



GREEN SYNTHESIS OF ZINC OXIDE NANOPARTICLES USING WILD GARLIC (*ALLIUM URSINUM*) AND EVALUATION OF THEIR ANTIBACTERIAL POTENTIAL

¹Chamakura Malla Reddy, ²Md Habeeb Ahmed, ³Akkiraju Pavan Chand

¹Student, Department of Biology, The Gaudium School, Hyderabad, India

²Assistant Professor, Research Department, School of Allied and Healthcare Sciences,
Malla Reddy University, Hyderabad, India

³Associate Professor, Medical Biotechnology, School of Allied and Healthcare Sciences,
Malla Reddy University, Hyderabad, India

Abstract:

Green synthesis of nanoparticles has emerged as a promising research area due to their wide potential applications in antimicrobial, anticancer, and other biomedical fields, providing a safer and more eco-friendly alternative to conventional-chemical synthesis methods. Their structural versatility further broadens their functional utility. In current study, zinc oxide (ZnO) nanoparticles were synthesized using an aqueous extract of single clove wild garlic (*Allium ursinum*). The nanoparticles obtained were evaluated for Anti-microbial activity against *Escherichia coli* and *Staphylococcus aureus*, the anti-microbial activity efficacy was compared with commercially available Antibiotics, viz. Tetracycline, Azithromycin, Chloramphenicol and Streptomycin. Results presented a significantly higher and dominant anti-microbial activity of synthesized ZnO nanoparticles over the commercially available antibiotics.

Key Words: Zinc Nanoparticles, Wild garlic Nanoparticles, Antimicrobial Activity, Green synthesis

1. INTRODUCTION

Nanotechnology has become one of the emerging research focus in last decade, with diverse applications of nanoparticles in water treatment, biomedical engineering, textile industries and many emerging day by day (Rabiee et al., 2020), has impacted to the rise in cost of nanoparticles, Zinc oxide nanoparticle is one such material with diverse applications in optical, gas sensing, end-odontics (Khalid et al., 2021), possessing a significant anti-fungal & anti-microbial activity (Bondarenko, et al., 2013), this has triggered researchers to focus on low-cost sustainable approaches of nanoparticles synthesis (Abdellatif et al., 2022).

Various alternative nanoparticles-synthesis methods were explored, but out of all, the plant-extract based green synthesis was found to be more safer alternative, with lower cost involved (Darbasizadeh et al., 2023), green synthesis majorly involves water as an solvent, which adds an advantage to this method, posing no cellular toxicity and the plant extract, acting as an reducing and capping agent in synthesis process is completely biodegradable, this property makes green synthesis more reliable and demanding with higher yield and safer alternative. (Nour et al., 2019) single clove wild garlic (*Allium ursinum* Sp) is one such plant extensively being screened from the start of 2020 for its high amount of varied flavonoids and other medically important bioactive components presence (Szczepaniak et al., 2025). Based on these findings, current study was conducted on green synthesis of Zinc oxide nanoparticles using single clove wild garlic (*Allium ursinum* Sp) extract using as the precursor Zinc acetate solution. Novelty of this work include using wild garlic and the main objective is to test its antimicrobial nature through nanoparticles that are synthesized.

2. MATERIALS AND METHODS

Freshly available wild garlic fruits (~10-20g whole fruit) were obtained from the local market to conduct research. Zinc acetate procured from HiMedia Laboratories, India. The studies were conducted in the Laboratory of Medical Biotechnology Department, School of Allied & Healthcare Sciences, Malla Reddy University, Hyderabad. All the samples were studied as triplicate, and the results were noted accordingly.

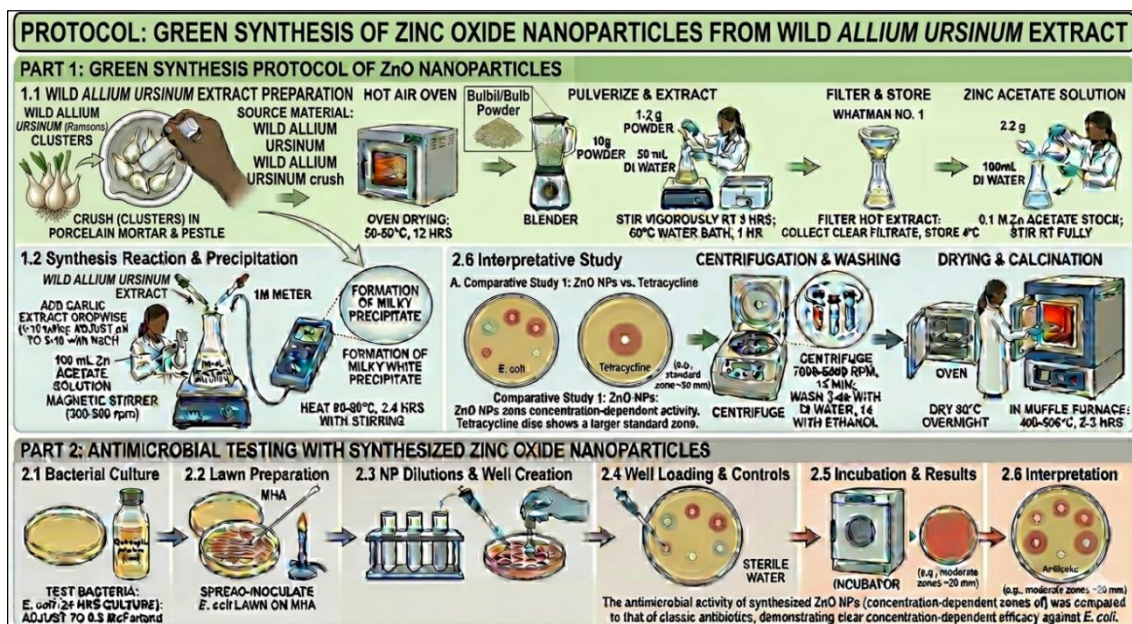


Figure.1: Nanoparticles synthesis procedure and their anti-microbial activities.

2.1 Wild Garlic Fruit Extract Preparation

Harvested and washed fresh wild garlic fruits thoroughly with tap and de-ionized water successively. Chopped and crushed 20g of whole fruits (including pulp; shell, inner bulb) into small pieces as shown in fig.1. The mixture was dried at 50-60°C for 12 hours until it becomes brittle. Ground 10g dried fruit into fine powder using a blender. Suspended 2g of powder in 50 mL de-ionized water as shown in fig. 2; stirred vigorously at room temp for 3 hours, then heated at 60°C in a water bath for 1 hour. Filtered hot extract through Whatman No. 1 paper (discarded the residue); collected ~40 mL clear, pale-yellow filtrate. Stored at 4°C for further studies (Rabiee *et al.*, 2020).

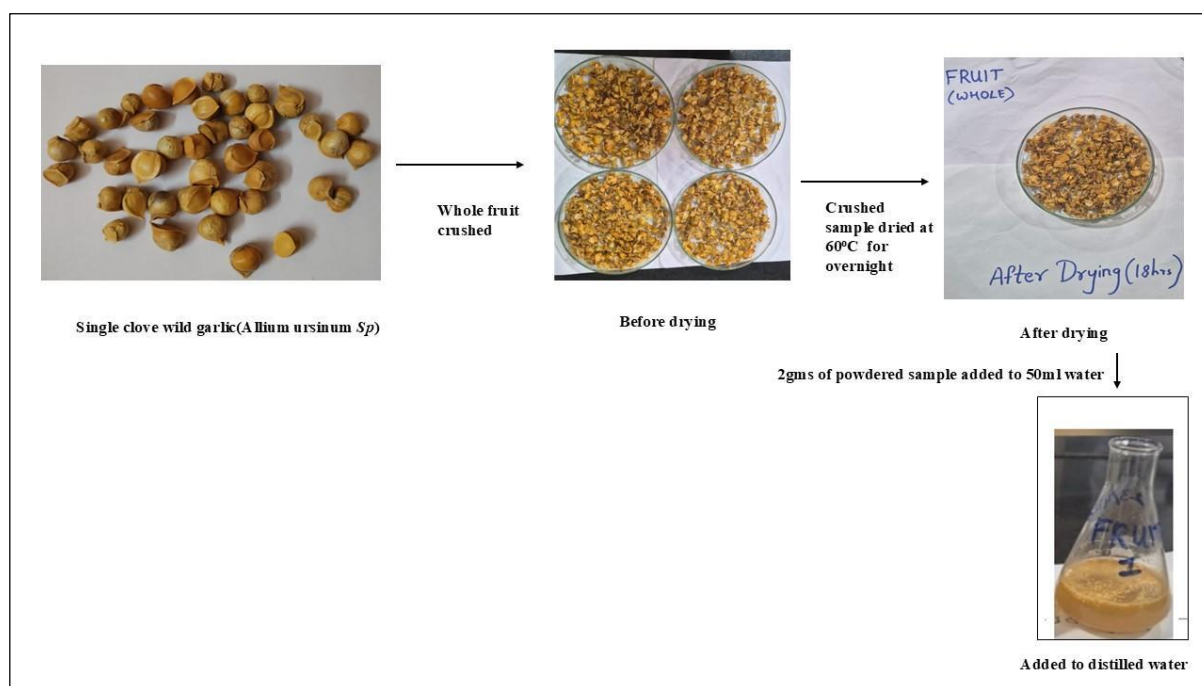


Figure. 2: Complete extraction and procedure for nanoparticles synthesis

2.2 Zinc Acetate Solution preparation

0.1 M zinc acetate dihydrate stock (2.2 g/100 mL de-ionized water) was prepared and 100 mL was used for nanoparticles synthesis; stirred thoroughly to dissolve fully at room temperature.

2.3 Synthesis Reaction

Under magnetic stirring (300-500 rpm), garlic fruit extract was added dropwise to zinc acetate solution (ratio 1:10 extract: precursor, e.g., 10 mL extract to 100 mL solution). The pH was adjusted to 8-10 dropwise with 1M NaOH while monitoring continuously, a milky white precipitate formed immediately due to fruit phytochemicals reducing Zn^{2+} to ZnO. The mixture was heated at 60-80°C for 2-4 hours while stirring to stabilize NPs.

2.4 Centrifugation and Washing

The mixture was centrifuged at 7000-8000 rpm for 15 minutes. Supernatant was decanted and resuspended pellet in de-ionized water; repeated wash/centrifuge 3 times, followed by one ethanol wash to remove excess organics.

2.5 Drying and Calcination

The pellet formed was air dried at 80°C overnight. The powder was annealed in muffle furnace at 400-500°C for 2-3 hours to yield pure wurtzite Nanoparticles. Cooled slowly; ground to fine white powder (~80% (Thi *et al.*, 2020).

2.6 Anti-microbial activity

To evaluate the antimicrobial activity of synthesized ZnO nanoparticles, zone of inhibition method or agar disk diffusion assay was followed. Antimicrobial activity was tested against *Escherichia coli* and *Staphylococcus aureus*, cultures were spread over nutrient agar plates and incubated at 37°C for overnight, to test the activity, circular wells were made with diameter of 6 mm to 8 mm and loaded with 20 μL of ZnO nanoparticles solution suspended in distilled water, simultaneously, commercially available antibiotics like Tetracycline, Azithromycin, Chloramphenicol and Streptomycin were also tested by disc diffusion method against both the organisms. All the testing plates were incubated for 24hrs at 37°C and observed for zone of inhibition formation, as shown in fig. 3.

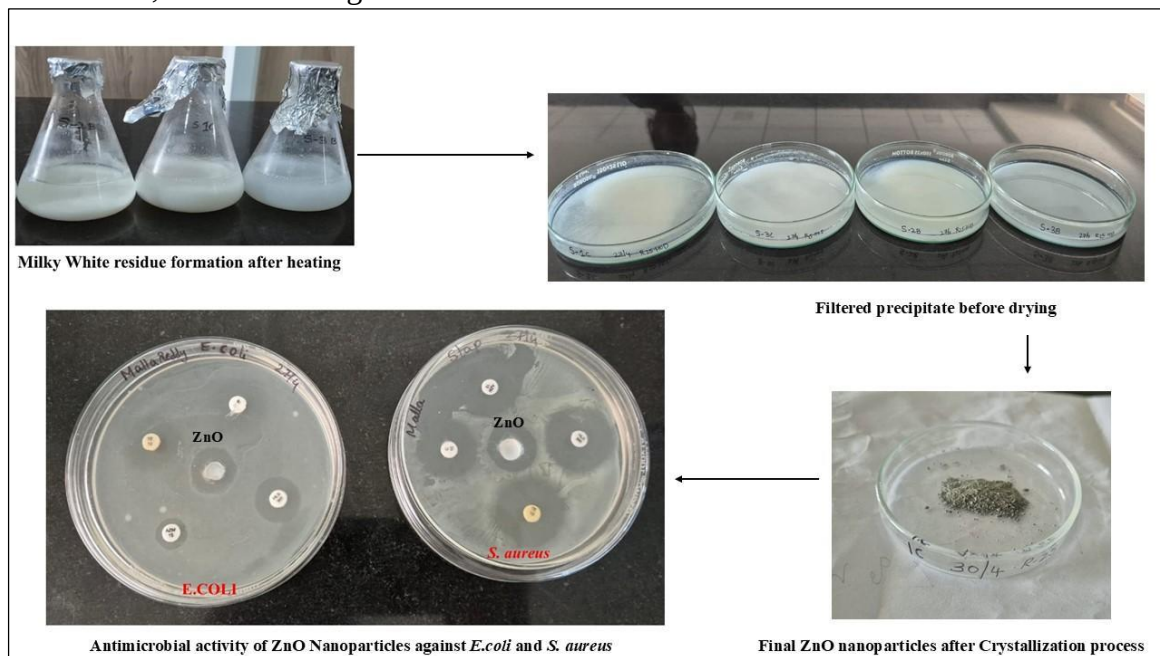


Figure. 3: ZnO nanoparticles crystallization process and its application of antimicrobial activity

3. RESULTS AND DISCUSSION

3.1 Synthesis Reaction

A white milky precipitation formation was seen while adding the extract drop wise to precursor zinc acetate solution under mixing condition. This white precipitate formation is a primary indication of successful reduction reaction of zinc acetate solution and ZnO nanoparticles formation. This could be attributed to thiopolysulfides (organosulfur compounds) present in the garlic (Szczepaniak *et al.*, 2025) acting as a strong reducing agent and a capping agent, guiding the reaction to successful ZnO nanoparticles formation and preventing agglomeration of ZnO nanoparticles.

3.2 Anti-microbial activity

The observed zones of inhibition as seen in fig. 3 and table1 (0.8 mm against *E. coli* and 1.0 mm against *S. aureus*) demonstrated that green-synthesized ZnO nanoparticles achieved antimicrobial efficacy comparable to other commercially available antibiotics (*Shahabadi et al., 2025; Khatana et al., 2021*). This is particularly significant given the global rise in antimicrobial resistance (AMR), where over-reliance on standard antibiotics has led to decreased therapeutic sensitivity, necessitating the development of robust, alternative agents (*Rabiee et al., 2020*). By providing a sustainable, plant-mediated synthesis route that yields nanoparticles capable of matching conventional antibiotic performance, these results highlight a vital opportunity to deploy this route of nanoparticles synthesis as powerful tools to mitigate current antibiotic limitations and address multidrug-resistant pathogens (*Khatana et al., 2021*).

Table1: zone of inhibition (cm) of ZnO nanoparticles and other antibiotics against *E. coli* and *S. aureus*

Antibiotics	<i>E. coli</i> (mm)	<i>S. aureus</i> (mm)
Tetracycline	0.6	1
Azithromycin	0.2	1.6
Chloramphenicol	0.8	0.9
Streptomycin	0.4	1
Zinc oxide Nanoparticles	0.8	1

4. CONCLUSION

From our current study it is evident that plant extracts like single clove wild garlic (*Allium ursinum* Sp), which has a high content of bioactive compounds could be potential and reliable source for green synthesis of nanoparticles. As demonstrated in this study, ZnO nanoparticles synthesized through green synthesis may serve as a promising approach for the future development of diverse nanoparticles, enabling the optimization of their structural properties and evaluation of their varying effects against emerging antimicrobial-resistant microbial strains. Further, Nanoparticle-based therapies could become a primary treatment option, decreasing reliance on antibiotics while enhancing safety, precision, and efficiency, thus reducing overall treatment duration.

5. ACKNOWLEDGMENT

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