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Reduction of Copper (II) To Copper and Copper (I) Oxide Nanoparticles for Antimicrobial Application Using Calamansi (*Citrus Microcarpa)* Peel Extract

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ABSTRACT: Calamansi (Citrus microcarpa) is a common citrus fruit in the Philippines that is widely used for food and medicine industries, but its peels are considered as wastes. This study aims to reduce copper (II) ions to copper and copper (I) oxide nanoparticles using calamansi peel extract (CPE). Copper sulfate solution containing 1000 ppm copper was mixed with calamansi peel extract at varying CPE to copper solution volume ratio (1:4, 1:2, 3:4). The reduction process involved adjusting the initial pH to 8 and having constant agitation rate of 185 rpm for 4 hours at varying temperatures (40ºC, 60 ºC, 80 ºC). The amount of copper reduced was calculated using the initial and final copper concentration in the solution. The produced solids were subjected to X-Ray Diffraction (XRD) and Scanning Electron Microscope with Energy Dispersive X-Ray Spectroscopy (SEM-EDX) to determine the composition and morphology of the solids. It was determined that % Cu reduction increases with temperature and volume ratio. The highest copper reduction of 67% is attained at 80ºC and 3:4 volume ratio. Solids produced were determined to be Cu and Cu_2O and observed to be spherical with diameter of 42 – 180 nm indicating that the particles are Cu and Cu_2O nanoparticles. The Cu/Cu2O nanoparticles exhibited a zone of inhibition of 11 mm against E.coli. Based on the results, calamansi peel extract can be an eco-friendly and cost-effective alternative in the reduction of copper (II) ions to antimicrobial Cu/Cu_2O nanoparticles.

KEYWORDS: Calamansi, Copper reduction, Antimicrobial, Nanoparticle

I. INTRODUCTION

Copper is known for its thermal conductivity, catalytic activity, and antimicrobial property. Because of its lower cost of production, copper is used as a substitute for very expensive precious metals (i.e. gold, silver, and platinum) that also exhibit some properties of copper [1]. Copper has been explored to be used in nanoscale because it has a higher surface area to volume ratio in the form of copper and copper oxide nanoparticles (CuNPs) which increases and improves its properties. CuNPs are synthesized in a wide variety of methods such as radiation, thermal reduction, electrolysis, vacuum vapor deposition, microemulsion, and aqueous solution reduction. However, these methods of preparing CuNPs require expensive materials and equipment and even use toxic and harmful chemicals [2]. Because of this, green synthesis of CuNPs have been studied using plant extracts such as aloe vera, eucalyptus, and pomegranate. This is because plant extracts contain different kinds of acids (i.e. ascorbic acid and gallic acid) and organic matter (i.e. flavonoids, alkaloids, terpenoids, and polyphenols) which reduce copper and serve as capping agent to prevent growth and agglomeration of the particles [3,4]. The most probable mechanism of the synthesis of nanoparticles in general is discussed by Mittal et al (Fig 1). Metal ion is reduced to its solid form. During reduction, metallic particles start to agglomerate and grow. However, the organic matter present in the plant extracts coat the agglomerated particles hindering its further growth, consequently, forming nanoparticles [4].

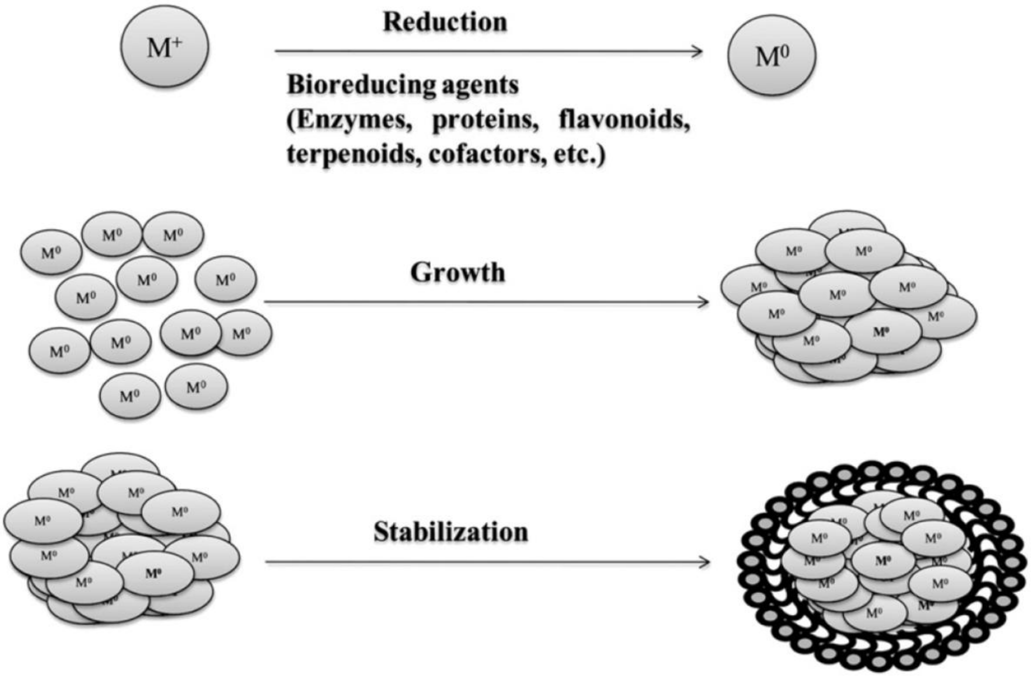

Figure 1. Mechanisms of nanoparticle synthesis [4]

The problem with the recent studied plant extracts is that these plants are still expensive in the Philippines and not common in the country. Also, application of these plants in nanotechnology competes with the food and medicine industries which are also fields where these plants are currently used.

Calamansi (*Citrus microcarpa*) is a citrus fruit common and abundant in the Philippines that is widely used for the food and medicine industries, but the peels are discarded and considered as wastes [5,6]. In 2023, calamansi production reached around 102,220 metric tons in the Philippines. Considering that calamansi consists of 43% peel, this results to around 44,000 metric tons of peel production in the Philippines [6]. Since the peels are rich in phenolic acids that act as antioxidant, these can be used to reduce copper ions from aqueous solutions and synthesize copper nanoparticles (CuNPs) [7]. Furthermore, synthesis of CuNPs reduces copper ions in aqueous solution, such as wastewater. The copper ions are removed as CuNPs which is very important since excessive consumption of copper ions by mammals causes headache, abdominal pain, respiratory problems, vomiting, liver and kidney failure, nausea, and gastrointestinal bleeding [8].

This study aims to reduce copper (II) ions to copper and copper (I) oxide nanoparticles using calamansi peel extract. Using calamansi peel extract will provide a cheaper and simpler removal of copper (II) ions from an aqueous solution that would be beneficial to industries that discharge wastewater containing high amounts of copper. It also has a potential to benefit calamansi farmers and users of the calamansi fruit. Calamansi peels that are considered as wastes can be recycled, reused, and turned into a profit.

II. MATERIALS AND METHODS

A. General Experimental Procedure and Solution Preparation

Figure 2 shows the schematic diagram for the methodology of this study. Calamansi peel extract was prepared by mixing 1:2 sliced calamansi peel to deionized water volume ratio and heated at 80°C for 15 minutes as shown in Figure 3. The solution was filtered then centrifuged to obtain the extract. Copper solution containing 1000 ppm Cu was prepared from copper sulfate pentahydrate ($CuSO_4 \bullet 5H_2O$). This concentration was used to simulate the copper concentration of electroplating wastewater [9].

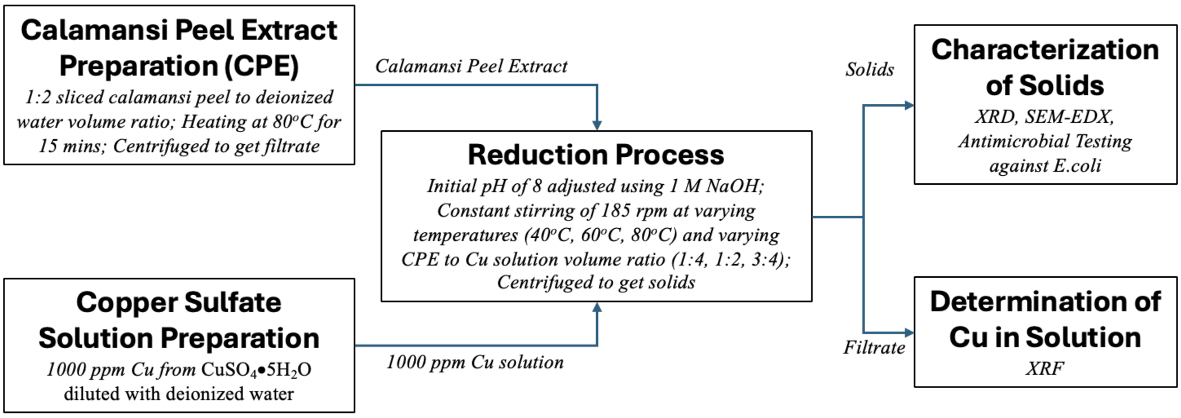

Figure 2. Schematic Diagram of the Experimental Procedure

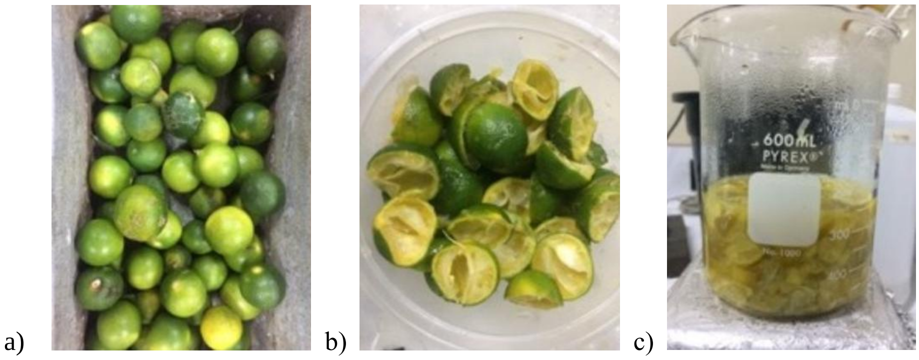

Figure 3. Calamansi Peel Extract Preparation a) Calamansi fruit; b) Sliced calamansi peels;

c) Calamansi peel extraction

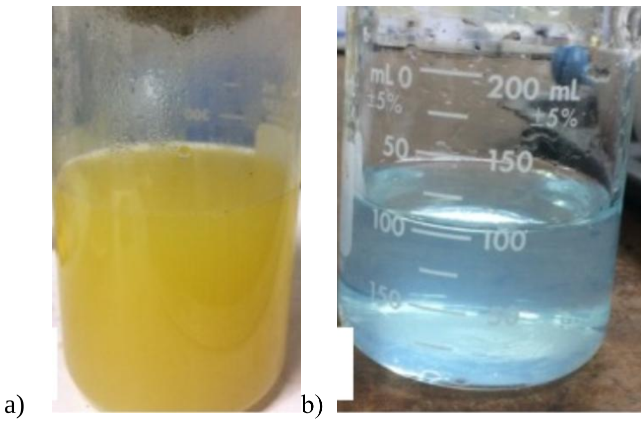

Figure 4. a) Calamansi Peel Extract; and b) Copper Sulfate Solution

B. Reduction Process

The calamansi peel extract and copper solution was mixed at varying volume ratio to form a 100-mL solution with initial pH of 8 and heated at varying temperatures for 4 hours with constant agitation rate of 185 rpm. The design of experiment is shown in Table 1 and done with 3 replicates.

Table 1. Design of Experiment for the Reduction Process

Run	Temperature, °C	CPE:Cu Solution Volume Ratio
1	40	1:4
2	40	1:2
3	40	3:4
4	60	1:4
5	60	1:2
6	60	3:4
7	80	1:4
8	80	1:2
9	80	3:4

An initial pH of 8 was set in the mixed solution using 1 M NaOH since this was determined to be the optimum pH that yields small copper nanoparticles based on the study conducted by Liu and Caroling [2,10,11]. The solution was filtered after the reduction process. Copper concentration of each liquid solution before and after reduction was determined using X-Ray Fluorescence (XRF). The data resulting from XRF were enough to immediately determine the percent change in copper in solution that can be related to copper reduction, thus the use of measurement with higher accuracy was not necessary. The equipment used provided an approximate detection limit for copper of 1 ppm (parts per million). The % reduction was calculated using Equation 1.

$$\%\ Cu\ reduction = \frac{C_i V_i - C_f V_f}{C_i V_i} \text{ x } 100 \quad (1)$$

where C_i = initial copper concentration of solution

V_i = initial solution volume

C_f = copper concentration of solution after reduction

V_f = final solution volume

C. Characterization of Reduced Solids

The solids were characterized using X-ray Diffraction (XRD) to determine the compounds present. Scanning Electron Microscope with Energy Dispersive X-Ray Spectroscopy (SEM – EDX) was also used to determine the size, shape, and composition of the synthesized particles.

Approximately 100μg/L copper nanoparticle suspension was submitted to the Natural Sciences Research Institute, UP Diliman to determine the antimicrobial property of the solid against *E.coli*. A sample of the calamansi peel extract and a sample of deionized water was also submitted to the institute for the same analysis to compare the results.

III. RESULTS AND DISCUSSION

A. Visual Observations

The reduction of copper using calamansi peel extract involved multiple observable color changes. As shown in Figure 5a, mixing the calamansi peel extract and copper solution produced a green solution with a pH of around 3.2. When the pH of the solution was increased to 8 by the addition of 1M NaOH, the color of the solution changed from light green to dark green. After 4 hours of heating the solution, the color changed to reddish brown with solids formed that changed to reddish brown upon heating.

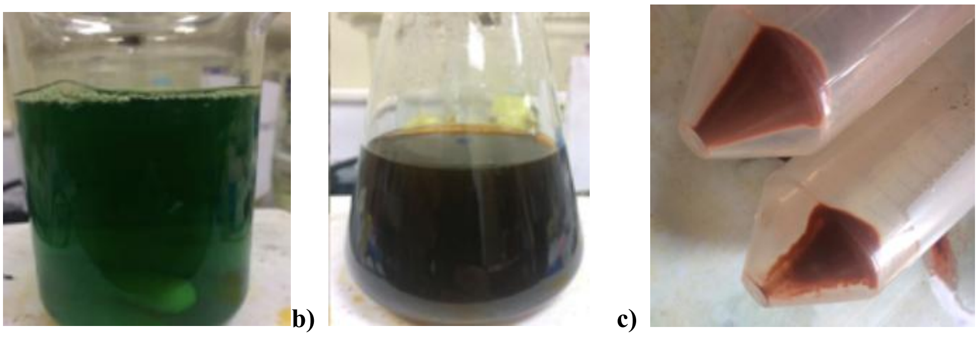

Figure 5. Visual Observations during Reduction a) mixed $CuSO_4$ and Calamansi Peel Extract b) heated solution c) collected solids

Reddish brown solids were observed to form after the process. The appearance of a reddish brown solution indicated that copper ions in $CuSO_4$ solution were possibly reduced, and copper solids were formed. Furthermore, the pH after synthesis decreased to around 7.4 which indicated that acids in the calamansi peel extract such as ascorbic acid has participated in the reduction of copper ions which releases H^+ ions represented in Equations 2, 3, 4, 5 [11].

$$Cu^{2+} + C_6H_8O_6 \rightarrow Cu + C_6H_6O_6 + 2H^+ \quad (2)$$

$$Cu^{2+} + 2OH^- \rightarrow Cu(OH)2 \quad (3)$$

$$Cu(OH)_2 + C_6H_8O_6 \rightarrow Cu + C_6H_6O_6 + 2H_2O \quad (4)$$

$$2Cu(OH)_2 + C_6H_8O_6 \rightarrow Cu_2O + C_6H_6O_6 + 3H_2O \quad (5)$$

B. Effect of Temperature and CPE to Copper Solution Volume Ratio on Copper Reduction

Using two-way ANOVA at 95% confidence level, it was determined that temperature (p-value = 2.45E-09) and CPE to copper solution by volume ratio (p-value = 5.6E-05) have significant effect on the percent reduction of copper. Figure 6 shows that the % copper reduction increases with increasing temperature and CPE to copper solution by volume ratio. The amount of copper reduced increases with temperature because increasing the temperature increases the kinetics of the reaction. However, an almost equal % reduction was observed for reduction at 40ºC and 60ºC. According to the studies of M.Biçer et.al. [11] and S.Jain et.al. [12], copper nanoparticles are not formed below 60ºC because the growth temperature of Cu is higher than that of Cu_2O which is 60ºC. Moreover, increasing the CPE to copper solution by volume ratio increases the amount of citric acid that act as reducing agent [10]. Thus, increasing the amount reducing agents would promote the formation of copper solid.

a)

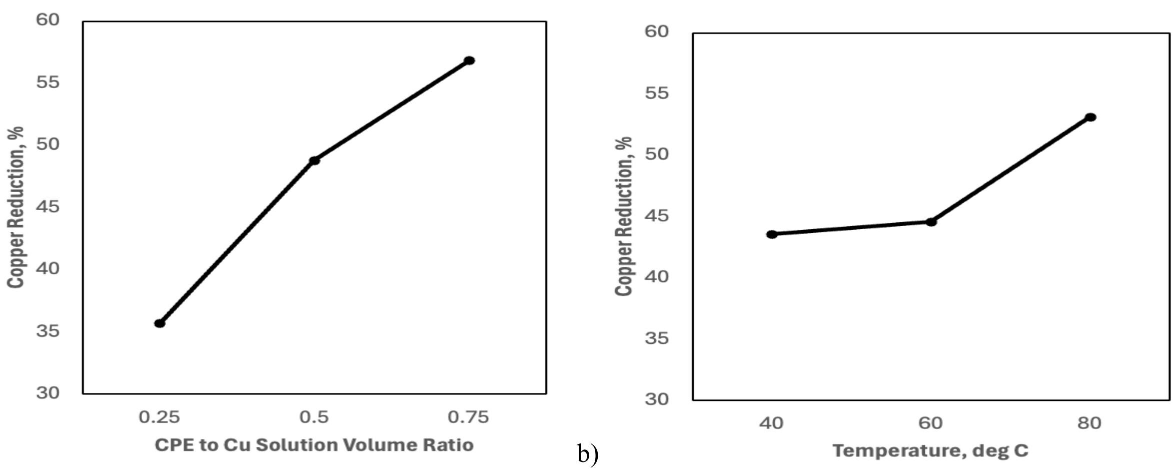

Figure 6. Main Effects to Copper Reduction, % of a) Calamansi Peel Extract (CPE) to Copper solution volume ratio and

b) Temperature, degrees Celsius

As shown in Figure 7, the highest percent copper reduction was around 67% which was at 80^{o}C at 3:4 ratio. It can be observed that at 80^{o}C, there was a significant increase in the reduction at 3:4 CPE to Cu solution by volume ratio. However, at 40 and 60 degrees Celsius, percent reduction started to plateau at 1:2 ratio. This shows that reduction of copper is more favorable at 80^{o}C.

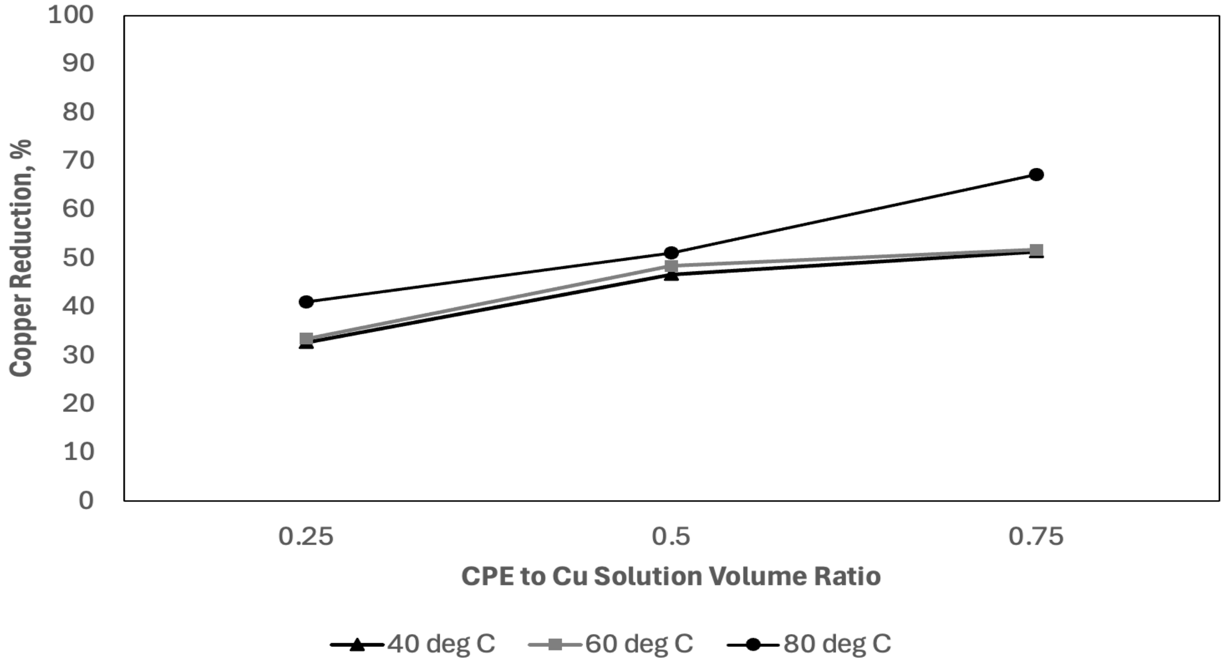

Figure 7. Copper Reduction, %, at varying temperature and volume ratio

C. Characterization of Reduced Solids

The XRD pattern of the solids shown in Figure 8 indicate diffraction peaks for copper (Cu) and copper (I) oxide (Cu_2O) indicating that the solids formed during the reduction process are copper and copper (I) oxide solids. Scanning electron microscopy (SEM) micrographs in Figure 9 show that high-intensity parts, indicating metallic copper, are surrounded with nanorods of lesser intensity which are indicative of intermetallic Cu_2O. The nanorods were determined to have diameters ranging from 260-400 nm and heights with range of 550-4000 nm. Furthermore, Figure 9b shows that the metallic copper nanoparticles are agglomerated. The copper nanoparticles are spherical having diameters ranging from 42-180 nm, and 92.51% of the particles fall under the range of 1-100 which is the diameter range for a particle to be considered as nanoparticle.

The EDX analysis, shown in Figure 9c, further confirmed that the nanoparticle is composed mainly of copper and copper oxide. Results showed that the atomic weight percentage of copper is around 35.2%. Moreover, EDX confirmed the presence of oxygen with an atomic concentration of 46.8%. This coincided with the results from the XRD where metallic copper and copper (I) oxide were detected.

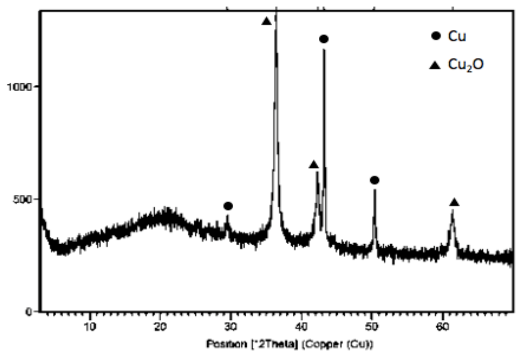

Figure 8. XRD pattern of solids from reduction

a) b) c)

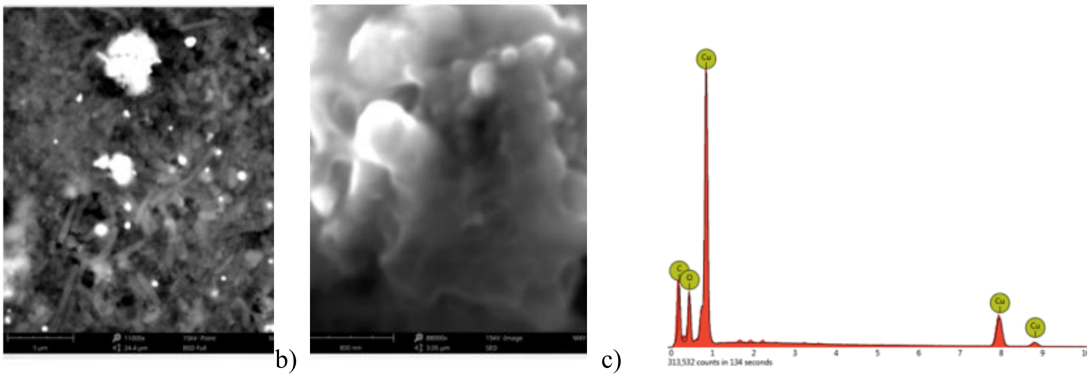

Figure 9. SEM Micrograph of Synthesized Copper Nanoparticles a) Cu nanoparticles and Cu2O nanorods at 11,000X b) agglomerated spherical Cu nanoparticles at 88,000X c) EDX Spectra of Copper Nanoparticles

The antimicrobial activity of the solids produced was determined on *E.coli* which is gram negative. Results illustrated in Table 2 showed that the copper nanoparticle suspension had a zone of inhibition of 11 mm, while calamansi peel extract had none. This shows that the copper nanoparticle has an antimicrobial activity against *E. coli*. The calamansi peel extract did not display any zone of inhibition.

Table 2. Antimicrobial Analysis

Sample	Zone of Inhibition, mm
Calamansi Peel Extract	0
Copper Nanoparticles	11

IV. CONCLUSIONS

This study investigated the reduction of copper (II) to copper and copper (I) oxide nanoparticles for antimicrobial application using calamansi (*Citrus microcarpa*) peel extract. Calamansi peel extract reduces copper ions in an aqueous solution. The percent reduction of copper increases with increasing temperature and CPE to copper solution by volume ratio. At 40ºC, % reduction already plateaus above 1:2 ratio which is also the same trend observed at 60ºC. At 80ºC, percent copper reduction significantly increases up to 67% reduction at 3:4 ratio. Solids produced were determined to be Cu and Cu_2O that are spherical with diameter of 42 – 180 nm. Moreover, the copper/copper (I) oxide nanoparticles are effective against *E.coli*. Based on the results, calamansi peel extract can be an eco-friendly and cost-effective alternative in the reduction of copper (II) ions to Cu/Cu2O nanoparticles for antimicrobial applications. Calamansi peels that are considered as wastes can be recycled, reused, and turned into a profit.

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