

Antibacterial Activity of Green Synthesized Silver Nanoparticles Using Vasaka (*Justicia adhatoda* L.) Leaf Extract

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Abstract There is an increasing demand for silver nanoparticles due to its wide applicability in various area of biological science such as in field of antimicrobial and therapeutics, biosensing, drug delivery etc. To use in bio-process the silver nanoparticles should be biocompatible and free from toxic chemicals. In the present study we report a cost effective and environment friendly route for green synthesis of silver nanoparticles using Vasaka (*Justicia adhatoda* L.) leaf extract as reducing as well as capping agent. This plant has been opted for the present study for its known medicinal properties and it is easily available. The biosynthesized silver nanoparticles are characterized by UV–Vis spectroscopy and TEM analysis. It is observed the nanoparticles are well shaped and the average particle size is 20 nm in the range of 5–50 nm. The antibacterial activity of these nanoparticles against *Pseudomonas aeruginosa* MTCC 741 has been measured by disc diffusion method, agar cup assay and serial dilution turbidity measurement assay. The results show green synthesized silver nanoparticles, using Vasaka leaf extract, have a potential to inhibit the growth of bacteria.

Keywords Antibacterial activity · Vasaka (*Justicia adhatoda* L.) leaf extract · *Pseudomonas aeruginosa* MTCC 741 · Silver nanoparticles · Green synthesis

Introduction

In terms of resistance, the battle between bacteria and antibacterial forced the researchers to have a re-look on the ways to attack bacteria. It is revealed that bacteria within biofilm are responsible for more than 80 % infectious diseases as well as more resistance to antibiotics [1]. In recent years, due to unique physical and chemical properties of nanoparticles, its uses to disrupt biofilm of bacteria are revolutionary steps in antimicrobial research [2–13]. The uses of silver and silver compounds are very well known from ancient time in case of dental work, catheters and burn wounds etc. Thus, among the various inorganic metal nanoparticles, silver (Ag) nanoparticles have received substantial attention in the field of biological system, living organisms and medicine [3–5, 7–13]. To use in biological field the metal nanoparticles should be biocompatible and free from toxic chemicals which are used to synthesis nanoparticles in traditional chemical process. The need of biocompatible silver nanoparticles opens the various bio-synthesis routes of nanoparticles. Among those, the silver nanoparticles synthesis using plant parts extract become an emerging field because these methods are very efficient in the production of silver nanoparticles due to of their simplicity, accuracy and cost effectiveness, and also from the green chemistry perspective and their biomedical application [3–5, 9, 13–20]. Environment friendly reducing and capping agents such as carbohydrates, polysaccharides, alkaloids, flavonoids present in the plant extract plays an important role in case of green synthesis of silver

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nanoparticles [21]. The synthesis of silver nanoparticles using various plant leaf extract are widely reported but antibacterial activities of few of those biosynthesized silver nanoparticles are reported [3–5, 7, 9, 11, 12, 15, 18].

In this present study an attempt has been done to synthesis of silver nanoparticles using Vasaka (*Justicia adhatoda* L.) leaf extract from the green chemistry perspective and to investigate the antibacterial activities of this biosynthesized silver nanoparticles on *Pseudomonas aeruginosa* MTCC 741 strain as a model gram negative bacteria.

Materials and Method

Synthesis of Ag Nanoparticles Using Plant Extract

About 5 g of fresh matured leaves of Vasaka was crashed and mixed with 60 ml of double distilled water. Adding 0.2 ml of vasaka leaf extract in 20 ml of 1 mM of AgNO_3 (Meark) solution, the mixture was stirred for 30 min at 60 °C. The colour of the solution was gradually changed from light yellow to reddish brown colour (Fig. 1).

Characterization of Ag-Nanoparticles

The formation of Ag nanoparticles was confirmed by recording the UV–visible spectra (SYSTRONICS, India). The morphology and size are analyzed by TEM (CM 12 PHILIPS) using standard method.

Microbiological Assays

The model gram negative organism *P. aeruginosa* MTCC 741 strain was maintained in both Nutrient agar (Hi-media, India) and Nutrient Broth medium (Hi-media, India) at 37 °C. For the disc diffusion method, 100 μl of 24 h old bacterial culture was uniformly spread over the nutrient agar surface. Freshly prepared Ag nanoparticles solution soaked sterile paper discs of 5 mm diameter were placed on that agar surface. The zones of inhibition were measured after 24 h incubation (at 37°). In case of agar cup assay, 100 μl of 24 h old bacterial culture was uniformly spread over the nutrient agar surface. Then, 50 μl of Ag nanoparticles solutions synthesized from three different concentrations of AgNO_3 solution were added into the wells (1 cm diameter) on that nutrient agar surface. The zones of inhibition (ZoI) were measured after 24 h incubation (at 37 °C). For serial dilution turbidity measurement assay, nutrients broths were prepared and distributed in several culture tubes. Each tube contains 10 ml of nutrient broth. The stock solution of the Ag



Fig. 1 Ag nanoparticle solutions, synthesized by reducing AgNO_3 using Vasaka leaf extract

nanoparticles solution was serially diluted at 10^{-1} , 10^{-2} , 10^{-3} level. The tubes were inoculated with 50 μl of 24 h old bacterial suspension. Each tube was mixed with 1 ml of Ag nanoparticles solution of different concentration. The tubes were incubated for 24 h at 37 °C and OD were taken at 540 nm to measure the turbidity against a sterile blank. A negative control without bacterium and a positive control without Ag nanoparticles were incubated for comparative purpose. All the experiments were done in triplicate.

Results and Discussion

The vasaka is easily available and well known medicinal plant. Leaf extract of it is used to treat cough, chronic bronchitis, asthma, phytisis etc. [22]. It contains a large number of secondary metabolites which includes vasicine, vasicinine, vasicinol, vasicinone, essential oil, maiontone, deoxyvasicinone, vasicol, glucoside sitosterol, kaempferol. It is reported that the alkaloids [23] present in leaf extract are responsible for reducing Ag^+ ions and capping for Ag nanoparticles. In this study some of the alkaloids present in working leaf extract may act as reducing and/or capping agent to synthesis Ag nanoparticles.

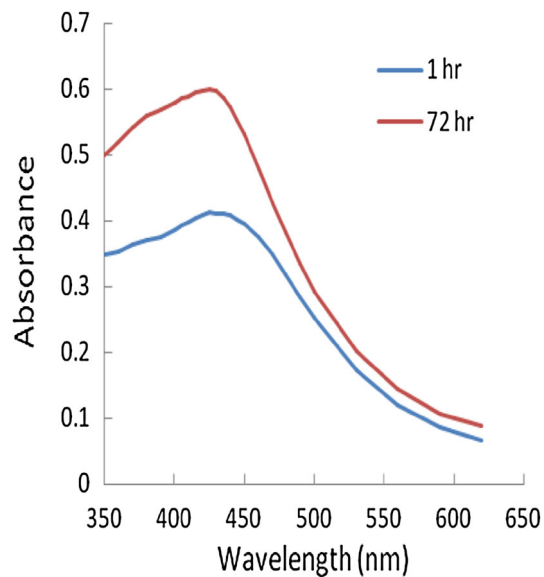


Fig. 2 UV–Vis spectra of Ag nanoparticle, synthesized by reducing AgNO_3 using Vasaka leaf extract

UV–visible Spectroscopy

Reduction of Ag^+ ion into Ag nanoparticles using leaf extract of vasaka was evidenced by visual change of colour of reaction mixture from colourless to yellow then to yellowish brown (Fig. 1). Figure 2 shows the UV–Vis spectra which confirmed the formation of Ag nanoparticles. The surface plasmon resonances (SPR) of silver nanoparticle were observed at 430 nm. The intensity of the SPR peaks increase as reaction time increase, which indicates the increase concentration of the silver nanoparticles. However, the overall result reflects that the Ag nanoparticles prepared by leaf extract are very stable without aggregation.

TEM

From Fig. 3 it is observed that most of the Ag nanoparticles spherical in shape, a few agglomerated Ag nanoparticles were also observed in some place. Figures also show that there is variation in particle size, the estimated average particle size is 20 nm in the range is from 5 to 50 nm. The size of these biosynthesized Ag nanoparticles is conformity with the size of earlier reported biosynthesized Ag nanoparticles using the leaf extract of *Mangifera indica* (50–65 nm), *Eucalyptus tereticornis* (60–150 nm), *Carica papaya* (25–40 nm), *Musa paradisiaca* (10–50 nm) [5].

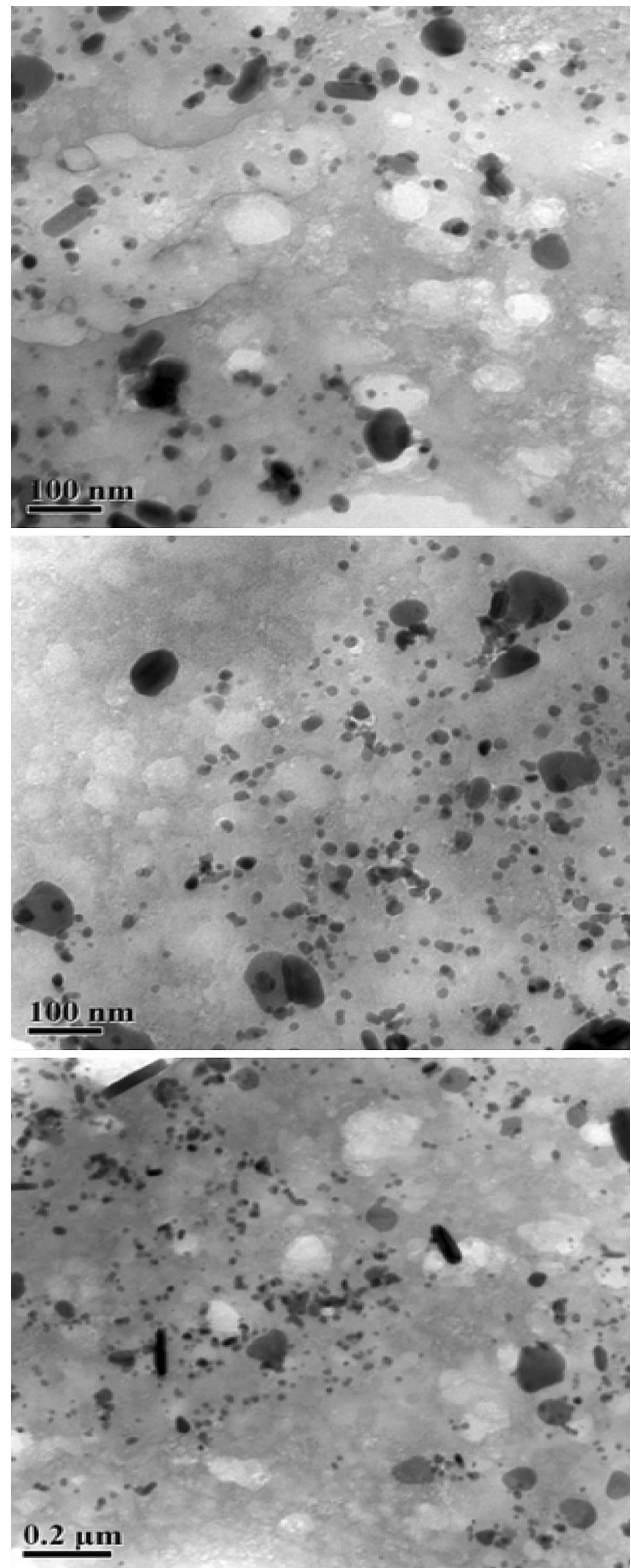


Fig. 3 TEM images Ag nanoparticles synthesized from AgNO_3 using Vasaka leaf extract

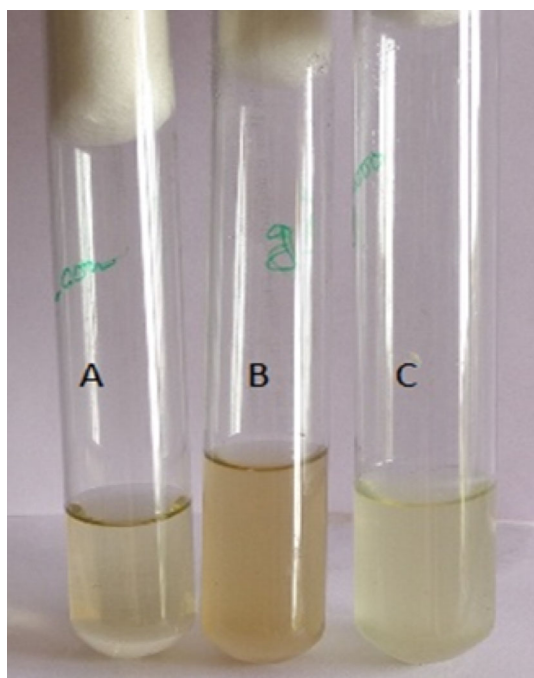


Fig. 4 Serial dilution method of anti-microbial activity assay of Ag-nanoparticles synthesized from Vasaka leaf extract

Microbiological Assays

In the present study, the potentiality of silver nanoparticles as a common antimicrobial substance has been evaluated against *P. aeruginosa* MTCC 741 by standard methods. *P. aeruginosa* is an opportunistic human pathogen and the genus itself is a potent plant pathogen also. It produces soft rot disease in *Arabidopsis thaliana* [24] and *Lactuca sativa* [25]. The results of disc diffusion assays indicate that the antimicrobial activities of silver nanoparticles (ZoI = 7–9 mm) are as effective as silver ions (ZoI = 8–10 mm) whereas the raw leaf extract has no visible inhibitory effect on *P. aeruginosa* MTCC 741 strain. However, the result of disc diffusion assays is too preliminary to conclude about the antibacterial properties of silver nanoparticles. The Agar-cup assay method and serial dilution turbidity measurement assay methods are more sensitive than disc diffusion assay method. The results of agar-cup assay reveal that biosynthesized silver nanoparticles using vasaka leaf extract have strong efficiency to inhibit (average ZoI = 1.36–1.4 cm) the bacterial growth. The serial dilution method (Fig. 4) has selective advantage over other method of assay. The results of serial dilution method are illustrated in Fig. 5. The nanoparticles when added to the liquid medium start their antibacterial activities in terms of inhibition of the growth of bacteria. This inhibition is measured in terms of optical density values (OD). The MIC value indicates that the green synthesized silver nanoparticles using the leaf extract of vasaka can inhibit the growth of *P. aeruginosa* MTCC 741 strain at the concentration of 10^{-5} (M). This study is tuned

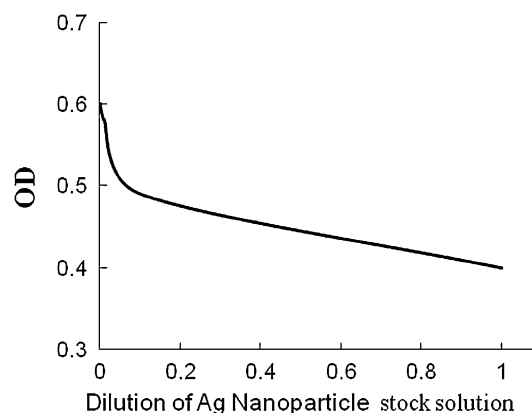


Fig. 5 Calculation of MIC of Ag nanoparticles (synthesized by reducing AgNO₃ using Vasaka Leaf extract)

with earlier reported studies: anti bacterial activity of Ag nanoparticles on fish pathogen and other microbes [3–5].

Several mechanisms regarding antimicrobial activity of Ag nanoparticles have been proposed. It seems that sulphur and phosphorus containing proteins or enzymes or phosphorous moiety of DNA of bacterial system may be affected by the Ag nanoparticles which lead to the inhibition of enzyme system of the organism [9]. Le et al. [26] clearly demonstrated the Ag nanoparticles get attached to the cell surface of *E. coli* and *V. Cholera* and then penetrated into the cell, destroyed the cell cytoplasm and killed the organism. Le et al. [26] also found that Ag nanoparticles significantly increase the cell permeability and affect the proper transport through plasma membrane. Thus the regulation of transport through the membrane become disrupted which leads the cell death.

In conclusion, this study revealed an economical, efficient and environment friendly route for green synthesis of Ag nanoparticles and evaluated the efficacy of the inhibitory effect of this biosynthesized Ag nanoparticles against *P. aeruginosa*. If further inhibitory studies of this biosynthesized Ag nanoparticles against other plant pathogenic pseudomonades like *P. syringae*, *P. tolaasii* and *P. agarici* agree with our present finding, then the green synthesized Ag nanoparticles could be more economical and promising alternate antibacterial agent in the field of agriculture.

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