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Phytogenic Synthesis of Copper Nanoparticles Using Curcuma longa Leaves: Structural Profiling and Functional Evaluation

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Abstract:

The secretion of enzymes and proteins as reducing agents from leaf extract can be used for synthesis of metal nanoparticle from metal salts.The production of nanoparticles on large scale from various fungal strain has great potential since they have grown in vitro. Several approaches have been made to expand the yield of nanoparticles of different shape, size and stability. Characterization of these nanoparticles is been carried out with the help of Transmission Electron Microscopy(TEM), UV-spectrophotometer, Fourier Transform infrared (FTIR).

Keywords: Metal nanoparticles, Green synthesis, Leaf extract.

1. INTRODUCTION

The emerging field of science that not only includes synthesis but also the developments of various nanomaterial's is termed as Nanotechnology. The term Nanoparticles which comes from this nanotechnology is defined as object which ranges from 1-100nm in size which may even differ from bulk material. There a various nanomaterial produced from different metals using copper, silver, gold, titanium, magnesium etc. Thus the properties of a nanoparticle metal are determined by its size, composition, shape, structure and crystallinity. Out of all other metals copper nanoparticle (CuNPs) shows several applications in terms of medical diagnosis, diverse purposes, cosmetics and many more[1]. Copper nanoparticle has also been known for it antimicrobial activity & anti-inflammatory agents which helps in wound healing. Leaf extract when gets exposed to metals it results into production of metabolites & other enzymes which helps to protect itself from unwanted foreign material & while this process is being carried out these metal gets reduced to nanoparticle. Fungal cell when get bonded to metal ion it undergoes reduction reaction resulting metal nanoparticle with oxidation of organic molecule like protein, enzyme, peptide etc. Leaf extract are gaining the central stage of studies in the generation of biological metal nanoparticles due to their ability of toleration and bioaccumulation of metals, being compartiviely economic, effortless synthesis method. Since the leaf extract has capability of bearing and bio accumulating metals also it is economically resourceful and downstream processing thus in this paper we have synthesized CuNP with fungal filtrate (A.niger)[2,3]. C. longa is a most common soil filamentous leaf extract with huge range of hydrolytic & oxidative enzymes. A range of such enzymes are one of the most important in biotechnology industry [4]. C. longa plays an important

role of absorbing metal ions from the aqueous solution [5].The synthesis of nanoparticles can be either physically, chemically, biologically. Other adverse effects due to chemical and physical syhnthesis methods have been associated due to presence of toxic chemical absorbed on the surface. Thus the solution to these alternatives of chemical and physical methods are Green shynthesis i.e Biological ways of nanoparticles using living organisms such as plant extract or plants[6,7], enzyme[8],microorganism[9,10], leaf extract [11]. The development of such eco friendly nanoparticles from these nanotechnology is developing worldwide interest and evolving a branch in nanoworld which has several applicatons.

BIOSYNTHESIS :

One of the green and eco friendly technology is biosynthesis of microorganism, which can be either intracellular or extracelluar according to the location of nanoparticle. The transportation of ions into microbial cells in presence of enzymes to form nanoparticles ,such nanoparticles are developed inside the organism. Due to enormous secretary components which are involved in reduction and capping of nanoparticles leaf extract are known to produced extracelluraly. Copper oxide or copper as a metal shows broad range as biocidal activity and hence several studies says that copper shows remarkable antimicrobial activity at nanoscale level. The most important character of this metal is that copper is 10 times cheaper than any other metal in market and therefore using copper becomes quite cost-effective . Also a limited number of studies have been published for the biosynthesis of copper nanoparticles of fungal strain of copper nanoparticles [12]. Leaf extract such as Curcuma longa has been reported to synthesize copper nanoparticle. The peak absorbance of copper nanoparticle was between at 254 nm indicates formation of CuNPs. In order to go for more confirmatory test to check the presence of copper nanpparticlesand to study their size, structure and shape TEM,FTIR was been carried out. In addition, to these metals copper nanoparticles have been showing antimicrobial activity .

2. EXPERIMENTAL DETAILS:

2.1 BIOSYNTHESIS OF CuNPs:

2.1.1 Culturing and Preparation of Leaf extract.

The Curcuma longa tubers were collected from local residential areas and thoroughly washed to remove adhering soil particles and other impurities. The cleaned tubers were then dried under sunlight for one week to ensure complete removal of moisture. After drying, the tubers were ground into a fine powder and sieved to obtain a uniform particle size. The sieved powder was used for all subsequent experiments.

For the preparation of the plant extract, 10 g of C. longa tuber powder was mixed with 100 mL of ethanol in a 200 mL conical flask. The mixture was heated at 70°C with continuous stirring for 4 hours using a hot plate. After extraction, the solution was filtered through Whatman No. 40 filter paper to remove solid residues. The resulting filtrate was used as the reducing and capping agent for the green synthesis of copper nanoparticles (CuNPs).

A microwave irradiation system (Sharp R-219T (W), 2.450 GHz) was used for the synthesis of copper nanoparticles (Cu NPs). A 0.1 M copper acetate dihydrate solution (100 mL) was prepared in a 200 mL

beaker, and 50 mL of Curcuma longa extract was gradually added to it with gentle mixing. The resulting reaction mixture was then exposed to microwave irradiation at a power of 200 W for 180 seconds.

During the irradiation process, the colour of the reaction mixture changed from yellow to brick brown, indicating the successful formation of Cu NPs (Supplementary Information S1). The synthesized nanoparticles were subsequently collected as a powder and further characterized to evaluate their morphology, crystallinity, microstructure, particle size, stability, and the presence of functional groups, as described in Supplementary Information S2[18].

2.1.2 Synthesis of CuNPs

A solution of 1mM $CuSO_4.5H_2O$ (0.249 g of Cu salt into 1000ml distilled water). Leaf extract was added in 1mM of $CuSO_4.5H_2O$ with the ratio of 1:1 at room temperature. On addition of Cu ion solution a colour change was observed and this was monitored under UV Spectrophotometer [15].

2.3 Characterization of CuNPs:

The newly synthesized Copper nanoparticles were characterized by UV Spectrophotometer, X-RAY Diffraction, Fourier transform infrared (FTIR), Transmission electron microscopy (TEM). The nanoparticle solution was sonicated 3 minutes before analysis.

2.3.1 UV-Visible Spectrum Analysis

To study the rate of formation of copper nanoparticles UV-visible absorbtion spectroscopy was carried out with varying concentration of leaf extract and Cu ion solution were mixed at room temperature.The concentration were as follow:

1. 1:1 (5ml of leaf extract and 50 ml of 1mm Cu ion solution)
2. 1:4 (2ml of Leaf extract and 8ml of 1mm Cu ion solution)
3. 1:9 (1ml of Leaf extract and 9ml of 1mm Cu ion solution)

The absorbance range was checked between 200-600nm wavelength through UV-visible spectrophotometer at 1nm resolution.,[12,16].

2.3.2 Fourier Transform Infrared

FTIR of A.niger leaf extract 1Mm $CuSO_4.5H_2O$ and CuNPs was carried out to determine various functional group and the changes that took place in the nanoparticle solution.

2.3.3 Transmission Electron Micrscopy

The size and shape of nanoparticles was been carried out by TEM analysis. TEM analysis shows biosynthesized CuNPs

2.4 RESULT AND DISCUSSION

The study says that Aspergilus niger synthesizes copper nanoparticle which was observed.

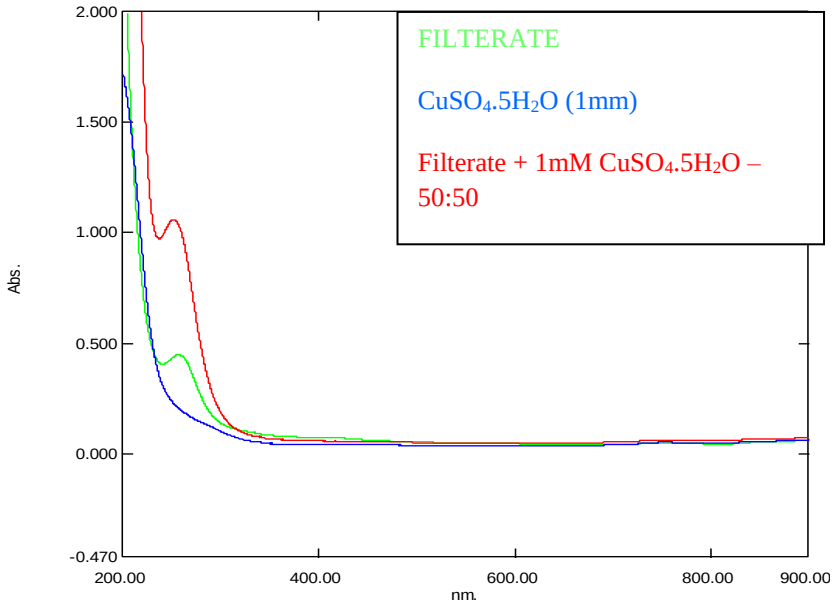

Fig. 1UV Spectrum of synthesis of copper nanoparticle

UV-spectroscopic analysis confirmed the stability of nanoparticles in the broth. The wavelength range of filtered broth was between 200-900nm, the maximum absorbance was observed at 248nm. The spectra analysis was performed after 24,48,72 hrs of incubation, and the maximum absorbance was been observed at same wavelength for all other incubation period as shown in fig.1 showing wavelength on x-axis and absorbance on y-axis. Thus, UV-spectroscopy has gave the confirmation for presence of nanoparticle extracellularly and their stability.The absorbace peak at 248nm indicated the presence of nanoparticle in the broth,since no variation was observed at maximum absorbance ,hence the copper nanoparticles was confirmed to be stable.

TRANSMISSION ELECTRON MICROSCOPY [TEM]

Transmission electron microscopy[TEM] is to characterize the shape,size and the morphology of copper nanoparticles. The best precursor which gives the best result among the other salts for copper nanoparticles is copper sulphate pentahydrate which has good particle size control and also act aas good capping agent of Curcuma longa. The average size of CuNPs is around 2nm. The nanoparticles are dispersed,small,spherical in shape. Since the nanoparticles are formed on thesurface of the mycelia, the first step which involves the reduction of copper ions by enzyme secreted extracellularly by the leaf extract and the second step which involves that the fungal cell wall is attached by copper nanoaprticles. The TEM image of CuNPs synthesized using $CuSO_4.5H_20$ stabilized by Curcuma longa is shown in fig.2

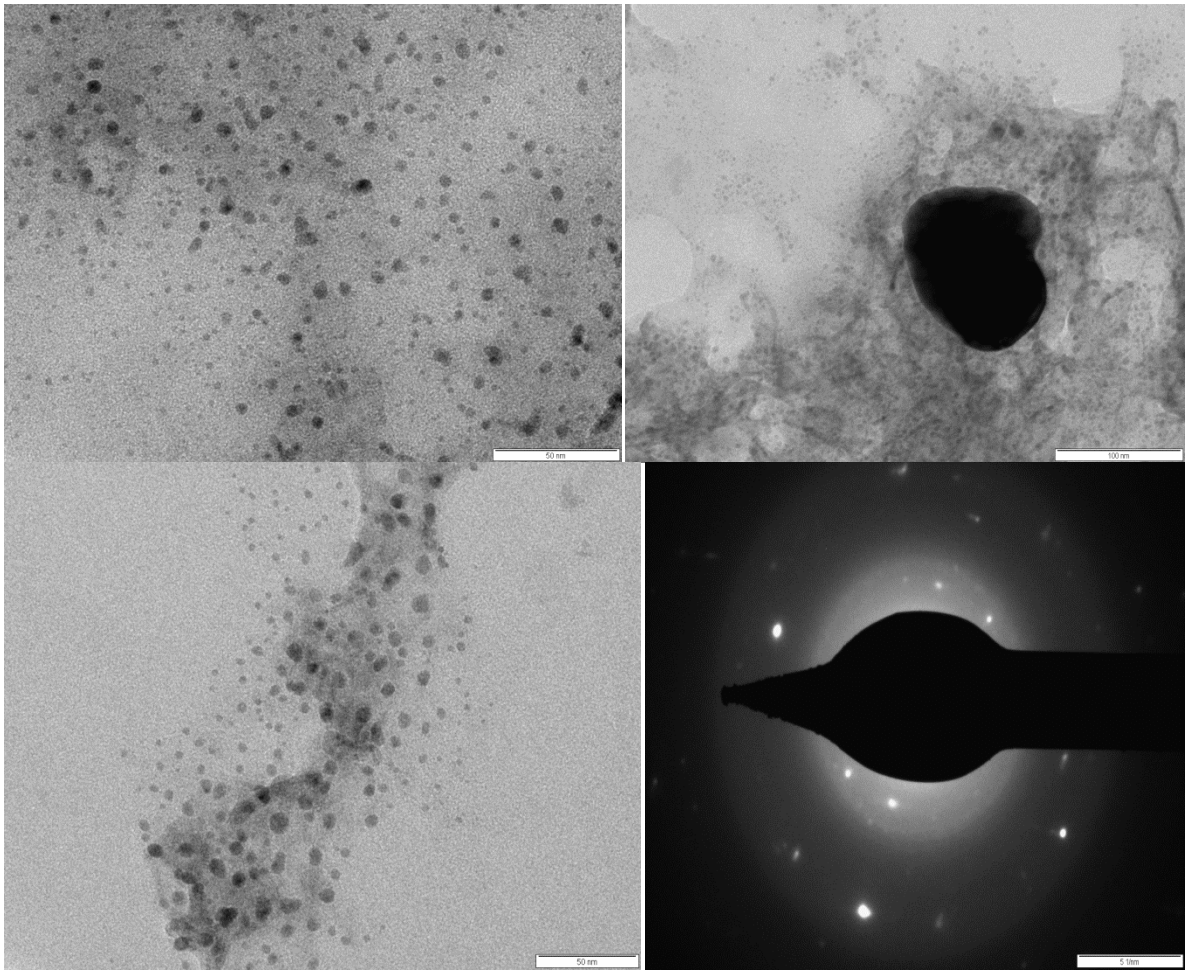

Fig2. TEM images of copper nanoparticles synthesis and their diffraction pattern

FTIR (Fourier Transform Infrared)

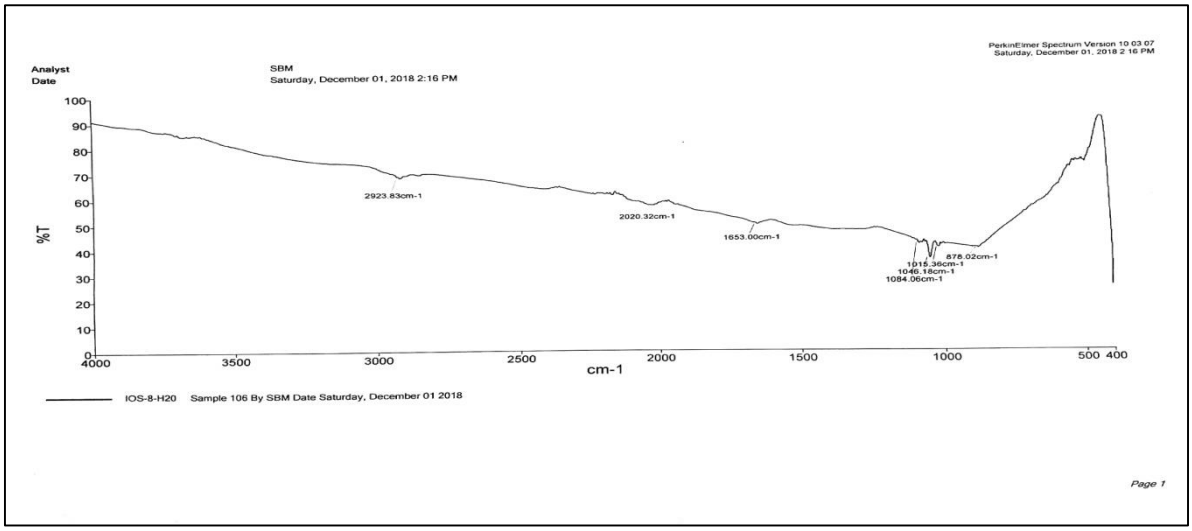

Fig1. FTIR Spectra of *C. longa*

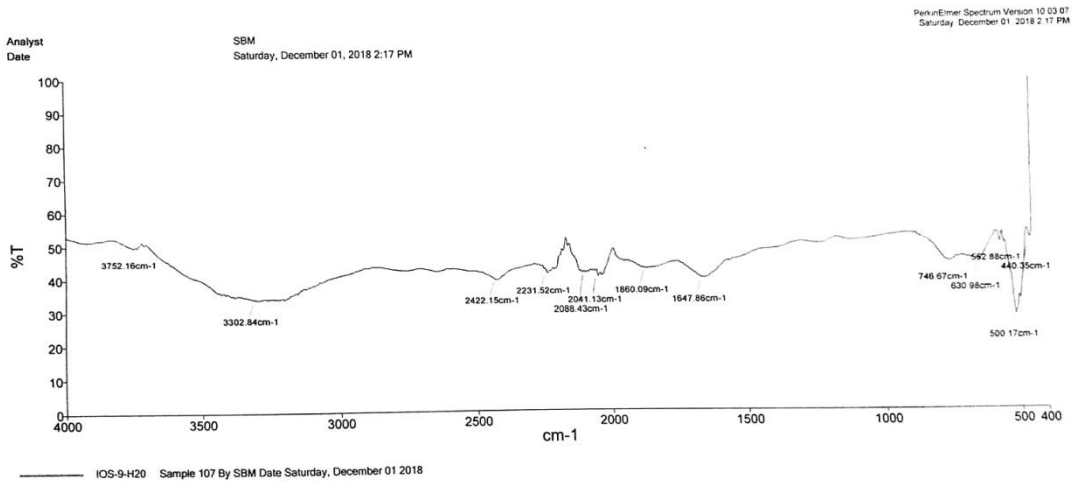

Fig2. FTIR Spectra of $CuSO_4.5H_2O$

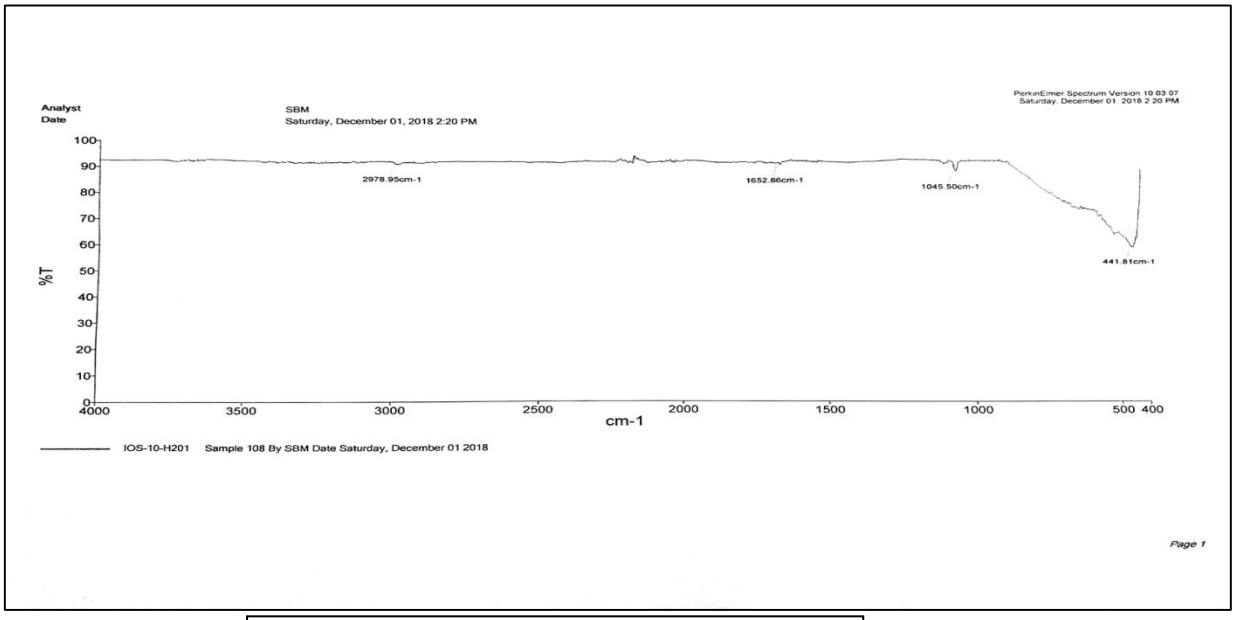

Fig 3. FTIR spectra of *CuNPs*

Figure 3. shows that the peak at 2976.95 cm-1 corresponds to O – H carboxylic acid stretch. The peak 1652.86 cm-1 corresponds to C=O carbonyl stretching of the carbonyl group. The peak at 1045.50 cm-1 corresponds to C – O – C stretch. Thus the nanoparticles that were synthesised are surrounded by proteins and metabolites having functional groups such as carboxylic acids, ketones, aldehydes, ethers,

etc. This shows that the biological functional groups can play dual role in forming the nanoparticles and stabilising them.

CONCLUSION:

In current times there has been a genuine need in developing methods for the synthesis of nanoparticles that are ecofriendly, time conserving, cost effective and simple .We can conclude that the method we applied in synthesizing copper nanoparticles from C. longa extract is easier to carry out and satisfies the above mentioned criteria. Also they can be further studied for its application and properties.

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