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Novel and Efficient Synthesis of Silver Nanoparticles Using Curcuma Longa and Zingiber Officinale Rhizome Extracts

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Abstract

Silver particles of nanometric size were prepared from silver sulphate solution using Curcuma Longa and Zingiber Officinale rhizome extracts. The reaction was completed at 3 hours on using Curcuma Longa rhizome extract as reducing agent, making it one of the fastest biosynthesis route reported so far. The plasmon bands at 420 nm in the UV visible spectra are broad with an absorption tail in the longer wavelengths, which could be due to the size distribution of the particles. TEM images indicated the presence of silver nanoparticles in the range of 20-50 nm. FTIR spectra suggest that the biological molecules possibly perform dual functions of formation and stabilization of silver nanoparticles in the aqueous medium. Silver NPs produced from 5mM silver salt solutions showed greatest antibacterial activity against Staphylococcus aureus pathogen (gram positive).

Keywords: *Biosynthesis, Silver nanoparticles, Curcuma longa, Zingiber officinale.*

1. INRODUCTION

Increasing environmental awareness has led researchers to focus on green chemistry principles during the last decade. These efforts aim at the total elimination or at least the minimization of generated waste and the implementation of sustainable processes through the adoption of twelve fundamental principles. Utilization of nontoxic chemicals, environmentally benign solvents and renewable materials are some of the key issues that merit consideration in a green synthesis strategy. Recently, researchers have used biological molecules as templates for generation of inorganic structures and materials. Bioinspired synthesis of nanoparticles is a better method compared to chemical and physical methods. It is cost effective and environment friendly because there is no need to use high pressure, energy, temperature and toxic chemicals [1, 2, 3]. One of the fundamental themes involved in biological synthesis is the use of organic matrix (proteins and/or other biological macromolecules) for controlling the nucleation and growth of the inorganic structure in the nanolevel. Biological systems form sophisticated mesoscopic and macroscopic structures with tremendous control over the placement of nanoscopic building blocks within extended architectures. Several studies have demonstrated that proteins identified from biological organisms can be used as enzymes or templates for nanomaterial synthesis [4, 5].

Noble metal nanoparticles (NPs) are well known for important applications in the fields of electronic, magnetic, optoelectronics, and information storage. Silver NPs, as a significant member of the noble metal NPs, are excellent substrates for surface enhanced Raman scattering (SERS) to probe single molecules, and are excellent as catalysts for accelerating some chemical reactions [6]. The intrinsic properties of a metal nanoparticle are mainly determined by its size, shape, composition, crystallinity, and structure. A number of approaches are available for the

synthesis of silver NPs. For example, silver ions are reduced by chemical [7, 8] electrochemical [9], radiation [10], photochemical methods [11], and biological techniques [12, 13]. Among these methods, biological synthesis is a superior method to fabricate benign nanostructure materials since it reduces the use or generation of hazardous substances to human health and the environment.

Zingiberaceae or the ginger family consist of aromatic perennial herbs with creeping horizontal or tuberous rhizomes, comprising about 52 genera and more than 1300 species, distributed throughout tropical Africa, Asia, and the Americas. Curcuma Longa and Zingiber Officinale are two prominent members of this family that are used as spices and medicinal plants. They contain several active ingredients like volatile oils, lipids, proteins, starch, vitamins, minerals, amino acids resins and a lot more. Curcumin, the active compound of Curcuma Longa has been documented to have a wide range of biological effects including anti-inflammatory, antioxidant, antitumour, anti bacterial and antiviral activities where as gingerol, the most active compound in Zingiber Officinale has analgesic, sedative, antipyretic and antibacterial properties.

In the present study, Curcuma Longa and Zingiber Officinale rhizome extracts are used for bioreducing sliver sulphate solution to silver nanopaticles (Ag NPs). The course of the synthesis has been studied extensively by different standard techniques such as UV Visible Spectrophotometry (UVS), Fourier Transform Infra Red (FTIR) spectroscopy, and High Resolution Tunneling Electron Microscopy (HRTEM). The effect of concentration of initial sliver sulphate solution on the synthesis procedure has also been compared. The antibacterial studies of the synthesized silver nanoparticles are done using the Agar well diffusion assay method.

2. MATERIALS AND METHODS

5 g home grown Curcuma Longa or Zingiber Officinale rhizomes were washed several times with distilled water to remove the dust particles, cleaned and cut in to small pieces. It was then boiled in a 500ml glass beaker along with 250 ml sterile distilled water for 30 minutes. The aqueous extract was separated by filtration with Whatman 1 filter paper. 20ml of the respective rhizome extract was added to 5ml of 0.5mM, 1mM and 5mM aqueous silver sulphate solutions respectively, stirring magnetically at room temperature. The extract acted as the reducing agent as well as capping agent.

The reduction of pure silver ions was observed by recording the UV-Visible spectra of the samples withdrawn from the reaction mixture periodically on a Perkin Elmer spectrophotometer from 200 to 700nm, at a resolution of 1nm. 1ml of the sample taken at regular intervals was diluted to 10 times with Millipore*TM* water to avoid errors due to high optical density of the solution and compared with 1ml distilled water as blank solution. The surface morphology of the prepared samples was evaluated using JEOL JSM-6390 LV Tunnelling Electron Microscope with a dynamic light scattering technique at an accelerating voltage of 80kV. A drop of the sample was placed on a staining mat. Carbon coated copper grid was inserted into the drop with the coated side upwards and air dried. The Fourier Transform Infra Red studies were carried out in KBr medium using Thermo Nicolet, Avatar 370 model FTIR spectrometer in the range of 400-4000 cm^{-1} with a resolution of 4 cm^{-1}. The sample was mixed with KBr and a thin sample disc was prepared and placed in FTIR spectrometer for the analysis of the nanoparticles.

Antibacterial activity of the synthesized silver nanoparticles was determined, using the Agar well diffusion assay method. Approximately 20ml of molten and cooled media (Nutrient agar) was poured in

sterilized petri dishes. The plates were left overnight at room temperature to check for the appearance of contamination. The bacterial test organisms were grown in nutrient broth for 24 hours. A 100ml nutrient broth culture of each bacterial organism ($1x10^5$cfu/ml) was used to prepare bacterial lawns. Agar wells of 5mm diameter were prepared with the help of a sterilized stainless steel cork borer. Wells were prepared in the agar plates. The wells were filled with 15µl of samples and allowed to diffuse at room temperature. The plates containing the bacteria and silver nanoparticles were incubated at 37˚C. The plates were examined for evidence of zones of inhibition which appear as a clear area around the wells. The diameter of such zones of inhibition was measured using a meter ruler and expressed in millimeter.

3. RESULTS AND DISCUSSIONS

3.1 UV-Visible spectroscopy

Ag NPs exhibit yellowish brown colour in aqueous solution due to excitation of surface plasmon vibrations in Ag NPs. The characteristic brown color of silver solutions provides a convenient spectroscopic signature to indicate their formation. As the Zingiber officinalis and Curcuma longa extract was mixed in the aqueous solution of silver sulphate, it started to change the colour from watery to yellowish brown due to reduction of silver ions; which indicated the formation of Ag NPs. Figures 1a, 1b and 1c represents the UV-Visible spectra of aqueous component as a function of reaction time of Curcuma longa extract with 0.5mM, 1mM and 5mM aqueous Ag_2SO_4 solution respectively. Figure 2 represents the UV-Visible spectrum of aqueous component as a function of time of Zingiber officinalis extract with 5mM aqueous Ag_2SO_4 solution.

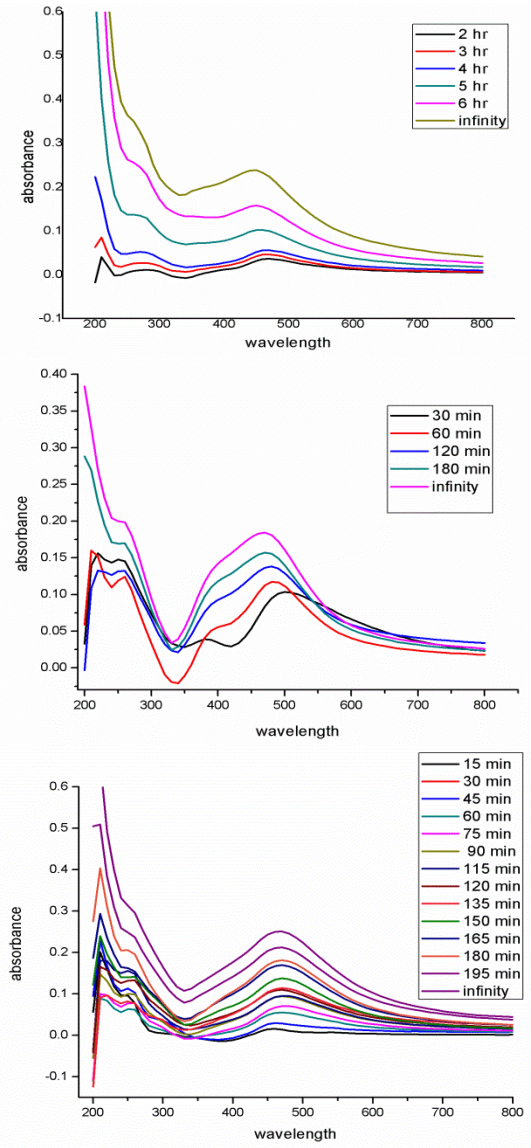

Figure 1. *UV-visible spectra of aqueous component as a function of reaction time of Curcuma longa extract with 1a) 0.5mM, 1b) 1mM and 1c) 5mM Ag_2SO_4 solution*

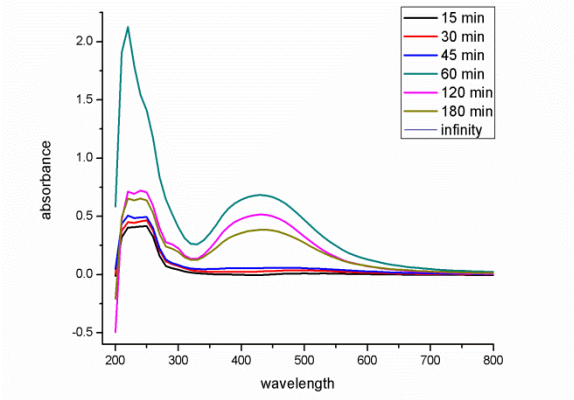

Figure 2. *UV-visible spectra of aqueous component as a function of reaction time of Zingiber officinalis extract with 5mM Ag_2SO_4 solution*

The UV-visible spectra of the Curcuma Longa and Zingiber Officinale rhizome extract shows peaks typical of the absorptions of proteins. The peak at 210 nm can be assigned to the strong absorption of peptide bonds in the extract. The absorption at 280 nm indicates the presence of tyrosine, tryptophan, and/or phenylalanine residues in the proteins, which are known to interact with silver ions [14]. As the reaction time increased intensity of the peaks at 210 nm and 270 nm decreases indicating their involvement in the production of silver NPs. The strong resonance centered at 420 nm is clearly observed and increased in intensity with time. It is well known that colloidal silver nanoparticles exhibit absorption at the wavelength from 390 to 420 nm due to Mie scattering [15]. Hence, the band at 420 nm can be attributed to the property of Mie scattering. The plasmon bands are broad with an absorption tail in the longer wavelengths, which could be in principle due to the size distribution of the particles. [13]. At higher concentrations, the completion of the reaction is faster with Curcuma Longa rhizome extract compared to Zingiber Officinale rhizome extract. The reduction of silver ions and the formation of stable nanoparticles occur rapidly with Curcuma Longa extract, making it one of the fastest bio-reducing methods to produce silver nanostructures reported till date.

3.2 Transmission Electron Microscopy

Transmission Electron Microscopy (TEM) images of silver nanoparticles derived from Curcuma Longa extract, used to visualize the size and morphology of the biosynthesized Ag NPs are shown in figure 3. The morphology of nanoparticles is spherical in nature. On careful observation, it is evident that the silver nanoparticles are surrounded by a faint thin layer of protein material, which we suppose are capping organic material from Curcuma Longa extract. The obtained nanoparticles are in the range of 20- 50nm. Few agglomerated silver nanoparticles are also observed in some places, indicating possible sedimentation at a later time.

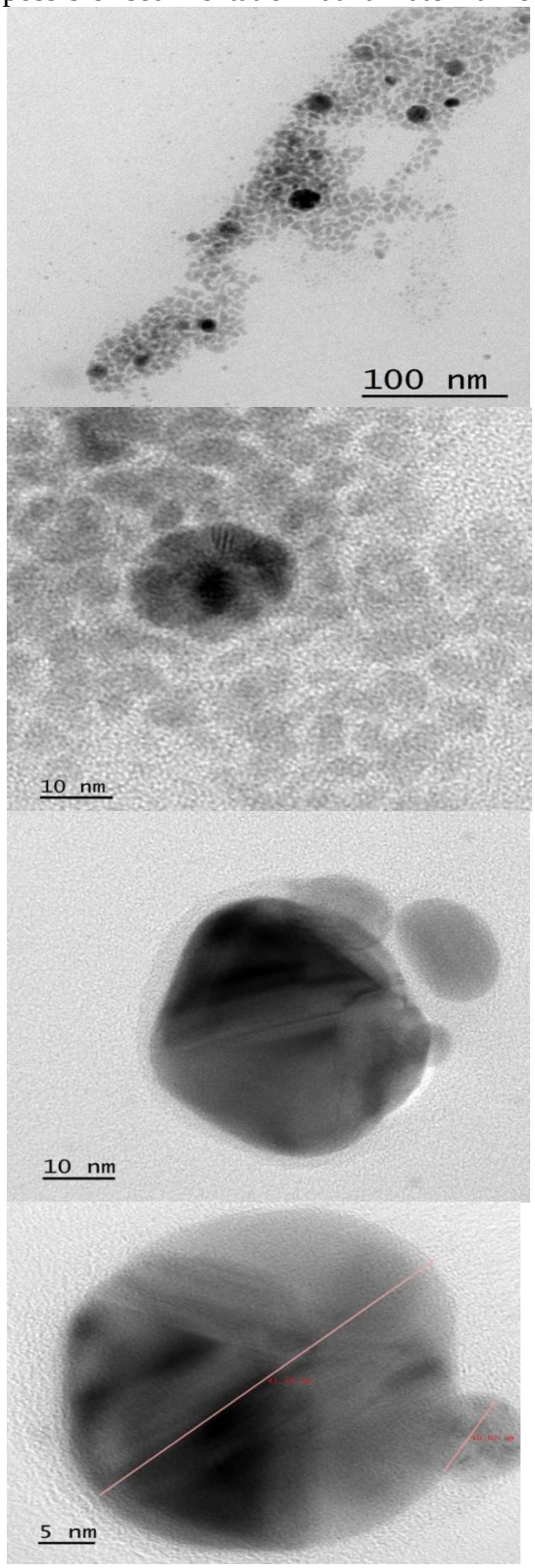

Figure 3. *TEM images of silver nanoparticles derived from Curcuma Longa extract*

3.3 Fourier Transform (FT-IR) spectroscopy

FTIR analysis was used for the characterization of the extract and the resulting nanoparticles. FTIR measurements were carried out to identify the biomolecules for capping and efficient stabilization of metal nanoparticles synthesized by plant root extract. FTIR absorption spectra of Zingiber officinalis and Curcuma longa extract before and after reduction of Ag ions are shown in figures 4a and 4b respectively.

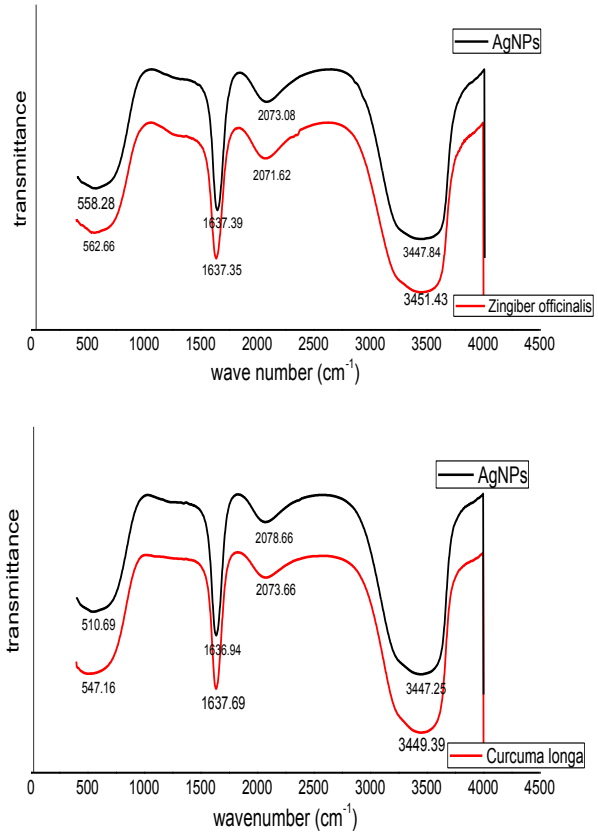

Figure 4. *FTIR absorption spectra before and after reduction of Ag ions with 4a) Zingiber officinalis extract and 4b) Curcuma longa extract.*

Absorption bands in figure 4a observed in the region of 500-4000 cm^{-1} are 3447 cm^{-1}, 2073 cm^{-1}, 1637 cm^{-1} and 558 cm^{-1} and that of figure 4b are 3447 cm^{-1}, 2078 cm^{-1} , 1636 cm^{-1} and 510 cm^{-1} respectively. The absorption peak at 1637 cm^{-1} may be assigned to amide I bond of proteins arising from carbonyl stretching in proteins and the peak at 3447 cm^{-1} can be assigned to OH stretching in alcohols and phenolic compounds. FTIR spectra reveal the presence of different functional groups like alcohol (OH stretch, H-bonded), alkene (C=C stretch) and primary amines (NH bend). Comparison between spectra of untreated sample to the treated samples Ag NPs reveal only minor changes in the positions as well as magnitude of absorption band. From the analysis of FTIR spectra, we conclude that the carbonyl group from the amino acid residues and proteins has strong ability to bind with silver ions. This suggests the formation of a layer covering silver nanoparticles that acts as a capping agent thereby preventing the agglomeration of particles. This also provides stability to the medium. Thus the biological molecules possibly perform dual functions of formation and stabilization of silver nanoparticles in the aqueous medium.

3.4 Antibacterial activity

Silver NPs are being used increasingly in wound dressing, catheters and various household products due to its antibacterial activity. Antibacterial activity of biosynthesized AgNPs were determined using Agar well diffusion assay method against Staphylococcus aureus pathogen (gram positive). Figure 5 shows the antibacterial activity of Curcuma longa, Zingiber officinalis extract and biosynthesized silver NPs using the test bacterium Staphylococcus aureus. The figure indicates that the extract alone did not exhibit considerable antibacterial effect against Staphylococcus aureus pathogen (gram positive). Silver NPs produced from 5mM silver salt solutions show greatest antibacterial activity against tested micro organism. Zone of inhibition observed is 14mm for 1mM Ag and 16mm for 5mM Ag for Curcuma Longa extract. For Zingiber officinalis extract it is 10mm for 1mM Ag and 16mm for 5mM Ag respectively.

Figure 5. *Antibacterial activity of Curcuma longa extract, Zingiber officinalis extract and biosynthesized silver NPs.*

Zone of inhibition increases with increase in concentration of the silver nanoparticles as expected (Table 1). Comparing the antibacterial activity with standard antibiotics like ampicillin, methicillin and pencillin at standard conditions it can be observed that silver nanoparticles are more effective against Staphylococcus aureus pathogen than the standard antibiotics. Several studies propose that antibacterial activity of silver NPs is due to the slow release of silver ions which impede in several ways. Silver ions can either react with the thiol groups of proteins or interfere with the DNA replication of the bacteria. Also silver NPs may get attached to cell membrane surface which in turn damage or disturb the functions of the cell leading to bacterial death.

Table 1. *Antibacterial activity of biosynthesized silver NPs towards Staphylococcus aureus pathogen.*

Plant root extract		Zone of inhibition in mm		
		Ag NPs	Antibiotics (mcg)	
Curcuma Longa	1mM	14	Ampicillin	12
	5mM	16		
Zingiber Officinalis	1mM	10	Methicillin	10
	5mM	16	Pencillin	11

4. CONCLUSIONS

From the foregoing discussion it can be concluded that the Zingiber officinalis and Curcuma Longa rhizome extracts are capable of producing silver nanoparticles extracellular which are quite stable in solution. It is a fast, eco friendly and convenient biochemical method for the synthesis of Ag NPs. TEM images show the spherical structure of Ag NPs and size in the range of 20-50 nm. FTIR spectra suggest that the biological molecules possibly perform dual functions of formation and stabilization of silver nanoparticles in the aqueous medium. Biologically synthesized silver nanoparticles are found to have superior antibacterial activity against Staphylococcus aureus pathogen than the standard antibiotics.

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