ORIGINAL ARTICLE

Plant-mediated synthesis of Silver nanoparticles by two species of *Cynanchum L.* (Apocynaceae): A comparative approach on its physical characteristics

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Abstract

The present study evaluates the biosynthesis of silver nanoparticles (SNPs) mediated by xerophytic plants, *Cynanchum viminale* and *Cynanchum sarcomedium*. The reaction between plant extracts and silver nitrate solution resulted in a yellowish brown/dark brown colored solution which suggests the formation of SNPs. Physical characteristics of synthesized SNPs were determined using UV-Vis spectral, Scanning electron microscopy (SEM) and Energy dispersion X-ray spectroscopy (EDAX) analyses. The UV-Vis spectrum showed a maximum absorbance of SNPs at 500 nm for SNPs synthesized by *C. viminale* whereas maximum absorbance of 1.87 was observed at 400 nm for *C. sarcomedium*. Agglomerated nanoparticles were synthesized by *C. viminale* with an average diameter of ~ 60-68 nm as depicted by SEM. Nearly spherical nanoparticles of average size of ~ 60-85 nm were obtained by *C. sarcomedium* extract. A strong signal of silver at 3 KeV was confirmed by EDAX analysis for SNPs produced by both plants. Thus, the present green synthesis offers a viable and an ecofriendly way of fabrication of benign SNPs without any huge inputs in terms of energy and waste.

Keywords: *Cynanchum sarcomedium; Cynanchum viminale; SEM-EDAX; Silver nanoparticle; UV-Vis.*

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INTRODUCTION

Nanotechnology is the most captivating field of research in the area of material science and is gaining remarkable impetus in the present scenario due to its capability of modulating metals into their nanosize, which drastically changes the chemical, physical and optical properties of metals. The synthesis of nanomaterial and their characterization is an emerging trend in nanotechnology due to their diverse applications in physics, biology and medicine. Recently fabrication of SNPs has drawn considerable attention due to their physical and chemical properties and tremendous applications in diagnostics, therapeutics, catalysis etc. Furthermore, among nanoparticles, much attention has been paid towards silver nanoparticles by their wide applications. Keeping this view in mind, the present study made an attempt to evaluate the facile synthesis of silver nanoparticles mediated by plants *viz., C. viminale and C. sarcomedium*. Silver has been in use for last few decades in the form of metallic silver, silver nitrate, silver sulfadiazine for various applications. Metallic silver in the form of SNPs has made a remarkable comeback as a potential antimicrobial agent [1]. Hence, SNPs have emerged up with diverse medical applications ranging from silver-based dressings, silver coated medicinal devices, such as nanogels, nano lotions etc. Despite their antimicrobial potential, cytotoxicity on various test systems and cancer cell lines also have been documented [2].

A number of approaches are available for the synthesis of nanoparticles viz., chemical reduction, thermal decomposition, microwave assisted method etc. Generally, these non-biosynthetic methods are low-cost for high volume; however, their drawbacks include contamination from precursor chemical

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compound, use of toxic solvents and generation of hazardous by-products [3]. Here arises the need for a bio-inspired route for the synthesis of metallic nanoparticles. Microorganisms like bacteria, fungi, actinomycetes and a wide variety of plants have been involved in the biosynthetic approach. Plant-mediated syntheses are always reliable over other modes of biosynthesis, because of the difficulty in maintaining the microbial cultures [4]. It can also be suitably scaled up for large-scale synthesis of nanoparticles.

To date, there is no report on the synthesis of SNPs utilizing the medicinal shrubs, *Cynanchum viminale* and *Cynanchum sarcomedium* (Apocynaceae) as biosubstrates. The plants are xerophytic, laticiferous, perennial shrub; least explored with regard to their bioactivities. Since molecular analyses have demonstrated that *Sarcostemma* R. Br. is deeply nested in the predominantly Madagascan stem-succulent clade of *Cynanchum* L., the genus has been treated as a synonym of *Cynanchum* as a result of the formal transfer of *Sarcostemma* genus into the genus *Cynanchum* [5]. Pre-apoptotic, genotoxic and modulatory activities of the plant have been reported recently [6, 7]. Also, the plant is rich in phytochemical constituents like terpenoids, hydrocarbons, fatty acid esters etc.

Based on the aforementioned background, the present study investigates the comparison of biosynthesis and characterization of SNPs using the two species of *Cynanchum* (Apocynaceae).

EXPERIMENTAL

Plant materials

C. viminale (L.) Bassi subsp. *viminale* and *C. sarcomedium* Meve & Liede were collected from Karnataka and Kerala, India. The specimens were authenticated and voucher specimens (CALI No. 123742 & CALI No. 123741) were deposited at the Herbarium of Department of Botany, University of Calicut, Malappuram, Kerala, India.

Plant extracts preparation

About 10 mg of the methanolic residue of plant extract was dissolved in 20 mL of deionized water. The plant material residue was blended uniformly with deionized water and filtered using Whatman No. 1 filter paper.

Synthesis of silver nanoparticles

About 10 mL of the collected filtrate was treated with 90 mL of silver nitrate (1 mM) and boiled at 80 ˚C for 10 minutes, resulting in the formation of brownish yellow coloured solution indicating the synthesis of silver nanoparticles.

Collection of silver nanoparticle pellets

The solution was centrifuged at 12,000 rpm for 10 min and redispersed in sterile distilled water. The centrifugation and redispersion were repeated three times to ensure the complete separation of silver nanoparticles. The pellet thus obtained was stored at room temperature for further characterization studies.

Characterization of silver nanoparticles

- UV-Vis spectral analysis

About 1 mL of the solution was monitored to detect the reduction of Ag^+ ions, followed by dilution of the sample with 2 mL of sterile distilled water at a wavelength range of 300-700 nm and resolution of 1 nm in UV-Vis spectrophotometer.-

- SEM/EDAX analysis

The dried pellet of silver nanoparticles was mounted on an aluminum stub using carbon tape and dried by keeping it in a desiccator overnight and examined using a scanning electron microscope (Hitachi SU 6600, Japan) equipped with energy dispersive spectrometer (HORIBA-EMAX EDS).

RESULTS AND DISCUSSION

The present study evaluates a novel strategy for the biogenic synthesis and characterization of SNPs by the two plant species *viz., C. viminale* and *C. sarcomedium.* The ability of plant extracts to reduce metallic salts is exploited for the synthesis of SNPs. The plant extract act as both stabilizing as well as capping agent in the synthesis of nanosilver. The reductions of silver nitrate into SNPs by plant extracts were estimated and the synthesized nanoparticles were characterized by its colour formation, surface plasmon resonance, SEM and EDX analysis. The optimal conditions for rapid generation of silver nanoparticles were fixed to be (incubation time 10 min, temperature 80 °C, metal ion (silver nitrate) concentration 1mM, pH of the reaction mixture 8, the stoichiometric proportion of 1mM silver nitrate and methanolic extract 9 : 1).

The reduction of silver nitrate into SNPs by the action of plant extracts resulted in the gradual colour change of clear silver nitrate solution into light yellowish brown or dark brown solution. The appearance of the yellowish brown colour in the reaction flask suggested the formation of silver nanoparticles. In the case of SNP synthesis by *C. viminale*, a dark brown coloured solution was gradually formed upon the reaction of the plant extract with 1 mM silver nitrate solution at 80 °C and later the nanoparticles tended to settle or agglomerate at the bottom of the reaction tube (Fig. 1a & c), whereas in the case of *C. sarcomedium* extract, the reaction was quite rapid and the resultant solution is a light yellowish brown solution (Fig. 1d & f). The blank solution remained colourless on the addition of silver nitrate solution (Fig. 1b & e).

The difference in colour tinge may attribute to the variation in the intensity of nanoparticle synthesis. For better synthesis, the reaction medium was tuned to alkaline pH to upsurge the availability of the plant active compounds [8]. The appearance of yellowish brown color in the reaction mixture of *C. sarcomedium* and 1mM silver nitrate indicated the formation of silver nanoparticles, also due to the excitation of surface plasmon resonance (SPR) and broad SPR excitation peak in UV-Vis spectra indicated the polydispersive nature of silver nanoparticles [9, 10].

The synthesized nanoparticles in aqueous solution of both plant species were subjected to optical measurements by UV-Vis spectrophotometry at a wavelength range of 200-800 nm. The UV-Vis spectrum of the synthesized silver nanoparticles by *C. viminale* showed a broad peak at 500 nm. According to the distribution of the size of nanoparticles, the synthesized silver nanoparticles showed absorbance (λ_{max}) from 460 to 505 nm and the maximum absorbance centered at 500 nm with an absorption value of 3.21 (Fig. 2a). In the case of silver nanoparticles by *C. sarcomedium*, a prominent optical peak was observed at the wavelength of 400 nm with the absorption of 1.87 (Fig. 2b). Here, the peak was so much specific and narrow, not as the broad peak observed in the case of *C. viminale*.

Metallic nanoparticles scatter and absorb light at certain wavelengths due to the resonant collective

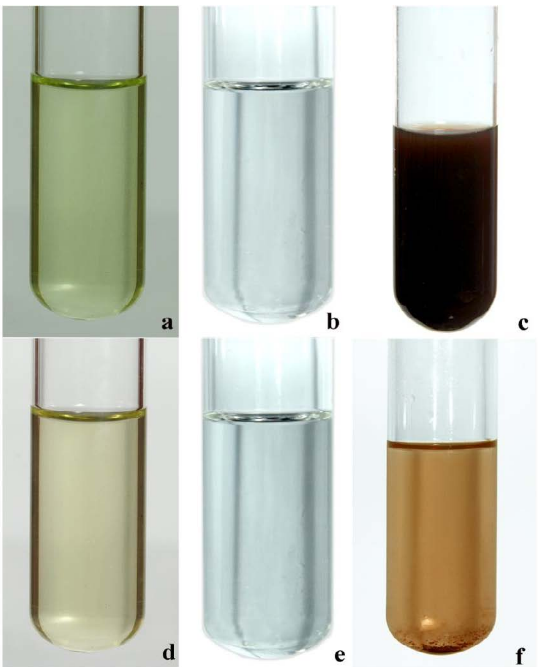

Fig. 1: Colour change pattern during the formation of nanoparticles by selected taxa of Cynanchum. a) *C. viminale* extract; b) 1 mM silver nitrate solution; c) brown colouration indicating the formation of silver nanoparticles; d) *C. sarcomedium* extract; e: 1 mM silver nitrate solution; f) yelloeish brown colouration indicating synthesized silver nanoparticles.

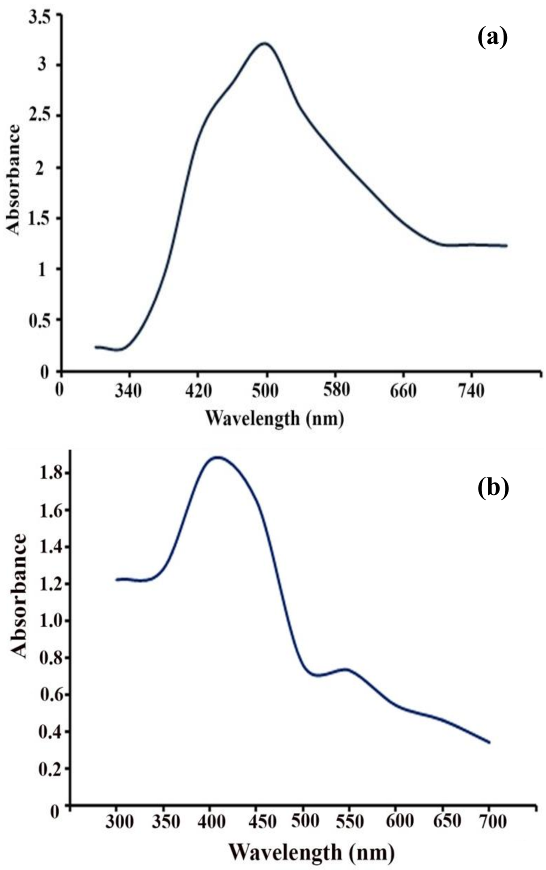

Fig. 2: UV-Vis spectra of silver nanoparticles synthesized by selected taxa of Cynanchum. a) spectrum of *C. viminale*; b) spectrum of *C. sarcomedium.*

excitations of charge density at the interface between a conductor and an insulator, phenomena known as SPR [11]. It is also well identified that the absorbance of SNPs mainly depends upon the size and shape of nanoparticles and their inner particle distance and the surrounding media. SNPs have optical properties and are sensitive to size, morphology, agglomeration state and refractive index near the nanoparticle surface, which makes UV-Vis spectroscopy an efficient tool for identifying, characterizing and studying these silver nanoparticles [12].

The absorbance peak between 400 and 450 nm by UV-Vis analysis is the characteristic of SNPs [13]. Spherical nanoparticles contribute absorption peak range of around 400-420 nm and broadening of absorption peak attributes to the polydispersion of nanoparticles [14, 15]. This λ_{max} shift to a larger value and the excessive broadening can be attributed to the increased crystalline size of the nanoparticles due to particle aggregation which decreases the sensitivity to plasmon response [16]. On the other hand, the nearly sharp peak of SNPs by *C. viminale* without λ_{max} shift is the best peak for SNPs. A high absorption value and sharpness of the SPR band results in a better sensing resolution for bioimaging applications [17].

In the present study, the size of the silver nanoparticles biosynthesized by *C. viminale* (60 - 68 nm) and *C. sarcomedium* (60 - 85 nm) seems to be optimal, when compared with nanoparticles produced *via* green synthesis by various other plant species. The size and shape of the silver nanoparticles varies, which depend on different conditions for the synthesis. The spherical shape of silver nanoparticles also depends on single SPR band as observed by UV-Visible spectroscopy, which is the evidence of the Mie theory, due to the presence of some anisotropic nanoparticles [18]. The appearance of SPR band corresponds

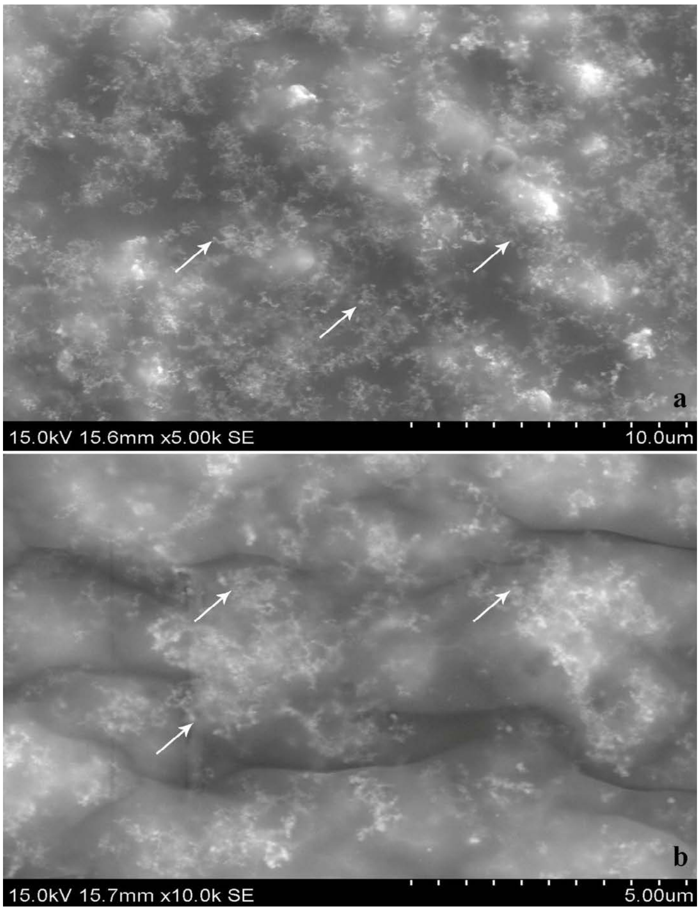

Fig. 3: Scanning electron micrographs of silver nanoparticles produced by *C. viminale*; a) normal view; b) enlarged view. Arrows indicates the agglomerated silver nanoparticles.

to the absorbance change of the nanoparticles, reduced from their corresponding metallic precursor solutions [19]. Mie theory predicts the appearance of single SPR band in the absorption spectra of spherical nanoparticles and. It is believed that the size, distribution, dispersibility as well as morphology of the silver nanoparticles can be effectively controlled by changing the concentration of the stabilizer. SEM images of silver nanoparticles biosynthesized by *C. viminale* extract were obtained, although the exact shape of the nanoparticles cannot be clearly predicted (Fig. 3a & 3b). A small percentage of resulting nanoparticles were nearly spherical shaped with the average range of particle size distribution from ~60-68 nm and they were found to be agglomerated without even distribution. Most of the nanoparticles were aggregated but only a few were dispersed as observed under SEM. Formation of silver nanoparticles may be due to interactions of hydrogen bond and electrostatic interaction between the biomolecules capping with silver [20]. Increasing the temperature of the reaction mixture from 40-80 °C increases the absorption intensity. However, boiling at high temperatures beyond 90 °C decreases the absorption intensity. It is also speculated that high temperatures may cause the denaturation of plant metabolites and aggregation of nanoparticles.

The size of the nanoparticle biosynthesized by *C. sarcomedium* extract was variable, as analyzed by SEM and the shape is found to be nearly spherical in the present study (Fig. 4a & 4b). A wide spectrum of literature is available, where the shape of the biologically synthesized silver nanoparticles is spherical. The size and shape of silver nanoparticles vary in different plant extracts. The size and shape of silver nanoparticles vary in different plant extracts as reviewed by [21]. It is reported that size, morphology, and properties are

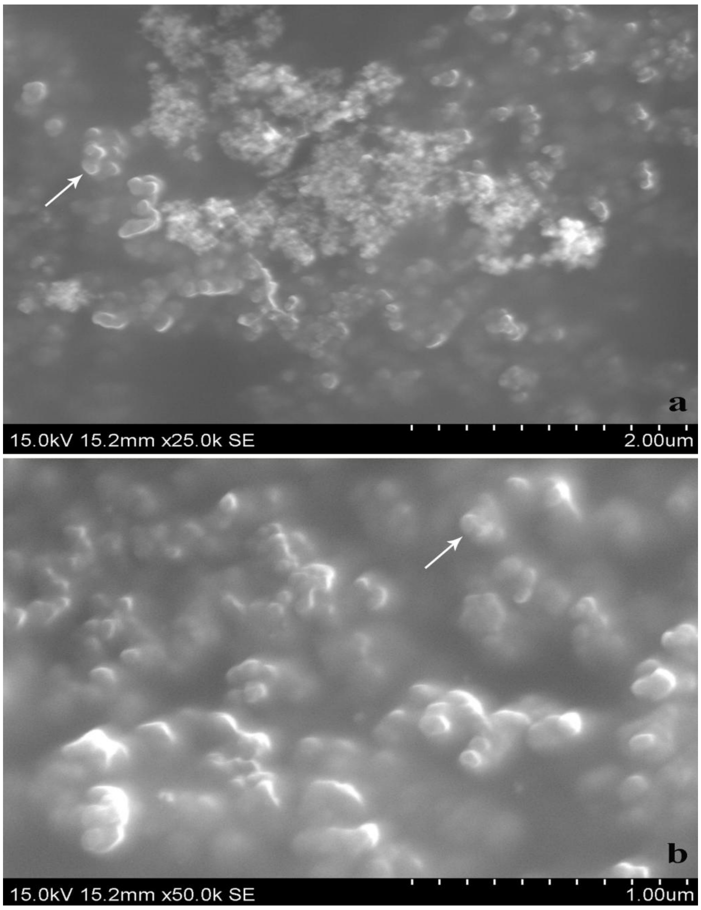

Fig. 4: Scanning electron micrographs of silver nanoparticles synthesized by *C. sarcomedium* extract: a) normal view; b) enlarged view. Arroes indicates the nearly spherical silver nanoparticles.

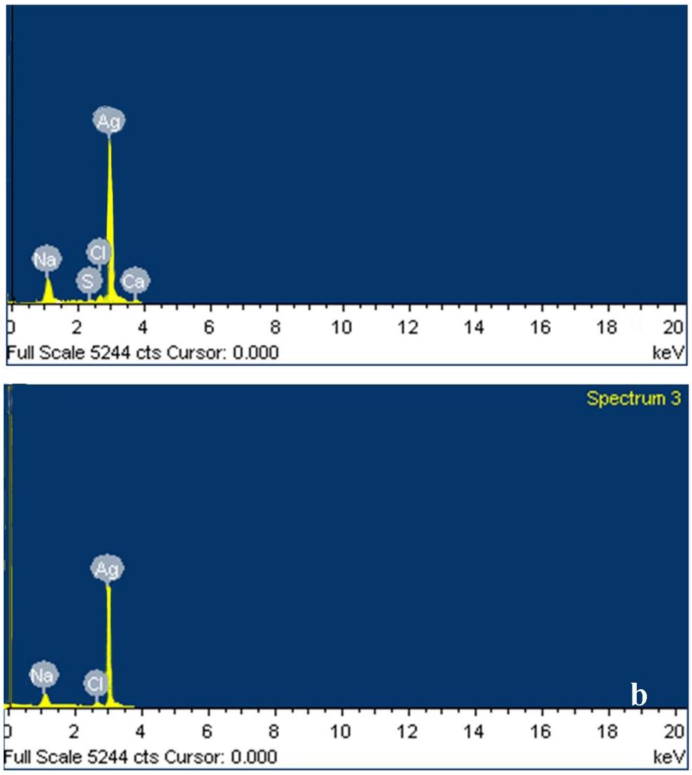

Fig. 5: Elemental composition of silver nanoparticles by selected taxa of *Cynanchum*.
a) EDX spectrum of *C. viminale*; b) EDX spectrum of *C. sarcomedium.*

dependent on reaction conditions like substrate concentration, bio-catalyst concentration, pH, temperature, light, reaction time *etc.* [22, 23]. So for a scale-up production of silver nanoparticles, these parameters need to be optimized. Biomolecules like proteins and aminoacids provide the dual function in silver reduction and shape-control in the synthesis of nanosilver [24]. The primary and secondary metabolites of *C. sarcomedium*, such as terpenes, fatty acid esters, phenols, glycosides and flavonoids, could possibly form a protective capping layer around the metallic nanoparticles which prevented the agglomeration of the nanoparticles [17].

In EDAX analysis of SNPs synthesized *via C. viminale* extract, a strong signal of silver was observed at 3 keV by elemental analysis which designates the elemental composition and chemical purity of nanoparticles (Fig. 5a). It confirmed the elemental constituents as silver (87.65%), sodium (6.49%), chlorine (2.51%), sulphur (1.5%) and calcium (1.86%) respectively. The strong peak of silver indicates that the biosynthesized nanoparticles were indeed made up of silver. The EDAX pattern of SNPs synthesized by *C. sarcomedium* extract clearly showed that the SNPs are crystalline in nature (Fig. 5b). The elemental analysis showed a strong signal of silver at 3 keV, which indicates the presence of silver nanoparticles (89.26%) along with weak background signals of Na (5.29%) and Cl (5.45%). A principal sharp signal was observed at 3 keV for silver [25], which is distinctive for the absorption of crystalline nature of biosynthesized SNPs [26]. In the case of *C. sarcomedium* mediated nanoparticles, the weak signals of Na and Cl may have originated from biomolecules bound to the surface of SNPs [27, 28]. The current approach thus demonstrated the biosynthesis of SNPs by *C. viminale* and *C. sarcomedium*.

CONCLUSIONS

As a goal to widen the scope of plants in the synthesis of nanomaterials, the potential of two species of *Cynanchum* to reduce and accumulate silver ions to silver nanoparticles is explored by the rapid and bio inspired synthesis of SNPs by *C. viminale* and *C. sarcomedium* extracts.

This investigation suggests that environmental friendly approach for the production of metallic nanoparticles will greatly help for the creation of an important symbiosis between material science and biology. The present work is also an effective pilot study to synthesize silver nanoparticles by an eco-friendly approach and physical characteristics of the synthesized nanoparticles were demonstrated. The upshot of study is the development of value-added products from *C. viminale* and *sarcomedium* in the biomedical field.

CONFLICT OF INTEREST

No conflicts declared.

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