

Synthesis of Copper Nanoparticles by Using *Eclipta Alba* Hassk Leaves Extract as Bioreducing Agent

Dr Than Htike¹, Dr Htay Htay Myint², Dr Tu Tu Wai³, Dr Aye Aye Khaing⁴, and Htay Htay Nwe⁵

Dr Than Htike¹, Lecturer in Chemistry Department, Mandalay University, Myanmar

Dr Htay Htay Myint², Lecturer in Chemistry Department, Yadanabon University, Myanmar

Dr Tu Tu Wai³ and Dr Aye Aye Khaing⁴ Lecturers in Chemistry Department, Mandalay University, Myanmar

Htay Htay Nwe⁵, Assistant Lecturer in Chemistry Department, Mandalay University, Myanmar

ABSTRACT

The chemical reaction usually involves organic compounds like flavonoids, alkaloids, terpenoids, polyphenols etc, reacting with metal ions to create a metal nanoparticles. The chemical constituents of plant extracts in the process act as reducing agent as well as stabilizing agent for the nanoparticles. There are a number ways of synthesizing nanoparticles; one way is using biomolecules from plant extract to reduce metal ions to nanoparticles in a single step. In this work, copper nanoparticles were synthesized by using *Eclipta alba* Hassk leaves extract as bio-reducing agent. Phytochemical constituents of sample leaves were detected by chemical methods and also chemical compositions of the sample were analyzed by EDXRF. Synthesized copper nanoparticles were characterized by FT-IR, SEM and XRD techniques.

Keywords : Component; Formatting; Style; Styling; insert (keywords)

1 INTRODUCTION

Nanotechnology is the science that deals with matter at the scale at 1 billion of a meter and is also the study of manipulating matter at the atomic and molecular scale. The term nanoparticle is used to describe a wide variety of materials submicron size. Nanoparticles are the particles with one or more dimensions at nanoscale. They have defined the nanoscale as dimensions of the order of 100nm or less. A nanoparticle is the most fundamental component in the fabrication of nanostructure and is far smaller than the world of everyday objects that are described by Newton's laws of motion, but bigger than an atom or a simple molecule that are governed by quantum mechanics.

In general, the size of a nanoparticle spans the range between 1 and 100 nm. Metallic nanoparticles have different physical and chemical properties from bulk metals properties that might prove attractive in various industrial applications. However, how a nanoparticle is viewed and is defined depends very much on the specific application.

A copper nanoparticle is a copper based particle 1 to 100 nm in size like many other forms of nanoparticles; a copper nanoparticle can be formed by natural processes or through chemical synthesis. These nanoparticles are of particular interest due to their historical application as coloring agents and their modern-day biomedical ones. Copper nanoparticles (CuNPs) have been used various field including agricultural, industrial engineering and technological fields (Shobha G. *et.al*, 2014).

CuNPs synthesis has attracted particular interest, compared with other NPs, as their useful properties are achievable at costs lower silver and gold (Han WK. *et.ai*, 2006).

Although the biosynthesis of CuNPs by plants has previously been reported, the potential of plants as biological materials for the synthesis of nanoparticles is yet to be fully explored. The bioactivities of *Eclipta alba*, which is a widely used traditional medicine and functional food, have been extensively explored (Zhong XK. *et.al*, 2009). The phytochemical screening of *Eclipta alba* were examined. The present study aimed to develop a method of rapidly synthesizing CuNPs using ethanol leaf extract of *Eclipta alba*. These copper particles were characterized by FTIR, SEM and XRD methods.

2. EXPERIMENTAL

2.1 Sample Collection and Preparation

The leaves of *Eclipta alba* Hassk were collected from Shwebo Township, Sagaing Region. These leaves were washed with water to remove dust from surface and cut with a steel knife into small pieces and dried at room temperature. Then the dried samples were percolated with 98% ethanol for about 1 month.

2.2 Preliminary Phytochemical Test for *Eclipta alba* Hassk Leaves

Preliminary phytochemical tests were carried out to detect the presence or absence of organic constituents in the *Eclipta alba* Hassk leaves. These results were shown in Table 1.

Test for alkaloids

The dried sample (2 g) was boiled with 1% hydrochloric acid (25 cm³) for 10 minutes. The solution was cooled and filtered. The filtrate was added to a few drops of Dragendroff's reagents. The formation of a precipitate indicates the presence of alkaloids.

Test for Flavonoids

The dried sample (2g) was boiled with 95% ethanol (25 cm³) for about 10 minutes. The solution was cooled and filtered. A few drops of concentrated hydrochloric acid and pieces of magnesium turning were added to the filtrate. The formation of pink color indicates the presence of flavonoids.

Test for Glycosides

The dried sample (2g) was boiled with distilled water (250 cm³) for about 10 minutes. The solution was cooled and filtered. About 2 drops of 10% lead acetate was added to the filtrate solution. The formation of white precipitate indicates the presence of glycosides.

Test for Saponins

The dried sample (2g) was boiled with distilled water (25 cm³) for about 10 minutes. The solution was cooled and filtered. The filtrate was added 3 drops of distilled water and then shaken for a few minutes, allowed to settle for 10 minutes. The formation of stable froth indicates the presence of saponins.

Test for Steroids

The dried sample (2g) was boiled with 95% ethanol (25cm³) for about 10 minutes. The solution was cooled and filtered. 2 drops of chloroform were added to the filtrate solution. Add then 2 drops of acetic anhydride solution and 2drops of concentrated sulphuric acid were added to this solution. The formation of green color indicates the presence of steroid.

Test for Phenolic compound

The dried sample (2g) was boiled with distilled water (25cm³) for about 10 minutes. The solution was cooled and filtered. A few drops of 10% FeCl₃ were added to solution. The greenish blue color indicates the presence of phenol.

Test for Polyphenol

The dried sample (2g) was boiled with distilled water (25cm³) for about 10 minutes. The solution was cooled and filtered. A few drop of 1% FeCl₃ and 1% K₃Fe(CN)₆ were added to the filtrate. The formation of blue-green color indicates the presence of polyphenol.

Test for Terpenoids

The dried sample (2g) was boiled with 95% ethanol (25cm³) for about 10 minutes, cooled and filtered. 2ml of filtrate was added to 2ml of chloroform. It was evaporated and then dryness. And then, it was 2ml of concentrated sulphuric acid at heat for 2 minutes which give cherry red color solution. This color indicates the presence of terpenoids.

Test for Lipophilips

The dried sample (2g) was boiled with distilled water (25cm³) for about 10 minutes. The solution was cooled and filtered. A few drop of KOH solution was added to the filtrate. The formation of deep color indicates the presence of lipophilips.

2.3 Determination of Elemental Analysis of *Eclipta alba* Leaves

The amount of element in dried powder of *Eclipta alba* leaves were measured by EDXRF spectrophotometer at Department of Chemistry, Monywa University.

2.4 Synthesis of Copper Nanoparticles

Chemicals

- (i) Copper acetate
- (ii) Distilled water
- (iii) 98% ethanol
- (iv) Acetone

Apparatus

- (i) 250mL beaker
- (ii) 100mL measuring cylinder
- (iii) Magnetic stirrer
- (iv) Centrifuge
- (v) Petridis
- (vi)

Preparation of 3mM copper acetate solution

0.06g of copper acetate was dissolved in 100ml of distilled water. This solution was stirred with magnetic stirrer for about 2 hours.

Procedure

The 80ml of 3mM copper acetate solution were mixed with 20ml of ethanol extracts. This mixture was stirred on a magnetic stirrer with 300 rpm for about 24 hours. The mixture was centrifuged with 3000 rpm for 1 hour. The precipitates of copper particles were obtained. The resultant copper particles was washed with ethanol and acetone and then dried in Petridis (Harne S. et.al, 2012).

2.5 Characterization of Synthesized Copper Nanoparticles

The synthesized copper nanoparticles were analyzed by Fourier Transform Infrared (FT-IR) at Department of Chemistry, Monywa University. The synthesized copper nanoparticles were analyzed by Scanning Electron Microscopy (SEM) at Universitie's of Research Center, University of Yangon, Yangon. The synthesized copper nanoparticles were analyzed by X-ray Diffraction (XRD) Universitie's of Research Center, University of Yangon, Yangon.

3. RESULTS AND DISCUSSION

3.1 Preliminary Phytochemical Test for Leaves of *Eclipta alba* Hassk

The *Eclipta alba* Hassk leaves were tested by phytochemicals screening and these results are shown in Table 1.

Table 1 Results of Phytochemical Test for Leaves of *Eclipta alba* Hassk

No	Tests	Extract	Reagents Used	Observation	Result
1	Alkaloids	1% HCl	Dragendroff's	Orange ppt	+
2	Flavonoids	EtOH	Conc: HCl, Mg	Pink color	+
3	Glycosides	EtOH	10% lead acetate	White ppt	+
4	Saponin	Water	Distilled water	Front	+
5	Steroids	EtOH	Acetic anhydride, conc: H ₂ SO ₄ , CHCl ₃	Green color	+
6	Phenolic	D/W	10% FeCl ₃	Blue -black color	+
7	Polyphenol	D/W	1% FeCl ₃ , K ₃ Fe(CN) ₆	Greenish blue	+
8	Terpene	EtOH	Acetic anhydride, conc: H ₂ SO ₄ , pet-ether	cherry red color	+
9	Lipophilip	Water	0.5M KOH	deep color	+

(+) = presence, (-) = absence

According to table (4.1), all tested compounds were found in the air dried sample of Kyeik-hman leaves.

3.2 Elemental Analysis of Dry *Eclipta alba* Hassk Leaves

The content of elements in *Eclipta alba* Hassk leaves powder was determined by EDXRF analysis at Department of Chemis try, Moywa University. The result was shown in Table 2.

Table 2 Elemental Analysis of *Eclipta alba* Hassk Leaves Powder

No	Element	Symbol	Concentration (%)
1	Silicon	Si	2.773
2	Potassium	K	2.236
3	Chlorine	Cl	1.023
4	Calcium	Ca	0.860
5	Sulfur	S	0.483
6	Phosphorus	P	0.208
7	Iron	Fe	0.023
8	Manganese	Mn	0.016
9	Strontium	Sr	0.003
10	Copper	Cu	0.003
11	Titanium	Ti	0.003
12	Zinc	Zn	0.002
13	Boron	Br	0.001
14	Silver	Ag	0.001

From Table 2, the higher amount of silicon (2.773%) and potassium (2.236%) were observed in the air dried sample of Kyeik-hman. And the contents of chlorine (1.023%), calcium (0.860%), sulphur (0.483%) and phosphorus (0.208%) were found in the selected sample.

3.3 Characterization of Synthesized Copper Nanoparticles

3.3.1 FT-IR Analysis

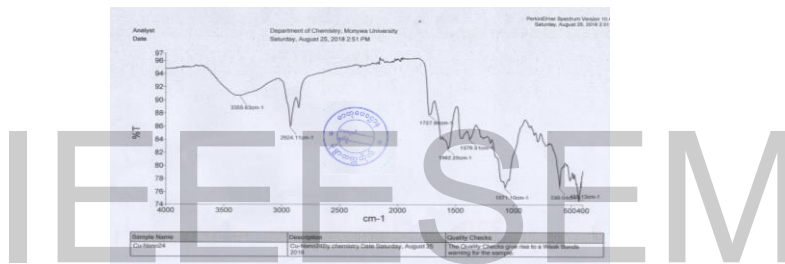


Fig 1 FT-IR spectrum of copper nanoparticles

The FT-IR spectrum of Cu nanoparticles is shown in figure 1. The IR spectrum of CuNPs shows band at 3355 cm⁻¹, 2924 cm⁻¹, 1729 cm⁻¹, 1635 cm⁻¹, 1562 cm⁻¹, 1376 cm⁻¹ and 1071 cm⁻¹ corresponds to O-H stretching H-bonded alcohols and phenols, C-H stretching, C=O stretching, carbonyl stretching, N-H bend primary amines, corresponds to C-N stretching of the aromatic amino group and C-O stretching alcohols, ethers respectively. FT-IR spectrum of CuNPs suggested that CuNPs were surrounded by different organic molecules such as terpenoids, phenols, alcohols, ketones, aldehydes and carboxylic acid.

3.5 SEM Analysis

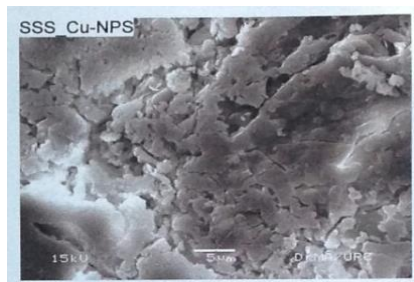


Fig 2 SEM micrograph for copper nanoparticles

SEM micrograph of the copper nanoparticles synthesized by the reduction of copper acetate revealed spherical, hexagonal and cubical nanoparticles ranging from nm to nm, with an average size of 500 nm due to Cu ions. It was observed that they were approximately spherical in shape with a smooth surface Fig 2.

3.6 XRD Analysis

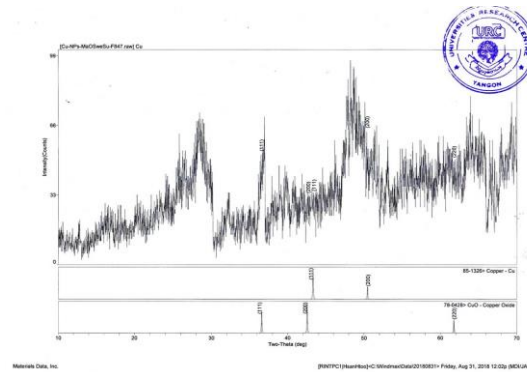


Fig 3 XRD diffractogram of copper nanoparticles

X-ray powder diffraction (XRD) is a powerful technique used uniquely identify the crystalline phases present in materials and to measure the structural properties of these phases. The XRD pattern of the copper nanoparticles was recorded by using a powder x-ray diffractometer with a diffraction angle between 20 and 80. Fig 3 shows three peaks (111), (200) and (220) planes of copper respectively.

The crystalline size was determined from the broadenings of the corresponding X-ray spectral peaks by using Scherrer's formula. The average nano-crystalline size was calculated using the Scherrer formula,

$$L = \frac{\kappa\lambda}{\beta\cos\theta} \text{ \AA}$$

- Where, L = the average crystalline size in Å
 K = the shape factor
 λ = the wavelength of X-ray (1.5406Å)
 β = the corrected line broadening of the Nps
 θ = the Bragg angle

Table 3 Calculation Parameters of Copper Nanoparticles

2θ (°)	FWHM (β in radians)	d-spacing (Å)	Cos θ	Crystalline Size (nm)
36.562	0.0096	2.4556	0.9495	15.21
42.647	0.0098	2.1183	0.9315	15.19
43.391	0.0050	2.0837	0.9291	25.76
50.360	0.0012	1.8104	0.9047	126.09
61.740	0.0118	1.5013	0.8583	13.69
Average				39.51

According to this table, the crystalline size of copper nanoparticles was found within the range of 13.69 nm to 127.72nm. The average crystalline size was found to be 39.51 nm.

4.CONCLUSION

The green synthesis of CuNPs was successfully done by using *Eclipta alba* Hassk extract as reducing and capping agent. The *Eclipta alba* Hassk were examined by phytochemical test and EDXRF analysis. Synthesized CuNPs were characterized using FT-IR spectrum of CuNPs was surrounded by different organic molecules such as terpenoids, phenol, alcohols and carboxylic acid. SEM micrograph of synthesized CuNPs showed that they were approximately spherical in shape with a smooth surface. The XRD pattern showed a crystallinity of Cu sample. The average crystalline size of synthesized CuNPs was found to be 39.51 nm.

ACKNOWLEDGMENT

The authors would like to acknowledge Rector Dr Thida Win, University of Mandalay and Professor Dr Yi Yi Myint, Head of Department, Department of Chemistry, University of Mandalay, for their allowing to carry out the research program and valuable suggestions.

REFERENCES

- [1.] Daniel, M.C.A., D., (2004), "Gold nanoparticles assembly supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology". Vol.104, pp.293-346.
- [2.] Heiligtag, FlorianJ; Niederloerger, Markus, (2013), "The fascinating world of nanoparticle research". Vol.16 (7-8). pp.262-271.
- [3.] Harne S, Sharma A Dhaygude M, Joglekar S, Kodam k, Hudlikar M, (2012), " Novel route for rapid biosynthesis of copper nanoparticles using aqueous extract of *Calotropis procera* L. latex and their cytotoxicity on tumor cells". Colloids Surf B Biointerfaces. Vol.95, pp.284-288.
- [4.] Han WK, Choi jW, Hwang GH, Hong Sj, Lee jS, Kang SG, (2006), "Fabrication of Cu nano particles by direct electrochemical reduction from CuO nano particles". pp.252-2832-2838.
- [5.] Johnson, MD PhD, Larry E, ed. (2008). "Copper" Merck Manual Home Health Handbook, Merck Sharp & Dohme corp, a subsidiary of Merck & Co, Inc.
- [6.] Khan, F.A,(2011), " Biotechnology Fundamentals".
- [7.] Lee H.Y.L., Z., Chen, K.; Hsu, A.R.; Xu, C.; Xie, J.; Sun, S.; chen, X., (2008), " PET/MRI dualmodality tumor imaging using arginine-glycine-aspartic (RGD)-conjugated radiolabeled iron oxide nanoparticles". J Nucl Med, vol.49, pp.1371-1379.
- [8.] Mc Henry, Charles,ed, (1992), "The New Encyclopedia Britannica". Vol. 3(15ed.) Chicago: Encyclopedia Britannica, Inc. pp. 612.ISBNO-85229-553-7.
- [9.] Richardson, H, Wayne, "copper compounds," Ulmann's Encyclopedia of Industrial Chemistry, Weinheim: Wiley-VCH.
- [10.] Shobha G, Vinutha M, Anande S, (2014), "Biological synthesis of copper nanoparticles and its impact a review". Int J Pharmaceut Sci Invent. Vol. 3, pp.28-38.
- [11.] Tan, M.W., G; Ye, Z. & Yuan, J, (2006), "Synthesis and characterization of titania-based monodisperse fluorescent europium nanoparticles for biolabeling". Journal of Luminescence, vol.117, pp.20-28.
- [12.] Xu ZP, Z.Q.P., Lu G Q and Yu AB, (2006), "Inorganic nanoparticles As Carrier for Efficient Cellular Delivery". Chemical Engineering Science. Vol.61, pp.1027-1040.
- [13.] Zhong XK, Li DC, Jiang JG, (2009) , "Identification and quality control of Chinese medicine based on the fingerprint techniques". Vol.16. pp.3064-3075.

IEEESEM