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GREENER APPROACH TO SYNTHESIZE CUO NPS USING LEAF EXTRACTS OF EUCALYPTUS GLOBOULUS AND THEIR ANTIMICROBIAL POTENTIAL

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Abstract

The construction of plant-based nanoparticles (NPs) has several benefits over traditional physico-chemical techniques and useful in both biology and medicinal field. This study compared the synthesis of CuO nanoparticles (CuO NPs) using chemical methods (using polymers such as PEG and PVP) and from leaf extract using medicinal plant *Eucalyptus globoulus* (*E. globulus*) by green approaches. Using a simple precipitation process, cupric nitrate served as a precursor for the synthesis of CuO NPs. X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy, Scanning electron microscopy (SEM) and Ultraviolet-visible spectrophotometer (UV-Vis) were used to examine the structural and optical characteristics of NPs.

When compared to conventional medications, the *E. globulus* mediated CuO NPs (Eu-CuO NPs) demonstrated remarkable antibacterial action against clinical infections, indicating that plant-based NPs synthesis can be an immense way to create biomedical products that are both adaptable and environmentally friendly. Antibacterial studies of NPs tested against *Escherichia coli* (*E. coli*) and antifungal activities were treated against *Aspergillus niger* (*A. niger*), *Aspergillus flavus* (*A. flavus*). Research on antibacterial and antifungal properties revealed that Eu-CuO NPs prepared through green approach photo dynamically inactivated more efficiently than those prepared from chemical approach.

Keywords: CuO nanoparticles, Eu-CuO NPs, *Eucalyptus globoulus*, green synthesis, antibacterial activity, antifungal activity.

1. Introduction

Plant mediated synthesis of NPs is an innovative method that has wide range of applications in food industry, medicine and agriculture. NPs combined via conventional methods have constrained uses in clinical domain due to their toxicity. Owing to the physio-chemical properties of green synthesised NPs, this method also offers an added gain of increased life span of NPs that overcome the limitations of conservative chemical and physical methods of NPs synthesis [1-3]. Plants acquire rich genomic adaptability with respect to number of biomolecules, metabolites and organic molecules like vitamins, proteins, flavonoids, phenols, co-enzymes based intermediates and carbohydrates. These plant metabolites contain carbonyl, amine and hydroxyl functional groups that interact with metal ions and cut down their size into nano range. More explicitly, flavonoids have numerous functional groups and it is believed that -OH group of flavonoids is responsible for the reduction of metal ions into NPs [4]. These molecules not only help in bio-reduction of the ions to the nano scale size, but they also play a major role in the capping of the NPs which is important for stability and biocompatibility [5]. In a single process, reducing agents such as phenolic compounds, sterols and alkaloids are capable of turning metal ions into NPs [6].

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The kind and composition of the metal utilized to make NPs the term "biosynthesis" mostly refers to end-user industry NPs. Some metals such as gold (Au), silver (Ag), zinc (Zn) and copper (Cu) and many others have been broadly used for the bio-synthesis of NPs applying plant extracts of various plant species extracts [7–9]. However, a significant barrier to their application in the medical industry is their greater toxicity to animals and humans. It has been discovered that CuO NPs have more potential benefits and effectiveness than other metals when it comes to the biosynthesis of NPs for medical applications. Many studies have demonstrated that CuO NPs can be synthesized using different plant extracts. The kind of plant or source species from which the plant extract used for NPs synthesis also influence the size of the particles.

Plant mediated green synthesis of NPs has recently gained popularity as an eco-friendly and low-cost replacement to chemical and physical methods. In this regard, many plants have been investigated for the synthesis of CuO NPs viz., *Ruelliatuberosa* leaf extract [10], *Drypetessepiaria* Leaf extract [11], *Banana* peel extract [12], *Nerium oleander* leaves extract [13], *Aloe Vera* leaf extract [14] and *Ixirococcinea* leaves extract [15]. Furthermore, plant based CuO NPs have been successfully used in a variety of biological and non-biological activities, including cytotoxicity [16] antibacterial activity [17] and photocatalytic activity [18].

E. globulus holds great promise for the effective production of NPs that may be used to treat clinical infections because of the wide range of these phytochemicals and their therapeutic qualities. There is not much literature documented for the synthesis of CuO NPs utilizing leaf extract of *E. globulus*. A member of the *rutaceae* family, *E. globulus* is widely utilized in the traditional medicine of the Khasi tribe in northeastern India and other places like South-East Asia. Traditional medical uses for *E. globulus* include treating cholera, managing hyperglycemia, treating helminth infections, tonic for fever, treating skin conditions, treating oral and dental problems and possessing antioxidant, anti-diabetic, antifungal and antibacterial properties [19-21]. In order to compare the antibacterial capability of CuO NPs, we present here a straight forward and environmentally friendly technique using plant extracts of *E. globulus* as reducing agents and cupric nitrate as a precursor. In this study, novel CuO NPs using *E. globulus* extract were characterized using XRD, FTIR spectroscopy, SEM and UV-Vis spectroscopy. The antibacterial and antifungal properties of Eu-CuO NPs were established evidently. The prospective application of plant-based NPs in the biomedical industry will be enhanced by this research.

2. Materials and methods

2.1. Glassware and Apparatus

All glass wares such as measuring cylinders, beakers, conical flasks, funnel, test tubes, 96 well plate, petri plates, filter paper (Whatmann 40), eppendorf tubes and microfuge tips, etc. were purchased from Borosil, India.

2.2. Chemicals

Analytical grades of Cupric nitrate trihydrate ($Cu(NO_3)_2.3H_2O$), Sodium hydroxide (NaOH), Polyethylene glycol (PEG) (MW : 500), Polyethylene pyrrolidone (PVP) (MW : 500) and ethanol were purchased from SD fine chemicals, India. All other compounds were used as received. All chemicals were purchased from SD fine chemicals and were used without any further purification.

2.3. Collection of fruits

Eucalyptus globulus (blue gum) fresh leaves were collected from Ooty forest, Nilgiri District of southern India.

2.4. Preparation of *E. globulus* leaf extract

The leaves of the *E. globulus* plant were washed with tap water followed by distilled water. The leaves were cut into small pieces and kept for shade drying and then finely powdered using a mixer.

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The powdered leaves (20gm) was added to 100 mL of distilled water in 250 mL beaker and heated at 60ºC for 30 min. The *E. globulus* leaf residue was separated from pale yellow extract formed by centrifugation at 10,000 rpm for 10 min. and the fresh supernatant obtained was used for NPs synthesis.

2.5. Synthesis of bare CuO NPs

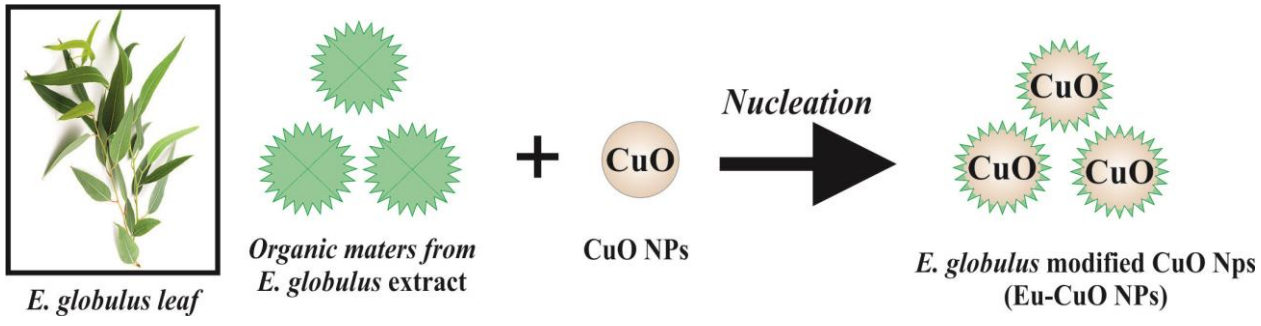
Bare CuO NPs were synthesized by simple precipitation method using copper nitrate ($Cu(NO_3)_2.3H_2O$). Precursor was dissolved in 100 ml deionized water to form 0.5 M concentration. NaOH solution (0.5 M) was slowly dropped in to the reaction mixture with vigorous stirring until to reach pH 14. Black precipitates were obtained and repeatedly washed with deionized water and absolute ethanol for several times to reach pH 7. Subsequently, the washed precipitates were dried at 80ºC for 16 hrs. and then calcinated using muffle furnace at 400ºC to obtain bare CuO NPs.

2.6. Synthesis of Polymer modified CuO NPs

Polymer modified CuO NPs were synthesized by simple precipitation method using polymer such as PEG and PVP. Each polymer was added to 0.75 mL of 0.5 M aqueous cupric nitrate solution in a glass reaction flask. NaOH solution (0.5 M) was slowly dropped under vigorous stirring. Black precipitates were obtained and repeatedly washed with deionized water and absolute ethanol for several times to reach pH 7. Subsequently, the washed precipitates were dried at 80ºC for 16 hrs. to obtain the different polymer-modified CuO NPs.

2.7. Synthesis of Eu-CuO NPs

The powdered *E. globulus* leaf extract was mixed with a 1 mM concentrated copper nitrate solution (1:9 ratio) to prepare Eu-CuO NPs. The mixtures were combined and stirred for 2 hrs. at room temperature. The brown colour of the solution indicates that Eu-CuO NPs was formed. The colloidal was incubated for 24 hrs., centrifuged for 10 mins. at 10,000 rpm to obtain a pellet and then washed three to five times with double distilled water to remove impurities. The final product was calcined in a muffle furnace at 400ºC to eliminate any remaining organic compounds, then ground into a powder and stored for further use (scheme 1). Finally, chemically and green approach synthesised CuO NPs was investigated by X-ray powder diffraction studies. Functional groups of CuO NPs was investigated by Fourier transform infrared spectroscopy. The morphology was monitored by scanning electron microscope analysis.



Scheme 1. Schematic diagram of formation of green synthesised Eu-CuO NPs using leaf extract of *E. globulus*

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2.8. Characterization Techniques

2.8.1. Bacterial Culture

A single *Escherichia coli* (*E. coli*) cell was removed from a primary bacterial plate, soaked in Luria - Bertani (LB) medium and cultured at 37ºC in incubator with continuous shaking for 16 hrs. to obtain the experimental *E. coli* culture solution. The solution was then diluted to a concentration of cfu count 1.4 × 109 cells/mL using LB medium for the antibacterial experiment.

2.8.2. Antibacterial Experiment

Bare CuO NPs, CuO/PEG NPs, CuO/PVP NPs, free extract and Eu-CuO NPs were separately suspended in deionized water at different concentrations (50, 100, 250 and 500 µg/mL). A 1mL aliquot of each CuO NPs samples suspension was added to an *E. coli* solution (1 mL, cfu count 1.4 × 109 cells) and incubated at 37ºC with continuous shaking at different time interval (0, 1, 3, 5, 6, 7 and 8 hrs.). Finally, the bacterial suspensions were added to a 96 - well culture plate (100 µL/well) and a BioTek Eon microplate spectrophotometer was used to measure their optical density (OD) under visible light irradiation (at 570 nm) with different time duration [22]. The bacterial inhibition rate of each antibacterial agent was calculated by the following methods: (1) subtracting the initial OD (i.e., OD at 0 h) from the OD at 8 hrs. to eliminate background contributions to the OD values and (2) determining the ratio of the OD in the presence of antibacterial agent to the OD in the absence of antibacterial agent (control group) using Equation (1) [23], as follows:

$$\text{Inhibition rate } (\%) = \left[1 - \frac{\text{OD}(8\text{ h, with CuO}) - \text{OD}(0\text{ h, with CuO})}{\text{OD}(8\text{ h, without CuO}) - \text{OD}(0\text{ h, without CuO})}\right] \times 100\% \quad \text{- Equ.(1)}$$

2.9. Antifungal studies

Clinical Laboratory and Standard Institute (CLSI) method was used to analyze the fungal strains (*A. flavus* MTCC 873 and *A. niger* MTCC 282). *A. flavus* and *A. niger* fungal strains were inoculated into 100 mL of PDB (Potato Dextrose Broth) that had been sterilized in an autoclave. In a room-temperature incubator, 1 mg/mL test samples were gently stirred at 120 rpm. After two weeks, the strains were collected and the fungal biomass was separated, filtered and dried. We stored the dried biomass and continued our research using carbendazim as a reference [24,25]. The below equation was used to get the fungal biomass mortality rate (Equation 2)

$$\text{Mortality percentage } (\%) = \frac{\text{Weight of the control - Weight of the test}}{\text{Weight of the control}} \text{ x } 100 \quad \text{-- Equ. (2)}$$

3. Results and discussion

3.1. XRD studies

The crystallographic structure of the products was determined by using X-ray powder diffraction in Fig. 1 a, and it can be indexed to synthesized (both chemical & green method) CuO NPs in a monoclinic structure. According to the standard data (JCPDS, No. 80-1916), the diffraction peaks in both spectra correspond to the lattice constants of CuO NPs, which are a = 3.249 and c = 5.206 Å. The sharpness of the peaks in XRD spectrum reveals their crystalline structure and no any other impurity were detected [26].

From Fig. 1 b & c, the XRD spectra of PEG and PVP, it can be observed that the characteristic peaks matched the diffraction peaks of bare CuO NPs, showing that complete CuO nanocrystal plane structures were retained in the surface modified CuO NPs [27]. It indicates that CuO NPs were capped with polymer molecules.

In Fig. 1.d, XRD spectra of green synthesised Eu-CuO NPs showed the diffraction peaks at 32.63, 35.67, 38.78, 48.88, 53.50, 58.34 and 65.02º [28,29], that was related to the (110), (-111), (111), (-

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202), (020), (202) and (-113) planes respectively [30]. The existence of diffraction peaks (between 2θ = 35-39º) indicated the formation of Eu-CuO NPs [31]. This confirms the growth of monoclinic crystalline morphology and crystalline nature [32].

From the XRD peak, the average crystallite size (D) was estimated using the scherrer formula. The estimated crystallite sizes were around 95, 80, 70 and 55 nm for bare CuO, CuO/PEG, CuO/PVP and Eu-CuO NPs respectively. The crystallite size of polymer and *E. globulus* leaf extract modified CuO NPs were smaller than bare CuO NPs, which can be attributed to the Cu^{2+} ions forming a complex with the polymer and organic compounds of *E. globulus* leaf extract [33]. All the diffraction peaks in Fig. 1, it indicates that CuO NPs were capped with polymer and organic molecules of *E. globulus* leaf extract, which is reliable with SEM and FTIR results.

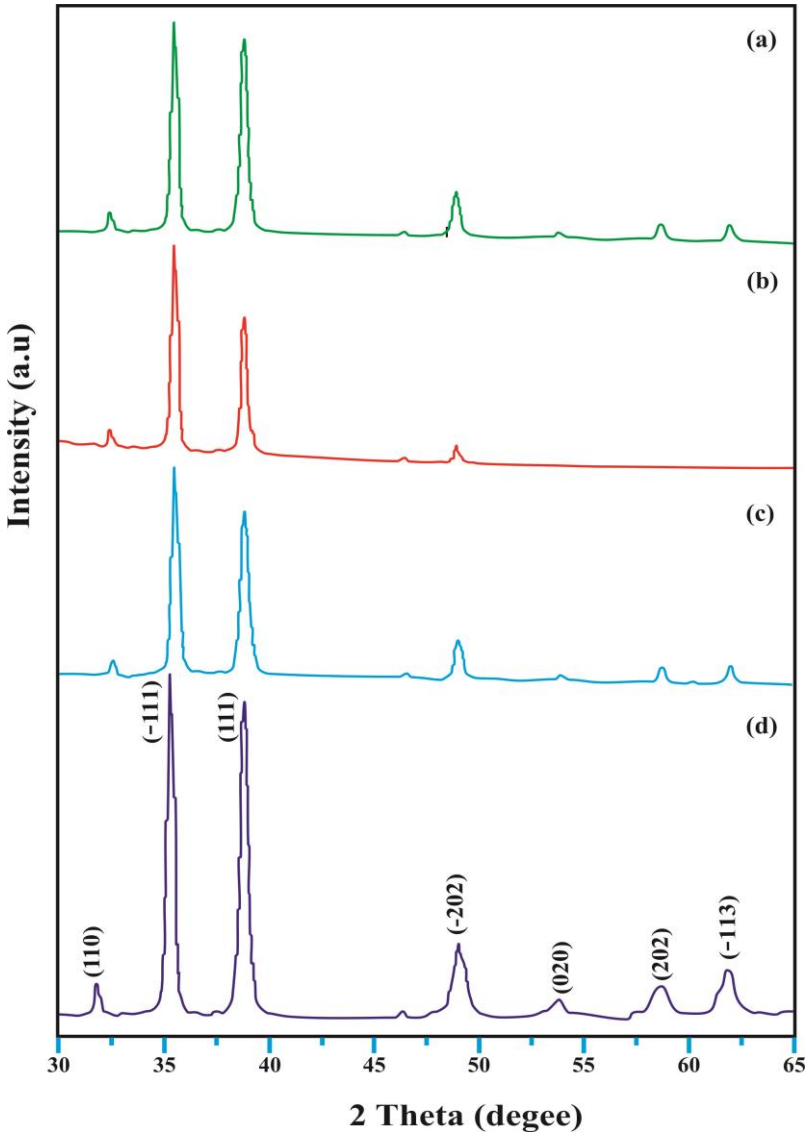

Fig. 1. X-ray diffraction spectra of (a) Bare CuO, (b) CuO/PEG, (c) CuO/PVP and (d) green synthesised Eu-CuO NPs

3.2. FTIR studies

FTIR spectroscopy was used to identify the functional groups on the surfaces of the various CuO NPs. As shown in Fig. 2, three clear peaks can be observed in the spectrum of the bare CuO NPs. These peaks correspond to stretching vibrations of Cu-O in the (-202) direction at 610 and 495 cm^{-1}

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and stretching vibrations of Cu-O in the (202) direction at 416 cm^{-1} (Fig. 2 a) [34,35]. As observed in the spectrum of the PEG modified CuO NPs (Fig. 2 b), characteristic signals for PEG were observed at 1061, 1411 and 2940 cm^{-1}, which correspond to C-O vibrations, C-H bending and CH_2 stretching, respectively [36,37]. Fig. 2c, representing the spectrum of PVP modified CuO NPs, clearly displays the characteristic signals of PVP, corresponding to C-N stretching (1019 cm^{-1}), C-O vibrations (1288 cm^{-1}), CH_2 bending (1373 cm^{-1}), C=O vibrations (1658 cm^{-1}) and CH_2 stretching (2940 cm^{-1}). In Fig. 2 d, peaks observed at 530 and 580 cm^{-1} can be attributed to Cu-O vibrations and peaks at 1630 and 3350cm^{-1} are assigned for O-H signal, confirming the green synthesis of Eu-CuO NPs [38-40]. Based on these results, FTIR analysis was performed on biomolecules extracted from *E. globulus* leaf that could have been utilized for the reduction of Eu-CuO NPs.

In Fig. 2 a-d, the O-H signal appeared at ≈3400 cm^{-1} in the spectra of most of the NPs may have been caused by the adsorption of atmospheric water vapour onto the CuO surfaces [41]. In consideration of the above results, it was confirmed that the synthesized NPs were CuO and that the intended surface modifications had been successfully achieved.

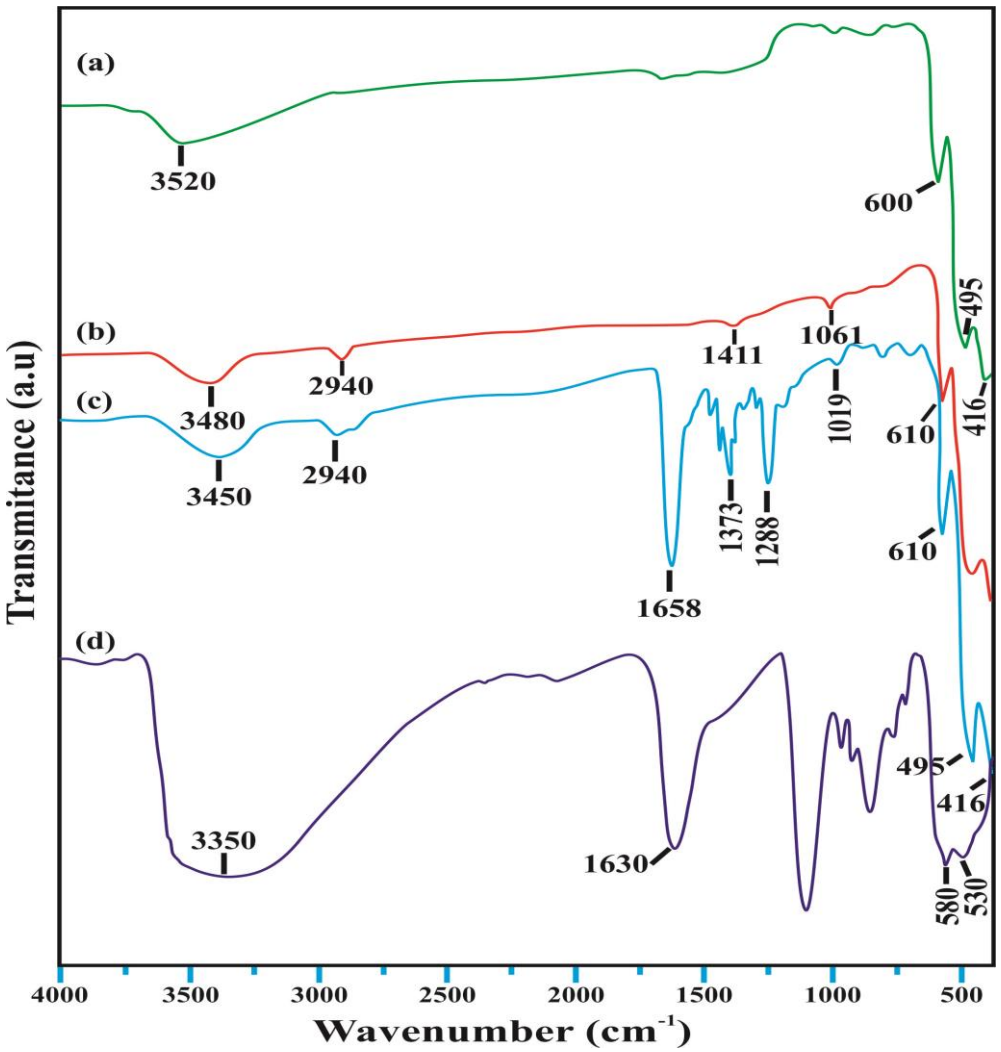

Fig. 2. FTIR spectra of (a) Bare CuO, (b) CuO/PEG, (c) CuO/PVP and (d) green synthesised Eu-CuO NPs

3.3. SEM studies

The morphological properties of the synthesized NPs (bare CuO, CuO/PEG, CuO/PVP and Eu-CuO NPs) by simple precipitation method were evaluated using SEM (Fig. 3). The images were recorded at magnification of 100nm. Direct information about the size and typical morphologies of the as-

synthesized products were observed by SEM. The SEM image of bare CuO, CuO/PEG, CuO/PVP and Eu-CuO NPs were formed in spherical shape [40] with diameter range around 90-100, 75-85, 60-70 & 40-60 nm respectively. The result indicates that the presence of the polymer and organic molecules modifying agents affected the shape and size of the NPs. The images show that bare CuO NPs were aggregated and compressed while PEG, PVP and *E. globulus* leaf extract modified CuO NPs have a better distribution and homogeneity.

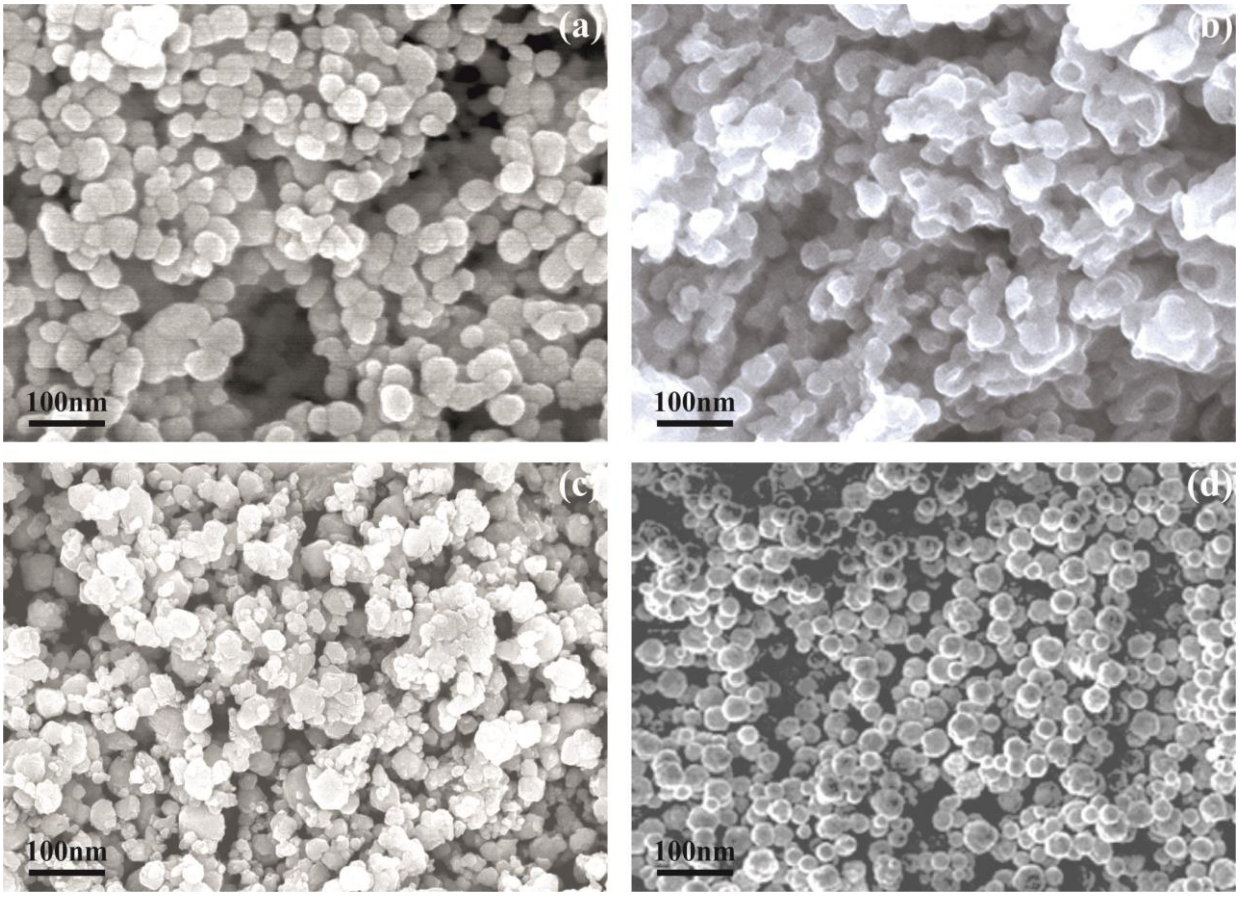

Fig. 3. Scanning electron microscopy of (a) Bare CuO, (b) CuO/PEG, (c) CuO/PVP and (d) green synthesised Eu-CuO NPs

3.4. UV-Vis spectral studies

UV-Vis absorption spectra of the bare CuO NPs, *E. globulus* leaf extract and its mediated Eu-CuO NPs were presented in Fig. 4. The light absorbance properties of CuO NPs synthesized by different method were investigated using UV–vis spectroscopy was measured in the wavelength range of 300 - 800 nm. The formation of CuO NPs between 200 and 350 nm agrees with previous studies [44]. As shown in Fig. 4, the absorbance peak of CuO NPs was found to be in the UV region at 340 nm [45-47]. Absorbance drops about zero at 610 nm, as shown in Fig. 4. This may be because UV-vis absorbance spectroscopy has a hard time detecting and classifying light-absorbing phytochemicals [48-50] or because metal oxide particles reflect light. However, despite these limitations, UV-vis absorbance spectroscopy is still a useful tool for the initial confirmation of metal oxide NPs existence. The presence of metal oxide NPs is confirmed by the peak at 340 nm and the peak at 610 nm may indicate the presence of a spherical particle.

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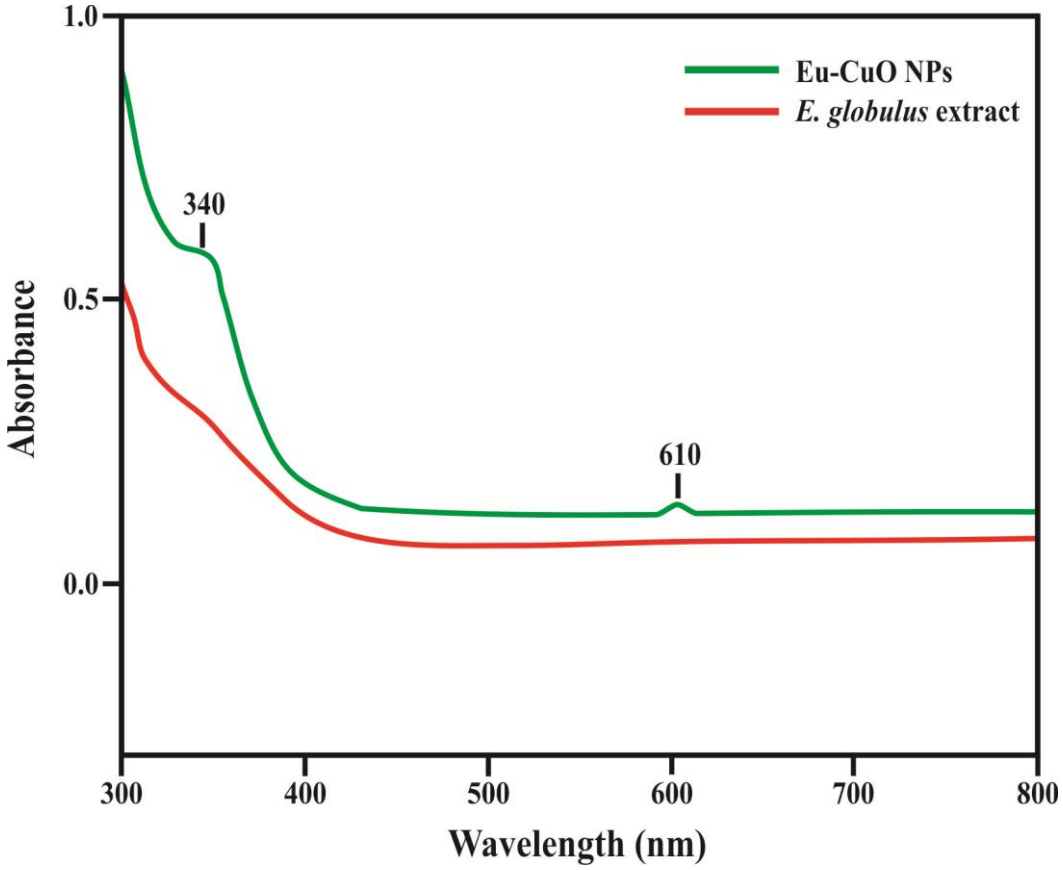

Fig. 4. UV-visible spectra of *E. globulus* leaf extract and Eu-CuO NPs.

3.5. Antibacterial activity of the CuO NPs

The bactericidal effect of the chemically synthesised bare CuO, CuO/PEG, CuO/PVP NPs, *E. globulus* leaf extract and green synthesised Eu-CuO NPs on Gram-negative (*E. coli*) were shown in Fig. 5. The antibacterial activity of NPs was tested against *E. coli* and were recorded at different concentrations (50, 100, 250 and 500 µg/mL) and the optical density (OD) values at different time intervals were recorded under visible light irradiation (570 nm).

Compared to the control group (Fig. 5 a), the bacterial inhibition effects of the bare CuO NPs increased with increasing antibacterial agent concentration. This preliminarily demonstrated that the inhibition of bacterial growth could be achieved through the addition of bare CuO NPs, with the rate of inhibition being positively correlated with concentration. The results indicated that the bacterial inhibition rates of the bare CuO NPs at concentrations of 50, 100, 250 and 500 µg/mL were 16, 20, 23 and 39% respectively.

From the Fig. 5 b & c, when the both polymer (PEG and PVP) modified CuO NPs were used as antibacterial agents, the experimental results indicated significantly superior antibacterial effects compared to bare CuO NPs, showing an increase with increased concentrations of CuO/PEG and CuO/PVP NPs. In these results, the negatively charged CuO/PVP NPs demonstrated a smaller particle size than CuO/PEG NPs and the electrostatic repulsion that occurred with the negatively charged surfaces of *E. coli* decreased its antibacterial effects [51-53].

From the Fig. 5 d & e, the relationships between antibacterial activity and both surface charge and particle size in two different types of *E. globulus* leaf extract and its modified CuO NPs were investigated. When the both *E. globulus* extract and Eu-CuO NPs were used as antibacterial agents, the experimental results indicated significantly superior antibacterial effects compared to other NPs (such as Bare CuO, CuO/PEG and CuO/PVP NPs), showing an increase with increased concentrations (500 µg/mL) of *E. globulus* extract and Eu-CuO NPs.

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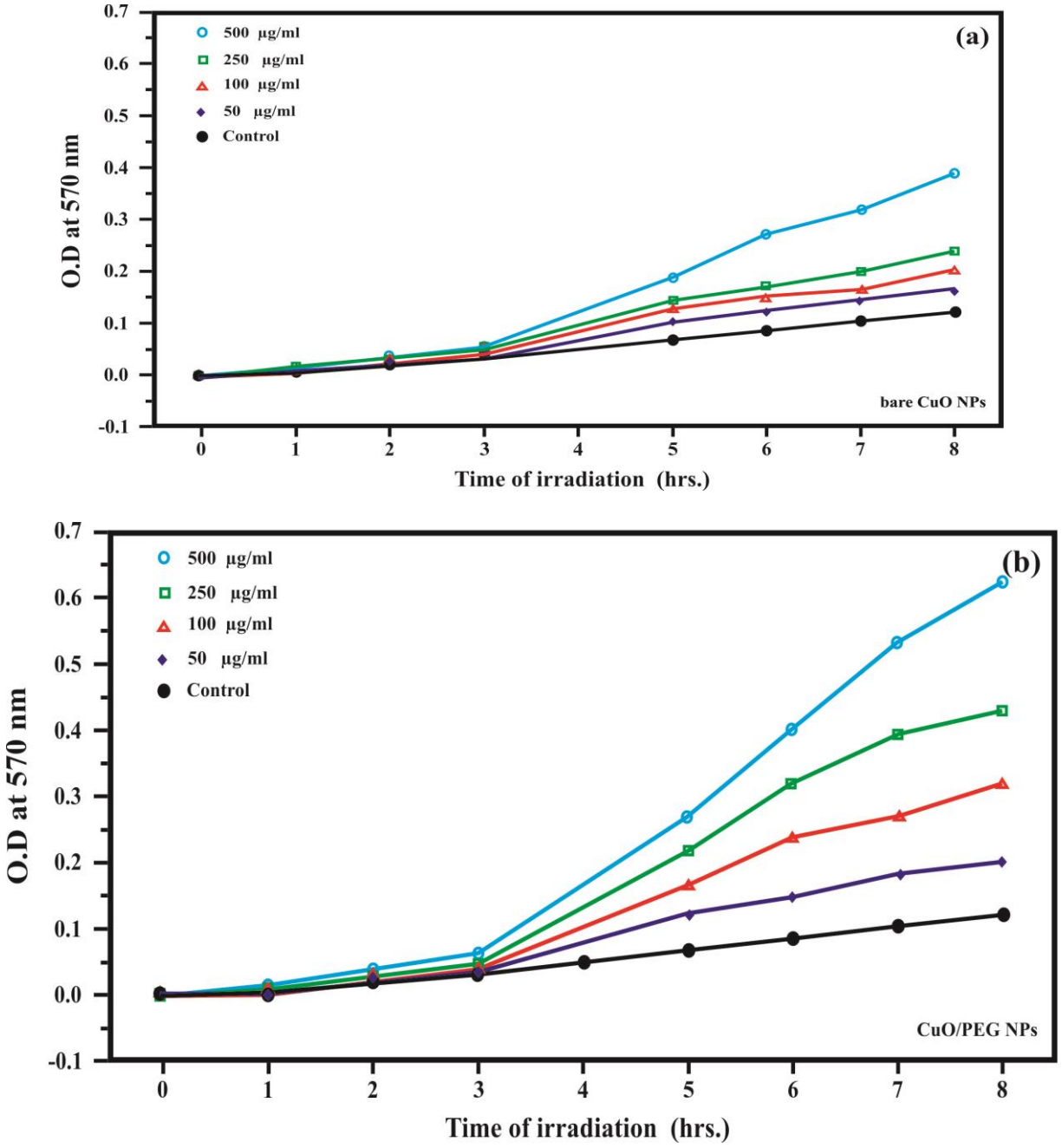
The bacterial inhibition rates calculated based on the ratio of the OD value of each experimental group to that of the control group (Equ. 1), were shown in Fig. 5 f. In Fig. 5 f, the bacterial inhibition rates of bare CuO, CuO/PEG and CuO/PVP NPs, *E. globulus* extract, Eu-CuO NPs at the highest dose level (500 µg/mL at after 8 hrs. irradiation) were 39, 63 and 68%, 50 and 72% respectively.

In these results, the bacterial growth curve of Eu-CuO NPs (Fig. 5 e) at high concentration shows a significantly extended latent period in *E. coli* growth, resulting from the inability of the bacteria to rapidly enter the exponential growth phase, thereby hindering bacterial growth and resulting in good bacterial inhibition effects.



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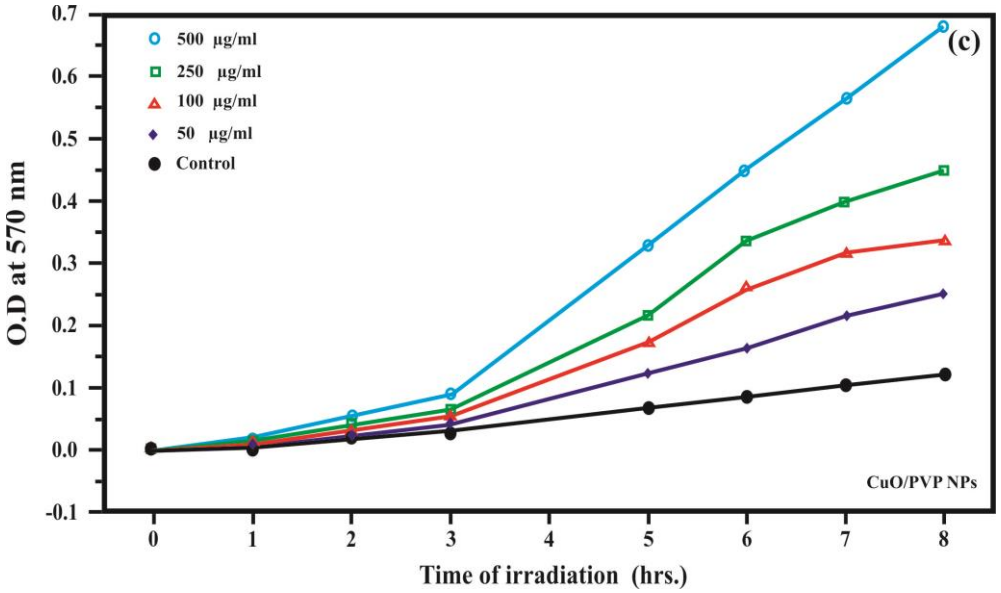

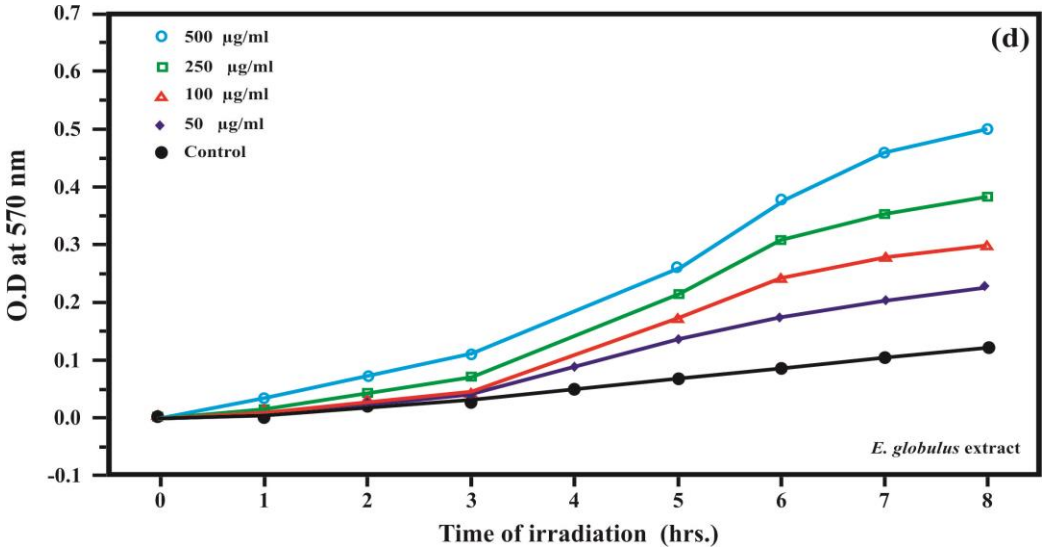

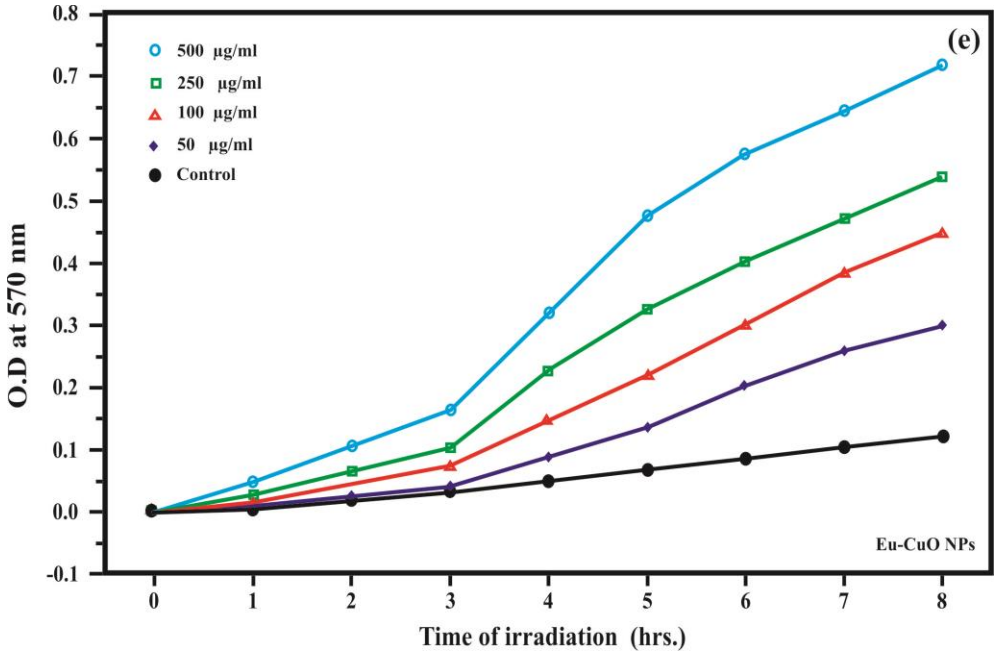

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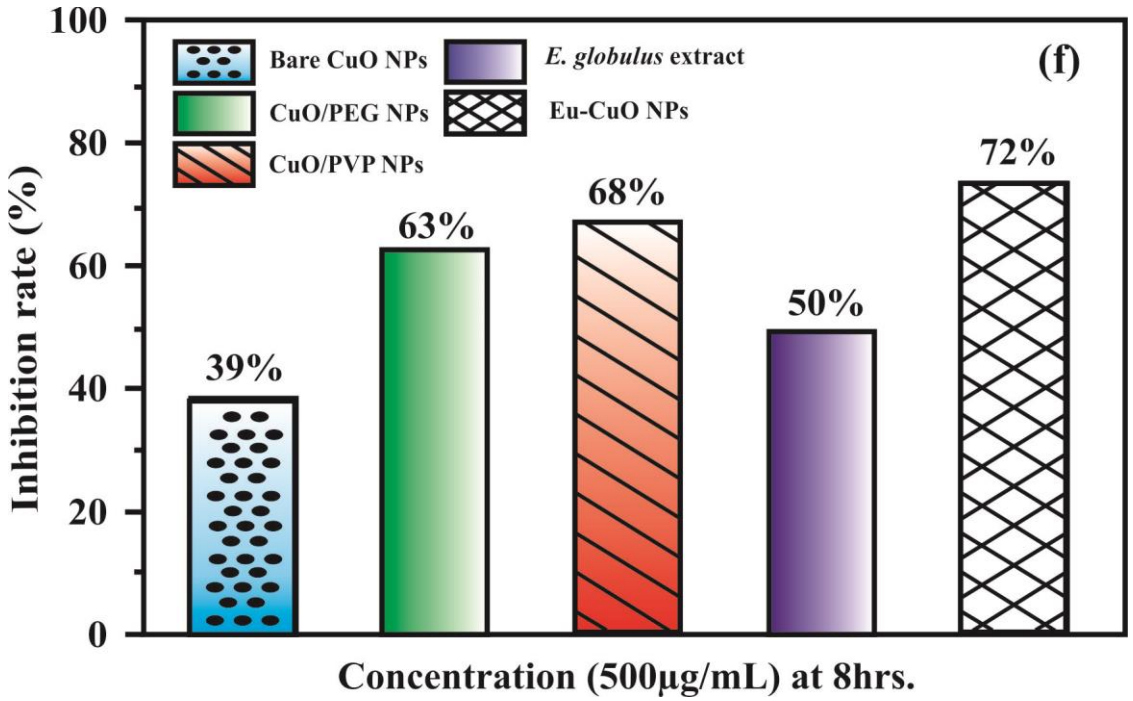

Fig. 5. Bacterial growth inhibition curves for (a) bare CuO NPs, (b) CuO/PEG NPs, (c) CuO/PVP NPs, (e) *E. globulus* leaf extract, (e) Eu-CuO NPs and (f) Bacterial inhibition rate of NPs at 500 µg/mL concentrations at 8 hrs. irradiation.

3.6. Antifungal studies

The antifungal activity of the investigated photosensitizers, particularly the green synthesized Eu-CuO NPs, was evaluated against two different fungal pathogens: *A. flavus* and *A. niger*. Eu-CuO NPs significantly inhibited the growth of *A. flavus* at concentrations of 250, 500 and 1000 ppm, with rates of inhibition 60%, 72% and 80% respectively. In contrast, *A. niger* had some resistance, as shown graphically in Fig. 6 (inhibition rates of 38%, 48% and 55% respectively at different concentration) [25]. The synthesised CuO NPs to have appreciable antifungal action against either *A. flavus* or *A. niger*, greater concentrations were required. The results show that Eu-CuO NPs are superior to carbendazim in terms of their ability to enhance the photodynamic inactivation of microorganisms (Table 1). The irreversible inhibition of synthetase function leads to the demise of the microorganism since its cells can no longer replicate (scheme 2).

Table 1. Comparative studies of antifungal studies of green synthesised Eu-CuO NPs with carbendazim.

Antifungal agent	***A. flavus***			***A. niger***		
	250 ppm	**500 ppm**	**1000 ppm**	**250 ppm**	**500 ppm**	**1000 ppm**
Carbendazim	35	45	53	32	40	43
Eu-CuO NPs	60	72	80	38	48	55

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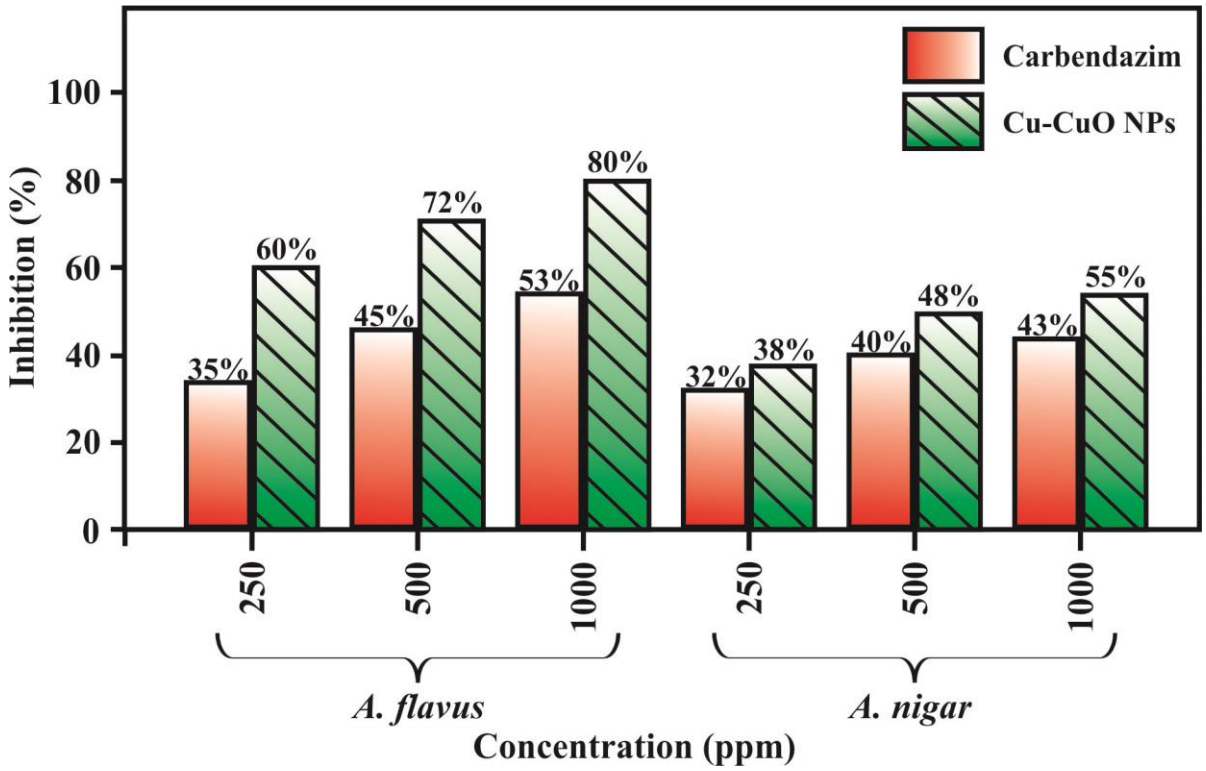
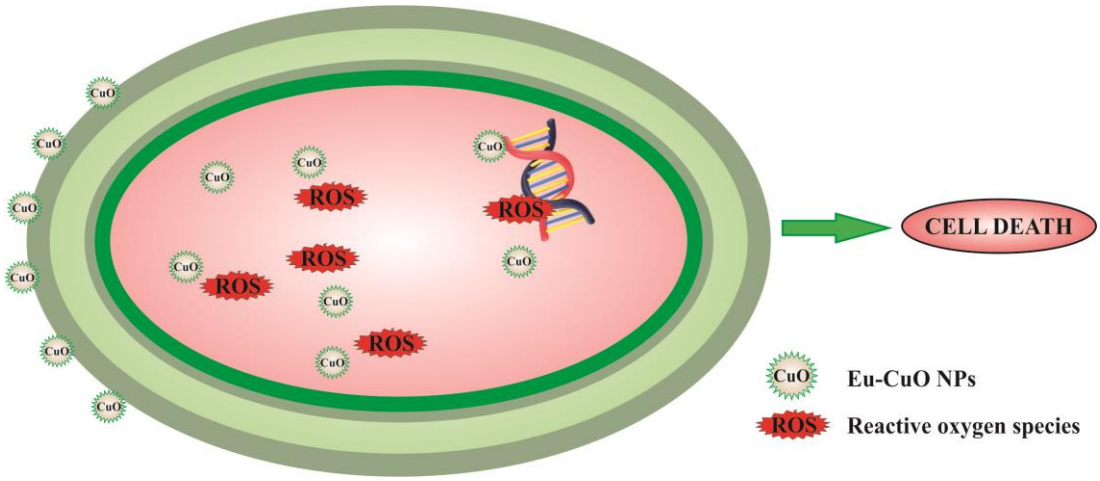


Fig. 6. Anti-fungal activity of green synthesized Eu-CuO NPs against *A. flavus* and *A. niger*.



Scheme 2. Mode of action of green synthesised Eu-CuO NPs on microbes.

5. Conclusion

The present work explored an environmentally-friendly method for synthesizing Eu-CuO NPs utilizing aqueous extracts of *E. globulus* leaves. The bare CuO NPs, polymer modified CuO NPs (using PEG and PVP) and Eu-CuO NPs were successfully synthesised by simple precipitation method. The formation of these NPs was further validated through various characterization techniques, including XRD, FTIR, SEM and UV-Vis spectroscopy. The extract of *E. globulus* leaves served as a reducing agent for reducing particle size. Compare to others, Eu-CuO NPs have been highly capable of bacterial inhibition of Gram-positive microorganisms under visible light at high concentration. Bacterial growth inhibition rates of up to 72% were achieved on *E. coli*. Eu-CuO NPs played a critical role in boosting photodynamic inactivation of the *E. coli* upon exposure

to visible light at high concentrations. The bioengineered Eu-CuO NPs could potentially open new avenues in various medical applications.

In terms of antifungal activity, the NPs displayed a superior inhibition rate against *A. flavus* compared to *A. niger*. Therefore, the study indicates that Eu-CuO NPs generated using green methods have potential as regulating agents against pathogenic bacteria and fungus, with implications for the pharmaceutical and biomedical industries.

6. Reference

1. K.S. Kavitha, K.S. Syed Baker, D. Rakshith, H.U. Kavitha, H.C. Yashwantha Rao, B.P. Harini, S. Sathish, Plants as green source towards synthesis of nanoparticles. *Int. Res. J. Biol. Sci.* 2, **2013**, 66-76.
2. P. Malik, R. Shankar, V. Malik, N. Sharma, T.K. Mukherjee, Green chemistry based benign routes for nanoparticle synthesis, *J. Nanopart*. 2014, **2014**, 1-15.
3. V.N. Kalpana, V.D. Rajeswari, A review on green synthesis, biomedical applicati-ons and toxicity studies of ZnO NPs, *Bioinorg. Chem. Appl.* 2018, **2018**, 1-12.
4. V.V. Makarov, A.J. Love, O.V. Sinitsyna, S.S. Makarova, I.V. Yaminsky, N.O. Kalinina, "Green" nanotechnologies: synthesis of metal nanoparticles using plants, *Acta Naturae*, 6, **2014**, 35-44.
5. V. Arya, Living systems: Eco-friendly nanofactories, *Dig. J. Nanomater Bios*., 5, **2010**, 9–21.
6. B. Biswas, K. Rogers, F. Mclaughlin, D. Daniels, A. Yadav, Antimicrobial activities of leaf extracts of Guava (Psidium guajava L.) on two Gram-negative and Gram-positive bacteria, *Int. J. Microbiol*., **2013**, 1-7.
7. A.K. Mittal, Y. Chisti, U.C. Banerjee, Synthesis of metallic nanoparticles using plant extratcs. *Biotechnol. Adv*., 31(2), **2013**, 346-356.
8. R. Rajan, K. Chandran, S.L. Harper, S.I. Yun, P.T. Kalaichelvan, Plant extract synthesized silver nanoparticles: An ongoing source of novel biocompatible materials, *Ind. Crop Prod.*, 70, **2015**, 356–373.
9. S. Ahmed, M. Ahmad, B.L. Swami, S. Ikram, A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise, *J. Adv. Res.*, 7, **2016**, 17–28.
10. J. Singh, V. Kumar, K.H. Kim, M. Rawat, Biogenic synthesis of copper oxide nanoparticles using plant extract and its prodigious potential for photocatalytic degradation of dyes, *Env. Res.*, 177, **2019**, 1-44.
11. P. Narasaiah, B.K. Mandal, N.C. Sarada, Biosynthesis of Copper Oxide nanoparticles from Drypetes sepiaria Leaf extract and their catalytic activity to dye degradation, *IOP Conference Series: Mater. Sci. Eng.*, 263, **2017**, 022012-21.
12. M. Aminuzzaman, L.M. Kei, W.H. Liang, Green synthesis of copper oxide (CuO) nanoparticles using banana peel extract and their photocatalytic activities, *AIP Conf. Proc.*, 1828, **2017**, 020016-21.
13. N. Sebeia, M. Jabli, A. Ghith, Biological synthesis of copper nanoparticles, using Nerium oleander leaves extract: Characterization and study of their interaction with organic dyes, *Inorg. Chem. Comm.* **2019**, 1-32.
14. P.P.N.V. Kumar, U. Shameem, P. Kollu, R.L. Kalyani, S.V.N. Pammi, Green synthesis of copper oxide nanoparticles using *aloe vera* leaf extract and its antibacterial activity against fish bacterial pathogens, *BioNanoScience*, 5 (3), **2015**, 135–139.
15. K. Vishveshvar, M.V. Aravind Krishnan, K. Haribabu, S. Vishnuprasad, Green synthesis of copper oxide nanoparticles using *ixiro coccinea* plant leaves and its characterization, *BioNanoScience*, 8 (2), **2018**, 554–558.

16. S. Saif, A. Tahir, T. Asim, Y. Chen, Plant Mediated Green Synthesis of CuO Nanoparticles: Comparison of Toxicity of Engineered and Plant Mediated CuO Nanoparticles towards Daphnia magna, *Nanomaterials*, 6 (11), **2016**, 205.
17. V.V. Thekkae Padil, Miroslav Cernik, Green synthesis of copper oxide nanoparticles using gum karaya as a biotemplate and their antibacterial application, Int. J. Nanomedicine, 8, **2013**, 889-898.
18. P. Narasaiah, B.K. Mandal, N.C. Sarada, Biosynthesis of copper oxide nanoparticles from *drypetes sepiaria* leaf extract and their catalytic activity to dye degradation, *IOP Conference Series: Mater. Sci. Eng.*, 263(2), **2017**, 022012-21.
19. A.K. Tyagi, A. Malik, Antimicrobial potential and chemical composition of Eucalyptus globulus oil in liquid and vapour phase against food spoilage microorganisms, *Food Chem*., 126 (1), **2011**, 228–235.
20. A. Jerbi, A. Derbali, A. Elfeki, M. Kammoun, Essential oil composition and biological activities of *Eucalyptus globulus* leaves extracts from tunisia, *J. Ess. Oil Bear. Plants*, 20 (2), **2017**, 438–448.
21. W.A. Saka, R.E. Akhigbe, A.F. Ajayi, L.O. Ajayi, O.E. Nwabuzor, Anti-diabetic and antioxidant potentials of aqueous extract of Eucalyptus globulus in experimentally - induced diabetic rats, *African J. Trad., Comp. Alt. Med.*, 14 (6), **2017**, 20–26.
22. H. Bai, Z. Liu, D. Delai Sun, Hierarchical ZnO/Cu ‘‘corn-like’’ materials with high photodegradation and antibacterial capability under visible light, *Phys. Chem. Chem. Phys.*, 13, **2011**, 6205-6210.
23. W. Wolkers, H. Oldenhof, In situ FTIR assessment of dried *Lactobacillus bulgaricus*: KBr disk formation affects physical properties, *Spectroscopy*, 19, **2005,** 89-99.
24. T.V. Surendra, S.M. Roopan, N.A. Al-dhabi, M.V. Arasu, G. Sarkar, K. Suthindhiran, Vegetable peel waste for the production of ZnO nanoparticles and its toxicological efficiency, antifungal, hemolytic and antibacterial Activities. *Nanoscale Res. Lett.*, 11, **2016**, 546–556.
25. P. Basnet, T. Inakhunbi Chanu, D. Samanta, S. Chatterjee, A review on bio-synthesized zinc oxide nanoparticles using plant extracts as reductants and stabilizing agents, *J. Photochem. Photobiol. B*, 183, **2018**, 201–221.
26. S. Yallappa, J. Manjanna, M.A. Sindhe, N.D. Satyanarayan, S.N. Pramod, K. Nagaraja, Microwave assisted rapid synthesis and biological evaluation of stable copper nanoparticles using T. arjuna bark extract, *Spectr. Acta Part A: Mol. Biomol. Spect.*, 110, **2013**, 108–115.
27. U.S. Shenoy, A.N. Shetty, A simple single-step approach towards synthesis of nanofluids containing cuboctahedral cuprous oxide particles using glucose reduction, *Front. Mater. Sci.*, 12, **2018**, 74-82.
28. D.E. Diaz-Droguett, R. Espinoza, V.M. Fuenzalida, Copper nanoparticles grown under hydrogen: Study of the surface oxide, *Appl. Surf. Sci.*, 257, **2011**, 4597-4602.
29. J.W. Ko, S.W. Kim, J. Hong, J. Ryu, K. Kang, C.B. Park, Synthesis of graphene-wrapped CuO hybrid materials by CO_2 mineralization, *Green Chem.*, 14, **2012**, 2391-2394.
30. K. Premkumar, N.D. Dinesh, S. Murari, Synthesis of CuO and Ag doped CuO nanoparticles form *Muntingia calabura* leaf extract and evaluation of their antimicrobial potential, *Int. J. Nano and Biomater*., 8, **2019**, 228-252.
31. S. Naz, A. Mukhtiar, M. Zia, On the toxicity copper oxide nanoparticles: a review study, *IET Nanobiotech.*, 14, **2019**, 1-13.
32. W.M. Rangel, R.A.A. Boca Santa, H.G. Riella, A facile method for synthesis of nanostructured copper (II) oxide by coprecipitation. *J. Mater. Res. Tech*., 9, **2019**, 994–1004.

33. M. Ghareib, W. Abdullah, M. Abu tahon, A. Tallima, Biosynthesis of copper oxide nanoparticles using the preformed biomass of *aspergillus fumigatus* and their antibacterial and photocatalytic activities, *Dig. J. Nanomater. Biostruct*., 14(2), **2019**, 291-303.
34. S. Konar, S. Sen, A. Pathak, Morphological effects of CuO nanostructures on fibrillation of human serum albumin, *J. Phys. Chem. B*, 121, **2017**, 11437 - 11448.
35. T. Morioka, M. Takesue, H. Hayashi, M. Watanabe, R.L. Smith, Antioxidation properties and surface interactions of polyvinylpyrrolidone-capped zerovalent copper nanoparticles synthesized in supercritical water, *ACS Appl. Mater. Interfaces*, 8, **2016**, 1627-1634.
36. R. Javed, M. Ahmed, I.U. Haq, S. Nisa S, Zia M, PVP and PEG doped CuO nanoparticles are more biologically active: Antibacterial, antioxidant, antidiabetic and cytotoxic perspective, *Mater. Sci. Eng. C*, 79, **2017**, 108-115.
37. B.W. Chieng, N. Ibrahim, W. Yunus, M. Hussein, Poly(lactic acid)/Poly(ethylene glycol) polymer nanocomposites: Effects of graphene nanoplatelets, *Polymers*, 6, **2013**, 93-104.
38. S. Saif, A. Tahir, T. Asim, Y. Chen, Plant mediated green synthesis of CuO nanoparticles: comparison of toxicity of engineered and plant mediated CuO nanoparticles towards daphnia magna, *Nanomaterials*, 6 (11), **2016**, 205-220.
39. M. Aminuzzaman, L.M. Kei, W.H. Liang, Green synthesis of copper oxide (CuO) nanoparticles using banana peel extract and their photocatalytic activities, *AIP Conf. Proc*., 1828, **2017**, 020016-21.
40. E. Darezereshki, F. Bakhtiari, A novel technique to synthesis of tenorite (CuO) nanoparticles from low concentration $CuSO_4$ solution, *J. Min. Metall. Sect. B-Metall.* 47 (1), **2011,** 73-78.
41. Mallakpour S, Jarahiyan A, Enhancement of Poly(Vinyl Alcohol)-Poly(Vinyl Pyrrolidone) blend properties using modified Copper (II) oxide and ultrasonic irradiation, *Polym. Plast. Technol. Eng.*, 56, **2017**, 1059-1087.
42. F. Ijaz, S. Shahid, S.A. Khan, W. Ahmad, S. Zaman, Green synthesis of copper oxide nanoparticles using Abutilon indicum leaf extract: Antimicrobial, antioxidant and photocatalytic dye degradation activitie. Tropical J. Pharm. Res. 16 (4), **2017**, 743-753.
43. S. Tavakoli, M. Kharaziha, S. Ahmadi, Green synthesis and morphology dependent antibacterial activity of copper oxide nanoparticles. J. Nanostr. 9, **2019**, 163–171.
44. K.S. Khashan, M.S. Jabir, F.A. Abdulameer, Preparation and characterization of copper oxide nanoparticles decorated carbon nanoparticles using laser ablation in liquid, *IOP Conf. Series: Journal of Physics: Conf. Series*, 1003, **2018,** 012100-107.
45. J. Jeevanandam, Y.S. Chan, M.K. Danquah, Biosynthesis and characterization of MgO nanoparticles from plant extracts via induced molecular nucleation, *New J Chem.*, 41, **2017**, 2800–2814.
46. R.M. Alwan, Q.A. Kadhim, K.M. Sahan, R.A. Ali, R.J. Mahdi, N.A. Kassim, A.N. Jassim, Synthesis of Zinc oxide nanoparticles via sol-gel route and their characterization, *Nanosci Nanotechnol*., 5(1), **2015**, 1–6.
47. A.A. Umar, M.Y.A. Rahman, R. Taslim, M.M. Salleh, M. Oyama, A simple route to vertical array of quasi-1D ZnO nanofilms on FTO surfaces: 1D-crystal growth of nanoseeds under ammonia-assisted hydrolysis process, *Nanoscale Res Lett.*, 6(1), **2011**, 1-12.
48. J. Klaas, G. Schulz-Ekloff, N.I. Jaeger, UV-vis diffuse reflectance spectroscopy of zeolite-hosted mononuclear titanium oxide species. *J Phys Chem B*, 101(8), **1997**, 1305–1311.
49. A.E. Morales, E.S. Mora, U. Pal, Use of diffuse reflectance spectroscopy for optical characterization of un-supported nanostructures. *Rev Mex Fis S*, 53(5), **2007**, 18-22.
50. A.S. Dacome, C.C. Da Silva, C.E. Da Costa, j.D. Fontana, J. Adelmann, S.C. Da Costa, Sweet diterpenic glycosides balance of anew cultivar of Stevia rebaudiana (Bert.) Bertoni: isolation and quantitative distribution by chromatographic, spectroscopic, and electrophoretic methods. Process Biochem (Oxford, UK) 40(11), **2005**, 3587–3594.

51. V.V. Kumar, S.P. Anthony, Antimicrobial studies of metal and metal oxide nanoparticles, *Elsevier: Amsterdam, The Netherlands*, 12, **2016**, 265-300.
52. M.D. Balakumaran, R. Ramachandran, P. Balashanmugam, D.J. Mukeshkumar, P.T. Kalaichelvan, Mycosynthesis of silver and gold nanoparticles: Optimization, characterization and antimicrobial activity against human pathogens, *Microbiol. Res*., 182, **2016**, 8-20.
53. Y.G. Yuan, Q.L. Peng, S. Gurunathan, Effects of silver nanoparticles on multiple drug-resistant strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa* from mastitis-infected goats: An alternative approach for antimicrobial therapy, *Int. J. Mol. Sci*,. 18, **2017**, 569-547.