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Original article

Bio-fabricated silver nanoparticles preferentially targets Gram positive depending on cell surface charge

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ABSTRACT

Recently bio-inspired experimental processes for synthesis of nanoparticles are receiving significant attention in nanobiotechnology. Silver nanoparticles (Ag NPs) have been used very frequently in recent times to the wounds, burns and bacterial infections caused by drug-resistant microorganisms. Though, the antibacterial effects of Ag NPs on some multi drug-resistant bacteria specially against Gram positive bacteria has been established, but further investigation is needed to elicit its effectiveness against Gram negatives and to identify the probable mechanism of action. Thus, the present study was conducted to synthesize Ag NPs using *Andrographis paniculata* leaf extract and to investigate its antibacterial efficacy. After synthesis process the biosynthesized nanoparticles were purified and characterized with the help of various physical measurement techniques which raveled their purity, stability and small size range. The antimicrobial activity of Ag NPs was determined against both Gram-positive *Enterococcus faecalis* and Gram-negative *Proteus vulgaris*. Results showed comparatively higher antibacterial efficacy of Ag NPs against Gram positive *Enterococcus faecalis* strains. It was found that greater difference in zeta potential values between Gram positive bacteria and Ag NPs triggers better internalization of the particles. Thus the cell surface charge played vital role in cell killing which was confirmed by surface zeta potential study. Finally it may be concluded that green synthesized Ag NPs using *Andrographis paniculata* leaf extract can be very useful against both multi drug resistant Gram-positive and Gram-negative bacteria.

1. Introduction

Nanotechnology is now creating a growing sense of excitement in life sciences especially in biotechnology and biomedical application field. This area of impetus is an inter-phase between engineering with biological applications to recognize, functionalize and organize the nano-materials which is applicable to field of medicine [1]. Different biological approaches for nanoparticles synthesis have been reported till date including bacteria [2–5], fungi [6,7] and plants [8–11]. Plants extracts mediated nanoparticles synthesis is gaining importance due to its ease, low cost synthesis and eco-friendliness [12]. The use of plant extract as reducing and capping agent serves various advantageous over traditional methods (physical and chemical process) for the synthesis of nanoparticles [13]. Traditional Ag NPs synthesis includes mostly by physical appearance and chemical approach. In physical approach Ag NPs can be synthesized using tube furnished through evaporation and condensation reaction but this methods have several draw back as this set up require large working space, high energy requirements and high cost apparent [14]. Jung et al. [15] reported the synthesis of Ag NPs via small seramic heater that involves heating of a local area. Though this method is effective of synthesis of small nanoparticles in high concentration but there are several limitation were observed which include inhalation toxicity etc. In case of chemical synthesis approach, though the developed Ag NPs are more stable but commonly used reductant like borohydrate, citrate, and elemental hydrogen are used. Not only that, a variety of surface stabilizing agent, organic solvents are used in fabrication process. After synthesis the removal of this surfactant and organic solvent from synthesized Ag NPs is very difficult work and it elevates the chances of unwanted toxicity in biological system [14].

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The use of silver nanoparticle is also important, as several pathogenic bacteria have developed resistance against various antibiotics. Hence, medical application of Ag NPs are emerging specially in biomedicine to produce silver based dressings, silver coated medicinal devices, such as nano-lotions, nano-gels etc [16]. Like various antibiotics Ag NPs does not prefers a specific target sites of microbes, thus it is not quickly confers resistance property. Beside medical application, Ag NPs also played beneficial role in biotechnology [17], optics [18,19], microelectronics [20] etc. It is eminent that silver possesses a strong activity against bacteria, viruses and fungi, while the mechanism of action is still not fully recognized [21]. Ag NPs have become the focus of broad research areas, because of its good antimicrobial efficacy against multidrug resistant bacteria, viruses and other micro-organisms [22]. Ag NPs was found to be most effective against *E. coli, Staphylococcus aureus, V. cholera, P. aeruginosa and S. typhus* bacteria [23,24,9]. As for example, silver sulfadazine cream made of Silver nitrate combined with sulfonamide, used as a broad-spectrum antibacterial, antifungal and antiviral agent and also used for the treatment of burns [25]. These applications strongly depend on the properties of Ag NPs, such as particle size, shape, size distribution and the surface charges [26]. Toxicity of Ag NPs in mammalian cells also depends on the synthesis procedure, particles size, shape and ion dissolution ability. It was also found that Ag-NPs appear to cause much stronger damages to chromosome [27].

In this article, we intended a rapid biosynthesis Ag NPs with a simple, non-toxic, cost-effective and eco-friendly method at ambient conditions using *Andrographis paniculata* leaf extract. utilizing the reduced property of *Andrographis paniculata* leaves extract, which shows greater efficacy against Gram positive and comparatively less efficacy against Gram negative bacteria and toxicological impacts on cells of these materials.

2. Materials and methods

2.1. Culture media and chemicals

All the chemicals and culture media were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), Merck Ltd. (India), pure silver nitrate ($AgNO_3$) was from Sigma-Aldrich (St Louis, MO, USA).

2.2. Bacterial strains used in this study

Multi drug resistant *Proteus vulgaris* [28] and *Enterococcus faecalis* bacterial strains isolated from urinary tract infection (UTI) patients were used in this study. The clinically isolated *Enterococcus faecalis* strain was received as a gift from Dr. Manideepa Sengupta, Professor and HOD, Medical College, Kolkata, West Bengal, India. The strains were subculture and used throughout the whole study.

2.3. Preparation of the leaves extract

The Indian medicinal plant, *Andrographis peniculata* (Kalmegh) was collected from Midnapore Local area, West Bengal, India, on the basis of cost-effectiveness, medicinal property and ease of availability. Fresh and healthy leaves of *A. peniculata* were collected and rinsed thoroughly by distilled water to remove all the dust particles. Then the leaves were cut into small pieces and dried at room temperature. About 10 g of completely dried finely incised leaves dust were weighed separately and transferred into 250 mL beaker containing 100 mL distilled water and placed on a shaking incubator at 37 °C for 48 h with 200 rpm speed. Then the extracts were filtered 2–3 times through Whatman No. 1 filter paper placed over a conical beaker (250 mL) to get clear solutions and to remove particulate matter. In each and every steps of the experiment, sterility was maintained for the accuracy and effectiveness in results without contamination. Filtrate samples were collected and lyophilized accordingly to obtained water extract as powder form.

2.4. Synthesis of Ag NPs

Aqueous solution of $AgNO_3$ (1 mM) of was prepared in 250 mL Erlenmeyer flasks using 100 mL distilled water. Then the water extract of *Andrographis peniculata* leaf (1 mg/mL) was added to the $AgNO_3$ for reduction into Ag+ ions. The mixture composite was then kept for complete bio-reduction at shaking incubator for overnight at 37 °C. In the mean time, the colour change of the mixture from faint brown to reddish brown to colloidal brown was monitored from time to time (time and colour change were recorded along with cyclic sampling and scanning by UV–vis spectrophotometry) for maximum 30 min. The reactions were carried out in dark (to avoid photo activation of $AgNO_3$) at 37 °C incubator. Complete reduction of $AgNO_3$ to Ag NP was confirmed by the change in colour from colourless to brown. After the reaction, the diluted colloidal solution was bring to room temperature (25 °C) and kept aside for 24 h for complete bio-reduction and saturation denoted by UV–vis spectrophotometric scanning. Then, the mixture was sealed and stored properly for future use. The formation of Ag NPs was furthermore confirmed by spectrophotometric analysis. [29].

2.5. Purification and production yield measurement

Purification of Ag NP mixer was done following the Density gradient centrifugation method according to the W. Wu et al., 2013 [30]. The production yield was calculated from the following formulae.

$$\% \text{ of yield} = (\text{Actual yield}/\text{Theoretical yield}) \times 100$$

2.6. Characterization of Ag NPs

2.6.1. UV–vis spectroscopy

To observe the optical property of biosynthesized Ag NPs, samples were analyzed for UV visible spectroscopic studies (Shimadzu UV/vis 1800 spectrophotometer) at room temperature operated at a resolution of 1 nm between 190 and 1100 nm ranges [31].

2.6.2. Fourier transform infrared spectroscopy

Silver nanoparticle was investigated by Fourier transform Infrared spectroscopy (FT-IR) by the help of Perkin Elmer Spectrum RX I FT-IR system with a frequency ranging from 500 to 4000 cm^{-1} and a resolution of 4 cm^{-1}. The samples were prepared using the KBr pellet method [31].

2.6.3. Dynamic light scattering (DLS) and zeta potential

DLS analysis was done with the help of Zetasizer Nano ZS (Malvern Instruments) following the method [31], with few modifications. The concentration of the 100 μg/mL of AgNP was sonicated about 2 min and dynamic particle sizes were measured by two drops (30 μL) of an aqueous suspension of NPs in 10 mL of Millipore water. The NPs were analyzed with a DLS analyzer when the NPs were completely detached in water. To obtain the average size of the NPs of the experiments were repeated several times. The zeta potential of the Ag NPs was measured by using a Zetasizer-Nano ZS (Malvern, Malvern Hills, U.K.) Instrument with 1 mg/mL of NPs. The NPs, dissolve in Millipore water solution was filtered by using whatman No 1 filter paper for this experiment. [32].