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Bio-reduction potentials of *Gentiana kurroo* Royle for single-step bio-genic production of silver nanoparticles

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ABSTRACT

Bio-genic route for the generation of silver nanoparticles (Ag-NPs) is the most preferred method due to its cost-effectiveness and eco-friendly behavior. In this study, Ag-NPs were prepared in the presence of the aqueous extract of *Gentiana kurroo* Royle and precursor silver nitrate salt. Moreover, the optimization of Ag-NPs was accomplished by varying factors like temperature, sample pH, incubation time, a variable quantity of salt and plant extract. Various structural properties of synthesized Ag-NPs were investigated via various characterization analysis including Fourier transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS/EDX) technique. FTIR spectrum of both rhizome extract and prepared Ag-NPs showed excellent stabilization of NPs. EDS analysis confirmed elemental analysis of the prepared sample. SEM displayed spherical shaped Ag-NPs with an average diameter of >20 nm.

Keywords: Bio-reduction; Green synthesis; *Gentiana kurroo* Royle; Silver nanoparticles; Nanotechnology

1. Introduction

Currently, nanotechnology has gained immense interest from scientists owing to the proficiency to compete with the daily challenges of environmental problems and the formulation of excellent scientific ideas [1,2]. Moreover, the main concept of nanotechnology research is the generation of nanomaterials with uniform morphology. Recently, due to the high content of organic and inorganic pollutants in the environment, scientists are trying to focus on a more eco-friendly approach. Green synthesis is considered to be an alternative, efficient, time-saving, non-toxic and ecofriendly approach for the synthesis of metallic NPs [3,4]. Although some traditional chemical processes have been reported for the synthesis of metallic NPs, these methodologies possess certain limitations such as toxicity, harmful by-products, costly chemicals and safety issues which lead to unacceptable levels of damage to the environment. Green synthesis offers more benefits over conventional chemical and physical methods. Moreover, a plant-mediated green route for the synthesis of Ag-NPs utilizes plant extract which naturally behaves as an excellent reducing agent due to the presence of the poly-phenol group [5,6]. In this study, *Gentiana kurroo* Royle was utilized for the reduction and stabilization of silver ions. *Gentiana kurroo* Royle is mostly found in the Himalayas, Kashmir where it is known as “Nilkanth”. This plant has been generally used for the treatment of hepatitis, fever, anti-inflammation and other soreness [7,8].

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Phytochemical study for *Gentiana kurroo* Royle revealed the presence of high content of biological molecules such as phenolic and sulfur (dimethyl sulfide, 2-ethylfuran) containing groups which help in the reduction of nanomaterials. Among various nanomaterials, metallic silver nanoparticles have gained huge attention due to the presence of characteristic properties that help in antimicrobial, anti-plasmodial, anticancer, pupicidal activities, or aid in diagnostic, imaging processing and other drug delivery applications [9–12].

Green technology for the production of nanoparticles has been widely reported in the literature but for the biogenic synthesis of Ag-NPs very limited literature is present [13–15]. To the best of our knowledge, no work has been published on the preparation of Ag-NPs using rhizomes of *Gentiana kurroo* Royle. In this work, single-step production of highly stable Ag-NPs using rhizomes of *Gentiana kurroo* Royle extract is reported with a particular focus on the biogenic synthesis and optimization of Ag-NPs.

2. Experimental

2.1. Biogenic synthesis of Ag-NPs using Gentiana kurroo Royle extract

Gentiana kurroo rhizomes as shown in Fig. 1 was collected from the local market. The rhizomes of *Gentiana kurroo* Royle thoroughly washed and then dried in an oven for complete removal of moisture. Afterward, these dried rhizomes were ground to a fine powder. 25 g of plant powder was dissolved in 400 mL distilled water at high temperature with constant stirring until completion of the extraction was attained. The extract was then filtered followed by centrifugation to remove further impurities and finally stored below 4°C in a refrigerator for further processing.

24 ml of 0.01 M silver nitrate solution was added in 6 ml of 20% *Gentiana kurroo* Royle. The pH of the sample was adjusted using NaOH and HCl solution. The color of the composite mixture was colorless. The sample was continuously stirred for 15 min on a hot plate to achieve complete bio-reduction. The mixture was then placed in an oven at 35°C for 45 min. Complete bio-reduction of $AgNO_3$ to Ag^+ ions was established and the color changed to colloidal brown as shown in Fig. 2.

Fig. 1. Images of *Gentiana kurroo* Royle.

3. Results and discussion

3.1. UV-Visible spectrum for biogenically synthesized Ag-NPs

Fig. 3 shows the UV-Visible spectrum for biogenically synthesized Ag-NPs. The results confirm the presence or formation of Ag-NPs attributed by the strong absorption peak at 430 nm [16,17]. The presence of a single peak in the spectra confirmed the presence of stable or spherical shaped Ag-NPs. Ag-NPs possess excellent surface and optical properties due to surface plasmon resonance λ_{SPR} [18].

3.2. Optimization of biogenically synthesized Ag-NPs

The surface and optical properties of Ag-NPs can be illustrated using UV-Visible spectroscopic techniques. The size, shape, and color of synthesized Ag-NPs are dependent on salt and extract concentration as reported in earlier papers for different nanoparticles [19,20]. Different

Fig. 2. Solution of silver nitrate (0.01 M) before (left) and after (right) addition of plant extract solutions.

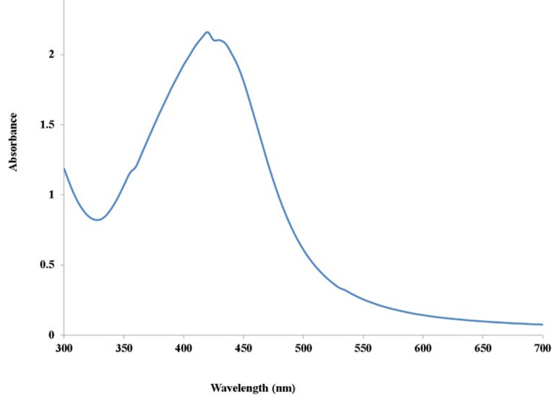

Fig. 3. Absorption spectra of AgNPs.

concentrations of $AgNO_3$ salt were added to the plant extract and four samples were prepared separately. Results revealed that 0.01 M silver nitrate($AgNO_3$) provides maximum yield along with the formation of uniform spherical shaped Ag-NPs while higher salt concentration (0.015, 0.02, and 0.025 M) possesses a broader peak for poly-dispersed particles (Fig. 4a). The UV-Visible spectra of Ag-NPs prepared by varying *Gentiana kurroo* Royle extract concentration showed that higher concentration extract provides more absorption intensity greater reduction of the metal salt occurs which leads to the favorable formation of Ag-NPs. Fig. 4b illustrates the UV/Vis spectra of samples with varying extract concentrations, that is, 12%, 16%, 20%, and 28%. It was observed that the sample containing 20% extract concentration showed an optimum yield. Along-with salt and extract concentration, pH is the most important factor

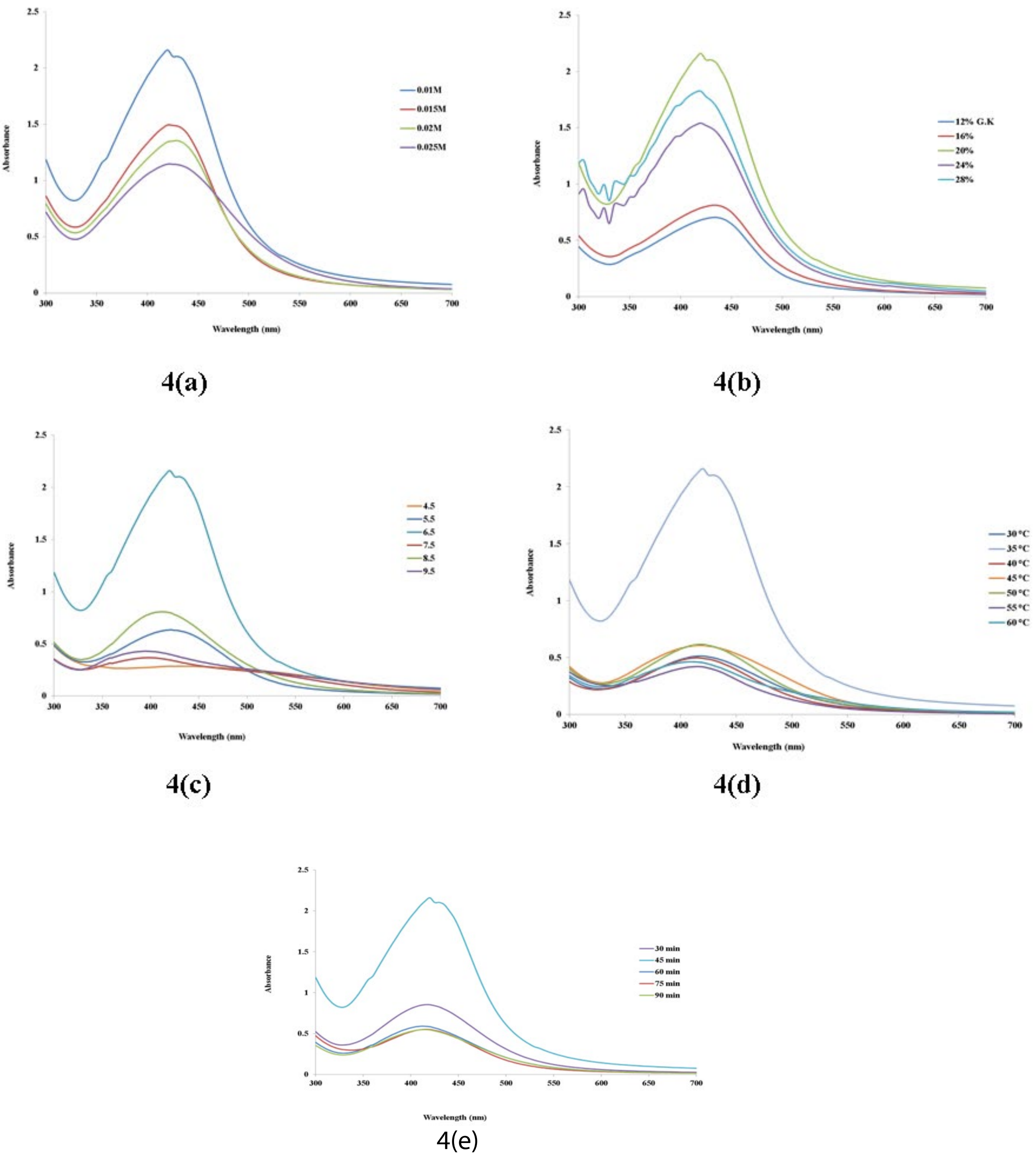

Fig. 4. Absorption spectra of AgNPs with different (a) $AgNO_3$ concentrations, (b) plant extract concentration, (c) pH, (d) Temperature, and (e) incubation time.

for the favorable reaction of Ag-NPs synthesis. Fig. 4c displays result for the synthesis of the Ag NPs by varying pH from 3.5 to 9.5. At an optimum pH, 6.5 maximum yield was attained, which revealed that highly acidic and basic pH leads to the inactivation of biomolecules which help in the reduction and stabilization of NPs [21]. Further to this, the reaction mixture was incubated at different temperatures and maximum absorption intensity was observed at 35°C (Fig. 4d). Results revealed that higher temperature leads to the aggregation of metallic nanoparticles and growth of crystals around the nucleus which restricts complete absorption. Similar behavior was observed by Veerasamy et al. [22] and they utilized *Garcinia mangostana* leaf extract for Ag-NPs synthesis. By varying the reaction mixture incubation time (30–90 min) it was found that maximum reduction of silver ions and higher potential of *Gentiana kurroo* extract biomolecules was observed at 45 min of incubation time (Fig. 4e).

3.3. Fourier transform infrared spectroscopy, scanning electron microscopy, energy-dispersive X-ray spectroscopy analysis for biogenically synthesized AgNPs

Fourier transform infrared (FTIR) spectroscopy is useful for determining the chemical composition of silver nanoparticles [23]. FTIR spectral bands of *Gentiana kurroo* Royle extract (Fig. 5a) indicate free hydroxyl groups in the molecule forming hydrogen bonding or O–H stretching of phenol at 3,300.60 cm^{-1} while the peak at 1,116 or 1,101 cm^{-1} specifies C–OH or C–H stretching vibrations. The peak at 1,644.70 cm^{-1} is associated with C=C stretching of the aromatic compound. All of these peaks showed the presence of multiple organic compounds, for example, phenol or flavonoids inside the *Gentiana kurroo* Royle extract. FTIR analysis of AgNPs as shown in Fig. 5b is represented by peaks at 2,920; 1,383–1,371; 107.4; 1,508; and 1,148 cm^{-1}. The peak at 2,920 cm^{-1} is owing to the presence of C–H stretching of the alkene group. The band at 1,383–1,371 cm^{-1} is attributed to the existence of C–N stretching for aromatic amino groups and at 107.4 cm^{-1} the presence of the ether (C–O) group is confirmed. Additionally, the bands at 1,508 and 1,148 cm^{-1} indicate that various groups of alkene, aliphatic amine, phenol and tertiary ammonium ions shown by C=C and C–H stretching, respectively. Moreover, the band at 1,508 cm^{-1} also confirms the existence of Ag-NPs.

Scanning electron microscopy, as shown in Fig. 6, depicts the morphology of silver nanoparticles using *Gentiana kurroo* Royle plant extract. Scanning electron microscopy (SEM) for Ag-NPs reveals that synthesized NPs were spherical and homogenously distributed without any aggregation [24]. Moreover, the diameters range for silver nanoparticles was 20–60 nm. The elemental identification was established by the energy-dispersive X-ray spectroscopy (EDS/EDX)

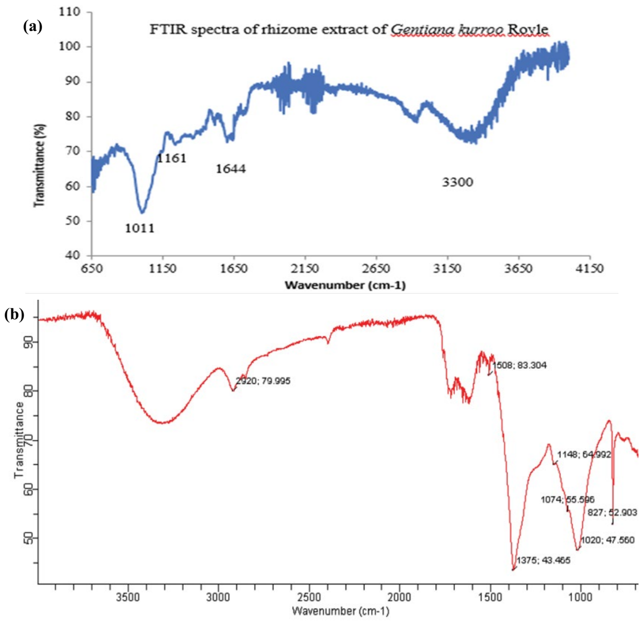

Fig. 5. Fourier transform infrared (FTIR) spectrum of (a) rhizome extract of *Gentiana kurroo* Royle and (b) AgNPs.

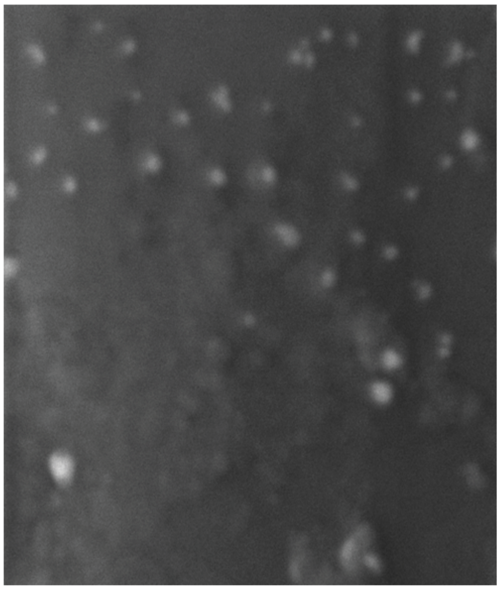

Fig. 6. Scanning electron micrograph showing the morphology of silver nanoparticles using *Gentiana kurroo* plant extract.

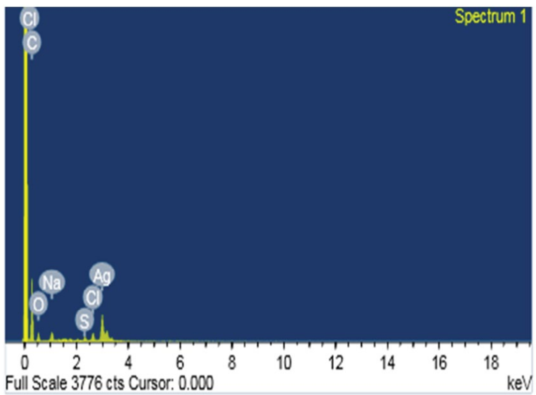

Fig. 7. SEM-EDX micrograph for confirmation of silver nanoparticles.

technique which showed the presence of silver in the sample. The EDX spectra exhibited strong signals for silver at 3 KeV as shown in Fig. 7. Moreover, an optical absorption band at 3 KeV is the main characteristic feature for AgNPs owing to the presence of SPR [13,25,26]. Additionally, some other peaks for weak signals of C and O were also observed.

4. Conclusion

Silver nanoparticles were synthesized by using plant-mediated biogenic synthetic route in the presence of precursor silver nitrate salt and extract of *Gentiana kurroo* Royle rhizomes. The UV-Visible spectra showed a peak at 430 nm revealing the successful generation and stabilization of Ag-NPs. Additionally, Ag-NPs showed optimum yield when using 0.01 M precursor salt, 20% extract concentration, pH 6.5 and temperature 35°C along-with 45 min incubation time. Successfully optimized Ag-NPs were further characterized by FTIR, SEM and EDX techniques which confirmed the presence of uniform spherical and homogenous Ag-NPs.

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