Research Square

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Green synthesis, characterization of copper nanoparticle synthesis from the leaf extract of Aegle marmelos and their biological activities

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Research Article

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Abstract

Over the past few decades, the development of nanomaterial applications in numerous fields has been known to be significantly aided by green methods for the manufacture of metallic and metallic oxide nanoparticles. In comparison to several current physical and chemical approaches, the green production of copper and copper oxide nanoparticles (Cu and CuO, NPs) proved more practical and cost-effective. Applications for green-fabricated Cu and CuO Nanoparticles include energy, biomedicine, pharmaceutics, photocatalysis, electrocatalysis, organic dye degradation, and cosmetics. The bioactive elements of the plant extracts and the preparation circumstances have a significant impact on the shape and topology of biologically active Nanoparticles. The goal of the current review is to raise awareness of the numerous plants used in the biologically active manufacture of copper and its oxide nanoparticles (NPs) for use in catalysis and biological activity testing like Antimicrobial, antioxidant and anticancer. It has also been studied how to characterise biogenic copper nanoparticles for their chemical, morphological, and topographical properties using various analytical methods.

Introduction

Studying materials at the nanotechnology is the intriguing and expanding field of research known as nanoscience (1–100 nm). When contrasted to their bulk counterparts, the nanomaterials have unique characteristics. Due to their distinctive characteristics, such as a high surface-to-volume ratio, form, size, and composition, nanomaterials have a tremendous potential for a variety of applications [1]. In recent years, metal and metal oxide nanoparticles have significantly improved biomedical sensing, imaging, diagnosis, and treatment. The three metals that are used the most frequently today are silver, copper, and gold. A low-cost, high-yielding material that can be exploited in biomedical and environmental remediation applications among these metal nanoparticles is copper [2]. Due to their interesting characteristics, copper and copper oxide-based nanomaterials have attracted a lot of attention recently. These materials can be used for a variety of purposes, including sensors [3,4], catalysts [5, 6], electronics [7], optics [8], solar cells [9], environmental remediation [10,11], antimicrobials, etc.

Cost-effective, dependable, sustainable, and energy-efficient strategies are used in green approaches for the synthesis of nanomaterials. Plant extract-based green synthesis techniques are preferred because they are easily accessible, manageable, non-toxic, economical, and environmentally friendly. The variety of phytochemicals also makes it possible to make nanomaterials with a variety of properties [12–15]. Several viruses, bacteria, and fungi are evolving resistance to the antimicrobials that are now accessible. New microbial species are also developing for unknown reasons. Microbiological diseases had become a serious threat to humanity [16–19]. This may be due to the ineffective use of current antimicrobial medications or unsustainable development that is bad for the environment and ecology. The discovery of novel antibiotics is essential in the event of pandemic infections. The answer to all of the world's issues has been maintained by nature. In the past, people used a variety of plant extracts to make very efficient medicines called "bhasmas."

A. marmelos, a traditional herb from the Rutaceae family, has been used to treat asthmatic disputes, catarrh, diabetes, ophthalmia, deafness, and other conditions. Stomach pain, loose bowels, and cardiovascular disorders are all treated with natural medicines. ZnO-NPs have a wide range of potential applications that have been rationally approved, including their antibacterial, hypoglycemic, anthelmintic, antispasmodic, antidysentery, antiulcer, stress, lessening, antiparasitic, injury-healing, larvicidal, photochemical, and neuroprotective properties. The current work provides a quick and straightforward process for making silver nanoparticles that is environmentally friendly by reducing silver nitrate solution with an aqueous leaf extract of *Aegle marmelos*. evaluation of copper nanoparticles' biological properties, including as their antimicrobial, anticancer effects [20–23].

Methodology

Copper chloride solution preparation

0.1245grams of copper chloride was dissolved in distilled water to make 500 ml of a 1 mM solution of copper sulphate.

Preparation of the plant extract

The 25 grams of fresh herbs that were gathered from the community to make the *Aegle marmelos* leaf extract were used. Fresh leaves were completely rinsed with pure water, then given a final wash two or three times with pure water to remove any remaining dust and other visible particles. The leaves were cut into little pieces and dried for two to three days in the shade (Fig. 1). The pieces of little leaves that had been shade-dried were then given 100 mL of distilled water, and they were boiled on a water bath for fifteen minutes. The solution was heated, cooled, and then filtered through Whatman Filter Paper No. 1 to remove contaminants. It was then held at 4°C until it was used again within a short period of time.

Copper nanoparticles synthesis

Crude extract from the leaves of *A. marmelos* were used to make copper nanoparticles. 100 ml of a 1 mM *A. marmelos* leaf extract and 1 ml of a 250 ml Erlenmeyer flask containing an aqueous copper chloride solution were used to make copper nanoparticles. The following day, the flask was kept at room temperature. By repeatedly centrifuging for fifteen minutes at 12,000 rpm to purify the produced Cu nanoparticle combination, the pellet was then redistributed in deionized water. The Cu nanoparticles were then dried in an oven set to 800^{o}C.

Characterization of Copper nanoparticles

Copper nanoparticles that have been synthesised can be examined using UV-VIS spectroscopy to determine their properties. In Spectral data, a characteristic surface plasmon resonance (SPR) absorption band is produced by free electrons in metal nanoparticles. This peak in the Ultra violet spectrum results from the strong interaction of the free electrons present on the outermost layer of metallic nanoparticles with specific wavelengths of light. The shape and size of metal nanoparticles influence their colour. Using

an Equiptronics microprocessor-based Single-beam spectroscopy, the Ultra violet spectrum of the solvent from area 300-600nm were used to confirm the decrease of Cu++ (EQ-825A). The substances that have their substituents coupled with the produced nanoparticles are identified by FT-IR characterization. To analyse the morphological properties, sizes, and shapes of nanoparticles, high resolution SEM investigation is performed. XRD (Shimadzu, XRD-6000) was used to analyse the crystalline nature and establish the average size of the particles.

In vitro antibacterial activity

Six human gram positive and gram-negative bacteria *Corynebacterium glutamicum* (MTC2745), *Salmonella typhimurium* (MTCC-98), *S. flexneri*, (ATCC-12022), *V. parahaemolyticus* (ATCC-17802), *Clostridium perfringens* (ATCC 13124), *Enterococcus faecalis* (MTCC-6845) were maintained on nutrient broth. Bacterial sensitivity to nanoparticles is commonly tested using a well diffusion assay. Copper nanoparticles solution that had been prepared was introduced to the wells. In order to allow for diffusion, the samples were first incubated for 15 min at 4 C and then for 24 h at 37 C. When a zone of inhibition was shown around the well after the overnight incubation, the experiment was deemed successful.

In vitro antifungal activity

The agar well diffusion method was used to assess the antifungal efficacy of CuO nanoparticles against *Aspergillus flavus* (MTCC-3396), *Aspergillus fumigatus* (MTCC-961), *Aspergillus niger* (MTCC-281), and *Candida albicans* (MTCC-277). Potato dextrose agar culture media plates were made, infected with the fungi, and incubated for 3 days at 25°C to encourage fungus development. Wells were made in the agar plate with a core-borer and filled with 100µL of CuO nanoparticles that had been dissolved in DMSO. The plates were incubated at 25°C for 3 days after being left at 37°C for 1 hour to allow the test sample to diffuse. 50µL of the antibiotic Nystatin was employed as a positive control. After the incubation period, the zone of inhibition was measured against tested fungal microorganisms.

In vitro anticancer activity of copper nanoparticles

Preparation of HCT 116 cell lines suspension

Trypsin was used to digest a subculture of the HCT 116 cell lines in DMEM (Dulbecco's Modified Eagle's Medium). 10% FCS (Fetal Calf Serum) with 25 mL of DMEM was added to the flask's dispersed cells. Via gently pipetting with the pipette, the cells were suspended in the medium and homogenised.

Seeding of cells

Various sampling quantities (10 to 200 µg/mL) were put to each well of a 24 well culture plate along with one millilitre of the homogenised cell suspension, and the plate was then maintained at 37°C in a pressurised CO_2 incubator with 5% CO_2. After 48 hours of incubation, the cells were examined at x40 magnification using an inverted tissue culture microscope from Olympus.

Cytotoxicity assay

The present work was performed by using (3, 4, 5, dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT). Live cells' mitochondrial succinate dehydrogenase and reductase convert MTT into measurable purple formazan. This formazan production is directly connected with the quantity of viable cells and negatively correlated with the degree of cytotoxicity. MTT was added to the wells after 48 hours of incubation, and they were then kept at room temperature for 3 hours. The contents of each well were taken out using a pipette. After dissolving the formazan crystals with 100 mL of DMSO, each well's absorbance was measured at 540 nm with a Robotnik microplate reader. The following calculation was used to compute the percentage growth inhibition:

$$\text{Percentage of growth inhibition} = \frac{\text{OD of Test}}{\text{OD of Control}} \text{X } 100$$

The optimal biological indicator of cytotoxic activity is typically determined to be the inhibitory concentration at 50% (IC_{50}). The purified chemical concentration at which 50% of cell inhibition resulted is known as the IC_{50} value.

Results

Evaluation of Biosynthesis of Copper Oxide Nanoparticles

The visual transformation in the colour of the reaction mixture was first used to confirm the formation of copper nanoparticles, and then an examination using UV-visible spectroscopy was done for additional confirmation. The copper ions were converted to copper nanoparticles using the extract from *A. marmelos.* Comparatively, no colour change was seen when aqueous Cu-SO4 was pre - treated under the same conditions without cellfree supernatant, indicating that the biosynthesis of copper nanoparticles was not taking place. This was demonstrated by the soft blue reaction blend turning green after 1% (v/v) of 1mM aqueous CuSO4 was added to the cell-free *A. marmelos* extract (Fig. 2). A subtle but noticeable shift in the colour of the copper sulphate solution was noticed after 24 hours at ambient temperature, going from a very light blue to a greenish brown, suggesting the creation of copper nanoparticles, according to Ashtaputrey et al., 2017 [13].

UV–visible absorbance spectroscopy

The Ultra violet absorbance spectrophotometer is used to identify synthetic metallic oxide nanoparticles (NPs). The fundamental idea behind this method is extremely sensitive to detecting the peak position and spectral form of a particle bulk. Figure 3 displays the copper nanoparticle solution's UV-visible spectrum as recorded after 24 hours. The 320 nm spectra show a prominent and high peak, indicating the stability and size of copper nanoparticles [14]. In copper solution, the strength of an SPR peak increased, which showed that copper ions had formed CuO NPs. The greatest absorption peak was seen at 280 nm, and

this can be attributed to the twisted SPR of CuO NPs [15]. The sample's UV-vis absorption spectra were obtained between 200 and 700 nm for produced copper oxide nanoparticles. According to the spectra, the absorbance peak at 310 nm corresponds to the band that copper oxide nanoparticles are produced [16].

X-ray diffraction analysis

The commonly used technique of X-ray diffraction (XRD) analysis was used to determine if a particle's structure was amorphous or crystalline. The XRD was utilised to study the copper nanoparticle's structure, and the first Bragg's diffraction technique was employed to determine the points. Such peak studies imply that the cubic assembly's centre is where copper oxide nanoparticle production occurs [16]. Some of the unassigned peaks shown on the copper nanoparticles include impurities from the production process [17]. The sample displayed a high level of crystalline phase with diffraction patterns of 22.09°, 27.8°, 28.20°, and 33.10°, which correlate to the distinctive face centred cubic (fcc) of copper lines indexed at (111), (211), (211), and (220), respectively. The XRD pattern of Cu nanoparticles prepared by using *A. marmelos* leaf extract is shown in Fig. 4. The crystalline phase of copper oxide nanoparticles is confirmed through the clear and precise copper oxide responses in the measured XRD patterns [18].

SEM Analysis

Copper nanoparticles made from extract of *A. marmelos* showed shell-like sheet structure. Copper nanoparticles in the size range of 15 to 26 nm (Fig. 5) were produced without agglomeration and with reasonably good monodispersed and almost spherical shapes [17]. Copper nanoparticles are morphologically characterised by SEM. It has several limitations in terms of morphological analysis because it only yields a small amount of data on the actual population and the dispersion of average size. Several nano polymers have reportedly been harmed by SEM. Thus, the NPs must be able to endure hoover pressure in order for a morphological examination using SEM to be effective [19].

FTIR Analysis

The main purpose of FTIR analysis is to find structural characteristics. The presence of potentially bioactive compounds is caused by the reduction in copper ions and the capping ability of copper nanoparticles. As illustrated in Fig. 6, the presence of various functional groups of copper nanoparticles was determined using the FT-IR absorption spectra. For the *A. marmelos* leaf extract, a large peak at 3233 cm1 is hydrogen-bonded -OH stretching was discovered. In IR spectra, the C-H bending vibration are represented by the bands' maxima at 2824 and 2756 cm1. Bands operating at 1429 and 1253 cm^{-1} were attributed to the materialisation of oxygen functional groups approximating strongly conjugated (C-O) stretching in carboxylic groups. The high of 1066 cm^{-1} was attributed to C-O stretching. C-N-C is credited with the peak at 657 cm1. The peak at 532 cm^{-1} is consistent with the production of copper nanoparticles [20]. The produced copper oxide nanoparticles' FT-IR measurement revealed prominent peaks at 3205 cm^{-1}, 1633 cm^{-1}, 1121 cm^{-1}, and 443 cm^{-1}. The C-O-H or C-N-H groups in the plant extract, which help in the conversion of copper acetate to copper oxide nanoparticles, are represented by the bands at 3405 cm^{-1} and 1633 cm^{-1} [16].

Antibacterial activity of CuO-NPs

Antibacterial performance of copper nanoparticles was performed against different bacterial strains *Corynebacterium glutamicum* (MTC2745), *Salmonella typhimurium* (MTCC-98), *S. flexneri* (ATCC-12022), *V. parahaemolyticus* (ATCC-17802), *Clostridium perfringens* (ATCC 13124), *Enterococcus faecalis* (MTCC-6845). The antibacterial effect of copper nanoparticles for diverse microorganisms in a well diffusion assay is shown in Fig. 8. The results showed that a variety of bacteria responded very well to copper nanoparticles remarkable antibacterial activity as in Table. 1. The size of the inhibitory zone represents how susceptible the microorganisms are. In contrast to resistant strains, those that were vulnerable to copper nanoparticles showed a greater zone of inhibition. *Vibrio parahaemolyticus* and *E. faecalis* showed the maximum sensitivity to copper nanoparticles, whereas *Salmonella typhimurium* showed the lowest sensitivity among the studied microorganisms, according to zone of inhibition. The antimicrobial properties and solution concentration differed from sample to sample. Gram-negative bacteria have a smaller effective zone than gram-positive bacteria. This might have happened because of the complicated structure of gram-negative bacteria. Gram-negative cell walls are thicker than gram-positive cell walls, which is another factor that contributes to the outcome [16].

Table 1 Antibacterial activity of CuO nanoparticles

S.No	Test Organism	Zone of inhibition (mm)			
		Copper nanoparticles (µg/mL)			
		80	100	150	Standard (Chloramphenicol) 30µg/mL
1	*Vibrio parahaemolyticus* (ATCC-17802)	17	18	20	22
2	*Shigella flexneri* (ATCC-12022)	-	20	20	22
3	*Corynebacterium glutamicum* (MTC2745)	-	-	-	18
4	*Enterococcus faecalis* (MTCC-6845)	12	14	16	19
5	*Clostridium perfringens* (ATCC 13124)	12	14	18	21
6	*Salmonella typhimurium* (MTCC-98)	10	12	14	16

Antifungal activity of CuO nanoparticles

According to Shamaila et al., 2016 [21], a well diffusion approach using agar plates was used to measure the zone of neutralisation. Using potato dextrose agar plates, clinical fungal pathogens were grown and constantly maintained at 37°C during the whole night. These mushrooms were employed in the

experiment after being cultured for 24 hours. This approach required that the potato dextrose gar plate be sterile and that an Agar plate be made for each fungus. The strains that were susceptible to copper nanoparticles had a larger zone of inhibition than the resistant strains. *Aspergillus fumigatus* and *Aspergillus flavus* showed the maximum sensitivity to copper nanoparticles, whereas *Candida albicans* showed the lowest sensitivity among the studied microorganisms, according to zone of inhibition as shown in Fig. 9 and further numeric details are given in Table. 2..

Table 2 Antifungal activity of copper nanoparticles

S.No	Test Organism	Zone of inhibition (mm)			
		Copper nanoparticles (µg/mL)			
		100	150	200	Standard (Nystatin) 50µg/mL
1	*Aspergillus flavus* (MTCC-3396)	10	12	12	15
2	*Aspergillus fumigatus* (MTCC-961)	12	14	16	18
3	*Aspergillus niger* (MTCC-281)	8	10	12	14
4	*Candida albicans* (MTCC-277)	6	8	9	11

In vitro anticancer activity

The anticancer potential of the copper nanoparticles was examined in the current work using HeLa cell lines. A typical MTT experiment was used to assess cell viability, and the outcomes are shown in Table 3. By increasing the concentration of fractions, the dead cells increased. Copper nanoparticles at a concentration of 200µg/mL were found to have the lowest viability in HeLa cell lines (39.12 ± 0.16). Copper nanoparticles demonstrated a strong cytotoxic effect with an IC_{50} value of 129.08 ± 0.11µg/mL on HeLa cell lines, which were used to estimate the IC_{50} values. The cytotoxic effects of copper nanoparticles was stronger against HeLa cell lines, according to the IC_{50} values, and it was thought to have significant antitumor activity. After being exposed to purified fractions for 48 hours, HeLa cell lines had changes in morphology, as observed under an inverted tissue culture microscope. HeLa cell lines used as a control (untreated with fractions) developed as erratic confluent aggregates with rounded and polygonal cell shape (Fig. 10). Copper nanoparticles at a concentration of 200 µg/mL caused the development of polygonal cells that started to shrink and take on a spherical shape after 48 hours of treatment (Fig. 10 (h)).

Table 3 *In vitro* cytotoxicity of copper nanoparticles on HeLa cell lines Mean of three replicates ± Standard deviation

S. No	Fraction Conc.(mg/mL)	% Cell viability of HeLa cell lines
		copper nanoparticles
1	0	100.00±0.00
2	12.5	93.32±0.24
2	25	85.12±0.04
3	50	74.70±0.01
4	75	64.30±0.01
5	100	56.21±0.04
6	150	47.22±0.26
7	200	39.12±0.16
8	IC_{50}	**129.08±0.11**

Discussion

An inexpensive and cost-effective approach was used to extract copper nanoparticles from *A. marmelos* extract. The diameters of nanoparticles were investigated using SEM examination, and it was discovered that the copper nanoparticles had a 15nm diameter and a 15–26nm length range. Using XRD patterns, single-crystalline and evenly distributed structures have been observed. Results from the UV-Vis and FTIR spectra indicated the presence of nanoparticles. Also, the findings of copper nanoparticle physicochemical characterizations added to the findings of in vitro research. The green produced Copper nanoparticles have potential uses in the realm of nanomedicine and might be utilised to create specialised treatments for cancer cell lines, germs, and fungi. Also, the current work encourages researchers to assess their antibacterial or anticancer potential in addition to enabling them to design pilot-scale techniques for ecological nanoparticle synthesis from natural resources. There are comparatively few investigations on the interactions of microorganisms with copper nanoparticles compared to other nanometals. Hence, they may discover new directions in the field of nanomedicine by investigating the processes of interaction of copper nanoparticles on long-term antibacterial activity.

Declarations

“Conflicts of interest/Competing interests”

The author has no relevant financial or non-financial interests to disclose.

The author has no conflicts of interest to declare that are relevant to the content of this article.

The author certifies that she has no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

The author has no financial or proprietary interests in any material discussed in this article.

Data cannot be shared openly but are available on request from authors:

The data that support the findings of this study are available from the author but restrictions apply to the availability of these data, which were used under specific laboratory conditions for the current study, and so are not publicly available. Data are, however, available from the authors upon reasonable request and with permission from the author.

References

1. R.A. Bapat, C.P. Joshi, P. Bapat, T.V. Chaubal, R. Pandurangappa, N. Jnanendrappa, B. Gorain, S. Khurana, P. Kesharwani, The use of nanoparticles as biomaterials in dentistry, Drug Discov. Today 24 (2019) 85–98.
2. S. Honary, H. Barabadi, E.G. Fathabad, F. Naghibi, Green synthesis of Copper oxide nanoparticles using Penicillium Aurantiogriseum, Penicillium citrinum and Penicillium waksmanii, Dig J. Nanomater. Bios 7 (2012) 999–1005.
3. Z. Haghparas, Z. Kordrostami, M. Sorouri, M. Rajabzadeh, R. Khalifeh, Fabrication of non-enzymatic electrochemical glucose sensor based on nano-copper oxide micro hollow spheres, Biotechnol. Bioproc. Eng. 25 (2020) 528–535.
4. V. Ambardekar, P.P. Bandyopadhyay, S.B. Majumder, Plasma sprayed copper oxide sensor for selective sensing of carbon monoxide, Mater. Chem. Phys. 258 (2021) 123966.
5. A. Muthuvel, M. Jothibas, C. Manoharan, Synthesis of copper oxide nanoparticles by chemical and biogenic method: photocatalytic degradation and in vitro antioxidant activity, Nanotechnol. Environ. Eng. 5 (2020).
6. T. Begildayeva, S.J. Lee, Y. Yu, J. Park, T.H. Kim, J. Theerthagiri, A. Ahn, H.J. Jung, M.Y. Choi, Production of copper nanoparticles exhibiting various morphologies via pulsed laser ablation in different solvents and their catalytic activity for reduction of toxic nitroaromatic compounds, J. Hazard Mater. 409 (2021) 124412.
7. N. Sarwar, S.H. Choi, G. Dastgeer, U.B. Humayoun, M. Kumar, N. Ali, D.I. Jeong, S.F.A. Zaidi, D.H. Yoon, Synthesis of citrate-capped copper nanoparticles: a low temperature sintering approach for the fabrication of oxidation stable flexible conductive film, Appl. Surf. Sci. 542 (2021) 148609.
8. S. Sahai, A. Varshney, Solar absorbance enhancement in perovskite solar cells with the inclusion of copper nanoparticles: an architectural study, Opt. Quant. Electron. 53 (2021).

9. H. Siddiqui, M.R. Parra, P. Pandey, M.S. Qureshi, F.Z. Haque, Utility of copper oxide nanoparticles (CuO-NPs) as efficient electron donor material in bulkheterojunction solar cells with enhanced power conversion efficiency, J. Sci.: Adv. Mater. Dev. 5 (2020) 104–110.
10. S. Kumar, R.S. Brar, J.N. Babu, A. Dahia, S. Saha, A. Kumar, Synergistic effect of pistachio shell powder and nano zero valent copper for chromium remediation from aqueous solution, Environ. Sci. Pollut. Res. 28 (2021) 63422–63436.
11. S.M.E. Sepasgozar, S. Mohseni, B. Feizyzadeh, A. Morsali, Green synthesis of zinc oxide and copper oxide nanoparticles using Achillea Nobilis extract and evaluating their antioxidant and antibacterial properties, Bull. Mater. Sci. 44 (2021) 129.
12. K. Barman, P. Dutta, D. Chowdhury, P.K. Baruah, Green synthesis of copper oxide nanoparticles using waste *Colocasia esculenta* leaves extract and their application as recyclable catalyst towards the synthesis of 1,2,3-triazoles, BioNanoSci 11 (2021) 189–199.
13. S. D Ashtaputrey, P. D. Ashtaputrey and Neha Yelane (2017). Green synthesis and characterization of copper nanoparticles derived from *Murraya Koenigii* leaves extract. Journal of Chemical and Pharmaceutical Sciences. 10(3):1288-1291.
14. Gopinath M, Subbaiya1 R, Masilamani Selvam M, Suresh D, Synthesis of Copper Nanoparticles from *Nerium oleander* Leaf aqueous extract and its Antibacterial Activity, Int.J. Curr. Microbiol. App. Sci, 3 (9), 2014, 814-818.
15. Md. Johurul Islam, M. T. Khatun, Md. Rezwanur Rahman and M. M. Alam (2021). Green synthesis of copper oxide nanoparticles using Justicia adhatoda leaf extract and its application in cotton fibers as antibacterial coatings. AIP Advances 11, 125-223
16. Ahid M. Al-Fa,ouri, Mahmoud H. Abu-Kharma and Akl M. Awwad (2021). Green synthesis of copper oxide nanoparticles using Bougainvillea leaves aqueous extract and antibacterial activity evaluation. Chemistry International 7(3) (2021).
17. M. Rafique, A. J. Shaikh, R. Rasheed, M. B. Tahir, S. S. A. Gillani, A. Usman, M. Imran, A. Zakir, Z. Khan, and F. Rabbani, “Aquatic biodegradation of methylene blue by copper oxide nanoparticles synthesized from Azadirachta indica leaves extract,” J. Inorg. Organomet. Polym. Mater. 28, 2455 (2018).
18. P. Sharma, S. Pant, V. Dave, K. Tak, V. Sadhu, and K. R. Reddy, “Green synthesis and characterization of copper nanoparticles by Tinospora cardifolia to produce nature-friendly copper nano-coated fabric and their antimicrobial evaluation,” J. Microbiol. Methods 160, 107–116 (2019).
19. A. Husen and M. Iqbal, Nanomaterials and Plant Potential: Plant-Mediated Synthesis of Copper Oxide Nanoparticles and Their Biological Applications (2019), Chap. 8, pp. 221–237.
20. A. Folorunso, S. Akintelu, A. K. Oyebamiji, S. Ajayi, B. Abiola, I. Abdusalam, and A. Morakinyo, “Biosynthesis, characterization and antimicrobial activity of gold nanoparticles from leaf extracts of Annona muricata,” J. Nanostruct. Chem. 9, 111–117 (2019)
21. S. Shamaila, N. Zafar, S. Riaz, R. Sharif, J. Nazir, and S. Naseem, “Gold nanoparticles: An efficient antimicrobial agent against enteric bacterial human pathogen,” J. Nanomater. 6(4), 71 (2016).

22. Gopa, Dinesh Reddy and Pullapukuri, Kalyani. "Green synthesis of silver nanoparticles from *Aspergillus flavus* and their antibacterial performance" Chemical Product and Process Modeling, 2023. https://doi.org/10.1515/cppm-2022-0054
23. Kalyani, P., Botsa, S.M. & Kumari, K.S. Purification, structural elucidation and anticancer activity of a compound extracted from Aspergillus fumigatus strain MF-1. Arch Microbiol 203, 4635–4640 (2021). https://doi.org/10.1007/s00203-021-02453-w

Figures



Figure 1

Preparation of plant extract



Figure 2

Extracellular synthesis of Cu-NPs

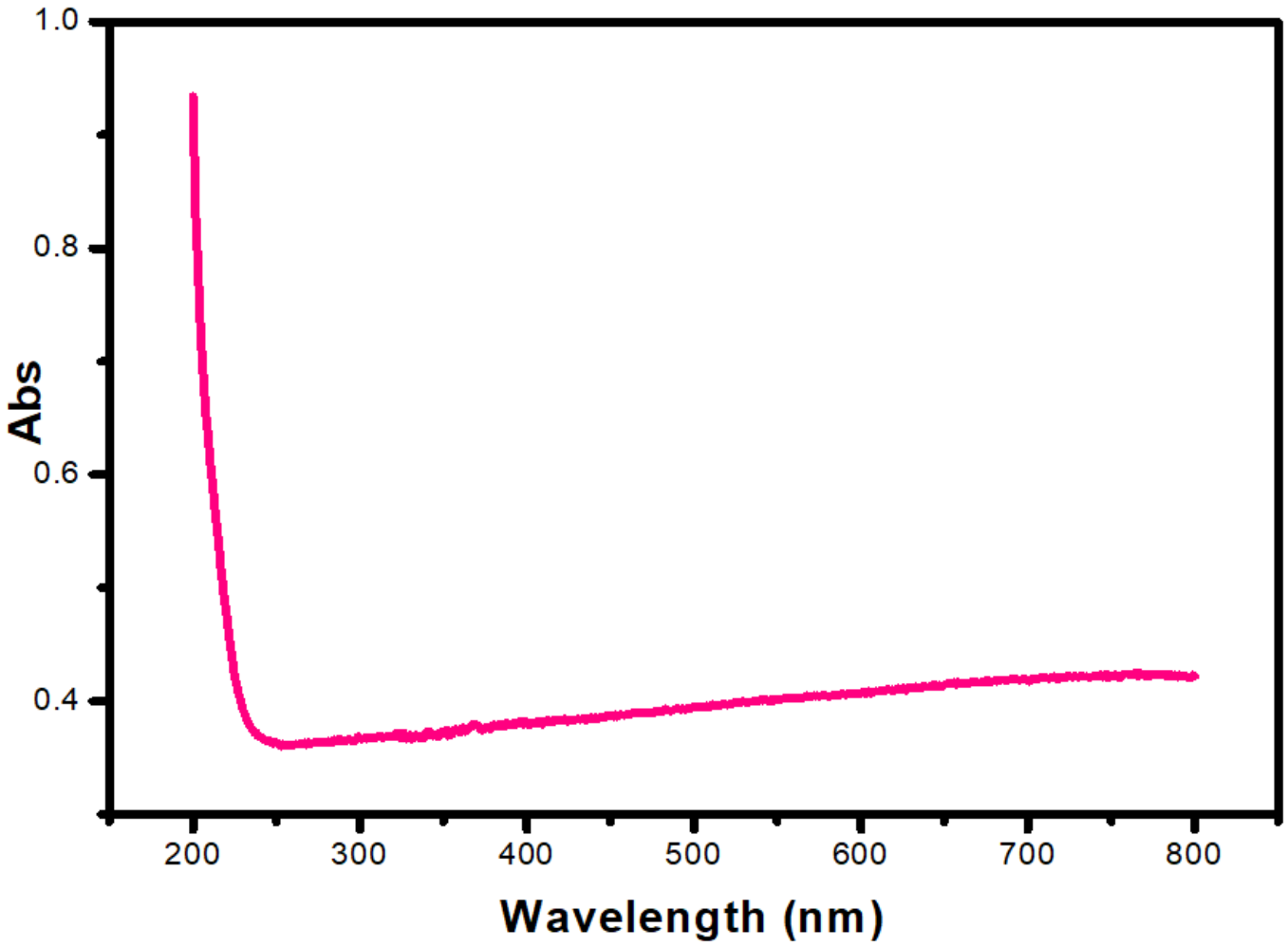


Figure 3

UV Spectrum analysis of Cu-NPs

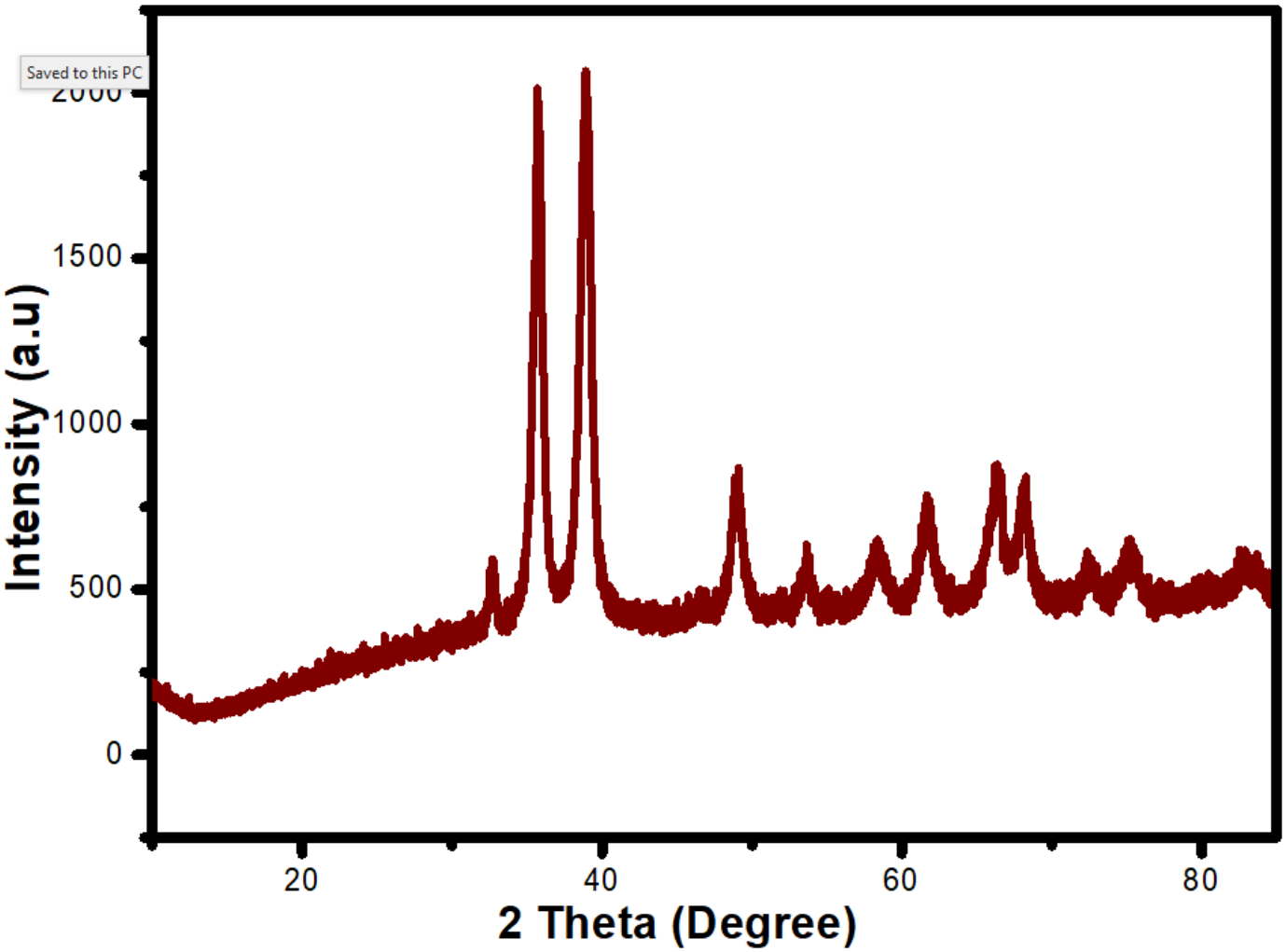


Figure 4

XRD Analysis of Cu-NPs

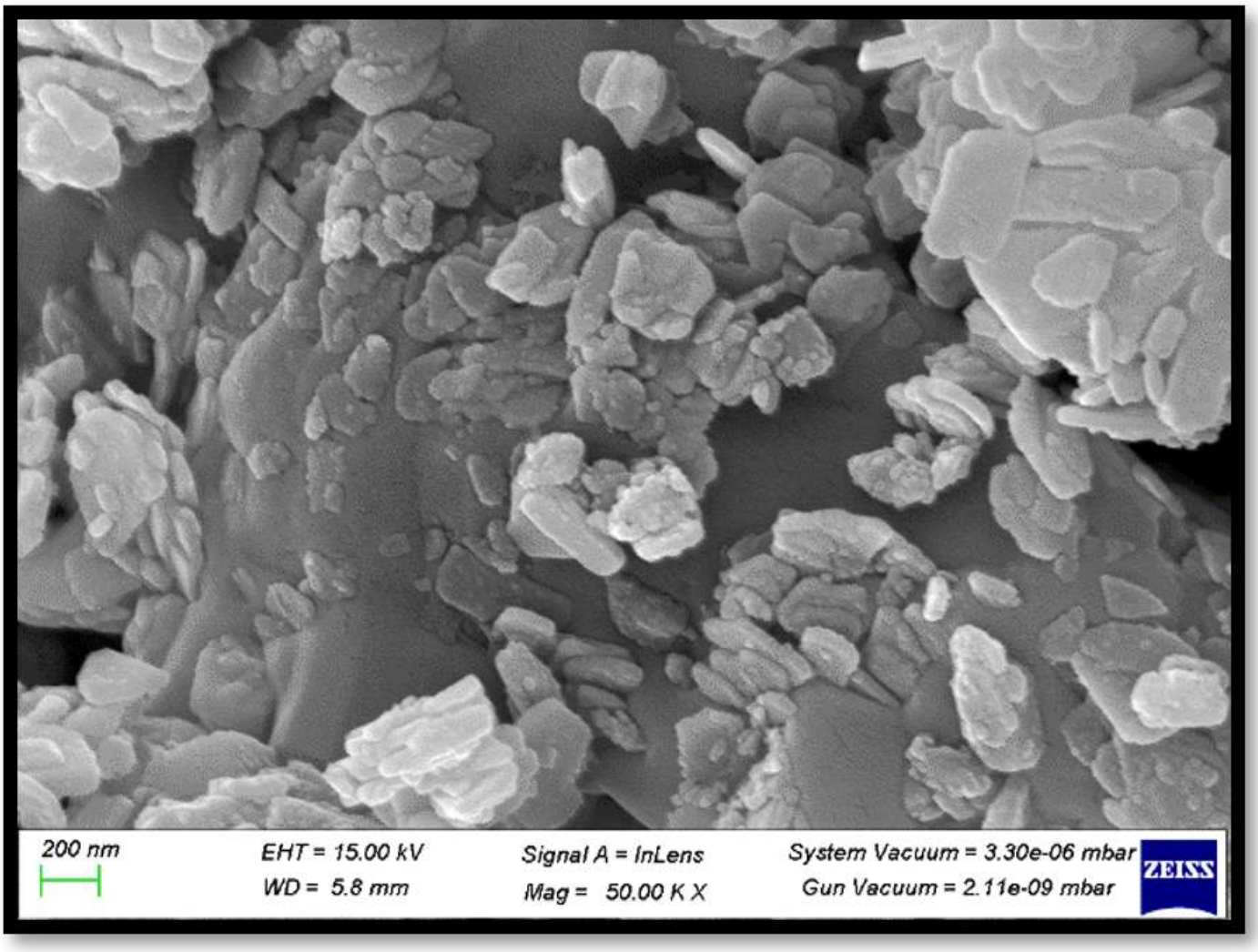


Figure 5

SEM image of Cu-NPs

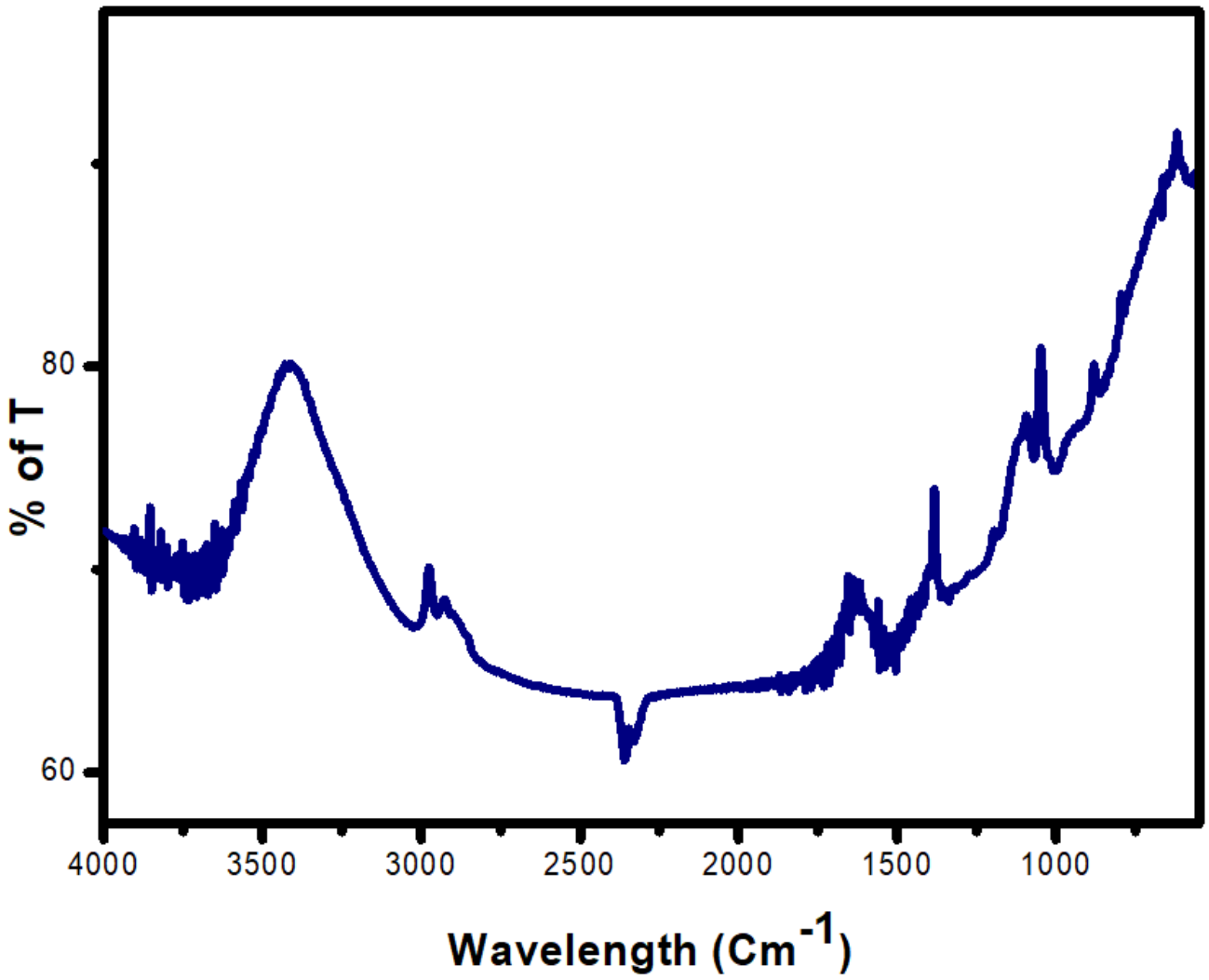


Figure 6

Fig. 7 FTIR analysis of Cu-NPs

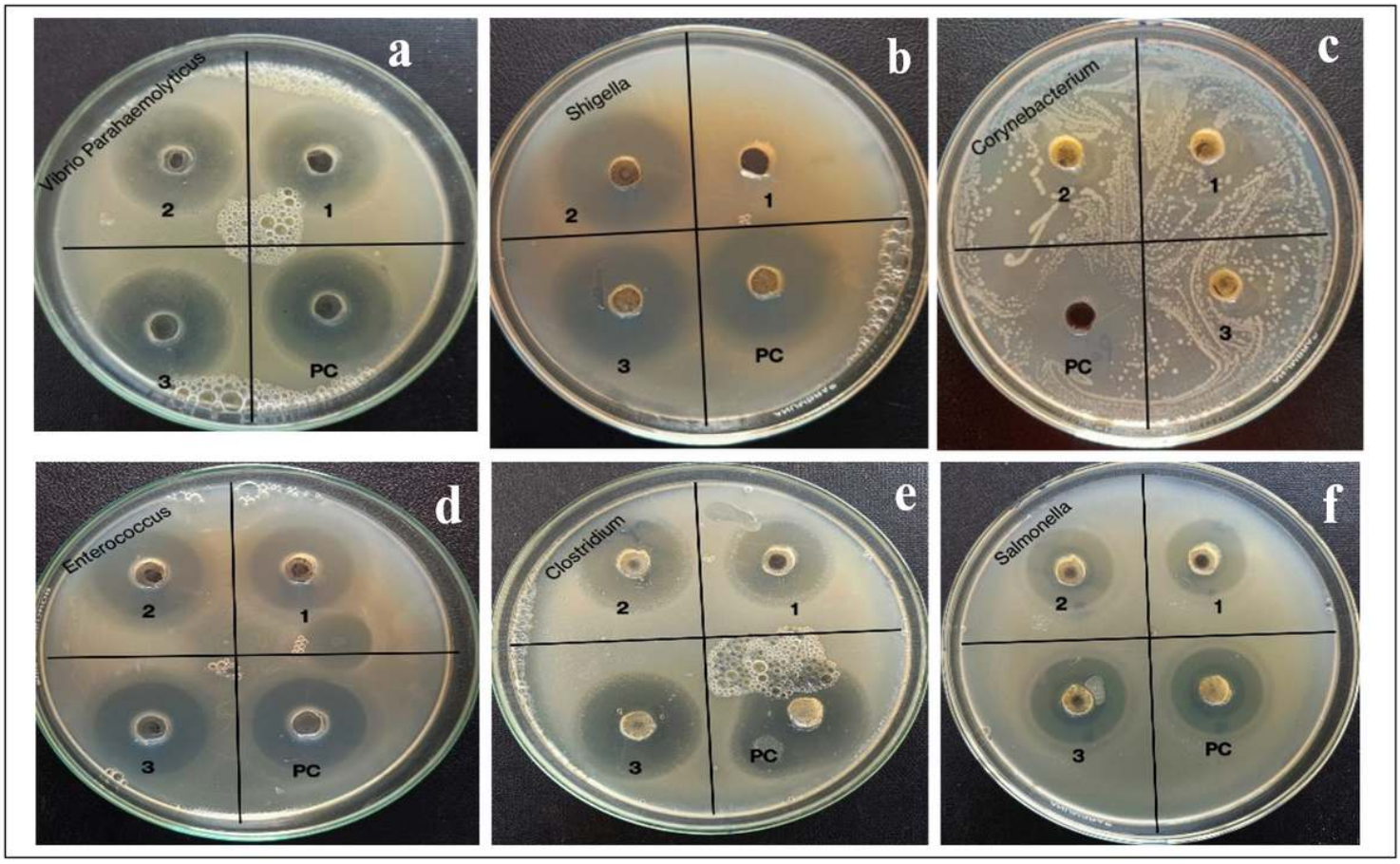


Figure 7

Fig. 8 Antibacterial activity of CuO nanoparticles against a) *Vibrio parahaemolyticus*

b) *Shigella flexneri* c) *Corynebacterium glutamicum* d) *Enterococcus faecalis*

e) *Clostridium perfringens* f) *Salmonella typhimurium*

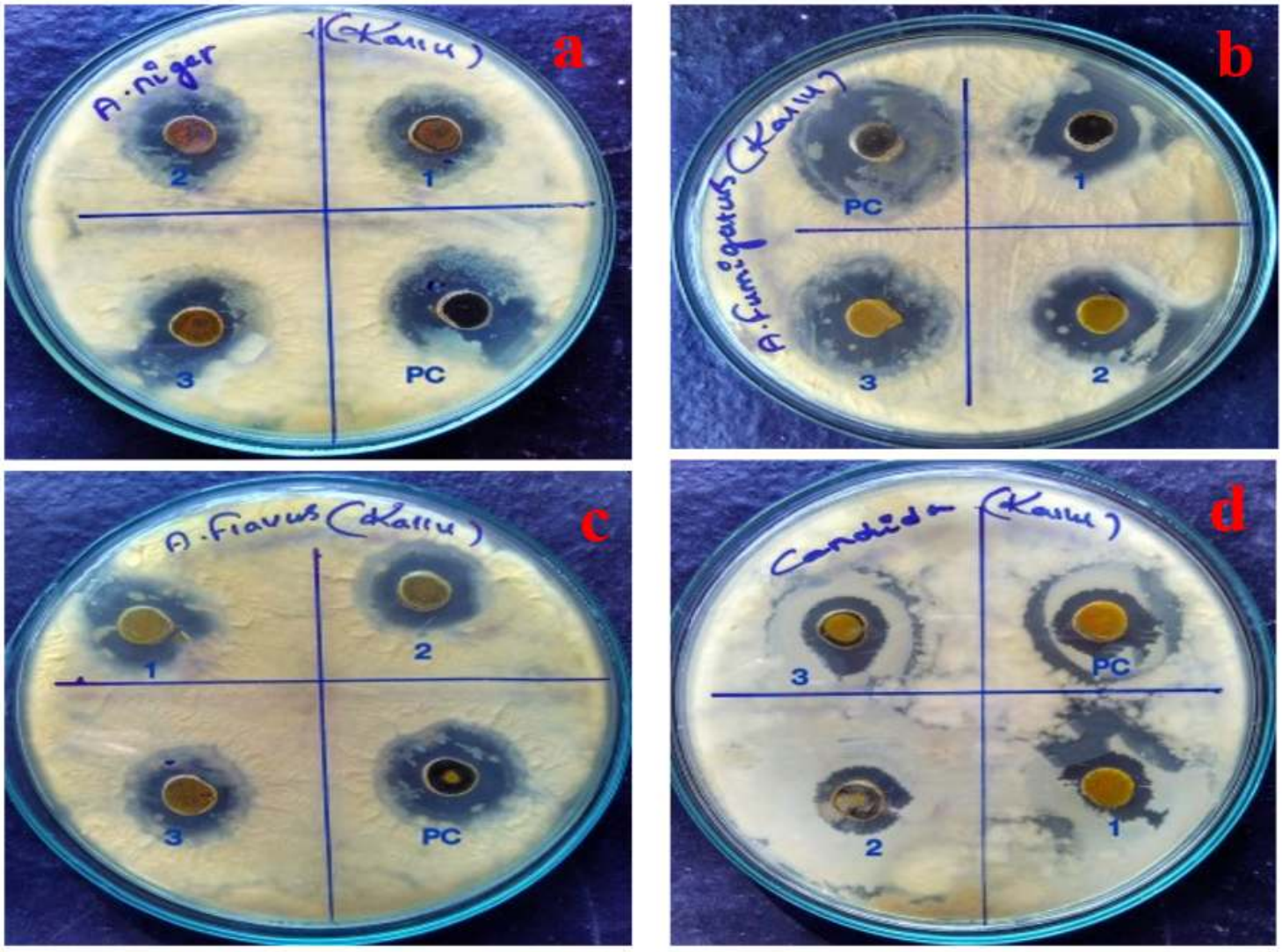


Figure 8

Fig. 9 Antifungal activity against a) *Aspergillus niger* b) *Aspergillus fumigatus*

c) *Aspergillus flavus* d) *Candida albicans*

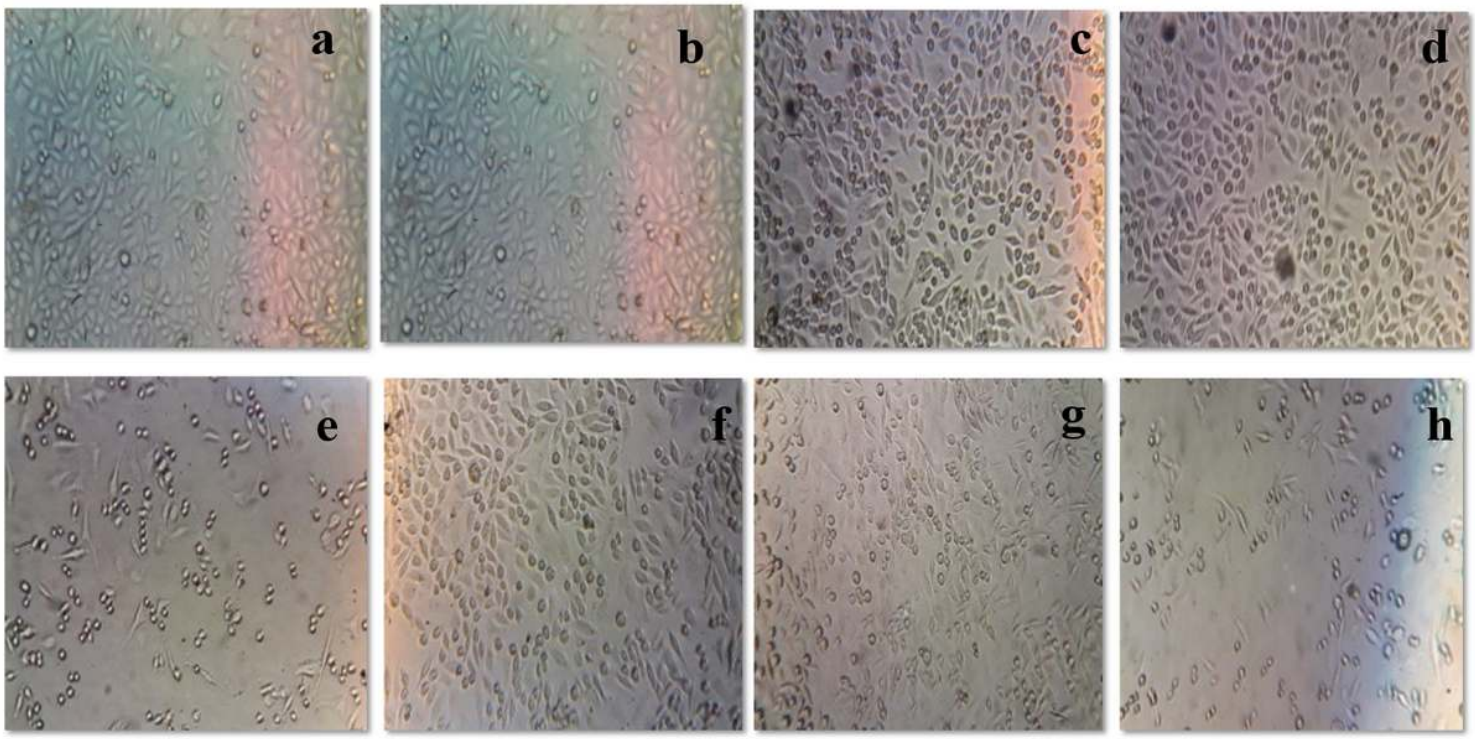


Figure 9

Fig. 10 Anticancer activity of copper nanoparticles against HeLa cell lines a) Control (Without treatment), b) Treatment with Cu-NPs (12.5µg/mL), c) Treatment with Cu-NPs (25µg/mL), d) Treatment with Cu-NPs (50µg/mL), e) Treatment with Cu-NPs (75µg/mL), f) Treatment with Cu-NPs (100µg/mL), g) Treatment with Cu-NPs (150µg/mL), h) Treatment with Cu-NPs (200µg/mL)