

# Antibacterial activity of eco-friendly zinc nanoparticles prepared from leaf extract of *Mentha piperita* L.

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**Abstract:** The study aims to prepare reliable, ecofriendly and cost efficient zinc nanoparticles (Zn NPs) by *Mentha piperita* L. leaf extract and zinc sulfate heptahydrate, for four pathogenic bacteria. Synthesized NPs were investigated by using X-ray diffraction (XRD), Fourier Transform Infrared spectroscopy (FTIR), Scanning Electron Microscopy (SEM), and Zeta Potential. Disc diffusion method was used to check their antibacterial activity. For synthesizing Zn NPs leaf extract was used as a biological reducing agent. During reaction, appearance of cloudy white solution due to reduction in the number of zinc ions, indicated the Zn NPs formation. XRD determined the average NPs size i.e. 9.8 nm. FTIR confirmed the presence of functional groups in the leaf extract sample. Spherical shape of the NPs was confirmed by the SEM analysis. Antibacterial activity of the Zn NPs was measured by the inhibition zones against various bacteria (*E. coli*, *P. aeruginosa*, *S. aureus* and *S. pneumoniae*). Inhibition zones were observed in the range of 2.9 to 12.9 mm.

**Keywords:** Green synthesis, characterization, antimicrobial activity, nanotechnology.

## INTRODUCTION

Nanobiotechnology, is a rising field with the aim of production of new materials at nano scale dimensions. Nanoparticles are of different types and sizes with dimensions less than 100 nm (Ashraf *et al.*, 2019). Based on the characteristics (i.e. morphology, distribution and size) nanoparticles have very precise properties. They play vital role in gene delivery, drug diagnostics, sensing and tissue culturing etc (Zafar *et al.*, 2020). Nanoparticles can be prepared by chemical and biological method, however, nanoparticles synthesis by plants is one of the most easiest and authentic way (Zafar *et al.*, 2020).

Green synthesis approach of nanoparticles, is totally non-toxic, safe and shows quite different absorption spectra (Yadav *et al.*, 2006). Zinc oxide nanoparticles (ZnO NPs) are the most important in having unique optical and semiconducting properties and have lots of applications in medical and industrial field (Zhang and Yu, 2014). In green synthesis approach, the biological agent used can be plant extract, microorganisms like fungi and bacteria enzymes are used for nanoparticles synthesis but extracts obtained from plants are considered as the most effective one. Plant extract contain antioxidant and reducing properties to reduce the metal to the respective NPs used for the production of stable nanoparticles.

The aim of the present study was the production of Zn NPs through bio-reduction method by using *M. piperita*

L. fresh leaves by using various analytical approaches. Synthesized nanoparticles were characterized and their antibacterial action was also investigated.

## MATERIAL AND METHODS

### Extraction of plant material

Fresh leaves of *M. piperita* were washed to remove all the impurities, shade dried and censored. Dried leaves (5 g) were dispensed in the 50 ml deionized water. After boiling for 20 minutes at 75°C, plant extract was acquired and put in storage at 4 °C (Zafar *et al.*, 2018).

### Preparation of Zn NPs

Zinc nanoparticles (Zn NPs) were acquired by adding filtrate i.e. plant extract (20 ml) in 1mM zinc sulfate heptahydrate (80 ml) solution. Sonicate the solution at 60 °C for 1 hour and later centrifuge at 10,000 rpm. Synthesis of Zn NPs was confirmed by the change of solution color to cloudy white (Saravanakkumar, 2016).

### Characterization

The size and structure of the Zn NPs was calculated by using the X-Ray diffraction by the Scherrer's equation i.e.

$$t = k\lambda / \beta \cos\theta$$

Where, t = size of the crystallite, k = shape factor,  $\lambda$  = wavelength of radiation

### SEM and FTIR

Scanning Electron Microscope was used to scan the surface of the sample. The chemical nature of zinc

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synthesized nanoparticles was developed by the FTIR RX1-Perkin Elmer in the wavelength range 4000-400  $\text{cm}^{-1}$ . It categorizes the functional groups and provide qualitative and quantitative examination of the sample (Zafar *et al.*, 2020).

### Zeta potential

The technique was used to observe the morphological and polydispersity index (Saranya *et al.*, 2017).

### Antibacterial potential

The antibacterial efficiency of the prepared NPs was analysed using Kirby-Bauer disc diffusion method (Velusamy *et al.*, 2015). Various dilutions 50 $\mu\text{g/mL}$ , 100 $\mu\text{g/mL}$ , 150 $\mu\text{g/mL}$  and 200 $\mu\text{g/mL}$  of Zn NPs against different species of bacteria exhibited considerable results. Bacterial suspension was applied and kept overnight on the nutrient agar plate. Antibacterial disc, Zn NPs coated discs and extract were placed on the inoculated plates and kept for 24 hours in the incubator at 37°C. After incubation, zone of inhibition was observed around each disc.

## RESULTS

### XRD Analysis

An X-ray diffractometer analyzed the shape and size of Zn nanoparticles. Scherrer formula was used to estimate size of the ZnNPs and was found to be 28nm.

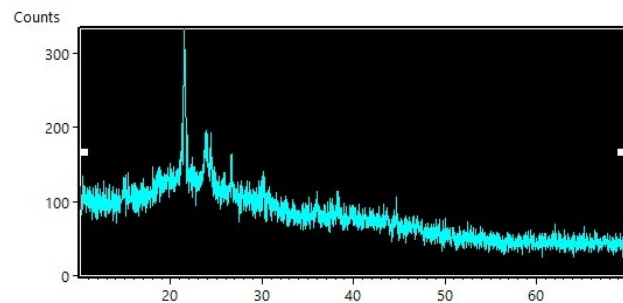


Fig. 1: XRD pattern of the Zn NP

The peaks of ZnNPs were found near 2θ values of 21° with interplanar spacing  $d=0.42269$  nm exhibiting the crystalline structure as calculated by Bragg's law:  $2d\sin\theta=n\lambda$ ,  $n=1$

### FTIR Analysis

FTIR is a technique used to determine the functional groups present in the sample. FTIR pattern exhibited peaks at 427.71, 722.35, 1018.12, 1461.45, 1561.06, 2845.74, 2912.09 and 3398.56 $\text{cm}^{-1}$  confirmed the presence of Zn NPs due to the presence of different stabilizing and reducing agents (fig. 2). At 427.71 $\text{cm}^{-1}$ , absorption band gave an indication about the presence of the Zn NPs. Peaks detected at, 722.35 and 1018.12 $\text{cm}^{-1}$ , revealed C-H bending, while other two peaks at, 1461.45

and 1561.06 $\text{cm}^{-1}$  confirmed the N-H and C-H bending. Similarly, absorption bands at 2845.74, 2912.09 and 3398.56  $\text{cm}^{-1}$  exhibited, C-O, C=O stretching of amides and O-H stretching respectively. Leaf extract of *M.piperita* act as a capping agent. It was determined that carbonyl groups possess strong ability to fix the metal expressive for the protein. Presence of carbonyl group revealed that flavanones and terpenoids could be engrossed on the surface of the Zn NPs. Reducing sugars could reduce the metal ions as well as terpenoids could also cause reduction of metal ions by the fragmentation of the carboxylic acids and oxidation of the aldehyde groups.

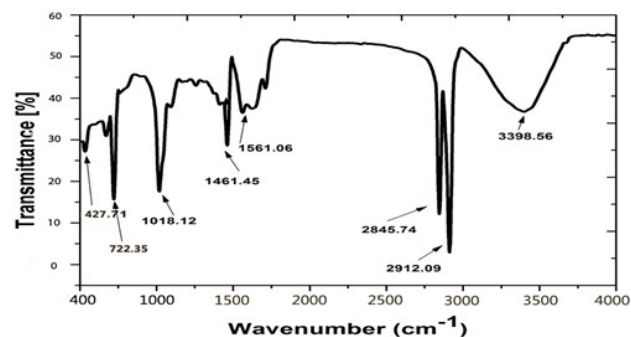


Fig. 2: FTIR spectrum of Zn NPs

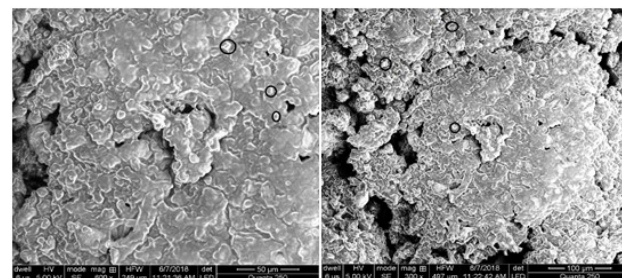


Fig. 3: Scanning electron microscopy images of spherical shaped Zn NPs

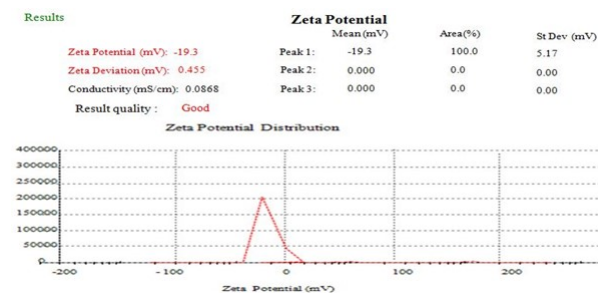


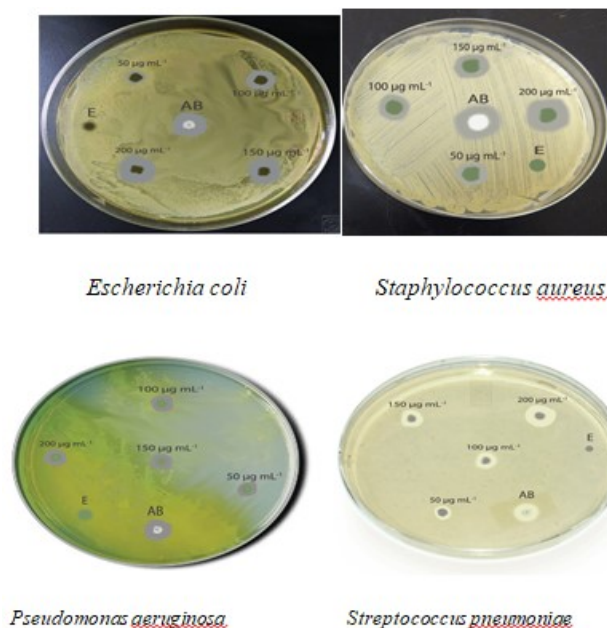
Fig. 4: Zeta potential of the Zn NPs

### SEM Analysis

Scanning electron microscopy is used to determine the size and structure of the NPs. It was confirmed that NPs synthesized by using leaf extract of the *M. piperita* L. were spherical shaped with an average size about 75 nm. The shape and size of the ZnNPs directly depends on the concentration of the extract and on the ratio of zinc sulfate to the leaf extract (fig. 3).

**Table 1:** Inhibition activity of the Zn NPs

Bacteria		Gentamicin	50 $\mu\text{g mL}^{-1}$	100 $\mu\text{g mL}^{-1}$	150 $\mu\text{g mL}^{-1}$	200 $\mu\text{g mL}^{-1}$
<i>Escherichia coli</i>	A	13.2 (mm)	-----	5.6	11.2	12.9
<i>Pseudomonas aeruginosa</i>	B	12.6	4.1	9.7	10.4	11.1
<i>Staphylococcus aureus</i>	C	14	4.2	6.3	9.6	10.3
<i>Streptococcus pneumoniae</i>	D	12.6	2.9	6.9	8.7	10.1

**Fig. 5:** Inhibition zones against the bacteria (AB stands for gentamicin, E as extract and  $\mu\text{g/ml}$  are the dilutions of the Zn NPs)

### Zeta Potential

Zeta potential verified the surface charges of the NPs which ultimately used to detect the stability (fig. 4). Zeta potential value of the Zn NPs was  $-19.3\text{ mV}$  (a measure of the stability of the NPs). The constancy of the NPs was identified by the negative charge to avoid accumulation of the NPs (Desai *et al.*, 2015). Biomolecules present in the leaf extract of the *Mentha piperita* L. could be responsible for the negative charge. It is a proved fact that there is a direct relation between zeta potential and stability (Mittal *et al.*, 2013).

### Antibacterial Potential

The Zn NPs were tested against *E. coli*, *P. aeruginosa* (gram negative), *S. aureus* and *S. pneumoniae* (gram positive) bacteria. Zones of inhibition (fig. 5) helped to select the best dilution. All the dilutions exhibited remarkable results but 200  $\mu\text{g/mL}$  dilution was the most effective one (table 1) against all the bacteria. Zones of inhibition of Zn NPs were quite nearer to zones that were showed by the gentamycin (antibiotic) as a standard. Observed inhibition zones fall in the range of 2.9 to 12.9 mm. The NPs changes the structure of their target and causes them severe damage. Greater zones of inhibition were observed in case of *E.coli* and *P.aeruginosa* as

compared to other two bacteria. Zinc ions penetrate the cell wall and cause severe damage to bacteria. Results clearly proved the efficiency of *M.piperita* based Zn NPs against bacteria.

## DISCUSSION

In this research work, a very effective and simple green method was used to prepare Zn NPs from the leaf extract of *M. piperita* L. (Karakose and Çolak, 2018). Formation Zn NPs showed cloudy white color appearance due to movement of surface plasmon sensation in the NPs (Kavitha *et al.*, 2017). A color change was the first indication for the formation of the NPs (Rajiv *et al.*, 2013; Zafar *et al.*, 2020). Zn NPs prepared by using the extract of *M. piperita* showed enhanced antibacterial activity as reported by Zafar *et al.* (2018) in *Ficus benjamina* leaf extract. NPs were characterized by using Zeta potential, SEM, XRD (fig. 1) and FTIR (Osman and Mustafa, 2015; Bai *et al.*, 2017).

Results of the zeta potential were constant by using *M. piperita* L. leaf extract to synthesize Zn NPs (Kavitha *et al.*, 2017). Biological molecules involved in stabilization

of Zn NPs were identified by FTIR the peaks at 427.71  $\text{cm}^{-1}$  along with other peaks correspond to the presence of the C-H bending of aromatic, N-H bending, C-O stretching vibrations, C=O stretching of amides and O-H stretching in the sample (Santhoshkumar *et al.*, 2017). Other peaks suggested the presence of ketones, aldehydes and carboxylic acids. XRD analysis showed different peaks, results of which revealed the average size of the Zn NPs as 9.8 nm (Santhoshkumar *et al.*, 2017). *M. piperita* based Zn NPs exhibited strong antibacterial activity. In this study, gram positive and gram negative strains of bacteria were used to determine the inhibitory potential of Zn NPs. Synthesized Zn NPs were effective against all the bacteria but they showed strong inhibition against *E. coli* and *P. aeruginosa*. Zn NPs are capable of adhering the cell wall, causing a change in membrane permeability ultimately leading to cell death. Zn NPs totally change and ultimately destroy the structure of the target bacteria (Xie *et al.*, 2011).

## CONCLUSION

Green synthesis method using *M. piperita* L. to synthesize Zn NPs is advantageous over other chemical procedures. Nature and size of the NPs was revealed by characterization through XRD. Results confirmed that size and shape of NPs, acquired direct relation with the concentration of leaf extract and the ratio of the leaf extract to zinc sulfate heptahydrate. SEM analysis revealed the spherical shape of Zn NPs. FTIR determined the functional groups as well as molecules that were involved in the stabilization and reduction of the NPs. Zeta potential exhibited the stability of the Zn NPs. Measurements of zones of inhibition confirmed the antimicrobial potential of the NPs against different bacteria (*E. coli*, *P. aeruginosa*, *S. aureus* and *S. pneumoniae*). Biological synthesis of Zn NPs possesses greater antibacterial activity against infectious bacteria exhibiting an innovative way to a wide array of applications.

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