# GREEN SYNTHESIS AND CHARACTERIZATION OF ZINC OXIDE NANOPARTICLES USING FLESH EXTRACT OF *Luffa acutangula* AND ITS ANTIBACTERIAL ACTIVITY

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***Abstract :*** Plants have been used in the synthesis of metallic nanoparticles because they are more ecofriendly. The ZnO nanoparticles are of significant interest as they provide many practical applications worldwide. The most important application of ZnO nanoparticles would be as antibacterial agents. Zinc Oxide Nanoparticles (ZnO NPs) can be synthesized from the aqueous flesh extract of Ridge gourd(*Luffa acutangula*)and to evaluate their antibacterial activity against gram positive and gram negative. The biosynthesized nanoparticles were characterized by UV-Vis spectroscopy, FTIR, XRD and SEM. Moreover, the outcomes of the synthesis certainly contribute to the developing a better understanding of simple, low-cost, green and non-toxic synthesis method.

***Keywords***- Metallic Nanoparticles, Green synthesis, ZnO nanoparticles, Ridge gourd flesh extract, Antibacterial activity.

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## I. INTRODUCTION

In Science and engineering, Nanoscience and Nanotechnology are considered as currently growing and innovating field which are budding at very leap. A theory progressive in the beginning of nanotechnology is frequently accredited by Richard P. Feynman (Father of Nanotechnology). From the Greek noun the term Nano is devised and it signifies Dwarf (Moghimi*et al.,* 2005).The route of synthesizing nanoparticles can varies from physical to chemical methods, until now they are regarded as bulky, lethal to the environment and expensive. In recent times, the use of biological system is probably exposed as recyclable and cost-effective. The reduction of metal ions is supported by the phytochemicals which are present in plant extracts. For the production of nanoparticles, plant extracts are used widely. It may be beneficial and plays a vital role in the synthesis of large scale Nanoparticles (Jeeva*et al.,* 2014).

Zinc Oxide nanoparticles is a fascinating semi-conductor. It can be synthesized by consuming various raw materials as chemical compounds such as Zinc sulfate, Zinc acetate and Zinc nitrate. The other name of Zinc Oxide is Zincite. The nature is crystalline and it is an inorganic compound. In various fields such as cosmetics, agrochemicals, optical, Ceramics and pharmaceutical, it has an extensive application. Further, as an antibacterial agent it gained more attention by the scholars. They are also used for the target drug delivery, molecular diagnostics and an evolving a new therapeutic preparation in the medical field. In the treatment of carcinoma and cancer cell it is also used (Anandraj and Jayalakshmy 2015).

*Luffa acutangula L.* is a common vegetable in Indian food and it comes under the family of Cucurbitaceae. It is an usually developing somatic creeper. It has several altered names in dialect languages. Most common it is mentioned as Ridge gourd in English. From the time when the Indian traditional classification of medicines originated the complete plant of *Luffa acutangula* is said to be pharmaceutically significant. It possess a quite a lot of medicinal properties. Selectively, it has diuretic properties. It is used in the treatment of cough, dysentery, ringworms, piles and leprosy. It is also act as hypoglycemic mediator and a laxative and cleansing agent. There are numerous phytochemicals present in this plant. Some of them include saponins, flavonoids, triterpenes, glycoside, cucurbitacin B (Anitha andMiruthula 2014).

# II. MATERIALS AND METHODS

### 2.1. Collection of *Luffa acutangula* flesh sample

The flesh of *Luffa acutangula* used in this study was purchased from the local market of Housing board, Tirupattur, Vellore district.

**Figure 1: Ridge gourd *(Luffa acutangula*)**

**Figure 2:Flesh of *Luffa acutangula***

### 2.2. Preparation of aqueous Ridge gourd (*Luffa acutangula*) flesh extract

After collection of *Luffa acutangula,* (Ridge gourd) flesh were separated and sliced. Then dried in sunshade to remove the residual moisture. The extract used for the reduction of Zinc ions ($Zn^{2+}$) to Zinc Oxide Nano particle (ZnO) was prepared by placing 50 g of dried fine flesh powder along with 1 litre of sterile distilled water and then boiled for 20 minutes at 60 ºC until the color of the aqueous solution changes from watery to brown. The extract was allowed to cool and kept in room temperature and filtered using Whatman filter paper. The extract was stored in the refrigerator in order to be use in further experiments.

### 2.3. Synthesis of Zinc Oxide Nano particle

For the synthesis of zinc Oxide Nanoparticle, 500 ml of *Luffa acutangula* flesh extract was taken in a clean conical flask. And 10 grams of Zinc nitrate was added to the solution and mixed thoroughly and kept in shaker incubator for 2 hours at 60˚C. After incubation that mixture was allowed to cool down to room temperature. And the solution was centrifuged for 20 minutes at 4000 rpm. After centrifugation supernatant was discarded and obtained pellet were separated and kept in Hot air oven for 6 to 7 hours at 80˚C. The resultant sample was collected and smashed in a mortar and pestle so as to get a finer nature for further characterization of zinc Oxide nano particles and stored in air tight container (Mishra and Sharma 2015).

### 2.4. Characterization studies of Zinc Oxide Nano particle

#### 2.4.1. UV- Visible spectrum Analysis

The sample was measured for its maximum absorbance using UV-Vis spectrophotometer. The optical property of ZnO nano particles was analyzed via ultraviolet and visible absorption spectroscopy (UV-Vis-Ais- Model 60 Bio) in the range of 200-600nm.

#### 2.4.2. FTIR analysis

The FTIR spectrum was taken in the mid-IR region of 400-4000 $cm^{-1}$. The spectrum was recorded using ATR (Attenuated Total Reflectance) technique. The dried sample was mixed with the KBr (1: 200) crystal, and the spectrum was recorded in the transmittance mode (PERKIN ELMER- Spectrum- 2).

#### 2.4.3. XRD Analysis:

The phyto-reduced Zinc Oxide Nanoparticles were characterized to reveal their crystal structure using X-ray diffraction technique. The XRD pattern was recorded using computer controlled XRD-system (JEOL, and Model: JPX-8030) with CuKa radiation (Ni filtered = 13418 A˚) in the range of 40 KV, 20A. The built in software (syn master 7935) program was used for the identification of XRD peaks corresponding to Bragg's reflections. The estimation of the size of particles was performed by Scherrer's formula.

**2.4.4. SEM analysis**

Scanning electron microscope (SEM) analysis was done by using Hitachi S -2500 SEM Machine. Thin film of the sample was prepared on a carbon coated copper grid by just dropping a very small amount on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid was allowed to dry putting it under a mercury lamp for 5 minutes.

**2.5. Antibacterial assay**

Many systematic methods have been adopted to evaluate the antibacterial activity of ZnO Nano particles. One of the most used is broth dilution method, followed by colony count, in which the plates serial culture broth dilutions containing the bacteria and ZnO Nano particles incubated at proper condition, in suitable agar medium. Presently, *Escherichia coli* (gram negative), *Staphylococcus aureus* (gram positive), *Enterococcus faecium* (gram positive), *Bacillus cereus* (gram positive), *Proteus mirabilis* (gram negative), *Micrococcus luteus* (gram positive) are mainly chosen as model bacteria to evaluate the antibacterial activity of ZnO nano particles.

**2.5.1. Inoculum preparation**

Twenty four hour old culture of selected bacterial broth prepared by inoculating a loop full of mother culture in to the test tubes containing 5 ml of broth (nutrient agar) which were incubated at appropriate time and temperatures (37 ºC for 24 hours).

**2.5.2. Preparation of test solution**

The test solution was prepared with known weight of sample dissolved in 5% dimethyl Sulphoxide (DMSO) (Corresponding to 50, 100 and 150µl).

**2.5.3. Determination of antibacterial activity**

Agar well diffusion method was trailed for antibacterial activity. The Muller Hinton agar was prepared, poured on petriplates and allowed to solidify. After solidification, 0.1 ml of standardized microbial inoculum suspension was poured and uniformly spread. The excess inoculum was drained and the plates were allowed to dry for 5 minutes. After drying, samples were placed on the surface of the plates with sterile pipette. Biosynthesized sample (50 µl, 100 µl, and 150 µl) were used as the positive controls and the DMSO (5 %) was used as a blind control. Finally the inoculated plates were incubated at 37 ºC for 24 hours. The zone of inhibition was observed and measured in millimeters. This experiment was repeated for four times.

# III. RESULTS AND DISCUSSION

**3.1. UV-Vis Spectra Analysis**

The synthesized Zinc Oxide nanoparticle illuminates the optical properties by UV-Vis Spectrum and photoluminescence spectroscopy at room temperature as displayed n Figure 3. The UV band around 200-600 nm express the exhaustive absorption which can be seen in Figure 3. The peak of maximum absorption was visualized approximately at 279 nm which indicates analogous to the results of (Belay *et al.,* 2017) where they had absorbance at 278 nm. The reducing activity of our flesh extract may be the reason for difference in UV absorption.

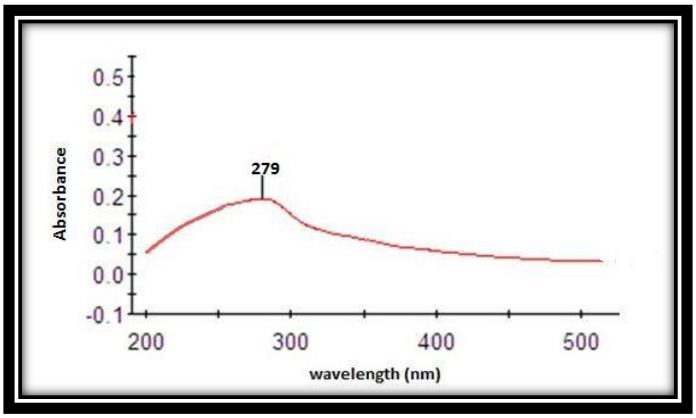


**Figure 3: UV-Vis Spectra analysis**

### 3.2. FTIR Analysis

In FTIR analysis the bands were observed at 3852.45 $cm^{-1}$, 3745.11 $cm^{-1}$, 3326.79 $cm^{-1}$, 3211.35 $cm^{-1}$, 2884.81 $cm^{-1}$, 2692.92 $cm^{-1}$, 1756.02 $cm^{-1}$, 1511.93 $cm^{-1}$, 1213.77 $cm^{-1}$, 1044.68 $cm^{1}$, 1011.47 $cm^{-1}$, 904.94 $cm^{-1}$, 803.57 $cm^{-1}$, 774.43 $cm^{-1}$, 723.38 $cm^{-1}$, 684.43 $cm^{-1}$, 639.02 $cm^{-1}$, 518.26 $cm^{-1}$. The band arise in the middle of 400-600 $cm^{-1}$ is only allocated to ZnO region (Yuvakumar*et al.*, 2015).

The peaks at 3852.45 $cm^{-1}$, 3745.11 $cm^{-1}$, 3326.79 $cm^{-1}$, 3211.35 $cm^{-1}$ corresponds to N-H stretching of primary amide. The band exist at 2884.81 $cm^{-1}$, 904.94 $cm^{-1}$, 803.57 $cm^{-1}$ specifies Alkanes. 2692.92 $cm^{-1}$ Signifies the O-H Stretch (Carboxylic acid). The band at 1756.02 $cm^{-1}$ is identified as C=O Carboxylic acid & derivatives. The medium band of aromatic C=C bend was noted at 1511.93 $cm^{-1}$. The band at 1213.77 $cm^{-1}$ implies O-C Carboxylic acid & derivatives. The bands seen at 1044.68 $cm^{-1}$, 1001.47 $cm^{-1}$were allocated to C-N (Amines). 774.43 $cm^{-1}$ indicates N-H (Amines) and 723.38 $cm^{-1}$ indicates CH=CH (Alkenes). The peak at 684.43 $cm^{-1}$, 639.02 $cm^{-1}$ reveal the presence of C-H (Alkynes). Likewise, the band positioned at 518.26 $cm^{-1}$ signifies the presence of Zinc oxide nanoparticles. It is detected owing to reduction and stabilization of metal group ZnO. The above result shows similarity to (Belay *et al.*,2017) also got FTIR band of ZnO at 510 $cm^{-1}$.

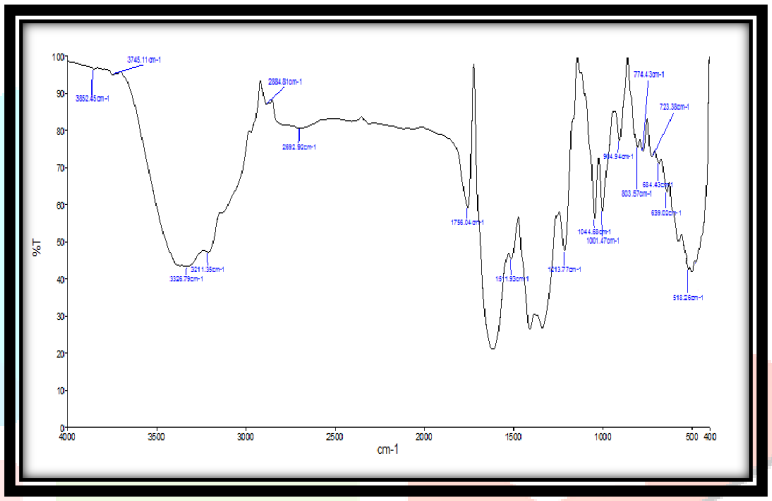


**Figure 4: FTIR Analysis**

### 3.3. XRD Analysis:

The pure phase and crystal structure of the synthesized Zinc oxide Nanoparticles was further confirmed by using X-Ray Diffraction. Figure 5 shows the XRD pattern of Zinc Oxide nanoparticles. Mainly, four peaks were observed. Therefore, the peak of XRD were identified as (100), (110), (002) & (101) with the help of JCPDS (36-1451).

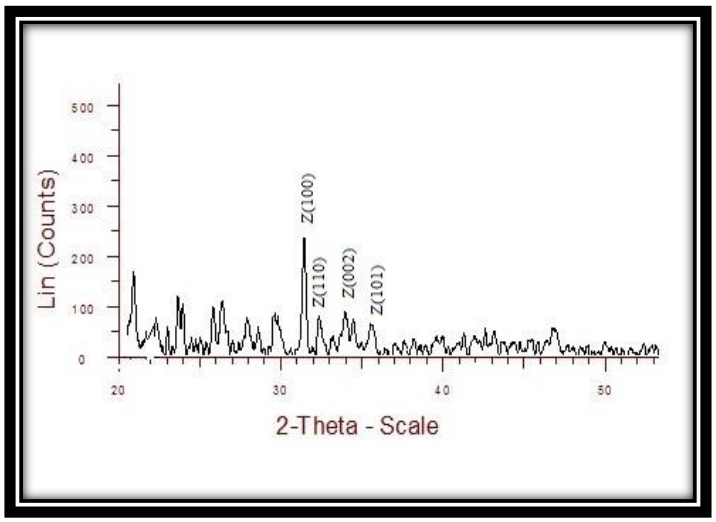


**Figure 5: XRD Analysis**

### 3.4. SEM Analysis

The size and shape of Zinc Oxide nanoparticles were visualized and revealed by SEM analysis which is shown in Figure 16. The size of the synthesized Zinc Oxide nanoparticles was observed to be 117.3 nm. In this present work, the obtained Zinc Oxide nanoparticles were appear in cubic shape which shows the similar result of Samzadehkermani*et al.,* (2016).

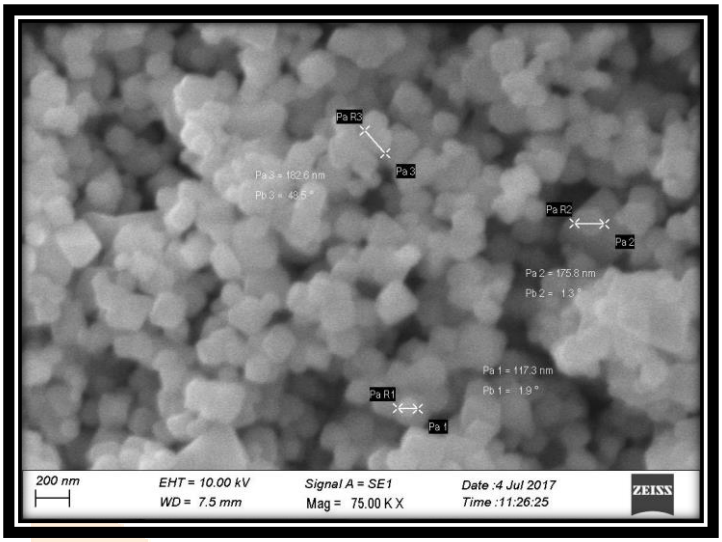


**Figure 6: SEM Analysis**

## 3.5. Antibacterial Activity

**Table 1: Antibacterial activity of biologically synthesized Zinc Oxide Nanoparticles**

| S.No | Bacteria | Zone of inhibition (mm in dm) | | | Blind control (5 % DMSO) |
|---|---|---|---|---|---|
| | | 50 µl | 100 µl | 150 µl | |
| 1 | *Staphylococcus aureus* | 9 mm | 13 mm | 14 mm | No Zone |
| 2 | *Micrococcusluteus* | 9 mm | 12 mm | 16 mm | No Zone |
| 3 | *Enterococcus faecium* | 8 mm | 9 mm | 11 mm | No Zone |
| 4 | *Bacillus cereus* | - | 9 mm | 11 mm | No Zone |
| 5 | *Proteus mirabilis* | 8 mm | 9 mm | 11 mm | No Zone |
| 6 | *Escherichia coli* | 8 mm | 9 mm | 10 mm | No Zone |

The above results shows the antibacterial activity of biosynthesized Zinc Oxide nanoparticles which was found very toxic against bacterial human pathogen. Zinc Oxide nanoparticles exhibited antibacterial activity against four gram positive and two gram negative bacteria. The bacteria which are chosen for this study include *Staphylococcus aureus, Micrococcus luteus, Enterococcus faecium, Bacillus cereus, Proteus mirabilis* and *Escherichia coli*. These bacterias shows a clear inhibition zone at the concentration of 150 µl which contains 1 mg of our synthesized nanoparticles. The stability in the culture medium primarily contribute the bacterial activity of particles.For the formulation of innovative type of antibacterial agents, the particles which are prepared in an easy, low cost and quick manner is suitable and that can be confirmed from the result.

# IV. CONCLUSION

When compared to chemical process, the synthesis of Zinc Oxide Nanoparticles by green method is very much safer and ecofriendly. From the current work, it determines that aqueous flesh extract of *Luffa acutangula* used as an active reducing as well as stabilizing agent for

ZnO Nanoparticle synthesis. The synthesized ZnO Nanoparticle shows optical property and its maximum absorption peak was noted at 279 nm by using UV-Vis spectroscopy. Fourier transform infrared (FTIR) analysis depicts the various functional groups present in the flesh extract of Luffa acutangula. The band at 518.26 $cm^{-1}$ was observed and it is considered as a region of ZnO metal group. X-Ray Diffraction (XRD) is further used to confirm the ZnO Nanoparticles and four peaks were identified. The appearance of cubic shape confirmed the ZnO nanoparticles using SEM analysis. Antibacterial activity was made by using flesh extract of *Luffa acutangula*. It clearly shows the zone of inhibition against bacterial human pathogens. ZnO Nanoparticles shows sufficient inhibition against four gram positive bacteria and two gram negative bacteria. Research have been supported for the green synthesis of Nanoparticles due to their ecofriendly nature, cost effective and less harmful. In future prospects, the contribution of Zinc Oxide Nanoparticles will be very high in many fields.

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