Research Article

Synthesis of Silver nanoparticles using *Ficus palmata* leaves extract: Characterization and evaluation for its Antimicrobial and Antioxidant activities

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https://doi.org/10.31024/ajpp.2018.4.2.15

Received: 15 January 2018 Revised: 18 February 2018 Accepted: 21 February 2018

Abstract

Objective: Metallic nanoparticles are usually synthesized by chemical methods which require toxic chemicals. The objective of present study was to prepare silver nanoparticles of *Ficus palmata* leaves extract and evaluated for antimicrobial and antioxidant activities. **Materials and methods:** In this current research article we report the eco-friendly green synthesis of silver nanoparticles from leaf extract of *Ficus palmata* without using any external reducing or capping agent. The synthesized nanoparticles were characterized by UV-Vis Spectroscopy, X-ray diffraction (XRD), Scanning Electron Microscopy and Transmission Electron Microscopy (TEM) techniques. **Results:** UV-Vis Spectroscopy studies showed the absorbance peak at 452 nm. The XRD patterns indicated the formation of crystalline silver nanoparticles having face-centered cubic geometry. The spherical nature and size of synthesized nanoparticles was further confirmed by TEM. **Conclusion:** The synthesized nanoparticles showed considerable antibacterial and antioxidant activities.

Keywords: Nanochemistry, *Ficus palmata*, antimicrobial, antioxidant

Introduction

Nanobiotechnology is one of the most current and promising areas of research in modern medical science. Nanotechnology deals with the nanoparticles having a size of 1-100 nm. Based on specific characteristics such as size, distribution and morphology, nanoparticles exhibit completely new, unique or improved properties (Veerasamy et al., 2011). Nanoparticles represent a higher surface to volume ratio with decreasing size (Singh et al., 2010). Nanotechnology is the rising area of manufacturing in the world today and there is an increasingly frenetic hunt for new nanomaterials and methods to make them. Nanoparticles are synthesized using different methods and more routinely used chemical methods (Thakkar et al., 2010). However, chemical methods cannot avoid the use of toxic chemicals in the synthesis protocol. Hence, the need of the hour is to develop high-yielding, low-cost, non-toxic and environmentally friendly procedures. Thus, there is an increasing demand for green nanotechnology. Therefore, the biological approach for the synthesis of nanoparticles becomes imperative (Parashar et al., 2009).

Recently several biological approaches have been reported using microorganisms including bacteria (Nair and Pradeep, 2002), fungi (Mukherjee et al., 2001) and plants (Chandran et al., 2006; Li et al., 2007) which have emerged as a very easy and feasible substitute to more complex synthetic procedures to obtain nanomaterials. Synthesis of nanoparticles by means of eco-friendly methods have become popular amid researchers due to its low cost, non-toxicity, environmental compatibility etc. and ease of applications since the resulting particles are highly soluble in water, biocompatible, and devoid of toxic stabilizers. Plant extracts are incredibly best candidates for facile synthesis of nanoparticles via green routes and they are also appropriate for large scale synthesis of nanoparticles. Synthesis of nanoparticles using plant extracts has several advantages over other environmentally green synthesis methods, because plants are broadly distributed, readily

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scalable, easily available, safe to handle and less expensive (Mittal et al., 2013).

During the last two decades, the biosynthesis of nobel metal nanoparticles have received considerable attention due to the rising need to develop eco friendly technologies in material synthesis. Among these metallic nanoparticles, silver nanoparticles (AgNPs) have become the center of rigorous research owing to several important applications such as their use in bio-labelling, sensors, drug delivery system, antimicrobial agents and filters (Kathiresan et al., 2009; Sridhara et al., 2013) Silver nanoparticles are also widely used for its exceptional properties in catalysis, chemical sensing, biosensing, photonics, electronics, and pharmaceuticals (Sarkar et al., 2010). In the field of biological systems and medicine, silver is the metal of preference (Parashar et al., 2009). Its disinfectant property is being exploited for hygienic and medicinal purposes, such as treatment of mental illness, nicotine addiction and infectious disease like syphilis and gonorrhea (Gulbrason et al., 2000).

As the plants contain different phytochemical products (Bhati-Kushwaha and Malik, 2014), which can breakdown the hazardous silver nitrate complex, into Ag^+ and NO_3^- ions. In the process, the toxic Ag^+ions are further reduced to the nontoxic Ag^0 metallic nanoparticles through the use of different functional groups on the surface of the extract (Ahmad et al., 2010).

Recently the bio synthesis of silver nanoparticles from many natural products such as alfalfa sprouts (Gardea-Torresdey et al., 2003) green tea (Vilchis-Nestor et al., 2008) and guava leaf extract (Raghunandan et al., 2011) have been reported.

In present investigation we have reported the synthesis of silver nanoparticles by an ecofriendly method using *Ficus palmata* leaves extract. *Ficus palmata*, commonly known as Bedu, is a deciduous tree of mostly 12 meters height with smooth bark. The phytochemical screening of the *Ficus palmata* plant extracts showed the presence of alkaloids, tannins, flavonoids, terpenoids and cardiac glycosides (Chauhan et al., 2014). The synthesized silver nanoparticles were characterized and evaluated for antimicrobial and antioxidant activities.

Materials and methods

Materials

Bedu leaves were collected from the Garhwal region and authentication of the plant was carried out by expert taxonomists of department of Botany in HNB Garhwal University, Srinagar, Pauri- Garhwal, Uttrakhand, whereas silver nitrate was procured from Qualigens Fine Chemicals, India. Millipore water was used throughout the study. The silver nitrate solution was freshly prepared using Millipore water and the glassware used during the experiment was also washed with Millipore water.

Preparation of leaves extract

The fresh and healthy leaves of *Ficus palmata* were collected and washed thoroughly with millipore water to remove dust from the leaves. The collected leaves were dried in shade. The dried leaves of bedu tree were crushed using a mortar and 10 g of crushed leaves were extracted with 100 mL of millipore water in a clean round bottom flask at 70^o C for 60 minutes. It was then cooled to room temperature and the supernatant liquid was filtered through a Whatman filter paper. The filtrate was used for the synthesis of AgNPs and stored in refrigerator (4^0C) for further experiments.

Synthesis of AgNPs

Ten mL of the aqueous plant extract was added to 90 mL of 3 mM AgNO3 solution in 250 mL conical flask. The total reaction volume was fixed to 100 mL at room temperature. The colour of mixture changed to dark brown which indicated the formation of nanoparticles (Kagithoju et al., 2015).

Characterization of synthesized Silver Nanoparticles

UV Analysis of Silver Nanoparticles

The UV–Vis spectroscopy analysis was performed with dilution of 0.5 ml of the sample with 2.5 ml of millipore water. The absorbance was calculated over the wavelength range, 200–800 nm (scan rate 2 nm/s).The dual beam spectrophotometer (Perkin Elmer Lambda 25) was used to attain the absorbance vs wavelength curve of silver nanoparticles.

XRD Analysis of Silver Nanoparticles

The X-ray diffraction, using Smartlab (Rigaku), was used to determine the particle size and nature of the AgNPs and it was operated at 40 kV, 40 mA with Cu K α radiation at 2θ angle ranging from 20˚C to 80˚C. 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

FESEM (Sigma, Carl Zeiss) instrument was utilized to observe the morphology of the synthesized AgNPs. 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. Other particulars concerning applied voltage, magnification used and size of the contents of the images were implanted on the images itself.

TEM analysis of Silver Nanoparticles

Transmission electron microscopy (TEM) was used to confirm the size, shape and surface morphology of the particle. 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 AgNPs on a carbon-coated copper grid.

Antibacterial property of Silver Nanoparticles

The disk diffusion method using a Mueller-Hinton agar culture medium (Hi-media) was used to assess the antibacterial activity of the silver nanoparticles against the human pathogenic bacteria *viz. Bacillus cereus* and *Klebsiella pneumonia* (Samy and Ignacimuthu, 2000) 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 (dimethyl sulfoxide). 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 inhibition zone that appeared around the disc was measured and recorded as the antibacterial effect of silver nanoparticles and the various concentrations of nanosilver stabilized in bedu extract.

Antioxidant activity

Antioxidant activity of synthesized nanoparticles was done by DPPH Scavenging method. The detail protocol for DPPH Free Radical Scavenging Activity (Mir et al., 2016) is given below:

Preparation of stock solution of the sample: 100 mg of sample was dissolved in 100 ml of DMSO to get 1000 µg/ml solution. (1) Dilution of test solution: 10, 20, 30, 40, 50,60,70,80,90,100 µg/ml solution of test were prepared from stock solution.

Preparation of DPPH solution: 15 mg for DPPH was dissolved in 10 ml of methanol. The resulting solution was covered with aluminium foil to protect from light.

Estimation of DPPH scavenging activity: 75 µl of DPPH solution was taken and the final volume was adjusted to 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 75 µl of DPPH and 100 µl of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Absorbance at zero time was taken in UV-Visible at 517 nm for each concentration. Final decrease in absorbance of DPPH with sample of different concentration was measured after 15 minute at 517 nm. Percentage inhibitions of DPPH radical by test compound were determined by the following formula.

% Reduction = Control absorbance – Test absorbance/ Control absorbance x 100

Calculation of IC50 value was done using graphical method

Results and discussion

UV Analysis

UV–Vis spectroscopy is one of the most frequently used techniques for structural characterization of silver nanoparticles. The UV-vis spectra results are an indirect but most competent method to detect the formation of nanoparticle. The progress of the reaction leading to the change of Ag^+ from $AgNO_3$ to reduced nanosilver was examined by observing the color change and absorbance maxima peak. The colour change from light to dark brown was observed within 10 minutes of addition. The aqueous leaf extract helped in reduction of silver ions to silver metal and change in color indicated the formation of nanoparticles. In the present work, the UV–vis spectra of synthesized silver nanoparticles displayed a strong broad peak around 452 nm (figure 1). This peak corresponded to the surface plasmon resonance of the synthesized AgNPs (Velusamy et al., 2015). The peak broadening at the base indicated that the synthesized nanoparticles are poly dispersed (Prasad and Swamy, 2013).

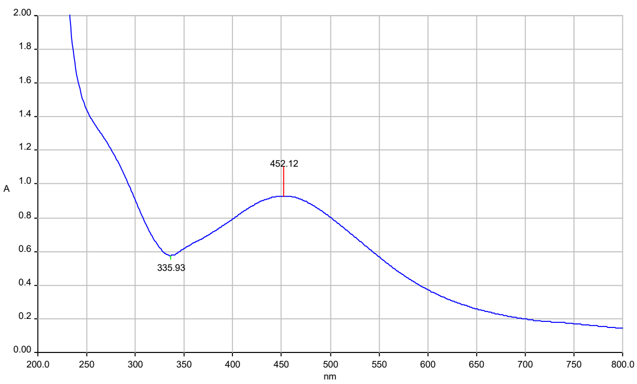

Figure 1. UV-vis absorption spectrum of synthesized AgNPs using *Ficus palmata* leaf extract

XRD Analysis

The XRD patterns of the prepared sample indicated the formation of the silver nanoparticles. Figure 2 clearly shows well defined diffraction peaks at 38.10, 46.319, 64.43 corresponding to the (111), (200), (220) crystallographic planes of face centered cubic (FCC) silver crystals, respectively. The diffraction peaks data obtained were in accordance with the reports of FCC structure from Joint Committee Powder Diffraction Standards (JCPDS) file No. 04–0783.

The average size of synthesized nanoparticles can be calculated by using scherrer equation.

$D = 0.94\ \lambda/\beta \cos \Theta$ Where D = Crystalline size of silver nanoparticles, λ = wave length of x-ray source (1.54 A), β is full width at half maximum of diffraction peak, k is scherrer constant with a value of 0.9 to 1and Θ is Bragg angle (Allafchian and Jalali, 2015). A number of small unassigned peaks were also recorded that might be due to the crystallization of bioorganic phases present in plant extract on the surface of the silver nanoparticles.

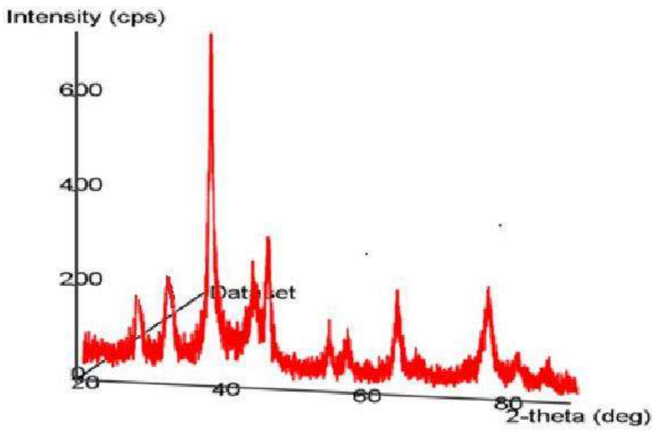

Figure 2. XRD of synthesized AgNPs using *Ficus palmata* leaf extract

Scanning Electron Microscope (SEM) and EDS Analysis

SEM technique is employed to find out the surface morphology and the topography of synthesized silver nanoparticles. SEM photomicrograph shows sample is highly concentrate and particle shows spherical surface morphology (Figure 3).

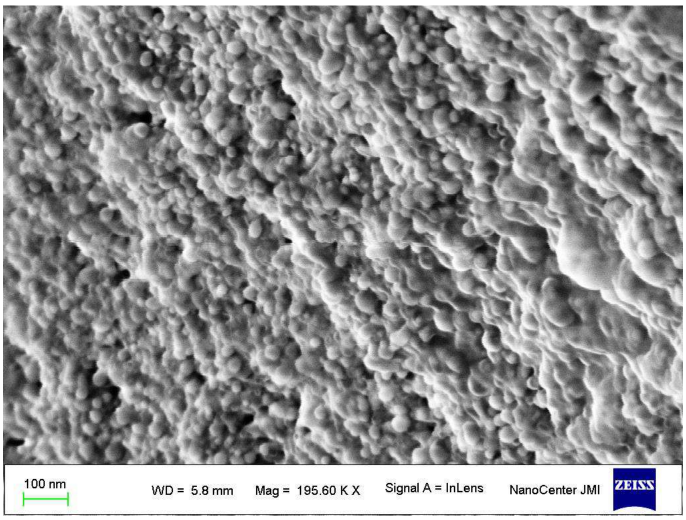

Figure 3. SEM Analysis of synthesized silver nanoparticles using *Ficus palmata* leaf extract

Elemental analysis is done which illustrated the presence of metallic silver nanoparticles and their homogenous distribution. It shows an intense signal at 3keV confirming the existence of metallic silver due to the Surface Plasmon Resonance. EDS pattern shows the presence of high amount around 58.9% of silver in sample. The peaks for chlorine and oxygen are due to the biomolecules which are bound to the surface of silver nanoparticles (Kasturi et al., 2009), while silicon and aluminium peaks are from sample grid holder (Figure 4).

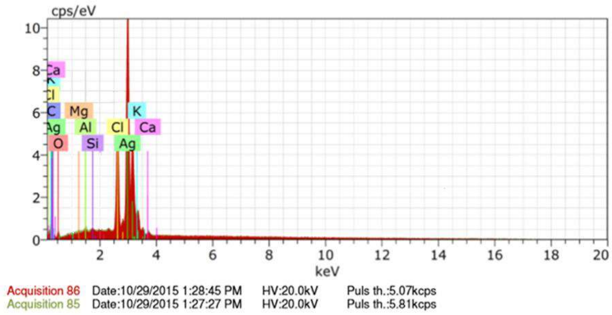

Figure 4. Energy Dispersive X ray Spectroscopy (EDX) of synthesized silver nanoparticles using *Ficus palmata* leaf extract

TEM Analysis

The shape and size of the biosynthesized AgNPs is further analyzed by TEM. TEM image shows particles with average size of nearly 30 nm. TEM micrograph clearly shows that particles were spherical with elongated morphology (Figure 5). Nearly similar results were reported by Perugu with *Saraca indica* leaf extract (Perugu et al., 2016). Particles were polydisperse and have two faces in surface morphology.

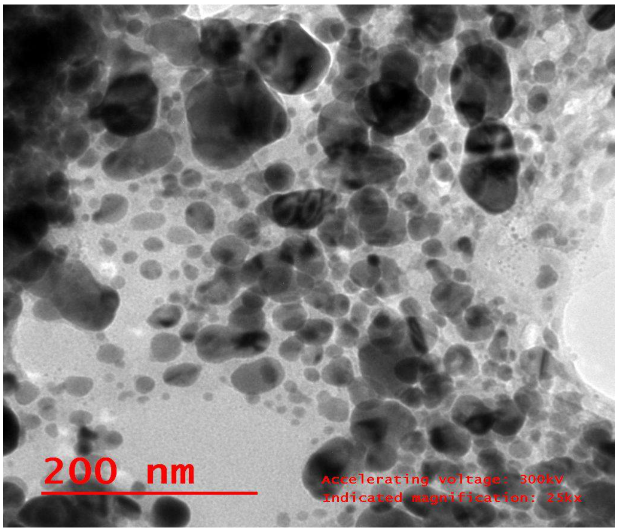

Figure 5. Transmission electron microscopy(TEM) of silver nanoparticles

The selected area electron diffraction pattern (SAED) of the silver nanoparticles is demonstrated in figure 6. Here silver nanoparticles were found crystalline. SAED spots corresponded to the different crystallographic planes of face-centered cubic (FCC) structure of elemental silver (Song & Kim, 2009).

Antibacterial activity

Recently, nanoparticles have gained significance in the field of Biomedicine. The most significant and distinguishing property of nanoparticles is that they exhibit

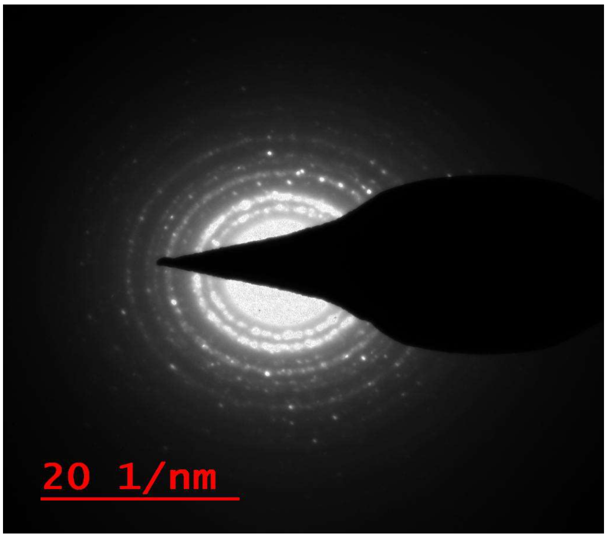

Figure 6. Selected Area Electron Diffraction (SAED) of silver nanoparticles

larger surface area to volume ratio. Surface area corresponds to the various properties such as the catalytic reactivity, antibacterial activity etc. When surface area of the nanoparticles gets increased, their surface energy will be getting increased and hence their biological effectiveness will also be increased (Srivastava et al., 2011). Smaller nanoparticles with a larger surface area to volume ratio provide a more effective antibacterial activity even at a very lower concentration.

Table 1. Antimicrobial activity of Nano Particles (at neat) against different bacteria

S. No.	Organisms	Zone of inhibition in mm	Positive Control (Streptomycin-25µg/ml)
1.	*B. cereus*	24	22.6
2.	*K. pneumoniae*	8.3	18

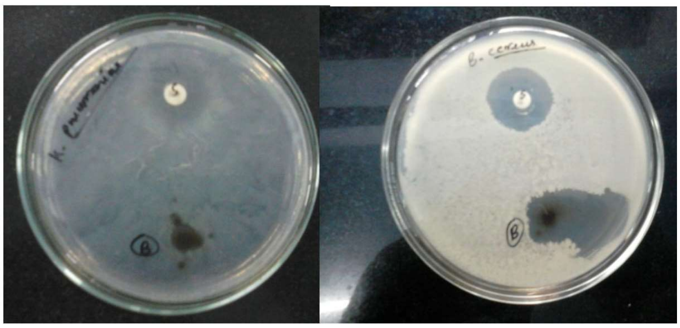

Figure 7 (a&b). Antimicrobial activity of silver nanoparticles against (a) *K. pneumoniae* and (b) *B. cereus*

The antibacterial activity of silver nanoparticles synthesized from *Ficus palmata* leaves extracts was studied against different bacteria by disc diffusion method. Silver nanoparticles synthesized from leaf extract of *Ficus palmata* showed efficient antimicrobial activity against *B. cereus* (24mm) and *K. pneumoniae* (8.3mm) (Table 1). The zones of inhibition were formed in antimicrobial screening test, indicating that synthesized nanoparticles have efficient antimicrobial activity against pathogenic bacteria (Guzman et al., 2012) (Figure 7a & b). Here Streptomycin 25µg/ml was used as positive control.

Antioxidant activity

An antioxidant is a molecule that slows or prevents the oxidation of the molecules. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves.. Plants produce a huge amount of antioxidants and they can represent a potential source of new compounds having antioxidant properties with fewer side effects (Mishra et al., 2007). In the present study silver nanoparticles prepared from plant extract was investigated for free radical scavenging activity by DPPH method.

Table 2. Antioxidant Activity of AgNPs of *Ficus palmata*

S. No.	Conc. (µg/ml)	Absorbance	% Reduction	IC_{50} Value
1.	10	0.285	42.82	
2.	20	0.263	45.93	
3.	30	0.242	48.38	
4.	40	0.231	53.65	
5.	50	0.212	58.14	
6.	60	0.182	60.22	29
7.	70	0.161	65.32	
8.	80	0.140	70.42	
9.	90	0.116	72.26	
10.	100	0.071	76.55	

Antioxidant activity of silver nanoparticles was assessed by DPPH scavenging assay with ascorbic acid as a positive control as observed in another study (Gomma, 2017). The antioxidant activity was tested using DPPH and was found to increase with the concentration of nanoparticles. At the concentration of 10µg/ml silver nanoparticles from leaves of *Ficus palmata* showed 42.82% reduction. On parallel examination it was found to be 40.61 % for standard compound Ascorbic acid. Though upon increasing concentration upto 100µg/ml, the free radical scavenging potential was also increased. Silver nanoparticles from leaves of *F. palmata* and standard ascorbic acid at 100µg/ml showed percent inhibition of 76.55 % and 82.14% respectively. This clearly indicates that antioxidant activity is concentration dependent. The efficiency of the antioxidant activity can be evaluated by IC 50 value. IC 50 can be described as the amount of sample needed for scavenging 50% of the free radicals. Lower is the IC 50 value, better is the antioxidant property. These IC 50 values can be calculated from absorbance value. The IC 50 value of standard ascorbic acid was found to be 25.5µg/ml (Figure 8) while that of nanoparticles was observed 29µg/ml (Figure 9) which was very near to the IC 50 Value of standard.

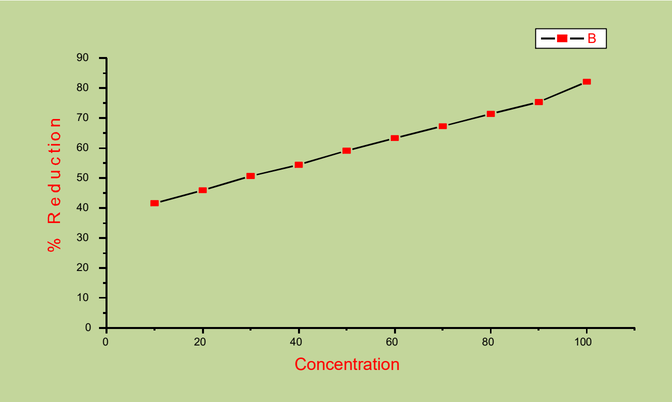

Figure 8. DPPH Free Radical Scavenging Activity of Ascorbic Acid

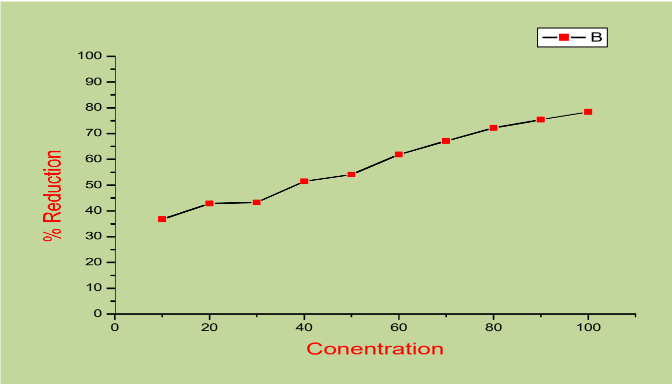

Figure 9. Antioxidant Activity of AgNPs of *Ficus palmata*

Conclusion

Green synthesis of silver nanoparticles is advantageous over other methods of synthesizing nanoparticles.In the present study silver nanoparticles are synthesized using *F. palmata* leaves extract without using synthetic reagents. Here we investigated an eco-friendly, nontoxic, cost effective and convenient green method for the synthesis of silver nanoparticles. The synthesised nanoparticles were characterized by different methods such as UV-Vis spectrophotometer, XRD, SEM and TEM. The observations suggest that nanoparticles are crystalline, polydispersed and spherical in shape with nearly 40nm size. These green synthesized nanoparticles exhibited potential antibacterial activity against *Bacillus cereus* and *Klebsiella pneumoniae* bacteria. Further these silver nanoparticles showed excellent antioxidant property. This green easy and inexpensive method can be used as alternative to chemical methods for the synthesis of silver nanoparticles.

Conflicts of interest

The authors declare any conflict of interest.

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