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Synthesis of Copper Nanoparticles by Using *Solenostemma argel* Extract and Its Application as an Antimicrobial Agent

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Abstract
In this study, metallic nanoparticles of copper were synthesized by the green method using an aqueous solution of *Solenostemma argel* extract. The extract was mixed well with cupric nitrate trihydrate. The plant extract acts as both a reducing and stabilizing agent. The prepared nanoparticles are found to be stable in the aqueous solution of the plant extract over seven months at room temperature (25 °C). The prepared copper nanoparticles were characterized by scanning electron microscopy (SEM) and UV-vis spectrum analysis. The scanning electron micrograph showed spherical nanoparticles in 30-50 nm size. These nanoparticles exhibited surface Plasmon absorption resonance (SPR) in the visible region. Also, they exhibited interesting anti-bacterial activity against Gram-positive and Gram-negative bacteria.

Keywords
synthesis of copper, *solenostemon argel*, nanoparticles

1. Introduction

Nanotechnology is the ability to build materials, devices, and systems with atomic accuracy. A brief and general definition of nanotechnology is the US National Science and Technology Council statement: "Nanotechnology is the ability to work at the molecular level, atom by atom, to create large structures with the fundamentally new molecular organization. The aim is to exploit these properties by gaining control of structures and devices at atomic, molecular, and supramolecular levels and to learn manufacture and use of these devices efficiently" [1-4]. The United States National Science Foundation defines nanoscience/nanotechnology as studies dealing with materials and systems with dimensions of 1–100 nanometers (nm) [5-7].

The promise and essence of nanoscale science and technology are based on the demonstrated fact that materials at the nanoscale have properties (chemical, electrical, magnetic, mechanical, and optical) quite different from the bulk materials. Presently, nanotechnology applications include bottom-up technology in biology, medicine, pharmaceuticals, electronics, energy, and environmental industries, which are rapidly increasing. In the synthesis of nanoparticles, some important manufacturing methods can be of natural or synthetic origin, and they both exhibit unique properties at the nanoscale. This includes green synthesis methods, where the biological method, an alternative to chemical and physical processes, provides an environmentally friendly way of synthesizing nanoparticles. Moreover, this method does not require expensive, harmful, and toxic chemicals. Metallic nanoparticles can be synthesized with various shapes, sizes, contents, and physicochemical properties. Synthesis can be done in one step using biological organisms such as bacteria, actinobacteria, yeasts, molds, algae, and plants or their products. Molecules in plants and microorganisms, such as proteins, enzymes, phenol compounds, amines, alkaloids, and pigments, perform nanoparticle synthesis by reduction. In the traditional chemical and physical methods, reducing agents involved in the reduction of metal ions and stabilizing agents used to prevent undesired agglomeration of the produced nanoparticles carry a risk of toxicity to the environment and the cell.

In recent years, copper nanoparticles have attracted much attention from researchers due to their unique physical and chemical properties and low-cost preparation. Copper nanoparticles have diverse applications as heat transfer systems, like super-strong materials, sensors, and catalysts. Their other properties, such as antimicrobial activity, disinfecting property, and stability as matrix-bound particles, can be further exploited in wall paints and plasters to coat hospital equipment. The synthesis of copper nanoparticles is an active area of academic and, more importantly, application research in nanotechnology. Various chemical and physical procedures such as chemical reduction, electrochemical reduction, chemical vapor deposition, thermal decomposition, and solving thermal reduction have been reported for synthesizing metallic nanoparticles [8].

However, these methods have many problems, including toxic solvents, generating hazardous byproducts, high energy consumption, and non-eco-friendly. Considering this aspect, there is essential to develop clean, reliable, biocompatible, cost-effective, environmentally friendly, and sustainable procedures for synthesizing nanoparticles. Considering the vast potentiality of plants as sources for the green synthesis of different nanoparticles, especially copper nanoparticles, researchers worked with plant extracts, some specific plant parts, or whole plants for the green synthesis. Many reported that extracts from plants like *Nerium oleander*, *Punica granatum sanctum*, and *Zingiber officinale* efficiently yielded copper nanoparticles concentrated and wet leaf extracts of *Ricinus communis*, *Punica granatum*, *Psidium guajava*, *Eucalyptus globulus*, and *Phyllanthus emblica* as both reducing and stabilizing agent. The antimicrobial activity of synthesized copper nanoparticles was studied against Gram-positive bacteria like *Staphylococcus aureus*, which was very potent. This is significant when drug-resistant infections of *Staphylococcus aureus* are becoming more prevalent [9].

Due to quantum effects and a large surface-to-volume ratio, metal NPs possess fascinating ultraviolet-visible sensitivity and electrical, catalytic, thermal, and anti-bacterial properties. A large number of atoms are present on the surface due to the smaller particle sizes. The surface-area-to-volume ratio of particles varies depending on the shape and size of nanoparticles, including the ultraviolet-visible sensitivity and conductivity. The characteristic properties, including the electronic energy levels, electron affinity, electronic transitions, magnetic properties, phase transition temperature, melting point, association to polymers, and biological and organic molecules, are also modulated by the change in surface area. Quantum effects are due to the combination of quantum size and Coulomb charging effects that impart the charge to nanoparticles. When the Coulomb charge effect is coupled with the quantum size, various fascinating properties are obtained that are not observed for the same bulk material. Quantum effects are prominent in spherical particles and particles with sharp edges. Due to these effects and their size-dependent nature, nanoparticles are used in catalysis, sensing, and imaging. The availability of copper has made it a better choice to work with because it shares properties similar to those of other expensive noble metals, including silver and gold. The choice of copper in the present research is attributed to the factors mentioned above; copper nanoparticles are reported to have antimicrobial activity against several species of bacteria and fungi [8, 10-13].

Copper nanoparticles can be produced using different techniques, typically classified as bottom-up or chemical and top-down or physical methods. In the bottom-up approach, the structure of nanoparticles is constructed by atoms, molecules, or clusters. In top-down approaches, a bulk piece of required material is reduced to nanosized dimensions using cutting, grinding, and etching techniques. Nanomaterials are prepared from larger entities without atomic-level control. Chemical reduction, microemulsion (colloidal) techniques, sonochemical reduction, electrochemical, microwave-assisted, and hydrothermal syntheses are the main techniques for the synthesis of nanoparticles through the chemical approach. Biological or biosynthesis techniques are also considered bottom-up or chemical processes. Physical methods for nanoparticle synthesis are laser (pulse) ablation, vacuum vapor deposition, pulsed wire discharge (PWD), and mechanical milling. A wide range of nanoparticles can be produced using physical methods with minor modifications for different metals. However, the main disadvantages of these methods are the quality of the product, which is less compared to nanoparticles produced by chemical methods. Usually, these methods require expensive vacuum systems or equipment to prepare nanoparticles (plasmas). During the chemical synthesis process of copper nanoparticles, the growth and morphology can be controlled by optimizing reaction conditions, such as the surfactant's temperature and concentration, precursor, capping/stabilizing agent, and the type of solvent. Narrow size distribution during chemical synthesis can be achieved using these optimum reaction conditions. These methods for producing copper nanoparticles are appropriate for laboratory-scale synthesis but are not economical for a large-scale or commercial setup. However, this method leads to byproducts that are of environmental concern. Compared to these methods, the green synthesis of the copper nanoparticles is safer and environmentally friendly [14-22].

Green Nanotechnology is about doing things right, making green nano-products, and using nano-products to support sustainability, encompassing nanoscale science, engineering, and technology. Nanotechnology involves imaging, measuring, modeling, and manipulating matter at this length scale. At this level, materials' physical, chemical, and biological properties differ fundamentally from those of individual atoms and molecules or bulk matter. Nanotechnology aims to understand and create improved materials, devices, and systems that exploit these new properties. It is expected to result in cleaner and less wasteful methods of manufacture, more robust and lighter building materials, smaller yet faster computers, and more powerful ways to detect and treat disease. Nanotechnology promises an exciting breakthrough and a sustainable future. The uses of metal nanoparticles and nanoclay as additives in polymer nanocomposites have been done by many researchers. Green nanotechnology is used to develop clean technologies to minimize human health and potential environmental risks. It is associated with the use of nanotechnology products and manufacturing processes. Green nanotechnology encourages substituting existing products to develop new nano-products. The production of new nano-products makes the environment friendlier. The term 'technology' refers to the application of knowledge for practical purposes. The field of 'green nanotechnology' encompasses a continuously evolving group of methods and materials, from techniques for generating energy to non-toxic cleaning products. The current expectation is that this field will bring innovation and changes in daily life, similar to the 'information technology' explosion over the last two decades. In these early stages, it is impossible to predict what 'green nanotechnology' may eventually encompass. Green nanotechnology provides tools for transforming biological systems into green approaches to nanomaterial synthesis while preventing associated toxicity [22-24].

2 Materials and Methods

Synthesis *Solenostemma argel* (Figure 1) has been used to synthesize metallic nanoparticles due to their availability, cost-effectiveness, environmentally friendly nature, and nonhazardous byproducts. They are using $CuSO_4$ as a precursor and with capping agents in an acidic solution. Various plant extracts were used as a stabilizing agent for copper nanoparticles in the presence of L-ascorbic acid as the antioxidant and NaOH as a catalyst [7].

Fig. 1. *Solenostemma argel* [25]

The essential medicinal plants are primarily extracted from herbs, shrubs, and trees. Herbs are defined as small plants with soft stems (people have discovered the benefit of using plants for medical purposes along with time and age. An estimated half a billion people use medicinal plants in various traditional ways worldwide. Herbal medicine is becoming popular now a day because they are available, cheap, and have no side effect on one hand. Sudan is a large country with varied topography and different ecological conditions. These factors have created a suitable condition for the growth of various species and plants, of which a high percentage are of medical value. For most people in Sudan, traditional medicine remains the primary source of health care or even the only one, especially in remote rural areas [26].

Among the enormous number of these medicinal plants belongs to the family *Asclepiadaceae*. Extract from the leaves of *S. argel* was used for the treatment of *diabetes mellitus* and renal inflammation. Other uses of *S. argel* are purgative and antipyretic for treating cough, colds, renal and colic, and inflammation, as well as treating chaplains internally. Externally the powder of *S. argel* leaves is claimed to treat inflammatory wounds. It is a leaf that is reported to be rich in saponin, flavonoid, and glycoside, showing an apparent positive antimicrobial activity [27-29].

The plant was reported to have antimicrobial properties, and the antifungal and anti-bacterial properties of the aqueous extracts of *Aspergillus niger*, *Pennicilium italicum*, *Escherichia coli*, and *Salmonella typhi* were investigated [30]. Various researchers have used the green root of Cu-NP synthesis [31, 32].

Copper nanoparticles were used as antimicrobial agents due to their high surface-to-volume ratio and easy interaction with other particles to enhance their antimicrobial efficiency. Copper nanoparticles are highly reactive compared to other metallic nanoparticles [33-36].

Synthesis of copper NPs: The *Solenostemma argel* leaves were purchased from the local market in Sudan, cupric nitrate trihydrate ($Cu(NO_3)_2{\cdot}3H_2O$) from Sigma Aldrich and Three liters of distilled water were used without further purification.

Preparation of plant leaves extract: The *Solenostemma argel* leaves extract was prepared as described: weighted (5, 10) g from plant leaves using a sensitive balance and then washed with distilled water to remove any contaminant or dust particles, then put on the sterile container and added 100 ml boiled distilled water and maintained for 24 h at room temperature. After a soaking period, the extract was filtered using filter paper, and the filtrate was used immediately to prepare the nanoparticles.

Preparation of copper solution: first, all glass was cleaned, weighed 1.2 g of cupric nitrate trihydrate, carefully dissolved in a small beaker, then poured into the volumetric flask and added distilled water to complete the volume to 50 ml. The solution was diluted by taking (5, 30, 25) ml from the solution and adding distilled water to complete the volume to 50 to prepare different concentrations of cupric nitrate trihydrate (0.01, 0.03, 0.05).

Biosynthesis of copper nanoparticles: Copper nanoparticles were synthesized by using the following procedure (Figure 2): the plant extract of *Solenostemma argel* (20 ml) was mixed with (10 ml) of (0.01, 0.03, 0.05 M) aqueous cupric nitrate trihydrate immediately the color changed from blue to green. Then put in autoclave, as shown in Figure 2, at 121 °C, 0.2 MPa, for 2 h, the color changed from green to dark (brown), indicating the formation of copper nanoparticles. The solution was then centrifuged, shown in Figure 3, at 1000 rpm for 10 min and then re-dispersion of the pellet in deionized water to remove any unwanted biological materials and dried at room temperature for room temperature 24 hours.

Scanning electron microscope: The scanning electron microscope (SEM) shown in Figures 4 and 5 of the test sample surfaces is obtained by scanning it with a high-energy beam of electrons in a vacuum chamber. When the beam of electrons strikes the surface of the specimen and interacts with atoms of the sample, signals in the form of secondary electrons and backscattered electrons are generated that contain information about the sample's surface morphology. The morphological features of copper nanoparticles were studied using SEM.

UV visible spectroscopy: Nanoparticles have unique optical properties that are sensitive to the size, shape, concentration, agglomeration state, and refractive index near the surface of the nanoparticles, which makes them visible for identifying, characterizing, and studying the nanoparticles. Bio-synthesized copper nanoparticles were characterized using a UV-visible spectrophotometer (Figure 6).

Staphylococcus aureus ATCC 25923 (gram-ve bacteria). *Escherichia coli* ATCC 25922 (gram-ve bacteria). American Type Culture Collection (ATCC) Rockville, Maryland, USA. MRSA (Methicillin-resistant *Staphylococcus aureus*).

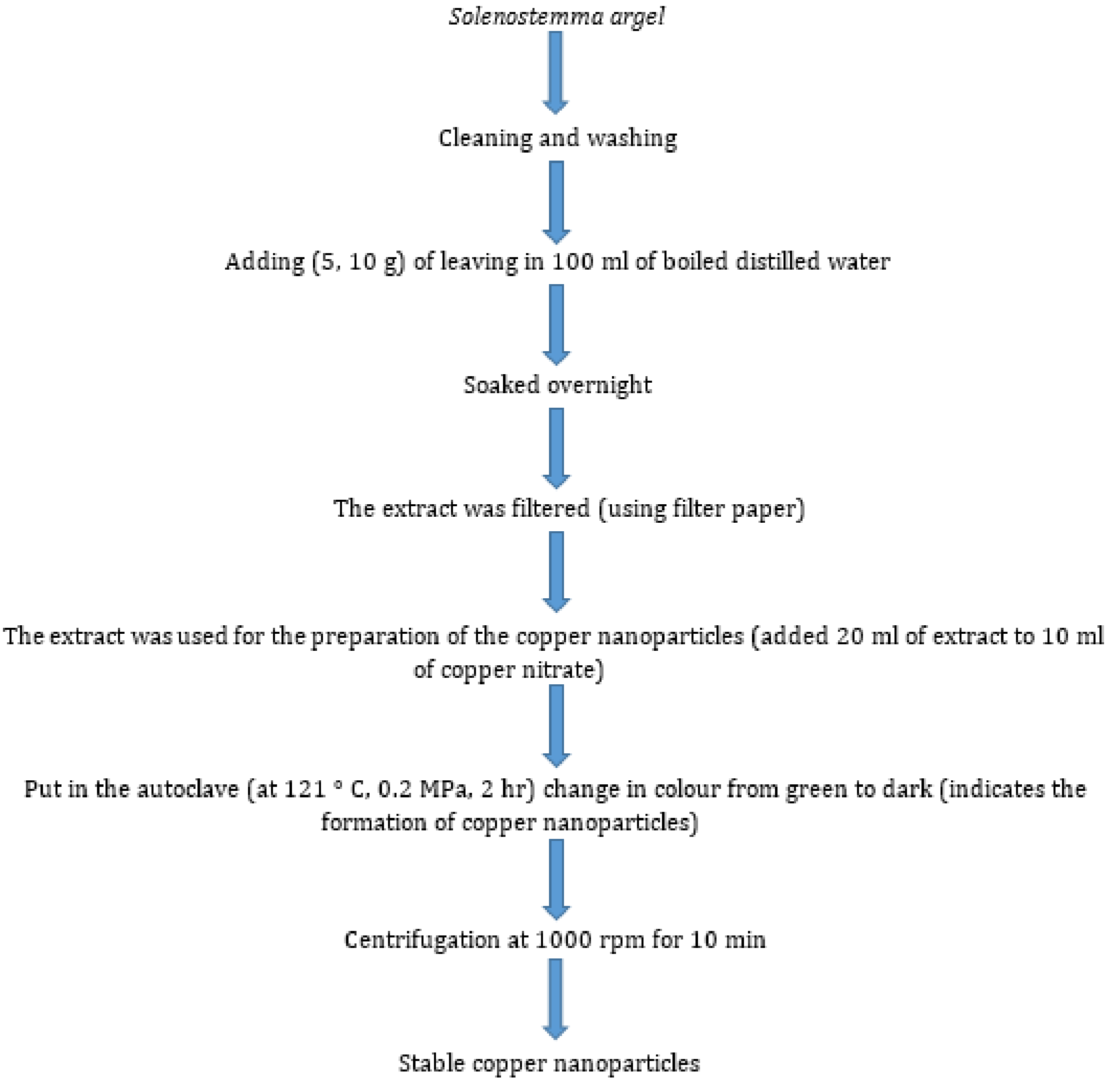
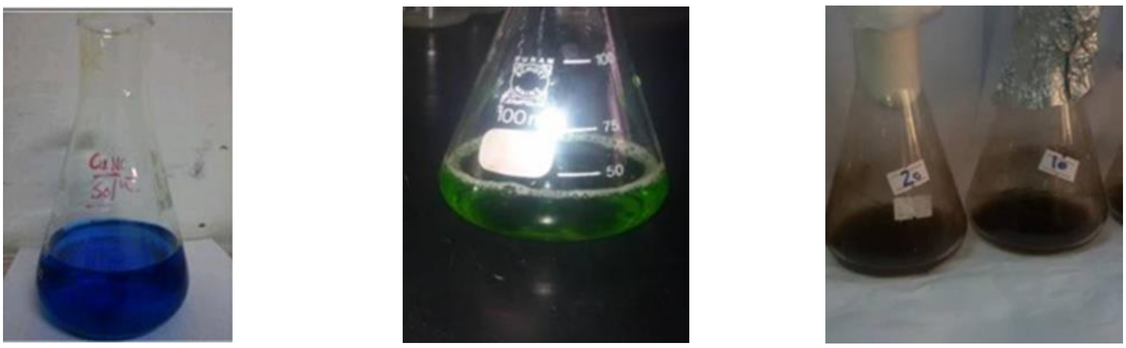


Fig. 2. Process flow chart for bio synthesize of Copper NPs



Copper solution | Copper + extract | Copper NPs

Fig. 3. Biosynthesis of copper nano particles (changing in color)

Preparation of bacterial suspensions: One ml aliquots of a 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37 °C for 24 hours. The bacterial growth was harvested and washed with 100 ml sterile normal saline to produce a suspension containing about 108-109 CFU/ml. The suspension was stored in the refrigerator at 4 °C till used. The average number of viable organisms per ml of the stock suspension was determined using the surface viable counting technique. Serial dilutions of the stock suspension were made in sterile standard saline solution, and 0.02 ml volumes of the appropriate dilution were transferred by micropipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37 °C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colonies forming units per ml suspension. Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

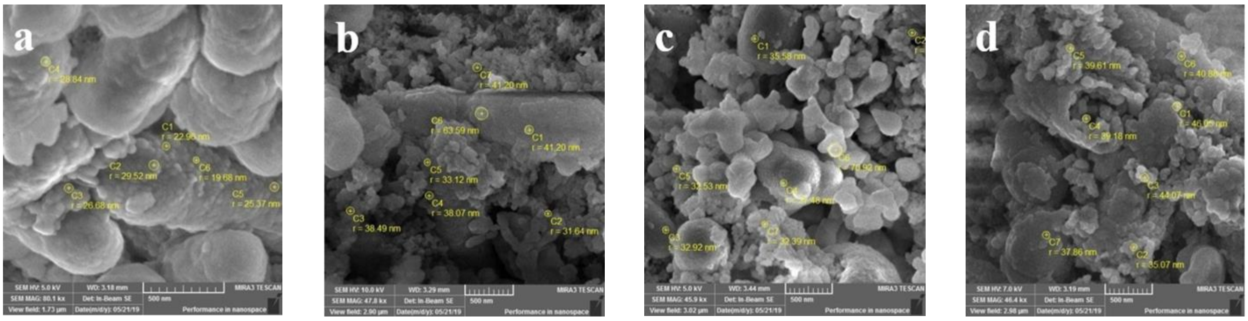


Fig. 4. The SEM photographs of the synthesized Copper Nano Particles
using extract of *Solenostemma argel* (5g)
(a) 0.01 [range 19.68-29.52 nm]; (b) 0.1 [range 31.64-63.59 nm];
(c) 0.03 [range 32.53-70.92 nm]; (d) 0.05 [range 35.07-46.05 nm]

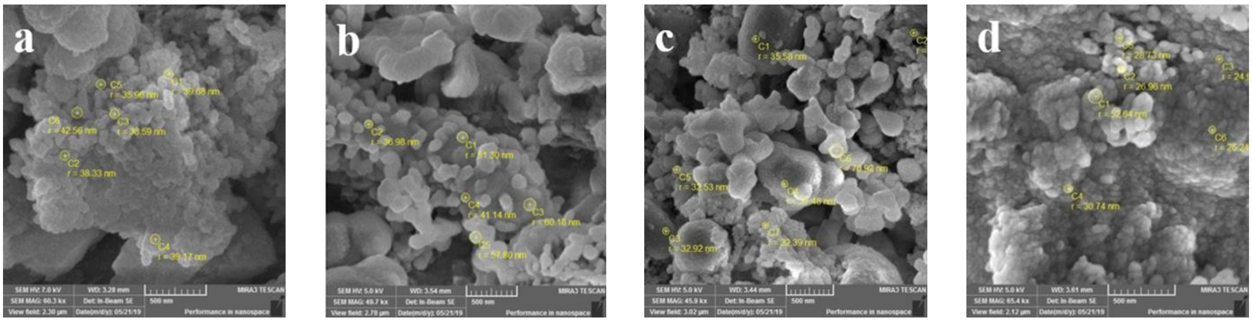


Fig. 5. The SEM photographs of the synthesized Copper Nano Particles
using extract of *Solenostemma argel* (10g)
(a) 0.01 [range 35.96-42.56 nm]; (b) 0.1 [range 36.98-60.18 nm];
(c) 0.03 [range 25.24-36.69 nm]; (d) 0.05 [[range 24-52.64 nm]

Testing of anti-bacterial susceptibility disc diffusion method: The disc diffusion method was used to screen the anti-bacterial activity of Copper NPS by using Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines (NCCLS, 1999). The bacterial suspension was diluted with a sterile physiological solution to 108 CFU/ml (turbidity = McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on the surface of MHA, and the inoculums were allowed to dry for 5 min. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20 µl of a solution of each plant extract. The inoculated plates were incubated at 37 °C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured.

• National Committee for Clinical Laboratory Standards (NCCLS) (1999). Performance standards for antimicrobial susceptibility testing; ninth informational supplement. Wayne, Pennsylvania document M100-S9, Vol. 19.

• The anti-bacterial activity results were expressed in terms of the diameter of the zone of inhibition, and < 9mm zone was considered inactive; 9-12 mm was partially active, while 13-18 mm was active and >18 mm was very active.

• The results were expressed in terms of the diameter of the inhibition zone: < 9 mm, inactive; 9-12 mm, partially active; 13-18 mm, active; >18 mm, very active [16].

In addition, the plant extract in the copper solution. The color changed from blue to green and to black (Brown). It is shown in Figure 3, indicating the formation of copper nanoparticles.

3 Results and Discussions

Characterization of Synthesis Copper Nano Particles (Cu-NPs)

The scanning electron microscopy image of the nanoparticles presented the particles' topography, shown in Figures 4 (a, b, c, and d) and 5 (a, b, c, and d). The TESCAN version MİRAJ took the SEM image at 7.0 kV at 3.28 mm, magnification of 60.3 kx and a view field of 1.73 to 2.38 micrometres at a scale of 500 nm.

In Figure 5a: Seven points have been on the image taken to determine the size of the particles. The minimum particle radius detected was 35.96 nm, while the maximum size was 42.56 nm giving an approximate average of 40 nm. This indicates that the copper has been synthesized at the nano level.

In Figure 5b: The SEM image taken by the TESCAN version MIRAJ at 7.0 kV at 3.28 mm, magnification of 49.7 kx, and a view field of 2.78 micrometres at a scale of 500 nm. Five points have been on the image taken to determine the size of the particles. The minimum particle radius detected was 36.98 nm, while the maximum size was 60.18 nm giving an approximate average of 50 nm. This clearly indicates that the copper has been synthesized at the nano level.

In Figure 5c: The SEM image taken by the TESCAN version MIRAJ at 5.0 kV at WD 3.44 mm, magnification of 45.0 kx, and a view field of 3.08 micrometres at a scale of 500 nm. Five points have been on the image taken to determine the size of the particles. The minimum particle radius detected was 32.39 nm, while the single maximum point was 70.92 nm, but four points lie within 32 nm giving an approximate average of less than 35.0 nm. This indicates that the copper has been synthesized at the nano level.

In Figure 5d: The SEM image taken by the TESCAN version MIRAJ at 5.0 kV at WD 3.61 mm, magnification of 65.4 kx, and a view field of 3.61 micrometres at a scale of 500 nm. Five points have been on the image taken to determine the size of the particles, and the minimum particle radius detected was the special about this image is that all points taken are of size below 30 nm this indicates that the copper has been synthesized at the nano level.

Sometimes, the spectrum obtained from the UV-Vis spectrophotometer must be different every time. The result may have needed to be corrected. Reproducibility is affected if parameters like temperature, pH, and concentration change. The variables affect the spectrum obtained from UV-Vis spectroscopy for measurement of absorbance and studies, and numerous factors impact the results in ultraviolet and visible spectroscopy. The factors may include the effect of sample temperature, where laboratory temperature control is out of control. The effect of sample concentration may change due to time laps or sample pH. These factors may have affected peaks in Figures 6d and 7.

For the stability of copper nanoparticles, see Figure 8. The stability of nanoparticle dispersions is a critical factor in their applications. Several capping agents have been added to reaction media to prevent the agglomeration of nanoparticles [14]. In this work, *Solenostemma argel* was used as a reducing and capping agent without any other special capping agent. The CuNPs was kept at room temperature for seven months and checked the stability was using UV-Vis.

Staphylococcus aureus: S. aureus, Escherichia coli: E. coli, Methiliclin-resistant: MRSA shown in Figure 9.

Table 1. The effects of copper NPs (different concentrations) on the studied bacteria

Copper NPs	0.1	0.05	0.03
Zone of inhabitation			
MRSA	-	-	-
S. aureus	13 mm	10 mm	10 mm
E. coli	11 mm	9 mm	7 mm

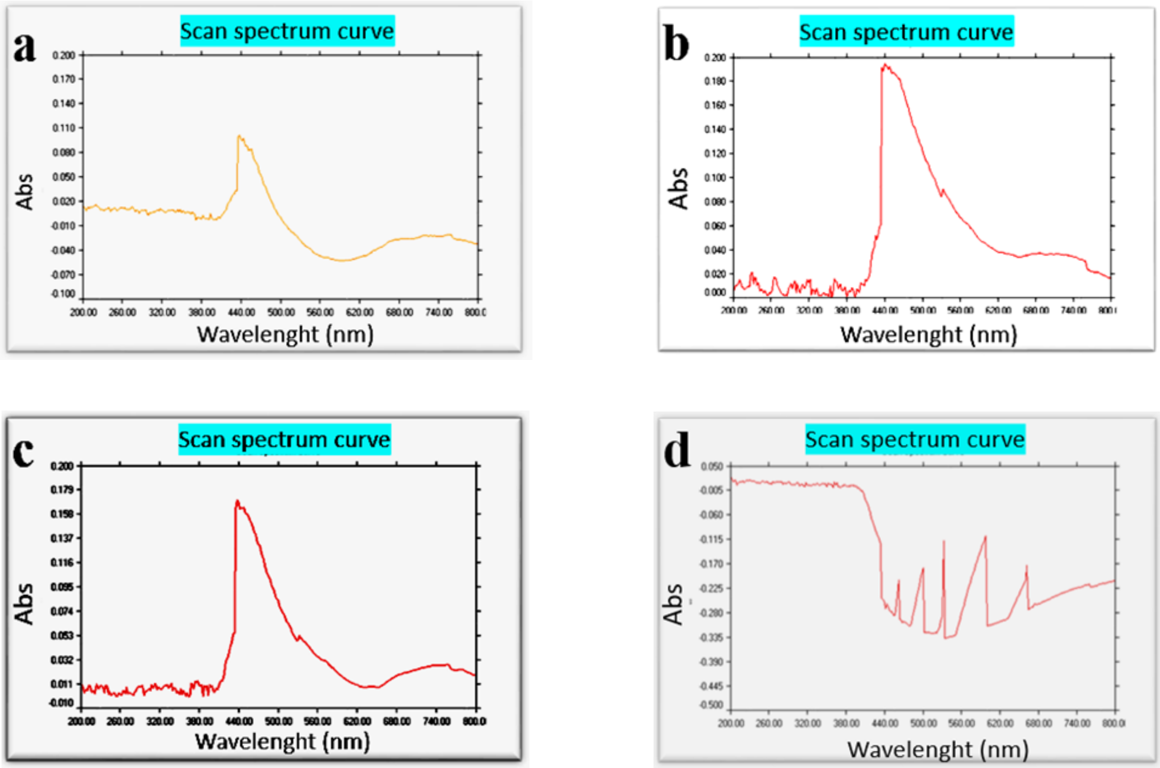


Fig. 6. The UV-Vis absorbance spectra of the synthesized copper NPs sample 0.01M
(a) at 15 min [400-560 nm]; (b) at 30 min [400-560 nm];
(c) at 45 min [400-560 nm]; (d) 60 min [400-560 nm]

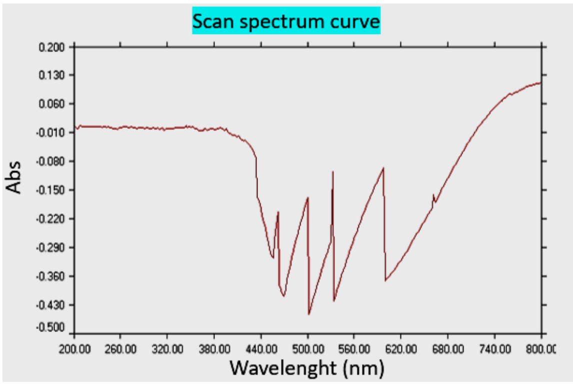


Fig. 7. The UV-Vis absorbance spectra of the synthesized copper NPs sample 0.03 M [450-600 nm]

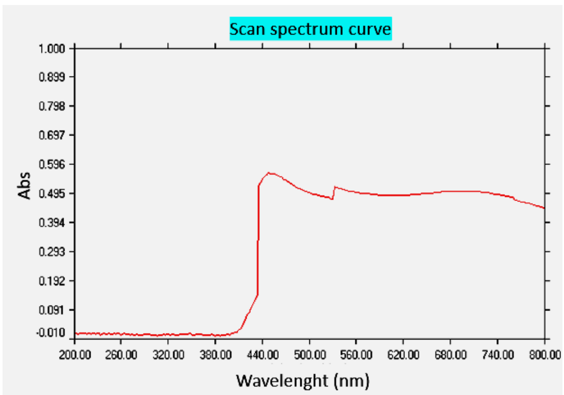


Fig. 8. UV-Vis absorbance spectra of the synthesized copper NPs sample 0.01 M (the stability checked after seven months) range [400-560 nm]

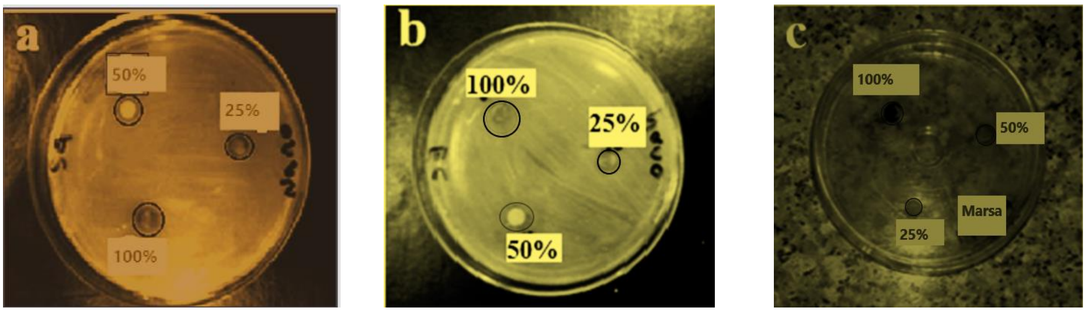


Fig. 9. Anti-bacterial activity against Gram positive and Gram negative
(a) (*S.aureus*) the CuNPs active; (b) (*E.coli*) the CuNPs active;
(c) (MRSA) the CuPNs inactive (no result)

The results showed a zone of inhibition proportional to the concentration of the CuNPs remarkably in the case of *S. aureus* and *E. coli*, where showed no inhibition zone in the case of MRSA.

4 Conclusion

In the present work, the green synthesis of copper nanoparticles using plant extract as a reducing and capping agent has many advantages. As mentioned, it is an eco-friendly, cost effectiveness, and simple route. The process can be performed in a basic chemistry laboratory, is renewable, and gives good scope for scale-up. It minimizes the side effects of chemical-physical methods by preventing toxic chemicals and forming harmful/dangerous by-products. The results have shown that the plant leaves extract is taken a shorter time to acquire the copper nanoparticles in 15 min. Leave extract of *Solenostemma argel* can perform dual functions of reducing and stabilizing copper nanoparticles. SEM and UV-Vis absorption characterized the size and structure of obtained nanoparticles. Copper nanoparticle synthesized by the green synthesis method has shown anti-bacterial activities against gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) while showing very weak or negligible effect on methilicllin-resistant MRSA.

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