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BIOSYNTHESIS OF SILVER NANOPARTICLES USING *PREMNA HERBACEA* LEAF EXTRACT AND EVALUATION OF ITS ANTIMICROBIAL ACTIVITY AGAINST BACTERIA CAUSING DYSENTERY

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ABSTRACT

Aqueous silver nanoparticle was synthesized in a single step by a green biosynthetic method using aqueous leaf extracts of *Premna herbacea*, a local herb of Bodoland, Assam, India which acts as reducing as well as capping agent. Absorption spectra of synthesized nanoparticles were taken by UV spectrophotometer which showed a characteristic surface plasmon resonance (SPR) peak centred at a wavelength of 425 nm. The TEM analysis showed the particles were spherical and size between 10-30 nm. The bactericidal activity of the nanoparticles was tested against two gram negative bacteria (*Shigella dysentrieae* and *E.coli*) causing human dysentery. Antimicrobial property of the synthesized molecules was evaluated by calculating MIC values, disc diffusion assay and time kill kinetics based on nanoparticles concentration. MIC values were for *Shigella dysentrieae* and *E.coli* were 55 and 70 µg/ml respectively. The efficiency of antimicrobial activity by silver nanoparticles on the above mentioned microorganisms propose the possibility of a more cost effective antibacterial agent against dysentery causing microbes.

KEYWORDS: Antimicrobial, *Premna herbacea*, *Shigella dysentrieae,* Silver nanoparticles, *E.coli*

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INTRODUCTION

In developing countries dysentery is most dreadful childhood disorder. *Shigella sp*. is a gram negative bacterium which is the cause for most of the cases of dysentery in tropical countries[1]. The search for effective anti-microbial agents is essential especially for reducing the incidence of *Shigella sp.* and other microorganisms such as *E.coli* causing dysentery in children. The WHO recommends ciprofloxacin or any one among pivmecillinam, azithromycin and ceftriaxone for dysentery treatments[2]. Seeing the present scenario, where most of the pathogenic microorganisms become resistance to preexisting antibiotics, need for new therapeutic molecules is a must. A novel approach to the prevention of antibiotic resistance of pathogenic species is the use of new compounds that are not based on existing synthetic antimicrobial agents[3, 4]. Antimicrobial study of metal nanoparticles is gaining importance as because of increase in multiple drug resistant strains of bacteria and its unique properties. Nanoparticles are in the size ranges from 10-100 nm. The most important and distinct property of nanoparticles is that they possess larger surface area to volume ratio. Nanoparticle can be synthesized by physical, chemical and biological method. Biological synthesis of nanoparticle emerges as an ecological method and exciting approach. In biological synthesis the reducing agent can be any bio source. Many plants are becoming probable sources of reducing and stabilizing agents for green synthesis of naoparticles[5-7]. Silver is well-known antimicrobial materials from ancient time and it is less toxic to human cells and environmentally friendly at low concentration[8]. In this study, silver nanoparticle was synthesized using *Premna herbacea* leaf extract as a reducing agent by green biosynthesis method at optimam temperature in a short period of time. *Premna herbacea*, commonly known as kharadhafhani and masgaldav locally in bodoland (Assam) and belongs to Verbenaceae family[9]. This plant has no height and mostly found near hilly area in ground, they are available in the month of February, March and April[10]. This plant leaves is mostly used for making curry in bodoland area (Assam), so it is also known by curry patta. Reports of various researches show that silver nanoparticles synthesized by green technology exhibited antimicrobial activities against various pathogenic microbes[11-18]. The objective of this study includes the synthesis of silver nanoparticles by plant extract and investigating the efficacy of the same as an antimicrobial agent on the pathogenic bacteria species causing dysentery.

MATERIALS AND METHODS

Premna herbacea leaves were collected from local field of Kokrajhar, Assam, India (Figure 1). Silver nitrate (AgNO3) was procured from SRL Chem, Mumbai, India. UV-Vis-spectrophotometer is procured from Perkin Elmer lembda-25. Transmission electron microscope (TEM) (JEOL JEM 2100) was used to investigate morphology and size of the particles.

(i) Preparation of the leaves Extract

Young, fresh and green leaves of *Premna herbacea* (Figure 1) were used to make the aqueous extract. 10 g of fresh green leaves were thoroughly washed thrice with distilled water followed by double distilled water to remove contaminant particles. Then the clean leaves ware cut into fine pieces and were crushed in a crucible by adding 50 ml sterile distilled water and filtered two times through Whatmann No.1 filter paper in a 100 ml Erlenmeyer conical flask. The filtrate was stored and used within five days as a reducing as well as capping agent for synthesis of silver nanoparticles.

Premna herbacea

Figure 1
Plant of Premna herbacea in its natural habitat.

(ii) Synthesis and Characterization of Silver nanoparticles

Firstly, 1mM solution of silver nitrate solution was prepared using double distilled water. Then for synthesis of silver nanoparticles 10 ml of siver nitrate solution has taken in five different sealed Teflon container of having 30 ml volume. Each container has placed on a hot plate magnetic stirrer and heated at a temperature of 40^{o}C. Then addition the plant leaves extract drop wise has been done in five different containers with differ number of drops separately and heated continuously until the color change observed. Color change (colorless to yellowish-brown) indicates the reduction of silver ions and time required for the colour change was noted, which remain stable for more 3 months without any changes in the absorption spectrum.The reduction of pure silver ion (Ag+) into silver (Ag^0) was monitored by measuring the UV-Vis spectrum of the reaction medium (sample) after diluting a small aliquot of the sample into distilled water. Aliquots of the reaction solution were measured on a Perkin Elmer UV spectrophotometer (model λ-25) using a quartz cell (1 cm path), operated at a resolution of 1nm. The size, morphology and distribution of silver nanoparticle was analysed by TEM.

(iii) Antimicrobial Analysis
Culture and culture maintenance

The pathogenic bacteria used in this study were clinical isolates of *Shigella dysentriea,* and *E.coli.* Bacterial strains stock cultures were maintained at 4 °C on nutrient agar medium. Active cultures were prepared by inoculating fresh nutrient broth medium with a loopful of cells from the stock cultures at 37 °C for overnight. At 37 °C, subculture of overnight grown bacterial cells was done to get desirable cells number for antimicrobial assays.

Determination of MIC by visual analysis

The silver nanoparticles were tested to determine the Minimal Inhibitory Concentration (MIC) for each bacterial strainaccording to Datta et al., (2011)[19]. Freshly, grown bacterial strains 100 µL (10^6 cells/mL) in nutrient broth was inoculated in tubes with nutrient broth supplemented with different concentrations (0 – 100 µg) of silver nanoparticles colloidal suspensions, incubated for 24 h at 37 °C. Presence of turbidity denoted presence of micro organism in the test tube after the period of incubation whereas the complete absence of any turbidity indicates complete inhibition of microbial growth. The test tube showing no bacterial growth when treated with the lowest dilution of nanoparticles was considered as MIC of test sample, here nanoparticles.

Disc diffusion method

The antimicrobial activity of silver nanoparticles was screened using disc diffusion technique[19]. On the agar plates 0.1 % inoculum suspension was swabbed uniformly with sterile cotton and was allowed to stand for 15 minutes. The different dilutions of nanoparticles (0, 50 and 80 %) from stock were loaded on 6 mm autoclaved filter paper discs. The disc containg specific amount of silver nanoparticles was placed on the surface of agar medium and placed at low temperature for 5 min to allow test sample to diffuse into the medium. After this the plates were incubated at 37°C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with ruler in millimetre.

(iv) Statistical analysis

All experiments were carried out in triplicate. Data points were represented by the mean of the measured values. Statistical analysis was carried out using MS-Excel software.

RESULTS AND DISCUSSIONS

This work explores the biosynthesis of AgNPs using *leaves extract of Premna herbacea*. The change in color from colorless solution of silver nitrate solution to dark brown in solution is a clear indication of the formation of AgNPs in the reaction mixture. The change in the color of the solution is the excitation of surface plasmon vibrations in the AgNPs, which is characteristic property of the nanoparticles[20]. The UV-Vis spectra of the aqueous reaction mixture were recorded (Figure 2). Synthesis of silver nanoparticles using our experimental plant was dose dependent. The UV-visible spectrophotometer absorbance band was observed at 425 nm in our study. The size, morphology and distribution of silver nanoparticle was analysed by TEM. The size of the nanoparticles was around 10 to 30 nm and observes to be spherical (Figure-3).

UV-Vis Spectra

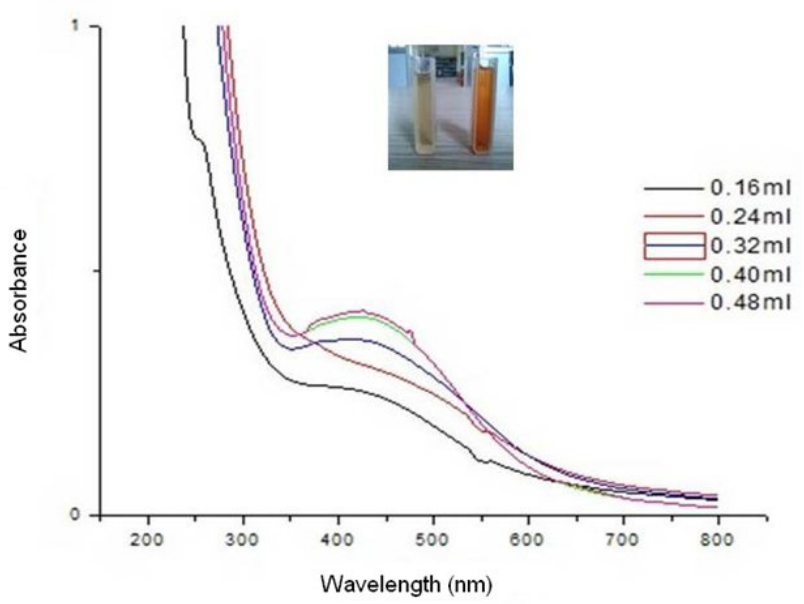

Figure 2

UV-Visible absorption spectrum of silver nanoparticles synthesized by treating 1mM aqueous AgNO3 solution with varying concentration of Premna herbacea leaf extract (0.16 – 0.48 ml). Inset showed synthesized silver nanoparticles (right) and plant leaf extract (Left).

TEM micrograph of synthesized silver nanoparticles

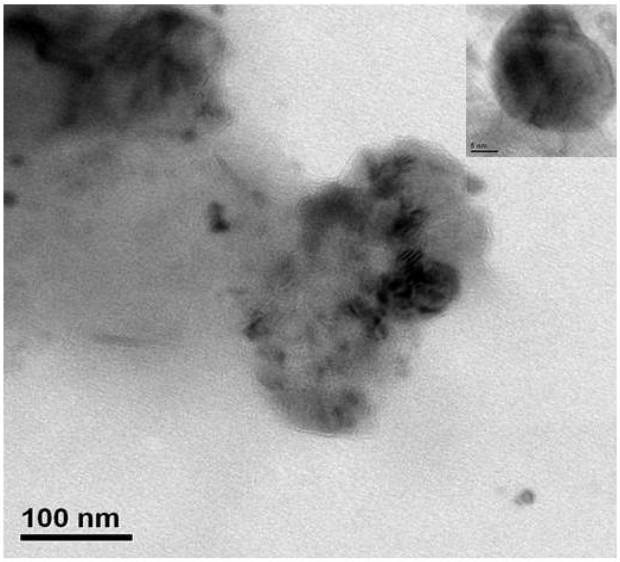

Figure 3
TEM image of Ag nanoparticles synthesized by leaves extract of Premna herbacea. Inset showed single nanoparticles of the same.

The antimicrobial effects were studied for two bacteria viz *Shigella dysentriea,* and *E.coli.* The minimum inhibitory concentration of the silver nanocolloids was determined by visual observations and it was observed that the MIC for the bacterial strain was 55 and 70 µg/ml respectively. The efficacy of nanoparticles on bacterial strains based on concentration is illustrated in Figure 4. The disc diffusion assay demonstrates the zone of inhibition for the tested bacteria (Figure 5). This report correlates with previously reported work on biofabricated silver nanopaerticles having antimicrobial property[21, 22].

Growth Inhibition of Bacteria

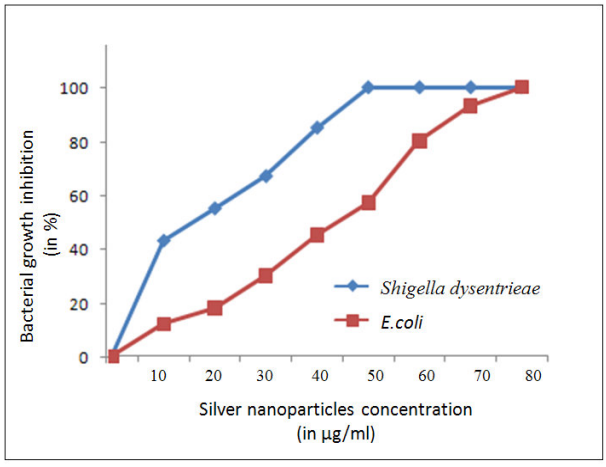

Figure 4
Growth inhibition curves of the tested clinical isolates of bacteria (Shigella dysentriea and E.coli). Isolates were grown in LB medium supplemented with different concentrations from the stock ANPs sample.

Zone of Inhibition of Bacteria

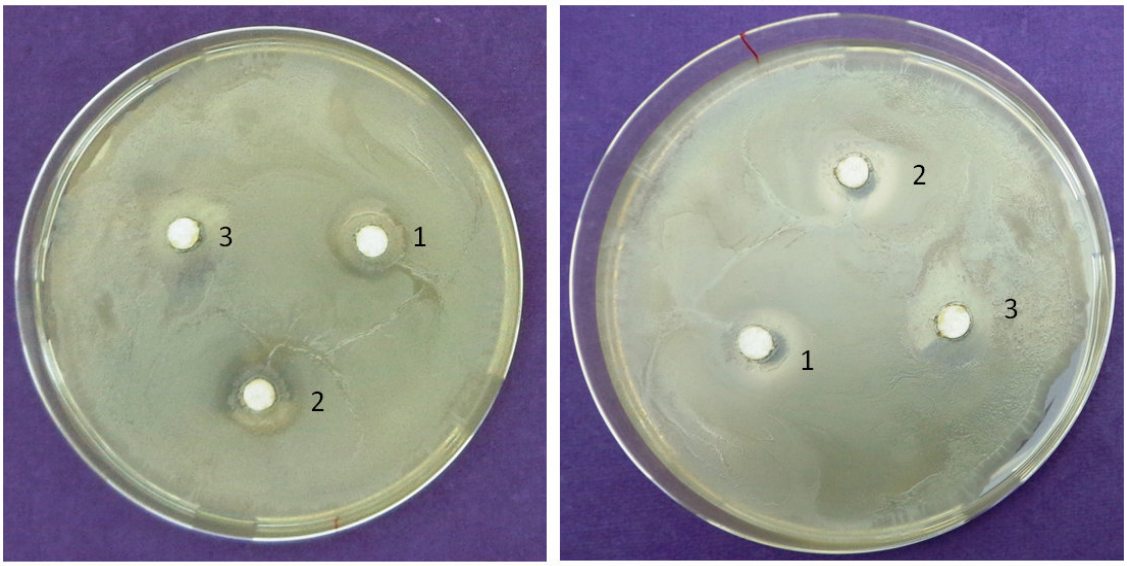

Figure 5
Agar plate exhibits zones of inhibition by different dilution of ANPs (0, 50 and 80 %) against Shigella dysentriea and E. coli. Disc 1, 2 and 3 contain 80, 50, and 0 % dilution from the stock nanoparticles suspension.

CONCLUSION

Comparing the tested bacteria, it was found that *E. coli* showed least effective and *Shigella dysentrieae* was most susceptible to the silver nanoparticles. The efficiency of antimicrobial activity by silver nanoparticles on the above mentioned microorganisms propose the possibility of a more cost effective antibacterial agent against dysentery causing microbes. Our synthesized silver nanoparticles did not hemolyzed the human blood cell when tested for RBC hemolysis assay (data not shown). The results obtained support the facts that more work needs to be done on the toxicity and compatibility with the view of their use for *in vivo* studies.

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