



# Glucoxylan-mediated green synthesis of gold and silver nanoparticles and their phyto-toxicity study



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## ABSTRACT

A green synthesis of gold and silver nanoparticles having exceptional high stability is reported. The synthesis involves the use of glucoxylans isolated from seeds of *Mimosa pudica* and excludes the use of conventional reducing and capping agents. The average particle sizes were 40 and 6 nm for gold and silver, respectively. The size of gold particles obtained in this work is suitable for drug delivery as they are non-cytotoxic. In phyto-toxicity tests the gold and silver nanoparticles did not show any significant effect on germination of radish seeds, whereas in radish seedling root growth assay the two particles behaved differently. The silver nanoparticles exhibited a concentration-dependent stimulatory effect on root length, whereas the gold nanoparticles had no significant effect in this test. The likely mechanism of these effects is discussed.

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## 1. Introduction

Carbohydrate polymers isolated from various plant materials, having versatile properties, find applications in drug delivery (Sinha & Kumria, 2001; Sinha et al., 2004), tissue engineering (Goldberg, Langer & Jia, 2007; Matricardi et al., 2013; Prabakaran & Mano, 2006; Senni et al., 2011), biosensors and electronics (Finkenstadt, 2005). Some of the carbohydrate polymers have been used in green synthesis of noble metal nanoparticles (NPs) (Park et al., 2011). Very recently, synthesis of gold and silver NPs with exceptional stability has been reported by use of arabinoxylan as self reducing and stabilizing agent (Amin et al., 2013). This study demonstrated the potential of carbohydrate polymers in synthesis of metal particles without the additional use of any reducing or stabilizing agents. It was hypothesized that the reducing action is because of the presence of some amount of free reducing sugars released by the polymer in the suspension. The polymers also cause dispersion of particles into their matrix, which results in high stability (Amin et al., 2013). In the present work we wish to report the

isolation of a glucoxylan fraction from *Mimosa pudica* and its use in green synthesis of gold and silver NPs having exceptional high stability. *M. pudica*, commonly known as touch-me-not or sensitive plant, is widely found in tropical regions of Pakistan, India and other parts of the world. Its seeds produce a mucilage when soaked in water. The mucilage is mainly composed of D-xylose and D-glucuronic acid (Aggarwal & Karimullah, 1945; Farooqi, Kapoor & Khan, 1977; Saraswat & Pokharkar, 2012) and as such can be classified as a glucoxylan (GX). The seeds are commercially available at very low price in Asian markets. Like arabinoxylans (Amin et al., 2013) the GX mucilage is expected to produce metal NPs without the additional use of any reducing or stabilizing agents. Being a natural biomaterial the mucilage is highly biocompatible and non-toxic. The objective of present work was to evaluate the potential of this material in synthesis of gold and silver NPs for biomedical applications.

## 2. Experimental

### 2.1. Materials

Chloroauric acid,  $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$  (E. Merck, Germany), silver nitrate,  $\text{AgNO}_3$  (E. Merck, Germany), sodium hydroxide,  $\text{NaOH}$  (E. Merck, Germany), and hydrochloric acid,  $\text{HCl}$  (E. Merck, Germany)

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were of analytical grade and used without further purification. *M. pudica* seeds were obtained from local market. Nanopure® water was used in this work.

## 2.2. Isolation of glucoxyylan from *M. pudica* seeds

*M. pudica* seeds (10.0 g) were soaked in Nanopure® water (500 mL) for 10 h in a clean environment at room temperature and blended by using a kitchen blender to separate the fiber and germs from the mucilage. The fiber and germs were separated out by passing the translucent mucilage through a muslin cloth under vacuum. The volume of the clear mucilage was made up to 500 mL with Nanopure® water. In order to determine the reproducibility, three batches were prepared from the seeds obtained from three different sources.

## 2.3. Synthesis of NPs

Varying amounts (1–20 mL) of the mucilage were added to 20 mL solutions of 1.0 mM  $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$  and  $\text{AgNO}_3$  separately, and total volume was made up to 40 mL with water under constant stirring. A change in color (yellow to brown to purple in case of AuNPs; colorless to yellow to brown in case of AgNPs) was observed in 15–180 min (in case of AuNPs) and 60–240 min (in case of AgNPs) depending upon the amount of the mucilage added, pH and temperature. The optimum conditions were determined by varying the amount of mucilage, pH (3–12), temperature (25–100 °C) and time. The NP suspensions thus obtained were washed several times with Nanopure® water in order to remove any unreacted gold or silver salts.

### 2.3.1. Characterization

**2.3.1.1. Surface plasmon resonance spectroscopy.** The reduction of the gold and silver salts was monitored by recording the surface plasmon resonance (SPR) spectrum in the 300–800 nm range by use of Pharmaspec UV-1700 spectrophotometer (Shimadzu, Japan). The spectra were recorded by three-time dilution of the sample with a blank in the reference using a matched pair of 1 cm quartz cuvettes.

## 2.4. Powder X-ray diffraction

The NP suspensions were purified by repeated centrifugation at 4000 rpm for 20 min followed by redispersion of the pellet of NPs into 10 mL of deionized water. After freeze-drying the purified particles were characterized by powder X-ray diffraction (pXRD). The spectra were recorded on Bruker D8 Discover (Germany) diffractometer using monochromatic  $\text{Cu-K}\alpha$  radiation ( $\lambda = 1.5406 \text{ \AA}$ ) operated at 40 kV and 30 mA. The data were collected in the  $2\theta$  range of 10–80°.

## 2.5. Atomic force microscopy

The atomic force microscopic (AFM) images were obtained from a  $5.0 \mu\text{m} \times 5.0 \mu\text{m}$  film of the samples using scanning probe microscope SPM-9500 J3 (Shimadzu, Japan) in the contact mode under normal atmospheric conditions. A freshly prepared sample was deposited on a fine metal surface in dust-free environment for this analysis.

### 2.5.1. Scanning electron microscopy

Scanning electron microscopic (SEM) images of selected NPs were obtained by using SEM S-3700N (Hitachi Japan) without sputter coating because the NPs were self-conducting.

### 2.5.2. Transmission electron microscopy

Thin films of ultrasonically dispersed samples were prepared by dropping a very small amount of sample on a carbon-coated copper grid followed by drying at room temperature. The transmission electron microscopic (TEM) images were obtained by using JEM-1200EX (JEOL, Japan) microscope at an accelerating voltage of 120 kV.

## 2.6. Phyto-toxicity assay

The phyto-toxicity assay was performed by using radish (*Raphanus sativus L.*) according to a reported method (Turker & Camper, 2002). In this assay root length and percent seed germination were determined. Briefly, 2 mL of the NPs suspension (four different concentrations) and a control (a suspension without NPs) were transferred to sterile Whatman 1 filter papers placed in sterile plates. Ten radish seeds were placed in each plate. The plates were incubated at  $25 \pm 2^\circ\text{C}$  in dim light and root length was measured for 5 days and compared with the controls. In another experiment four different concentrations of the NPs and a control were used to determine the percent germination under similar conditions. The germination was recorded for 5 days. These experiments were performed in triplicate and data were statistically analyzed.

## 3. Results and discussion

### 3.1. Synthesis of NPs

The Au and Ag NPs were obtained by reduction of  $\text{HAuCl}_4$  and  $\text{AgNO}_3$  with the *M. pudica* mucilage; where the mucilage, characterized to be a GX, served as reducing as well as dispersing agent. Formation of the NPs was monitored by SPR absorptions at 530 nm and 412 nm for AuNPs and AgNPs, respectively. The smallest particle size was obtained after optimizing the reaction conditions with respect to amount of mucilage, temperature, pH and time. The spectral variations with varying amount of polymer, temperature and pH are shown in Fig. 1. The optimum conditions for nearly monodisperse AuNPs having size approximately 40 nm were: amount of mucilage 16 mL/40 mL, amount of 1 mM  $\text{HAuCl}_4$  20 mL/40 mL, temperature 90 °C, pH 4.5, time 25–45 min; whereas the conditions for AgNPs having a mean size of 6 nm (range 5–10 nm) were: amount of mucilage 4–8 mL/40 mL, amount of 1 mM  $\text{AgNO}_3$  20 mL/40 mL, temperature 90 °C, pH 12, time 20 min.

### 3.2. Powder X-ray diffraction

The pXRD spectra of the synthesized AgNPs and AuNPs are shown in Fig. 2. The spectra are characteristic of face-centered cubic phase (JCPDS File No. 87-0720). The diffraction peaks in the spectrum of AgNPs at  $2\theta$  values 44.4°, 51.2°, 63° and 73.6° can be assigned to (1 1 1), (2 0 0), (2 2 2) and (3 1 1) planes respectively. Similarly the peaks in the spectrum of AuNPs at  $2\theta$  values 44.2°, 48°, 64° and 76.5° can be assigned to (1 1 1), (2 2 0), (2 2 2) and (3 1 1) planes respectively. The sizes of the particles as calculated by use of Debye–Scherrer equation (Scherrer, 1918) were found to be 5–10 nm and 10–50 nm for silver and gold, respectively. The Bragg reflections (2 0 0), (2 2 0) and (3 1 1) were weak and broadened relative to the intense (1 1 1) reflection. This feature indicates that the nanocrystals are mainly oriented along (1 1 1) plane. There were some unidentified peaks in the spectra which may be due to crystallization of organic materials from GX on the surface of the NPs.

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