

**How to Cite:**

Fentie, M., Chouhan, G., Moges, M., & Tyagi, P. (2022). Green synthesis of copper oxide nanoparticles using *Bryophyllum pinnatum* leaf extract and its antibacterial potential against *Listeria monocytogenes*. *International Journal of Health Sciences*, 6(S2), 5349–5367. <https://doi.org/10.53730/ijhs.v6nS2.6345>

## **Green synthesis of copper oxide nanoparticles using *Bryophyllum pinnatum* leaf extract and its antibacterial potential against *Listeria monocytogenes***

**Molla Fentie**

Department of Biotechnology, School of Engineering and Technology, Sharda University, Knowledge Park III, Greater Noida, Uttar Pradesh, India  
Corresponding author email: [mollafentie3@gmail.com](mailto:mollafentie3@gmail.com)

**Garima Chouhan**

Department of Biotechnology, School of Engineering and Technology, Sharda University, Knowledge Park III, Greater Noida, Uttar Pradesh, India

**Meron Moges**

Department of Biotechnology, School of Engineering and Technology, Sharda University, Knowledge Park III, Greater Noida, Uttar Pradesh, India

**Priya Tyagi**

Department of Biotechnology, School of Engineering and Technology, Sharda University, Knowledge Park III, Greater Noida, Uttar Pradesh, India

**Abstract**---Green synthesis nanoparticle preparation is a biocompatible, safe, and ecologically friendly method of nanoparticle preparation. In the green manufacture of nanoparticles, plants and other microorganisms such as bacteria, algae, and fungi are employed. In terms of efficiency, speed, affordability, non-toxicity, simplicity, and environmental friendliness, it outperforms physicochemical techniques. Nanoparticle production mediated by plant biomass is a fast expanding topic of research around the world. Copper oxide nanoparticles were made from the aqueous leaf extract of the *Bryophyllum pinnatum* plant in this work. The antibacterial activity of the produced copper oxide nanoparticles was investigated against *Listeria monocytogenes*. To make copper oxide nanoparticles, 1ml aqueous extract of *Bryophyllum pinnatum* was mixed with 20ml (0.1M)  $\text{Cu}(\text{NO}_3)_2$  and centrifuged at 10,000 rpm for 15 minutes before being rinsed with distilled water and dried. UV-Visible spectroscopy, High-resolution field emission scanning electron microscope (HR FESEM), X-ray diffraction (XRD), Fourier transform infrared

spectroscopy (FTIR), and Dynamic light scattering (DLS) techniques were used to evaluate the produced copper oxide nanoparticles. The antibacterial potential of the compound was next tested utilizing disk diffusion and agar well diffusion methods against *Listeria monocytogenes*. A UV-Vis absorption peak at 266nm was seen in the absorption spectra of copper oxide nanoparticles made from leaf extracts. Rod-shaped copper oxide nanoparticles with a size range of 61.48-112.4nm and an average particle diameter of 96.8 nm were produced, according to SEM results. It also possesses strong antibacterial properties against *Listeria monocytogenes*. Copper oxide nanoparticles made from *Bryophyllum pinnatum* leaf extract have been discovered to be a viable antibacterial agent for treating *Listeria monocytogenes*-related foodborne illnesses. However, more research is needed to figure out how it affects the target microorganism.

**Keywords**---bryophyllum pinnatum, green synthesis, copper oxide nanoparticles, antibacterial potential, listeria monocytogenes.

## Introduction

On several biomedical devices, copper oxide nanoparticles (CuONPs) have been used as antibacterial and antibiofilm agents (Chari, Felix, Davoodbasha, Sulaiman Ali, & Nooruddin, 2017; LewisOscar et al., 2015; Nabila & Kannabiran, 2018). It has a longer shelf life and is more stable than other organic antimicrobials (Raffi et al., 2010; Ren et al., 2009). CuONPs' antibacterial action is related to their size and surface volume ratio and their peculiar crystal shape, which allows CuONPs to engage easily with bacterial cell membranes and kill the bacterial cell by metal ions released inside the cell (Laha et al., 2014; S. Sathiyavimal et al., 2018). Precipitation (Mayekar, Dhar, Radha, & Research, 2014; Phiw dang, Suphankij, Mekprasart, & Pecharapa, 2013; Rahimi-Nasrabadi, Pourmortazavi, Davoudi-Dehaghani, Hajimirsadeghi, & Zahedi, 2013), sonochemical (Silva, Ramirez, Diaz, Garcia, & Hassan, 2019), thermal breakdown of copper salts (Shahsavani, Feizi, & DEHNO, 2016), electrochemical (Katwal, Kaur, Sharma, Naushad, & Pathania, 2015), heat treatment (Baquer et al., 2018), nonionic water-in-oil microemulsions (Dodoo-Arhin, Leoni, & Scardi, 2012), and sol-gel synthesis methods (Dorner et al., 2019) are some of the ways to make CuONPs. The best way to make nanoparticles is to use plants or components of plants. It is a more environmentally friendly, cost-effective, biologically safe, and stable method of nanoparticle manufacturing. (Balazs, Emrick, & Russell, 2006; Chatzimitakos & Stalikas, 2016; Shameli et al., 2012)

Plant biomolecules make non-toxic, stable, and cost-effective copper oxide nanoparticles (Laha et al., 2014; LewisOscar et al., 2015; Nabila & Kannabiran, 2018; Ren et al., 2009). *Cynodon dactylon* and *Cyperus rotundus* Grass Extracts (S. Suresh, 2020), *Ailanthus altissima* leaf extract (Akl M. Awwad1, 2020), *Asparagus racemosus* roots extract (Pammi5, 2019), *Populus ciliata* (Muhammad Hafeez\*1, 2019), fenugreek leaves and fruit (ARDEEP KUMAR1, 2019), *Terminalia belerica* (AKHTER, 2019), *Terminalia belerica* (Ahmad2, 2019), Sugarcane juice (A.P. Angeline Mary a, 2019), *Ruellia tuberosa* (Seerangaraj Vasantharaj et al.,

2018), *Malva sylvestris* Leaf Extract (Awwad, 2015), and *Acalypha indica* (Rajeshwari Sivaraj a, 2014) are some of the plants being used to synthesize CuONPs.

The current study used *Bryophyllum pinnatum* (*B.pinnatum*) plant to synthesize an environmentally friendly CuONPs. CuONPs were prepared using *Bryophyllum pinnatum* leaf extract, and their antibacterial potential was tested against *Listeria monocytogenes* (*L.monocytogenes*). *B.pinnatum* is a vegetatively propagated wild herb found in the tropical countries including India (Debabrat Baishya\*, 2012a). It is widely used by the traditional practitioners for treating various ailments like renal calculi, hypertension, asthma, cold, abscesses, bleeding disorders (Odangowei I Ogidi, 2019). The methanol and aqueous extracts of *B. pinnatum* leaf showed strong antibacterial activity against *Streptococcus* species and *Staphylococcus* species (Etim, Obande, Aleruchi, & Bassey, 2016).

The silver nanoparticles synthesized using *B. pinnatum* leaf showed antibacterial activity against *E. coli* and *Staphylococcus aureus* (Debabrat Baishya\*, 2012b). The qualitative phytochemical result showed that plant samples contain alkaloids, tannins, saponin, terpenoid, glycoside, phenols and flavonoid (Odangowei I Ogidi, 2019). Therefore, considering its potential herbal remedies and antimicrobial application, CuONPs were prepared using *B.pinnatum* leaf extract, and its antibacterial potential was tested against *L.monocytogenes*. The antibacterial potential of *B.pinnatum* leaf extract mediated CuONPs has never been tested against *L.monocytogenes*. Advanced techniques were used to characterize the prepared CuONPs and its antibacterial potential against the test microbe was also assessed.

## Materials and Methods

The *B.pinnatum* plant (Figure 1) was purchased from Godawari Farms and Services and Nursery, Greater Noida, and the plant authenticity certificate was received from CSIR-National Institute of Science and Communication Information Resources (Ref. NISCAIR/RHMD/Consult/2020/3706-07). The chemicals, solvents, and reagents employed in the experiment were all of analytical quality, and all of the solutions were produced from scratch with distilled water.



**Figure 1.** *Bryophyllum pinnatum* plant

### Preparation of plant extracts

To remove dust particles, fresh plant leaves were thoroughly cleansed with tap water and then rinsed with distilled water. To remove any remaining moisture, the cleaned leaves were dried in the oven. The dry plant material was ground into fine powder using a mixer grinder (Sujata). After that, 5 grams of plant powder were dissolved in 50 millilitres of distilled water to extract the plant material. After boiling for 30 minutes at 65°C in a water bath, the aqueous extract was filtered using WhattmanNo. 1 filter paper after cooling (Figure 2). After that, the filtrate was maintained in the refrigerator at 4°C for further testing.



Figure 2. Aqueous plant extract preparation

### CuONPs synthesis

CuONPs were produced by dropping 1 mL of plant extract into a 20 mL aqueous copper nitrate (0.1 M) solution while stirring. The solution was stored in the darkroom overnight by wrapping it in aluminum foil. The original blue tint of the solution quickly turned green. Nanoparticle generation was preliminarily validated by visually detecting the color shift of the solution to green (Figure 3).

The solution was centrifuged at 10,000 rpm once it turned green. After discarding the supernatant, which contained unreacted herb extract, CuONPs were washed with distilled water.

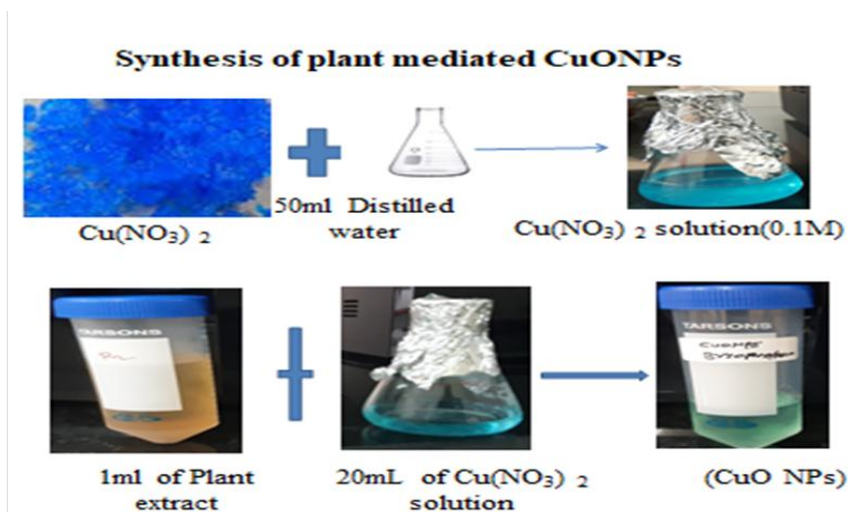


Figure 3. Synthesis of plant mediated CuONPs

### Nanoparticle Characterization

Different techniques were used to analyze the optical characteristics of CuONPs produced, as well as the plant biomolecules involved in their synthesis, crystallinity, surface charges, nanoparticle size and shape. A UV-Vis spectrophotometer was used to validate the synthesis of CuONPs (Shimadzu, UV 1800). Between the spectral ranges of 200-800nm, it was subjected to UV-Vis spectroscopic testing. The peak of CuONPs' surface plasmon resonance was determined by plotting the wavelength on the X-axis and the absorbance on the Y-axis (SPR). Dynamical Light Scattering (DLS) was used to determine the hydrodynamic diameter and zeta potential of the CuONPs.

Fourier-transform infrared spectroscopy (FTIR) analysis was done by FTIR Spectrometer (Agilent, Cary 630 FTIR-ATR including choice of KBr or ZnSe engine), an analysis of the probable functional groups in leaf extract involved in synthesizing and stabilizing CuONPs. The spectra were collected between 650 and 4000  $\text{cm}^{-1}$ . CuONPs' morphological characteristics (form and size) were studied using NOVA NANOSEM-450 FEI, Netherlands) High-resolution field emission scanning electron microscope (HR FESEM). The crystallinity of CuONPs was measured using Rigaku Smart Lab Guidance X-Ray Diffractometer (Rigaku Company) at JMI University. Cu K radiation was used in the X-ray diffractometer (Rigaku SmartLab X-Ray Diffractometer) at a voltage of 40 kV and a current of 30 mA. The information gathered was examined with the help of origin pro (Version 2019b). After determining the width of XRD peaks, the Debye-Scherrer equation was used to calculate the average crystallite diameter based on the half-width of the diffraction peaks:

$$D = k\lambda / \beta \cos\theta$$

Where D is the mean particle size,  $\lambda$  is the wavelength of Cuka,  $\beta$  is the full width at half maximum (FWHM),  $\theta$  is the Bragg diffraction angle, and k is a constant.

## **Antibacterial Activity**

The CuONPs antibacterial activity was investigated using *L.monocytogenes* (MTCC 1143) bacterial culture. Both agar wells and discs were used to investigate the antibacterial capability of CuONPs. The bacterial culture was purchased from Microbial Type Culture Collection and Gene Bank (MTCC), CSIR - Institute of Microbial Technology, Sector 39-A Chandigarh-160036, India. Before usage, the bacteria were kept onto BHI media (Hi-Media, India) and sub-cultured. Both agar wells and discs were used to investigate the antibacterial capability of CuONPs.

### **Disc diffusion test**

BHI agar (5.2gm) was dissolved in 100 ml of distilled water and autoclaved at 121°C for 15 minutes. After cooling, approximately 20 mL of medium was aseptically transferred to sterile Petri plates and allowed to solidify. By two-fold serial dilution from an overnight culture of the test organism (*L.monocytogenes* MTCC 1143) in sterile distilled water, bacterial suspension with 0.5McFarland turbidity standard ( $10^8$  CFU $mL^{-1}$ ) was prepared. The inoculum was evenly distributed over the surface of BHI agar plates using a sterile cotton swab. Then, on the surface of the BHI agar plate, which was divided evenly into four quadrants, sterile filter paper discs (6mm in diameter) loaded with CuONPs, plant extract, copper nitrate, and chloramphenicol were placed. Before incubation at 37°C for 24 hours, plates were kept for 20 minutes at room temperature to allow diffusion of the solutions outward from the discs, establishing a concentration gradient. The plates were then incubated at 37°C for 24 hours, and the inhibitory zone (diameter in mm) was measured using a scale. Triplicates of the test were run. CuONPs inhibition zones were compared to a positive control (Chloramphenicol). Antimicrobial activity was expressed as the mean inhibition zone (mm)  $\pm$  SD.

### **Agar well diffusion method**

Inoculation of bacterial suspension with 0.5McFarland turbidity standard ( $10^8$  CFU  $mL^{-1}$ ) prepared by two-fold serial dilution from an overnight culture of the test organism (*L.monocytogenes* MTCC 1143) was done on BHI media using a sterile cotton swab. Four wells were punched into the agar plate that was well separated and equidistant using a 6 mm cork borer. After that, 10 $\mu$ L of CuONPs, chloramphenicol, Cu (NO<sub>3</sub>)<sub>2</sub>, and plant extract solutions are poured into each well. After allowing samples to diffuse into the agar by keeping them at room temperature for 20 minutes, the plates were incubated for 24 hours at 37°C. After 24 hours, the diameter of the zone of inhibition was evaluated and measured in mm with a scale. The test was performed in triplicate, and the inhibition zones of CuONPs were compared with the positive control (Chloramphenicol). Antimicrobial activity was expressed as the mean inhibition zone (mm)  $\pm$  SD.

### **Determination of minimum inhibitory concentration (MIC)**

The strength of antibacterial activity can be assessed by characterizing microorganism inhibition at a particular time point. It can be calculated using MIC, the lowest concentration at which no growth occurs in nutritional media.

Micro broth dilution was used for MIC and MBC determination. CuONPs in various concentrations (17.862, 8.927, 4.463, and 2.231, 1.115, 0.558, 0.279, and 0.138mgmL<sup>-1</sup>) were synthesized by two-fold serial dilution using distilled water 100L of each sample was transferred to a 96-well microtiter plate.

Each well containing varied concentration of CuONPs received an identical volume (100µL) of overnight developed bacterial cultures adjusted to 0.5McFarland turbidity standard. Each well had a total volume of 200µL. Chloramphenicol, Cu (NO<sub>3</sub>)<sub>2</sub>, and plant concentrations were all studied in the same way. As a control, BHI broth with CuONPs and bacterial culture without sample was introduced to separate wells. Inside the temperature-controlled incubator, the plates were incubated for 24 hours at 37°C. In the wells of the plates, bacterial growth (turbidity) was found. The MIC value was determined as the lowest concentration of CuONPs that prevented observable bacteria growth (with no turbidity). All the experiments were carried out in triplicates.

### **Statistical Analysis**

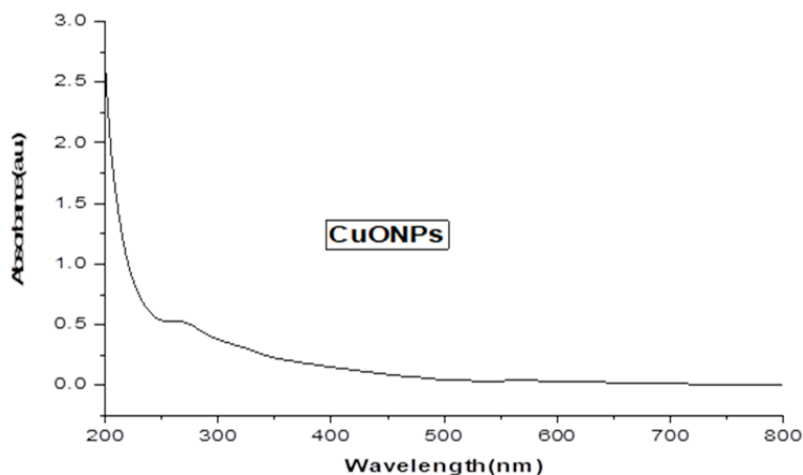
Data analysis was done using one-way analysis of variance (ANOVA), and p values of < 0.05 were considered statistically. All the experiments were done in triplicate

### **Results and Discussion**

#### **UV-Vis analysis**

The color shift of the solution to green after the reaction of plant extract with copper ions can be used to illustrate the synthesis of CuONPs. This outcome closely resembles the findings of previous studies (Gebremedhn, Kahsay, Aklilu, & Pharmacology, 2019; Sepasgozar, Mohseni, Feizyadeh, & Morsali, 2021; Shaheen et al., 2021). In addition, the absorbance was examined, and the peak value of CuONPs was observed at 266nm (Figure 4), which is consistent with previous study. The absorption peaks of CuONPs prepared by combustion technique using ascorbic acid as a capping agent appeared at wavelengths of 266 nm(Sagadevan et al., 2019) and CuONPs synthesized using leaf extract of *Enicostemma axillare* (Lam.) had absorption peak of 264nm(Chand Mali, Raj, & Trivedi, 2019) while CuONPs made from *Musa acuminata* peel extracts had peak at 268nm(Chaudhary, Rohilla, Umar, Kaur, & Shanavas, 2019). CuONPs synthesized by hydrothermal method had absorption peaks at 258nm(Dutta, Kar, Bose, & Mukherjee, 2015) and CuONPs made from *Pterospermum acerifolium* leaf extract have shown UV absorption at 274 nm (Saif, Tahir, Asim, & Chen, 2016). CuONPs mediated by *Enicostemma axillare* (Lam.) leaf extract revealed a peak at 234nm (Mali, Raj, Trivedi, & reports, 2019) where as CuONPs produced with *Magnolia champaca* were validated by a large peak in the 250-350 nm range(Santhosh Kumar & Shanmugam, 2020). *Acalypha indica* mediated CuONPs had an optical absorbance around 220 nm (Sivaraj et al., 2014). Similarly, the CuONPs synthesized from *Camellia sinensis* extract and *Prunus africana* bark extract had UV vis surface plasmon resonance peaks between 272 and 286 nm(Ssekatawa et al., 2022). *Phoenix dactylifera* L extract mediated CuONPs has shown peak at 275nm(Berra et al., 2018).CuONPs synthesized by hydrothermal method had absorption peaks at 258nm(Dutta et al., 2015). Other researches

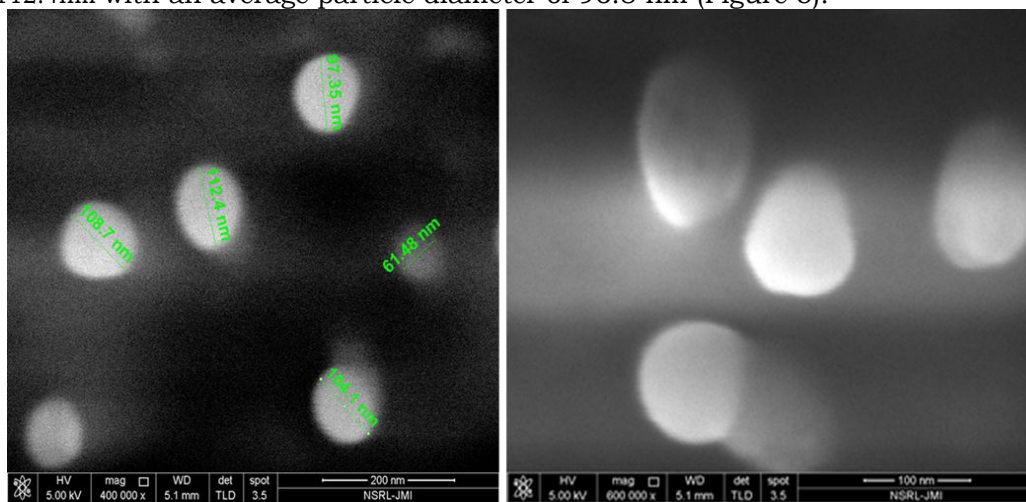
have also shown similar results with our findings (Badawy, Abdelfattah, Salem, Awad, & Fouda, 2021; Bhavyasree & Xavier, 2020; Davari, Hakimzadeh, Mahdian, & Shahidi, 2021; Ghidan, Al-Antary, Awwad, & Management, 2016; Kumar, Smita, Cumbal, Debut, & Angulo, 2017; Rajivgandhi et al., 2019; Selvam Sathiyavimal et al., 2021; Sepasgozar et al., 2021). An intense surface Plasmon resonance between 250–300 nm in the UV–vis spectrum clearly reveals the formation of CuONPs (Renu Sankar et al., 2014).



**Figure 4.** UV–Visible absorption spectrum of CuO NPs prepared from *Bryophyllum pinnatum* leaf extract

### SEM analysis

The morphological features of produced CuONPs were determined using HR FESEM images. The SEM images of the synthesized CuONPs revealed that the CuONPs with nearly oval morphology were formed and have size range of 61.48–112.4 nm with an average particle diameter of 96.8 nm (Figure 5).



**Figure 5.** SEM image of synthesized CuONPs at different magnifications

### FT-IR characterization

FT-IR characterization was also done to analyse the plant phytochemicals involved in reducing copper ions to CuONPs and subsequent capping.  $3350\text{cm}^{-1}$ ,  $3327\text{cm}^{-1}$ ,  $3312\text{cm}^{-1}$ ,  $2355\text{cm}^{-1}$ ,  $2322\text{cm}^{-1}$ ,  $1640\text{cm}^{-1}$ , and  $776\text{cm}^{-1}$  are the peaks in the spectra of *Bryophyllum pinnatum* leaf extract (Fig. 6A). The hydroxyl (-OH) group was identified by FTIR analysis with a peak at  $3350\text{cm}^{-1}$  whereas the peak at  $3327\text{cm}^{-1}$  denotes N-H. The peak at  $3312\text{cm}^{-1}$  is for C=C-H and the peak at  $2,355\text{ cm}^{-1}$  was attributed to C=N. C=O stretch has shown peak at  $1640\text{ cm}^{-1}$ . The peculiar characteristic bands at  $3231$ ,  $1633$ ,  $1383$ ,  $1339$ ,  $1153$ ,  $1119$ ,  $1097$ ,  $1074$ ,  $974$ , and  $773\text{cm}^{-1}$  correlate to the *Bryophyllum pinnatum* leaf extract bands in the FTIR spectrum of CuONPs. The peak at  $3231\text{cm}^{-1}$  is assigned to the N-H extension vibration,  $1633$  indicates C=O,  $1383$  denotes C-N,  $1339$  indicates C-C,  $1153$ ,  $1119$  denotes C-O. This indicates that biomolecules which are found in plant extract were used to synthesise CuONPs.

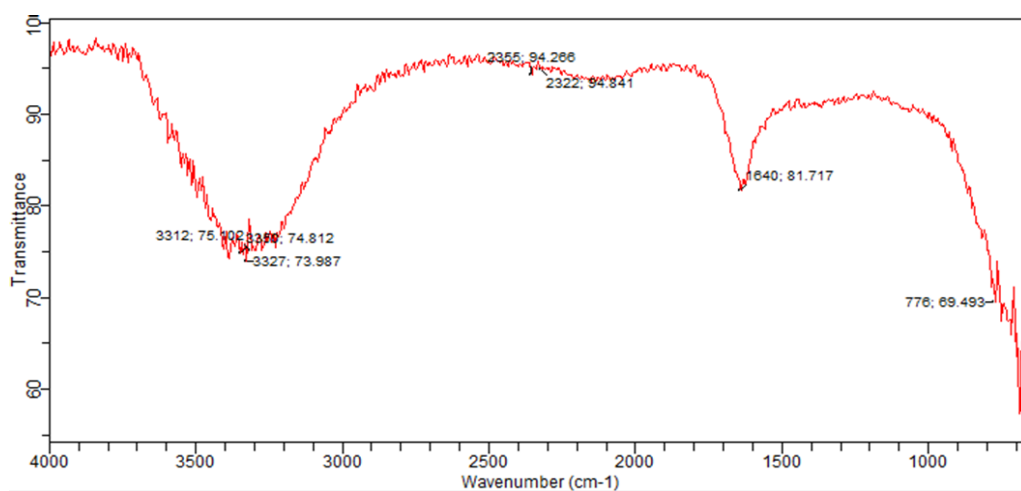


Figure 6A. FT-IR spectrum of *Bryophyllum pinnatum* leaves extract.

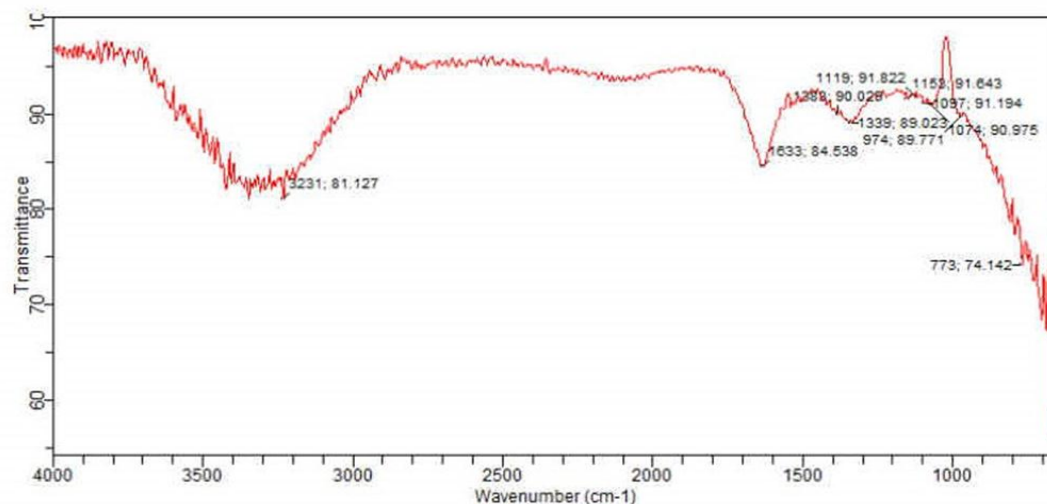


Figure 6B. FT-IR spectrum of prepared CuONPs

## XRD Analysis

Figure 6 shows the XRD analysis of copper oxide nanoparticles generated with *Bryophyllum pinnatum* leaf extract. Diffraction peaks were found at  $2\theta = 11.763^\circ$ ,  $12.838^\circ$ ,  $20.782^\circ$ ,  $25.777^\circ$ ,  $42^\circ$ , and  $57.06^\circ$ . CuO nanoparticles produced by reducing Cu ions by *Bryophyllum pinnatum* leaf extracts are crystalline, as seen by strong peaks in X-ray diffraction patterns. Using Jade software, the miller indices and peaks of CuO nanoparticles were compared to JCPDS/ICDD database and published literatures found to be relatively similar. The (113), (030), (036), (436) planes of the crystalline phase of cuper oxide ( $\text{Cu}_2\text{O}$ ) are related to the diffraction peaks positioned at  $2\theta$  values  $20.78^\circ$ ,  $25.77^\circ$ ,  $42^\circ$ , and  $57.06^\circ$ , respectively. The result agrees closely with powder CuO obtained from the Joint Committee on Powder Diffraction Standards (JCPDS file no. 77-1898), while the peaks positioned at  $2\theta$  values of  $11.763^\circ$ , and  $12.83^\circ$  are assigned to the (110), (110), and (220) planes of the crystalline phase of cuprous oxide ( $\text{Cu}_2\text{O}$ ), respectively, and are matched with reported literature (AKHTER, 2019) and the cubic phase of  $\text{Cu}_2\text{O}$  values with the corresponding "JCPDS" (Joint Committee on Powder Diffraction Standards card no. 34-1354). The existence of peaks in the XRD spectra corresponding to CuO and  $\text{Cu}_2\text{O}$  suggests that both oxides are present in the crystalline phase of CuO nanoparticles (AKHTER, 2019). The CuONPs has an average crystal size of 65.776nm. A few studies have previously reported various crystalline sizes according to XRD analysis for CuONPs biosynthesized using plants extracts.

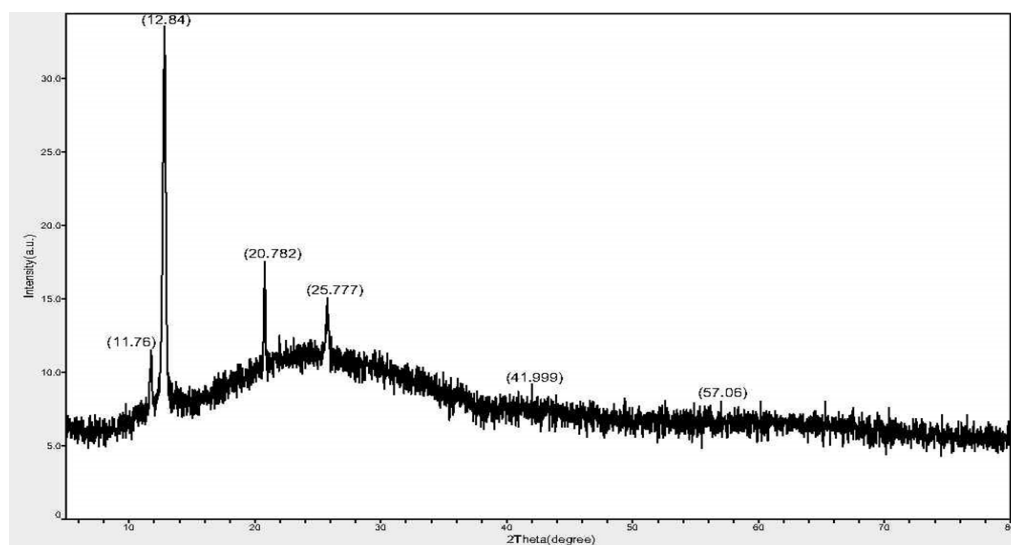


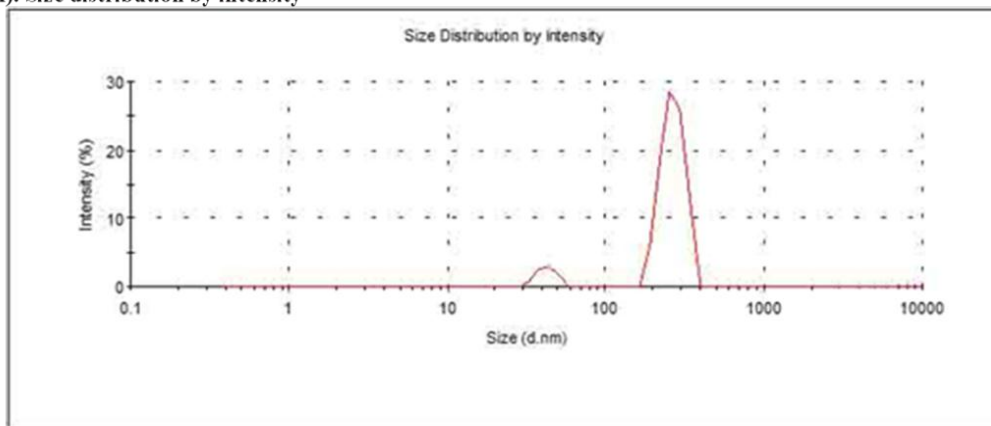
Figure 7. XRD of synthesized CuONPs

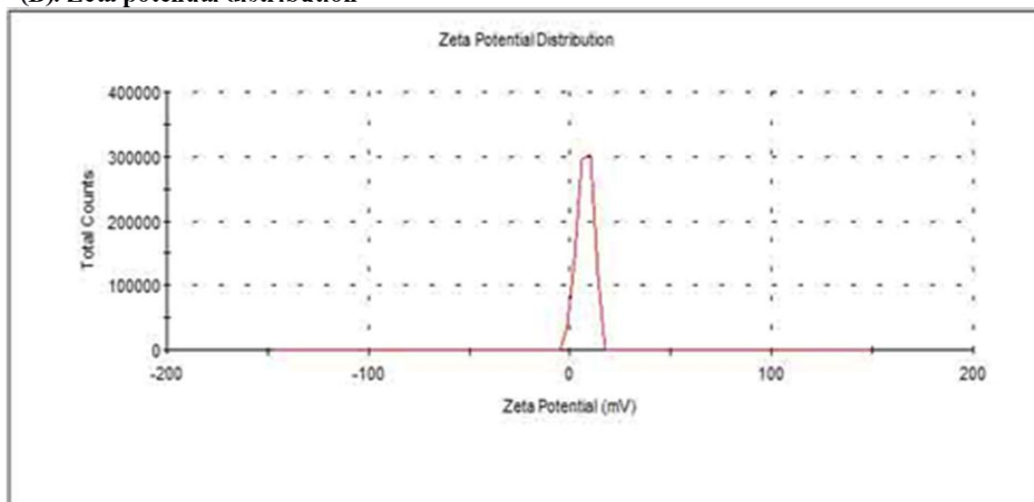
## DLS study

DLS analysis was done to determine nanoparticles' size and surface charge through colloidal solutions. According to the DLS results, the CuONPs had an average particle size of 398 nm. Because DLS evaluates the hydrodynamic size of the produced nanoparticles, the particle size is bigger than that reported by SEM (Pallela et al., 2019). Biomolecules and the aqueous layer covering nanoparticles'

surfaces were included in the measurement (Bhakya et al., 2015). DLS analysis revealed that *Origanum vulgare* plant synthesized CuONPs having a particle size of 551 nm (R. Sankar, B. B. Prasath, et al., 2014). Copper oxide nanoparticles synthesized from *Ficus religiosa* and *O. vulgare* leaves were measured at 577 nm and 551 nm, respectively, using dynamic light scattering analysis (Sankar, Maheswari, Karthik, Shivashangari, & Ravikumar, 2014). 4 to 620 nm (Ssekatawa et al., 2022). The charge distribution and size improve CuONPs' biological activities (R. Sankar, R. Maheswari, et al., 2014). According to the findings, CuONPs with a positively charged surface had a zeta potential of +12 mV. If the hydrosols have a high negative or positive zeta potential, they will resist each other, preventing the particles from clumping together. On the other hand, if the particles have low zeta potential levels, there will be no force to keep them from colliding and flocculating (Sorbiun1 & 2018). However, the green synthesized CuONPs showed a net surface charge of +12 mV in this study. Therefore, clustering of the CuONPs revealed by FESEM micrographs may be due to this low net charge. *Camellia sinensis* extract and *Prunus africana* bark extract green synthesized Copper oxide nanoparticles (CuONPs) have shown almost similar result with a zeta potential value of +12.5 mV (Ssekatawa et al., 2022).

(A). Size distribution by intensity



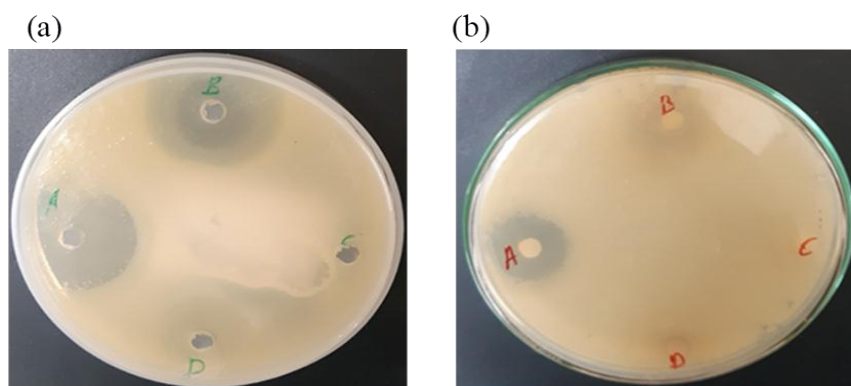
**(B). Zeta potential distribution**

**Figure 8.** DLS analysis of CuO NPs (A) Size distribution, (B) zeta potential

**Antibacterial activity**

The antibacterial activity of the CuONPs against *L.monocytogenes* was investigated using an agar well and disc diffusion assay. For the agar well and disc diffusion procedures, respectively, a 28.33mm and 17.67mm zone of inhibition was found after 24 hours of incubation with the CuONPs. The result indicates that a higher inhibition zone was recorded in the agar well diffusion method than in the disc diffusion technique. Different researches have shown similar results with greater inhibition zones in agar wells diffusion than in disk diffusion test(Gunalan, Sivaraj, & Rajendran, 2012; Hamelian, Hemmati, Varmira, & Veisi, 2018). The synthesized CuONPs were found to have a MIC of  $1.115\text{mgmL}^{-1}$ . The antibacterial properties of the synthesized CuONPs against the target microbe may be due to the shape of the nanoparticles. Spherical CuONPs prepared from *Camellia sinensis* extract and *Prunus africana* bark extract exhibited potent antibacterial activity (Ssekatawa et al., 2022). In Contrary to this, rod-shaped copper nanoparticles have potent antimicrobial activity against both gram-positive and gram-negative bacteria. This is because rod-shaped nanoparticles present a large surface area to volume ratio that enhances their interaction with the bacterial cells and therefore helps to inhibit its growth(Qamar, Rehman, Chauhan, Tiwari, & Upmanyu, 2020). This agrees with other studies that green synthesized uniformly shaped copper nanoparticles (Qamar et al., 2020; Siddiqi, Rashid, Rahman, Husen, & Rehman, 2020; Wu, Rajeshkumar, Madasamy, Mahendran, & Biotechnology, 2020) whereas studies showed that biosynthesized polymorphic copper nanoparticles reported lower MICs (Chandraker et al., 2020; Murthy, Desalegn, Kassa, Abebe, & Assefa, 2020). In addition to this, the antibacterial activity of the *P.incarnata* plant-mediated CuONPs may be due to its positive zeta potential/charge(+12mV). The bacterial cell membrane exhibits a net negative charge that greatly attracts positively charged materials(Ssekatawa et al., 2020). Because of this electrostatic interaction, adsorption and consequently penetration of bacterial cells by CuONPs is enhanced(Ssekatawa et al., 2022). CuONPs synthesized from *Rosmarinus*

*officinalis L.*, *Syzigium aromaticum* and *Cinnamomum verum* (Osaili et al., 2019), *walnut shells*(Mehdizadeh, Zamani, & Abtahi Froushani, 2020), *Capsicum frutescens* (K, S, P, S, & S, 2021), and *Lemon Extract*(Tshireletso, Ateba, & Fayemi, 2021) have shown antibacterial activities against *L.monocytogenes* at different concentrations.



**Figure 9.** Antibacterial assay images of CuONPs against *L.monocytogenes*: (a) Agar well diffusion and (b) Disc diffusion technique.

[Where, A = chloramphenicol, B = CuONPs, C= Plant extract, D = Cu (NO<sub>3</sub>)<sub>2</sub>]

Table 1  
Inhibition zone of CuONPs against *L monocytogenes*

	Zone of inhibition (mm)	
	Well diffusion	Disk diffusion
CuONPs	28.83 ± 0.57	17.5 ± 1
Cu(NO <sub>3</sub> ) <sub>2</sub>	18 ± 1.52	15 ± 0
Chloramphenicol	33.17 ± 0.57	25.33 ± 0.44
Plant extract	NA	NA

Data are means of triplicates (n =3). NA: no Activity

Table 2  
MIC value of copper oxide nanoparticles against *L.monocytogenes*

Copper nanoparticles	MIC value (mgmL <sup>-1</sup> )
CuONPs	1.115
Cu(NO <sub>3</sub> ) <sub>2</sub>	2.344
Chloramphenicol	0.015625
Plant extract	NA

## References

A.P. Angeline Mary a, A. T. A. b., †, R. Subramanian c. (2019). Sugarcane juice mediated synthesis of copper oxide nanoparticles, characterization and their antibacterial activity. doi:10.1016/j.jksus.2019.03.003

- Ahmad2, S. M. H. A. F. M. S. (2019). Terminalia belerica Mediated Green Synthesis of Nanoparticles of Copper, Iron and Zinc Metal Oxides as the Alternate Antibacterial Agents Against some Common Pathogens. doi:10.1007/s12668-019-0601-4
- AKHTER, S. M. H. (2019). GREEN SYNTHESIS OF NANOMETALS USING MEDICINAL PLANT EXTRACT AND EVALUATION OF THEIR ANTIBACTERIAL EFFICACY.
- Akl M. Awwad1, a. M. W. A. (2020). Biosynthesis of copper oxide nanoparticles using Ailanthus altissima leaf extract and antibacterial activity. doi:10.5281/zenodo.3670918
- ARDEEP KUMAR1, A. G. N., SANDEEP PUNIA3, MANOJ KUMAR4, ZAHOOR ABBAS4, FALAK THAKRAL3, HARDEEP SINGH TULI3. (2019). SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF CUO NANOPARTICLES. doi:10.22159/ijap.2020v12i1.36271
- Awwad, A. M., Albiss, B.A2, Salem N.M.3. (2015). Antibacterial Activity of synthesized Copper Oxide Nanoparticles using Malva sylvestris Leaf Extract.
- Badawy, A. A., Abdelfattah, N. A., Salem, S. S., Awad, M. F., & Fouda, A. J. B. (2021). Efficacy assessment of biosynthesized copper oxide nanoparticles (CuO-NPs) on stored grain insects and their impacts on morphological and physiological traits of wheat (Triticum aestivum L.) plant. *10*(3), 233.
- Balazs, A. C., Emrick, T., & Russell, T. P. (2006). Nanoparticle polymer composites: where two small worlds meet. *Science*, *314*(5802), 1107-1110. doi:10.1126/science.1130557
- Baqer, A. A., Matori, K. A., Al-Hada, N. M., Kamari, H. M., Shaari, A. H., Saion, E., & Chyi, J. L. Y. J. J. o. M. S. M. i. E. (2018). Copper oxide nanoparticles synthesized by a heat treatment approach with structural, morphological and optical characteristics. *29*(2), 1025-1033.
- Berra, D., Laouini, S., Benhaoua, B., Ouahrani, M., Berrani, D., Rahal, A. J. D. J. o. N., & Biostructures. (2018). Green synthesis of copper oxide nanoparticles by Pheonix dactylifera L leaves extract. *13*(4), 1231-1238.
- Bhakya, S., Muthukrishnan, S., Sukumaran, M., Muthukumar, M., Kumar, S. T., Rao, M. J. J. o. B., & Biodegradation. (2015). Catalytic degradation of organic dyes using synthesized silver nanoparticles: a green approach. *6*(5), 1.
- Bhavyasree, P., & Xavier, T. J. H. (2020). Green synthesis of Copper Oxide/Carbon nanocomposites using the leaf extract of Adhatoda vasica Nees, their characterization and antimicrobial activity. *6*(2), e03323.
- Chand Mali, S., Raj, S., & Trivedi, R. (2019). Biosynthesis of copper oxide nanoparticles using Enicostemma axillare (Lam.) leaf extract. *Biochem Biophys Rep*, *20*, 100699. doi:10.1016/j.bbrep.2019.100699
- Chandraker, S. K., Lal, M., Ghosh, M. K., Tiwari, V., Ghorai, T. K., & Shukla, R. J. N. E. (2020). Green synthesis of copper nanoparticles using leaf extract of Ageratum houstonianum Mill. and study of their photocatalytic and antibacterial activities. *1*(1), 010033.
- Chari, N., Felix, L., Davoodbasha, M., Sulaiman Ali, A., & Nooruddin, T. (2017). In vitro and in vivo antibiofilm effect of copper nanoparticles against aquaculture pathogens. *Biocatalysis and Agricultural Biotechnology*, *10*, 336-341. doi:10.1016/j.bcab.2017.04.013
- Chatzimitakos, T. G., & Stalikas, C. D. (2016). Qualitative Alterations of Bacterial Metabolome after Exposure to Metal Nanoparticles with Bactericidal Properties: A Comprehensive Workflow Based on (1)H NMR, UHPLC-HRMS, and Metabolic

- Databases. *J Proteome Res*, 15(9), 3322-3330. doi:10.1021/acs.jproteome.6b00489
- Chaudhary, S., Rohilla, D., Umar, A., Kaur, N., & Shanavas, A. (2019). Synthesis and characterizations of luminescent copper oxide nanoparticles: Toxicological profiling and sensing applications. *Ceramics International*, 45(12), 15025-15035. doi:10.1016/j.ceramint.2019.04.239
- Davari, A., Hakimzadeh, V., Mahdian, E., & Shahidi, N. M. (2021). Investigation Of Structural And Antimicrobial Properties Of Copper Oxide Nanoparticles Produced Using Iranian Violet Extract.
- Debabratt Baishya\*, N. S. a. R. B. (2012a). <Bryophyllum pinnatum.pdf>.
- Debabratt Baishya\*, N. S. a. R. B. (2012b). Green Synthesis of Silver Nanoparticle using Bryophyllum pinnatum (Lam.) and monitoring their antibacterial activities.
- Dodoo-Arhin, D., Leoni, M., & Scardi, P. (2012). Microemulsion Synthesis of Copper Oxide Nanorod-Like Structures. *Molecular Crystals and Liquid Crystals*, 555(1), 17-31. doi:10.1080/15421406.2012.634357
- Dorner, L., Cancellieri, C., Rheingans, B., Walter, M., Kagi, R., Schmutz, P., . . . Jeurgens, L. P. H. (2019). Cost-effective sol-gel synthesis of porous CuO nanoparticle aggregates with tunable specific surface area. *Sci Rep*, 9(1), 11758. doi:10.1038/s41598-019-48020-8
- Dutta, B., Kar, E., Bose, N., & Mukherjee, S. J. R. a. (2015). Significant enhancement of the electroactive  $\beta$ -phase of PVDF by incorporating hydrothermally synthesized copper oxide nanoparticles. 5(127), 105422-105434.
- Etim, L., Obande, G., Aleruchi, C., & Bassey, V. (2016). Antibacterial Potential of Bryophyllum pinnatum Leaf Extracts on Bacteria Obtained from Infected Infant Respiratory Tract. *British Journal of Pharmaceutical Research*, 10(6), 1-8. doi:10.9734/bjpr/2016/24757
- Gebremedhn, K., Kahsay, M. H., Aklilu, M. J. J. o. P., & Pharmacology. (2019). Green synthesis of CuO nanoparticles using leaf extract of catha edulis and its antibacterial activity. 7(7), 327-342.
- Ghidan, A. Y., Al-Antary, T. M., Awwad, A. M. J. E. N., Monitoring, & Management. (2016). Green synthesis of copper oxide nanoparticles using Punica granatum peels extract: Effect on green peach Aphid. 6, 95-98.
- Gunalan, S., Sivaraj, R., & Rajendran, V. J. P. i. N. S. M. I. (2012). Green synthesized ZnO nanoparticles against bacterial and fungal pathogens. 22(6), 693-700.
- Hamelian, M., Hemmati, S., Varmira, K., & Veisi, H. J. J. o. t. T. I. o. C. E. (2018). Green synthesis, antibacterial, antioxidant and cytotoxic effect of gold nanoparticles using Pistacia Atlantica extract. 93, 21-30.
- K, V., S, S., P, M., S, M., & S, S. (2021). Ecofriendly green synthesis, characterization and biomedical applications of CuO nanoparticles synthesized using leaf extract of Capsicum frutescens. *Journal of Environmental Chemical Engineering*, 9(5). doi:10.1016/j.jece.2021.106299
- Katwal, R., Kaur, H., Sharma, G., Naushad, M., & Pathania, D. (2015). Electrochemical synthesized copper oxide nanoparticles for enhanced photocatalytic and antimicrobial activity. *Journal of Industrial and Engineering Chemistry*, 31, 173-184. doi:10.1016/j.jiec.2015.06.021

- Kumar, B., Smita, K., Cumbal, L., Debut, A., & Angulo, Y. J. J. o. S. C. S. (2017). Biofabrication of copper oxide nanoparticles using Andean blackberry (*Rubus glaucus* Benth.) fruit and leaf. *21*, S475-S480.
- Laha, D., Pramanik, A., Laskar, A., Jana, M., Pramanik, P., & Karmakar, P. (2014). Shape-dependent bactericidal activity of copper oxide nanoparticle mediated by DNA and membrane damage. *Materials Research Bulletin*, *59*, 185-191. doi:10.1016/j.materresbull.2014.06.024
- LewisOscar, F., MubarakAli, D., Nithya, C., Priyanka, R., Gopinath, V., Alharbi, N. S., & Thajuddin, N. (2015). One pot synthesis and anti-biofilm potential of copper nanoparticles (CuNPs) against clinical strains of *Pseudomonas aeruginosa*. *Biofouling*, *31*(4), 379-391. doi:10.1080/08927014.2015.1048686
- Mali, S. C., Raj, S., Trivedi, R. J. B., & reports, b. (2019). Biosynthesis of copper oxide nanoparticles using *Enicostemma axillare* (Lam.) leaf extract. *20*, 100699.
- Mayekar, J., Dhar, V., Radha, S. J. I. J. o. S., & Research, E. (2014). Synthesis of copper oxide nanoparticles using simple chemical route. *5*, 928-930.
- Mehdizadeh, T., Zamani, A., & Abtahi Froushani, S. M. (2020). Preparation of Cu nanoparticles fixed on cellulosic walnut shell material and investigation of its antibacterial, antioxidant and anticancer effects. *Heliyon*, *6*(3), e03528. doi:10.1016/j.heliyon.2020.e03528
- Muhammad Hafeez\*1, R. A., Jahanzeb Khan2, Bilal Akram2, Muhammad Naem Ahmad1, Muhammad Usman Hameed3, Sirajul Haq. (2019). *Populus ciliata* mediated synthesis of copper oxide nanoparticles for potential biological applications. doi:10.1088/2053-1591/ab0601
- Murthy, H., Desalegn, T., Kassa, M., Abebe, B., & Assefa, T. J. J. o. N. (2020). Synthesis of green copper nanoparticles using medicinal plant *hagenia abyssinica* (Brace) JF. Gmel. leaf extract: Antimicrobial properties. *2020*.
- Nabila, M. I., & Kannabiran, K. (2018). Biosynthesis, characterization and antibacterial activity of copper oxide nanoparticles (CuO NPs) from actinomycetes. *Biocatalysis and Agricultural Biotechnology*, *15*, 56-62. doi:10.1016/j.bcab.2018.05.011
- Odangowei I Ogidi, N. G. E. a. O. G. D. (2019). Phytochemical, proximate and mineral compositions of *Bryophyllum Pinnatum* (Never die) medicinal plant Odangowei I Ogidi, Ngozi G Esie and Oluchi G Dike.
- Osaili, T. M., Albiss, B. A., Al-Nabulsi, A. A., Alromi, R. F., Olaimat, A., Al-Holy, M., . . . Holley, R. (2019). Effects of metal oxide nanoparticles with plant extract on viability of foodborne pathogens. *Journal of Food Safety*, *39*(5). doi:10.1111/jfs.12681
- Pallela, P. N. V. K., Ummey, S., Ruddaraju, L. K., Kollu, P., Khan, S., & Pammi, S. J. S. A. S. (2019). Antibacterial activity assessment and characterization of green synthesized CuO nano rods using *Asparagus racemosus* roots extract. *1*(5), 1-7.
- Pammi5, P. N. V. K. P. S. U. L. K. R. P. K. S. K. S. V. N. (2019). Antibacterial activity assessment and characterization of green synthesized CuO nano rods using *Asparagus racemosus* roots extract. doi:10.1007/s42452-019-0449-9
- Phiwdang, K., Suphankij, S., Mekprasart, W., & Pecharapa, W. (2013). Synthesis of CuO Nanoparticles by Precipitation Method Using Different Precursors. *Energy Procedia*, *34*, 740-745. doi:10.1016/j.egypro.2013.06.808

- Qamar, H., Rehman, S., Chauhan, D. K., Tiwari, A. K., & Upmanyu, V. J. I. J. o. N. (2020). Green synthesis, characterization and antimicrobial activity of copper oxide nanomaterial derived from *Momordica charantia*. *15*, 2541.
- Raffi, M., Mehrwan, S., Bhatti, T. M., Akhter, J. I., Hameed, A., Yawar, W., & ul Hasan, M. M. (2010). Investigations into the antibacterial behavior of copper nanoparticles against *Escherichia coli*. *Annals of Microbiology*, *60*(1), 75-80. doi:10.1007/s13213-010-0015-6
- Rahimi-Nasrabadi, M., Pourmortazavi, S. M., Davoudi-Dehaghani, A. A., Hajimirsadeghi, S. S., & Zahedi, M. M. (2013). Synthesis and characterization of copper oxalate and copper oxide nanoparticles by statistically optimized controlled precipitation and calcination of precursor. *CrystEngComm*, *15*(20). doi:10.1039/c3ce26930b
- Rajeshwari Sivaraj a, b., ↑,1, Pattanathu K.S.M. Rahman a,1, P. Rajiv b,2, S. Narendhran b,2, R. Venckatesh c,3. (2014). Biosynthesis and characterization of *Acalypha indica* mediated copper oxide nanoparticles and evaluation of its antimicrobial and anticancer activity. doi:10.1016/j.saa.2014.03.027
- Rajivgandhi, G., Maruthupandy, M., Muneeswaran, T., Ramachandran, G., Manoharan, N., Quero, F., . . . Song, J.-M. J. M. p. (2019). Biologically synthesized copper oxide nanoparticles enhanced intracellular damage in ciprofloxacin resistant ESBL producing bacteria. *127*, 267-276.
- Ren, G., Hu, D., Cheng, E. W., Vargas-Reus, M. A., Reip, P., & Allaker, R. P. (2009). Characterisation of copper oxide nanoparticles for antimicrobial applications. *Int J Antimicrob Agents*, *33*(6), 587-590. doi:10.1016/j.ijantimicag.2008.12.004
- S. Suresh, R. I., G. Kalaiyan, S. Thambidurai, P. Kannan, K.M. Prabu, N. Suresh, R. Jothilakshmi, S. Karthick Kumar, M. Kandasamy. (2020). Green Synthesis of Copper Oxide Nanostructures using *Cynodon dactylon* and *Cyperus rotundus* Grass Extracts for Antibacterial Applications. doi:10.1016/j.ceramint.2020.02.015
- Sagadevan, S., Vennila, S., Marlinda, A. R., Al-Douri, Y., Rafie Johan, M., & Anita Lett, J. (2019). Synthesis and evaluation of the structural, optical, and antibacterial properties of copper oxide nanoparticles. *Applied Physics A*, *125*(8). doi:10.1007/s00339-019-2785-4
- Saif, S., Tahir, A., Asim, T., & Chen, Y. J. N. (2016). Plant mediated green synthesis of CuO nanoparticles: comparison of toxicity of engineered and plant mediated CuO nanoparticles towards *Daphnia magna*. *6*(11), 205.
- Sankar, R., Maheswari, R., Karthik, S., Shivashangari, K. S., & Ravikumar, V. (2014). Anticancer activity of *Ficus religiosa* engineered copper oxide nanoparticles. *Mater Sci Eng C Mater Biol Appl*, *44*, 234-239. doi:10.1016/j.msec.2014.08.030
- Sankar, R., Manikandan, P., Malarvizhi, V., Fathima, T., Shivashangari, K. S., Ravikumar, V. J. S. A. P. A. M., & Spectroscopy, B. (2014). Green synthesis of colloidal copper oxide nanoparticles using *Carica papaya* and its application in photocatalytic dye degradation. *121*, 746-750.
- Sankar, R., Prasath, B. B., Nandakumar, R., Santhanam, P., Shivashangari, K. S., & Ravikumar, V. (2014). Growth inhibition of bloom forming cyanobacterium *Microcystis aeruginosa* by green route fabricated copper oxide nanoparticles. *Environ Sci Pollut Res Int*, *21*(24), 14232-14240. doi:10.1007/s11356-014-3362-1

- Santhosh Kumar, J., & Shanmugam, V. J. I. J. R. T. E. (2020). Green synthesis of copper oxide nanoparticles from magnolia champaca floral extract and its antioxidant & toxicity assay using Danio Rerio. *8*, 5444-5449.
- Sathiyavimal, S., Vasantharaj, S., Bharathi, D., Saravanan, M., Manikandan, E., Kumar, S. S., & Pugazhendhi, A. (2018). Biogenesis of copper oxide nanoparticles (CuONPs) using *Sida acuta* and their incorporation over cotton fabrics to prevent the pathogenicity of Gram negative and Gram positive bacteria. *J Photochem Photobiol B*, *188*, 126-134. doi:10.1016/j.jphotobiol.2018.09.014
- Sathiyavimal, S., Vasantharaj, S., Veeramani, V., Saravanan, M., Rajalakshmi, G., Kaliannan, T., . . . Pugazhendhi, A. J. J. o. E. C. E. (2021). Green chemistry route of biosynthesized copper oxide nanoparticles using *Psidium guajava* leaf extract and their antibacterial activity and effective removal of industrial dyes. *9*(2), 105033.
- Seerangaraj Vasantharaj, S. S., Elayaperumal, Manikandan, PII:, P., Reference:, D., date:, T. a. i. R., date:, R. d. A., . . . Arivalaga. (2018). Synthesis of ecofriendly copper oxide nanoparticles for fabrication over textile fabrics: Characterization of antibacterial activity and dye degradation potential. doi:10.1016/j.jphotobiol.2018.12.026  
10.1016/ACCEPTED
- Sepasgozar, S. M. E., Mohseni, S., Feizyadeh, B., & Morsali, A. J. B. o. M. S. (2021). Green synthesis of zinc oxide and copper oxide nanoparticles using *Achillea Nobilis* extract and evaluating their antioxidant and antibacterial properties. *44*(2), 1-13.
- Shaheen, M., Kalwar, N. H., Intisar, A., Batool, Z., Rasheed, S., & Kousar, R. J. O. M. (2021). Efficient surfactant modified copper oxide nanoparticles for solar light driven water purification. *122*, 111688.
- Shahsavani, E., Feizi, N., & DEHNO, K. A. (2016). Copper oxide nanoparticles prepared by solid state thermal decomposition: synthesis and characterization.
- Shameli, K., Bin Ahmad, M., Jaffar Al-Mulla, E. A., Ibrahim, N. A., Shabanzadeh, P., Rustaiyan, A., . . . Zidan, M. (2012). Green biosynthesis of silver nanoparticles using *Callicarpa maingayi* stem bark extraction. *Molecules*, *17*(7), 8506-8517. doi:10.3390/molecules17078506
- Siddiqi, K. S., Rashid, M., Rahman, A., Husen, A., & Rehman, S. (2020). Green synthesis, characterization, antibacterial and photocatalytic activity of black cupric oxide nanoparticles.
- Silva, N., Ramirez, S., Diaz, I., Garcia, A., & Hassan, N. (2019). Easy, Quick, and Reproducible Sonochemical Synthesis of CuO Nanoparticles. *Materials (Basel)*, *12*(5). doi:10.3390/ma12050804
- Sivaraj, R., Rahman, P. K., Rajiv, P., Narendhran, S., Venckatesh, R. J. S. A. P. A. M., & Spectroscopy, B. (2014). Biosynthesis and characterization of *Acalypha indica* mediated copper oxide nanoparticles and evaluation of its antimicrobial and anticancer activity. *129*, 255-258.
- Sorbiun1, M., & , E. S. M., Ali Ramazani1,2 \*, Asemeh Mashhadi Malekzadeh1. (2018). <NCR\_Volume 3\_Issue 1\_Pages 1-16.pdf>. doi:10.22036/ncr.2018.01.001
- Ssekatawa, K., Byarugaba, D. K., Angwe, M. K., Wampande, E. M., Ejobi, F., Nxumalo, E., . . . biotechnology. (2022). Phyto-Mediated Copper Oxide Nanoparticles for Antibacterial, Antioxidant and Photocatalytic Performances. *10*.

- Ssekatawa, K., Byarugaba, D. K., Kato, C. D., Ejobi, F., Tweyongyere, R., Lubwama, M., . . . Wampande, E. M. J. J. o. N. R. (2020). Nanotechnological solutions for controlling transmission and emergence of antimicrobial-resistant bacteria, future prospects, and challenges: a systematic review. *22*(5), 1-30.
- Tshireletso, P., Ateba, C. N., & Fayemi, O. E. (2021). Spectroscopic and Antibacterial Properties of CuONPs from Orange, Lemon and Tangerine Peel Extracts: Potential for Combating Bacterial Resistance. *Molecules*, *26*(3). doi:10.3390/molecules26030586
- Wu, S., Rajeshkumar, S., Madasamy, M., Mahendran, V. J. A. C., Nanomedicine,, & Biotechnology. (2020). Green synthesis of copper nanoparticles using *Cissus vitifolia* and its antioxidant and antibacterial activity against urinary tract infection pathogens. *48*(1), 1153-1158.