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Biological and electrical properties of biosynthesized silver nanoparticles

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Abstract. In this work, silver nanoparticles (AgNPs) were synthesized biochemically at room temperature using aqueous extract of rhizome of *Rheum australe* plant. The as-synthesized AgNPs were further studied for their morphological, biological and electrical characterization. The morphological studies, such as scanning electron microscopy, X-ray diffraction and UV–vis spectrum confirmed their successful synthesis. Biological analysis revealed their antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Electrical characterization showed that the conductivity of the mixture of AgNPs with DPPH assay is more than the AgNPs dispersed in distilled water. The obtained results may have potential applications as sensors.

Keywords. Silver nanoparticles; antioxidant activity; morphology; rhizome; *Rheum australe*.

1. Introduction

The studies related to nano-science are of great interest due to their potential applications. During recent times, synthesis of nano-wires, nanoparticles and carbon nanotubes is widely studied and developed due to their wide range of applications in microelectronics, medicine, biology, catalysis and sensing technology.[1] The scope of this paper is limited to the synthesis and characterization of silver nanoparticles (AgNPs).

Nanoparticles can be synthesized physically, chemically or biologically. Biological synthesis of nanoparticles is regarded as ecofriendly as they do not use toxic chemicals for the synthesis protocol.[2,3] Biological methods of nanoparticle synthesis involve use of microorganisms, enzymes and plants/plant extracts. Using biological method, metallic nano-particles, such as, Au, Ag, Cu, etc., have been reported to be synthesized by various researchers.[4–13] The silver (Ag) metal is well renowned for its toxicity against several microorganisms from the ancient times. In this work, we used silver nitrate ($AgNO_3$) and *Rheum australe* extract for synthesizing AgNPs.[14,15]

R. australe (Polygonaceae), an Indian/Himalayan Rhubarb, is a leafy perennial herb distributed in altitudes ranging from 2800 to 3800 m in the temperate and subtropical regions of Himalayas from Kashmir to Sikkim in India. Traditionally, the Rhubarb is used as a laxative, diuretic to treat kidney stones, gout, liver diseases, to heal skin sores and scabs.[16] Scientifically, their roots are reported to have antioxidant,[17] antibacterial, antifungal and hepatoprotective activities. The major constituents known to be present in rhubarb rhizomes are anthraquinones, flavonoids, anthraquinone glycosides, tannins, volatile oils and saponins.[18]

In the present study, AgNPs are synthesized by using aqueous solution of $AgNO_3$ and *R. australe* extract and are investigated to show free radical scavenging activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Structural characteristics were examined using X-ray diffraction (XRD) and scanning electron microscopy (SEM) that confirmed the formation of AgNPs. Further, UV–vis spectrum and electrical characterization have also been carried out.

2. Materials and methods

2.1 *Preparation of the AgNPs*

Fresh plant material (rhizome) of *R. australe* was collected from the Bhaderwah area of Jammu and Kashmir, India. The material was shade dried and grounded to a fine texture (powder form) in a grinding machine. Figure 1 shows the schematic procedure for the biosynthesis of AgNPs.

As shown in figure 1, the aqueous extract of plant material was then prepared by mixing plant powder with double distilled water. It was kept for 24 h. The mixture

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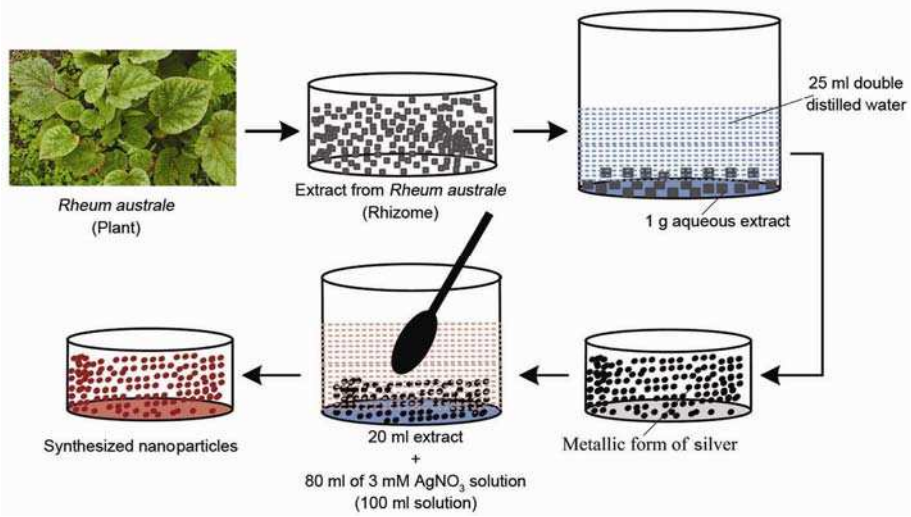

Figure 1. Biosynthesis of silver nanoparticles from rhizome extract.

was then filtered and the obtained filtrate was dried. For the synthesis of AgNPs, 1 g aqueous extract of *R. australe* rhizome was dissolved in 25 ml double distilled water. In this procedure, the plant extract acts as reducing agent that reduces $AgNO_3$ and converts it into metallic form of silver. Twenty millilitres of this extract was mixed with 80 ml of 3 mM $AgNO_3$ solution. The prepared solution was kept in dark for about 24 h. The formation of reddish brown colour was the evidence for the formation of nanoparticles. After 24 h, the reaction mixture was centrifuged at 10,000 rpm for 20 min at 20°C temperature. The supernatant thus obtained was discarded and pellet was washed twice with double distilled water using centrifugation. The pellet obtained was collected using ethyl acetate. Finally, the synthesized nanoparticles were dried at room temperature for further morphological, biological and electrical studies.

2.2 *Free radical scavenging ability on DPPH*

The free radical scavenging activity of Rheum extracts and AgNPs was determined with slight modifications in the method adopted by Abe *et al.*[19] One millilitre from a 0.5 mM methanol solution of the DPPH radical was mixed to four different concentrations (50, 100, 150 and 200 µl) of plant extract and AgNPs and to this 2.0 ml of 0.1 M sodium acetate buffer (pH = 5.5) was added. The final volume was made to 4 ml with methanol. The mixtures were well shaken and kept at room temperature in the dark for 30 min. The absorbance was measured at 517 nm using a double beam UV–vis spectrophotometer. Methanol was used as a negative control. Briefly, 50% inhibition (IC_{50}) of the samples was calculated from the graph by plotting percentage inhibition against sample concentrations. The radical scavenging activity (RSA) was calculated as a percentage of DPPH radical discolouration, using the equation

$$\%\text{RSA} = \frac{A_0 - A_S}{A_0} \times 100, \quad (1)$$

where A_0 is the absorbance of the control and A_S the absorbance of the test compound.

2.3 *Ferric reducing ability (FRAP)*

The FRAP assay was carried out according to the method followed by Benzie and Strain.[20] FRAP reagent was prepared in acetate buffer (300 mM) by adding 10 mM 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) solution in 40 mM HCl and 20 mM $FeCl_3$ solution in proportion of 10 : 1 : 1 (v/v), respectively. FRAP reagent was prepared fresh at the time of use. Fifty microlitres of the sample was added to 1.5 ml of the FRAP reagent and after 3–4 min, absorbance was measured at 593 nm. The standard curve was prepared by using $FeSO_4$ (100–2000 µM) and the result was expressed as µmol Fe(II) per g dry weight of extract.

2.4 *Membrane integrity assay using lipid peroxidation inhibition*

Mice brain homogenate was used to estimate the membrane integrity potential. Mice were selected randomly and killed by cervical dislocation. Briefly, 10% whole brain homogenate was prepared in cold buffered saline (pH 7.4). Brain homogenate (10%) was used to study lipid peroxidation inhibition potential of aqueous extract of *Rheum emodi* and its AgNPs. Reactive oxygen species (ROS) produced in the body disrupt membrane by destroying its lipids. The AgNPs combat this process by

scavenging ROS produced and thus protect the membrane from being destroyed.

Thiobarbituric acid reactive substances (TBARSs) estimation is the most commonly used method to detect lipid peroxidation with slight modifications in the method. Brain homogenate (100 µl) was mixed with different concentrations of extracts and its nanoparticles (5 mg ml^{-1}) and the volume of mixture was then raised to 1.0 ml using PBS.[21] The reaction mixture was then incubated at 37°C for 15 min. After incubation, 500 µl of Fenton reagent was subjected to the reaction mixtures. Followed by an incubation of 1 h at 37°C, the equivalent volume of 10% trichloroacetic acid (TCA) and 0.7% thiobarbituric acid (prepared in 0.025 N NaOH) was added. The resultant mixture was incubated at 80°C for 1 h in water bath. A pink-coloured chromogen complex formed was read at 532 nm. The protection index of the extract and nanoparticles was compared based on percentage inhibition in the colour chromogen complex formation corresponding to the reduction in absorbance at 532 nm. The protection index was estimated using the same formula as shown in equation (1).

3. Results and discussion

The characterization techniques such as SEM and XRD were done to study the morphological and structural properties of the nanoparticles. For biological characterization, antioxidant activity is studied. The current–voltage (*I–V*) characterization of AgNPs is also studied by the two-point probe method. UV–vis spectrum analysis shows interesting results. Further, the as-synthesized AgNPs were demonstrated as a temperature sensor.

3.1 *SEM characterization*

The morphological properties of the as-synthesized AgNPs were studied using Zeiss EVO 40 SEM. After the preparation of the nanoparticles, the suspension of nanoparticles in double distilled water was used for SEM analysis by fabricating a drop of suspension onto a clean electric stubs and allowing water to completely evaporate.

The dried sample was then mounted on a clean aluminium stub. The sample was then viewed under SEM at an accelerating voltage of 20 kV. Figures 2 and 3 show the SEM micrograph (top view) of as-synthesized AgNPs. SEM studies confirmed the development of nanoparticles with almost same shapes and dimensions and have diameter 100 nm (approx.).

3.2 *XRD characterization*

The structural studies of as-synthesized AgNPs were done using PAN Anatyical X-ray diffractometer machine operating at 40 kV. The patterns were scanned for the scanning angle of 20–80° with a step size of 0.0170° per second using wavelength (K_a) radiation 4.087 Å. The crystalline nature of AgNPs was confirmed by the peaks obtained in XRD diffraction patterns as shown in figure 4. The diffraction peaks in the patterns match with the standard ICDD data file (JCPDS number 04-0783).[22]

The high intense peak in XRD pattern indexed as (111) suggests a preferential growth of nanoparticles. The intensity of peaks reflects the crystalline nature of AgNPs. However, the diffraction peaks are broad indicating that the crystal size is exceptionally small. Few diffraction peak patterns were observed at 38.33°, 44.48°, 64.64° and 77.56° corresponding to the planes (111), (200), (220) and (311), respectively. Some low intensity peaks were also observed. These may be due to some precursors involved in the process as stabilizing agents.

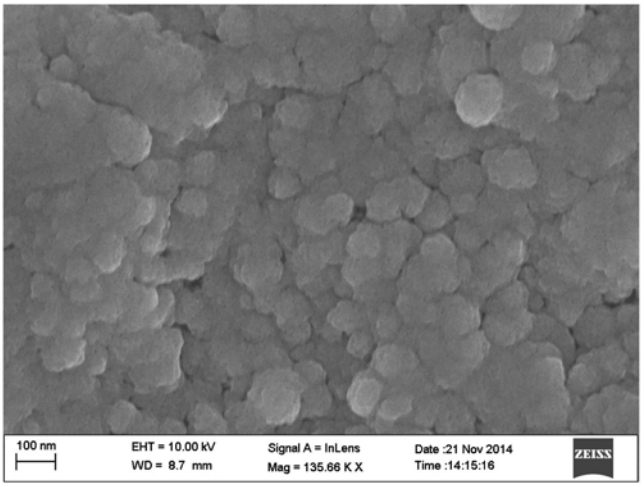

Figure 2. SEM of silver nanoparticles with X = 135.66 K.

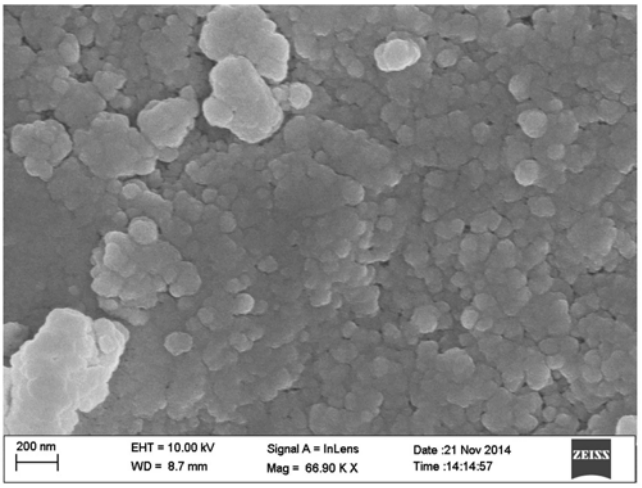

Figure 3. SEM of silver nanoparticles with X = 66.90 K.

3.3 *UV–vis spectrophotometer characterization*

The reduction of silver ions into AgNPs by using rhizome extract was analysed by UV–vis Spectrophotometer (GENESYS™ 10S UV–vis Spectrophotometer, United States). Scanning is done from 300 to 500 nm range with wavelength speed 4200 nm min^{-1}. Wavelength accuracy is ± 1.0 nm. Figure 5 shows the UV absorption spectra of the synthesized AgNPs recorded as a function of reaction time.

Absorption spectrum shows that the peak positioned in-between 410 and 420 nm indicated the formation of AgNPs. During initial reaction time, the desired region was broad and the peak positioned at 410 nm due to the early formation of nanoparticles. Broadening of the peak indicates that the particles are poly-dispersed. After 1 h of incubation, the region shifts to the wavelength of 420 nm and a very swift reaction took place. As the reaction time increases, the reaction rate shows gradual increase. Hence, the formation of AgNPs was confirmed using UV–vis spectral analysis.

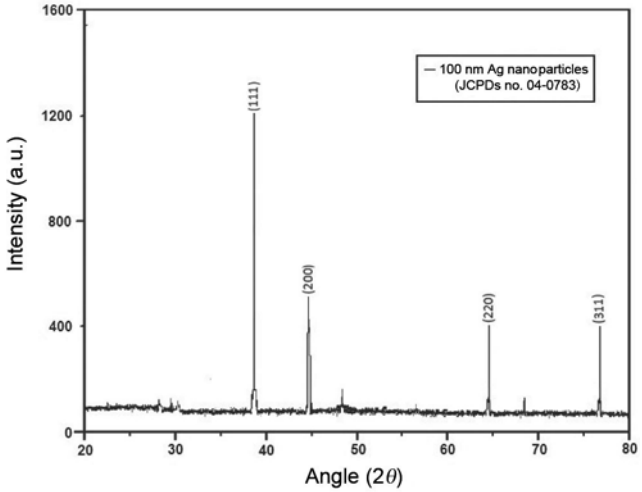

Figure 4. XRD pattern of as-synthesized silver nanoparticles.

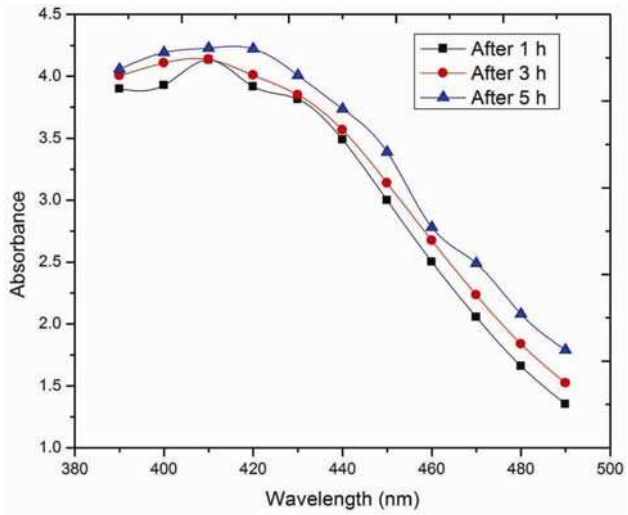

Figure 5. UV spectroscopic analyses of synthesized silver nanoparticles recorded as a function of time.

3.4 *Antioxidant activity*

3.4a *DPPH assay:* The free radical scavenging activity of aqueous extract of *R. australe* rhizome and its nanoparticles on DPPH is summarized in table 1. The AgNPs were found more potent antioxidant with IC_{50} value of 73.79 ± 1.5, whereas the plant extract had IC_{50} value of 96.46 ± 2.1. The obtained results showed that the nanoparticles of an extract increase the radical scavenging activities by folds. It is pertinent to mention that $AgNO_3$ showed negligible free radical scavenging activity. Figure 6

Table 1. Antioxidant activity of the plant extract and its nanoparticles using DPPH assay.

	Percentage inhibition	
Conc. (μg ml^{-1})	Extract	Nanoparticles
50	24.25	36.09
100	43.27	66.47
150	62.93	81.22
200	78.48	96.46
IC_{50}	118.87	73.79

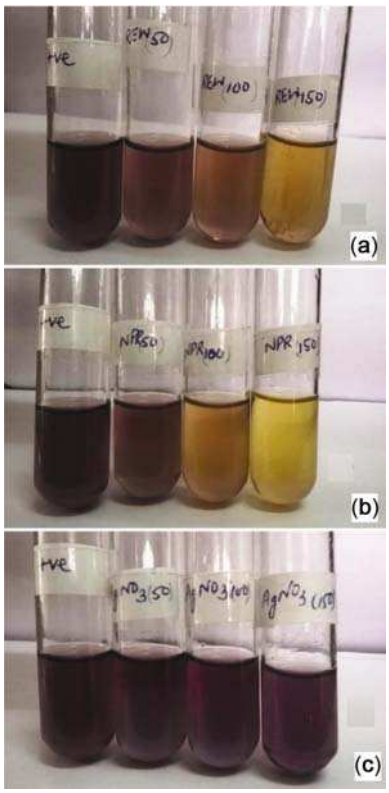

Figure 6. Change in colours by DPPH radical scavenging activity at different concentrations: (**a**) *Rheum australe* water extract, (**b**) AgNPs of *Rheum australe* and (**c**) silver nitrate ($AgNO_3$).

confirms the antioxidant potential of AgNPs indicated by the change in colours of the solution at different concentrations.

3.4b *FRAP assay:* Moreover, the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} in the presence of TPTZ, forming an intense blue (Fe^{2+}–TPTZ complex), was investigated. Reducing power was expressed in μmol Fe(II) per g dry weight of plant material. Higher antioxidant activity to reduce the ferric ions (Fe^{3+}) was observed in AgNPs (997.4 ± 5.2 μmol Fe(II) g^{-1}) followed by aqueous Rheum extract (959.4 ± 8.3 μmol Fe(II) g^{-1}).

3.5 *Membrane integrity potential*

The membrane integrity potential of silver was examined by comparing the lipid peroxidation inhibition of plant extract and its AgNPs and the result is tabulated in table 2. Cell membrane is more susceptible to free radicals that reacts rapidly with the unsaturated fatty acids (like linoleic acid and arachidonic acid) embedded in the membrane which results in lipid peroxidation. The IC_{50} values of extract and its AgNPs were obtained to be 302.44 ± 2.4 and 105.45 ± 3.01 μg ml^{-1}, respectively. By comparing the IC_{50} values of both the samples, it was calculated that AgNPs showed higher ability to maintain membrane integrity by inhibiting the lipid peroxidation of the membranes.

3.6 *Role of Ag in inducing ROS*

Silver ions have been widely used to inhibit bacterial growth by inhibiting the essential enzymatic functions of the microorganisms. However, silver ion has raised the possibility of ROS generation. This induction of free radicals leads to oxidative stress. But when we synthesize AgNPs using plant extracts, $AgNO_3$ gets reduced and loses its free radical generating potential.[23]

3.7 *Electrical characterization*

The current–voltage (*I–V*) characteristics of the as-synthesized AgNPs dispersed in distilled water are studied for its electrical characterization. The schematic of the apparatus employed for electrical characterization is shown in figure 7.

The currents (μA) is noted with respect to the corresponding dc voltage (mV) using the two-point probe method and a graph is plotted as shown in figure 8. The *I–V* characteristics were studied for two different sub-experiments, i.e., for the solution of (i) AgNPs dispersed in DPPH solution and (ii) AgNPs in distilled water.

From figure 8, it is clear that the current for the AgNPs dispersed in oxidant solution (DPPH) increases more

Table 2. Lipid peroxidation inhibition of the plant extract and its silver nanoparticles.

	Percentage inhibition	
Conc. (μg ml^{-1})	Extract	Nanoparticles
50	13.56	45.53
100	22.82	48.01
150	25.83	54.25
200	59.95	59.95
IC_{50}	302.44	105.45

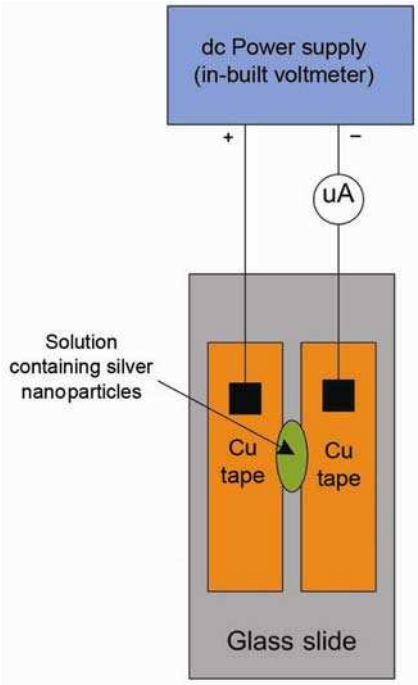

Figure 7. Schematic for electrical characterization.

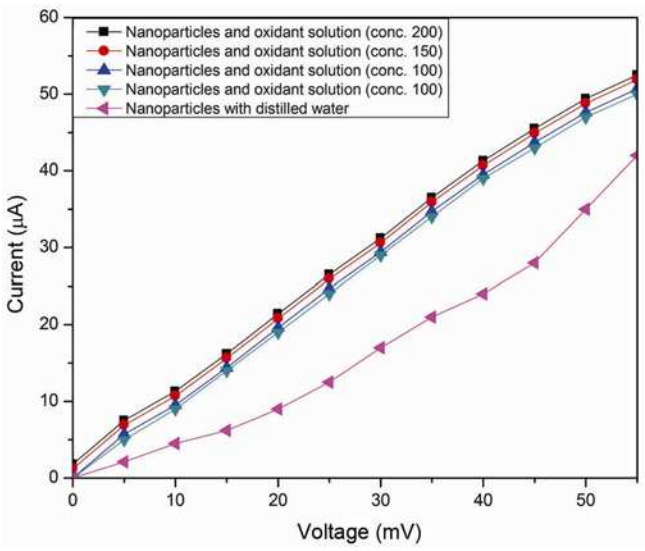

Figure 8. *V–I* characteristics showing conductivity of nanoparticles for different studies.

rapidly with respect to the voltage applied than that of the nanoparticles dispersed in distilled water. This indicates that, for AgNPs dispersed in oxidant solution, the current increases rapidly with the increase in their concentration. Thus, *I–V* graph revealed that the increase or decrease in current depends on the DPPH dose concentration in combination with dispersed nanoparticles and this follows the same trend as shown in table 1.

4. Conclusion

In this work, AgNPs have been synthesized by a simple method using aqueous extract of *R. australe* rhizome. Results confirmed that plant extracted AgNPs showed a higher antioxidant activity as compared to $AgNO_3$. The SEM analysis showed that the successful synthesis of AgNPs ranged with particle size upto 100 nm. XRD confirmed the elemental composition. UV–vis spectra have been studied to confirm the synthesis of nanoparticles in-between 410 and 420 nm spectral window. Biological characterization showed strong antioxidant activity of as-synthesized AgNPs. Electrical characterization confirmed that the current increases with the increase in the antioxidant property of the nanoparticles. Thus, it is possible to estimate the antioxidant property of the synthesized nanoparticles by studying their electrical properties.

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