroscopy results confirmed the significant presence of pure silver with no other contaminants. Metallic silver nanocrystals are generally show typical optical absorption peak approximately at 3 keV due to SPR.³³

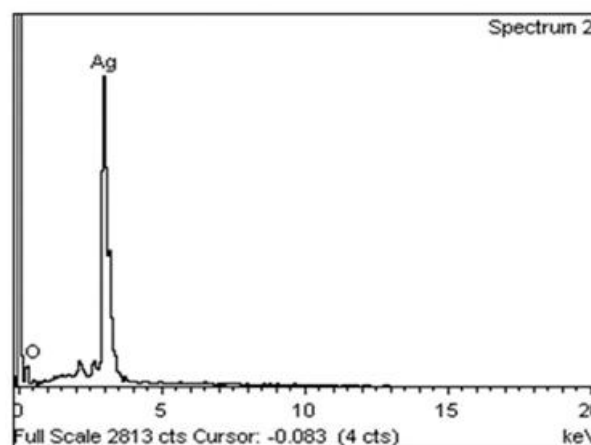


Figure 5: EDX spectrum of silver nanoparticles

Fourier transform infra red spectroscopy (FTIR)

FTIR measurement was carried out to identify the possible biomolecules of plant extract responsible for capping leading to efficient stabilization of the silver nanoparticles. The FTIR spectrum obtained for raspberry leaf extract (Fig.6) displays a number of absorption peaks, reflecting its complex nature due to biomolecules. The IR spectrum of silver nanoparticles manifests prominent absorption bands at 3969 cm^{-1} , 3878 cm^{-1} , 3771 cm^{-1} , 3431 cm^{-1} , 2930 cm^{-1} , 2675 cm^{-1} , 2575 cm^{-1} , 2149

cm⁻¹, 1711 cm⁻¹, 1369 cm⁻¹, 1229 cm⁻¹, 1043 cm⁻¹ and 537 cm⁻¹. The strong band at 3431 cm⁻¹ may result from the N-H stretching vibration band of amino groups and indicative bond of -OH hydroxyl group. The well-known band at 2930 cm⁻¹, 2675cm⁻¹, 2575cm⁻¹, 2149 cm⁻¹, 1711 cm⁻¹ and 1369 cm⁻¹ can be assigned as absorption bands of -C-H, -O-H, -S-H, -N=C=N, -C=O and -S=O stretching vibration respectively. These are derived from water soluble compounds such as alkaloids, polyphenols and flavonoids, present in the plant leaves. Therefore, we conclude that any or mixture of the above mentioned organic constituents could act as reducing and stabilizing agents. This result suggested that the biological molecules possibly perform dual function of formation and stabilization of silver nanoparticles.³⁴

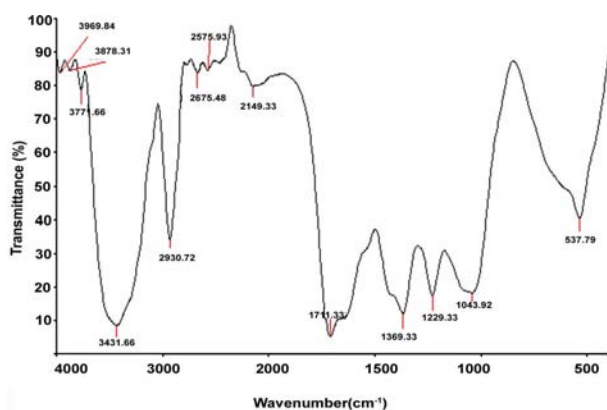


Figure 6: FTIR spectrum of silver nanoparticles

Antibacterial Screening

Since Roman times silver has been used for its antimicrobial properties. The more advances in generating silver nanoparticles have made possible to use silver as a powerful bactericide against various clinical pathogens.³⁵ In the present study, zone of inhibition of biologically synthesized silver nanoparticles was evaluated at the concentrations of 50µl, 75µl and 100µl against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* where, raspberry leaf extract (100 µl) was used as a control. Figure 7 depict synthesized silver nanoparticles showed a clear well defined zone of inhibition (1.3cm) against all the bacterial species at 75µl and 100µl concentrations whereas, 50µl concentration of synthesized silver nanoparticles was showed 0.7cm of zone of inhibition when compared to control plant extract (0.5cm). The mechanism of inhibitory action of silver nanoparticles on microorganisms is based on interaction between silver ions and thiol groups of vital enzymes of bacteria thus inactivating them.³⁶ At the initial stage of the interaction, silver nanoparticles were found to adhere to the wall of the bacteria due to its charge of the functional group and disturb the permeability and respiration functions of the cell which leads to loss of cell viability and eventually resulting in cell death.³⁷⁻³⁹ Thus, in the present investigation, the application of silver nanoparticles was proved as an antibacterial agent by green route method.

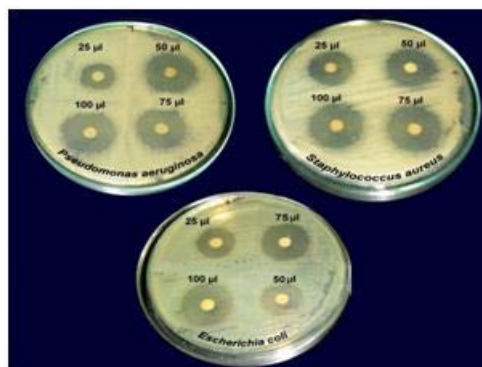


Figure 7: Zone of inhibition of silver nanoparticles against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*

CONCLUSION

For the first time, bio-reduction of silver ions by the leaf extract of the raspberry plant has been demonstrated. The reduction of the metal ions through leaf extract leading to the formation of silver nanoparticles of fairly well-defined dimensions. The formation of silver nanoparticles by bio-inspired route method as an eco-friendly and low cost method than chemical methods. Since the synthesised silver nanoparticles using raspberry leaf extract shows antibacterial activity against tested bacterial pathogens, it could be targeted for the promising potential to prepare silver nanoparticles impregnated wound dressing material for healing wounds effectively in future.

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