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Biogenic synthesis of silver nanoparticles from *Dodonaea viscosa* Linn. and its effective antibacterial activity

P. Balashanmugam * and P. T. Kalaichelvan

Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai, Tamil Nadu, India 600025

Abstract

A green rapid biogenic synthesis of silver nanoparticles (Ag NPs) using *Dodonaea viscosa* (*D. viscosa*) aqueous extract was demonstrated in this present investigation. Cubic shape was observed from biosynthesized SNPs in the range of 30 to 50 nm. The formation of Ag NPs was confirmed by Surface plasmon resonance (SPR) at 435 nm using UV–VIS spectrophotometer. The reduction of silver ions to Ag NPs by *D. viscosa* extract was completed within 24 hrs which was evidenced potentiometrically. Synthesised AgNPs was characterised using UV–vis spectroscopy, Fourier transformed infrared spectroscopy (FT-IR), Scanning Electron Microscopy (SEM), Energy dispersive X-ray Analysis (EDX), Powder X-ray diffraction (XRD) and also antibacterial activity using clinically important pathogens. Plant derived proteins or polyphenols are used as reductants and stabilizers as well. In addition, it showed significant antibacterial activity towards two Gram-positive bacteria (*Bacillus subtilis, Enterococcus faecalis*) and two Gram-negative bacteria (*Proteus vulgaris*, *Klebsiella pneumonia*). The highest zone of inhibition was observed in *Klebsiella pneumonia* (19 mm in diameter) and followed by *Enterococcus faecalis* (18 mm in diameter).

Key words: biosynthesis, *Dodonaea viscosa,* AgNPs**,** antibacterial activity

INTRODUCTION

The field of nanotechnology is one of the most active areas of research in modern materials science. Nanotechnology refers broadly to a field of applied science and technology whose unifying theme is the control of matter on the atomic and molecular scale (Anima Nanda and Saravanan, 2009). Nanotechnology is highly interdisciplinary by nature and requires close collaboration between biologists, physical scientists and engineers (Singh et al., 2011). The term "nano" is adapted from the Greek word meaning "dwarf." When used as a prefix, it implies 10–9. A nanometer (nm) is one billionth of a meter, or roughly the length of three atoms side by side. Nanoparticles, generally considered as particles with a size of up to 100 nm, exhibit completely new or improved properties as compared to the larger particles of the bulk material. As specific surface area of nanoparticles is increased, their biological activity of the nanoparticles are also increased. (Jhan, 1999). Nanoparticles are often referred to as clusters, nanospheres, nanorods and nanocups are just a few of the shapes at the small end of the size ranges from 1 to 100nm. Metal nanoparticles are used prominently because of their antimicrobial activity.

Metal nanoparticles such as silver, gold, platinum, lead, copper etc are used for the nanoparticles synthesis. Among these silver nanoparticles are predominantly used because of their high antimicrobial activity, good conductivity and chemical stability. New applications of nanoparticles are emerging rapidly. nanoscale materials possess unique electrical, optical as well as biological properties and are thus applied in catalysis, bio-sensing, imaging, drug delivery, nanodevice fabrication and medicine, shampoos, soaps detergent, shoes, cosmetic products , and toothpaste, besides medical and pharmaceutical applications. (Mohanpuria et al., 2008). Silver nanoparticles are used in clothing, food industry, sunscreens and cosmetics (Jhan ,1999).

Nature has devised various processes for the synthesis of nano- and micro- length scaled inorganic materials which have contributed to the development of relatively new and largely unexplored area of research based on the biosynthesis of nanomaterials (Raveendran et al., 2003). An important aspect of nanotechnology concerns the development of experimental processes for the synthesis of nanoparticles of different size, shape and controlled dispersity. A number of approaches is available for the synthesis of silver nanoparticles viz., chemical and photochemical reactions in reverse micelles, thermal decomposition of silver compounds, radiation assisted, electrochemical, sonochemical and microwave assisted process and via green chemistry route(Parashar et al., 2009 ; Vorobyova et al.,1999). With the development of new chemical or physical methods, the concern for environmental contaminations are heightened as the chemical procedures involved in the synthesis of nanomaterial generate a large amount of hazardous by products. Chemical synthesis methods lead to the presence of some toxic chemical species adsorbed on the surface that may have adverse effects in medical applications. This is not an issue when it comes to biosynthesized nanoparticles as they are eco friendly and biocompatible for pharmaceutical applications. Thus, there is a need for green chemistry that includes a clean, nontoxic, and environment

*Corresponding Author :
e-mail: biobala17@gmail.com

friendly method for nanoparticles synthesis. (Mukherjee *et al.*, 2008). Plants synthesize nanoparticles with controlled size and shape due to its slower kinetics, better manipulation etc. The three main steps in the preparation of nanoparticles that should be evaluated from a green chemistry perspective are the choice of the solvent medium used for the synthesis, the choice of an environmentally benign reducing agent and the choice of a non toxic material for the stabilization of the nanoparticles. Most of the synthetic methods reported to date rely heavily on organic solvents. This is mainly due to the hydrophobicity of the capping agents used (Taleb *et al*., 1998).

Dodonaea viscosa is belonged to the family *Sapindaceae*, distribution in tropical, subtropical and warm temperate regions of Africa, the Americas, southern Asia and Australia. In the traditional system of medicine, various plant parts such as stem, leaves, seeds, roots, bark and aerial parts are used as antibacterial, analgesic, antiviral, anti-inflammatory, antiulcer and antioxidant. The present study was designed to synthesize and characterize silver nanoparticles and to investigate their antimicrobial activity against gram positive and gram negative bacteria.

MATERIALS AND METHODS

Preparation of *Dodonaea viscosa* Extract

All the chemicals and reagents used are analytical grade. Silver nitrate was obtained from Hi-media Laboratories (Mumbai, India). All the glasswares were washed in dilute $K_2Cr_2O_7$ solution and rinsed thoroughly with distilled water prior to use and dried in hot air oven. In order to prepare *D. viscosa* extract, 5 gm of powdered sample was boiled with 100 ml of sterile distilled water for 15 min at 50°C. The crude extract was passed through Whatman No.1 filter paper and the filtrates were stored at 4°C for further use.

Synthesis of Ag NPs

Five ml of *D. viscosa* aqueous extract was added into 45 ml of glass distilled water containing 1 mM silver nitrate. The solution was kept for incubation for room temperature in darkroom for 24 hrs. Suitable controls, only with plant extract, were maintained. The colour change from yellowish colour to dark brown colour indicated the formation of AgNPs.

Characterization

UV-visible spectroscopy

The initial characterization of synthesized AgNPs was carried out using UV-vis spectroscopy. The reduction of silver ions was monitored between 300 and 700 nm with Hitachi-u-2900 spectrometer (Japan).

Fourier transformed infrared spectroscopy (FTIR)

Biosynthesized AgNPs powder was analyzed using FT-IR spectrum for which function groups are responsible for synthesize of AgNPs. The FTIR spectroscopy using JASCO FT-IR 4100 instrument in the diffuse reflectance mode at a resolution of 4 cm"1 in KBr pellets.

SEM And EDX Analysis

Purified AgNPs in suspension were characterized for their size using Scanning Electron Microscope (SEM) (Hitachi S-3400n, Japan). EDX (Energy Dispersive X-ray) analysis of purified Ag NPs was carried out using the same instrument for confirming the elemental composition of the sample.

X-ray diffraction

Biosynthesized AgNPs were analyzed by X-ray diffraction (XRD) using SEIFERT JSO- DEBYEFLEX 2002 was used to study the crystalline nature of AgNPs. XRD pattern was observed for AgNPs with Cu Ká radiation of wavelength 1.5406 Å with operating condition as 40 kV, 30 mA. The XRD pattern was scanned in the 2è range from 30° to 70° with step size 0.04° per second.

Antibacterial Activity of Ag NPs

The AgNPs synthesized using *D. viscosa* leaf extract was tested for antibacterial activity by agar well- diffusion method against *Proteus vulgaris*, *Klebsiella pneumonia* (Gram-negative bacteria), *Bacillus subtilis* and *Enterococcus faecalis* (Gram-positive bacteria) (Prasad et al., 2011, Mudasir et al., 2012). The pure cultures of bacteria were swabbed uniformly on the individual plates using sterile cotton swabs on the Mueller Hinton Agar. Four wells were made on 6 mm in diameter in Muller Hinton agar plates with help of gel puncture using a micropipette, 40µl of synthesized silver nanoparticle solution'A', Plant extract 'B', Antibiotic 'C' (Streptomycin) and Silver Nitrate 'D', Plates were incubated at 37°C for 24 hrs to observe formation of zone of inhibition.

RESULT AND DISCUSSION

Synthesis of Nanoparticles from *D.viscosa* leaf extract

The study on extracellular green synthesis of silver nanoparticles through plant extracts were carried out. It is well known that silver nanoparticles exhibit yellowish to dark brown colour (Fig.1) in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles (Thirumurugan *et al.*, 2010). This important observation indicates the extracellular reduction of the Ag+ ions. The appearance of the dark brown colour in solution containing the *D. viscosa* aqueous extract is a clear indication of the formation of silver nanoparticles in the reaction mixture.

UV–vis Spectroscopic analysis

The formation and stability of the reduced AgNPs in the *D. viscosa* aqueous extract was monitored by using UV–vis spectral analysis. This technique has proved its validity for the analysis of nanoparticles (Sastry *et al.*, 2003). Silver nanoparticles suspension exhibits dark brown colour due to the surface Plasmon resonance (SPR), which results from collective oscillations of their conduction band electrons in response to electromagnetic waves. Under the UV region, $AgNO_3$ gives a characteristics absorbance band due to the excitation mode of their surface Plasmons, which is dependent on the size of nanoparticles. These SPR bands undergo red-shift or blue-shift depending on the quantum size effects (Shankar *et al*., 2004).

Absorption spectra of silver nanoparticles formed in the reaction solution has absorbance peak at 435 nm (Fig 2) broadening of peak indicated that the particles are mono dispersed and spherical silver particles.

Fourier Transform Infrared spectroscopy (FTIR)

The FTIR spectrum shows several absorption bands **(**Fig. 3) which indicate the presence of active functional groups in the synthesized silver nanoparticles. The intensity peaks are slightly increased for the period of silver nanoparticles synthesize like 3853.2, 3436.0, 2933.7, 1384.4, cm^{-1} as well as some intensity peaks decreased like 1384.4, 1075.9, and 668.8 cm^{-1}.The band at 3436 cm^{-1} corresponds to O-H stretching H-bonded alcohols and phenols. The peak at 2933.7 cm^{-1} corresponds to O-H stretch carboxylic acids. The assignment at 1636.0 cm^{-1} corresponds to N-H bend primary amines. The peak at 1384.4 cm^{-1} corresponds to C-N stretching of aromatic amine group and the bands observed at 1384.4, 1075.9, 668.8 cm^{-1} correspond to C-N stretching alcohols, carboxylic acids, ethers and esters (Sathyavathi *et al*., 2010). Therefore the synthesized nanoparticles were surrounded by proteins and metabolites such as terpenoids having functional groups of alcohols, ketones, aldehydes and carboxylic acids.

SEM and EDX studies

Scanning Electron Microscopy (SEM) image recorded the drop coated films of silver nanoparticles synthesized by *D. viscosa* leaf extract. The SEM image showed spherical shape of nanoparticles range 50 - 60 nm (Figure 4.a). Energy dispersive X-ray analysis (EDX) spectrometers confirmed the presence of elemental silver signals (Fig 4.b). Similar result has also been reported by Marimuthu Vivek *et al*. (2011) confirms the metallic nanoparticles.

X-ray Diffraction Analysis (XRD)

XRD patterns were analyzed to determinethe peak intensity, position and full width at half maximum (FWHM). The distinct Bragg reflections corresponding to the peak of pure silver was attained (111), (200) (220), and 2è= 38.0°, 44.2°, 64.4° respectively, and the lattice planes were manifested in the X-ray diffraction patterns are shown in (Fig.5). They may be indexed on the basis of face-centered cubic (FCC) structure of silver. (Yaminisudhalakshmi *et al.,* 2011).

Antibacterial activity

Biologically synthesized silver nanoparticles was tested for antibacterial activity and compared with positive control, and against *Proteus vulgaris*, *Klebsiella pneumonia* (Gram-negative bacteria), *Bacillus subtilis* and *Enterococcus faecalis* (Gram-positive bacteria) (Plate 1). The highest zone of inhibition was observed in *Klebsiella pneumonia* 19 mm and followed by *Enterococcus faecalis* 18 mm, *Proteus vulgaris*10 mm and *Bacillus subtillis* 9mm. There was no zone of inhibition observed in *D. viscosa* extract and silver nitrate alone treated well for throughout the experiment (Table 1) with all the four organisms, except *K. pneumonia which* showed activity in silver nitrate and the positive control streptomycin showed highest activity for tested bacteria.

Among the different types of nanomaterials like copper, zinc, titanium, magnesium, gold and silver which have antimicrobial properties, especially silver nanoparticles are more efficient. These materials exhibit antimicrobial activity against bacteria, viruses and other eukaryotic microorganisms. The actual mechanism of formation, for instance, of silver nanoparticles, in all of these microorganisms and plants, is still an open question, even though much research has been attempted to find different ways to investigate the possible mechanisms (Jain *et al.,* 2009). Similar aspects have occurred with the antibacterial activity of silver nanoparticles. Small particles have larger surface areas to be in contact with the bacterial cells, showing a larger activity (Prabhu *et al.,* 2010). The antimicrobial efficacy of the nanoparticles also depends on the shape or morphology of the nanoparticles. Furthermore, the biologically synthesized silver nanoparticles have been clearly demonstrated about their antibacterial activity against various bacteria.

Table 1 The antibacterial activity

Name of the organism	Zone inhibition (mm in diameter)			
	Ag NPs	*D. viscosa* Extract	Positive control	Silver nitrate
Enterococccus faecalis	18	-	24	-
Klesiella pneumonia	19	-	30	8
Proteus vulgaris	10	-	22	-
Bacillus subtilis	9	-	18	-

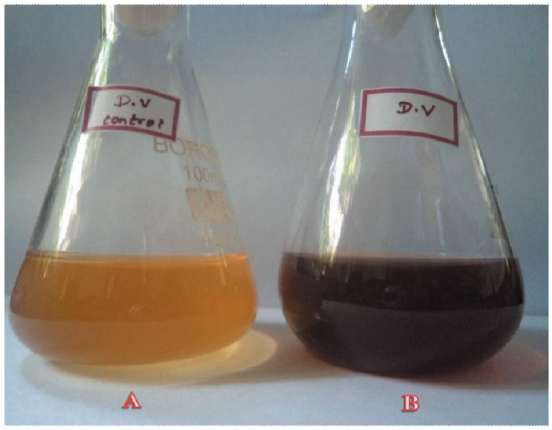


Figure 1 - Biosynthesis of silver Nanoparticles

A. without silver nitrate (Blank), B. Silver nitrate (1mM) Reduction after 24 hrs

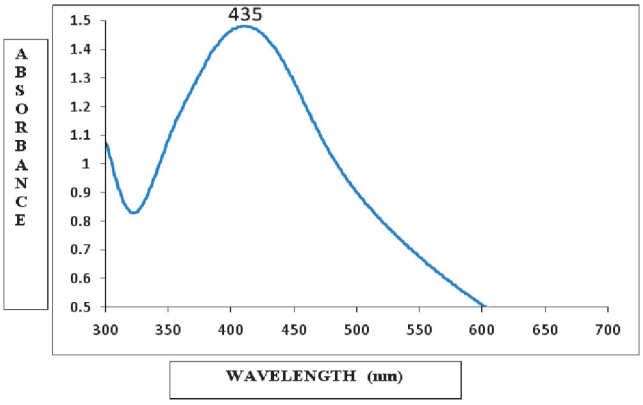


Fig.2 UV – Vis Spectrum of Ag NPs

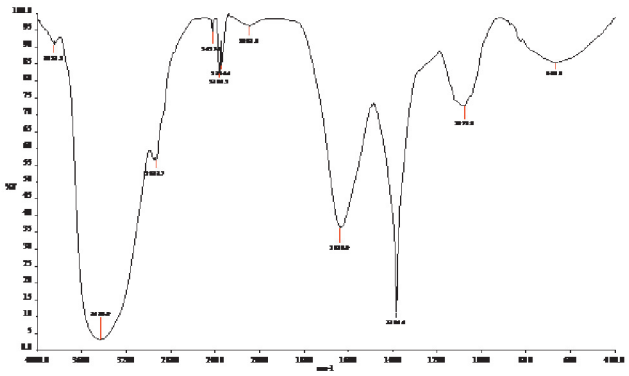


Fig .3 Fourier Transform Infrared spectroscopy (FTIR)

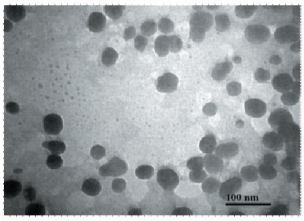


Fig 4.a SEM

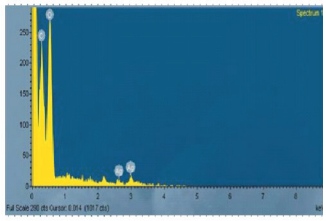


Fig 4.b EDX

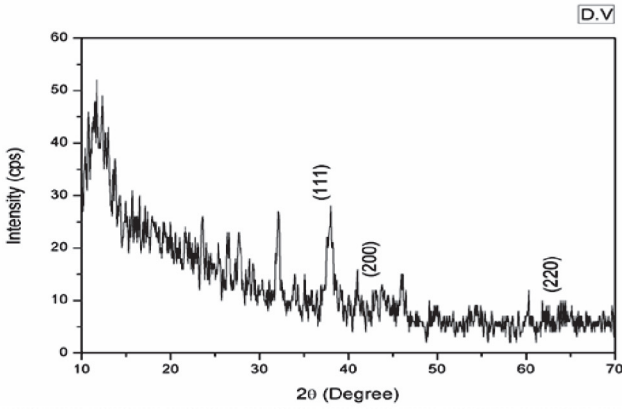
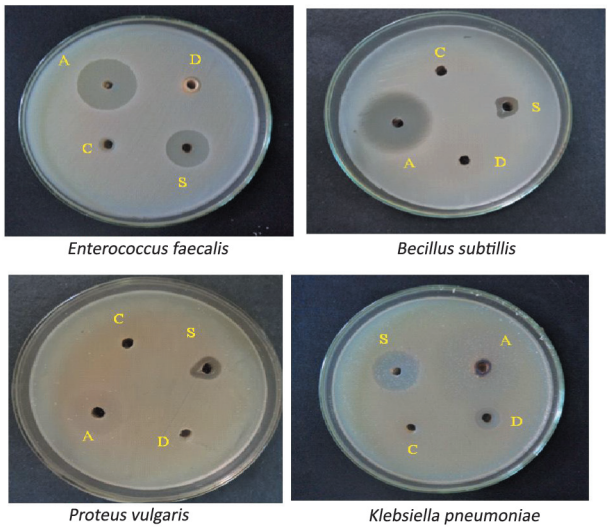


Fig. 5 XRD

PLATE 1



Enterococcus faecalis *Becillus subtillis*

Proteus vulgaris *Klebsiella pneumoniae*

S - Biologically synthesized silver nanoparticles. C - plant extract
A - Streptomycin D - Silver Nitrate

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