# ANTIBACTERIAL ACTIVITY OF ZINC OXIDE NANOPARTICLES PRODUCED FROM *Kappaphycusalverezii*

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**Abstract :** The synthesis and antibacterial activity of zinc nanoparticles from *Kappaphycus alverezii* (*K. alverezii*), a marine algae, has been studied in the present paper. Fresh *K. alverezii* was collected from the Gulf of Mannar, southeast coast of Indiafor the synthesis of zinc nanoparticles (NP) at room temperature. The characterisation of zinc NP was carried out using X-ray diffraction (XRD)and Scanning Electron Microscope (SEM). The synthesized zinc NPswere subjected to antibacterial activity against human pathogenslike *B. subtilis, E. coli, Klebsiella sp. and Staphylococcus sp.*It showed remarkable antibacterial activity indicating the sea weed as a good source of antibacterial component which was further enhanced when used in the form of nanoparticles. The above eco-friendly synthesis procedure of zinc NPcan be scaled up in future for industrial and therapeutic needs.

**Keywords:** Zinc nanoparticles, green synthesis,*Kappaphycusalverezii*, antibacterial activity

## 1.0 Introduction

Nanotechnology implies the creation of nanoparticles in size, shape, chemical compositions and controlled diversity and their potential uses for human benefits.[1]Nanoparticles are used in various fields of science and has potential applications which is classified based on its morphology andsize.[2]Green synthesis of metal nanoparticles has an important role to play in the future of medicine and the biosynthesis of metal nanoparticles using plants is a current focal point in nanomaterial studies.[1]It was noted that synthesis process was considerably rapid and nanoparticles were generated within few minutes of ions coming in contact with the algal extract.[3] Nanoparticles produced by plants are more stable and the rate of synthesis is faster than in the case of microorganisms.[4]

Theantibacterial activity of synthesized nanoparticles exhibited potential inhibitory activity against pathogenic bacteria has been reported in several studies.[3]Nanoparticles of metals are the most potential agents as they show excellent antibacterial activities due to their large surface area-to-volume ratio, which is getting up as the current interest in the researchers due to the growing microbial resistance against metal ions, antibiotics and the growth of resistant strains.[5]

Of all the different types of nanoparticles, some of the metal oxide like ferric oxide, titanium oxide, cupric oxide and zinc oxide are thoroughly being investigated for their various biological activity.[6] Nanoparticles have remarkable application in micro-electronics, diagnostics, optoelectronic devices, biomolecular detection, surface acoustic wave devices like laser devices,electromagnetic coupled sensor.[4]Green synthesis of nanoparticles that have environmentally acceptable solvent systems and eco-friendly reducing agents is of great importance. Also, some algal species are used for the extraction of sugars (polysaccharides).[7]

Some of the proposed mechanism responsible for the antibacterial activity of zinc oxide (ZnO) nanoparticles (NP) includes attack the respiratory chain cell division finally leading to cell death. The nanoparticles releaseions in the bacterial cells which enhance their bactericidal activity.[8] The present study reports the synthesis of zinc (NP) with sizes in the range of 15-34 nm using *Kappaphycus alverezii* extract and also assessing their antagonistic effect against few Gram-positive and Gram-negative bacteria.

## 2.0 Materials and Methods

**2.1 Sample collection**: Red seaweed (*K. alverezii*) was collected from the Gulf of Mannar, southeast coast of India. To maintain the freshness, the seaweed samples are instantly kept in a plastic air tight container with natural seawater.

**2.2 Preparation of seaweed extract**: The samples were thoroughly washed with milli Q water, cut into small pieces and then shade dried for 10 days. Dried seaweed was made into fine powder using kitchen blender following which 2 g of sample in 100 ml of milli Q water was kept in a magnetic stirrer for 1 hour at 60°C.Then, the extract was filtered with Whatmann no:3 filter paper and stored in a cold room for further analysis.

**2.3 Biosynthesis of zinc nanoparticles:**For the biosynthesis of ZnO NP,10 g of zinc acetate was added in 100 ml of seaweed filtrate and kept in a magnetic stirrer for 1 hour at 60°C. The solution was observed for precipitation but due to unprecipitatedparticles, it was poured in a petridishe and heated to 100°C. The solution was scrubbed and the sample was stored. The sample was heated to 450°Cfor 2 hours in a muffle furnace to obtain ZnNP.

**2.4 Characterization of zinc oxide nanoparticles:**

XRD analysis was conducted with ka-XRD-862 diffractometer using monochromatic Cu radiation running at 40 kV and 30 mA. The scanning was done in 2theta from 5-80° 10degree/min. SEM of ZnO nanoparticles was done which showed thin films of the sample were made on a carbon-coated copper grid by immersing a very small amount of the sample on the grid. Excess solution was removed with the help of a blotting paper and then the film on the SEM grid was allowed to dry by putting it under a mercury lamp for 5 min. For energy-dispersive X-ray spectroscopic (EDAX) analysis, the ZnO NP were dehydrated and drop coated on to a carbon film.

**2.5 Antibacterial activity:** The antibacterial activity of synthesized ZnO NPs was determined by agar disc diffusion method against *B. subtilis, E. coli, Klebsiella pneumonia, and Staphylococcusaureus* Fresh overnight culture of each strain was swabbed uniformly onto the individual plates. The 0.10 mg, 0.20 mg and 0.30 mg of ZnO NPs solution were loaded on the wells and incubated for 24 h at 37 °C. Then 0.10 mg ZnO NP solutionused as positive control and water as negative control were placed. After incubation period of 24 hours, different levels of zoneswas formed around the wells which was measured.

## 3.0 Results and Discussion

**3.1 XRD analysis:** The development of a single-phase compound was confirmed by XRD by comparingthe ZnO NP with the standard powder diffractometer card of Joint Committee on Power Diffraction Standards (JCPDS). Intense diffraction peaks due to ZnO NP were clearly observed at 36.47, 31.98 and 34.65 degrees pertaining to 1359, 850 and 580 planes of Bragg's reflection based on the structure of ZnONP as shown in Figure 1. In addition, the acquired reflections are sharp with good intensity which confirms that the structure of synthesized NP were well crystallized. Our findings match with the reports suggested by Govindaraju *et al*. (2009) who found that the metal losses its ions and they turn crystalline in nature after exposure to XRD.

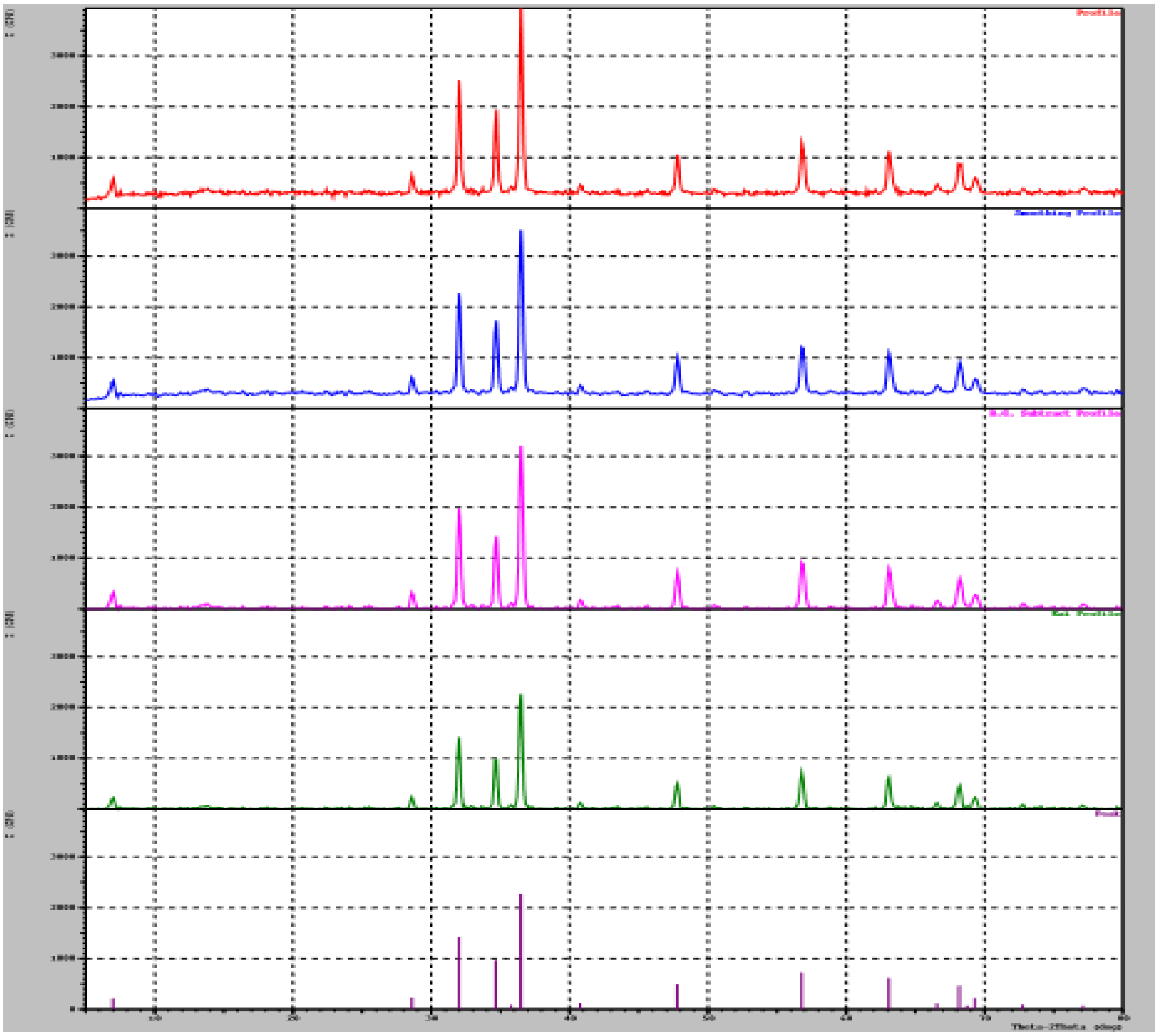

**Fig. 1** XRD pattern analysis of synthesized ZnO nanoparticles

**3.2 SEM analysis:** It showed colloidal ZnO NP (Fig. 2). The cubical and hexagonal-shaped ZnO NP were observed and it was found to be 15-34 nm range in sizes. Singh et al. (2010) reported that particle size between 10 and 50 nm was cubic and hexagonal in shape synthesized from *Argemonemexicana* leaf extract. Chandran *et al.* (2016) reported that the SEM can be employed for characterization based on the size, shape and morphologies of NP.

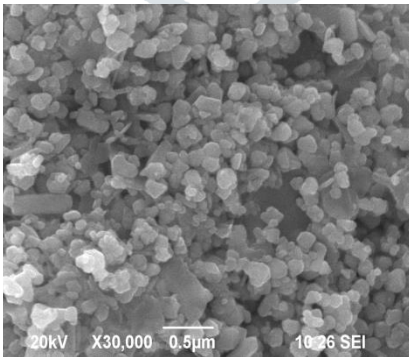


Fig. 2 SEM Analysis of ZnO Nanoparticle

**3.3 EDAX analysis:** Fig 3. shows the EDX spectrum of Zn NP. The spectrum revealed distinct signals in the zinc region and confirmed the formation of ZnO NP and its elemental nature. In the spectrum, the first zinc (Zn) and oxygen (O) peaks may be due to presence of zinc as impurities coming from the sample substrate and oxygen peak is from the chamber of EDS.EDX spectrum shows four peaks which are identified as zinc and oxygen. Hence, it can be seen that pure ZnO NP canbe prepared by precipitation method. Our findings match with the report suggested by Swati *et al.* (2012) who have obtained similar results on EDAX with 50% ZnO-Bentomite nanocomposite.

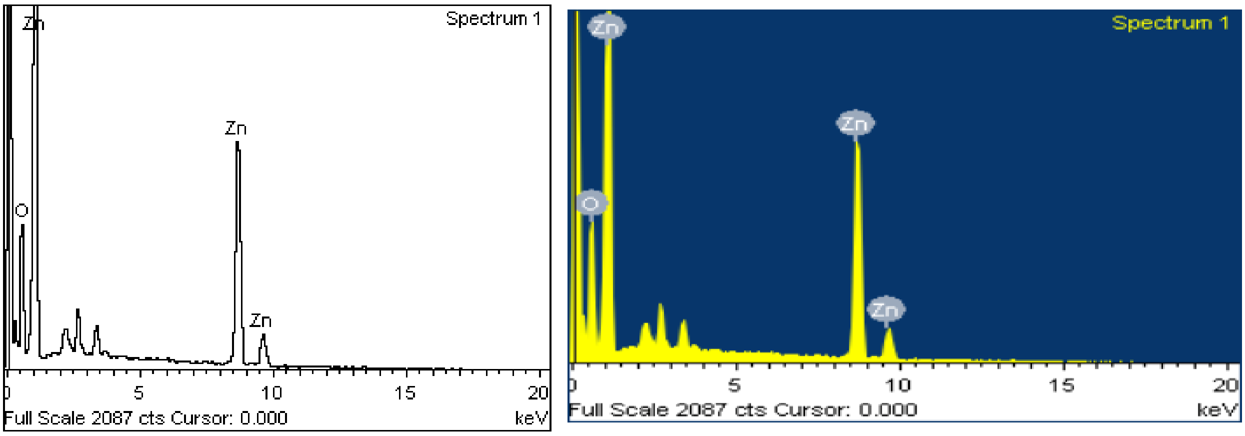


**Fig. 3** EDS spectrum of *K. alverezii*ZnO nanoparticles

**3.4 Antibacterial activity:**The antibacterial effect of ZnO NP was determined against *B. subtilis*, *E. coli*, *Klebsiellapneumoniae*and *Staphylococcus aureus*. The results clearly demonstrated that the ZnO NP synthesized from *K. alverezii* showed antibacterial effect in a dose-dependent manner (Table 1). The maximum zone of inhibition was observed against Gram+ve bacteria like*Staphylococcus aureus*and *B. subtilis*followed by Gram-ve*Klebsiellapneumoniae* and *E.coli.*The organism showed resistance towards the ZnO NP with increasing concentration (0.10, 0.20, 0.30) of the same. Maximum resistance was rendered towards *S.aureus* at the highest concentration of 0.30 mg of *K. alverezii* ZnO NP with the least protection at minimum concentration of 0.10 mg of the same. Different mechanism of action of nanoparticle against Gram+ve and Gram-ve bacteria has already been reported in previous literature because of difference in structural composition. Therefore, the antibacterial activity of *K. alverezii*ZnO NP has proved that these can be used as potent antibacterial agent against diseases.Our findings match with the reports suggested by Zarrindokht *et al.* (2011) who found that Gram -ve bacteria seemed to be more resistant to ZnO nanoparticles than Gram +ve bacteria.

| **Sample** | **Zone of inhibition (mm) at different concentrations of *K. alverzii* ZnO NP** | | |
|---|---|---|---|
| | **0.10 mg** | **0.20 mg** | **0.30 mg** |
| *E.coli* | 9 | 9 | 11 |
| *B. subtilis* | 9 | 10 | 11 |
| *Staphylococcus aureus* | 15 | 17 | 17 |
| *Klebsiella pneumoniae* | 9 | 10 | 12 |

Table 1: Antibacterial activity of *K. alverezii*ZnO Nanoparticle

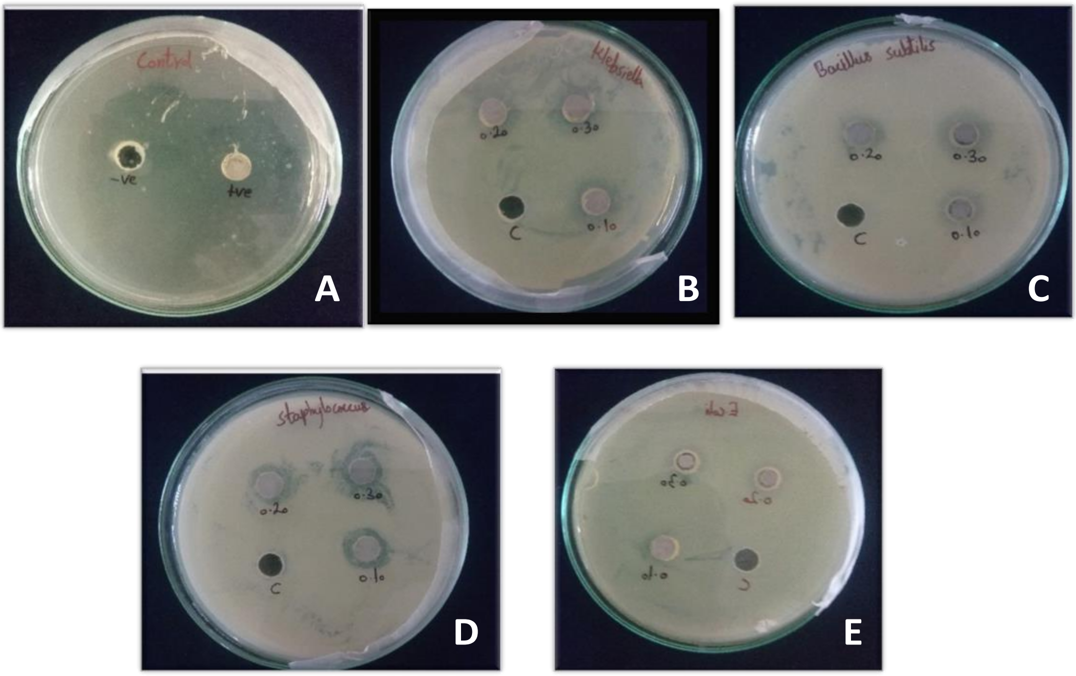


**Fig 4**. Antibacterial activity of K. *alverezii ZnO nanoparticles showing zones of inhibition* (A) *Control* (B) *Klebsiella pneuomiae* (C) *Bacillus subtilis* (D) *Staphylococcus aureus* (E) *E.coli*

4.0

**Conclusion:** In thepresent studyit wasfound that green synthesis of ZnO NP produced from *K. alverezii* showed significant antibacterial activity against human pathogens. The synthesis of nanoparticles was confirmed by characterization studies. It indicates that *K. alverezii* can be used for the synthesis of nanoparticles and tested at various levels to check for antibacterial activity. This can lead to a synthesis of antibacterial drugs against pathogenic bacteria in the future.

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