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Green synthesis of silver nanoparticles using medicinal plant and its characterization

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ABSTRACT

Ecofriendly or Green synthesis of metal nanoparticles has become an important branch of nanotechnology and there is an increasing commercial demand for nanoparticles due to their wide applications. In the present study, we report an eco-friendly and economical way for the synthesis of silver nanoparticles using leaf extract Azadirachta indica. The plant properly known as 'neem' belongs to the family Meliaceae. For the synthesis of silver nanoparticles (SNPs) using the leaf extract of Azadirachta indica as a reducing agent from 1 mM silver nitrate (AgNO3) has been investigated. The resulting SNPs are characterized using UV–Vis, TEM. Silver nanoparticles were synthesized within 24 hours of incubation period and synthesized SNPs showed an absorption peak at around 400 nm in the UV-visible spectrum. The morphological study of Silver nanoparticles using TEM suggests that the nanoparticles are spherical in shape with a diameter around 50-nm. This route is rapid, simple without any hazardous chemicals as reducing or stabilizing agents and economical to synthesized SNPs.

Key words: *Azadirachta indica*, Green synthesis, Silver nanoparticles, UV-Vis, TEM.

INTRODUCTION

Nanotechnology, and alongside nanostructured materials, play an ever increasing role in science, research and development as well as also in every days life, as more and more products based on nanostructured materials are introduced to the market. The advance and very applicable technology is nanotechnology and it was derived from the term of nano it is the billionth of meter or 10^{-9} m. The Nano come ultimately from the Greek word for dwarf, and is also related to the Spanish word Nino [1]. The synthesis of silver nanomaterials or nanoparticles extensively studied by using chemical and physical methods, but the development of reliable technology to produce nanoparticles is an important aspect of nanotechnology. Biological synthesis process provides a wide range of environmentally acceptable methodology, low cost production and minimum time required. At the same time the biologically synthesized silver nanoparticles has many applications includes catalysts in chemical reactions [2]. Microbial source to produce the silver nanoparticles shows the great interest towards the precipitation of nanoparticles due to its metabolic activity. Of course the precipitation of nanoparticles in external environment of a cell, it shows the extracellular activity of organism. Extracellular synthesis of nanoparticles using cell filtrate could be beneficial over intracellular synthesis, the fungi being extremely good candidates for extracellular process and also environmental friendly. There are few reports published in literature on the biosynthesis of silver nanoparticles using fungal as source [3]. The use of bacterial strain in the bio-manufacturing process has the advantage that ease of handling than the fungal sources [4-7]. Plant biotechnology has opened up unexpected new ways for finding new way for trapping their potential resources. Silver nanoparticles (AgNPs), are the noble metal nanoparticles that has being studied extensively due its various biological properties [8]. Silver is a nontoxic, safe inorganic antibacterial agent used for centuries and it has the capability of killing different type of diseases causing microorganisms [9]. Silver has been known to be a potent antibacterial, antifungal and antiviral agent, but in recent years, the use of silver as a biocide in solution, suspension, and especially in nano-particulate form has experienced a dramatic revival. Due to the properties of silver at the nano level, nanosilver is currently used in an increasing number of consumer and medical products. The remarkably strong antimicrobial activity is a major reason for the recent increase in the development of products that contain nanosilver. The main challenge in nanomaterials synthesis is the control of their physical properties such as obtaining uniform particle size distribution, identical shape, morphology, chemical composition and crystal structure. For the production of nanoparticles, one needs to know the physical and chemical principles of nanoscale materials and also know how to commercialize them. Generally, metal nanoparticles can be prepared and stabilized by chemical, physical, and biological methods; the chemical approach, such as chemical reduction, electrochemical techniques, photochemical reduction [10-12], and pyrolysis [13], and physical methods, such as Arcdischarge and physical vapor condensation [14], are used. Living organisms have huge potentials for the production of nanoparticles of wide applications. A promising move towards to reach this objective is to develop the array of biological resources in nature. Indeed, over the past several years, plants, algae, fungi, bacteria and viruses have been used for production of inexpensive, energy-efficient and eco-friendly metallic nanoparticles. Many bacterial cultures were used for different kind of nanoparticles some are gold nanoparticles using *Shewanella algae*; it's a kind of marine bacterium [15], silver nanoparticles by *Cynobacteria Plectonema boryanum* [16], cadmium nanoparticles biosynthesis was done by *Clostridium thermoaceticum*, magnetite nanoparticles by *Actinobactor* sp., *Shewanella oneidensis* used for uranium nanoparticles [17].

Existing literature also reports successful synthesis of silver nanoparticles through a green route where the reducing and capping agent selected was the latex obtained from Jatropha curcas [18]. Ag NPs were also obtained using Aloe vera [19], Acalypha indica [20], Garcinia mangostana [21] leaf extracts. Crataegus douglasii fruit extract [22] as well as various other plant extracts [23] as reducing agent. Here we have developed a rapid, eco-friendly and convenient green method for the synthesis of silver nanoparticles from silver nitrate using leaf extracts of three Indian medicinal, namely, Musa balbisiana (banana), A. indica (neem) and O. tenuiflorum (black tulsi), by microwave irradiation method. In this research, the plant mediated synthesized Ag NPs were characterized and studied in details with all of their properties significant to current science and prevailing technologies. However, the biosynthesis of silver nanoparticles by free cell system and culture filtrate has not been investigated yet. In this paper, we report on the synthesis of silver nanoparticles by the reduction of aqueous Ag+ ion by simultaneous reduction of aqueous Ag+ with the leaf extract of medicinal plant. Through our screening process involving a number of plants we observed that were potential candidate for rapid synthesis of silver nanoparticles.

MATERIALS AND METHODS

Materials
Silver nitrate used for the synthesis of silver nanoparticles was procured from E. Merck, (India) Limited, Mumbai, India. *A. indica* (Neem) used in this work were collected from the garden. Cultures Dehydrated Luria broth and Nutrient agar media used for bacterial growth study were the products of E. Merck, (India) Limited, Mumbai, India.

Preparation of the leaf extract
Indian medicinal plant A. indica (neem) was selected from Lucknow, India, on the basis of cost effectiveness, ease of availability and medicinal property. Fresh and healthy leaves were collected locally and rinsed thoroughly first with tap water followed by distilled water to remove all the dust and unwanted visible particles, cut into small pieces and dried at room temperature. About 5 g of leaves were weighed separately and transferred into 100 mL beakers containing 50 mL distilled water and boiled for about 10 min. The extracts were then filtered thrice through Whatman No. 1 filter paper to remove particulate matter and to get clear solutions which were then refrigerated (4°C) in 100 mL Erlenmeyer flasks for further experiments.

Synthesis of silver nanoparticles
For the synthesis of silver nanoparticles, 1 mM silver nitrate and leaf extract were taken. For the reduction of Ag+ ions, 5 ml of leaf extract was added drop wise to 5 ml of 1 mM silver nitrate solution. A distinct color change was

observed after 24 hrs as the solution turned into brown from yellow solution at room temperature suggesting formation of silver nanoparticles. The color became brown and turned into dark brown after 48 hrs.

The reduction of Ag+ was confirmed from the UV–Vis spectrum of the solution. The nanoparticles were separated out from the mixture by ultracentrifugation (at 10000 rpm for 4 hrs).

Characterization of silver nanoparticles
UV-Vis Spectroscopy
The reaction mixture was subjected to UV-Vis Spectrophotometric Measurements (Model UV-1600 PC). According to this technique many molecules absorb ultraviolet or visible light. The percentage of transmittance light radiations determines when light of certain frequency passed through the samples. This spectrophotometer analyses records the intensity of absorbance or optical density (O.D) as a function of wavelength. Absorption is directly proportional to the concentration of the absorbing species (Beer's law).

Formation of silver nanoparticles is easily detected by spectroscopy because the colored nanoparticle solution shows a peak ~400 nm. In this study, spectrophotometer was used to measure the optical density of solutions or suspensions.

Transmission Electron Microscopy (TEM)
This study was undertaken to know the morphology and particle size distribution of silver nanoparticles . TEM shows the shape and crystal structure as well as size of the particles. The grid for TEM analysis was prepared by placing a drop of the nanoparticle suspension on a carbon-coated copper grid and allowing the water to evaporate inside a vacuum dryer. The grid containing silver nanoparticles was scanned by a Transmission Electron Microscope.

RESULTS AND DISCUSSION

For the synthesis of nanoparticles, report an eco-friendly and economical way using leaf extract *Azadirachta indica* (Neem) **[Figure 1]**.

Fig 1: ***Azadirachta indica*** **(Neem)**

Fig 2: Synthesis of silver nanoparticles

After reduction for 48 hours, culture filterate color changed from yellow to brown. Formation of brown is due to the surface plasmon resonance property of silver nanoparticles. Aqueous silver nitrate ions were reduced during exposure to the Neem leaf extract. The color of the reaction mixture changed from yellow to brown indicates the formation of silver nanoparticles **(Figure 2)**. Due to excitation of surface Plasmon vibration in metal nanoparticles, silver nanoparticles exhibit brownish color in water.

UV-Vis Spectroscopy

The production of silver nanoparticles by reduction of silver ions due to the addition of Neem leaf (*Azadirachta indica*) extract was followed by UV–Vis spectroscopy. The UV-Vis absorption spectrum of 'Green' silver nanoparticles in the presence of Neem leaf extract is shown in **Figure 3**. The band in silver nanoparticles solution was found to be close to 400 nm throughout the observation period as the nanoparticles were dispersed in the solution without possibility for aggregation in UV-Vis spectrum. The high OD of the solution suggests a high conversion of Ag^{+} to Ag^{0} as nanoparticle.

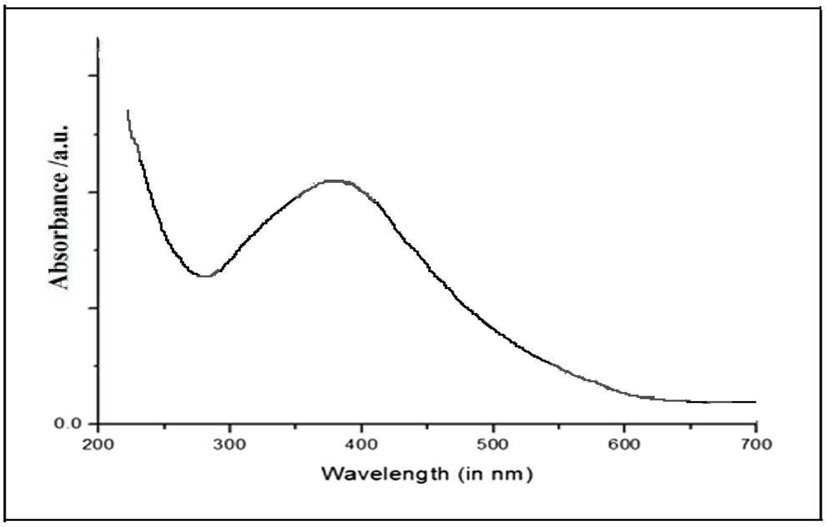

Fig 3: UV-visible absorbance spectra obtained from silver nanoparticles

Transmission electron microscopy studies

TEM analysed the silver nanoparticles coated on carbon coated copper TEM grid. This micrograph showed that they are well-disperse and size ranging from 20-50 nm. The morphology of nanoparticles is essentially spherical. The results are shown in **Figure 4**.

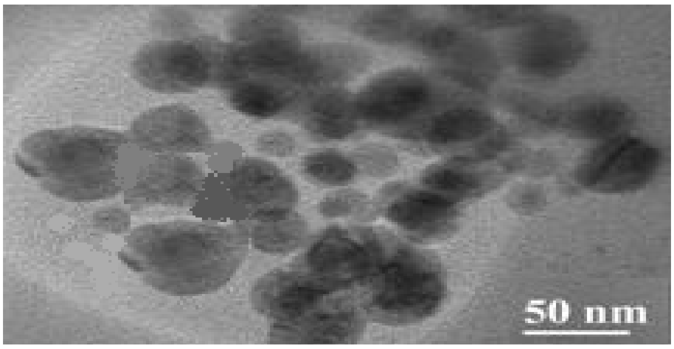

Fig 4: TEM image of Silver Nanoparticles

CONCLUSION

Silver nanoparticles (AgNPs) were successfully obtained from bioreduction of silver nitrate solutions using neem leaf extracts. In summary, visual observations, UV–Vis and TEM spectroscopic techniques confirmed the formation of silver nanoparticles by neem leaf extracts. This work indicates that neem leaf extract had a good valuable potential in the future for production of silver nanoparticles. Hence, due to their benign and stable nature these Silver nanoparticles (AgNPs) may be well utilized in industrial and remedial purposes. However, plant uptake and utilization of Silver nanoparticles (AgNPs) require more detailed research on many issues like uptake potential of various species, process of uptake and translocation and the activities of the AgNPs at the cellular and molecular levels.

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