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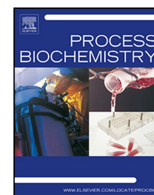

Green synthesis of silver nanoparticles with *Dalbergia spinosa* leaves and their applications in biological and catalytic activities

N. Muniyappan, N.S. Nagarajan*

Department of Chemistry, Gandhigram Rural Institute – Deemed University, Gandhigram 624302, Tamil Nadu, India

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ABSTRACT

The phytosynthesis of silver nanoparticles (AgNPs) by *Dalbergia spinosa* leaves (DSL) in aqueous extract was investigated. AgNPs were characterized by UV–visible absorption spectroscopy (UV–vis), transmission electron microscopy (TEM) and Fourier transform infra red spectrophotometry (FTIR). The results showed that the increase in the initial extract concentration at room temperature increased the mean size and widened the size distribution of the AgNPs, leading to a red shift and broadening the surface plasmon resonance absorption (439 nm). The results showed that the reducing sugars and flavonoids were primarily responsible for the bioreduction of silver ions and that their reductive capability was promoted at 36 °C. TEM analysis showed that the AgNPs were nearly spherical in shape with an average size of 18 ± 4 nm. When evaluated for *in vitro* antioxidant activity by DPPH, NO, hydrogen peroxide radicals, reducing power and CUPRAC assay methods in addition to anti-inflammatory activity by HBRC method, the silver nanoparticles exhibited considerably enhanced antioxidant and anti-inflammatory activity at the test doses when compared with that of the standards and the plant extract. Finally, the antibacterial activity of the AgNPs against two Gram-positive bacteria and two Gram-negative bacteria showed moderate antibacterial activity when compared with the standard and the plant extract. The synthesized silver nanoparticles were also effective in the catalytic reduction of 4-nitrophenol (4-NP) into 4-aminophenol (4-AP).

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1. Introduction

Natural products from plants, animals and minerals have been serving as sources of drugs for treating human diseases since time immemorial. Our ancestors derived therapeutic materials from thousands of plants. Considerable research on phytochemistry, pharmacology, toxicology and to a lesser extent on clinical pharmacology is being performed on medicinal plants [1]. Many major pharmaceutical industries have renewed their interest in favor of plant drugs, and numerous drugs have entered the international pharmacopeia through the study of ethnopharmacology and traditional remedies.

Of the 135 known *Dalbergia* species in the world, only 35 are reportedly present in India [2,3]. *Dalbergia* species possess a wide range of medicinal properties [4]. The phytochemical examination of various *Dalbergia* species has resulted in the isolation and characterization of a large number of compounds such as flavonoids, isoflavonoids, neoflavonoids, steroids, terpenoids, *etc.*, and a number of phytochemical reviews have been published on *Dalbergia* species [5–8].

Flavonoids are a large family of 4000 secondary metabolites [9] that occupy a prominent position among the polyphenols. A characteristic feature of this group of compounds is its ability to interact with primary metabolites such as proteins and polysaccharides. The pivotal role played by nearly 8000 flavonoids identified from vascular plants and their medicinal properties has become an important research subject in recent years [10]. Because flavonoids and isoflavonoids reportedly possess varied biological activities, introducing these compounds into modern medicine might be a valuable alternative because the therapeutic efficacy of isoflavonoids is well-supported.

Dalbergia is a source of a large number of compounds including flavonoids and isoflavonoids. *Dalbergia* species that have been exhaustively studied and noted for various pharmacological activities include *Dalbergia spinosa*, *Dalbergia paniculata*, *Dalbergia sissoo*, *Dalbergia sissoides*, *Dalbergia volubilis*, *Dalbergia parviflora*, *Dalbergia nigra*, *Dalbergia sympathetic*, *Dalbergia horrida etc.* The pharmacological studies performed to date include antimicrobial, antihypertensive, immunomodulatory, anti-inflammatory,

* Corresponding author. Tel.: +91 9442029933; fax: +91 451 2454466.
E-mail addresses: nsnrajan@yahoo.co.in, natesan.nagarajan@gmail.com (N.S. Nagarajan).

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antioxidant, anti-diabetic, anticancer, anti-arthritic, antifungal, antibiotic, hematinic, anti-hyperlipidemic, anti-androgenic, anti-anxiolytic, antiulcer activities, *etc.* [9–11]. The inflammatory response involves a complex array of enzyme activation, fluid extravasation, cell migration, tissue breakdown and repair, which are aimed at host defense and are usually activated under most disease conditions [12].

Our previous phytochemical evaluation of *D. spinosa* has resulted in the isolation of prunetin, 7-O-methyl tectorigenin, dalspinin, dalspinosin, prunetin-4′-O-β-D-galactoside and 7-O-methyltectorigenin-4′-O-β-D-galactoside [13].

The antibacterial activity of a number of plants and plant products has been investigated by many researchers [14,15]. *Dalbergia monetaria* and some of the phytoconstituents of *Dalbergia candenatensis* reportedly possess high antibacterial activity [16]. The three primary methods involved in the screening of antibacterial activity include the diffusion method, dilution method and bioautography method. In the diffusion method, the zone of inhibition is measured by using a disk, a hole or a cylinder as a reservoir from which the sample is allowed to diffuse in an inoculated medium [17,18]. The dilution method involves a homogenous dispersion of sample into the broth culture required for the organism to be assayed and the transmittance measurement at 530 nm [19]. In the bioautography method, a suspension of microorganisms in a liquid nutrient medium is sprayed on a developed chromoplate (using TLC) and the zone of inhibition is observed after incubation [20]. The adverse effects of modern drugs and the lower availability of proper medicines for emerging diseases has resulted in more emphasis on the development of potent, safe and novel drugs from natural sources.

The creation of useful functional materials, devices and systems by controlling and manipulating matter on the nanometer length scale (1–100 nm) and exploiting novel phenomena and properties such as the physical, chemical, biological, mechanical, and electrical characteristics at the nanoscale has gained importance [21]. Nanoparticles exhibit totally new or improved properties based on specific characteristics such as size, distribution and morphology [22]. Nanoparticles exhibit varying interactions in electrical, optical, magnetic and chemical properties. AgNPs possess antifungal, antibacterial, antiviral and antioxidant properties [23]. Most of the methods used to synthesize nanoparticles involve a high energy requirement in addition to low material conversion and unnecessarily wasteful purifications. The methods also involve hazardous chemicals. Silver nanoparticles have diverse *in vitro* and *in vivo* applications. Recently, biosynthetic methods employing either microorganisms, such as bacteria [24] or fungi [25], or plant extracts [26,27] have emerged as simple and viable alternatives to more complex chemical synthetic procedures for obtaining nanomaterials. Although many routes are available for the synthesis of silver nanoparticles, biological synthesis from plant sources offers several advantages such as cost-effectiveness, non-toxic and eco-friendly products *etc.* [28], obviating the need to design methods that are more suitable for pharmaceutical and biomedical applications.

With the abundant availability of isoflavones in *Dalbergia* species and their therapeutic potential in mind, we report the phytosynthesis of silver nanoparticles at room temperature by using an aqueous extract of *Dalbergia spinosa* leaves and screening test drugs for their varied biological and catalytic activities.

2. Materials and methods

2.1. Materials

All chemicals were of analytical grade, and they were obtained commercially and used without further purification. Silver nitrate (99.9%), DPPH, phosphate buffer, peptone, streptomycin sulfate, diclofenac and ascorbic acid were purchased from Sigma–Aldrich, Mumbai, Maharashtra, India. All apparatus were rinsed with aqua regia (a 3:1 solution of $HCl:HNO_3$) and then washed with Millipore water before use. All solutions were prepared in Millipore water.

2.2. The phytosynthesis of silver nanoparticles

D. spinosa is a delicate shrub belonging to the *Leguminosae* family. The leaves of *D. spinosa* (DSL) used in this study were collected from the Nagamali Hills near Madurai Kamaraj University campus, Madurai, Tamil Nadu. The leaves were primarily cleaned with water, washed and dried by pressing with blotting paper. They were then shade-dried and cut into small pieces. Ten g of leaves in 100 ml of Millipore water were microwave-irradiated for 5 min and the extract was filtered and stored at 4 °C for further experiments.

A 50 ml portion of aqueous 0.1 M $AgNO_3$ was transferred to a conical flask and 5 ml of aqueous DSL extract was added with vigorous magnetic stirring for 10 min. The reduction of silver ions into silver particles at room temperature was completed in 30 min, as observed by the changing color of the aqueous extract from greenish yellow to dark brown to indicate the formation of silver nanoparticles (Fig. 1).

For the purification of AgNPs, the fully reduced solution was centrifuged at 8000 rpm for 30 min. The supernatant liquid was discarded and the residue was dispersed in Millipore water. The samples were centrifuged five times to wash off any substances that had been absorbed onto the surface of the silver nanoparticles.

2.3. Characterization

The absorption spectra were measured with a Perkin Elmer Lambda 35 UV-Visible spectrophotometer with a 1 cm quartz cell. FTIR spectra were recorded at room temperature using a (JASCO FTIR 400). The spectra of the capped silver nanoparticles were measured with a small amount of AgNPs dried at 60 °C for 4 h and mixed with KBr to form a round disk suitable for FTIR measurements. The silver nanoparticle solution was prepared by dropping AgNPs onto the var-coated copper grid and air-dried. The morphology and composition of the product were identified by High Resolution Transmission Electron Microscopy (HR-TEM). Images of the AgNps were obtained from a JEOL TEM 3010 operating at 200 kV.

2.4. Antibacterial activity of the test drug

This study involves the antibacterial activity screening of the test drugs, that is, the DSL crude extract and the AgNPs evaluated by disk diffusion method [17]. The microorganisms used for the experiment were standard cultures of Gram-positive bacteria *Bacillus subtilis* and *Pseudomonas aeruginosa* and gram negative bacteria *Staphylococcus aureus* and *Escherichia coli*, which were procured from the Department of Biology, GRI, Gandhigram. The microorganisms were identified by standard staining techniques and biochemical reactions. The microorganisms were maintained by sub-culturing at regular time intervals on nutrient agar medium. The microorganism suspension was prepared by following the McFarland nephelometer standard.

2.5. Standard culture method

A suspension of microorganisms containing approximately 1 × 108 cells per ml was obtained by adjusting the optical density of the suspension to that of 0.033 ml of 1.75% $BaCl_2$ in 10 ml of 1% H_2SO_4. A 24-h-old culture was used to determine the zone of inhibition for the test drugs. Nutrient agar medium was prepared by dissolving beef extract (3 g), peptone (5 g) and agar (15 g) in 1000 ml of distilled water for the preliminary antibacterial study to evaluate the zone of inhibition. All the necessary precautions with regards to sterilization were adopted by using standard methods. Sterile molten agar medium (30 ml) was allowed to solidify in sterile Petri dishes. A suspension of the bacterial inoculums of interest (1 × 108 CFU per ml) was inoculated with sterile cotton swabs. Five holes were made in the medium with a sterile borer. A 0.1 ml portion of each test drug was added to the holes and they were allowed to diffuse by keeping the Petri dishes at 4 °C in the refrigerator for 1 h.

Streptomycin sulfate (0.1 ml, 10% solution) was used as the standard reference in all the experiments. After the diffusion, the Petri dishes were incubated at 37 ± 1 °C for 1 h, and the zone of inhibition was observed and measured by using a scale. The antibacterial activity of all of the test drugs was evaluated for all four types of microorganisms in a similar manner. The observations and data are shown in Table 1.

2.6. Minimum inhibitory concentration (MIC) determination

The antibacterial activity of natural products was studied by employing a microdilution method by using two different culture media, namely Mueller-Hinton broth and Luria Bertani (LB). The inoculum was prepared as previously described. Natural products were dissolved in DMSO (10% of the final volume) and diluted with culture broth to a concentration of 100–300 mg per mL.

Additional 1, 2 serial dilutions were performed by adding culture broth to reach concentrations ranging from 100 to 300 mg per mL, and 100 μL of each dilution was distributed in 96-well plates, along with a sterility control and a growth control (containing culture broth plus DMSO without antimicrobial substances). Each

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