

Research Article

Green Synthesis of CuO Nanoparticles Using *Carissa edulis* Stem Bark Extract, and the Antibacterial Evaluation

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Abstract

In this work, copper oxide nanoparticles (CuO NPs) were efficiently synthesized using an environmentally friendly and simple process by applying the aqueous stem bark extracts of *Carissa edulis*. During synthesis of CuO the following parameters were varied so as to achieve the optimum conditions. The first parameter to be varied was precursor ($\text{Cu}(\text{NO}_3)_2 \cdot 2\text{H}_2\text{O}$) concentration. Secondly, was the ratio of extract to precursor salt. Thirdly, the pH was investigated between 6 and 11. Lastly, effects of synthesis temperature was investigated from 25 °C to 70 °C. To characterize the synthesized CuO NPs, Fourier transform infrared (FTIR) spectroscopy, powder X-ray diffraction (XRD), UV-visible spectrophotometer, and scanning electron microscopy (SEM) analysis were used. The average particle size was 20.84 ± 10.19 nm as determined using the XRD technique, which was mainly spherical in shape. The XRD also revealed a monoclinic crystal system of the synthesized CuO nanoparticles. Ultraviolet-Visible analysis showed characteristic peak at 630 nm indicating formation of the CuO NPs. The Tauc plot was used to calculate the optical band gap of CuO NPs from the absorption spectra, which was found to be approximately 2.7 eV. The FTIR peak at 420 cm^{-1} is associated with Cu-O-H stretching suggesting the formation of CuO NPs. Further, the CuO NPs antibacterial potentials were assessed using a standard disc diffusion method. Variety of microorganisms, including *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus* were used to test the antibacterial activity of the synthesized CuO NPs. The synthesized CuO NPs demonstrated highest antibacterial activity against *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus* compared to both positive control (Erythromycin) and carissa edulis extract. The findings suggest that the CuO NPs synthesized using *Carissa Edulis* may be used as an alternative medication to fight bacterial infections.

Keywords

Green Synthesis, Copper Oxide, Nanoparticles, Antibacterial Activity

1. Introduction

Feynman (1960) said that "there is plenty of room at the bottom" when describing the beauty of nanomaterials. As he predicted, the science and technology underlying miniaturization have created novel approaches to the synthesis, characterization, and use of nanomaterials. Because NPs act as bridges

to govern the distance between bulk materials and atomic or molecular assemblies, they have attracted scientific attention. Compared to most materials, NPs' high aspect ratio allows for better reactivity and efficacy [1]. There are two definitions that spring to mind when considering nanomaterials. In a

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broader sense, materials smaller than 100 nm would be referred to as nanomaterials. According to the second definition, which is far more precise and limited, nanomaterials acquire properties based on their size [2].

Thousands of researchers worldwide started investigating and taking advantage of the many opportunities provided by nanotechnology in general and the special qualities of nanoparticles in various fields. The unique mechanical, magnetic, electric, catalytic, and thermal properties of CuO nanoparticles, along with their wide range of applications in agriculture, industry, the environment, and medicine, draw a lot of attention among different kinds of nanoparticles [3]. Cu, Fe (0), Au, Ag, Pt, and other metallic nanoparticles have been synthesized using several techniques so far, although numerous metallic NPs have been employed as antibacterial and anticancer agents [4].

To formulate metal nanoparticles, a number of methods have been devised, including physical and chemical processes. Chemical, physical, and conventional methods of synthesizing nanomaterials have a number of drawbacks, including extremely high temperatures and pressures, the use of costly and dangerous chemicals, longer reaction times, and the production of harmful by-products on the surfaces of the nanomaterials [5]. Green chemistry has been progressively promoted in recent years as environmental conservation has gained greater attention in society. Many scientists have used non-toxic chemicals and solvents in their biosynthesis of copper nanoparticles to meet the requirements [6]. Green synthesis of metal oxide nanoparticles has emerged as an alternative to conventional chemical methods, it offers reduced environmental impact, cost effective, and biocompatible. Numerous plant extracts have been employed as reducing and stabilizing agents in nanoparticle synthesis, each botanical source contributes unique phytochemical profiles that influence the quality of the as synthesized nanoparticles [7].

The large amount of phytochemical components in plant extract that function as stabilizing and reducing agents, transforming metal ions into metal nanoparticles, makes the synthesis of nanoparticles from plant extract possible [8]. Numerous plant species have been studied for the green synthesis of CuO NPs, including *Camellia sinensis* (green tea), *Azadirachta indica* (neem), *Aloe vera*, *Ocimum sanctum* (holy basil) among others. Numerous bioactive compounds found in these plants reduce copper ions and stabilize the resulting nanoparticles [9]. The genus *Carissa edulis* belongs to the *Apocynaceae* family. Many *Carissa* plants have been used in traditional medicine to treat a variety of conditions, including rabies, gonorrhea, syphilis, edema, rheumatism, headaches, and chest complaints. Additionally, the plants have been used to treat worm infestation, ulcers, sickle cell anaemia, fever, cough, and toothaches. Due to their accessibility, ease of preparation, and potential to treat many illnesses, these medicinal plants have been utilized since ancient times [10]. The most significant source of medication is the bark of the stem or roots.

Alkaloids and phenols are the most identified active components with pharmacological significance in these species' well-characterized chemical composition [11].

In this study, *Carissa edulis* stem bark extract is introduced for the first time to synthesize CuO nanoparticles. *Carissa edulis* stem bark is rich in phenolic compounds, flavonoids, tannins, and saponins, which provide abundant hydroxyl and carbonyl groups which are responsible for reduction of Cu²⁺ ions to CuO and simultaneously capping the nanoparticles to prevent agglomeration.

The specific contribution of this work lies in demonstrating that the phytochemical composition of *Carissa edulis* not only facilitates nanoparticle formation hence expanding the library of green synthesis routes and provides comparative insights into how different plant-derived biomolecules influence CuO nanoparticle properties. The synthesized CuO NPs are examined for potential antibacterial properties and their application in medicine.

2. Materials and Methods

2.1. Materials

HCl (35%), (Cu(NO₃)₂·2H₂O) (98%), and NaOH (98%) were purchased from *Precise Lab Africa Ltd., Kenya*. The *Carissa edulis* stem barks were collected from Murkutwa, Elgeyo Marakwet, Kenya. The four bacterial strains tested included: *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus*. The solvents and chemicals used were all of analytical grade.

2.2. Preparation of *Carissa edulis* Stem Bark Extracts

Fresh *Carissa edulis* stem bark gathered from Murkutwa, Elgeyo Marakwet, Kenya, was first chopped into small pieces. The stem bark pieces were then thoroughly cleaned with distilled water to get rid of any remaining dust particles. The cleaned stem bark was then air dried under shade for five days. After drying, it was pounded in a mortar and pestle into a fine powder and kept in a dry place. The powder and distilled water (1:10 weight ratio) were combined to form the *Carissa edulis* stem bark extract, which was heated at 80 °C for 60 minutes in an Erlenmeyer flask until the solution turned dark brown, indicating phytochemical extraction. After cooling to room temperature, Whatman No. 1 filter paper was used to filter the mixture. The filtrate was used to synthesize the CuO nanoparticles [12].

2.3. Biosynthesis of CuO Nanoparticles

0.01 M, 0.1 M and 1 M of copper nitrate solution were freshly made for use. The varied concentration of the precursor salt was mixed with varied amounts of *Carissa edulis*

aqueous extracts in the ratios of 1:1, 5:1 and 1:5. During synthesis the pH of the solution was varied at 6, 8, 9 and 11 using 1 M NaOH and 1 M HCl so as to obtain the optimum pH. To obtain optimum temperature the mixture was heated at 25 °C, 45 °C, 60 °C and 70 °C for one hour while continuously stirring, until the color changed from blue to brown, indicating the formation of CuO nanoparticles. The precipitate (CuO nanoparticles) was washed using distilled water for three cycles by centrifugation at 6000 revolutions per minute (rpm) for 20 minutes. The cleaned CuO NPs were oven-dried at 40 °C overnight and later stored at 4 °C for further characterization [13].

2.4. Antibacterial Activity

The biosynthesized CuO NPs were tested for their antibacterial activity against a variety of microorganisms, including *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus*. CuO NPs' antibacterial potentials were assessed using a standard disc diffusion method. Before being used for the antibacterial tests, the test bacteria were cultivated in nutrient broths overnight. Nutrient agar plates were sterilized and set. Following solidification, 100 µL of each organism's overnight culture was spread onto the Petri plates using a sterile glass rod to generate bacterial lawns. Sterile discs with a diameter of 5 mm were placed on these plates, and CuO

NPs, plant extract, and erythromycin were loaded onto the appropriate discs and kept at 37 °C for 24 hours. After the incubation period, zones of inhibition around the discs were measured in mm [14].

2.5. Statistical Analysis-One-Way ANOVA

Three runs of the antibacterial tests were conducted (in triplicate), and the standard deviation and average of the results obtained were calculated. An Excel spreadsheet program was used to examine the results and determine whether they were statistically significant ($p \leq 0.05$).

3. Results and Discussion

3.1. Synthesis of CuO NPs Using *Carissa Edulis* Stem Bark Extract

The cupric nitrate solution turned from blue to dark-brown on the addition of aqueous stem bark extracts of *Carissa edulis*, which indicated the formation of CuO nanoparticles (Figure 1). As CuO NPs form, their size and surface chemistry changes compared to bulk CuO resulting in a color shift [15]. The phenomenon of colour change is dependent on size and shape transformations.

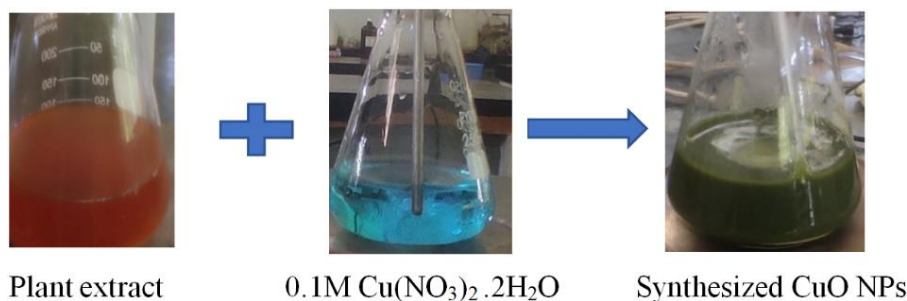


Figure 1. The graphical flowchart for the green synthesis of CuO NPs using *Carissa edulis* stem bark extract.

3.2. Optical Analysis

The maximum absorption peaks were observed between 552 nm to 638 nm (Figures 2-6) at varied synthesis parameters and at optimized synthesis conditions. The band-gap energy plot (Figure 7) for the CuO nanoparticles was 2.72 eV. Green-synthesized CuO nanoparticles with band gap energies of 1.75 eV to 2.72 eV were also reported [16]. The increased catalytic active sites, greater light harvesting, and higher electron transfer ability are all attributed to the critical role of bandgap tailoring enhanced photocatalytic activity and other properties [17]. The maximum absorbance of green-synthesized copper nanoparticles at 580 nm [18] and 670 nm [16] was also similarly observed. The peaks at 784 and 802 nm shown in Figure

2 and Figure 3, respectively, represent the absorbance of the excess unreacted cupric nitrate salt. The salt to extract ratio of 1 to 1 gave a characteristic peak at 552 nm (Figure 3). At the pH of 11, an absorption peak at 632 nm (Figure 4) was observed. At 70 °C (Figure 5), a blue shift to a lower wavelength of 620 nm was observed, signifying reduced sizes of the CuO NPs. When the optimized conditions were used to synthesize CuO NPs, a sharp peak was obtained at 630 nm (Figure 6). This blue shift can be explained by the production of copper nanoparticles in the reaction mixture during stirring and an increased nucleation rate brought on by a higher reduction in Cu^{2+} ions [19]. According to research by [20], the red shift may have been produced by the aggregation of nanoparticles formed.

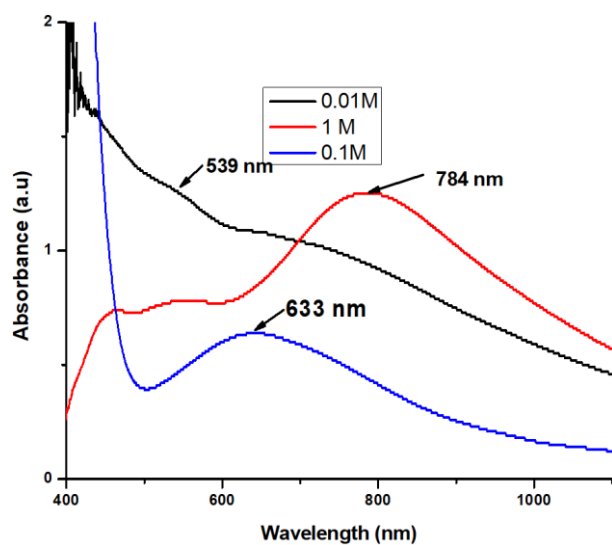


Figure 2. The biosynthesis of CuO NPs at varied precursor molar concentrations.

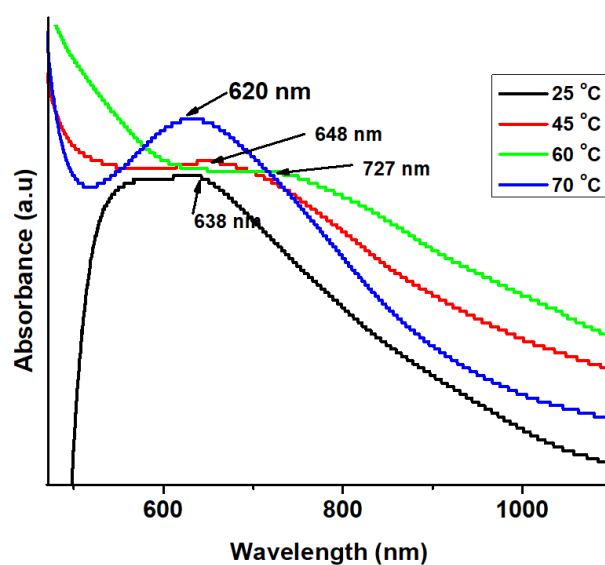


Figure 5. The biosynthesis of CuO NPs at varied temperatures.

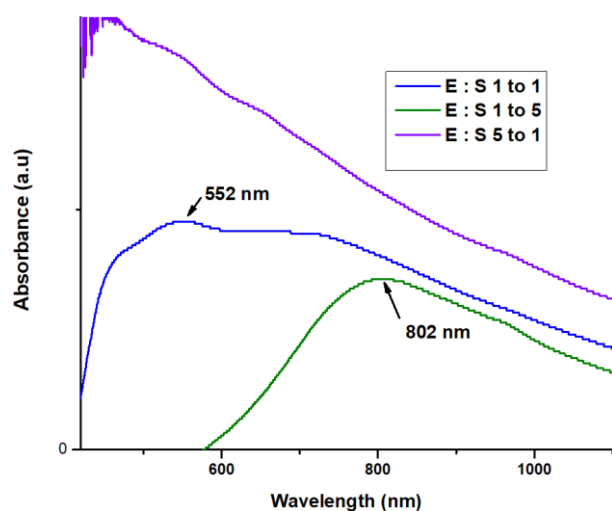


Figure 3. The biosynthesis of CuO NPs at varied concentration ratios of precursor salt and plant extract.

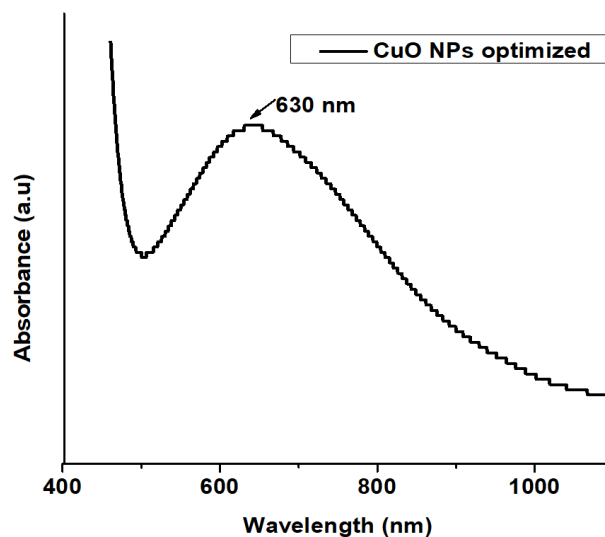


Figure 6. The optimized CuO NPs.

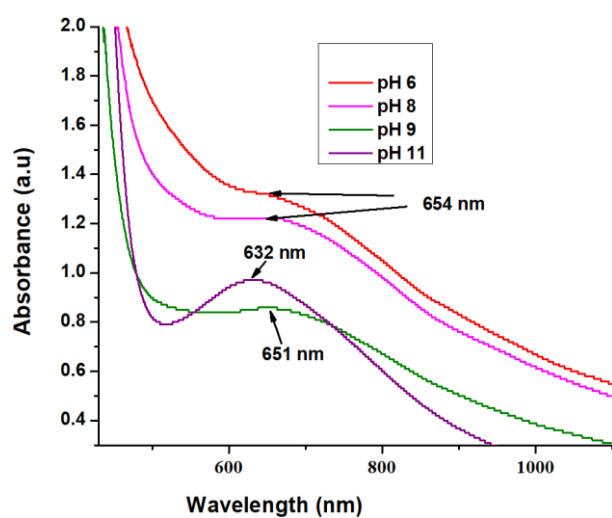


Figure 4. The biosynthesis of CuO NPs at varied pH values.

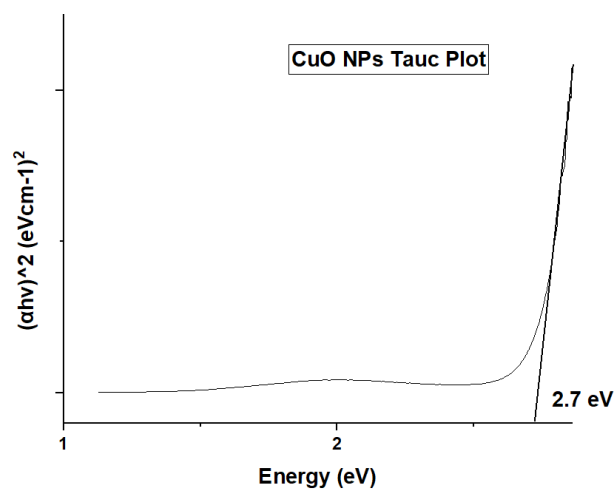


Figure 7. The Tauc plot of used to calculate the band gap of CuO NPs.

3.2. FTIR Analysis

The CuO NPs were examined through FTIR in the 400 cm^{-1} to 4000 cm^{-1} range to identify the functional groups capping them. The phytochemicals responsible for the capping and stabilization of the CuO NPs and the reduction of cupric nitrate solution were identified using FT-IR analysis (Figure 8). The majority of the absorption peaks found in the *Carissa edulis* stem bark extract were also found in the FT-IR spectrum of CuO NPs, often at the same locations or with just slight variations in the peak's intensity and location. The peak at 3431 cm^{-1} in the FTIR spectrum corresponds to the O–H stretching vibration of hydroxyl groups (from alcohols, phenols, or water) of *Carissa edulis* stem bark extract. When *carissa edulis* is used to synthesize CuO nanoparticles, these hydroxyl groups interact with the nanoparticle surface, leading to a reduction in intensity of the peak compared to the pure extract. This indicates that the biomolecules present in the plant act as capping and stabilizing agents for the nanoparticles [14]. The redox reactions for the creation of NPs include spectrum reduction, increase, and alteration [21]. The band at 1643 cm^{-1} shows the aliphatic and aromatic amides' C=O stretching vibration as well as the potential binding of CuO NPs with the extract's proteins. The location of the out-of-plane O-H bending or N-H wagging vibration is 748 cm^{-1} [22]. Cu–O–H stretching can be linked to the peak at 420 cm^{-1} , suggesting the formation of CuO NPs [23]. Nitrile or alkyne functional groups may be connected to the signal at 2101 cm^{-1} , indicating potential stabilizing processes [24].

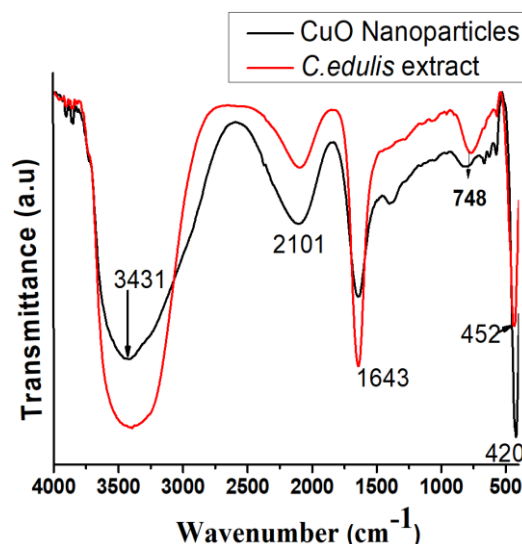


Figure 8. FT-IR spectra of (a) CuO NPs and (b) *Carissa edulis* stem bark extract.

3.3. XRD Analysis

The CuO NPs' XRD pattern shows crystal planes at 37.99° with Miller indices of (1 1 1) in line with the JCPDS (01-089-5895) and strong diffraction peaks at 2θ values (Figure 9) [24]. The XRD data show that the NPs are crystalline and have a distinctive structure. The 110, 111, 220, and 311 planes are represented by the peaks at 2θ values of 30.12° , 37.99° , 41.38° , and 74° in the primary diffraction peaks of CuO NPs. The XRD also revealed a monoclinic crystal system of the NPs. In addition, the crystallite sizes of CuO NPs after synthesis were calculated using the Scherrer equation and found to be $20.84 \pm 10.19\text{ nm}$ (Table 1).

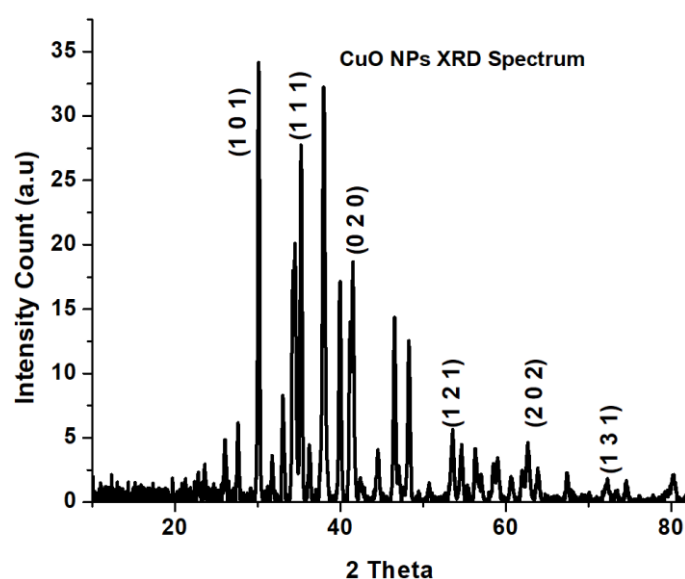


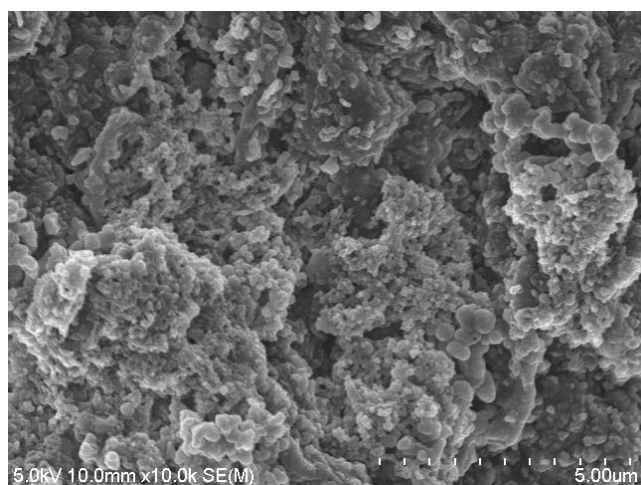
Figure 9. XRD spectrum of CuO NPs.

Table 1. CuO nanoparticle XRD data.

Peak NO	2 θ values (°)	FWHM	Particle size (0.9*0.154)/($\beta \sin \theta$)(nm)
peak 1	30.12	0.25	32.89
peak 2	33.75	0.58	14.31
peak 3	34.4	1.92	4.33
peak 4	37.99	0.37	22.70
peak 5	41.38	0.58	14.64
peak 6	46.53	0.29	29.81
peak 7	48.28	0.32	27.19
Average particle size (nm)			20.84 $\pm$ 10.19

3.4. SEM Analysis

SEM was used to study the surface morphology of the shape and the existence of copper nanoparticles (Figure 10). The sample comprised spherical copper nanoclusters that were present in an agglomerated state, according to the SEM image of the green-synthesized CuO NPs [25]. The enhanced surface energy and surface area of the CuO NPs are responsible for some of the large-size grains that cause the NPs to aggregate. The enhanced surface area to volume ratio allowed the nanoparticles to clump or aggregate together because of the physical forces that attracted them to one another [20].

**Figure 10.** SEM image of CuO NPs.

3.5. Antimicrobial Activity

Table 2 and Figure 11 illustrate the prominent antibacterial activity of CuO NPs against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. The *Staphylococcus aureus* bacteria showed high sensitivity to CuO NPs, indicated by a larger zone of inhibition (12.00 ± 0.82 mm), while the *Pseudomonas aeruginosa* bacteria showed less sensitivity against the CuO NPs (9.00 ± 0.82 mm). In comparison with the plant extract and the positive control antibiotic, CuO NPs were shown to be more effective against all the test microbes, as shown in Table 2. By attracting microbial cells, a nanoparticle's surface charge can enhance its antibacterial efficacy. Microbes with negatively charged cell walls are known to be electrostatically attracted to positively charged nanoparticles [26]. The primary cause of the samples' varying antibacterial activity against the various test microorganisms is the variations in the bacteria's cell walls [27]. The thicker wall of Gram-positive bacteria serves as a barrier, reducing the amount of interaction between nanoparticles and cell walls but in other cases allows small-sized nanoparticles to pass through easily. Lipopolysaccharide covers the outermost layer of Gram-negative bacteria. This attraction causes an accumulation and intake of ions, which damages cells inside [2, 28]. Furthermore, all bacterial species are susceptible to the antimicrobial effects of metallic nanoparticles. Their small size and high surface-to-volume ratio make them bactericidal [29]. Copper ions are released when CuO NPs interact with microbial cells. The microbial cells may die as a result of these ions' ability to cross the cell membrane and cause oxidative stress, in which the Cu^{2+} species react with the sulfhydryl groups of the membrane proteins and DNA to damage their functions [30, 31]. Additionally, these nanoparticles can prevent biofilm formation [32].

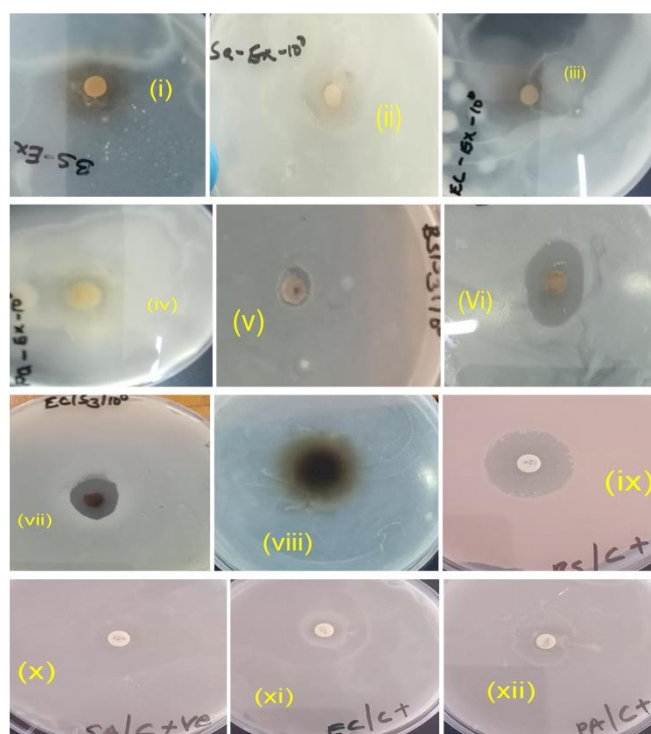


Figure 11. Zones of inhibition of (a) plant extract against (i) *Bacillus subtilis*; (ii) *Staphylococcus aureus*; (iii) *Escherichia coli*; (iv) *Pseudomonas aeruginosa*, and (b) CuO NPs against (v) *Bacillus subtilis*; (vi) *Staphylococcus aureus*; (vii) *Escherichia coli*; (viii) *Pseudomonas aeruginosa*, and (c) erythromycin against (ix) *Bacillus subtilis*; (x) *Staphylococcus aureus*; (xi) *Escherichia coli*, and (xii) *Pseudomonas aeruginosa*.

Table 2. Zones of inhibition values of CuO NPs, plant extracts, and erythromycin against four bacterial strains (*E. coli*, *B. subtilis*, *P. aeruginosa*, and *S. aureus*) according to the disc diffusion method.

Microbial Strains	Sample Name				
	CuO NPs (50 µg/mL)	CuO NPs (100 µg/mL)	<i>Carissa edulis</i> extract (5% (w/v))	<i>Carissa edulis</i> extract (10% (w/v))	Positive Control (15 µg/mL)
<i>Bacillus subtilis</i>	6.33 ± 1.15	9.67 ± 2.52	5.33 ± 0.58	7.00 ± 1.00	10.67 ± 0.47
<i>Pseudomonas aeruginosa</i>	8.00 ± 0.10	11.67 ± 1.53	6.33 ± 1.15	8.83 ± 0.47	7.67 ± 0.47
<i>Escherichia coli</i>	6.33 ± 1.15	9 ± 1.00	5.0 ± 0.00	5.33 ± 0.58	7.83 ± 0.24
<i>Staphylococcus aureus</i>	7.33 ± 0.82	12.00 ± 1.00	5.00 ± 0.00	5.33 ± 0.58	5.67 ± 0.47

3.6. Statistical Analysis-One-Way ANOVA

Table 3. The ANOVA analysis of antibacterial (Zones of Inhibition) results.

Anova: Single Factor				
Groups	Count	Sum	Average	Variance
CuO NPs	4	43	10.75	1.8826
Plant extract	4	26.49	6.6225	2.785558
Positive control	4	31.84	7.96	4.229733

ANOVA						
Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	35.47902	2	17.73951	5.981026	0.022266	4.256495
Within Groups	26.69368	9	2.965964			
Total	62.17269	11				

The acquired anti-bacterial and antifungal results are significant because the p-value is less than 0.05 (Table 3).

4. Conclusions

In this study, green-synthesized CuO NPs were formulated through an economical, simple, and environmentally convenient process using the aqueous stem bark extract of *Carissa edulis*. The synthesized CuO NPs were spherical in shape as observed in SEM images. The results also show that the formulated nanoparticles are stable and monoclinic, with average particle diameters of 20.84 nm as reported in XRD analysis. The nanoparticles were tested against four microbes (*Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus*). The isolates of *Staphylococcus aureus* were more susceptible to CuO NPs than the *Pseudomonas aeruginosa* isolates. According to the study's findings, the CuO NPs synthesized using *Carissa Edulis* may be used as an potential antibacterial agent.

Abbreviations

CuO	Copper Oxide
HCl	Hydrochloric acid
NaOH	Sodium Hydrochloride
UV-Vis	Ultra Violet-Visible Spectroscopy
XRD	X-Ray diffractometer
ZnO	Zinc Oxide
JCPDS	Joint Committee on Powder Diffraction Standards
NPs	Nanoparticles
ANOVA	Analysis of Variance
DNA	Deoxyribonucleic Acid
FWHM	Full Width at Half Maximum

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Conflicts of Interest

The authors declare no conflicts of interest.

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