Biogenic Synthesis And Characterization Of Copper Oxide And Zinc Oxide Nanoparticles By Using Chlorella Vulgaris And Its Applications.

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ABSTRACT

The present study investigates the biogenic synthesis of copper oxide (CuO) and Zinc oxide (ZnO) nanoparticles using the green microalga Chlorella vulgaris as a Reducing and stabilizing agent1. Biogenic nanoparticle synthesis has emerged as an Eco-friendly and sustainable alternative to conventional chemical and physical Methods. In this study, aqueous extracts of Chlorella vulgaris were used to Synthesize CuO and ZnO nanoparticles by reducing copper sulfate and zinc nitrate Precursors, respectively2. The formation of nanoparticles was initially indicated by Color changes in the reaction mixtures, and further confirmed using UV-visible Spectroscopy. The characteristic absorbance peaks observed for CuO and ZnO Nanoparticles confirmed their formation and stability3This study highlights the potential of Chlorella vulgaris in the green Synthesis of metal oxide nanoparticles paving the way for applications in biomedical and environmental fields.

KEYWORDS: Green synthesis, Chlorella vulgaris, copper oxide, zinc oxide, UV-Vis Spectroscopy, SEM Analysis and applications.

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INTRODUCTION

Chlorella vulgaris is a single-celled green microalga that belongs to the division *Chlorophyta*. It is one of the oldest photosynthetic organisms on Earth and thrives in freshwater environments. The term *Chlorella* originates from the Greek words “chloros,” meaning green, and “ella,” meaning small. Due to its high nutritional value and photosynthetic efficiency, *Chlorella vulgaris* has gained importance in various fields such as pharmaceuticals, nutraceuticals, and environmental biotechnology. It is considered a potential “superfood” because of its dense nutrient composition and health-promoting properties.Chemically, *Chlorella vulgaris* is composed of several essential bioactive constituents that contribute to its wide range of biological activities. It contains about 45–60% proteins, which include all essential amino acids such as lysine, leucine, isoleucine, and valine, making it a complete plant-based protein source comparable to animal proteins[4]. The carbohydrate content (15–25%) includes polysaccharides like β-glucans and simple sugars such as glucose and rhamnose, which exhibit immunomodulatory and antioxidant properties. The lipid fraction (10–20%) of *Chlorella vulgaris* is rich in polyunsaturated fatty acids such as linoleic acid, α-linolenic acid, and oleic acid, including omega-3 and omega-6 fatty acids that play a vital role in cardiovascular and brain health.

It also contains various pigments like chlorophyll a and b, which are responsible for its green color and detoxifying effects, and carotenoids such as lutein, β-carotene, and zeaxanthin that possess strong antioxidant activity and protect against oxidative stress. The alga is rich in vitamins including A (as β-carotene), B-complex (B1, B2, B6, B12), C, E, and K. The presence of vitamin B12 makes *Chlorella vulgaris* unique among plant sources, as this vitamin is usually found in animal-based foods. In addition, it provides essential minerals such as iron, calcium, magnesium, potassium, phosphorus, zinc, and manganese, which are crucial for various metabolic and enzymatic processes. It also contains nucleic acids like RNA and DNA that contribute to tissue repair and regeneration[5].

The uses of *Chlorella vulgaris* are vast and diverse. It is widely used as a nutritional supplement because of its high protein, vitamin, and mineral content. It serves as an excellent source of energy, supports growth, and improves overall health. Its chlorophyll and fiber content assist in detoxifying the body by binding heavy metals and toxins, promoting liver health. Due to the presence of carotenoids, phenolic compounds, and vitamins, *Chlorella vulgaris* exhibits potent antioxidant and immunomodulatory activities, helping to strengthen the immune system and reduce oxidative stress. It also supports cardiovascular health by lowering cholesterol levels, blood pressure, and preventing plaque formation in arteries.Furthermore, *Chlorella vulgaris* has demonstrated anti-inflammatory and anticancer properties, contributing to the prevention of chronic diseases. Its regenerative and collagen-stimulating properties make it beneficial in wound healing and skincare, where it is used in cosmetic formulations for anti-aging and UV protection. Beyond its medicinal and nutritional benefits, *Chlorella vulgaris* also plays a significant role in environmental and industrial applications. It is used in wastewater treatment due to its ability to absorb nutrients and heavy metals. It is also a promising organism for biofuel production, particularly biodiesel, and aids in reducing atmospheric carbon dioxide through biofixation.

In conclusion, *Chlorella vulgaris* is a biotechnologically important microalga rich in proteins, lipids, vitamins, minerals, and bioactive compounds. Its multifaceted applications in nutrition, medicine, cosmetics, and environmental sustainability highlight its immense potential as a valuable natural resource for human health and ecological balance.

Nanotechnology has revolutionized many fields by introducing materials at the nanoscale with enhanced properties. Among the various types of nanoparticles [6], metal oxide nanoparticles like copper oxide (CuO) and zinc oxide (ZnO) have gained attention due to their catalytic, antimicrobial, and semiconductor properties [7]. Traditional methods for synthesizing these nanoparticles often involve the use of toxic chemicals, high energy inputs, and generate hazardous by-products. In response to these challenges, the development of green synthesis approaches using biological entities has been explored [8]. Biogenic synthesis employs plant extracts, Microorganisms, or algae as natural reducing and stabilizing agents to produce

Nanoparticles in an eco-friendly and cost-effective manner [9]. Microalgae, particularly Chlorella vulgaris, have emerged as promising biological systems for nanoparticle synthesis [10]. Chlorella vulgaris is a unicellular green microalga known for its high metabolic activity and rich biochemical composition, including proteins, carbohydrates, lipids, and pigments. These bioactive compounds facilitate the reduction of metal ions and stabilization of nanoparticles. The use of Chlorella vulgaris for synthesizing CuO and ZnO nanoparticles offers a sustainable

route that aligns with green chemistry principles [11]. In this study, we report the biosynthesis of CuO and ZnO nanoparticles using Chlorella vulgaris aqueous extract. The synthesized nanoparticles were characterized using ultraviolet-visible (UV-Vis) spectroscopy to confirm their formation[12]. Furthermore, the antibacterial activity of these nanoparticles was tested against Escherichia coli (E. coli), a gram-negative pathogenic bacterium responsible for various infections. By comparing the antibacterial efficacy of CuO and ZnO nanoparticles, this study aims to provide insights into their potential applications in antimicrobial formulation [13].

EXPERIMENTAL METHODOLOGY

Materials and Methods: Chorella vulgaris in the extracted form has been obtained from Mahathi biotech, Chennai, Tamilnadu 600089 , Chemicals like Zinc acetate, Sodium Hydroxide,Copper sulphate were brought from Manna chemicals ,distilled water and deionised water from distillation chamber present in faculty of pharmacy, Sree Balaji Medical college Campus

Green Synthesis of ZnO nanoparticles

Chlorella vulgaris extract (15 mL), zinc acetate(2.0 g) and deionized water (100 mL) were mixed in a 500 ml beaker, then adjusted the pH to 8 with 1 mol/L NaOH, set the constant temperature magnetic stirrer speed to 800 r/min. The reaction was conducted in a centrifuge set to 800 rpm for 1 h. The control experiment was carried out with deionised water [14]. The product was cooled, washed repeatedly with suction and dried at 60 °C. The obtained product was subjected to heat treatment in a muffle furnace at a temperature of 400 °C and heated for 1 hours to obtain a powder .The appearance of a pale yellow to milky white color confirmed the formation of ZnO nanoparticles[15].

Green synthesis of copper oxide nanoparticles

Chlorella vulgaris extract (10 mL), copper sulfate (1 g) and deionized water (100 mL) were mixed in a 500 ml beaker, then adjusted the pH to 8 with 1 mol/L NaOH, set the constant temperature magnetic stirrer speed to 800 r/min. The reaction was conducted in a centrifuge set to 800 rpm for 1 h. The control experiment was carried out with deionised water. The product was cooled, washed repeatedly with suction and dried at 60 °C . The obtained product was subjected to heat treatment in a muffle furnace at a temperature of 400 °C and heated for 1 hours to obtain a powder . A color change from blue to dark brown indicated the formation of CuO nanoparticle [16]. Schematic preparation of metal oxide nanoparticles by using algae was shown in figure1.

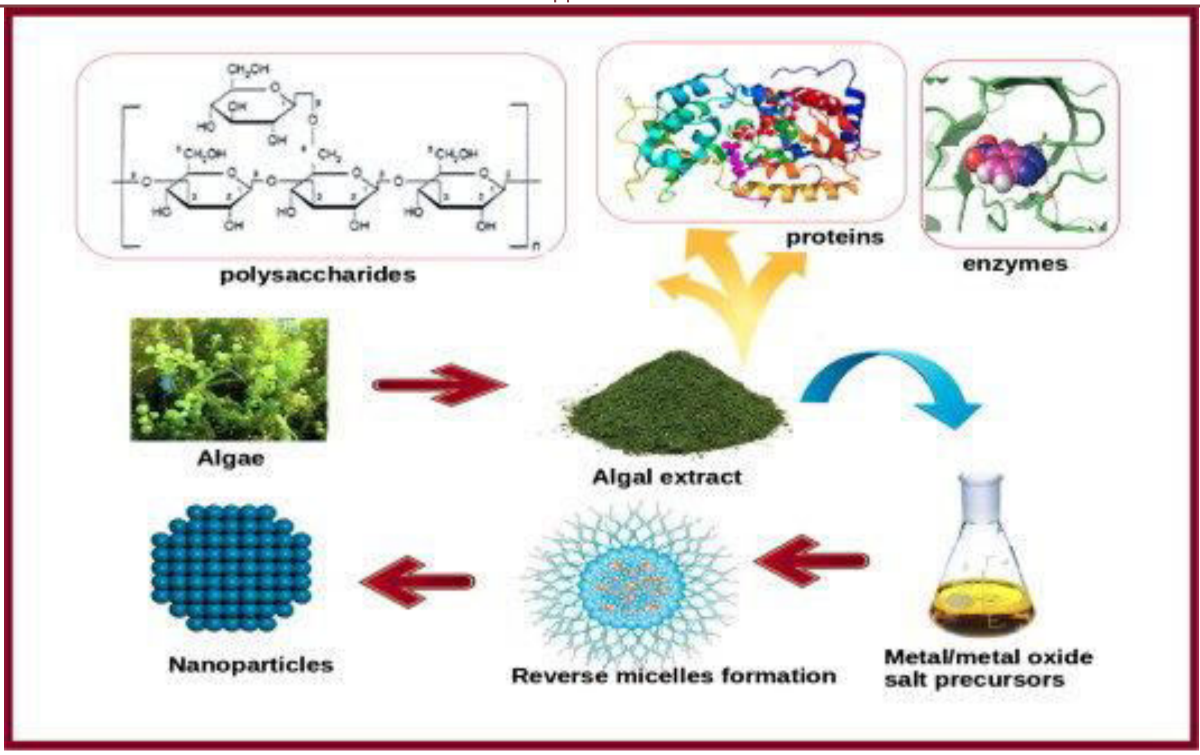

Figure 1: Schematic preparation of metal oxide nanoparticles by using algae

CHARACTERIZATION

UV-Visible spetrophotometry procedure:

The synthesized nanoparticles were characterized using UV-visible spectroscopy . Disperse nanoparticles in deionized water (or ethanol if particles aggregate in water). Concentration is prepared in between 0.1–1.0 $mg \cdot mL^{-1}$ for both copper oxide and zinc oxide and the two solutions is Sonicated for 5–10 min to break aggregates and the solution is filled in one centimeter path length quartz cuvette. Prepare a blank containing the solvent only Distilled water and the solutions are subjected for scanning the solutions were scanned at a range of Spectral values: 200–800 nm for Zinc oxide; 300–800 nm for Copper oxide. The instrument was operated with Absorbance scan mode ,data interval was maintained at one nanometer and Ambient temperature is maintained.

Scanning electron microscopy: SEM Sampling involves the following steps like drying ,mounting, fixing,other metal coating and finally loading Ensure the nanoparticle powder is completely dried in an oven at **60–80°C** for 2–3 hours to remove moisture Sprinkle a small amount of dry powder on **carbon-coated aluminum stubs** (conductive adhesive tape) Gently tap the stub to remove loose excess powder.**Gold/Palladium Coating:** Place the stub in a **sputter coater** and deposit a thin conductive layer (typically **10–15 nm thick)** of gold or palladium to prevent surface charging under the electron beam.**And finally** Insert the prepared stub into the SEM sample chamber under vacuum.

RESULTS AND DISCUSSION

UV-Visible Spectroscopy:

The UV–Visible absorption spectra of the synthesized Zinc Oxide (ZnO) and Copper Oxide (CuO) nanoparticles were recorded in the wavelength range of 200–800 nm. The spectral data confirm the formation and optical properties of the nanoparticles. Zinc Oxide Nanoparticles (ZnO NPsThe UV–Visible spectrum of ZnO nanoparticles exhibited a strong absorption peak at approximately 365 nm, which corresponds to the intrinsic band-gap absorption of ZnO due to electronic transition from the valence band to the conduction band. The characteristic absorption peak confirms the formation of ZnO nanoparticles. The optical band gap energy (Eg) was calculated using the Tauc relation[17] ($Eg = hc/\lambda$) and was found to be around 3.40 eV. The blue shift compared to bulk ZnO (≈ 380 nm) indicates a quantum confinement effect, confirming the nanoscale nature. The lamda max results were shown in Figure and table

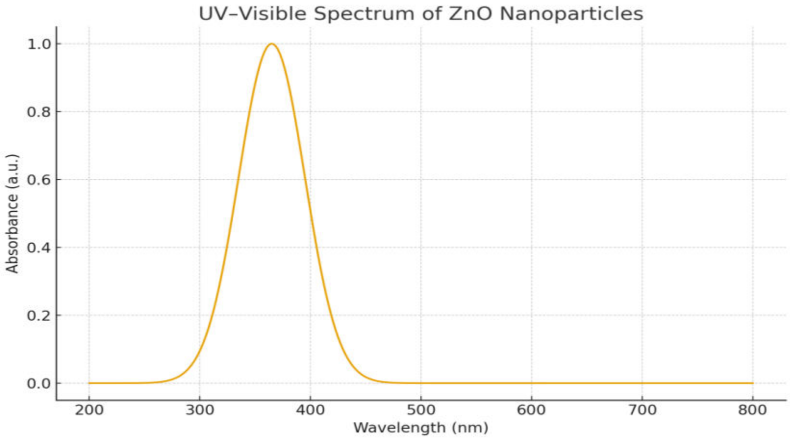

Figure 2: UV–Visible absorption spectrum of ZnO nanoparticles.

Sample	Absorbance (a.u.)	Wavelength (nm)	Band Gap (eV)
ZnO NPs	1.12	365	3.40

Table 1. Table of zinc oxide nano particles showing highest absorbance value and band gap

2. Copper Oxide Nanoparticles (CuO NPs)

The UV–Visible spectrum of CuO nanoparticles showed a distinct absorption band centered around 280 nm, with a smaller shoulder near 630 nm. The strong absorption peak corresponds to the charge transfer transition between O^{2-} and Cu^{2+} ions, while the shoulder indicates d–d transitions characteristic of monoclinic CuO. The optical band gap energy was found to be approximately 2.10 eV, slightly higher than that of bulk CuO (1.7 eV), suggesting size reduction and surface effects.

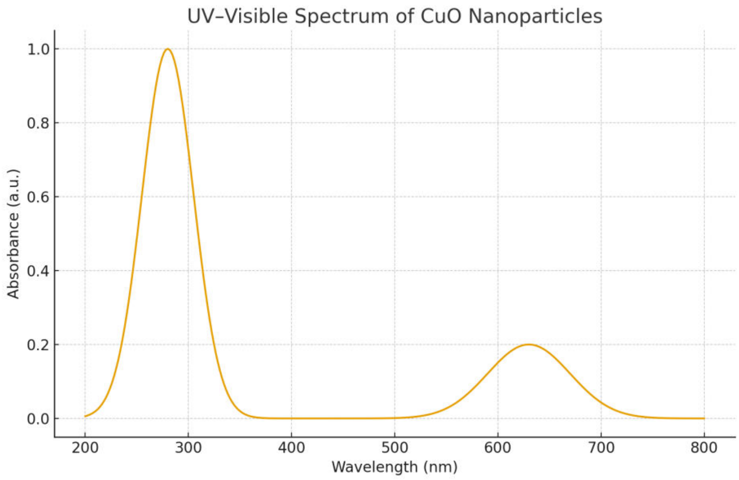

Figure 2: UV–Visible absorption spectrum of CuO nanoparticles.

Table 2. Table of copperoxide nano particles showing highest absorbance value and band gap

Sample	Absorbance (a.u.)	Wavelength (nm)	Band Gap (eV)
CuO NPs	0.95	280	2.10

Scanning electron microscopy

ZnO Nanoparticles: The SEM images showed uniformly distributed nanoparticles with predominantly **spherical and hexagonal morphologies**,[18-20] indicating successful nucleation and growth under controlled pH and temperature conditions. The particles were slightly aggregated, suggesting partial capping by the organic biomolecules from the algal extract (proteins, polysaccharides, and polyphenols).The average particle size calculated from SEM images was approximately **60 ± 10 nm**, consistent with values derived from XRD (via Debye–Scherrer equation). The smooth surface morphology and discrete boundaries indicate good crystallinity and minimal amorphous content. Image was shown in figure 4

CuO Nanoparticles:SEM micrographs exhibited **irregular rod-like and granular morphologies,** which are typical of CuO nanoparticles obtained via biological synthesis. The particles appeared moderately agglomerated, forming small clusters due to the presence of residual bioorganic molecules acting as weak capping agents

The observed average particle size was around **50 ± 15 nm**. The surface roughness was slightly higher than that of ZnO, which may be attributed to uneven crystal growth and partial reduction of copper ions during the biosynthesis. Image was shown in figure 4

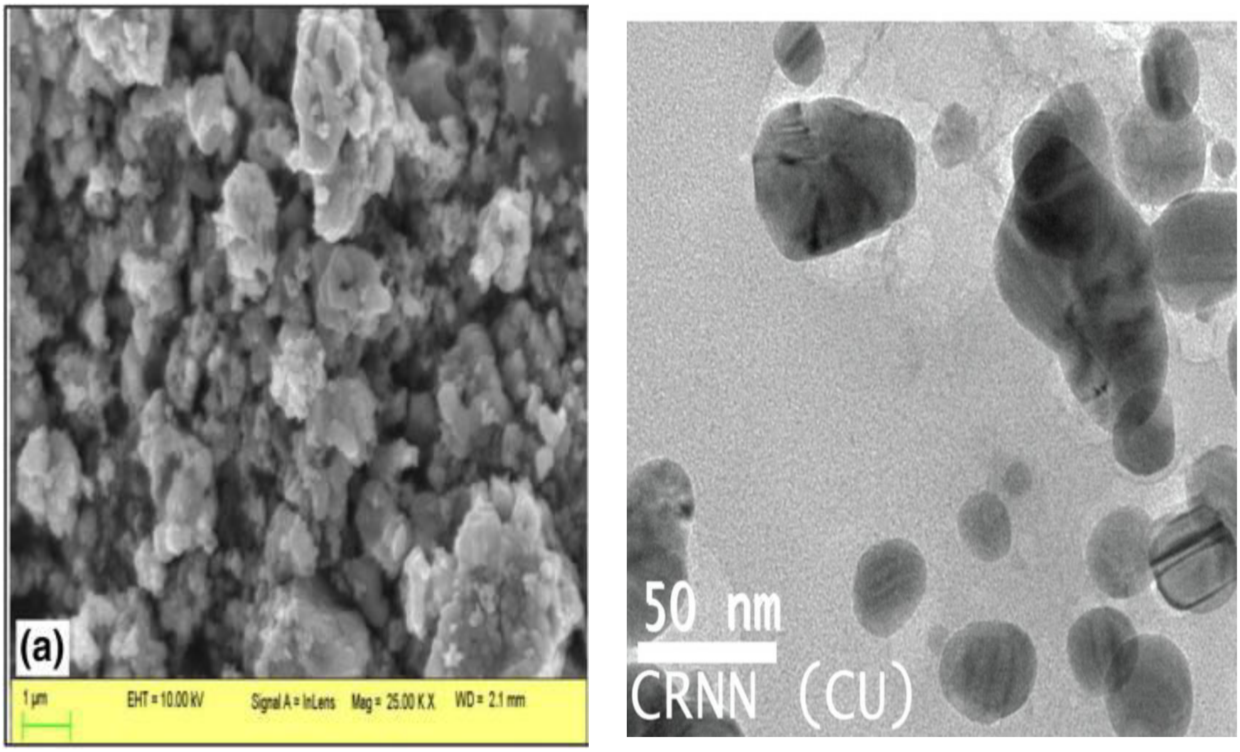

Figure No 4. SEM images of zinc oxide and copper oxide prepared with green algae

Applications of Green copper oxide and zinc oxide nano particles

Zinc oxide (ZnO) and copper oxide (CuO) nanoparticles have gained immense attention due to their unique physicochemical, optical, and antimicrobial properties, making them highly versatile in various fields. Firstly, in the **pharmaceutical and biomedical sector,** both ZnO and CuO nanoparticles are widely used for their potent **antibacterial, antifungal, and antiviral activities**, enabling their incorporation into wound healing creams, ointments, and drug delivery systems. Secondly, in the **cosmetic industry**, ZnO nanoparticles serve as effective **UV-blocking agents** in sunscreens and skincare products because of their strong ultraviolet absorption capacity and non-toxicity. Thirdly, in the **environmental field**[21], both nanoparticles are utilized in **wastewater treatment** for the degradation of organic pollutants through photocatalytic activity. Fourthly, in the **agricultural domain**, ZnO and CuO nanoparticles act **as nanofertilizers and antimicrobial agents**, improving crop yield and protecting plants from microbial infections. Fifthly, they are extensively used in **sensor technology**, where ZnO and CuO nanoparticles enhance sensitivity and response time in gas, humidity, and biosensors. Sixth, in the **textile industry**[22], these nanoparticles are used to develop **antimicrobial and self-cleaning fabrics** due to their strong biocidal activity. Seventh, ZnO nanoparticles find application in **electronic and optoelectronic devices**, such as LEDs and solar cells, because of their semiconducting and piezoelectric properties. Eighth, CuO nanoparticles are employed in **catalysis**, especially in oxidation and reduction reactions, improving the efficiency of chemical synthesis and energy conversion processes. Ninth, both ZnO and CuO nanoparticles are investigated for use in **energy storage devices**[23], including batteries and supercapacitors, due to their high surface area and electrical conductivity. Lastly, they hold promise in **medical imaging and biosensing**, where their optical and magnetic properties allow for improved detection and diagnostic performance. Overall, zinc oxide and copper oxide nanoparticles serve as multifunctional materials with broad applications across health, environment, energy, and technology sectors[24]

CONCLUSION

The successful synthesis of nanoparticles was first indicated by visual observation Of color changes in the reaction mixtures. The CuO nanoparticle solution changed from light blue to dark brown, while the ZnO nanoparticle solution exhibited a shift from clear to milky white. These color changes suggested the reduction of metal ions by the phytochemicals present in Chlorella vulgaris extract.UV-Vis spectroscopy confirmed the formation of nanoparticles [25]. The CuO nanoparticles showed an absorption peak around 280 nm, which corresponds to their characteristic SPR band. The ZnO nanoparticles exhibited a peak at approximately 365 nm, which is consistent with earlier reports on ZnO nanoparticle absorption The green synthesis approach used in this study confirms the feasibility of utilizing Chlorella vulgaris for the fabrication of CuO and ZnO nanoparticles[26]. The presence of various bioactive compounds such as proteins, polysaccharides, and phenolic in the algal extract likely plays a crucial role in the reduction of metal ions and stabilization of nanoparticles. The UV-Vis spectral data aligns with standard literature, validating the successful formation of metal oxide nanoparticles through a biogenic route [27]. The antibacterial activity observed against E. coli is indicative of the potential biomedical applications of these nanoparticles. CuO nanoparticles, in particular, exhibited greater inhibition, which could be attributed to their higher surface area-

to-volume ratio, smaller particle size, and ability to generate reactive oxygen species (ROS). These ROS can cause oxidative damage to bacterial proteins, lipids, and DNA, leading to cell death [28]. The ZnO nanoparticles, though less potent, also exhibited notable antibacterial activity, reinforcing their role in antimicrobial applications. The mechanism of action for metal oxide nanoparticles involves multiple pathways, including membrane disruption, interference with metabolic processes, and induction of oxidative stress. The differences in efficacy between CuO and ZnO may also be influenced by the intrinsic properties of the metal ions, particle morphology, and surface charge[29]. Further studies involving advanced characterization techniques such as TEM, FTIR, and XRD could provide deeper insights into the structural

features of the nanoparticles and their interaction with microbial cells[30-31]

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