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Biosynthesis of Silver Nanoparticles by *Plumeria rubra* Flower Extract: Characterization and Their Antimicrobial Activities

Pinaki Mandal
Department of Chemistry, Bankura Sammilani College, Kenduadihi, Bankura-722102, WB, India.
Corresponding Author: Pinaki Mandal

Abstract: *The main objective of this work is focused on to propose a simplified and efficient green synthesis of silver nanoparticles with proven antibacterial properties. In the present study silver nanoparticles were biosynthesized by the biological approach using Plumeria rubra (red frangipani) flower extract at room temperature. AgNPs were rapidly synthesized from bio-reduction of silver ions using aqueous extract of Plumeria rubra flower and was observed when the light yellow coloured flower extract solution turned to brown colour with the addition of silver nitrate solution. The biosynthesized AgNPs were characterized by ultraviolet-visible (UV-Vis) and transmission electron microscope (TEM). The UV spectra showed the characteristic surface plasmon resonance band for AgNPs in the range of 425–465 nm. The prepared AgNPs were effective broad spectrum antimicrobial agent, which exhibited an effective inhibitory activity against Escherichia Coli and Bacillus sp. Therefore synthesis of AgNPs using environmentally benign resources such as flower extract suggests a potential tool to combat against increasing antibiotic resistance.*

Keywords: *Plumeria rubra flower, green synthesis, silver nanoparticles, TEM, antimicrobial property.*

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I. Introduction

Over the past few decades' nanotechnology and nanoscience is an emergent interdisciplinary field of research interposing biotechnology, material science and bionanosciences [1]. Presently improvement of green synthesis of different metallic nanoparticles like silver, gold, copper etc. and their applications is one of the most important areas of research. Nanoparticles exhibit completely new or advanced properties due to specific characteristics such as smaller particle size ranging from 1 to 100 nm, various shapes and increased surface area [2]. Recently, resistance to commercially available antimicrobial agents by pathogenic bacteria and fungi is increasing at an alarming rate and has become a global threat [3]. Many researchers are now engaged in developing new effective antimicrobial reagents with the emergence and increase of microbial organisms resistant to multiple antibiotics, which will increase the cost of health care. To circumvent this, novel methods or novel strategies are required to develop new bactericides. The successful approach is the use of natural antimicrobials, and more recently use of metal nanoparticles [4]. The strong toxicity of silver against wide range of microorganisms is well known and silver nanoparticles have been recently shown to be a promising antimicrobial material [5]. Several new applications of nanoparticles and nanomaterials are emerging rapidly [6, 7]. Nanocrystalline silver particles have found tremendous applications in the field of medicine, biosensing, imaging, drug delivery, nanodevice fabrication [8, 9], water treatment, cosmetics, electronics, household appliances [10] and also as catalyst [11]. They are reported to possess anti-inflammatory, antiplatelet, anti-viral activity [12].

Nanoparticles could be derived from various sources of gas, liquid or solid phases. There are many approaches for the synthesis of silver nanoparticles such as chemical synthesis, thermal decomposition, electrochemical, photochemical and biological synthesis. Chemical and physical methods for synthesizing nanoparticles are hazardous to environment, required high energy consumption and expensive [13]. For the synthesis of silver nanoparticles, the development of a reliable green process is very important in the current nanotechnology research. In chemistry and chemical technologies, the 'green' environment friendly processes are becoming increasingly popular and are much needed as a result of worldwide problems associated with environmental concerns [14]. The techniques for obtaining nanoparticles using naturally occurring reagents such as biodegradable polymers (chitosan, etc.), microorganisms, plant extracts as reductants and capping agents could be considered attractive for nanotechnology [15-17]. Greener syntheses of nanoparticles has many advantages over other methods as they are simple, efficient, one –pot, fast, cost-effective, stable, environment friendly, relatively reproducible and easily scaled up for large scale synthesis [18]. Reports on the synthesis of green silver nanoparticles using plant extracts such as *Populus alba*, *Hibiscus arboreous* and *Lantana camara*

[19], latex of *Plumeria rubra* [20] are available. Further, anti-bacterial properties of green silver nanoparticles synthesized from *Betua monosperma* (Palash) [21] flower extract have been reported.

Plumeria rubra L. species is grown throughout the tropical and subtropical world. About eight species are reported from India, but it is very difficult to fix their identity because of the overlapping character of some species [22]. *Plumeria rubra* L. (syn. P. acutifolia Poiret), commonly known as 'red Frangipani' is one of the commonly distributed member of this genus in India [23]. *Plumeria rubra* produces fragrant flowers generally red pink or purple centre rich with yellow with 5 spreading petals. Leaves are lance or oval shaped, and about 20cm to 30cm long. Extracts of the different plant parts like flowers, leaves, bark and latex are known to possess some important biological activities of such as antioxidant, antiulcer, antitumor, antimicrobial, abortifacient, anti-inflammatory, analgesic, anthelmintic, antipyretic, antifertility, and hypolipidemic [24-26]. The flowers contain several bioactive compounds, which includes plumieride, plumeric acid, β-sitosterol, lupeol, plumieride, amyrin, fulvoplumierin, plumieride and coumarate glucoside etc. [27]. In the present study, I established that an aqueous extract of *Plumeria rubra* flower was used in reduction of Ag(I) and in the formation of stable silver nanoparticles and tested the effect of antimicrobial activities. According to my knowledge this is the first report for the synthesis of AgNPs using *P rubra* flower extract.

Figure 1: *Plumeria rubra* flower

II. Materials And Methods

2.1. Preparation of flower extract

Fresh *Plumeria rubra* flowers were collected from the Bankura District, West Bengal, India (Figure 1). Fresh flowers were washed thoroughly with tap water to remove the dust and dirt particles and then washed with double distilled water and shade dried. Dried flowers were powdered in a mixer and stored in a container to prevent from moisture, and then this powder was preserved for experimental work. 10 g of flower powder was mixed with 100 ml double distilled water in 250 ml Borosil beaker and heated for 30 minutes. Then the extract was filtered through Whatman filter paper (pore size >0.5μm), collected and stored in refrigerator until being used.

2.2. Preparation of metal solution

Initially 1M $AgNO_3$ solution was prepared (4.25 g silver nitrate was dissolved in 25 ml double distilled water). From it 0.25 M $AgNO_3$solution was prepared.

2.3. Green Synthesis of Silver Nanoparticles using *Plumeria rubra* flower extracts

The aqueous *P rubra* flower extract had been used for the bio-reduction process. At first 1 ml of the flower extract was mixed with 5 ml of 0.25M $AgNO_3$ solution and kept at room temperature for 24 hours. Colour of the solutions ware changed from very light yellow to dark brownish indicating the formation of silver nanoparticles.

2.4. Characterization of Synthesized AgNPs

The synthesized AgNPs was primarily detected by visual observation of colour change. The bio-reduction of pure Ag^+ ions and formation of AgNPs from *P rubra* was monitored by measuring the UV–Vis spectrum of the reaction medium after diluting a small aliquot of the sample into deionized water. UV–Vis

spectral analysis was done by using Shimadzu UV–visible spectrophotometer (UV-1800). Deionised water was used as reference for background correction of experiments. The reduction of silver was measured periodically at 200–800 nm. A spectrum of silver nanoparticles was plotted with wave length on x-axis and absorbance on y-axis. The absorbance peaks can be observed in Figure 3. Transmission electron microscopy (TEM) technique was used to visualize the morphology of the AgNPs. TEM was done by using a JEOL JEM1400 plus microscope, operated at an accelerated voltage of 120 kV. TEM's grid size was 3 mm diameter which was prepared by spreading 5µl of the silver nanoparticles solutions on carbon-coated copper grids and drying under mercury lamp and then analysed.

2.5. Antimicrobial Assessment

The antibacterial activities of synthesized silver nanoparticle were carried out by standard agar well diffusion method. The antibacterial activities of the silver nanoparticle were tested against gram negative *Escherichia Coli (E. coli)* and gram positive *Bacillus sp*. Fresh overnight grown bacterial suspension of *E. coli* and *Bacillus sp*. were swabbed on the respective plates. Then, agar was punched with the help of sterile borer to create 6 mm well on each plate. 50µl of AgNPs solutions were placed on wells of agar plates respectively. Similar experiment was also carried out with the solution of *P rubra* flower extract alone to make the reference plate. All the plates were incubated at 37^{o}C for 48 hours in bacteriological incubator and after incubation the zone of inhibition was calculated by measuring the diameters of the inhibition zone around the well. All the experiments were carried out in triplicate.

III. Results And Discussion

Just after the addition of aqueous silver nitrate solution to the *P rubra* flower extract, a preliminary visual observation showed that the initial colour of the reaction mixture was light yellow. But remarkably the colour of the reaction mixture changed from light yellow then brown to dark brown exponentially with reaction-time (Figure 2) confirming the formation of silver nanoparticles due to excitation of surface plasmon vibration. Previous studies reports [28-30] similar colour changes which confirmed the completion of reaction between flower extract and $AgNO_3$.

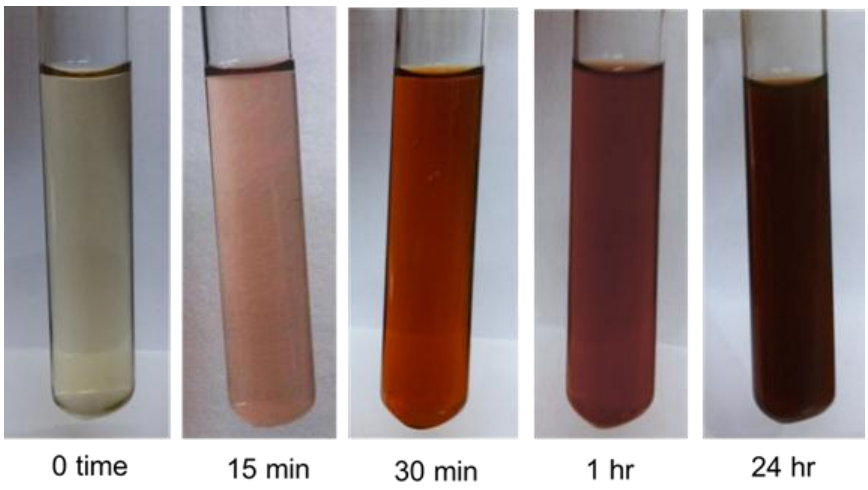

Figure 2: Digital photograph of bio-synthesized AgNPs from a mixture of aqueous *P rubra* flower extract and $AgNO_3$ solution at different time intervals.

The UV-vis spectra recorded after time intervals of 15 min, 30 min, 1 hr and 24 hr from the initiation of reaction are shown in Figure 3. The maximum absorbance peak at 450 nm in the UV-absorption spectrum indicated the presence of surface plasmon resonance peak of silver nanoparticles. The increase in intensity could be due to increasing number of nanoparticles formed as a result of bio-reduction of silver ions presented in the aqueous solution [31]. The spectra of silver nitrate solution and plant extract did not show any SPR band.

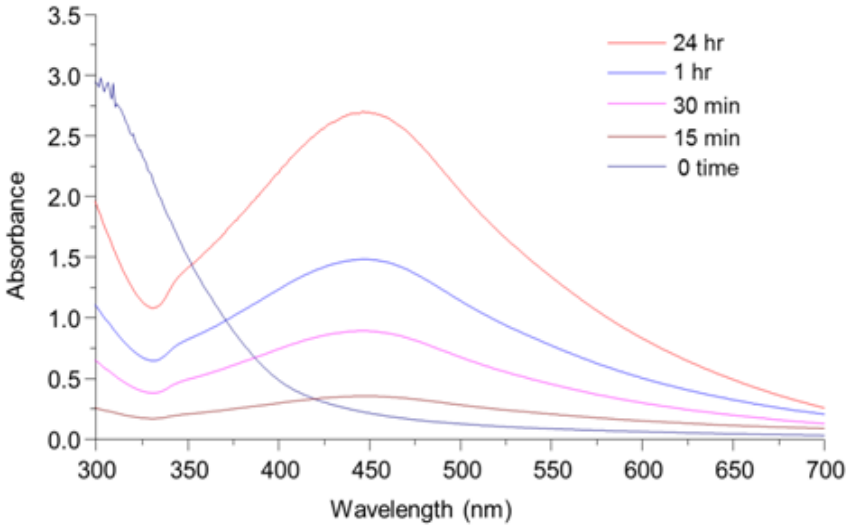

Figure 3: UV–visible absorption spectra of silver nanoparticles synthesized by aqueous flower extract of *P rubra* at different reaction time.

The shape, size and morphology of the synthesized silver nanoparticles were elucidated with the help of transmission electron microscopy (TEM) which further confirms the formation of silver nanoparticles. The size was in the range of 20-80 nm and the shape of the nanoparticles was spherical and irregular in shape with moderate variation in size.

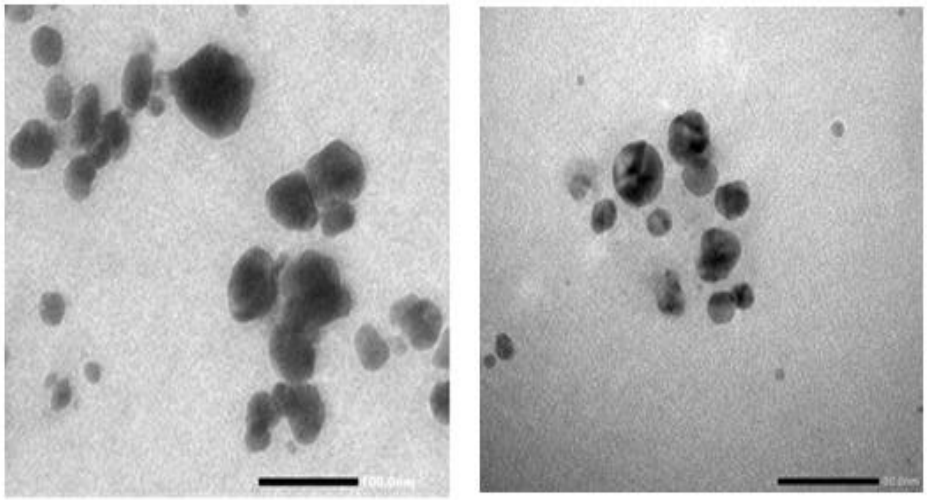

Figure 4: Transmission Electron Microscopy images of Silver Nanoparticles synthesized using *P rubra* flower extract

The antimicrobial activity of synthesized AgNPs were analysed by growing *Bacillus sp.* and *E. coli* colonies on agar plates. Figure 4(a) & 4(b), clearly showed growth of *Bacillus sp.*& *E. coli* and inhibition zones of 28 and 09 mm which indicates maximum antibacterial activity of the prepared AgNPs solution. Results obtained in previous studies [21, 32] also support the antibacterial potential of AgNPs. On the other hand, Figure 4(c) showed that *P rubra* flower extract alone did not exhibit any inhibition zone. The mechanism of the bacterial effect of AgNPs as proposed is due to the attachment of AgNPs to the surface of the cell membrane, thus disrupting permeability and respiration functions of the cell [33]. It is also proposed that AgNPs not only interact with the surface of a membrane but can also penetrate inside the bacteria [34].

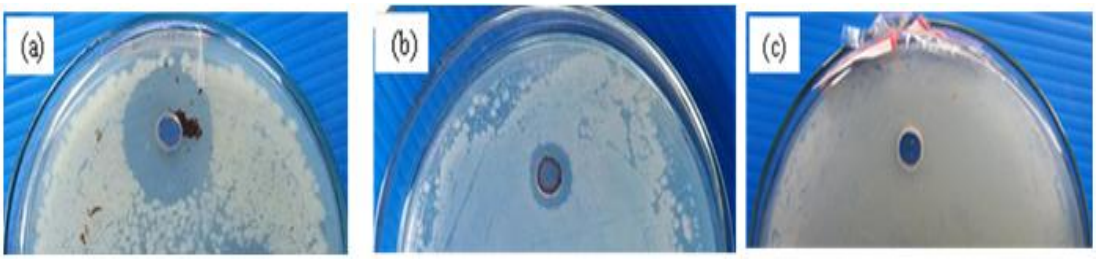

Figure 5: Antibacterial activity of AgNPs solution against (a) *Bacillus sp.* and (b) *E. coli*. *P rubra* flower extract alone did not exhibit any inhibition zone (c)

IV. Conclusions

Silver nanoparticles (AgNPs) were successfully synthesized from bioreduction of silver nitrate solutions using *Plumeria rubra* flower extracts. Different phytoconstituents which were present in the flower extracts, supposed to involve in the reduction of Ag(I) ions to Ag(0) and capping of silver nanoparticles. AgNPs have been appropriately characterized visually and further confirmed by UV-vis spectroscopy and TEM analysis. This type of phyto-mediated synthesis appears to be cost effective, one pot, eco-friendly and easy alternative approach to conventional physical and chemical methods. Therefore, this green method would be proving of developing a biological process for large-scale production of AgNPs. The synthesized silver nanoparticles showed efficient antimicrobial activities against Gram-positive and Gram-negative bacteria. Further studies should be conducted to better understand the mechanisms of action for improving biomedical properties of these silver nanoparticles.

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