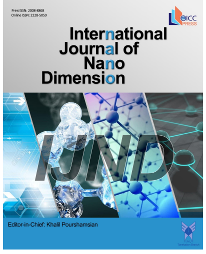

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Green synthesis, characterization, and biological activities of copper nanoparticles using *Clitoria ternatea* leaf extract

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Original Research

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Abstract:

Nanotechnology has a broad spectrum in various fields such as chemistry, biology, physics, material sciences, and engineering. Due to the unique features of nanoparticles, it has a wide range of biological applications like Nanomedicine, biosensors, agriculture, and industry. The current study uses an environmentally friendly method of green synthesis of copper nanoparticles using *Clitoria ternatea* leaf extract. By using sophisticated instruments like UV-visible spectroscopy, FTIR, SEM, and zeta potential, the copper nanoparticles were characterized. The FTIR spectrum analyzed the functional groups in leaf broth. Scanning Electron Microscope (SEM) confirmed the size and morphology (spherical) of copper nanoparticles. The charge and size of CuNPs were confirmed by Zeta potential. The copper nanoparticles exhibited good antibacterial activity on Gram-positive (*Bacillus subtilis*) and Gram-negative (*E. coli*) bacteria and antifungal properties on fungal strain *A. niger*. The free radical scavenging activities of copper nanoparticles were studied with DPPH and Hydrogen Peroxide in vitro assay methods. The biogenic CuNPs act as good antioxidants and larvicidal agents. The antiviral activity of CuNPs was investigated on *Sesbania mosaic virus* (SeMV), which infects *Sesbania* plants. The green synthesized CuNPs act as good antiviral agents for decreasing the mosaic symptoms caused by the *Sesbania Mosaic Virus* in the *Sesbania* plant, and it indicates the antiviral efficacy of copper nanoparticles in the Agricultural sector.

Keywords: Antimicrobial activity; Antioxidant activity; Antiviral activity; Characterization; Copper nanoparticles; Larvicidal activity

1. Introduction

Nanotechnology is a multidisciplinary emerging science with a wide range of applications in various fields, including medicine, agriculture, optics, biosensors, and pharmaceuticals [1]. Nanoparticles include metallic and non-metallic materials, which can be synthesized by physical, chemical, and biological processes [2]. In the chemical process, nanoparticles can be synthesized by the electrochemical method [3], precipitation [4], sol-gel method [5, 6], chemical reduction [7], hydrothermal composition, and sonication [8, 9]. Whereas in physical methods like plasma, mechanical milling, gamma radiation, and laser ablation [10–12], nanoparticles are used to explore fields like medical technology, biotechnology, modern science, and medical imaging due to their prospective applications and characteristic properties regarding their size, shape, and morphology [13–15]. Because of their small size, nanoparticles readily interact with cell membranes, receptors, and proteins, making them valuable in medicine and research, particularly for their antimicrobial properties [16]. Researchers nowadays obtain fabricated materials at the nanoscale by physical and chemical methods, which require energy, time, and cost, and are monotonous processes to get nanoparticles [17]. Both processes are harmful to the ecosystem and human health [18], and in the chemical process, catalysts can be used that pose environmental conflicts and hazards. However, in biological processes, plant extract is one of the vital branches of synthesizing nanoparticles, which act as capping and reducing agents, so like the green synthesis method are natural, environmentally friendly, cost-effective, and non-toxic, and the green nanoparticle acts as a heterogeneous catalyst, which is eco-friendly [19–27]. The green metal nanoparticles, metal oxide nanoparticles, possess great potential for antibacterial, antifungal, antilarval, and bioremediation, and biological applications because of their special mode of action, whereas in antibiotics, they can penetrate the cell wall, but the nanoparticles can make direct contact with the bacteria cell wall. When the metal atom is fused with the oxygen atom the metal oxide nanoparticles and nanometals structure can be invariable, and they mainly have use and adverse effects on tissues and cells in biological applications [28]. Due to their medicinal, optoelectronic, magnetic, and catalytic properties, as well as their surface area, size, and physical and chemical properties of metal oxide nanomaterial to produce bulk amounts so more intense research attention by researchers as well as they are nontoxic which is widely used in the face made products, ointments for infections, wounds, and plant protection [29]. The biological method can be carried out by bacteria [30, 31], fungi [32], actinomycetes [33], algae [34], and plants like *Sesbania grandiflora* [35], *Ocimum* leaf extract [36], *Ziziphus nummularia* [2], etc.

Due to its high thermal conductivity, good electrical conductivity, high corrosion resistance, improved malleability, good ductility, low-cost reactivity, and strong catalytic activity, copper is one of the eight most common metals on Earth and is classified as a transition metal [37–44]. In the ancient world, copper was used for ornaments, weapons, coins, etc. [45]. A trace amount of copper is needed for plants and animals. Humans require a minimum amount ($<$100 mg/day), which can be obtained from various sources such as shellfish, nuts, cereals, and grains [43]. In contrast, the plant is also necessary for their metabolisms, protein regulation, electron transport, etc. Various physical and chemical methods like pulsed laser ablation [46], plasma decomposition, pulsed wire discharge [47], vacuum vapor decomposition [48], sonochemical reduction [49], mechanical milling, and electrolysis [50–54] are used to synthesize copper nanoparticles. These end products are hazardous and toxic and require a long time to synthesize. Therefore, researchers are exploring the green route to the synthesis of copper nanoparticles. Various parts of plants, such as roots, leaves, stems, and fruit, can be used. Through biological methods, the nanoparticles show good antimicrobial activities since they can directly interact with microbes at the cellular level and perform various functions [55, 56]. The phytochemicals present in plants are used to reduce metal ions to nanoparticles. By using the green method, copper nanoparticles are synthesized through various plant extracts like *Ziziphus spina-christi* (L) Wild [57], *Syzygium alternifolium* [58], etc. The plant extracts are used for the capping and stabilization of copper nanoparticles. The biological synthesis of copper nanoparticles demonstrates anti-cancer and antioxidant activities.

The plant chosen for the study is *Clitoria ternatea*, which belongs to the family Fabaceae and is also called "Butterfly Pea". In many foreign countries, plant flowers are used for ornamental purposes, fodder, and medicinal purposes. In ancient times, each part of the plant, such as roots, flowers, and leaves, was used in Ayurvedic medicine. The whole plant has high medicinal value. In the past, the *Clitoria ternatea* flower was used to treat snake bites, and its roots were used to treat arthritis and various diseases like leprosy, fever, and tuberculosis. The leaves of *Clitoria ternatea* are useful for treating otalgia (ear pain), hepatopathy (liver disease), and neurological disorders [59]. According to Devi et al., this plant has also been used to treat bronchitis and regulate respiratory issues (asthma) [60]. The plant contains secondary metabolites and phytochemicals like phenols and flavonoids. These secondary metabolites play a key role in the green synthesis process [61, 62]. *Clitoria ternatea* leaf extract is utilized for the synthesis of copper nanoparticles, with characterization conducted via UV-visible spectroscopy, FTIR, SEM, and Zeta potential analysis. Additionally, its antimicrobial, antioxidant, antiviral, and larvicidal properties have been thoroughly investigated.

2. Experimental

2.1 Collection of plant material

The plant *Clitoria ternatea* was collected from Uyyalawada village, Alagadda Mandal, Kurnool district, Andhra Pradesh, India. Commonly, the plant is called Shankupushpam (local name), as shown in Fig. 1.

2.2 Preparation of leaf extract

Five grams of fresh *Clitoria ternatea* leaves were taken and washed under running tap water. The leaves were chopped into small pieces and macerated using a mortar and pestle. The ground leaf extract, consisting of 100 mL of distilled water, was filtered through muslin cloth followed by Whatman No.1 filter paper.

2.3 Preparation of copper sulphate

For the preparation of CuNPs, 5 mM copper sulfate was weighed and dissolved in 100 mL of distilled water [63].

2.4 Synthesis of copper nanoparticle

For the biosynthesis of copper nanoparticles, the copper sulfate solution is added to the plant extract slowly. After

Figure 1. Clitoria ternatae plant.

a few minutes, color change was observed, indicating the formation of copper nanoparticles.

2.5 Characterization of copper nanoparticles

2.5.1 UV-Visible spectroscopy

The primary characteristic of CuNPs is determined by UV-visible spectroscopy at SV University, Tirupati. The reduction of CuNPs from copper sulfate using plant extract was monitored at the absorption peak in the wavelength range of 200 – 800 nm.

2.5.2 Fourier-transform infrared spectroscopy (FTIR) analysis

From the plant extract biosynthesized copper nanoparticles to analyze by FTIR, the presence of the functional groups such as amino and carboxyl in the nanosuspension can be confirmed. The FTIR spectral analysis of the copper nanoparticle was performed using the Perkin Elmer spectrophotometer.

2.5.3 Scanning electron microscopy (SEM) analysis

The biosynthesized copper nanoparticles extract from the plant *clitoria ternate* was prepared and the sample was kept in a sterile tube and packed tightly without any leakage. A scanning electron microscope was used to confirm the surface morphology, size, and shape of CuNPs.

2.5.4 DLS particle size and Zeta potential

With Zeta Potential, the CuNPs' potentiality and stability charge were investigated. The size of the CuNPs was ascertained using dynamic light scattering. At the DST Purse Centre, Sri Venkateswara University, Tirupati, Zeta Potential and Nanoparticle Size Analysis were performed via Laser Diffractometry with an SZ-100 apparatus.

2.6 Antibacterial activity

The antibacterial activity of CuNPs was evaluated using the agar-well diffusion method [64]. According to this method, a nutrient agar medium was prepared with peptone, NaCl, agar, and distilled water. The medium was sterilized in an autoclave. The media were poured into sterile Petri plates and allowed to solidify. Bacterial strains such as Gram-positive (*B. subtilis, S. aureus*) and Gram-negative (*E. coli, K. pneumoniae*) were spread onto the media using a sterile L-shaped rod. Later, four wells were made using a sterile borer loaded with different concentrations (25, 50, 75, and 100 µL) of copper nanoparticles. The values are taken as means of duplicate sets. Streptomycin antibiotic was used as a standard control. All plates were incubated at 37 °C for 24 – 48 hours. The formation of the zone of inhibition was then measured using a centimeter scale.

2.7 Antifungal activity

The antifungal activity of copper nanoparticles was studied against the fungal strain A. niger by using agar-well diffusion method [65]. For this, potato dextrose agar media was prepared and sterilized in an autoclave maintained at 121 °C. The media was poured into sterile Petri plates and allowed to solidify. The fungal spore suspension was spread onto the PDA agar plate. Borers were used to create four wells. Different concentrations of CuNPs (25, 50, 75, and, 100 µL) were loaded into the wells of the Petri plate and incubated for 48 – 72 hours. The experiment was performed by duplicate sets of values. The zone of inhibition around the wells was measured in cm.

2.8 Antioxidant activity

Antioxidant activity was assessed using DPPH and hydrogen peroxide scavenging activities.

2.8.1 Hydrogen Peroxide scavenging activity

The technique described by Ruch et al. [66] was used to perform the hydrogen peroxide scavenging activity. For this, 43 mM of hydrogen peroxide and 0.1 M of phosphate buffer (pH-7.4) were used. A solution of 0.6 mL H_2O_2 and, 3.4 mL PBS was prepared to make a total volume of 4 mL. Various concentrations of CuNPs (100, 150, 200, and, 250 µL) were added to the solution slowly and incubated for 10 minutes at room temperature. The absorbance value was measured at 230 nm using a UV spectrometer. The experiment was performed in duplicate sets. Ascorbic acid was used as a standard. The radical scavenging activity was computed using the following formula:
Percentage of RSA = [(Abs of control − Abs of the test sample)/Abs of control]$\times$100

2.8.2 DPPH radical scavenging activity

The antioxidant activity of copper nanoparticles was determined by DPPH [67]. To do this, 4 mg of DPPH was dissolved in 100 mL of methanol and then kept at 20 °C. One milliliter of DPPH was taken from the stock solution, then 1 mL of methanol and various concentrations of copper nanoparticles (0.5, 1, 1.5, and, 2 mL) were added. The reaction mixture was stored in a dark area. Following the incubation time, absorbance at 517 nm was measured using UV-Spectroscopy. Methanol served as a blank. Ascorbic acid was used as the standard. The mean values of duplicate sets were taken as a percentage of radical scavenging activity was determined using the following formula:
Percentage of RSA = [(Absorbance of control − Absorbance of the test sample)/Abs of control]$\times$100

2.9 Larvicidal activity

The larvicidal action of copper nanoparticles was specifically investigated on the second and third instar larvae of the *Aedes aegypti* mosquito, which were collected from stagnant water surrounding the Sri Venkateswara University campus in Tirupati. Different concentrations of CuNPs (50, 100, 150, and, 200 µL) were added to each Petri plate containing ten larvae in water. At regular intervals, observations were made. The percentage mortality rate was then recorded using the formula:
Percentage of mortality = (No. of dead larva / No. of larva introduced)$\times$100

2.10 Antiviral activity

2.10.1 Sample collection

The *Sesbania grandiflora* plant, infected with the *Sesbania Mosaic Virus*, was collected from the Department of

Virology, Sri Venkateswara University, Tirupati, Andhra Pradesh.

2.10.2 Preparation of virus inoculum

The diseased leaves of the *Sesbania grandiflora* were removed and pulverized with 0.1 M phosphate buffer (pH 7.0) using a mortar and pestle. This process prepared the virus inoculum. The sap was then passed through a muslin cloth filter. The acquired sap solution was utilized for the experiment.

2.10.3 Anti-viral activity

CuNP antiviral efficacy was investigated using *Sesbania grandiflora* as the host plant. In a suitable environment, the seeds were grown in plastic glasses with pore diameters. Next, *Sesbania grandiflora* plant leaves were used for the antiviral activity. A mixture of copper nanoparticle concentrations (100 and, 200 µL) was added to the infected virus inoculum. Using a mechanical sap inoculation method, the samples were treated on the host plant at different intervals of incubation (30 min, 60 min, 90 min, and, 120 min.) by dusting carborundum powder and rubbing slowly without damaging the leaf. Following that, the symptoms were closely monitored and recorded daily.

3. Result and discussion

3.1 Conformation of nanoparticle

The immediate confirmation of CuNPs was verified as the color shifted from green to pale green. A similar report shows that copper nanoparticles are formed [68, 69]. Clitoria ternatea plant extract acts as the reducing agent for the formation of CuNPs, as shown in Fig. 2. Due to their surface plasmon resonance, a color change was observed [70]. The plant contains secondary metabolites and secretes NADPH-dependent nitrate reductase enzymes. The enzyme is responsible for the reduction of nanoparticles [71, 72].

3.2 Characterization

3.2.1 UV-Visible spectroscopy

The green synthesized CuNPs were initially assessed using UV-visible spectroscopy, which confirmed their identity through the observation of an absorption peak at 418 nm.

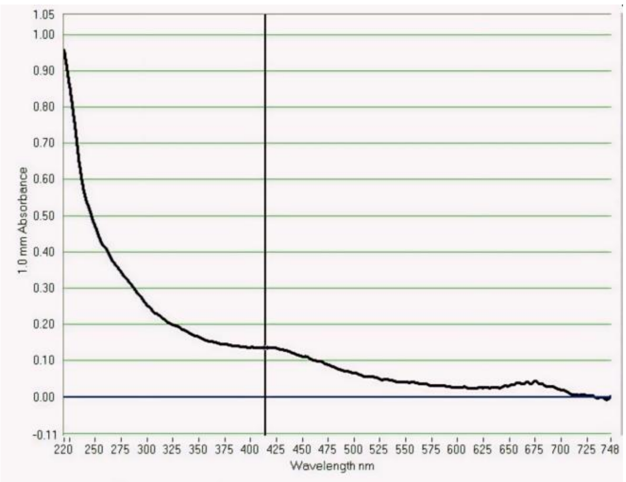

Figure 3. UV-visible spectroscopy of copper nanoparticles.

In a UV-visible spectrophotometer, the surface plasmon resonance (SPR) of metallic nanoparticles produces an absorption band [73]. The characteristic peak of surface plasmon resonance (SPR), as illustrated in Fig. 3, is also evident in the copper nanoparticle showing a peak at around 400 nm [74, 75].

3.2.2 FTIR

The biomolecules (functional groups) within the plant extract are responsible for both the reduction and stabilization of nanoparticles. This was confirmed through FTIR analysis, illustrated in Fig. 4, where the spectra of CuNPs displayed a peak stretching at 3334.94 1/cm, indicating the presence of alcohol and phenol hydroxyl functional groups. Additionally, broad-spectrum peak bands were observed at 1635.85 1/cm (O-H bending) and at 645.17 1/cm, suggesting the presence of secondary metabolites like flavonoids and phytochemicals, which contribute to the stability of copper nanoparticles [76]. Similar findings have been reported for copper nanoparticles derived from *Celastrus paniculatus* Willd leaf extract [77].

3.2.3 Scanning electron microscope

Scanning electron microscopy analyses the size and shape of copper nanoparticles. The biosynthesized CuNPs exhibit

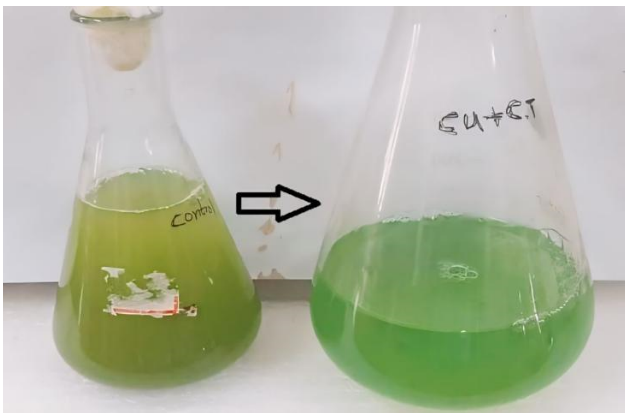

Figure 2. Green synthesis of copper nanoparticles.

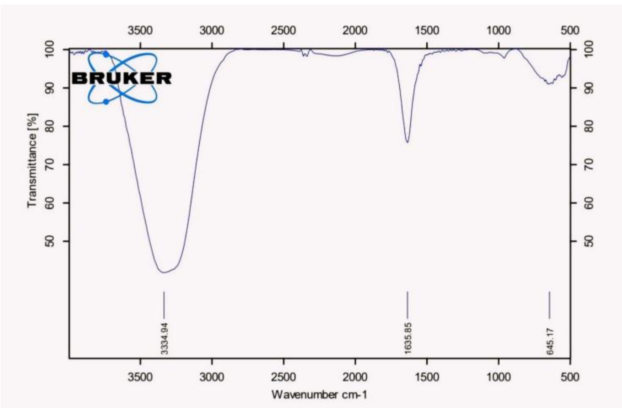

Figure 4. Shows FTIR analysis of copper nanoparticles.

spherical particles with some instances of agglomeration, and irregular shapes as depicted in Fig. 5. The size of copper nanoparticles was observed to fall within the range of 1 to 10 nm and also above 100 nm as per particle analyzer and the average is 55.2 nm Similar reports the average range around 50 to 60 nm seen in *Fortunella margarita* leaves [78]. The nanoparticle structures are heterogeneous and complex. The shapes like cylindrical spherical, polygonal, and irregular, which indicate the different facets and orientations of copper nanoparticles, it may influence the properties and features of nanoparticles [79].

3.2.4 DLS particle size and Zeta potential:

The Zeta potential determines the charge stability of nanoparticles, influenced by electrostatic repulsion between the charged particles, giving rise to the zeta potential values [4]. Dynamic Light Scattering (DLS) is employed to examine the average particle size in polydisperse suspensions, as it accounts for the Brownian motion of nanoparticles [54]. The Zeta potential and DLS particle size of copper nanoparticles were measured at 0.8 mV and 55.2 nm, respectively, as illustrated in Fig. 6 and Fig. 7. Similar findings regarding the average length of nanoparticles have been reported for copper nanoparticles using leaves of *Azadirachta indica* [4] and *Syzygium alternifolium* [58].

3.3 Antibacterial activity

The antibacterial activity was assessed against the bacterial strains, including *E. coli*, *B. subtilis*, and *K. pneumoniae*. The zone of inhibition, indicative of nanoparticle efficiency, was measured and documented in Table 1. Fig. 8(a-d) illustrates that different concentrations of CuNPs resulted in varying zones of inhibition. Increasing the concentration of CuNPs led to a corresponding increase in the zone of inhibition. For instance, in *E. coli*, 100 µL of CuNPs exhibited a substantial zone of inhibition measuring 2 cm, whereas 25 µL showed lesser inhibition with 1 cm. Similarly, in *B. subtilis*, notable antimicrobial activity was observed with a 2.5 cm zone of inhibition at a higher concentration (100 µL) of CuNPs, while lower concentrations (25 µL) of CuNPs resulted in a smaller zone of inhibition with 1.2 cm. *K. pneumoniae* also displayed significant inhibition zones, particularly with 100 µL of CuNPs showing a 3 cm zone of inhibition and exhibiting a 1.2 cm zone of inhibition at 25 µL of CuNPs. Among the three bacterial strains, *K. pneumoniae* showed the most prominent antibacterial activity with a 3 cm zone of inhibition when treated with 100 µL of CuNPs. This activity was compared to that of the antibiotic-resistant strain (streptomycin) as shown in Fig. 9. The biosynthesized CuNPs can penetrate the cell, disrupt and cleave the

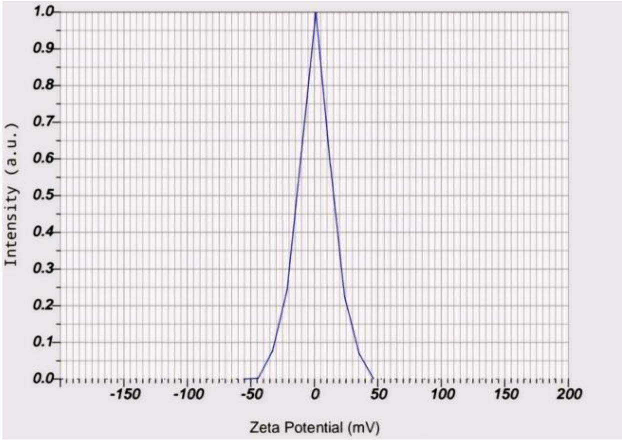

Figure 6. Zeta potential of copper nanoparticles.

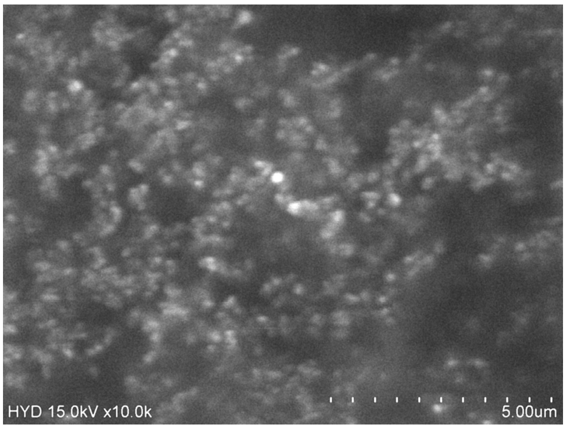

Figure 5. SEM image of copper nanoparticles.

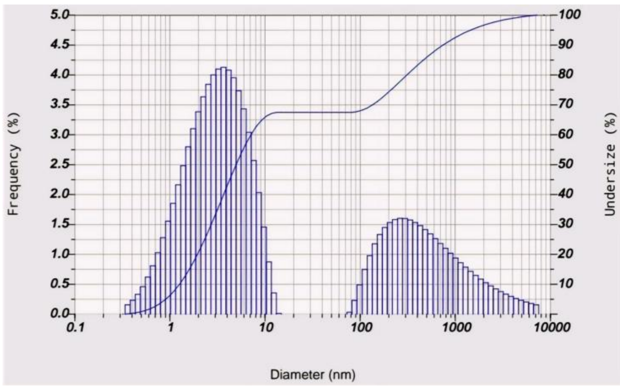

Figure 7. Particle size analysis of CuNPs.

Table 1. shows the copper nanoparticles' antibacterial efficacy against several bacterial strains.

S.No	Concentration of CuNPs (µL)	Zone of Inhibition (cm)			
		E.coli	*B.subtilis*	*K.pneumoniae*	Streptomycin
1.	25	1	1.2	1.4	3.2
2.	50	1.6	1.7	1.6	3.3
3.	75	1.8	2.0	2.5	3.4
4.	100	2.0	2.5	3.0	3.6

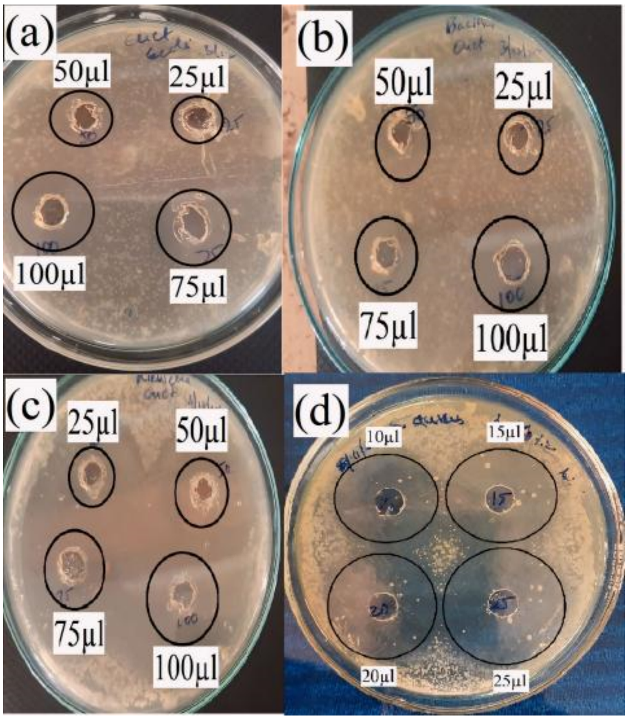

Figure 8. Antibacterial study of copper nanoparticles investigates on (a) *E.coli*, (b) *B.subtilis*, (c) *K.pneumonia*, (d) Antibiotic (Streptomycin).

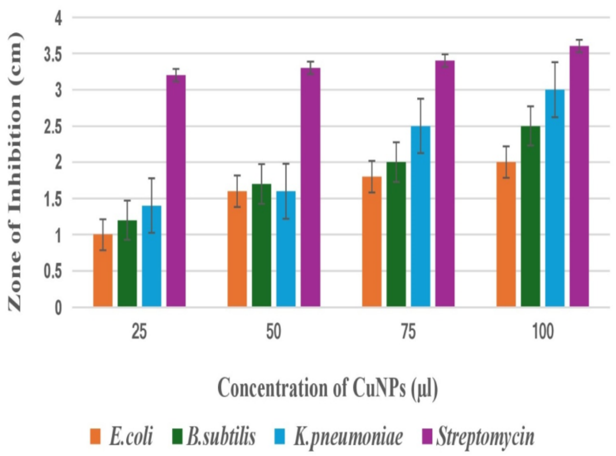

Figure 9. A Comparison of the different bacterial strains of zone of inhibition.

Table 2. Antifungal activity of CuNPs against the fungal strain *A.niger*.

S.No	Concentration of CuNPs (µL)	Zone of Inhibition (cm)
1.	25	0.7
2.	50	2
3.	75	2.2
4.	100	2.8

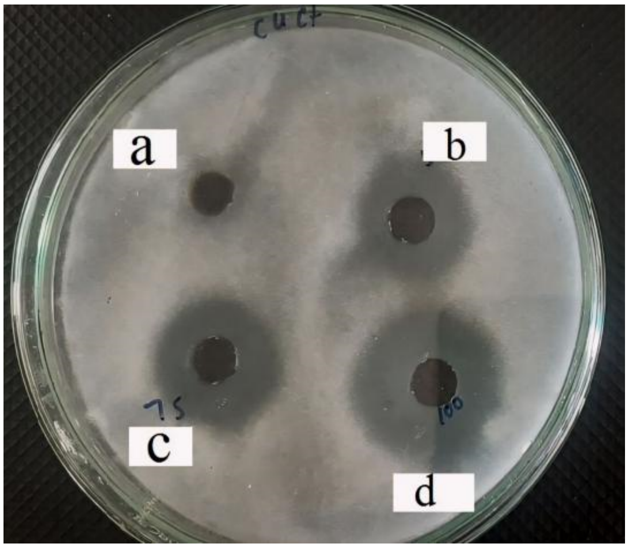

Figure 10. Depicts the antifungal activity of different concentrations of CuNPs, (a) 25 µL, (b) 50 µL, (c)75 µL, (d) 100 µL.

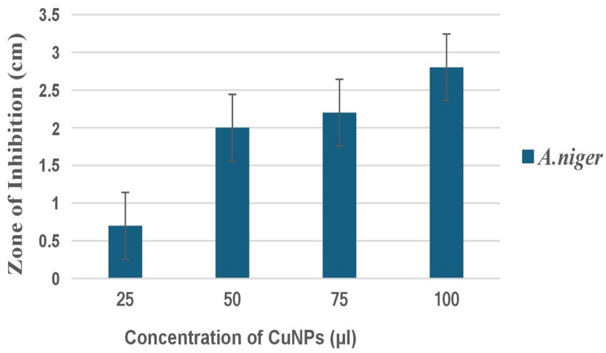

Figure 11. Antifungal activity of CuNPs against *A.niger*.

genome, and inhibit cellular mechanisms, ultimately leading to cell killing [80]. Similar findings have been reported with *Tilia* plant extract [81].

3.4 Antifungal activity

The antifungal activity was assessed against the fungal strain *A. niger*, with results summarized in Table 2. The biosynthesized CuNPs exhibited significant antifungal activity. Specifically, at a concentration of 100 µL, CuNPs displayed a zone of inhibition of 2.8 cm, while at 25 µL, it was 0.7 cm. At concentrations of 50 µL and 75 µL, the maximum zones of inhibition observed were approximately 2 cm and 2.2 cm, respectively, as illustrated in Fig. 10(a-d). The formation of maximum zones indicates the high fungicidal efficacy of CuNPs, as depicted in Fig. 11. The antifungal mechanism of biosynthesized CuNPs involves nanoparticle penetration of the cell wall, disruption of cell structure and functions, and inhibition of DNA replication and protein synthesis, ultimately leading to cell death [77]. Due to the adsorption of biomolecules onto their effective size and large surface area, they can destroy the fungal cell membrane [82]. Similarly, the zone of inhibition against the fungal strain was studied in *Grewia asiatica* [83].

3.5 Antioxidant activity

3.5.1 Hydrogen peroxide scavenging activity

Antioxidant activity was determined using the Hydrogen peroxide scavenging assay, with results presented in Table 3. The biosynthesized CuNPs demonstrated good antioxidant activity. Specifically, 250 µL of CuNPs showed

Table 3. Hydrogen Peroxide scavenging assay of CuNPs and Ascorbic acid.

S.No	Concentration of CuNPs (µL)	Percentage of antioxidant activity	
		CuNPs	Ascorbic acid
1.	100	70.9	87.6
2.	150	80.6	88.8
3.	200	83	93.7
4.	250	85.4	94.5

85% radical scavenging activity, whereas 100 µL showed 70% antioxidant activity. These activities are depicted in Fig. 12. In water and air, microorganisms naturally contain low levels of H_2O_2, but when it decomposes, it forms free hydroxyl radicals which can damage DNA. The phenolic groups present in plant extracts can donate electrons, neutralizing and stabilizing H_2O_2 [84]. Copper nanoparticles have shown superior hydrogen peroxide activity as compared to other plants, such as *Spondias pinnata* [85].

3.5.2 DPPH radical scavenging activity

The DPPH activity was used to determine the antioxidant capacity of the biosynthesized CuNPs, as presented in Table 4. For this, CuNPs were added to DPPH and methanol, and after 24 hours, a slight color change from brown to light yellow was observed. The antioxidant activity increased proportionally with the concentration of CuNPs, with significantly good antioxidant activity noted at higher concentrations. Specifically, 2 mL of CuNPs exhibited 94% antioxidant activity, while 0.5 mL showed 87%. Ascorbic acid was used as a standard. These results are elucidated in Fig. 13. According to [65], during the metabolic process, free radicals are released in the body, leading to oxidative stress and various health issues such as severe illnesses and

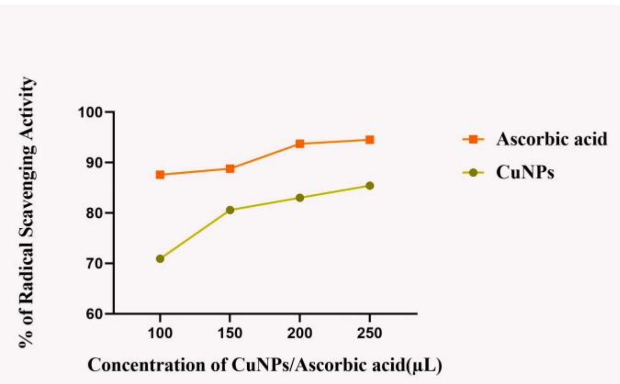

Figure 12. Percentage of H_2O_2 radical scavenging activity of CuNPs and Ascorbic acid.

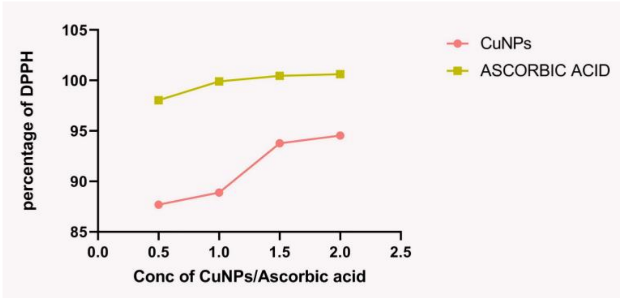

Figure 13. Percentage of DPPH activity of CuNPs and Ascorbic acid.

cardiovascular diseases. Antioxidant properties found in medicinal plants can help control or reduce diseases by neutralizing free radicals [86]. Recent research has indicated that metal nanoparticles possess antioxidant properties [87]. Plant extracts containing secondary metabolites contribute to stabilization and electron donation [32]. The antioxidant properties in plants can inhibit lipid oxidation and maintain cell structure and function by neutralizing free radicals [88].

3.6 Larvicidal activity

The larvicidal activity was tested against Aedes aegypti mosquito larvae, as detailed in Table 5. Within 24 hours, 200 µL of CuNPs showed a high mortality rate. Notably, with 50 µL of CuNPs, six larvae have died. Meanwhile, 100 µL and, 150 µL of CuNPs exhibited the maximum mortality rate, as observed in Fig. 14 and, Fig. 15(a-d). This indicates that increasing the concentration of CuNPs is directly proportional to the death rate. After 48 hours, a concentration of 50 µL CuNPs resulted in the death of ten larvae. 50% of the mortality rate is shown in 50 µL concentration within 24 hrs whereas LC_{90} depicts 100% of mortality in other concentrations (100 µL, 150 µL, and 200 µL). This suggests that the mortality rate increases based on the dose dependence of nanoparticles. Upon introduction to mosquito larvae, CuNPs bind to cell membranes, integrate into the cell, inhibit growth, and damage DNA [68]. These CuNPs interact with cellular proteins, inhibiting enzymatic reactions, lead-

Table 4. Explains the DPPH radical scavenging activity of CuNPs and Ascorbic acid.

S.No	Concentration of CuNPs (µL)	Percentage of DPPH scavenging activity	
		CuNPs	Ascorbic acid
1.	0.5	87.6	98.03
2.	1.0	88.8	99.91
3.	1.5	93.7	100.4
4.	2.0	94.5	100.6

Table 5. Illustrate the larvicidal activity of copper nanoparticles.

S.No	Concentration of CuNPs (μL)	Percentage of mortality	
		24 hrs	48 hrs
1	50	60	100
2	100	100	100
3	150	100	100
4	200	100	100

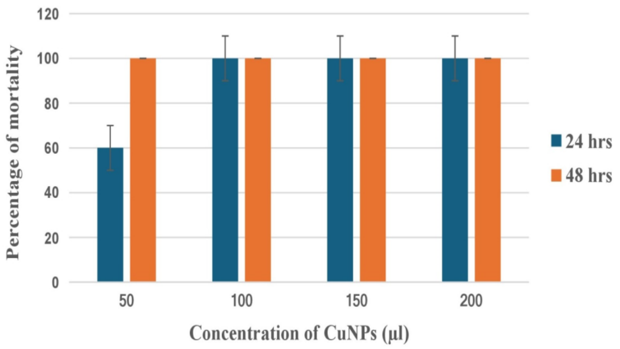

Figure 14. The graph shows the percentage of mortality rate of *Aedes aegypti* larvae.

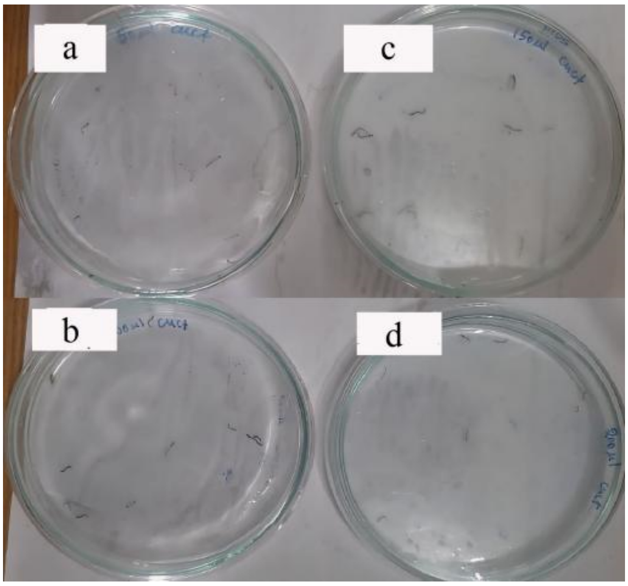

Figure 15. Elucidates the larvicidal activity of different concentrations of CuNPs (a) 50 μL,(b) 100 μL, (c) 150 μL, (d) 200 μL against *Aedes aegypti* mosquito larvae.

ing to organ damage and cellular stress, ultimately resulting in larval death [89, 90]. In comparison to the larvicidal activity of marine actinomycetes, biosynthesized copper nanoparticles demonstrate excellent activity against *Aedes aegypti* [91].

3.7 Antiviral activity

Biosynthesized CuNPs exhibit excellent antiviral activity against the Sesbania Mosaic Virus. The tested plants were monitored daily for symptoms. In the third week of inoculation, plants treated with 100 μL of CuNPs along with virus sap exhibited signs of mosaic appearance on leaves, stunted growth, and molting of leaves after 30 minutes of incubation, whereas no symptoms appeared after 120 minutes. Plants treated with a high concentration of 200 μL of CuNPs along with virus sap remained healthy and erect after 120 minutes of incubation, with increased plant growth. However, after 30 minutes of incubation, slight yellowing of the leaf base, stunted growth, bending of the plant, and mottling of some leaves were observed. This indicates that as the concentration of copper nanoparticles increases, the rate of infection decreases. The positive control exhibited symptoms of mosaic appearance, yellowing of leaves, stunted growth, etc., within one week, as shown in Fig. 16(a-c). This means that nano-treated plants are more resistant to infection compared to the positive control. The primary infection that spreads the disease is responsible for the accumulation of plant viruses [92]. Thus, when copper nanoparticles enter plant cells, they can activate an antiviral reaction. These nanoparticles bind to the viral receptors and genome, preventing interaction with cellular receptors. Consequently, viral replication and reproduction are blocked, halting polymerase activity [93, 94]. Flavonoids, secondary metabolites present in plants, help in the defense mechanism against pathogens. Biologically synthesized nanoparticles increase the production of flavonoids and enhance the immune system to develop acquired resistance [95–97]. In recent years, the agriculture industry has increasingly used nanoparticles in nanopesticides and nanofertilizers to improve plant growth, development, and quality production [98]. By assisting in the regulation of the stress response, these nanoparticles boost crop yield and seed production [99].

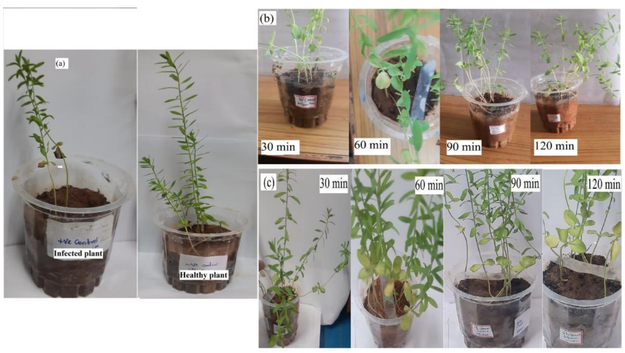

Figure 16. The pictures elucidate the antiviral activity of CuNPs against *Sesbania Mosaic Virus*, showcasing symptoms such as leaf mottling, mosaic patterns, stunted growth, and yellow discoloration of leaves at different concentration: (a) Healthy plant (negative control) and infected plant (positive control), (b) Nano treated plants with 100 μL of CuNPs incubated for 30 min, 60 min, 90 min, and 120 min, (c) Nano treated plants with 200 μL of CuNPs incubated for 30 min, 60 min, 90 min, and 120 min.

4. Conclusion

In the present work, copper nanoparticles synthesized by a biological method are eco-friendly and non-toxic green approaches. Secondary metabolites like flavonoids and tannins are responsible for the reduction of copper nanoparticles. The nanoparticles were confirmed using UV-visible spectroscopy, with a peak at 418 nm, and FTIR was used to analyze functional groups such as carboxyl and amino. The spherical shape of copper nanoparticles was determined by SEM. The particle size with spherical and zeta potential describes the stability and charge of nanoparticles. The CuNPs showed diverse biological activities, such as antibacterial, antifungal, larvicidal, and antioxidant activities. The green synthesized CuNPs showed antiviral efficacy on the *Sesbania Mosaic Virus* in the *Sesbania* plant, and it could be used as an antiviral agent for the eradication of viral diseases in the Agricultural sector.

Authors contributions
Authors have contributed equally in preparing and writing the manuscript.

Availability of data and materials
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interests
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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