Phyto-mediated synthesis of copper oxide nanoparticles using *Artemisia abyssinica* leaf extract and its antioxidant, antimicrobial and DNA binding activities

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**ABSTRACT**

Copper oxide nanoparticles (CONPs) are one of the most important metal oxide nanoparticles (MONPs) in the emerging field of nanomedicine due to their remarkable features. Novel CONPs were synthesized using ethanolic (50%, \(\text{v/v}\)) leaf extract of the indigenous plant *Artemisia abyssinica* for the first time. The precursor salt concentration, extract volume, \(\text{pH}\) of the solution, and synthesis temperature were optimized with the UV-Vis spectrophotometer. Using UV-visible spectroscopy, the SPR peaks were observed at 408 nm, and the band gap was found to be 3.039 eV, revealing its semiconductor nature. The presence of phytochemicals on the surface of CONPs was confirmed by FTIR, TGA/DTA, and EDX analysis. The spherical nature and average crystal sizes of the particle 18.4 and 24.6 nm were determined using TEM and XRD analysis. CONPs showed promising antimicrobial activity against selected drug-resistant pathogenic bacterial and fungal strains. The highest inhibition zone was exhibited on *Staphylococcus aureus* (32.5 ± 0.02 mm with MIC value 10 \(\mu\)g/mL) among bacterial strains and *Aspergillus flavus* (22 ± 0.34 mm with MIC value 25 \(\mu\)g/mL) in fungal strains. CONPs revealed the strong antioxidant potential (88.81 ± 0.02%, at 200 \(\mu\)g/mL) with an IC50 value of 5.75 \(\mu\)g/mL. Furthermore, CONPs remarkably exhibited DNA binding activity with CT-DNA.

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1. Introduction

Nanotechnology has recently sparked tremendous research, with applications in science, engineering, agriculture, biotechnology, food science, environment, energy, electronics, space industry, medicine, and biology (1–4). Because of their diverse, physicochemical and structural properties include as small size, large surface area, optical and electrical activity, and mechanical and magnetic features (5, 6). Metal oxide
Copper is needed by our body for a variety of metabolic and physicochemical functioning including serving as a cofactor for numerous enzymes. Therefore, CONPs are highly reactive and easily combine with several molecules, resulting in a wide range of biological functions (36). Consequently, CONPs have a great impact in biomedical applications recently, such as antimicrobial (37, 38), antioxidant (39), antifungal (40), anticancer (41), anti-inflammatory and antidiabetic (42), antimarial (43), and antiviral activities (44).

The Ethiopian indigenous Artemisia abyssinica Sch. Bip. ex A. Rich (Family-Asteraceae; Chikung-Amharic) is a short-lived annual, aromatic, gray, silky hairy plant frequently utilized in traditional medicine and rituals (45). It is a well-known stimulant and reliever, with ternal, grey-green leaves that grow up to 10 cm long. The stems are superiorly grooved and sparsely branching. Infectious diseases (bacterial, viral, protozoan), bronchitis and other inflammatory disorders, cold and fever, anorexia, colic, headache, amenorrhea, and dysmenorrhea are treated by Artemisia abyssinica traditionally (46). In addition, the plant has also been used as an antimarial, antispasmodic, antirheumatic, antioxidant, and antitumor agent (47–49). The major phytochemicals screened in Artemisia abyssinica are alkaloids, flavonoids, saponins, phenolic compounds, tannins, and essential oils (50).

The main objective of this study was to carry out green synthesis of novel CONPs using leaf extract of indigenous medicinal plant and to evaluate its biological activities. Instead of toxic reducing and stabilizing agents, using versatile phytochemicals that make synthesis of eco-friendly and biocompatible nanotherapeutics. Moreover, integration of bioactive molecules of medicinal plant with the CONPs is supposed to be tremendously important for enhancement of bioavailability and bioactivity due to the synergistic effect in varieties of ailments. Therefore, this study attempts to synthesize CONPs by greener route using the leaf extract of Artemisia abyssinica through optimizations of different reaction parameters. The biosynthesized CONPs were characterized by using UV-visible, FTIR, TGA, SEM-EDX, and TEM/HRTEM-SAED techniques. The comprehensive reports of antimicrobial activity of biosynthesized CONPs on drug-resistant pathogenic bacterial and fungal strains, its antioxidant and DNA binding activities was also provided.

2. Materials and methods
2.1 Collection and preparation of plant extract

The fresh leaves of Artemisia abyssinica were collected from Jimma University garden, Oromia region, Ethiopia,
and authenticated at the Addis Ababa University Herbarium (Voucher No. AUGH004). The leaves were frequently cleansed and rinsed with tap water, followed by distilled water to eliminate dust particles, and then air-dried for 15 days in the shade to eliminate moisture contents. Dry samples were mashed in a mechanical blender before being packed into brown bottles. The extractions were prepared using 10 g of powdered plant material in 250 mL conical flasks containing 100 mL deionized water, 50% ethanol (water & ethanol, 1:1 v/v), and n-hexane. The flasks were subsequently covered with aluminum foil to avoid the effect of light. The mixtures were then shaken for 90 min at 120 rpm and 50 °C in a mechanical shaker, allowed to warm for 30 min on a mechanical stirrer at 60 °C and then allowed to cool to room temperature overnight. To obtain a clear solution, the prepared solutions were filtered through Whatman No.1 filter paper, centrifuged, and then stored at −4 °C for the next works.

2.2 Phytochemical analysis of the extracts
The aqueous, 50% Ethanol, and n-hexane leaf extracts of Artemisia abyssinica were subjected to phytochemical screening, and major secondary metabolites such as alkaloids, polyphenols, flavonoids, terpenoids, saponins, protein, and amino acids, anthraquinones, glycosides, and tannins were analyzed by following the reported procedures (46, 51).

**Test for alkaloids:** To 1 mL of extract in test tube 3 mL distilled water and 1 mL 35% HCl were added. The mixture was warmed for 15 min in a water bath, then cooled and filtered. After that, 1 mL of the filtrate was tested with 0.5 mL each of Mayer’s and Dragendorff’s reagents.

a) **Mayer’s Test:** A few drops of Mayer’s reagent (solution of Potassium Mercuric Iodide) were added to 2 mL of extracts. The presence of alkaloids is indicated by the appearance of a light yellow color precipitate.

b) **Dragendorff’s Test:** Two drops of Dragendorff’s reagent (solution of Potassium Bismuth Iodide) were added to 2 mL of extract. The presence of alkaloids is indicated by the production of a crimson precipitate.

**Test for flavonoids:** Flavonoids were determined by two alternative tests:

a) **Alkaline reagent test:** A few drops of sodium hydroxide solution were added to 2 mL extracts. The presence of flavonoids is revealed by producing a bright yellow color that fades to colorless when dilute acid is added.

b) **Lead acetate test:** A few drops of lead acetate solution were added to the 2 mL extracts. The presence of flavonoids is shown by the production of a yellow color precipitate.

**Test for proteins and free amino acids**

**Biuret test:** Equal amounts of 5% sodium hydroxide and 1% copper sulfate solutions were added to the 2 mL extracts. The presence of proteins and free amino acids is observed by the formations of pink or purple color.

**Test for phenols**

**Ferric Chloride Test:** To 3 mL of warmed extract in a water bath 2 mL ferric chloride solution was added. The presence of phenols was determined by the appearance of blue color.

**Anthraquinones test:** A few drops of diluted sulfuric acid extracted with benzene were added to 5 mL of extract to hydrolyze the solution. Finally, it was treated with 1 mL of dilute ammonia. The mild rose pink coloring suggested the minor reactions for anthraquinones.

**Tannins test:** To 5 mL of pure extract in a test tube, a few drops of 0.1% ferric chloride (FeCl₃) were added and allowed to stand sometimes. The brownish-green color observed indicates the existence of tannins.

**Test for Saponins**

**Foam test:** A drop of sodium bicarbonates solution was added to a test tube holding around 5 mL of extracts. The test tube was firmly shaken for 3 min. The presence of saponins is indicated by the appearance of honeycomb-like foam.

**Test for Terpenoids**

**Salkowski test:** To 5 mL of the extracts in a test tube 2 mL chloroform and 3 mL of saturated H₂SO₄ was added. A reddish-brown coloring confirmed the presence of terpenoids near the interface.

**Test for Glycosides**

**Keller-killing test:** To 0.5 mL extract in a test tube 1 mL glacial acetic acid containing traces of ferric chloride and 1 mL concentrated sulfuric acid were mixed. The creation of a reddish-brown color observes the presence of glycosides at the junction of two layers and the upper layer turning bluish-green.

2.3 Biosynthesis of copper oxide nanoparticles
Different concentrations (0.01, 0.1, 0.3, 0.5, and 1M) of Cu (NO₃)₂·3H₂O solutions with varying volumes of the ethanolic leaf extract (10, 20, 30, 40, and 50 mL) at different pH (3, 5, 7.5, 9, and 11) and temperature (25, 40, 50, 60,
70 and 80 °C) were used to synthesize CONPs. Visual observation of blue to brown color was used to assess the formation of CONPs in the solution. The reduction of copper ions and formation of CONPs was assessed periodically by using UV–visible spectrophotometer. The precipitated particles were isolated by centrifuging at 6000 rpm for 20 min and washed with deionized water. The precipitated pellets were dried in a hot-air oven at 80°C for six to eight hours, and stored in proper containers. The purified CONPs were then subjected to characterization.

2.4 Characterizations

The Surface Plasmon Resonance (SPR) peak in the wavelength range of 200–800 nm was determined using an ultraviolet–visible (UV–Vis) JENWAY 6405 Spectrophotometer. The possible biomolecules responsible for the reduction of copper ions into CONPs were tested using Fourier transform infrared spectroscopy (FTIR Shimadzu, Japan 8400S) with the potassium bromide (KBr) disk method over the wavenumber regions between 400 and 4000 cm⁻¹. Moreover, the existing biomolecules and thermal stability of biosynthesized CONPs were analyzed by TGA/DTA (DTG 60H Shimadzu, Japan), heated 0–800 °C. The size and crystalline nature of biosynthesized CONPs were analyzed by A BRUKER D8 Advance XRD, AXS GMBH, and Karlsruhe, West Germany, equipped with a Cu target for generating a Cu Kα radiation (wavelength 1.5406 Å) at GSE. The measurements were conducted at room temperature with an accelerating voltage of 40 kV and an applied current of 30 mA, respectively. The instrument was used in a step scan mode with a step time of 1 s and a degree (2θ) of 0.0200, respectively, spanning a temperature range of 10–180°. External morphology and surface features of CONPs were analyzed using a scanning electron microscope (Tescan Mira 3 LMU), and their elemental composition was assessed using an energy-dispersive X-ray (EDX) spectroscope with a resolution of 1 nm and a voltage of 15 kV. Morphology, particle size, and crystalline nature of CONPs were also characterized using TEM instrument JEOL, JEM-2100 (accelerating voltage up to 200 kV, LaB6 filament), EDS-1.5 Å TEM resolution.

2.5 Antioxidant activity test

Antioxidant activities of the precursor salt, extract, and bio-synthesized CONPs were assessed by DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging assay (RSA) (52). The solution composed of DPPH radical was stored uninterrupted for 3 h in order to confirm its stability. Constant λ max of the solution at 517 nm was observed, which confirms the solutions stability throughout the experiment. Then, 1 mL methanolic DPPH solution (0.1 mM) was mixed with 1 mL methanolic CONPs solutions (200, 150, 100, 50, and 25 g/mL). The reaction solution was mixed and incubated for 30 min at 27 ± 2 °C in the dark. The absorbance of the solutions was read at 517 nm using a UV–vis spectrophotometer. Ascorbic acid and methanol were used as a positive and blank, respectively. Free radical scavenging activity was calculated using the following formula:

\[
\text{DPPH scavenging activity (\%) = } \frac{AB - AS}{AB} \times 100 \tag{1}
\]

where AB absorbance of the blank, and AS absorbance of the sample.

2.6 Antimicrobial activity of extract and synthesized nanoparticles

2.6.1 Antibacterial activity

Antibacterial activity of extract and CONPs was evaluated against Escherichia coli (E.coli, ATCC 25922), Pseudomonas aeruginosa (P.aeruginosa, ATCC 27823), Streptococcus pneumoniae (S. pneumoniae, ATCC 11778), and Staphylococcus aureus (S. aureus ATCC 25923). The antibacterial activity of the extract and CONPs was tested using the agar medium disc-diffusion technique by using Mueller Hinton Agar and Muller Hinton Broth as solid and liquid mediums, respectively, according to the Clinical and Laboratory Standards Institute’s standard protocols (CLSI) (53). The bacterial density was visibly adjusted to 0.5 McFarland standard reagents and swabbed on a plate containing Mueller Hinton culture media from a suspension of fresh bacterial culture added to the liquid media to obtain turbidity similar to the McFarland standard. The disks were soaked with 50 μL of leaf extract and green CONPs dissolved by DMSO at various concentrations (25, 50, 100, and 200 μg/mL). Chloramphenicol impregnated standard disc (30 μg) and DMSO solvent were used as standard antibiotics and negative control, respectively. The cultures were incubated at 37°C for 24 h before measuring the inhibition zone and then measured with a ruler (in mm) to estimate the bacterial strains’ susceptibility to the samples. Also, every experiment was conducted in duplicate petridish, and the results were expressed as mean standard deviation using statistical analysis software SPSS (version 20).

2.6.2 Antifungal activity

The antifungal activity of extract and CONPs against Aspergillus flavus, Aspergillus niger, and Candida albicans was analyzed by the agar well diffusion method (54).
Sabouraud dextrose agar (SDA) culture media plates were made, inoculated with the fungal strains, and incubated at 25 °C for 10 days to see if fungus growth occurred. After 10 days, a core-borer was used to punch wells in the agar plate, and different concentrations (25, 50, 100, and 200 g/mL) of samples dissolved in DMSO were injected into each well. Fluconazole and DMSO were used as positive and negative controls, respectively. The plates were left at 25 °C for 1 h to allow the test sample to diffuse before being incubated at 25 °C for three days. The zone of inhibition against the examined fungus was evaluated after incubation.

2.7 DNA binding activity

UV–visible spectral analysis was performed to examine the interaction between CONPs and calf thymus DNA (CT-DNA) (55). CT-DNA solution was prepared in 0.1 M Tris–HCl buffer (pH = 7.2) with 12 h of stirring at 4 °C. Absorption experiments were carried out by keeping a constant concentration of CONPs (0.2 mg/mL) and varying the CT-DNA concentration (15–240 μL). At different concentrations of CT-DNA, spectral changes of CONPs were observed in the 200–800 nm range of UV–visible absorption.

2.8 Statistical data analysis

Data obtained from the analysis of the Artemisia abyssinica leaf extract and copper oxide nanoparticles (CONPs) samples were analyzed using one-way analysis of variance (ANOVA) of statistical package of social science (SPSS) version 20 and denoted as mean ± SD for triplicate experiments. Also, data analysis was carried out using Origin software (Originpro 9.0 64bit), Gatan Microscopy Suite ® software (GMS 64bit) version 2.x and ImageJ (imagej153-win java8\imagej\imagej.exe).

3. Results and discussion

3.1 Biosynthesis synthesis of CONPs

Biosynthesis of CONPs was performed by using ethanolic (50%, v/v) leaf extract of Artemisia abyssinica. Reaction parameters (precursor salt concentration, extract volume, pH, and temperature) were optimized as described in Figures 1–4. The change in color from blue (aqueous salt solution) and dark brown (pure extract color) to reddish-brown indicates the formation of CONPs. Aside from the color transition, developing a distinctive absorption band associated with CONPs at 406–410 nm also validates the nanoparticles’ formation via the green route (as indicated in Figures 1–4 (37, 56, 57). The characteristic band in the prescribed reported range for CONPs was noticed in all of the synthetic reactions carried out to optimize the synthesis approach of the CONPs (marked in Figures 1–4).

3.2 Optimization of the reaction parameters

3.2.1 Optimization of Cu(NO₃)₂·3H₂O concentration

The effect of precursor salt concentration on the biosynthesis of CONPs was investigated using different concentrations (0.01, 0.1, 0.3, 0.5, 1 M) of Cu(NO₃)₂·3H₂O by fixed volume of Artemisia abyssinica leaf extract. The distinctive absorption peak of CONPs at a wavelength of 400–410 nm was observed in all of five tests of the UV–Vis spectrum (Figure 1), indicating that the concentration range of 0.01–0.3 M was appropriate for CONPs biosynthesis. However, it is not recommended to using the salt beyond this threshold concentration is not recommended because any further increment in the salt concentration in the medium effect inhibits the formation of CONPs. Moreover, the feasible concentration of parent salt among the three for the biosynthesis of CONPs was revealed to be 0.1 M. Because the absorbance at 0.1 M (408 nm) generated by using the 0.1 M salt is the sharpest.

Hence, the maximum yield (58) of CONPs was obtained in this concentration compared to 0.01 and 0.3. Exceedingly high salt contents (0.5 and 1 M) were also found to be unfavorable, as CONPs could not be produced under these concentrations. This was linked to the fact that the existing biomolecules from the extract could not eliminate a large number of precursor ions present in the reaction media. In order to achieve surface stability, the reduced nanostructures were agglomerated on the unreacted salt molecules present in the reaction media. As a result, very big clusters evolved in the reaction media, leaving this reaction situation inappropriate for the biosynthesis of CONPs.

The UV–Vis spectrum of the CONPs was further utilized to calculate the Eg values (eV) of the synthesized nanostructures by utilizing the following empirical formula: \((\alpha h ν)^2 = (h ν – E_g)\); where \(h ν\) represents the optical energy, while \(\alpha\) represents the absorptivity coefficient of the material (59). The Tauc plots (in Figure 1(b–d)) indicated that band gap energy (Eg) values for 0.01, 0.1, and 0.3 M were 3.048, 3.039, and 3.032 eV, respectively. Hence at 0.01 and 0.1, more small nanoparticles were produced compared to 0.3 M, which has the smallest band gap. However, by 0.1 M, small nanoparticles with a high yield have been expected. Accordingly, using 0.1 M of Cu(NO₃)₂·3H₂O solution by considering other parameters is optimum for the biosynthesis of CONPs using leaf extract of Artemisia abyssinica.
3.2.2 Optimization of extract volume
As described in Figure 2, the effect of extract volumes was assessed by varying the ratio of precursor salt to extract volume while keeping all other parameters constant (temperature 25 ± 1 °C, pH 5, and precursor salt 0.1 M). Surface plasmonic resonance peaks were observed with a volume ratio of 10:40, 20:40 which implied the formation of CONPs. As the volume of

Figure 1. (a) UV–Vis spectrum for CONPs prepared at different precursor concentrations; Tauc plot for CONPs prepared at precursor concentration of (a) 0.01 M, (b) 0.1 M and (d) 0.3 M.

Figure 2. UV–Vis spectrum for CONPs prepared at different extract concentrations; Tauc plot for CONPs at (a) 10:40 and (b) 20:40 mL extract ratio.
extract in the reaction media increased, the characteristic peak shifted to longer wavelengths. The surface plasmonic resonance peak of CONPs was not shown at higher (30:40 and 40:40) and lower (5:40) volumes of extract. According to the findings, a moderate amount of the extract is required to synthesize CONPs successfully. Because a sufficient amount of phytochemicals was available in the reaction medium to enable the reduction and stability of CONPs at moderate volumes of extracts. At a lower volume of extract (5:40), the amount of phytochemicals that exist could not stabilize the particles. However, by raising the phytochemical concentrations; to higher volume (30:40 and 40:40) of extract the small-sized nanostructures owing to their higher instability aggregated to give large-sized CONPs.

As provided in Tauc plots (Figure 2(b,c)), the band gap energy (Eg) values for 10:40 and 20:40 were 3.032 and 3.024 eV, respectively. Hence at 10:40 larger band gap indicates the formation of more small nanoparticles compared to 20:40, which has a smaller band gap. However, by 10:40, small nanoparticles with a high yield have been expected. Consequently, using a 10:40 extract to salt volume ratio is optimum for the biosynthesis of CONPs using leaf extract of *Artemisia abyssinica* (60).

### 3.2.3 Optimization of pH

The reaction process was also optimized to determine what pH value was required for CONPs synthesis. The UV–Vis spectral investigation results at various pH values (3, 5, 7.5, 9, and 11) in Figure 3 revealed that at lower pH values (3 and 5) greater absorbance with sharp peaks was observed, indicating the formation of CONPs. Abandoned surface plasmonic resonance peaks were recorded at high pH (7.5, 9, and 11), indicating the inhibition of the synthesis of CONPs. This is because changes in pH affect the type of charge in the extract’s secondary metabolites (56). This change in charge nature impacts phytoconstituents binding and reducing capacities.

Because of the constituent metabolites in the extract of *Artemisia abyssinica*, the ability to reduce the precursor salt concentration was observed at low pH levels. However, due to neutralization and hydrolytic reactions, the acidic nature of metabolites such as polyphenols, flavonoids, and tannins was suppressed in the basic conditions.

![Figure 3](image-url)
medium. As a result, the increased hydroxyl ion concentration in the medium favored agglomeration rather than reduction processes, prohibiting metabolites from producing CONPs at higher pH levels (61). As revealed by SPR absorption peaks and Tauc plots Figure 3(b–d) among lower pH, at pH 5 observed more intense peak with moderate band gap than the others, which means the small size of CONPs with high yield have been produced (62).

3.2.4 Optimization of temperature
Temperature is also an important factor that can determine the medium and yield of the reaction. Consequently, the effect of temperature on the biosynthesis of CONPs is also optimized by varying its value from 25 to 70 °C with keeping all other reaction variables constant. Moreover, the spectral analysis of the synthesis reaction at various temperatures is described in Figure 4. Temperature variation altered the reaction properties of the medium in the same way as other parameters affected (63). Therefore, the heat stability of the phytochemicals was critical for the biosynthesis of CONPs. If the appropriate temperature were not used during the synthesis, the phytochemicals would decompose, and the process of nanostructure reduction and stabilization would suppress (64).

Temperatures of 50, 60, and 70 °C were effective in the biosynthesis of CuONPs, as evidenced by the formation of the typical absorption peaks at 409, 408, and 407 nm, respectively. However, at a temperature of 70 °C maximum absorbance was observed as more yields with small particles were expected. Nevertheless, moderate and low absorbance was observed at lower (25 °C) and higher (80 °C) temperatures; hence they were not optimum for the effective synthesis of CONPs. At higher temperature conditions (80 °C), the phytochemicals become destabilizing, and the collision frequency of the nanocrystals will be increased, which leads to the aggregation of the nanostructures and hence lower absorbance has been detected. The Eg values (Figure 4(b–g)) also demonstrated the optimum temperature range for the biosynthesis of CONPs. The temperature of 70°C was considered optimum due to having higher band gap energy which produces a small size with more yield (64).

3.3 FTIR analysis of synthesized CONPs
FT-IR spectroscopy is an effective technique for the detection of responsible functional groups of biomolecules that act as capping and stabilizing agents in the biosynthesis of CONPs. Absorption bands at 3436, 2072, 1638, and 588 cm$^{-1}$ have been observed in the FT-IR spectrum of Artemisia abyssinica leaf extract (Figure 5(a)). The stretching and bending vibrational frequencies of phenolic –OH are assumed to be involved in the broad band at 3436 cm$^{-1}$ (65). The small peak observed at 2072 cm$^{-1}$ also attributed due to the stretching and bending C–H of methylene groups. The intense peak at 1638 cm$^{-1}$ could be responsible for the stretching vibration of C=O of phenolic compounds. Broad band at 588 cm$^{-1}$ could be attributed due to stretching of C–O or C–O–C (66).

Biosynthesized CONPs were shown in absorption bands at 3334, 3224, 2907, 2072, 1110, and 588 cm$^{-1}$, as indicated in Figure 5(b). The appearance of prominent IR peaks of Artemisia abyssinica at 3443–3224, 2072 and 588 cm$^{-1}$ indicates the contribution of phytochemicals to the biosynthesis of CONPs. The generation of CONPs was also verified by a minor shift in the FT-IR bands of Artemisia abyssinica extract from 1638 to 1385 cm$^{-1}$, as well as the presence of new peaks at 2907 and 1110 cm$^{-1}$. The peak at 2907 cm$^{-1}$ was due to the stretching vibration of atmospheric CO$_2$. The peak at 1385 cm$^{-1}$ corresponds to the stretching vibrations of the Cu–O bond (37). The FT-IR spectra demonstrated that nanoparticles are coated with biomolecules, particularly with the OH residues of alcohols and polyphenols. Polyphenols’ OH residues have a great ability to bond with metal by coating their surface and inhibiting aggregation, which are essential for stabilization (66).

3.4 TGA analysis of CONPs
Thermogravimetric analysis was also performed to confirm the presence of biomolecules on the surface of CONPs, which are used as capping and reducing agents, as illustrated in Figure 6. The DTA thermogram revealed two distinct peaks in the temperature range of 41°C to 800°C. The first endothermic peak at 41°C to 202°C, which corresponds to the first 5.46 % weight loss in the TGA curve. The exothermic peak at 202°C to 345°C observed is linked with the second 16.18 % weight loss in the TGA curve. The first weight loss (5.32 %) observed is associated with the desorption of physically as well as chemically adsorbed water molecules on the surface of CONPs. The second weight reductions on the thermogram were found to be 16.18 % which is due to the decomposition of biomolecules involved in stability and reduction of CONPs. As a result, biomolecules such as alkaloids, flavonoids, and polyphenols are expected to be present in the Artemisia abyssinica leaf extract, which is responsible for the reduction and stabilization of CONPs, as previously mention in (Table 1 and Figure 5). The overall weight
loss detected in the thermogram was 21.64 percent, indicating that the manufactured CONPs contain 78.36 percent copper oxide at a given temperature range.

### 3.5 XRD analysis of synthesized CONPs

Figure 7 shows the X-ray diffractometer (XRD) analysis of biosynthesized CONPs. It exhibited characteristic diffraction peaks at (002), (111), (202), (220) and (311) planes with 2 theta values of 36.4, 38.7, 48.7, 61.5, and 73.6°, which are similar to reflections revealed by various literature. In addition, some small peaks of Cu$_2$O nanoparticles have been shown with characteristic diffraction peaks at (200) plane and 2 theta values of 42.6°, which might result from the reduction of copper nanoparticles. The formation of face-centered cubic
Crystals of copper oxide nanoparticles (CuONPs) were observed, as determined by the JCPDS (card No. 00-048-1548, Tenorite-C2/c). The average crystallite size was calculated to be 24.6 nm by using the Debye-Scherrer equation:

\[ D = \frac{0.9 \lambda}{\beta \cos \theta} \]

where \( \lambda \) is the wavelength of X-ray (0.1541 nm), \( \beta \) is FWHM (full width at half maximum), \( \theta \) is the diffraction angle, and \( D \) is crystallite size.

### 3.6 SEM-EDX analysis of synthesized CONPs

Scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM-EDX) techniques was used to characterize the morphology, structures and composition of the synthesized nanoparticles. As shown in Figure 8(a,b), SEM images of CONPs at low and high resolution were found to be nearly spherical. Moreover, the particles are dispersed across the surface without agglomeration, which might be associated with the presence of bioactive molecules from plant extract. The formation of crystalline CONPs was confirmed by EDX analysis (Figure 8(c)) which revealed an intense optical absorption peaks around 0.5 and 1 and 8 keV energy, confirming the desorption of copper nitrate and the generation of crystalline copper oxide nanoparticles. The minor peaks with neglected weight percentages observed are carbon and nitrogen, which could come from biomolecules bonded to the CONPs’ surface.
3.7 TEM analysis of synthesized CONPs

Figure 9 shows Transmission Electron Microscope (TEM) images of biosynthesized CONPs with nearly spherical shapes and sizes ranging from 11.6 to 26.7 nm, with an average particle size of 18.4 nm as analyzed by ImageJ software. The five spots on the SAED pattern (Figure 9 (d)) were found to correspond to specific CONPs crystal planes observed in XRD measurements in Figure 6. Colored concentric circles were used to indicate the most prominent five planes, which correspond to the (002), (111), (200), (202) and (311) planes, as well as the XRD result. Figure 9(b,c) also demonstrates the monocrystalline character of CONPs with an inter-planer spacing (IPS) of 0.271 nm in the CuO (002) plane.

3.8 Antioxidant assay

In-vitro antioxidant potential of *Artemisia abyssinica* extract, precursor salt (Cu(NO₃)₂·3H₂O) and biosynthesized copper oxide nanoparticles were evaluated against DPPH free radical. The DPPH free radical scavenging potential of each sample was determined compared to ascorbic acid (AA). The acquired results in Table 2 and Figure 10 revealed that the activity of the samples against DPPH radical was dose-dependent, in which the DPPH scavenging activity percentage was directly correlated with concentrations. The precursor salt (Cu(NO₃)₂·3H₂O) revealed comparatively least anti-DPPH activity percentage of 36.78 ± 0.01 at 200 µg/mL with an IC₅₀ value of 1998.19 µg/mL. The extract showed better antioxidant activity of 65.02 ± 0.02 (36.97 µg/mL of IC₅₀) at the same concentration due to the presence of phenolic compounds as mentioned in (Table 1) that have an assured free radical scavenging nature. The bio-synthesized CONPs exhibited the highest DPPH radical scavenging activity (88.81 ± 0.21) with respective an IC₅₀ values of 5.75 at 200 µg/mL concentrations. Compared to the observed anti-DPPH potency of precursor salt and extract at similar concentrations, the inhibitory activity against the free radical of the CONPs was found to be advanced. However, it still needs some modifications on CONPs concerning ascorbic acid activity.

3.9 Antimicrobial activity

This study analyzed in vitro antimicrobial activity of *Artemisia abyssinica* extract and green synthesized CONPs against selected bacterial and fungal strains using the Mueller–Hinton agar disc-diffusion method (53). The
antibacterial activity of extract and CONPs were evaluated against drug-resistant bacterial strains such as *Staphylococcus aureus* (+), *Streptococcus pneumoniae* (+), *Pseudomonas aeruginosa* (-) and *Escherichia coli* (-) by measuring the zones of inhibition and chloramphenicol used as a positive control. In addition, the antifungal activity of extract and CONPs were also evaluated using drug-resistant human and plant pathogenic fungal strains (*Aspergillus flavus*, *Aspergillus niger* and *Candida albicans*).

### 3.9.1 Antibacterial activity

As shown in Table 3 and Figure 11, both extract and green synthesized copper nanoparticles have shown viable *in vitro* antibacterial activity in all selected drug-resistant bacterial strains. In most cases, based on their concentrations, the efficacy of extract and CONPs was shown to be substantial when compared to Chloramphenicol (standard drug) for all bacterial strains. In the case of *S. aureus*, the highest zone of inhibition was identified. Following that, antibacterial activity was shown against *S. pneumoniae*, *P. aeruginosa*, and *E. coli*, as provided in Table 3.

**Table 2.** DPPH radical scavenging percentages (mean ± in mm) of *Artemisia abyssinica* extract, precursor salt, CONPs and ascorbic acid.

| Concentration (μg/mL) | % scavenging activity (mean ± SD) against DPPH radical of *Artemisia abyssinica* extract and Salt, CONPs and AA | CuNO$_3$.3H$_2$O | Extract | CONPs | Ascorbic acid |
|-----------------------|------------------------------------------------------------------------------------------------------------|-----------------|--------|-------|---------------|
| 12.5                  | 19.57 ± 0.02                                                                                               | 39.88 ± 0.04    | 58.67 ± 0.00 | 63.91 ± 0.03 |
| 25                    | 23.13 ± 0.04                                                                                               | 46.66 ± 0.01    | 65.86 ± 0.02 | 70.83 ± 0.00 |
| 50                    | 31.07 ± 0.01                                                                                               | 53.49 ± 0.02    | 73.32 ± 0.01 | 78.31 ± 0.06 |
| 100                   | 31.15 ± 0.02                                                                                               | 58.75 ± 0.01    | 81.26 ± 0.03 | 87.58 ± 0.01 |
| 200                   | 36.78 ± 0.01                                                                                               | 65.02 ± 0.02    | 88.81 ± 0.02 | 94.56 ± 0.02 |
| IC$_{50}$ (μg/mL)     | 1998.19                                                                                                    | 36.97           | 5.75    | 3.78           |

**Figure 9.** TEM micrograph of CONPs (a) at 50 nm, (b) Magnified lattice fringes, (c), IFFT patterns with d-spacing (d) SAED pattern, and (e) Profile of IFFT with d-spacing distance.

**Figure 10.** DPPH radical scavenging activity of precursor salt, *Artemisia abyssinica* extract, CONPs and Ascorbic acid (AA).
Artemisia abyssinica extract has revealed antibacterial activity (>10 mm to <21 mm zone of inhibition) against all bacterial strains with the lowest and highest concentrations used. It showed the highest zone of inhibition (21 ± 0.00) against S. aureus and lowest zone of inhibition (18.5 ± 0.22) against E.coli bacterial strain with MIC (25 and 50 μg/mL), respectively.

CONPs showed the highest antibacterial activity against S. aureus (+), then S. pneumoniae (+), P. aeruginosa (−) and E.coli (−). At 200 μg/mL CONPs showed highest zone of inhibition (32.5 ± 0.02) with MIC (10 μg/mL) against S. aureus strains which slightly proceeded chloramphenicol (31 ± 0.11). Among the tested, it showed the lowest zone of inhibition (27.5 ± 0.22 with MIC 25 μg/mL) with the same concentration (200 μg/mL) against E. coli which is also comparable to chloramphenicol (27.5 ± 0.22). The results confirmed that antibacterial activity of the biosynthesized nanoparticles were toughly higher than the extract against both tested Gram-positive and negative bacterial strains.

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As most studies reported properties of nanoparticles such as size, shape, surface area, and charge distribution can influence the pass of biological barriers and interactions of nanoparticles with cellular environment (67–69). The biosynthesized CONPs was revealed auspicious antibacterial activities in selected standard bacterial cell lines. This may be due to distinctive physicochemical properties of the synthesized nanoparticles including small size, large surface area, and high charge distributions. In addition, controlling parameters, including precursor salt concentrations, plant extract, pH, temperature, and reaction time, could contribute for these aspects. The precise mechanism underlying CONPs antibacterial activity is unspecified; however, adhesion of these particles to bacteria’s surfaces, penetration inside the cell and destruction of bacterial biomolecules and intracellular structures, production of reactive oxygen species (ROS) and free radicals that cause cellular toxicity and oxidative stress, and modulation of the bacterial signal transduction pathway are among the most proposed mechanisms for their action in general (70).

### Table 3. Antibacterial activity inhibition zone (mean ± SD, in mm) of extract, CONPs and Chloramphenicol against standard E. coli, S. pneumoniae, P. aeruginosa and S. aureus bacterial strains.

| Samples  | Concentrations (μg/mL) | S. pneumoniae | P. aeruginosa | E. coli | S. aureus |
|----------|------------------------|---------------|---------------|--------|----------|
| Extract  | 25                     | 13 ± 0.21     | 12 ± 0.13     | 11 ± 0.11 | 14 ± 0.11 |
|          | 50                     | 15 ± 0.00     | 15.5 ± 0.01   | 14 ± 0.33 | 16 ± 0.04 |
|          | 100                    | 16.5 ± 0.12   | 17 ± 0.02     | 16.5 ± 0.14 | 18.5 ± 0.12 |
|          | 200                    | 19 ± 0.14     | 20.5 ± 0.01   | 18.5 ± 0.22 | 21 ± 0.00 |
| MIC      | µg/mL                  |               |               |        |          |
| CONPs    | 25                     | 25            | 30            | 50     | 25       |
|          | 50                     | 25 ± 0.17     | 20 ± 0.33     | 17.5 ± 0.28 | 22 ± 0.21 |
|          | 100                    | 27 ± 0.45     | 23 ± 0.23     | 21.5 ± 0.18 | 24 ± 0.27 |
|          | 200                    | 29 ± 0.31     | 28 ± 0.11     | 27 ± 0.34 | 32.5 ± 0.02 |
| MIC      | µg/mL                  |               |               |        |          |
| Chloramphenicol | (30 μg/disc) | 29 ± 0.04     | 30 ± 0.03     | 27.5 ± 0.06 | 31 ± 0.11 |

Figure 11. Antibacterial activity extract and CONPs against selected bacteria strains.

Artemisia abyssinica extract has revealed antibacterial activity (>10 mm to <21 mm zone of inhibition) against all bacterial strains with the lowest and highest concentrations used. It showed the highest zone of inhibition (21 ± 0.00) against S. aureus and lowest zone of inhibition (18.5 ± 0.22) against E.coli bacterial strain with MIC (25 and 50 μg/mL), respectively.

CONPs showed the highest antibacterial activity against S. aureus (+), then S. pneumoniae (+), P. aeruginosa (−) and E.coli (−). At 200 μg/mL CONPs showed highest zone of inhibition (32.5 ± 0.02) with MIC (10 μg/mL) against S. aureus strains which slightly proceeded chloramphenicol (31 ± 0.11). Among the tested, it showed the lowest zone of inhibition (27.5 ± 0.22 with MIC 25 μg/mL) with the same concentration (200 μg/mL) against E. coli which is also comparable to chloramphenicol (27.5 ± 0.22). The results confirmed that antibacterial activity of the biosynthesized nanoparticles were toughly higher than the extract against both tested Gram-positive and negative bacterial strains.

As most studies reported properties of nanoparticles such as size, shape, surface area, and charge distribution can influence the pass of biological barriers and interactions of nanoparticles with cellular environment (67–69). The biosynthesized CONPs was revealed auspicious antibacterial activities in selected standard bacterial cell lines. This may be due to distinctive physicochemical properties of the synthesized nanoparticles including small size, large surface area, and high charge distributions. In addition, controlling parameters, including precursor salt concentrations, plant extract, pH, temperature, and reaction time, could contribute for these aspects. The precise mechanism underlying CONPs antibacterial activity is unspecified; however, adhesion of these particles to bacteria’s surfaces, penetration inside the cell and destruction of bacterial biomolecules and intracellular structures, production of reactive oxygen species (ROS) and free radicals that cause cellular toxicity and oxidative stress, and modulation of the bacterial signal transduction pathway are among the most proposed mechanisms for their action in general (70).

### 3.9.2 Antifungal activity

As provided in Figure 12 in vitro antifungal activity of Artemisia abyssinica extract and biosynthesized copper oxide nanoparticles against conventional human and plant pathogenic fungal strains. In comparison with Fluconazole (positive control), CONPs showed promising antifungal activity while the extract showed the lowest inhibition zones against all fungal strains. Plant extract
in Table 4 revealed the highest zone of inhibition (13 ± 0.22 with MIC value 30 μg/mL) against A. flavus and the lowest zone of inhibition (11 ± 0.01 with MIC value 35 μg/mL) against C. albicans. CONPs showed the highest zone of inhibition (25 ± 0.34 with MIC value of 15 μg/mL) against A. flavus and the lowest (22 ± 0.11 with MIC value 20 μg/mL) against C. albicans.

However, the biosynthesized COPNs showed inspiring ability in both fungal and bacterial strains, it is demonstrating less activity in fungi when compared to bacteria. This could be linked to fungi having chitin made up of polysaccharides with N-acetylglucosamine and a nitrogen group, and a firmer cell wall. As a result, samples cannot easily pass from the outside layer of the cell wall to the interior layer. Nevertheless, bacteria’s cell wall is composed of peptidoglycan (a polymer containing sugars and amino acids), which is less rigid and allows for easier passage of samples.

### 3.10 DNA binding activity

One of the most fundamental approaches for testing a compound’s DNA binding ability is UV–visible spectral titration. CONPs’ DNA binding capacity was examined using CT-DNA, one of the most important features in the most therapeutic molecule. The stability of CT-DNA was tested at room temperature for 1 hr at 10-min intervals, and the absorption peak stayed consistent (1.78 at 280 nm). The binding efficiency was observed by spectral changes of the CONPs during titration with an increasing amount of CT-DNA. The SPR maxima of CONPs revealed the blue shift (408–435 nm) by increasing the concentrations of CT-DNA from 15 to 240 μL. As shown in Figure 13, the absorption wavelengths of CONPs were 408, 411, 416, 423, 429, and 441 for concentrations of CT-DNA 0, 15, 30, 60, 120, and 240 μL. The blue shift in absorption spectra and lowering the absorbance of the CONPs indicate the strong stacking interactions of nanoparticles with CT-DNA (71).

### 4 Conclusion

In this study, constituent phytochemicals from Artemisia abyssinica leaf extract were employed as natural reducing and capping agents for the biosynthesis of novel CONPs in a simple and eco-friendly way. The generation of viable CONPs in the reaction mixture was confirmed by the revealed Surface Plasmon Resonance (SPR) peak at 408 nm UV–visible spectroscopy. The synthesized nanoparticles have band gap energy of 3.039 eV with

### Table 4. Antifungal activity inhibition zone (mean ± SD, in mm) of extract, CONPs and Fluconazole against standard Aspergillus flavus, Aspergillus niger and Candida albicans fungal strains.

| Name of sample | Concentrations (μg/mL) | Aspergillus flavus | Aspergillus niger | Candida albicans |
|----------------|------------------------|-------------------|------------------|------------------|
| Extract        | 25                     | 8 ± 0.11          | 7 ± 0.01         | 6 ± 0.13         |
|                | 50                     | 9.5 ± 0.33        | 8.5 ± 0.21       | 7.5 ± 0.01       |
|                | 100                    | 12 ± 0.14         | 10 ± 0.22        | 9 ± 0.02         |
|                | 200                    | 13 ± 0.22         | 11.5 ± 0.03      | 11 ± 0.01        |
| MIC (μg/mL)    | 30                     | 35                | 35               |                  |
| BCZONPs        | 25                     | 12 ± 0.28         | 11 ± 0.07        | 10 ± 0.33        |
|                | 50                     | 16.5 ± 0.8        | 15.5 ± 0.15      | 14.5 ± 0.23      |
|                | 100                    | 21.5 ± 0.22       | 19 ± 0.16        | 17.5 ± 0.27      |
|                | 200                    | 25 ± 0.34         | 23.5 ± 0.31      | 22 ± 0.11        |
| MIC (μg/mL)    | 15                     | 15                | 15               | 20               |
| Fluconazole    | (30 μg/disk)           | 29 ± 0.02         | 26 ± 0.21        | 27.5 ± 0.11      |
proper size and optical property for biological activity. The constituent functional groups from the extract phytochemicals were responsible for reducing and capping biosynthesized CONPs, as confirmed by the FTIR spectrum and TGA/DTA. According to XRD, SEM-EDX and TEM/HRTEM-SAED analysis, spherical CONPs with average particle and crystal sizes of 18 and 24 nm, respectively, and face-centered cubic geometry were investigated. The biological activity of synthesized green copper nanoparticles had tested and shown inspiring ability in vitro by scavenging DPPH radicals, inhibiting the growth of different drug-resistant bacterial and fungal strains, and interacting with CT-DNA. In conclusion, biosynthesized CONPs from Artemisia abyssinica leaf extract could be promising candidates for the new therapeutic agent for microbial infection, antioxidant therapy, and DNA targeting drugs.

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