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Synthesis of silver nanoparticles using *Cardiospermum halicacabum* L. leaf extract and their characterization

Mahipal S. Shekhawat, M. Manokari, N. Kannan, J. Revathi, R. Latha*

Mahipal S. Shekhawat
Biotechnology Unit, K.M. Centre for P.G. Studies, Pondicherry 605008, India

M. Manokari, N. Kannan
Biotechnology Unit, K.M. Centre for P.G. Studies, Pondicherry 605008, India

J. Revathi, R. Latha
Biotechnology Unit, K.M. Centre for P.G. Studies, Pondicherry 605008, India

Correspondence:
Dr. Mahipal S. Shekhawat
Biotechnology Unit, K.M. Centre for P.G. Studies, Pondicherry 605008, India
Phone: +914802735737
Fax: 04802735837
E-mail:
annshinepaul@gmail.com

Abstract

Present study reports the biogenesis of silver nanoparticles from the leaves of *Cardiospermum halicacabum* L. and their characterization. *C. halicacabum* has been used in traditional medicines but so far it has not been tested for synthesis of silver nanoparticles (AgNPs). The aqueous silver ions exposed to the herbal extract, which were reduced and the nanoparticles were synthesized. The presence of nanoparticles was confirmed by the formation of brown color of the reaction mixture. The brown color was observed after 25 minutes. The silver nanoparticules qualitatively characterized by UV-Visible spectrophotometer. A sharp peak was observed in between 422nm to 447nm indicates formation of silver nanoparticles.

Keywords: *Cardiospermum halicacabum* L., Characterization, Green synthesis, Silver nanoparticles.

Introduction

Herbal-mineral formulations of Ayurvedic system constituting Bhasma as ingredients are as superior as it is even today. Manufacturing methods of Bhasma are in tune of nanotechnology of contemporary era and proved advancement of Rasashastra, a branch of Ayurvedic system, which may cover scientific validation of today. These medicines are safe in therapeutics. There are various methods for AgNPs formation such as sol-gel process, chemical precipitation, reverse micelle method, hydrothermal method, microwave, chemical vapour deposition and biological methods.[1, 2] Biological methods are currently gaining importance because they are eco-friendly, cost effective and free from any toxic chemicals.[3, 4]

There have been several reports on the synthesis of AgNPs using medicinal plants such as *Helianthus annus*, *Saccharum officinarum*, *Sorghum bicolour*, *Zea mays*[5], *Medicago sativa*[6], *Capsicum annuum*[7], *Turnera ulmifolia*, *Couroupita guianensis*[8, 9] *Magnolia kobus*[10] and *Geranium sp.*[11] etc. for pharmaceutical and biological applications. Recently, some studies have shown that specially formulated AgNPs have good antibacterial activity.[12] Shekhawat et al., reported that the silver nanoparticles from *Turnera ulmifolia* leaf and *Couroupita guianensis* plant extracts were active against human pathogenic bacteria and fungi.[8, 9]

C. halicacabum L. is a highly important medicinal plant belongs to the family Sapindaceae. It is also known as Ballon vine, Love in a Puff, Heart seed, Modakathon etc. It is used as a natural healer for hay fever, allergies, sneezing, watery eyes, and allergic reactions. It is also one of the ingredients in "Allergy Relief Liquid™"

and “Bioforce Pollinosan® Tabs”. Balloon vine is considered to be a traditional medicinal herb[13] and the leaves are used to treat diarrhea, dysentery, Oedema, Nephritis and Oliguria haemorrhoids, asthma, pyodermas and carbuncles, earaches, ophthalmias and muscular pains, rheumatism and arthritis.[14-16] The interaction of nanoparticles with biomolecules and microorganisms is an expanding field of research. In this study, we demonstrate the synthesis and characterization of silver phyto-nanoparticles from *C. halicacabum* leaf extract using green methods.

Materials and methods

Collection of plant materials and extract preparation: *Cardiospermum halicacabum* L. is popularly known as Modakathon in South India because of its anti-arthritic property. It is an important medicinal climber in India. The plant material was collected from the coastal area of Pondicherry, India. Fresh, green and mature leaves were harvested and thoroughly washed with distilled water. The leaves were finely cut in small pieces. The plant leaf broth solution was prepared by using 5 gm of washed and cut leaves in a 250 ml Erlenmeyer flask with 50 ml of sterile distilled water and then boiling the mixture for 5 min. The herbal aqueous extract was collected in separate conical flasks by standard filtration method and stored at 4^{o}C.

Preparation of 1mm aqueous solution of silver nitrate: 0.017g of Silver Nitrate ($AgNO_3$) (Himedia, Mumbai) was added to the 100ml of distilled water and the solution was stirred well continuously until the silver nitrate is dissolved completely. This 1mM Silver Nitrate solution is stored in brown bottle at 4oC for further use for the synthesis of Silver Nanoparticles from Cardiospermum leaf aqueous extract.

Synthesis of silver nanoparticles: 1mM aqueous solution of Silver nitrate was used for synthesis of silver nanoparticles. For the synthesis of AgNPs, two boiling tubes were taken, one containing 10 ml of1mM $AgNO_3$ solution as control and the second containing 9.0 ml of 1mM Silver nitrate solution and 1.0 ml of plant leaf extracts as test solution. These were incubated at room temperature for 12 hrs. The color change of the leaf extracts from yellowish green to dark brown was checked periodically. The reaction mixtures were centrifuged at 5000 rpm for 15 minutes in order to obtain the pellet which is used for further study. Supernatant is discarded and the pellet is dissolved in deionised water.

Characterization of silver nanoparticles: Due to the unique optical properties of silver nanoparticles, a great deal of information about the physical state of the nanoparticles can be obtained by analyzing the spectral properties of silver nanoparticles in solution. So it is necessary to characterize them. The formation of Ag Nanoparticles were characterized visually with naked eyes by the color change in greenish yellow to dark brown and qualitatively characterized by UV-Visible spectrophotometer.

UV- Vis spectral analysis: Carefully monitoring the UV-Visible spectrum of the silver nanoparticles with time is a sensitive technique used in determining if any nanoparticle aggregation has occurred. The reduction of metallic Ag+ ions was monitored by measuring the UV-Vis spectrum using a UV-VIS Spectrophotometer (Systronics Double beam spectrophotometer, Model 2202, Systronics Ltd.). A small aliquot was drawn from the reaction mixture and a spectrum was taken on a wavelength from 300 nm to 700 nm. The time duration of change in color varies from plant to plant. The protocol was repeated for three times with regular time intervals in order to correlate the data.

Results and discussion

Nanoparticles are often referred to as particles with a maximum size of 100 nm, and they exhibit unique properties, which are quite different than those of larger particles. New properties of nanoparticles related to variation in specific characteristics like size, shape and distribution have been demonstrated.[17] Among the noble metals, silver (Ag) is the metal of choice for potential applications in the field of biological systems, living organisms and medicine.[18] Synthesis and characterization of AgNPs from plant extracts may act as reducing and capping agents in silver nanoparticles synthesis.

C. halicacabum is a medicinal plant. People are using this plant in their day-to-day life all around the world (Fig. 1). Green synthesis of silver nanoparticles using aqueous leaf extract of *C. halicacabum* with help of 1mM $AgNO_3$ was proved significant in this study. The fresh suspension of *C. halicacabum* was yellowish-green in color. However, after addition of $AgNO_3$ and shaking for 25 minutes at room temperature, the emulsion turned dark brown (Fig. 2A). This confirmed the synthesis of AgNPs in the mixture solution. It is well known that silver nanoparticles exhibit dark brown color in aqueous solution due to excitation of surface Plasmon vibrations in silver nanoparticles.[19]

Figure 1: *Cardiospermum halicacabum* in nature and the fresh braches with leaves used in the present study

The color was changed in the cell free extract when challenged with 1mM AgNO3 from pale yellow to dark brown, attained maximum intensity after 10-12 hrs with intensity increasing during the period of incubation indicative of the formation of silver nanoparticles (Fig. 2B). Control (without silver ions) showed no change in color of the cell filtrates when incubated under same conditions. *Boswellia ovalifoliolata* aqueous extract could synthesize silver nanopartcles within 10 min whereas *Shorea tumbuggaia* and *Svensonia hyderobadensis* took 15 min to synthesize nanoparticles.[20]

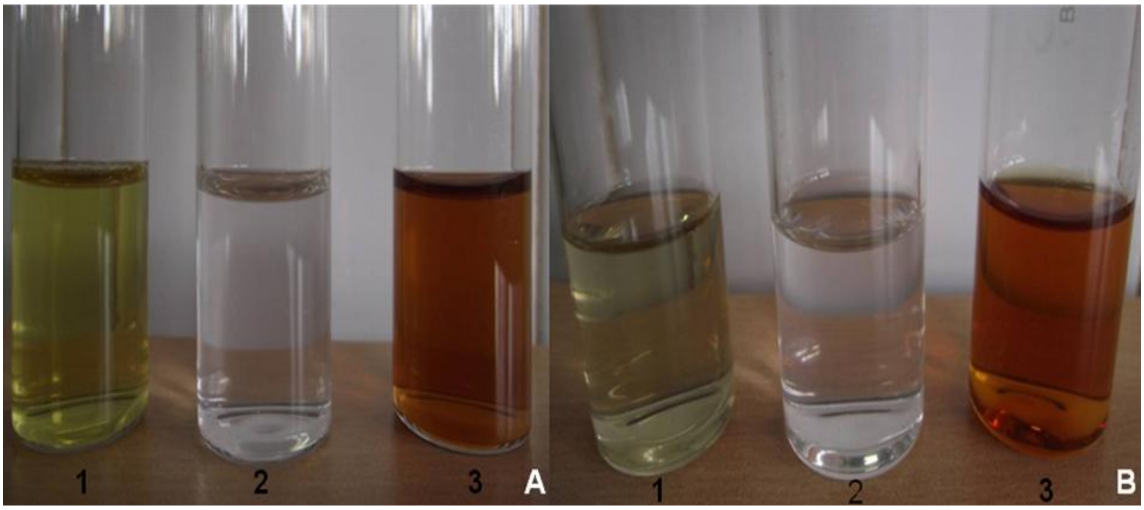

Figure 2: (A1). *Cardiospermum halicacabum* leaf aqueous extract, (A2) Silver Nitrate solution, (A3) Silver Nitrate + *Cardiospermum halicacabum* emulsion after 25 min. (B1, B2 and B3) Experimental solutions after 12 hrs

The important aspect of AgNPs is that their optical properties depend upon the particle size and shape. These optical properties are dominated by the collective oscillation of conduction electrons resulting from the interaction with electro-magnetic radiation.[21]

The extract of lower plants (algae, fungi etc.) was also used to synthesize AgNPs at room temperature. Proteins in the extract provide dual function of Ag+ reduction and shape control in the nanoparticles synthesis. The carboxyl groups in aspartic and/or glutamine residues and the hydroxyl groups in tyrosine residues of the proteins were suggested to be responsible for the Ag+ ion reduction.[7]

The formation of silver nanoparticles was followed by measuring the surface plasmon resonance (SPR) of the *Cardiospermum halicacabum* and Silver Nitrate + *Cardiospermum halicacabum* emulsion at the wavelength range from 300–700 nm. The characteristic silver SPR bands were detected around 400–450 nm (Fig. 3). These absorption bands were assumed to correspond to the silver nanoparticles extra fine and smaller than 25 nm. UV–Vis absorption spectra (Fig. 3B) showed that the broad SPR band contained two peaks. These two peaks illustrate the presence of two broad distributions of hydrosol silver nanoparticles.

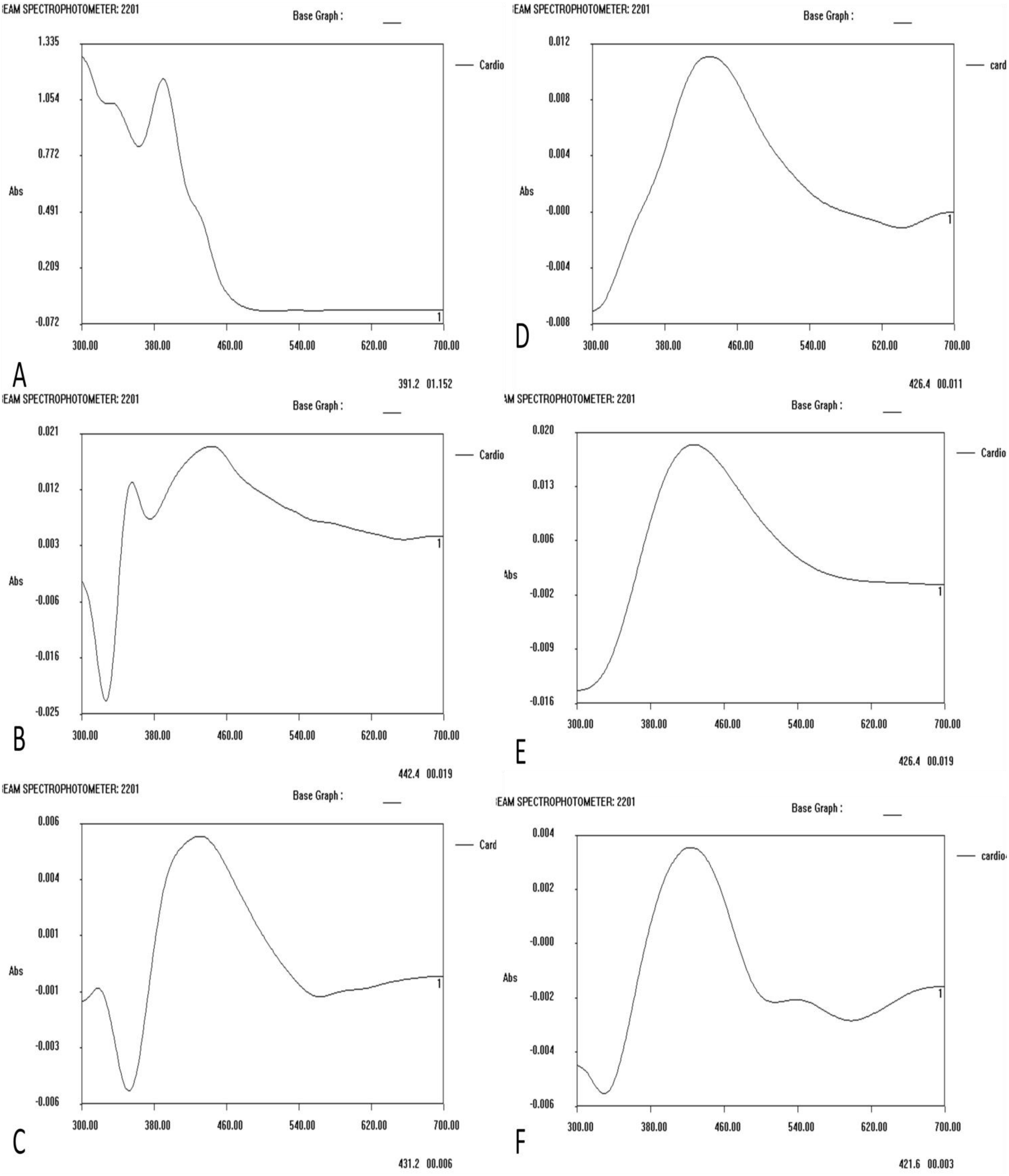

Figure 3A-F: UV-Vis spectrum of silver nanoparticles synthesized using *Cardiospermum halicacabum* aqueous leaf extract after every one hr time interval.

The reduction of Ag+ ions by combinations of bio molecules found in these extracts (e.g. enzymes/proteins, amino acids, polysaccharides, vitamins etc.) is environmentally benign, yet chemically complex.[22] Due to their exclusive properties, silver nanoparticles (AgNPs) may have several applications, such as catalysts in chemical reactions[23], electrical batteries and in spectrally selective coatings for absorption of solar energy[24, 25] pharmaceutical components and in chemical sensing and biosensing[26].

Conclusion

Hazardous organic solvents and surfactants which are often employed in chemical synthesis of nanoparticles can be avoided through green synthesis techniques. Due to the increasing prevalence of silver nanoparticles in consumer products, there is a large international effort underway to verify silver and other metals nanoparticles safety and to understand the mechanism of action for antimicrobial effects. The present protocol is an eco-friendly and cost-effective way of synthesis of silver nanoparticles under laboratory as well as room conditions.

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