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# A Review on Antibacterial Activity of Green Synthesis Zinc Oxide Nanoparticle

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**Abstract:** This study assesses the antibacterial efficacy of synthesized zinc oxide nanoparticles produced using pomegranate leaves and flowers, Zingiber Officinale and Phyllanthus Niruri (Keezhanelli) leaves extract. In the study, zinc oxides were successfully employed to fabricate nanoparticles using leaves extract. The nanoparticles obtained were characterized spectroscopically. X-ray diffractive analysis (XRD) revealed the purity and crystalline of the synthesized particles. The functional were confirmed using Fourier Transform Infra-Red (FTIR) Spectroscopy and the microstructure and composition of the nanoparticles is shown by scanning electron microscopy (SEM). By transmission electron microscope (TEM), the shape and size distribution of the synthesized particles is shown. Evaluation of the antibacterial efficacy of the fabricated nanoparticles showed that zinc oxide nanoparticle was effective against all selected pathogenic strains such as Staphylococcus aureus, Proteus Vulgaris and Escherichia coli used in the analysis. The effectiveness of these nanoparticles could be linked to their zone of inhibition as obtained using an optical microscope. This study revealed that the zone of inhibition of the nanoparticles was exist thus suggesting that zinc oxide nanoparticle can effectively be used as alternative antibacterial agents.

**Keywords:** Zinc Oxide, Nanoparticle, Bacteria, Leaves Extract, Antibacterial

## 1. Introduction

Bacterial infections are widely recognized as a significant public health problem across the world. Antibiotic resistance is on the increasing and pathogenic strain outbreaks are becoming more frequent. As

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a result, there is an urgent needed for the creation of new effective antibacterial agents [1]. Zinc oxide has antibacterial effects for time immemorial. Since of their antibacterial properties, they are used as antimicrobial agents because they are stable, long-lasting, and strong [2]. Non-toxic or less toxic nanoparticle green synthesis attracts researchers' interest nowadays because of their features such as their being nontoxic or having less toxicity, which have the similar unique properties [3]. Alternative than physical, chemical, and microbial technique productions, the plant extract-mediated nanoparticle synthesis is one of the most stable and appropriate methods. Green synthesis improved hygiene by providing a sanitary environment with less pollution and a wider variety of stable chemicals. The present study reports that zinc oxide nanoparticles can be used effectively for the control of microorganism and prevention of infections caused by Staphylococcus Aureus, Escherichia Coli and Proteus Vulgaris [4]. As more diseases are spreading in the world, it is very important to carry out this study to substitute antibiotics with leaf extract. This will save human beings from continuing to suffer from serious diseases and it can also reduce the reliance on human-made antibiotics. It is therefore important to carry out this study to develop a new vaccine for certain diseases using plant extracts.

## 2. Materials and Methods

Zinc nitrate hexahydrate was used as a precursor to synthesize zinc oxide nanoparticles. Distilled water was used to prepare all possible solutions. Staphylococcus Aureus, Proteus Vulgaris and Escherichia Coli were the bacteria strain used in this study.

### 2.1 Preparation of Leaves Extract

The raw leaves are collected from Maiza Nursery. The raw leaves are washed separately to remove dust particle at the leaves surface. After that, the leaves are dried in the vacuum dried oven. The dried leaves grinded to a fine powder. Ten grams of powdered leaves were mixed with 100 ml of distilled water. The leaves mixture was boiled at 60°C for 20 min.

### 2.2 Synthesis of Zinc Oxide Nanoparticles

A solution of 0.1 M zinc nitrate hexahydrate in 300 ml distilled water was prepared by dissolving 6.58 g of zinc nitrate hexahydrate throughout the water. 10 ml of aqueous leaves extracts were added dropwise under magnetic stirring to a solution that was at 60°C for 2 hours to obtain complex formation. The mixture of leaves extract and zinc nitrate hexahydrate will be put in a centrifuge machine at 1600 rpm for 10 minutes. Next, the precipitate will be undergoing drying process in the vacuum dry oven at 80°C for 8 hours.

### 2.3 Characterization of the Synthesized Zinc Oxide Nanoparticle

The synthesized zinc oxide nanoparticles were characterized by X-ray diffraction (XRD), Fourier transform infrared spectrophotometry (FTIR), Scanning electron microscope (SEM) and Transmission electron microscope (TEM). The XRD analysis was done to obtain information about the purity and crystalline nature of the formed particles. FTIR analysis was carried out to detect the surface functional groups in the leaves extract and the nanoparticle samples. The microstructure and the composition of the synthesized zinc oxide nanoparticles was done using SEM analysis. TEM analysis was done to provide information on the shape and size distribution of synthesized nanoparticles.

### 2.4 Antibacterial Activity of the Synthesized Zinc Oxide Nanoparticles

Antibacterial activity of green synthesis zinc oxide nanoparticles against bacterial strain was seen by agar well diffusion method. The mechanism of antibacterial activity from morphology impact, surface modification and particle size as well as the diameter of zone of inhibition of bacteria are observed by using optical microscope.

## 3. Results and Discussion

Figure 1 shows XRD pattern for the zinc oxide nanoparticles synthesized by way of pomegranate leaves and flower extract. The results obtained in pomegranate leaves extract in diffraction peaks at  $2\theta$

values were 31.71°, 34.34°, 36.25°, 47.54°, 56.62°, 62.76°, 68.00°, 69.10°, 72.43° and 72.43° while for the ZnO NPs synthesized with pomegranate flower extract were 31.74°, 34.42°, 36.13°, 47.54°, 56.53°, 62.84°, 67.93°, 69.05°, 72.69° [5]. It is considered that these peak values correspond to the crystal or lattice plane of (100), (002), (101), (102), (110), (103), (200), (112), (201) and (202).

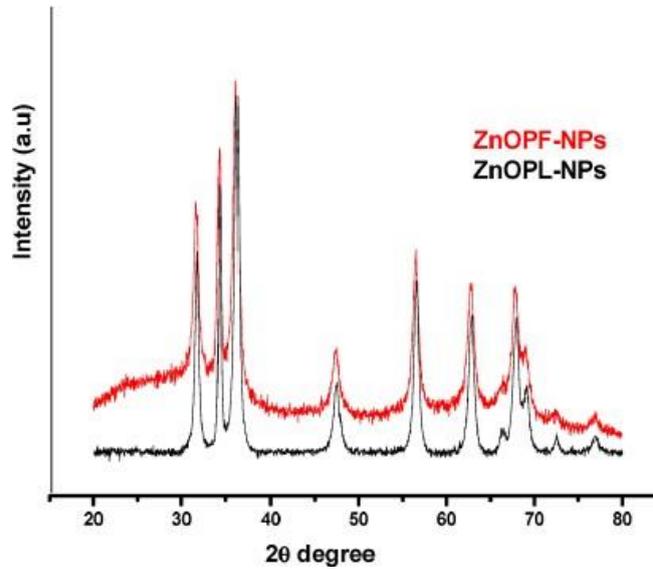


Figure 1: XRD spectra of ZnO–NPs–PL and ZnO–NPs–PF [5]

The average particle size was calculated using the Debye-Scherrer equation as ascribed in Eq. 1.

$$D = \frac{K\lambda}{\beta \cos \theta} \quad \text{Eq. 1}$$

where D denotes the average particle size (nm), K is the Scherrer constant, somewhat arbitrary value that falls in the range 0.8-1.0 (it was assumed to be 0.9);  $\lambda$  is wavelength of X-ray radiation;  $\theta$  is the diffraction angle and  $\beta$  is full width at half maximum. From Debye-Scherrer equation, the average particle size for the pomegranate leaves with zinc oxide nanoparticle was about 57.75 nm while for the pomegranate flower with zinc oxide nanoparticle was about 52.50 nm.

Figure 2 shows XRD pattern between Zingiber Officinale leaves extract and ZnO NPs. The result shows that the diffraction peaks values correspond to the crystal or lattice plane of (101), (002), (100), (110), (103), (112), (102), (202) planes in hexagonal type structure. The unique peak at about 36° correspond to (100) plane. When using Debye-Scherrer equation, the average particle size for Zingiber officinale leaves mediated with zinc oxide nanoparticle was about 4 nm [4].

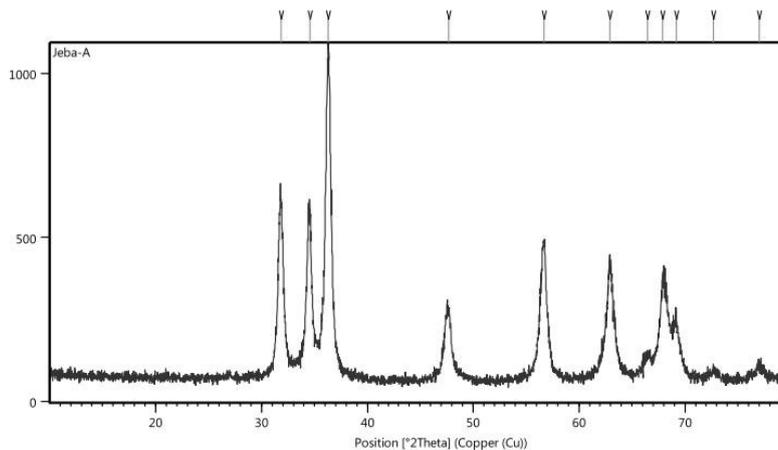


Figure 2: XRD spectra between Zingiber Officinale leaves extract and ZnO NPs [4]

Figure 3 shows the result of *Phyllanthus niruri* (keezhanelli) leaves extract with ZnO NPs. The unique peak obtained were  $26.91^\circ$ ,  $30.52^\circ$ ,  $39.61^\circ$ ,  $47.56^\circ$ ,  $51.77^\circ$  and corresponding to the reflection plane was (100), (101), (102), (110), (103). The strong unique peak at  $26.91^\circ$  correspond to (100) plane. The average crystalline particle size was found 74 nm when calculate using Debye-Scherrer equation [6].

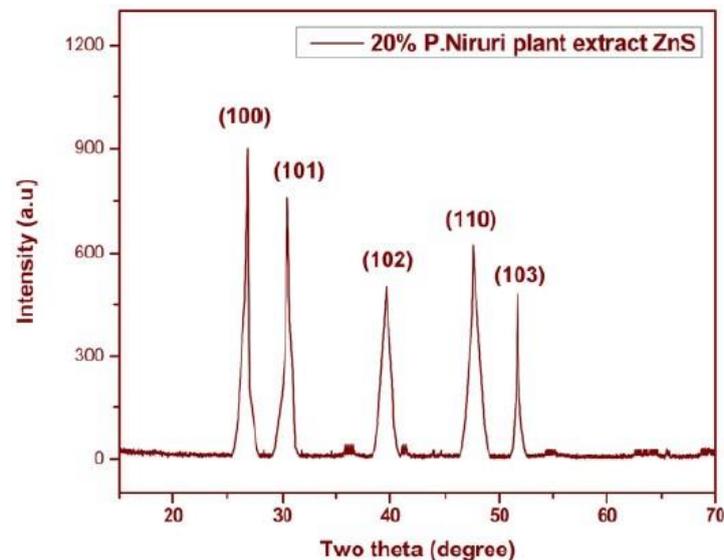


Figure 3: XRD spectra of *Phyllanthus Niruri* (Keezhanelli) leaves extract and ZnO NPs [6]

Figure 4 shows FTIR spectrum of the pomegranate leaves and flower extract synthesized with ZnO NPs and ZnO sample. The spectral range was  $4000$  to  $400\text{ cm}^{-1}$ . At  $3560$  and  $3700\text{ cm}^{-1}$  which fall within the range of  $3000$  until  $3700\text{ cm}^{-1}$ , strong peaks that show chemical structures for pomegranate leaves extract were observed [5]. This absorption spectrum indicates the existence of free hydroxyl groups with an O-H stretch bond, indicating that the pomegranate leaf has flavonoids, polyphenols, and alcohol functional groups with different hydrogen bonds.

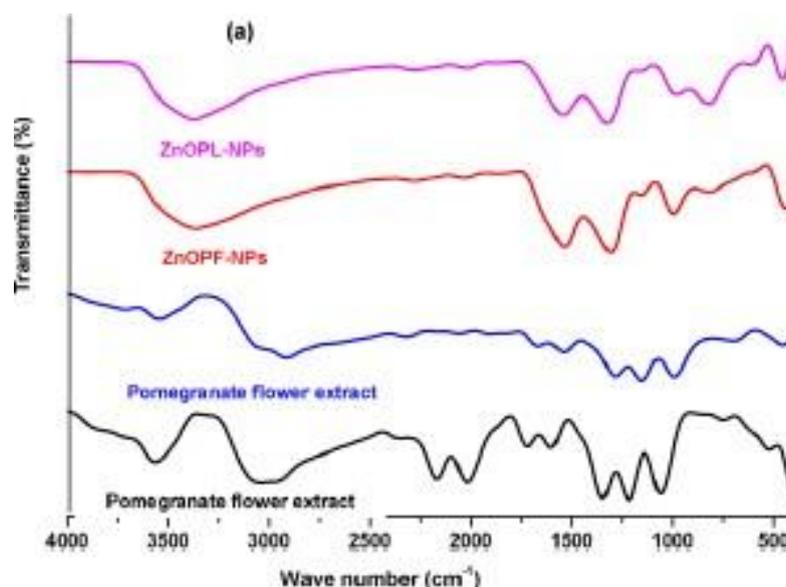


Figure 4: FTIR spectrum of the PL extract, PF extract, (ZnO–NPs–PL) and (ZnO–NPs–PF) [5]

The result of the synthesis of zinc oxide with *Zingiber Officinale* leaves extract shows in Figure 5 state that the FTIR spectral range were within the range of 450 until 4000  $cm^{-1}$ . There was a significant absorption at 528  $cm^{-1}$  and peaks at 3451, 1552, 2170 and 1399  $cm^{-1}$  which corresponding to the stretching of the -OH, N-H, C=C and C-H bonds. Alkenes included along with *Zingiber Officinale* leaves extracts may have aided in the minimization of ZnO nanoparticles [7].

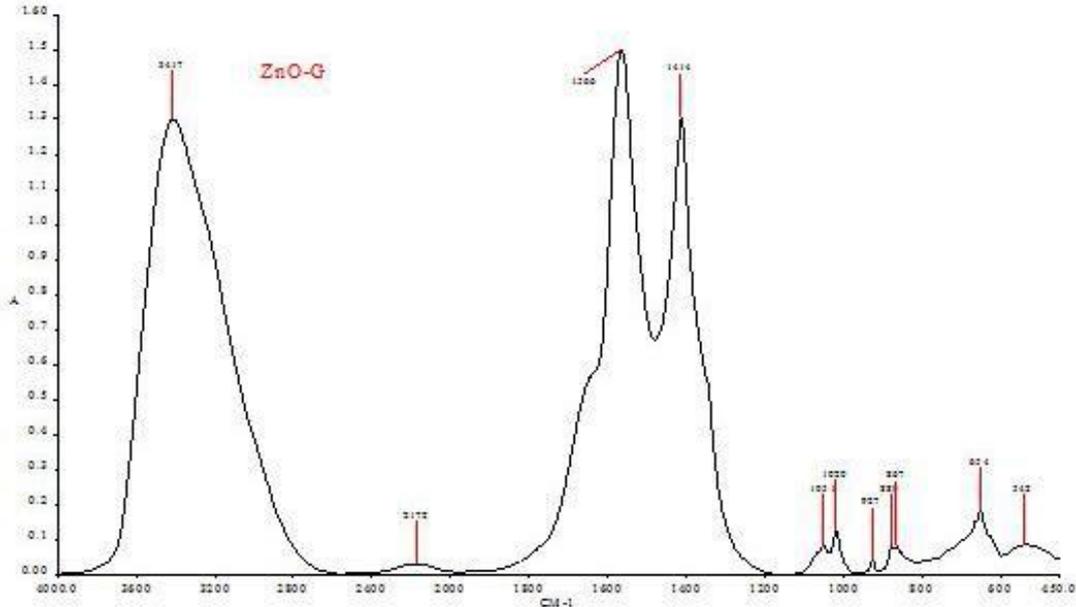


Figure 5: FTIR pattern of the synthesized ZnO NPs from *Zingiber Officinale* leaves extract [7]

Figure 6 shows the FTIR spectra analysis of *Phyllanthus niruri* (keezhanelli) leaves extract with zinc oxide nanoparticles. The results obtained the spectral range from 4000 until 400  $cm^{-1}$ . The aliphatic aldehydes (C=H) have a strong peak at 1426  $cm^{-1}$  thus it indicates the formation of zinc bonds while in tertiary butyl 1240  $cm^{-1}$  (O=H) had a strong peak at 978  $cm^{-1}$  and cycloalkanes (C=C) had a medium peak at 978  $cm^{-1}$  hence it is indicating the formation of nanoparticle[6].

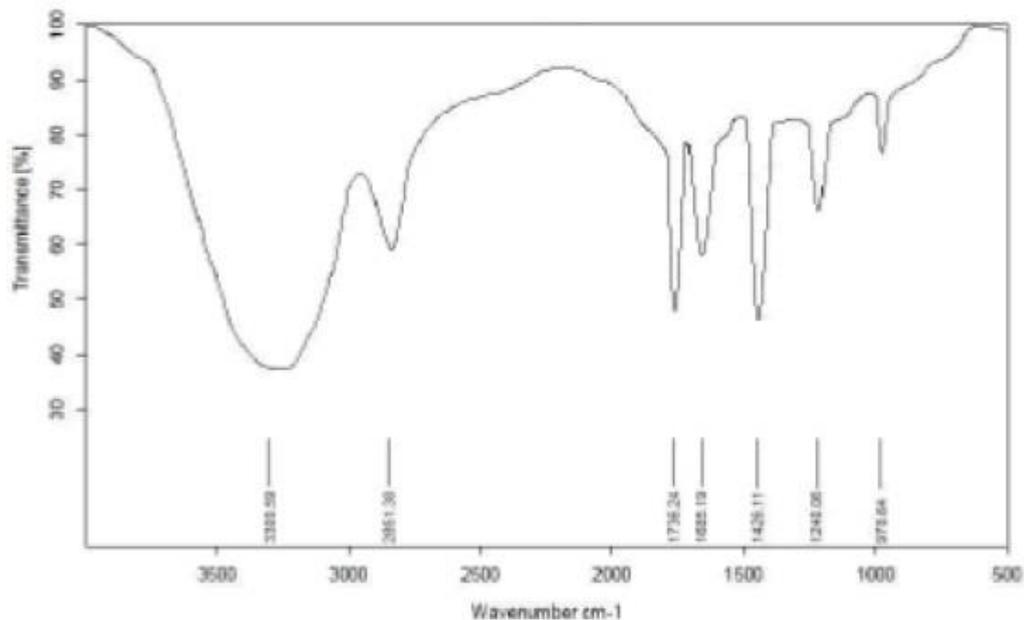
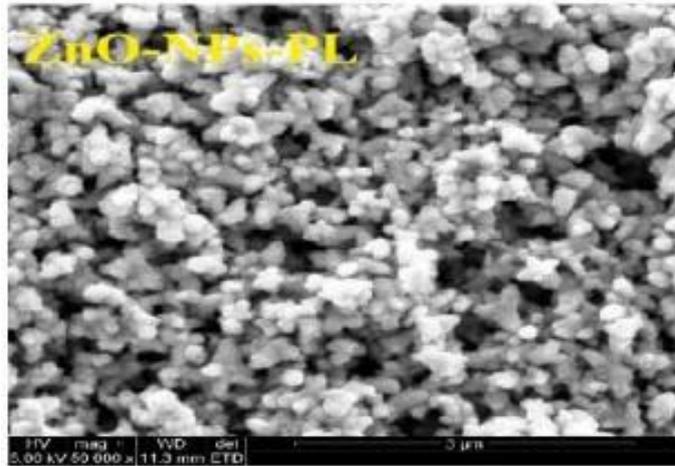


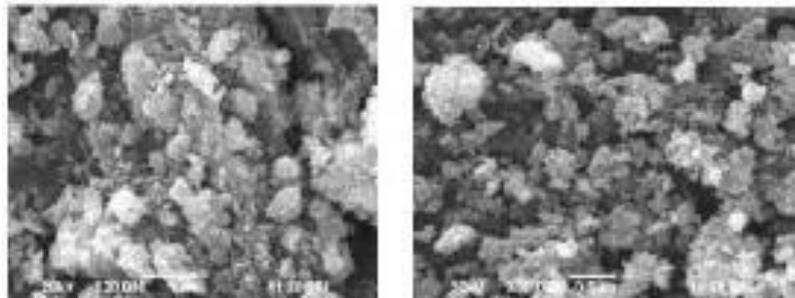
Figure 6: FTIR spectra analysis of *Phyllanthus niruri* leaves extract with ZnO NPs [6]

Figure 7 shows zinc oxide nanoparticles synthesized with pomegranate leaves had an irregular spherical shape with the presence of agglomerated nanoparticles visible when observed by using scanning electron microscope (SEM). It is similar results that have been obtained by W. Muhammad [8] that were using *Papaver somniferum* L leaves extract.



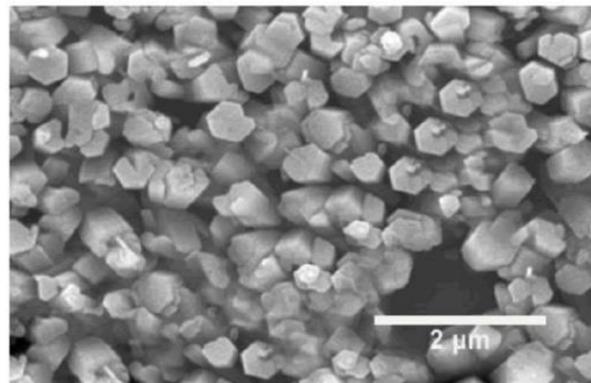
**Figure 7: SEM image of pomegranate leaves synthesized with ZnO NPs [5]**

Figure 8 shows the SEM images of the zinc oxide nanoparticles synthesized by the *Zingiber Officinale* leaves extract. The surface morphology of this specimen was spherical in shape with individual particles along with the number of aggregates. Additionally, the average particle size of the selected individual particles is in the range of 90 nm for the green synthesis method [4].



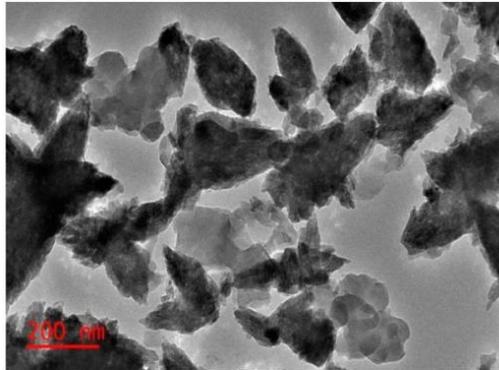
**Figure 8: Different magnification of the zingiber officinale leaves synthesized with ZnO NPs [4]**

By Sathishkumar [6] Figure 9, the results state that the specimen of the zinc oxide nanoparticles synthesise by the *Phyllanthus niruri* (keezhanelli) leaves extract showed that the different hexagonal shape and size less than the 2 μm.



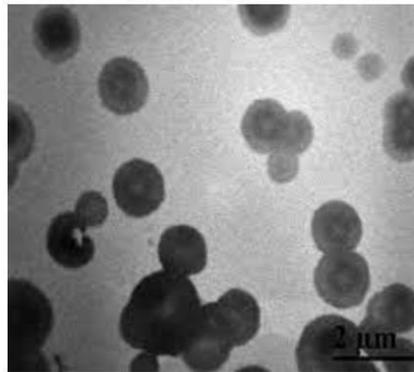
**Figure 9: SEM analysis of *Phyllanthus niruri* (keezhanelli) leaves extract ZnO-NPs[6]**

Figure 10 shows the TEM images of zinc oxide nanoparticles synthesized by the pomegranate leaves. It shows that the particle was in aggregation process and this phenomenon can be attributed to the presence of biological components in the leaves extract that wrap around the nanoparticles causing the particles to stick together [5].



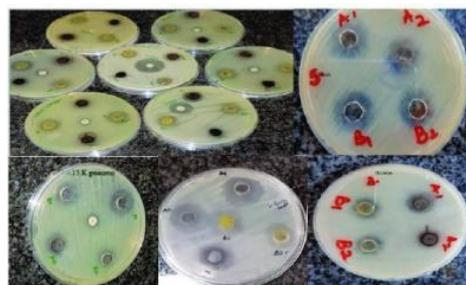
**Figure 10: Image of synthesized ZnO NPs by pomegranate leaves in TEM [5]**

Figure 11 shows the TEM images of zinc oxide nanoparticles synthesized by the *Phyllanthus niruri* (keezhanelli) leaves extract. The results of the TEM analysis give image that the majority of the particles were spherical in shape with the average size of 2  $\mu\text{m}$ . Moreover, these nanoparticles were uniformly distributed and exhibited little agglomeration [9].



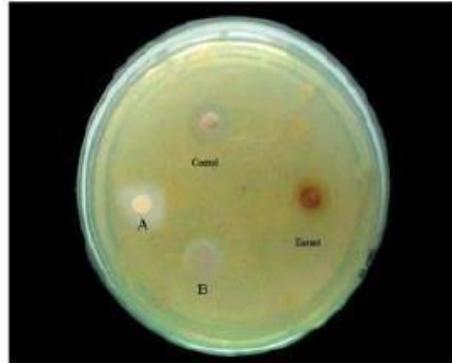
**Figure 11: : TEM analysis of green synthesized ZnO NPs using *Phyllanthus niruri* (keezhanelli) leaves extract [9]**

Different concentration of the zinc oxide nanoparticles synthesis by pomegranate leaves were prepared to observe the antimicrobial activity against the pathogenic stains examined might be a result of an oxidative stress produced by the ROS production which results in protein and bacterial DNA damage. The antimicrobial activity of the synthesized nanoparticle which inhibits bacterial growth may be observed in the form of a distinct zone of inhibition as shown in Figure 12. It is similar to the Nazoori [10] who are investigated the antibacterial activity of ZnO NPs and reported that the less concentration of ZnO that can prevent the growth of the bacteria.

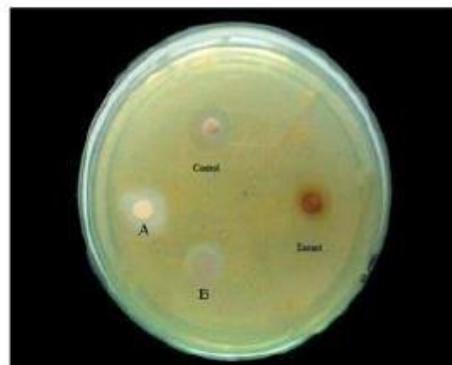


**Figure 12: Zone of inhibition after synthesized of ZnO NPs against bacteria [5]**

Figure 13 and 14 shows antibacterial activity of ZnO NPs by using zingiber officinale leaves extract against various types of bacteria. The existence of an inhibitory zone demonstrates clearly that the antibacterial activity of ZnO NPs includes rupture of the membrane with a high rate of production of surface oxygen species which ultimately results in the pathogen's death [4].



**Figure 13: Staphylococcus aureus in ZnO NPs from zingiber officinale leaves extract [4]**



**Figure 14: Proteus Vulgaris in ZnO NPs from zingiber officinale leaves extract [4]**

The results showed that the plates of the ZnO NPs synthesis by the Phyllanthus niruri (keezhanelli) leaves extract was formed around by the most widespread zone of inhibition during the incubation period that clearly shown in the Figure 15 and 16 [6].



**Figure 15: Staphylococcus aureus in ZnO NPs from Phyllanthus niruri (keezhanelli) leaves extract [6]**



Figure 16: Escherichia coli in ZnO NPs from Phyllanthus niruri (keezhanelli) leaves extract [6]

#### 4. Conclusion

Recently published literature has been critically reviewed in this paper on the antibacterial activity of green synthesis zinc oxide nanoparticle. Several new understandings have been perceived through the review process, which could have significant inputs for future works. It was observed that synthesizing zinc oxide nanoparticles by using different types of leaves extract is quite promising as there are several similar characteristics between the leaves extract. Antibacterial efficacy testing of the produced nanoparticles revealed that zinc oxide nanoparticles were efficient against selected pathogenic bacteria. From the previous research, it can be concluded that:

- Green synthesis approaches employ chemicals free of pollutants since it has no harmful particles that can help produce a better result.
- The presence of zinc oxide nanoparticles can be obtained by various methods such as XRD, FTIR, TEM and SEM.
- The existence of inhibition zone for the nanoparticles implying that zinc oxide nanoparticles can be used as antibacterial agents.

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