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SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL POTENTIALS OF GRAPE BASED COPPER NANOPARTICLES

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Abstract

Here in, copper nanoparticles/nanomaterials were formulated using grape extract. The formulated nanomaterials was distinguished with the aid of UV-VIS, FTIR, SEM, EDX and XRD approach. The outcome of UV-Visible analysis revealed a Plasmon sorption culmination at 561 nm. FTIR results shows Functional groups around 3433 cm^{-1} and 2923 cm,$^{-1}$ 1629 cm,$^{-1}$ 1459 and 1380 cm^{-1}. The XRD pattern of the synthesized CuNPs revealed diffraction peaks at 2θ which are 43.50, 50.60 and 74.10 that correlates to (111), (200), and (220) lattice planes of face centered cubic (FCC) of copper nanomaterials structure. Variable experimental factors results such as temperature, plant extract volume and reaction time shows that the surface plasmon resonance (SPR) peaks for reactions conducted at various temperatures increase in absorption intensity as temperature increases. At room temperature, the SPR peak is weak but as the temperatures increases the SPR peak became more intense. It was also found that as the reaction time increases (120 seconds – 80 minutes), the absorption gets better. The effect of grape extract volume on the synthesis of the CuNPs shows that at a volume of 3 mL, there was no obvious peak and the optimum volume was found to be 5 mL. The antimicrobial efficacy of the synthesized CuNPs under optimized experimental conditions was examined with the use of Kirby–Bauer disk diffusion sensitivity testing procedure. The outcome indicate that the synthesized CuNMs effectively inhibit E. coli and S. aureus. It is recommend for future studies to consider comparing aqueous solvent extraction with alternative methods like Soxhlet extraction or cold pressing for higher yield and purity of active bio-compounds and expand testing to cover a broader range of mosquito species prevalent in different regions to improve generalizability.

Keyword: CuNPs, Grape, Synthesis, Characterization, Antibacterial activity.

Introduction: Fruits are well known for having a high dietary flavonoid contents that are beneficial to human health by killing or suppressing the growth of microorganisms, they also have the ability to neutralize free radicals, and prevent other types of disease. Plants also function as antimicrobial agents and chemotaxonomic markers. Furthermore, they are essential for maintaining the water cycle, maintaining ecosystem balance, oxygen generation, and advancing drug research. They also provide necessary resources for the home and furniture, such as wood and timber Utilizing phytochemicals found in plants for nanotechnology applications has recently gained popularity. Particles in the size range of 10−7 to 10−9 meters are the focus of this cutting-edge field, which has numerous applications in environmental science, bio-nanotechnology, applied microbiology, medicine, and other fields (Amjad, Mubeen, Ali, Alshehri, Ghoneim, Alzarea, Rasool, Ullah, Nadeem and Kazmi, 2021).

Among the suitable techniques for synthesizing nanoparticles, bio-nanotechnology-specifically, green synthesis—stands out as an economical and environmentally beneficial strategy. This technique makes use of sophisticated eukaryotic plants or simple prokaryotic bacteria cells to create nanoparticles without the use of perilous chemicals. It is ideal for large-scale production and has benefits like lower energy, temperature, and pressure requirements. On the other hand, perilous substances that may adsorb onto nanoparticles and result in unhealthy reactions. The production of stable and well-characterized nanoparticles is also guaranteed by green synthesis, which prioritizes important elements like choosing the best organism, refining reaction conditions, and using the right characterization instruments. It's crucial to take into account the plant's capacity as well as the particular reaction conditions like volume and temperature, when selecting the ideal plant for green synthesis (Iravani, 2011). Globally, extracts from a diverse range of plants have been effectively utilized to synthesize copper nanoparticles. The rich presence of bioactive compounds in leaves, fruits, and whole plants has made these plant parts suitable for the green synthesis of CuNPs. (Murthy *et al*., 2020).

Mosquito-borne diseases such as malaria, dengue fever, and yellow fever continue to present severe public health challenges, particularly in tropical and subtropical regions. The widespread use of chemical mosquito repellents, including products containing synthetic compounds like N,N-diethylmata-toluamine (DEET), has been a primary strategy for mitigating mosquito bites. However, these repellents pose significant health risks, such as skin irritation and potential long-term toxicity, in addition to environmental concerns stemming from chemical residues. Natural plant-based alternatives, such as extracts from Hyptis spicigera (black sesame), hold promising potential due to their eco-friendly nature and lower health risks. However, their use has been underexplored and underutilized, necessitating comprehensive research to validate their efficacy and develop effective formulations. Addressing this gap by formulating and evaluating a natural mosquito repellent derived from Hyptis spicigera could provide an accessible and environmentally sustainable solution to mosquito control and disease prevention.

Methodology: Materials: The grapefruit used for this study were gotten from the Zuru central market in Kebbi State, Nigeria. Representative samples were selected using random sampling.

Sample Preparation: The representative sample was peeled and 20 g was carefully weighed, using a mortar and pestle, the peeled fruit was smashed and finely grounded. The resultant mixture was brought to a boil in a water bath maintained at 60°C for quarter of an hour after being combined with 100 cm^3 of deionized water. The extract was immediately utilized for nanoparticle synthesis after it cooled and was filtered through Whatman filter paper and muslin cloth. Additionally, 2.4955 g of the salt was solubilized in 10 cm^3 of demineralized water to create a 0.01 M solution of $CuSO_4{\bullet}5H_2O$.

Green synthesis of copper nanomaterials: After thorough mixing, about 0.01 liter of the grape extract was combined with 0.1 liter of a 0.01 M CuSO4·5H2O aqueous solution. The combination was heated for six hours to 80°C while being constantly stirred. After centrifuging the suspension for one-sixth of the hour at 3,000 rpm, the supernatant was poured away, and the remaining residue was washed multiple times with 10 cm^3 of deionized water to remove surface impurities from the copper nanoparticles. The final precipitates was stored for characterization and antimicrobial testing after being dried for 24 hours at 50°C in an oven.

Instrumentation: In this work, Thermo Scientific Genesys 10S UV-Visible Spectrophotometer with VISION Lite software for UV-Vis analysis was used, the Rigaku Ultima IV X-ray Diffractometer for X-ray diffraction, the Quattro S SEM was used for detailed imagery study, and the Thermo Electron Nicolet 6700 FTIR Scope was used for FTIR analysis.

Optimization of Experimental Variables: In order to evaluate their relativity and interactive effects on the synthesis of the grape-based CuNPs, reaction variables were optimized. Three experimental factors were analyzed. The parameters of the experiment are: (i) Sample extract volume in cm^3; for the analysis, the extract volume was varied between 1 cm^3, 5 cm^3, 10 cm^3, 15 cm^3, and 20 cm^3. The CuSO4.5H2O(aq) concentration was maintained at 0.01 M, and the reaction temperature was kept at 80°C. (ii) $CuSO_4.5H_2O$(aq) concentration; 0.01 M, 0.02 M, 0.03 M, 0.04 M, and 0.05 M were utilized in the analysis for the optimization, keeping the reaction temperature at 80°C and the sample extract volume at 10 cm^3, respectively. (iii) Reaction operating temperature in degrees Celsius; operating temperature was varied between 60 °C, 70 °C, 80 °C, 90 °C and 100°C, whereas concentration of $CuSO_4.5H_2O_{(aq)}$ and quantity of extracted samples was kept at 0.01 M and 10 cm^3, respectively.

Results and Discussion: Reaction Time Effect: The incubation period of Cu salt and reducing Cuents results in the reduction of Cu^{2+} ions to Cu atoms. The reaction time do differ, sometimes lasting only a few minutes to hours subject to the quality of the reducing agents and other conditions. As the reaction time increases (2 minutes – 80 minutes), the absorption gets higher because more nanoparticles are present in solution.

Impact of the amount of plant extract: The impact of the amount of plant extract on CuNPs concentration, at a volume of 3 mL there was no obvious peak which means the extract volume was not sufficient to form a significant amount of CuNPs, and the optimum volume was found to be 5 mL subsequent increases in volume (7 mL and 10 mL) resulted in absorptions with lower intensity and this is attributed to the dilution of CuNPs colloid as the volume of plant extract increases above the optimal volume.

Effect of temperature: It was observed that the surface plasmon resonance (SPR) peaks for conducted reactions at varying temperatures shows increase in inbibition force as temperature increases. At room temperature, the SPR peak was weak and almost linear but as the temperatures increases, the SPR peak became more intense. This suggested that room temperature is not a suitable temperature for the synthesis of grape based CuNPs while higher temperature are the best conditions for the synthesis of the said nanoparticles.

UV-Visible spectroscopy: In this study, UV-visible spectroscopy was employed to confirm the formation of nanomaterials. 4.0 cm^3 of deionized water was used to dilute 1.0 cm^3 of the sample fraction of suspension for the analysis, and the UV-visible spectrum was captured. The results of UV-Visible analysis (Figure 1) showed that Reduction of Cu^{2+} to Cu nanomaterials utilizing sample distillate was revealed by changing colour from pale red to reddish brown. The process in reduction of copper ions to Cu nanomaterials utilizing the fruit extract was demonstrated by the enhanced force of surface Plasmon absorption peak observed at 561 nm. The appearance of this peak, is within the range of 550 nm–600 nm which is typical of copper nanomaterials absorption peak (Khodaie and Ghasemi, 2018). This suggest that the synthesized nanoparticle is CuNPs.

Fourier transform infrared spectroscopy (FT-IR): Bioactive molecules involved in the diminution of copper sulfate to CuNPs, along with the agents that stabilize and cap the nanoparticles, were identified using FTIR analysis. After the copper nanoparticles were synthesized, the extract's precipitate was dried for 24 hours at 50°C in an oven. After being dried, the nanoparticles were crushed with KBr, formed into pellets, and examined with an FTIR spectrophotometer set to 4 cm^{-1} resolution. The result of the FTIR studies (Figure 2) shows Functional groups in the FTIR spectra of the grape based CuNPs, peaks around 3433 $cm^{-1}$and 2923 cm^{-1} which are attributes of a carboxylic COOH (or N-H stretching mode) and alkynic ≡C-H stretching. The band at 1629 cm^{-1} corresponds to amide, arising primarily to carbonyl stretching in proteins. The peaks at 1459 and 1380 cm^{-1} correspond to methylene scissoring vibrations from the proteins in the solution and C-N stretching vibrations of amine (Wang *et al*., 2004). This suggests that free carbonyl and NH_2^- groups from amino acid residues and proteins have capacity to stick to a metal indicating that the proteins could thus form a layer engulfing the metal. The FTIR spectra indicated that the flavonoids, alkaloids, and protein entities in the grape extract contribute to the diminution of copper ions and the stabilization of Cu nanomaterials. The FTIR data conforms to earlier reports (Xie *et al*., 2004; Kalainila *et al*., 2014; Valodkar *et al*., 2018).

X-Ray Diffraction Analysis (XRD): The XRD method was applied to assess the phase and crystalline structure of the nanomaterials. Here, the synthesized CuNPs were ground into a fine powder, and a clean glass plate was dipped into the powder to create a thin layer of the powder. Monochromatic Cu Kα radiation (θ = 1.5406 Å) working at 40 kV with 25 mA current of and 25℃ was used for the XRD analysis. With a time constant of one second and a scan rate of 0.02°/min, the XRD configurations were gathered over a 2θ range of 10° to 80°. To determine the nature of the nanoparticles, the resulting plots were matched against the Joint Committee on Powder Diffraction Standards (JCPDS) reference database (card no: 53-61386). XRD pattern of the synthesized grape based CuNPs (Figure 3) revealed diffraction peaks at 2θ which are 43.50, 50.60 and 74.10 which relates to (111), (200), and (220) lattice planes of face centered cubic structure (FCC) of copper nanomaterials. These figures of diffraction data were in accordance with Inorganic Structure Data Base (ICSD); file no. 04-0836 (Rajesh, et al., 2018) also with International Centre for Diffraction Data (ICDD) standard for figures of Copper nanomaterials; file number 04-0836 (Theivasanthi and Alagar, 2011). Scherrer equation was used to diameter of the synthesized CuNPs. The result obtained was 54 nm.

Scanning Electron Microscopy (SEM): A scanning electron microscopy technique was used to evaluate the morphological structure of the produced CuNPs. In this instance, an aliquot of the colloidal CuNPs was put on a copper slide coated in carbon, left to dry in the air, and then moved to a microscope. A resolution of 1 nm and X10 magnification were used in the 130 kV SEM analysis. Figure 4 presents SEM images of the grape based CuNPs, The image revealed dispersed copper nanoparticles with uneven edges. The synthesized CuNPs appeared to have flake-like shapes. The results align with the findings reported by (Jaehoon *et al*., 2006; Arya *et al*., 2018)

Energy Dispersive X-ray (EDX): For assessing the grape-based CuNPs' elemental composition, an instrument used in conjunction with a SEM was used to perform the EDX analysis. Result of EDX analysis (Figure 5) indicates the EDX spectra of the newly formed grape based CuNPs. There was an identifiable peak at 1 keV, which corresponds to that of typical metallic

copper nanoparticles. Thus, spectra suggest the

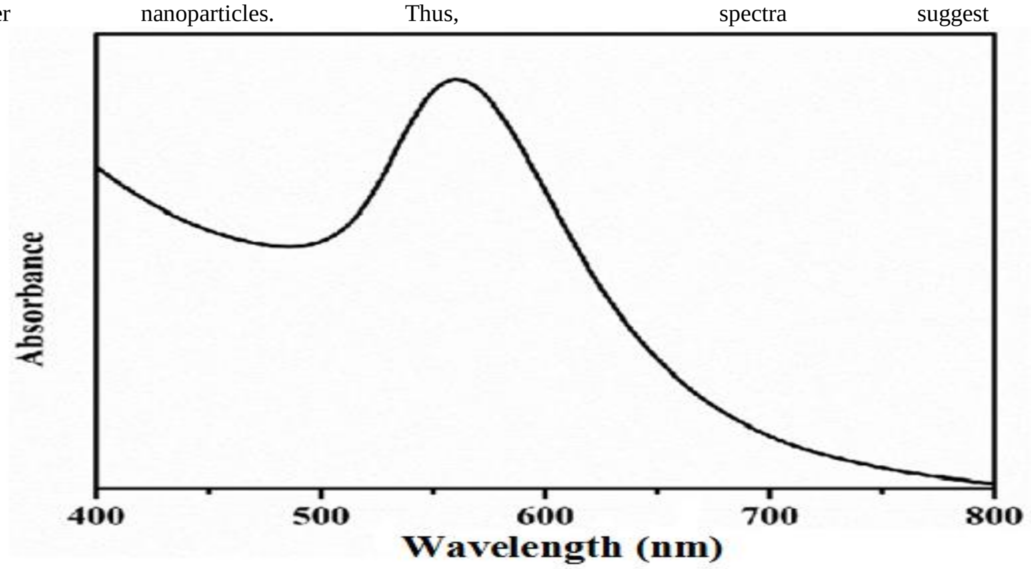

Figure 1: UV-Vis spectrum of Grape based CuNPs

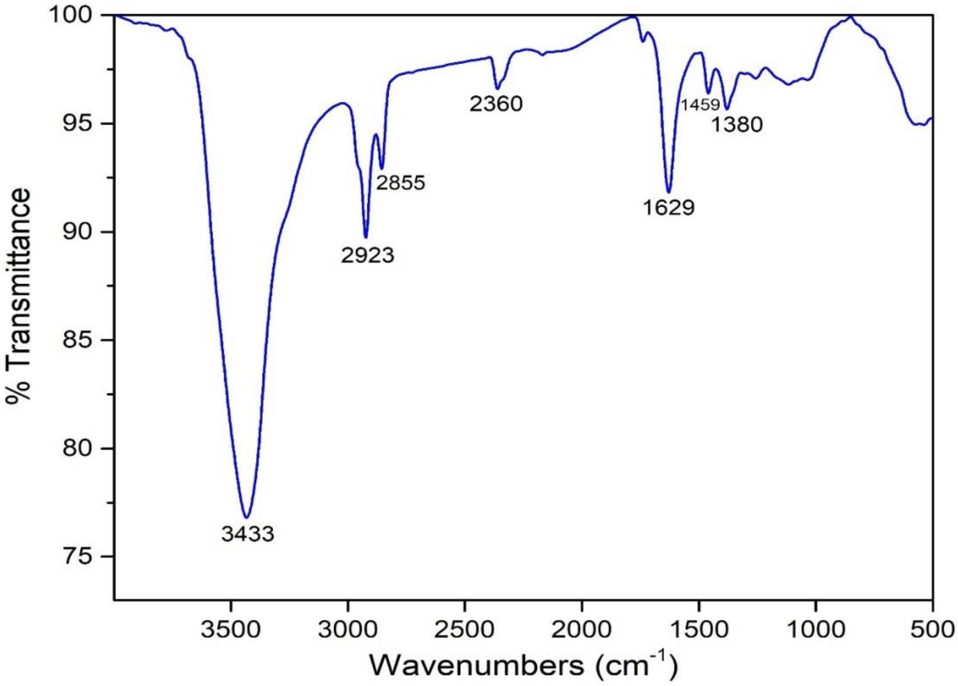

Figure 2: FTIR spectra of grape based CuNPs

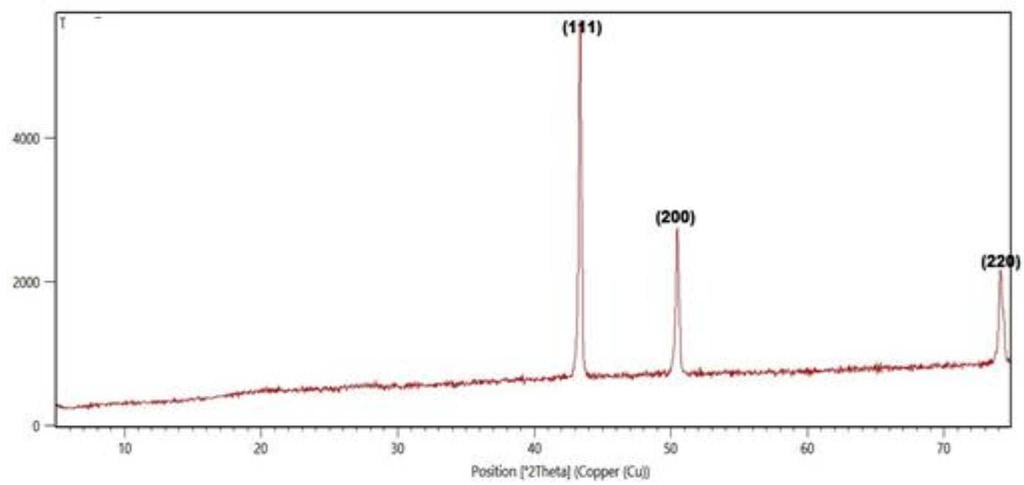

Figure 3 XRD pattern of the synthesized grape based CuNPs

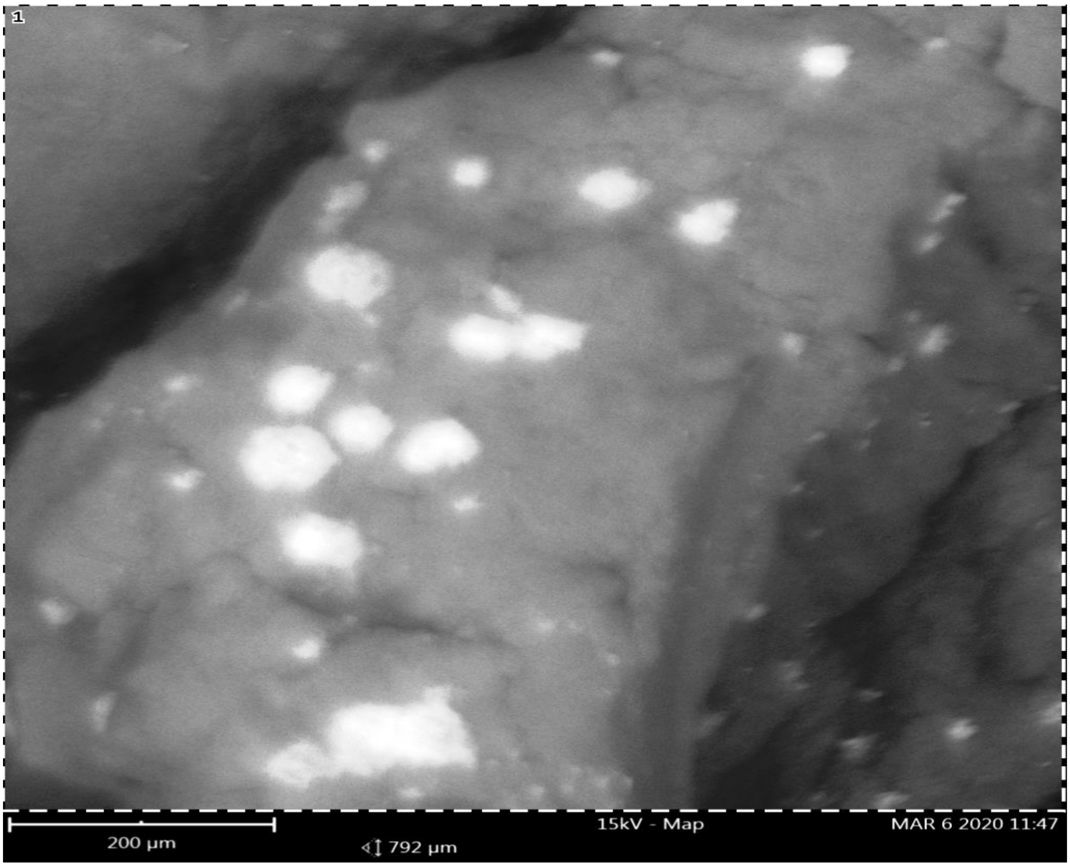

Figure 4 SEM image of Grape based CuNPs

presence of metallic copper in the nanoparticle.

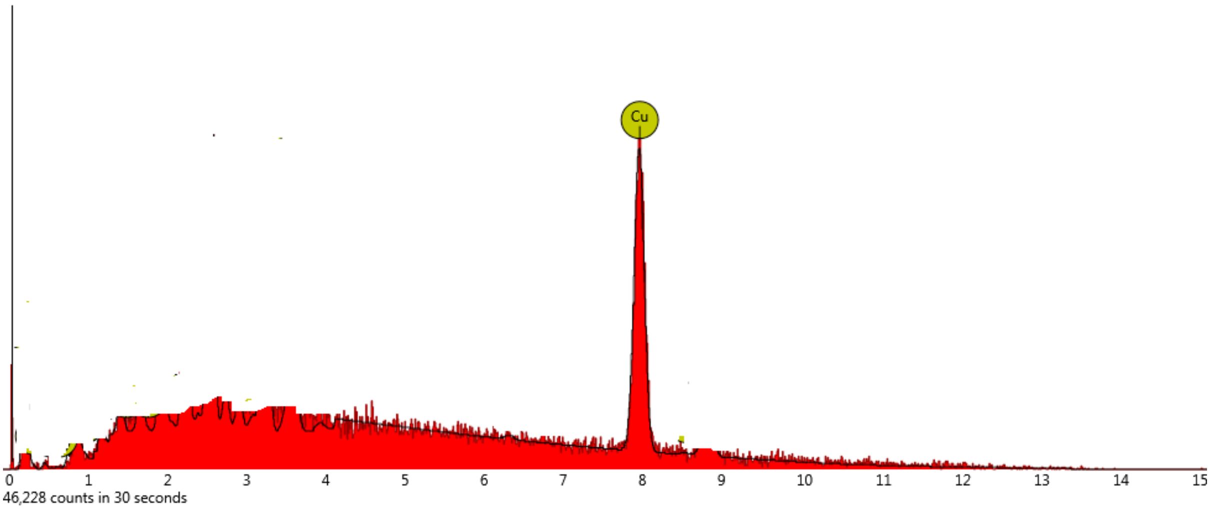

Figure 5: EDX spectra of the synthesized Grape based CuNPs

3.9 Test of antibacterial activity

The antimicrobial effectiveness of grape-based synthesized CuNMs was evaluated by the use of Kirby–Bauer disk diffusion susceptibility test method (Hudzicki and Kirby, 2009). Microorganisms for testing including Gram-negative *E. coli* and Gram-positive *S. aureus* were selected according to the 2010 National Committee for Clinical Laboratory Standards guidelines (Clinical Laboratory Standards Institute, 2010). Amoxicillin clavulanate-impregnated antimicrobial discs were used as a positive control. All bioevaluation were conducted using 30 μL of a solution containing redissolved CuNMs in deminiralized water, grape extract, and copper sulphate solution following the criteria of the positive control (amoxicillin clavulanate). After incubating for 18-hours period, the breadth of the zone of inhibition (ZOI) was estimated in millimeters and recorded. The weakness or resistance of each test organism to the drugs was gotten based on the Clinical Laboratory Standards Institute (CLSI) guidelines. The ZOI was categorized as susceptible (S), intermediate (I), or resistant (R) in accordance with CLSI interpretive criteria. The result showed that the grape based CuNMs had suppressive action against *E. coli* and *S. aureus*. Pearson's product-moment correlation was done to examine the interconnection between the size of CuNMs and ZOI of CuNMs in respect to *E. coli* and *S. aureus* ($n = 13$). The assessment showed a negative correlation between the size of CuNMs and the zone of inhibition of *E. coli* ($r = -0.76$; $p \geq 0.01$). A negative correlation was observed between the size of CuNPs and the zone of inhibition of *S. aureus* by the CuNPs ($r = 0.76$; $p \geq 0.05$). The greater inhibition of Gram-negative bacteria by CuNMs can partly be attributed to the easier penetration of smaller nanoparticles into the cell wall of Gram-negative bacteria, which consists of a unique outer membrane and a single peptidoglycan layer, unlike Gram-positive bacteria that have multiple peptidoglycan layers. Additionally, CuNMs are believed to attach to Gram-negative bacterial cell walls primarily due to electrostatic interactions, while copper ions accelerate DNA deterioration and reduce bacterial respiration. In certain Gram-negative strains, copper ions are thought to change the structure and electron transfer of associated reductases, leading to the blockage of cytochromes within the membrane.

Conclusion: The research shows that grape is an excellent precursor for the production of copper nanomaterials, it also demonstrated that; temperature, reaction time and volume of the grape extract are factors to be considered when synthesizing CuNps.The synthesized nanoparticles has antimicrobial inhibitory effectiveness against Gram-negative bacteria, such as *E. coli*, and Gram-positive bacteria, such as *S. aureus*.

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