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The Utilization of Curcumin in the Environmentally Friendly Green Synthesis of Zinc Oxide-Curcumin Nanoparticles and Their Antibacterial Activity

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Abstract

In this study, Curcumin (Cur) was utilized as a green reducing and capping agent for the synthesis of zinc oxide-curcumin (ZnO/Cur) nanoparticles (NPs) via an eco-friendly approach. The physicochemical properties of the synthesized nanocomposites were systematically characterized using UV-visible spectrophotometry, X-ray diffraction (XRD), and field-emission scanning electron microscopy (FESEM). UV-Vis analysis confirmed the successful formation of ZnO/Cur NPs, exhibiting a characteristic absorption peak at 377 nm, which falls within the typical range for ZnO nanoparticles (330–460 nm). XRD analysis revealed the hexagonal wurtzite crystalline structure of ZnO with a crystallite size of approximately 23.6 nm. FESEM images demonstrated that the nanoparticles possessed a spherical to hexagonal morphology with particle sizes ranging from 50.06 to 86.78 nm. The antibacterial efficacy of ZnO/Cur NPs was evaluated against Staphylococcus aureus (Gram-positive) and Moraxella species (Gram-negative) using the agar disk diffusion method. The nanocomposites exhibited significant antibacterial activity, with inhibition zones of 24 mm and 22 mm against S. aureus and Moraxella sp., respectively, at a concentration of 1000 µg/mL. The results suggest a synergistic antibacterial effect between ZnO and Curcumin, attributed to reactive oxygen species (ROS) generation and cell membrane disruption. These findings highlight the potential of ZnO/Cur NPs as promising antibacterial agents for biomedical and environmental applications.

Keywords
Green synthesis,
Zinc oxide nanoparticles,
Curcumin,
Staphylococcus aureus,
Moraxellaceae.

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Introduction

The field of nanotechnology has made remarkable strides in contemporary scientific innovation over the past few years. It primarily deals with materials that are measured at the nanoscale, which spans from 1 to 100 nanometers. The distinctive properties of these nanoscale materials stem from its high specific surface area and diminutive stature, structural configuration, chemical makeup, suggesting numerous possible uses in biomedicine, including medication delivery, bioimaging, and diagnostics [1, 2, 3, 4.5]. Because of their exceptional properties, metal nanoparticles are widely used in electrical engineering, biomedicine, agriculture, and optics. These nanoparticles are synthesized using a wide range of metals, such as Fe_2O_3, ZnO, TiO_2, In_2O_3, SnO_2, SiO_2, Au, Ag, CeO_2, and CuO [6].

Among the various types of nanoparticles, zinc oxide has drawn considerable attention because of its distinctive properties [7,8]. Zinc is a crucial metallic element for human health. Its absence renders numerous enzymes, including carboxypeptidase and alcohol dehydrogenase, ineffective. Compared to cadmium and mercury, zinc exhibits lower toxicity and plays a vital role in various cellular functions within eukaryotic cells [9]. Zinc oxide nanoparticles are recognized for their antimicrobial properties against numerous pathogenic and food spoilage microorganisms. When compared to other metal nanoparticles, ZnO nanoparticles exhibit a broader spectrum of applications as antimicrobial agents. Their antimicrobial action is facilitated by oxidative stress mechanisms, which result from the buildup of ZnO nanoparticles, causing Reactive oxygen species generation and cell membrane degradation [10]. Both Gram-positive and Gram-negative bacteria can be efficiently inhibited from growing by the use of nanotechnology, along with spores that are resistant to high temperatures and pressures. Furthermore, it is selectively toxic to bacterial cells, exhibiting low toxicity levels towards human cells. This characteristic makes it particularly advantageous for the food industry, especially given the rising necessity for less harmful and cost-effective solutions to tackle food and environmental contaminants and pathogens [11].

It is known that zinc acts as an activator for over 300 of the body's enzymes [12]. This metal is vital for diabetes treatment. Zinc is instrumental in promoting glucose production in the liver by modulating the insulin pathway, which in turn enhances the utilization of glucose [13]. From the earliest days of civilization, plants have provided a vital source of numerous pharmaceuticals and remedies, in addition to their essential function in addressing various diseases. Evidence from studies and references highlights turmeric as one of the plants commonly employed for medicinal and therapeutic uses [14]. Turmeric *(Curcuma longa)*, a perennial herbaceous plant, belongs to the Zingiberocidaceae family and is used in traditional medicine as a home remedy for many health disorders, such as bile disorders, cough, diabetes, liver diseases, rheumatism, and sinusitis [15].

Turmeric extract comprises sesquiterpene compounds that result from the decomposition of terpene molecules, alongside polyphenols called curcuminoids and derivatives of ferulic acid. These constituents are chiefly responsible for the extensive medicinal and therapeutic qualities of turmeric. In addition to their anti-sclerotic effects, antioxidants may also inhibit tumor growth and development, supported by numerous studies that have demonstrated their antibacterial properties [16].

2. Materials and methods

2.1 Materials

For this study, all chemicals used in the experiments were sourced from Sigma-Aldrich, Germany. Curcumin powder, zinc oxide, and deionized water were obtained periodically during the experimental process.

2.2 The methodology for the synthesis of material nanoparticles.

Using a modified version of the method described by Bashi et al. (2013), nanocomposites with curcumin and zinc oxide layers are synthesized. First, a zinc oxide solution is made by dissolving one gram of zinc oxide in fifty milliliters of deionized distilled water, to which one gram of curcumin is added. At room temperature, this combination is magnetically swirled for a full day. It is then put in a shaking incubator that is kept at 40 degrees Celsius, which causes it to turn from light yellow to reddish yellow. After being separated by centrifugation for 20 minutes at 3000 rpm, the precipitate is repeatedly cleaned with deionized distilled water. A mortar and pestle are used to grind the precipitate into a fine powder after it has been cleaned and dried at 50 degrees Celsius in an electric oven. Until the study requires it, the powder is kept in a refrigerator.

2.3 Muller- Hinton agar

Following the instructions provided by the manufacturer, dissolve 38 grams of the medium in one liter of distilled water. The medium should then be sterilized using an autoclave. Once sterilization is complete, allow the medium to cool to a temperature range of 45 to 50 °C, and subsequently pour it into sterile Petri dishes. This medium serves the purpose of growing bacterial isolates and evaluating the inhibitory effects of antibiotics.

2.4 Estimation of the inhibitory activity of antibiotics

The disk diffusion test was used to determine the susceptibility of isolated bacteria (two types of *Moraxellaceae and Staph. aureus*) to antibiotics according to Morello et al. (2006) using the following procedure:

1. Nutrient agar medium was utilized to activate the bacterial isolates, which were then incubated at $37C^0$ for 24 hours.
2. A minimal volume of the developed bacterial colony was carefully transferred using a loop into a saline tube and mixed adequately. The turbidity of this growth was subsequently compared to the turbidity of the solid solution, which corresponds to the McFarland standard 0.5.
3. Transfer 20 microliters of the bacterial suspension with a micropipette and spread it evenly on the Mueller-Hinton agar surface with a glass spatula. Then, allow the plate to dry at room temperature (10-15 minutes).
4. Transfer the antibiotic disk to the plate using sterile forceps, and then incubate the plate at $37C^o$ for 24 hours.
5. Employ a ruler to ascertain the diameter of the inhibition zone that

encircles the antibiotic disc in millimeters and document the findings.

3. Results and discussion

3.1 Optical absorbance

The optical properties of curcumin, Std-ZnO, and Green-ZnO nanoparticles were investigated between 200 and 1000 nm using a UV-Vis spectrophotometer (see Figure 1). The Green-ZnO nanoparticles showed an absorption peak at 377 nm. The appearance of the 377 nm peak indicates that curcumin was successful in helping to create the nanoparticles. ZnO nanoparticles are known to have a characteristic absorption peak that ranges from 330 to 460 nm. [17]. This peak is linked to the basic band gap absorption of zinc oxide and is the result of electronic transitions triggered by UV light [18]. The low-energy β-excitation feature of curcumin may be linked to the broadband in its absorption spectra, which peaks at 425 nm [19]. ZnO nanoparticles can be produced using curcumin because it can bioaccumulate metal ions and enhance the stability of the synthesis process [20].

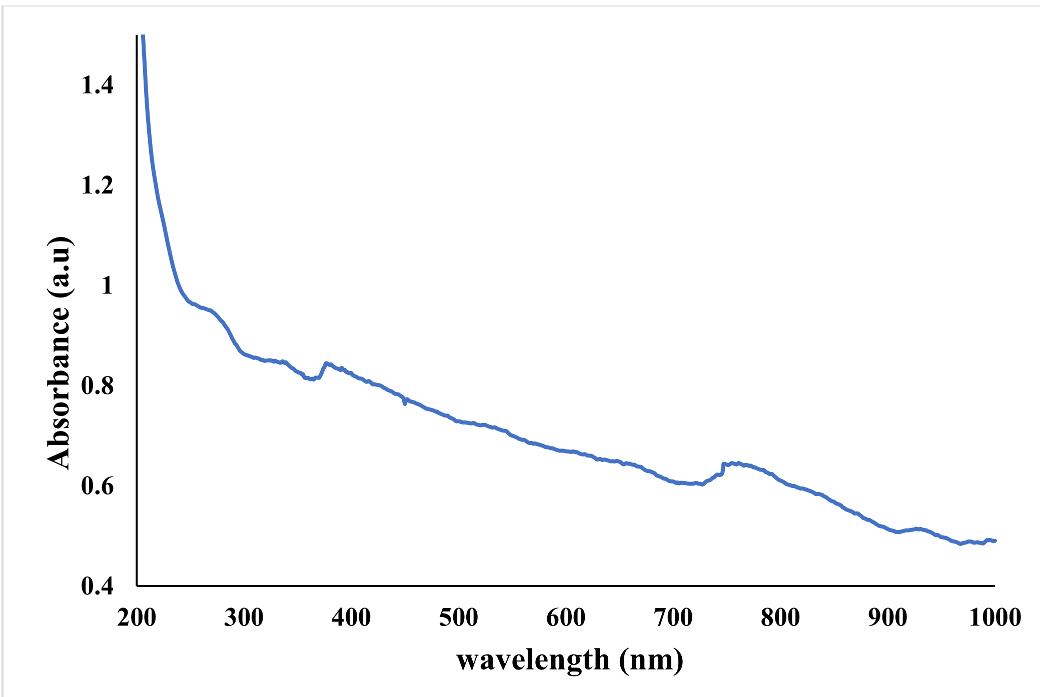

Figure 1: The absorbance spectrum of ZnO NPs-curse nanocomposite was examined using UV-vis spectroscopy.

3.2 Structure analysis

X-ray diffraction (XRD) was used to examine the crystallinity of curcumin, conventional ZnO nanoparticles, and green ZnO nanoparticles. As shown in Fig. 2, the diffraction pattern of the green ZnO nanoparticles was consistent with the properties of a typical hexagonal ZnO structure. The ZnO diffraction peaks were found at 31.51°, 34.24°, 36.07°, 47.34°, 56.43°, 62.65°, 67.76°, 68.91°, 71.83° and 76.81°

respectively. These correspond to the lattice planes (110), (002), (101), (102), (110), (103), (200), (112), (004) and (202). The obtained peaks are consistent with those observed in the hexagonal phase of wurtzite-structured conventional ZnO nanoparticles. The hexagonal phase of ZnO is effectively represented by all diffraction peaks, as shown in JCPDS map No. 36-1451 (space group P63mc) [21]. The preferred orientation is along the crystal plane (101). The observed diffraction peaks were characterized by their sharpness, intensity, and narrow width, reflecting the high crystallinity of the green ZnO nanoparticles. Furthermore, the absence of peaks associated with impurities or other ZnO phases suggests the compound's purity [22]. The structure parameters and the lattice constant were calculated using Sherrer, interplanar spacing, crystallite size, and the micro-strain of ZnO/Cur nanostructure for the preferred orientation (101) using the following equations (1, 2, 3 and 4). All results are illustrated in Table (1) [23, 24,25]:

$$2d_{hkl}sin\theta = n\lambda \qquad (1)$$

$$\frac{1}{d_h^2kl} = \frac{4}{3}\left(\frac{h^2+hk+k^2}{a^2}\right) + \frac{l^2}{c^2} \qquad (2)$$

$$D = \frac{K\,\lambda}{\beta\,cos\theta} \qquad (3)$$

$$\varepsilon = \frac{\beta cos\theta}{4} \qquad (4)$$

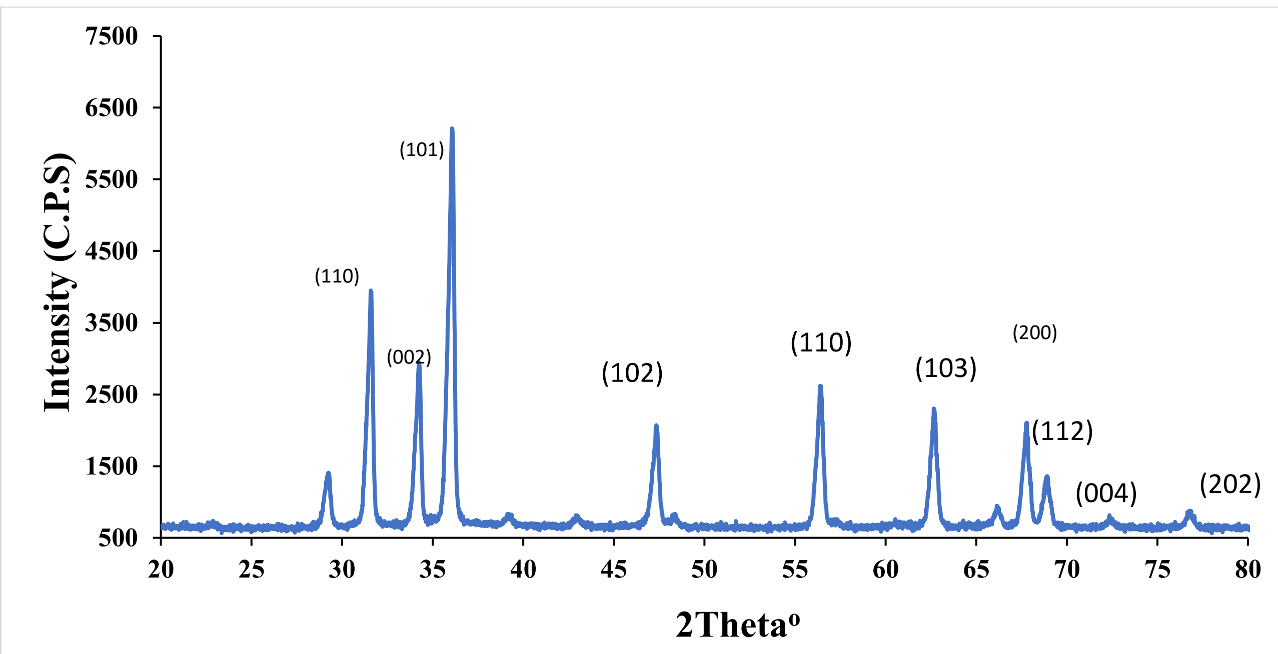

Figure (2): The X-ray diffraction (XRD) spectrum of nano zinc oxide (ZnO/Cur) synthesized using curcumin.

Table 1: Structure parameters values of ZnO/Cur for preferred orientation along (101) plane.

2θ°	d_{hkl} Å	Lattice constants Å		D nm	E
		a	c		
36.07	2.493	3.305	5.244	23.56	1.53×10^{-3}

3.3 Surface morphology of ZnO/Cur nanocomposite

Scanning electron microscopy (FESEM) was employed to examine the ZnO/Cur nanoparticles, as presented in Figures A, B, C, and D4. The figures provide insights into the shape and surface morphology of these nanoparticles. Analysis of images taken at different magnifications indicated that the ZnO particles associated with curcumin formed hexagonal spherical nanoparticles. Furthermore, they exhibited irregular morphology and were observed to be aggregated **[23].** In the representation provided in Figure 4A, It was found that the nanoparticles' average size ranged from 50.06 to 86.78 nm. Granules or semi-particles were the most common shape of the green ZnO NPs, however some also had a spherical appearance. The images marked 4A, B, C, and D imply the existence ZnO layers, which may include curcumin interleaved within them, yet this is not fully clarified **[24].**

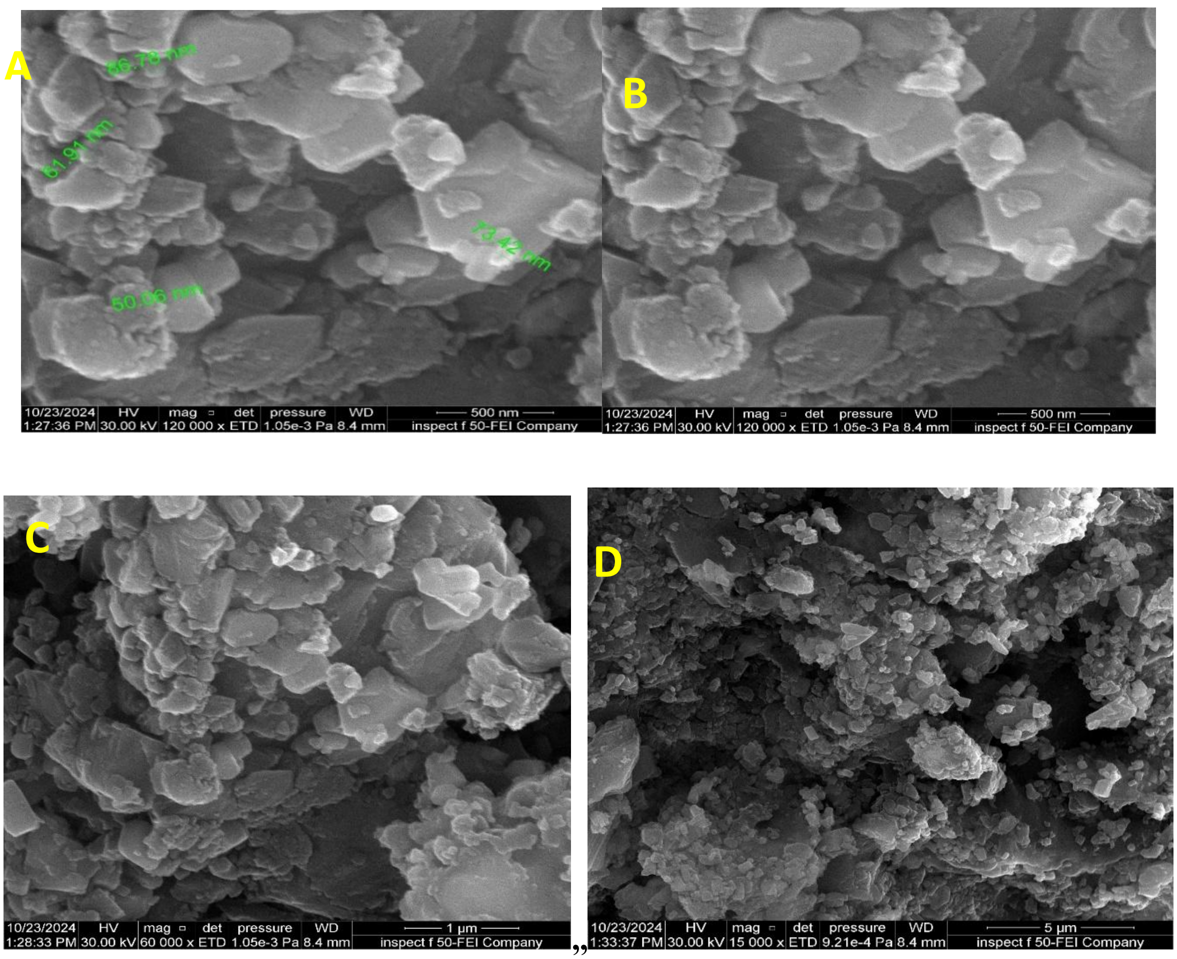

Figure (3): Field emission scanning electron microscope (FESEM) images of ZnO-Cur NPs .

3-4 Antibacterial activity of ZnO/Cur NPs

The agar inhibition method was employed to assess serial concentrations of nanoparticles (ZnO- Cur) as illustrated in Figure 4, specifically at concentrations of 1000, 500, 250, and 125 µg/ml. The results revealed an inhibition diameter of 24 mm at 1000 µg/ml, while at 500 µg/ml, the diameter was 17 mm. At 250 µg/ml, the inhibition diameter decreased to 13 mm, and no antibacterial effect was noted at 125 µg/ml. These findings are relevant to the Staphylococcus ratio. For the bacteria Moraxellaceae (A.B), the inhibition diameter was 22 mm at 1000 µg/ml, decreasing to 18 mm at 500 µg/ml. At 250 µg/ml, the diameter was 14 mm, and at 125 µg/ml, it measured 11 mm. The efficacy of the nanomaterials is linked to their capacity to inflict damage on the cell membrane wall via reactive oxygen species (ROS), which adhere to and penetrate both the membrane and the cell's interior. **[25-26].**

The findings regarding the inhibition of (ZnO/Cur) nanoparticles are impressive, indicating a synergistic effect between zinc oxide and curcumin. This combination is promising as an antibacterial and medicinal agent. The hydrophobic nature of curcumin allows it to attach firmly to bacterial membranes, which enhances the activity and effectiveness of the nanoparticles **[27].** The principal mechanisms proposed for (ZnO/Cur) consist of the annihilation of bacterial cells through the creation of reactive oxygen species and the discharge of zinc ions **[28].** Consequently, the combination of curcumin and zinc oxide may exhibit a synergistic action that results in antibacterial and inhibitory properties.

Table (2): Inhibition zone of nanostructures (ZnO/Cur) as antibacterial agents in mm at different concentrations.

Bacteria	1000 µg/ml	500 µg/ml	250 µg/ml	125 µg/ml
Staph. aureus	24	17	13	Zero
moraxellaceae	22	18	14	11

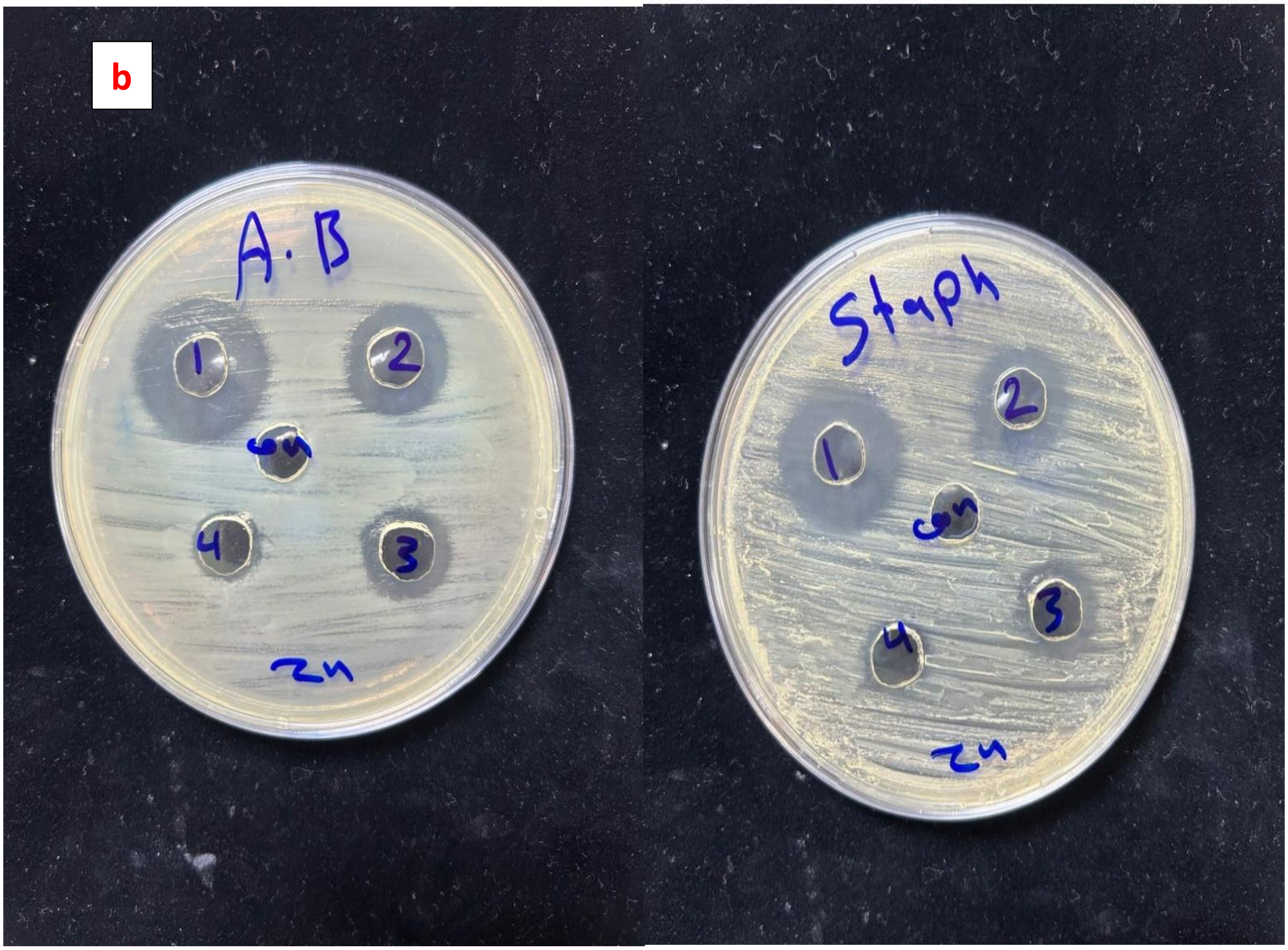

Figure 4: The Estimated Inhibition Zones of (ZnO/cur) Nanoparticle (a). *moraxellaceae* (b). *Staph. Aureus*,

4. Conclusions

Curcumin is used in this study's biosynthesis of ZnO nanoparticles to produce ecologically safe ZnO nanoparticles. Because of their hexagonal form, the produced nanoparticles demonstrated exceptional crystalline characteristics. According to photos from scanning electron microscopy (FESEM), images indicated the ZnO NPs are spherical, hexagonal-shaped NPs with irregular morphology and aggregated NPs from 50.06 to 86.78 nm. In addition, the green ZnO nanoparticles exhibited significant antimicrobial properties, with the inhibition diameter reaching 24 mm at a concentration of 1000 µg/mL, 17 mm at 500 µg/mL, and 13 mm at 250 µg/mL. No antibacterial effect was observed at 125 µg/mL. In the case of Moraxellaceae (A.B), the inhibition diameters were recorded at 22 mm for 1000 µg/mL, 18 mm for 500 µg/mL, 14 mm for 250 µg/mL, and 11mm for 125 µg/Ml. The results revealed an inhibition diameter of 24 mm at 1000 µg/ml, while at 500 µg/ml, the diameter was 17 mm.

At 250 μg/ml, the inhibition diameter decreased to 13 mm, and no antibacterial effect was noted at 125 μg/ml. These findings are relevant to the Staphylococcus The findings show that employing curcumin as a reducing and capping agent in the green synthesis of ZnO nanoparticles has several advantages, such as cost-effectiveness for large-scale production, accessibility, and environmental safety.

Enhancing the competitiveness and economic viability of this strategy requires a thorough grasp of the biochemical processes behind the creation of ZnO nanoparticles mediated by Curcumin is hydrophobic and therefore binds strongly to the bacterial membrane, providing an opportunity to increase the activity and efficacy of nanocomposites. The main mechanisms proposed for ZnO/Cur Nps include the destruction of bacterial cells through the generation of reactive oxygen species and the release of zinc ions (Zn2). Thus, both Curcumin and zinc oxide may act synergistically to exert antibacterial and inhibitory effects.

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