

Green synthesis of copper oxide nanoparticles using *Spinacia oleracea* leaf extract and evaluation of biological applications: antioxidant, antibacterial, larvicidal and biosafety assay

Gomathi Thandapani (✉ drdrgoms1@gmail.com)

D.K.M. College for Women (Autonomous), Affiliated to Thiruvalluvar University

Arthi K

D.K.M. College for Women (Autonomous), Affiliated to Thiruvalluvar University

Pazhanisamy P

Sir Theagaraya College, Affiliated to University of Madras

John Joseph J

VIT University

Vinothini C

D.K.M. College for Women (Autonomous), Affiliated to Thiruvalluvar University

Rekha V

D.K.M. College for Women (Autonomous), Affiliated to Thiruvalluvar University

Santhanalakshmi K

IFET College of Engineering (Autonomous)

Sekar Vijayakumar

Shandong University

Research Article

Keywords: *Spinacia oleracea*, Green synthesis, Copper oxide nanoparticles, Analytical tools, biological applications

Posted Date: October 17th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-2150620/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Copper nanoparticles (Cu-NPs) recently engrossed the attention of investigators due to their incredible features and numerous applications in the pharmacological industry. This research work focuses on the manufacture of copper oxide nanoparticles in an aqueous media utilizing *Spinacia oleracea* leaf extract. Various analytical methods like UV/Vis Spectroscopy, FT-IR spectroscopy, X-Ray diffraction studies, SEM analysis were utilized to identify structure of the CuO nanoparticles produced. The CuO nanoparticles exhibited significant bactericidal action towards *Staphylococcus aureus*, *Escherichia Coli*, *proteus*, *klebsiella* & potential antifungal activity towards *A.flavus*, *Rhizopus* & *A.fumigant*. Furthermore, the synthesized CuO nanoparticles have significant larvicidal activity towards *Culex. Quinquefasciatus*. This research shows that CuO can be synthesized in a simple, environmentally friendly way and has a wide range of uses.

1. Introduction

Nanoparticle stand out in the medicine field, nutrition and energy sector because of their diverse capabilities and compelling applications [1]. Nanomaterials are distinguished from other comparable materials by their exceptional characteristics. The shapes and sizes of the NPs have an impact on their optical, stimulative and conductive capabilities [2]. Nanoparticles and Nanostructured Materials have acquired significance in scientific breakthroughs over their bulk counterparts because of their controllable physicochemical features like melting point, catalytic action, absorption of light and scattering of light, electrical conductivity, thermal conductivity [3]. Nanoparticles were widely used in biomedical research in recent years for antioxidant, antibacterial, antiinflammatory, antiviral, cytotoxic, anticancer, and anti-HIV effects [4]. The learning of the biological characteristics of metal nanoparticle is becoming more popular. In recent years, Nanoparticles of noble metal have gained a lot of attention because of their uses in many industries and improved physical, chemical & biological properties [5].

When compared to other noble metals like Gold, Platinum and Silver, copper is a less expensive material, but it is prone to microscopic oxidation [6–8]. Several researchers have recently begun evaluating the biological activities of nanoparticles comprising metal oxide like Copper Oxide & demonstrated superior biological and photo catalytic activities over those derived from metal nanoparticles have been reported [9–11]. Nanoparticles of Silver and copper have revealed to exhibit potential inhibitory and antibacterial properties. Copper is now used in the metallic nanoparticles synthesis in the place of noble metals as they are low cost than gold and silver [12]. Wide-ranging antibacterial properties of copper are present. While this might be employed therapeutically, it might also negatively impact symbiotic bacteria in the environment, on the skin or in the intestine. Cu nanoparticles have been used as disinfectants in water treatment facilities [13], food processing [14], wound healing ointments [15], bandages [16], and other applications due to their antibacterial and antiviral properties [17, 18].

The utilization of plant extract in green synthesis techniques is gaining popularity to generate metal based nanoparticles. Green synthesis techniques often employ relatively benign resources to produce

nanomaterials such as water, biological extracts & microwave assisted synthesis [19, 20]. With regard to efficiency, cleanliness, simplicity, non-toxicity, cheap cost and environmental friendliness, biosynthesis of nanoparticles using bio-green technologies has a number of benefits over chemical and physical synthesis approaches [21–23].

The majority of the methods include non-biodegradable stabilizing agents, organic solvents and dangerous chemicals as reducing agents, all of which are risky for the environment and biological systems. These operations are generally expensive since they require non-standard conditions and are complicated. Thus, it has been shown that biosynthesis of nanoparticles is a more practical and sustainable method of producing them than chemical & physical methods [24–29]. Utilizing plant extracts rather than microorganisms to produce Nano-particles stands more profitable due to the difficult process of maintaining cell cultures [30]. Metal nanoparticles have been produced consuming plant extracts is a quick and economical biosynthetic method for producing stable metallic nanoparticles in large quantities [31, 32]. In this paper, copper nanoparticles were produced from *Spinacia oleracea* leaf extract by bio-green technologies and characterized using several spectroscopic techniques before being tested for antibacterial, antioxidant, antifungal, larvicidal and in-vitro biodegradation assay.

2. Materials And Methods

2.1. Materials

The leaves of “*Spinacia oleracea*” (Tamil- Pasalaikeerai) were collected from Thorapadi, Vellore District, Tamil Nadu, India. Copper sulphate analytical grade were procured from Sigma- Aldrich, USA. Other chemicals utilized are of analytical grade.

2.2. Preparation of *Spinacia oleracea* leaf extract

To make the *Spinacia oleracea* leaf extract, 20 g of their leaves were taken and washed thoroughly, dried out and excellently chopped. The final sliced product was boiled in 200 mL of double-distilled water for 15 minutes at 80°C before being permitted to cool to room temperature. The resultant solution was filtered to eliminate solid particles & again filtered. The brown colour solution is finally produced.

2.3. Green synthesis of Copper Oxide NPs

About 200 mL of CuSO_4 solution (10 mm) was taken in a 500 mL beaker to make Copper Oxide NPs. This was continually mixed using a magnetic stirrer at 80°C with the addition of 50 mL of leaf extract. The reaction mixture's colour changing from dark green to light green is proof that copper oxide nanoparticles have been produced (Fig. 1). After the complete reduction, the light green coloured solution was centrifuged and the supernatant part was removed. The resultant black coloured precipitate is baked to dry, and the dried copper oxide nanoparticles are gathered and used for more research.

<Insert Fig. 1>

2.4. Characterization of CuO NPs

Copper oxide particles prepared by the bio reduction process were analyzed regarding their size and chemical composition [33, 34]. The initial detection of CuO NPs creation was witnessed by the colour change visually Using a UV-VIS spectrometer, CuO NPs were examined by UV-Vis Spectroscopy from 200 to 800 nm (HITACHI-U2800) [35]. The functional group present in the Green synthesized CuO NPs was founded by FT-IR spectrometer [36]. SHIMAZU Spectrometer was used to record Fourier transform infrared images in the frequency range of 400–4000 cm⁻¹. X-ray powder diffractometer (XRD- SHIMAZU XD-D1), Ni-filter and Cu K radiation source were utilized to acquire the X-ray diffraction of the materials. In the scattering range 2θ, the relative intensity was measured and ranged from 10° to 90°. SEM was used to examine and take pictures of the surface morphology of the obtained green synthetic copper oxide nanoparticles [37].

2.5. In vitro antioxidant activity

The antioxidant action of the created Copper NPs was investigated by DPPH and hydrogen peroxide scavenging analyses [38].

2.6. Scavenging action for hydrogen peroxide

One of the finest methods for examining the antioxidant properties of produced nanoparticles is hydrogen peroxide scavenging activity [39–42]. The phosphate buffer was used to create a hydrogen peroxide solution (40 mM) (50 mM, pH 7.4). A spectrophotometer was used to measure the hydrogen peroxide content at 230 nm [43]. After 10 minutes, the absorbance at 230 nm was assessed in comparison to a control solution made up of phosphate buffer and hydrogen peroxide after the addition of synthetic compounds at a concentration of 2 mg per 1 mL of double-distilled water. The following formula is used to determine the amount of hydrogen peroxide scavenging [44].

$$\% \text{of inhibition} = \frac{A_c - A_s}{A_c} \times 100$$

2.7. DPPH scavenging activity

The stock solution was produced up to 50 ml in a standard flask by combining 10 mg of the material with 10 mL of distilled water. To obtain the concentrations of solution from 20 µg/mL to 120 µg/mL, 1, 2, 3, 4, and 6 mL from the 50 mL of stock solution were taken and built up to 10 mL in the test tubes using distilled water. One millilitre of 2,2,2-diphenyl-1-picrylhydrazyl (DPPH) with a normality of 0.001 N was added to three millilitres of solutions of various concentrations [45]. After this, it was placed in a dark room nearly for 30min & absorbance was measured using a spectrophoto colorimeter (420 nm), using DPPH as control. The following formula was used to get the scavenging percentage [46].

$$\% \text{scavenging} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}$$

2.8. Antibacterial activity

Using the Muller Hinton Agar (MHA) medium, the disc diffusion method was used to assess the produced sample's antibacterial activity against the two bacterial strains [47]. *Staphylococcus aureus* (One gram positive bacteria) & *Escherichia Coli, proteus, klebsiella* (three gram negative bacterias) were used as a test organisms. The One gram positive and gram negative bacteria were originally infected on Muller Hinton Agar (MHA) medium and disseminated uniformly using a sterile spreader in petri plates to examine the antibacterial activity of the produced samples [48]. After the MHA medium had set up for the disc diffusion technique, a little quantity of the sample was placed on many cultivated agar plates. Before the growth of the organism could begin, the plates were incubated on individual racks for 24 hours at 37°C to allow the chemicals to diffuse. The antibacterial activity of the prepared sample was then evaluated by determining the diameter of the zone of inhibition.

2.9. Antifungal activity

Using disc diffusion method and Sabouraud Dextrose Agar medium, the produced sample's antifungal activity was evaluated against the fungal strains [49]. *A.flavus, Rhizopus, and A.fumigant* were utilized as test organisms. To examine the sample's fungal resistance, primarily the microorganisms were inoculated on Sabouraud Dextrose Agar (SDA) medium and dispersed uniformly using sterile spreader in the petri plates. For the disc diffusion procedure, a sample little portion was located on cultured agar plates after the SDA Medium solidified. The plate was permitted to stand for 1 hour at ambient temperature for the substances to diffuse, and then the plates were incubated on individual racks for one day at 37°C prior to the growth of organism. Then, using a ruler, the zone of inhibition diameter encircling the discs towards test microorganisms was calculated to find the fungal resistance of the manufactured sample.

2.10. Larvicidal activity

The larvae culture was done on *Culex. quinquefasciatus*. The *Culex quinquegasciatus* eggs and egg rafts were acquired from Zonal Entomological unit, Vellore, Tamil Nadu. To hatch out larvae, eggs and *Culex quinquegasciatus* (Cx. quinquefasciatus) egg rafts were submerged in a plastic container filled with de-chlorinated water (500 mL) nearly for 30–45 minutes. They were kept in the lab using the techniques described by Kamaraj et al. in 2009 [50]. Once a day, powdered nutritional soup was given to mosquito larvae. The newly born larvae were used in subsequent experiments after four days when they reached the early stages of the fourth stage.

2.11. Larvicidal bioassay test

Larvicidal activity was evaluated using a modified version of the WHO guidelines [51]. A whole of 30 nurtured Mosquito larvae were located in 150 mL of twin distilled sterilized water comprising different concentration (0.10g, 0.06g and 0.02g) of synthesized compounds. The commercial larvicide with test solution was utilized as the positive control, whereas the sterilized distilled water complex was utilized as the negative control. Percentage of mortality was assessed after 0.5 to 4 h of incubation. Some dead larvae in each batch were counted.

2.12. Safety of compounds

The brine shrimp (*Artemia salina*) bioassay was used to conduct the brine shrimp toxicity test [52]. The Brine Shrimp eggs were allowed to hatch in a 1 L sterile bottle that was filled with synthetic seawater (33 g/L of sea salt adjusted to a 8.5 pH by consuming 1M Na₂CO₃ solutions) [53]. This took place for 48 hours with continual aeration where enough times were provided to all dynamic nauplii to break free of their egg shells. Then, the energetic nauplii were gathered from the hatching chamber's brighter area and employed in the bioassay. The procedure involves transferring 10 shrimp into sterile test tubes after pulling them via a glass capillary. Each test tube enclosed 5 mL of Brine solution. Different sample concentrations (250, 500, 1000, 2000, 6000, and 10,000 g/mL) were introduced to test tubes containing brine solution (5 mL) in each experiment. The tubes were kept for 1, 2, 3, 4 and 24 hours at 37 degrees Celsius under visible light. The amount of dead larvae was calculated, and the rate of mortality was determined. For a test substance to be deemed non-lethal, it must result in less than 50% of brine shrimp deaths. The formula below was used to compute the percentage of mortality [54].

$$\% \text{ mortality} = (\text{No. of death } \textit{artemia nauplii}) / (\text{total No. of live } \textit{artemia nauplii}) \times 100$$

2.13. Statistical Analysis

After being analysed, all of the data were reported as the average of three different counts of individual observations. Mortality was rearranged according to the mean proportion of *Artemia nauplii* that died following a 24-hour exposure. The results were presented as the mean minus the standard deviation.

3. Results

3.1. Phytochemical Screening Test

For the extracted *Spinacia oleracea* leaf extract, phytochemical screening of secondary metabolites such as alkaloids, flavonoids, sterols, triterpines, proteins, saponins, phenols & glycosides was conducted in accordance with recent methodologies of plant analysis [55]. The results of phytochemical analysis of *Spinacia oleracea* leaf extracts were presented in Table 1. The findings showed that alkaloids, flavonoids, proteins, tannins, saponins, steroids, phenols, reducing sugars, carbohydrates & proteins are among the biomolecules included in the *Spinacia oleracea* leaf extract. These phytochemicals are in charge of the quick ion reduction and nanoparticle production.

Table 1
Phytochemical screening of *Spinacia oleracea* leaf extract [56]

Test	<i>Spinach</i> leaf Extract
Flavonoids	+
Tannins	++
Glycosides	+
Steroids	+
Saponins	++
Phenols	+++
Triterphenoids	+
Reducing sugars	+
Anthocyanins	
Carbohydrates	++
Proteins	++
Alkaloids	+
Fatty acid	
Lignins	
Anthraquinones	
+: Present - : Absent	

<Insert Table 1>

3.2. UV–Vis Analysis

Spinacia oleracea extract and copper acetate were used in the biosynthesis of Cu-NPs. Within 10 minutes following the addition, the suspension's colour changed from brown to green. The green manufactured CuO NPs and leaf extract was subjected to UV-Visible spectroscopy analysis. In these works, broad bands were observed for the synthesized CuO NPs (Fig. 2). While the band at around 790 nm corresponds to the cupric oxide band edge transition, the core electron transition of copper metal's band at about 270 nm is induced by an inter-band transition (CuO) [57].

<Insert Fig. 2>

The solution's colour changed to green, with a max wavelength of 700–800 nm, signifying the production of CuO NPs as seen in the UV–Visible spectrum. The CuO Nanoparticles created using this process are

quite stable, and even one month later, no significant changes in the absorption peak's form, location, or symmetry are seen, demonstrating the stability of CuO NPs.

3.3. FT-IR Analysis

FTIR spectroscopy was used to investigate the relations between various species and the chemical make-up of the mixtures. The presence of multifunctional groups in the green synthesized CuO NPs using *Spinacia oleracea* is studied by FT-IR analysis (Fig. 3). The band at 1103 cm^{-1} confirms that green synthesised CuO NP has formed.

<Insert Fig. 3>

The O-H stretching of H-bonded alcohols, phenols, C-H stretching of aldehydes and other hetero compounds are responsible for the unusually broad band at 3392 cm^{-1} , while the stretching of groups with the C = C, N, and O atoms is responsible for the bands at 1647 cm^{-1} . The bands lie between 600 and 400 cm^{-1} are due to M-O (Cu-O). The band at 621 cm^{-1} indicates copper oxide nanoparticles stretching vibration and confirms the formation of CuO NPs from green synthesis. According to the FTIR data, the proteins, acids, flavonoids, polyphenols, carboxylic acid and alkaloids that are present around the Cu-NPs cause copper ion reduction into NanoParticles [58].

3.4. XRD Analysis

To confirm that the CuO NPs are crystalline, the XRD pattern was used. The XRD analysis of the CuO nanoparticles demonstrates that the Copper oxide is crystalline in nature [33]. The pattern is the same as that of pure CuO, confirming the creation of monoclinic single-phase CuO (JCPDS-05-0661) (Fig. 4). The reflection lines of monoclinic CuO nanoparticles were identified by the diffraction patterns, which were detected at $2\theta = 32.47, 35.49, 38.68, 48.65, 53.36, 58.25,$ and 61.45 .

<Insert Fig. 4>

3.5. SEM Analysis

SEM micrographs of the CuO NPs which was synthesized by the reduction of copper sulphate using *Spinacia oleracea* leaf extract revealed oval, spherical, hexagonal and cubical in shape ranging from 20 to 80 nm (Fig. 5). The biosynthesis of Cu-NPs is the result of electrostatic interactions and molecular bonds between the bio-organic capping molecules. The images exposed that the particles underwent agglomeration. The CuO nanoparticles are organized in a cylindrical shape over one another in the SEM image, with a narrow size distribution and nanomaterial size. The size and the size estimated from the XRD data were also found to be in good agreement.

<Insert Fig. 5>

3.6. Antioxidant assay

3.6.1. Hydrogen peroxide methods

The change in the optical density of hydrogen peroxide free radicals was observed in order to examine the antioxidant potential through free radical scavenging by copper oxide nanoparticles at various concentrations.

<Insert Fig. 6>

Figure 6 represent the scavenging action of the prepared CuO NPs. The findings demonstrate that the scavenging percent increases along with the solution concentration. The solution taken in the concentrations of 2 mg/mL shows 86.80% scavenging activity. The concentrations of 4, 6, 8 and 10 mg/mL shows of 88.34%, 90.18%, 91.41% and 93.86% scavenging respectively.

3.6.2. DPPH assay

The most used method for determining a material's antioxidant potential is the DPPH scavenging assay [59]. The colour of DPPH, a stable nitrogen-centered free radical, changes from violet to yellow as it is reduced through the processes of hydrogen reduction or electron donation.

<Insert Fig. 7>

Figure 7 represent the scavenging action of the prepared Copper oxide nanoparticles. According to the findings, the scavenging percentage rises together with the concentration of CuO NPs. The solution taken in the concentration of 20 µg/mL shows about 49.38% and the concentrations of 40 µg, 60 µg, 80 µg, 100 µg and 120 µg shows of 53.75%, 56.25%, 60.62%, 62.62% and 70.18% scavenging respectively.

3.7. Antibacterial Activity

All the synthesized Copper oxide nanoparticle was examined in vitro to identify their antibacterial activity towards Gram-positive bacteria like *Staphylococcus aureus* and Gram-negative bacteria like *klebsiella pneumonia*, *proteus* and *E.coli* by the well diffusion method. The zone of inhibition values of the synthesized nanoparticle against the growth of the selected bacteria was measured in mm. The results of screening of antibacterial activities of the nanoparticle are shown in Figs. 8 and 9. When the zone of inhibition is less than 10 mm, the bacterium is thought to be resistant. As a control, DMSO was utilized as a solvent.

<Insert Figs. 8 and 9>

3.8. Antifungal Activity

By using the well diffusion method, the produced nanoparticles are tested for their in vitro antifungal effectiveness against fungi such *A. flavus*, *A. fumigant*, and *Rhizopus*. Figures 10 and 11 provides the findings in relation to the antifungal activity of the produced complexes. DMSO served as the control. According to the zone of inhibition values for the produced nanoparticles, the copper oxide nanoparticles has strong antifungal activity against *Rhizopus* and *A. flavus*.

<Insert Figs. 10 and 11>

According to the zone of inhibition values for the produced nanoparticles, copper oxide nanoparticles are effective against *Rhizopus* and *A. flavus*.

3.9. Larvicidal Activity

Synthesized nanoparticles' larvicidal activity was tested on *Culex quinquefasciatus*, and the results are tabulated. The synthesized Cu oxide nanoparticles show moderate to stronger toxic effect against *Culex quinquefasciatus*. However, the maximum mortality was originated in Copper oxide nanoparticles after 24 h of the exposure period.

<Insert Fig. 12>

The results of larvicidal activity reveal that when the concentration of copper oxide nano particles increases from 2 to 10 mg/mL the percentage of mortality also gets increased. From the Fig. 12, it can be concluded that when the concentration of nanoparticles increases the percentage of mortality also increases, thereby acting as the best larvicidal agent.

3.10. Safety of compounds (Toxicity assay)

Due to the different chemical characteristics of molecules in this small size range compared to their larger counterparts, nanoparticles require specific consideration [2, 60]. Due to their widespread use as a nutrient-rich live food source for the larvae of a variety of marine organisms, artemiasalina is one of the most valuable test organisms for marine ecotoxicity testing. Additionally, because of their high levels of interaction with the environment through nonselective filter feed, they are the most practical and labor-efficient live food available for aquaculture and will be significantly impacted by the release of nanoparticles into aquatic systems [61, 62].

<Insert Table 2>

Table 2
Percentage calculation of copper oxide NPs

Compd.	Concentration µg/mL					
	250	500	1000	2000	6000	10000
CuO NPs	1.5 ± 0.2	5.33 ± 0.3	9.22 ± 0.5	13.33 ± 0.6	23.33 ± 0.6	46.67 ± 1.15

<Insert Fig. 13>

According to the current research, considerable mortality was observed within 24 hours after exposure to guts that had high nanoparticle aggregate levels. As the concentration increased to the maximum concentration of 10,000 g/mL, more than half of the nauplii population was discovered to be dead. But given that the stomachs were entirely filled with Copper oxide nanoparticle aggregates, these consequences were most likely brought on by a lack of food absorption. The previous study found that *Artemia franciscana* cysts exposed to mercury at concentrations as low as 10 nM Hg had a lower

hatching percentage (Table 2 and Fig. 13). Cysts exposed to higher quantities of mercury saw a longer delay in the emergence and hatching curves crossing than controls did. This study shows that cysts as well as living animals are affected by copper oxide nanoparticles. Thus, the findings of the present investigation showed that the cytotoxic effect rises with increasing concentrations of copper oxide nanoparticles (250, 500, 1000, 2000, 6000, and 10,000 g/mL).

4. Conclusions

Copper nanoparticles were bio-synthesized from *Spinacia oleracea* leaf extract in a quick, environmentally friendly and step-by-step approach that included plant-based bio-resources that also served as potential reducing agent and stabilizing agents. Surface plasmon resonance, which develops as a result of the reaction between the compounds in the plant leaf extract, is what changes the colour. The development of Copper nanoparticles was supported by UV–Vis, FTIR, XRD and SEM analysis. Cu-nanoparticles are made without the use of any hazardous chemicals in the current process. Cu-NPs displayed greater antioxidant, antibacterial, antifungal, larvicidal activity, and toxicity assay. In recent years, NPs have been employed in modern medicine for a number of functions, such as a different transducer in medical imaging and a carrier for the transport of genes into biological systems. However, before assigning to the biological system, the injuriousness of nanoparticles must be adjusted suitably for manufactured nanoparticles. This led to the successful preparation and completion of clinical studies for a number of manufactured nanoparticles. This infiltrates the sick cell while leaving healthy cells in the body alone. As a result of the findings, green produced copper oxide nanoparticles will operate as an excellent biological activity agent and also can be used economically in a variety of medicinal usages. Future research issues have been brought on by the aggregation of nanoparticles, which prevents their entry into bacterial cells and interferes with their ability to carry out biological actions, as well as by variations in production, processing and storage which can result in oxidation and undesirable forms.

Declarations

Author Contributions: Conceptualization, T.G., C.V. and K.A.; methodology, T.G. and J.J; investigation, V.R., K.S., S.V., and P.P; writing—original draft preparation, T.G. and K.S.; All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors would like to acknowledge the DST-FIST facility offered by the management of D.K.M. College for Women, Vellore.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. M. Thiruvengadam, I.-M. Chung, T. Gomathi, M.A. Ansari, V. Gopiesh Khanna, V. Babu, G. Rajakumar, *Bioprocess Biosyst. Eng.* **42**, (2019)
2. J. Jeevanandam, A. Barhoum, Y.S. Chan, A. Dufresne, M.K. Danquah, *Beilstein J. Nanotechnol* **9**, 1050 (2018)
3. M.F. Al-Hakkani, *SN Appl. Sci.* **2**, 505 (2020)
4. X. Zhao, L. Zhou, M.S. Riaz Rajoka, L. Yan, C. Jiang, D. Shao, J. Zhu, J. Shi, Q. Huang, H. Yang, M. Jin, *Crit. Rev. Biotechnol.* **38**, 817 (2018)
5. I. Khan, K. Saeed, I. Khan, *Arab. J. Chem.* **12**, 908 (2019)
6. G. Kianpour, M. Salavati-Niasari, H. Emadi, *Ultrason. Sonochem* **20**, 418 (2013)
7. S.E.-D. Hassan, S.S. Salem, A. Fouda, M.A. Awad, M.S. El-Gamal, A.M. Abdo, *J. Radiat. Res. Appl. Sci.* **11**, 262 (2018)
8. B. Klębowski, J. Depciuch, M. Parlińska-Wojtan, J. Baran, *Int. J. Mol. Sci.* **19**, (2018)
9. S. Drummer, T. Madzimbamuto, M. Chowdhury, *Materials (Basel)*. **14**, (2021)
10. A.A. Yaqoob, H. Ahmad, T. Parveen, A. Ahmad, M. Oves, I.M.I. Ismail, H.A. Qari, K. Umar, M.N. Mohamad, Ibrahim, *Front. Chem.* **8**, 1 (2020)
11. D. Sharma, S. Kanchi, K. Bisetty, *Arab. J. Chem.* **12**, 3576 (2019)
12. A. Pujari, T. Thomas, *Mater. Chem. Phys.* **253**, 123419 (2020)
13. L. Al-Issai, W. Elshorbagy, M.A. Maraqa, M. Hamouda, A.M. Soliman, *Water* **11**, (2019)
14. N. Zamindar, E. Sadat Anari, S. Sadat Bathaei, N. Shirani, L. Tabatabaei, N. Mahdavi-Asl, A. Khalili, S. Paidari, *Acta Sci. Nutr. Heal* **4**, 14 (2020)
15. M. Tiwari, K. Narayanan, M.B. Thakar, H.V. Jagani, J. Venkata Rao, *IET Nanobiotechnol.* **8**, 230 (2014)
16. M. Senthamarai Kannan, P.S. Hari Haran, K. Sundar, S. Kunjiappan, V. Balakrishnan, *Prog Biomater.* **11**, 229 (2022)
17. G. Borkow, J. Gabbay, *Curr. Chem. Biol.* **3**, 272 (2009)
18. G. Borkow, J. Gabbay, *FASEB J.* **18**, 1728 (2004)
19. M. Gondwal, G. Joshi Nee Pant, *Int. J. Biomater.* **2018**, 6735426 (2018)
20. P. Malik, R. Shankar, V. Malik, N. Sharma, T.K. Mukherjee, *J. Nanoparticles* **2014**, 302429 (2014)
21. A.A. Mohamed, A. Fouda, M.S. Elgamal, S. EL-Din Hassan, T.I. Shaheen, S.S. Salem, *Egypt. J. Chem.* **60**, 63 (2017)
22. B. Salimath, S.D. Balaji, A. Lagashetty, R. A H, and V. Abbaraju, *Mater. Bull.* **43**, 1164 (2007)
23. A.-R. Phull, Q. Abbas, A. Ali, H. Raza, S.J. kim, M. Zia, I. Haq, *Futur J. Pharm. Sci.* **2**, 31 (2016)
24. J. Chaudhary, G. Tailor, B.L. Yadav, and O. Michael, *Heliyon* **5**, e01878 (2019)

25. S. Zinatloo-Ajabshir, M. Salavati-Niasari, M. Hamadani, RSC Adv. **5**, 33792 (2015)
26. M. Salavati-Niasari, F. Farzaneh, M. Ghandi, J. Mol. Catal. A Chem. **186**, 101 (2002)
27. M. Salavati-Niasari, M. Dadkhah, F. Davar, Inorganica Chim. Acta **362**, 3969 (2009)
28. M. Salavati-Niasari, Chem. Lett. **34**, 244 (2005)
29. M. Salavati-Niasari, Inorg. Chem. Commun. **8**, 174 (2005)
30. H. Bahrulolum, S. Nooraei, N. Javanshir, H. Tarrahimofrad, V.S. Mirbagheri, A.J. Easton, G. Ahmadian, J Nanobiotechnol. **19**, 86 (2021)
31. K. Bogunia-Kubik, M. Sugisaka, Biosystems. **65**, 123 (2002)
32. Willems and van den Wildenberg, Methodology 1 (2005)
33. V.V. Thekkae Padil, M. Černík, Int. J. Nanomedicine **8**, 889 (2013)
34. Y. Abboud, T. Saffaj, A. Chagraoui, A. El Bouari, K. Brouzi, O. Tanane, B. Ihssane, Appl. Nanosci. **4**, 571 (2014)
35. P. KUMAR, A.G. NENE, S. PUNIA, M. KUMAR, Z. ABBAS, F. THAKRAL, H.S. TULI, Int. J. Appl. Pharm. **12**, 17 (2020)
36. M. Subhani, N. Wahab, M. Ibrahim, H. Rehman, M. Asad, W. Ullah, M. Kamil, M. Ikramullah, M. Arif, M. Zubair, W. Shah, M. Shuja, Int. J. Biosci. **14**, 243 (2019)
37. S.A. Akintelu, A.S. Folorunso, F.A. Folorunso, A.K. Oyebamiji, Heliyon **6**, e04508 (2020)
38. Z. Bedlovičová, I. Strapáč, M. Baláž, A. Salayová, Molecules **25**, (2020)
39. X. Ma, H. Li, J. Dong, W. Qian, Food Chem. **126**, 698 (2011)
40. N. Sreejayan, M.N. Rao, Arzneimittelforschung. **46**, 169 (1996)
41. J. Vaijanathappa, S. Badami, S. Bhojraj, J. Heal Sci. **54**, 524 (2008)
42. V.P. Veerapur, K.R. Prabhakar, V.K. Parihar, M.R. Kandadi, S. Ramakrishana, B. Mishra, B.S. Satish Rao, K.K. Srinivasan, K.I. Priyadarsini, M.K. Unnikrishnan, Evid. Based Complement. Alternat Med. **6**, 317 (2009)
43. C.-C. Hsu, Y.-R. Lo, Y.-C. Lin, Y.-C. Shi, P.-L. Li, Sensors **15**, 25716 (2015)
44. F. Shahidi, Y. Zhong, J. Funct. Foods **18**, 757 (2015)
45. S.S. Alharthi, T. Gomathi, J.J. Joseph, J. Rakshavi, J.A.K. Florence, P.N. Sudha, G. Rajakumar, M. Thiruvengadam, J. King Saud Univ. - Sci. **34**, 102177 (2022)
46. M.M. Rahman, M.B. Islam, M. Biswas, and A. H. M. Khurshid Alam. BMC Res. Notes **8**, 621 (2015)
47. S. Karimi, F. Azizi, M. Naye-Aghae, L. Mahmoodnia, Electron. Physician **10**, 6548 (2018)
48. S. Jillani, M. Jelani, N.U. Hassan, S. Ahmad, M. Hafeez, Mater. Res. Express **5**, 45006 (2018)
49. N.R. Bhalodia, V.J. Shukla, J. Adv. Pharm. Technol. Res. **2**, 104 (2011)
50. A.A. Zahir, A.A. Rahuman, C. Kamaraj, A. Bagavan, G. Elango, A. Sangaran, B.S. Kumar, Parasitol. Res. **105**, 453 (2009)
51. S. Marimuthu, A.A. Rahuman, G. Rajakumar, T. Santhoshkumar, A.V. Kirthi, C. Jayaseelan, A. Bagavan, A.A. Zahir, G. Elango, C. Kamaraj, Parasitol. Res. **108**, 1541 (2011)

52. A.O. Jaiyeola, K. Anand, K. Kasumbwe, M. Ramesh, R.M. Gengan, J. Photochem. Photobiol B Biol. **166**, 136 (2017)
53. K.F. Chai, L.S. Chang, N.M. Adzahan, R. Karim, Y. Rukayadi, H.M. Ghazali, Food Chem. **271**, 298 (2019)
54. B.N. Meyer, N.R. Ferrigni, J.E. Putnam, L.B. Jacobsen, D.E. Nichols, J.L. McLaughlin, Planta Med. **45**, 31 (1982)
55. M. Skoula, J.B. Harborne, in *Oregano* (2002), p. 42
56. P.P.N.V. Kumar, U. Shameem, P. Kollu, R.L. Kalyani, S.V.N. Pammi, Bionanoscience **5**, 135 (2015)
57. V. Kumar, V. Kumar, N. Upadhyay, S. Sharma, 3 Biotech. **5**, 791 (2015)
58. V.V. Makarov, A.J. Love, O.V. Sinitsyna, S.S. Makarova, I.V. Yaminsky, M.E. Tiliansky, N.O. Kalinina, Acta Naturae **6**, 35 (2014)
59. I.I. Koleva, T.A. van Beek, J.P.H. Linssen, A. de Groot, L.N. Evstatieva, Phytochem Anal. **13**, 8 (2002)
60. J.F. Banfield, H. Zhang, Rev. Mineral. Geochemistry **44**, 1 (2001)
61. Y. Lu, in edited by J. Y. E.-H. E.-D. Saleh (IntechOpen, Rijeka, 2019), p. Ch. 2
62. R. Rajasree, S.R.,G. Kumar, S. Abraham, I. Dhinakarasamy, Int. J. Appl. Bio-Engineering **5**, 35 (2011)

Figures



Figure 1

Leaf extract synthesized CuO nanoparticles

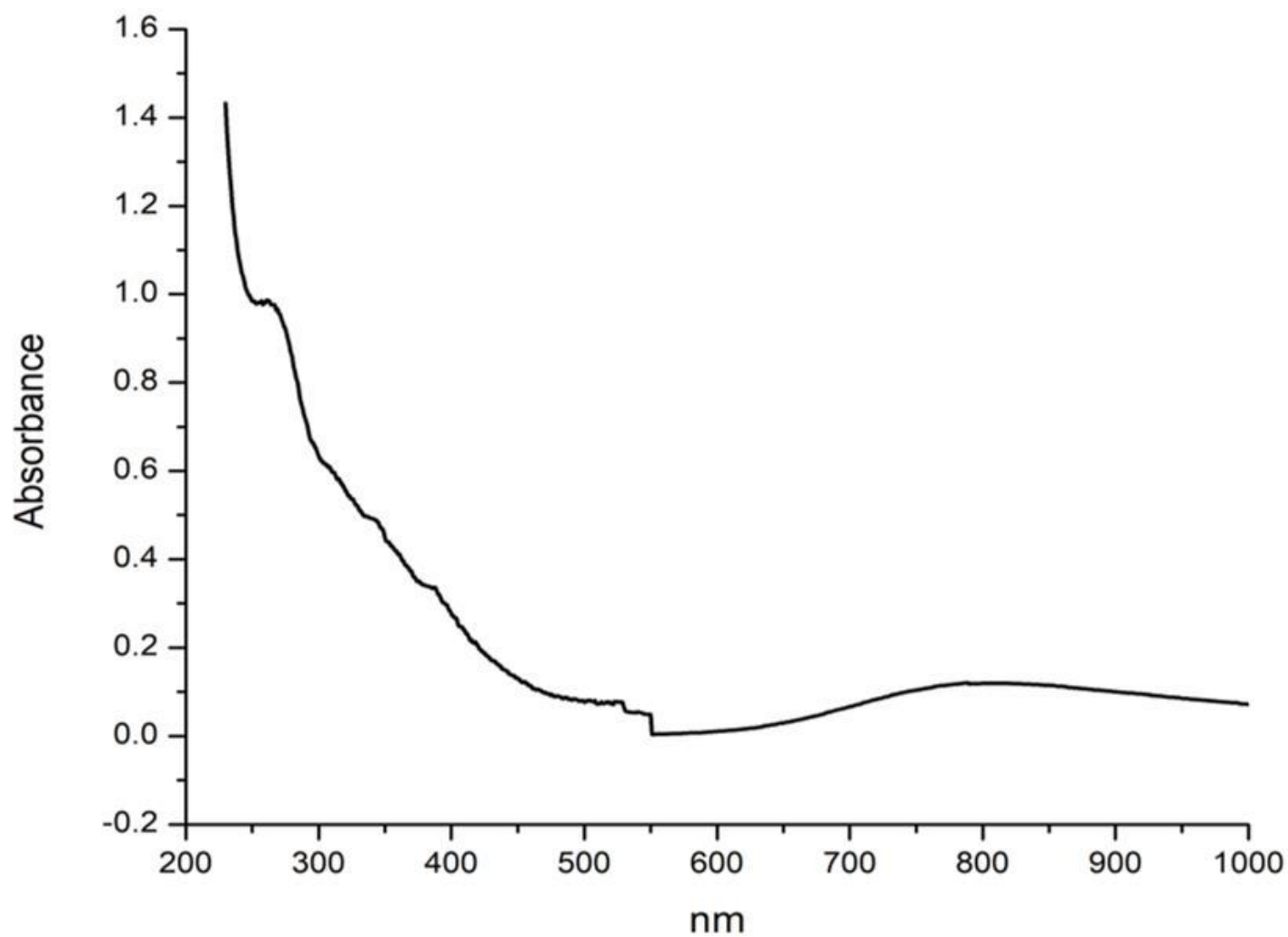


Figure 2

UV-Visible spectrum of synthesized CuO nanoparticles

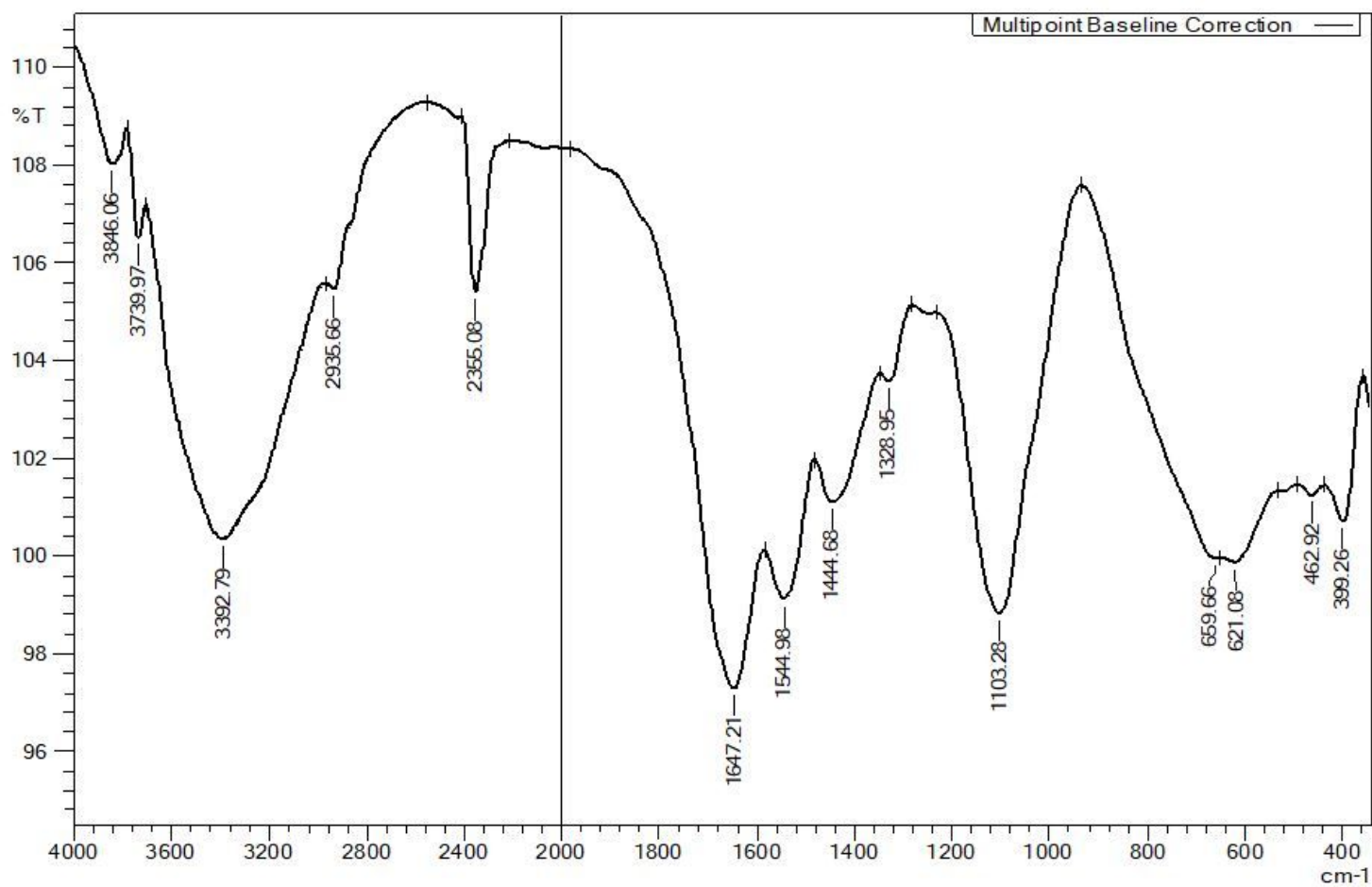


Figure 3

FT-IR spectrum of CuO nanoparticles

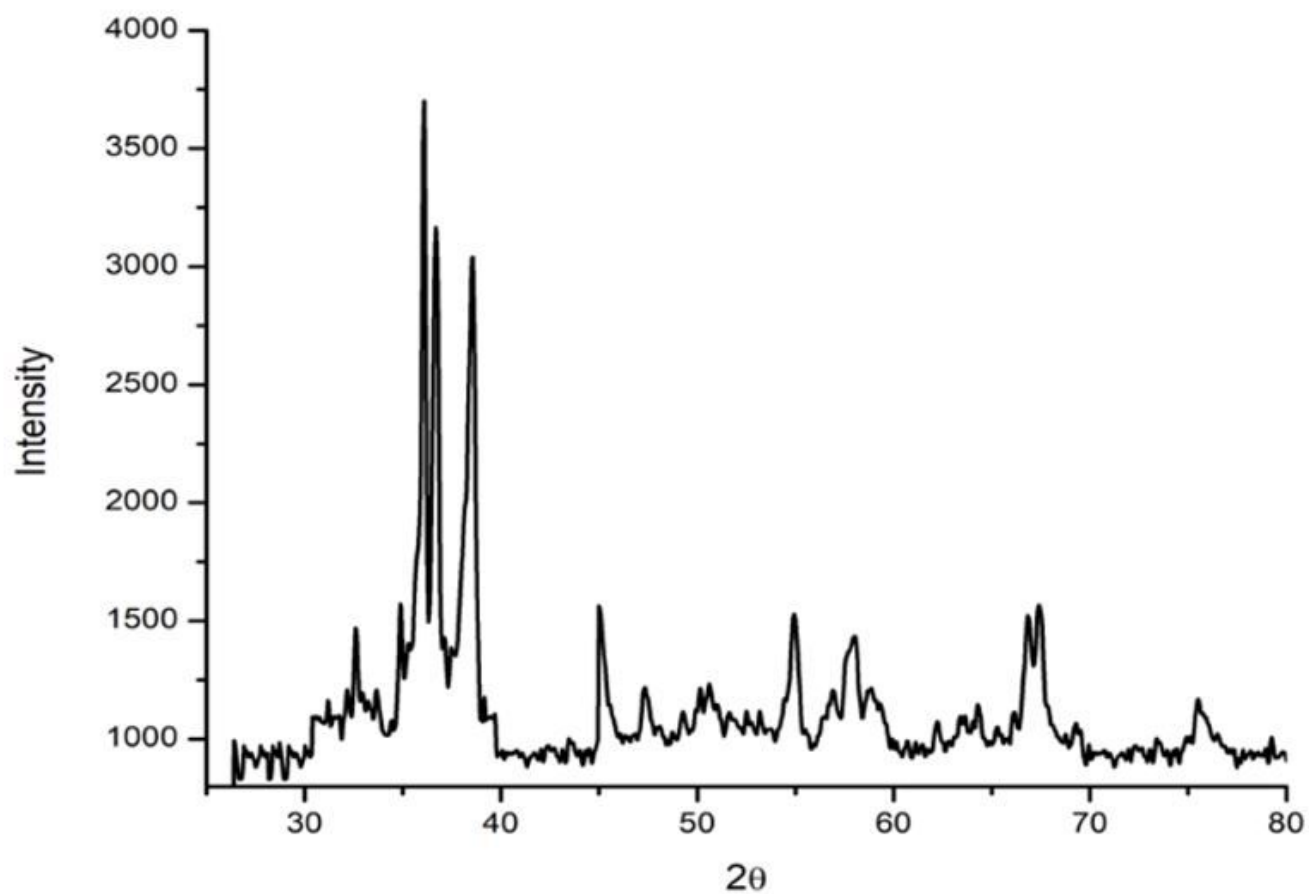


Figure 4

XRD pattern for CuO NPs

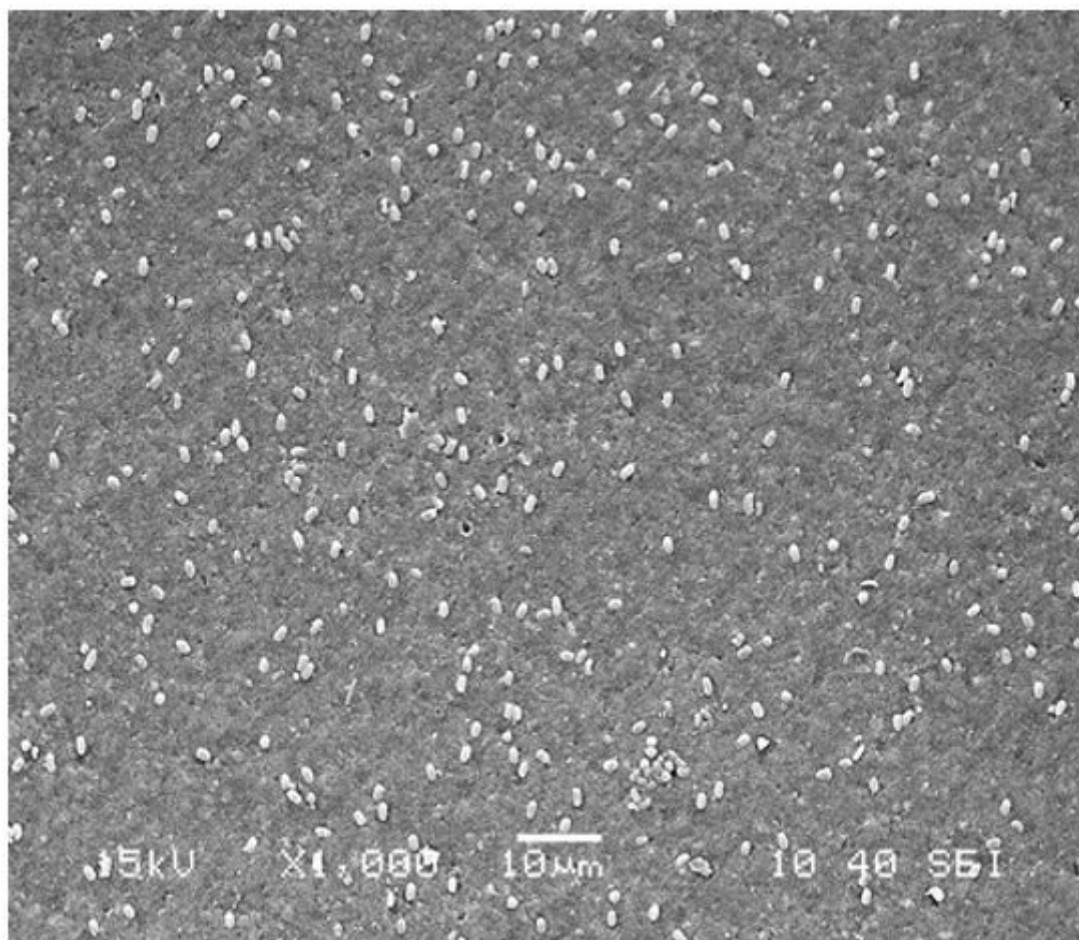


Figure 5

SEM images of CuO NPs

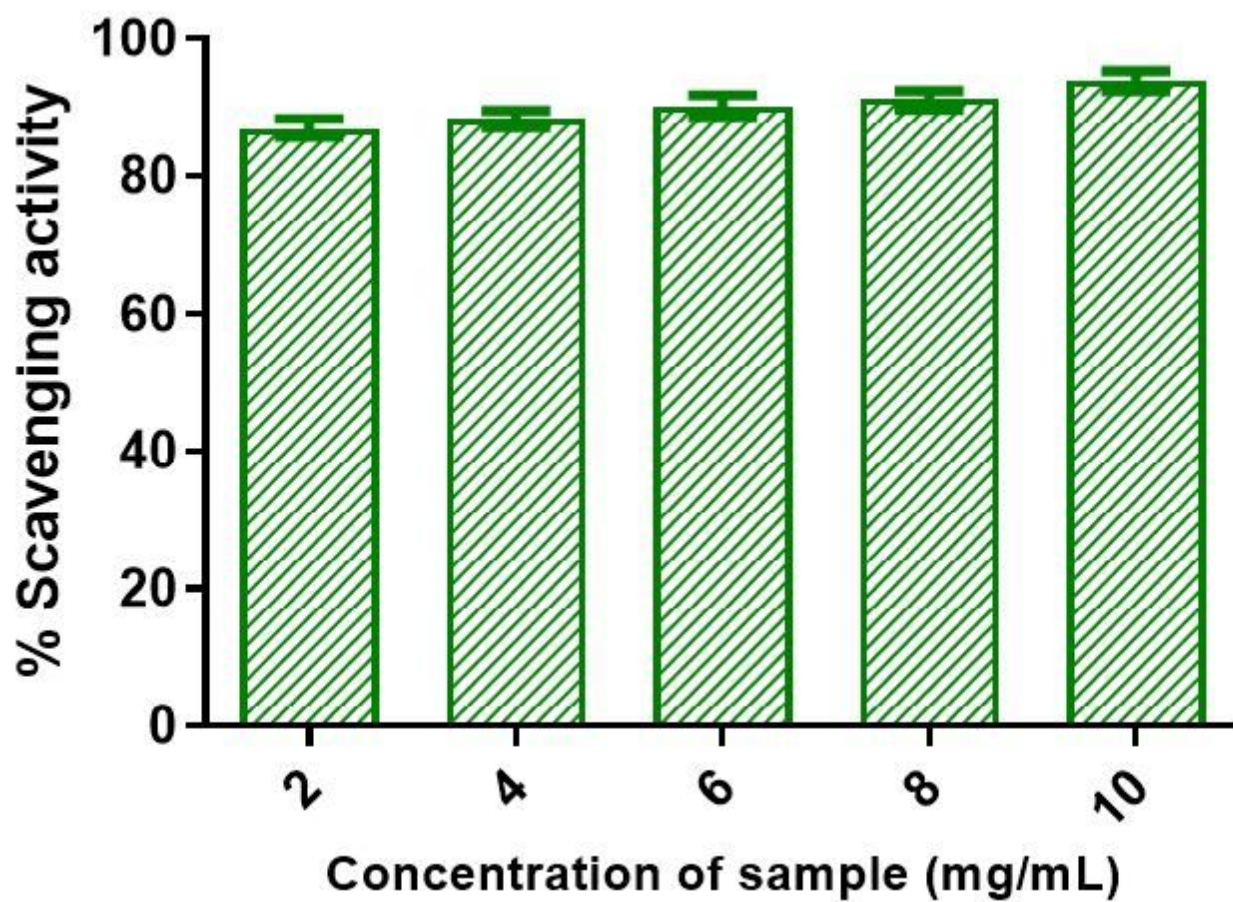


Figure 6

Scavenging activity of Copper oxide nanoparticles

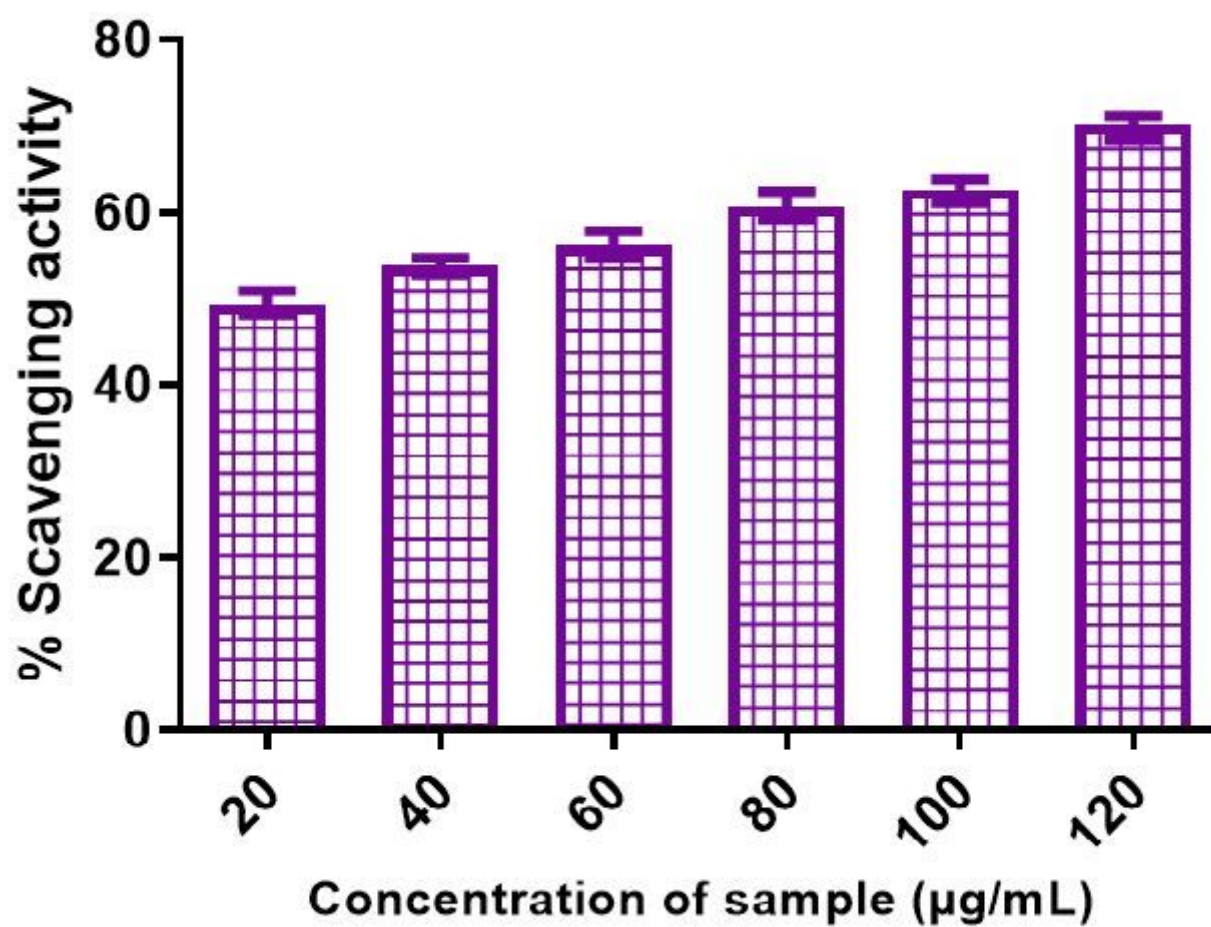


Figure 7

Scavenging activity of Copper oxide nanoparticles

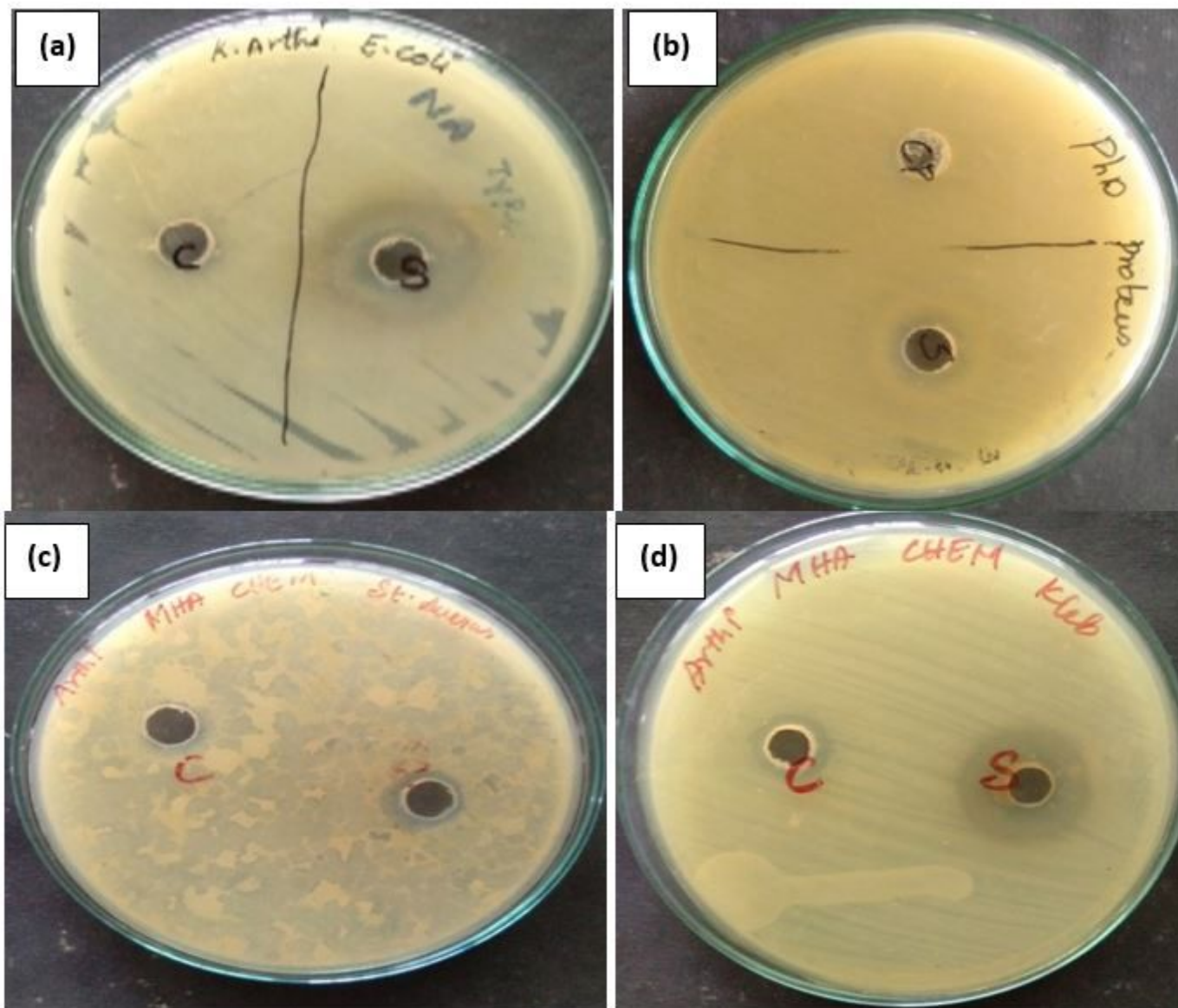


Figure 8

Antibacterial activity of CuO nanoparticles towards (a) *E. coli*; (b) *Proteus*; (c) *Staphylococcus aureus*; (d) *Klebsiella pneumoniae*

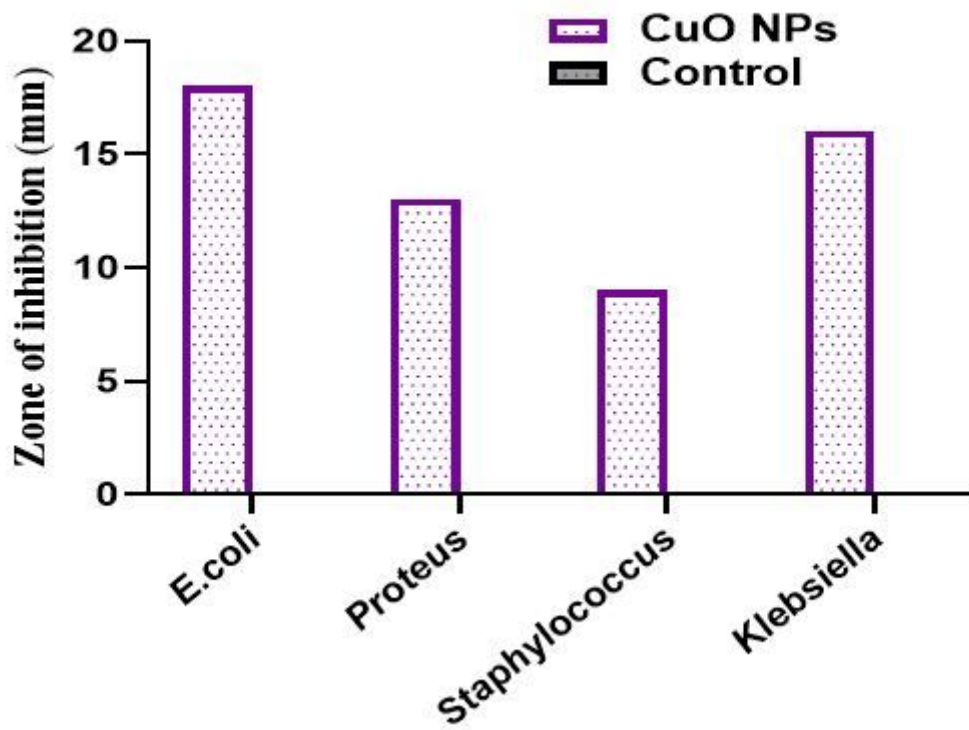


Figure 9

Antibacterial Activity of Copper Oxide Nanoparticles

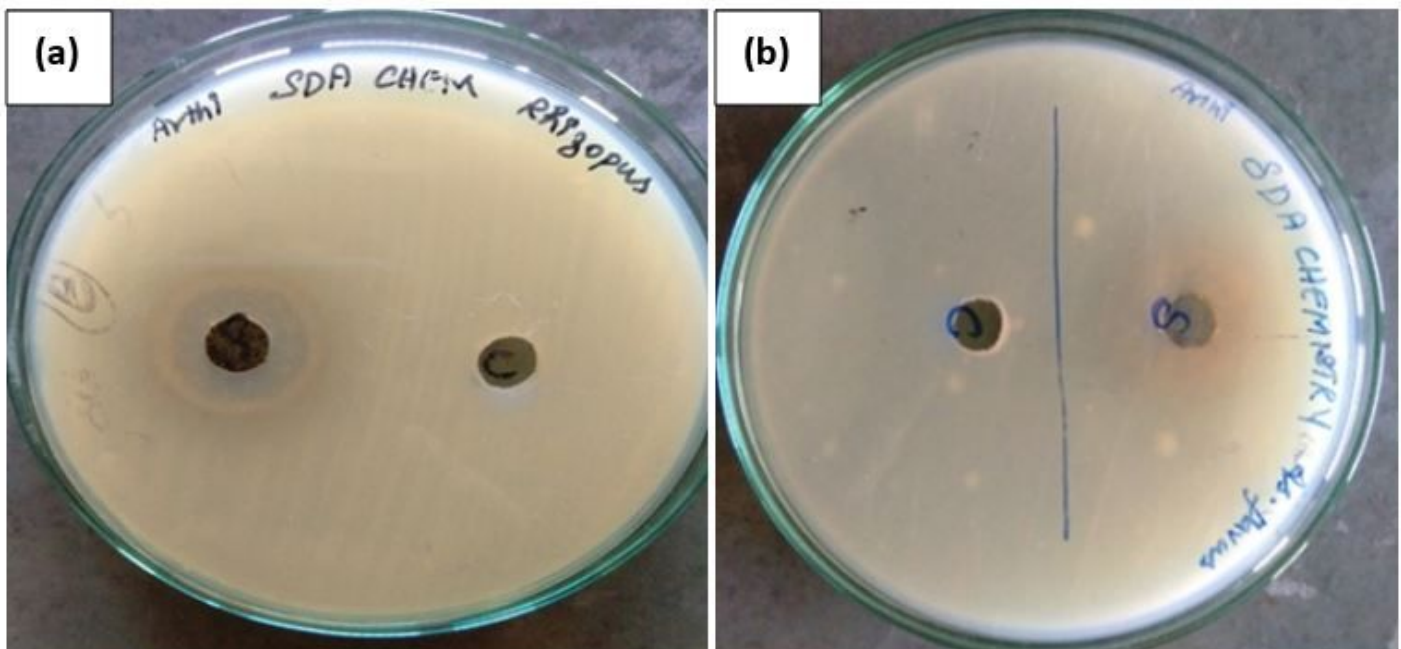


Figure 10

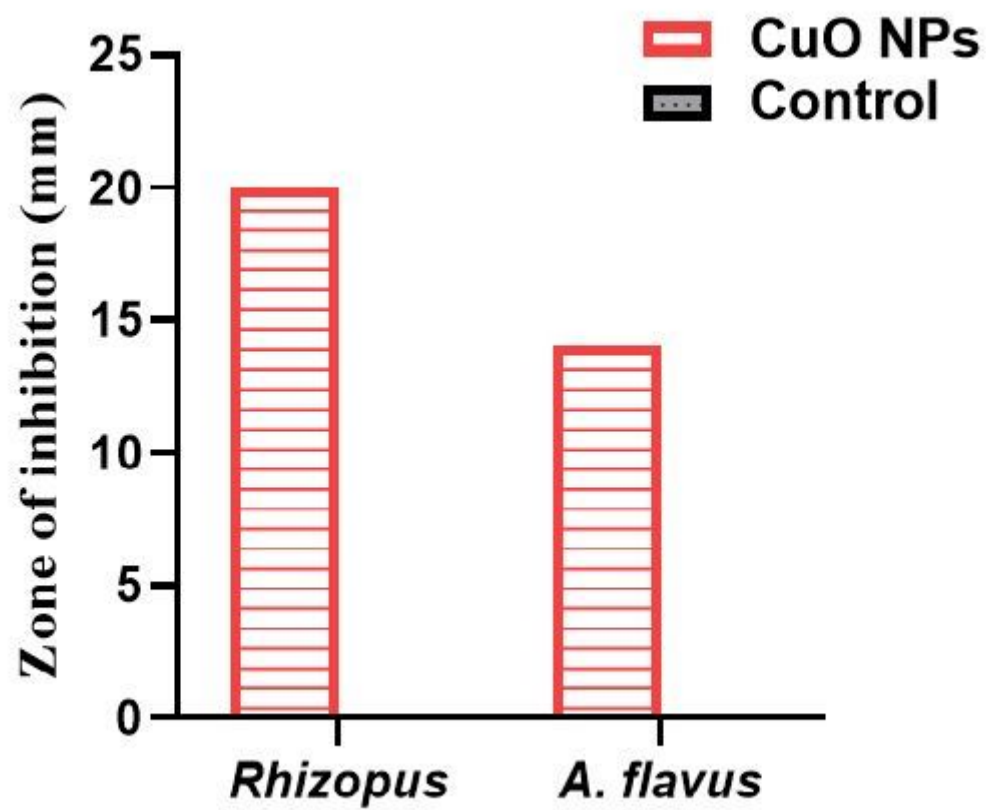


Figure 11

Antifungal activity of CuO nanoparticles

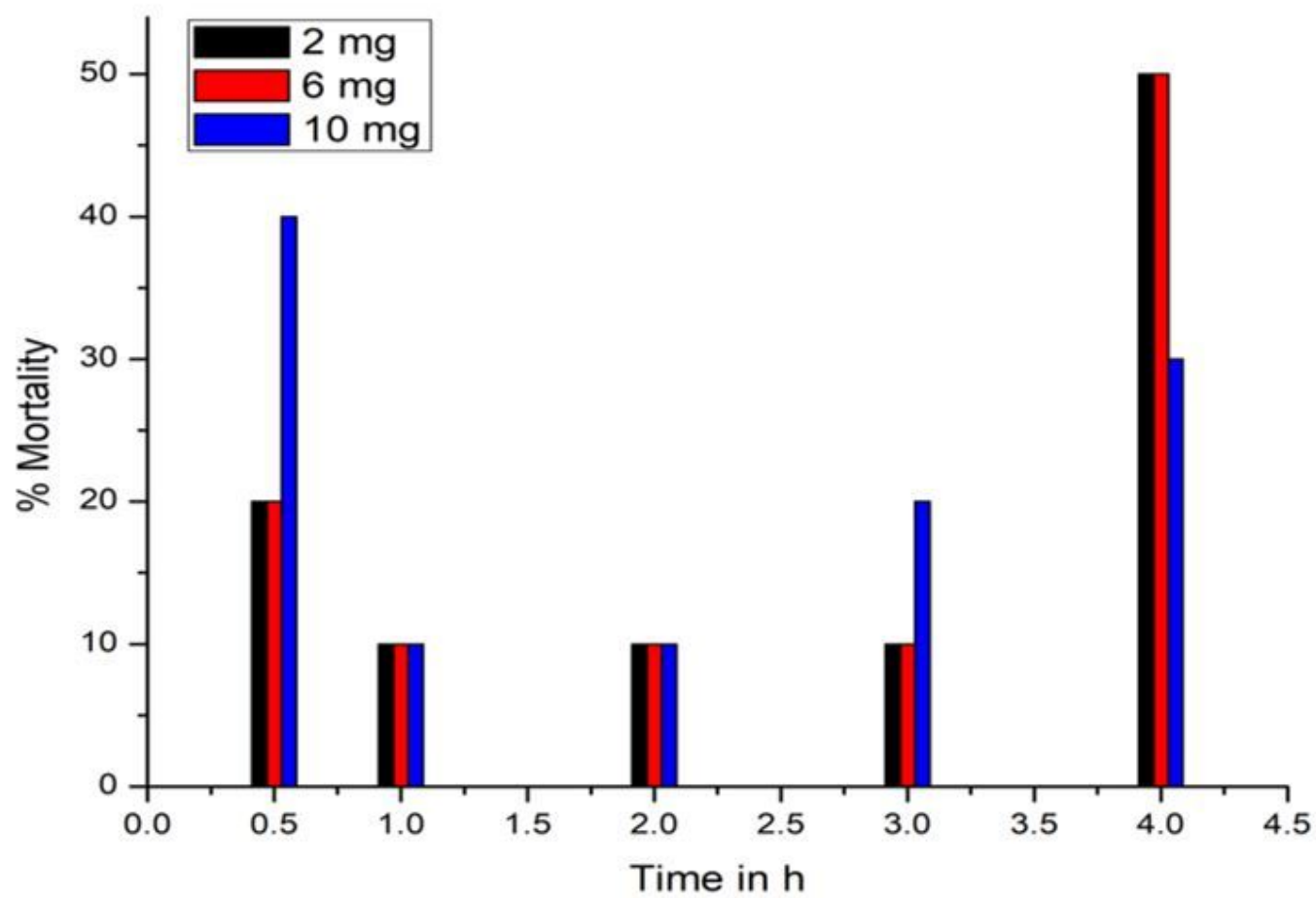


Figure 12

Larvicidal activity of CuO nanoparticles

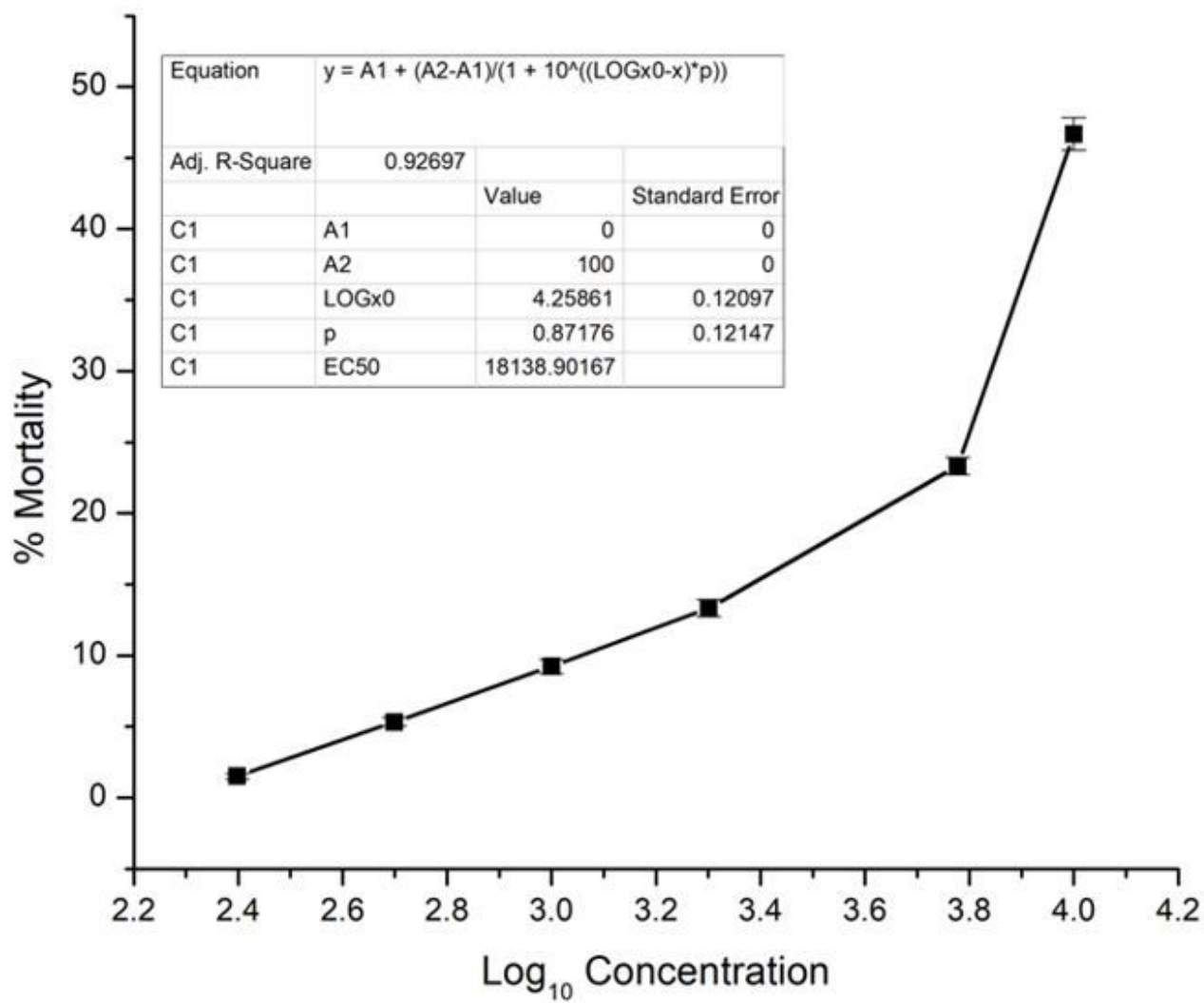


Figure 13

Half maximal effective concentration test