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Green synthesis of zinc nanoparticles: *Brassica juncea* extract-mediated approach and antimicrobial assessment

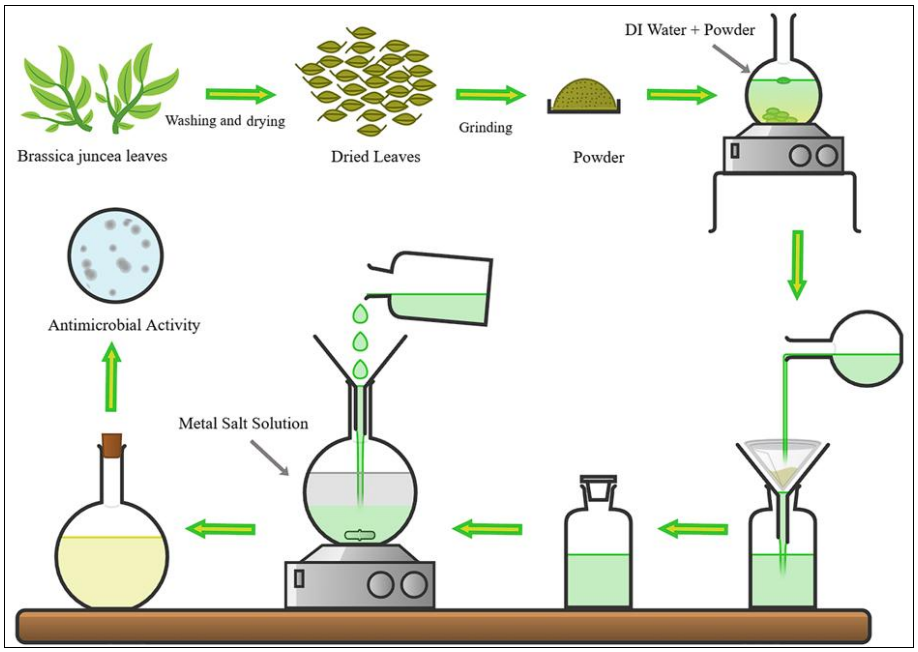
Jaivir Singh, Kavita Yadav, Rajesh Thakur, Rakesh Dhar, Sandeep Yadav and Pallavi Bhardwaj

Abstract
Persent reasearch work describes the green synthesis of zinc nanoparticles by the *Brassica juncea* leaf extract using Zn $(NO_3)_2$ as a precursor material. Green synthesis is a synthetic approach that does not require very high temperature, high pressure, hazardous chemicals, or inert environments. Present research work also test synthesised nanoparticles against both gram +ve & -ve bacteria (Micrococcus & Salmonella enterica) to understand their antimicrobial properties. BJZnNPs demonstrate good antimicrobial activity against both type of bacteria i.e. gram-positive & gram-negaive. The green synthesized zinc nanoparticles (BJZnNPs) were characterized using various analytical tools, such as UV-Vis, FTIR, X-ray Diffraction, FESEM, EDX, Zeta potential and DLS measurements. UV-Vis spectra indicates the synthesis of the zinc nanoparticles by displaying a peak near 370 nm. The FESEM analysis revealed a mean size of 48.49 nm. XRD pattern indicates the presence of hexagonal wurtzite structure. The EDX spectrum verified the presence of Zn in the newly synthesized BJZnNPs. Hydrodynamic diameter comes out to be 85.34 nm from DLS measurments confirmed the synthesis of nanoparticles.

Keywords: Zinc nitrate, *Brassica juncea*, green synthesis, *B. juncea* zinc nanoparticles (BJZnNPs)

1. Introduction
The term "nanometer" was first introduced in 1914 by Richard Adolf Zsigmondy. In 1959, Richard Feynman, an American physicist and Nobel Prize laureate, conceptualized nanotechnology in his speech at the American Physical Society's annual meeting. In 1974, Norio Taniguchi coined the term "nanotechnology," defining it as the processing of materials by manipulating individual atoms and molecules [1]. Evidence of the use and application of various nanoparticles, such as gold, silver, copper, and carbon, can be traced to the period before the Common Era. Some of the famous examples of nanoparticles in history include the Lycurgus Cup, which was invented by the Romans, Mayan Blue, a blue pigment that is corrosion resistant in nature, and Damascus steel, which makes swords non-breakable and razor sharp. Nanotechnology was not confined to artefacts only because it was also employed in Indian traditional medicine system in the form of Bhasmas. The Bhasmas as a medicine is one of the oldest examples of nanomedicine in Ayurveda [2].
The Charak Samhita, an ancient ayurvedic manuscript written by Maharshi Charaka and his disciples around 2000 years ago, constitutes the foundation of Ayurvedic medicine system [3, 4]. A significant aspect of Ayurveda involves the therapeutic application of metal-mineral formulations known as Bhasmas, which literally translates to “ashes” [5, 6]. These Bhasmas are produced using traditional Ayurvedic techniques known as Bhasmikarana [7], which are complex and lengthy processes by which non-bio-compitable substances are converted into biocompatible substances [8,9], requires no advanced knowledge of sophisticated equipment, and is considered both economical and safe for health [5]. Various Bhasmas have been employed in Ayurvedic medicine, including Makardwaja (an alloy of gold, sulfur, and mercury), Tamra Bhasma (copper), Rajata Bhasma (silver), Mandura Bhasma (iron oxide) Loha Bhasma (iron), Jasada Bhasma (zinc), and Swarna Bhasma (gold) [5,9,10]. For example, Jasada Bhasma, which is a preparation derived from zinc, has been employed for managing of diabetes [11] and arthritis [12]. Similarly, iron-based formulations have been employed to treat conditions such as anemia, diabetes, and rheumatism [5], whereas silver-based Rajata Bhasma is utilized for muscle wasting, nerve disorders, brain diseases [13, 14] and diabetes [15].

Copper ash (Tamra Bhasma) has long been used to address acidity, ascites, and asthma [16], while Swarna Bhasma (gold ash) is employed for the treatment of rheumatoid arthritis and other conditions [17].



In the last fifty years, material researcher have meticulously explored the applications of nanoparticles and nanostructured materials across various domains. Nanotechnology has emerged as a significant scientific achievement of the 21st century, encompassing the production, manipulation, and application of materials with at least one dimension smaller than 100 nm. When the particle size approaches the nanoscale, the periodic boundary conditions of the crystalline particle are disrupted, resulting in significant deviations in their physical properties compared to bulk materials. This phenomenon unlocks a plethora of novel applications for nanoparticles in various fields [18]. The primary aim of nanotechnology is to manipulate materials at the molecular level to achieve specific desired properties. By regulating the size and shape of nano materials at the nanometer scale, we can create new materials with innovative uses [19].

Nanoparticles find extensive applications across various sectors such as space industries, surface coatings, optoelectronics, environmental science, natural health products, single-electron transistors, food technology, catalysis, Pesticides, biomedical sciences, scratch-resistant paints, cosmetics, chemical industries, sports, sensors, photoelectrochemical, tissue engineering, energy storage devices, nonlinear optical devices and antibacterial agents [18-22]. The distinctive characteristics of nanoparticles, including their biocompatibility, anti-inflammatory properties, efficacy in drug delivery, bioactivity, bioavailability, tumour-targeting capabilities, and bio-absorption, have propelled their usage in biotechnological and applied microbiological applications [18].

Optical properties are one of the primary and essential characteristics of nanoparticles. Like Silver nanoparticles exhibit a yellowish-grey hue. Gold nanoparticles, when sized around 20 nm, have a distinct red wine color. Platinum (Pt) and palladium (Pd) nanoparticles are characterized by their black color [23].

Nanotechnology-based tools have the potential to rapidly detect diseases at early stage and also indicates the possible treatments. Nano devices, through the examination of cells and molecules, can enhance our understanding of an individual's susceptibility to diseases, thereby facilitating the identification of optimal treatment strategies [24]. Furthermore, it can facilitate tissue regeneration [20]. The use of various nanoparticles, particularly metallic ones, in producing biofuels such as biohydrogen, biodiesel, bioethanol, and biogas, can significantly enhance production efficiency and performance [25].

Nanoparticles can be classified into various categories, including inorganic, organic, ceramic, and carbon-based. Additionally, nanoparticles can be categorized based on their dimensionality into one-dimensional, two-dimensional, and three-dimensional nanoparticles. Nanoparticle synthesis involves two primary approaches: Top-down and Bottom-up approaches. Top-down synthesis involves the breakdown of bulk materials into nanoparticles. This approach includes techniques, such as grinding, milling, and laser ablation. For instance, grinding coconut shells produces nanoparticles, while bottom-up synthesis assembles nanoparticles from smaller units, such as atoms or molecules, to form nanoparticles. This approach includes techniques such as Chemical Vapour Deposition (CVD), sol-gel processes, and biological synthesis. Biological Synthesis is a technique which utilizing plant extracts (various parts such as roots, stems, leaves, and fruits) and microorganisms (bacteria, fungi, and yeast) to reduce metal salts into nanoparticles. This method is eco-friendly, cost-effective, and avoids the use of toxic chemicals, making it a sustainable alternative to the conventional chemical and physical synthesis methods [23, 26].

Kumari *et al*. synthesized silver nanoparticles (Ag-NPs) using the extract of *Azadirachta indica*. Silver nanoparticles (Ag-NPs) were formed within 40 min and characterized by TEM, UV, and DLS. Synthesized Ag-NPs have particle sizes in the range of 10-60 nm [27]. Vijaya kumar *et al*. presented an environmentally friendly and cost-efficient method for synthesizing hexagonal ZnO nanoparticles with significant antimicrobial properties, utilizing the leaf extract of *A. monophylla* [28].

Phytochemicals, such as polyols, unsaturated fatty acids, terpenoids, amino acids, and polyphenols found in plant extracts, function as reducing and stabilizing agents for metallic ions. One notable benefit of utilizing plant based nanoparticle synthesis is that the reaction kinetics of this method are significantly faster than those of other biosynthetic approaches, including chemical nanoparticle preparation. Bacterial infections are a major contributor to chronic illnesses and mortality. Although antibiotics have been the go-to treatment owing to their affordability and effectiveness, extensive research has shown that their widespread use has led to the rise of multidrug resistant bacterial strains. Consequently, the misuse of antibiotics has resulted in superbacteria that are resistant to nearly all antibiotics. Nanoparticles (NPs) typically have dimensions under 100 nm. Their higher surface area to mass ratio leads to unique properties such as increased cell interaction. Nanoparticles (NPs) are largely unaffected by most antibiotic resistance mechanisms, as their mechanism of action involves direct interaction with the bacterial cell wall, eliminating the need for cell penetration. This suggests that NPs are less likely to promote bacterial resistance than are antibiotics. Consequently, researchers have focused their attention on novel NP-based materials with promising antibacterial properties [29, 30].

Metal nanoparticles such as zinc oxide (ZnO), magnesium oxide (MgO), Ag, silver oxide (Ag_2O), titanium dioxide (TiO_2), Cu, copper oxide (CuO), Au, calcium oxide (CaO) and silicon (Si) have demonstrated significant antimicrobial properties. *In vitro* studies have shown that these metal nanoparticles effectively inhibit a variety of microbial species[31].

Brassica juncea, also known as Indian mustard, is an essential oilseed crop grown widely in South Asia and other regions worldwide for its remarkable adaptability and rich nutritional bounty [32]. Indian mustard is a source of various natural products such as proteins, vitamin c and e, sterols, β-carotenoids, triterpene alcohols, glycosides, flavonoids, phenols, and carbohydrates. *B. Juncea* leaves have many therapeutic effects such as they help diabetic patients with comorbid anxiety disorders, also show antihyperglycemic and anti-depressant effects. These leaves are used in production of stimulants, diuretics and expectorants medicines. Glucosinolates and isothiocyanates present in the leaves of *B. Juncea* are anticancer and antimicrobial substances [64, 36]. An *in vitro* study on leaf extracts of *B. Juncea* showed anticancer properties against both lung and colon cancers[37].

2. Materials and Methods

2.1 Chemicals

All chemicals employed during the current research work were of analytical grade and utilized without any additional purification and standardization. Ethanol & Zinc nitrate was purchased from Loba Chemie Private Limited (India). Nutrient Agar was procured from Himedia Laboratories Pvt. Ltd., India. *Brassica juncea* leaves were collected from beautiful crop fields located on the outskirts of Hisar city (Haryana, India). Double distilled water was used during the whole research work to attain the accurate result and to avoid unnecessary contamination.

2.2 Plant material

First, Brassica juncea leaves were washed multiple times with tap water to remove dirt and sandy particles. The leaves were then washed thrice with DI water to remove other soluble impurities. Then BJ leaves undergone drying process in vacuum oven at 60 °C for 48 hr. After this, leaves were grind in the mortar and pastel to make powder. BJ leaf powder (20 g) was dissolved in 250 ml of DD water and heated at 85 ℃ with stirring for 2 h to obtain the extract. After cooling solution was filtered using vacuum filtration unit. Residue left on filter paper was discarded and extract was placed in refrigerator at 4 °C for further work [38].

2.3 Green synthesis of BJZnNPs

150 ml of 0.05M solution of Zinc nitrate was taken in a 500 ml flat bottom flask. Next, 75 ml of BJ extract was added dropwise to a flat bottom flask containing a zinc nitrate solution, and the reaction mixture was stirred on a magnetic stirrer for 2 h at 70 ℃. After 2 h, the reaction mixture exhibited a colour change from greenish to pale yellow which indicates the successful synthesis of BJZnNPs. Following centrifugation of the final product at 10,000 rpm for 15 minutes, the precipitates were subjected to three successive washes with ethanol to eliminate any unreacted organic components. The Washed product dried at 60°C in a vacuum oven for 6 h, dried product was stored in air tight container for further use and characterization.

2.4 Characterization of synthesized metal nanoparticles

Synthesized BJZnNPs were characterized using various characterization techniques like Ultraviolet-visible spectrophotometry, FTIR, XRD, SEM and Dynamic Light Scattering. The Ultraviolet-visible absorption spectra were obtained using a SHIMADZU UV-2450 spectrophotometer, encompassing a wavelength range of 300-700 nm. The particle size distribution and zeta potential of the newly synthesized BJZnNPs were determined using a Litesizer 500, Anton Paar, Austria. The FTIR spectrum was captured within the range of 4000-400 cm^{-1} using a Perkin-Elmer Spectrum BX1 Spectrophotometer. The diffraction patterns were recorded using a Malvern Panalytical Empyrean diffractometer with a Cu-Kα wavelength of 1.540598, a scan range of 4.99° to 90°, and a scan rate of 0.0001°. The surface morphology of BJZnNPs was analyzed using FESEM (Merlin Compact 6073, Carl Zeiss, Germany), and EDX analysis was performed with an Oxford maxN system.

2.5 Antibacterial Behaviour

To understand the antimicrobial efficacy, the synthesized BJZnNPs was investigated using the agar well diffusion method[39]. Prepared nutrient media plates were inoculated with bacterial cultures, evenly spread (approximately 30 µl). A sterile borer was used to create four wells per plate, each with a diameter of 6 mm. BJZnNPs, leaf extract, and Zn $(NO_3)_2$ (20 µl) were dispensed into the wells following the application of an antibiotic disc (streptomycin) on the plate. After a 24-hour incubation at 37℃, the inhibitory zones were measured. Present research work assessed the antimicrobial activity against both type of bacteria i.e. Gram-positive and Gram-negative.

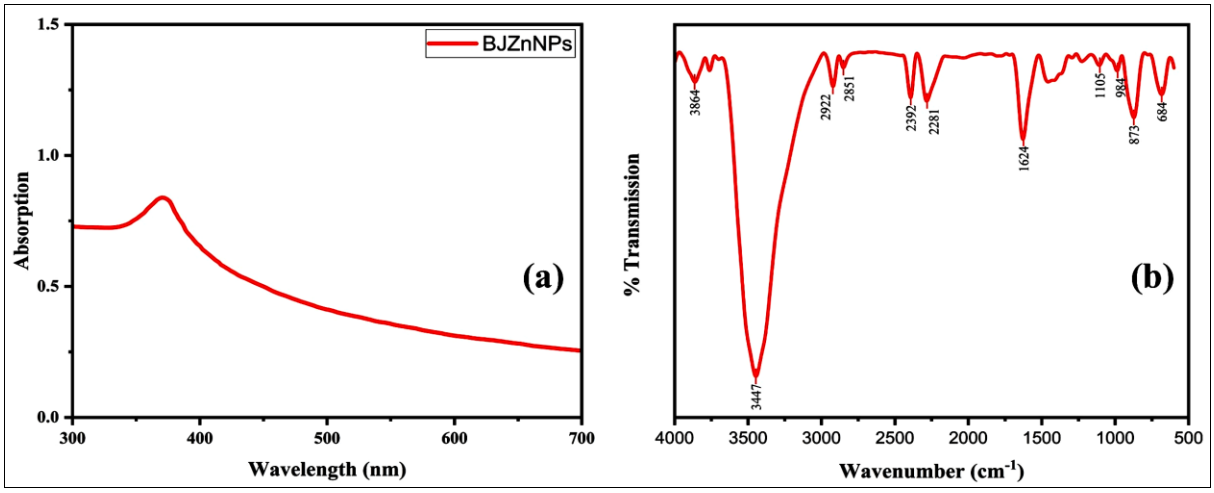


Fig.1 (a) UV-vis graph of BJZnNPs **(b)** FTIR spectra of BJZnNPs

3. Results

3.1 UV-Vis spectroscopy

First of all, to prepare a homogeneous solution of the synthesized ZnO nanoparticles (NPs) were uniformly dispersed in deionized (DI) water using an ultrasonicator for 10 minutes. The UV-visible analysis results revealed a prominent absorption peak around 370 nm [40, 41], confirming the successful synthesis of ZnO nanoparticles using the *B. juncea* extract. Fig.1 (a) illustrates the UV-Vis spectra of the newly synthesized nanoparticles. The optical absorbance was analyzed using a UV-Vis spectrophotometer operating within a wavelength range of 300-700 nm.

3.2 FTIR Analysis

The synthesized nanoparticles were subjected to analysis through Fourier-transform infrared (FTIR) spectroscopy, with the objective of identifying the functional groups and bio constituents present within the samples. The spectrum was recorded in the range of 4000 to 600 cm^{-1}. Fig.1 (b) represent the FTIR spectrum of BJZnNPs. Peaks at 3864 and 3447 cm^{-1} corresponds to the -NH and -OH stretching vibration [42,43]. Peaks at 2922 and 2851 cm^{-1} indicates the presence of aliphatic CH\$CH_2$\$CH_3$ groups. Peak at 2392 cm^{-1} due the OH stretching of carboxylic acid[44]. Peak at 2281 cm^{-1} attributed to the stretching of alkyne group (-C≡C-) due the presence of flavonoids and terpenoids[45]. Peak at 1624 cm^{-1} may be due to the presence of alkene (-C=C-) or carbonyl (-C=O) group[46-48]. Peaks at 1105 & 984 cm^{-1} corresponds to the -C-OH and -C-O-C- groups [49, 50]. Two bands at 873 & 684 cm^{-1} confirms the formation of ZnO nanoparticles i.e. Zn-O stretching vibrations [43, 51].

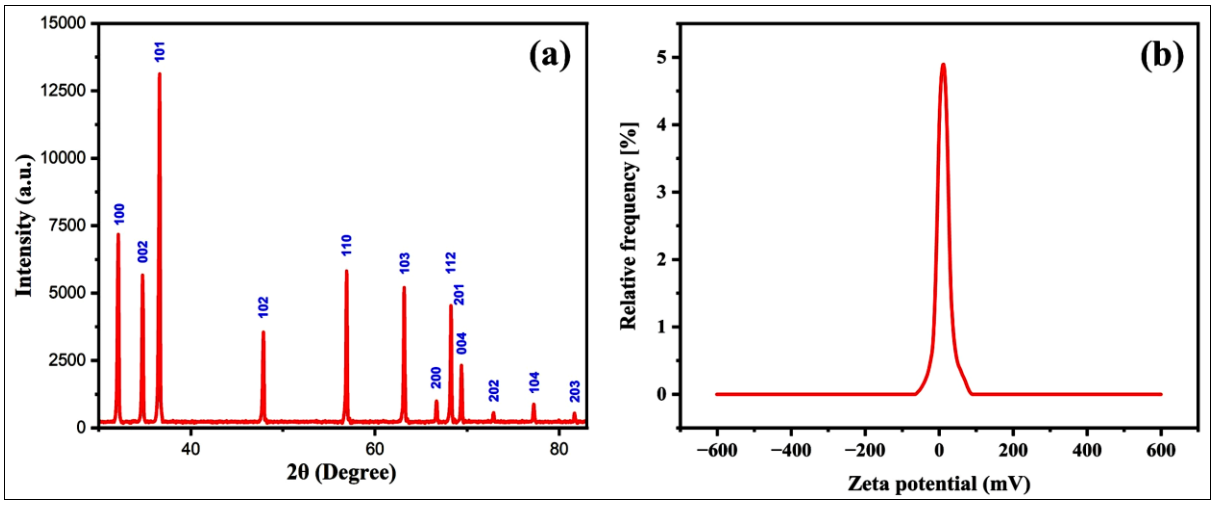


Fig2: (a) Diffraction pattern of BJZnNPs **(b)** Zeta potential plot of BJZnNPs

3.3 Diffraction Analysis

The measurement conditions for the XRD analysis were as follows: 2θ ranged from 4.994599997° to 90.00005004°, with a time per step of 36.465 seconds and a scan step size of 0.0262606°. The K-Alpha1 and K-Alpha2 wavelengths were 1.540598 Å and 1.544426 Å, respectively. The minimum step sizes for 2θ and Omega were both set to 0.0001°. The generator operated at a voltage of 45 kV and a tube current of 40 mA. The XRD analysis shown in Fig.2 (a) revealed the crystalline nature of BJZnNPs, producing diffraction peaks at 32.10°, 34.75°, 36.69°, 47.85°, 56.90°, 63.17°, 66.68°, 68.24°, 68.63°, 69.38°, 72.86°, 77.26°, and 81.65°. These peaks correspond to the (100), (002), (101), (102), (110), (103), (200), (112), (201), (004), (202), (104), and (203) planes. The observed XRD pattern closely matched the JCPDS card number 01-080-0075, confirming the hexagonal wurtzite structure [52-54].

3.4 Field Emission SEM and Energy Dispersive X-ray Spectroscopy

Field Emission Scanning Electron Microscopy (FESEM) serves as a crucial tool for examining the morphological characteristics of newly synthesized nanoparticles. The FESEM images Fig. 3 (a) reveal the presence of nanoparticles within the sample, with average size is 48.49 nm. Energy Dispersive X-ray Spectroscopy (EDX) analysis further confirms the presence of zinc (Zn) in the sample. Additionally, the FESEM images indicate agglomeration of the synthesized zinc nanoparticles [55, 56].

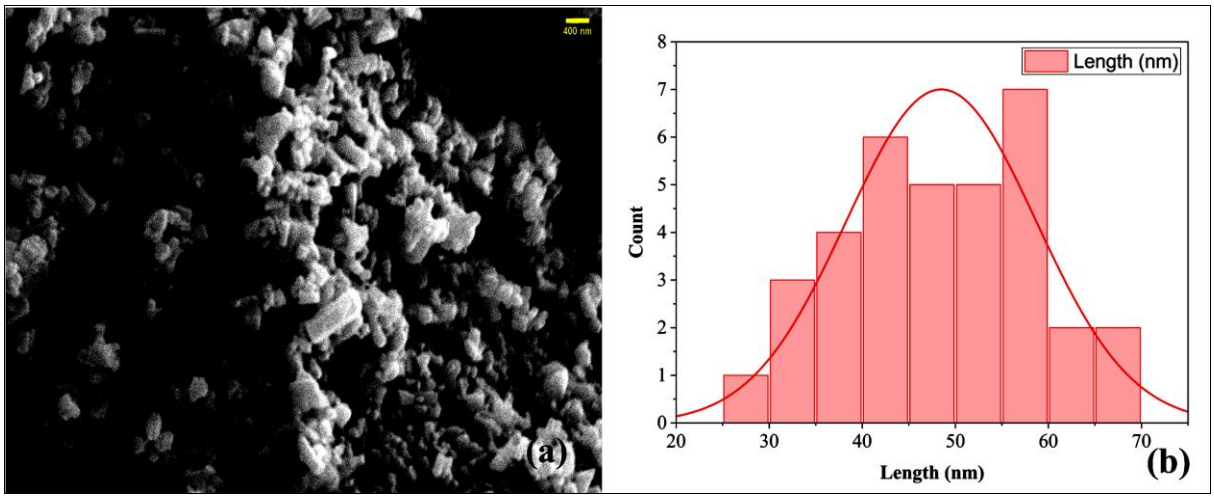


Fig 3: (a) SEM image of BJZnNPs (b) SEM histogram of BJZnNPs

3.5 Zeta Potential & Hydrodynamic Diameter

The zeta potential's magnitude determines how strongly similarly charged particles in a dispersion repel each other electrostatically. A higher absolute value means stronger repulsion, aiding stability, while a lower value can lead to aggregation or coagulation [57, 58]. Fig. show the Zeta potential distribution graph which show change in relative frequency with zeta potential of BJZnNPs. Zeta potential of synthesized BJZnNPs comes out to be 7.451563055 mV while distribution peak at 11.75953591 mV. It shows BJZnNPs carries positive charge. Fig shows the size distribution graph of synthesized nanoparticles disclosing the hydrodynamic diameter of 85.34 nm with a 0.23 PDI value. Low PDI (>0.3) value indicates the low polydisperse nature of synthesized nanoparticles [59].

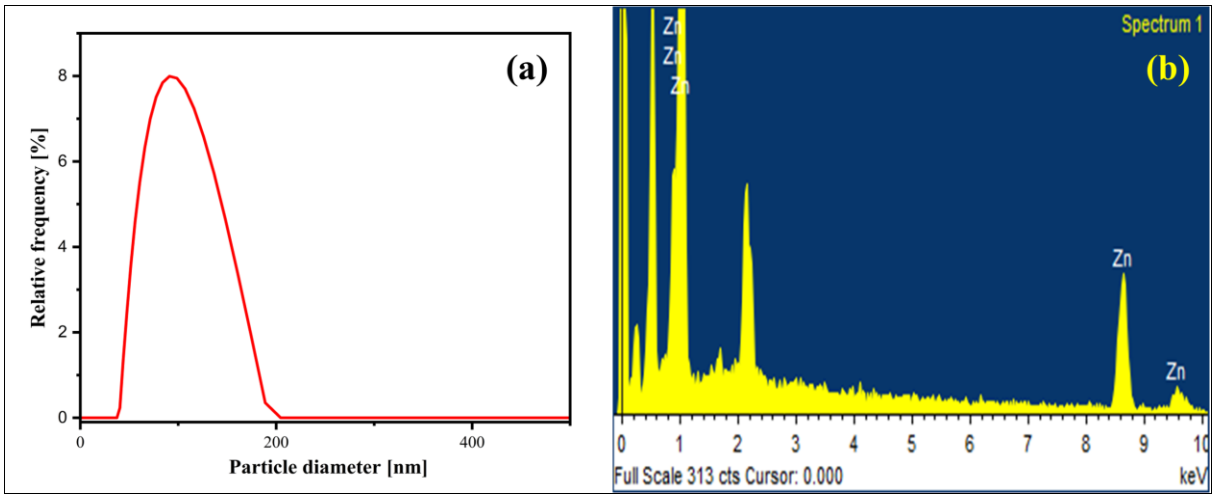
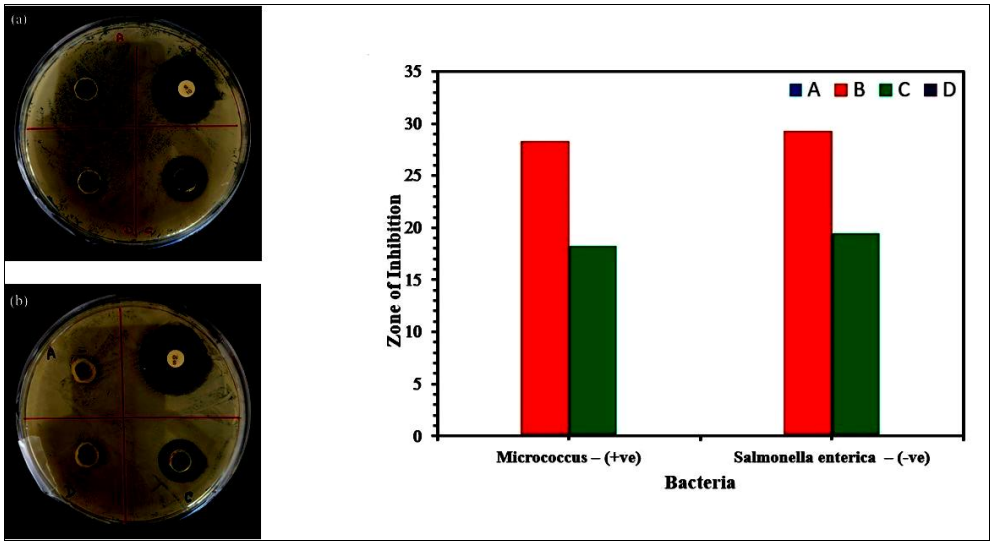


Fig 3: (a) DLS size distribution graph of BJZnNPs **(b)** EDX spectrum of BJZnNPs

3.6 Antibacterial Study

Green synthesized BJZnNPs showed very good antimicrobial behaviour towards both type of bacteria (Micrococcus and Salmonella enterica i.e. gram positive & negative. The results shown in Table 1 reveal that synthesized BJZnNPs exhibited antimicrobial activity while plant extract and their corresponding metal salt do not show any antimicrobial activity. Here, "A" represents plant extract, "B" represents control (streptomycin), "C" represents BJZnNPs, and "D" represents corresponding metal salt. Zones of inhibition (ZOI) by control, BJZnNPs, metal salt, and plant extract are shown in Fig. 5(a-b). Graph shown in Fig. 5c describe the antimicrobial activity of BJZnNPs against both (+ve & -ve) types of bacteria.

Bacteria	A	B	C	D
Micrococcus - (+ve)	0	28.27	18.26	0
Salmonella enterica - (-ve)	0	29.26	19.47	0



4. Proposed mechanism of antimicrobial behaviour of metal nanoparticles

The complete mechanism of the antimicrobial behavior of metal nanoparticles is still unclear, but the most commonly accepted antibacterial activity mechanism by which metal nanoparticles inhibit or destroy bacteria is ROS generation. Reactive oxygen species (ROS) contain several oxygen-containing species such as radicals (superoxide, singlet oxygen, and hydroxyl radical), peroxide (H_2O_2), acid (HOCl), and hydroxide (LOOH). Reactive oxygen species (ROS) contribute to oxidative stress, which ultimately leads to bacterial cell death [60, 61]. Another reason are direct contact with cell wall causing destruction of cell and liberation of Zn^{2+} ions which penetrate into the cell and disturb the cell function [62,63].

5. Conclusion

Green synthesis of zinc nanoparticles was done with the *brassica juncea* leaf extract in a single reaction vessel. The synthesized BJZnNPs characterized using several characterization technique like FTIR, UV-Vis, XRD, FESEM, EDX, Zeta Potential and DLS. BJZnNPs showed antimicrobial behaviour against both gram +ve and -ve bacterium. Green, economic and fast synthesis of metal nanoparticles is removed the energy, financial, and pollution burden from the earth because traditional synthesis of metal nanoparticles involves the hazardous chemical, lot of energy and time.

6. Declaration of conflicts of interest

The authors declare no conflicts of interests.

7. Acknowledgement

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