International Journal of Scientific Research in Science and Technology
Print ISSN: 2395-6011 | Online ISSN: 2395-602X (www.ijsrst.com)
doi : https://doi.org/10.32628/IJSRST52310271

Effect of Various Concentrated Copper and Plant Extract Concentrations on the Antibacterial Activity

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ARTICLEINFO

Article History:

Accepted: 01 April 2023
Published: 12 April 2023

Publication Issue
Volume 10, Issue 2
March-April-2023

Page Number
543-551

ABSTRACT

Copper nanoparticles (Cu NPs) were created using two methods, one with reducing and stabilizing agents and the other with green tea extract. The formation of Cu NPs has been described by spectra of UV-Vis absorption, which showed the surface plasmon resonance (SPR) at 620 and 630 nanometers, respectively. The position and shape of the surface plasmon resonance and plasmon absorption bands strongly depend on the reaction conditions. The crystalline morphology and size of the nanoparticles were determined by XRD, FESEM, and TEM studies. In both ways, the average particle size of Cu nanoparticles was found to be in the range of 28 nm and 142 nm for the chemical and green methods, respectively. The effectiveness of the materials prepared by both methods was high, and the antibacterial activity of the products prepared by both ways was against E. coli and S. aureus pathogens represented by inhibition zones ranging from 14–20 mm, 30–38 mm, and 11–18 mm, 20–24 mm, respectively.

Keywords : copper nanoparticles, green method, plant extract, reduction agents, inhibition zone.

I. INTRODUCTION

Specific characteristics of nanoparticles in many fields include Because of their unique mechanical, magnetic, electric, and thermal properties, as well as their catalytic properties and their broad range of uses in areas such as agriculture, industry, the environment, and medicine; copper nanoparticles stand out among other types of nanoparticles. Moreover, applications for copper nanoparticles include catalysis, sensing, dye degradation, and fungicidal and nematocidal [1.2] activities. In nanotechnology, synthesis methodologies are considered to be of paramount relevance. In this regard, three basic approaches are used to create Cu NPs: physical, biological, and chemical. The availability of three crucial components—a precursor to providing copper ions, a reducing agent to give the electrons required to reduce the copper ions into copper atoms, and copper—is the foundation of the fundamental principle underlying the synthesis of metallic nanoparticles in general and copper nanoparticles in particular, and a surfactant to control the formation of copper nanoparticles with a specific size under the proper pH and temperature conditions. The method is chemical when a chemical component acts as the reducing agent or an electron source. [3, 4], whereas When an organism or a physical source, such as an electric current, is the source of the electrons, the procedure is physical and biological, respectively. Cu has a significant tendency to oxidize, making its nanoparticle synthesis difficult. The oxide phases are more thermodynamically stable, although they are highly air-sensitive. Applications for Cu nanoparticles may be constrained by their rapid oxidation. [5]. Chemical reduction is often preferred among these techniques since it is simple; by maximizing the experimental parameters, including the percentage of the reducing agent with the precursor salt and the molar ratio of the stabilizer with the precursor salt, it may be possible to anticipate enhanced size and size dispersion control at low cost. The reduction of metal salts in various solvents with reducing agents is a common component of chemical reduction techniques. [6]. Ascorbic acid was used to decrease Cu ions to produce metallic copper nanoparticles; it functioned as an agent for reduction. Lowering chemicals such as cetyltrimethylammonium bromide (CTAB) [7]. As a result, the reaction has a low driving force, and it is difficult to apply the physical, thermal, and chemical reduction techniques for nanoparticle production to be widespread. These techniques are complicated, expensive, and potentially harmful. [8]. Plant extract-based green synthesis nanoparticles provide an alternate strategy for solving these issues. It was an easy, sustainable, and affordable green synthesis process. The plant extract was highly affordable and resistant to adverse environmental conditions. [9] Redox processes involving the reduction of metal ions to generate nanoparticles are facilitated by plant extracts. When metal ions are converted into stable nanoparticles while being reduced to a small size, metabolites such as sucrose, terpenoids, polyphenols, alkaloids, phenolic acids, and proteins play crucial roles. [10] In terms of culture and maintenance times, environmentally friendly green NP synthesis using plants outperformed microbe-mediated synthesis [11]. In this environmentally friendly process, as a powerful natural reducing agent and Cu NP stabilizer, the plant extract serves its purpose. The phytoconstituents flavonoids, phenolic acids, tannins, saponins, glycosides, alkaloids, and polysaccharides transform copper metal ions into copper nanoparticles (Cu NPs). The two primary benefits of this method are that (1) the extract solution and the metal salt precursor aqueous solution may be mixed easily, and (2) the produced Cu NPs are less poisonous and biocompatible; they exhibit strong antibacterial action against harmful microorganisms. The optical, catalytic, mechanical, and electrical characteristics of the CuNPs have garnered a great deal of interest. [12]. Cu NP synthesis via chemical reduction yields good results, but the process can become toxic in some cases due to the use of expensive, harmful, and protective agents. We employed ascorbic acid in our chemical reduction method to prevent toxicity and create Cu NP in a green environment. The technique is affordable, safe, and environmentally benign because ascorbic acid serves as both a reducing and a protective agent. In the current study, Cu NPs were made using both chemical and environmentally friendly approaches [13, 14]. synthesizing them using tea extract, characterizing them with UV-vis, FTIR, XRD, FESEM, and TEM methods, and testing their antibacterial efficacy against two pathogenic microorganisms, E. coli and Staphylococcus aureus.

II. METHODS AND MATERIAL

Copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$, 98%) Sigma-Aldrich, **ascorbic** acid $C_6H_8O_6$ (0.2 M) >99%, cetyltrimethylammonium bromide (C T A B 98%), and sodium hydroxide NaOH (25% China) were also employed to raise the pH and quicken the water's reduction process. were bought at Sigma Aldrich. Deionized water was used in all studies.

Equations used to analyze the X-ray results [1]
The d-spacing (the distance between atoms in the **[2]** lattice plane) was calculated using the Bragg law, represented by equation no.1, based on the results of the XRD analysis, and the average crystallite size was determined using the Debye-Scherrer equation. denoted in equation no.2

$2d \sin\theta = n \lambda$ ……. (1)

$$D = \frac{K\lambda}{FWHM \cos\theta} \quad ……. (2)$$

where n is an integer (n = 1), λ= 0.15418 nanometers for Cu Ka, k is an integer (k = 0.15418 nm for CuKa), and d is the interplanar distance between atoms, , FWHM is the full width at half maximum, θ is the diffraction angle, and D is the mean crystallite size. The lattice constant a was used to determine the cubic crystals by equ.3. [15]

$$a = d\sqrt{h^2 + k^2 + l^2} \quad …. (3)$$

For cubic crystals, Miller's index (h k l) represents cartesian coordinates.

Preparation of the extract

A dry green tea plant was taken, weighing approximately 25 grams, after washing, drying, grinding, and placing it in a baker containing 150 ml of deionized water and heating it to a temperature of 60–70 degrees for 10 minutes, after which the extract was cooled and filtered several times until all impurities and sediments were removed. It remains a homogeneous golden-yellow color and clear solution, as shown in Figure.1 A: It was kept in a cool place until it was used in the experiment.

Copper nanoparticle synthesis

The initial step in producing copper nanoparticles is to create a blue solution by dissolving copper sulfate pentahydrate salt, $CuSO_4.5H_2O$ (0.72 g) (3 mM), in deionized water. CTab, 0.04 g (0.14 mM), was added to the copper solution along with 10 ml of vigorously stirred CTab over 30 minutes. The ascorbic acid was then added to the aqueous solution while being briskly stirred, weighing 0.52 g (2 mM).second step was to add 10 ml of the extract to the key that had been formed. Finally, we added a few drops of sodium hydroxide (NaOH) solutions (1 M) with continuous stirring for two hours. The appearance of a dark brown color may have been the end of the preparation. (Figs. 1B, C).

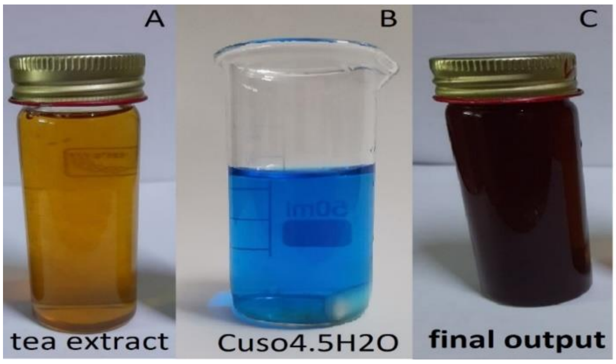


Figure1 : preparation of the nanocopper with the green tea extract

III.Characterization of Copper nanoparticles

The following procedures were used to characterize the produced nanoparticles. With the use of scanning electron microscopy (FeSEM) and transmission electron microscopy (TEM), the form and size of the made copper NPs were examined on microscopy (TEM), and the shape and size of the produced Cu NPs were studied. X-ray diffraction proved the CuNPs powder's crystalline form (XRD). AUV spectrophotometer was used to confirm the production of Cu NPs. The FTIR spectrometer and particle size distribution were employed to identify the functional chemical groups.

Antibacterial activity.

Using the disc diffusion technique, the antibacterial study of Cu NPs was assessed against bacterial pathogens, including E. coli and S. aureus. Bacteria were cultured in LB broth for 24 hours at 30 degrees Celsius and 300 rpm, yielding 10-8 colony-forming units (CFU)/mL. 100 µL of each bacterial solution was dispersed over a nutrient agar medium using the spread plate method. The lawn of bacterial culture and standard was covered with discs containing four different concentrations of CuNPs colloidal solution (50, 100, 150, and 200) µL. All agar plates were incubated for 24 hours at 37°C in the bacteriological incubator. Using a measuring scale, the inhibition zone around the discs was identified in mm. The present study's findings are consistent with prior research that shows copper NP has better efficacy against gram-negative microorganisms [16, 17].

IV. RESULTS AND DISCUSSION

FTIR spectra of copper nanoparticles with chemical agents are shown in Figure 2a, corresponding to O-H stretching vibrations (alcohol or phenolic), C-H asymmetric stretching, C=C stretching, C=C aromatic ring stretching, C-OH stretching vibrations, and C-OH bending, with the following values: 1606.70, 1452.55, 1343.69, 1134.39, and 1095.44 cm-1. Respectively, [18] The peak in the image is an example of how a copper peak may form at the FTIR peak for Cu 625 cm-1 [19]. At 3427, 2924, 2048, 1604, 1396, 1284, 1076, 779, and 570 cm-1, absorption peaks have been discovered. Fig. 2b shows that the mountains at 2924, 1604, and 1076 cm-1 correspond to C-H asymmetric stretching, C=O aromatic vibrations, and C=C stretching, respectively. The signal at 2048 cm-1 indicates that the ions are carboxylates. While the peak positions at 1396 and 1284 cm-1 are related to the organic and aromatic molecule derivatives such as phenols, alkaloids, flavonoids, tannins, aldehydes, and ketones contained in the plant extract, the peak sites at 779 and 570 cm-1 were identified as the aromatic ring of amino acids. Possible causes of the 3427 cm-1 peak include phenolic compounds stretching with OH or NH. In addition to the extract, the spectra revealed additional bands that indicated the synthesis of CuNPs, including the absorption of Cu-O and Cu-O-H. Cu-O-H bonds led to bending absorptions in the 870–880 cm–1 range, whereas Cu-FTIR typical peaks in the 550–570 cm–1 content were confirmed. [20], The OH group absorption is caused by the functional groups on the surface of nanoparticles made from the extract.

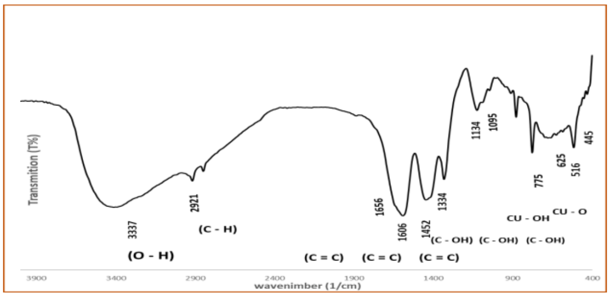


Figure2 a : FTIR spectra of the synthesized CuNPs with chemicals agents.

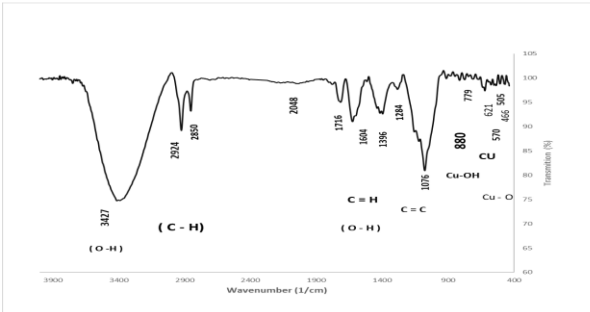


Figure 2 b : FTIR spectra of the synthesized CuNPs by using the plant extract

A. *UV-vis. Spectroscopy*

Copper NPs were successfully prepared and displayed in Figure 3 depicts their distinctive surface plasmonic resonance peak at 620 nanometers. The simultaneous vibration of the metal nanoparticle *electrons in resonance with a light wave results in the surface plasmon resonance (SPR) absorption band, which is detected. In this context, surface plasmonic resonance (SPR) peaks in the range (of 620–635 nanometers are often seen in copper nanoparticles. Additionally, it was seen at a range of 630–634 nanometers when we created it by adding reducing and stabilizing chemicals. (Fig. 3a, b) After adding a green tea extract, this resonance peak may have shifted toward longer wavelengths due to bigger particles. [21]. Based on the characteristics of each particle, such as its shape, size,* *capping agent, and precise chemical composition, the location of the SRP peak may be altered.*

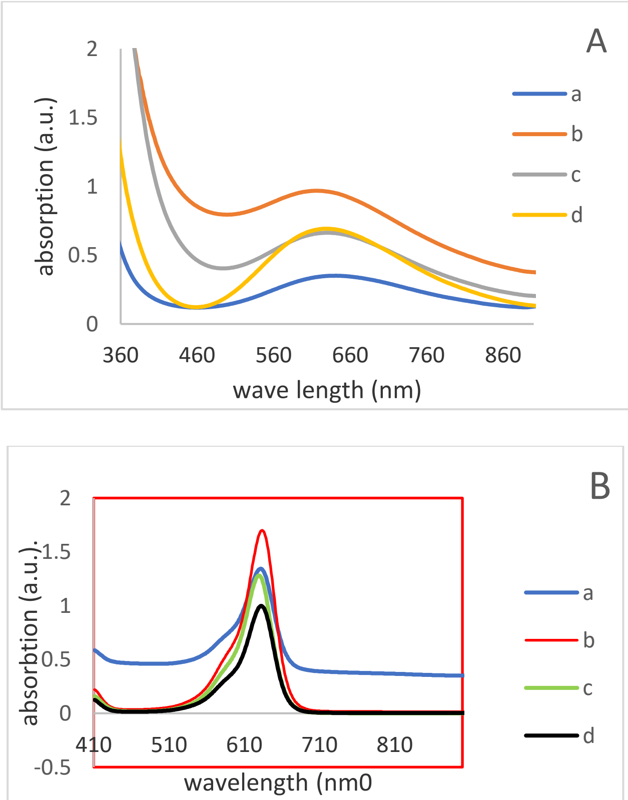


Figure 3. UV-vis absorption spectra of copper nanoparticles A - different concentrations without green tea extract, B- with different concentrations of green tea extract

X-ray diffraction analysis

Copper nanoparticles produced using ascorbic acid and CTAB (cetrimonium bromide) as stabilizing agents were analyzed using XRD (Fig. 1b).

The XRD pattern's firm peaks demonstrated that CuNPs are crystalline. The copper plane of the face center cubic structure is represented by the Miller indices (111), (200), and (220). respectively, correspond to the maxima at 43.6, 50.8, and 74.4 (JCPDS card no. 04-0836). [21]. Figure 4a shows the XRD pattern of the produced green tea leaf extract-based CuNPs. The copper nanoparticles' XRD pattern created by the green technique is demonstrated in Fig. 4b. Peaks detected at two values of 30.47, 43.56, 65.21, and 73.24, respectively, correspond to (110), (111), (300), and (220). These Cu peaks in the extract were quite similar to those in the standard (JCPDS Card No. 045-0131). According to the X-ray diffractogram analysis results, the created copper NPs have a face-centered cubic structure (FCC), the crystal structure of Cu with a lattice constant of 0.36 nm that is compatible with the standard lattice parameter (a = 0.3615 nm). JCPDS card no. 04-0836 [22] shows that the Scherrer equation was used to compute the mean size of the crystalline of CuNps with agents and CuNps for green synthesis (D), which came out to be around 47.08 and 161.43, respectively.

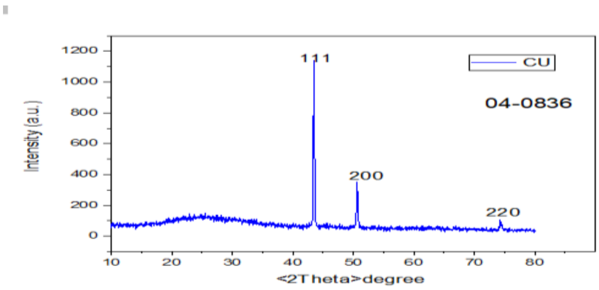


Figure4 (a) XRD patterns of Cu

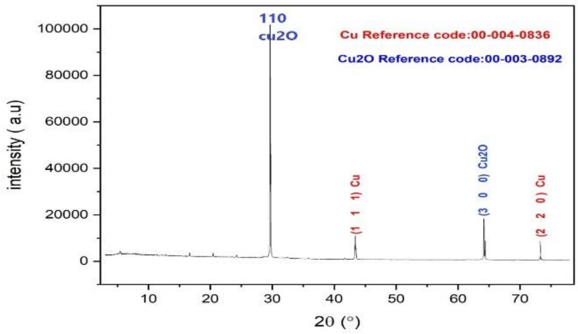


Figure 4 (b): XRD patterns of Cu with extract of green tea

Fesem Test:

FESEM and TEM analyses were used to assess the shape of CuNPs. The produced CuNPs were spherical and tended to congregate randomly, according to FESEM analyses. Images of as-produced copper nanoparticles with and without extract showed that they formed into somewhat agglomerated, spherical nanoparticles. The presence of copper atoms in CuNps, According to the energy dispersive X-ray analysis (EDX) of copper nanoparticles (Fig. 5), minor weight percentages of oxygen are present in addition to most copper. Additionally, spherical copper nanoparticles with linked structures can be seen in the fence picture of the calcined copper nanoparticles. The average copper nanoparticle size was 134 nm, and most of the particles looked larger (186 nm) than the calcined (80 nm) particles. Since no capping agent was utilized, the greater particle size of the as-produced copper nanoparticles in FeSEM analysis may be due to particle agglomeration. The amounts of phytochemicals used in manufacturing Cu NPs and plant extract affect the particle size. Fig. 6 displays a variety of particle sizes, with diameters ranging from 37 to 171 nm. These Cu nanoclusters are made up of smaller nanoparticles with good homogeneity and an average diameter of 115 nm, according to a high magnification examination.

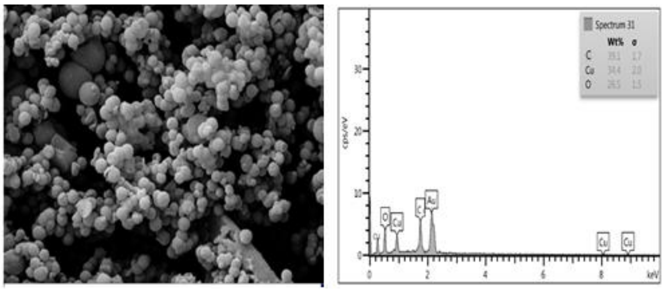


Figure 5: Fesem image &Edx of copper nanoparticles without extract.

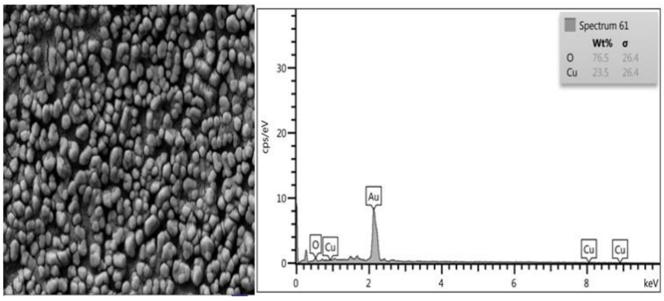


Figure 6:Fesem image &Edx of copper nanoparticles with green tea extract

TEM Test:

The shape, size, and size distributions of copper nanoparticles were revealed by TEM investigation. The size of naturally occurring copper NPs produced without the use of extract ranged from 21 to 73 nm; the histogram depicted in the figure shows the particle size distribution and the mean size of Copper NPs is 28 nanometers. Figure 7. generated copper NPs utilizing green methods and plant extracts mainly were consistent and had a spherical shape, making it easy to see their size and shape. The produced Cu particles showed a spherical shape, and their diameters varied from 64 nm to 304 nm. According to the histogram depicting the particle size distribution, the average size of Cu NPs is 142 nm. Amounts of plant extract and phytochemicals necessary for the synthesis of copper NPs affect the size of the particles. as seen in Fig.8 .The organic shell plays a crucial function in preventing the chemical oxidation of metallic Cu nanoparticles, making them stable and suitable for coatings or biotechnological applications.

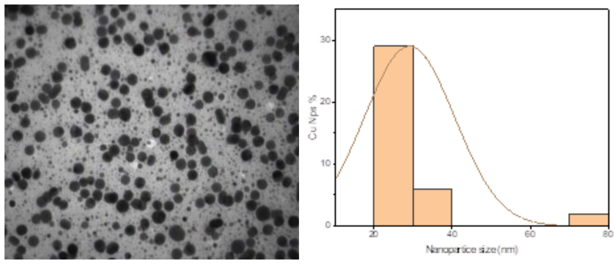


Figure 7 : Tem image & histogram of copper with chemical agents.

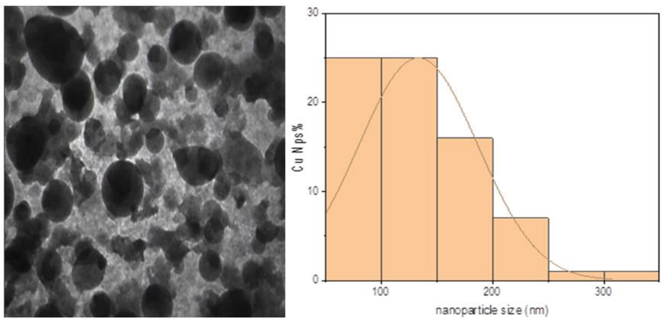
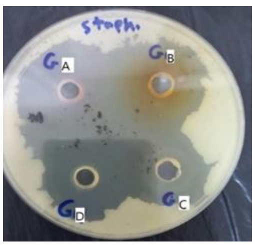
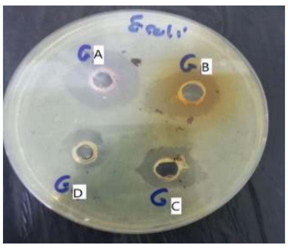


Figure 8 : Tem image & histogram of copper prepared with green tea .

Antibacterial activity:

The antibacterial activity of copper nanoparticles made using the agent technique without extract and the green method, another approach, was investigated using two pathogenic bacteria isolates, S. aureus, and E. coli. Figure 9 shows the first method's high effectiveness for all concentrations ranging from 14–20 mm for E. coli bacteria and 30–38 mm for S. aureus bacteria. Figure 10 shows the green method using green tea extract. Collecting samples, diluted concentrations (12.5, 25, 50, and 100%) were used. In addition to the second approach, the green system, For E. coli and S. aureus bacteria, the produced nanomaterials were practical, and the inhibition zones were 10–18 mm and 10–24 mm, respectively. The well's hole, considered the control, is 10 mm in diameter. In the first technique, nanocopper was effective against both types of bacteria at all concentrations, and as the copper concentration increased, so did its effectiveness against bacteria. The inhibition zone is, therefore, inversely proportional to the attention of copper nanoparticles; in the green approach, the inhibition zones for S. aureus and E. coli were 8–18 mm and 8–24 mm, respectively. From Fig. 10, it can be shown that efficiency rises as the extract's concentration increases, resulting in larger inhibition zones. These results show that the chemical method is more effective than the green method. Still, the green process has also demonstrated good effectiveness and is not bad because the extract concentrations here are low. The greenway is also safer, less toxic, easier to use, requires less preparation time, and is less expensive. Sample No. 3 did not demonstrate any effects due to adding the extract. Due to their large surface-to-volume ratio, nanoparticles can interact with the surfaces of bacteria., with tiny size and excellent dispersion [23]. The increased surface area of cube-wide NPLs improves their ability to interact with bacteria and perform broad-spectrum antibacterial actions [24, 25]. Figure 9 depicts the CuNps inhibition zone. Diluted concentrations (100, 50, 25, 12.5%): Fig. 10: Inhibition zone of diluted extract concentrations (100, 50, 25, 12.5%)





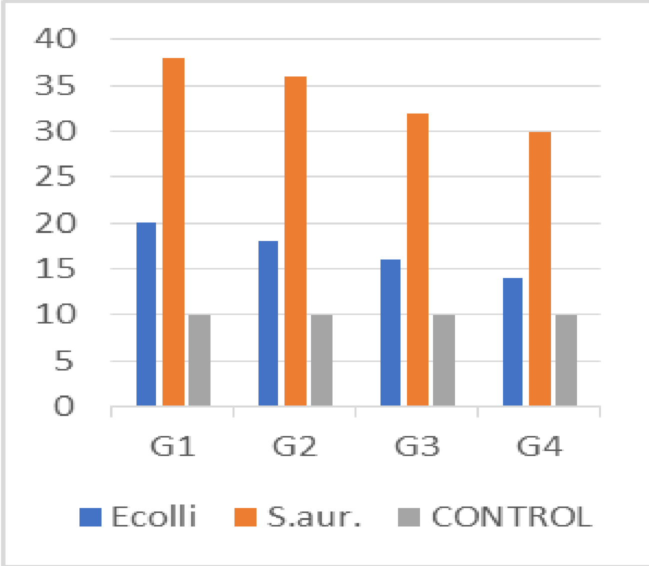
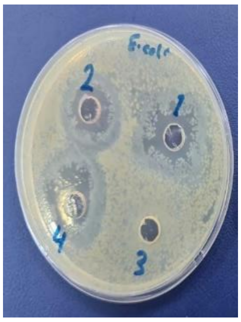


Figure 9 : Inhibition zone of CuNps Diluted concentrations (100, 50, 25,12.5) %.



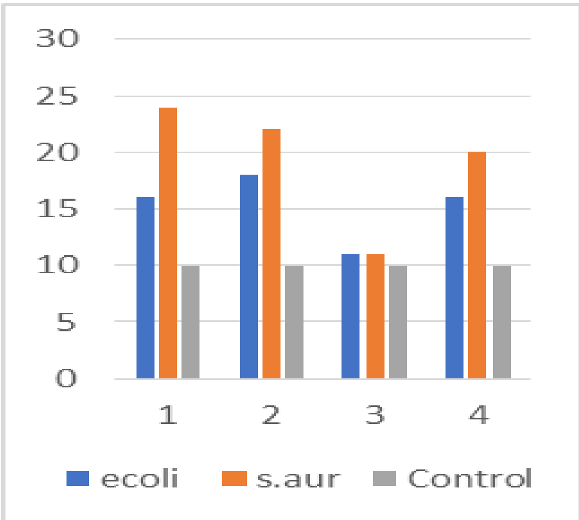


Figure 10 : Inhibition zone of Diluted extract concentrations (100, 50, 25,12.5) % .

V. CONCLUSION

The first method involved adding chemical agents, and the second method involved a plant extract. FTIR, UV-vis, X-ray diffraction, FESEM, and TEM were used to characterize the resultant copper. The average particle size of the nanoparticles was 142 nm with chemical agents and 28.4 nm with the extract. The product of the two procedures was spherical in form, and The samples exhibited vigorous antibacterial activity against the pathogens Staphylococcus aureus and Escherichia coli. With higher concentrations, the movement grew when the antibacterial pieces were effective. The agent process is more efficient, but the green approach was also advantageous, non-toxic, promising, stable in a particular solvent, and showed antibacterial efficacy. Finally, the two methods are effective against the two types of bacteria. Still, the chemical method with agents is more effective with potential toxic risks. The greenway is environmentally friendly and safe, although less effective than the chemical method.

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Cite this article as :

Alaa F. Hashim, Khalid H. Razeg, Fuad T. Ibrahim, "Effect of various concentrated copper and plant extract concentrations on the antibacterial activity.", International Journal of Scientific Research in Science and Technology (IJSRST), Online ISSN : 2395-602X, Print ISSN : 2395-6011, Volume 10 Issue 2, pp. 543-551, March-April 2023. Available at doi : https://doi.org/10.32628/IJSRST52310271

Journal URL : https://ijsrst.com/IJSRST52310271