



Antibacterial activities of *Hibiscus cannabinus* stem-assisted silver and gold nanoparticles

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ABSTRACT

The synthesis of silver (Ag) and gold (Au) nanoparticles (NPs) using *Hibiscus cannabinus* stem extract was studied. The synthesized NPs were characterized using UV-visible spectroscopy (UV-vis), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), Transmission electron microscopy (TEM) and Energy Dispersive X-ray analysis (EDX). The surface plasmon resonance (SPR) peak of synthesized NPs was observed at 444 nm and 547 nm corresponding to AgNPs and AuNPs respectively. The prepared AgNPs and AuNPs were almost spherical in shape with the average particle size of 10 nm and 13 nm respectively. The FTIR study reveals that the carboxylic acid present in *H. cannabinus* stem extract has been used as reducing agent. The observed antibacterial properties, suggest the possible utilization of prepared NPs in water purification.

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1. Introduction

Purity of water is compromised due to its pollution by the existence of pathogenic bacteria and carcinogenic chemicals present in it. Diseases causing microorganisms, that characteristically are waterborne significantly contain protozoa and bacteria. The removal or inactivation of pathogenic microorganisms is the important step in the treatment of wastewater. In recent years, microorganisms have evolved to be drug resistant due to the changes in their chromosomes or genetic materials. These problems demand for the development of novel effective antibacterial agents against new generation bacteria.

Metal nanoparticles are used for the purification of water which is one of the essential enablers of life on earth. Among various metal nanoparticles, AgNPs and AuNPs play a profound role in the field of biology and medicine due to their attractive physiochemical properties. Increasing the awareness towards green chemistry and other biological processes, that has led to a desire to develop an eco-friendly approach for the synthesis of nanoparticles which has several advantages such as simplicity, cost-effectiveness, compatibility for antibacterial, antioxidant, and antitumor activity of natural products. Biosynthesis of AgNPs and AuNPs using *Centella asiatica*, *Daucus carota*, *Solanum lycopersicum*, *Hibiscus cannabinus* leaf, *M. flower*, *Ananas comosus* fruit, *Bacopa monnieri*, *Citrus unshiu* peel, coriander and *Bischofia javanica* (L.) has been reported [1–13]. Main objective of this present study is to synthesize AgNPs and AuNPs using *H. cannabinus*

stem extract as reducing agent and to demonstrate its function as a antibacterial agent so that it may be utilized for water purification.

2. Experimental details

An aqueous solution of HAuCl_4 was added to 5 ml of stem extract and stirred for 5 min at room temperature. During the synthesis, it initially becomes colorless and turned into purple indicating the formation of AuNPs. Similarly, an aqueous solution of AgNO_3 (3 mM) was added to 5 ml of fruit extract and stirred for 5 min at room temperature. Upon addition of the extract, the colorless solution changes color from light yellow to reddish orange indicating the formation of AgNPs. The absorption spectra of the prepared NPs were measured using a Shimadzu spectrophotometer (UV 1700). XRD analysis of the NPs was done using PANalytical X'pert – PRO diffractometer with $\text{Cu K}\alpha$ radiation operated at 40 kV/30 mA. FTIR measurements were obtained on a Nexus 670 FTIR instrument with the sample as KBr pellets. TEM analysis was done using a JEOL JEM 2100 HRTEM operating at 200 kV. A disc diffusion method was used to evaluate the antibacterial action of prepared NPs against bacteria namely *Pseudomonas aeruginosa* (gram negative) and *Staphylococcus aureus* (gram positive).

3. Results and discussion

Fig. 1(i) shows the UV-vis spectra of AgNPs and AuNPs. Fig. 2(i) and (ii) shows the TEM image of AgNPs and AuNPs respectively. The appearance of SPR peak at 444 nm and 547 nm confirms the

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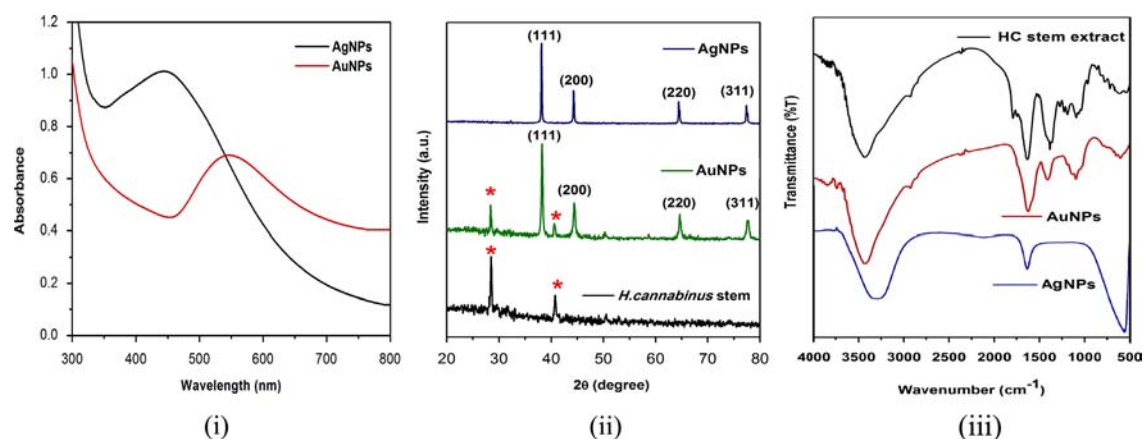


Fig. 1. UV-vis spectra (i), XRD pattern (ii) and FTIR (iii) of prepared NPs.

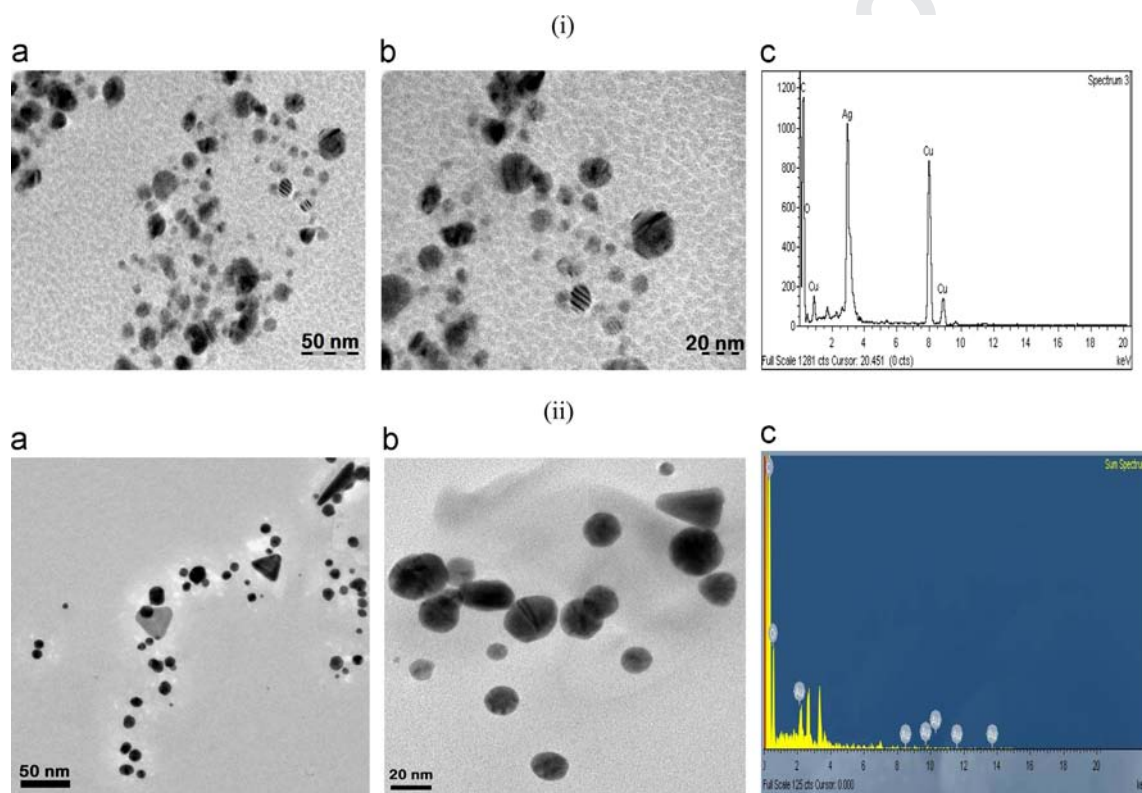


Fig. 2. TEM images at different magnifications (a,b) and EDX spectrum (c) of (i) AgNPs and (ii) AuNPs.

formation of AgNPs and AuNPs, respectively. The position of the SPR peak depends on the particle size and shape. The observed symmetric sharper SPR band with a single peak is due to spherical particles. The TEM image also confirms this result. AgNPs have sizes ranging from 5 to 20 nm with an average diameter of 10 nm and AuNPs having 3 to 31 nm sized nanoparticles with an average size of 13 nm. In comparison to the size and shape of particles, majority of the nanoparticles are smaller sized and spherical shaped particles. This may possibly be due to the control in size and shape of the particles as they are protected by biomolecules responsible for capping and efficient stabilization, which provides steric hindrances between neighboring nanoparticles preventing aggregation by overcoming the Vander Waals of force of attraction between them. There was no obvious change in the peak position even after three months and this indicates the better quality and stability of the formed nanoparticles. The prepared AgNPs exhibits high SPR peak intensity when compared to AuNPs, as Ag has a low

imaginary part of dielectric permittivity [14]. This also evidences higher number of NPs observed in TEM images of AgNPs in comparison to AuNPs. Fig. 2(i)(c) and (ii)(c) shows EDX elemental profile of synthesized nanoparticles. Metallic Ag and Au nanocrystals show typical optical absorption peak approximately at 3 keV and 2.2 keV respectively due to SPR, and confirms the formation of AgNPs and AuNPs.

XRD pattern of *H. cannabinus* stem extract, AgNPs and AuNPs is shown in Fig. 1(ii). Diffraction peaks at 28.48°, 40.72° and 50.4° represent the presence of ascorbic acid (JCPDS 22-1560 and 32-1637), malic acid (JCPDS 23-1631) and citric acid (JCPDS 22-1568). The peaks at 38.09°, 44.28°, 64.42° and 77.37° can be indexed to the crystallographic planes of (111), (200), (220) of face centered cubic (fcc) structure of metallic Ag respectively with space group of Fm-3 m (JCPDS file no. 04-0783). The peaks observed at 38.2°, 44.39°, 64.6° and 77.59° correspond to (111), (200), (220) and (311). Bragg's reflections were in good agreement with fcc structure of metallic Au with space

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