

Green Synthesis of Copper Oxide Nanoparticles Using Leaf Extract of *Justicia Schimperiana* and their Antibacterial Activity

Walelign Wubet Melkamu (✉ wwalelign@gmail.com)

University of Gondar College of Natural and Computational Sciences, Central Gondar Zone

Eskedar Getachew Feleke

University of Gondar College of Natural and Computational Sciences, Central Gondar Zone

Research Article

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Abstract

A fast, efficient, simple and environmentally-friendly, green synthesis method was employed for the synthesis of copper oxide nanoparticles (CuONPs) using easily available, low-cost and non-toxic *Justicia Schimperiana plant leaf extract (JSPLE)* from Ethiopia. Ultraviolet-Visible (UV-Vis) spectrophotometer, X-ray Diffraction (XRD) technique and Fourier-Transform Infrared (FTIR) Spectroscopy were used to characterize the synthesized CuONPs. The UV-Vis confirmed the formation of CuONPs and the maximum absorbance for the synthesized CuONPs in solution at 212 nm. The different parameters like penta hydrated copper sulphate concentration, volume of leaf extract, temperature, pH and time were optimized spectrophotometrically. The FTIR analysis confirmed the existence of various functional groups responsible for reducing and stabilizing during the biosynthesis process. The XRD result confirmed that the synthesized CuONPs were crystalline in nature with the monoclinic structure and the average crystallite sizes of the particles were 21.8 nm. The synthesized CuONPs showed antimicrobial (gram-negative bacteria (*Salmonella typhimurium*, *Escherichia coli* and *Klebsiella pneumoniae*) and grampositive bacteria (*Staphylococcus aureus*)) activity. It has been noted that the green synthesized CuONPs showed efficient antibacterial activity against gram-negative (*salmonella typhimurium*) bacteria with maximum ZOI of 18.6 mm with respect to the highest concentration (150 µg/mL). The developed method for the CuONPs synthesis using JSPLE is an eco-friendly and convenient method. In the near future, the synthesized CuONPs could be used in the fields of biomedicine, biosensor, water treatment and nanotechnology.

Introduction

Nowadays, the potential applications of nanomaterials in catalysis, electric, optical, photonic, textile, nanofluid, and antibacterial activity make it to become an extensive area of research [1, 2]. Physical, chemical, and biological processes are among the verities of ways that generated nanostructures. Due to their usage of harmful substances, high cost, and energy requirements, physical and chemical procedures are not chosen. To address the side effects of physical and chemical approaches, green methods have arisen [3]. For the synthesis of metal oxide nanoparticles, ecofriendly methods such as the use of plant extract, microorganisms, and algae have attracted much attention. Due to its easy handling, easy accessibility, cheapness, and compatibility with the biomedical applications, plant extract was more selective than other green methods for the synthesis of metal nanoparticles [4].

Manifestations of unique and phenomenal properties of the metal nanoparticles make them to be of particular interest. The metal nanoparticles found to have merits arising because they exhibit properties like good electrical conductivity, large surface area, chemical and optical properties, and their small size. Among the various categories of metal nanoparticles, copper oxide nanoparticles (CuONPs) are viewed as an alternate to noble metals due to its cost-effectiveness, economically attractive and promising, nontoxicity and easy preparation [5, 6]. The various methods like chemical methods, solution combustion methods, thermal oxidation route, hydrothermal approach, thermal decomposition, reverse microemulsion methods, and precipitation methods have been employed for the preparation of copper oxide

nanoparticles. However, these methods involve toxic chemicals harmful to the environment and exclude their applications in biology, medicine, and clinical applications [7, 8]. Developing simplistic and environmentally friendly procedures for preparing copper oxide nanoparticles are of importance and still a trouble for materials researchers [8].

Different parts of herb extracts have been used for the preparation of copper oxide nanoparticles for various applications, namely Vasaka (*Justicia adhatoda* L.) Leaf Extract [9], *Tabernaemontana divaricate* leaf extract [10], Seedless dates [11], *Ocimum basilicum* leaf extract [12], *Punica granatum* peels extract [13], *Psidium guajava* leaf extract [14], *Cassia fistula* and *Melia azedarach* leaf extracts [15], *Cordia sebestena* (*C. sebestena*) flower aqueous extract [16], Mint leaves and orange peels extracts [17], *Cedrus deodara* aqueous extract [18], *Achillea millefolium* leaf extracts [19], *Abutilon indicum* leaf extract [20], *Malva sylvestris* Leaf Extract [21], *Leucas aspera* and *Morinda tinctoria* leaf extracts [22], *Justicia gendarussa* leaf extract [23], *Justicia adhatoda* leaf extract [24], *Juglans regia* leaf extract [25], *Ixiro coccinea* plant leaves [26], *Aloe Vera* leaf extract [27], *Gloriosa superba* L. extract [28], *Pistia stratiotes* leaf extract [29], *R. tuberosa* plant leaf extract [30], *Carica papaya* leaf extract [31], seed varieties of rice (*Oryzasativa* L.) [32], *C. persicus* leaf extract [33], *Catha edulis* leaf extract [34], and *Annona squamosa* seed extract [35] are among the herb part extracts that have been reported in literatures.

The nanoparticles biosynthesis mechanism by different plant extracts is mysterious, it has been suggested that the phytochemicals in plant extract play a significant role in the reduction of metals ions and capping the biosynthesized nanoparticles [36]. In addition, in the majority of cases, a reducing agent from the plant extract plays a role as both the capping and stabilizing agents [37].

Lists of antibiotic-resistant priority pathogens have been published by the World Health Organization (WHO) in 2017. These pathogens present a great threat to humans and to which new antibiotics are urgently needed. Gram-negative bacterial pathogens are the majority of the WHO list. Due to their peculiar structure, Gram-negative bacteria are more resistant than Gram-positive bacteria, and cause remarkable morbidity and mortality worldwide [38].

Therefore, the present study focused on the green synthesis of CuONPs using the JSPLE and evaluating its anti-bacterial activities. *Justicia schimperiana* belongs to the family of Acanthaceae and this species is known from Ethiopia, Eritrea, Somalia and Kenya (Fig. 1). Previous studies showed that each part of the plant is used in the treatment of various diseases such as skin irritation, stomachache, wound, asthma, common cold, hepatitis B (jaundice), rabies, diarrhea, tapeworm infestation, anthrax, external parasite, malaria, and ascariasis [39, 40]. To the best of our knowledge and literature survey, there has been no study on the green synthesis of CuONPs using leaf extract of *Justicia Schimperiana* and no studies have been conducted on the green synthesis of CuONPs using JSPLE for any application except using the crude extract of the plant. Our investigation was therefore focused on the characterization of the biosynthesized nanoparticles using UV-Visible spectrophotometer, XRD and FTIR. In addition, the anti-bacterial activity of the synthesized CuONPs against gram-negative bacteria (*Salmonella typhimurium*,

Escherichia coli and *Klebsiella pneumoniae*) and grampositive bacteria (*Staphylococcus aureus*) were evaluated by standard agar well diffusion method.

Materials And Methods

In this study, analytical grade chemicals and reagents and double distilled water were used throughout the experiment. Copper(II) sulphate pentahydrated ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 98.5%), ferric chloride (FeCl_3 , 99%), hydrochloric acid (HCl, 35.4%), and sulphuric acid (H_2SO_4 , 98%) were from Loba Chemie Pvt. Ltd, India, Gentamicine were from Abcek adtek Pvt. Ltd; potassium bromide (KBr) was from Uvasol, Germany; chloroform (99.9%) was from Fisher Scientific UK Limited, UK; sodium hydroxide (NaOH, 98%) and ammonia solution (25%) were from Blulux Laboratories Pvt. Ltd; Benedict's solution, Magnesium chloride (MgCl_2 , 98%), iodine solution (80%), potassium Iodide (KI, 99%) were from Abron Chemicals, India; and *Justicia schimperiana* leaf extract.

Plant material and preparation of the extract

The plant *Justicia schimperiana* was collected from its Natural habitat in January, around Azezo, Central Gondar Zone, Amhara region, Ethiopia. Identification of the plant specimen was done by Dr. Getnet Masresha, Botanist at Department of Biology College of Natural and Computational Sciences, University of Gondar and Confirmed by Mr. Abiyu Enyew Molla (M.Sc.), Assistant Professor of Botanical Sciences, Department of Biology, University of Gondar, Ethiopia. Additionally, the identity of the plant was assessed from the book entitled Flora of Ethiopia and Eritrea, Volume 5 [41]. Tap water was used to remove any dust particles on Fresh leaves of *Justicia schimperiana* and rinsed with distilled water. To remove moisture contents from the leaves, it was allowed to dry under shadow room temperature for 20 days. To get a fine powder of the sample, an electrical grinder was used to powder the dried leaf. Plant powdered leaves of 50 g in a 1000 mL Erlenmeyer flask containing 400 mL of distilled water was taken to carry out the extraction. To prevent the effect of light on the solution, it was covered with aluminum foil. Mechanical shaker was used to shake the mixture for 30 min and allowed to warm at 60 °C for 20 min on a hot-plate with magnetic stirrer, and then it was allowed to cool down at room temperature. To get the clear extract solution, the prepared solution was filtered by Whatman No.1 filter paper. For future experiments, the filtrate was stored at 4 °C [42].

Preliminary phytochemical screening

The absence or presence of phytochemicals like alkaloids, flavonoids, saponins, tannins, terpenoids polyphenols, cardiac glycosides, anthraquinones and carbohydrates in the leaves of *Justicia schimperiana* was evaluated using standard testing methods [43, 44, 45]. **Tables 1** and **2** showed the details of the methods used for the qualitative phytochemical screening of JSPL and the results on phytochemical screening test respectively.

Table 1

Qualitative phytochemical screening of JSPLE.

Phytochemicals	Reagent/Test
Test for Alkaloids	Mayer's reagent test: 6 mL of aqueous JSPLE was added to 3 mL 1% of HCl and it was stirred and heated for 20 min on water bath. The resulted reaction mixture was cooled at room temperature and used to perform Mayer's test. To the cooled reaction mixture in a test tube, 1 mL of Mayer's reagent was added drop by drop. The formation of Turbidity of the resulting precipitate was taken as an evidence for the presence of alkaloid. Further addition of a few drops of olive oil to form an emulsion confirmed the presence of alkaloids.
Test for Tannins	Ferric chloride test: To 3 mL JSPLE solution, 3 mL of distilled water and 2 drops of 1% ferric chloride solution were added and the observed brownish green color confirmed the presence of tannins.
Test for Saponins	Frothing test: 3 mL of aqueous JSPLE was dissolved in 5 mL of distilled water. Then, the mixture was shaken vigorously. The formation of stable persistent froth confirmed the presence of saponins.
Test for Flavonoids	Alkaline reagent test: 2 mL of the aqueous JSPLE were treated with few drops of 1 mL of 10% NaOH. The formation of an intense yellow color was indicated and it confirms the presence of flavonoids.
Test for Terpenoids	Salkowski test: 3 mL of the aqueous JSPLE solution was mixed with 2 mL of chloroform and 2 mL of concentrated sulfuric acid. The formation of a reddish brown colour in the interface indicated the presence of terpenoids.
Test for Cardiac glycosides	Killer killiani test: 2.5 mL of distilled water and 1 mL of glacial acetic acid containing a few drops of ferric chloride was added to 0.5 mL of aqueous JSPLE. Then, 1 mL of concentrated sulfuric acid was added. The formation of brown ring at the interface was indicated for the presence of cardiac glycosides.
Test for polyphenols	Ferric chloride test: 1 mL of the aqueous JSPLE and 2 mL of distilled water was mixed. Then, it was treated with 2 mL of 10% ferric chloride solution. The formation of blue color in the reaction mixture was an indication for the presence of phenols or polyphenols.
Test for anthraquinones	Bontrager's test: 1 mL of JSPLE was dissolved in 5 mL of concentrated sulfuric acid and it was filtered. Then, the filtrate was dissolved in 2 mL of chloroform. Chloroform layer was pipetted into a tube and 0.5 mL of 10% diluted ammonia was added. The formation of pink red colour was an indication for the presence of anthraquinones.
Test for carbohydrates	Molisch's test: 1 mL of Molisch's reagent was added to 2 mL of JSPLE. Then, few drops of concentrated sulphuric acid were added. The presence of purple-reddish color was an indication for the presence of carbohydrates.

Green Synthesis of CuONPs

To synthesize CuONPs, an optimized concentration of aqueous solution of penta hydrated copper sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) (3 mM) was prepared in 250 mL amber volumetric flask. An optimized volume of JSPLE (5 mL) was mixed with optimized volume (45 mL) & concentration (3 mM) of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ solution for the bio-reduction of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ to CuONPs. Optimal temperature of 31 °C and an incubation time of 60 min were used to carry out the synthesis of CuONPs in a closed incubator. After optimized reaction conditions, a color change from brown red to greenish yellow was observed and this was an indication for the formation of CuONPs.

Table 2

Results on Phytochemical screening of JSPLE.

S.No.	Bioactive Phytochemicals	Test/Reagents	Result
1	Alkaloids	Mayer's reagent test	+
2	Polyphenols	Ferric chloride test	+
3	Flavonoids	Alkaline reagent test	+
4	Tannins	Ferric chloride test	+
5	Cardiac Glycosides	Killer killiani test	+
6	Saponins	Frothing test	—
7	Terpenoids	Salkowski test	—
8	Anthraquinones	Bontrager's test	—
9	Carbohydrates	Molisch's test	+
Note: + = Present, and — = Absent			

The UV–Vis spectrum of the reaction solution confirmed the bio-reduction of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ to CuONPs. Repeated centrifugation method at 3000 rpm for 30 min separated out the CuONPs from the mixture, followed by re-dispersion of the pellet in deionized water to remove any unwanted biological materials. For further characterizations, the separated CuONPs were collected and dried in an oven at 80 °C for 2h and stored at room temperature [46]. Effects of concentration, reaction time, volume ratio, temperature, and pH on the formation of CuONPs were investigated. The study was monitored using double beam UV-Vis spectrophotometer (Agilent technologies, Cary 60 UV-Vis; USA). The experiment was carried out by optimizing the concentrations of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ solution (1 mM, 2 mM, 3 mM, 4 mM, and 5 mM); reaction time (10 min, 25 min, 40 min, 55 min, and 70 min); volume ratio with respect to $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ to JSPLE (1:1, 1:2, 1:3, 1:4 and 1:5); temperature of the reaction mixture (25 °C, 35 °C, 45 °C, 55 °C and 65 °C); and effects of pH (pH 6, pH 7, pH 8, pH 9, and pH 10).

Characterization of CuONPs

UV-vis Spectroscopy Analysis

The biosynthesis of CuONPs and the bio-reduction of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ to CuONPs were examined for surface plasmon resonance (SPR) by using double beam UV-Vis spectrophotometer (Agilent technologies, Cary 60 UV-Vis; USA) in the wavelength range of 200–800 nm. The samples were prepared in cuvettes that are made using quartz so that they do not absorb any light and will not affect our measurements. Deionized water was used as a blank [47].

FTIR analysis

The FTIR measurement was carried out using FTIR spectrometer (PerkinElmer spectrum LS-65-Luminescence; USA). The FTIR spectra of the synthesized CuONPs were obtained in the range of 4000–400 cm^{-1} . The FTIR spectrometer was used to determine the functional groups linked to the surface of CuONPs which are responsible for the reduction, stabilizing and capping agents. For FTIR studies, all the colloidal nanoparticles were dried at 75 °C in the form of nanopowders and palletized with KBr [2].

XRD Analysis

The crystalline structure, nature of the phase, and crystalline grain size of CuONPs were determined by using advanced X-ray Diffractometer (Rikagu Minflux 600 Powder diffractometer (USA). The instrument was adjusted with a Cu tube and a graphite monochromator with $\text{CuK}\alpha$ radiation ($\lambda=1.5406\text{\AA}$) and a scan speed of 1°/min in the range between $2\theta = 10$ to 80° [2, 47].

Antibacterial Activity

The antibacterial activities of biosynthesized CuONPs were performed by following the procedure [48]. Agar well diffusion method was used to evaluate it. The antibacterial activity was studied using three gram-negative bacteria (*Salmonella typhimurium*, *Escherichia coli*, and *Klebsiella pneumoniae*) and one gram-positive bacteria (*Staphylococcus aureus*) clinically isolated pathogenic bacteria. The media used was Mueller-Hinton Agar (MHA) and it was prepared according to the manufacturer's instruction, where 35 g of powder media was mixed with one liter of distilled water and enclosed in a container and autoclaved at 121 °C for 15 min. The media were later dispensed into sterile Petri dishes. After the media was solidified, 100 μL of the working stock culture was spread with sterile cotton swab and wells were made in each Petri dish by a stainless steel cork borer. Then different concentrations of CuONPs and JSPLE (50 $\mu\text{g/mL}$, 100 $\mu\text{g/mL}$, and 150 $\mu\text{g/mL}$) were prepared separately from 100 mg/mL stock solution. Wells were filled with the prepared solutions of CuONPs, Dimethylsulfoxide (DMSO) (negative control), and gentamycin (positive control) and kept in refrigerator for 30 min until it diffuses. The petri dishes were incubated at 37 °C for 24 h. Finally, zone of inhibition of green synthesized CuONPs against the pathogenic bacteria were measured in millimeter.

Results And Discussion

Phytochemical analysis of the plant leaf extracts

To determine the presence of some phytochemicals in the leaves of this medicinal plant, qualitative phytochemical screening analysis was done on JSPLE. The presence and/or absence of useful bioactive compounds such as Alkaloids, Tannins, Saponins, Flavonoids, Terpenoids, Cardiac glycosides, Polyphenols, Anthraquinones, and Carbohydrates in leaf extracts were revealed by the confirmatory test, involving color changes, precipitate formation and other confirmations. **Fig. 2** below and **Table 2** above show the summary of phytochemical characteristics of the JSPLE. The results revealed that bioactive compounds like Alkaloids, Polyphenols, Flavonoids, Tannins, Cardiac glycosides, and Carbohydrates are present in JSPLE while Saponins, Terpenoids and Anthraquinones were absent in this plant leaf extract. The presence of these bioactive components may be responsible for the reducing, capping and stabilization of CuONPs.

CuONPs Biosynthesis

Successful synthesis of CuONPs were performed using an optimized volume (45 mL) & concentration (3 mM) of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ solution and the optimized volume (5 mL) of JSPLE as a reducing and capping agent, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ as a precursor and with other optimized parameters. When the brown-red colored JSPLE was added on the solution of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, the solution turned green-yellow, while no color change was observed in the absence of plant extract (**Fig. 3**). Color change in the reaction mixture could be taken as first hand information for the formation of CuONPs. The prepared CuONPs (**Fig. 3C**) were later put through to various characterization methods.

Reducing agents in the green synthesis of metal nanoparticles using plants are believed to be Phytochemicals/secondary metabolites present in plants. Secondary metabolites are responsible for the reduction of metal ions to zerovalent metals or the stabilization of metal/metal oxide nanoparticles. **Fig. 4** illustrated some proposed bio-reduction mechanism for CuONPs by phytochemicals [49].

Characterization of synthesized CuONPs

UV-Vis spectroscopy analysis

UV-Vis spectroscopy was used to monitor the process of the bio reduction of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ to CuONPs and to monitor the formation and optimization of CuONPs. Color change was the first-hand information for the formation of CuONPs which was further confirmed by the appearance of characteristic Surface Plasmon Resonance (SPR) peak between 206 to 212 nm [26]. Formation of the dark-brown color of the synthesized CuONPs in aqueous solution was an indication for the bioreduction of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ to CuONPs. The observed peaks in the range of 206 to 212 nm confirmed the formation of CuONPs (**Fig. 5a-e**). The synthesis of CuONPs was affected by the different parameters like temperature, pH, time, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ concentration and volume of leaf extract.

Effects of operational parameters for the synthesis of CuONPs

Operational parameters like reaction time, temperature, concentration, volume of JSPLE and pH of the reaction mixture influence the synthesis of CuONPs. These parameters influence the nanoparticles synthesis irrespective of the technique used. In this study, evaluation of several important experimental factors, including reaction time (10 min, 25 min, 40 min, 55 min and 70 min); temperature (25 °C, 35 °C, 45 °C, 55 °C and 65°C); concentration of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ solution (1 mM, 2 mM, 3 mM, 4 mM, and 5 mM); volume of JSPLE (1 mL, 2 mL, 3 mL, 4 mL, and 5 mL); and pH of the reaction mixture (pH 6, pH 7, pH 8, pH 9, and pH 10) were studied. UV–Vis spectrophotometer was used to monitor each of these experimental factors.

Effects of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ concentration on the synthesis of CuONPs

Fig. 5a shows the effect of concentration on the synthesis of CuONPs from JSPLE and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$. The experiment was carried out on the following series of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ concentration: 1 mM, 2 mM, 3 mM, 4 mM, and 5 mM at fixed amount of 5 mL of JSPLE as both reducing and stabilizing agent. The intensity increases with an increase in the concentration of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$. For a concentration of 3 mM $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ solution, a distinctive SPR peak and maximum absorbance was observed at a wavelength of 212 nm and this was considered as an optimal value for the synthesis of CuONPs. As the concentration of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ increased, the height of the absorption peak of UV-Vis spectrum around 208 nm intensifies. The production of CuONPs was confirmed by peaks of different heights at 208 nm. The size and shape of the CuONPs produced were affected by varying the concentration of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ solution. From the previous studies, it has been confirmed that as the concentration of nanoparticles increases the size of nanoparticles decreases. This is due to the creation of an excess number of nuclei at high concentrations, which causes nanoparticles to assemble together and expands in size [50, 51].

Effects of volume of the JSPLE

We have studied the effects of volume ratio by varying the volume of the leaf extract (1 mL, 2 mL, 3 mL, 4 mL, and 5 mL) and by keeping constant the volume (45 mL) and concentration (3 mM) of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ solution. For the volume 5 mL of JSPLE, an absorption peak at 206 nm with maximum absorbance was recorded and it was taken as an optimized condition for the synthesis of CuONPs. As shown in **Fig. 5b**, the absorption peaks were slightly broader and lower absorbance was recorded for the JSPLE volumes of 1 mL, 2 mL, 3 mL, and 4 mL which indicate a slow bio-reduction of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ to CuONPs. Previous studies showed that when a small amount of leaf extract was utilized to make CuONPs inadequate absorption was observed, indicating insufficient copper ion reduction. In this study, it has been proved that lower amounts of JSPLE were insufficient to complete reduction of 3 mM $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ concentration to CuONPs. The other optimization parameters were carried out using this optimum volume (5 mL) of JSPLE. When the leaf extract volume was increased to 5 mL, the strength of

the Surface Plasmon Resonance (SPR) peak increased and this indicated a higher conversion rate but the reverse has happened during our trial (i.e., when the volume of JSPLE increased to 6 mL) which was a saturated level. The result was in a good agreement with the previously studied and biosynthesized CuONPs [42, 50, 51].

Effects of temperature for the synthesis of CuONPs

Fig. 5c showed the UV–Vis absorption spectra of CuONPs obtained from aqueous solution of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ with JSPLE. By keeping other optimal conditions constant, the spectra were recorded at 25 °C, 35 °C, 45 °C, 55 °C and 65 °C. The color of the CuONPs was changed from green-yellow to black for the different temperatures used. The maximum absorbance has been recorded at 65 °C with maximum wave length of 212 nm and the absorption intensity was slightly increased in comparison to others. During our experimentation, the reverse happened when the temperature rises above 65 °C. Some researchers working on green synthesis of nanoparticles recommended the ideal synthesis temperatures to be less than 100 °C and some others recommended the synthesis temperature to be usually kept at ambient temperature. Other researchers also recommend temperatures above 70 °C are inappropriate for the production of nanomaterials. This is due to the very volatile nature of plant phytochemicals [51]. The favorable temperature for the formation of CuONPs was found to be 65 °C. This indicated that high temperature is not recommended for the reduction and stabilization of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ by JSPLE. The temperature value of 65 °C was used for further optimization of other operational parameters.

Effects of reaction time for the synthesis of CuONPs

Reaction time effect was studied by monitoring the reaction of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ solution and the aqueous plant extract for 10 min, 25 min, 40 min, 55 min and 70 min at 65 °C. A color change was observed from green-yellow to red-brown within 10 min of reaction at the moment JSPLE reacts with the solution of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$. As time increases, the color intensified. **Fig. 5d** showed the record of UV–Vis measurement at different time intervals. Because of the slow conversion of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ to CuONPs by the JSPLE, the SPR band was slightly broadened between zero and 10 min. As the reaction time increases, excellent SPR band and maximum absorption was observed and this may be due to the conversion of large amount of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ to CuONPs. The measured UV–Vis absorption spectra of the synthesized CuONPs showed best SPR peak within 209 nm at 55 min reaction time. The rate of conversion or nucleation of nanoparticles increases with time, and after reaching the optimum time, the reaction rate does not alter considerably. Previous researchers achieved the greatest reduction of metal ions after 60 min manufacture of Copper nanoparticles using howthorn berries extract [52].

Effects of reaction pH for the synthesis of CuONPs

Adjustment was made for the pH of green synthesized CuONPs without any pH optimization using 1 M HCl and 1 M NaOH to a final pH of pH 6, pH 7, pH 8, pH 9, and pH 10 and their absorbance was measured by double beam UV-Vis spectrophotometer as depicted in **Fig. 5e**. The SPR peaks are shifted towards a

longer wavelength at ~208 nm in the basic medium (pH 8 and pH 9) while the SPR peaks for acidic condition (pH 6) and neutral condition (pH 7) appear at a relatively short wavelength of around 203 nm. The longer wavelength shift in a basic medium indicates relatively large CuONPs sizes compared with neutral and acidic conditions. Moreover, the intensity of SPR peaks for acidic conditions decreases with increasing pH whereas the intensity of SPR peaks tends to increase when the pH value increases. Enhanced synthesis of CuONPs under alkaline conditions may be due to more availability of bio-reductants. This could be attributed to changes in the dissociation constant values of the functional groups present in the biomolecules involved in the reduction of Copper ions. Furthermore, the decreased reduction rate in acidic medium could be ascribed to the denaturation and/or degradation of bioactive compounds present in the plant leaf extract [38]. From the previous studies, the best result for synthesis of CuONPs using Leaf Extract of *Catha edulis* was obtained at pH = 11. They have obtained stronger plasmon resonance for pH 9 to 12 [42].

FT-IR Analysis

FT-IR analysis was used to investigate the presence of some functional groups in both the JSPLE and the synthesized CuONPs which are responsible for reducing as well as capping agent. **Fig. 6** showed the FT-IR absorption spectra of green synthesized CuONPs and JSPLE in the range of 400 cm^{-1} to 4000 cm^{-1} .

Fig. 6(a) revealed the FT-IR spectrum of JSPLE. The major and strongest vibrational modes in the JSPLE spectrum are those located at 3379.51, 2932.26, 2850.18, 1610.16, 1446.01, 1072.59, 844.11, 762.77, and 671.08 cm^{-1} . A band around 3379.51 cm^{-1} is due to the N–H stretching vibration of NH_2 group and O–H group of the overlapping of the stretching vibration attributed to water and phenolic compounds present in JSPLE. The result obtained by qualitative phytochemical analysis of JSPLE was in line with this FTIR result. The phytochemical analysis confirmed the presence of polyphenols, flavonoids, alkaloids and other similar phytochemicals containing O-H and N-H bonds in the JSPLE [13]. The bands at 2932.26 and 2850.18 cm^{-1} are assigned to CH_2 and C-H stretching mode in alkanes. The presence of carboxylic, aldehydes, esters or ketone containing compounds arising from flavonoids and tannins were suggested by the stretching mode of carbonyl group (C=O) observed at 1610.16 cm^{-1} . The N–H stretching vibration present in the amide linkages were assigned for the bands at $\sim 1446.01\text{ cm}^{-1}$. The intense band at 1072.59 cm^{-1} could be assigned to the C–N stretching vibrations of aliphatic amines and bands at 1446.01 cm^{-1} is due to the C–O group of aliphatic esters [47,53]. The major and strongest vibrational modes at around 3415.85, 2923.39, 1637.52, 1418.65, 1081.47, 835.24, 780.52 and 634.85 cm^{-1} as shown in **Fig. 6(b)** showed the green synthesized CuONPs. A broad band around 3415.85 cm^{-1} corresponds to –OH stretching of phenolic compounds and N–H stretching vibrations of amino groups present in the plant leaf extract. The peak at 2923.39 cm^{-1} corresponds to C–H stretching modes and the band at 1637.52 cm^{-1} corresponds to C=O stretching and N–H bending of primary amides. An intense peak around 1418.65 cm^{-1} shows the presence of –COO carboxylic acid and the absorption peak at 1081.47 cm^{-1} corresponds to the C– NH_2 primary aliphatic amines present in JSPLE. The formation of pure

CuONPs was confirmed by the narrow band at 634.85 cm^{-1} . This indicates the phytochemicals present in JSPLE have a dual function of stabilization and formation of CuONPs. **Fig. 6(b)** showed a little change in position and intensity in the peaks for the FTIR band of synthesized CuONPs. The displacement at 3415.85 cm^{-1} may be related to hydrogen bond breakage, which plays a role in reducing copper ions into CuONPs. The similarity of spectral pattern for the synthesized nanoparticles and plant leaf extract may be a confirmation for the existence of the identified phytochemicals in the synthesized CuONPs [6].

XRD pattern analysis

Fig. 7 displays an XRD pattern for the prepared CuONPs using JSPLE. To evaluate the crystalline structure, size, and purity of the prepared CuONPs, the XRD analysis was performed. In the XRD pattern, five major peaks were observed at $2\theta = 32.06^\circ$, 35.69° , 38.996° and 48.93° corresponding to (110), (002), (111) and (202) planes, respectively. The resulted peaks and their corresponding Bragg's reflections are strongly agreed with the Joint Committee on Powder Diffraction Standards (JCPDS, file no. 801268). The observed diffraction peaks can be indexed as the typical monoclinic structure and some extra diffraction peaks and noises may be due to the oxidation of the sample. Moreover, the peak corresponding to the (002) plane is more intense than the other planes and also other CuO reflections in the observed XRD patterns verify the crystalline nature of CuONPs [2].

All the diffraction peaks were attributed from the monoclinic structure of pure Bragg's reflections of CuO powder phase. These planes confirmed the crystalline nature of the green synthesized CuONPs. The highest peak intensity of (002) plane with narrow full width at half maximum (FWHM) illustrates the good crystalline nature of synthesized CuONPs as observed from the XRD patterns. The resulted peaks and their corresponding Bragg's reflections are strongly agreed with the Joint Committee on Powder Diffraction Standards (JCPDS, file no. 801268). The major peaks of the green synthesized CuONPs indicate the purity of the synthesized nanoparticles. The average crystallite sizes of the particles were calculated by using Debye-Scherrer's equation:

$$D = \frac{K\lambda}{\beta \cos\theta}$$

where, D is the estimated crystal size in nanometere (nm) from XRD patterns, K is the shape factor or Scherrer constant (0.9) of Debye-Scherrer's equation, λ is the wavelength of X-ray source used ($\text{CuK}_\alpha = 1.5406\text{ \AA}$), β is the angular width at the half maximum of the diffraction peak in radians and θ is the Bragg angle in radians. The estimated average crystalline size (D) of the synthesized silver nanoparticles is found to be 21.8 nm.

Anti-bacterial studies of green synthesized CuONPs

The leaf extract alone as a comparison and the biosynthesized CuONPs from JSPLE were studied for their antibacterial activity against clinically isolated three gram-negative bacteria (*Salmonella typhimurium*, *Escherichia coli*, and *Klebsiella pneumoniae*) and one gram positive bacteria (*Staphylococcus aureus*) by following standard agar well diffusion method, and Zone of Inhibition (ZOI) was recorded.

Antibacterial activity was shown by ZOI which was characterized by a clear zone between the wells (containing samples) and a certain distance. Formation of ZOI around the wells showed bacterial sensitivity to antibacterial and antibiotic drugs (which are used as positive controls). The positive control used in the well was a gentamycin and functioned as a control of the test solution by comparing the diameter of the ZOI formed. On the contrary, DMSO as a negative control was used to determine the effect of solvents in the test solution on the growth bacteria. The ZOI on all bacterial strains was measured after 24 h of incubation time at 37 °C.

As clearly described by **Fig. 8** and **Table 3**, the synthesized CuONPs showed anti-bacterial activity against all bacterial strains tested. However, it was more effective against gram-negative (salmonella typhimurium) bacteria in both the synthesized nanoparticle. As the concentration of the synthesized CuONPs increases the anti-bacterial activity also increased. The anti-bacterial activity of green synthesized CuONPs against gram-negative (salmonella typhimurium) bacteria was maximum with ZOI of 18.6 mm with respect to the highest concentration (150 µg/mL) (**Table 3**). Based on this result, one can conclude that the green synthesized CuONPs had highest anti-bacterial activity against gram-negative bacteria strains specially salmonella typhimurium as compared to the plant leaf extract. The studies of this result correlate with the previous report [30]. By the same procedure, the antibacterial activity of the JSPLE was tested. Compared to CuONPs, the JSPLE possessed a little anti-bacterial activity with the highest concentration of 150 µg/mL.

The highest anti-bacterial activity of green synthesized CuONPs against gram-negative bacteria (salmonella typhimurium) was probably due to large surface area of the nanoparticles which provide better contact with the microorganisms and will have stronger bactericidal effect than larger particles. This anti-bacterial difference can also be due to the difference in the composition of their cell wall, as the cell membrane of gramnegative bacteria consists of a single layer of peptidoglycan, while multiple layers of peptidoglycan are present in the membrane of grampositive bacteria, which makes them more rigid [54].

Table 3

Antibacterial activity of green synthesized CuONPs against clinically isolated human pathogenic bacteria

Concentration (µg/mL)	CuONPs			
	Mean diameter of ZOI (in mm) for the tested bacteria			
	<i>S. aureus</i>	<i>K. pneumoniae</i>	<i>E. coli</i>	<i>S. typhimurium</i>
50	14.3	10	15.4	15
100	15.2	12.3	16	17.2
150	17	13	17.4	18.6
genta	21	15	20	23
DMSO	0	0	0	0

genta = gentamycin

Staphylococcus aureus = *S. aureus*

Klebsiella pneumonia = *K. pneumonia*

Escherichia coli = *E. coli*

Salmonella typhimurium = *S. typhimurium*

Conclusion

In this study, an eco-friendly and simple method was used for the successful synthesis of CuONPs from *Justicia Schimperiana* plant leaf extract for the first time. The synthesized CuONPs were fully characterized by UV-Visible spectroscopy, FTIR and XRD. The FT-IR and phytochemical screening test confirmed the presence of bioactive-molecules present in the plant leaf extract. Surface Plasmon resonance with intense peak was observed at 212 nm by UV-Vis spectrophotometric measurements. The XRD result confirmed the monoclinic crystal structure with an average crystallite size of 21.8 nm. The synthesized CuONPs exhibited significant antibacterial activity against both gramnegative (*Klebsiella pneumonia* and *Salmonella typhimurium*) and grampositive bacteria (*Streptococcus pneumonia*). The plant leaf extract showed insignificant antibacterial activity at the highest concentration. From this study,

it can be inferred that the synthesized CuONPs could be used in the fields of water treatment, biomedicine, biosensor and nanotechnology.

Declarations

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References

1. Varughese A, Kaur R, & Singh P. Green Synthesis and Characterization of Copper Oxide Nanoparticles Using Psidium guajava Leaf Extract. *IOP Conf. Ser.: Mater. Sci. Eng.* 2020;961:012011. IOP Publishing.
2. Padil VVT, & Černík M. Green synthesis of copper oxide nanoparticles using gum karaya as a biotemplate and their antibacterial application. *Int J Nanomedicine.* 2013;8: 889–98.
3. Bhavyasree PG, & Xavier TS. Green synthesized copper and copper oxide based nanomaterials using plant extracts and their application in antimicrobial activity: Review. *CRGSC.* 2022;5:100249.
4. Amin F, Khattak B, Alotaibi A, Qasim M, Ahmad I, Ullah R, Bourhia M, Gul A, Zahoor S, Ahmad R. Green Synthesis of Copper Oxide Nanoparticles Using Aerva javanica Leaf Extract and Their Characterization and Investigation of In Vitro Antimicrobial Potential and Cytotoxic Activities. *Evid.-Based Complementary Altern. Med.* 2021;2021:1-12.
5. Nabila MI, Kannabiran K. Biosynthesis, characterization and antibacterial activity of copper oxide nanoparticles (CuO NPs) from actinomycetes. *Biocatal. Agric. Biotechnol.* 2018;15:56-62.
6. Sukumar S, Rudrasenan A, Nambiar DP. Green-Synthesized Rice-Shaped Copper Oxide Nanoparticles Using Caesalpinia bonducella Seed Extract and Their Applications. *ACS Omega.* 2020;5:1040–51.
7. Siddiqui VU, Ansari A, Chauhan R, Siddiqi WA. Green synthesis of copper oxide (CuO) nanoparticles by Punica granatum peel extract. *Mater. Today.* 2021;36:751-55.
8. Awwad AM, Amer MW. Biosynthesis of copper oxide nanoparticles using *Ailanthus altissima* leaf extract and antibacterial activity. *Awwad and Amer / Chemistry International.* 2020;6:210-17.
9. Bose D, Chatterjee S. Antibacterial Activity of Green Synthesized Silver Nanoparticles Using Vasaka (*Justicia adhatoda* L.) Leaf Extract. *Indian J Microbiol.* 2015;55:163–67.

10. Sivaraj R, Rahman PKSM, Rajiv P, *Abdul Salam H, Venckatesh R*. Biogenic copper oxide nanoparticles synthesis using Tabernaemontana divaricate leaf extract and its antibacterial activity against urinary tract pathogen. *Spectrochim. Acta A Mol. Biomol.* 2014;133:178–81.
11. Mohamed EA. Green synthesis of copper & copper oxide nanoparticles using the extract of seedless dates. *Heliyon.* 2020;6:e03123.
12. Altikatoglu M, Attar A, Erci F, Cristache CM, Isildak I. Green synthesis of copper oxide nanoparticles using *ocimum basilicum* extract and their antibacterial activity. *Fresenius Environ. Bull.* 2017;26:7832-37.
13. Ghidan AY, Al-Antary TM, Awwad AM. Green synthesis of copper oxide nanoparticles using Punica granatum peels extract: Effect on green peach Aphid. *Environ. Nanotechnol. Monit.* 2016;6:95–98.
14. Sathiyavimal S, Vasantharaj S, Veeramani V, Saravanan M, Rajalakshmi G, Kaliannan T, Al-Misned FA, Pugazhendhi A. Green chemistry route of biosynthesized copper oxide nanoparticles using *Psidium guajava* leaf extract and their antibacterial activity and effective removal of industrial dyes. *J. Environ. Chem. Eng.* 2021;9:105033.
15. Naseer M, Ramadan R, Xing J, Samak NA. Facile green synthesis of copper oxide nanoparticles for the eradication of multidrug resistant *Klebsiella pneumonia* and *Helicobacter pylori* biofilms. *Int. Biodeterior. Biodegradation.* 2021;159:105201.
16. Prakash S, Elavarasan N, Venkatesan A, Subashini K, Sowndhanya M, Sujatha V. Green synthesis of copper oxide nanoparticles and its effective applications in Biginelli reaction, BTB photodegradation and antibacterial activity, *Adv Powder Technol.* 2018;29:3315-26.
17. Mahmoud AED, Al-Qahtani KM, Alflaij SO, Al-Qahtani SF, Alsamhan FA. Green copper oxide nanoparticles for lead, nickel, and cadmium removal from contaminated water. *Sci. Rep.* 2021;11:12547.
18. Ramzan M, Obodo RM, Mukhtar S, Ilyas SZ, Aziz F, Thovhogi N. Green synthesis of copper oxide nanoparticles using Cedrus deodara aqueous extract for antibacterial activity. *Mater. Today.* 2021;36:576-81.
19. Rabiee N, Bagherzadeh M, Kiani M, Ghadiri AM, Etessamifar F, Jaberizadeh AH, Shakeri A. Biosynthesis of Copper Oxide Nanoparticles with Potential Biomedical Applications. *Int. J. Nanomedicine* 2020;15:3983–99.
20. Ijaz F, Shahid S, Khan SA, Ahmed W, Zaman S. Green synthesis of copper oxide nanoparticles using Abutilon indicum leaf extract: Antimicrobial, antioxidant and photocatalytic dye degradation activities. *Trop. J. Pharm. Res.* 2017;16:743-53.
21. Awwad AM, Albiss BA, Salem NM. Antibacterial Activity of synthesized Copper Oxide Nanoparticles using *Malva sylvestris* Leaf Extract. *SMU Medical Journal.* 2015;2:91-101.
22. Radhakrishnan R, Ali Khan FL, Muthu A, Manokaran A, Savarenathan JS, Kasinathan K. Green Synthesis of Copper Oxide Nanoparticles Mediated by Aqueous Leaf Extracts of *Leucas aspera* and *Morinda tinctoria*. *Lett. Appl. NanoBioScience* 2021;10:2706–14.

23. Vasantharaj S, Shivakumar P, Sathiyavimal S, Senthilkumar P, Vijayaram S, Shanmugavel M, Pugazhendhi A. Antibacterial activity and photocatalytic dye degradation of copper oxide nanoparticles (CuONPs) using *Justicia gendarussa*. *Appl. Nanosci.* 2021;1-8. <https://doi.org/10.1007/s13204-021-01939-9>
24. Islam MdJ, Khatun MT, Rahman MdR, Alam MM. Green synthesis of copper oxide nanoparticles using *Justicia adhatoda* leaf extract and its application in cotton fibers as antibacterial coatings. *AIP Adv.* 2021;11:125223.
25. Asemani M, Anarjan N. Green synthesis of copper oxide nanoparticles using *Juglans regia* leaf extract and assessment of their physico-chemical and biological properties. *Green Process. Synth.* 2019;8:557–67.
26. Vishveshvar K, Krishnan MVA, Haribabu K, Vishnuprasad S. Green Synthesis of Copper Oxide Nanoparticles Using *Ixiro coccinea* Plant Leaves and its Characterization. *BionanoScience.* 2018;8:554-58.
27. Tavakoli S, Kharaziha M, Ahmadi S. Green Synthesis and Morphology Dependent Antibacterial Activity of Copper Oxide Nanoparticles. *J Nanostruct.* 2019;9:163-71.
28. Naika HR, Lingaraju K, Manjunath K, Kumar D, Nagaraju G, Suresh D, Nagabhushana H. (2015) Green synthesis of CuO nanoparticles using *Gloriosa superba* L. extract and their antibacterial activity. *J. Taibah Univ. Sci.* 2019;9:7-12.
29. Traiwatcharanon P, Timsorn K, Wongchoosuk C. Flexible room-temperature resistive humidity sensor based on silver nanoparticles, *Mater. Res. Express* 2017;**4**:085038.
30. Vasantharaj S, Sathiyavimal S, Saravanan M, Senthilkumar P, Gnanasekaran K, Shanmugavel M, Manikandan E, Pugazhendhi A. Synthesis of ecofriendly copper oxide nanoparticles for fabrication over textile fabrics: Characterization of antibacterial activity and dye degradation potential. *Jpb* 2019;191:143-49.
31. Turakhia B, Divakara MB, Santosh MS, Shah S. Green synthesis of copper oxide nanoparticles: a promising approach in the development of antibacterial textiles. *J. Coat. Technol. Res.* 2020;17:531-40.
32. Anwaar S, Maqbool Q, Jabeen N, Nazar N, Abbas F, Nawaz B, Hussain T, Hussain SZ. The Effect of Green Synthesized CuO Nanoparticles on Callogenesis and Regeneration of *Oryza sativa*. L. *Front, Plant Sci.* 2016;7:1-9.
33. Hosseinzadeh R, Mohadjerani M, Mesgar S. Green synthesis of copper oxide nanoparticles using aqueous extract of *Convolvulus periclus* L. as reusable catalysts in cross-coupling reactions and their antibacterial activity. *IET Nanobiotechnol.* (2017;11: 725-30.
34. Andualem WW, Sabir FK, Mohammed ET, Belay HH, Gonfa BA. Synthesis of Copper Oxide Nanoparticles Using Plant Leaf Extract of *Catha edulis* and Its Antibacterial Activity. *J. Nanotechnol.* 2020;2020:1-10. <https://doi.org/10.1155/2020/2932434>
35. Singh P, Singh KR, Singh J, Das SN, Singh RP. Tunable electrochemistry and efficient antibacterial activity of plant-mediated copper oxide nanoparticles synthesized by *Annona squamosa* seed extract

- for agricultural utility, *RSC Adv.* 2021;11:18050–60.
36. Pirtarighat S, Ghannadnia M, Baghshahi S. Green synthesis of silver nanoparticles using the plant extract of *Salvia spinosa* grown in vitro and their antibacterial activity assessment. *J. Nanostruc. Chem.* 2019;9:1–9.
 37. Salayová A, Bedlovičová Z, Daneu N, Baláž M, Bujňáková ZL, Balážová L, Tkáčiková L. Green synthesis of silver nanoparticles with antibacterial activity using various medicinal plant extracts: morphology and antibacterial efficacy. *Nanomaterials.* 2021;11:1–20.
 38. Melkamu WW, Bitew LT. Green synthesis of silver nanoparticles using *Hagenia abyssinica* (Bruce) J.F. Gmel plant leaf extract and their antibacterial and anti-oxidant activities. *Heliyon* 2021;7:e08459.
 39. Mekonnen B, Asrie AB, Wubneh ZB. Antidiarrheal Activity of 80% Methanolic Leaf Extract of *Justicia schimperiana*, *Evidence-Based Complementary and Alternative Medicine* 2018;2018:1-10. <https://doi.org/10.1155/2018/3037120>
 40. Abdeta D, Amante M, Tamiru Y. Survey on Ethno Botany and Medicinal Animals at Sayo and Hawa Gelan Districts of Kelem Wollega Zone, Western Ethiopia. *preventive medicine.* 2020;28:21408-20.
 41. Hedberg I, Kelbessa E, Edwards S, Demissew S, Persson E, eds. *Flora of Ethiopia and Eritrea*. Volume 5: Gentianaceae to Cyclocheilaceae. Addis Ababa, Ethiopia: The National Herbarium, Addis Ababa, Ethiopia, and Department of Systematic Botany. Uppsala, Sweden; 2006 Pp. 468.
 42. Gebremedhn K, Kahsay MH, Aklilu M. Green Synthesis of CuO Nanoparticles Using Leaf Extract of *Catha edulis* and Its Antibacterial Activity. *J. Pharm. Pharmacol.* 2019;7 327-42.
 43. Pradeep A, Dinesh M, Govindaraj A, Vinothkumar D, Ramesh Babu NG. Phytochemical analysis of some important medicinal plants. *Int J Biol Pharm Res.* 2014;5:48-50.
 44. Jayapriya G, Shoba FG. Phytochemical analysis, antimicrobial efficacy and determination of bioactive components from leaves of *Justicia adhatoda* (Linn). *Asian J Plant Sci Res.* 2015;5:43-51.
 45. Uzoekwe NM, & Hamilton-Amachree A. Phytochemicals and nutritional characteristics of ethanol extract of the leaf and bark of *Njangsa* (*Ricinodendron Heudelotii*) plant. *J. Appl. SCI. Environ. Ma.* 2016;20:522-527.
 46. Khan TM, Mateen A. Synthesis of CuO nanoparticles by using leaf extracts of *Melia azedarach* and *Morusnigra* and their antibacterial activity. *J Innov Sci.* 2018;4:120-29.
 47. Sankar R, Manikandan P, Malarvizhi V, Fathima T, Shivashangari KS, Ravikumar V. Green synthesis of colloidal copper oxide nanoparticles using *Carica papaya* and its application in photocatalytic dye degradation. *Spectrochim. Acta A Mol. Biomol. Spectrosc.* 2014;121:746-50.
 48. Kumar P, Nene AG, Punia S, Kumar M, Abbas Z, Thakral F, Tuli HS. Synthesis, Characterization and Antibacterial activity of CuO nanoparticles. *Int. J. Appl. Pharm.* 2020;12:17-20.
 49. Anjum NA, Adam V, Kizek R, Duarte AC, Pereira E, Iqbal M, Lukatkin AS, Ahmad I. Nanoscale copper in the soil–plant system–toxicity and underlying potential mechanisms, *Environ. Res.* 2015;138:306-25.
 50. Prasad KS, Patra A, Shruthi G, Chandan S. Aqueous extract of *Saraca indica* leaves in the synthesis of copper oxide nanoparticles: finding a way towards going green. *Journal of Nanotechnology.*

2017;2017:1-6.

51. Nagar N, Devra V. Green synthesis and characterization of copper nanoparticles using *Azadirachta indica* leaves. *Mater. Chem. Phys.* 2018;213:44-51.
52. Długosz O, Chwastowski J, Banach M. Hawthorn berries extract for the green synthesis of copper and silver nanoparticles. *Chem. Pap.* (2020;74:239-52.
53. Sutradhar P, Saha M, Maiti D. Microwave synthesis of copper oxide nanoparticles using tea leaf and coffee powder extracts and its antibacterial activity. *J NANOSTRUCTURE CHEM.* 2014;4:1-6.
54. Hemlata PRM, Singh AP, Tejavath KK. Biosynthesis of silver nanoparticles using *cucumis prophetarum* aqueous leaf extract and their antibacterial and antiproliferative activity against cancer cell lines. *ACS omega.* 2020;10:5520.

Figures



Figure 1

Justicia schimperiana plant in its natural habitat



Figure 2

The color change observed when the leaf extract of *Justicia schimperiana* was tested for the presence and absence of (A) Alkaloids, (B) Favonids, (C) Tannins, (D) Terepenoids, (E) Saponins (F) Polyphenols (G) Cardiac glycosides, (H) Carbohydrates, (I) Anthraquinones

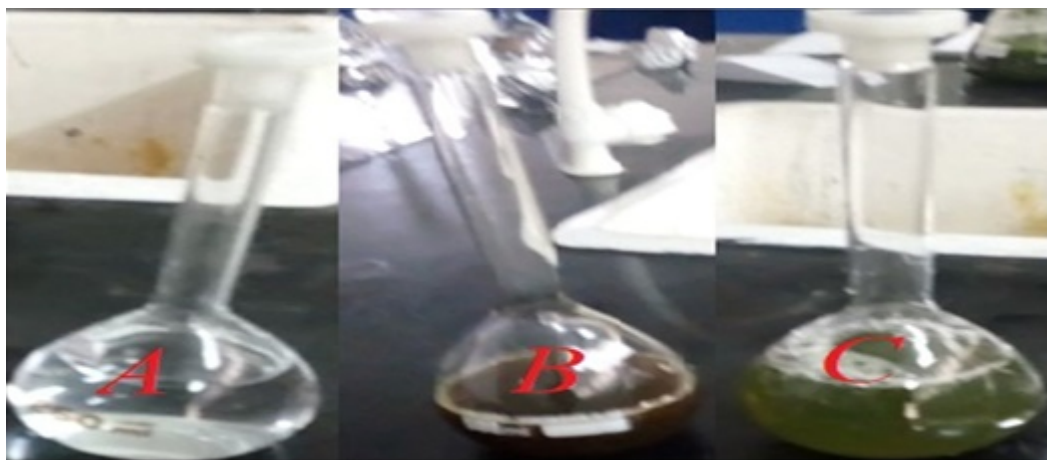


Figure 3

Green synthesis of CuONPs from JSPLE and colour change observation (A) $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ precursor, (B) JSPLE after filtration and (C) Colloidal CuONPs

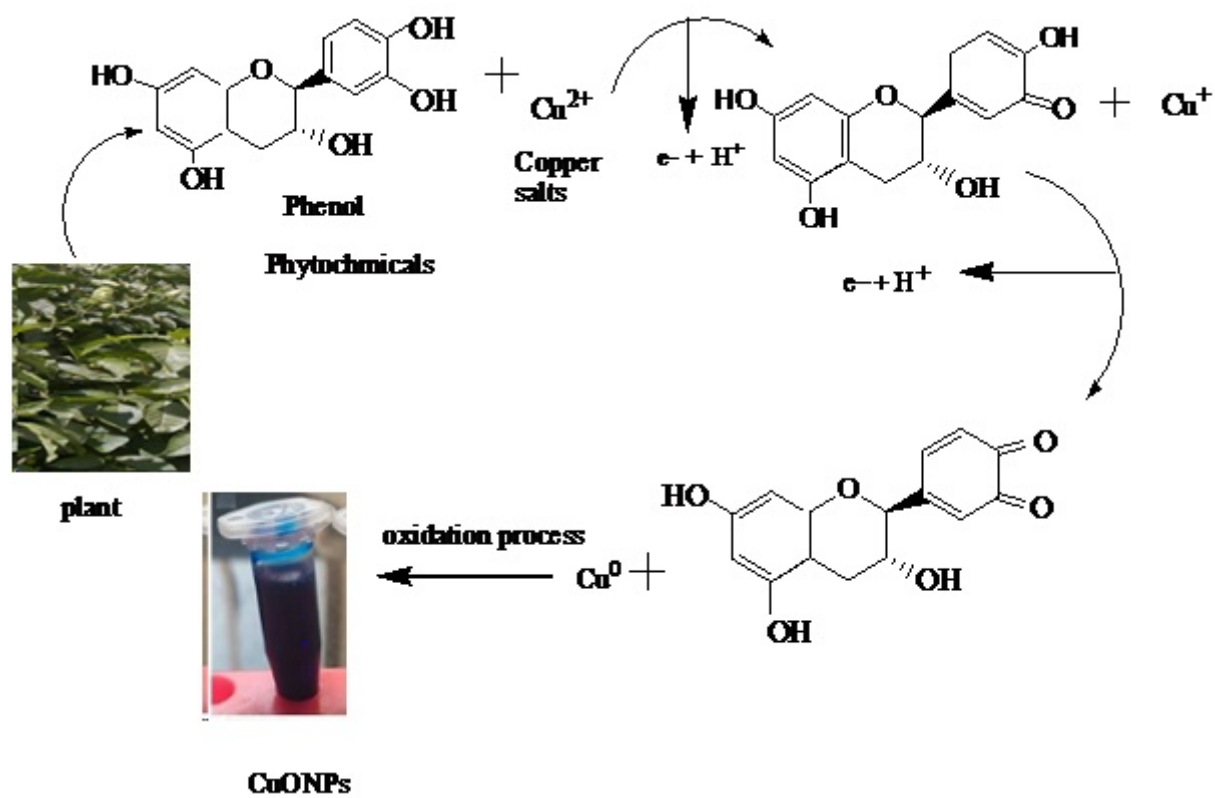


Figure 4

Mechanism of CuONPs formation by plant extract

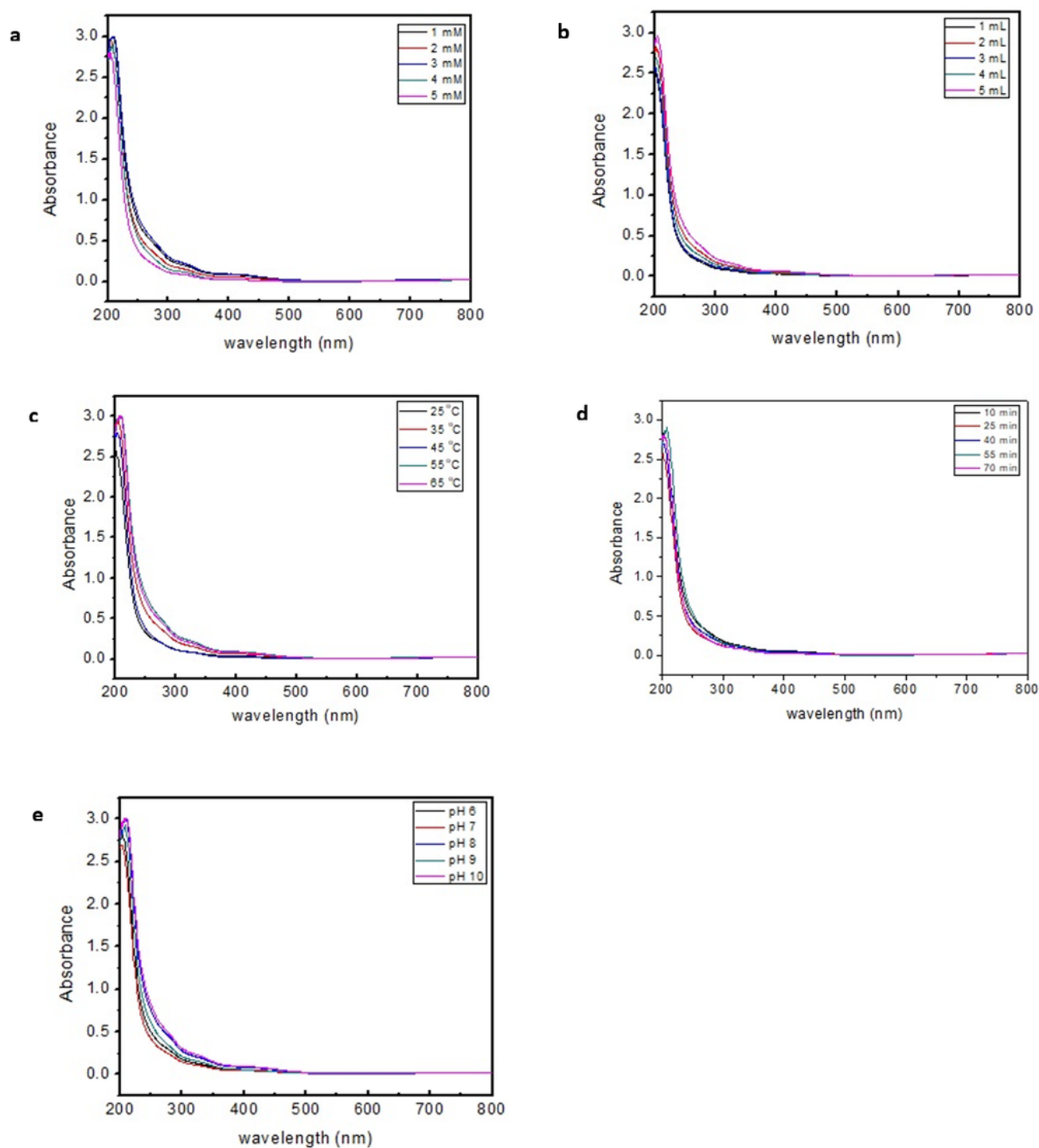


Figure 5

(a) UV-Vis absorption spectra of CuONPs synthesized by JSPLE at different $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ concentration, (b) UV-Vis absorption spectra of CuONPs synthesized by varying volume ratio of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ solution to JSPLE by keeping constant the concentration & volume of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ solution, (c) UV-Vis absorption spectra of temperature optimization for the synthesis of CuONPs from JSPLE, (d) UV-Vis absorption

spectra of reaction time optimization for the synthesis of CuONPs from JSPLE, and (e) UV-Vis spectra of pH optimization for the green synthesis of CuONPs from JSPLE

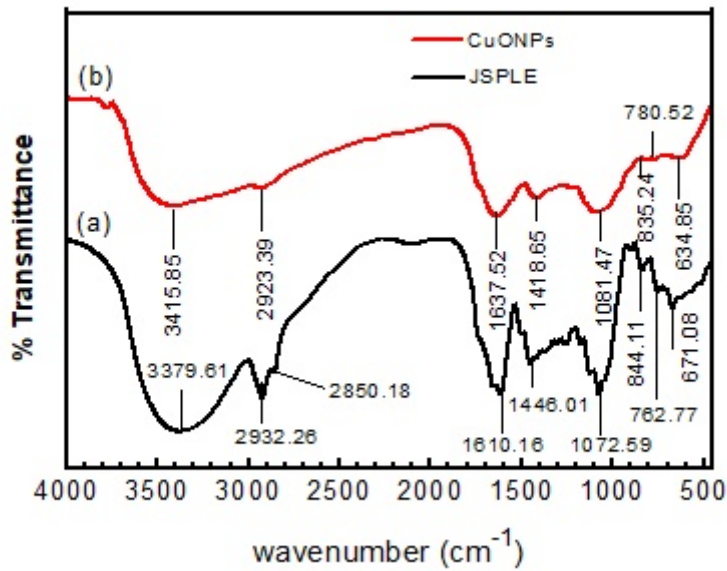


Figure 6
FTIR spectra of (a) JSPLE and (b) Synthesized CuONPs

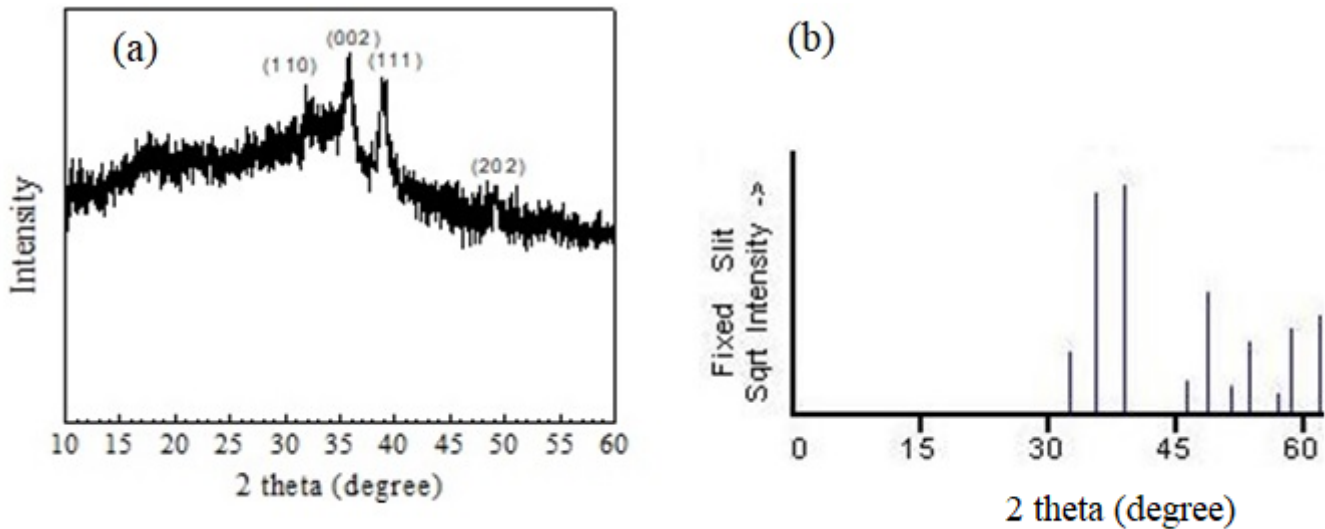


Figure 7
(a) XRD patterns of green synthesized CuONPs from JSPLE (b) Reference Pattern (JCPDS, file no. 801268)

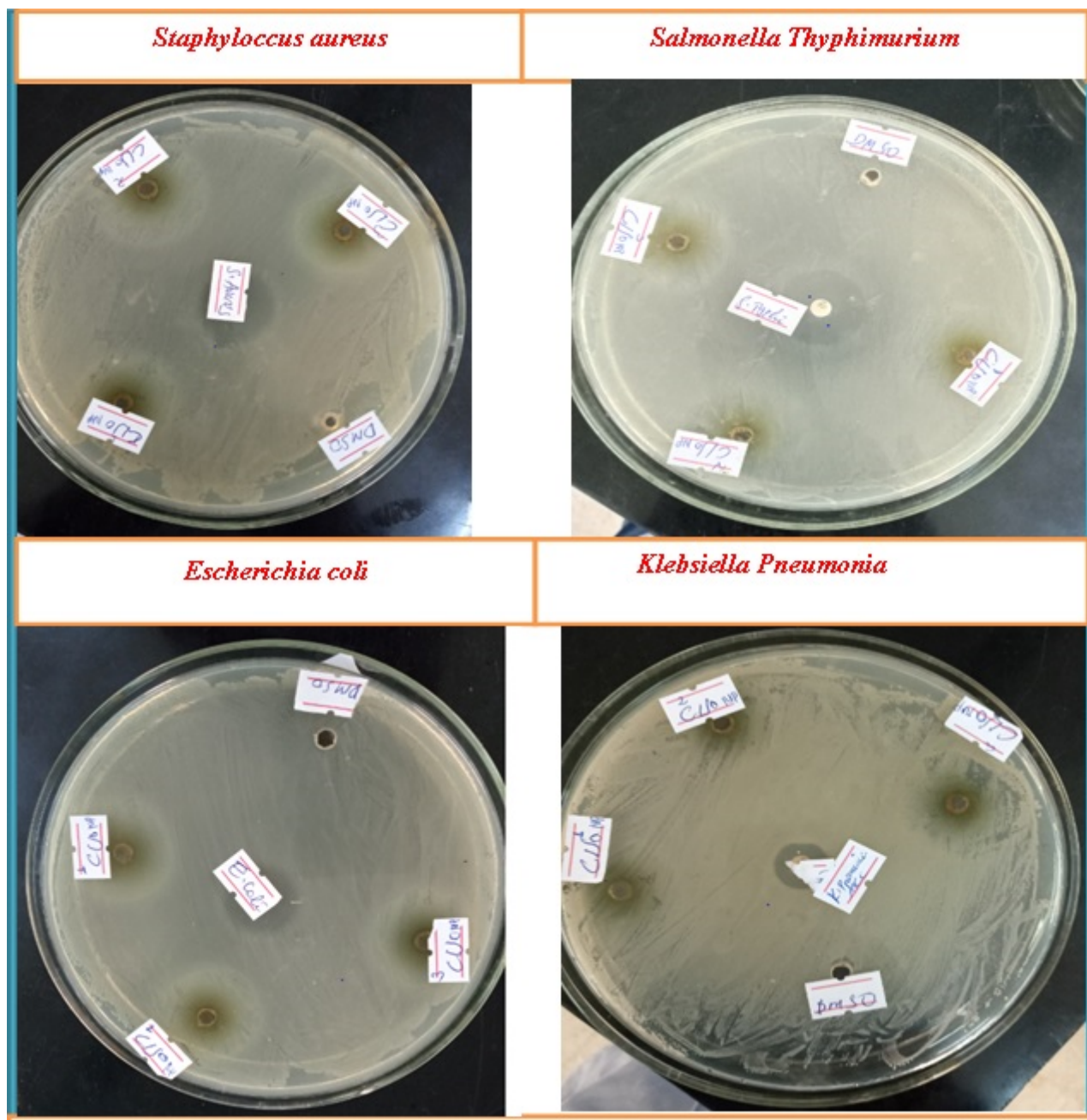


Figure 8

Antibacterial activities of synthesized CuONPs (1 = 50 $\mu\text{g/mL}$, 2 = 100 $\mu\text{g/mL}$, 3 = 150 $\mu\text{g/mL}$, gentamycin (positive control) and DMSO (negative control))