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Research Article

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Phytochemical Screening, Biogenic Synthesis, Characterisation and Antibacterial activity Investigation of Zinc oxide nanoparticles (ZnO - NPs) Using *Biancaea decapetala* Leaf extract

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Abstract

In this study, zinc oxide nanoparticles were synthesized using various ratios of the *Biancaea decapetala* Leaf extract and zinc acetate dehydrated in solution. The phytochemical analysis of the leaf extract confirms the presence of contains flavonoids, saponins, Steroidal glycosides, Triterpenoids, Phenolic compound and steroids bioactive compounds present in methanol Extract. The total phenol content and total flavonoid of *Biancaea decapetala* leaves are methanolic 375.74± 0.16, 330.34± 0.15 and aqueous extract 285.30± 0.33, 220.52± 0.25 respectively. The above results showed that aqueous extract contain less phenolic and flavonoids content than the alcoholic extract.
The synthesized zinc oxide nanoparticles (ZnO-NPs) were characterized by using UV–Vis, FT-IR, XRD, DLS, SEM and TEM spectroscopic techniques. The average crystal size of the synthesized nanoparticles was found to be 21.6 nm. The in vitro antibacterial activities of the synthesized nanoparticles were tested on two Gram-positive (Bacillus, and S. aureus) and two Gram-negative (*E. coli,* and *P. aeruginosa*) bacteria strains using the disc diffusion method. The antibacterial activities of zinc oxide nanoparticles showed comparable results with the antibiotic standards. The significant antibacterial zone of inhibition was recorded in S. aureus (27 ± 0.32 mm) followed by B. cereus (23 ± 0.16 mm), *P. aeruginosa* (20 ± 0.30 mm) and E. coli (15± 0.21 mm). Therefore, the eco- friendly synthesis of zinc oxide nanoparticles using the *Biancaea decapetala* Leaf extract is a promising approach for developing new drug products with efficient antibacterial properties, and it could be evaluated as an opportunity for in vivo biomedical applications with positive environmental impacts.

Keywords: *Biancaea decapetala* Zinc oxide Nanoparticles, Phytochemicals Screening, Biogenic synthesis, Characterisation, Antibacterial activity.

1. Introduction

Nanotechnology is now considered to be a proven state-of-the-art technology with numerous branches embedded in industrial fields such as chemical, pharmaceutical, mechanical, and food processing industries. Nanotechnology also plays an interesting role in the areas of computing, power generation, optics, drug delivery, and environmental sciences [1].Novel utilization of nanoparticles and nanomaterials is developing on different fronts due to their new or improved properties dependent on size, distribution and morphology [2]. It is quickly acquiring redesign in many fields, for example, medical care, beauty care products, biomedical, drug-quality conveyance, climate, synthetic enterprises, gadgets and photograph electrochemical applications [3].

Among the various nanoparticles that have been synthesized so far, zinc oxide nanoparticles (ZnO NPs) have attracted a lot of interest due to their unique physical and chemical characteristics [4]. The attention of the scientific and medical community was drawn to ZnO nanoparticles because of their crucial significance in biomedical and cancer applications [5]. The antibacterial activity may include the accumulation of ZnO NPs in the bacterial cells' outer membrane, which would induce the release of Zn2+ and lead to the disintegration of the bacterial cell membrane, damage to the membrane proteins, and genetic instability, ultimately leading to the death of the bacterial cells [6].

The primary mechanisms of ZnO NPs' antibacterial toxicity were based on their capacity to generate excess reactive oxygen species (ROS), including hydrogen peroxide, superoxide anion, and hydroxyl radicals [7]. As a result, cells enter an oxidative stress state that finally results in death by denaturing proteins, impairing mitochondrial action, and increasing cell metabolic activity [8]. Nanoparticles, with unique size, morphology, and shape, are synthesized using chemical, physical, and green/microbiological methods, offering alternative solutions to bulk materials shown in Figure 1[9] .

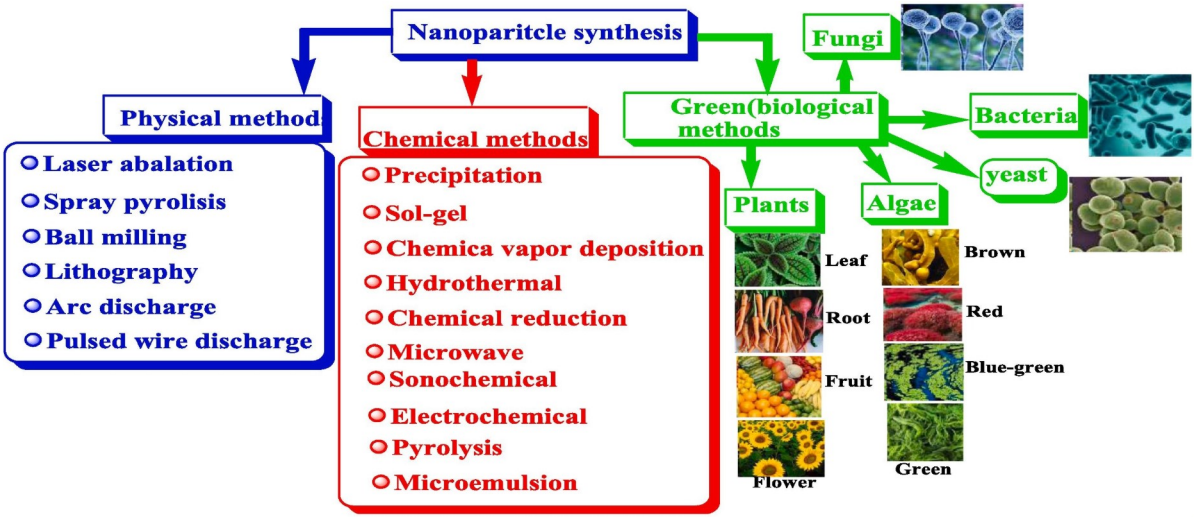

Fig. 1. Fabrication of nanoparticles with physical, chemical, and biological methods

Biancaea decapetala (Roth) O.Deg. (Fabaceae) is used to treat colds, fever, and rheumatic pain caused by inflammation. However, the mechanism underlying its anti-inflammatory properties remains unclear. In recent years, research on the medicinal properties of *B. decapetala* has mainly focused on its chemical components and pharmacological activities. In a previous study, it was found that the 70% ethanol extract of *B. decapetala* showed a significant inhibitory effect on the increase in peritoneal capillary permeability mediated by acetic acid in mice and showed good anti-inflammatory activity. In addition, compounds such as protosappanin B, liquiritigenin, and 3-deoxysappanchalcone have been isolated for the first time [10].

Fig.1(a) *Biancaea decapetala* (Roth) Flowering Plant

Here, we report plant-based synthesis of zinc oxide nanoparticles using the methanol extracts of *Biancaea decapetala* leaves. Green synthesis of ZnO-NPs has eco-friendly aspects and various biomedical applications. The metabolites found in the aqueous extract of *Biancaea decapetala* act as an oxidizing, reducing, and capping agent for the synthesis of biogenic ZnO-NPs. The green

synthesized nanoparticles will be characterized using modern techniques such as Fourier transform infrared (FTIR) spectroscopy, ultraviolet (UV) spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM), transmission electron microscopy (TEM) and dynamic light scattering (DLS). The NPs will be checked for their antimicrobial, antidiabetic, antioxidant, anti-inflammatory and protein kinase inhibitory potential.

2. Materials and Methods

2.1 Plant Collection and Authentication:

Biancaea decapetala leaves were collected from the Mainpat Hiis Surguja Chhattisgarh . The plants were authenticated by Prof. Rijwan Ulla, Department of Botany, Rajeev Gandhi Govt. Autonomous Post Graduate College Ambikapur, Surguja, Chhattisgarh, India. The plant materials were dried under shade by placing in a single layer and coarsely powdered by hand mixer and pass through sieve no 60.

2.2 Preparation of Plant Extract

The collected samples were washed with clean water and dried in the shed for about three weeks. The dry samples were chopped into pieces and ground into powder by using a mechanical grinder. The powdered materials were stored in clean plastic bottles until the use. The materials were subjected to Soxhlet's extraction using methanol and aqueous solvent. The extracts were concentrated in a rotary evaporator. The extracts were stored at 4˚C, till used for analysis.

2.3 Qualitative Phytochemical Tests:

Each extract was subjected to qualitative tests for identification of various constituents like alkaloids, carbohydrates, glycosides, steroids, saponins, flavonoids, tannins and phenolic compounds and proteins. The preliminary phytochemical screenings of extracts were performed according to standard procedure [11-13].

2.4. Quantification analysis of Phytochemicals

Quantitative analysis is an important tool for the determination of quantity of phytoconstituents present in plant extracts. For this TPC and TFC are determined. Extracts obtained from Flowers of Jasminum mesnyi plant material of subjected to estimate the presence of TPC and TFC by standard procedure.

(i) Determination of Total phenolic content (TPC)

The total phenolics content of the extract was estimated according to the method described by Singleton and Rossi.[14]. The concentration of methanolic and butanol extracts solution was 10 mg/10 mL. From this solution, 1mLwas taken in test tubes and by dilution with same solvent up to 10 mL. This is stock solution. From stock solution different concentrations were taken in different test tubes. This same procedure was used for standard. Gallic acid was used as a standard; 1 mL of Folin–Ciocalteu reagent was added in this concentration and the content of the flask was mixed thoroughly and 5 min later 4 mL of 20% sodium carbonate was added, and the mixture was allowed to stand for 30 min with intermittent shaking. The absorbance of the blue color that developed was read at 765 nm in UV spectrophotometer.

(ii) Determination of Total flavonoid Content (TFC)

The Total Flavonoid Content Was Determined Using the Method Described By Olufunmiso [15]. 1 Ml of 2% $AlCl_3$ Methanolic Solution Was Added To 1 Ml of Extract or Standard And Allowed To Stand For 60 Min At Room Temperature; The Absorbance Of The Reaction Mixture Was Measured At 420 Nm Using UV/Visible Spectrophotometer The Content Of Flavonoids Was Calculated Using Standard Graph Of Quercetin And The Results Were Expressed As Quercetin Equivalent (Mg/G).

2.5 Green Synthesis of ZnO Nanoparticles

After heating 20 ml of *Biancaea decapetala* leaf methanolic extract at 50 °C for 10 min, 50 ml of 0.1 M zinc acetate dihydrate ($Zn(CH_3COO)_2 \cdot 2H_2O$) (1.095 g of zinc acetate dihydrate was dissolved in 50 mL of d.H_2O) was added drop-by-drop to it under stirring at 800 rpm that resulted in cream-colored zinc hydroxide precipitate formation. For the complete reduction in zinc hydroxide, the reaction mixture was left for 30 min. Then the precipitate was centrifuged (Sigma Laborzentrifugen 2k 15, Osterode, Germany) at 16,000 rpm for 10 min at 4 °C by dH2O followed by ethanol repeatedly in order to remove the impurities. The precipitate was dried overnight in an oven at 100 °C. The obtained dried powder was calcined in a muffle furnace at 600 °C for 2 h and the white powder of ZnO NPs was obtained after calcination as shown in figure 2. The resulted powder was used for characterization [16].

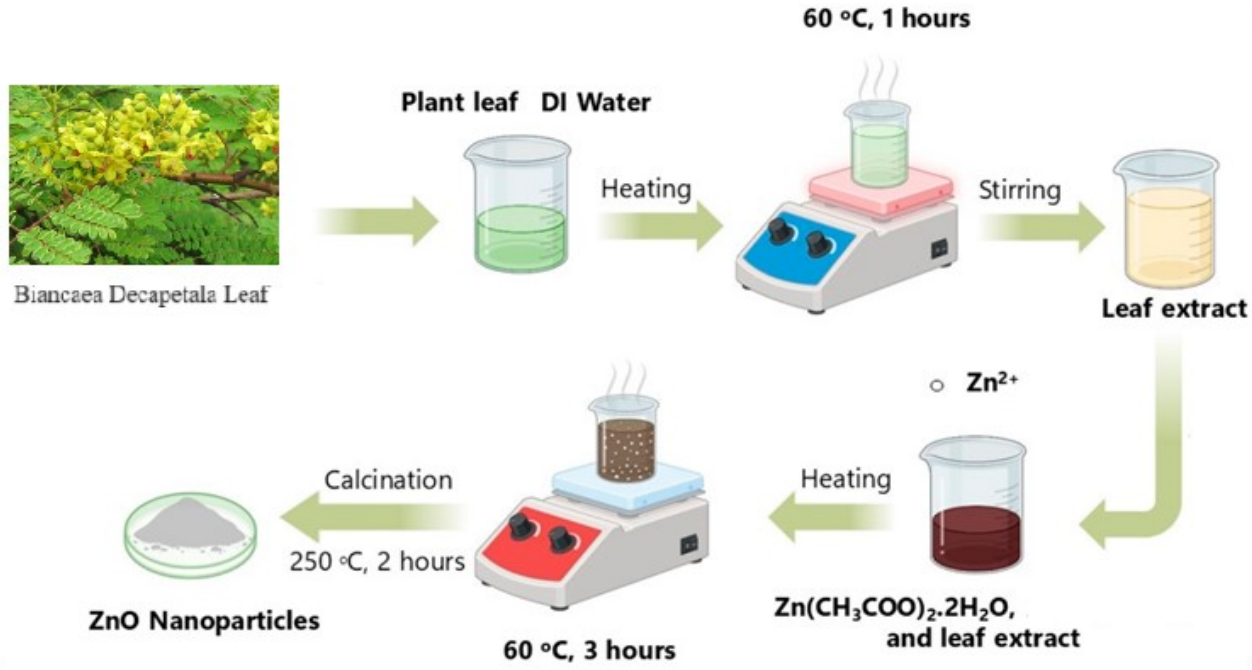

Fig 2. Represent (pictorial) the synthesis of ZnO NPs via *Biancaea decapetala*

2.8. Characterization Methods of ZnO NPs

2.8.1. UV-Vis Spectroscopy

In order to study the optical characteristics of green synthesized ZnO NPs, a known amount of ZnO NPs (0.05 g) was dispersed in 5 mL of ethanol (96%). The absorption spectrum was recorded by using a UV-Vis (U-2900) double beam spectrophotometer (Hitachi, Tokyo, Japan) in between a wavelength scan of 200–800 nm.

2.8.2. Dynamic Light Scattering (DLS)

A particle size analyzer (Zetasizer V 2.2, Worcestershire, Malvern, UK) was utilized to determine the particle size distribution (PSD) of ZnO NPs obtained using methanol extract. The zeta potential of ZnO NPs was carried out in the water as a dispersant through a Zeta sizer (V 2.3, Worcestershire, Malvern, UK) to identify the stability of the synthesized NPs.

2.8.3. Fourier Transform Infra-Red Spectroscopy (FTIR)

FTIR analysis (Bruker, Berlin, Germany) was employed to identify the functional groups (FGs) involved in biosynthesized ZnO NPs. At a wavelength of 4000–400 cm−1, the FTIR spectra were scanned with a resolution of 4.0 cm−1.

2.8.4. X-ray Diffraction (XRD)

The crystalline structure of ZnO NPs was analyzed by an X-ray diffractometer (Bruker D8 DISCOVER, Bruker, Germany) with Cu-Kα radiation (λ = 1.54060 Angstrom). The relative intensity data were collected over a 2θ range of 5°–80°, 2θ values and relative intensities (I/Io) were determined from the chart, and the minerals of core materials were identified with JCPDS carts.

2.8.5. Field Emission-Scanning Electron Microscopy (FE-SEM)

The topography and surface morphology of the biosynthesized ZnO NPs were examined using FE-SEM (Carl- ZEISS Sigma 500 VP, Sigma, Osterode, Germany) equipped with an energy dispersive X-ray spectrometer (EDX, Bruker, Germany) for the element composition present in the powder of ZnO NPs. A portion of the sample was set on a carbon-coated copper (CCC) grid, and the film on the FE-SEM grid was then dried by fixing it under gold for 5 min.

2.8.6. High-Resolution Transmission Electron Microscopy (HR-TEM)

The shape and size distribution of powdered ZnO NPs were studied by using HRTEM (JEM-2100, JEOL, Tokyo, Japan) at an accelerated voltage of 200 kV.

2.9 Estimation of Antibacterial Activity

2.9.1. Bacteria Strains

The antibacterial effect of the biosynthesized ZnO NPs with Biancaea decapetala methanol extract was established against two Gram-positive bacteria (GPB), Bacillus cereus and Staphylococcus aureus , and two Gram-negative bacteria (GNB), Escherichia coli and Pseudomonas aeruginosa . These four strains were acquired from the Biotechnology Dept., Govt. Digvijay Autonomous P.G. College Rajnandgaon Chhattisgarh. The bacterial strains used were maintained in the Luria–Bertani (LB) agar at 30 °C for 24 h and then kept at 4 °C in a refrigerator. During this study, LB media was used for all bacterial cultures.

2.9 Antibacterial Assay

The antibacterial effect against the examined bacterial strains was determined using the agar disc diffusion method (ADDM) described by Bauer et al. [17]. In this method, three different ZnO NPs concentrations (10, 20 and 30 µg mL−1) and methanol (20 µg mL−1) were dissolved in ethanol and then used to fill sterilized Whatman filter paper discs of approximately 40 µL with the proper volume containing the tested ZnO NPs concentrations and methanol and left to totally dry. A disc containing only solvent was used as a negative control and a disc containing zinc acetate dihydrate was employed. A positive control ciproflaxin (10 µg mL−1) was used. Overnight bacterial cultures were prepared in LB broth for obtaining tested bacterial suspensions for the assay. The discs were then placed on the plates having the tested bacterial cultures and diluted to obtain about 1 × 10−7 colony-forming unit (CFU). The inoculated plates were incubated at 37 °C for 24 h and then the activity was assayed by measuring the inhibition diameter in millimeters (mm). All tests were performed in triplicate.

3. Results and Discussion

3.1 Phytochemical Screening

The study of the chemical constituents and the active principles of the medicinal plants have acquired a lot of importance all over the world [18]. The present study includes the phytochemical screening of the plants *Biancaea*

decapetala. The qualitative chemical tests for the methanolic extracts were performed. The investigation showed that *Biancaea decapetala* contains flavonoids, saponins, Steroidal glycosides, Triterpenoids, Phenolic compound and steroids present in methanol and Flavonoid, Steroidal Glycoside, Triterpenoids, Steroids, Phenolic compound, in present aqueous extract but tannins, Saponins, Glycoside, alkaloids, amino acid and protein were absent in aqueous extract.

Table 3.1: Phytochemical Screening of *Biancaea decapetala* leaf Methanolic and Aqueous extract

S.N	Phytochemicals	Methanolic Extract	Aqueous Extract
1	Flavonoid	+	+
2	Saponins	+	-
3	Tannins	-	-
4	Steroidal Glycoside	+	+
5	Triterpenoids	+	+
6	Glycoside	+	-
7	Carbohydrate	-	-
8	Alkaloid	-	-
9	Steroids	+	+
10	Phenolic compound	+	+
11	Amino acid and Protein	-	-

(+): Presence, (-): Absent

3.2 Estimation of total phenolics and total flavonoids content

The results given in table 3.2 show that the total phenol content and total flavonoid of *Biancaea decapetala* leaves are methanolic 375.74± 0.16, 330.34± 0.15 and aqueous extract 285.30± 0.33, 220.52± 0.25 respectively. The above results showed that aqueous extract contain less phenolic and flavonoids content than the alcoholic extract. It may due to the solubility of principle contents presence be higher in case of alcoholic solvent, thus it has been accepted that it is a universal solvent for the extraction of plant constituents.

Table 3.2**:** Estimation of total phenolics and total flavonoids content in *Biancaea decapetala* leaf extract

S.N.	Extract	Test Parameter	Results(mg/g) (±SEM)
1.	Methanol	Total phenolic Total Flavonoids	375.74± 0.16 330.34± 0.15
2.	Aqueous	Total phenolic Total Flavonoids	285.30± 0.33 220.52± 0.25

3.3 Characterization of ZnO-NPs

3.3.1 UV-Vis Spectroscopy

To confirm the synthesis of ZnO NPs, UV/Vis spectrophotometry was performed in order to examine the optical characteristics of green synthesized ZnO NPs using *Biancaea decapetala* methanol extract . The UV-Vis spectrum recorded the maximum absorbance peak at 370 nm as shown in figure 5, which verified the synthesis of ZnO NPs via *Biancaea decapetala,* which is consistent with earlier studies [19], who examined the ability of *Biancaea decapetala* to synthesize ZnO NPs with surface plasmon resonance (SPR) at 370 nm. Additionally, there are no other peaks

recorded in the spectrum which means that the biosynthesized ZnO NPs are a pure product.

Furthermore, the high absorption band seen at 378 nm might be attributed to ZnO's inherent band-gap absorption caused by electron transitions from the valence band (EV) to the conduction band (EC) (O2p–Zn3d) [20]. The formula for calculating the energy bandgap (EG) of ZnO NPs was used as follows:

$$EG = hc/\lambda$$

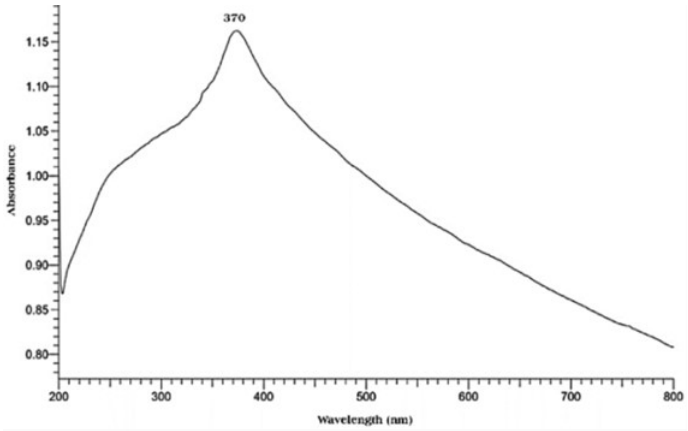

Fig 3. UV/Vis spectrum of ZnO NPs biosynthesized using Biancaea decapetala .

Where h is Planck's constant (6.626 × 10−34 Js), c is the velocity of light (3 × 108 m/s) and λ is the wavelength (378 nm). In total, 3.28 eV was found to be the bandgap energy of ZnO. The significant UV absorption of ZnO NPs demonstrates the product's suitability for a variety of medicinal applications, including sun-screen protectors and antibacterial ointments [21].

3.3.3 Dynamic Light Scattering (DLS)

The Z-average diameter (nm) and PSD of the biosynthesized ZnO NPs were measured using the DLS technique. As shown in Fig. 4 A, the measurements demonstrated that the average size (nm) of the ZnO NPs with *Biancaea decapetala* methanol extract was about 76 nm. The result obtained from the PSD profile of the ZnO nanoparticles revealed two notable peaks with intensities of 98.7% and 1.3%. Additionally, the ZnO NPs have a polydispersity index (PDI) of 0.241. This indicated that ZnO nanoparticles are very homogeneous and have a uniform size range. This finding is completely compatible with Badran, Chen et al. and Putri et al.[22] who reported that PDI values of 0.3 and below are considered to be monodisperse. Because of the hydrodynamical shell, the DLS technique is known to produce significantly higher values than HR-TEM size analyses. Additionally, the size of the hydrodynamical shell is influenced by particle structure, particle shape, and roughness.

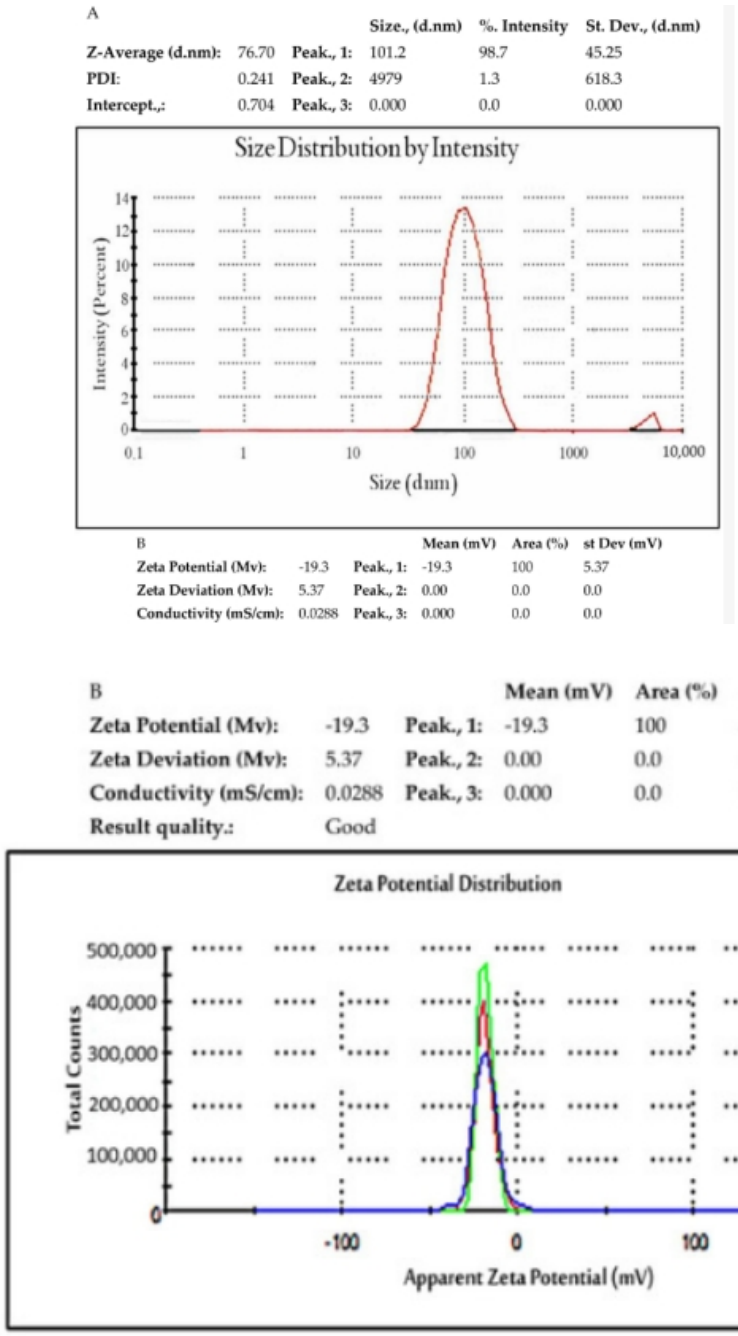

Fig 4. PSD (A) and ZP (B) of green synthesized *Biancaea decapetala* -ZnO nanoparticles.

The surface charges and stability of biosynthesized ZnO NPs have been assessed through zeta potential (ZP) analysis. The ZP graph of ZnO nanoparticles is presented in fig. 4 B. As shown in fig 4 B, the ZP was found to be −19.3 mV which indicates the potential stability of the examined NPs. As a result, the reducing agents (i.e., phenolic and flavonoid components) found in the leaf extract (LE) are probably responsible for the negative charge potential of the produced ZnO NPs. It also confirms that the produced substance contains substantial electrostatic forces [23].

3.3.4. FTIR Analysis of Biosynthesized ZnO NPs

The FTIR technique was used in order to detect possible FGs present in the methanol extract of *Biancaea decapetala* that contribute to the reduction in and stabilization of ZnO NPs. Fig. 5 a,b represents the FTIR spectra of biosynthesized ZnO nanoparticles. The peaks of of *Biancaea decapetala* methanol extract and biosynthesized ZnO nanoparticles are displayed. The broad stretch peak at 3409 cm−1 and 3417 cm−1 indicates the presence of an O-H stretch band for the extract and ZnO NPs which are corresponded to the O-H stretching of alcohol, phenolic and flavonoid constituents [24]. The low-intensity peaks that arise at 2923 cm−1 and 2920 cm−1 were assigned to –CH stretching vibration of the hydroxyl compounds. The absorption peaks at 2356 cm−1 and 2356 cm−1 were ascribed to O=C=O (stretching vibration). The peaks observed at 1616 cm−1 and 1621 cm−1 indicate the stretching C=C vibration of the aromatic ring system. The absorption peaks at 1400 cm−1 and 1403 cm−1 correspond to the C-N stretching vibration of amino acids.. The strong intensity peaks at 1068 cm−1 and 1072 cm−1 are due to the C-O stretching bond of the aromatic ring and may also be related to phenols and flavonoids found in the *Biancaea decapetala* methanol extract. The bands at 852 cm−1 and 855 cm−1 are attributed to –CH stretching vibration of aromatics. The absorption band observed at 435 cm−1 confirmed the successful formation of Metal-Oxygen (ZnO). The ZnO absorption peak obtained by FTIR analysis of biosynthesized ZnO NPs has been detected at wavelengths 436 cm−1, 442 cm−1, 450 cm−1 and 485 cm−1, in the range 400 to 500 cm−1 which are consistent with our findings. The similarity of bands in *Biancaea decapetala* - synthesized ZnO NPs could be attributable to capped biomolecules on the surface of green synthesized ZnO nanoparticles.

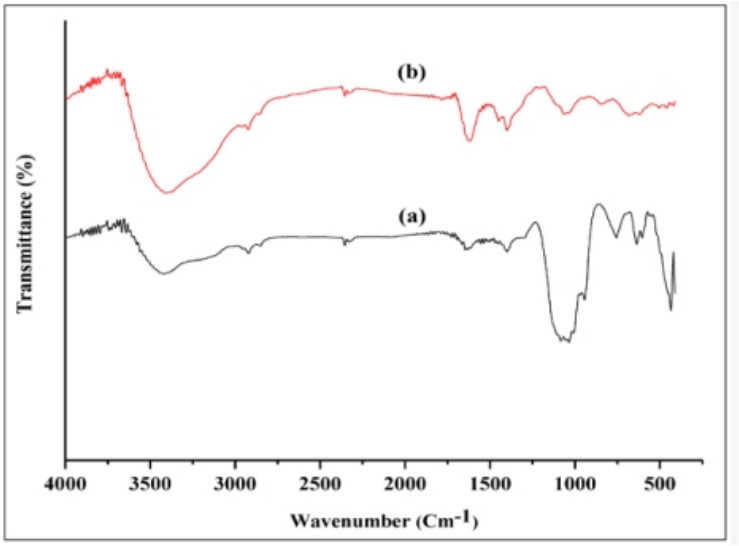

Fig. 5. FTIR absorption spectra of (a) ZnO NPs and (b) Methanol extract of *Biancaea decapetala*

3.3.5. X-ray Diffraction (XRD) Analysis of ZnO NPs

The XRD pattern of biosynthesized ZnO NPs using methanol extract of *Biancaea decapetala* is illustrated in Figure 8. The XRD diffraction peaks existed at 2θ angles of 31.85°, 34.55°, 36.35°, 47.69°, 56.75°, 63.09°, 66.56°, 68.17°, 69.29°, 72.87° and 77.21° corresponding to lattice planes (100), (002), (101), (102), (110), (103), (200), (112), (201), (004) and (202), respectively [31] . These peaks are in accordance with those of (JCPDS card No: 36-1451), which is indicating the confirmation of the hexagonal wurtzite structure of ZnO NPs formation . The average crystalline size (ACS) of biosynthesized ZnO NPs was calculated using Deby-Scherrer's formula [25] and the ACS of the ZnO NPs was estimated

to be 14 nm, which is derived from the full width at half maximum (FWHM) of the most intense peak corresponding to (101) plane located at 36.35°. Furthermore, the XRD pattern revealed no additional peaks other than the characteristic ZnO peaks, confirming the purity of the produced ZnO NPs. Additionally, the narrow and strong diffraction peak clearly indicates that the ZnO NPs have an optimal crystalline structure.

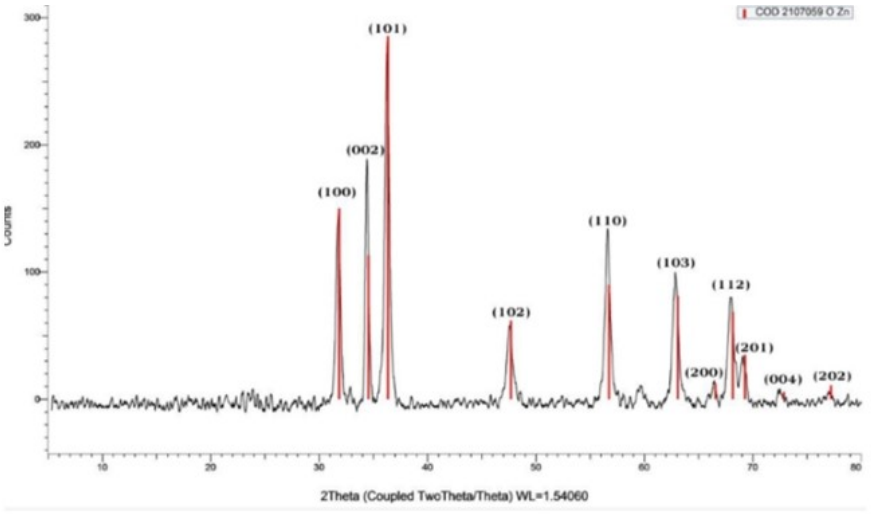

Fig 6: XRD pattern of biosynthesized ZnO NPs

3.3.6. FE-SEM of ZnO NPs

The size and the morphology of the biosynthesized ZnO nanoparticles were imaged via FE-SEM (Fig 7), and the chemical composition of the biosynthesized ZnO nanoparticles was determined using EDX. The FE-SEM image demonstrated that the ZnO NPs were spherical and hexagonal in the morphology shape with good distribution. FE-SEM examination showed that the average size of ZnO NPs was 21.6 nm [26].

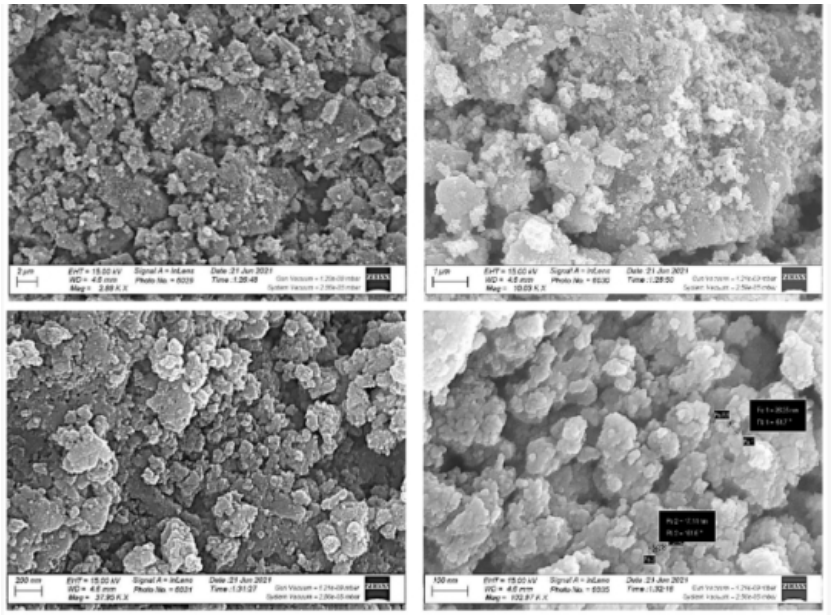

Fig.7: FE-SEM image of biosynthesized ZnO-NPs.

3.3.7 HR-TEM of ZnO NPs

The high-resolution TEM analysis (fig. 8 a–f) was carried out to confirm the formation of the biosynthesized ZnO NPs. Based on the results obtained and also clearly reveal lattice fringes without any distortion, indicating that ZnO NPs have high crystallinity. The selected area electron diffraction (SAED) (fig.8) pattern revealed a series of rings with bright spots, indicating that ZnO nanoparticles are crystalline in nature [27]. Additionally, the hexagonal wurtzite crystalline structure of ZnO NPs is also proven by the diffraction rings on the SAED image and the peaks in the XRD pattern.

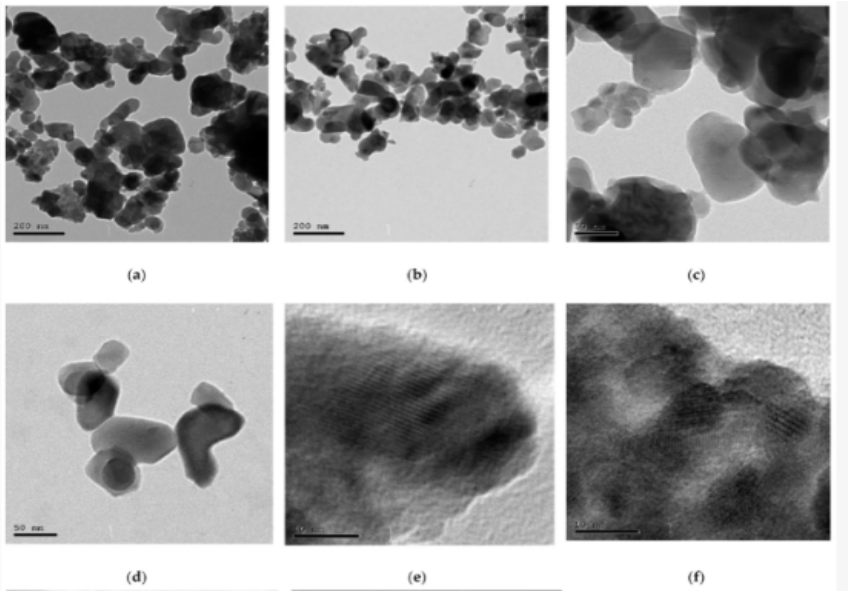

Fig 8:. (a–f) HR-TEM images of biosynthesized ZnO NPs,

5. Antibacterial Activity

The antibacterial effect of the biosynthesized ZnO NPs was evaluated by disc diffusion assay against *S. aureus, B. cereus* as Gram Possitive Bacteria (GPB) , and *E. coli* and *P. aeruginosa* as Gram Negative Bacteria (GNB). The results are represented in fig. 9. Generally, the results revealed that the biosynthesized ZnO NPs using *Biancaea decapetala* methanol extract possessed a significant antibacterial effect against all tested bacterial strains. The significant antibacterial zone of inhibition was recorded in S. aureus (27 ± 0.32 mm) followed by *B. cereus* (23 ± 0.16 mm), *P. aeruginosa* (20 ± 0.30 mm) and *E. coli* (15± 0.21 mm). Furthermore, compared to gentamycin as a positive control and methanol of *Biancaea decapetala* , biosynthesized ZnO NPs displayed higher antibacterial activity. The antibacterial activities of ZnO NPs differ depending on the cell wall nature of GPB or GNB [28-29]. In the present study, the biosynthesized ZnO NPs showed higher antibacterial activity against GPB (*S. aureus* and *B. cereus*) compared to GNB (*P. aeruginosa* and *E. coli*). A similar trend was obtained by Vijayakumar et al. [30] who stated that ZnO NPs synthesized from *Laurus nobilis* leaf extract displayed greater antibacterial activity against GPB (*S. aureus*) than GNB (*P. aeruginosa*). This is maybe owing to the structure and the components of GPB (i.e., peptidoglycan layer) and may improve the ZnO NPs' attachment to the cell wall, while the components of GNP avoid this attachment [31].

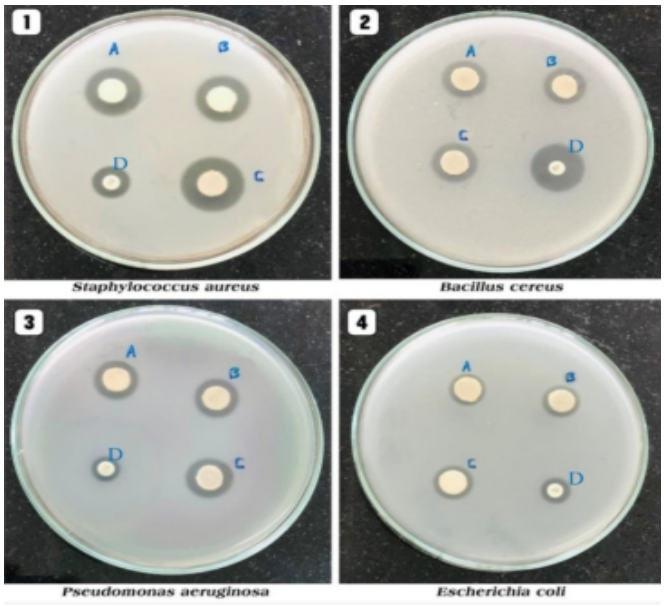

Fig.9: Antibacterial effects (zone of inhibition (mm)) at different concentrations of ZnO NPs (A: 10 µg mL−1; B: 20 µg mL−1; C: 30 µg mL−1 and D: standard) towards various pathogens.

Table 3.3: Evaluation of the antibacterial activity toward pathogenic bacteria

Pathogenic Bacteria	Diameter of Inhibition Zones (mm)			Positive Control Ciprofloxacin 10 µg mL−1
	ZnO NPs			
	10 µg mL−1	20 µg mL−1	30 µg mL−1	
S. aureus	22 ± 071	24 ± 1.40	27 ± 0.32	12 ± 0.25
B. cereus	16 ± 0.55	17±0.58	23 ± 0.16	21 ± 0.50
E.coli	12 ± 0.82	14 ± 0.52	15± 0.21	11 ± 0.85
P. aeruginosa	18 ± 0.35	20± 0.70	20 ± 0.30	13 ± .0.75
Mean of ZnO NPs	17.75 ± 4.7(C)	19.5 ± 5.2 (B)	22.23 ± 3.5 (A)	15 ± 5.00 (D)

Additionally, the results indicated that the inhibitory effect of biosynthesized ZnO NPs using *Biancaea decapetala* leaf extract increased when the concentration of ZnO NPs was increased. This was in agreement with Gunalan [32] who reported that increasing the concentration of ZnO NPs in discs and wells consistently increased the growth inhibition due to optimal NPs diffusion in the agar medium.

For the effect of ZnO NPs, there are some proposed bactericidal mechanisms (fig.10) that have been suggested by scientists. Some suggested that the released Zn from ZnO NPs possess toxic properties that are leading to inhibiting a lot of bacterial cell activities such as bacterial metabolism, and enzyme activity resulting in cell bacterial death [33]. The other suggested mechanism is the formation of reactive oxygen species (ROS) that activates oxidative stress which subsequently leads to cell death [34]. Another proposed mechanism is the lethal activity of the ZnO NPs due to the attachment of the NPs to the bacterial cell membranes, and the accumulation inside the cytoplasm resulting in damaging the cell membrane integrity and loss of cell contents because of the leakage ending up with cell death [35].

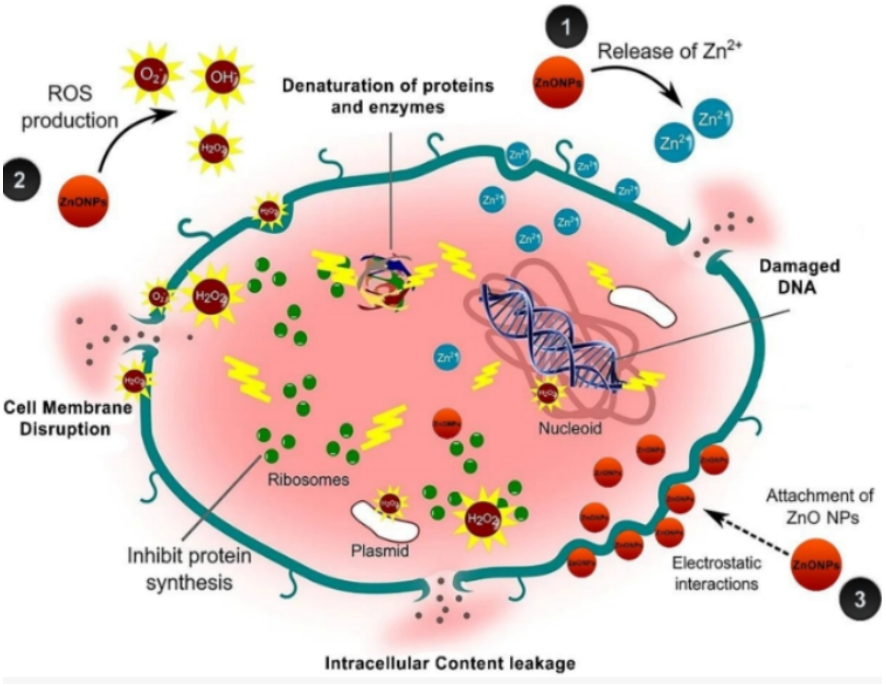

Fig. 10: Bactericidal mechanisms

4. Conclusion

Various methods have been utilized for the synthesis of ZnO NPs. However, biological methods have been advanced over physical and chemical approaches due to their eco-friendly, and eco-friendliness. In this study ZnO NPs were synthesized using *Biancaea decapetala* leaf extract. The bio-active organic components of *Biancaea decapetala* leaf extract are used as reducing, and capping, agents for the synthesis of ZnO nanoparticles.

This study presents the biosynthesized ZnO NPs for the first time using an methanol extract of *Biancaea decapetala* via a simple green route. The biosynthesized ZnO NPs showed a characteristic Uv-Vis absorption peak at 370 nm. The XRD pattern also indicated the hexagonal pure Wurtzite structure. FE-SEM coupled with EDX, HR-TEM, FTIR, and DLS, confirmed the formation of NPs with an average size of 34.12 nm as obtained from HR-TEM analysis. Our findings suggest the possibility of using the aqueous leaf extract of *Biancaea decapetala* for synthesizing stable ZnO NPs.

The in vitro antibacterial activity of the produced ZnO NPs was tested on *S. aureus, Bacillus*, and *P. aeruginosa* strain. As a result, ZnO NPs shows an excellent antibacterial activity on the tested strain. This contributed to the biocompatibility and in vivo biological potency of ZnO NPs. The biosynthesized ZnO NPs possess a significant antioxidant, antibacterial against foodborne pathogenic bacteria that can be used as a safe and stable alternative to synthetic substances in the fields of pharmaceutical and biomedical research. Further investigation should be done on isolation of active constituents and necessary to identify the metabolites responsible for pharmacological activities of the crude methanolic extract.

5. Conflicts of interest

The authors declare no conflict of interest.

6. Acknowledgments

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