Tinosporacordifolia **reduced copper oxide nanoparticles synthesis, characterisation, and its antibacterial investigation**

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Abstract

Tinosporacordifolia is a plant that has been found to be a rich source of bioactive compounds that can be used to synthesize copper nanoparticles (CuNPs). Spectrophotometric analysis confirms the presence of these nanoparticles, and their size and structure have been confirmed using energy dispersive x-ray (EDX), Fourier-transmission infrared spectroscopy (FTIR), X-ray diffraction (XRD), and scanning electron microscopy (SEM). The study found that these CuNPs were able to control the growth of a specific bacterial species, *Klebsiella pneumoniae,* at a minimum concentration of 125 µg, while other bacteria such as *Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa* were not affected at doses as high as 1000 µg. This suggests that these CuNPs have selective antibacterial activity and could be used as a safer alternative for future nanodrugs.

Keywords: *Tinosporacordifolia,* Growthinhibition, Copper oxide nanoparticles, Bacteria.

1. Introduction

The use of bio-enzymes, microorganism's by-products, and plant extracts is considered as one of the eco-friendly alternatives to chemical and physical methods for synthesizing nanoparticles. This method is cost-effective and does not require detailed process optimization or scale-up processes as compared to non-biological methods. In addition, plant-derived nanoparticles can be synthesized without the need for detailed process optimization or scale-up processes, which makes this method more beneficial than other methods(Kim and Song, 2009; Mohanpuria et al., 2008; Patil et al., 2012; Shankar et al., 2004; Tyagi et al., 2012). *Tinosporacordifolia*, also known as guduchi or giloy, is a dioecious plant that grows in various regions including India, Sri Lanka, Myanmar, and China. The plant has been found to contain a variety of bioactive compounds, including berberine alkaloids, giloin, and glucosoids such as tinosporine, heptacosanol, clerodanefuranoditerpene, columbin, diterpenoidfuranolactone, tinosporidine, and b-sitosterol. These compounds have been found to have a variety of medicinal properties, including anti-diabetic, anti-inflammatory, anti-periodic, anti-arthritic, anti-spasmodic, anticancer, anti-HIV, anti-leprotic, anti-oxidant, anti-allergic, anti-stress, anti-malaria, hepatoprotection, immunomodulation and anti-neoplastic properties(Chowdhury, 2021; Dhama et al., 2017; Tyagi, 2016). Research has shown that *Tinosporacordifolia* has a variety of biological functions, including the regulation of blood sugar levels and the improvement of therapeutic outcomes in conditions such as osteoporosis and osteoarthritis. In ayurvedic medicine, the plant extract has traditionally been used to treat fever, jaundice, chronic diarrhea, cancer, dysentery, bone fractures, pain, asthma, skin diseases, urinary diseases, allergic conditions, poisonous insect bites, snake bites, and eye disorders. Given these properties, it is important to further investigate the potential of *T. cordifolia* as a source of new bio-nanomaterials(Ghosh and Saha, 2012; Jassim et al., 2016; Mittal et al., 2022). Many research conducted by various scientists have focused on the antibacterial properties of *Tinosporacordifolia* (giloy) extract and biogenic copper nanoparticles. These studies have shown that the CuNPs and extract can inhibit the growth of various pathogenic microorganisms such as *S. aureus*, *E. coli*, *B. subtilis*, *A. niger*, and *Candida spp*. In the current work, we investigated the synthesis of CuNPs and their antibacterial capabilities against the pathogenic bacteria *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *KlebsiellaPneumoniae*.

2.Materials and methods

2.1 Chemicals and Microorganism

The analytical grade chemicals such as copper sulphate pentahydrate procured from Himedia, India. The given solutions prepared in sterile distilled water in mM concentrations.The pathogenic bacteria employed in this study were isolated from chicken faeces; include *Escherichia coli* (Acc. No. LC747145), *Klebsiellapneumoniae* (Acc. No.LC747146), *Staphylococcus aureus* (Acc. No.LC747148), and *Pseudomonas aeruginosa* (Acc. No. LC747147).

2.2 Collection of plantsand preparation of extract

The mature stems of*Tinosporacordifolia*(Giloy) were collected from Nagpur district, Maharashtra for the preparation of copper nanoparticles (Fig. 1).1g of stem dried powder was kept in the sterile distilled water (100ml) for the 48 hours and extract filtered with muslin cloth used for CuNPs synthesis

2.3 Synthesisof copper nanoparticles

To produce copper nanoparticlesstem powder of *T. cordifolia* rich in polyphenol extract (1% aqueous) mixed with 20mM $CuSO_4.5H_2O$ in 1:1 ratio in an amber coloured bottle. The preparationsthen kept in dark for 24 hours. The change in colour noted as preliminary formation of copper nanoparticles. The content then pelleted by centrifugation at 15,000 RPM for 30 minutes. The pellet devoid of supernatant was then added with pure ethanol to remove traces of water content and kept drying in an oven at 60 °C for next 72 hours. The dried content then stored in a 1.5 ml centrifuge tube in a dark condition till further analysis.

2.4 Characterization of copper nanoparticles

Initially spectrophotometric analysis carried out at 200-600 nm for CuNPs to record plasmon resonance up to 72 hours of formation. The XRD- X ray diffraction performed with Braker EcoD8 advance using nickel filteredCuKa (λ = 1.5405 A°) radiation. The average crystalline size (t) determined using line broadening using Scherrer's relation: $t= 0.9 \lambda / ß \cos\Theta$, here λ is X- ray wavelength and ß is full width of half maximum (FWHM). In addition, scanning electron microscopy (Zeiss) caried out by once glass slide mounted with powdered sample exposed to capture surface structural details at a resolution of 1 µm to 10 nm to record nanoparticles by their shapes and sizes along with EDX analysis. Fourier transform infra-red spectroscopy for CuNPs noted with KBr pellets Shimadzu IR trace100 as facility availed fromKalasalingam Academy of Research and Education in Tamil Nadu, India.

2.5 Antibacterial activity of CuNPs

CuNPs synthesized from *T. cordifolia*were evaluated for potential growth inhibitory activity employing antibacterial activity against*Escherichia coli* (Acc.No. LC747145), *Klebsiella pneumoniae*(Acc. No. LC747146), *Staphylococcus aureus* (Acc.No. LC747148) and *Pseudomonas aeruginosa* (Acc.No. LC747147)isolated from chicken faecal matter. The testing concentrations set on 62, 125, 500 and 1000 µg/ml in a nutrient broth preloaded with 0.5 O.Disolates by MacFarland as per minimum inhibitory assay. The reaction was allowed to incubate for 24 hours along with control sets. Upon incubation change in O.D. for growth inhibitory concentration recorded at 560 nm to determine the minimum inhibitory dose concentration in each group.

3. Result

3.1. Spectrophotometric Analysis

The CuNPs prominently developed after 24 hours of reaction was confirmed by spectroscopic analysis (Fig. 2). As per spectral analysis, Giloy stem based CuNPs noted with absorption maxima at 320 nm with absorbance of 0.971 while at 0 hr it was 0.516 absorption noted at 300 nm. Further only giloy stem extract absorption maxima noted at 300 nm with 0.812 absorption and of $CuSO_4.5H_2O$. (2mM) at 290 nm with 0.318 absorption. In a similar finding CuNPs derived from leaf extract *Catha edulis* found to be showing absorption maxima at 333 nm which is very close to our finding when common $CuSO_4.5H_2O$ used as precursor(Kiflom Gebremedhn et al., 2019).

3.2. XRD study of copper oxide nanoparticles

XRD based measurement mainly been used for determining crystalline nature of the nanoparticles and their respective phases. The CuNPs produced by *T. cordifolia* recorded with XRD diffraction pattern of 2ϴ= 32.254, 35.2,85, 38.518, 48.463, 53.264, 53.419, 58.176, 58.079, 61.165, 61.272, 61.363, 65.363, 65.758, 65.959, 67. 893, 72.133, 74.944 and 75.104 were assigned to 307, 2341, 2755, 632, 213, 211, 270, 279, 338, 341, 351, 550, 630, 462, 172, 181 and 231 net intensities, respectively of monoclinic CuNPs (J CPDS-05-0661). Hence, the XRD spectrum evident to form crystalline nature of CuNPs synthesized from the plant extract of *T. cordifolia*(Fig. 3)(Kumar et al., 2015; Sarkar et al., 2020; Vigneshwaran et al., 2007).

3.3. FTIR analysis of copper nanoparticles

FTIR analysis of *T. cordifolia*based CuNPs in the range of 500- 4000 cm^{-1} with KBr pellet carried out in Shimadzu IRtracer-100 device. FTIR analysis able to detect many functional groups and chemical bonds available in synthesis NPs (Fig. 4). Peaks at 451.31, 569 and 1060 cm^{-1}noted with established stretching bond of O atom in CuNPs structure(Calvo-De La Rosa and Segarra Rubí, 2020; Kombaiah et al., 2018). The weak peak transfer related to 451 cm^{-1} to other region of 569 and 642 cm^{-1} confirmed an early transfer of the stretching bond from the tetrahedral space to the octahedral location(Dayana et al., 2022; Manikandan et al., 2021). The peak at 3664.75 cm^{-1} attributed to stretching vibration of O-H group of plant phenolic compounds. Hence it can be related that plantsplays a reducing role for the CuNPs synthesis(Raeisi et al., 2021). The available peaks at 3664, 1708, 1543, 1060 cm^{-1}linked with synthesized copper nanoparticles must be surrounded by polyphenols or proteins. Thus, these biomolecules are stabilizing the copper nanoparticles as noted earlier by(Luo et al., 2014; Mohanraj et al., 2014; Rengasamy et al., 2016).

3.4. SEM and EDX analysis of copper nanoparticles

The SEM image at 300 nm resolution represented for copper nanoparticles reduced by *T. cordifolia* (Fig.5). The synthesized copper nanoparticles recorded as amorphous in nature and noted to agglomerate upon storage. As per EDX analysis elemental copper nanoparticles having Kα line at 7.8 and Lα at 0.08 (X- ray energies of copper) (Fig.6). The Kα1 label noted to oxygen with value of 0.05. these two elements generally appear from sulphur – rich phytochemicals which remains capsulated into the nanomaterial. The peaks are pure in EDX hence confirms absence of impurities of the sample. As per SEM analysis (Fig 5) formed nanoparticles of synthesized CuNPs ranging less than 100 nm at least in one dimension as noted earlier also by(Das et al., 2020)that strengthens our findings.

3.5. Antibacterial activity

As tested for potential antibacterial activity against four bacterial pathogens once concentrations of nanoparticles set at 62, 125, 500 and 1000 µg/ml, it has been observed that CuNPs only able to control *Klebsiella pneumoniae* strain having multidrug resistant feature with as low as 125 µg/ml concentration.Here other species recordedto be resistant towards synthesized CuNPs and hence can be stated as species specific drug controlling *K. pneumoniae* only which could be mentioned as specific drug reported. In a similar kind of study,*Ocimumamericanum*aqueous leaf extract(Manikandan et al., 2021, 2017), leaf extract of *Sidaacuta*(Sathiyavimal et al., 2018)also able to control *Klebsiella pneumoniae* acting as human pathogens like present study.

4. Conclusion

Nano world research is putting forward the hidden mysteries of nanoparticles. Its success can be gauzed from many angles as nanoparticles becoming an internal part of material research. Here we have reported one of the nanoparticles-based success stories by synthesizing *Tinosporacordifolia*reduced CuNPs which areamorphous in nature controlling specific species of multidrug resistant bacterial kingdom. Here minimum of 125 µg/ml of concentration of *Tinosporacordifolia*reduced CuNPs controlling particlular*Klebsiellapneumoniae*strains only and thus can be presented as specific drug molecule for further course of research. Need of the action specific drug is in demand, whereour CuNPs fulfilling the utmost demand of specificity and activity both and hence can be put forward for further research to elucidate its hidden potential in greater detail.The entire process of producing copper oxide nanoparticles, their characterisation, and their antibacterial investigation are shown in Fig. 7.

Acknowledgement

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Figures

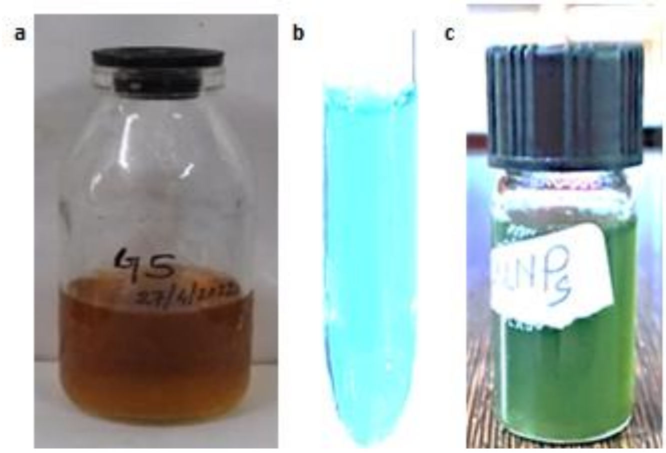


Fig. 1: *Tinosporacordifolia* stem extract(a),Copper(II) sulfate pentahydrate(b)and CuNPs (c).

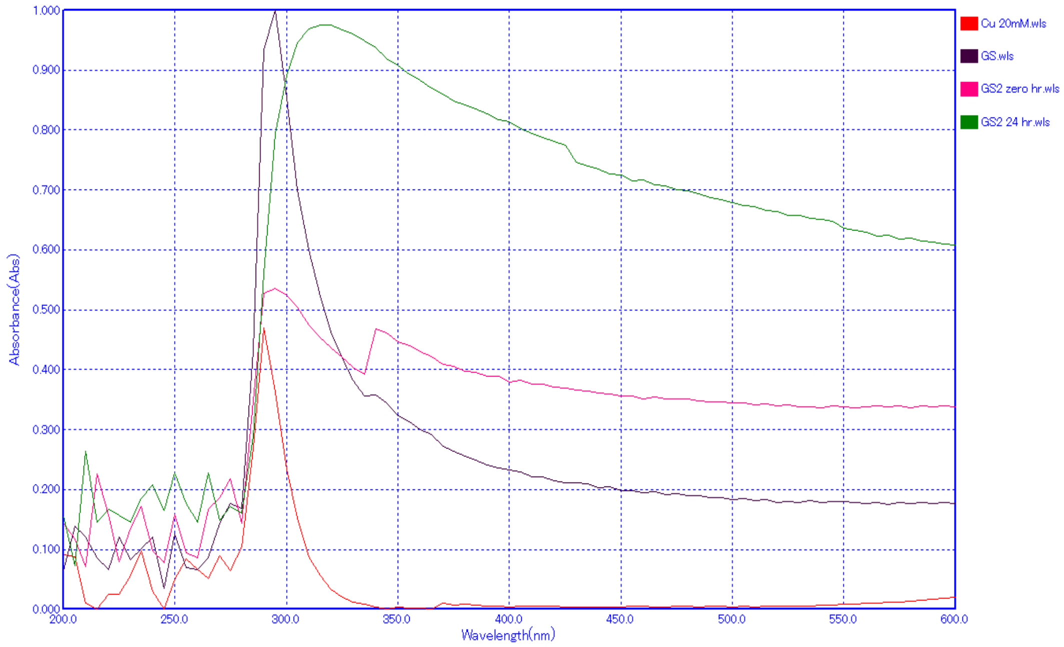


Fig. 2: Spectroscopic analysis of CuNPs along with pure copper solution, Giloy stem extract, and 0-hour preparation reading along with 24 hours reading.

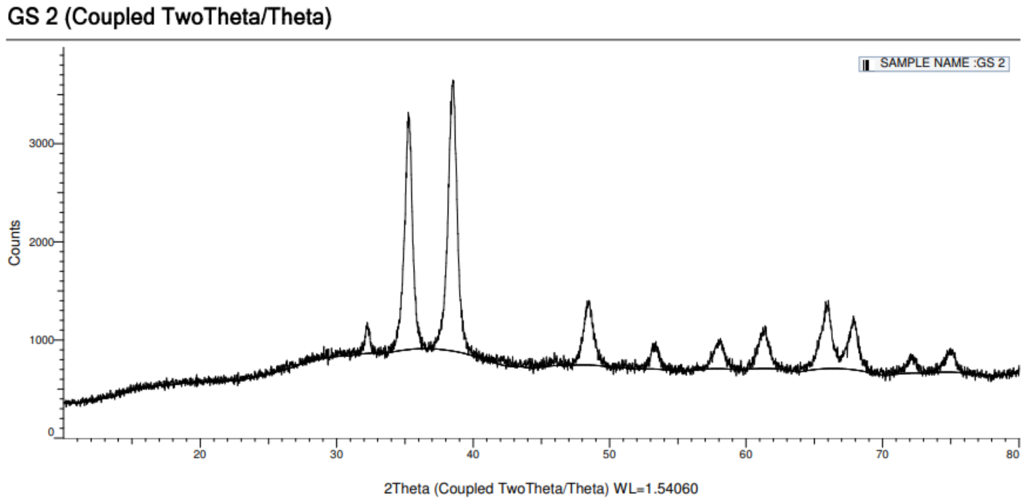


Fig. 3: XRD spectrum of copper nanoparticles prepared from *Tinosporacordifolia*

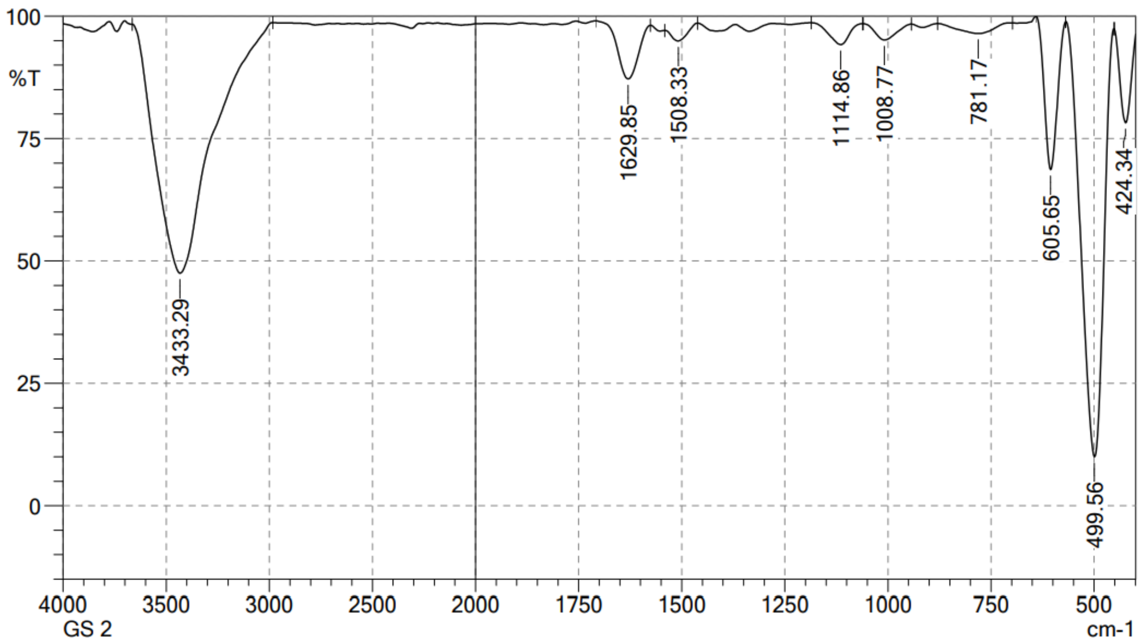


Fig. 4: FTIR absorption spectra of copper nanoparticles prepared from *Tinosporacordifolia*

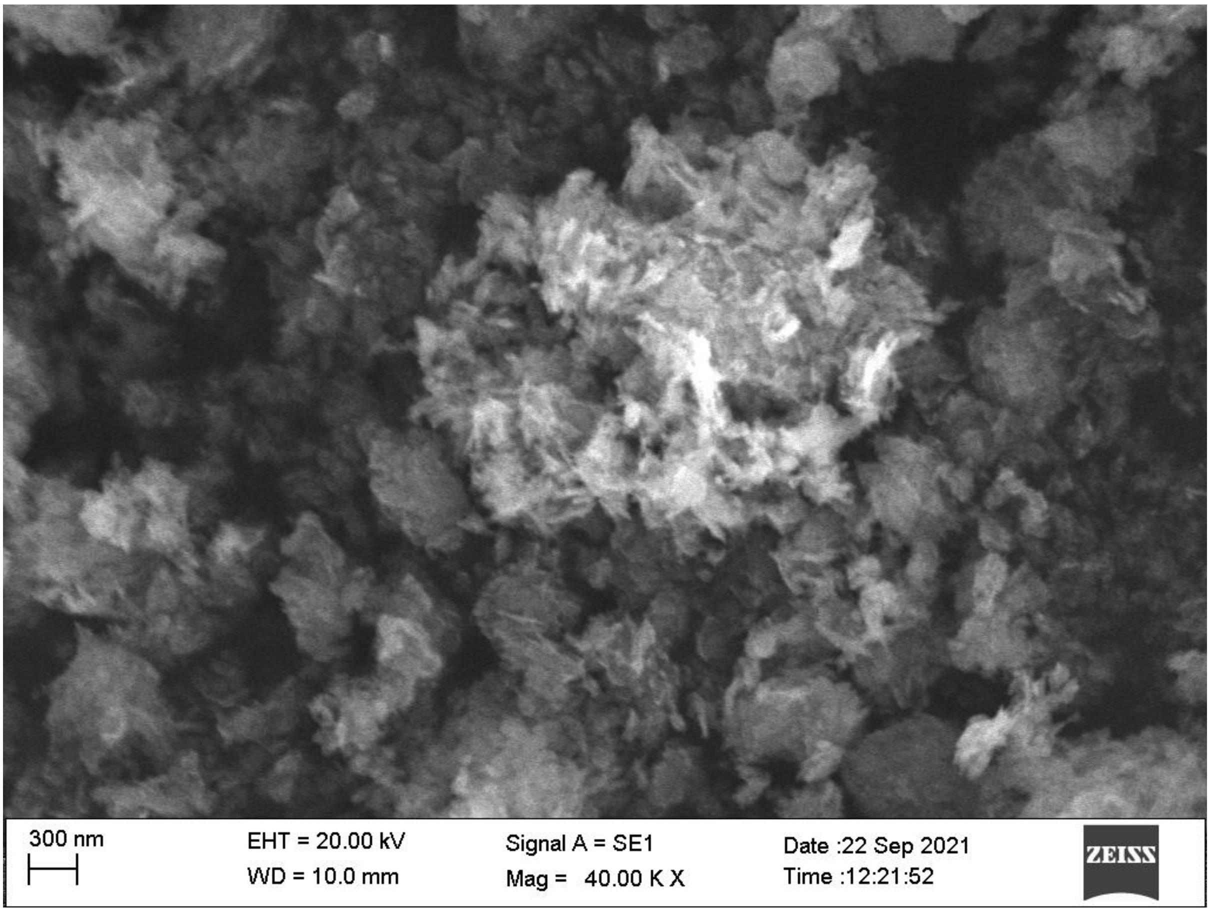
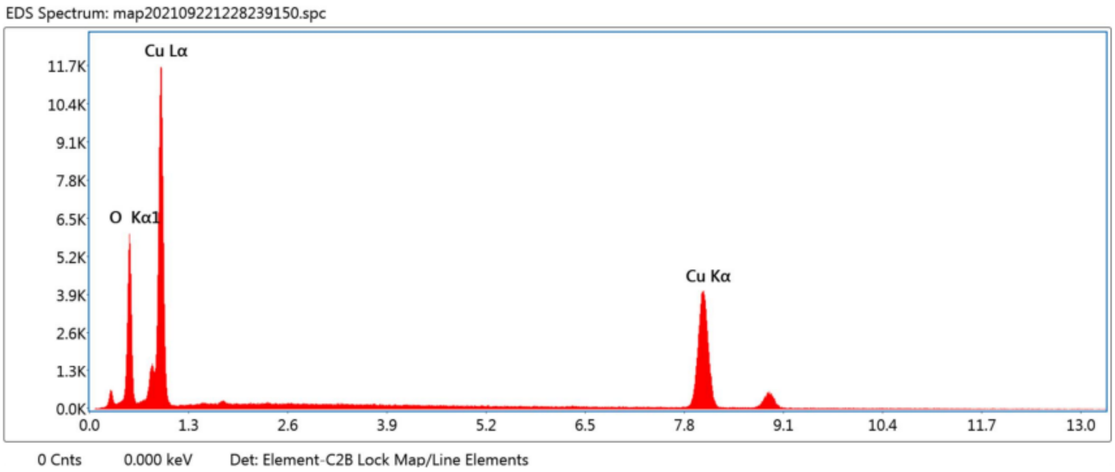


Fig. 5: SEM of *Tinosporacordifolia* reduced CuNPs at 300 nm scale resolution



Smart Quant Results

Element	Weight %	Atomic %	Error %	Kratio
O K	20.7	50.9	7.4	0.1058
CuK	79.3	49.1	2.1	0.7418

Fig. 6: EDX spectra of *Tinosporacordifolia* reduced copper nanoparticles recorded with presence of copper and oxygen atoms.

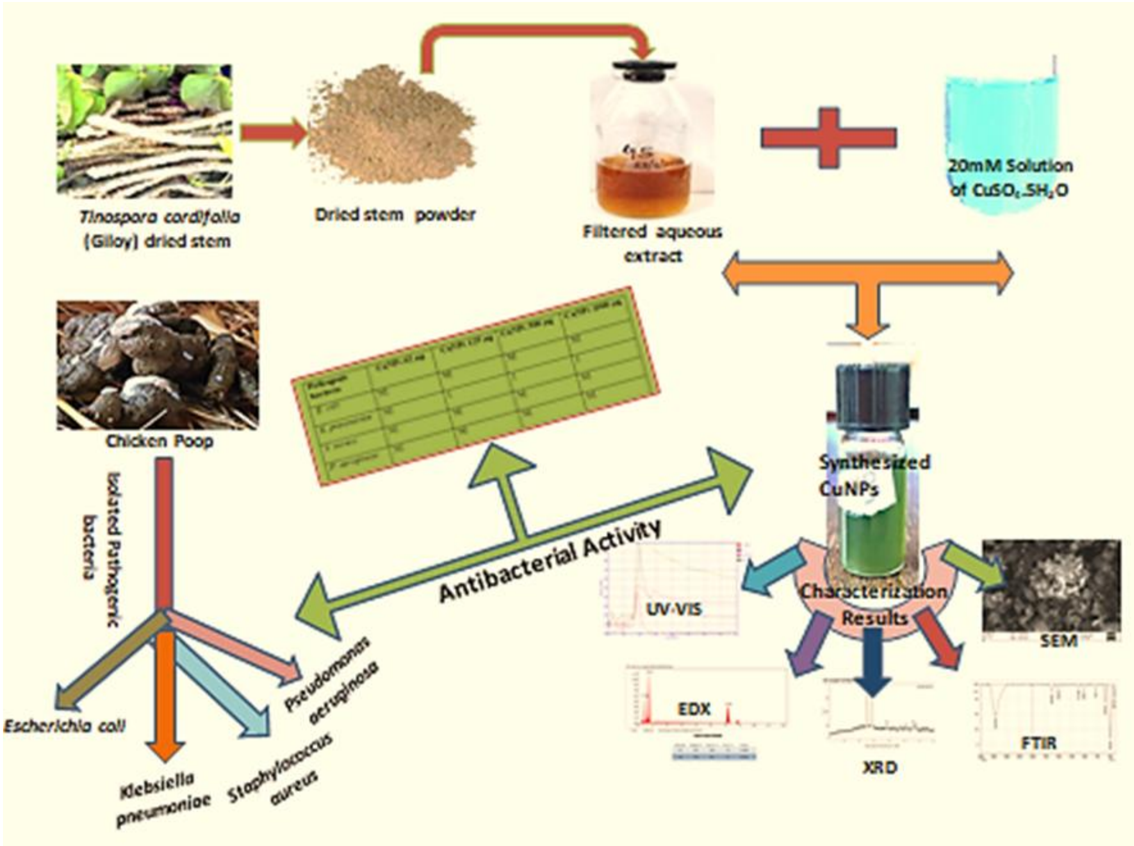


Fig. 7: The entire process of producing copper oxide nanoparticles, their characterisation, and their antibacterial potential.

Table

Table 1: Antibacterial activity of CuNPs against pathogenic bacteria

Pathogenic bacteria	**CuNPs 62 μg**	**CuNPs 125 μg**	**CuNPs 500 μg**	**CuNPs 1000 μg**
E. coli	NI	NI	NI	NI
K. pneumoniae	NI	I	I	I
S. aureus	NI	NI	NI	NI
P. aeruginosa	NI	NI	NI	NI

NI- No inhibition; I- inhibition