Original Research Article

Antibacterial Activity of CuO Nanoparticles Synthesized by *Justicia Adhatoda* Leaf Extract

ABSTRACT

Aims: To determine the antibacterial activity of CuO nanoparticles (CuO NPs) synthesized by *Justicia Adhatoda* leaf extract
Study design: Synthesis, characterization and antibacterial activity determination of CuO NPs.
Place and Duration of Study: PG and Research Department of Chemistry, V.O.Chidambaram College, Tuticorin, Tamilnadu, India, between April 2019 and April 2020.
Methodology: CuO NPs were synthesised using *Justicia adhatoda* leaf extract. UV-Visible spectroscopy was used to characterize CuO NPs. The role of biomolecules in plant extract in the formation of the CuO NPs was identified using Fourier transform infrared spectroscopy. The particle size and lattice properties of CuO NPs were determined using XRD. The surface morphology of CuO NPs was studied using SEM. The presence of Cu and O in CuO NPs was confirmed using EDAX analysis. Bacterial antimicrobial activity was investigated.
Results: An absorbance band at 285 nm in the UV–visible spectrum clearly revealed the formation of CuO NPs. In the FTIR, CuO NPs had a distinct absorption peak at 608 cm^{-1}, confirming metal–oxygen stretching in Cu-O nanostructures. The presence of crystalline CuO NPs was confirmed by XRD patterns, and they were found to be spherical in shape with a size range of 23.21–37.57 nm, as evidenced by FESEM. The biosynthesized CuO NPs exhibited a strong antibacterial activity against *Staphylococcus epidermidis* and *Bacillus cereus* bacteria.
Conclusion: *Justicia adhatoda* leaf extract mediated CuO NPs have shown significant antibacterial activity and they are considered potent antibacterial agents.

Keywords: Green synthesis, Copper oxide nanoparticles, Justicia Adhatoda, antibacterial activity

1. INTRODUCTION

Copper oxide nanoparticles (CuO NPs) have attracted a lot of attention due to their sensing, catalytic, electrical, conducting, optical, and antimicrobial properties. Copper oxide nanoparticles have found applications as antimicrobial agents, metal adsorbents, gas sensors, catalysts, lithium ion electrode materials, solar energy converters, magneto resistance materials, field emission emitters, and high-temperature superconductors [1, 2].

CuO NPs have been synthesised using a variety of methods, including electrochemical reduction, sonochemical, precipitation, and microwave radiation. These methods, on the other hand, are limited to the synthesis of CuO NPs due to the use of hazardous chemicals and their high cost. CuO NPs made by chemical methods are limited in their applications in biological systems and pollute the environment by discharging toxic wastes [3]. Researchers focused on developing an eco-friendly biosynthesis of NPs after learning about the limitations of physio-chemical methods. The biological synthesis of NPs uses enzymes, microorganisms, and plants as environmentally friendly resources [4, 5].

Plant extract-mediated NP synthesis is a widely accepted method for a variety of reasons, including cost effectiveness, ease of use, ease of scaling up, abundant availability, and environmental safety. By acting as excellent reducing and capping agents, phytochemicals found in plant matter play a critical role in improving reduction rate and stabilisation.

Justicia adhatoda (L.) is a shrub that is commonly known as Vasaka or Malabar nut and belongs to the Acanthaceae family. It is a branched, perennial, evergreen shrub with a height of 1.0 m to 2.5 mm, an unpleasant odour, and a bitter taste. It has white, purple, or pink flowers on opposite ascending branches [6]. *Justicia adhatoda* [7] has anti-periodic, antispasmodic, expectorant, diuretic, astringent, and purgative properties. It's a highly valuable medicinal plant that's used to treat coughs, colds, pneumonia, bronchitis, asthma, fever, blood spitting, jaundice, catarrh, and tuberculosis [8]. Vasicine and vasicinone, the plant's most important alkaloids, have been discovered to be biologically active.

The current study used Justicia adhatoda leaf extract to fabricate copper oxide nanoparticles (CuO NPs) in a simple and environmentally friendly manner.

2. MATERIALS AND METHODS

2.1 Chemicals used

E. Merck, Germany, provided the copper sulphate pentahydrate used in the green synthesis of CuO NPs. The leaves of *Justicia adhatoda* used in this study were collected locally (Muthiahpuram, Thoothukudi, Tamilnadu, India).

$CuSO_4 \cdot 5H_2O$
Copper sulphate pentahydrate

Justicia adhatoda leaves

Fig 1. Chemicals used

2.2 Preparation of *Momordica charantia* leaf extract

About 10g of fresh *Justicia adhatoda* leaves were taken, and the leaves were surface cleaned with running tap water, then double distilled water. *Justicia adhatoda* leaves were washed and cut into small pieces, then boiled for 1 hour in 100 mL of distilled water in a round-bottom flask with a condenser. To obtain clean *Justicia adhatoda* leaf extract, the leaf extract was filtered using Whatman No. 41 filter paper (Fig. 2).

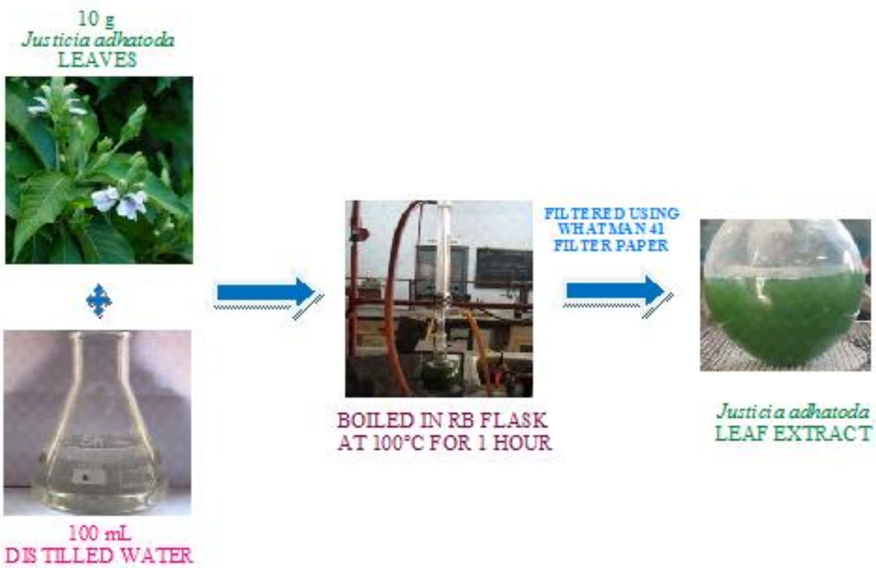


Fig. 2. Preparation of *Justicia adhatoda* leaf extract

2.3 Green synthesis of copper oxide NPs

In a round-bottom flask with a condenser, 30 mL of freshly prepared *Justicia adhatoda* leaf extract was added to 70 mL of 0.1M copper sulphate pentahydrate solution for the synthesis of CuO NPs. For 2 hours, the above mixture was refluxed at 100°C. After that, the biosynthesized CuO NPs were centrifuged and dried (Fig.3).

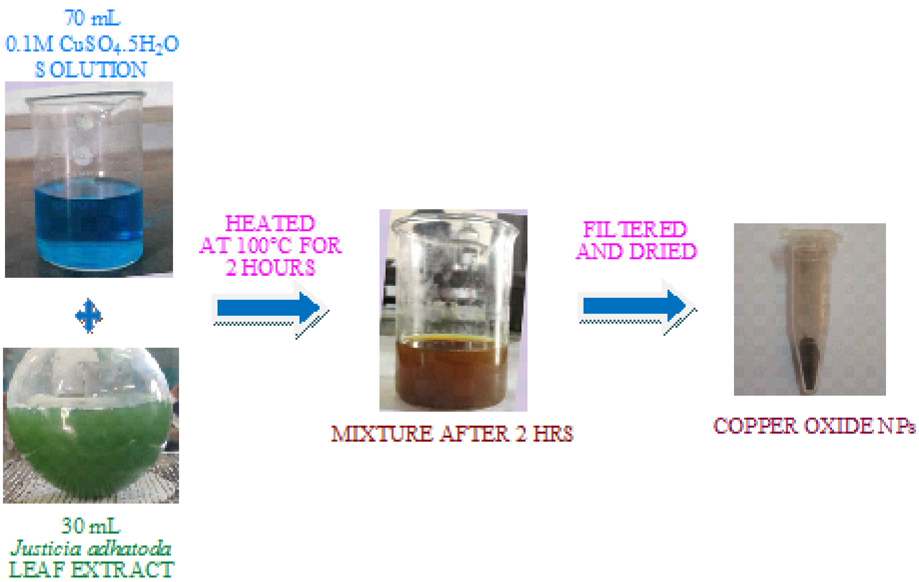


Fig. 3. Biosynthesis of CuO NPs

2.4 Characterization of copper oxide nanoparticles

The UV-Visible spectra of the CuO NPs and *Justicia adhatoda* leaf extract were recorded on JASCO UV-Visible spectrometer. FTIR measurements were performed on a Thermo Scientific Nicolet iS5 instrument in the diffuse reflectance mode at a resolution of 4 cm^{-1} in KBr pellets. The average particle size of CuO NPs was determined by using XPERT-PRO X-ray diffractometer operating at a voltage of 40 kV and a current of 30 mA with Cu Kα radiation. FESEM analysis and EDX analysis were performed on a TESCAN MIRA3 XMU model at Avinashilingam Institute, Coimbatore.

2.5 Antibacterial activity

The antibacterial activity of the samples was determined using the disc well-diffusion method on an agar plate against the test bacteria. Briefly incubated bacteria cultures were swabbed uniformly on individual plates of Muller Hinton Agar using sterile cotton swabs, and 1 mg/ml of sample was loaded onto sterile discs. The plates were incubated for 24 hours at 37°C. The presence of inhibition zones around the discs was measured in millimetres to confirm the inhibition effect.

3. RESULTS AND DISCUSSION

Characterizations and applications of CuO NPs were done by various techniques. The results obtained are discussed in detail as follows:

3.1 Green synthesis of CuO NPs

The colour change in the mixture during the synthesis indicated the reducing action of *Justicia adhatoda* leaf extract on copper sulphate solution for the synthesis of CuO NPs. During the heating process, the colour changed. The dark brown colour of the solution indicates that the phenolics and other phytochemicals found in *Justicia adhatoda* leaf extract act as good reducing agents for the synthesis of CuO NPs. According to previous research on green NP synthesis, phytochemicals in plant parts play a critical role as effective reducing agents for NP synthesis.

3.2 UV-Visible Spectroscopic analysis

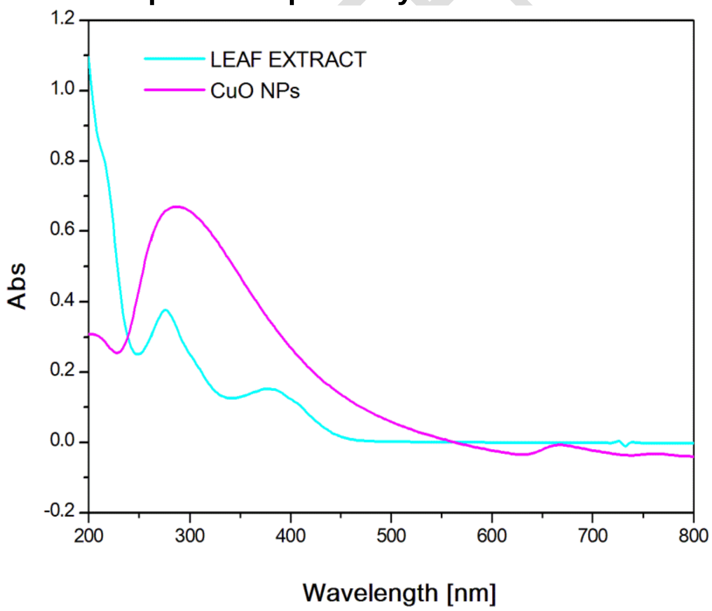


Fig. 4. UV-Vis spectra of CuO NPs and *Justicia Adhatoda* leaf extract

The absorbance band at around 285nm confirms the formation of CuO NPs, as shown in the UV-Vis spectra (fig. 4). The UV-Vis spectrum of the leaf extract reveals bands at 270nm and 375nm arising from π → π* transitions, revealing the presence of phenolic compounds as antioxidants in the *Justicia Adhatoda* leaf extract [10].

3.3 FTIR analysis

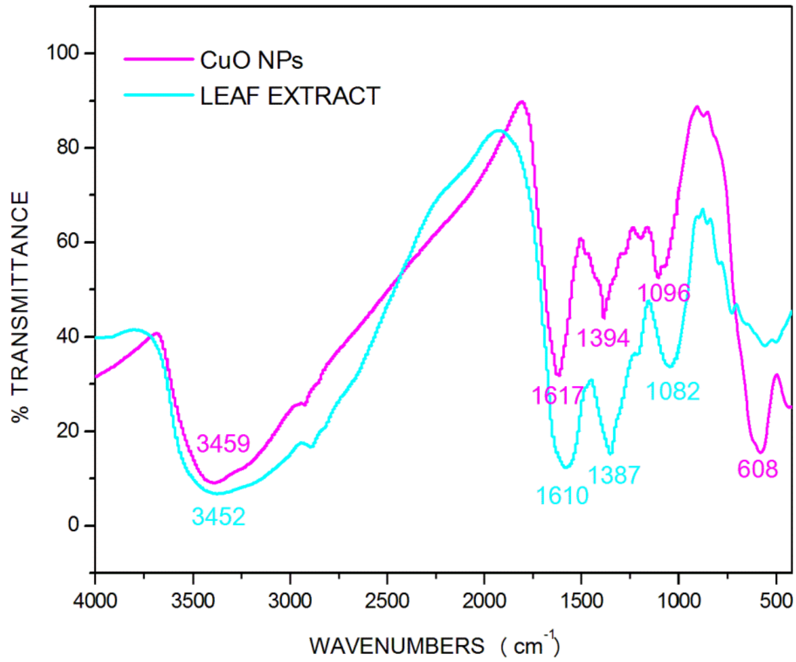


Fig. 5. FTIR spectra of CuO NPs and *Justicia Adhatoda* leaf extract

The FTIR spectrum of *Justicia Adhatoda* leaf extract shows peaks at 1082, 1387, 1610 and 3452 cm^{-1} due to –C-O-C stretching vibration, aromatic C-H group/heteroatom containing C-C group, carbonyl/ amine and O–H stretching mode respectively. These FTIR bands are characteristic of phenolic compounds present in the *Justicia Adhatoda* leaf extract.

The FTIR spectrum of CuO NPs shows peaks at 1096, 1394, 1617 and 3459 cm^{-1} due to –C-O-C stretching vibration, aromatic C-H group/heteroatom containing C-C group, carbonyl/ amine and O–H stretching mode respectively. The spectrum of CuO NPs shows a characteristic absorption peak at 608 cm^{-1}, which confirms the formation of metal–oxygen stretching of Cu-O nanostructure [11].

3.4 X-ray diffraction analysis

The crystallite size of CuO NPs is between 23.21 and 37.57 nm NPs as estimated using the Scherrer formula. CuO NPs have an average size of 29.27 nm.

The (110), (002), (111), (202), (020), (202), and (113) crystalline planes are responsible for the XRD peaks at 32.71°, 34.51°, 39.63°, 52.53°, 57.69°, 63.94°, and 68.16°, which correspond to JCPDS No. 48-1568 [12].

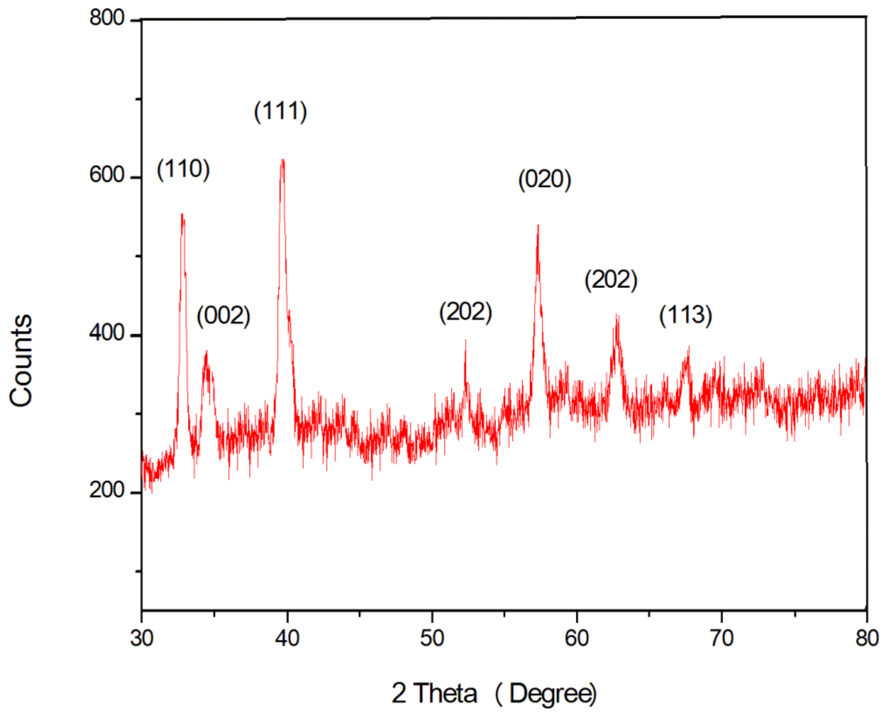


Fig. 6. XRD pattern of CuO NPs

3.5 Field Emission Scanning Electron Microscopy (FESEM)

The size and morphology of the green synthesised CuO NPs by *Justicia Adhatoda* leaf extract were determined using FESEM. The CuO NPs obtained are less than 100 nm in size and spherical in shape, as shown by FESEM images in Figures 7 and 8.

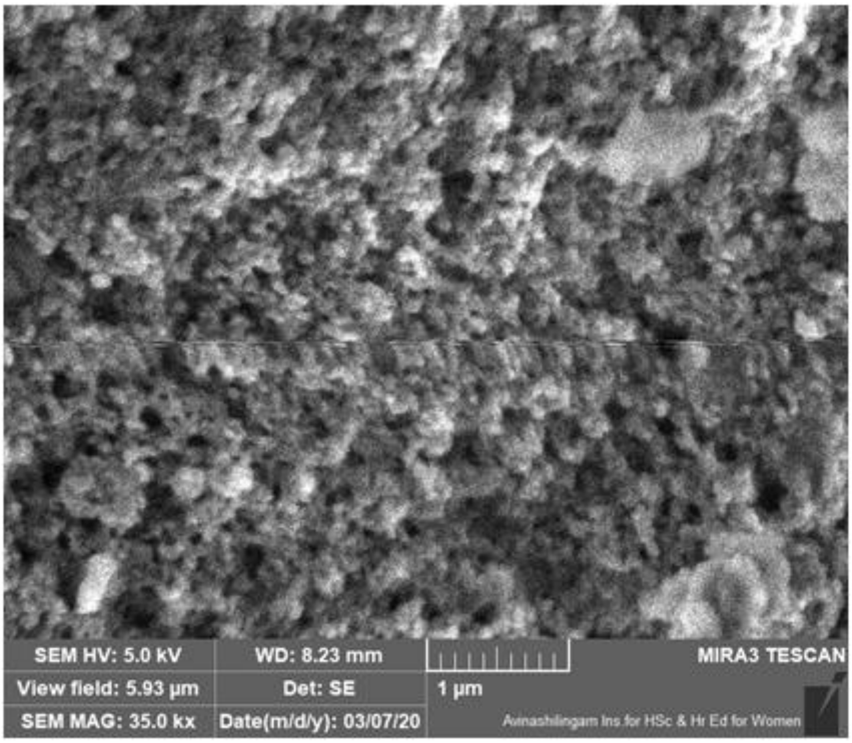


Fig. 7. FE SEM image of CuO NPs in 1 µm scale

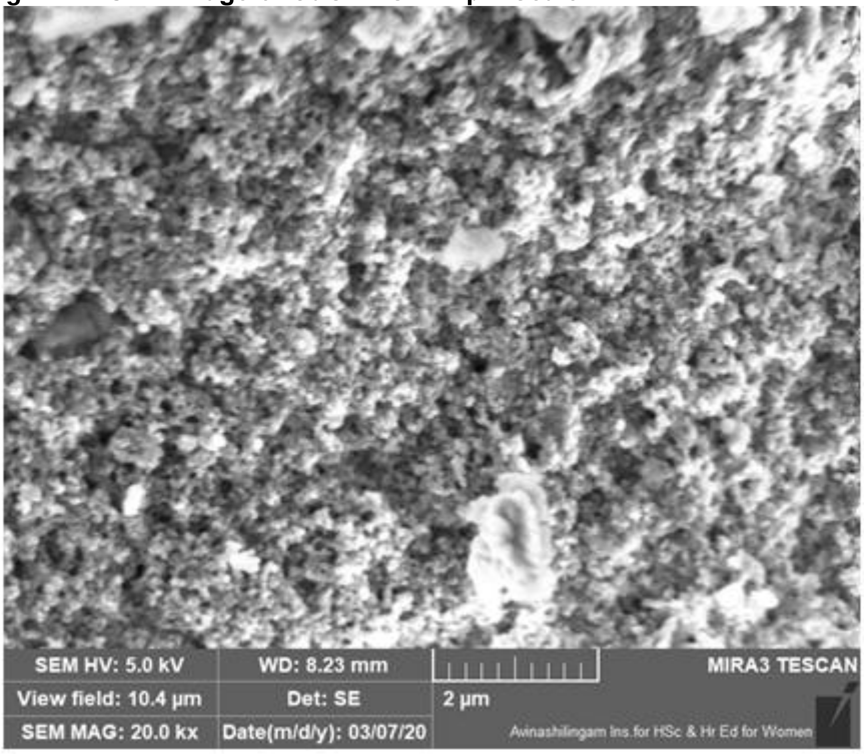


Fig. 8. FE SEM image of CuO NPs (A) in 2 µm scale

3.6 Energy dispersive X - ray analysis (EDAX)

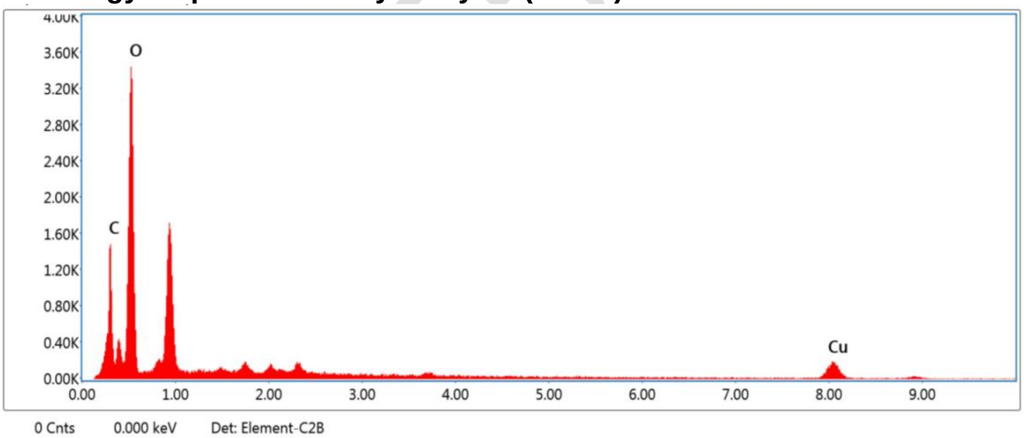


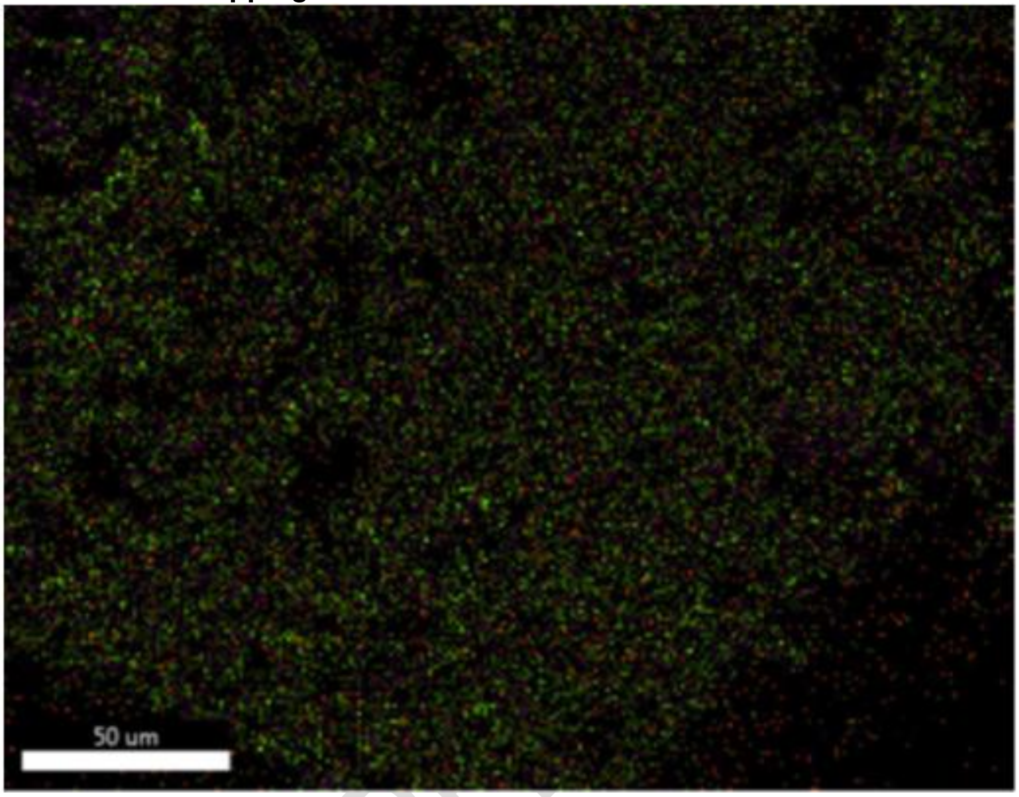
Fig. 9. EDAX spectrum of CuO NPs

Table. 1. EDAX data of CuO NPs

Element	Weight %	Atomic %
C	10.15	15.18
Cu	57.91	49.12
O	31.94	35.7
TOTAL	100.00	100.00

The elemental composition of the synthesised CuO NPs was confirmed using EDAX analysis. In the EDAX spectrum, the presence of copper and oxygen signal peaks confirms that the synthesised NPs are CuO NPs (Fig. 9).

3.7 Elemental mapping



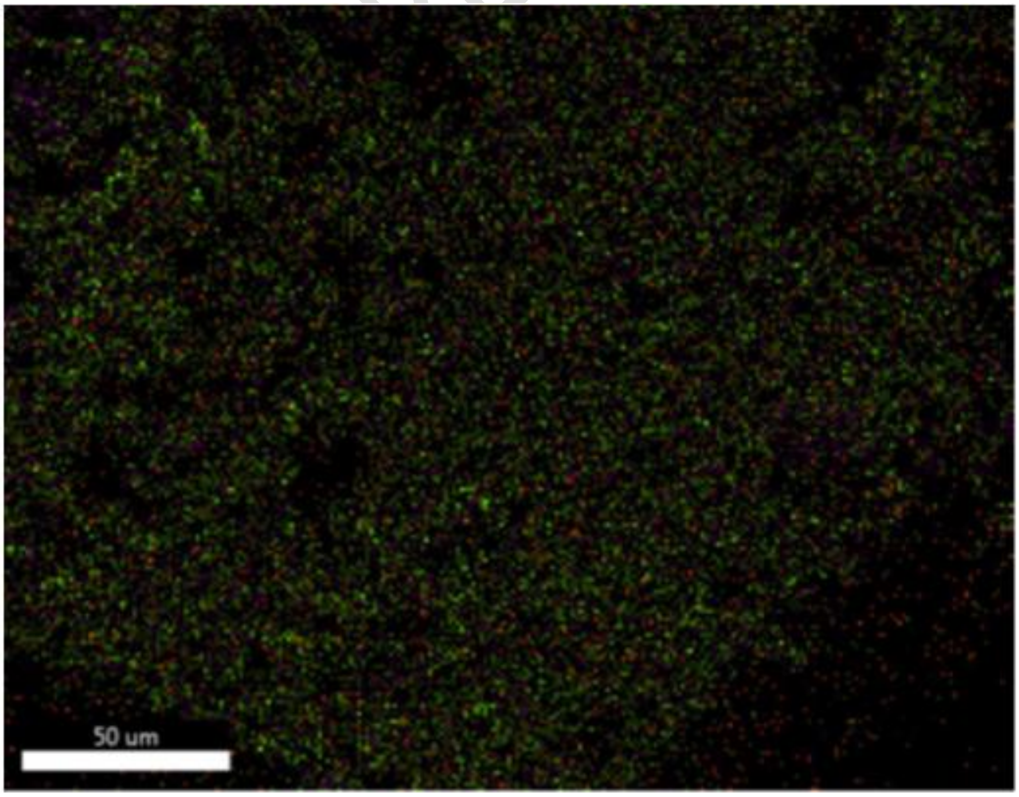


Fig. 10. Elemental map of (•) Oxygen (•) Copper

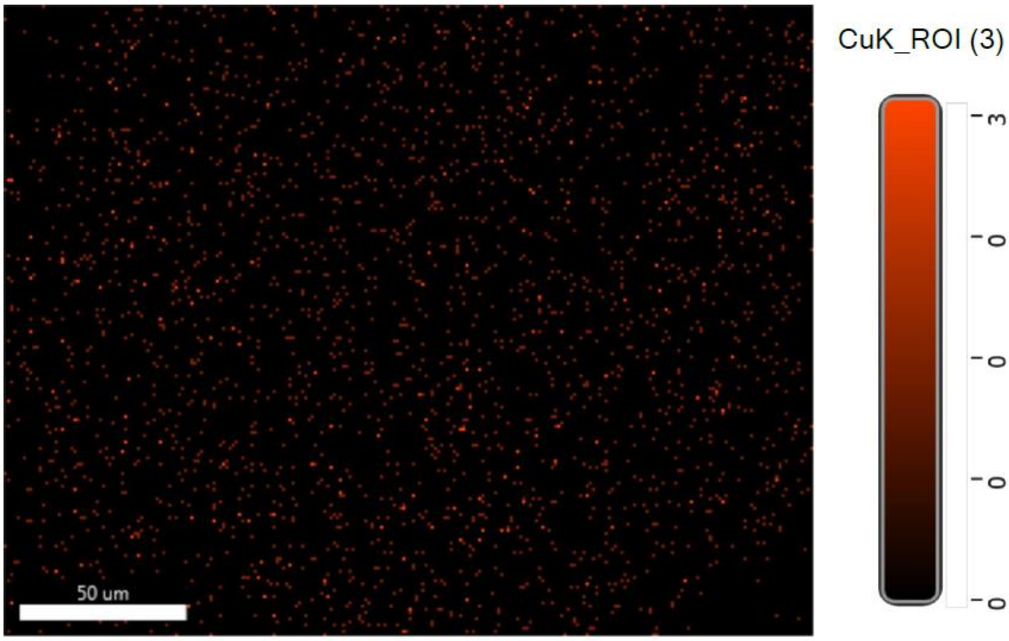


Fig. 11. Elemental map of copper

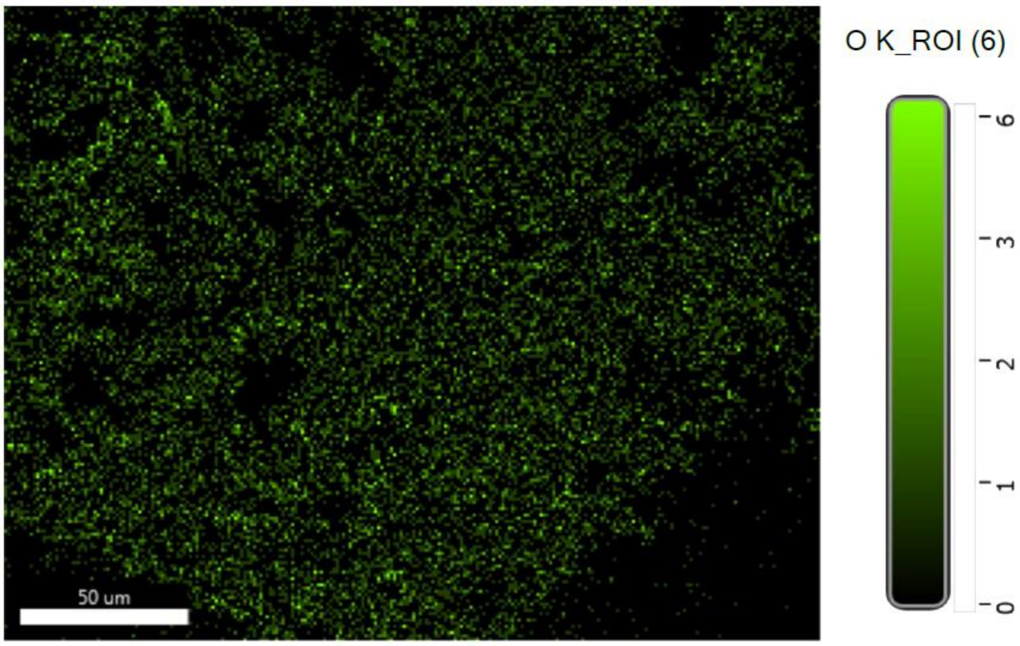


Fig. 12. Elemental map of oxygen

The uniform distribution of copper and oxygen in CuO NPs is confirmed by elemental mapping.

3.8 Antimicrobial activity

Figure 13 depicts the antibacterial activity of biosynthesized CuO NPs against *Staphylococcus epidermidis* and *Bacillus cereus* bacteria.

According to the findings of this study, biologically synthesised CuO NPs have significant antimicrobial properties against *Staphylococcus epidermidis* and *Bacillus cereus* bacteria. CuO NPs' antibacterial activity may be attributed to their ability to bind to the bacterial cell membrane and inhibit the active transport process, causing cell lysis.

Fig.13. Antibacterial activity of CuO NPs on (I) *Staphylococcus epidermidis* (II) *Bacillus cereus*

Table. 2. Antibacterial activity data of CuO NPs

Name of Species	**Zone of Inhibition (mm)**	
	Ampicillin	**CuO NPs**
Staphylococcus epidermidis	17	18
Bacillus cereus	13	14

4. CONCLUSION

The use of Justicia Adhatoda leaf extract in the green synthesis of copper oxide nanoparticles is demonstrated, with the potential role of phenolic compounds as a reducing agent. The formation of CuO NPs is confirmed by a band at 285 nm in UV – Visible spectroscopy. The presence of phenolic compounds in the *Justicia Adhatoda* leaf extract is confirmed by FTIR. CuO NPs with an average crystallite size of 28.47 nm are confirmed by XRD patterns. CuO NPs are spheroid in shape and less than 100 nm in size, as determined by FESEM. The presence of copper and oxygen in CuO NPs is confirmed by EDAX analysis. CuO NPs have high antimicrobial activity against the bacteria *Staphylococcus epidermidis* and *Bacillus cereus*.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge.

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