M.Tech Industrial Biotechnology [SHRUTIKA CHAUDHARY] 2023

# PHYCOSYNTHESIS OF ZINC OXIDE NANOPARTICLES FROM *CHLORELLA SP.* AND ITS CHARACTERIZATION AND APPLICATION

A DISSERTATION

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OF

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Submitted by

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**MAY 2023**

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## CANDIDATE'S DECLARATION

I, Shrutika Chaudhary, Roll no. 2K21/IBT/09, student of M.Tech Industrial Biotechnology, hereby declare that the project Dissertation titled **"Phycosynthesis of Zinc Oxide Nanoparticles from *Chlorella sp.* and its characterization and application"** which is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfilment of the requirement for the award of the degree of Master of Technology is original and not copied from any source without proper citation. This work has not previously formed the basis for the award of any Degree, Diploma Associateship, Fellowship or other similar title or recognition.

Shrutika

**Place: Delhi**

**Shrutika Chaudhary**

**Date: 30/05/2023**

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## CERTIFICATE

I hereby certify that the project dissertation titled **"Phycosynthesis of Zinc Oxide Nanoparticles from *Chlorella sp.* and its characterization and application"** which is submitted by Shrutika Chaudhary, Roll no. 2K21/IBT/09 Department of Biotechnology, Delhi Technological University, Delhi in partial fulfilment of the requirement of the award of the degree of Master of Technology, is a record of the project work carried out by the students under my supervision. To the best of my knowledge this work has not been submitted in part of or full for any Degree of Diploma to this University or elsewhere.

08/06/2023

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## ABSTRACT

*Chlorella sp.* are bio-factory of several bioactive compounds which aid in the formation of many nanoparticles (NPs). The bioactive compounds present in algae are fucoxanthin, carotenoid, phycocyanin, etc. These bioactive compounds help in the diminution of metal precursor to their respective metal/metal oxide NPs. NPs synthesized from *Chlorella sp.* are biocompatible, ecofriendly, and non-toxic. “Phycosynthesis” is the term given to the synthesis NPs from algal species. ZnO NPs are very versatile because they possess biomedical properties like antimicrobial, anti-cancerous, and anti-diabetic activity. Moreover, they also have the ability to degrade pollutants such as heavy metals and dyes. In this report, biological synthesis ZnO NPs is performed from extract of *Chlorella sp.* and characterized using UV-vis spectrophotometer, Zeta sizer to identify the charge and the hydrodynamic size of the ZnO NPs. These ZnO NPs were then used to identify their antibacterial property against *E.coli*.

## ACKNOWLEDGEMENT

A formal declaration of appreciation is insufficient to communicate the depth of my gratitude to everyone who supported me and assisted me in writing my dissertation for Master of Technology. I'd like to express my sincere thanks to Dr. Navneeta Bharadvaja, Assistant Professor in the Department of Biotechnology at Delhi Technological University, for being such a wonderful and helpful supervisor. Without her valuable guidance and encouragement, I would never have finished my dissertation. I'd want to express my gratitude to my seniors, particularly Mr. Lakhan Kumar, Ms. Harshita Singh, Mr. Sidharth Sharma, and Ms. Anuradha for their constant guidance and cooperation because of which I was able complete this project successfully. I would like to express my gratitude to the Head of the Department Prof. Pravir Kumar and the technical staff members Mr. Chhail Bihari, Mr. Jitendra Singh, and Mr. Lalit. I'd also like to thank my college fellows Mr. Abhishek Singh, Sukrit Kashyap, Tanya Singh, and Ms. Ramsha Usman who have been tremendously helpful during the course of my research. Personally, I'd like to thank my family for all their support they've given me throughout my life.

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## LIST OF ABBREVIATIONS

| ABBREVIATIONS | FULL FORMS |
|---|---|
| Au | Gold |
| BG11 | Blue Green 11 |
| CuO | Copper oxide |
| DLS | Dynamic Light Scattering |
| DPPH | 2,2-Diphenyl-1-picrylhydrazyl |
| DW | Distilled water |
| LAF | Laminar Air Flow |
| NPs | Nanoparticles |
| ROS | Reactive Oxygen Species |
| Rpm | Rotations per minute |
| ZnO | Zinc Oxide |
| $ZnSO_4.7H_2O$ | Zinc sulphate heptahydrate |

# CHAPTER 1- INTRODUCTION

Algae are photosynthetically active unicellular/multicellular microorganisms having many beneficial and important bioactive compounds. Depending on size, structure, and components, algae can be both macro/micro ranged. They possess both the properties of plants and microorganisms but do not have well defined structure like plants [1]. They are considered as the bio-factory because of the bioactive compounds present in it such as catechins, astaxanthin, canthaxanthin, lutein, phlorotannins, lycopene etc. which possess many medicinal properties [2]. Therefore, algae can be considered as a good source for in the fields of feedstock, biomedicine, environmental remediation, nutraceuticals, cosmetics industry etc. Apart from these, they have also been used in the past few years as a good source of biodiesel production and in the synthesis of nanoparticles [3].

Nanoparticles are nanoranged materials possessing size range from 1nm to 100 nm. They are synthesized both chemically and biologically. Nanoparticles have huge potential in different sectors for pollution control, nutraceutical industry, disease treatment, activity against microbes, cosmeceutical industry etc. [4]. In recent decades, biological synthesis of nanoparticles attracted many researchers because of its non-toxicity and harmless nature [7]. Biological synthesis of nanoparticles involve synthesis using roots of plants, leaf, and stem, bacteria, fungi, and micro/macroalgae. Biological synthesis was found to be safe, toxins-free, and biocompatible as compared to chemical synthesis of nanoparticles. Microalgae/macroalgae have been exploited for different metal/metal oxide nanoparticles (NPs) synthesis [5]. The proteins, pigments and

bioactive compounds present in algae are of huge importance and responsible for nanoparticles synthesis as well. Apart from green micro/macroalgae, brown (phaeophytes) and red (rhodophytes) also have the ability to synthesize nanoparticles [6]. In various research, it has been reported that algae are good source for nanoparticles synthesis and follow two mechanisms for their production: Intracellular synthesis, and extracellular synthesis. If we talk about intracellular synthesis, synthesis takes place inside the cell via enzymes and proteins present inside the algae whereas, in extracellular synthesis, the whole synthesis takes place at the cell surface or cell wall of the algae [8]. Nanoparticles produced from the extract of micro/macroalgae are the easiest way to synthesize because they do not require any specific culture conditions for growth. The term "PHYCOSYNTHESIS" refers to the synthesis of nanoparticles using algae. Different species of algae have been used so far for the biogenic synthesis of nanoparticles such as *Chlorella sp., Pseudomonas sp., Sargassum etc*. [9].

The three significant stages involved in the Phycosynthesis of ZnO NPs are: (i) preparation of algal extract (ii) metal precursor preparation, (iii) the incubation of extract with the salt solution under optimized conditions [12], [13]. There are many different algal compounds that contribute to the reduction and stability of metal ions [14]. Some examples of these biomolecules include carbohydrates, proteins, mono/polysaccharides, catechins, etc. If we compare this method with other green synthesis methods, the time required for algal-based NPs synthesis is significantly less [15]. Synthesis of algal NPs can take place both outside and inside of the cell [1]. The recovery of produced NPs inside the cells is an extra step that needs to be completed in order for intracellular production of NPs to be possible [16].

Various metal, and metal oxides nanoparticles have been synthesized so far like ZnO NPs, Au NPs, CuO NPs, $Fe^{2+}/^{3+}$ NPs, etc. Among these, ZnO NPs possess many important and beneficial properties as it is an excellent semiconductor, it has electronics, optics, and optoelectronics properties as well [10]. ZnO NPs are well known for its property to work against tumor cells by generating reactive oxygen species (ROS), thus acting as anti-tumor agent. Zinc is considered as an essential nutrient therefore, it has been also been used in sunscreens and other cosmetics products as the protector against ultraviolet radiations (UV Rays) [11].

Green NPs synthesis uses leaves, stems, roots, flowers, and fruits of plants. Plant-based manufacturing is also fast, harmless, environmentally friendly inexpensive, and one-step. Amino acids, vitamins, proteins, and flavonoids reduce, cap, and stabilize nanoparticles [6]. For NPs manufacturing, at first plant extract is prepared and then it is subjected to high temperature after mixing it with precursor metal salt solution for few hours. The period of incubation and NPs structure depend on the nature of extract, metal salt, and amount of salt, pH, contact time, light exposure, and stirring intensity [12]. Bacterial cells need enzymes, protein molecules, and nucleic acids to stabilize, reduce, and cap NPs. Bacterial cell walls have functional groups that remove heavy metals. NPs are synthesized by growing bacterial cultures, adding metal precursor, and stirring or incubating at static conditions [13]. UV-Vis spectrophotometers track NPs formation [9]. *Pseudomonas aeruginosa*, *Escherichia coli*, *Lactobacillus*, etc. are often used to synthesize ZnO NPs [14]. Proteins in fungi reduce and stabilize NPs during production. Fungi are easy to handle, yield well, and leave low-toxic residues [15]. Fungal metabolites boost nanoparticle longevity and biological activity. Fungi and yeasts are

metal-tolerant and easy to work with. Fungi possess 6400 bioactive compounds [10]. Fungi synthesize NPs intracellularly or extracellularly. Mycelia absorb metal salt during NPs synthesis.

Algae are one of the best choice for the production of ZnO NPs due to their simplicity of cultivation, low nutrient requirements, and wide range of bioactive components. The greatest option is algal strains because they can be grown in wastewater. They have also been used in feedstock production, wastewater treatment, NPs synthesis, etc. Figure 1 describes the potential applications, advantages, and limitations of ZnO NPs synthesized from algae.

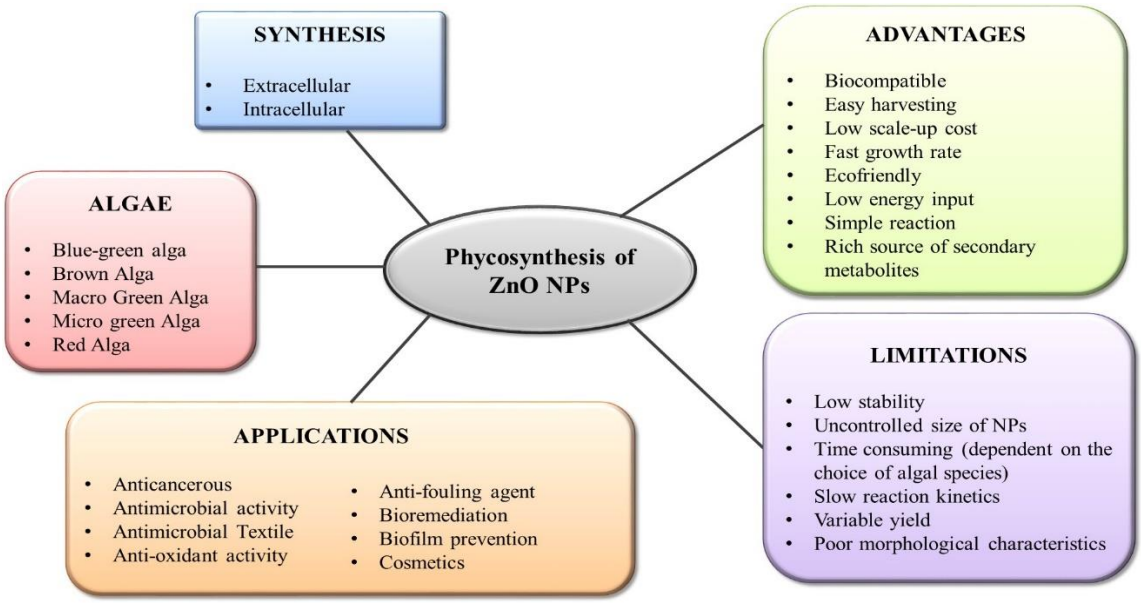


**Figure 1:** Phycosynthesis of ZnO NPs, their advantages, limitations, and applications.

**Aim/Objective of this study:**

1. Phycosynthesis of ZnO NPs using *Chlorella sp.* algal extract.
2. Characterization of phycosynthesized ZnO NPs.
3. Anti-bacterial activity of ZnO NPs against *E. coli*.

# CHAPTER 2: REVIEW OF LITERATURE

## 2.1 Algae: A Bio-factory

There are different types of algae found like Brown, red, and green algae also named as Phaeophyceae, Rhodophyceae, and Chlorophyceae, respectively [16]. The bioactive compounds present inside these algae possess a very pivotal character in the conversion of metal salts to NPs. Algae are very good source for biodiesel production, act as food supplements, consist of important pigments, and act as a nanobiofactory for NPs synthesis [17]. There are many bioactive compounds present in algae such as Pholorotannins, Polyphenols, Diterpenes, and Carotenoids etc. [18]. Algae are considered as the best source for β-carotene production. Overall, it can be concluded that algae have all the qualities to be considered as a "Bio-factory".

## 2.2 Phycosynthesis of ZnO NPs

Synthesis of nanoparticles (NPs) using algae is known as Phycosynthesis of NPs. The two basic methods followed for the synthesis of NPs are: (a) Top-down, and (b) bottom-up approach [19]. In the first approach, synthesis/ formation of NPs takes place by reducing the bulk material to its nanoranged form. On the other hand, the second process comprises the assembly of nanoranged particles to form a nanomaterial. The harmful chemicals used in chemical synthesis of NPs are considered as highly toxic [20]. The physical methods like pyrolysis or attrition require energy but produce very less NPs. Therefore, there was a requirement of an alternative method for the NPs synthesis which is widely known as biological synthesis or biogenic synthesis of NPs [25]. Among all the methods followed world-wide for the NPs synthesis, algae are considered as one of

the most biocompatible and easy source for NPs synthesis. This method of biogenic synthesis is more cost-effective in addition to being more environmentally friendly because it removes the need of high set-ups, huge energy requirements, harmful chemicals, extreme temperatures, and pressure [23]. As a result, this technique is more suited for the manufacture of nanoparticles. Intracellular and extracellular synthesis are the two different routes that might be taken in order to reach the final product. The production of nanoparticles that are generated inside the cell is referred to as intracellular synthesis [21]. This method is dependent on the metabolic pathway of algae, which is the process by which cell components or enzymes lower the valence of metal ions to zero. On the other hand, during the process of extracellular production, algae expel enzymes that help in the conversion of metal ions into NPs [32]. The basic steps followed in the synthesis of NPs are:

a) Algal extract preparation from algal biomass grown in water by heating or sonication.
b) Preparation of metal salt precursor ($ZnSO_4.7H_2O$).
c) Addition of algal extract drop-wise to the $ZnSO_4.7H_2O$ solution under stirring conditions.
d) Isolation and purification of ZnO NPs via centrifugation and then washing with ethanol.
e) Drying the NPs at 80℃ for 3-4 hrs.

## 2.3 Factors influencing ZnO NPs synthesis

### 2.3.1 pH

The morphology of the NPs synthesised from microalgae can be affected by the pH of the reaction [52]. It can change the shape of cells and the charges on their functional groups [53]. A higher pH is preferable since it boosts the NPs' reducing and stabilising abilities. The enormous size of the NPs led to their aggregation at lower pH [54]. Particles generated at a broad Surface Plasmon Resonance (SPR) are enormous and can take on a variety of geometric forms (e.g., square, triangle, cylinder, etc.). This occurs at acidic pH, but at basic pH, algae generate tiny spherical NPs [55]. Maximum productivity is achieved when ZnO NPs are synthesized in an alkaline environment (high pH). One probable explanation is that NPs are stable and protected by biomolecules released from algal cells [56].

### 2.3.2 Temperature

Temperature is yet another critical component that performs a major part in the production of NPs. Not only does it impact the dimension of the NPs, but it additionally alters their monodispersity. The morphology of the NPs are particularly linked to the temperature at which the process is carried out. The reaction rate is increased by raising the temperature, which in turn makes the production of NPs more efficient [57]. As a result, one might reach the conclusion that temperature performs a pivotal role in the formation of NPs.

### 2.3.3 Reactant concentration

The formation of NPs is also controlled by the amount of extract added to the solution. Above a particular concentration of reactant, the nanoparticles start agglomerating. Therefore, one should be specific while adding metal salts or extracts for the formation of NPs [58].

### 2.3.4 Type of algal strain and reaction time

Time is crucial because it, too, may yield excellent outcomes if used wisely. The number of NPs formed and their size can be modified by changing the rate of reaction. It is important to take into account the cell/organism's inherent features before choosing an algal strain for NPs synthesis [59]. The alga's activity of enzymes, the pathways it takes, and its growth rate are all examples of its inherent features. The size of the inoculums and the choice of biocatalyst are additional factors that should be taken into account [60]. This is significant since certain species of algae have very sluggish processes and require many weeks to generate NPs. As a result, while selecting species, it's important to prioritize speed of synthesis.

## 2.4 ZnO NPs Potentials

The important parameters to be taken care of while synthesizing ZnO NPs are temperature, pH, and period of incubation, concentration of metal salts, and concentration of extract [22]. They have widely been used in past few years as an agent in environmental agent as well as in biomedicinal applications. ZnO NPs possess antimicrobial property, antioxidant property, anticancerous property, antidiabetic property, and many more. It has widely been used in the environmental remediation as

well [24]. The properties such as dye degradation, and heavy metal removal are the most common applications of ZnO NPs.

## 2.5 Applications of ZnO NPs

### 2.5.1 Antimicrobial Property

As it has been observed over past few years that bacteria has overcome antibiotics and gained antibiotic resistance properties. In order to overcome this drawback, ZnO NPs are a good source to work against bacterial species as it possess antibacterial properties. The benefit of utilizing ZnO NPs as antibacterial agent is that this method is cheap and environmental friendly as well [25]. The possible mechanism behind the antibacterial property can be the interaction of cell wall or cellular components of bacteria leading to their rupturing and destruction. Moreover, they also generate ROS which can lead to destruction of cells of bacteria [26]. According to Abdulwahid et al. ZnO NPs synthesized from *Cladophora glomerata* possessed antifungal property. They possessed antifungal property against two fungi *Rhizoctonia solani* and *Fusarium oxysporum* [27]. Extract prepared from *Tetraselmis indica* possessed antibacterial activity against *E. coli* and *S. aureus* [28].

### 2.5.2 Antioxidant activity

Different scavenging assays such as DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay, Hydroxyl radical ($OH^-$) scavenging, Hydrogen peroxide scavenging, and ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) have been used to demostrate the antioxidant property of ZnO NPs. The functional groups present on the ZnO NPs are responsible for the antioxidant activity of ZnO NPs [28]. ZnO NPs synthesized from

*Tetraselmis indica* possessed antioxidant activity as well. ZnO NPs acted as excellent radical scavengers [28].

### 2.5.3 Anti-cancerous activity

Cancer refers to the uncontrolled division of cells and is definitely a toxic disease. But, ZnO NPs have anticancer property as well. The ROS generation inside cancer cells can lead to their death [29]. ZnO NPs synthesized from *Gracilaria edulis* possessed anticancerous activity against *PC3* cell lines [30]. Similarly, Fouda et al. demosrated the anticancerous property of ZnO NPs against Caco-2 cells and clone09 cells [31]

### 2.5.4 Environmental remediation

There are many applications where ZnO NPs has also shown good environmental remediation properties [32]. Harmful chemicals, dyes, heavy metal released from industries can cause harm to human and aquatic lives. Thus, there was a need to implement a technique which is both environment and eco-friendly both. Heavy metals like cadmium, arsenic, mercury, nickel, etc. can cause cancer inside human body and may also lead to death of both humans and aquatic animals [32]. Dyes used for coloring of textiles, plastics, leather cosmetic, etc. are very harmful. They get easily released in water bodies and need to be eradicated. They can damage the agricultural fields, can cause soil erosion, and can enter food chains leading to fatal diseases as well [33]. ZnO NPs possess huge surface-area to volume ratio, and have very high surface activity. Therefore, it can be used to adsorb pollutants like heavy metals and dyes on its surface and may lead to removal of pollutants at a very good scale [34]. For the eradication of methyl orange dye, Selvam et al. used ZnO NPs synthesized from red alga *Hypnea*

*musciformis.* ZnO NPs successfully degraded methyl orange dye after 10 hrs. [35]. Similarly, *Ulva lactuca* was used to synthesize ZnO NPs and helped in the reduction of methyl orange dye [36].

### 2.5.5 Other Applications

Sunscreens and other cosmetics have started using ZnO NPs because of their ability to block UV-A and UV-B rays [37]. Organosilanes provide a stable coating for ZnO NPs, allowing them to be employed in sunscreens. They also have hemolytic characteristics in addition to their UV-protective ones. Hemoglobin is released from these nanoparticles upon interaction with red blood cells in the blood. The effects of ZnO NPs were studied using osmotic fragility, morphological characteristics, and hemolysis [38]. ZnO NPs were used to increase astaxanthin synthesis. The highest yield of astxanthin was produced when *Haematococcus pluvialis* was treated to ZnO NPs [39]. ZnO NPs can also be used to speed up the curing process for nitrile and natural rubbers. Swelling ratios for both NR and NBR decreased, indicating enhanced mechanical and dynamic mechanical qualities [40], and tensile strengths for both rubbers increased by 80 and 70 percent, respectively [41]. They're also useful since they operate as antifouling agents, stopping fouling agents from growing on the substrate [42]. Their ability to generate ROS had an effect on the bacterial biofilm as well. ZnO NPs in the 10-15 nm size range were reported to have antibacterial activity against the *Pseudomonas aeruginosa* strain of bacterium [43]. Table 1 shows different sources used for ZnO NPs synthesis and their major applications.

**Table 1:** Various methods followed for ZnO NPs synthesis and the applications

| S. No. | NPs synthesized | Source used for NPs synthesis | Applications | References |
|---|---|---|---|---|
| 1. | ZnO NPs | *Gracilaria edulis* (Algae) | Anticancer activity | [31] |
| 2. | ZnO NPs | *Bacillus megaterium* (Bacteria) | Antimicrobial activity | [43] |
| 3. | ZnO NPs | *Terminalia chebula* (Plant) | Photocatalytic activity | [44] |
| 4. | ZnO NPs | *Tetraselmis indica* (Algae) | Antimicrobial activity | [28] |
| 5. | ZnO NPs | *Pichia kudriavzevii* (Fungi) | Antioxidant activity | [45] |
| 6. | ZnO NPs | *Pseudomonas sp.* (Bacteria) | Antibacterial activity | [46] |
| 7. | ZnO NPs | *Anabaena* strain (Algae) | Sunscreen | [47] |
| 8. | ZnO NPs | *Theobroma cacao* (Plant) | Antibacterial activity | [48] |
| 9. | ZnO NPs | *Alternaria tenuissima* (Fungi) | Antibacterial activity | [49] |
| 10. | ZnO NPs | *Brown Sargassum* | Antimicrobial | [32] |

| | | | | |
|---|---|---|---|---|
| | | *myriocystum* (Algae) | activity | |
| 11. | ZnO NPs | *Nocardiopsis flavascens* (Actinomycetes) | Cytotoxic activity | [50] |
| 12. | ZnO NPs | *Physalis alkekengi* (Plant) | Soil contamination | [51] |
| 13. | ZnO NPs | *Pterocladia Capillacea* (Algae) | Dye degradation | [40] |

# CHAPTER 3- MATERIALS AND METHODOLGY

## 3.1 Media Preparation for algae growth

- Three flasks of 1000 ml/ 1L was taken and 500 ml of distilled water (DW) was added.
- Add 0.821 g of BG11 media in each flasks having 500 ml DW.
- Maintain pH of the medium at 8 using pH meter.
- Autoclave the media at 121 ℃ for 20 min.

## 3.2 Algae Cultivation

- Take the media out of the autoclave, cool the media, and keep it in Laminar Air Flow (LAF).
- 10% inoculum of *Chlorella sp.* was added to each flask containing media.
- Keep the flasks at 25℃ for 15-20 days with a photoperiod of 16:08 in a growth chamber having light intensity 50-100 μmol photons $m^{-2}s^{-1}$.

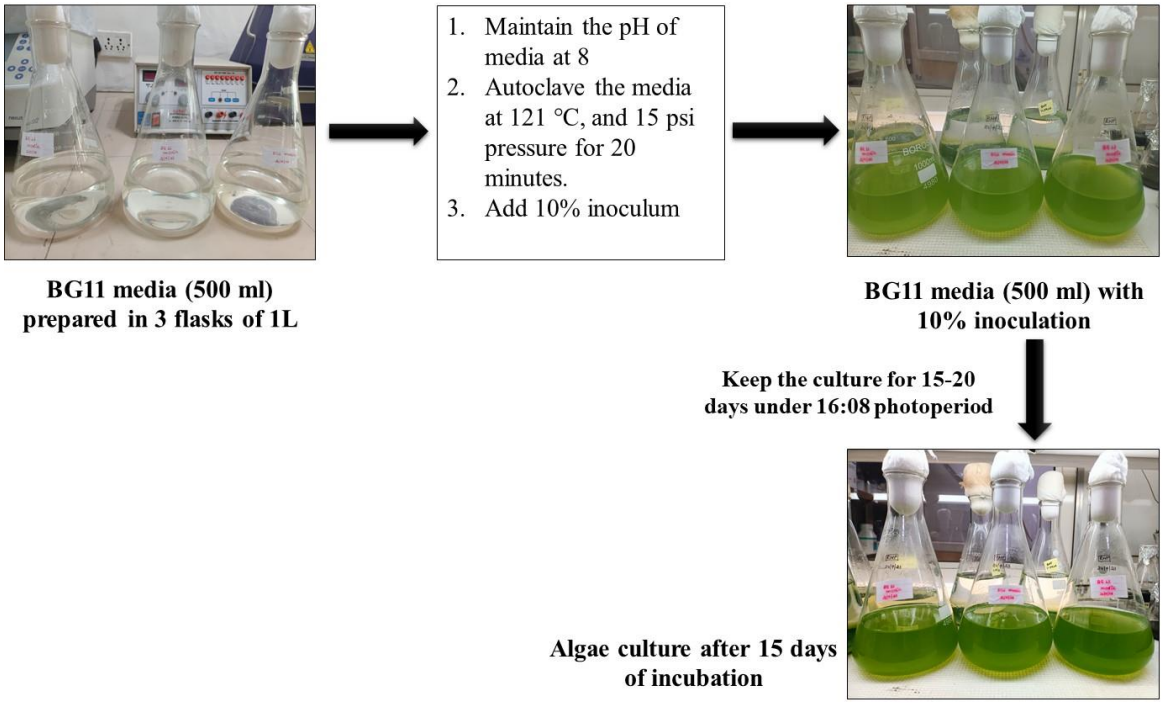


**Figure 2:** Media preparation, inoculation and incubation of Chlorella sp. for 15 days

### 3.3 Biomass harvesting

- After 15-20 days, biomass is accumulated by performing centrifugation at 10000 rpm for 10 minutes.
- The biomass harvested is washed 2 to 3 times using DW.
- The biomass is then dried inside hot air oven at 70-80°C
- The obtained biomass is weighed to determine its concentration and productivity.
- Biomass productivity can be obtained by formula:

$$Biomass\ productivity = \frac{Total\ dry\ weight\ (mg)}{Number\ of\ days\ of\ cultivation(days)\ X\ Volume(ml)}$$

- Biomass concentration can be obtained by formula:

$$Biomass\ Concentration = \frac{weight\ (mg)}{volume\ of\ culture\ (L)}$$

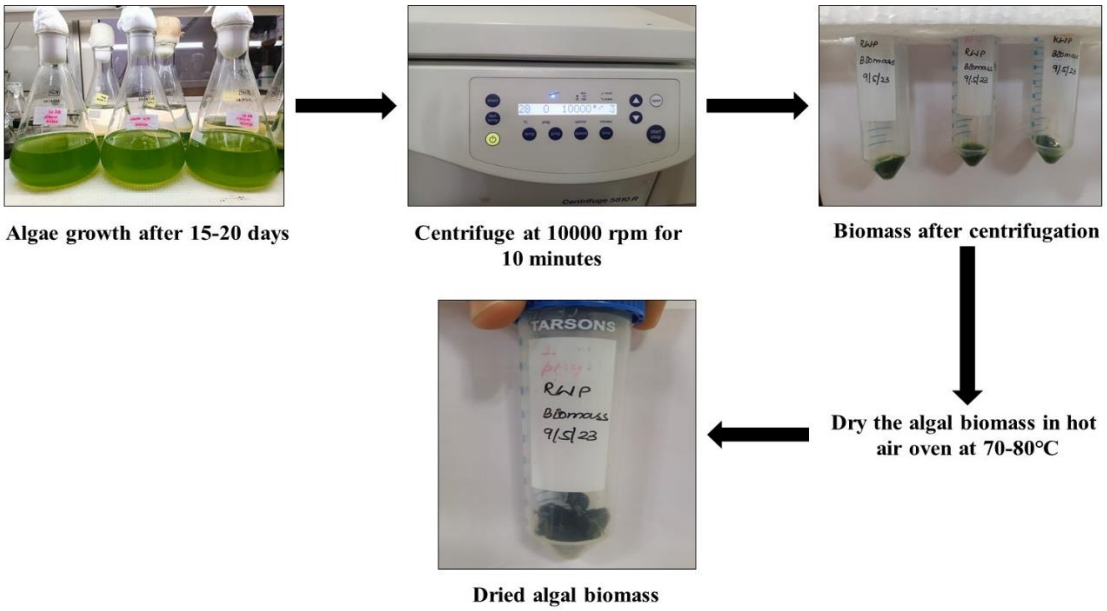


**Figure 3:** Biomass accumulation via centrifugation and collection of dried algal biomass by putting them in hot air oven at 70-80℃ for 7-8 hours.

### 3.4 <u>Phycosynthesis of ZnO NPs</u>

#### 3.4.1 Extract preparation

a) The obtained pellet is again mixed in DW and boiled at 80-100℃.

b) The supernatant obtained is collected after performing centrifugation at 10000 rpm.

c) The extract is then stored in fridge at 4℃ for further applications.

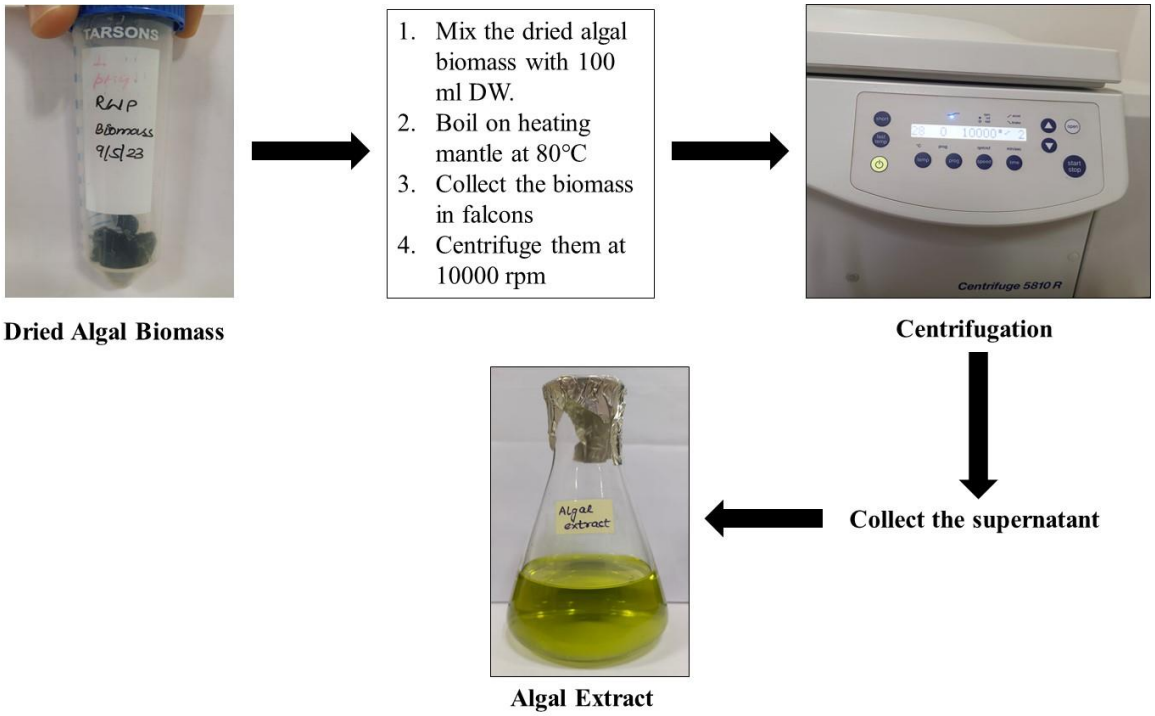


**Figure 4:** Extract preparation procedure from dried algal biomass.

### 3.4.2 Phycosynthesis of ZnO NPs

a) 10mM of $ZnSO_4.7H_2O$ was prepared and mixed under continuous stirring conditions with 10 ml algal extract at 25℃, and 500 rpm for 7-8 hours

b) Formation of ZnO NPs can be identified by change in color from transparent to white color.

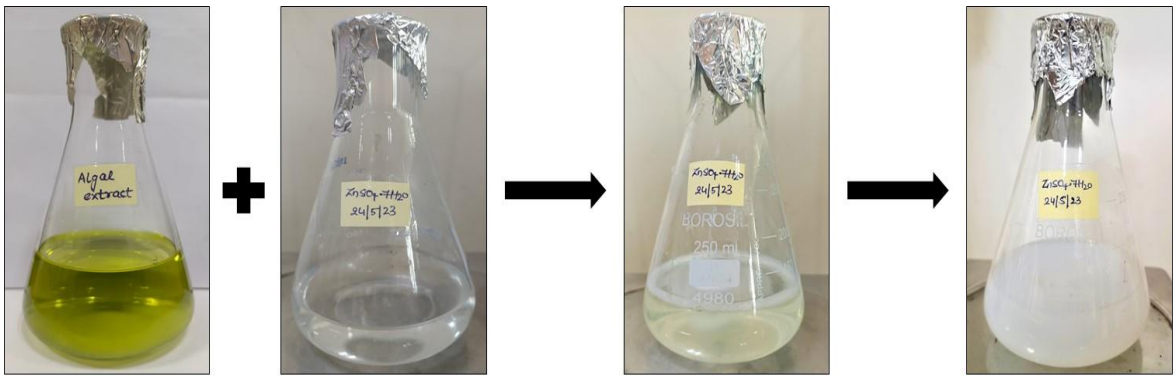


**Figure 5:** Formation of white color confirms the synthesis of ZnO NPs.

### 3.5 Characterization of ZnO NPs

Characterization of the ZnO NPs were done by taking absorbance via UV-Visible spectrophotometer (PerkinElmer). The hydrodynamic size and zeta potential of the ZnO NPs were measured from Malvern Zetasizer Nano series (ZEN3600).

### 3.6 Antibacterial activity of ZnO NPs against *E. coli*

i. Take two falcons and add 10 ml of LB media in each falcon.
ii. Keep 1 as control and suspend 10 µl of *E.coli* stock in another falcon having 10 ml media.
iii. Keep the falcons in a beaker and place it in incubator shaker at 37℃ for 12-16 hours.
iv. Prepare master plates by streaking the *E.coli* culture from stock by using inoculum loop in LAF.
v. Pick one colony of *E.coli* from the master plate, inoculate it in 10 ml of LB media and keep it in incubator shaker for 12-14 hours at 37℃, 200 rpm (Primary Culture).

vi. Take 100 µl from the primary culture, inoculate it in 10 ml of LB media and keep it in incubator shaker at 37℃, 200 rpm (Secondary culture).

vii. Take O.D. of the secondary culture regularly in an interval of 40-45 minutes till it reaches up to 0.4 to 0.6. This O.D. ensures that the bacteria are in log phase.

viii. After this, make bacterial dilutions by adding 10 µl of secondary culture in 10 ml of LB media (1000 times dilution).

ix. Take 6 MCTs (Micro-centrifuge tubes), and label them as 1, 2, 3, 4, 5, and 6.

x. Take 500 µl diluted media in 1$^{st}$ MCT as control.

xi. Take 490 µl diluted media and add 10 µl ZnO NPs to maintain the volume 500 µl and label it as 2$^{nd}$ MCT.

xii. Take 475 µl diluted media and add 25 µl ZnO NPs to maintain the volume 500 µl and label it as 3$^{rd}$ MCT.

xiii. Take 450 µl diluted media and add 50 µl ZnO NPs to maintain the volume 500 µl and label it as 4$^{th}$ MCT.

xiv. Take 400 µl diluted media and add 100 µl ZnO NPs to maintain the volume 500 µl and label it as 5$^{th}$ MCT.

xv. Take 300 µl diluted media and add 200 µl ZnO NPs to maintain the volume 500 µl and label it as 6$^{th}$ MCT.

xvi. Prepare 2 agar plates, take 100 µl from first 3 MCTs and add a drop of each on the first agar plate.

xvii. Similarly, take 100 µl from other 3 MCTs and add a drop of each on the second agar plate.

xviii. Keep the plates in incubator for 12 hours.

xix. Analysis of the growth inhibition of bacteria after 12 hours of incubation.

# CHAPTER 4- RESULTS AND DISCUSSION

## 4.1 Biomass concentration and productivity

The dry weight biomass obtained after culturing algae in 3 flasks of 1000 ml was 780 mg. The biomass concentration obtained was:

$$Biomass\ Concentration = \frac{780\ (mg)}{1.5\ (L)} = 520\ mg/L$$

The biomass productivity obtained was:

$$Biomass\ productivity = \frac{780\ (mg)}{20\ (days) X\ 1500\ (ml)} = 0.026\ mg/ml/day$$

## 4.2 ZnO NPs synthesis

Formation of white colored particles indicate the successful synthesis of ZnO NPs. The ZnO NPs formed were centrifuged and washed twice with DW and then collected in falcon for further characterization and application.

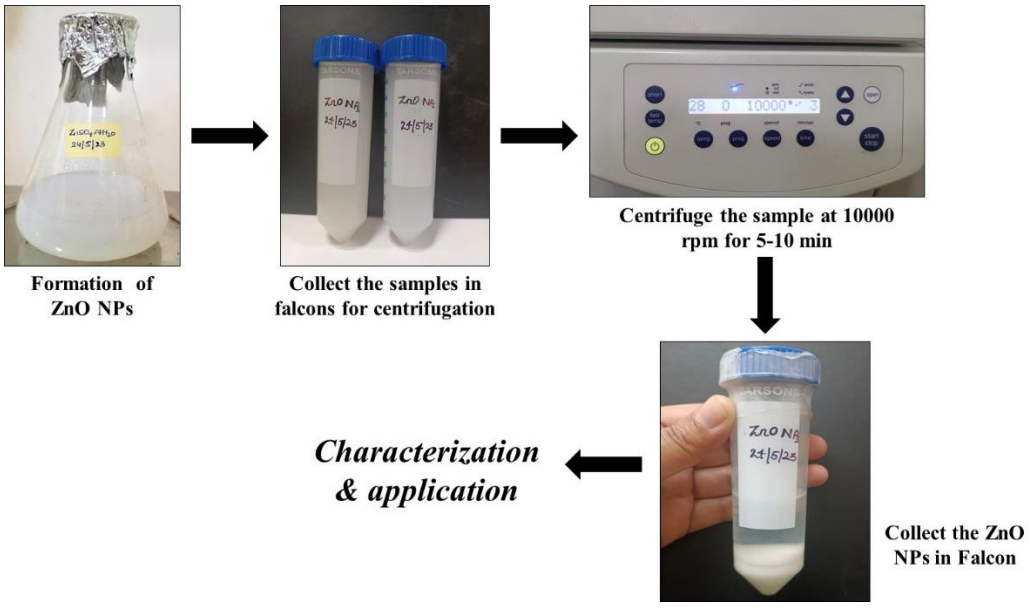


**Figure 6:** The ZnO NPs formed are centrifuged and collected in a falcon for further application.

## 4.3 Characterization

### 4.3.1 The hydrodynamic size and zeta potential of ZnO NPs

The hydrodynamic size of the ZnO NPs was around 310nm - 320nm when measured using Malvern Zetasizer Nano series (ZEN3600).

| | | | Size (d.nm): | % Intensity: | St Dev (d.nm): |
|---|---|---|---|---|---|
| Z-Average (d.nm): | 496.1 | Peak 1: | 168.4 | 100.0 | 18.68 |
| PdI: | 0.523 | Peak 2: | 0.000 | 0.0 | 0.000 |
| Intercept: | 0.696 | Peak 3: | 0.000 | 0.0 | 0.000 |
| Result quality : | Refer to quality report | | | | |

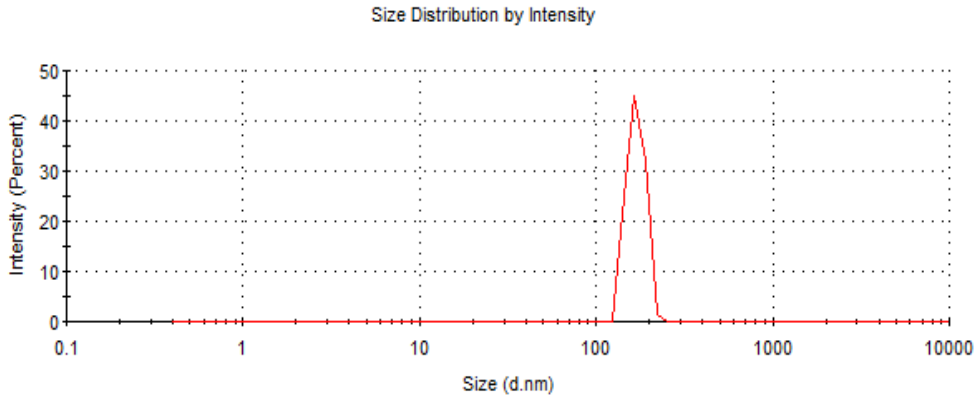


**Figure 7:** Size distribution by intensity of the ZnO NPs measured from Malvern Zetasizer Nano series (ZEN3600).

The zeta potential of ZnO NP was around 0 mV when measured using Malvern Zetasizer Nano series (ZEN3600).

| | | Mean (mV) | Area (%) | St Dev (mV) |
|---|---|---|---|---|
| Zeta Potential (mV): -22.1 | Peak 1: | -22.1 | 100.0 | 5.80 |
| Zeta Deviation (mV): 5.80 | Peak 2: | 0.00 | 0.0 | 0.00 |
| Conductivity (mS/cm): 0.00686 | Peak 3: | 0.00 | 0.0 | 0.00 |

Result quality : Good

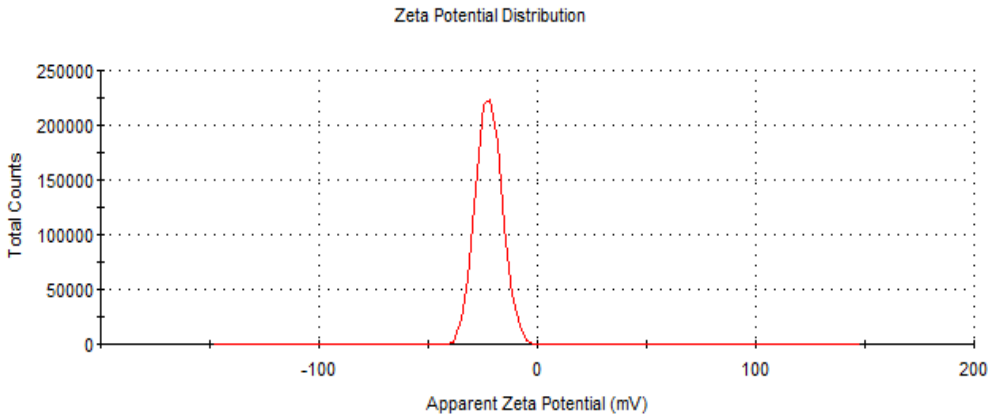


**Figure 8:** Zeta potential distribution of the ZnO NPs using Malvern Zetasizer Nano series (ZEN3600).

### 4.3.2 Absorbance of ZnO NPs determined from UV-Visible spectrophotometer

The absorbance of ZnO NPs was measured by using PerkinElmer spectrophotometer. The absorbance peak was observed at 338 nm indicating the successful synthesis of ZnO NPs.

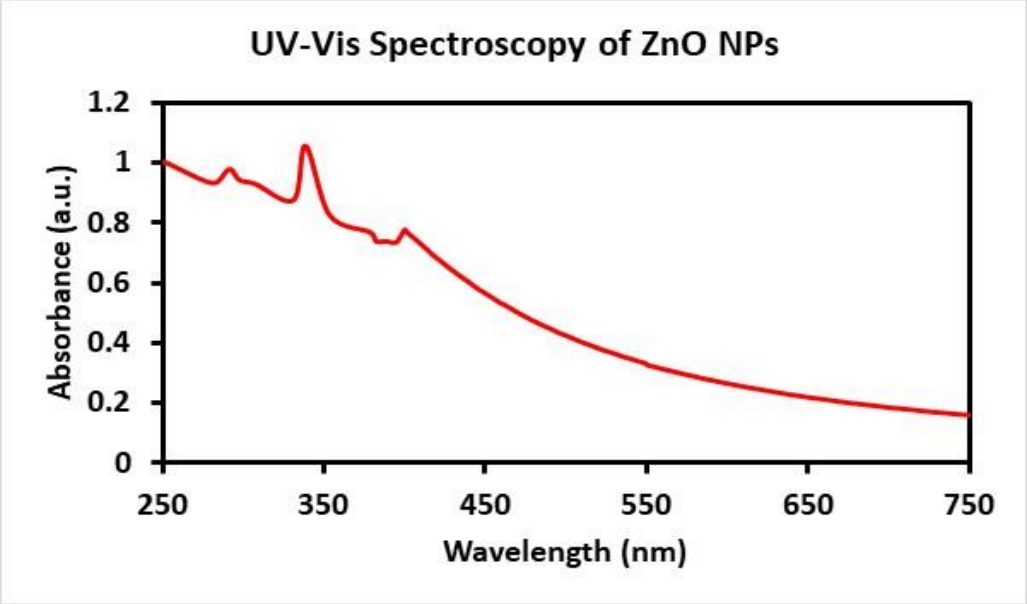


**Figure 9:** Absorbance of ZnO NPs measured from UV-Visible Spectrophotometer

## 4.4 Antibacterial activity of ZnO NPs against *E.coli.*

When *E.coli* bacterial dilution was mixed with different concentration of ZnO NPs, inhibition in growth was observed as the concentration was increased from 10 µl to 200 µl. The maximum inhibition in growth was observed when 200 µl of ZnO NPs was used.

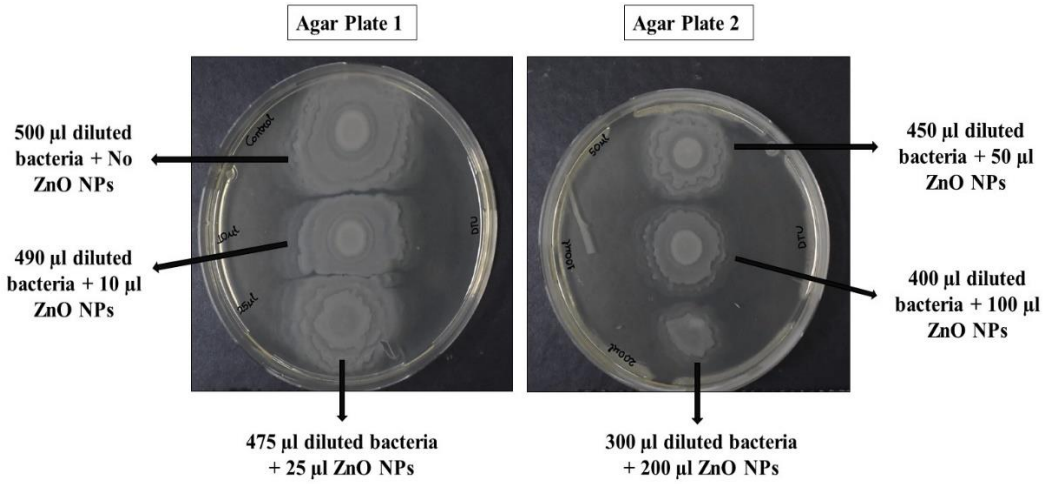


**Figure 10:** Antibacterial activity of ZnO NPs against E.coli at different concentrations of ZnO NPs

# CHAPTER 5- CONCLUSION AND FUTURE PROSPECTS

*Chlorella sp*. possess the capacity to synthesize ZnO NPs in a very ecofriendly, and biocompatible manner. Phycosynthesis of ZnO NPs require algal extract and a metal salt precursor to be stirred at a particular rpm for a specific time period. The results have revealed a successful formation of ZnO NPs by the occurence of white color from a transparent solution. The characterization of ZnO NPs was done via UV-visible spectrophotometer, DLS, and zeta potential instrument to check the absorbance, size, charge of the NPs respectively.

ZnO NPs have many potentials like anticancerous activity, antimicrobial property, antioxidant activity, and many more. The antibacterial property of ZnO NPs was tested against *E.coli.* An inhibition in the growth of *E.coli* was seen when the amount of ZnO NPs was increased to 200 µl. So, it can be concluded that the ZnO NPs possess antibacterial property.

There are many other applications which can be explored further. The ZnO NPs work against UV-rays thus, can be an asset for humans to be utilized in sunscreens. The photocatalytic property of ZnO NPs have now been used for dye eradication purposes. In few researches, it was found that the anticancer property of ZnO NPs are utilized to treat cancer cells in in-vitro conditions. Therefore, we can reach a conclusion thta phycosynthesized ZnO NPs possess both biomedical and environmental properties. These ZnO NPs have huge futuristic applications in treatment of water and many diseases.

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# LIST OF PUBLICATIONS

1. Shrutika Chaudhary and Navneeta Bharadvaja (2023). "**Recent Developments in Phycosynthesis of Zinc Oxide NPs for Biomedicine and Environmental Applications**". Advances in Natural Sciences: Nanoscience and Nanotechnology (ANSN) **[ACCEPTED]**.

Advances in Natural Sciences: Nanoscience and Nanotechnology

**Decision Letter (ANSN-2023-0072.R2)**

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