Section A-Research paper

Green Synthesis, Characterization and Antibacterial Potential of Zinc Oxide Nanoparticles Synthesized from Bryophyllum pinnatum (Lam.) Oken Leaf Extract

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Abstract

Antibiotic resistance is a public health issue since it might result in treatment failure. Despite being susceptible to a wide range of antibiotics, *Listeria monocytogenes* has developed resistance to several conventionally used antibiotics. Alternative therapeutics that can either complement or replace antibiotics is highly desired to resolve the issue of drug resistance. Thus, the search for antibacterial agents against such emerging drug-resistant bacteria is currently an area of research worldwide. In this regard, several green synthesized nanoparticles have been known to exhibit for their antibacterial properties and, therefore, may aid in alleviating the problem of bacterial resistance in *Listeria monocytogenes*. Therefore, the present study aimed at the evaluation of the antibacterial potential of *Bryophyllum pinnatum (Lam.)* Oken leaf extract mediated zinc oxide nanoparticles (ZnO-NPs) against *Listeria monocytogenes*. ZnO-NPs synthesis was confirmed using UV-Visible spectroscopy, Scanning Electron Microscopy (SEM), X-ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), and Dynamic Light Scattering (DLS) techniques and the antibacterial activity was evaluated against *Listeria monocytogenes* using agar well diffusion method. Spherical shaped ZnO-NPs with an average crystalline size of 34.62 nm were prepared. The prepared ZnO-NPs have shown good antibacterial activity against *Listeria monocytogenes.* Hence, from the present work, it could be concluded that *Bryophyllum pinnatum* mediated ZnO-NPs can be used as potential antibacterial agent for treating *Listeria monocytogenes*-related infections. However, more research work is needed to figure out the mechanism of action of the synthesized nanoparticles against the target microbe.

***Keywords*:** Antibacterial potential, *Bryophyllum pinnatum*, green synthesis, *Listeria monocytogenes*

Introduction

L.monocytogenes is a gram-positive bacteria that causes human listeriosis disease which is characterized by septicemia, gastroenteritis, spontaneous abortions, and infections of the central nervous system such as meningitis, encephalitis, or meningoencephalitis (Goldenberg & Thompson, 2003; Souza et al., 2008). Although infection and development of disease by Listeria is not much common, due to the high rate of mortality associated with the disease, the human listeriosis is considered one of the major foodborne illnesses(WHO, 2018). It is alarming for human health since listeriosis has a high mortality rate of 20–30% due to the brain damage the bacterial illness causes (Santos et al., 2019). Listeriosis is particularly prevalent in pregnant women, infants, the elderly, and people with weakened immune systems, such as those who have HIV or certain respiratory infections (Bekondi et al., 2006; Ferreira et al., 2014; David & Cossart, 2017; Gandra et al., 2019; H. H. Yu et al., 2019; Liu et al., 2020).Pregnant women with listeriosis run the risk of miscarriage or early delivery (Lamont et al., 2011).

L.monocytogenes is susceptible to a variety of drugs, but it is resistant to different antibiotics(Hof & Microbiology, 2003). To alleviate multidrug resistance problem, new medicines are needed that can either work with or instead of antibiotics. In this regard, it has been found that some nanoparticles display potent antibacterial action; hence, they may help in resolving the issue of bacterial resistance in *L. monocytogenes*. Since NPs employ fundamentally distinct antibacterial activity pathways from those used by antibiotics, they present an alluring substitute(Álvarez-Chimal et al., 2022). The majority of processes underlying antibiotic resistance do not apply to NPs since they directly interact with bacterial cell walls rather than penetrating them(Imade et al., 2022). The chemistry, size, and structure of the NPs affect how effective they are against bacteria(Seil & Webster, 2012; Carrouel et al., 2020; M. Ahmed et al., 2021). The particular surface area of NPs increases as they become smaller in size and therefore can interact more closely with microbial structures more actively and demonstrate their antibacterial action (Álvarez-Chimal et al., 2022). The greater surface area to volume ratio also causes the release of ions and the creation of reactive oxygen species (ROS), which negatively affect the proteins and DNA of the bacteria because bacteria lack particularly good defense mechanisms(Sirelkhatim et al., 2015; Carrouel et al., 2020). Metal and metal oxide NPs can be used to kill microbes and stop the development of biofilm, as evidenced by the antimicrobial activity of metallic nanoparticles like Zn-NPs and ZNO-NPs, Cu-NPs and CuO-NPs and Ag-NPs (Kanematsu et al., 2009; Sadiq et al., 2009). Compared to other nanoparticles, metal oxide nanoparticles are more promising (Danish et al., 2021), and ZnO-NPs is the third most utilized metal-containing nanomaterial (Faizan et al., 2020).

Due to their great biocompatibility, environmental friendliness, and low toxicity, ZnO-NPs are suitable for biological use(L. Yu et al., 2015).ZnONPs have shown their application in biomedical sectors like anticancer, antibacterial, and antioxidant activities (Ata et al., 2018; Safawo et al., 2018). ZnO-NPs can be found in many commercial cosmetics like sunscreen, ointments, and lotions today and are thought to be safe (Suresh et al., 2018). ZnO-NPs have inhibitory effects on bacterial cell growth and antibacterial activity against spores (Sirelkhatim et al., 2015). It can affect gram-positive bacteria more easily than gram-negative bacteria (Bhuyan et al., 2015; Zare et al., 2017).

ZnO-NPs, results in the production of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide anion radicals (O_2•), hydroxide radicals (HO_2•), and hydroxyl radicals (HO•) (Espitia et al., 2012; Joe et al., 2017; Król et al., 2017; Siddiqi et al., 2018). The production of ROS by ZnO-NPs damages the microbial cell membrane, causing the contents to leak out and cell death. ZnO-NPs' strong reactivity and oxidizing capabilities are thus linked to ROS's antibacterial actions (Abbasi et al., 2017). Through an electrostatic contact with the cell membrane, ZnO-NPs enter the bacterial cell by damaging and disorganizing bacterial cell walls and, once within the bacterial cytoplasm, release zinc ions (Zn^{2+}). These Zn^{2+} ions damage DNA and cause oxidative stress through their interactions with cellular components and this is thought to be another mechanism of action of ZnO-NPs(Joe et al., 2017).

NPs can be prepared by physical, chemical, and biological synthesis approaches. Chemical techniques are frequently employed and widespread (Botha et al., 2019); but they carry the risk of creating substances that are hazardous (Elemike et al., 2019).The biological nanoparticle synthesis method is environmentally friendly, cost-effective, biologically safe, and stable (Razavi et al., 2015) and can be performed by using fungi, algae, bacteria, and plants. Nanoparticle synthesis using plants or components of plants is the best way because it uses a simple, one-step biosynthesis method and is a more environmentally benign, cost-effective, biologically safe and energy-efficient (Ibrahim & sciences, 2015; Elemike et al., 2021). It is also important to note that the biological effectiveness of NPs may be enhanced by the presence of active medicinal compounds in plants that serve as capping and reducing agents(Bharadwaj et al., 2021).Plant parts like leaves, fruits, roots, stems, and seeds are used to make nanoparticles(Narayanan et al., 2011). Plant biomolecules make non-toxic, stable, and

cost-effective NPs (Ren et al., 2009; Laha et al., 2014; LewisOscar et al., 2015; Nabila & Kannabiran, 2018). The plant extract functions in the green path as a capping and reducing and can serve as a biological template that limits the NPs' size, morphology, and shape(Khalil et al., 2018). Plant extracts could be used to synthesis ZnO-NPs which would make them more effective against bacteria and safer for the environment (Akbar et al., 2020; Rasli et al., 2020).

Therefore, considering its potential herbal remedies and antimicrobial application, ZnO-NPs were prepared using *B. pinnatum* leaf extract, and its antibacterial potential was tested against *L. monocytogenes*. Advanced techniques were used to characterize the prepared ZnO-NPs and its antibacterial potential against the test microbe was also assessed.

Materials and Methods

The plant *B. pinnatum* was procured locally and authenticated at CSIR-National Institute of Science and Communication Information Resources (Ref. NISCAIR/RHMD/Consult/2020/3706-07).The Microbial Type Culture Collection and Gene Bank at the CSIR-Institute of Microbial Technology in Sector 39-A, Chandigarh-160036, India, is where the freeze-dried ampoule of *L. monocytogenes* (MTCC 1143) strain was purchased. ZnO-NPs were synthesized by a green synthesis method from the aqueous leaf extracts of *B. Pinnatum.* UV-Vis, HR FESEM, XRD, FT-IR, and DLS were used to characterize the produced ZnO-NPs. The antibacterial effect of ZnO-NPs was evaluated against *L. monocytogenes* (MTCC 1143) using agar well diffusion method.

Results and Discussion

UV-Vis analysis: ZnO-NPs production was preliminarily validated by observing the solution's color change from colorless to yellow. The zinc sulfate suspension changed from being clear less to having a yellowish colour when the surface plasma vibrations were activated(Chaudhary et al., 2019). The yellow color of the solution following the reaction serves as evidence that ZnO-NPs were produced (Bala et al., 2015; Akhter et al., 2019).

UV absorbance peaks of ZnO-NPs were observed at a wavelength of 236 and 396 nm with the maximum peak observed at 236 nm (Figure 1). The peaks observed further confirmed the formation of biosynthesized ZnO-NPs. This result agrees with other findings which are found in different literatures. ZnO-NPs prepared from *Aloe vera* peel extract had a variety of peaks between 200 and 300 nm with maximum absorbance of 240 nm (Chaudhary et al., 2019). ZnO-NPs synthesized using *Mimosa pudica has* maximum absorption peaks occurred at 235 nm, 250 nm, 270 nm, and 300 nm (Balogun et al., 2020).Biosynthesized ZnO-NPs using Plumeria leaf extract showed two strong absorption peaks at 232.5 nm and 242.5 nm (Halanayake et al., 2021). ZnO-NPs Synthesized using leaf extract of *Azadirachta indica* had UV-Vis absorption peaks at 263nm and 304nm(J. Ahmed et al., 2022). Plants called *Nephelium lappaceum L.* and *Garcinia mangostana L*. were used to make ZnO-NPs, which showed a distinctive absorption band between 362 and 368 nm (Kuruppu et al., 2020).

The absorption peak for the biosynthesized of ZnO-NPs from *Calotropis gigantean* leaf extract was around 350 nm(Chaudhuri & Malodia, 2017). ZnO-NP prepared using The electrical spark discharge method has absorption peaks at 345nm (Tseng et al., 2022). Hydrothermally, ZnO nanoparticles were produced and show UV–visible absorption edge at 372 nm (Mishra et al., 2010). ZnO-NPs are UV photosensitive if their absorbance is at or below 250 nm (Ahmad2, 2019). ZnONPs with an absorbance at or below 250 nm are photosensitive in the UV area (Akhter et al., 2019).

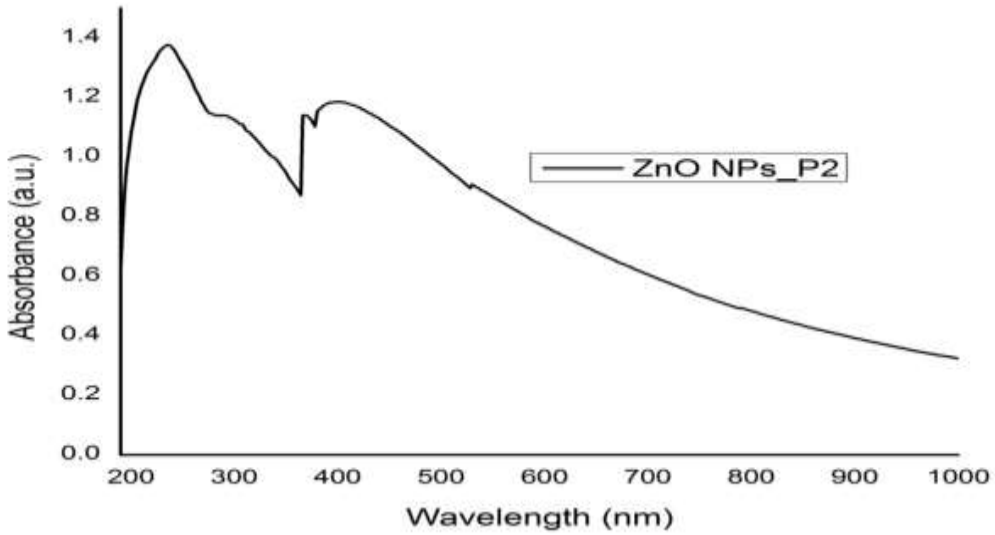


Figure 1.UV-Vis absorption spectrum of ZnO-NPs prepared from *B. pinnatum* leaf extract

SEM analysis: To describe the morphology of produced ZnO-NPs, SEM was used and the comparatively spherical shaped nanoparticles were prepared by the extract of *P. incarnata* (Figure 2)

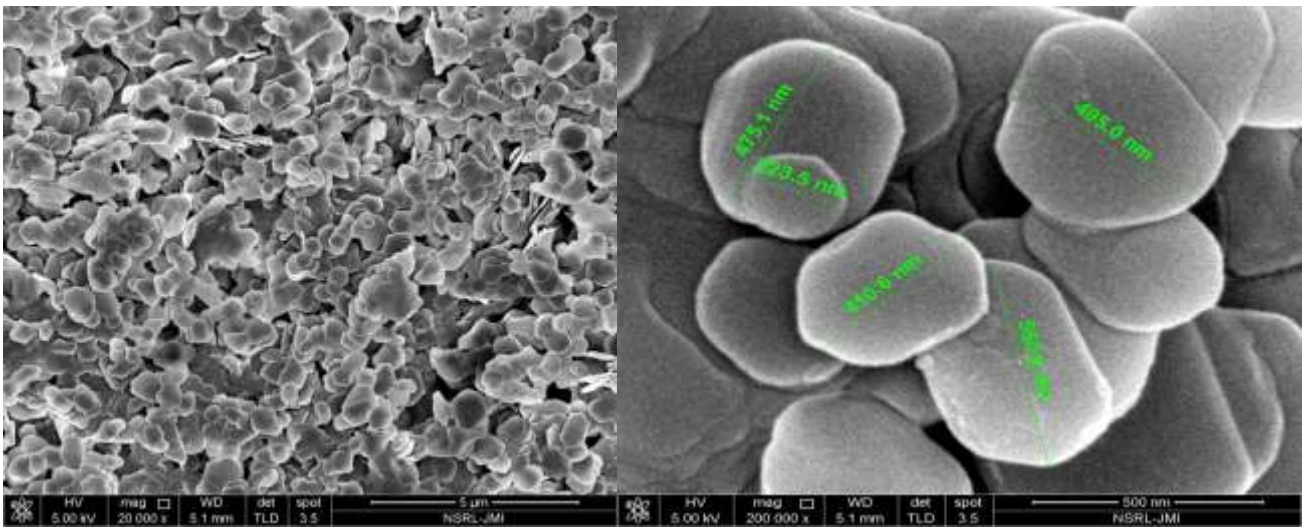


Figure 2. SEM images of synthesized ZnO-NPs at different magnifications

FT-IR characterization: The ZnO-NPs' FTIR spectra shows bands at wavelengths of 3337 cm^{-1}, 1635 cm^{-1}, and 1101 cm^{-1}(Figure 3). The peak at 3337 cm^{-1} is due to O-H, whereas the peaks at 1635 cm^{-1} and 1101 cm^{-1} are caused by C=O and C-O which were sourced from *B. pinnatum* leaf extracts indicating the presence of the Hydroxyl, Carbonyl and Amide groups in green synthesized ZnO-NPs respectively.

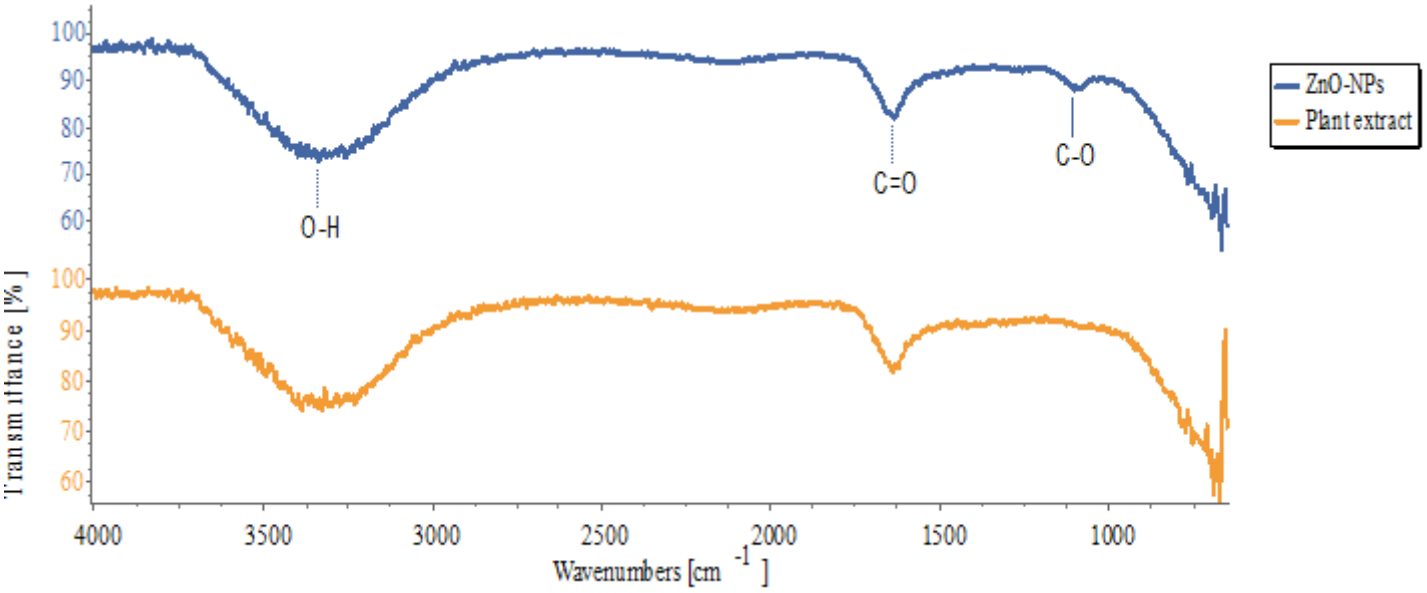


Figure 3. FT-IR spectrum of green synthesized ZnO-NPs

XRD Analysis: Diffraction peaks were found at 2θ=20.20°, 21.97°, 27.88°, 37.06°, 44.70°, 65.68°, 69.38°, and 85.44° (Figure 4). The peaks of ZnONPs were compared to the JCPDS database and with those published literatures and found to be relatively similar. The diffraction peaks positioned at 2θ values 20.20°, 21.97°, 27.88°, 37.06°, 44.70°, 65.68°, 69.38°, and 85.44° correspond to the XRD planes of [111], [200], [211], [310], [321], [432], [440], and [542] the crystalline phase of ZnO respectively.The result agrees with powder ZnO obtained from Joint Committee on Powder Diffraction Standards (JCPDS file no. 21-1486), confirming the formation of ZnO-NPs.

According to the Scherrer equation, the crystalline size of green synthesized ZnO-NPs made with *B. pinnatum* leaf extract was 34.62 nm.

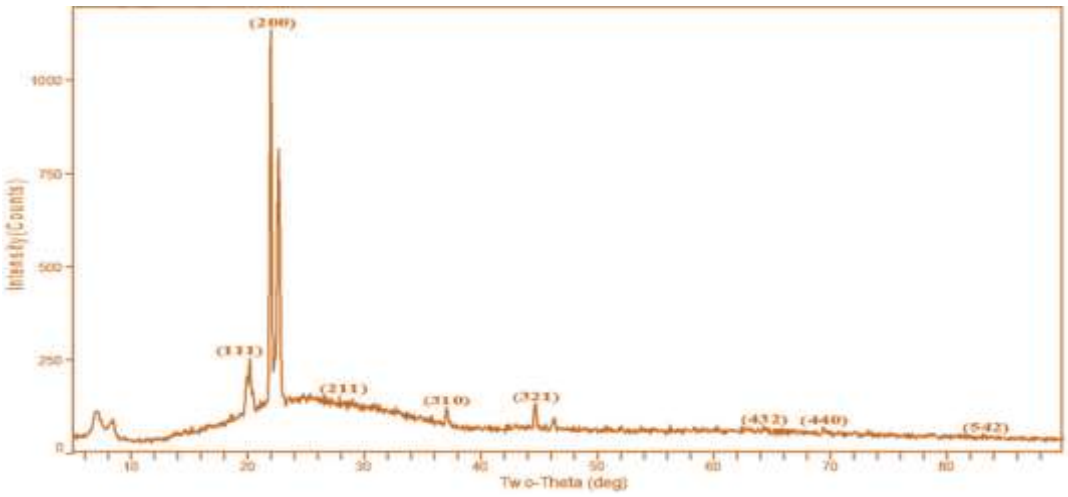
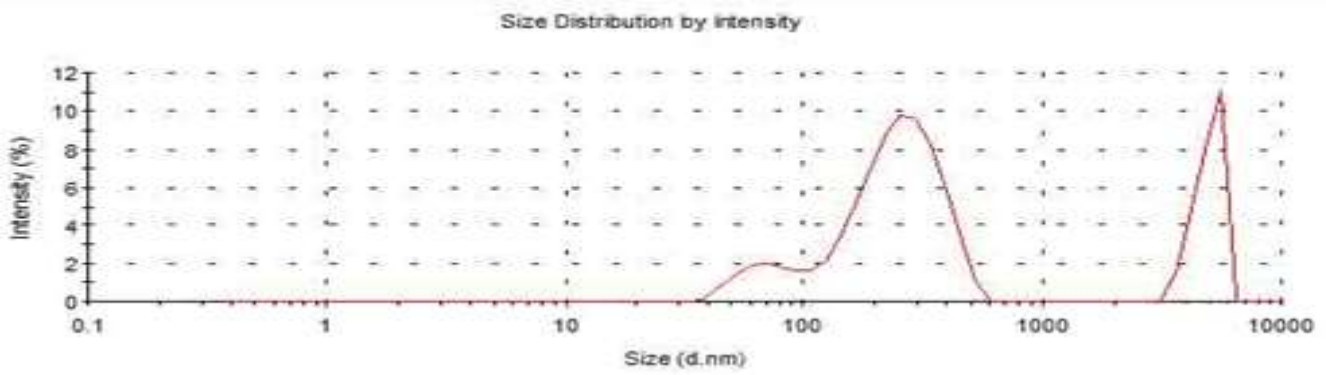


Figure 4. XRD pattern of prepared ZnO-NPs

DLS analysis: DLS was used to figure out the size and surface charge of NPs by using colloidal solutions. The average particle size distribution of the ZnO-NPs that were created was 659 nm, as shown in (Figure 5a). Due to extra hydrate layers on molecules' or ions"surfaces, DLS analysis may detect larger sizes. This indicates that when determining the size, the water layer on the surface of the NPs and biomolecules was taken into account(Bhakya et al., 2015). ZnO Std NPs and ZnO-R NPs had hydrodynamic sizes of 909.00 ± 65.18 nm and 804.67 ± 68.86 nm, respectively. As compared to the size determined through TEM analysis, the size determined through dynamic light

scattering appeared to be larger (Saleemi et al., 2022). The investigation's positive zeta potential was found to be +39 mV. (Fig.5b). Larger sizes are visible in DLS analysis when there are more hydrate layers on the surface of the molecules or ions. This suggests that the water layer covering the surfaces of the NPs and biomolecules was considered while measuring the size (Bhakya et al., 2015). A high repelling force between the particles is shown by the significantly positive zeta potential value, which also prevents agglomeration (Lin et al., 2008; Sankar et al., 2013). This result is agrees with other findings. The highly positive zeta potential value indicates a strong force of attraction between the particles, which also avoids agglomeration (Lin et al., 2008; Sankar et al., 2013). When compared to the other results, it shows that the surface electrical charge of ZnO-NPs is in good condition. The size and charge distribution of the NPs affect the biological properties of ZnO-NPs (Sankar et al., 2014).

(a) Size distribution by intensity



(b) Zeta potential distribution(mV)

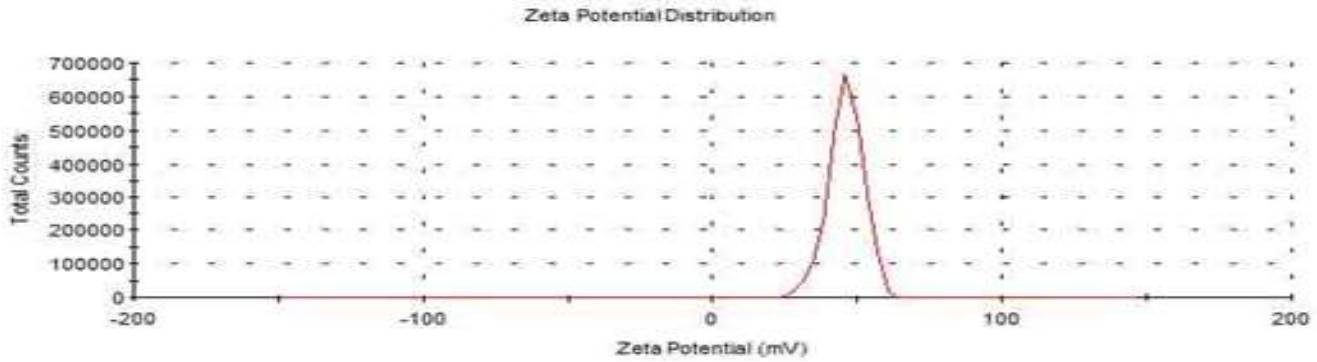


Figure 5. DLS analysis of prepared ZnO-NPs (a) size distribution, (b) zeta potential

Antibacterial activity: The antibacterial activity of the ZnO-NPs against *L.monocytogenes* was investigated using an agar well diffusion assay (Figure 6). A 14.67±0.58 mm zone of inhibition was found after 24 hours of incubation with the ZnO-NPs (Table 1), and the MIC value was 62.50 µM. ZnO-NPs synthesized from *Punica granatum* (Ifeanyichukwu et al., 2020), *Cinnamomum verum* (Osaili et al., 2019), *Peganum harmala* (Mehar et al., 2019), *Ochradenus baccatus* (Nasser A. Al-Shabib et al., 2018), *Lagenaria siceraria* (Kalpana et al., 2017), *Allium sativum*(Vodnar2, 2016), *Nigella sativa*(Nasser A Al-Shabib et al., 2016), and *Rosmarinus officinalis* (Stan et al., 2016) have shown antibacterial activities against *L. monocytogenes* at different concentrations. Similarly, different strains of *L. monocytogenes* have been inhibited by ZnO-NPs synthesized using *Ocimum basilicum* (Stan et al., 2016), *Rosa canina* (Jafarirad et al., 2016), and *Petroselinum crispum* (Stan et al., 2015).

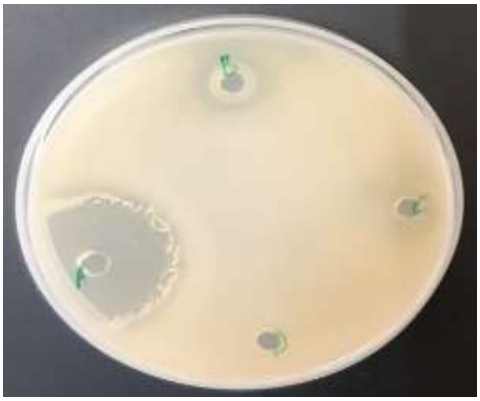


Figure 6. Antibacterial activity of ZnO-NPs against *L. monocytogenes* [where, A= chloramphenicol, B=ZnO-NPs, C=Sterile dist.H_2O, D=$ZnSO_4.H_2O$].

Table 1

Inhibition zone of ZnO-NPs against *L. monocytogens*

Samples	**Zone of inhibition (mm)**
ZnO-NPs	14.67± 0.58
$ZnSO_4.H_2O$	10.67 ±0.57
Chloramphenicol	33.33± 1.15

Conclusion

B. pinnatum leaf extract was used to successfully prepare ZnO-NPs by green synthesis approach. With the aid of FT-IR, SEM, DLS, XRD, and UV-Vis spectroscopy, the synthesized nanoparticles were characterized. The antibacterial activity of the produced ZnO-NPs was examined using agar well diffusion technique. ZnO-NPs has shown antibacterial activity against *L. monocytogenes.* The produced ZnO-NPs could be an alternative antibacterial agent against multidrug resistant *L. monocytogenes* strains. However, more study is necessary to properly comprehend how ZNO-NPs work against *L. monocytogenes.*

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