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In-vitro bio-fabrication of silver nanoparticle using *Adhathoda vasica* leaf extract and its anti-microbial activity

G.M. Nazeruddin [a], N.R. Prasad [a,b,*], S.R. Prasad [c], K.M. Garadkar [b], Arpan Kumar Nayak [d]

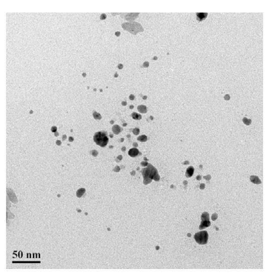
[a] *Poona College of Arts, Commerce & Science, Pune, India*
[b] *Solid State Research Laboratory, Department of Chemistry, Shivaji University, Kolhapur 416004, Maharashtra, India*
[c] *Walchand College of Engineering, Sangli, India*
[d] *Department of Material Science, Indian Institute of Technology, Kharagpur, India*

HIGHLIGHTS

- Green synthesis of nanoparticles.
- Antimicrobial activity of synthesized nanoparticles.
- Synergetic effective study of nanoparticles and/with common antibiotic medicine penicillin.

GRAPHICAL ABSTRACT

We have developed a novel method to synthesize silver nanoparticles by mixing silver salt solution with leaf extract of *Adhathoda vasica* (Adulsa) without using any surfactant or external energy. By this method physiologically stable, bio-compatible Ag nanoparticles were formed which could be used for a variety of applications. With this method rapid synthesis of nanoparticles was observed to occur; i.e. reaction time was 1–2 h as compared to 2–4 days required by microorganisms. TEM images shown reveal that there was polydisperse spherical particles with non-uniform distribution of nanoparticles in the prepared sample. It was observed that the nanoparticles formed were of different sizes and particle size was found to be 4.74 nm, 8.17 nm, 14.23 nm and 18.98 nm and the mean size of about 11.5 nm which lies in the nano-range.



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ABSTRACT

It is well known that on treating the metallic salt solution with some plant extracts, a rapid reduction occurs leading to the formation of highly stable metal nanoparticles. Extracellular synthesis of metal nanoparticles using extracts of plants like *Azadirachta indica* (Neem), and *Zingiber officinale* (Ginger) has been reported to be successfully carried out. In this study we have developed a novel method to synthesize silver nanoparticles by mixing silver salt solution with leaf extract of *Adhathoda vasica* (Adulsa) without using any surfactant or external energy. By this method physiologically stable, bio-compatible Ag nanoparticles were formed which could be used for a variety of applications such as targeted drug delivery which ensures enhanced therapeutic efficacy and minimal side effects. With this method rapid synthesis of nanoparticles was observed to occur; i.e. reaction time was 1–2 h as compared to 2–4 days required by microorganisms. These nanoparticles were analyzed by various characterization techniques to reveal their morphology, chemical composition, and antimicrobial activity. TEM image of these NPs indicated the formation of spherical, non-uniform, polydispersed nanoparticles. A detailed study of anti-microbial activity of nanoparticles was carried out.

* Corresponding author at: Department of Nanoscience and Technology, Solid State Research Laboratory, Department of Chemistry, Shivaji University, Kolhapur 416004, Maharashtra, India. Tel.: +91 940 4265521.
E-mail address: neeraj_prasad21@rediffmail.com (N.R. Prasad).

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1. Introduction

The nanoparticles are conventionally synthesized by various routes such as electro-chemical [1], photochemical reduction [2,3] and heat evaporation [4,5] but most of these strategies consist of utilization of high energy, hazardous chemicals and difficulty in purification. To switchover these technical hitches biological principles have been established recently and material scientists are trying to understand, mimic and deploy nature for this purpose [6].

Nature has devised ingenious and elegant way of creating the most efficient miniaturized functional material. There are abundant examples of nanoparticles within a biological system such as DNA, chromosomes, biomolecules and even biochemical reactions occur at nanoscale time range with nano-molar concentration. There is a rich and long history of gaining inspiration from nature for the design of noble materials and structures [7,8]. Therefore a growing number of interdisciplinary research themes have emerged at the frontier between biology and material sciences [9]. Biological methods for the synthesis of nanoparticles employ living organisms viz microorganism and plants, molecules of biological origin such as peptides and biological templates such as DNA [10]. Nanomaterials have a long list of applicability in improving human life and its environment. Metallic nanoparticles are intensely studied due to their unique optical, electrical and catalytic properties [11,12]. The way in which a biological system fabricates and reproduces structural and functional inorganic materials with precise dimensions and controlled morphology has attracted nanotechnologist [13].

It has been found that 5000 years old Indian System of Medicine *Ayurveda* had some knowledge of nano-scale fabrication used for medicinal purposes. Thus we can claim that nanotechnology was in existence even before the term nano was coined. A bulk material has constant physical properties regardless of its size, but at the nano-scale often this is not true. Several well characterized bulk materials have been found to possess most interesting properties when studied at nanoscale and this is mainly due to a very high aspect ratio possessed by nanoparticles [14]. Nanoparticles are of great scientific interest as they bridge the gap between bulk level and atomic-molecular level. To utilize and optimize chemical and physical properties of nanosized metal particles, a large spectrum of research has been focused to control the size and shape which is crucial in tuning their properties [15].

Nanoparticles can be produced either intra- or extra-cellularly by using living organism. It was demonstrated that parallel to the chemical process, it is also possible to synthesize gold nanoparticles of uniform size extra-cellularly using extremophilic actinomycete *Thermomonospora* sp. There are some species of Algae which are used as bio-factory for the synthesis of metallic nanoparticles; Singaravelu et al. have reported to synthesize the metallic nanoparticles by using *Sargassum*. Fungi such as *Verticillium, Fumigatus, Trichoderma, Asperellium,* and *Phapnerochaete chrysosporium* have been explored and exploited for noble metallic nanoparticles synthesis. Recently synthesis of metal nanoparticles using plants is being looked into with interest. While microorganisms continued to be investigated for bio-mineralization and metal nanoparticles synthesis, the use of plant extracts in similar nanoparticles biosynthesis methodologies is an exciting possibility and is relatively unexplored and under-exploited [16].

Plants have been known to bio-mineralize calcium carbonate, silica and even magnetite internally. Similar to micro-organisms, plants have also been used for purification of heavy metal ions from contaminated soil and water. Certain plants are known to hyper-accumulate these heavy metals or can even be induced to hyper-accumulate within different parts of plants. The internal accumulation of metal in plants can occur both via complexation of the metal ion with a suitable bio-ligand in its native oxidation state or after its reduction to a lower oxidation state. The possibility of reduction of metal ions by plants and the presence of metal complexing agents in them entices a materials scientist to use plants for the goal of synthesizing nanoparticles and controlling their size and shape and to experiment on the likelihood of forming nanoparticles of low reduction potential metals. The possibility of synthesizing nanoparticles of different compositions using plants would offer an environmental friendly alternative to the existing potentially toxic chemical and physical methods of preparations. This would also help stave off the growing apprehensions related to environmental degradation and biological hazards apart from being a cost-effective process with potential to scale up for large scale synthesis. In fact Jose-Yacaman and co-workers have shown that live alfalfa plants when supplied with Au^{3+} ions reduce them to Au^0 state and absorb them resulting in the internal formation of gold nanoparticles. Jose-Yacaman's group has also extended this to demonstrate the synthesis of assemblies of silver nanoparticles within alfalfa shoots by supplying the plants with Ag^+ ions.

While these reports demonstrated the possibility of synthesizing metal nanoparticles using plants, they suffer with the inherent complication of being intracellular, making the isolation of particles an additional difficult job. Thus to overcome this internalization problem, extract from well known commercial plants like *Coffea arabica*, and *Cymbopogon citrus* was used as green reagents in AgNPs synthesis resulting in extracellular synthesis. By reducing silver nitrate in solution of tea extract or epicatechin of varying concentrations, spherical silver nanoparticles were formed that had controllable size distribution depending upon the concentration of tea extract and epicatechin in the samples. Solution temperature, concentration of metal salt, pH, capping agent, reducing agent and reaction time all influence the shape and size of particle. High amount of poly-phenolic compounds in the plant extract is generally supposed to influence the reduction process and stabilize nanoparticles preventing agglomeration. These nanoparticles are studied for their antibacterial, antioxidant, and antitumor properties [17,18].

In this research work for metal nanoparticles synthesis an extra-cellular synthesis approach i.e. the extracts of plants instead of live plants are used as an alternative to chemical or microbiological synthetic processes. This approach does not require an elaborate process such as intracellular synthesis, multiple purification steps, and maintenance of microbial cell culture. In the green chemistry approach of synthesis the reaction is normally carried out at room temperature although in some processes a little heating below 100 °C was applied. It is well known that few medicinal plants exhibit anti-oxidant property. Thus they can act as biological source of reducing agent. On this belief choice of plants for this purpose was for those carrying medicinal and aromatic properties. In addition they also make the nanoparticles biologically more effective. Shankar et al. have reported synthesis of silver and gold nanoparticles using decoction solution of *Azadirachata indica*. However, the nanoparticles synthesized by them are in the range of 50–100 nm and are not well separated from each other and tend to form agglomerated structures [19].

In the present research work *Adhatoda vasica* leaf extract has been used as a reducing agent. The same extract also acts as a capping agent. *A. vasica* is widely available plant in tropical country like India. A thorough study of the literature reveals that the major components of the *A. vasica* leaf are Vasicine—$C_{11}H_{12}N_2O$, Adhatodic acid (about 0.25%), volatile oil, sucrose etc. In addition, there may be traces of some undiscovered compositions. Thus in the present state it is difficult to identify the exact chemical in Adhathoda which acts as a reducing agent. Still an interpretation about the ingredient responsible for reduction can