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BIOGENIC COPPER NANOPARTICLES FROM THE AQUEOUS STEM EXTRACT OF *CERIOPS TAGAL*

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ABSTRACT

Synthesis of cost effective, reliable and eco-friendly nanoparticles is an expanding branch of green nanotechnology. *Ceriops tagal*, a mangrove plant has wide range of medicinal properties. Synthesis of *C.tagal* mediated nanoparticles may further enhance its medicinal potency. The aqueous stem extract of *C.tagal* was used as a bio-reducing agent for synthesis of copper nanoparticles. Reduction of metal ions was confirmed by Ultraviolet-visible absorption spectroscopy, transmission electron microscopy, energy dispersive spectroscopy and X-ray diffraction. Functional groups involved in nanoparticle synthesis were recorded by ATR-FTIR spectra. Nanoparticle formation was dependent on time, temperature and metal salt concentration. UV-Visible spectroscopy demonstrated peak at 500 nm. TEM and XRD results revealed shape and structure of synthesized nanoparticles. These results have indicated formation of metallic copper nanoparticles. This is the first report on rapid biosynthesis of copper nanoparticles synthesized by bio-reduction of copper sulphate solution employing aqueous stem extract of *C.tagal*.

KEYWORDS: Mangroves, *Ceriops tagal*, aqueous extract, copper nanoparticles.

INTRODUCTION

Nanotechnology has wide applications in almost every field with the most exploitable properties being chemical, optical, mechanical, thermal, specific surface area, electrical, magnetic, diagnostics and medical etc.[1,2] It is playing an important role in global manufacturing and commercialization. Amongst various nanoparticles, copper nanoparticles are gaining increased attention because of their optical and electrical conducting properties along with its considerably lower cost than gold and silver.[3,4] Copper nanoparticles can be

efficiently used in / as catalyst, printed circuit board, flexible electronics, light emitting diode, biocompatibility.[5] They are added to lubricant oil to mend worn surface by reducing friction.[6] Bioactive coatings with copper fluroropolymer nanocomposite are capable of inhibiting growth of microorganisms such as *S. aureus, S. cerevisiae, E. coli*, and *Listeria.*[7] Copper oxide is used as antimicrobial in textiles.[8] Copper and silver ions are used to disinfect wastewater from hospitals.[9,10] Yoon *et al.* have reported higher activity of copper nanoparticles than silver nanaoparticles against single representative strains of *B. subtilis* and *E. coli.*[11] There are reports of bactericidal application of copper nanoparticles when supported on polyurethane foam, carbon, sepiotile and polymers.[12,16] Yet another report by Suparna *et al* has reported strain specific superior activity against *B. subtilis, S. aureus* and *E. coli* of copper nanoparticles.[17] Copper ions released may also interact with DNA molecules and intercalate with nucleic acid strands.[18] However, the difficulty of copper nanoparticles synthesis is its highly oxidizing property when exposed to air and water. Biological effectiveness of nanoparticles is directly proportional to its surface area. Nanoparticles exhibit new and improved properties based on their size and shape. Various methods such as direct electrochemical reduction, thermal reduction and decomposition, electro exploding wire (EEW), *in situ* synthesis in polymers, polyol process, mechano-chemical process, ion beam radiation and chemical reduction have been employed for synthesis of copper nanoparticles thus most of the reduction methods require high energy and chemical input.[5] Chemical synthesis of nanoparticles possesses the potential hazards like general toxicity, cytotoxicity, genotoxicity and carcinogenicity.[19] Therefore, there is a current drive to produce 'green' nanoparticles from plants because of its eco friendliness, energy efficiency and convenience. The Indian mangroves consist of nearly 65 species belonging to 31 families[20] out of 77 species all over the world.[21] Mumbai coast has 21 species of which most dominating genera include *Avicennia, Sonneratia, Rhizophora* and *Acanthus.*[22] Mangrove plants have distinctive characteristic like the ability to sustain in high salinity, extreme temperatures, anaerobic & unstable substrates thus forming unique environments and floral-faunal assemblages and therefore, might produce special types of bioactive compounds than terrestrial plants.[23] Recent research evidenced that Indian mangroves contained antiviral[24], antibacterial[25], antifungal[26], mosquito larvicidal[27] and antioxidant activity.[28] Among the different mangrove plants, the chemical constituents and bioactivities of *Ceriops tagal* have been studied extensively. Dolabrane diterpenes from *C. tagal* has exhibited significant anti-tumor effect.[29] Bark of *C. tagal* is a powerful astringent and is used for treating hemorrhage in defecation and malignant ulcers whereas leaves are used to heal

paludism and malaria; ethanolic extract of stem and twigs has strong feeding deterrent activity against *T. castaneum* adults.[30] Few of the compounds isolated from ethanolic embryo extract of *C.tagal* were effective to inhibit proliferation and growth of H-7402 and Hela cells.[31]

It has been reported to contain condensed and hydrolysable tannins, aliphatic carboxylic acids, indole alkaloids, polyphenols, proteins, tannins, fatty acids, hydrocarbons, inorganic salts, inositols, steroids, carotenoids, chlorophyll a, b, a+b, etc.[32] Thus, it is a rich source of bio-reductant and stabilizers. Currently there are reports of nanoparticles synthesis from only two species- *Rhizophora mucronata*[33] and *Avicennia marina*.[34] However, there is no such report on synthesis of copper nanoparticles from *Ceriops tagal*. With the view of this background, we had attempted to rejuvenate the use of traditional ethno-medicinal properties of *C. tagal* applying modern techniques at nano scale.

Here in, we report for the first-time synthesis of copper nanoparticles employing aqueous stem extract of *C. tagal* (CTSE) by reduction of aqueous Cu^{2+} ions. We also investigated effect of time course, temperature and metal salts on the rate of synthesis.

1. MATERIALS AND METHODS

1.1. Plant collection and extract preparation

C.tagal stem was collected from the Gorai creek, Mumbai, India. It was chopped, dried at 40º C and pulverized. Ultra pure water produced by MilliQ system was used throughout the experiment. Aqueous stem extract was prepared by soaking 5 gm of stem powder in 100ml MilliQ water, for 5 mins and then the mixture was boiled at 100º C for 5 mins. The freshly prepared extract was obtained by filtering it through Whatman filter paper No.1. and used for further study.

1.2 Synthesis of copper nanoparticles

Synthesis of copper nanoparticles was initiated by adding 5ml of CTSE in 95 ml aqueous $CuSO_4$ (Himedia, India) solution. The reaction was carried out at static condition. Reduction of Cu^{2+} was monitored as a function of time by measuring UV-Vis spectra using UV-1650CP Schimadzu spectrophotometer operated at 1nm resolution. Effect of temperature on rate of synthesis was studied by carrying out the reactions at Room temperature (RT), 40ºC, 50ºC and 60ºC in water bath. Concentration of copper sulphate salt varied from 1mM – 5mM.

1.3 TEM and energy dispersive spectroscopy measurements

Size and surface morphology of bioreduced nanoparticles was determined by transmission electron microscope (TEM, Tecnai 12 Cryo, FEI, Eindhoven, The Netherlands). Nanoparticle solution was drop coated on copper TEM grids, after which film was allowed to stand for 2 mins and excess solution was blotted. The grid was dried properly prior to measurement. An energy dispersive spectrum was recorded with the same instrument at the energy range 0-20 keV.

1.4 X-ray diffraction measurements

After complete reduction of metal ions by CTSE, the solution was centrifuged at 10,000 rpm for 15 minutes at room temperature. The pellet obtained was re-dispersed and centrifuged with MilliQ water. This process was repeated three times to get rid of any free entities. Phase formation of nanoparticles was studied by preparing thin film of thoroughly dried nanoparticles on glass slides. Diffraction data was recorded on Schimadzu XRD 7000 diffractometer with Cu Kα radiation (1.54 Å) source operating at 40 kV voltage and a current of 30 mA.

1.5 Attenuated Total Reflectance Fourier Transform Infrared (ATR - FTIR) Spectroscopy

ATR - FTIR spectrum of CTSE before and after reduction of metal ions was obtained using FTIR (Perkin Elmer) Frontier spectrophotometer. 5% plant extract and supernatant of bio-reduced samples was subjected to IR source 400 cm^{-1} - 4000 cm^{-1}.

2. RESULTS AND DISCUSSION

2.1 Visual observations and UV-Visible spectroscopy

Bio-reduction of salt to respective metal ions in presence of CTSE was monitored as a function of time using UV-Visible spectroscopy. The absorption band for copper nanoparticles are reported to be in the range of 500-600nm.[5] The surface plasmon resonance (SPR) peak of absorption spectra was found to be at 500nm (Fig.1) which is maintained over the time indicating capping of nanoparticles. However, with the passage of time, the intensity of SPR peak has decreased which may have occurred because of oxidation which is in agreement with earlier report.[35] A blue shift of absorbance peak was observed from 507 to 500nm. This shift is attributed to the decreasing size of particles as well as it can occur because of partial oxidation and oxidation of colloidal copper with dissolved oxygen in water.[5] Position, shape and intensity of plasmon absorption depends on several factors such

as dielectric constant of the surrounding medium, the electronic interactions between the stabilizing ligands and the nanoparticle, which alter the electron density inside the nanoparticle and the size, shape and monodispersity of the NPs.[36] Reduction of metal ions was very rapid; maximum reduction of Cu^{2+} ions took place between 3-4 hours displaying colour change within 15 min. Copper nanoparticles exhibited intense brown colour on complete reduction. The difference in the redox potential and solubility of metal ions are most likely to be considered for rate of synthesis of nanoparticles formation. According to Armendariz *et al.* pH of plant extract affects the binding trend of ions to functional groups of biomass and subsequently the shape and size of nanoparticles during synthesis.[37] The pH of CTSE extract was 7. The optimization study of different concentrations of metal salt against kinetics of reaction revealed the significant effect of metal salt concentration on synthesis of nanoparticles. The lower salt concentration showed comparatively low rate of synthesis whereas higher salt concentration resulted in precipitation which could be easily resuspended. The rate of synthesis was highest at 1mM concentration (Table 1). We also investigated the role of temperature on enhancement of the rate of synthesis by carrying the synthesis at R.T., 40ºC, 50ºC and 60ºC. Temperature plays an important role in the formation of nanoparticles. In the present investigation maximum bio-reduction of Cu^{2+} to Cu^{0} was achieved at RT. Although the reaction was fast at higher temperature and low at lower temperature with slight colour variation, indicating the dependence of temperature on bio-reduction process however no significant difference was observed in peaks. Thus in the present investigation copper nanoparticles are synthesized by reduction of 1mM copper sulphate solution at RT employing aqueous stem extract of *C.tagal*.

2.2 TEM and energy dispersive spectroscopy measurements

TEM micrograph confirmed the synthesis of nanoparticles (Fig 2). The nanoparticles showed a tendency to cluster with flower like outline as reported by Mott *et al.*; making it unfeasible to measure their diameter. A close examination (Fig 3) of some large particles reveals the subtle cluster features. This observation hints at the possibility of coalescence of small particles which is in agreement with Mott *et al.*[38] The coalescence process begins with the formation of 'neck' at the contacting planes when nanoparticles diffuse across the substrate. It can occur under the influence of high intensity electron beam as well as at RT or below RT. As coalescence proceeds, the planes start aligning which results in unique FCC crystal structure thus forming a new nanoparticle. It is well known that the shape and size of

nanoparticles considerably changes their properties, bioactivities and further applications. Figure 4 shows TEM diffraction pattern of copper nanoparticles.

For elemental analysis of copper nanoparticles, a signature spectrum from copper atoms present in the nanoparticles was obtained by EDS (Fig 5). The occurrence of weak signals from carbon, sodium (Table 2) were also seen which may have originated from the bio-molecules bound to the surface of the nanoparticles.[39] Despite the fact, that all the characterizations except UV-Visible spectroscopy, were done 8 months after nanoparticles synthesis, there are no other peaks attributable to copper oxides, this means our copper nanoparticles are fairly stable against oxidation.

It is reported that the organic molecules present in plants gives stability to nanoparticles by capping.[40] According to Hu *et al. C.tagal* stem is a rich source of monoterpenoids, diterpenoids, triterpenoids, flavonoids, alkaloids, polyphenolics and saponins also recently 43 diterpenes, 29 triterpenes and six new dolabranes named 'tagalsins' P–U (**1**–**6**)[41] had been isolated from *C.tagal*; thus forming the main constituents of the extract.[42] On close observation of TEM micrograph it can be seen that the nanoparticles are capped by a thin layer which can be the organic material from CTSE. This indicates that the bioactive principles present in plant are involved in capping of nanoparticles. Thus, TEM result has confirmed synthesis copper nanoparticles. The EDS profile indicates that the sample contains pure copper, with no oxide layer.

2.3 XRD

The crystalline nature of elemental Cu was confirmed by X-ray diffraction analysis. Figure 6 shows the XRD data of bio-reduced copper nanoparticles. The crystallite size of the nanoparticles was estimated from the Debye–Scherrer formula.[43]

$d = 0.9\lambda/\beta \cos \theta$

Where 0.9 is the shape factor, λ is x-ray wavelength, 1.54 Å, β full width at half the maximum intensity in radians, and θ is the Bragg angle.

Strong Bragg reflection was obtained corresponding to (111) plane, which is the principal diffracting plane of face centered cubic symmetry (Table 3). The crystallite size of copper nanoparticles synthesized was ~4.0 nm.

2.4 ATR - FTIR

ATR - FTIR spectroscopic studies were carried out to identify the functional group involved in capping and efficient stabilization of the metal nanoparticles. FTIR absorption spectra (Fig. 7) of CTSE showed prominent peaks at 3306.66 cm^{-1}, 2119.25 cm^{-1} and 1634.46 cm^{-1}. The intense broad stretching at 3306.66 cm-1 arises due to the free O-H groups present in alcohols and phenols[44], weak stretch at 2119.25 cm^{-1} is due to C≡C from alkynes while the IR peak at 1634.46 cm^{-1} could be assigned to characteristic asymmetrical stretch of carboxylate group.[45] While the spectrum of bioreduced sample exhibited peaks at 3271.77 cm^{-1}, 2111.01 cm^{-1} and 1634.35 cm^{-1} indicating the shift in peaks. Based on the band shift occurring at hydroxyl and carbonyl groups it can be concluded that both hydroxyl and carbonyl groups of *C.tagal* are involved in the synthesis of copper nanoparticles. This indicates that the copper nanoparticles are surrounded by metabolites such as terpenoids having functional groups of alcohols, phenols and carboxylic acids.

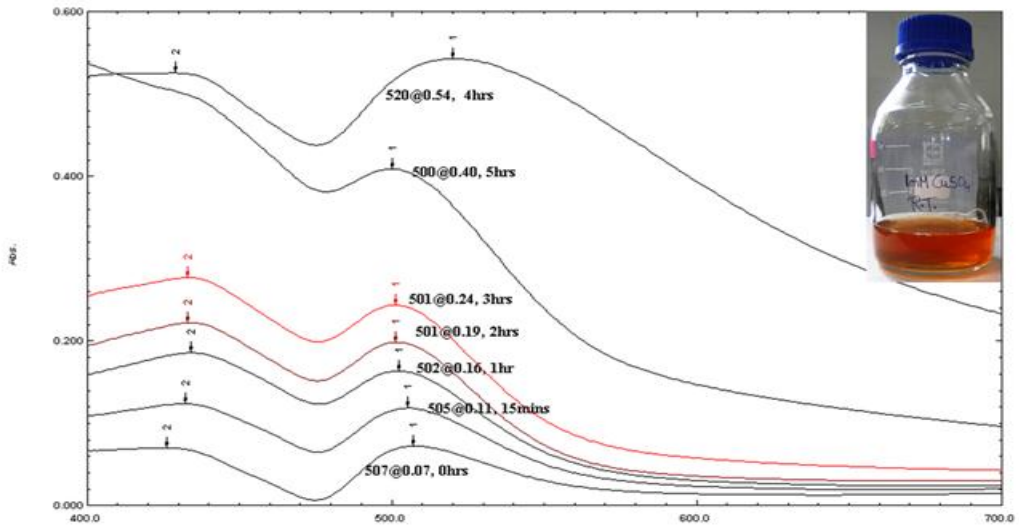

Figure 1: UV-Visible spectra recorded as a function of reaction time of 1mM CuSO4 solution with CTSE at RT.

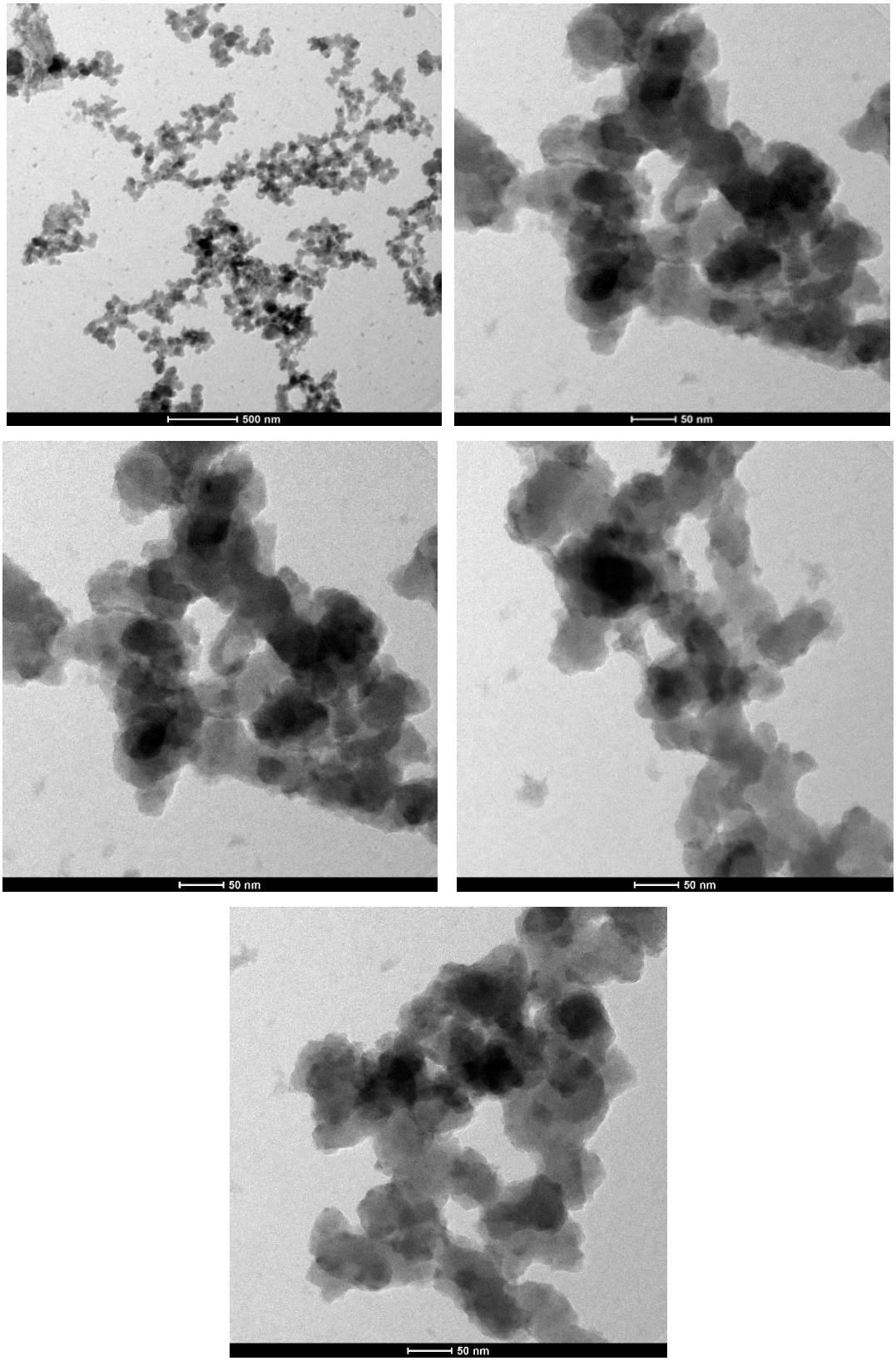

Figure 2: TEM micrographs of copper nanoparticles.

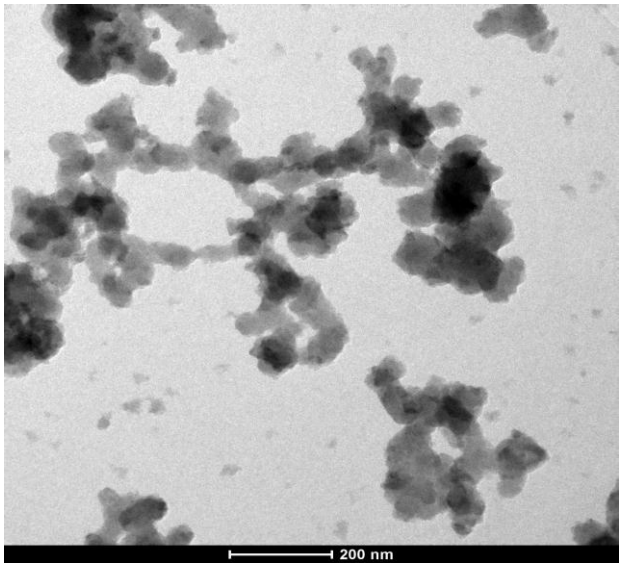

Figure 3: TEM micrograph showing strong aggregation of copper nanoparticles.

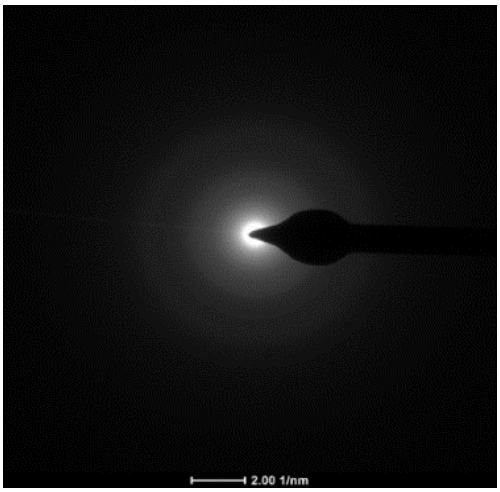

Figure 4: TEM Diffraction pattern of copper nanoparticles from $CuSO_4$ salt.

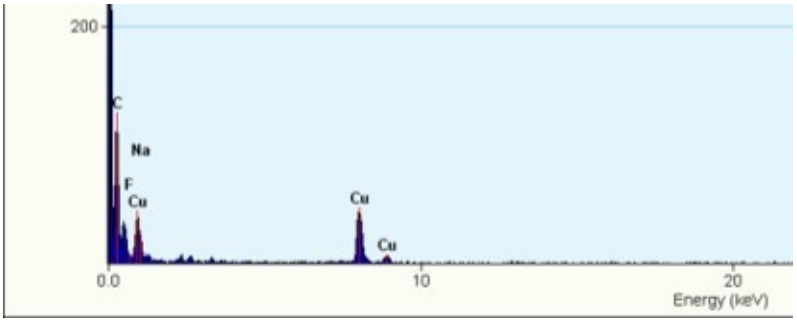

Figure 5: Representative spot EDS profile confirming the presence of copper nanoparticles.

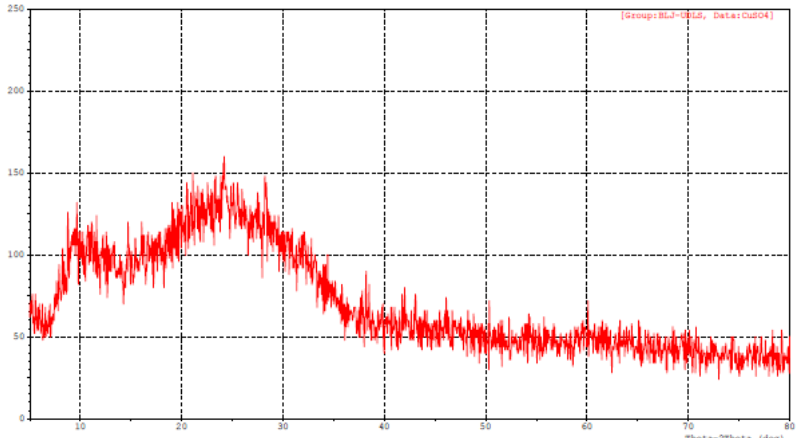

Figure 6: Representative XRD profile of thin film of copper nanoparticles from $CuSO_4$ salt.

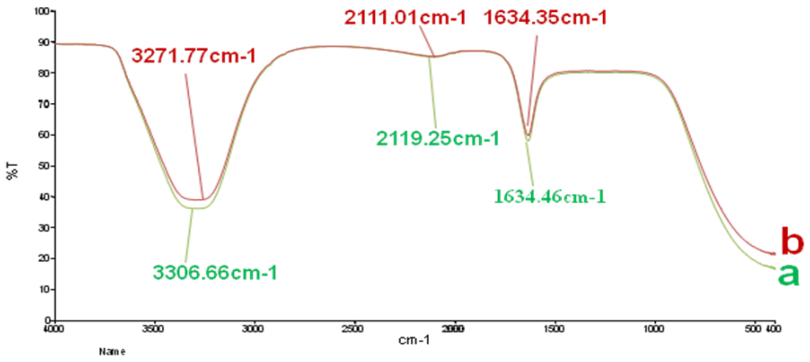

Figure 7: FTIR absorption spectra of CTSE before bioreduction (a), after complete bioreduction of $CuSO_4$ salt (b).

Table 1: Optimization of synthesis process.

Sr.No.	Salt concentration (mM)	Temperature (degrees)	Colour observed	Absorbance at λ max
1.	1 mM $CuSO_4$	RT	Intense brown	500 nm

Table 2: EDS table.

Element	Weight %	Atomic %
C(K) 77.32	93.05	2.49
F(K)	0.48	0.37
Na(K)	3.79	2.38
Cu(K)	18.39	4.18

Table 3: XRD details of silver nanoparticles.

Sr.No.	Nanoparticles Synthesized from salt	Crystal structure	2Θ of the intense peak (deg)	hkl	Crystallite size
1.	$CuSO_4$	FCC	09.35	(111)	~ 4.0 nm

CONCLUSION

In this paper we report rapid biosynthesis of copper nanoparticles from *C.tagal* stem extract. The active constituents from *C.tagal* extract are responsible for simple and efficient reduction of $CuSO_4$ to nanoparticles which could be further exploited to study its various properties. Apart from being eco-friendly, this process can be easily scaled up thus reducing the steps in downstream process and has economical viability providing an alternative to chemical synthesis. As *C.tagal* has a high number of terpenoids and flavonoids, believed to add stability and increased productivity of nanoparticles. From the present study it is found that the rate of synthesis of nanoparticles can be controlled by varying the temperature and concentration of metal salts. Further studies should be done to obtain monodispersed nanoparticles. Separation of nanoparticles on the basis of shape and size should also be focused to get target specific nanoparticles. Compounds responsible for reduction and capping of nanoparticles should be properly identified and separated.

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