Synthesis and Characterization of Copper Oxide Nanoparticles Using Rhizome Extract

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Abstract

The rhizome of Shwe-pan was collected from Pyipan village, Kyaukse Township, Mandalay Region. The preliminary detection of phytochemical compounds presents in rhizome extracted was carried out by phytochemical tests. The elemental analysis of rhizome extracted was also investigated by Energy Dispersive X-ray Fluorescence (EDXRF) Spectroscopy. An experiment has been synthesized the copper oxide nanoparticles using an aqueous solution extracted from the rhizome of Shwe-pan. The size distribution of nanoparticles surface and morphology of copper oxide nanoparticles were determined by X-ray diffraction (XRD) method and Scanning Electron Microscopy (SEM) method. The characteristic absorption peaks of copper oxide nanoparticles were analysis by Fourier Transform Infrared (FTIR) Spectroscopy. Antimicrobial activities of synthesized copper oxide nanoparticles were examined by agar well diffusion method on tested microorganisms. The synthesis of nanoparticles using the rhizome extract is an affordable method and it reduces the environmental pollution.

Key Words- EDXRF, SEM, XRD, FT-IR, Antimicrobial activities.

Introduction

Nanotechnology was believed first introduced by Richard P. Feynman in his lecture at the California Institute of Technology in 1959 (Saloni Chadha, 2013). The objective of nanotechnology is to gain atomic and molecular control over matter (Feynman, R.P., 1960). Nanotechnology is the study and application of small object which can be used across all fields such as chemistry, biology, physics, material science and engineering. As the name indicates Nano means a billionth or 10^{-9} unit (Nair, R. *et al.,* 2010).

Nanoparticles are materials at sub-micrometer scales, usually 1-100 nm, so they possess a large surface to volume ration. Production of nanoparticles can be achieved mainly through chemical, physical and biological methods (Sinjumol Thomas., *et al.,* 2014). Green synthesis is the most effective methods with respect to cost, eco-friendly, and no requirement of high temperatures or pressures and does not involve toxic chemicals (Mohammad Mansoob Khan, *et al.,* 2012).

Copper is the preferred material in the world due to its electrical, optical, catalytic, biomedical and antifungal, antibacterial applications. Copper nanoparticles can be used as antimicrobial agent in many fields. It is toxic to microorganisms such as bacteria (*E. coli, Staphylococcus aureus, Pseudomonas aeruginosa*) but non-toxic to animal cells. Due to this property, it is considered to be an effective bactericidal (Abbound, Y., *et al.,* 2013).

The nanoparticles synthesized from plant extract were found to be covered by the medicinal properties of plant extract which could be used in drug, targeted drug delivery and cosmetic applications (Mallikarjunaa K, Narasimhab G, *etal*., 2011).

The rhizome of *Hedychium flavescens* Carey ex Roscoe has been used for the treatment of analgesic, sprains and rheumatic anti-inflammatory activities of rhizome of this plant (Baby, S., 2007; Shrotriya, S., *et al.,* 2007).

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In this study, an aqueous solution extracted from the rhizome of *Hedychium flavescens* Carey has been used. This plant extract was used as reducing agent for the synthesis of copper oxide nanoparticles. The current investigation focused on the aqueous rhizome extract to synthesize copper oxide nanoparticles and to be used as an antimicrobial agent.

Figure 1. The Flower and Rhizome of *Hedychium flavescens* Carey ex Roscoe (Shwe-pan)

Family	-	Zingiberaceae
Botanical name	-	*Hedychium flavescens* Carey ex Roscoe
English name	-	Yellow ginger
Myanmar name	-	Shwe-pan
Genus	-	*Hedychium*
Species	-	*H. flavescens*
Part used	-	Rhizome

Materials and Methods

Sample Collection

The rhizome *Hedychium flavescens* Carey ex Roscoe (Shwe-pan) was collected from Kyaukse Township, Mandalay Region, Myanmar. Then, they were cut into small pieces and dried in the shades used for the experiment.

Preliminary Phytochemical Test for Rhizome

The preliminary phytochemical tests were carried out to detect the presence or absence of organic constituents in the *Hedychium flavescens* Carey ex Roscoe (Shwe-pan) rhizome (Harborne J, 1973).

Determination of Elemental Analysis

The elemental analysis of *Hedychium flavescens* Carey ex Roscoe (Shwe-pan) rhizome was determined by using EDXRF (Energy Dispersive X-ray Fluorescence) Spectroscopy.

Preparation of Rhizome Extract

The rhizome sample was washed with distilled water and chopped into small pieces. Then, they were dried at room temperature and motored into fine powder, 10 g of each washed sample was boiled in 200 mL of distilled water for 30 minutes to obtain the extract. The extract solution was cooled and filtered through Whatman filter paper. The filtrate was obtained (Devasenan, S., *et al*., 2016).

Synthesis of Copper Oxide Nanoparticles using 70 mM of Copper (II) Sulphate Solution and Rhizome Extract (1:4)

1.75 g of copper (II) sulphate was weighed accurately and dissolved in 100 mL of distilled water and stored. The 30 mL of this solution was mixed with 120 mL of rhizome extract. This mixture was stirred on a magnetic stirrer with 700 rpm. This reaction mixture was maintained at 80º C for reaction time 5 hours for the reduction of copper ions. The mixture was

centrifuged with 3000 rpm for 30 minutes. The precipitates of copper oxide nanoparticles were obtained. The resultant copper oxide nanoparticles were washed with distilled water. The obtained precipitate was placed in petridish and then it was dried in an oven at 80°C for 3 hours (Hailemariam Gebru, 2011).

Synthesis of Copper Oxide Nanoparticles using 50 mM of Copper (II) Sulphate Solution and Rhizome Extract (1:4)

1.25 g of copper (II) sulphate was weighed accurately and dissolved in 100 mL of distilled water and stored. The 30 mL of this solution was mixed with 120mL of rhizome extract. This mixture was stirred on a magnetic stirrer with 700 rpm. This reaction mixture was maintained at 80º C for reaction time 5 hours for the reduction of copper ions. The mixture was centrifuged with 3000 rpm for 30 minutes. The precipitates of copper nanoparticles were obtained. The resultant copper nanoparticles were washed with distilled water. And then they were dried in petridish for 3 hours (Hailemariam Gebru, 2011).

Synthesis of Copper Oxide Nanoparticles using 30 mM of Copper (II) Sulphate Solution and Rhizome Extract (1:4)

0.75 g of copper (II) sulphate was weighed accurately and dissolved in 100 mL of distilled water and stored. The 30mL of this solution was mixed with 120mL of rhizome extract. This mixture was stirred on a magnetic stirrer with 700 rpm. This reaction mixture was maintained at 80º C for reaction time 5 hours for the reduction of copper ions. The mixture was centrifuged with 3000 rpm for 30 minutes. The precipitates of copper nanoparticles were obtained. The resultant copper nanoparticles were washed with distilled water. And then, they were dried in petridish (Hailemariam Gebru, 2011).

Characterization of Copper Oxide Nanoparticles

Morphology and size distribution of nanoparticles were determined by Scanning Electron Microscopy (SEM) and the size of Nano crystallites were measured by X-ray diffraction (XRD) method. Estimation of particle size was carried out by using Debye-Scherrer's equation (Debye, *et al*., 2010).

Functional Group Determination of Copper Oxide Nanoparticles

Functional groups contained in synthesized copper oxide nanoparticles were measured by FT-IR spectroscopic method (Ved Prakash, *et al.,* 2015).

Determination of Antimicrobial Activities of Synthesized Copper Oxide Nanoparticles

For the measurement of antimicrobial activities, the synthesized Copper Oxide Nanoparticles from the rhizome extract were determined by agar-well diffusion method. After that, seven types of micro-organisms were used to screen the antimicrobial activities.

Results and Discussion

Determination of Phytochemical Test for Rhizome Extract

Hedychium flavescens Carey ex Roscoe (Shwe-pan) rhizome extract was tested by phytochemical screening. According to phytochemical results, polyphenol, terpene, flavonoid, steroid, tannin, saponin, lipophelic, phenolic, glycoside, reducing sugar and carbohydrate were present in rhizome of Shwe-pan but alkaloid was not present in this rhizome.

Determination of Mineral Content of Rhizome Extract

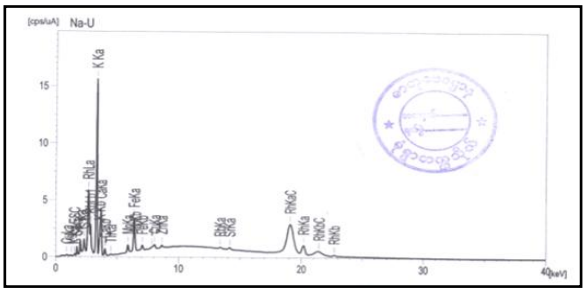

Figure 2. EDXRF Pattern of Rhizome Extract

According to experimental result, the higher amount of potassium and magnesium were found in rhizome of Shwe-pan. Moreover, silicon, phosphorus, calcium, sulfur, iron, manganese, titanium, copper, zinc were also found in that order. This elemental composition indicated that the rhizome of Shpwe-pan was a rich source of minerals.

The Yield Percent of Copper Oxide Nanoparticles Using Water Extract

The yield percent of copper oxide nanoparticles from selected rhizome extract samples was obtained by adding 120 mL of rhizome extract to 30mL of 70 mM, 50 mM and 30 mM copper II sulphate solution.

Table (1) The Yield Percent of Synthesized Copper Oxide Nanoparticles from Water Rhizome Extract

Sample	Amount of $CuSO_4$-$5H_2O$ (g)	Time (hr)	Amount of CuO NPs(g)	Yield (%)
Shwe-pan	1.75	5	0.18	10.3
	1.25	5	0.11	8.8
	0.75	5	0.06	8.0

According to this table (1) copper oxide nanoparticles were determined by using different concentration intervals. It was found that the amount of copper oxide nanoparticles depends on the contact concentration. When concentration increases, the amount of copper oxide nanoparticles also increases.

XRD Analysis of Copper Oxide Nanoparticles by using Rhizome Extract

The X-Ray diffraction was carried out to analyze the phase and estimate the crystallite size of the samples using X-ray diffractometer with 0.15405 nm Cu-Kα radiation source in the 2θ range from 10-50. The XRD patterns with diffraction intensity versus 2θ were recorded. The crystallite size is calculated by using Debye Scherrer equation, $D = K\lambda/\beta Cos\theta$. According to XRD, the CuO nanoparticles of average crystallite size from the water rhizomes extract of Shwe-pan were found to be range between 18.26 and 46.46 nm respectively (Ved Prakash, *et al.*, 2015).

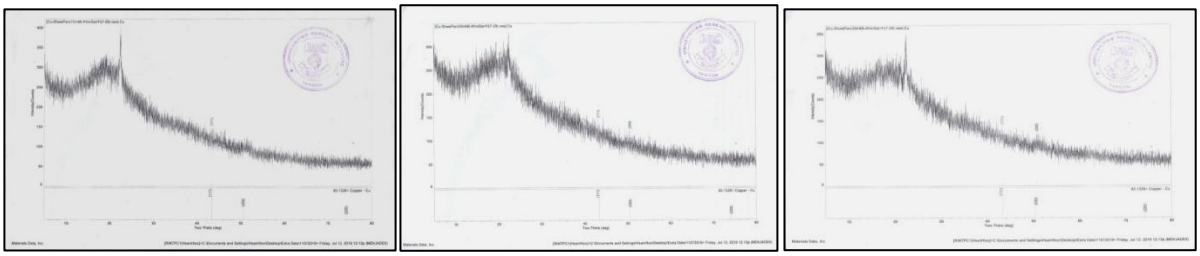

Figure 3. XRD Diffractogram of Copper Oxide Nanoparticles using 70, 50, 30 mM of $CuSO_4$ Solution and Rhizome Extract

Table (2) XRD Diffractogram of Copper Oxide Nanoparticles using 70, 50, 30 mM of $CuSO_4$ Solution and Rhizome Extract

Sample concentrations (mM)	2 θ value	FWHM (β in radians)	d-spacing (nm)	Crystlline size (nm)
70	43.250	0.277	0.2090	28.68
50	43.084 50.440	0.171 0.344	0.2097 0.1807	46.46 23.09
30	43.181 50.682	0.435 0.371	0.2093 0.1799	18.26 21.41

SEM Images of Copper Oxide Nanoparticles

Scanning Electron Microscopy determines the size distribution of nanoparticles surface and morphology of copper oxide nanoparticles. The surface morphologies of the sample Shwe-pan, were observed by Scanning Electron Microscopy images as shown in figure (4a), (4b), and (4c).

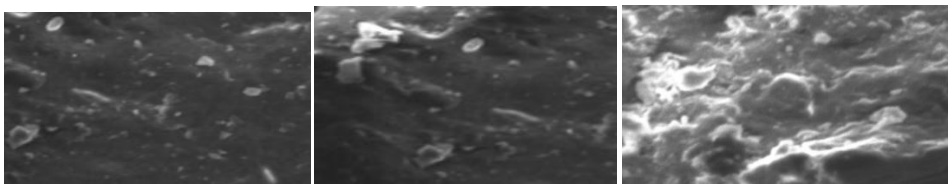

Figure (4a,4b and 4c) SEM Image of Copper Oxide Nanoparticles using 70, 50, 30 mM of $CuSO_4$ Solution and Rhizome Extract

FT-IR Measurement

The FT-IR spectrum reported the characteristic peaks of synthesized CuO from the water extract of rhizome. The water extract of rhizome was observed in the range 487.45-497.45cm^{-1}. The broad absorption peak at 3278.87 cm^{-1} was caused by absorbed water molecules since the Nano crystalline materials exhibit a high surface to volume ratio and thus absorbed moisture. The infrared spectrum (Figure 5) informs at 400-865cm^{-1}, which can be assigned to the vibration of copper oxide nanoparticles (Pricilla J, *et al*., 2017).

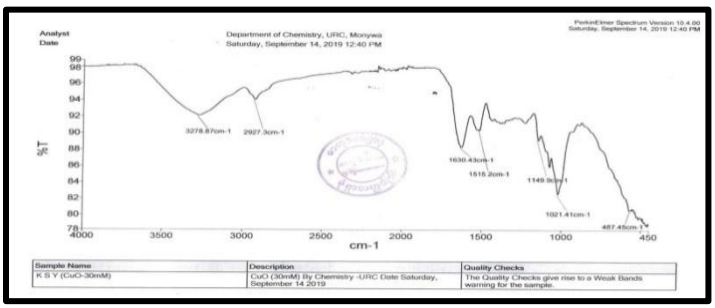

Figure5. FT-IR Assignment of Copper Oxide Nanoparticles using 30 mM $CuSO_4$ Solution and Water Rhizome Extract

From the FT-IR spectrum for 30 mM of copper (II) nitrate solution, it was found that copper oxide nanoparticles of 30 mM $CuSO_4$ solution and water extract of Shwe-pan rhizome extract contain O-H or N-H stretching vibration of hydroxyl or amine group, C = C stretching vibration of alkene group, O-H in plane bending vibration of alcohol group, Cu-O stretching vibration.

Determination of Antimicrobial Activities of Copper Oxide Nanoparticles by using Rhizome Extract

The study of antimicrobial activities of copper oxide nanoparticles from the rhizome extract of *Hedychium flavescens* Carey ex Roscoe (Shwe-pan) was performed by agar-well diffusion method on seven microorganisms.

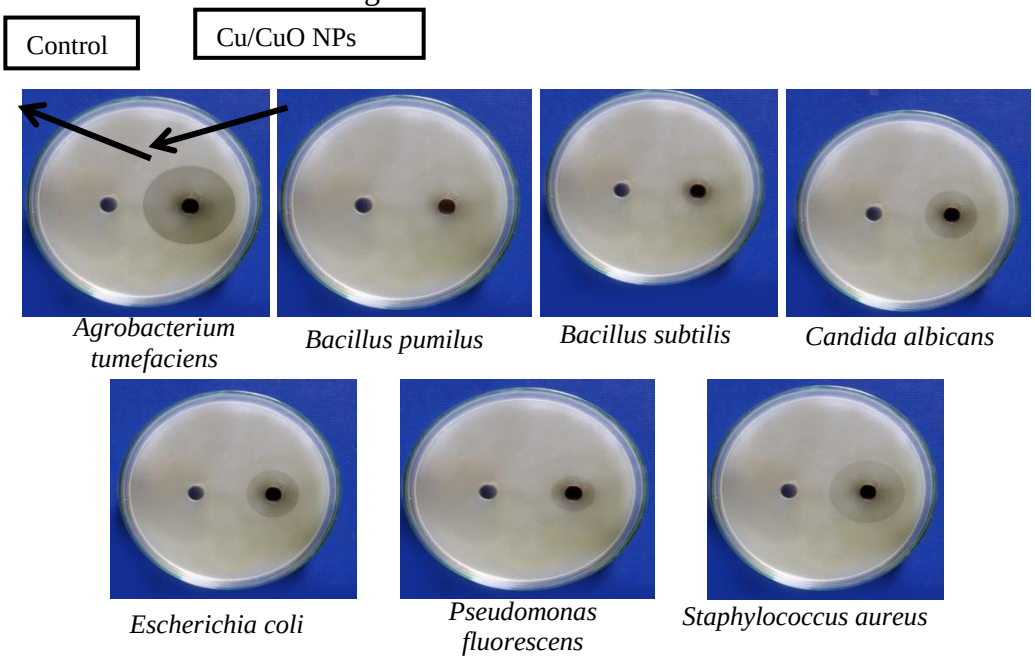

Figure 6. Antimicrobial Activities of Synthesized Copper Oxide Nanoparticles using Water Rhizome Extract

Table 3. Results of Antimicrobial Activities of Synthesized Copper Oxide Nanoparticles using Water Rhizome Extract

Sample	Solvents	Inhibition zone (mm)						
		I	II	III	IV	V	VI	VII
Shwe-pan	Distilled water	35 +++	17 ++	16 ++	31 +++	24 +++	22 +++	26 +++

Ager well - 8 mm
8 mm - 12 mm (+) - low activity
13 mm - 17 mm (++) - medium activity
18 mm above (+++) - high activity

Organisms
I. *Agrobacterium tumefaciens*
II. *Bacillus pumilus*
III. *Bacillus subtilis*
IV. *Candida albicans*
V. *Escherichia coli*
VI. *Pseudomonas fluorescens*
VII. *Staphylococcus aureus*

According to the results of antimicrobial activities of synthesized copper oxide nanoparticles using water extract of Shwe-pan rhizome showed the high activity on *Agrobacterium tumefaciens*, *Candida albicans*, *Escherichia coli*, *Pseudomonas fluorescences* and *Staphylococcus aureus* and medium activity on *Bacillus pumilus* and *Bacillus subtilis.*

Conclusion

The result of synthesis and characterization of copper oxide nanoparticles were gained from rhizome extract. The detection of phytochemical compounds presented in rhizome was carried out by phytochemical tests. According to phytochemical tests, the rhizome of Shwe-pan

consists of polyphenol, terpene, flavonoid, steroid, tannin, saponin, lipophilic, phenolic, glycoside, reducing sugar and carbohydrate but these rhizomes do not contain alkaloid. According to EDXRF spectroscopy, the higher amount of potassium and magnesium were found in rhizome of Shwe-pan. Copper oxide nanoparticles were synthesized by using copper (II) sulphate solution and aqueous rhizome extract of *Hedychium flavescens* Carey ex Roscoe as reducing agent. The yield percent of synthesized copper oxide nanoparticles from the water extract of Shwe-pan rhizome by using 70mM, 50mM and 30mM was found to be 10.3%,8.8% and 8.0 %. According to XRD the average crystallite size of copper oxide nanoparticles from the water extract of Shwe-pan was found to be range between 18.26 and 46.46 nm respectively. The resulting nanoparticles were characterization by FT-IR and the characteristic absorption peak of copper oxide was observed at 487.45-497.45 cm^{-1}. According to the results of antimicrobial activities of synthesized copper oxide nanoparticles using water extract of Shwe-pan rhizome showed the high activity on *Agrobacterium tumefaciens*, *Candida albicans*, *Escherichia coli*, *Pseudomonas fluorescences* and *Staphylococcus aureus* and medium activity on *Bacillus pumilus* and *Bacillus subtilis*. The synthesis of copper oxide nanoparticles using the plant extracts is an affordable method and it reduces the environmental pollution and has effective antibacterial activity.

Acknowledgements

We would like to express our deepest gratitude to Dr Thein Win, Rector, Dr Mi Mi Gyi, Dr Myint Zu Min, and Dr Kathi Tin, pro-rectors, University of Mandalay for their interest and encouragement on our research paper. We also wish to express our thanks to Dr Yi Yi Myint, Professor and Head, Department of Chemistry, University of Mandalay for her kind help and invaluable guidance for this research .

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