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EVALUATION OF ANTIMICROBIAL EFFICACY OF SILVER NANOPARTICLES SYNTHESIZED FROM *CURCUMA LONGA* EXTRACTS

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ABSTRACT
Synthesis of silver nanoparticles by plant sources has emerged as an essential part of nanobiotechnology due to its safe, eco-friendly and cost effective results. In the present study, plant extract of *Curcuma longa* leaves was prepared in water and treated with 1mM solution of silver nitrate to obtain nanoparticles. The synthesis of silver nanoparticles was confirmed by change in colour from faint yellow to dark brown. Further, a peak observation between 420-450nm obtained on UV-Vis spectrophotometery confirmed the biosynthesis of silver nanoparticles. Characterization of obtained nanoparticles was futher confirmed by various methods like Transmission Electron Microscopy, Fourier Transmission Infra Red, X-Ray Diffraction analysis. TEM micrographs revealed well dispersed silver nanoparticles with its size between 5-50 nm and spherical in morphology. X-ray diffraction showed that the particles are crystalline in nature, with a face-centered cubic structure. FTIR spectra helps to identify the possible biomolecules responsible for the reduction of the Ag^+ ions and capping of the bioreduced Ag-NPs synthesized by the *C. longa* extract Microbiology assays proved a great efficacy of silver nanoparticles against selected bacterial and fungal cultures. The most required outcome of the present work will be the development of value added products from *C.longa* for biomedical purposes and nanotechnology-based industries.

INTRODUCTION

Bionanotechnology is a field in current research work which implies the synthesis and application of different nanomaterials. Nanoparticles are nowadays of great scientific interest as they bridge the gap between bulk materials and atomic or molecular structures (Kaushik N Thakkar *et al.,* 2010). Among various metal nanomaterials, noble metal nanomaterials have demonstrated potential biomedical implications (Mahendra Rai *et al.,* 2015). Silver nanoparticles have a wide area of interest, as they have a large number of applications, such as in nonlinear optics, spectrally selective coating for solar energy absorption, biolabeling, intercalation materials for electrical batteries as optical receptors, catalyst in chemical reactions, and as antibacterial capacities(Kamyar Shameli *et al.,* 2012) . A number of synthesis techniques for silver nanoparticles have been developed including chemical reduction, photo-reduction of silver ions (Vishnudas *et al.,* 2012) but biological synthesis of silver nanoparticles by using plants, microorganisms and enzymes *etc.* have been suggested as more ecofriendly possible alternatives than using physical and chemical methods (Elumalai, S. and Devki, R. 2014). A survey of earlier literature suggests that leaf extracts from various plants such as *Azadirachta indica* (Shankar *et al.,* 2004), *Aloe vera,*(Chandran *et al.,*2006) *Bryophyllum* sp., *Cyperus* sp., *Hydrilla* sp. (Jha *et al.,* 2009), *Gliricidia sepium,* (Raut *et al.,* 2009), *Rosa rugosa* (Dubey *et al.,* 2010), *Chenopodium album* (Dwivedi and Gopal, 2010), *Cycas* (Jha and Prasad, 2010) and various others play a substantial role in extraction of silver nanoparticles. So in the present study, the green route is used to synthesise the silver nanoparticles which offers single step, easy extracellular synthesis of nanoparticles.

Silver is an effective antimicrobial agent exhibits low toxicity (Farooqui Arshad *et al.,* 2010).Antimicrobial capability of silver nanoparticles allows them to be suitably employed in numerous household products such as textiles, food storage containers, home appliances and in medical devices (Marambio-Jones and Hoek, 2010). Due to immense biomedical applications, silver nanoparticles are nowadays emerging as one of the fastest growing product categories in the nanotechnology industry.

The drug resistant microbes have been increasing with alarming rate. To overcome this problem, there is a great need of developing antimicrobial agents and so inspired the present investigation. The paper deals with silver nanoparticles synthesized from *Curcuma ionga* extracts as antibacterial agent.

MATERIALS AND METHODS

The *C. longa* leaves were taken from local garden, Nagpur. All reagents in this effort were analytical grade and were used as received without further purification. All solutions were freshly prepared using double-distilled water and kept in the dark to avoid any photochemical reactions. All glassware used in experimental procedures was cleaned in a fresh solution of

HNO_3/HCl (3:1, v/v), washed thoroughly with double-distilled water, and dried before use. The procedure proposed by Kamyar Shameli *et al.*, (20120; Farooqui Arshad *et al.* (2010) was adopted in the study with little modifications.

Preparation of plant extract

The leaves were washed to remove the adhering mud particles and possible impurities. Later they were dried under sunlight for a week to completely remove the moisture. The leaves were cut into small pieces, powdered in a mixer, and then sieved using a 20-mesh sieve to get uniform size range. The final sieved powder was used for all further studies. 25 grams of the leaves were thoroughly washed three times in distilled water for 15 min, air dried, cut into fine pieces, and were boiled in a Erlenmeyer flask with 100 mL of sterile distilled water for 5 min and were finally filtered to get the leaf extract through the Whattman filter paper No.1 and used for the synthesis of silver nanoparticles.

Synthesis of silver nanoparticles

After preparation of extract 10 ml of plant extract was added to 90 ml sterilized distilled water and later challenged with 1 mM $AgNO_3$ (final concentration) and incubated at room temperature. The change in colour from green/faint green to brown was observed visually. Aliquots of reaction solution were removed and measurements were observed in UV-Vis spectrophotometer.

Characterization Methods

The synthesis of silver nanoparticles was firstly observed due to the change in colour from faint yellow to dark brown which preliminarily indicates the synthesis of silver nanoparticles.

Instrumentation

The prepared silver nanoparticles were characterized by UV-visible spectroscopy. The reduction of pure silver ions was monitored by measuring the UV-Vis spectrum of the colloidal solution obtained after 10 min of adding 100 ì L of sample solution to 1 mL of deionized water. The absorbance spectra observed at around 420-450 nm is very specific for the silver nanoparticles. A graph of wavelength on X-axis and absorbance on Y-axis was plotted. The UV-visible spectra were recorded over the 200–800 nm range with a UV -visible spectrophotometer (Shimadzu-UV 1800, Japan). Further characterizations were done by X-ray diffraction (XRD), transmission electron microscopy (TEM), and Fourier-transform infrared (FT-IR) spectroscopy. The structures of the Ag-NPs produced were examined by XRD (Rigaku Miniflex II dextop X-ray diffractometer). The XRD patterns were recorded at a scan speed of 4°/minute. TEM observations were carried out on a (PHILIPS model CM 200). Meanwhile, the FT-IR spectra were recorded over the range of 400–4000 cm^{-1} using an Thermo Scientific; NICOLET-6700 model. Each sample was measured in a transmission mode at a resolution of 4 cm^{-1}.

Antimicrobial Assay

Antibacterial assay

Disc diffusion assays were carried out to check the antimicrobial activity of the silver nanoparticles. Silver nanoparticles synthesized in this study were tested for their antibacterial activity using agar disc diffusion assay, a method adopted from Bauer *et al.* (1996); Prasad, K. *et al.* (2011). Briefly, tested bacterial strains were taken from Dept. Of Microbiology, L.I.T. Campus, RTMNU, Nagpur. The standard commercial antibiotic discs (the standard disc containing specific concentration of antibiotic) purchased from Hi-Media, Mumbai were used. The assays were performed in triplicate.

Antifungal assay

For the purpose, tested fungal strain taken was *Phoma exigua* (MTCC-2315) (obtained from microbial type culture collection MTCC, Chandigarh, India). The strain was maintained on sabroud dextrose agar. The assays were performed in triplicate.

RESULTS AND DISCUSSION

It has been observed that reduction of silver ions to silver nanoparticles due to addition of plant extracts is followed by colour change as shown in figure no.1 where colour changed from faint colour to dark brown and so synthesis of Ag-NPs was further confirmed by performing UV-Vis spectroscopy as shown in figure no.2. Similar observations are observed in

Figure 1: Change in colour of plant extract solution after addition of AgNO3 1mM Silver nitrate solution from sample A to B

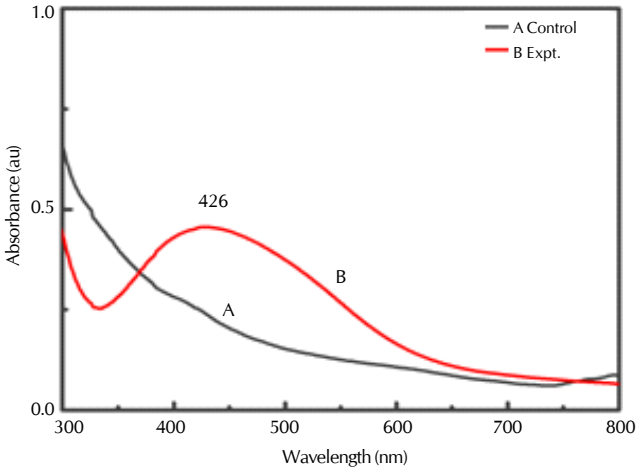

Figure 2 : depicts the UV-Visible absorption spectrum of the reaction solution taken after incubation period of 24 hours showing the maximum absorbance at ~450 nm

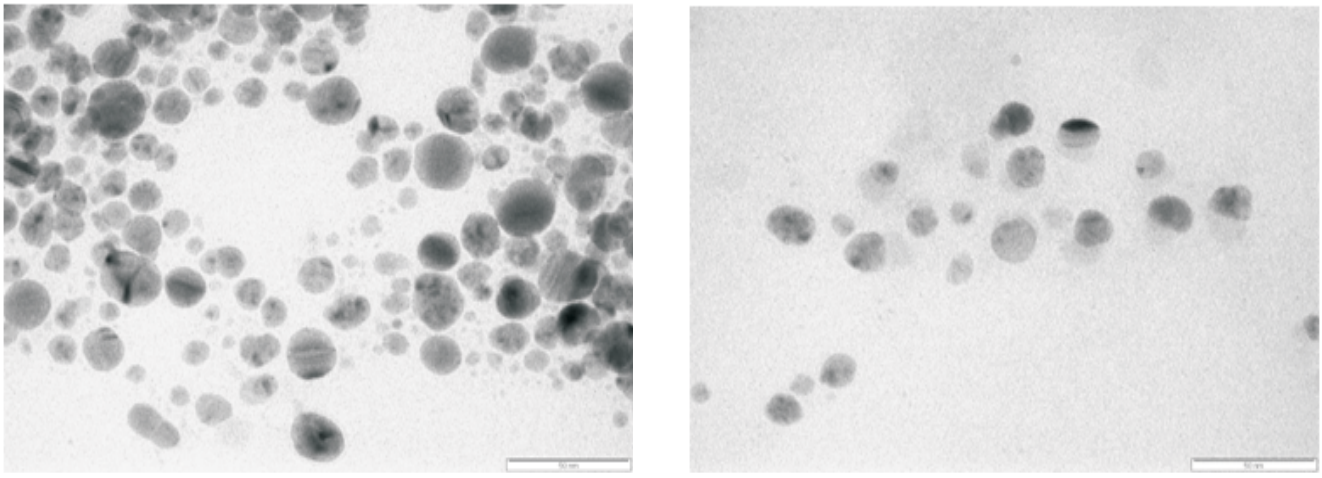

Figure3: Particle size confirmation by TEM

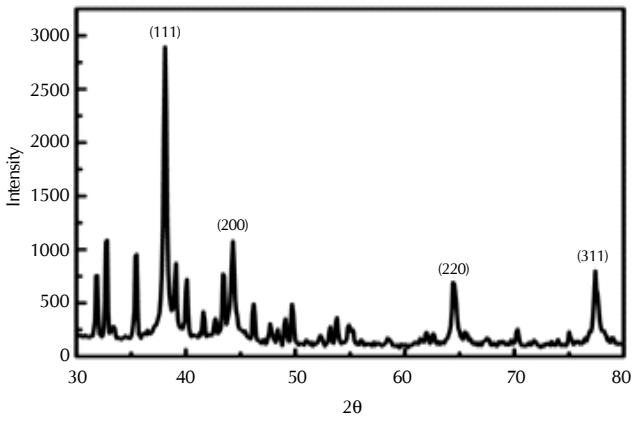

Figure 4 : X-RD pattern of synthesised Ag-NP

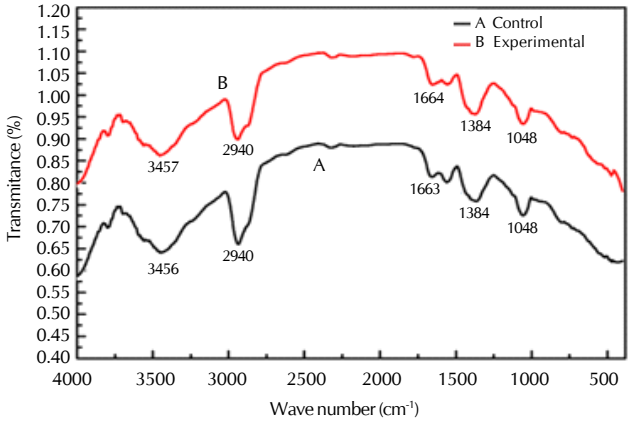

Figure5 : FTIR spectra depicts the presence of functional groups

Table 1 : Zone of Inhibition where extract is control; Ab is antibiotic;NP is Nanopartcile; NP + Ab gives synergistic effect

Test Bacteria	AgNPs Turmeric (zone of inhibition in mm)			
	Extract	Ab	NP	NP + Ab
E. coli	08	14	17	19
S. aureus	07	-	20	20
K. pneumoniae	09	15	15	19

Table 2 : Zone of Inhibition depicting antifungal activity : Ex- Extract; Ab: Antibiotic Fluconazole (10 μg);NP: Nanoparticles;NP + Ab: Antibiotic + Nanoparticles

Test Fungus	AgNPs Turmeric (zone of inhibition in mm)			
	Extract	Ab	NP	NP + Ab
P.exigua(MTCC-2315)	00	00	11	12

study of Kamyar Shameli *et al.* (2012). This colour change is due to excitation of surface Plasmon resonance phenomenon in silver nanoparticles.

The formation and stability of synthesised nanoparticles was observed by measuring SPR of *C.longa* and synthesised silver nanoparticles.It can be seen that the surface plasmon resonance (SPR) of AgNPs is 420-450nm. Previous studies Kelly *et al.* (2003); Stepanov *et al.* (1997) have shown that the spherical Ag-NPs contribute to the absorption bands at around 400–420 nm in the UV-visible spectra. UV-visible absorption spectra shown in figure no.2 showed that the broad SPR band contained one peak at 426 nm. This peak illustrates the presence of a homogeneous distribution of hydrosol Ag-NPs after 24 hours stirring. Nearly similar observation was found in study of Mostafa Khalil *et al.* (2014).

By performing Transmission Electron Analysis, the average size of synthesised Ag-NPS found to be in size range 5-50 nm (Spherical) given in figure no.3. (Elumalai and Devki, 2014). AFM measurements of Subbaiah, K.P.V. *et al.* (2013) also showed the size of SNPs was 46.60 nm spherical in shape.

Figure 6 : Antibacterial activity of Synthesised Ag-NPs against A: *E.coli*;B: *S.aureus*;C:*K.pneumoniae*

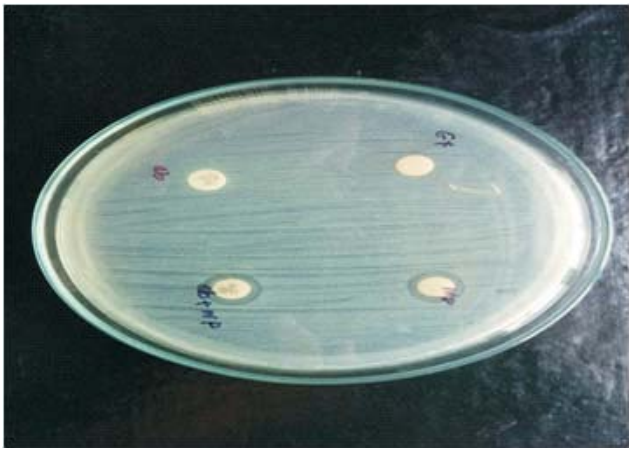

Figure.7 : Antifungal activity of Synthesised Ag-NPs against *P.exigua*

Similar phenomenon was reported by Chandran *et al*. (2006). The dark points in this figure represent the large-scale distribution of Ag-NPs as seen in the study of Kamyar Shameli *et al*. (2012).

The biosynthesis of silver nanoparticles from the *C.longa extract* was further demonstrated by the characteristic peaks observed in the X-Ray Diffraction (XRD) image. Figure no.4 shows the X-ray diffraction (XRD) patterns of dried silver nanoparticles synthesized using *C.longa* leaf extract at room temperature where three distinct diffraction peaks at 38.2æ %, 32.4æ % and 44.4æ %, were observed corresponding to diffraction from the 111, 101 and 200 planes 2Ÿ angle confirms the face-centered cubic structure of nanoparticles The 100% intensity was found at 2è value with 38.2æ %. The average nanocrystallite size calculated from Scherrer formula was found to be 3.5 nm. Similar results were found in Kamyar Shameli *et al*. (2012); Banerjee, J. and Narendhirakannan, R.T. (2011). In reference to investigation of Ahmad *et al*. (2009) 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.

Further, the presence of various biomolecules with extract and synthesised Ag-NPs was confirmed by Fourier Transform Infrared (FTIR) spectra given in figure no. 5. FT-IR analysis was carried out to identify the possible bio-molecules and cell-metal ions interaction responsible for formation and stabilization of silver nanoparticles as observed in study of Prasad kumar *et al*. (2011) ; Shameli, K. *et al*. (2012). The shifting of broad and strong bands at 3457 and 3456 cm-1 were due to bounded hydroxyl (-OH) showing the presence of alcohols or phenols as observed in study of Khalil *et al*. (2012). The peaks observed at 2940 cm^{-1} can be assigned to the C-H group as alkanes. The peaks at 1664 to 1663 cm-1 were attributed to stretching vibration of alkene l group (-C = C-). The adsorption at around 1384 cm^{-1} notably showed, NO3 - existed in residual amount. The peak observed at 1048 cm^{-1} showed the presence of aliphatic amines(-C-N- stretch).The peaks 3457 shifted to 3456 cm^{-1} and 1664 to 1663 cm^{-1} showed the involvement of hydroxyl and alkene group in the fabrication of silver nanoparticles.

Toxicity studies on pathogen opens a door for nanotechnology applications in medicine (Savitharamma, N. *et al.,* 2011). Biological synthesis of metal NPs is a traditional method and the use of plant extracts has a new awareness for the control of disease, besides being safe and no phototoxicity (Gardea, T. *et al.,* 2003). Ag-Nps were known to have strong antimicrobial activities proved by Furno *et al*. (2004) and as shown Table no.1 very well depicts the zone of inhibitions. The antibiotic used for the test was streptomycene (10 μg). The synergistic effect of nanoparticles with antibiotic showed the effective toxicity against *S.aureus* while moderate toxicity against *E.coli* and *K.pneumoniae* as shown in figure no.6. These results are agreed by Mostafa Khalil *et al*. (2014); Bindhu and Umadevi, (2013) and Li *et al*. (2011). The results of antifungal activity given in table no.2 also depict the potentiality of nanoparticles against *P.exigua,* a phtyopathogen as shown in figure no.7.

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