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Synthesis of Silver Nanoparticle from *Curcuma longa* Leaves Extract and its Antibacterial Activity against *E. coli* and *S. aureus*

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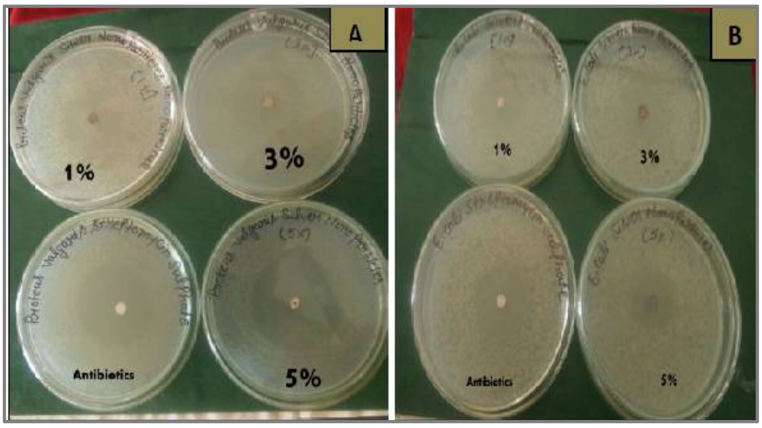
ABSTRACT

In this present study, silver nanoparticles were synthesized using hydroalcoholic extract of Curcuma longa leaves. Synthesized silver nanoparticles were characterized using UV-Visible spectroscopy (300-600 nm), SEM, TEM and XRD analysis. The comparative characterization analysis reveals that the synthesized nanoparticles were spherical in shape with the size of 31.07 nm. The antibacterial activities against different pathogens (E. coli, S. aureus) were reported. The zone of inhibition was observed both in gram positive and gram negative bacterial strains. They were found to have considerable inhibitory action against the mentioned microorganisms.

Graphical Abstract

Curcuma Plant



Antibacterial activity of silver Nanoparticle a) 1% b) 3% c) 5% d) antibiotics, A) *S. aurus* and B) *E. coli*

Keywords: *Curcuma longa*, Silver nanoparticle, Hydroalcoholic extract.

INTRODUCTION

Nanoparticles have wide range of applications in the fields of catalysis, photonics, optoelectronics, biological tagging, pharmaceutical applications environmental pollution control, drug delivery systems, and material chemistry. Various strategies are employed for synthesis of silver nanoparticles. Silver nanoparticles are synthesized by reduction in solutions, thermal decomposition of silver compounds, microwave assisted synthesis, laser mediated synthesis and biological reduction method [1]. Plant-mediated synthesis of silver nanoparticle is considered as an ecofreindly method [2]. The fascinating properties of silver nanoparticles mostly depend on the size and shape of the nanoparticles. It has also been widely reported that less aggregated, small, and spherical-shaped silver nanoparticles have proven more effective for most applications than silver nanoparticles with other morphologies [3]. Medicinal plants play a major role in the discovery of new therapeutic agents for drug development [4]. Curcuma longa, a member of the family Zingiberaceae and commonly known as turmeric, is a perennial, erect rhizomatous herb with yelowish rhizome of high economical importance because of its medicinal values. It is native to North-East and Central India. Rhizomes of the plant are aromatic with intense camphoraceous odor .The rhizomes are reported to contain anti-inflammatory agents, and the paste of fresh rhizomes is used as a remedy for insect and snake bite by the Khamti tribe of Lohit district of eastern Arunachal Pradesh [5]. Rhizomes of the plant are used for sprains and bruises and also employed in the preparation of cosmetics [6]. In this experiment, the rapid synthesis of stable silver nanoparticles has been demonstrated using the leave extract of. *Curcuma longa*.

MATERIALS AND METHODS

Sample collection: Freshly *Curcuma longa* leave were collected from potted turmeric plant (Figure 1).

Hydroalcoholic Extraction: 10 g of Curcuma longa leaves were cut into small pieces, placed in 100 mL of hydro alcoholic solvent (70 mL alcolhol, 30ml water) and left for 7 days with occasional shaking(cold maceration) [7]. The extract was filtered, concentrated, weighed and stored in cool place.

Figure 1. Curcuma Plant.

Silver nanoparticle synthesis: 10 mL of extract was added to 90 mL of 1mM silver nitrate solution and incubated at room temperature for 24 h [8]. The color changes from pale yellow to brown indicating that the silver nanoparticles are formed as a result of the reaction of extracts of *Curcuma longa* with silver metal ions.

Characterization of the synthesized silver nanoparticles: The reduction of pure Ag+ ions was monitored by UV-Visible spectrophotometer ((Shimadzu UV2700) [9] after diluting a small aliquot of the sample in distilled water. Scanning electron microscopy (SEM) analysis of synthesized AgNPs was done using a Hitachi S-4500 SEM machine. The TEM images of synthesized AgNPs were obtained by using TECHNAI 10 Philips. Transmission electron microscopy (TEM) technique was used to visualize the morphology of the Ag NPs [1]. For XRD analysis, the powdered nanoparticles were coated on the amorphous silica substrate. The spectra were recorded by using XDL 3000 powder X-ray diffractometer with 40 kV and a current of 30mA with Cu Ka (1.5405° A) radiation. The crystallite domain size was calculated from the width of the XRD peaks, assuming that they are free from nonuniform strains, using the Scherrer formula. $D= 0.94\ \lambda/\ \beta \cos\theta$ (1) where D is the average crystallite domain size perpendicular to the reflecting planes, λ is the X-ray wavelength, β is the full width at half maximum (FWHM), and θ is the diffraction angle[10].

Screening of green synthesized nanoparticles by Disc diffusion method: Soil cultures of *E.coli* and *S. aureus* was taken from the KEM. A synthesized silver nanoparticle was screened for their antibacterial activity against *E. coli* and *S. aureus* by disc diffusion method. Standard size Whatman No.1 filter paper discs 6 mm in diameter, sterilized by dry heat at 80°C in an oven for 1hour were used to determine antibacterial activity. Nutrient agar medium for disc diffusion test was prepared. After sterilization, it was poured in to sterilized petri plates and allowed to solidify. The culture suspension of each of the bacterial culture was prepared from 1-2 days old cultures separately. Duplicate plates were swabbed with the fresh cultures of selected pathogenic bacteria cultures. Sterilized filter paper discs were soaked in neat, diluted (100%) concentration of synthesized nanoparticles. A synthesized nanoparticles disc of 100 µg mL^{-1} per disc was placed on an agar plate containing bacterial cultures suspension. Similarly, solution of standared antibiotic (Streptomycine sulphate) of 100 µg mL^{-1} per disc concentration of antibacterial activity were prepared and impregnated in the filter- paper discs. These discs were then placed over the plates preceded with respective microorganism. The plates incubated at 37°C for 24 h. Two replicates were kept in each case and average values were calculated.

The diameter of the inhibition zones was measured in mm and the activity index was calculated on the basis of the size of the inhibition zone. The activity of synthesized nanoparticles was measured by the following formula [11].

Activity index= Inhibition zone of sample/Inhibition zone of standard.

RESULTS AND DISCUSSION

UV-Vis absorption spectroscopy is an important technique to monitor the formation and stability of metal nanoparticles in aqueous solution. The absorption spectrum of metal nanoparticles is sensitive to several factors, including particle size, shape, and particle–particle interaction (agglomeration) with the medium [3]. The yellow colour changed to brown colour indicates the synthesis of silver nanoparticle. The color change observed was due to excitation of surface Plasmon vibration in the silver nanoparticles. The surface plasmon resonance of AgNPs of Curcuma longa leave was found to be at 426 nm (Figure 2). The metal nanoparticles have free electrons, which give the surface plasmon resonance absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with light wave [1, 2]. SEM analysis revealed that synthesized silver nanoparticles were spherical in shape (Figure 3). The morphology of silver nanoparticles were further confirmed by TEM and XRD analysis. Shape is critical parameter which effects cell uptake and/or the rate and site specific drug delivery from the system. Preferential interaction with specific proteins could be achieved on proper shape selection of nanomaterials. Spherical nanoparticles are good option for drug delivery, however anisotropic structures could be the best option due to their large surface area e.g. dendrite [12]. This kind of structures can make good seating and binding arrangements for the drug which can be useful for sustained drug delivery. However, sharp edges of anisotropic structures can be responsible for injury of blood vessels. Figure 5 shows the XRD patterns of vacuum-dried Ag-NPs synthesized using *C. longa*. The XRD patterns of Ag/*C. longa* indicated that the structure of Ag-NPs is face-centered cubic. In addition, all the Ag-NPs had a similar diffraction profile, and XRD peaks at 2θ of 38.18°, 44.25°, 64.72°, and 77.40° could be attributed to the 111, 200, 220, and 311 crystallographic planes of the face-centered cubic silver crystals, respectively. The XRD pattern thus clearly illustrated that the Ag-NPs formed in this study were crystalline in nature. The main crystalline phase was silver, and there were no obvious other phases as impurities were found in the XRD patterns.

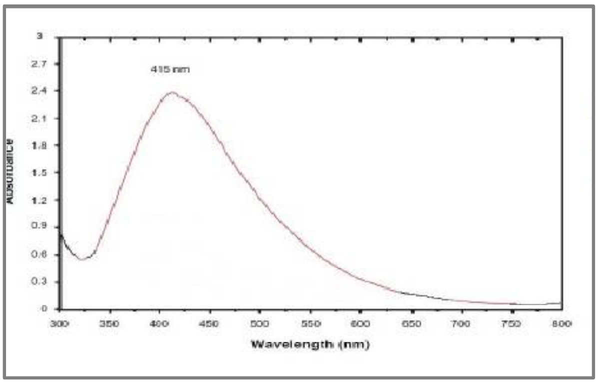


Figure. 2. UV–Visible spectrophotometer analysis of silver nanoparticle synthesized using extract of *Curcuma longa* leave.

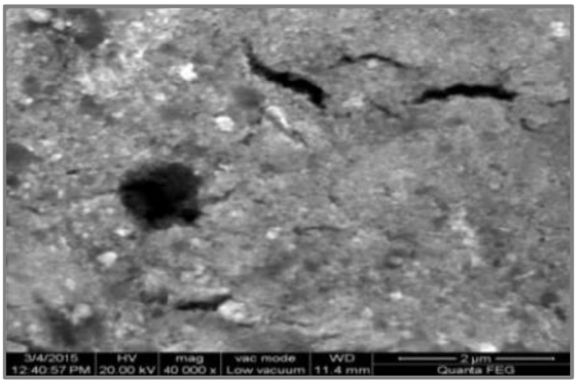


Figure 3. Scanning electron micrograph of the silver nanoparticle of *Curcuma longa.*

The TEM and XRD analysis also revealed that the size of the silver nanoparticles was 31.07 nm (Curcuma longa) (Figure 4, 5). Small size of nanoparticles are preferred, since after drug loading overall diameter should be more than enough to circulate though out the blood circulatory network for sustained period of time.

Figure 4. TEM micrograph o AgNPs synthesized using *Curcuma longa* leaves extract.

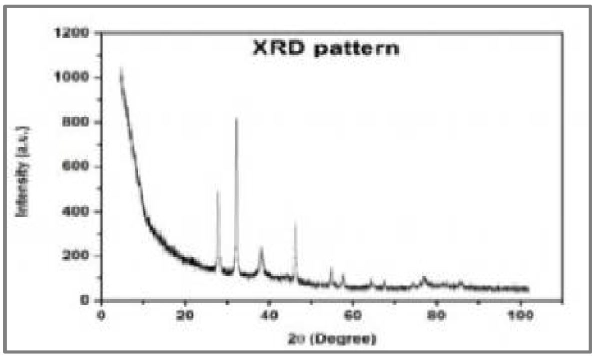


Figure 5. XRD Pattern of silver nanoparticle synthesized using *Curcuma longa* leaves extract.

Antibacterial assay: Antibacterial activity of the synthesized silver nanoparticles was examined against selected bacterial strains was investigated and represented in (Table 1). It reveals that the synthesized silver nanoparticles antibacterial activity against Staphylococcus aureus and Escherichia coli showed the maximum inhibition of bacterial zone. Silver nanoparticles exerted lowest effect on Staphylococcus aureus (1%, 3%, 5% synthesized AgNPs). Silver nanoparticles green synthesized at 5% concentration were found better on all the bacteria tested followed by 3%, 1% (Figure 6).

Table 1. Antibacterial activity of silver Nanoparticles from *Curcuma longa* leaves extract against *S. aureus* and *E. Coli*

S.No.	Bacterial Name	Zone of Inhibition			
		Silver Nanoparticles (100 µg mL^{-1})			Streptomycine sulphate (100 µg mL^{-1})
		1%	3%	5%	
1	*S. Aurus*	22 mm	23 mm	26 mm	35 mm
2	*E.Coli*	30 mm	31 mm	33 mm	30 mm

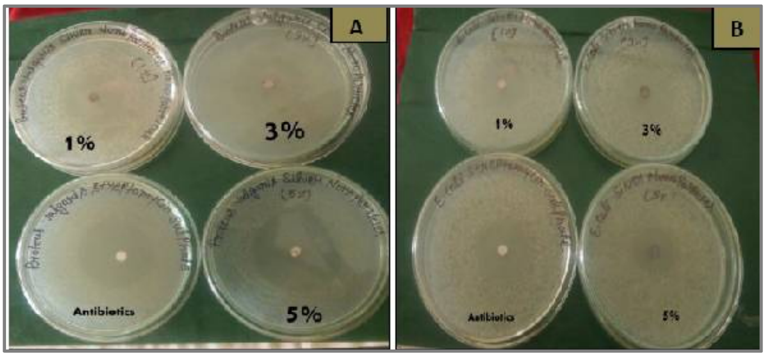


Figure 6. Antibacterial activity of silver Nanoparticle a) 1% b) 3% c) 5% d) antibiotics, A) *S. aurus* and B) *E. coli*

The maximum toxicity was observed in silver nanoparticles synthesized from 3% and 5% of leaf extract. The reason could be that the smaller size of the particles which leads to increased membrane permeability and cell destruction. Our results are in agreement with those of found in *Cassia auriculata*. Finally these reports suggest that silver nanoparticles can be synthesized from even green plant sources. Some report shows that many microorganism like algae, bacteria and fungi.

APPLICATION

The small, sized nano particles within the range of 0 to 40 nm found to be suitable in drug delivery system.

CONCLUSION

In the present observation, green synthesis shows that the environmentally benign and renewable source of *Curcuma longa* leaves are used as an effective reducing agent for the synthesis of AgNPs. This biological reduction of silver nanoparticles would be boon for the development of clean, nontoxic, and environmentally acceptable green approach to produce AgNPs, involving organisms even ranging to higher plants. The formed AgNPs synthesized *C.longa* from are highly stable and have significant activity against bacterial strains (*E. coli* and *S. aureus*).

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