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Biosynthesis of silver nanoparticles from *Flacortia jangomas* leaf extract and its bactericidal application against *E.coli*

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Abstract
Emerging rate of drug resistant microbes are creating major health issues now a days. Therefore, there is an urgent need to identify a new class of efficient biocides agents, which may least affected by resistant provoke system of microbes. Silver is one of the metals of choice for effective prevention of microbes. Development of eco-friendly routes for the synthesis of bactericidal nanoparticles is a burning topic in nanotechnology research. Here, we studied the effect of silver nanoparticles on pathogenic bacteria which was derived from *F. jangomas* plant leaf extract.Transmission electron microscopy analysis of these particles shows size range of ~4 nm to ~24nm.UV-visible spectroscopy and FTIR spectroscopy studies were also carried out to evaluate the formation silver nanoparticles. The well characterized silver nanoparticles were studied for antibacterial activity of pathogenic bacteria (*E.coli)* by standard disc diffusion method. Here, we investigated that AgNPs are potentially effective against disease causing microbes.

Keywords: Silver nanoparticles, *Flacortia jangomas*, antimicrobial activity.

Introduction

Nanomaterials exhibit several unique properties which differ from its own bulk materials. The properties of nanomaterials in different applications such as catalysis, sensors and in medicine critically depend on its size and composition [1]. Generally chemical, physical and biological methods have opted for nanomaterials synthesis. Novel metal nanoparticles, such as platinum, gold and silver are frequently applied to human contacting areas therefore there is an urgent need to develop eco-friendly synthesis routes of such nanomaterials. Biological methods denote bio-inspired compounds like microorganism secondary metabolites, enzyme, plant or plant extract which have been recommended as possible eco-friendly alternatives of chemical and physical methods [2].Specifically, different studies have been attempted to bio-reduction of silver ions and biogenesis of AgNPs using plant extracts, such as *A. indica*, *P. graveolens*, *E. hirta*, *M. Piperita* and *P. guajava* [3,4,5,6,7]. Silver is promising material for biological and medicinal concern among different metals such as lead, platinum and gold [9].Basically, silver nanoparticles are nano range particles in between 1 nm to 100 nm and paying attention enormously in research. Various studies have revealed that AgNPs may penetrate through cell membrane of bacteria and also can damage bio-molecules such as protein, enzymes, DNA which ultimately disturb biological functionality. Silver coated nano materials are employing in textile, food and other industries widely. That's highly enforced to explore new methodologies related to synthesis and fabrication of silver nanomaterials and its composites [10]. Biological synthesis is fascinating way to build up a "greener synthesis" technology to nanomaterials. Secondary metabolites of plants such as different glycosides, alkaloids corticosteroids, essential oils etc are good reducing agents. One of the plants, *Flacourtia jangomas* (Indian plum) belongs to family salicaceae which is widely cultivated in Asia regions [11, 12, 13]. It is extensively useful in diarrhea, piles, bleeding, gum toothache and stomatitis. The present work shows synthesis of AgNPs utilizing *F.jangomas* extract as reducing agent. Its potential antibacterial effect on pathogenic bacteria *E.coli* was also studied.

Methods

Preparation of leaf extract

F. jangomas leaves were collected from the D.D.U. GKP University Campus, Uttar Pradesh, India. Primarily they were washed and dried with water absorbent paper. 20 g, small pieces of *F. jangomas* leaves were boiled for half hour at 100ºC in 100 ml of sterile distilled water. The obtained aqueous extract was cooled at room temperature and filtered through Whatman No.1 filter paper. The extract was stored at 4°C for further analysis.

Synthesis of AgNPs

50 ml of aqueous solution of $AgNO_3$ (1.0mM) was taken and and stirred smoothly at 37°C. 100 µl of freshly prepared leaf extract was mixed for 10 minutes in solution. As synthesized AgNPs solution was stored at 4°C for characterization.

Characterization of AgNPs

Transmission electron microscopy (TEM, Tecnaii-G2F30 STWIN) operated at an accelerating voltage of 200 KeV has used for structural characterization of AgNPs. The UV-Visible absorbance spectroscopy has done with Perkin Elmer UV-Visible-Lambda 25 spectrophotometer. FTIR spectrum has recorded by Thermo scientific Fourier transform infrared spectroscopy (Thermo Nicolet-6700). Fluorescence spectroscopy has recorded with Perkin Elmer Fluorescence spectroscopy.

Anti bacterial Assay

The antibacterial assay was performed at *E. coli*, by standard disc diffusion method. Luria Bertani (LB) broth medium was used for bacterial growth and culture. Fresh culture of bacterial inoculums (100µl) was spread on to Muller Hinton Agar (MHA) Petri dishes. Different concentrations of silver nanoparticles as 4.0, 8.0, 16.0 and 32.0 µg ml^{-1} were administrated in each Petri dish over the sterilized paper disc of 5.0 mm diameter. Culture plates were incubated at 37ºC overnight. The inhibition zones produced around the discs were measured.

Results

The obtained AgNPs by biological reduction was firstly confirmed by visual observation. The color of solution was turned into reddish brown from transparent, due to reduction of silver ions (Figure 1). A reddish Brown color appearance of AgNPs in aqueous solution comes due to surface plasmon resonance phenomenon.

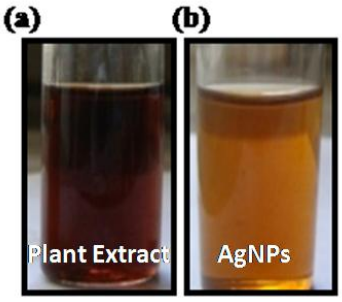

Figure1: (a) & (b) Optical photograph of the Crude extract of *F.jangomas* and colloidal solution of AgNPs.

The synthesized AgNPs by *F. jangomas* plant extracts were detected by UV-Vis spectrophotometer. Absorption spectra of AgNPs have shown absorbance peak at 422 nm which gradually decreased (Figure 2 (a). Figure 2(b) shows the FTIR spectra of the synthesized AgNPs. FTIR peaks represent the different functional groups present on nanoparticles after reduction of leaf extract such as, 3456.923083cm^{-1} (–OH), 2926.9230 cm^{-1} (C–H), 1743.0769 cm^{-1} (C=O),1643.0 cm^{-1} (C=C), 1396.1538 cm^{-1} (N–O), 1097.7 cm^{-1} (C–O), 724.6153 cm^{-1} (R–CH) and 560 cm^{-1} (M–O). Figure3 depicts fluorescence behaviour of AgNPs. Emission spectrum was recorded by exciting wavelength of 380 nm. The obtained emission spectrum shows a broader peak at ~440 nm. More interestingly, two smaller peaks have shown around ~455 nm and ~485 nm. The hump gave serrated and rough appearances. An emission spectra pattern of the AgNPs indicates that some organic bio-molecules from the leaf extract have adsorbed onto the AgNP surface, making its emission spectrum with wavy appearances. The morphology was carefully characterized using TEM spectroscopy, as shown in Figure 4(a) & (b). The AgNPs obtained through green route i.e. reduction by the leaf broth, showed nice morphology. Here, the size distribution of particles was in between ~4 nm to ~24nm range. Size distribution of silver nanoparticles has showed in bar diagram 4(c).

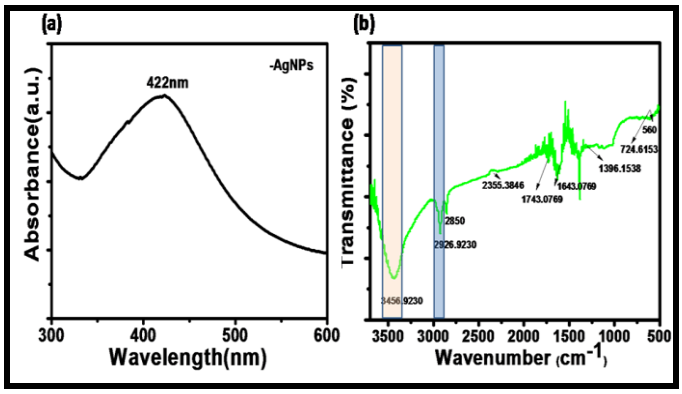

Figure 2: (a) UV–vis spectra of the AgNPs **(b)** FTIR Spectra of AgNPs.

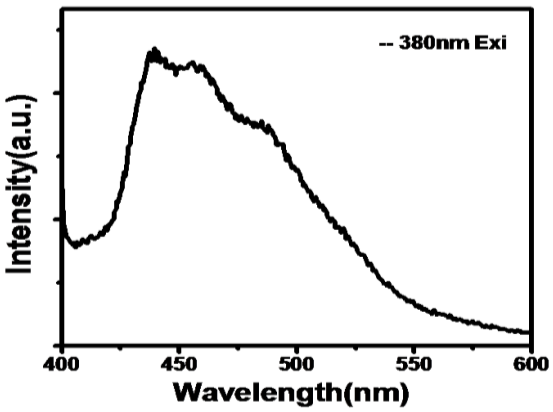

Figure 3: The emission spectra of AgNPs.

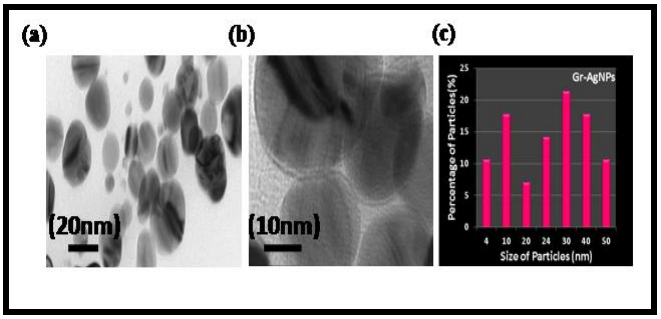

Figure 4: (a), (b) and (c) show TEM study of AgNPs of different size range with an average of ~24 nm.

Figure 5 depict the antibacterial activity of synthesized AgNPs against *E. coli*, As it showed a clear inhibition zone in Figure 5(a), the bio synthesized AgNPs were highly effective in their activity against bacteria. Bacterial cell membrane proteins enriched by sulphur element and DNA which acquires highly negative group phosphate are privileged sites for AgNPs interaction along with Ag+ released. The action mechanism of antimicrobial activity of AgNPs follows disruptions in membrane integrity as well as bio molecules such as enzyme and DNA. Fig-5(b) showed bar diagram of inhibition zones with 4.0, 8.0, 16.0 and 32 .0μg ml^{-1} doses of AgNPs exposed to *E. coli* lawns.It has seen that the zone of inhibition or area of affected region increased with the increased concentration of AgNPs.

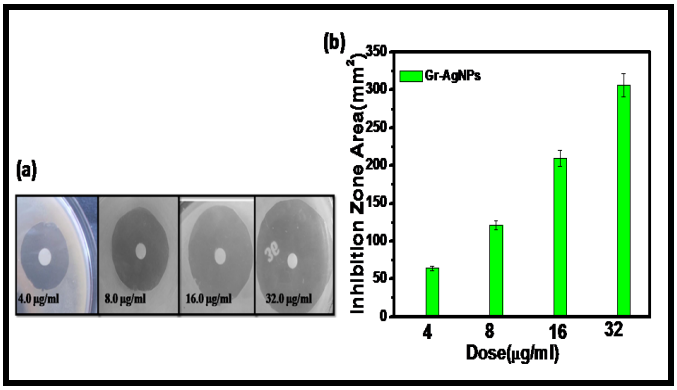

Figure 5: (a) Comparative inhibition zones with 4.0, 8.0, and 16.0 and 32 μg ml^{-1} diffusion of AgNPs into *E. coli* lawns through absorbent paper discs.**(b)** Graphical representation of inhibition zones with 4.0, 8.0, 16.0 and 32 μg ml^{-1} doses of AgNPs exposed to *E. coli* lawns.

Discussion

Here, we have reported green synthesis of AgNPs by bio reducing agent, which was extracted from leaves of *F. jangmas* plant containing high medicinal values. Aqueous solution of silver nitrate was used as source of silver ions. The obtained AgNPs showed a good colloidal stability and with narrow size distribution range. These AgNPs, were characterized physical and its antibacterial properties were seen at bacteria. Thus, this bio inspired route facilitates a simple, economically viable, and environmentally friendly technique to obtain AgNPs.

Conclusions

AgNPs were prepared by plant leaf. It was well characterized. It was found that the AgNPs show bactericidal behavior against *E.coli.* This work represents an eco-friendly method of AgNPs synthesis, which can employed for prevention of various microbial infections.

Acknowledgements

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