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Research Article

GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM AQUEOUS STEM EXTRACT OF *CERIOPS TAGAL*

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ABSTRACT

Biosynthesis of reliable and eco-friendly nanoparticles is an important branch of green nanotechnology. *Ceriops tagal*, a mangrove plant has various medicinal properties. Synthesis of *C.tagal* mediated nanoparticles may further enhance its medicinal potency. The aqueous stem extract of *C.tagal* was used as a bio-reducing agent for synthesis of silver nanoparticles Reduction of metal ions was confirmed by Ultraviolet-visible absorption spectroscopy, transmission electron microscopy, energy dispersive spectroscopy, and x-ray diffraction. Before and after bio-reduction changes in functional groups of plant extract was recorded by FTIR spectra. The kinetics of particle formation was dependent on time, temperature and metal salt concentration. UV-Visible spectroscopy demonstrated peak at 455 nm. TEM and XRD results revealed shapes, size and structure of synthesized nanoparticles. These results have indicated the formation of silver nanoparticles.

Keywords: Mangroves, *Ceriops tagal*, aqueous extract, silver nanoparticles.

INTRODUCTION

Nanotechnology has wide applications in almost every field with the most exploitable properties being mechanical, thermal, chemical, electrical, optical, magnetic, specific surface area etc[1]. It is playing an important role in global manufacturing along with rapid commercialization. Beside medical and pharmaceutical applications, noble metals nanoparticles are widely applied in shampoos, soaps, detergent, shoes, cosmetic products, and toothpaste[2]. Silver has been used as a healing and antibacterial agent since ages[3]. Nano silver has found application in diverse areas like catalysis, textile engineering, water treatment, therapeutics etc furthermore it is used as a strong antimicrobial, antifungal and anti-inflammatory agent[4]. It has been used in the formulation of dental resin composites[5,6,7] and ion exchange fibers[8] and in coatings of medical devices[9,10,11]. Nanocrystalline silver dressing is effective for treating wounds especially burns and chronic wounds which slowly releases small and highly reactive silver particles[12].

Biological effectiveness of nanoparticles is directly proportional to its surface area. Nanoparticles exhibit new and improved properties based on their size and shape. Chemical synthesis of nanoparticles possesses the potential hazards like carcinogenicity, genotoxicity, cytotoxicity, and general toxicity[13]. Therefore, there is a current drive to produce 'green' nanoparticles from plants because of its eco friendliness, energy efficiency and convenience. Mangroves are salt tolerant plant species surviving under stressful conditions such as high tides and salinity, extreme temperatures, anaerobic & unstable substrates thus forming unique environments and floral-faunal assemblages. India has a total mangrove cover of only 4,628 km2, 3% of the global mangrove area and 8% of Asia's mangroves[14]. Around 20 out of the 35 species of true mangroves found in India have been identified along the Maharashtra coast and 15 species of these are found in Mumbai; in which one of the major species is *C.tagal.* Among the different mangrove plants, the chemical constituents and bioactivities of *Ceriops tagal* have been studied extensively. Dolabrane diterpenes from *C. tagal* has exhibited significant anti-tumor effect[15]. Bark of *C. tagal* is a powerful astringent and is used for treating hemorrhage in defecation and malignant ulcers whereas leaves are used to heal paludism and malaria; ethanolic extract of stem and twigs has strong feeding deterrent activity against *T. castaneum* adults[16]. Few of the compounds isolated from ethanolic embryo extract were effective to inhibit proliferation and growth of H-7402 and Hela cells[17]. It has been reported to contain condensed and hydrolysable tannins, aliphatic carboxylic acids, indole alkaloids, polyphenols, proteins, tannins, fatty acids, hydrocarbons, inorganic salts, inositols, steroids, carotenoids, chlorophyll a, b, a+b, etc[18]. Thus it is a rich source of bio-reductant and stabilizers. There are reports of monometallic nanoparticles synthesis from mangrove species- *Rhizophora mucronata*[19] *Avicennia marina*[20] *Sonneratia apetala*[21] etc. Here in, we report for the first time synthesis of silver nanoparticles employing aqueous stem extract of *C. tagal* (CTSE) by reduction of aqueous Ag^+ ions. We also investigated effects of time course, temperature and metal salts on the rate of synthesis.

MATERIALS AND METHODS

Plant collection and extract preparation

C.tagal stem was collected from the Gorai creek, Mumbai, India. It was chopped, dried at 40º C and pulverized. Ultra pure water produced by MilliQ system was used throughout the experiment. Aqueous stem extract was prepared by soaking 5 g of stem powder in 100ml MilliQ water, for 5 min and then the mixture was boiled at 100º C for 5 min. The freshly prepared extract was obtained by filtering it through Whatman filter paper No.1 and used for further study.

Synthesis of silver nanoparticles

Synthesis of monometallic nanoparticles was initiated by adding 5ml CTSE in 95 ml aqueous $AgNO_3$ solution. The reaction was carried out at static condition. Reduction of Ag^+ was monitored as a function of time by measuring UV-Vis spectra using UV-1650CP Schimadzu spectrophotometer operated at 1nm resolution. Effect of temperature on rate of synthesis was studied by carrying out the reactions at Room temperature (RT), 40ºC, 50ºC and 60ºC in water bath. Concentration of metal salt varied from 1mM – 5mM for silver nitrate.

TEM and energy dispersive spectroscopy measurements

Size and surface morphology of bio reduced nanoparticles was determined by transmission electron microscope (TEM, Tecnai 12 Cryo, FEI, Eindhoven, The Netherlands) in the Department of Physics, Savitribai Phule Pune University, Pune as follows; Nanoparticle solution was drop coated on copper TEM grids, after which film was allowed to stand for 2 min and excess solution was blotted. The grid was dried properly prior to measurement. An energy dispersive spectrum was recorded with the same instrument at the energy range 0-20 keV.

X-ray diffraction measurements

After complete reduction of metal ions by extracts, the solutions were centrifuged at 10,000 rpm for 15 min at RT. The pellet obtained was re-dispersed and centrifuged with MilliQ water. This process was repeated three times to get rid of any free entities. Phase formation of nanoparticles was studied by preparing thin film of thoroughly dried nanoparticles on glass slides. Diffraction data was recorded on Schimadzu XRD 7000 diffractometer with Cu Kα radiation (1.54 Å) source operating at 40 kV voltage and a 30 mA current at Department of Chemistry, University of Mumbai, Mumbai.

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectrum of extracts before and after reduction of metal ions was obtained using FTIR (Perkin Elmer) Frontier spectrophotometer at Department of Chemistry, University of Mumbai, Mumbai. 5% plant extract and supernatant of bio-reduced samples was subjected to IR source 400 cm^{-1} - 4000 cm^{-1}.

RESULTS

Visual observations and UV-Visible spectroscopy

Bio-reduction of salt to respective metal ions in presence of extract was monitored as a function of time using UV-Visible spectroscopy. The surface plasmon resonance (SPR) peak of absorption spectra for CTSE was found at 455nm for nanoparticles synthesized using $AgNO_3$ (Figure 1a) salt solution (**Indian patent application 1320/MUM/2013 A**). The well-known intense brown colour was observed after silver nanoparticles formation. Complete reduction of Ag^+ metal ions was done in 120 min, while the colour change was observed after 15 min. The pH of CTSE was 7. Optimization of different concentration of metal salts against kinetics of reaction was observed (Table 1). The rate of synthesis was highest at 2mM for silver nitrate solution. The lower salt concentration showed comparatively low rate of synthesis whereas higher salt concentration resulted in precipitation. Maximum bio-reduction of Ag^+ to Ag^0 was achieved at RT.

TEM and energy dispersive spectroscopy measurements

TEM micrograph confirmed the synthesis of monometallic nanoparticles. For elemental analysis of nanoparticles, a signature spectrum corresponding to metal atom in the nanoparticles was obtained by EDS (Figure 1b). Weak signals from oxygen, chloride, carbon etc were also seen which may have originated from the bio-molecules bound to the surface of the nanoparticles, while copper peaks may have origin from the copper grid. TEM diffraction pattern of nanoparticles is shown in Figure 1c. All monometallic nanoparticles synthesized were almost spherical with a small percentage being ellipsoidal (Table 2). From the few particle images we found that the size (diameter) varied from 5nm to 22nm for silver nanoparticles synthesized employing $AgNO_3$ (Figure 1d). It was clear from the TEM measurements that the biosynthesized nanoparticles were poly-dispersed. TEM micrograph also indicates that the nanoparticles are capped by a thin layer which is supposed to be the organic material from CTSE.

XRD

The crystalline nature of nanoparticles was confirmed by X-ray diffraction analysis. The crystallite size of the nanoparticles was estimated from the Debye–Scherrer formula[22].

$$d = 0.9\lambda/\beta \cos \theta$$

Where 0.9 is the shape factor, λ is x-ray wavelength, 1.54 Å, β full width at half the maximum intensity in radians, and θ is the Bragg angle.

Strong Bragg reflections were obtained corresponding to (111), (200) and (220) planes for silver nanoparticles, which are the principal diffracting planes of face centered cubic symmetry (Table 2). High degree of crystallinity of the nanoparticles was reflected by the intensity of peaks. The crystallite size of silver nanoparticles synthesized using $AgNO_3$ (Figure 1 e) was ~6 nm.

FTIR

FT-IR spectroscopic studies were carried out to identify the functional group involved in capping and efficient stabilization of the metal nanoparticles. Representative absorption peaks of the nanoparticles obtained in the present study is presented in Table 4. The plant extract shows intense broad stretching at 3550-3200 cm-1 which arises due to the free O-H groups present in alcohols and phenols [22], weak stretch at 2260 – 2100 cm^{-1} is due to C≡C from alkynes while the IR peak at 1690-1630 cm^{-1} could be assigned to characteristic asymmetrical stretch of carboxylate group[23]. The shifting of peaks occurred after synthesis of silver nanoparticles (Figure 1f). Based on the band shift occurring at hydroxyl and carbonyl groups it can be concluded that both hydroxyl and carbonyl groups of *C.tagal* are involved in the synthesis of nanoparticles. As stated earlier, CTSE is mainly composed of terpenoids, flavonoids, alkaloids and ployphenols which may play an important role in reduction, stabilization and assembly of synthesized nanoparticles.

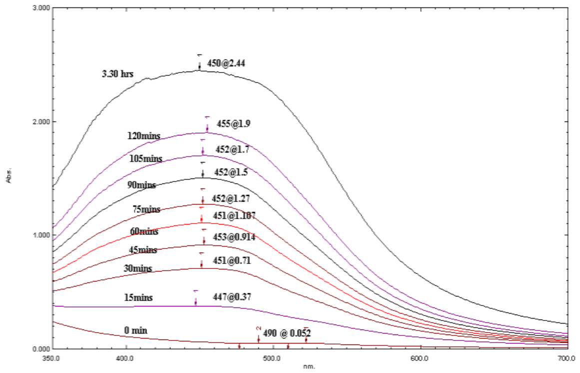

Figure 1a: UV visible spectra recorded as function of reaction time of 2mM AgNO3 solution with CTSE at RT.

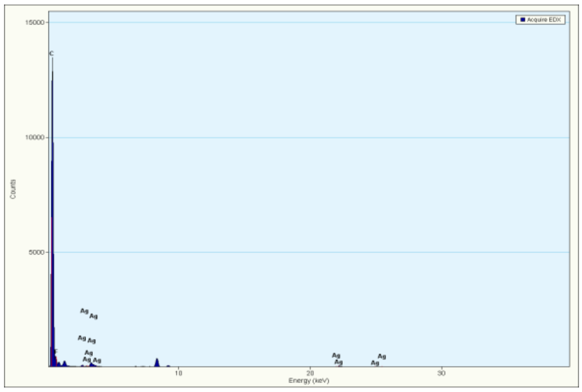

Figure 1 b: Representative spot EDS profile confirming the presence of silver nanoparticles biosynthesized by challenging CTSE with $AgNO_3$ salt.

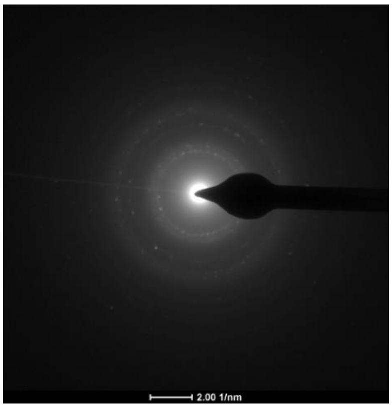

Figure 1 c: TEM Diffraction pattern of silver nanoparticles.

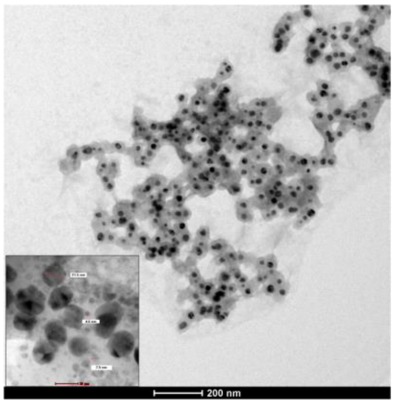

Figure 1.d: TEM micrograph of silver nanoparticles biosynthesized by challenging CTSE with AgNO3 salt

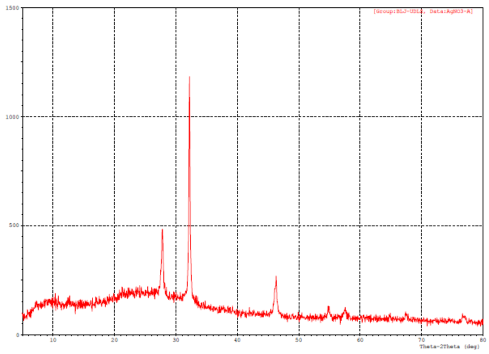

Figure 1e: Representative XRD profile of silver nanoparticles.

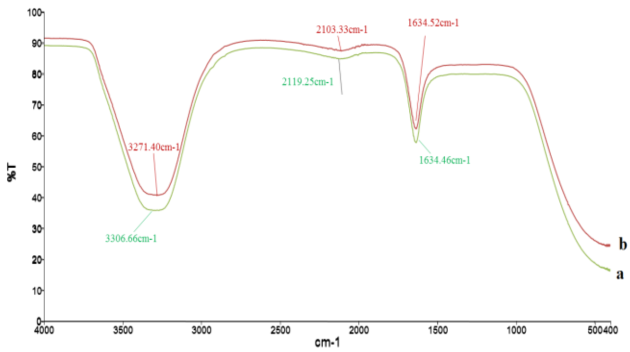

Figure 1.f: FTIR absorption spectra of CTSE before bioreduction (a), after complete bioreduction of $AgNO_3$ salt (b)

Table 1: Optimization of nanoparticle synthesis

Sr. No	Salts in mM conc.	Synthesis time in min.	Temp. ºC	Colour	λ max
1.	2mM $AgNO_3$	15-30	RT (38º C)	Intense brown	455 nm

Table 2: Size, shape and yield of NPs synthesized from *C.tagal* stem

Sr. No.	NPs	Size in nm	Shape	Yield mg/mL
1.	Silver NPs from silver nitrate	5-22	Predominately Spherical, few ellipsoidal	620

Table 3: XRD details of silver nanoparticles

Sr.No.	Nanoparticles synthesized from salt	Crystal structure	2Ɵ of the intense peak (deg)	hkl	Crystallite size
1.	$AgNO_3$	FCC	a. 27.756 b. 32.174 c. 46.300	(111) (200) (220)	~6 nm

Table 4: FTIR details of CTSE and bio-reduced $AgNo_3$

Peaks and functional groups	Free O-H groups from alcohols and phenols 3550 -3200 cm-1	C≡C from alkynes 2260 – 2100 cm-1	Asymmetrical stretch of carboxylate group 1690- 1630 cm-1
C.tagal stem extract	3306.66	2119.25	1634.46
Bio-reduced $AgNo_3$	3271.40	2103.33	1634.52

DISCUSSION

The optimization study revealed that the reaction gradually increased at higher temperature with slight colour variation indicating the dependence of temperature on bio-reduction process however no significant difference was observed in peaks. Silver nanoparticles synthesized in the present study are in the size range of 1−10 nm, there are reports that silver nanoparticles with this size have greatest biocidal activity against bacteria, it has been also reported that silver nanoparticles with this size range could attach to the HIV-1 virus and inhibit it from binding to host cells thus having a great promise in the treatment of HIV infection[24]. Therefore, it is suggested that the silver nanoparticles may be useful in health management of HIV. Capping of nanoparticles can be attributed to monoterpenoids, diterpenoids, triterpenoids, flavonoids, alkaloids, polyphenolics, saponins etc present in CTSE. Recently 43 diterpenes, 29 triterpenes and 6 new dolabranes named 'tagalsins' P−U (**1**−**6**)[25] had been isolated from *C.tagal*; thus forming the main constituents of the extract. Therefore, it is possible that the bioactive principles present in plant are involved in capping thus offering stabilization and inhibiting aggregation of nanoparticles.

CONCLUSION

In this paper we report rapid biosynthesis of silver nanoparticles from *C.tagal* stem extract. The active constituents from *C.tagal* extract are responsible for simple and efficient reduction of $AgNO_3$ to nanoparticles which could be further exploited to study its various properties. Apart from being eco-friendly, this process can be easily scaled up thus reducing the steps in downstream process and has economical viability providing an alternative to chemical synthesis. As *C.tagal* has a high number of terpenoids and flavonoids, believed to add stability and increased productivity of nanoparticles. From the present study it is found that the rate of synthesis of nanoparticles can be controlled by varying the temperature and concentration of metal salts. Further studies should be done to obtain monodispersed nanoparticles. Separation of nanoparticles on the basis of shape and size should also be focused to get target specific nanoparticles. Compounds responsible for reduction and capping of nanoparticles should be properly identified and separated. Long-term toxicity, mutagenicity and carcinogenicity studies must be done to elucidate any adverse effects of colloidal silver nanoparticles to support its safe use.

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