Biosynthesis and Characterization of Ipomea Leaf Extract Based Copper Nanoparticles and Its Antimicrobial Activities

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Abstract

Nanotechnology broadly refers to a field of applied science and technology with tremendous implications for society, industry and medicine. Biosynthesis is gaining attention due to its cost effective, eco-friendly and large scale production possibilities. Copper nanoparticles (CuNPs) with different structural properties and effective biological effects may be fabricated using new green protocols. The importance of copper nanoparticles, compared to other metal nanoparticles, is due to the high conductivity. Biological methods involve the use of plant extracts, bacteria and fungi. In this study, the leaf of *Ipomea sp*. L. was taken to investigate their potential for synthesizing copper nanoparticles. These synthesized CuNPs were characterized by using Fourier Transform Infrared (FT-IR) Spectroscopy, X-ray diffraction (XRD) and Scanning Electron Microscope (SEM). The crystalline size of synthesized copper nanoparticles was found in 35.79 nm. CuNPs were investigated antimicrobial activities by Agar-well diffusion method on seven microorganisms. CuNPs showed the medium activity on *Escherichia coli, Pseudomonas fluorescens* and *Staphylococcus aureus* and high activity on *Agrobacterium tumefaciens, Bacillus pumilus, Bacillus subtilis* and *Candida albicans.*

Keywords: Copper nanoparticle, XRD, FT-IR, SEM, antimicrobial activities

Introduction

Nanoparticles, compared to bulk materials, exhibit improved characteristics due to their size, distribution and morphology and are widely used in numerous scientific fields. Among metallic nanoparticles, copper nanoparticles (CuNPs) are very important mainly due to their physicochemical and antimicrobial properties which help in therapies, molecular diagnostics and in devices used for medical procedures (Anand K. S. and Dwivedi K. N., 2018).

The properties of nanoparticles often bridge the microscopic and macroscopic regimes, meaning that conventional theories do not necessarily allow us to predict their behavior. It is this uncertainity that lies at the heart of concerns surrounding the health and environmental impact of nanoparticles, but also to the excitement around opportunities for their application in new areas of science and technology. Therefore, it is important to have robust analytical approaches for characterizing nanoparticles, to maximize the benefit from them whilst mitigating their impact.

In literature, the copper nanoparticles are synthesized from (a) vapor deposition (Hyungsoo C., 2004) (b) electrochemical reduction (Huang L., *et al*., 2006), (c) radiolysis reduction (Joshi S., *et al*., 1998), (d) thermal decomposition (Aruldhas N., *et al*., 1998), (e) chemical reduction of copper metal salt (Hashemipour H., *et al*., 2011) and (f) room temperature synthesis using hydrazine hydrate and starch (Surmawar N. V., *et al*., 2011). Recently, green synthesis of Cu nanoparticles was achieved by using microorganisms (Honary S., *et al*., 2012) and plant extract (Gunalan S., *et al*., 2012).

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Biosynthesis of metal nanoparticles by plant is currently under development. The synthesis of metal nanoparticles using inactivated plant tissue, plant extracts, exudates and other parts of living plants is a modern alternative for their production (Huang, J., *et al*., 2007). It is a very cost effective method and therefore a prospective commercial alternative for large-scale production (Vellora, V., 2013). Biosynthesis of copper nanoparticles using microorganisms such as fungi, bacteria and, yeast has been reported in the literature (Singh, A. V., 2010).

Various species of *Ipomoea* have been used extensively, in many countries, in the traditional medicine for the treatment of several diseases (Pereda-Miranda R. And Bah M., 2003). Approximately 600-700 species of *Ipomoea*, Convolvulaceae, are found throughout tropical and subtropical regions of the world. Several of those species have been used as ornamental plants, food, medicines or in religious ritual.

The genus *Ipomoea* since time immemorial has been in continuous use for different purposes, such as nutritional, medicinal, ritual and agricultural ones. The knowledge constitutes a rich source of ethnomedical information for effective selection of plants to be evaluated by chemical studies (Pereda-Miranda R., *et al*., 2005). These species are used in different parts of the world for the treatment of several diseases, such as, diabetes, hypertension, dysentery, constipation, fatigue, arthritis, rheumatism, hydrocephaly, meningitis, kidney ailments and inflammations. Some of these species showed antimicrobial, analgesic, spasmolitic, spasmogenic, hypoglycemic, hypotensive, anticoagulant, anti-inflammatory, psychotomimetic and anticancer activities. Alkaloids, phenolics compounds and glycolipids are the most common biologically active constituents from these plants (Marilena Meira, *et al*., 2012). The most common use of the roots and leaf of *Ipomoea* species are to treat constipation (Marilena Meira, 2012).

To the best of our knowledge, the use of *Ipomea sp.* L. leaf extract at room temperature for biosynthesis of copper nanoparticles has not been reported in Myanmar. Hence, the present study was carried out to synthesize and characterize the copper nanoparticles using *Ipomea sp.* L. leaf extract.

The present study reports for the biosynthesis and characterization of copper nanoparticles using *Ipomea* extract and copper (II) sulphate. And, the antimicrobial characteristics of the synthesized copper nanoparticles are also presented.

Botanical Description

The botanical description of the sample is as follow;

Family	: Convolvulaceae
Botanical name	: *Ipomea sp.* L.
Myanmar name	: Kanzun
English name	: Water spinach, water morning glory
Common name	: Morning glory
Part used	: Leaves

Figure 1. Leaf of *Ipomea sp.* L.

Materials and Methods

Sample Collection

Ipomea sp. L. leaf was collected from Mandalay University campus. It was washed with water twice and kept under room temperature for 3 weeks. Then, it was made into the powder using blender.

Preparation of Leaf Extract

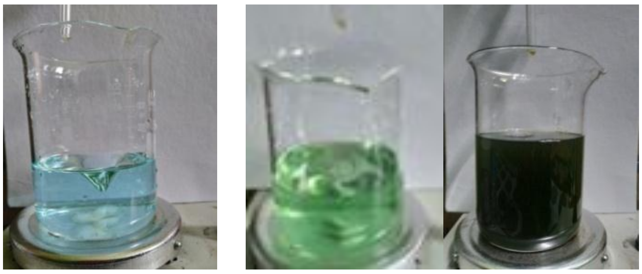
To prepare the plant solution, 10 g of dried leaf powder was taken in a 250 mL beaker with 200 mL of distilled water for 15 min in heating mental at temperature 80 ºC. The filtrate was used as reducing and stabilizing agents.

Preparation of 20 mM Solution of Copper (II) Sulphate Solution

Accurate concentration of 20 mM Copper (II) sulphate solution was prepared by dissolving 0.5 g $CuSO_4$ in 100 mL distilled water and stored in bottle.

Synthesis of Copper Nanoparticles using *Ipomea sp.* L. Leaf Extracts

For the synthesis of copper nanoparticles, 80 mL of 20 mM $CuSO_4$ solution was taken and was stirred using magnetic stirrer. 40 mL of leaf extract (2:1 volume/volume, optimum condition) was added slowly using separatory funnel to the $CuSO_4$ solution. The colour change was observed (Figure 2), which stands as a preliminary identification of the formation of copper nanoparticles. Here the leaf extract acted as a reducing agent. The copper nanoparticles thus obtained were purified by repeated centrifugation method at 3000 rpm for about 30 minutes. Then the synthesized copper nanoparticles were dried in oven at 60 ºC for 4 hours (Sankar, R., 2014).



$CuSO_4$ solution

Ipomea Leaf Extract

Leaf extract+ $CuSO_4$ solution

Figure 2. Color changes for the process of synthesis of copper nanoparticles

Characterization of Synthesized Copper Nanoparticles

Copper nanoparticles synthesized by this biosynthesis method were characterized by Fourier-transform Infrared (FT-IR) spectrum in the range 400-4000 cm^{-1}. The morphology was characterized by using Scanning Electron Microscopy (SEM). The size and crystal nature of the copper nanoparticles were determined using X-ray Diffraction (XRD).

Functional Groups Determination of Synthesized Copper Nanoparticles

FT-IR spectroscopy, a type of vibrational spectroscopy, is used to identify the stretching and bending frequencies of molecular functional groups attached to copper nanoparticles surface (Ndana. M., 2013).

Functional groups of synthesized copper nanoparticles were measured by FT-IR Spectroscopic method at Department of Chemistry at Monywa University.

Identification of Synthesized Copper Nanoperticles by XRD

X-Ray diffraction is a very important method to characterize the structure of crystalline materials and used for the lattice parameters analysis of single crystals, or the phase, texture or even stress analysis of sample. The synthesized copper nanoparticles were measured by X-ray diffraction (XRD) method at University Research Centre, Yangon. The size and crystal nature of the copper nanoparticles were calculated using Debye-Scherrer's equation.

$$D = K\lambda / \beta \cos \theta$$

Where, D is the average size of crystallite, K is the Scherrer constant with a value from 0.9 to , λ is the wavelength of the X-ray source (0.1541 nm) used in XRD, β is the full width at half maximum of the diffraction peak, and θ is the Braggis angle.

Scanning Electron Microsopy (SEM)

The morphology of the synthesized copper nanoparticles was examined at University Research Centre at University of Yangon.

Determination of Antimicrobial Activities

Antimicrobial activities of the synthesized copper nanoparticles were determined by Ager-well diffusion method at Department of Chemistry, Meikhtila University. The seven selected microorganisms were used to screen the antimicrobial activity. These microorganisms were *Agrobacterium tumefaciens*, *Bacillus pumilus, Bacillus subtilis, Candida albicans*, *Escherichia coli, Pseudomonas fluorescens* and *Staphylococcus aureus.*

Results and Discussion

FT-IR Assignments of Synthesized Copper Nanoparticles

The infrared spectrum of copper nanoparticles was carried out by FT-IR instrument and these results obtained were illustrated in Figure 3.

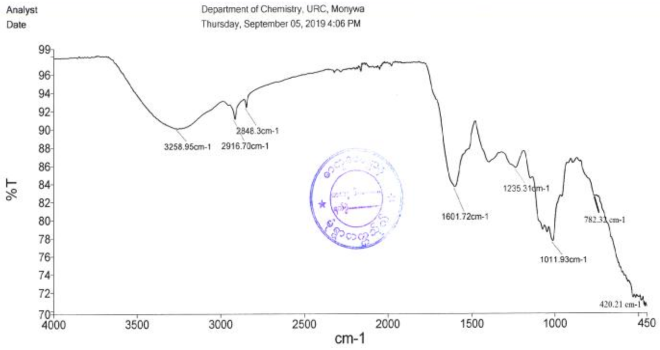


Figure 3. FT-IR Spectrum of copper nanoparticles

FT-IR spectroscopy, a type of vibrational spectroscopy, was used to identify the stretching and bending frequencies of molecular functional groups attached to copper nanoparticles surface. The peak at 3258.95 cm^{-1} was broad and strong band which ascribed to O-H (hydroxyl) stretching vibrations on the surface of copper nanoparticles. At 2916.70 cm^{-1}, it originates from C-H (hydrocarbon) stretching vibration in the molecule, 1601.72 cm^{-1}, it O-H bending vibration, 1011.93 cm^{-1}, it indicated the C-O-C stretching vibration of ether group, and 782.32, 420.21, they all indicated the formation of copper nanoparticles. CuNPs might be surrounded by any one of these bioactive molecules such as polyphenols, alkaloids and terpenoids which were in compliance with the already established facts in the literature (Saranyaadevi K, *et al*., 2014).

XRD Analysis of Synthesized Copper Nanoparticles

XRD pattern of copper nanoparticles using the leaf extract of *Ipomea sp.* L. was shown in Figure 4. Peak observed at d(Å) (1.8103), FWHM (0.238) and 2θ (diffraction angle) value of 50.3666° correspond to h,k,l value to the reflection from (200) plane of copper nanoparticles. This peak was quite consistent with those of the standard spectrum. The crystalline size of synthesized copper nanoparticles was found in 35.79 nm.

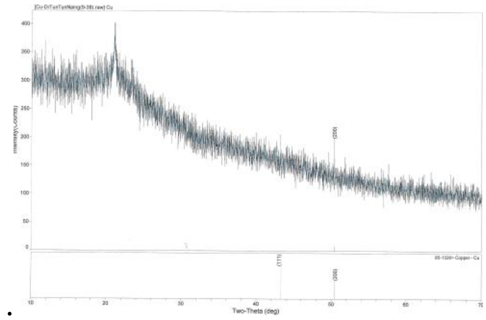


Figure 4. XRD Spectrum of synthesized copper nanoparticles

SEM Analysis of Synthesized Copper Nanoparticles

SEM analysis was used to provide information about the morphology and size of the synthesized copper nanoparticles. SEM spectrum of synthesized copper nanoparticles was showed in Figure 5.

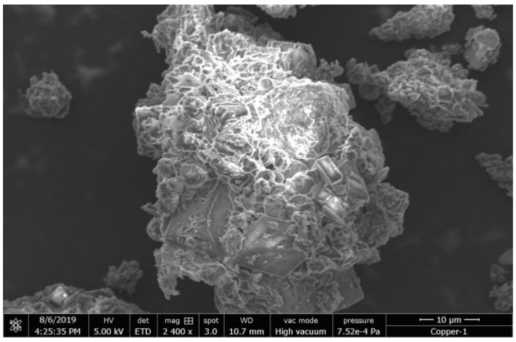
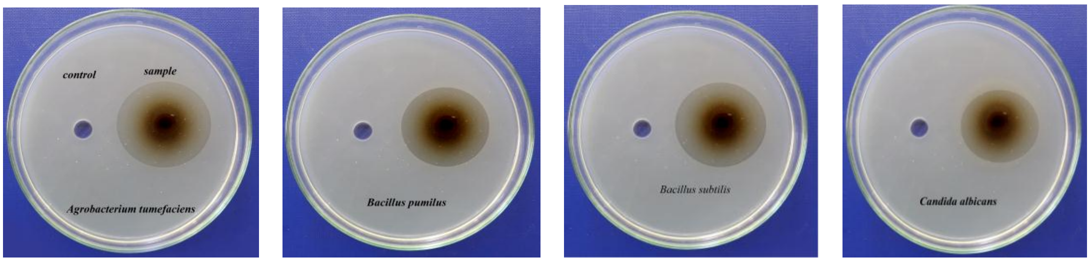


Figure 5. SEM images of synthesized copper nanoparticles

Antimicrobial Activities of Synthesized Copper Nanoparticles

The study of antimicrobial activities of synthesized copper nanoparticles was performed by Agar-well diffusion method onto seven microorganisms. These results were tabulated in Table (1).



(a)*Agrobacterium tumefaciens* (b) *Bacillus pumilus* (c) *Bacillus subtilis*(d) *Candida albicans*

(e) *Escherichia coli* (f) *Pseudomonas fluorescens* (g) *Staphylococcus aureus*

[1=Ethanol (control), 2=CuNPs (30 mg/ml), well=8 mm]

Figure 6. Antimicrobial activities of synthesized copper nanoparticles

Table 1. Antimicrobial Activities of Synthesized Copper Nanoparticles

Test microorganisms	Inhibition Zone (mm)	
	Control	Sample
A. tumefaciens	-	+++
B pumilus	-	+++
B. subtilis	-	+++
C. albicans	-	+++
E. coli	-	++
P. fluorescens	-	++
S. aureus	-	++

9-12 mm (+), 13-17 mm (++), 18 mm above (+++)

Conclusion

In conclusion, the leaf extract of *Ipomea sp.* L. was found efficient for the synthesis of copper nanoparticles. The crystalline size of CuNPs was found nanoparticles size (1-100 nm) at 35.79 nm. CuNPs was confirmed using FT-IR with literature references. Antimicrobial activities of CuNPs showed the medium activity on *Escherichia coli, Pseudomonas fluorescens* and *Staphylococcus aureus* and high activity on *Agrobacterium tumefaciens, Bacillus pumilus, Bacillus subtilis* and *Candida albicans.* This method had merits over other reported methods because it was easily available starting materials, inexpensive and procedure was easy to carry out in any laboratory. Moreover, the use of toxic reagent could be avoided and pollution was free. Synthesis of CuNPs has been demonstrated to be a rapid and environmentally benign route. Thus this rapid, ecofriendly and economical route can be used to synthesize CuNPs with wide biotechnological and chemical applications.

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