Eco-friendly synthesis and evaluation of biological activity of silver nanoparticles from leaf extract of *Indigofera barberi* Gamble: an endemic plant of Seshachalam Biosphere Reserve

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Abstract

The present study reports the simple, rapid and ecofriendly synthesis of silver nanoparticles (AgNPs) using aqueous leaf extract of *Indigofera barberi*. The aqueous leaf extract of *I. barberi* reduces the silver ions (Ag⁺) into AgNPs (silver nanoparticles synthesized from *I. barberi* leaf extract). Fourier transform infrared spectroscopy analysis showed the broad peaks at 1054, 1371, 1589, 2355, 2933, and 3257 cm⁻¹ which indicated the participation of polyhydroxy compounds and proteins respectively in the biosynthesis and stabilization of AgNPs. TEM analysis showed that the AgNPs synthesized in this study were spherical in shape and having a dimension of 5–20 nm. XRD analysis of AgNPs confirmed the crystalline nature with face centered cubic lattice. Particle size analysis showed that AgNPs were 2–10 nm in size with average hydrodynamic radius of 3.4 nm. Zeta potential value of the AgNPs was found to be – 13.3 mV. AgNPs showed effective antimicrobial activity against both Gram–ve and Gram+ve bacteria. AgNPs showed effective antioxidant activity by quenching DPPH and H₂O₂ radicals with IC-50 values of 67.37 and 72.04 µg/mL, respectively.

Keywords Antimicrobial activity · Antioxidant activity · *I. barberi* leaf · Silver nanoparticles · TEM · XRD

1 Introduction

Nanotechnology comprises of particle synthesis within the range of 1–100 nm at least in one dimension with high surface to volume ratios [1]. Owing to the decrease of particle size, the ratio of surface area to volume increases, which influence the biological, physical and chemical properties of the particles [2, 3]. Noble metal nanoparticles have drawn the attention of researchers world-wide due to their unique physico-chemical properties including optical, electronic, opto-electronic, catalytic and thermal properties [4, 5]. Among the noble metal nanoparticles silver occupies upfront due to their tunable photo physical attributes related with surface plasmon resonance (SPR) [6, 7]. Silver nanoparticles (AgNPs) have been reported to play a key role in the diagnosis, drug delivery, bioimaging [8], treatment of neurodegenerative (Alzheimer's and

Parkinson's), autoimmune (rheumatoid arthritis), cardiovascular and diabetic diseases [8–10]. AgNPs have been found to possess antimicrobial, antioxidant, and anticancer activities [11–13]. The antimicrobial activity of the AgNPs is an important application in the biomedicine and biotherapeutics to prepare medicines, injections, surgical vials, implants and various diagnostic kits [14]. The antioxidant activity of the AgNPs is also an important application in the nutraceutical field to protect the food and food additives from deterioration by oxidation [15–17].

Various physical and chemical methods including γ -irradiation assisted [18], laser ablation [19], thermal decomposition [20], microwave assisted [21], sonochemical assisted [22], lithography [23], polyol process [24] and chemical reduction methods have employed for AgNPs production [25]. But most of these methods are expensive and employs harmful radiations and hazardous chemicals.

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Hence, biosynthesis of AgNPs has significant potential that have used in different organisms like bacteria, fungi, yeast and plants [26]. Biosynthesis of AgNPs using bacteria, fungi and yeast involves laborious sterilized procedures and tedious processes including maintenance of optimal culture conditions for their growth etc., Further these organisms possess less phenolic compounds compared to plants [27]. Hence the biosynthesis of AgNPs using plants will be preferred over bacteria, fungi and yeast. There are several reports on the synthesis of silver nanoparticles using different plant parts such as leaves of *Andrographis paniculata* [28], *Achyranthes aspera* [29] *Morus alba* [30], *Priva cordifolia* [31], fruits of *Phoenix dactylifera* [32] *Cleome viscosa* [33], roots of *Cibotium barometz* [34], *Berberis vulgaris* [35] and fruit peel of *Punica granatum* [36].

Indigofera barberi Gamble is a high valued endemic medicinal plant belongs to Fabaceae family. The plant is used in folk system of medicine to cure various ailments such as liver diseases, renal diseases, diabetes, antihelminthic, peptic ulcers, skin diseases [37, 38]. Leaves exhibit antimicrobial activity against Gram-positive and Gram-negative bacteria [39], antifungal activity [40] and antioxidant activity [41].

To our knowledge, there have been two reports available on silver nanoparticle synthesis from *Indigofera species* such as *Indigofera tinctoria* [42] and *Indigofera hirsuta* [11]. However, no reports on silver nanoparticle synthesis using *I. barberi* aqueous leaf extract as a reducing agent. Therefore, for the first time we report biosynthesis of AgNPs using aqueous leaf extract of *Indigofera barberi*. The biosynthesized AgNPs (*I. barberi* leaf synthesized AgNPs) were characterized for their size, shape, crystallinity and stability using different physico-chemical techniques including UV–Vis spectroscopy, Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), Transmission electron microscopy (TEM) and Dynamic light scattering (DLS). Further the biosynthesized AgNPs were evaluated for their antimicrobial and antioxidant activities.

2 Materials and methods

2.1 Chemicals

Silver nitrate (AgNO_3) was purchased from Sigma Aldrich, USA. 1 mM of AgNO_3 solution was prepared by adding 169.8 mg of AgNO_3 into 1 L of sterile double distilled water.

2.2 Plant material collection

I. barberi plants were collected from Tirumala hills, Eastern Ghats, Andhra Pradesh, India (Latitude 13°39'12.152"N and Longitude 79°20'35.5"E). The plants were authenticated by

a taxonomist and the voucher specimens (SVUTYIB-01-05) were deposited in the Herbarium, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India.

2.3 Preparation of plant extract and biosynthesis of AgNPs

Leaves of *I. barberi* were collected and washed under running tap water to remove the dust adhered on the leaves and surface sterilized using sterile double distilled water. Thereafter, the leaves were shade dried completely for 5 days and then grounded into fine powder. Ten grams of fine leaf powder was added into 100 mL of sterile double distilled water, boiled for 30 min at 50–60 °C, cooled to room temperature and filtered. The filtrate named as aqueous leaf extract of *I. barberi* was used for the biosynthesis of AgNPs. Leaf extract (10 mL) was added to 90 mL of 1 mM AgNO_3 solution, which was heated for 15 min at 50 °C and then incubated for 4 h. After incubation, the color change was observed from light yellow to dark brown, which indicates the formation of AgNPs.

2.4 Characterization of AgNPs

UV–Vis analysis of the colloidal solution of AgNPs was carried out to confirm the biosynthesis. UV–Vis spectrum was recorded between 200 and 800 nm of wavelength. FTIR analysis of AgNPs was done to reveal the functional groups participated in the biosynthesis and capping of AgNPs between the wave number of 500–4000 cm^{-1} (Alpha Interferometer, Karlsruhe, Germany). XRD pattern of AgNPs was recorded using Cu K α as radiation source on an Ultima IV X-ray diffractometer with 2 θ scale between 0° and 100° range (Rigaku, Tokyo, Japan). TEM analysis was done to know the size and morphology of synthesized AgNPs (FEI Tecnai, G2 20 Twin, Philips Electron Optics, Holland). Particle size analysis and zeta potential measurement were carried out using DLS (Nanopartica, Horiba Scientifics, UK).

2.5 Antimicrobial activity of AgNPs

Antimicrobial activity of AgNPs was carried out against both Gram–ve (*Escherichia coli* and *Klebsiella pneumoniae*) and Gram+ve (*Bacillus subtilis* and *Staphylococcus aureus*) bacterial species by employing disc diffusion method [29]. 200 μL of actively growing bacterial inoculum was spread on the surface of nutrient agar (NA) plates. Each plate consists of three sterile paper discs. First disc is impregnated with 25 μL of AgNPs (1 mg/mL), second disc is impregnated with 25 μL of 1 mM of AgNO_3 solution and the third disc is impregnated with

aqueous leaf extract of *I. barberi*. NA plates were incubated for 24 h at 37 °C and then observed for inhibition zones.

2.6 In vitro antioxidant activity of AgNPs

2.6.1 DPPH free radical scavenging assay

In vitro antioxidant activity of the AgNPs was carried out by 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay [43]. Prepared the stock solution of DPPH by dissolving 4 mg of DPPH in 100 mL of methanol and stored at 5 °C. Different concentrations (25, 50, 75 and 100 µg/mL) of test samples (AgNPs, leaf extract, and ascorbic acid) were dissolved separately in methanol. 1 mL of methanolic solution containing different test samples was added to 2 mL of DPPH stock solution and incubated for one hour in dark at room temperature. After incubation, the absorbance was measured. The DPPH radical scavenging activity (RSA) was measured by taking the absorbance at 517 nm.

$$\text{RSA (\%)} = \frac{[(\text{control absorbance} - \text{sample absorbance}) / (\text{control absorbance})] \times 100}$$

The concentration of AgNPs required to scavenge 50% of the radicals (IC-50) was determined from the linear regression curve.

2.6.2 H₂O₂ radical scavenging assay

In vitro antioxidant activity of the AgNPs was further confirmed by hydrogen peroxide (H₂O₂) radical scavenging assay. The capability of AgNPs to scavenge H₂O₂ radical was assessed according to Patel et al. [44]. 1 mL of different concentrations of test samples (25, 50, 75 and 100 µg/mL) were added to 2 mL of H₂O₂ solution prepared in 40 mM phosphate buffer (pH 7.4) and then incubated for 10 min. After incubation, the absorbance was measured at 230 nm for each sample against a blank containing phosphate buffer without H₂O₂. Ascorbic acid was used as standard. The percentage of radical scavenging activity (RSA) was calculated using the formula. % RSA = [(Ac – As)/Ac] × 100. Where Ac is the absorbance of the control and As is the absorbance of the sample.

3 Results and discussion

In the present study, we report the biosynthesis of AgNPs using aqueous leaf extract of *I. barberi*. The leaf extract of *I. barberi* acts as reducing agent, which converts the silver ions (Ag⁺) into nanosilver (Ag⁰)/AgNPs. The phytochemicals present in the leaf extracts might be involved in this reduction process [45]. The biosynthesis of AgNPs was initially detected by color change of the reaction mixture from light yellow to dark brown (Fig. 1b2). The dark brown solution is known as colloidal solution of AgNPs. Further the biosynthesis of AgNPs was confirmed by UV–Vis testing of colloidal solution of AgNPs.

Fig. 1 **a** *Indigofera barberi* Gamble plant, **b** (1) *Indigofera barberi* aqueous leaf extract, (2) Silver nanoparticles (IB-AgNPs)

3.1 Characterization of AgNPs

3.1.1 UV-Vis analysis of AgNPs

UV-Vis analysis of reaction mixture containing leaf extract and 1 mM AgNO_3 initially did not show absorption peak (Fig. 2a). UV-Vis analysis of reaction mixture was carried

out after 4 h of incubation. UV-Vis spectrum shows a characteristic surface plasmon resonance (SPR) peak of AgNPs at 440 nm (Fig. 2b). The SPR peak is due to oscillations of metallic nanoparticles in the visible region [6, 7]. The broad and clear UV-Vis peak in this study indicates the formation of spherical shaped AgNPs with small size. The size and shape were further confirmed by TEM and DLS analysis.

Fig. 2 UV-Vis spectrum of reaction mixture containing 1 mM AgNO_3 and aqueous leaf extract of *I. barberi* **a** at initial time of reaction and **b** after 4 h of incubation

Fig. 4 XRD pattern of biosynthesized IB-AgNPs

Fig. 3 FTIR spectrum of biosynthesized IB-AgNPs

Fig. 5 TEM analysis of IB-AgNPs **a** 100 nm scale (magnification x 19 k), **b** 50 nm scale (magnification x 50 k), **c** crystal lattice fringes with 'd' space value of 0.222 nm and **d** SAED pattern shows debye–scherrer rings

Fig. 6 Particle size analysis of biosynthesized IB-AgNPs

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	3.4 nm	1.0 nm	3.2 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	3.4 nm	1.0 nm	3.2 nm

Cumulant Operations

Z-Average

: 3233.4 nm

PI

: 0.293

Fig. 7 Zeta potential measurement biosynthesized IB-AgNPs

3.1.2 FTIR analysis of AgNPs

FT-IR analysis showed the broad peaks at 1054, 1371, 1589, 2355, 2933, and 3257 cm^{-1} (Fig. 3). The peak at 1054 cm^{-1} is corresponding to primary amine (C–N stretch) of aromatic compounds and proteins [17]. The peak at 1371 cm^{-1} could be indexed to C=O stretching of carboxylates [46]. The peak at 1589 cm^{-1} might be stemmed due to secondary amine (–NH bend) of proteins. The peak at 2355 cm^{-1} could be due to C=O stretching vibrations of carboxylic compounds and proteins [47]. The peak at 2933 cm^{-1} could be indexed to C–H stretching of proteins and the peak at 3257 cm^{-1} is corresponding to O–H group of polyhydroxy compounds including flavonoids and triterpenoids [12]. The FTIR analysis clearly revealed the participation of polyhydroxy compounds in the bioreduction of silver (Ag^+) ions into AgNPs in this study. FTIR analysis also revealed the stability of AgNPs was due to coating/capping by proteins. Further the stability of AgNPs was proved by zeta potential measurement.

3.1.3 XRD analysis of AgNPs

XRD spectrum of AgNPs (Fig. 4) showed the four bragg's diffraction peaks at 2θ values of 38.7, 44.6, 65.2 and 77.6 which were assigned to the (111) (200) (220) and (311) crystalline planes respectively of the face centered cubic (FCC) lattice structure of AgNPs. Thus, XRD pattern showed that the biosynthesized AgNPs were pure crystalline in nature with FCC structural lattice. Crystalline nature is one of the important aspects of AgNPs for their effective biological functions. The results of XRD are in consistence with previous reports [11, 12].

3.1.4 TEM analysis of AgNPs

TEM analysis showed that the biosynthesized AgNPs in this study are roughly spherical shaped with 5–20 nm in size. TEM micrographs were represented at different magnifications (Fig. 5a, b). TEM was also used for the study of selected area electron diffraction (SAED) study of nanocrystal. Crystal analysis of AgNPs showed crystal lattice fringes with d spacing value of 0.222 nm (Fig. 5c).

Fig. 8 Antimicrobial activity of IB-AgNPs against gram-positive and gram-negative microorganisms using disc diffusion method. **a** *S. aureus*, **b** *B. subtilis*, **c** *E. coli*, and **d** *K. pneumonia*. [1. IB-AgNPs, 2. 1 mM of AgNO₃ solution and 3. Aqueous leaf extract of *I. barberi*]

Fig. 9 **a** DPPH Free radical scavenging activity and **b** H₂O₂ scavenging activity of Ascorbic acid, aqueous leaf extract and IB-AgNPs

SAED pattern of nanocrystal showed clear debye-schearrer rings of FCC crystal (Fig. 5d).

3.1.5 DLS analysis of AgNPs

Particle size analysis by DLS showed that the biosynthesized AgNPs are 2–10 nm in size with average hydrodynamic radius of 3.4 nm. Highest percentage of biosynthesized AgNPs were 5 nm in size. Polydispersity index value of AgNPs was determined as 0.293 (Fig. 6). Zeta potential value of AgNPs was found to be −13.3 mV (Fig. 7). This high negative surface charge indicates that AgNPs are long term stable without agglomeration. The non-agglomeration of the formed AgNPs was clearly observed in TEM micrograph. This non-agglomeration is due to capping of AgNPs by proteins present in the leaf extract of *I. barberi*.

3.1.6 Antimicrobial activity of AgNPs

Silver nanoparticles showed effective antimicrobial activity against both Gram+ve (*B. subtilis* and *S. aureus*) and Gram−ve (*E. coli* and *K. pneumonia*). AgNPs formed the inhibition zones (Fig. 8a–d) of 12.3, 11.2, 9.8 and 8.7 mm, respectively against *S. aureus*, *B. subtilis*, *E. coli* and *K. pneumoniae*. IB-AgNPs showed maximum inhibition against Gram+ve bacteria compared to Gram−ve bacteria. In this study, AgNPs showed highest inhibition against *S. aureus* compared to other bacterial species. AgNPs showed 2–3-fold inhibition compared to *I. barberi* leaf extract and AgNO₃. The effective antimicrobial activity of AgNPs is due to their small size and large surface area. Small sized AgNPs easy percolate through membrane and bind with important enzymes of bacterial respiration which leads to bacterial death [48, 49]. AgNPs form the pores/pits on the membrane which leads to bacterial membrane rupture.

3.1.7 In vitro antioxidant activity

In vitro antioxidant activity of the biosynthesized AgNPs was evaluated by DPPH and H₂O₂ free radical scavenging assays. AgNPs showed dose dependent inhibitory activity against both DPPH and H₂O₂ radicals (Fig. 9a, b). Increase in the concentration of AgNPs showed increment in radical scavenging activity. AgNPs showed maximum inhibition of 63.45 and 60.11, respectively against DPPH and H₂O₂ radicals. IC-50 values of AgNPs against DPPH and H₂O₂ radicals were found to be 67.37 and 72.04 µg/mL, respectively. Antioxidant activity of AgNPs is due to the participation of flavonoids and other polyphenolic compounds in the biosynthesis of AgNPs [50]. Antioxidant activity of the flavonoids is due to their ability to reduce free radical formation and to scavenge free radicals. *I. barberi* leaf constitutes flavonoids quercetin and pinitol which were proved to be

very good antioxidants that might take part in the antioxidant activity of AgNPs. The results are in consistence with many previous reports. *I. hirsuta* leaf synthesized AgNPs showed effective free radical scavenging activity against both DPPH and H₂O₂ radicals [11]. *Cibotium barometz* root mediated synthesized AgNPs showed effective free radical scavenging activity against DPPH radicals [34], *Bergenia ciliate* [51], *Chenopodium murale* [52] and *Rhododendron dauricum* [15] extract synthesized AgNPs showed effective antioxidant activity against DPPH radicals. Different free radicals such as superoxides, hydroxyls, epoxyls, peroxylnitrile (PAN) and singlet oxygen radicals generated in the human diseases causes severe oxidative stress [53]. The oxidative stress can be remediated by natural antioxidant compounds and drugs [13]. Scientists have focused on application of AgNPs as natural antioxidant systems in pharmaceutical and nutraceutical industries [16].

4 Conclusions

In this study, we have reported the simple, rapid, robust and eco-friendly synthesis of silver nanoparticles (AgNPs) using aqueous leaf extract of *I. barberi*. The phytochemicals, particularly flavonoids and proteins present in the leaf extract act as reducing and capping agents, respectively. The synthesized AgNPs are spherical in shape, crystalline in nature and sized between 2 and 20 nm. AgNPs showed effective radical scavenging activity against DPPH and H₂O₂. Thus, the AgNPs proved their biopharmaceutical importance.

Compliance with ethical standards

Conflict of interest The authors confirmed that there is no conflict of interest.

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