

Bio Fabrication of Zinc oxide nanoparticles using Dillenia indica (Elephant apple) Core extract and its Antimicrobial properties.

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Abstract

At present, the focus on bringing change via green synthesis of nanoparticles is peak field in research and development. In this study, biosynthesis of ZnO nanoparticles from fruit gel extracts of *Dillenia indica* natively known as "ou tenga" is used as a key reducing agents and reported as eco-friendly, rapid, and cost-effectiveness. The Characterization for synthesized nanoparticles were done by transmission electron microscopy (TEM), energy-dispersive X-ray spectroscopy (EDX), UV–visible spectroscopy (UV–vis), X-ray diffraction (XRD) and fourier transform infrared spectroscopy (FTIR). The nanoparticles were exclusively ZnO, beaned shape with dimensions scaling from 12 nm to 15 nm. In current work, the green synthesized ZnO nanoparticles have been engaged for antimicrobial activity. The antimicrobial activity of characterized samples was determined using different concentrations of biosynthesized ZnO nanoparticles 10 ml, 20 ml, 30 ml, and 50 ml against Gram-positive bacteria *Staphylococcus aureus* (ATCC-BAA976) and fungi *Aspergillus niger* (ATCC-10535) 50 ml via well diffusion technique, grown using broth incubation method. The analysis revealed that the bacterial inhibition escalates with increasing concentration of bio derived - ZnO nanoparticles. Likewise, fungi *Aspergillus niger* was apparently sensitive to a set proportion of the nanoparticles. In this research, the idea of amalgamating beneficiary gel with ZnO retaining the biochemical properties of the gel, post-synthesis, combining the theme of tradition and science, this can play a crucial role in the eliminating prime skin infection and dandruff causing microbes, and use in some sort of cosmetic and skin health products.

1. Introduction

Anything which is particle with dimensions greater than 1 nm and smaller than 100 nm in two or three dimensions is termed as "Nanoparticles".(1) The most rampant one of it, is the "Green Synthesis". This is a ground level approach is used since its user friendly, easy to use, low cost and low environmental risk. (2) Studies found that biological agents has proven to be exceptional reducing and capping agent for synthesis of nanoparticles,(3) whereas, the field of other conventional metal ion nanoparticles has shown significance, although these have several side effects such as emission of toxins reported recently.(4, 5) Zinc oxide (ZnO) belongs to a broad category of inorganic metal oxides that exhibit a variety of nanostructures. These oxides are distinguished by their ability to act as photocatalysts and photo-oxidizing agents, proving effective against a range of chemical and biological materials.(6)

Therefore, synthesising green nanoparticles, such as Zinc Oxide nanoparticles (ZnO-NPS) has proven its worth in research due to their applications in drug delivery, used as Nanomedicine, Cell Biology, Pharmacology, Microbiology, Food Industry, Antioxidants, Molecular Biology, Catalysts, Antimicrobial Agents. (7–9)

Moreover, Zinc oxide NPs has been given the status of "GRAS" (Generally Recognized as Safe) by the US FDA.(10) that is safe for human use.

The main ingredient used while synthesising the nanoparticles was a gel-based extract acquired from fruit core of Elephant apples, or *Dillenia indica* Linnaeus, are a valuable medicinal plant that may be

easily found in Assam, North East India. They belong to the Dilleniaceae family.(11) The portion of the fruit that cannot be used is the core, sometimes referred to as the pulp, and it must be removed.(12) but again traditionally, Assamese people in northeastern India have long utilized the fruit's mucilaginous material as a cosmetic to lessen dandruff (13), this fruit is found has shown phenomenal attributes carrying antioxidant attributes, phytochemical compounds such as β -sitosterol, kaempferol, stigmasterol, quercetin, rhamnetin, myricetin, dillenetin, and betulinic acid, rich in phenolics, ketones, phytosteroids, alcohols, anthraquinones, and triterpenoids and, again traditionally, native in northeastern India, Assam have long utilized the fruit's mucilaginous material as a cosmetic to lessen dandruff.(13–15) Also, *D. indica* is widely utilized for various medicinal purposes, including the treatment of hair loss. Additionally, its fruit are employed in managing skin conditions such as leukoderma, eczema, itching, and rashes. (16)

Existing records suggest the good antimicrobial affects of biosynthesized ZnO NPs with amalgamation of other plant and fruit based extract. And being a conductor able to retains the biochemical properties of the plant which again helps to create a synergy, often seen in-between those molecules is what provides the benefits from plant-based synthesis.(17–19) also previously studies proves that nano based materials derived from plant extracts retains the biochemical properties of the plant which again helps and there is evidence now that the synergy of those molecules is what provides the benefits of plant-based synthesis.(20)

Keeping this in mind we wanted study the inhibitory action of nanoparticles (ZnO NPs) against *Staphylococcus aureus* and fungi *Aspergillus niger* via quantitative evaluation of growth against the conjugated ZnO NPs in culture media.(21, 22) It mostly regarded that the identified active oxygen species formed by these metal oxide particles have a mechanism by which their antibacterial activity is observed. On other hand the fungal species, ZnO NPs have been witness many times as potential antifungal agents.(23–25)

Therefore, the primary goal of this study in earnest of a bio-based product which could help tackle the affects of skin affiliated disease caused by *S. aureus* and *A. niger*(26, 27)

2. Materials and Methodology

2.1 Collection of materials

The fruit “Elephant apple” (*Dillenia indica*) were gathered from the domestic market of Dispur, Kamrup Metropolitan district. Zinc Acetate Dihydrate [$\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$] and sodium hydroxide [NaOH], MH agar and MH Broth (Mueller Hinton), Czapek dox agar and PDA (potato dextrose agar) were bought from HiMEDIA.

2.2 Preparation of Aqueous Gel extract from fruit core of *D. indica*

The preparation of *Dillenia indica* gel extract via cutting the fruit to expose the core, followed by the disposal or repurposing of the skin.(12) The core was thoroughly washed 3–4 times with deionized water to remove any bugs or parasites, which are commonly found in these fruits. Ensuring the core's health, it was then pricked with a sterile pin washed in 70% ethanol. The gel was extracted from the incision point and collected in a sterile petri dish.

To separate the seeds from the gel, sterile lab tweezers were used manually. The viscous gel was subsequently diluted and blended with autoclaved distilled water at a 1:3 ratio (1 part gel to 3 parts deionized water). This mixture was blended at 3000 rpm for 20 minutes to achieve optimal consistency. The resulting solution was filtrated through Whatman filter paper and stored at 4°C for future.

2.3 Bio fabrication or green synthesis of zinc oxide nanoparticles using *Dillenia indica*

In an Erlenmeyer flask, a 25 mL, 2M solution $[\text{Zn}(\text{CH}_3\text{COO})_2] \cdot 2\text{H}_2\text{O}$ (Zinc Acetate dihydrate) was prepared. Separately, 25 mL of 2 M NaOH (sodium hydroxide) was prepared supplied by Hi Media and both solutions were mixed in a 200 mL beaker. Then, 5 mL of the gel extract was added dropwise to the solution while stirring continuously with a magnetic stirrer for about 2 hours, resulting in a foamy white precipitate. The solution in the beaker was covered with aluminium foil during the stirring process. The precipitate was filtered and washed repeatedly with deionized water followed by ethanol treatment to remove any contaminants. The resulting white powder was obtained after drying the purified precipitate at 60°C in an oven overnight via crushing the paper in a petri dish collected autoclaved vial.(6)

2.4 Characterization of Zinc oxide NPs

The preliminary characterization was done UV–vis spectrophotometer for optical properties of the biosynthesized zinc oxide nanoparticles were evaluated using (Model UV 1900 SHIMADZU) ranged set to 200–800 nm per use. For size and structural details ZnO NPs were investigated using transmission electron microscopy. (JEM-2100 PLUS (HR), Jeol). For TEM sample preparation, a small drop of zinc oxide nano powder solution were positioned onto a TEM grid coated with a continuous carbon film. Then the powdered NPs were dried under vacuum settings. To find elemental makeup of the bio fabricated ZnO nanoparticles was estimated by energy-dispersive X-ray spectroscopy (Bruker). The synthetic zinc oxide nanoparticles were dissolved in ethanol and then applied to copper grids to prepare samples for Energy Dispersive X-ray (EDX) examination. Using an X-ray diffractometer XRD (D8 Advance, Bruker) the crystalline structure of the biosynthesized zinc oxide nanoparticles was examined. CuK α radiation operated at 40 kV and 40 mA was used to record the XRD spectrum from 20°- 80° 2 θ angles. In order to investigate and identify surface functional groups, the biosynthesized zinc oxide nanoparticles' Fourier transformer infrared spectroscopy (FT-IR) spectra were recorded at room temperature using an FT-IR spectrometer (Model: Spectrum Two, Make: PerkinElmer). A little quantity of powdered zinc oxide nanoparticles was applied to a circular zinc selenide plate for FT-IR spectrum analysis in order to conduct FT-IR measurements. The range taken were 4000 – 400 cm^{-1}

2.5 Antimicrobial assay

Agar medium were readied by sterilizing through an autoclave in 15 psi and $121 \pm 2^\circ\text{C}$ for 30 minutes and then moved to a laminar air flow cabinet top sustain its sterility. Czapek Dox agar was decanted into one petri plate and Muller Hinton agar into another, then left for 10 minutes to solidify. An overnight culture of *Staphylococcus aureus* was smeared on the surface of Muller Hinton agar the plate, while *Aspergillus niger* was spread on the Czapek Dox agar plate. Wells were prepared using a cork borer. In the bacterial plate, 10 mg, 20 mg, and 30 mg of ZnO NPs were added to the wells. In the fungal plate, 50 mg of ZnO NPs was added to the wells. The plates were sealed and incubated at 37°C for 24 hours for the growth of bacteria and at 25°C for 48 hours for the growth of fungi.

3. Results and Discussion

3.1 Ultraviolet- Vis Spectroscopy of Bio fabricated ZnO NPs

The preliminary investigation of characterization was initiated by performing UV-vis spectroscopy, after successful fabricated 2M ZnO by *D. indica*, range set as 200 nm – 800 nm. Observation of various a no. of peaks from 300 to 366 nm which means the compounds associated with the fruits which falls in this ranage and the hight to be 365.9 which again perfectly falls within the range of ZnO, confirming the UV characterization (28). My research suggests that The UV-Vis spectrum of ZnO nanoparticles typically ranges from 350 to 380 nm, with peaks around 370 nm(29, 30), but this can vary depending on the fabrication technique and optimized parameters used. The absorption characteristics are influenced by factors such as doping concentration, method of preparation, and contaminants, which can extend the observable range from 250 nm to 390 nm. There is no single optimum exciton peak for ZnO nanoparticles. To further validate the optical properties, it is essential to measure the bandgap value using a Tauc plot and determine the Urbach energy level.(31, 32)

3.2 FTIR Spectrum Analysis of Biologically Synthesized Zinc Oxide Nanoparticles

The FTIR spectrum of biofabricated ZnO NPs unveils characteristic peaks that signify the occurrence of various functional groups and bonds. The peak at 3381.94 cm^{-1} is associated with O–H stretching vibrations, suggesting the presence of adsorbed water or surface hydroxyl groups on the ZnO nanoparticle.(33, 34). At 1549.86 cm^{-1} , the peak is attributed to N–H bending (amide II) and C = C stretching vibrations from aromatic rings, indicating protein residues or organic compounds as capping agents.(35) The peak at 1393.80 cm^{-1} corresponds to C–N stretching, O–H bending and S = O, which could be from aliphatic amines and residual organic molecules from the synthesis process.(36) The 1020.95 cm^{-1} peak signifies C–O stretching vibrations, typically associated with alcohols, ethers, or phenolic groups, consistent with biologically synthesized ZnO nanoparticles.(37) Peaks at 669.18 cm^{-1} and 440.89 cm^{-1} confirm the Zn–O stretching vibrations, characteristic of ZnO nanoparticles.(38, 39)

Table 1
FTIR analysis of ZnO NPs

Absorption (cm^{-1})	Groups	Compound class
3381.94	O-H stretching	Alcohol
1549.86	N-O stretching	nitro compound
1393.80	S = O stretching	sulfonyl chloride
1020.95	C-F stretching	fluoro compound
669.18	C = C bending	Alkene
440.89	C-C bending	Cycloalkane

3.2 XRD analysis

X-ray diffraction (XRD) analysis was meticulously carried out utilizing the sophisticated Bruker D8 Advance instrument complemented with a cutting-edge LynxEye detector and a precision-engineered Cu K-alpha X-ray source sourced from BRUKER AXS, a renowned entity based in Germany. The instrument calibration procedure was meticulously overseen by a skilled service engineer, ensuring optimal performance. Data acquisition was rigorously executed in Two Theta-Omega mode, employing a precise step size of 0.05 and a duration of 0.20 seconds per step, all meticulously controlled at a constant temperature of 24°C. The specimen under investigation, designated as SH ZN NPs, underwent thorough analysis following the established standard operating procedures recommended by the reputable company. The peaks (100) (101) (002) (102) (110) (201) were found after plotting graph which confirms the 2θ values listed for the common planes of ZnO (wurtzite structure) match those typically found in JCPDS (Joint Committee on Powder Diffraction Standards) cards for ZnO. Specifically, these values correspond to JCPDS card no. 36-1451.(40) (41)(42)(43)

Table 3
for XRD

Intensity (a.u)	2 θ	FWHM (in nm)	Peak reflection
920	31.06°	0.560053	$\approx$ (100)
3123.8	33.18°	1.4029	$\approx$ (101)
1226	34.94°	0.747925	$\approx$ (002)
1108.2	36.93°	1.78651	$\approx$ (101)
890.42	47.71°	2.69861	(102)
1624.2	59.20°	1.62376	(110)
564.12	69.57°	2.7179	$\approx$ (201)

Calculation of d-spacing

Using Bragg's Law ($n\lambda = 2d\sin\theta$ $\lambda = 2d\sin\theta$) with $\lambda = 1.54$ Å (Cu K α radiation):

Observed 2 θ (°)	Observed d- spacing (Å)	Theoretical d- spacing (Å)	Miller Indices (hkl)
31.06	2.838	Not listed	-
33.18	2.698	Not listed	-
34.94	2.563	Not listed	-
36.93	2.431	Not listed	-
47.71	1.906	1.912	(102)
59.20	1.560	1.538	(110)
69.57	1.345	Not listed	-

The observed peaks at 47.71° and 59.20° match well with the theoretical d-spacings for the (102) and (110) planes of an hcp structure, suggesting that your zinc sample has an hcp crystal structure. The other peaks need further investigation, potentially considering additional hcp planes or other factors.

3.3 EDX spectrum of Bio Fabricated ZnO NPs

Figure 8 shows the EDX pattern of the Zn NPs prepared by green synthesis method. The peaks 150 eV shows a strong EDX signal which correspond the binding energy of Zn. There were signals for Cu, which might suggest of some impurities present .

Table 4

Element	Atomic No.	Netto	Mass (%)	Mass Norm. (%)	Atom (%)	Abs. error (%) (1 sigma)	Rel. error (%) (1 sigma)
Carbon	6	3758	20.402	20.402	54.712	0.722	3.537
Oxygen	8	1086	3.419	3.419	6.883	0.171	4.999
Copper	29	22743	61.632	61.632	31.240	1.925	3.123
Zinc	30	5014	14.546	14.546	7.165	0.513	3.528

3.4 Transmission Electron Microscope (TEM)

The TEM assessment reports indicate the characteristics of the bio-fabricated ZnO nanoparticles, including their size, shape, and morphology. It shows that the zinc nanoparticles are mainly beaned-rod shaped and, in a bulk, although the crystalline structure could be seen in Fig. 7. but some of them, as shown in Fig. 6–7, were found to have irregularly shaped structures. Particle size ranged from 12 to 80 nm. Previous findings suggest that variations in bio fabrication techniques commonly result in differences in the size and shape of the synthesized nanoparticles.(44, 45)

Different sizes of cystalline structure of ZnO NPs seen and measured in Fig. 11.

Figure 9 Shows a similar yet confirming bright coloured rings along with the brightest dots compared it other ZnO NPs reports by (46)

3.5 Antimicrobial activity of copper nanoparticles and zinc nanoparticles

Antimicrobial properties of ZnO nanoparticles were scrutinized using Agar well diffusion method versus gram positive *Staphylococcus aureus* (BAA976) and *Aspergillus niger* (10535).

50 µL of culture (*S. aureus*) were smeared and with a sterile stainless stell spreader spread on the MHA (Mueller Hinton agar) plates, maintained for 15 minutes at 4°C to allow, and then then Bio- Fabricated ZnO NPs were incubate in the punched well respectively for 24 or 48 hours at 37°C in bacterial incubator (Digiquel DQ-BOD). Zones of inhibition were seen around the wells of zinc oxide3w nanoparticles after the incubation period, about 30 mg, 20 mg, and 10 mg ZnO NPs formed zones of 0.6 cm, 0.5 cm, and 0.4 cm approximately, respectively.

Similarly, for the fungi, the sample first incubated for 15 minutes at 4°C to allow diffusion, and then for 48–72 hours at 25°C in the Fungal BOD (Digiquant DQ-BOD). Zones of inhibition was formed and measured around 0.8 cm, after the incubation period.

Discussion

Here Gel extract from *Dillenia indica* was prepared and used for the synthesis of metal nanoparticles. The bio fabricated ZnO NPs are synthesized. Zinc oxide has the benefit of being inexpensive and widely available, making the cost of generating zinc nanoparticles affordable.

The characterization was also done using Ultraviolet-Visible Light Spectroscopy, FT-IR spectroscopy and TEM. It demonstrates that the ZnO NPs were in bulk and affixed, it varied and size in-between fluctuated, displayed TEM and XRD results, which may be due to agglomeration of powder(47) and which were primarily beaned-rod in morphology, however the ZnO NPs revealed to have inconsistent morphology, as observed in Fig. 7. The particle size ranges from 12 nm – 80 nm. The Miniature size range nanoparticle were then most observed, although agglomerated.

ZnO NPs were also tested for antimicrobial activity against the bacteria *Staphylococcus aureus* and fungi *Aspergillus niger*. In the present study both *Staphylococcus aureus* and *Aspergillus niger* were non-resistant towards ZnO NPs. Due to which the inhibition zones around the wells are found as shown in Figs. 10 and 11. The diameter of the inhibition zone was 16 mm against *A. niger*. Against *S. aureus*. similar results are reported by 8 mm, 10 mm and 12 mm with increasing concentrations.(48–50)

Also previous studies the fruit *Dillenia indica* has shown antioxidant attributes as well as as well as anti-inflammatory and pain-relieving properties.(51, 52) Tuning to that, extracting the core gel with methods shown by (12) has certain problem in making the gel dilute and thus with the golden ratio of 1:3, where 1 part of gel was blended with 3 parts of distilled water.

The gel is being used by the local people of Assam as a traditional remedy and cosmetic for hair.

4. Conclusion

The study revealed that “Gel Extract” from fruit core of *Dillenia indica* was utilized to make Bio fabricated ZnO nanoparticles in a simple, quick, and ecologically responsible way. The outcome of absorption and colour change, it turned the solvent slurry to white foam and then dried powder, the ZnO nanoparticles developed as a result. And the peak at 365nm in UV-vis spectroscopy proved it, after 24 hours from synthesis. The presence of several vibrational functional groups was evident in the FTIR spectra. And for further characterization XRD, EDX and TEM results indicate the formation beaned-rod shaped ZnO nanoparticles and their crystalline structure. The size of Bio fabricated ZnO NPs were ranged from 12–

80 nm. The synthesized ZnO nanoparticles displayed increasing zone of inhibition with increasing concentration against *S. aureus* and *A. niger*. ZnO nanoparticles serve as a promising medicine as the amalgamation of tradition and elemental science has potential for being an antimicrobial agent (drug) because of its susceptibility to different skin infections, bio-inspired manufacturing, and biocompatibility

Declarations

Author Contribution

S.H. and S.A wrote the main manuscript and D.S. and G.R.S. prepared figures 1-11.

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Data Availability

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Table 2

Table 2 is not available with this version.

Figures

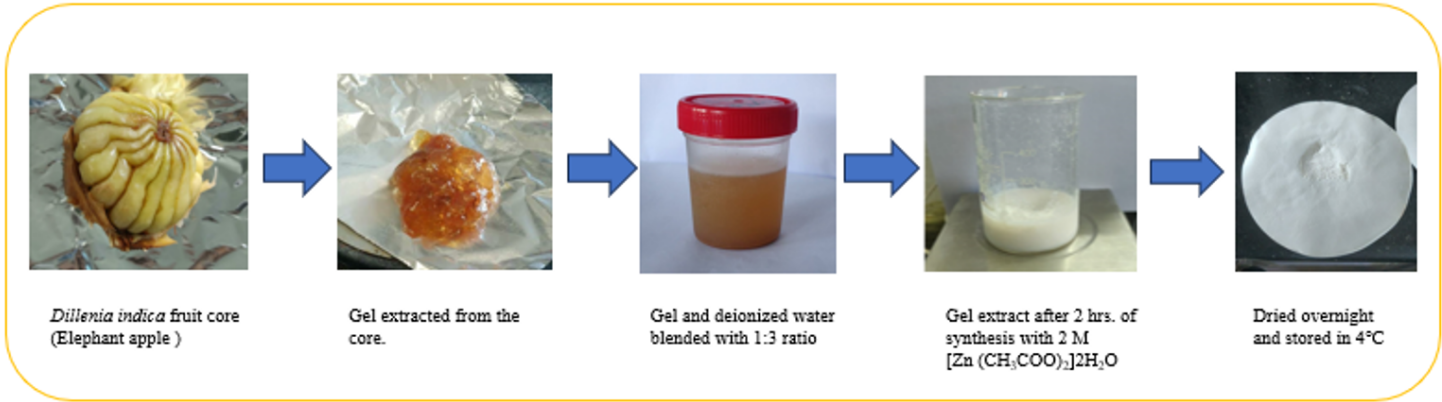


Figure 1

Bio fabrication of ZnO NPs by *Dillenia indica* gel extract.

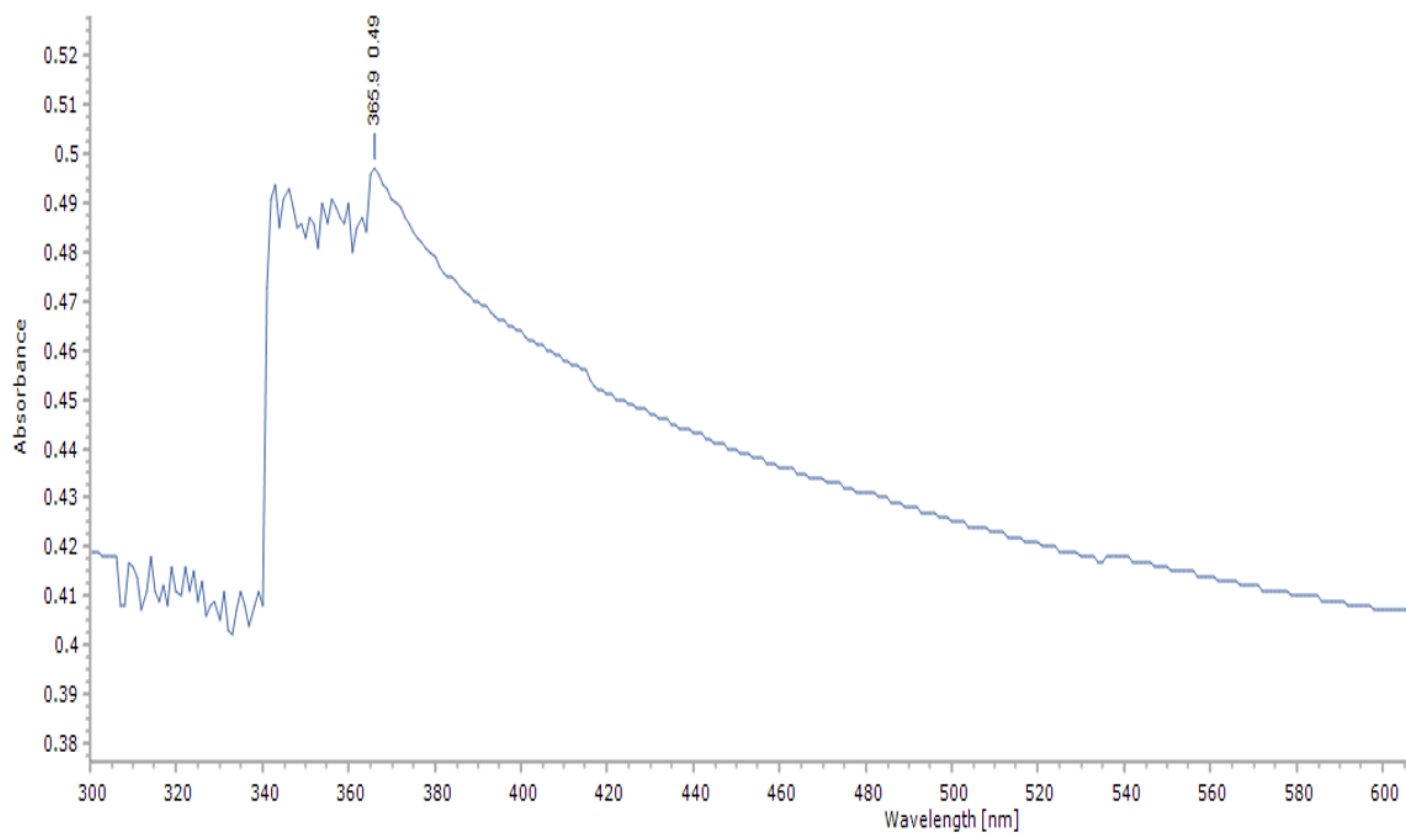


Figure 2

UV spectrophotometry spectrum of ZnO NPs

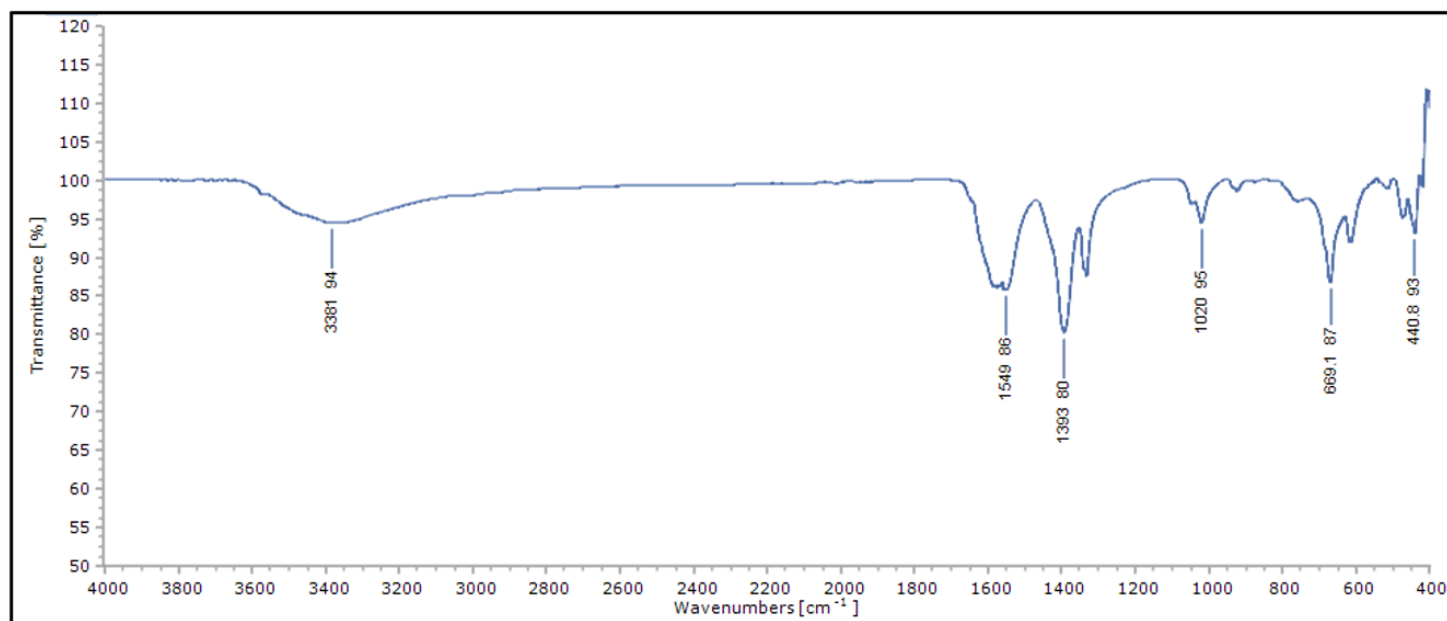


Figure 3

FT-IR spectrum of ZnO NPs

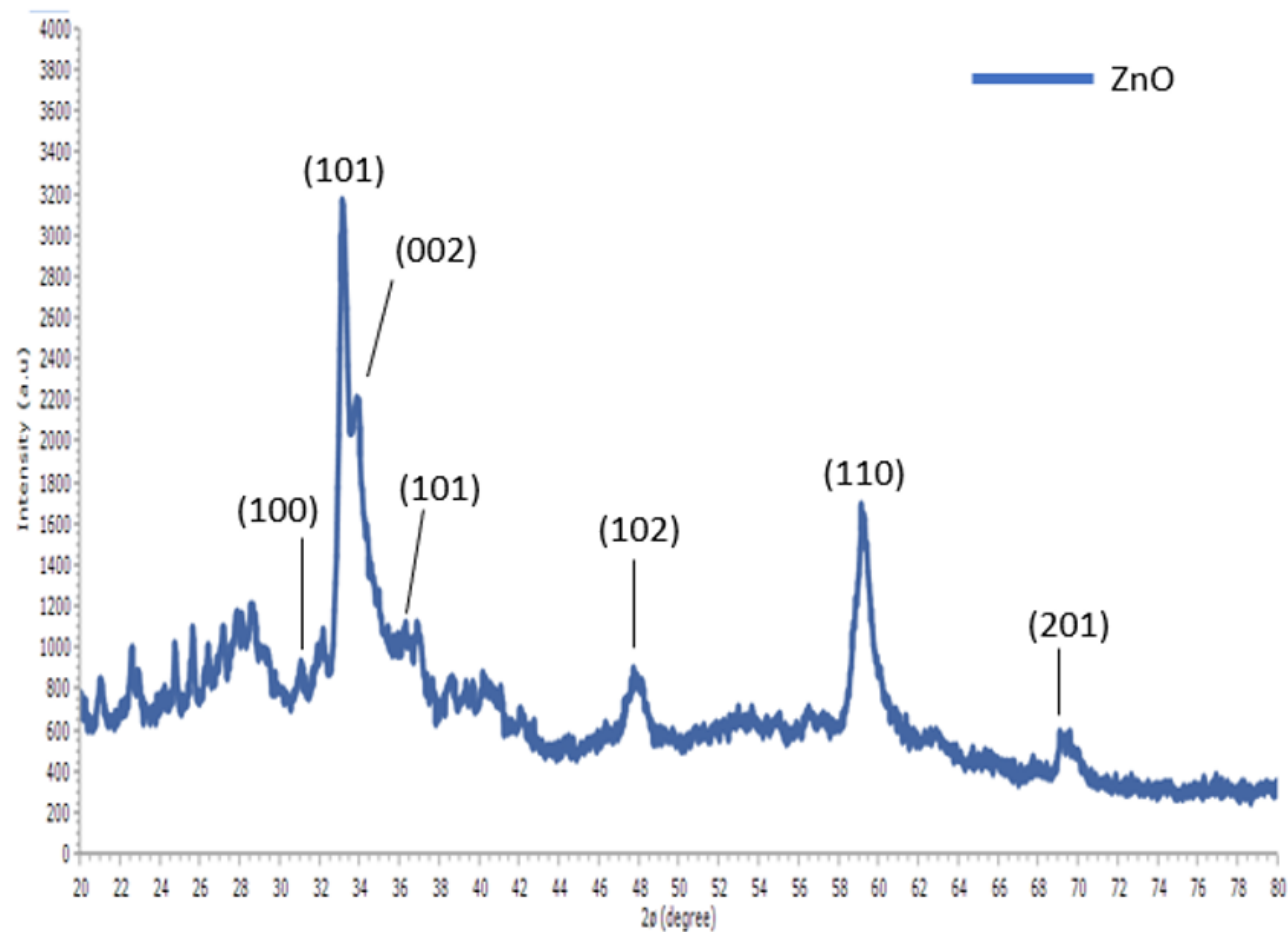


Figure 4

XRD pattern of Biofabricated ZnO NPs

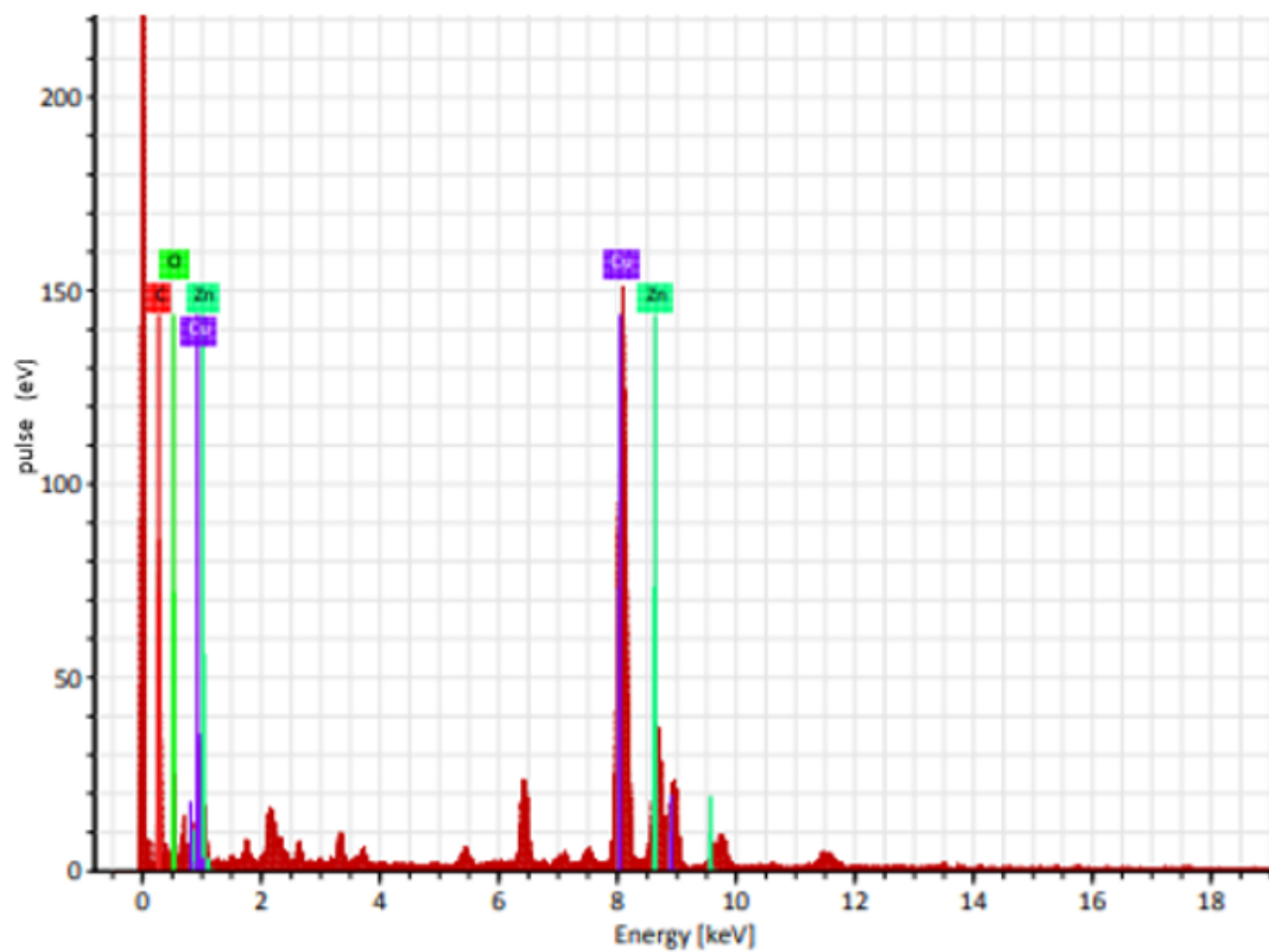


Figure 5

EDX of ZnO NP

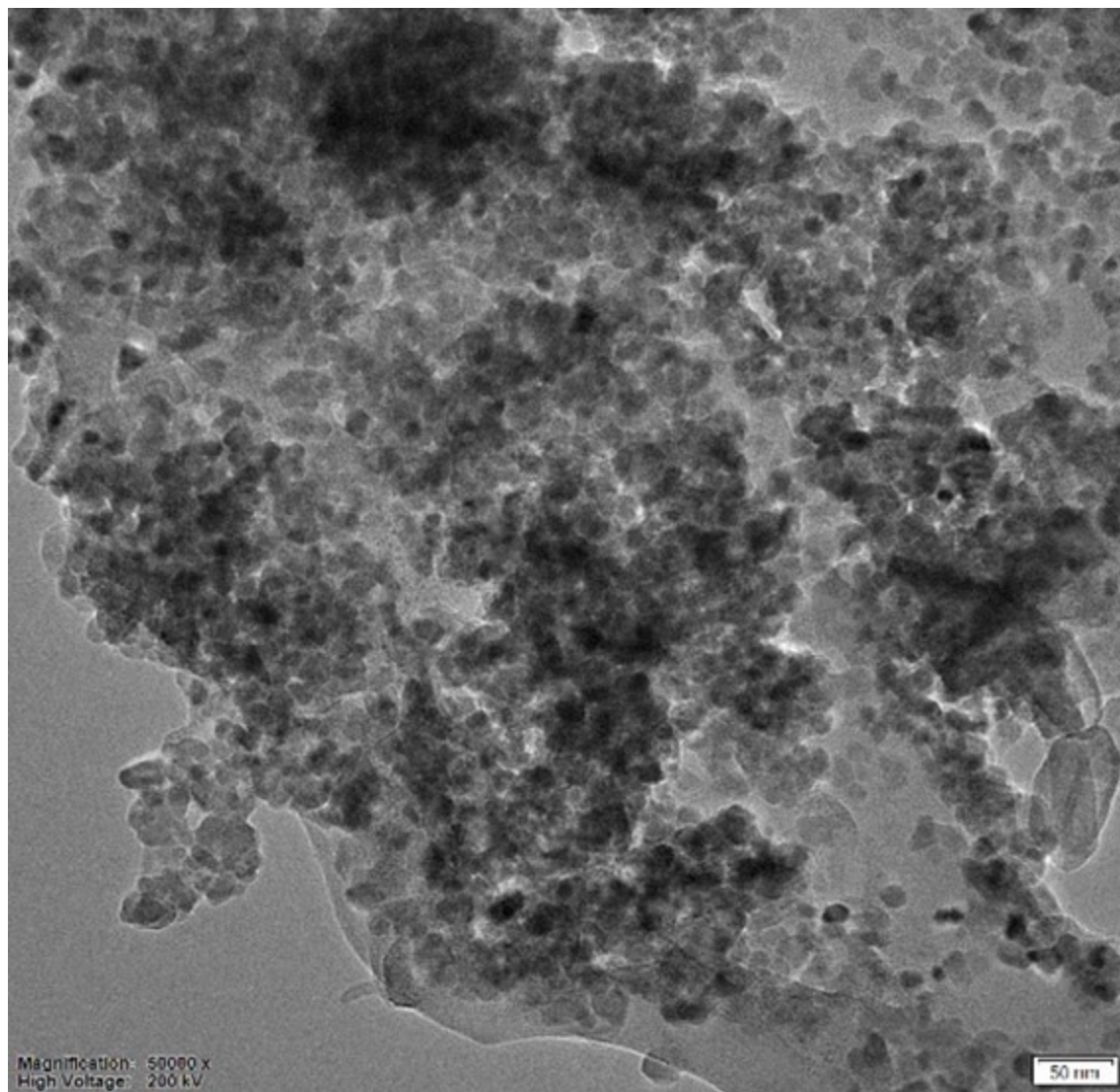


Figure 6

TEM image of ZnO NPs(in 50nm mag.)

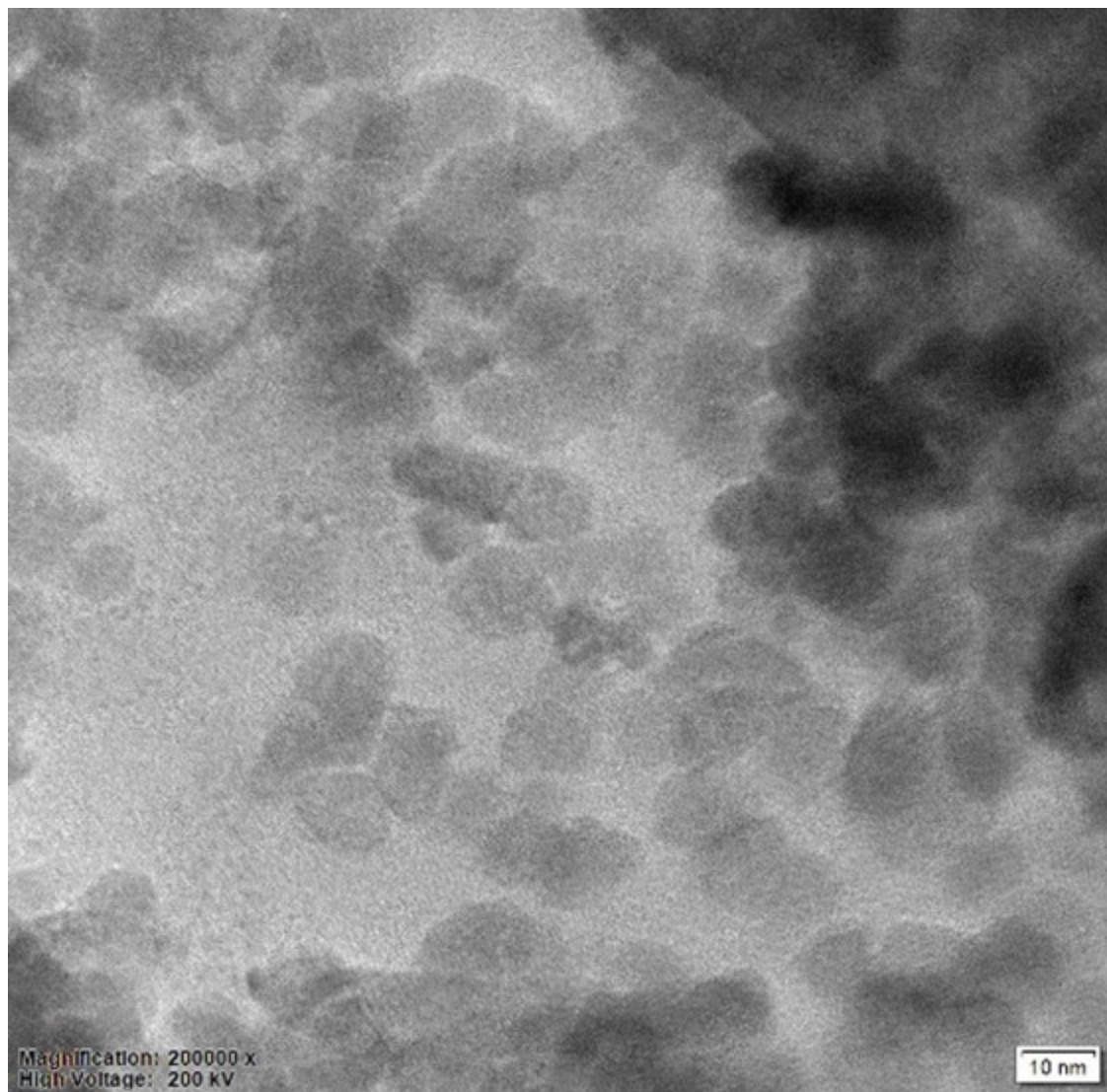


Figure 7

TEM image of ZnO NPs(in 10nm mag.)

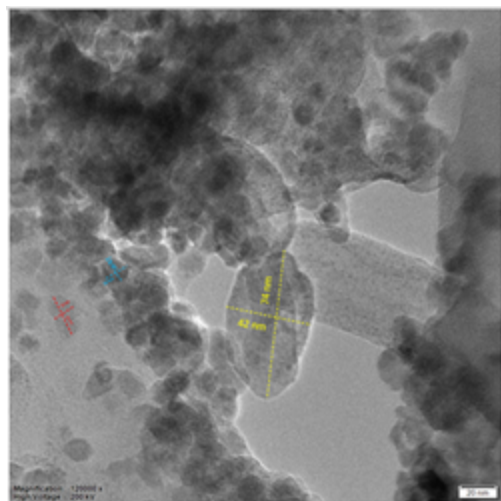


Figure 8

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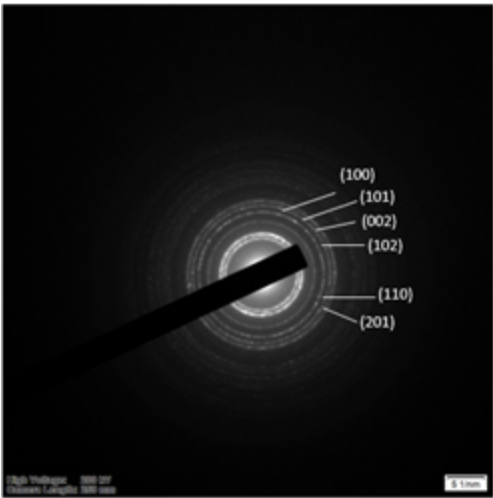


Figure 9

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Figure 10

ZnO NPs against *S. aureus*

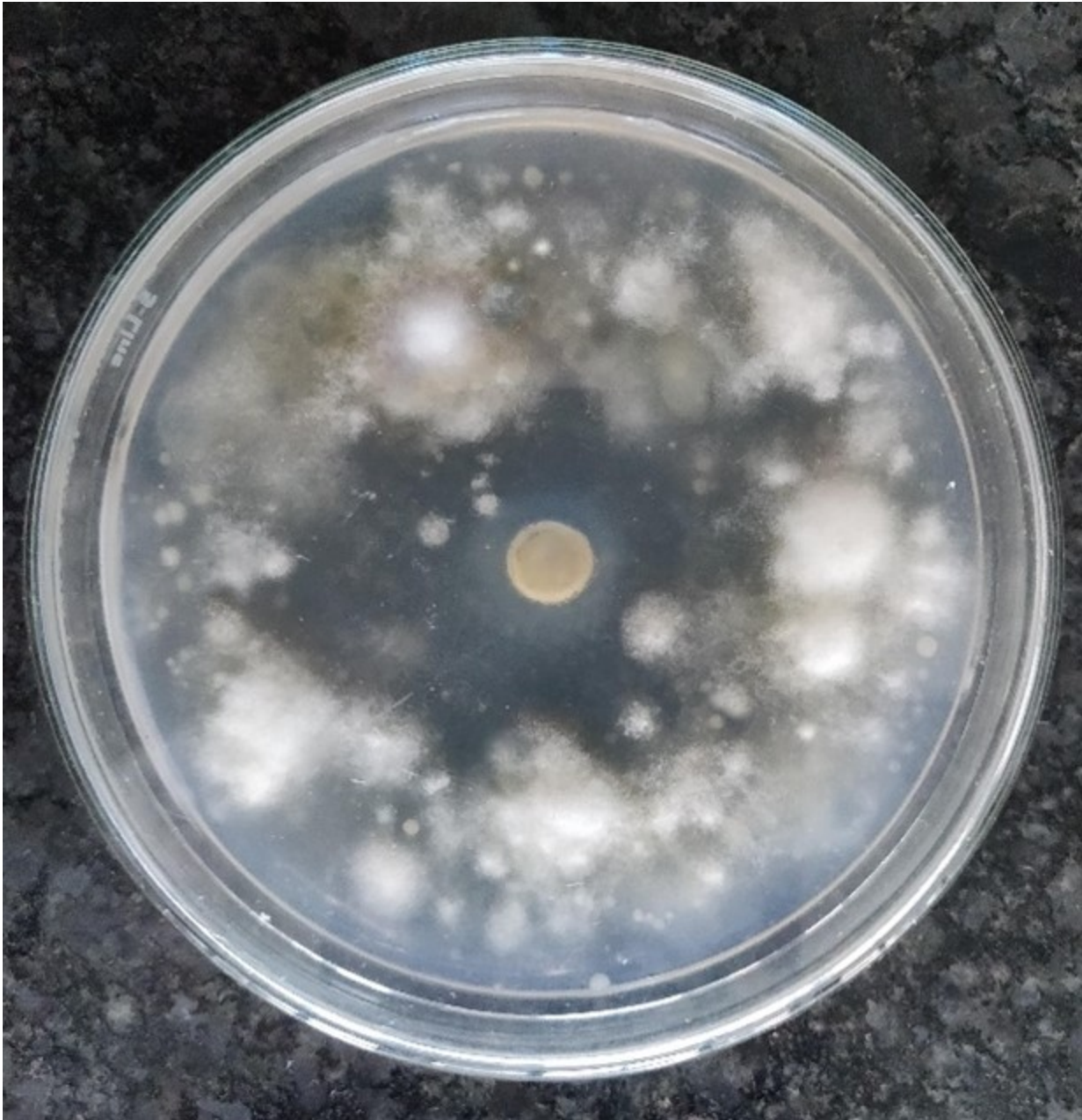


Figure 11

ZnO NPs against *A. niger*