"ECO-FRIENDLY SYNTHESIS OF HONEY-ENHANCED ZnO NANOPARTICLES: CHARACTERIZATION AND ANTIBACTERIAL POTENTIAL"

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Abstract:
Green ways have become an emerging field in nanotechnology as eco-friendly composite of NPs are less hazardous and more economic. Discover the fascinating results of our latest research project, where we utilised the natural properties of honey collected by the *Apis malifera* honey bee and zinc acetate to create ZnO NPs. Experience the precise characterization of our synthesised NPs through advanced techniques such as Ultraviolet-Visible spectroscopy (UV-Vis), Fourier Transform Infrared Spectroscopy (FT-IR), and Transmission Electron Microscopy (TEM). The distinctive absorption peak showcased in the UV-Vis spectra was on 286 nm. Owing to their significant excitation binding energy at ambient temperature, O-C-O and H-O. FT-IR analysis was used to confirm the synthetic material, and TEM examination was used to estimate the average size of the ZnO NPs particles, which came out to be between (100-200) nm. These synthesised NPs were also evaluated to check their antibacterial properties against the *E.coli* and *Bacillus megaterium.*

Keywords: UV-Visible, TEM, FT-IR, Honey, ZnO Nanoparticles, Green Synthesis, antibacterial.

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Introduction

As a new field of study, nanotechnology has the potential to alter several established disciplines. Nanomaterials, due to their size and shape, provide a wide range of potential applications and have been an important topic in both the pure and applied sciences (Gour, A *et al*., 2019). Because of their new features that is applications in optoelectronics, many researchers have focused their efforts on nanoscale semi-conductors in recent years (Jeremy J., 2016). ZnO NPs are a versatile type of semiconductor that exhibit exceptional features of UV-Visible that works on light transmission and emission. The remarkable chemical and thermal stability of nanoparticles has made them a topic of intense interest in recent years. (Vennila, S *et al*., 2017). Several methods for preparing ZNPs have been devised, among them are chemical vapour deposition, ultrasonic condition, precipitation, microwave-assisted methods, chemical vapour deposition, and spray pyrolysis (Islam *et al*.,2022). In addition to the high energy expenditures, there is also the risk to human health from the use of dangerous and hazardous substances in such preparations. Biological methods, on the other hand, are gaining popularity due to their simplicity, cleanliness, safety, and low cost (Fakhari et al., 2019). Nanoparticles made using biosynthetic processes have more uniform size and shape than those made via conventional physicochemical approaches (Kahraman *et al*., 2022). In order to create stable nanoparticles, scientists have turned to naturally occurring chemicals present in biological systems as capping agents and key building elements. Reviewing the available research shows that there are major advantages to employing plants instead of other biological systems. There is an improvement in stability when synthesising nanoparticles from plant extracts, and the plants themselves are easily accessible and non-hazardous to deal with. (Chikkanna *et al*., 2019). Multiple strategies have been used for the environmentally friendly manufacture of ZnO NPs to avoid harmful effect of chemically synthesised nanoparticles. In consideration of this, ZnO NPs have been effectively synthesized using eco-friendly methods. Bee honey is one of them who is a rich source of sugar and phenolic compound (Sharmila *et al*., 2021). Honey has been used as both a food and a medicine for a very long time now because of the unique physicochemical properties that allow it to do both of these things effectively. Using honey in its purest form for medical reasons presents a number of challenges, including the inability to preserve a sufficient therapeutic concentration for a sufficient period of time due to melting and seepage (Abolhassani *et al*., 2022) Hydrogels, dressings, ointments, pastes, and lozenges all benefit from honey's antibacterial properties, which has inspired scientists to include it into these products (Hixon *et al*., 2019). Honey's powerful osmotic activity, which pulls autolytic debridement relies on moisture from exudates and lymph fluid draining from the wound to the surface, may help reduce the disagreeable odour of wounds (Alam *et al*., 2014). The enzymatic breakdown of sugar releases hydrogen peroxide, which kills bacteria, and the acidic pH and osmotic impact of the high sugar concentration also kill bacteria. (Maringgal, 2020). In addition to phenolic chemicals, flavonoids, and methylglyoxal, honey may also include other molecules that have antimicrobial properties (Ismail, 2019). Overall, honey has qualities that make it helpful against many different kinds of bacteria, even antibiotic-resistant ones (Moses *et al*, 2023). UV-Vis, Both TEM and FT-IR can be used to examine a sample's structure have all been used to verify the structural properties of the synthesised ZNPs.

2. Materials and Methods:

2.1 Chemicals and Instrumentation:

We got all the solvents and chemicals from HIMEDIA. U.V.-Visible (3600 Plus, SHIMADZU, JAPAN), FT-IR (DST-PURSE, PERKIN ELMER, GERMANY), TELOS 2000Kv TEM (Fei, Electron Optics) were used to carry out NPs characterization. SONICS Vibra Cell^{TM}; Heater-Stirrer (REMI 2MLH MAGNETIC STIRRER); Hot Air Oven HAHNSHIN SCIENTIFIC (HS-2005 V); Centrifuge (REMI R-8C BL), Filter Papers (WhatmanTM), Micro Syringe filter paper (Biomed Scientific Sterile Syringe Filters Hydrophobic PTFE Membrane) made use of for the fabrication of ZnO NPs.

2.2 Preparation of Honey samples:

The measuring cylinder was filled with 20ml of collected honey. Now, the sample was mixed with 80ml of de-ionised water and allowed to heat and stir on the magnetic heater up to 75℃ for 10 mins and stored for the further use.

2.3 Fabrication and purification of ZnO NPs:

First, 40 gm of NaOH were dissolved in 500 millilitres of distilled H_2O using a magnetic stirrer until completely dissolved. Next, a solution of $ZnC_4H_6O_4$ was prepared in 50 millilitres of deionized water, and the NaOH solution made to add drop by drop while maintaining a pH of 12 using a micropipette. Now the transparent zinc solution turned into milky white on this period added 1ml of Honey into the solution pale yellow solution prepared now ultra-sonication was done of the sample for 15 mins on SONICS Vibra Cell^{TM}. Now this solution was filtered with Whatman`s Filter paper and then filtered from syringe micro filter for the best results. Now centrifugation was done for 15 mins on 10,000 rpm for 3 times, after this 3-time washing were also done because debris must be filtered out. This step involved drying the collected ppts in the hot air oven for 6 hrs at 60°C (Figure1). After an additional hour of stirring, the liquid had reached a solid state and had become a pale-yellow colour. Multiple re-dispersions in deionized water and subsequent centrifugation were used to clean the precipitates. After everything had been processed, a white powdery substance was observed as the result.

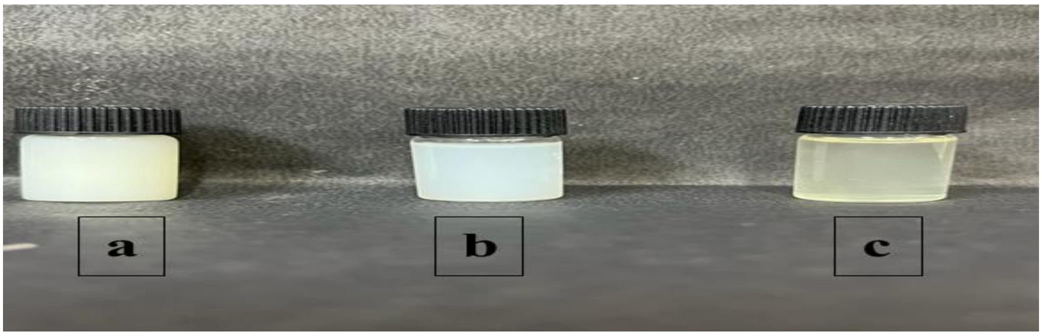

Figure 1: Synthesis of ZnO nanoparticles using honey a) ZnO NPs without any filtration. b) ZnO NPs with filter paper. c) ZnO NPs with micro syringe filter.

3. ZnO NPs: Characterization:

Several methods were used to characterise the synthesised nanoparticles. After the reaction was complete, 1 millilitre of the purified sample was sonicated at 10000 rpm for 15 minutes to provide a suspension suitable for UV-Visible examination. The ultraviolet-visible spectra were collected from 200 to 800 nanometres. The existence of different modes of vibration of different functional groups in the synthesised nanoparticles was studied using FOURIER TRANSFORM INFRARED SPECTROSCOPY (FT-IR) evaluation of the dried ZnO NPs utilising the Potassium Bromide, IR Spectroscopy grade, and Hydraulic Pellet Press technique. The synthesised nanoparticles were verified to be of the expected TEM size.

4. Discussion and Results

4.1 Ultraviolet-visible Spectroscopy

Nanoparticles' absorption spectra in the UV-Visible range after synthesis are displayed in Figure 2. ZnO NPs are distinguished by a sharp peak at 286 nm, the result of their high excitation binding energy even at ambient temperature. (Bhuyan *et al*., 2015). Absorption spectroscopy established long ago that, as particle size decreases, so does the band gap. Band gap and absorption wavelength have inverse relationships. (Arya *et al*., 2021) Absorptivity of bulk ZnO was measured as 1.151 at a wavelength of 286 nm. Synthesised ZNPs may have a greater blue shift absorption compared to bulk ZnO because of a significant decrease in particle size (Mallikarjunaswamy *et al*., 2020).

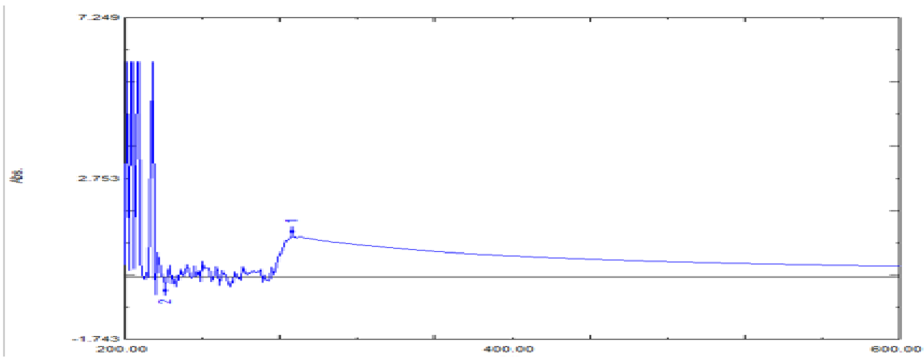

Figure 2. ZnO photonic crystals using Zinc acetate and Bee honey.

4.2 FOURIER TRANSFORM INFRARED SPECTROSCOPY:

FOURIER TRANSFORM INFRARED SPECTROSCOPY was used to identify the distinctive functional group associated with green-synthesised ZnO NPs (Ioannou, 2023, Mohamadzade, 2023; Gashti, 2023) The presence of distinctive functional groups in the synthesised nanoparticles were shown by the peaks. The samples absorb radiation between 3401.93 and 2928.04 cm^{-1} cm-1, 1560.30 and 1397.05 cm^{-1} , 1340.61 and 1046.19 cm^{-1}, 932.27 and 830.93 cm^{-1}, 679.46 and 616.16 cm^{-1}, and 471.84 and 471.85 cm^{-1} in the infrared spectrum. Metal-oxygen (ZnO stretching Vibrations) correspond to the absorption maxima between 700 and 400 cm^{-1}. (Mallikarjunaswamy, 2020) (Haque *et al*., 2020). The various peaks from FT-IR confirms the presence of carboxyl group the peak at 2928.04 cms^{-1} and 3401.93 cms^{-1} attributed to the hydroxyl group's vibrational modes (Gudkov *et al*., 2021)

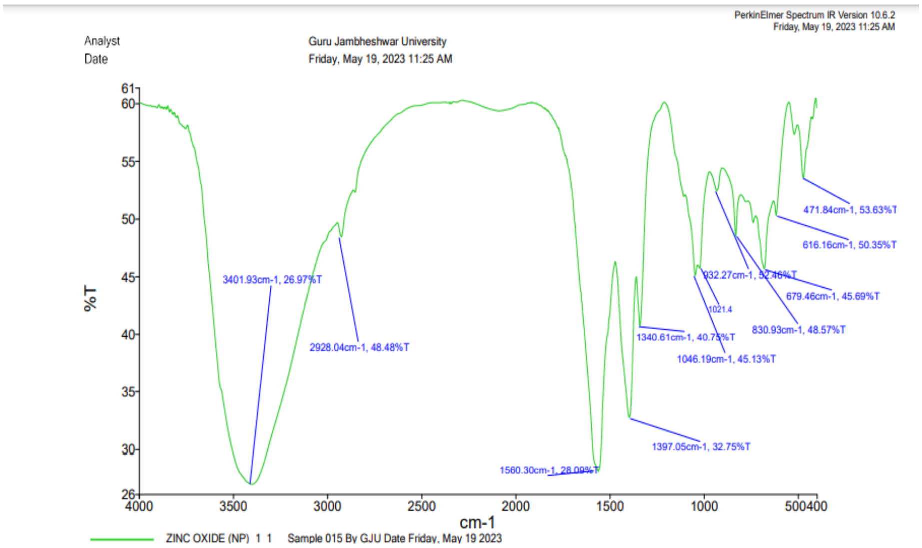

Figure 3. The Fourier-transform infrared (FT-IR) spectra of zinc oxide nanoparticles (NPs) synthesised using honey as a capping agent.

4.3 Microscopy by use of transmission electron beams (T.E.M):

Figure 4 displays transmission electron microscopy (TEM) images of ZnO (NPs). A Transmission Electron Microscopy (TEM) analysis was conducted to determine the size and crystalline structure of ZnO (NPs) fabrication through eco-friendly sustainable methods. The transmission electron microscopy (TEM) images illustrate the significant dimensions and spherical morphology of the nanoparticles. The data presented in the images suggests that the ZnO NPs exhibit a size range of approximately in between 100-200 nm.

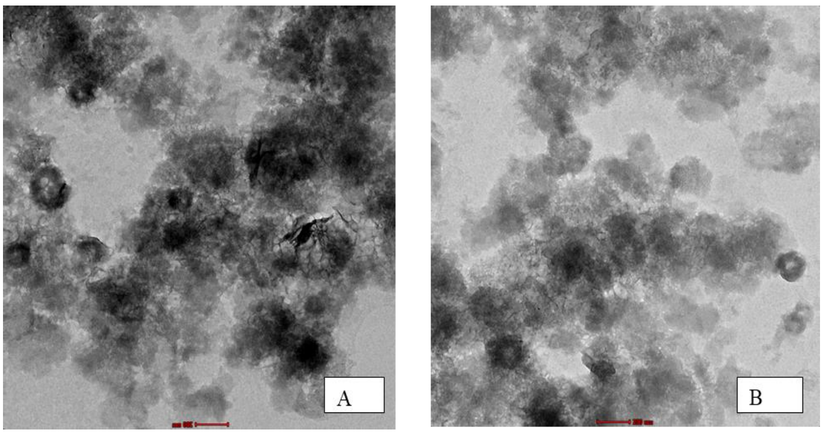

Figure 4. Illustrating pics of T.E.M of honey capped Zinc oxide nanoparticles a) T.E.M image of size 200nm, b) T.E.M image of size 100nm.

5. Antibacterial activity

Metal and metal oxide NPs can show antibacterial effects both directly by adhering to the bacterial membrane and indirectly by producing Reactive Oxygen Species (ROS) (Alavi *et al*., 2021 and 2022) the whole antibacterial process for ZnO NPs remains uncertain. However, many antibacterial executions of ZnO NPs have been investigated. Formation of Reactive Oxygen Species has been found to be an important component in the bactericidal action of ZnO NPs (Gold *et al*., 2021). ZnO NPs cause structural changes in the bacterial cell membrane also, electrostatic forces cause metal oxide NPs to adhere to the bacterial surface, where they can then cause instantaneous and fatal damage. Zinc oxide nanoparticles (NPs) have antibacterial properties that scale with particle size, and the release of antimicrobial ions like ZnO nanoparticles can compromise the integrity of bacterial cells. (Ansari *et al*., 2020). ZnO nanoparticles (NPs) were synthesised cheaply and their zones of inhibition were examined in a disc diffusion agar assay, and their average diameters were, For *E.coli* for 50 µg/mg ZOI (Zone of Inhibition) was 22mm wide and 21mm deep, but when solution poured was of 100µg/mg ZOI was 23mm wide and 21mm deep, so no much difference could be observed. For *Bacillus megaterium* for 50µg/mg ZOI were 17mm wide and 20 in depth and for 100µg/mg ZOI were 35mm wide and 25 in depth was observed (Figure 5).

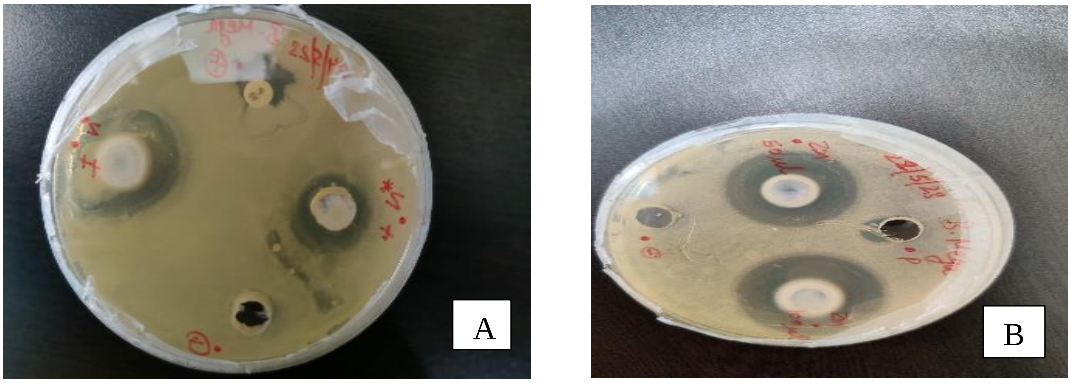

Figure: 5. Antimicrobial burst of ZnO NPs A. ZnO on *E. coli*, B. ZnO on *Bacillus megaterium*.

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