Advances in Bioresearch
Adv. Biores., Vol 15 (2) March 2024: 379-390
©2024 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.15.2.379390

Advances in Bioresearch

ORIGINAL ARTICLE

Biosynthesis and evaluation of metallic nanoparticles (ZnO-NPs) using polyphenol-containing *Ajuga macrosperma* (Ghonke ghas) leaf extract, along with anticancer activity and antimicrobial activity

Shama Parveen[1], Vikas Gupta[1], Veera Nagendra kumar[2], U. Srineetha[3], M. Rama Mohan[4], C. Narasimha Rao[5], Anuj Kandwal[6*], C. Nageswara Reddy[7], Akshay Gautam[6]

[1]Department of Chemistry, Faculty of Science, Motherhood University, District- Haridwar, 247661 Uttarakhand (India),

[2]Department of Zoology, Faculty of science, Government College for Men (Autonomous), Kadapa-516004, Andhra Pradesh, India

[3]Department of Zoology, Faculty of science, Government Degree college for women, Pulivendula, 516390, Andhra Pradesh, India

[4]Department of Zoology, Faculty of science, SV College of arts and computer science, Proddatur-51630, Andhra Pradesh, India

[5]Department of Zoology, Faculty of science, Government Degree College, Mydukur-516172, Andhra Pradesh, India

[6]Department of Chemistry, Harsh Vidya Mandir P.G.College, Raisi, District- Haridwar, 247671 Uttarakhand (India)

[7]Department of Chemistry, faculty of science, Government college for Men (Autonomous), Kadapa-516004, Andhra Pradeh, India

*Corresponding author's Email: anujkandwal4@gmail.com

ABSTRACT

Green chemistry is a relatively new discipline that promotes the use of a set of guidelines to decrease both the use of and the production of chemical waste. Accordingly, the use of environmentally friendly technology has a more beneficial effect on ecosystems than the use of factory workers. Plant extracts are seen as a greener and cheaper alternative to traditional methods of synthesizing metallic nanoparticles, and their usage is on the rise. In this study, an aqueous leaf extract of Ajuga macrosperma was used to biosynthesized metallic Zinc oxide nanoparticles. There is a limiting and capping effect caused by plants. Ultraviolet spectrophotometric analysis was used to monitor the biosynthesized nanoparticles in real-time. The incorporation of leaf extract resulted in a noticeable hue shift, which allowed for the visual detection of the creation of metallic nanop and articles. Scanning electron microscopy, Fourier transform infrared spectroscopy, X-ray diffraction, energy dispersive X-ray spectroscopy (EDX), and zeta potential were all used to learn more about the nanoparticles. The SEM scan reveals that the nanoparticles have a spherical form and measure between 10 and 100 nm in size. The XRD analysis verified that the synthetic ZnONPs have a Wurtzite crystalline structure. Results from FTIR and EDAX analysis reveal the nanoparticles' functional groups and elemental makeup. MTT and MTS assay was used to test the anticancer activity of created ZnONPs in MCF-7 (breast cancer), HeLa (human embryonic lung cancer), PC-3 (prostate cancer), and A549 (lung cancer). Additionally, research has also shown that produced ZnONPs are highly active against Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis and Escherichia coli. as well as against yeast (Candida albicans) showing no efficacy.

Keywords: *Zinc Oxide Nanoparticles, characterization, anticancer activity and antimicrobial activity.*

Received 24.01.2024 Revised 04.02.2024 Accepted 11.03.20243

How to cite this article:
Shama P, Vikas G, Veera Nagendra kumar, U. Srineetha, M. Rama Mohan, C. Narasimha Rao, Anuj K, C. Nageswara R. Akshay G. Biosynthesis and evaluation of metallic nanoparticles (ZnO-NPs) using polyphenol-containing *Ajuga macrosperma* (Ghonke ghas) leafextract, along with anticancer activity and antimicrobial activity. Adv. Biores., Vol 15 [2] March 2024: 379-390

INTRODUCTION

Tumor is a substantial cause of illness and death among noninfectious diseases. The use of nanomaterials in cancer therapy is one of the innovative options. As anticancer medicine carriers, nanoparticles and nano - structured gadgets have been employed. Release rate of anti-cancer prescription drugs is now possible thanks to the use of nanotechnology in medicine administration raising its in vivo evaluation. Nanoparticles with distinct chemical and physical properties have been shown to have anticancer properties. The anticancer effect of nanoparticles can be linked to specific qualities like antioxidant activity or the use of environmental stimulation like hyperthermia in answer to the injection of infrared rays or electromagnetism. They can manufacture reactive oxygen species in response to environmental stimuli, which can destroy tumors cells. Further, Zinc oxide nanoparticles are suitable for thin coating applications because they exhibit good antibacterial and antifungal activities even at lower concentrations [10-14]. As time goes on, every sector will come to rely more and more on developments in nanotechnology. Due to its cheap cost and benign impact on the environment, biosynthesis of nanoparticles has lately gained favor. Because of its unique physical and chemical features, nanotechnology is becoming vital to fields as diverse as industry, medicine, the environment, and health care. (1). Nanoparticles, which have a diameter of just 10^9 nm, may be transported and characterized much like larger particles but on a much smaller scale. In general, chemical, physical, and biological techniques have been employed to produce metallic nanoparticles. (2) (3). Synthesis techniques that rely on chemicals or physical processes are often costlier and result in waste that is harmful to the environment. Plant-based synthesis methods have expanded to include the biosynthesis of nanoparticles. Researchers are interested in metal oxide nanoparticles because of their distinct properties and potential uses in a variety of physical and chemical fields. Nanoparticles composed of metals and metal oxides have advanced medical imaging, diagnosis, and therapy in recent years. Silver, copper, and gold are now the most widely used metals in the world. With a lattice spacing of a = 0.325 nm and c = 0.521 nm, zinc oxide (ZnO) is a stable wurtzite structure that exists in the II-VI compound semiconductor family as mentioned in table 1 (4).

Table 1: lists of the basic physical properties of bulk ZnO [4].

"Physical Properties of Bulk ZnO	**Value**
Lattice constants (T = 300 K)	a0 = 0.32469 nm c0 = 0.52069 nm
Density	5.606 g/cm3
Melting point	2248 K
Relative dielectric constant	8.66
Gap Energy	3.4 eV,
direct Intrinsic carrier concentration	n < 106 cm-3
Exaction binding Energy	60 meV
Electron effective mass	0.24
Electron mobility	(T = 300 K) 200 cm2 /V s
Hole effective mass	0.59
Hole mobility	(T = 300 K) 5-50 cm2 /V s"

One kind of inorganic nanoparticle with antibacterial characteristics is zinc. In collaboration with other researchers, (5), (6), (7) Zinc oxide shows promise as a photocatalyst due to the size of its band gap energy and the durability of zinc nanoparticles. Applications where ZnO nanoparticles show promise include: (8) Nano generators (9) gas sensors (10) biosensors; (11) solar cells (12) site visitors (13) photodetectors and (14) photocatalysts. catalysts (14). Regardless of the kind of cancer, all tumor cells have the characteristic of unchecked cell growth and dramatic alterations in biochemical and enzymatic parameters. Via bio-based nanoparticles as innovative regulating agents, malignant cells' overexpression of cellular growth may be stopped and managed using systematic cell cycle processes (15). Moreover, nanoparticles mediated by plants have potent anticancer effects in Hep 2, HCT 116, and HeLa cell lines. Nanoparticles generated from plants have shown promise in recent years as a means of limiting the spread of malignant cells in the body. It is the secondary metabolites and other non-metal components in the synthesis medium that are responsible for the enhanced cytotoxic action (16), (17). Silver nanoparticles produced from Syzygium cumini were investigated for their anti-diabetic and cardio-protective properties (18). This research found that silver nanoparticles extracted from S. cumini effectively reduced heart stress brought on by a glucose overload. Mechanism of action research pointed to the protection of cell membranes and

suppression of oxidative stress (18). Several studies have also discovered that plant-mediated zinc oxide nanoparticles have anticancer properties against some of these cancer cell lines such as MCF-7,(19), (20), (21) , human liver cancer cells HepG2. (22), Human prostate cancer PC3, (23), (24), (25) human Kidney cancer Hek-293, (26), (27), lung cancer H1299, A549, (28), (29), (30), (31).

MATERIAL AND METHODS

Plant materials: Dr. Neelu Singh (Taxonomist), of the National Tropical Research Institute of Forests in Jabalpur, Madhya Pradesh, gathered the *Ajuga macrosperma* (Ghonke ghas) plant material used in this study. *Ajuga macrosperma* (Ghonke ghas) was identified as species number 11251.

Scientific description and traditional knowledge of Ajuga macrosperma: The tropical areas of India, Nepal, and the People's Republic of China are home to the perennial plant known as *Ajuga macrosperma* Wall. Ex Benth. Mainland of China is home to two distinct varieties of A. macrosperma: var. macrosperma and var. thomsonii. In traditional Chinese medicine, *A. macrosperma* var. macrosperma is used for the treatment of nephritis, as well as for the reduction of fever and the elimination of phlegm.

Leaves extract preparation:

Getting the extract from the leaves of the *Ajuga macrosperma* plant:

After being harvested from their natural habitat in Kotdwara, *Ajuga macrosperma* leaves were washed in distilled water to eliminate any remaining dust. After washing and sorting the leaves, they were dried in the shade for 8 or 9 days before being ground into a powder. A grinder mixer was used to reduce the dried leaves to a fine powder, which was then kept for further use in the tests (*Ajuga macrosperma* leaves powder). In a 500 ml round-bottom flask, 7 grams of *Ajuga macrosperma* leaves powder was combined with 100 ml of distilled water and cooked for 50 minutes at 80°C to extract the desired substance. After waiting 50 minutes, the watery extract solution became a dark brown, indicating that an extract had formed. After allowing the extract to cool to ambient temperature, it was filtered using Whatman No. 1 filter paper.

Biosynthesis of zinc oxide Nanoparticles (ZnO-NPs):

A 0.05 aqueous solution of zinc nitrate was made to produce zinc oxide nanoparticles. 4 mL of *Ajuga macrosperma* extract of leaves was added to 0.05 M zinc nitrate mixture and heated to 60 C while stirring with a magnetic stirrer for two hours. The mixture was then gradually coloured cream-colored by adding 0.02 M NaOH drop by drop. The mixture was then cooled until it reached room temperature. The resultant solution was centrifuged at 1000 rpm for 15 minutes to separate the NPs from it. To get rid of biological components, the particles went through two rounds of distilled water washing. kept the ZnONPs solution for later analysis**.**

Zinc nitrate + Plant extract → ZnO + byproducts.....[1]

Anticancer activity:

MTS Assay for PC-3 (Prostate Cancer Cell Line), Hela (Human Endometrial Cancer Cell Line) and A459 (Human Lung Cancer Cell Line).

Method: The anticancer efficacy of zinc oxide nanoparticles was evaluated using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide tetrazolium (MTT) reduction assay. According to the manufacturer's instructions, cell titer was measured using the cell titer 96 aqueous non-radioactive cell proliferation assay (Promega, WI, USA). In a proliferation or cytotoxicity experiment, the number of live cells may be measured colorimetrically using the Cell titer 96 aqueous one-solution assays. In this solution, both the MTS chemical and the PES electron-coupling reagent may be found. A colored formazan product that is soluble in tissue culture media is produced when cells bio-reduce the MTS chemical (3-(4,5-dimethyl thiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphonyl)-2H-tetrazolium). The cells were seeded at a density of 5,000 per well in growth media onto 96-well plates and allowed to adhere overnight. Subsequently, the cells were exposed to chalcones and their derivatives at the given doses (100, 33.33, 11.11, 3.70, 1.23, and 0.41 g mL-1). Twenty microliters of cell Titer 96 aqueous solution were poured into each well after 72 hours of treatment. The plates were put back into the incubator and the lights were turned off for two hours. Absorbance was determined at 490 nm against a 690 nm standard using a Spectra Max 340 microplate reader (Molecular Devices, USA). It was repeated three times to ensure accuracy.

Cancer cell cytotoxicity: Synthesized chalcones and derivatives are tested for their ability to kill cancer cells in comparison to the standard anticancer medicines cis-platin and doxorubicin. In an initial MTS assay using PC-3 (Prostate Cancer Cell Line), Hela (Human Endometrial Cancer Cell Line), A459 (Human Lung Cancer Cell Line), and prostate cancer cells, all of the complexes were able to inhibit cell viability at

micromolar concentrations (IC50 values), with Cisplatin 13 μM and Doxorubicin 4.1 M serving as positive controls.

MTT Assay for MCF-7 (Human Breast cancer)

Method: MTT assay was performed out the cytotoxic nature of MNPs in MCF-7 (human breast cancer) cell lines. In 96-well plate, 5,000 MCF-7 cells were seeded and incubated for a 24-hour period. Cells were also treated with ZnONPs at various concentrations (100, 33.33, 11.11, 3.70, 1.23, and 0.41 μg) and incubated for 72 hours. Then, the cells were revealed for 4 hours to 10 μl of brand-new yellow MTT reagents (0.5 mg/mL). Ultimately, 100 L of dimethyl sulfoxide (DMSO) was introduced and the UV absorbance at 570 nm of the purple formazan solution was measured (Multimode reader, Tecan, Austria). The cytotoxicity of produced ZnONPs against MCF-7 cells was tested in vitro at various doses (100, 33.33, 11.11, 3.70, 1.23, and 0.41 g/ml). The cytotoxic effect of synthesized ZnONPs, was greater at higher concentrations (11 to 100 μg/ml), but less at lower concentrations (0.41 to10 μg/ml).

Antimicrobial activity

The zinc oxide nanoparticles synthesized from *Ajuga macrosperma* leaf extract were tested for antimicrobial activity by Agar well disk diffusion method against five bacterial strain i.e., Bacillus Subtilis, *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and Candida albicans.

Antimicrobial assay of the schiff bases and metal chelates by Kirby –Bauer Disk diffusion method

Bacterial strain were cultured overnight at 37 °C in muller hintson medium. The inoculum as adjusted according to CLSI (clinical and laboratory standard institute) using a sterile sline to the final density of 0.5 McFarland standard (1.5 x 10^8 CFU/mL). microbial inoculum were spread over the entire surface of plates with growth medium and left for 15 minutes at room temperature to achieve a total absorption. Holes with a diameter of 7 mm have punched aseptically in inoculated plates using the sterile cork barer and the well is filled with 50 ul of nanoparticles solution. After that agar plates containing bacteria were incubated at 37 °C for 24 hour. Antimicrobial activities of synthesized nanoparticles was evaluated based on diameter (mm) of inhibition zones that occurs as the results of Schiff bases diffusion in the medium and inhibition zones of the microbial growth. The results obtained for the individual test organism to the different plant-based nanoparticles.

Characterization:

UV-Visible Spectroscopy: UV-Visible spectra of the synthesized ZnONPs nanoparticles was recorded by using a spectrophotometer instrument (Jasco V650) in the range of 200 to 800 nm.

Fourier Transformation Infra-Red Spectroscopy analysis (FTIR): Recorded FT-IR spectra in the range of 400 to 4000 cm^{-1} using a Fourier Transform Infra-Red spectrophotometer (Shimadzu, IR affinity 1A) indicated the presence of functional groups in produced ZnO-NPs.

Zeta potential: The zeta potential may be used to evaluate the charge stability of zinc oxide nanoparticles (ZnO-NPs). Charge density at a surface is expressed as the zeta potential. Zinc oxide nanoparticles that were biosynthesized exhibit excellent stability and a zeta potential of 42.12 mV. (32)

Scanning Electron microscope (SEM): Nanoparticles of ZnO was analyzed for their shape using a scanning electron microscope. The samples for the SEM pictures were made by drop-casting the solution onto a silicon- wafer and then drying it in a desiccator overnight. Scanning electron microscopy was used to get all of the pictures (JEOL JEM 2100, Japan).

Energy-dispersive X-ray spectroscopy (EDX) EDAX analysis: Elemental analysis of the synthesized nanoparticles was recorded on an energy-dispersive X-ray diffractometer (EDAX) (AMETEK team V.4.3 EDS detector)

X-Ray Diffraction analysis (XRD): The diffraction intensities of photo-induced ZnO-NPs were measured by using a powder X-ray diffractometer (Bruker D8 Advanced equipped with Cu, K- alpha radiation source) at an operating voltage of 40 kV and a current of 30 mA with CuKα radiation (λ= 1.5405) in the 2θ range of 10–80 to determine the crystal structure and size of ZnONPs by Using the Scherrer equation-

$$D = K\lambda/\beta\cos\theta \quad \text{..... equation [1]}$$

The crystal size of ZnONPs, FWHM (full-width half-length maximum) in radians, the X-ray wavelength (1.4506), the Bragg diffraction angle, and k are all constants (0.9).

RESULTS AND DISCUSSION

UV-Visible analysis:

Leaf extract from the *Ajuga macrosperma* plant has been shown to include polyphenols, flavonoids, quercetin, kaempferol, glycosides, and tannins. These phytochemicals have been linked to NP production.

The leaf extract acts as a stabilizing agent to keep the synthesized NPs from clumping together and as a reductant to reduce the size of metallic salts to NPs. The addition of the leaf extract causes a light-dark brown to yellow tint to appear in the reaction liquid, which is indicative of the creation of zinc oxide nanoparticles (ZnO-NPs). The ZnO-NPs absorption peaks occurred in the spectra at wavelengths in a restricted range of 340 nm (depict in figure 1), hence indicating the photosensitivity of the synthesized ZnONPs.

FTIR analysis:

The zinc oxide nanoparticle that was generated from *Ajuga macrosperma* was subjected to FTIR measurements to look for any potential changes to the functional group bonds that might have occurred during the reduction process. Figure 2 illustrates the FTIR spectrum for synthesized nanoparticles of *Ajuga macrosperma*, which showed multiple distinct bands. Peak absorption occurs at 3738.05 cm^{-1} and 3633.89 cm^{-1} are correlated with the stretching vibrations of polyphenolic species' hydroxyl (O-H) functional group. A functional group called (C-O) is depicted by the absorption peak at 2306 cm^{-1}. Carbonyl (C=O) stretching vibrations of highly conjugated systems are likely responsible for the peak at 1687 cm 1. C-O may be to blame for the peak at 1220 cm^{-1} (vibrational of Carboxylic acid and Alcohol). The C-H functional group may be represented by the plateau at 1068 cm^{-1}. Zinc oxide may be responsible for the peaks below 690 cm-1, which could imply nanoparticle formation.

XRD analysis:

XRD analysis was employed to investigate the crystalline nature of the ZnONPs. The yellow powderwas us ed to obtain the crystalline particles for XRD analysis. Zinc oxide pellets after
centrifugation, multiple itemswashing, and complete oven drying XRD information of biomolecule capped ZnONPs were gathered and discussed over a wide angular range of 20 to 80. The Xray diffraction pattern of Zinc oxide nanoparticles is depicted in Figure 3. A distinct line broadening of the XRD peaks implies im plies that the synthesized material is made up of nanoscale particles. We determine peak intensity positio n and width from this XRD pattern analysis, and the diffraction peaks at 10.01, 11.15, 22.07, 23.32, 29.44, and 31.61 have been cordially indexed at hexagonal wurtzile phase of ZnO (35), (36) with lattice constant a=b=0.324 and c= 0.521nm (JPCDS card number: 361451) (37).

Using the Scherrer equation, D = K/cos, we calculated that the average crystallite size of the ZnONPs is 18.04 nm from the FWHM of the diffraction peak.

SEM and EDAX Analysis:

The scanning electron microscope, is fully capable of resolving different particle sizes and th e surface morphology of the synthesized particles at the micro and Nano scales (38), (39), (40),(41)**.**

The SEM image of ZnO NPs is shown in (**figure 4**). **It clearly shows that the particles consist of agglomerated and nearly spherical**. Thus, the prior researcher stated that only metal oxides provide such findings (D. Suresha et al., 2015). The first picture (a) in Figure 8 shows the 1 µm at 20.00 K X magnification, whereas the second image (b) Displays the 2 µm with 10.00 K X magnification.

Energy Dispersive X-Ray Diffractive (EDX) Analysis:

The chemical compositions of the synthesized ZnO nanoparticles were determined using an Energy Dispersive X-ray Diffractive (EDAX) study. As shown in Figure 5, EDX demonstrates the presence of zinc and oxygen signals in zinc oxide nanoparticles, and this analysis revealed peaks corresponding to the optical absorption of the resulting nanoparticle. Nanoparticles consist of 67.56 percent zinc, 22.44% oxygen, and 11% additional elements, according to elemental analysis.

Particle size Analyzer

A particle size analyzer, which works based on dynamic light scattering, can be used to figure out the average size of nanoparticles. The mixture was ultrasonicated after dispersing ZnO-based basil nanoparticles in deionized for half an hour to increase their stability. The size of the particle's analyzer ascertained that the particles formed were 12.48 nm in size. (shown in figure 6)

Zeta potential

Using Zeta potential, one may evaluate the charge stability of zinc oxide nanoparticles (ZnONPs). Surface charge density may be shown as the zeta potential. Zeta potential measurements taken 72 hours following Ajuga macrosperma's manufacture of zinc oxide nanoparticles showed a value of -29.8 mV (Figure 7).

Anticancer (In vitro cytotoxicity study)

ZnO-NPs Cytotoxicity analysis against (PC-3) Prostate Cancer cells line

Leaf extracts of *Ajuga macrosperma* plants are also used to make **ZnO-NPs**. Synthesized NPs examined for anticancer effectiveness against PC-3 (prostate cancer). Zinc oxide nanoparticles of 50 nm revealed substantial antitumor action in PC-3 (p.05). The IC50 values for ZnO-NPs were 1.78 µM.

Cytotoxicity analysis of ZnO-NPs against Hela cancer cells line

Leaf extracts of *Ajuga macrosperma* were used to synthesis zinc oxide nanoparticles. In a manner that is reliant on concentration, ZnO-NPs suppressed the growth of HeLa carcinoma. ZnO-NPs have been found to exhibit anti-cancer characteristics against a wide variety of tumor cells on multiple times. The (IC50) of ZnO-NPs on HeLa cancer cells has been discovered. Its relative IC50 values was 3.0 µM. It showed promising results against cancer.

Cytotoxicity study of ZnO-NPs against A459 (Human Lung Cancer)

In vitro, synthetic ZnO-NPs were tested against A459 (Human Lung Cancer) at several doses (100, 33.33, 11.11, 3.70, 1.23, and 0.41 µg/ml). The cytotoxic effect of the ZnO-NPs fabricated was larger at higher doses (11 to 100 µg/ml) but decreased at lower concentrations (0.41 to 10 µg/ml). Copper oxide nanoparticles showed significant anticancer efficacy and inhibitory concentration levels in A459 cells (Human Lung Cancer). The cell viability with IC50 values in the micro molar range for zinc oxide nanoparticles was 6.54 µM.

Cytotoxicity study of ZnO-NPs towards MCF-7 cancer cells lines

After manufacturing, the ZnONPs were studied for anticancer effectiveness against MCF-7 human breast cancer cell lines. We carried out a variety of in vitro tests, including a cytotoxicity evaluation, for that reason. ZnO-NPs have considerable anticancer activity against MCF-7 cells at varied doses, according to the cytotoxic experiment. When ZnO-NPs were present in greater concentration, cell death in Mcf-7 cells increased substantially. In this study, extracts of *A. pindowroyle, L. cephalote, and A. mcrosperma* were used to create highly good ZnO-NPs with enhanced activity against cancer cells and IC50 value is 1.76 µM. According to calculated IC50 value of synthesized zinc oxide nanoparticles we observed that IC_{50} for ZnONPs was lowest 1.76 µM for MCF-7 and whereas showed highest IC_{50} 6.54 µM for A549 cancer cells.

Antimicrobial activity

Kirby –Bauer Disk diffusion method was used to perform the antimicrobial assay and the zone of diameter of inhibition (mm) was measured for all tested organisms i.e., *Staphylococcus aureus,* Bacillus Subtilis**,** *Escherichia coli and Pseudomonas aeruginosa and* Candida albicans (table 1). Sodium chloride solution was used as positive control. In the present study and it was observed that the synthesized ZnO nanoparticles showed antimicrobial potential against four test organisms. The maximum zone of inhibition was reported for *Bacillus Subtilis 20.00 mm and Staphylococs aureus 17.00* mm (Gram positive) and minimum zone of inhibition was reported for *Streptococcus pneumonia* 12.00 mm and *Escherichia coli.* 12.00 mm (Gram negative). And ZnONps show no activity towards *Candida albicans.* Thus from the results, it was confirmed that the synthesized nanoparticles have potential antibacterial activity. The number of other workers also reported the antibacterial and antifungal activity of plant based ZnO nanoparticles (42, 43, 44, 45, 46).

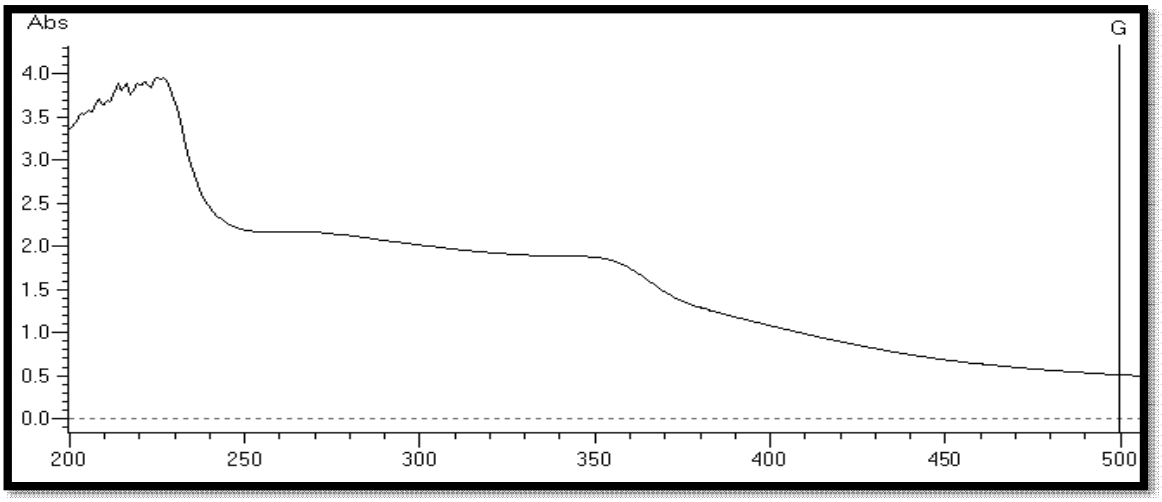

Figure1 : UV- Visible spectra of ZnO nanoparticles using plant extract of Ajuga

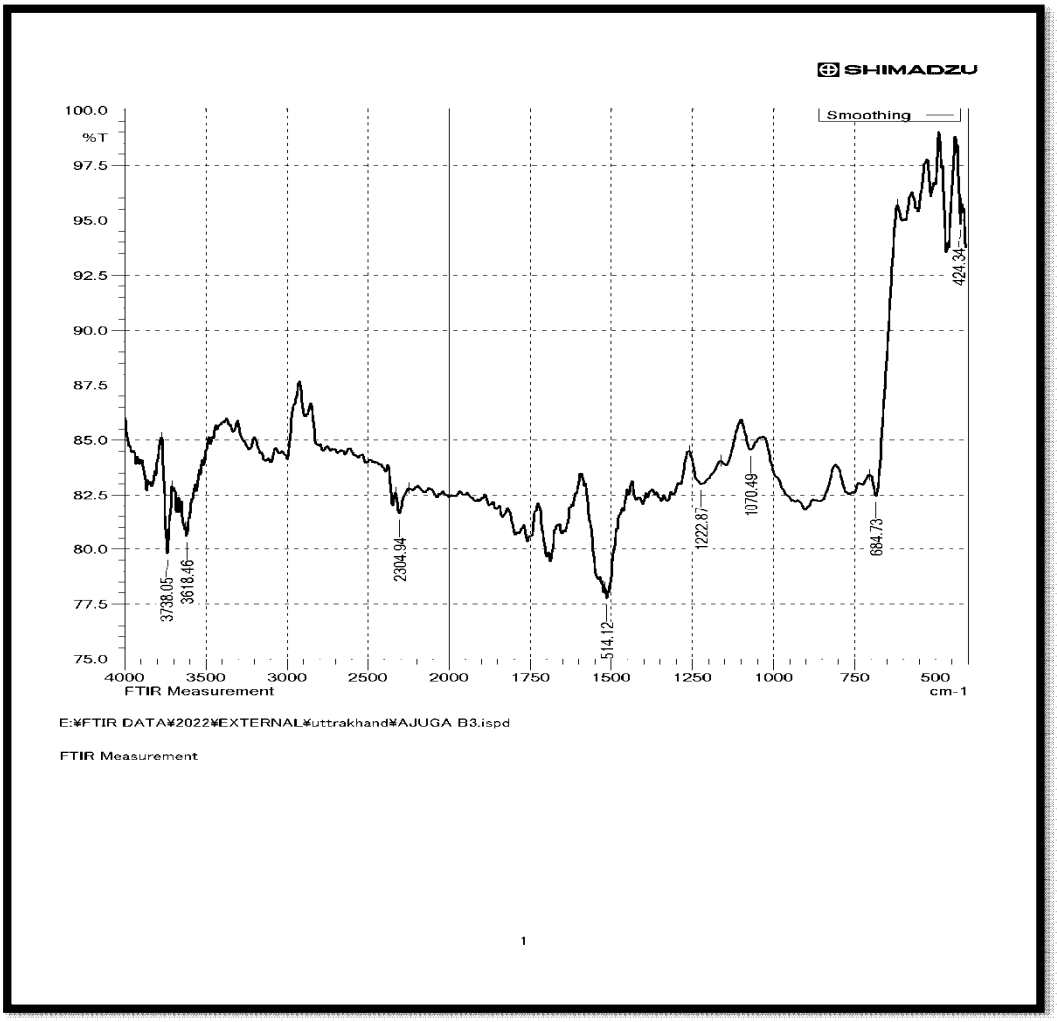

Figure 2: FTIR spectra ZnONPs synthesized using *Ajuga macrosperma* leaf extract.

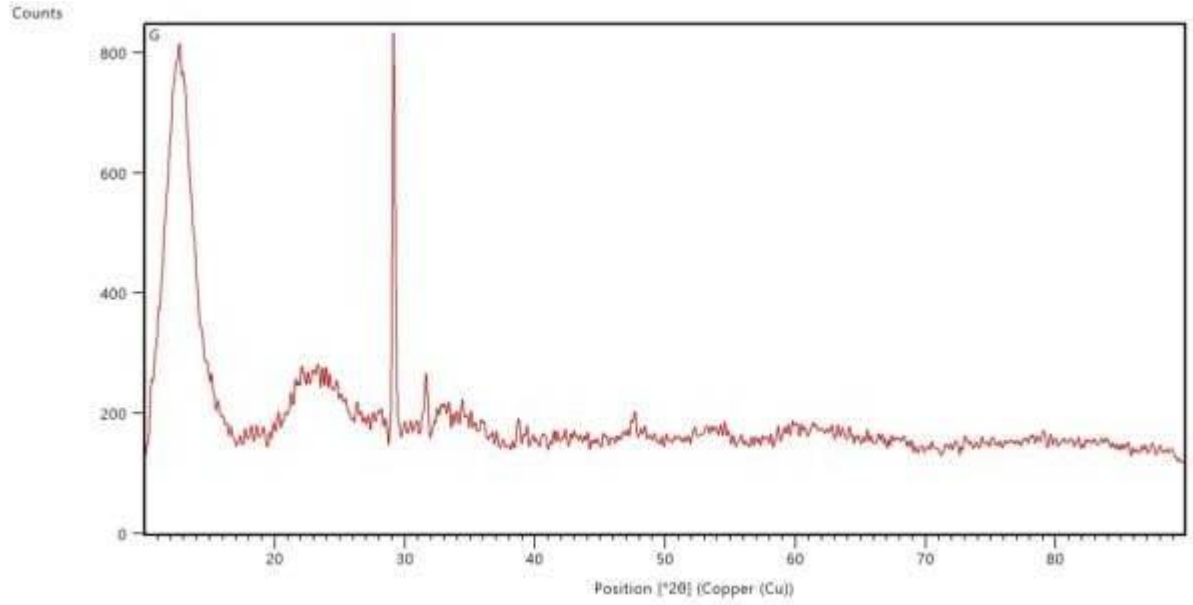

Figure3: XRD spectra ZnONPs synthesized using *Ajuga macrosperma* leaf extract

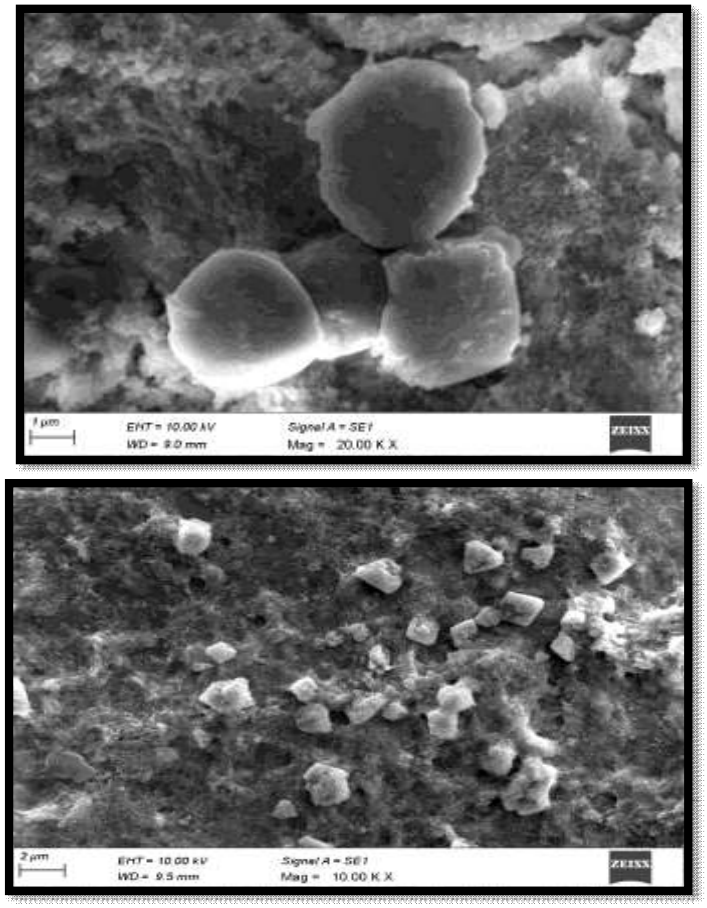

Figure: 4. SEM morphology of ZnO nanoparticles using plant extract of *Ajuga macrosperma* (a) using 20.00KX (b) using 10.00KX.

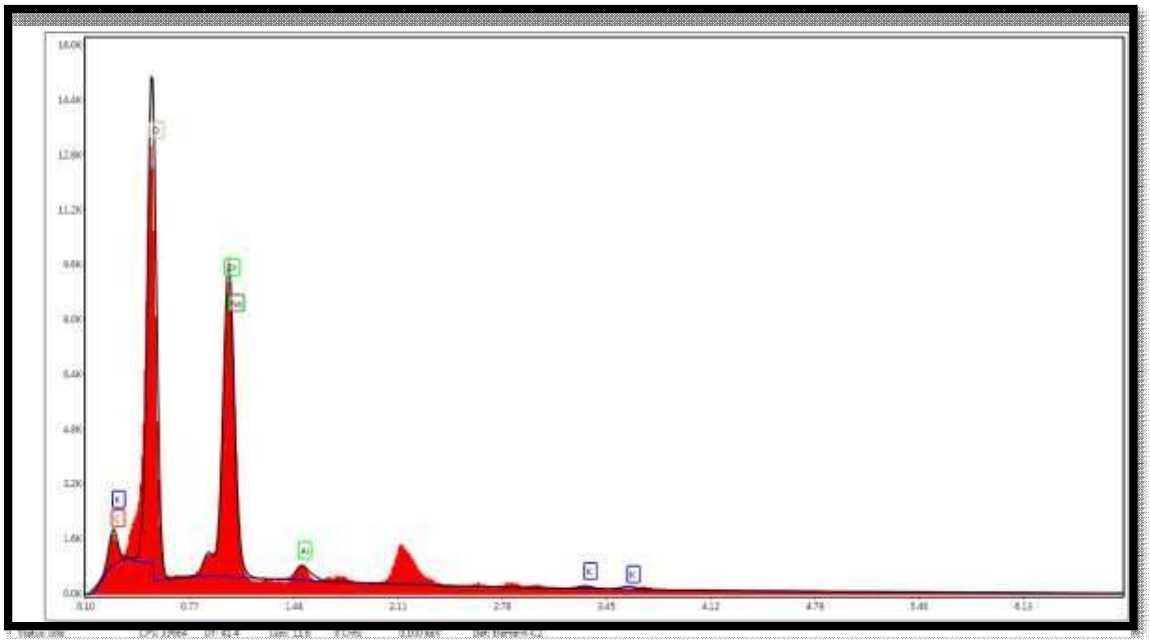

Figure5: EDAX of ZnO nanoparticles using plant extract of *Ajuga macrosperma*

Figure 6: Particle size analyser curve of ZnO nanoparticles using plant extract of *Ajuga macrosperma*

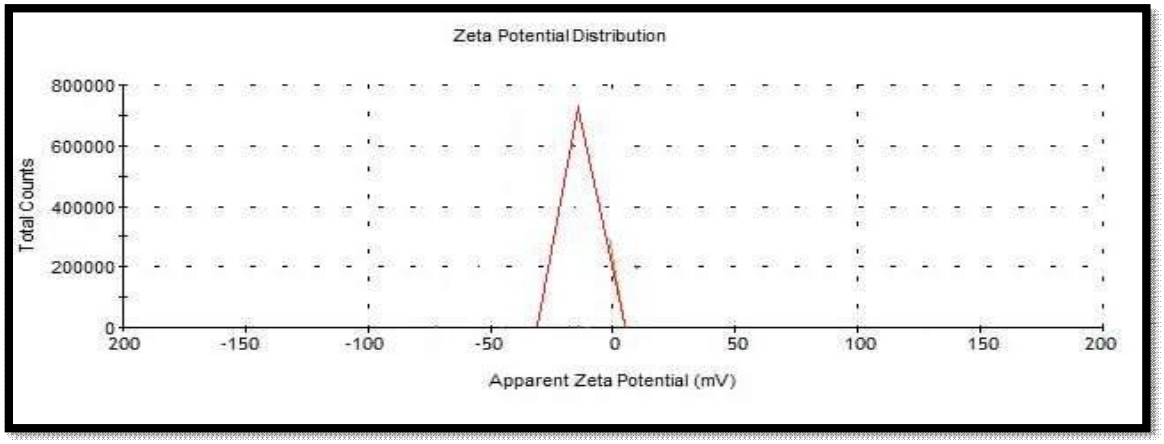

Figure7: Zeta potential curve of ZnO nanoparticles using plant extract of *Ajuga macrosperma*

CONCLUSION

Green synthesis was used to create ZnO nanoparticles from *Ajuga macrosperma* leaf extract. Zinc acetate was reduced by phytochemicals found in plants. We discovered a band at 340 nm using a UV visible spectrophotometer, which we named the "surface Plasmon resonance and," and this band is attributed to the excitation of valence electrons of ZnO arranged in nanoparticles. The size of the particle'sanalyzerascertained that the particles formed were 12.48 nm in size. According to XRD analysis, the particles are crystalline and have 102 atomic arrangements, indicating that they are arranged in Wurtzite crystal. The ZnO sample was analyzed by SEM, and the results showed that agglomeration had occurred. The particles are roughly spherical but of varying sizes. Based on their zeta potential, scientists determined that NPs' surface potential was 42.12 mV, indicating that they were beginning to exhibit instability. The bond vibration peaks at predetermined wavenumbers allowed FTIR to identify the potential biomolecules responsible for the ZnO reduction and the capping agent of bio-reduced ZnO NPs. It is also studied that synthesized zinc oxide nanoparticles show a cytotoxic effect against MCF-7 among prostate cancer, lung cancer, and HeLa cancer cell lines. Furthermore it is also studied that the Zinc oxide nanoparticles showed good and enhanced antimicrobial activity against tested microorganism and thus can serve as potential nano drugs in various industrial and biomedical applications.

Ethical Approval

Not applicable

Conflicts of Interest

The authors declare no conflict of interest in this work.

Availability of data and materials

Not applicable

REFERENCES

1. Farook MA and Mohamed MHS. (2017). Citrus limon Leaf extract mediated synthesis of Titanium Dioxide Nanoparticles. Life Science Archives. 3(7):901- 908.
2. O. V. Kharissova, H.V. RasikaDias, B. I. Kharisov, B. O. Pérez, and V. M. JiménezPérez, (2013). Trends Biotechnol. 31, 240.
3. B. Mirzayi, A. Nematollahzadeh, and S. Seraj, (2015). Powder Technol. 270, 185.
4. S. J. Pearton, D. P. Norton, K. Ip, Y. W. Heo, T. Steiner, (2005). Prog. Mater. Sci. 50, 293.
5. E. S. Nazoori and A. Kariminik, (2018). *In vitro* evaluation of antibacterial properties of zinc oxide nanoparticles on pathogenic prokaryotes, || Journal of Applied Biotechnology Reports, 5(4), 162-165.
6. S. Akbar, I. Tauseef, F. Subhan, N. Sultana, I. Khan, U. Ahmed and K. S. Haleem. (2020). An overview of the plant-mediated synthesis of zinc oxide nanoparticles and their antimicrobial potential, || Inorganic and Nano-Metal Chemistry, *50*(4), 257-271.
7. T. Gur, I. Meydan, H. Seckin, M. Bekmezci, and F. Sen, (2022). -Green synthesis, characterization and bioactivity of biogenic zinc oxide nanoparticles, || Environmental Research, 204, 111897

8. P. X. Gao, Y. Ding, W. Mai, W. L. Hughes, C. Lao, and Z. L. Wang, (2005). Materials science: conversion of zinc oxide nanobelts into superlattice-structured nanohelices, || Science, vol. 309, no. 5741, pp. 1700– 1704.
9. X. L. Cheng, H. Zhao, L. H. Huo, S. Gao, and J. G. Zhao, (2004). -ZnO nanoparticulate thin film: preparation, characterization and gas-sensing property,|| Sensors and Actuators, B, vol. 102, no. 2, pp. 248–252.
10. E. Topoglidis, A. E. G. Cass, B. O'Regan, and J. R. Durrant,(2001). -Immobilisation and bioelectrochemistry of proteins on nanoporous TiO2 and ZnO films,|| Journal of Electroanalytical Chemistry, vol. 517, no. 1-2, pp. 20–27.
11. Y. Hames, Z. Alpaslan, A. Kosemen, S. E. San, and Y. Yerli, ¨(2010). Electrochemically grown ZnO nanorods for hybrid solar cell applications,|| Solar Energy, vol. 84, no. 3, pp. 426–431.
12. W. Jun, X. Changsheng, B. Zikui, Z. Bailin, H. Kaijin, and W. Run, (2002). Preparation of ZnO-glass varistor from tetrapod ZnO nanopowders,|| Materials Science and Engineering B, vol. 95, no. 2, pp. 157–161.
13. P. Sharma, K. Sreenivas, and K. V. Rao, (2003). nalysis of ultraviolet photoconductivity in ZnO films prepared by unbalanced magnetron sputtering,|| Journal of Applied Physics, vol. 93, no. 7, pp. 3963– 3970, (2003).
14. P. V. Kamat, R. Huehn, and R. Nicolaescu, (2002). A -sense and shoot|| approach for photocatalytic degradation of organic contaminants in water,|| Journal of Physical Chemistry B, vol. 106, no. , pp. 788–794.
15. M. S. Akhtar, J. Panwar, and Y. S. Yun, (2013). Biogenic synthesis of metallic nanoparticles by plant extracts, || ACS Sustainable Chemistry & Engineering, 1(6), 591-602
16. D. Raghunandan, B. Ravishankar, G. Sharanbasava, D. Mahesh, V. Harsoor, M. S. Yalagatti, and Venkataraman, –A. Anti-cancer studies of noble metal nanoparticles synthesized using different plant extracts, || Cancer nanotechnology, 2(1), 57-65011)
17. S. Suman, T. P. Das, and C. Damodaran, (2013). Silencing NOTCH signaling causes growth arrest in both breast cancer stem cells and breast cancer cells,|| British journal of cancer, 109(10), 2587-2596
18. S. Kumar, S. Sharma, V. Kumar, A. Sharma, R. Kaur, and R. Saini, – Jamun (*Syzygium cumini* (L.) Skeels): The conventional underutilized multifunctional plant-an exotic gleam into its food and functional significance, || Industrial Crops and Products, 191, 115873 (2023)
19. M. Kalaimathi, S. Sisira, K. S. Hithisha, J. S. Sankar, and R. K. Vimalraj, – Facile Synthesis, Optimization of Zinc Oxide & Magnesium Oxide Nanoparticles Using Solanum Torvum and Their Activity Against MM2 and HeLa Cancer Cells." Optimization of Zinc Oxide & Magnesium Oxide Nanoparticles Using Solanum Torvum and Their Activity Against MM2 and HeLa Cancer Cells.
20. S. Rafique, S. Bashir, R. Akram, S. Jawaid, M. Bashir, A. Aftab, and Awan, S. U. Awan, (2022). In vitro anticancer activity and comparative green synthesis of ZnO/Ag nanoparticles by *Moringa oleifera*, *Mentha piperita*, and *Citrus lemon*." Ceramics International. http://dx.doi.org/10.1016/j.ceramint.2022.10.163.
21. S. A. Al-Ghamd, T. A. Alkathiri, A. E. Alfarraj, Alatawi, O. M. , Alatawi, A. S. Alkathiri, C. Panneerselvam, and S. Khasim, (2022). Green synthesis and characterization of zinc oxide nanoparticles using Camellia sinensis tea leaf extract and their antioxidant, anti-bactericidal and anticancer efficacy, || Research on Chemical Intermediates 48.11: 4769-4783.
22. A. D. Khalid, N. Ur-Rehman, G. H. Tariq, S. Ullah, S. A. Buzdar, S. S. Iqbal, and F. Sher, (2023). Functional bioinspired nanocomposites for anticancer activity with generation of reactive oxygen species, || Chemosphere, 310, 136885.
23. H. F. H. Hassan, A. M. Mansour, A. M. H. Abo-Youssef, B. E. Elsadek and B. A. S. Messiha, (2017). Zinc oxide nanoparticles as a novel anticancer approach; in vitro and in vivo evidence. Clinical and Experimental Pharmacology and Physiology, 44(2), 235-243.
24. R. I. Priyadharshini, G. Geetha, N. Prasannaraj, and P. Venkatachalam,(2014). Microwave-mediated extracellular synthesis of metallic silver and zinc oxide nanoparticles using macro-algae (*Gracilaria edulis*) extracts and its anticancer activity against human PC3 cell lines, || Applied biochemistry and biotechnology, 174(8), 2777-2790.
25. B. Naiel, M. Fawzy, M. W. A. Halmy, and A. E. D. Mahmoud, (2022). Green synthesis of zinc oxide nanoparticles using Sea Lavender (Limonium pruinosum L. Chaz.) extract: characterization, evaluation of anti-skin cancer, antimicrobial and antioxidant potentials, ||Scientific Reports, 12(1), 1-12.
26. M. S. Mthana, M. N. Mthiyane, A. C. Ekennia, M. Singh, and D. C. Onwudiwe, (2022). Cytotoxicity and antibacterial effects of silver doped zinc oxide nanoparticles prepared using fruit extract of Capsicum Chinense, || Scientific African, 17, e01365.
27. E. Hannachi, F. A. Khan, Y. Slimani, S. Rehman, Z. Trabelsi, S Akhtar, E. A. Al-Suhaimi, (2022). In Vitro Antimicrobial and Anticancer Peculiarities of Ytterbium and Cerium Co-Doped Zinc Oxide Nanoparticles, || Biology, 11(12), 1836. (2022)
28. N. Rani, K. Rawat, M. Saini, S. Yadav, A. Shrivastava, K. Saini, and D. Maity, (2022). *Azadirachta indica* leaf extract mediated biosynthesized rod-shaped zinc oxide nanoparticles for in vitro lung cancer treatment, || Materials Science and Engineering: B, 284, 115851.
29. A. Annapoorani, A. Koodalingam, M. Beulaja, G. Saiprasad, P. Chitra, A. Stephen, and R. Manikandan, (2022). Eco-friendly synthesis of zinc oxide nanoparticles using Rivina humilis leaf extract and their biomedical applications, || Process Biochemistry, 112, 192-202.
30. S. Khaleghi, J. Khayatzadeh, and A. Neamati,(2022). Biosynthesis of Zinc Oxide nanoparticles using Origanum majorana L. leaf extract, its antioxidant and cytotoxic activities, || Materials Technology, 1-10. (2022)
31. S. Rasheed, S. Arif, A. Ullah, W. Rehman, and M. H. Abdellatif, (2022).Brassica Species Mediated Green Synthesis of Nanoparticles and Its Potential Biological Applications. Perspective Chapter: Brassica Species Mediated.

32. S. Yedurkar, C. Maurya, and P. Mahanwar, (2016). Biosynthesis of zinc oxide nanoparticles using ixora coccinea leaf extract—a green approach, || Open Journal of Synthesis Theory and Applications, *5*(1), 1-14.
33. K. Punjabi, S. Mehta, R. Chitalia, V. Chitalia, D. Deogharkar, and S. Deshpande, (2018).Efficiency of biosynthesized silver and zinc nanoparticles against multi-drug resistant pathogens, || Frontiers in microbiology, 9, 2207.
34. Y. Gan, F. Gu, D. Han, Z. Wang, and , G. Guo, (2010).Biomimetic synthesis of zinc oxide 3Darchitectures with gelatin as matrix, || Journal of Nanomaterials. https://doi.org/10.1155/2010/289173.
35. J. Zhou, F. Zhao, Y. Wang, Y. Zhang, and L. Yang, (2007). Sizecontrolled synthesis of ZnO nanoparticles and their photoluminescence properties,|| Journal of Luminescence, vol. 122-123, no. 1-2, pp. 195–197.
36. Z. M. Khoshhesab, M. Sarfaraz, and M. A. Asadabad,(2011). Preparation of ZnO nanostructures by chemical precipitation method,|| Synthesis and Reactivity in Inorganic, Metal-Organic and Nano-Metal Chemistry, vol. 41, no. 7, pp. 814–819.
37. JCPDS, (1977). Powder Diffraction File, Alphabetical Index, Inorganic Compounds, International Centre forDi ffraction Data, Newtown Square, Pa, USA.
38. P.C. Lin, S. Lin, P.C. Wang, R. Sridhar, (2014). Techniques for physicochemical characterization of nanomaterials, || Biotechnol. Adv. 32, 711–726.
39. H. Fissan, S. Ristig, H. Kaminski, C. Asbach, M. Epple, (2011). Comparison of different characterization methods for nanoparticle dispersions before and after aerosolization. Anal. Methods 2014, 6, 7324– 7334. Johal, M.S. Understanding Nanomaterials; CRC Press: Boca Raton, FL, USA.
40. J. B. Hall, M. A. Dobrovolskaia, A. K. Patri, S. E. McNeil, (2007). Characterization of nanoparticles for therapeutics. Nanomed. Nanotechnol. Biol. Med. 2, 789–803.
41. B. D. Ranter, A. S. Hoffman, F. J. , Schoen, J. E. Lemons, (2004). An Introduction to Materials in Medicine, Biomaterials Science , Elsevier: San Diego, CA, USA.
42. Elumalai, K., & Velmurugan, S. (2015). Green synthesis, characterization and antimicrobial activities of zinc oxide nanoparticles from the leaf extract of *Azadirachta indica* (L.). *Applied Surface Science*, 345, 329-336.
43. Khajuria, A.K., Bisht, N.S., Manhas, R.K., & Kumar, G. (2019a). Callus mediated biosynthesis and antibacterial activities of zinc oxide nanoparticles from *Viola canescens*: an important Himalayan medicinal herb. *SN Applied Sciences*, 1(5), 1-13.
44. Sharma, D., Rajput, J., Kaith, B., Kaur. M. and Sharma, S.S. Synthesis of ZnO nanoparticles and study of their antibacterial and antifungal properties (2010). *Thin Soil Films*, 51, 1224-29.
45. Rajiv, P., Rajeshwari, S., & Venckatesh, R. (2013). Bio-Fabrication of zinc oxide nanoparticles using leaf extract of *Parthenium hysterophorus* L. and its size-dependent antifungal activity against plant fungal pathogens. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *112*, 384-387.
46. Khajuria, A. K., Kumari, M., Kandwal, A., Singh, A., & Bisht, N. S. (2020). Biofabrication of Zinc Oxide Nanoparticles from Two Different Zinc Sources and Their Antimicrobial Activity. *BioNanoScience*, 1-17