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# Comparison Between Green And Chemical Synthesis Of Copper Nanoparticles: Characterization And Antibacterial Activity

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## *ABSTRACT*

Nanoparticles are often associated with their small size and numerous applications. The synthesis process is equally important since it determines the size and properties of the nanoparticles. Additionally, green synthesis provides an economical, ecologically beneficial, and sustainable substitute. Researchers have focused on copper nanoparticles (NPs) due to their unique characteristics, which include optical, antibacterial, and electrical capabilities that depend on size and form. Two techniques are becoming more well-known in this field for producing silver nanoparticles. The green method and chemical reduction were used to create copper nanoparticles, and the two samples' optical, antimicrobial, and structural properties were examined. An extract derived from lemon fruit was incorporated into the environmentally friendly procedure. Furthermore, trisodium citrate was used as a reducing agent in the chemical procedure, and several techniques were used to characterize the nanoparticles. These techniques include X-ray diffraction (XRD), ultraviolet-visible spectroscopy (UV-Vis), scanning electron microscopy (SEM), and antibacterial activity. The XRD analysis revealed that the particles were crystalline in form and that the green-produce variation's crystal sizes (4.6 nm) were greater than those of the chemical reduction variant (2 nm). The shape of the chemically produced nanoparticles is unclear, whereas the green approach coats the generated nanoparticles with the biological materials in lemon extract. The antibacterial evaluation was completed using the agar well diffusion method. The particles' SEM images showed that they were aggregating into polyhedral and leaf-shaped particles. In the aqueous solution containing copper nanoparticles, the chemically generated absorption peak was located at 300 nm in the UV-visible spectrum, whereas the green synthesized absorption peak was located at 260 nm. This included Escherichia coli O157:H7 and Staphylococcus aureus as the bacteria in the medium. The green had a larger zone of bacterial growth inhibition. synthesized variant in different concentrations of 20 mg, 40 mg, and 60 mg, which were 22 mm, 25 mm, and 28 mm in Escherichia coli and 14 mm, 24 mm, and 26 mm in Staphylococcus aureus, respectively. Conclusions: The improved antibacterial responses of the green-produced versions were caused by the accelerated rate at which the organic chemicals in the lemon fruit extract stabilized the nanoparticles.

***KEYWORDS:*** Nanoparticles, green synthesis, chemical synthesis, antibacterial

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**1.INTRODUCTION**

Nanoparticles are particles that are between one and one hundred manometers in size. For any object that is 'one billionth,' or $10^{-9}$, of another, the prefix 'nano' is used. Ordinary materials often exhibit unusual and distinctive characteristics when reduced to the nanoscale, such as electrical conductivity, chemical reactivity, super paramagnetic behavior, exceptional strength, and other characteristics that the same material lacks at the macro- or microscale. Technology is being developed to limit any dangers to environmental factors and human health related to manufacturing (1-4). The application of nanotechnology products, as well as promoting the substitution of new, environmentally friendly nano-products for current ones. This is known as "green nanotechnology (5,6).

The branch of science and engineering that focuses on materials is called nanotechnology, according to (7). According to (8), nanoscience is the study of phenomena at particle sizes between 1 and 100 nm, and nanomaterials are materials with ordered components at least one dimension smaller than 100 nm. Nanoparticle-derived nanomaterials: In the past decade, nanoparticles have emerged as a unique class of materials. Because of their superior conductivity, metal nanoparticles have garnered more scientific interest than metal oxide nanoparticles (9). Metal nanoparticles are discrete particles with sizes ranging from one to one hundred nm; they are not part of a chemical bond between metals. In contrast to their equivalent in bulk metal, metal nanoparticles (NPs) differ greatly from their counterparts in bulk metal in that. In addition to being large and having a higher surface-to-volume ratio, these objects also have lower energy state densities (10, 11). As a result, they exhibit more specificity and chemical activity than bulk metals, making them desirable for use as catalysts (12, 13).

The mechanical, optical, magnetic, electrical, and chemical characteristics of metal nanoparticles differ greatly from bulk metals because of their size effects, often referred to as quantum effects, and high surface-to-volume ratio (14, 15). Nanoparticles have drawn significant interest from researchers because of their special qualities and wide variety of uses.

The "bottom-up" and "top-down" approaches are the two methods used to create nanoparticles (16, 17). A top-down approach uses size reduction from a suitable starting material for nanoparticle manufacturing (18). Size reductions are achieved using a variety of physical and chemical techniques. Imperfections created in a top-down manner in the nanoparticles' surface structure pose a substantial constraint because the chemistry of the surface and additional physical characteristics of the nanoparticles largely rely on the surface structure (19, 20). Bottom-up synthesis combines smaller entities to produce nanoparticles (21,22).

As per (18), this technique entails the initial creation of smaller entities that are subsequently put together to create final particles with sizes in the nanoscale range. The bottom-up approach is implemented through the use of chemical and biological methods. Although biological processes need very little energy and are conducted in ecologically benign settings, the production of nanoparticles via chemical and physical methods requires a lot of energy, which is harmful to the environment, and involves dangerous substances (23-25).

Although the synthesis of metal nanoparticles by biological methods takes longer than chemical methods, this time has been decreased by employing the appropriate bacteria or organisms (26). Thus, the low cost, environmental friendliness, and one-step, large-scale process are the benefits of biological approaches over chemical and physical procedures. It is possible to produce nanoparticles without using dangerous chemicals, high pressures, temperatures, or energy (27, 28). It is well known that bacteria can produce nanoparticles through biological processes (29). The

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cost of controlling and growing microorganisms is higher than that of plant extracts, although using microbes to synthesize nanoparticles is easily scalable, environmentally benign, and appropriate for use in medical applications. Due to its low cost, plant-mediated synthesis of metal nanoparticles can be a valuable and economical alternative to large-scale manufacturing (30, 31). Therefore, this study aims to develop an environmentally friendly, cost-effective, and simple synthesis method for metal nanoparticles, also the application of synthesized nanoparticles as antibacterial effect on Gram-negative bacteria (Escherichia coli) and Gram-positive bacteria (Staphylococcus aureus).

## 2.EXPERIMENTAL

### 2.1.Green Method

To create the lemon fruit extract, the lemon fruit was cut and transferred to a 250-mL beaker with 200 mL of deionized water. The mixture was heated at 80 °C for 10 minutes and subsequently filtered through 125-mm filter paper. Copper sulphate pentahydrate ($Cu_2SO_4$) is used to prepare green Nanoparticles of copper for the synthesis of nanoscale nanoparticles of copper from lemon fruit extract; $H_2O$ was utilized as a precursor. Eight grams of copper sulphate pentahydrate were added to 200 milliliters of lemon fruit extract, and this mixture was stirred magnetically for two hours, forming a dark green paste. The mixture was then calcined to produce a black powder of copper nanoparticles.

### 2.2.Chemical Method

Initially, a 100 mL aqueous solution of copper sulphate pentahydrate ($Cu_2SO_4.5H_2O$) at 0.01M was prepared. Next, 100 mL of tri-sodium citrate ($Na_3C_6H_5O_7.2H_2O$) at 0.1M was incorporated into the solution above and was stirred at 80°C for an hour. Green powder formed and was dried for half an hour at 200°C. Lastly, 100 mL of potassium hydroxide (KOH) 1M was progressively added to the mixture until the pH reached 12. After that, it was stirred for 30 minutes.

### 2.3.Antimicrobial Action:

Cultures of bacteria: The nutrients agar medium plate cultures were employed to cultivate the bacteria. 250 milliliters of sterile distilled water were used to dissolve 7 grams of powder to create the medium. Following a 15-minute autoclaving at 121 °C to sanitize the medium, the bacteria were grown and incubated for 24 hours at 37 °C. Antimicrobial test: Green synthesis and the antibacterial properties of chemical synthesis. By applying the agar disk diffusion technique, copper nanoparticles will be examined on two species of bacteria, Escherichia coli and Staphylococcus aureus, that were cultured for 72 hours in stable cultural media.

## 3.RESULTS AND DISCUSSION

### 3.1.Physical Properties

The physical properties of Green and Chemical Synthesized Copper nanoparticles are shown in Table 1

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**Table 1.** Physical properties of green and chemical copper nanoparticles

| Nanoparticle | Melting point | Solubility in water | Solubility in alcohol | Solubility in DMSO | Solubility in DMF | Color and texture |
|---|---|---|---|---|---|---|
| Green Cu nanoparticles | Over 350°C | Slow solubility | Non soluble | Slow solubility heating | Slow solubility with heating | Black hard powder |
| Chemical Cu nanoparticles | Over 350°C | Soluble | Non soluble | Slow solubility with heating | Slow solubility with heating | Pale Green soft powder |

### 3.2.Ultra Violet-Visible Spectroscopy

Each sample was diluted with 0.005 mg in 5 dimethyl form amides, sonicated for 20 minutes, and then scanned in the 200–400 nm wavelength range using a BMG LABTECH spectrophotometer (Ortenberg, Germany).

As seen in Figure 1 below, the absorption band in the UV-VIS spectroscopy of Chemical Copper nanoparticles and Green Copper nanoparticles was located at 300 nm, with absorption values of 1.5324 and 1.651, respectively. Copper nanoparticles formed in accordance with studies that suggested that copper nanoparticles created between 200 and 350nm, as figure 1 shows the absorption band at 300nm verified the formation of copper nanoparticles; The absorbance intensity dropped as the wavelength grew, suggesting that formation did not take place at a very long wavelength.

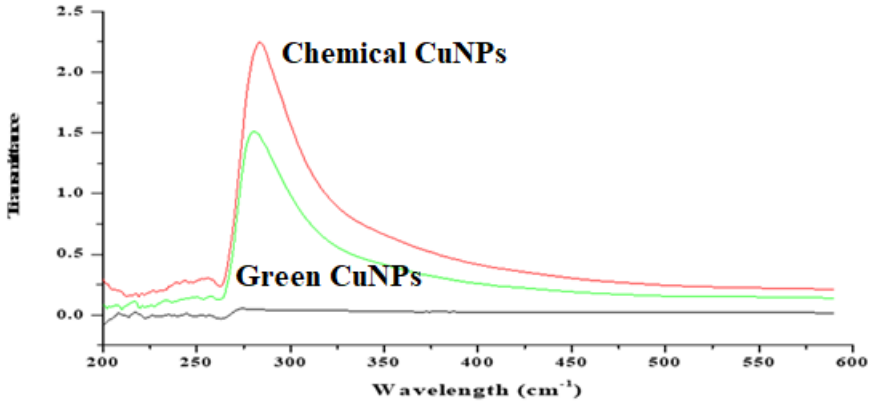



**Figure1.** UV-VIS spectrum analysis

### 3.3.Scanning Electron Microscopy

SEM was used to investigate the shape of artificially created copper nanoparticles. The SEM images of chemically synthesized copper nanoparticles and green synthesized copper nanoparticles are shown in Figure 2. The experimental results showed that the SEM images of the chemically synthesized nanoparticles showed aggregation of the particles with polyhedral and leaf-shaped particles. In contrast, the green method produced nanoparticles whose shape is obscured in SEM images because they are coated in biological molecules in the lemon fruit extract. However, it is evident from the images that green nanoparticles are smaller than chemically produced ones, which indicates a large ratio of surface area to volume. These results are consistent.

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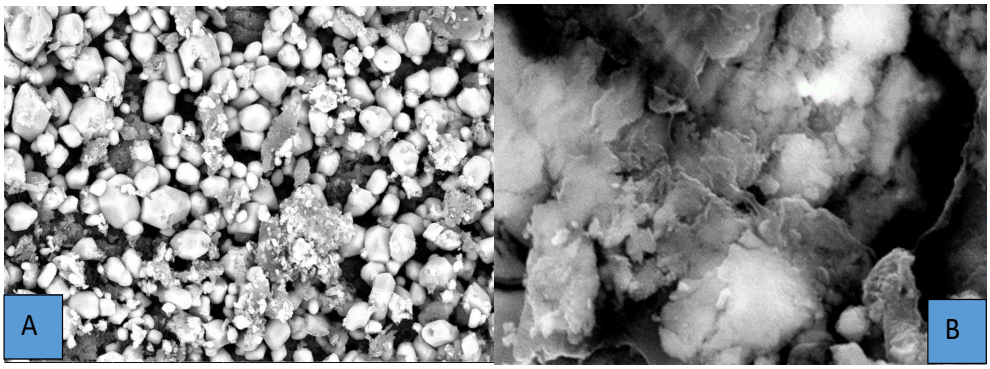



**Figure 2.** SEM Images of (A) chemical Copper nanoparticles and(B) Green Copper nanoparticles

**3.4.X-Ray Diffraction Analysis**

The copper nanoparticles' X-ray diffraction patterns produced by lemon fruit extract are shown in Figures 3 and 4. The copper nanoparticles synthesized in green exhibit a more acute diffraction peak value, indicating a higher percentage of crystalline phases. On the other hand, the size of the crystallite was established using the Debye Scherer equation:

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

Here, K is the dimensionless form factor (~0.94), μ is the radiation wave length, and θ is the Bragg angle. β is also the complete width at half maximum of the corresponding peak. D is primarily the ordered domains' primary size, which is regarded as being equivalent to the size of the particle (applicable for only particles less than 100 nm). From the values in the diffraction pattern of The Green copper nanoparticles, the particle size can be calculated as below.

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

$D=(0.94\times1.54)/(4.73 \cos 9 )$

$D=4.6$ nm

For less 2θ value, and for a higher one, it becomes as below.

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

$D=(0.94\times1.54)/(1.13 \cos 42.5)$

$D= 1.7$nm

So, from XRD, the particle size of the Green Copper Nanoparticle is from 1.7 to 4.6 nm, and from the X-ray diffraction pattern values of the chemical copper nanoparticles, the particle size of chemically synthesized copper nanoparticles is calculated as

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

$D=(0.94\times1.54)/(1.0 \cos 49)$

$D=0.3$nm

And for the less value of 2θ, the smaller particles the particle size:

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

$D=14.56/8.36$

$D=1.74 \sim 2$nm

From the results of XRD and by applying the Debye Scherer equation, the particle size of Green Copper nanoparticles is between 3nm-16nm, smaller than the chemically synthesized 0.3-2nm in size.

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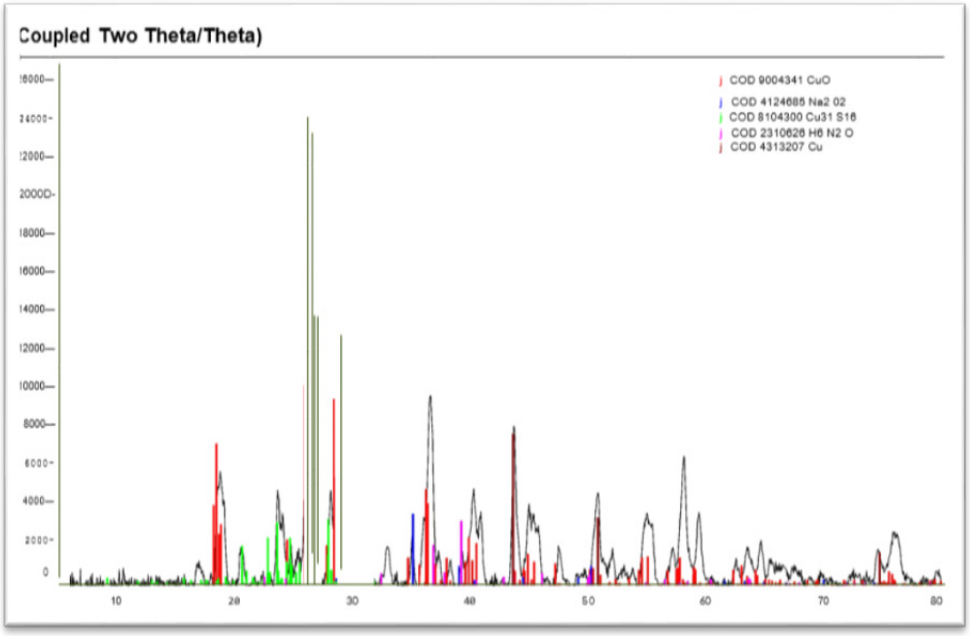



**Figure3.** XRD diffraction pattern of Green Synthesized Copper nanoparticles

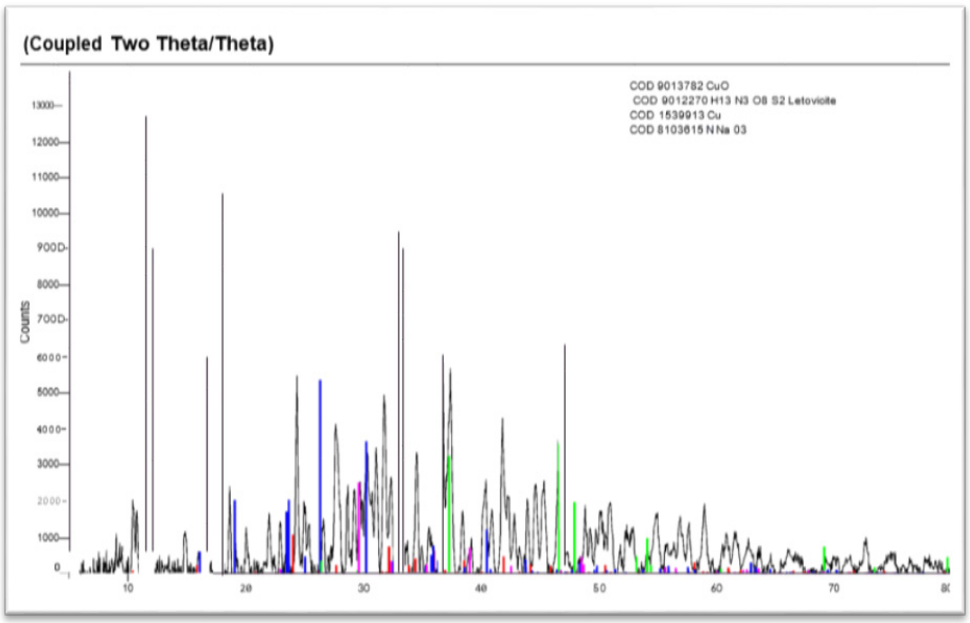



**Figure 4.** XRD diffraction pattern of chemically synthesized Copper nanoparticles

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**3.5.Antibacterial activity**

Two different species of bacteria were used to test the antibacterial activity. Gram-negative bacteria, Gram-positive Staphylococcus aureus, and Escherichia coli are all represented in the antibacterial action of chemical and green copper nanoparticles. Using the Agar diffusion method, the products were used in powder form at concentrations of 20, 40, and 60 mg. All the concentrations were applied to the surface of the culture plates, and the plates were then incubated upside down at 37°C for a whole day(35). After the inhibitory effects of the substances were identified, it was found that the zones of suppression of bacterial growth produced by green and chemical copper nanoparticles differed from one another. Nanoparticles' antibacterial efficacy is a size-dependent property that improves with a reduction in particle size; for E. coli (Gram-negative), green CuNPs produced inhibition zones of 22–28 mm, whereas chemically synthesized CuNPs produced 12–23 mm. A similar trend was observed for S. aureus (Gram-positive), with green CuNPs producing 14–26 mm inhibition zones compared to 10–28 mm for chemical CuNPs. The enhanced activity of green nanoparticles may be attributed to the presence of bioactive phytochemicals from the lemon extract, such as flavonoids, polyphenols, and citric acid, which can act synergistically with the copper ions to disrupt bacterial membranes more effectively (32,36) Such natural capping agents can improve nanoparticle stability, dispersion, and surface reactivity, all of which increase antibacterial efficiency (33,34). However, Green Copper nanoparticles increase the zone of inhibition more than Chemical Copper nanoparticles, and the measurements of the inhibition zone are tabulated in Tables 3 and 4. Green Copper nanoparticles exhibit more antibacterial potential than Chemical Copper nanoparticles because they have smaller particle sizes and higher surface areas. The tables (2–3) and figures (5–6) provided illustrations of the results.

**Table 2** Antibacterial measurements of green copper nanoparticles

| Sample | Bacteria | Weight of the sample (mg) | Bacteria type | Zone of inhibition D(mm) |
|---|---|---|---|---|
| Green Copper Nano-particles | Escherichia coli | 20 | Gram negative | 22 |
| | | 40 | | 25 |
| | | 60 | | 28 |
| | Staphylococcus aureus | 20 | Gram positive | 14 |
| | | 40 | | 24 |
| | | 60 | | 26 |

**Table 3** Antibacterial measurements of Chemical Copper Nanoparticles

| Sample | Bacteria | Weight of the sample (mg) | Bacteria type | Zone of inhibition D(mm) |
|---|---|---|---|---|
| Chemical Copper Nano-particles | Escherichia coli | 20 | Gram negative | 12 |
| | | 40 | | 15 |
| | | 60 | | 23 |
| | Staphylococcus aureus | 20 | Gram positive | 10 |
| | | 40 | | 20 |
| | | 60 | | 28 |

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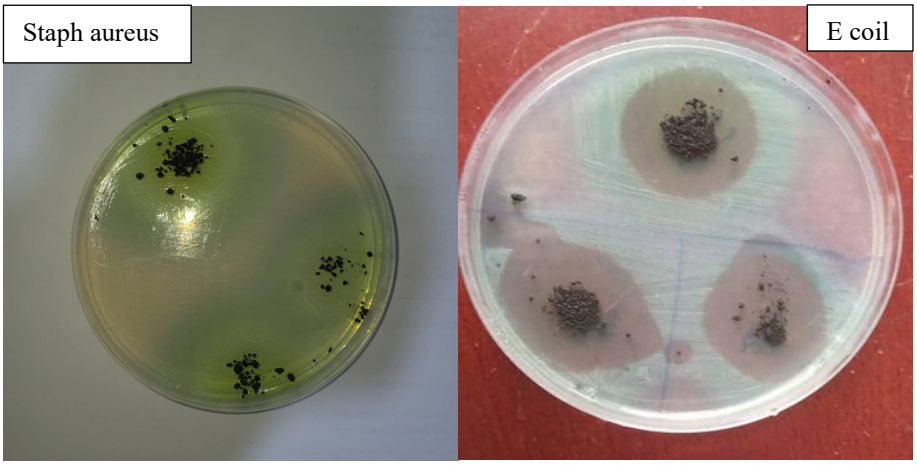



**Figure 5.** Antibacterial effect of green copper nanoparticles at three concentrations on E. coli and staph aureus.

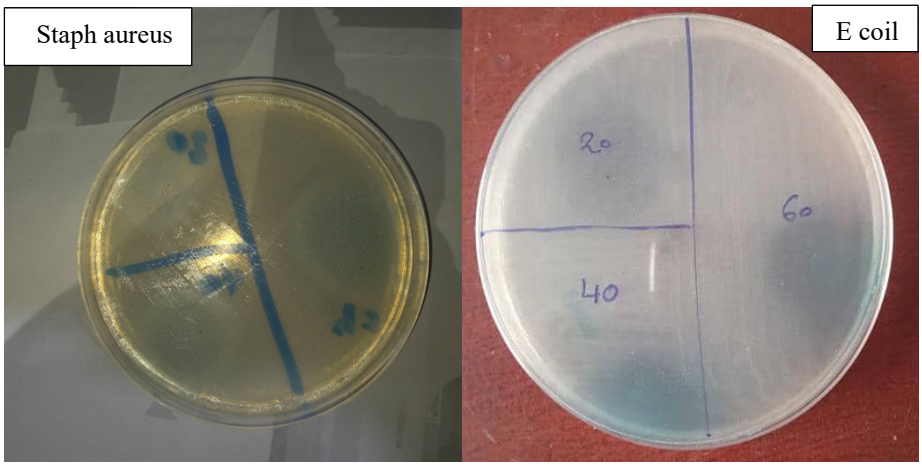



**Figure 6.** Antibacterial effect of chemical copper nanoparticles at three concentrations on E. coli and Staph aureus.

## 4.CONCLUSION

In summary, two different methods were employed to produce copper nanoparticles: the green method, which utilized the aqueous extract from lemon fruit, and the chemical reduction method, which used trisodium citrate. The aqueous extract from lemon fruit is free of the dangerous chemicals used in the chemical process. The phytochemicals in the lemon fruit extract, however, not only aid in lowering the particle sizes but also in adequately stabilizing them. Furthermore, the optical band gaps of the two processes were about the same, and the green approach's average copper nanoparticle size was greater than the chemical method's (2 nm). Additionally, using the green technique increased the lemon fruit extract antimicrobial effectiveness. Consequently, a comparison of the features of the two variations leads to the conclusion that green synthesis is more effective than chemical reduction in producing copper nanoparticles.

## 5.ACKNOWLEDGMENT

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