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FABRICATION OF COPPER NANOPARTICLE USING CATHARANTHUS ROSEUS BY GREEN SYNTHESIS AND ITS ANTIBACTERIAL ACTIVITY

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ABSTRACT

In the present study, fabrication of copper nanoparticle is achieved by Green Synthesis using the aqueous leaf extract of *Catharanthus roseus*. *Catharanthus roseus* is an evergreen shrub. The plant has various medicinal properties and used to make anti-carcinogenic and anti-diabetic drugs. Copper nanoparticle also exhibit antibacterial activity. The reduction of copper salt using the aqueous leaf extract resulted in the formation of copper nanoparticle. Immediate colour change was observed which indicates the formation of copper nanoparticle. It is a single pot synthesis, because the plant extracts functions as both a reducing and also as a capping agent. The fabricated nanoparticle is characterized using UV -Vis spectrophotometer. The UV-Vis absorbance maximum confirms the presence of copper nanoparticle. FT-IR technique is also used for characterization. SEM studies show that the size of nanoparticle ranges between 40nm – 47nm which looks like coral reef. The antibacterial activity of copper nanoparticle is also studied. This method proves to be cost effective, environment friendly and can be used in the synthesis of copper nanoparticles compared to other chemical reduction methods.

KEY WORDS: ***Catharanthus roseus,*** **Copper nanoparticle, Green Synthesis, Single pot synthesis**

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INTRODUCTION

Fabrication of metal nanoparticle by green synthesis has high potential application in the field of bio medical sciences because of their high stability, faster rate of synthesis and nanoparticle of various sizes and shapes can be prepared compared to other methods where other organisms are used[1-3]. The structure and function of biomolecules present in nature have specific properties which are still unknown to mankind. Synthesis of nanoparticle using green synthesis is one such method, which uses the compounds like terpenoids, alkaloids, phenols, amine present in plants as both reducing and stabilising agent[4]. Green synthesis is of more importance today compared to other processes, as it is simple, eliminates the usage of harmful chemicals which affect the environment, cost effective and forms stable product[5-7]. Synthesis of copper nanoparticles using ascorbic acid and red wine as stabilizing and reducing agents for the synthesis of copper nanoparticles are reported[3-5] but since they harm the environment, green synthesis finds advantage over the others[6,7]. Nanoparticles prepared by the present biogenic method has wide application in the field of medicine as drug delivery probes, to develop nano-engineering devices, as imaging probes and drug delivery vehicles[8]. Copper nanoparticles have been of interest in the fields of catalysis[9], biosensor[10], in nano-electronics and as antibacterial agent[11,12] The present method is a single pot synthesis wherein copper nanoparticle formation, stabilization and reduction occur in a single step. In this method aqueous leaf extract of the plant *Catharanthus roseus*, a traditional Chinese medicinal plant is used for the synthesis of copper nanoparticle. The leaf extract used, functions as both reducing and capping agent. The alkaloids vincristine and vinblastine present in the plant extract can be used in purified form to treat common types of leukaemia and lymphoma. The leaf has anticancer and antidiabetic property. The present approach is cost effective, reaction completes very quickly, no toxin reagents are used, environmental friendly and hence used in the field of biomedicine

MATERIALS AND METHODS

$CuSO_4.5H_2O$ was purchased from Sigma-Aldrich.

i) Preparation of leaf extract

Fresh *Catharanthus roseus* leaves were collected from the farm and in an around of Madras Christian College. The collected leaves were washed several times under running water and then washed with distilled water 3 to 4 times to remove dust particles. The leaves were dried in shade to remove the moisture content and powdered in a blender (Fig 1). About 10g of the powder was added to 500ml of distilled water and heated to 60°C with continuous stirring. The mixture was cooled to room temperature, filtered with Whatman No 1 filter paper to obtain the extract (100%). The extract was collected and stored at 4°C in refrigerator conditions. The extract functions as both the reducing agent and stabilizing agent.

ii) Synthesis of copper nanoparticle

For the synthesis of copper nanoparticle 1mM $CuSO_4$ was prepared. For the reduction of copper ions 75ml of extract was added to 25ml of $CuSO_4$ with continuous stirring. Reduction takes place immediately and is shown by the colour change from brown to sea green colour. The colour change given in Fig 1(c), 1(d) and 1(e) implies the reduction and formation of copper nanoparticle. The pH of the extract is 4.5

Figure 1(a)
Powdered Catharanthus roseus Leaves

Figure 1(b)
Preparation of Leaf Extract

Figure 1(c)
Leaf Extract

Figure 1(d)
$CuSO_4$ solution

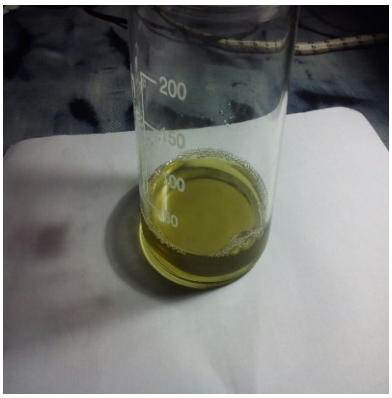

Figure 1(e)
Copper nanoparticle

iii) Antimicrobial activity (agar disc diffusion method) [13]

Preparation of Inoculums

Stock cultures were maintained at 4°C on the slant of nutrient agar. Active cultures for experiments were prepared by transferring of a loop full of cells from the stock cultures to test tubes of nutrient broth for bacteria that were incubated at 24hours at 37°C. The assay was performed by the agar disc diffusion method.

Antibacterial Activity

The disc diffusion method is employed to establish the antibacterial activity. The antibacterial activity of copper nanoparticle is determined by disc diffusion method on Muller Hinton agar (MHA) medium. The MHA medium is weighed as 3.8gms and dissolved in 100ml of distilled water and add 1gm of agar. Then the medium is kept for sterilization. After sterilization the media is poured in to sterile Petri plates, these Petri plates are allowed to solidify for twenty minutes. After the medium is solidified, the inoculums are spread on the solid plates with a sterile swab moistened with the bacterial suspension. The discs are placed on MHA plate and 20µl of sample is added. The plates are incubated for 24 hours, at 37°C. Then the antibacterial activity is determined by measuring the diameter of zone of inhibition.

RESULTS

UV-Visible Spectroscopy Analysis

UV-Visible spectral analysis is done using Erico Double Beam UV-Visible analyser. UV-Visible spectroscopy is an important technique to confirm the formation and stability of metal nanoparticles in aqueous solution. The reduction of copper to copper nanoparticle is confirmed using this technique. The absorbance of copper nanoparticle was observed at 450nm[12].

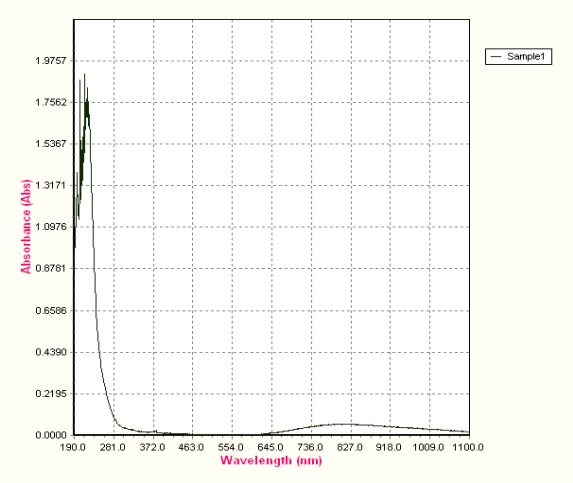

Figure 2(a)
UV-visible absorbance 1mM $CuSO_4$

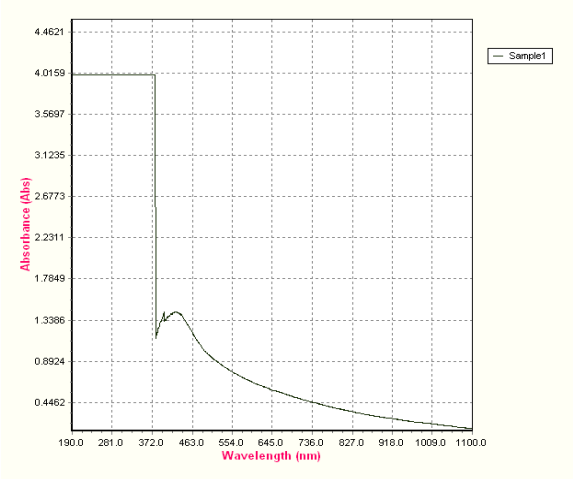

Figure 2(b)
UV-Visible absorbance of copper of nanoparticle (75ml of 100% extract + 25ml $CuSO_4$)

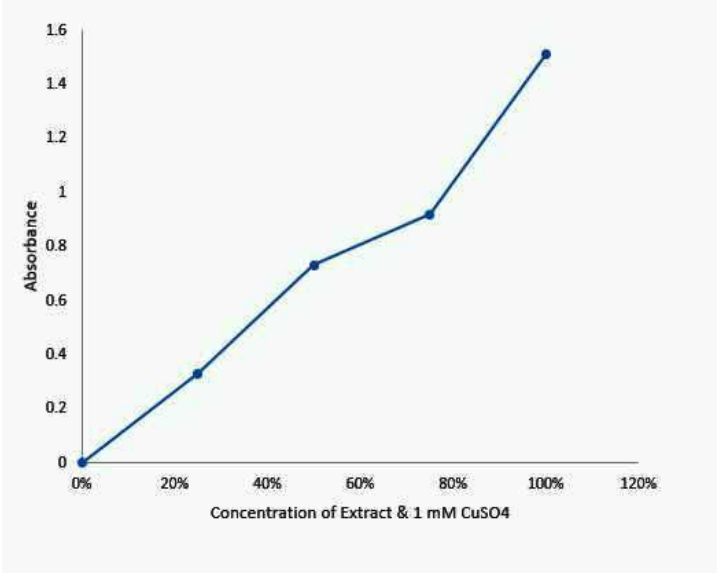

Figure 3
100% extract g*ives *maximum absorbance (UV-Visible analysis) with 1mM $CuSO_4$

Scanning Electron Microscopy (SEM) ***Analysis***

The Hitachi S-3400N Scanning Electron Microscope is utilized to study the surface morphology of copper nanoparticle formed which is shown in fig 4(a) and 4(b).

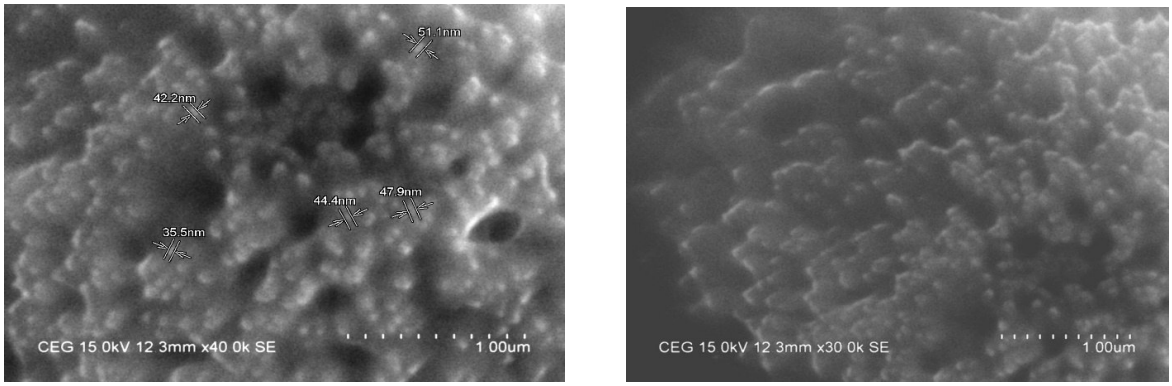

Figure 4(a) & 4(b)
SEM micrograph of Copper Nanoparticle

Fourier Transform Infrared Spectroscopic (FTIR) Analysis

The FT-IR response for the pure extract and the nanoparticle are observed using Shimadzu FT-IR Spectrometer shown in fig 5(a) and 5(b).

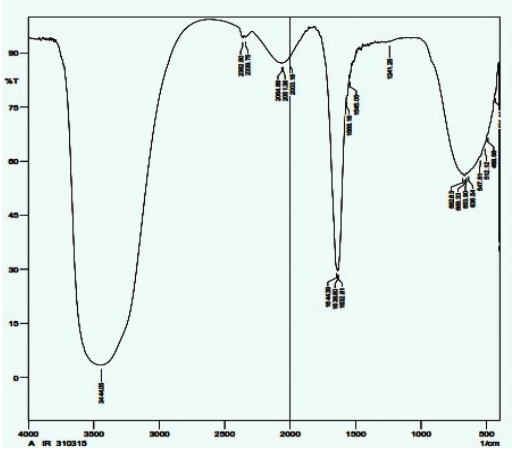

Figure 5(a)
FT-IR response of the Plant Extract

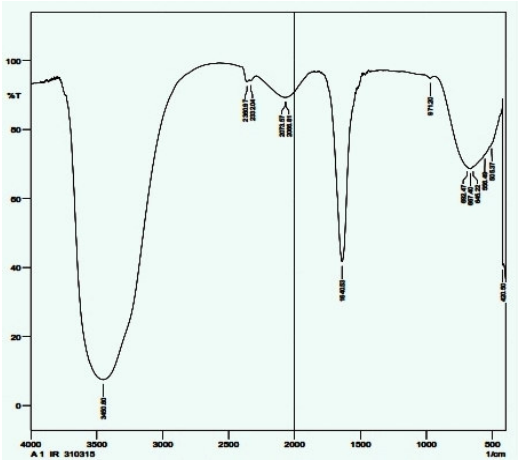

Figure 5(b)
FT-IR response of the Nano particle

DISCUSSION

The absorbance at 460nm observed from fig 2(b) and the colour change from brown to sea green in fig 1(a), 1(b) & 1(c) proves the formation of copper nanoparticle. The presence of biomolecules present not only stabilize the nanoparticle but also prevent the oxidation of nanoparticle after prepration[12]. It is an easy single pot synthesis wherein the nanoparticle is formed immediately and is found to be stable for 24hours under normal room temperature condition. With time, it turns darker in colour. This method is found to be environmental friendly, since no toxic chemicals are being used for reduction and stabilization of nanoparticle. The SEM images (fig 4(a) & 4(b)) clearly show that the average size of copper nanoparticle ranges between 40nm and 47nm. They are found to be spherical in shape having projections which look like a coral reef. It also has good uniformity. The FT-IR response (fig 5(a) & 5(b)) for the plant extract and nanoparticle confirms the presence of alkaloids, phenolic groups, polysaccharides, flavones and terpenoids. After the formation of nanoparticle it is observed that the peaks have become narrow and the intensity has also decreased. The decrease in intensity and the similarity of the two spectra confirms that the alkaloids, phenolic groups, polysaccharides, flavones and terpenoids present in the extract attached to the nanoparticle retains its essential structure and is responsible for capping and stabilization of copper nanoparticles. A strong peak at 3444cm^{-1} corresponds to the stretching vibration of hydrogen bonded O-H group in alcohols, phenols and also the presence of aromatic amines[14], the shift of the peak from 1644cm^{-1} peak in the pure extract in fig 5 is due to the C =O stretching of amide linkage is shifted to 1640cm^{-1} downfield in the synthesised copper nanoparticle confirming the formation of nanoparticle. The peak between 1620-1680cm^{-1} confirms the presence of C=C stretching vibration, which confirms the presence of alkene, the medium peak between 2210-2260cm^{-1} shows the presence of C-N stretching vibration of aliphatic and aromatic amines[11,14-17]. The shift of this band to higher frequency 2066cm^{-1} indicates the coordination of the amine group of the extract with copper nanoparticle which plays a major role in the stabilizing and capping of copper nanoparticle. It is also observed that there is a reduction in % transmittance as we compare the response of the copper nanoparticle to that of the pure extract. The presence of functional groups alkaloids, phenolic groups, polysaccharides, flavones and terpenoids in the extract not only function as reducing agent but also as stabilizing agent[12,14]. Hence the nanoparticles formed do not agglomerate to form larger particles and prevent in further oxidation of the copper nanoparticle.

Antibacterial activity

The antibacterial activity of the copper nanoparticle synthesised from *Catharanthus roseus* leaf extract was examined against Shigella bacteria using disc diffusion method. The maximum zone of inhibition zone by copper nanoparticle on Shigella is 7mm. (Fig 6)

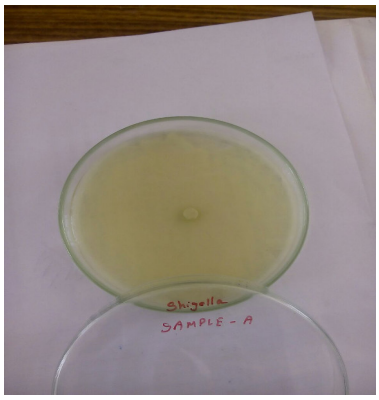

Figure 6
Antibacterial Assay: Zone of inhibition Against Shigella Bacteria

CONCLUSION

This biogenic method of using *Catharanthus roseus* for the formation of copper nanoparticle is a single pot synthesis. The SEM images show that copper nanoparticles of average size 40nm -47nm is obtained and they are found to be spherical and the projections look like a coral reef with uniform size. The colour change from red to sea green, the UV absorption at 450nm and the FT-IR response confirms the formation of copper nanoparticle. The antibacterial activity is also studied It is an inexpensive and fast process. The synthesised copper nanoparticle is stable and can be used in future as drug delivery units.

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Conflict of Interest

Conflict of interest declared none.

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