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Green Synthesis and Characterization of Zinc Oxide Nanoparticles Using *Cyperus pangorei* Grass Extract and its Antibacterial Activity

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ABSTRACT

Green synthesis of nanoparticles using plant-derived biomolecules has emerged as an eco-friendly and sustainable approach in nanobiotechnology. In the present study, zinc oxide nanoparticles (ZnO NPs) were biosynthesized using aqueous leaf extract of Cyperus pangorei, a biologically important grass species rich in phytochemicals such as flavonoids, phenolics, alkaloids, and terpenoids that act as natural reducing and stabilizing agents. UV–Visible spectroscopy confirmed nanoparticle formation with a characteristic absorption peak at 264 nm, while FTIR analysis revealed the involvement of hydroxyl, amine, phenolic, and flavonoid functional groups in nanoparticle stabilization. XRD studies confirmed the crystalline nature of ZnO nanoparticles, and SEM analysis demonstrated predominantly spherical particles with sizes below 30 nm.

The biosynthesized ZnO nanoparticles exhibited significant antibacterial activity against important human pathogens including Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Salmonella sp., indicating broad-spectrum antimicrobial potential against both Gram-positive and Gram-negative bacteria. Furthermore, the nanoparticles showed promising antioxidant and agricultural properties by enhancing plant growth and reducing environmental stress effects in crop plants. The use of Cyperus pangorei, an abundantly available dry grass species with rich bioactive constituents, highlights its biological significance as a sustainable resource for green nanomaterial synthesis. The study demonstrates that Cyperus pangorei-mediated ZnO nanoparticles are eco-friendly, cost-effective, and biologically active nanomaterials with potential applications in biomedical, agricultural, and agro-biotechnological fields.

Keywords: *Cyperus pangorei, UV-Vis, ZnO, FTIR, SEM,XRD ,E. coli.*

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Introduction

Green synthesis of nanoparticles using plant extracts has emerged as an ecofriendly, cost-effective, and sustainable approach in nanotechnology. Nanotechnology-based antimicrobial agents have gained considerable attention as promising alternatives to traditional antibiotics. Nanoparticles possess unique antimicrobial mechanisms that enable them to effectively target resistant microorganisms through multiple pathways simultaneously. The utilization of nanoparticles in commercial applications and nanotechnology has seen a sharp rise in attention. Because green synthesis methods employ fewer harmful chemicals, are environmentally friendly, and allow for the one-step synthesis of nanoparticles, their adoption by researchers is quickly increasing.

Cyperus pangorei is a perennial sedge belonging to the family Cyperaceous. It is both pantropical & temperature in distribution [5]. Phytochemical investigations of *Cyperus* species have revealed the presence of diverse bioactive secondary metabolites including sesquiterpenoids, coumarins, anthraquinones, flavonoids, terpenoids, cyperones, rotunols, limonoids, phenolic compounds, and other biologically active constituents [3,6,9]. These phytochemicals possess strong reducing, stabilizing, antioxidant, and antimicrobial properties, making the plant a promising biological resource for green nanoparticle synthesis. Such bioactive metabolites act as natural capping and reducing agents, thereby eliminating the requirement for hazardous chemical reagents commonly used in conventional nanoparticle synthesis methods. Consequently, *Cyperus pangorei* represents an ecofriendly, sustainable, and cost-effective botanical source for the fabrication of biogenic nanoparticles with potential biomedical, antimicrobial, agricultural, and environmental applications. *Cyperus pangorei* plays a crucial role in the bio reduction of metal ions and stabilization of synthesized nanoparticles during the photosynthesis process. Such bioactive metabolites act as natural capping and reducing agents, thereby eliminating the requirement for hazardous chemical reagents commonly used in conventional nanoparticle synthesis methods, metal oxide nanoparticles have been widely employed in medicine can function as catalysts to assist reduce or remove dangerous substances from the environment, they have applications in the environmental field [4,10].Metal nanoparticle synthesis has been described using a variety of techniques over the years, including chemical, biological, and physical techniques. There have been reports of the biosynthesis of ZnO NPS in a variety of bacterial and fungal taxa, including *Bacillus subtilis*, *Escherichia coli*, Ureolytic bacteria, and Lactobacillus plantarum, as well as in plants like *Aloe vera*, *Sargassum muticum*, *Eichhornia crassipes*, and *Borassus flabellifer* fruit [13]..Numerous researchers have examined the diverse morphologies and noteworthy antibacterial activity of nano-sized ZnO against a broad range of bacterial species [1.2].

The antimicrobial properties of zinc nanoparticles (NPs) indicate their efficacy in combating infections and the essential requirements for using zinc-based nanoparticles as nano-pesticides in farming include both the materials' basic characteristics and the surrounding environment. Comparing nano-pesticides with traditional formulations, the former offers greater stability, superior efficacy, excellent dispersion, and precise release against target species [30].

Every day researchers migrate away from synthetic approaches since green synthesis does not require high pressure, energy, temperature, or harmful substances. The synthesis of nanoparticles through biosynthesis has been offered as an environmentally friendly, low-maintenance, and inexpensive alternative to physical and chemical processes [31].Sustainable Development Goal (SDG) targets have been attained in part with bimetallic nanoparticles made using green synthesis [22]. Zinc oxide nanoparticles have attracted a lot of attention from researchers nowadays because of their unique properties [21]. These characteristics are potent photosensitivity, increased excitation binding energy, biocompatibility, eco-friendliness, low prices, simplicity of synthesis, thermal conductivity, and resistance to adverse circumstances [17]. Zinc oxide-based nanoproducts can be produced with greater efficiency via the growth of green nanotechnology [18].

Materials and Methods

Sample Collection and Preparation of Plant Extract:

Dry Grass samples of *Cyperus pangorei* were collected from vicinity of pond embankments and transferred in a sterile polyethylene bag for further analysis into laboratory. In laboratory the collected plant material thoroughly rinsed in running tap water to remove any physical contaminants and subsequently washed several times with distilled water. 5gm of cleaned air-dried samples was cut into small fragments and thoroughly mixed with 100 ml of distilled water in 250 ml of Erlenmeyer flask. The mixture was maintained at 70°C for 8 min to extract bioactive phytoconstituents responsible for nanoparticle synthesis. The obtained extract was allowed to cool to ambient temperature and filtered through Whatman No. 1 filter paper to remove particulate matter. The clear filtrate was collected and preserved at 4°C for subsequent biosynthesis experiments involving zinc oxide nanoparticles [14,15].

Biogenic Synthesis of Zinc oxide Nanoparticles :

50 milliliters of distilled water were used to dissolve 1 milligram of zinc acetate, which was then stirred for one hour. Next, 25 mL of plant extract was added to the zinc acetate solution after 20 mL of NaOH solution had been gradually added. After an hour of incubation, the reaction mixture color changed. Three hours were spent with the solution in the stirrer.

The synthesis of ZnO NPs was confirmed by the appearance of yellow color following the incubation period. Centrifugation was used to separate the precipitate from the reaction solution for 15 minutes at 60°C and 8000 rpm. The pellet was then collected. Pellet was kept in airtight vials for future research after being dried for two hours at 80°C in a hot air oven.

Characterization of Nanoparticles:

SEM:

Scanning electron microscope image shows the morphology of the green synthesized ZnO NPS using *Cyperus pangorei*. A Scanning Electron Microscopy (SEM) investigation was performed with a JEOL JSM-6390 model. By depositing a small amount of sample on the copper grid, a thin carbon-coated film was created. Before analysis, the sample film on the SEM grid was dried for 5 minutes under a mercury lamp [26] The observations revealed that the ZnO NPs were hexagonal in shape and more agglomerated (fig.1).

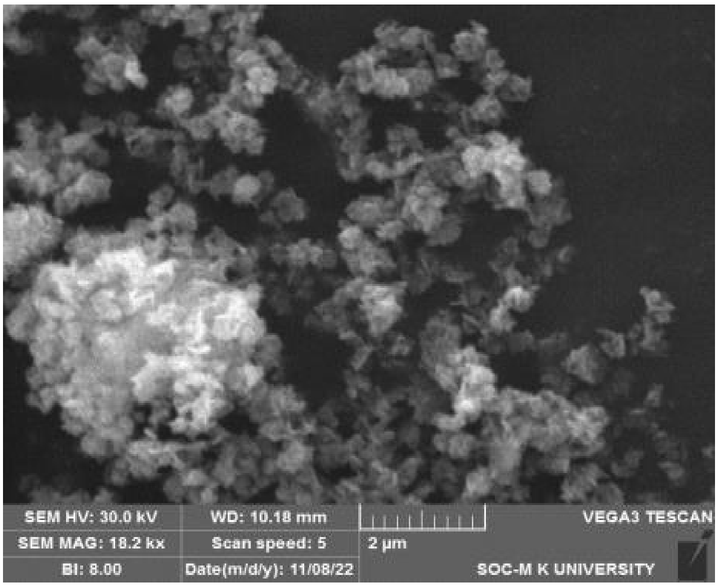

Fig. 1: SEM showing Hexagonalin shape

Utilizing FT-IR spectroscopy, the surface chemistry and involvement of bacterial proteins in the reduction and stability of NPs were investigated. The wavelength spectrum of the cell-free supernatant before and after the addition of metal ion solution ZnONPs was recorded using Perkin Elmer manufacture model spectrum 22575927 RX1 (wavelength range of 4000 cm-1 to 400 cm-1).. Blotting paper was used to get rid of any extra water. A small amount of the emulsified sample in the amount of 0.2 was diluted with 10 ml of distilled water for the UV-visible spectrophotometry assessment. This diluted sample was then placed in 1 ml into a 2 ml quartz cuvette. A horizontal slit that was 2 mm broad was placed at the front of the chamber to measure absorption. The UV absorption was further investigated over a spectrum of wavelengths from 100 to 1000 nm. The sample's maximum absorption wavelength was determined to be the wavelength at which the emulsion sample presented its highest level of absorption. The phase purity and particle size of ZnO NPs were determined using the X-ray diffractometer, Brucker AXS (Germany) D8 advanced diffractometer unit. Scherrer's equation (Equation (1)) was used to determine the crystalline size of the synthesized ZnO NPs (Holzwarth *et al.*, 2011).

$D = k\lambda / \beta\, COS\, \theta$

λ–Is the X-ray wavelength

β–Is the Full Width at Half Maximum (FWHM) and θ–Is the diffraction angle

$D = 0.9\lambda / \beta \, Cos\, \theta$

where D is the crystallite size, λ is the wavelength of the x-ray used (1.5406 Å), β is the full width at half maximum (FWHM), and θ is the Bragg's angle.

Characterization

UV spectroscopy and FTIR spectroscopy were applied to zinc oxide nanoparticles. The range of 4000-400cm-1 was used to record the infrared spectrum. The absorption peak for UV spectroscopic examination was found in each spectrum in the 200–400 nm region, which is a typical band for pure ZnO.

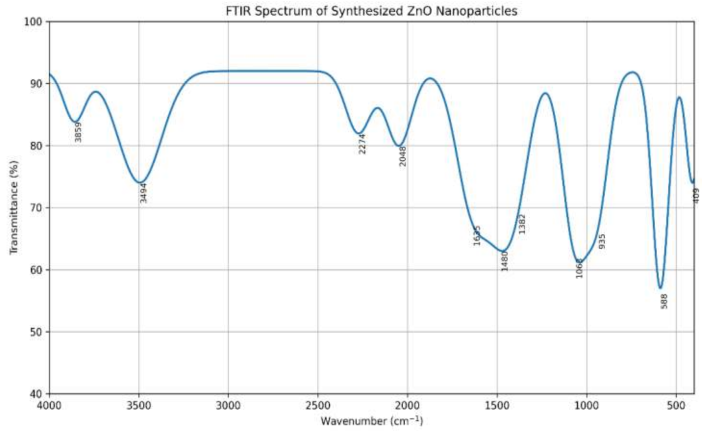

Fig.2 FTIR spectrum of Synthesized ZnO Nanoparticle

No.	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	408.93	1.0055	0.0005	410.86	400.25	21.1888	0.0009
2	588.31	0.5262	0.523	856.43	411.82	943.3609	65.2121
3	935.52	0.9822	0.0567	970.24	857.4	224.0261	1.2172
4	1063.79	0.8938	0.3771	1259.57	971.2	560.614	22.2074
5	1382.06	1.3524	0.1901	1432.21	1260.54	311.9491	4.4975
6	1480.43	1.369	0.1274	1570.12	1433.17	251.6354	2.8475
7	1635.71	1.3569	0.4098	1852.71	1571.09	490.0962	7.869
8	2048.49	2.1284	0.0563	2221.13	1853.67	612.1039	1.9818
9	2274.17	2.1533	0.0192	2337.83	2222.09	192.6494	0.1892
10	2349.4	2.1775	0	2354.22	2338.79	25.6472	0.0001
11	3494.2	0.9387	0.7521	3794.14	2355.19	2629.891	134.7148
12	3859.73	1.5365	0.011	3890.59	3795.11	172.9139	0.1678
13	3973.53	1.4926	0.0156	4000.54	3891.55	198.4032	0.2447

X-ray Powder Diffraction Analysis

The significant diffraction peaks observed in (010), (002), (011), (012), (110), (013), (020), (112), (021), (004), and (022) planes, and XRD- values represent the average size of 29.34 nm (JCPDS 98-002-7781).

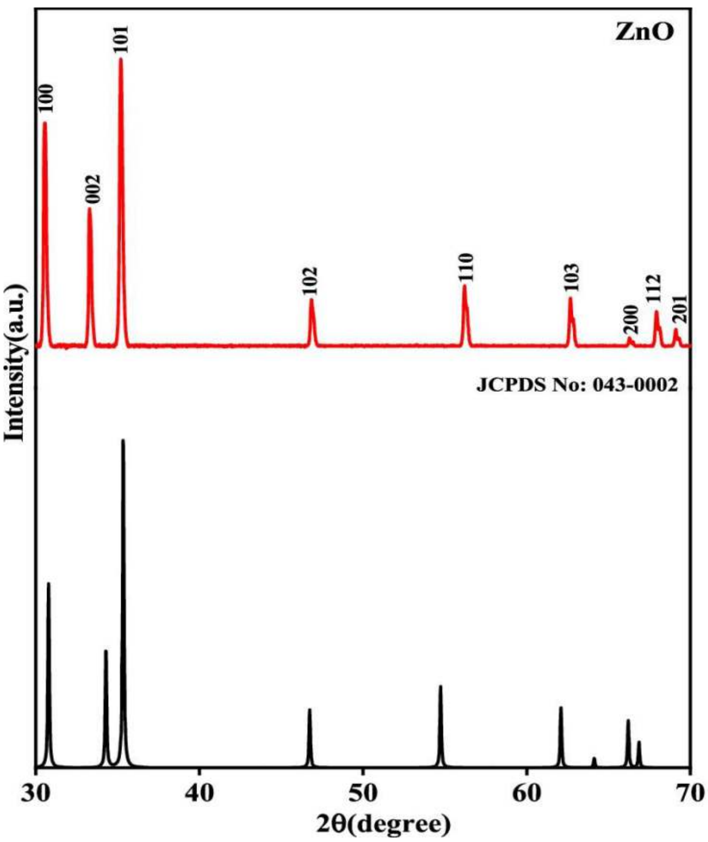

Fig.3: XRD analysis examines the crystalline Phases of green synthesized ZnO Nps.

Antibacterial Assay of ZnO Nanoparticle:

To evaluate antibacterial potential of biogenic synthesized bacterial strains were used namely *Salmonella* sp., *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus*. The bacterial cultures used in the present investigation were obtained from maintained stock cultures and subculture prior to experimentation to ensure active growth. The antibacterial assay was carried out using the standard disc diffusion technique. Fresh overnight cultures of each bacterial strain were uniformly spread onto sterile nutrient agar plates using sterile cotton swabs under aseptic conditions. Sterile discs impregnated with different concentrations (10 µL and 20 µL) of ZnO nanoparticle suspension were carefully placed on the inoculated agar surface. Commercially available antibiotic discs were used as positive controls for comparison of antibacterial efficacy. The inoculated plates were subsequently incubated at 37°C for 24 h. Following incubation, the antibacterial activity of the synthesized nanoparticles was determined by measuring the diameter of the clear inhibition zones formed around each disc. As a control, commercial antibiotic discs were used. Different degrees of zonation that developed around the disc once the incubation period was over were measured.

Result and Discussion

The produced zinc oxide nanoparticles; UV-Vis spectra revealed an absorption peak at about 264 nm. The beneficial applications of green synthesized zinc oxide nanoparticles in both agriculture and nanobiotechnology. Earlier reports indicated that ZnO nanoparticles can enhance plant growth and productivity, supporting the plant growth-promoting effects observed in the present study. Similarly, successful biosynthesis of ZnO nanoparticles using plant extracts has been widely reported, where phytochemicals present in botanical materials act as natural reducing and stabilizing agents.

The UV-visible absorption spectra of the biosynthesized ZnO nanoparticles using the leaf extract of Vernonia cinerea reveal an absorption peak at 360 nm [32]. functional groups can be identified by analyzing its FT-IR spectra. Higher extract volumes raise the absorbance peak strength, which in turn increases the yield of nanoparticles because the extract contains more phytochemicals. [27]. The synthesis of zinc nitrate to produce zinc oxide nanoparticles is aided by an abundance of hydroxyl (OH) groups present in flavonoids and phenol [29]. Biosynthesized CuO nanoparticles using *Rumex nepalensis* showed a crystalline monoclinic structure with particle sizes ranging from 21–97 nm. The synthesized CuO NPs exhibited significant antimicrobial and antioxidant activities, including effective DPPH free radical scavenging, highlighting their potential biomedical applications and the eco-friendly nature of the green synthesis approach [33]. Green synthesized ZnO nanoparticles using *Nicotiana plumbaginifolia* extract exhibited a hexagonal wurtzite crystalline structure with spherical particles ranging from 16–24 nm. The nanoparticles showed significant antibacterial activity against pathogenic bacteria and strong antioxidant potential with 75.59% DPPH radical scavenging activity, indicating promising biomedical applications [34]. Similar studies were reported in Sustainably synthesized cobalt oxide nanoparticles (CoO NPs) using Uraria picta extract demonstrated effective green synthesis through natural reducing and stabilizing phytochemicals. Characterization by UV–Vis, FTIR, XRD, EDS, SEM, and TEM analyses confirmed the structural and morphological properties of the nanoparticles. The biosynthesized CoO NPs exhibited significant antioxidant and antibacterial activities against selected bacterial strains, highlighting their potential biomedical and environmental applications through an eco-friendly and cost-effective synthesis approach [35,37].

The presence of phenolic and flavonoid group molecules plays a role in the inhibition process, stability, and capping agents, while the peak in the 400–600 cm^{-1} range is designated as Zn–O, suggesting the coordination of produced ZnO nanoparticles [28]. As shown in the SEM image, the synthesized ZnO nanoparticles, with an average particle size of 26 nm estimated by using Debye-Scherrer's formula, revealed efficiency in decreasing the effects of salt stress within Sorghum bicolor. The XRD tests examined the particles' hemispherical morphology and sizes below 30 nm. Most of the particles' shapes are spherical and aggregate into larger particles with uncertain shape [24]. Azadirachta indica nanoparticles have gained attention in nanotechnology due to their antioxidant and protective properties against reproductive toxicity. Recent studies suggest that neem-mediated nanoparticles may help reduce lead acetate-induced damage in male and female reproductive systems.This review highlights the therapeutic potential of neem-based nanomaterials as eco-friendly alternatives for reproductive health protection [36].

The production of nanoparticles (NPs) from plant extracts has grown into a popular field for research in the past few years. This pattern shows the increased awareness about the potential advantages of green-synthesized metal nanoparticles over the traditional alternatives used in the agricultural sector [20]. Silver nanoparticles are less harmed by zinc nanoparticles (Zn NPs), which have been indicated to be beneficial as a nano fertilizer and an anti-fungal properties agent. Penicillium expansum, Botrytis cinerea, and Aspergillus flavus are just a few of the fungi that zinc nanoparticles are effective against [19,22].

Plenty of nanomaterials help to reduce crop diseases while used in pest management applications. These materials include nano-containers, nano- encapsulates, nano-cages, and nano-emulsion [16]. Zinc oxide nanoparticles, applied to Vicia faba seeds extract had significant impacts on Meloidogyne incognita, resulting in mortality rates of 88.2%, 93.4%, and 96.72% after exposure times of 24, 48, and 72 hours, in that combination. were treated with five concentrations of ZnO-NPs (12.5, 25, 50, 100, and 200 µg/mL [25].The current findings are consistent with these observations, confirming that *Cyperus pangorei* extract can effectively mediate the formation of biologically active ZnO nanoparticles with promising antibacterial and agricultural applications[7,8] According to research published in 2019 by Gomathi and Sathya, zinc nanoparticles exhibit an absorption peak at 277 nm.

The ZnO nanoparticles FT-IR spectra revealed broad bands that occur at 3253.11cm-1 and correspond to the N-H stretching frequencies of amines. The N=O and C-C stretch is indicated by the bands at 1564.22 cm-1 and 1405.26 cm-1(Figure 1). The N-O and C-N are seen in the band at 1343.86 cm-1 and 1094.30 cm-1. The broad bands at 3377.41 cm-1, which correspond to the O-H and N-H stretching frequencies of alcohol and amine groups, were reported by Myint Khaing et al., in 2018. By evaluating the zone of inhibition, zinc oxide nanoparticles that were manufactured were found to have antibacterial activity against *Salmonella sp*., *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus*. The pathogens were clearly affected by the nanoparticles; anti-bacterial action, as the results plainly revealed. For 10µl (16mm, 17mm) and 20µl (17mm, 18mm), the maximum zone of inhibition was shown against *Escherichia coli* and *Pseudomonas aeruginosa*; for 10µl and 20µl, the minimum zone of inhibition was seen against *Staphylococcus aureus* (13mm, 13mm) and *Salmonella sp* (15mm, 14mm) (Table 1).The greatest zone of inhibition (32±0.050) against *Pseudomonas aeruginosa* and the minimum zone of inhibition (25±0.100) against *Staphylococcus aureus* [11].The plants branch length grew as the concentration of ZnO nanoparticles rose. The plant growth was higher in the *Cicer arietinum* seeds coated with 0.02g & 0.03g ZnO nanoparticles and the Vigna radiata seeds coated with 0.01g ZnO nanoparticles than in the control [12]. Study describes the green synthesis of multiphase iron oxide nanoparticles using *Dolichandrone falcata* leaf extract. The synthesized α-Fe_2O_3 nanorods and γ-Fe_2O_3 spherical nanoparticles were characterized by XRD, FTIR, SEM, and TEM, confirming their crystalline nature with an average size of ~21 nm. The nanoparticles showed significant antibacterial activity, indicating their potential for biomedical and environmental applications, similar study shows green synthesis of nanoparticles using plant extracts is an eco-friendly and cost-effective approach due to the natural reducing and stabilizing agents present in plants. In this study, copper oxide nanoparticles (CuO NPs) were synthesized using *Rivina humilis* L. extract and characterized by UV–Vis, FTIR, XRD, SEM, TEM, and EDS analyses. The biosynthesized CuO NPs exhibited significant antibacterial activity against selected bacterial strains and showed notable antioxidant activity through DPPH free radical scavenging assay. [38,39].

Statistical analysis revealed significant differences ($p < 0.05$) between the positive control and ZnO nanoparticle treatments for most bacterial strains. However, no significant difference was observed between ZnO nanoparticle concentrations of 10 µL and 20 µL in certain bacterial species, indicating comparable antibacterial efficacy at both concentrations.

The enhanced antibacterial activity may be attributed to the small particle size, large surface area, and generation of reactive oxygen species (ROS), which disrupt bacterial cell membranes and interfere with cellular metabolism. These findings demonstrate that green synthesized ZnO nanoparticles using Cyperus pangorei possess promising antibacterial potential and could be utilized as eco-friendly antimicrobial agents for biomedical and agricultural applications.

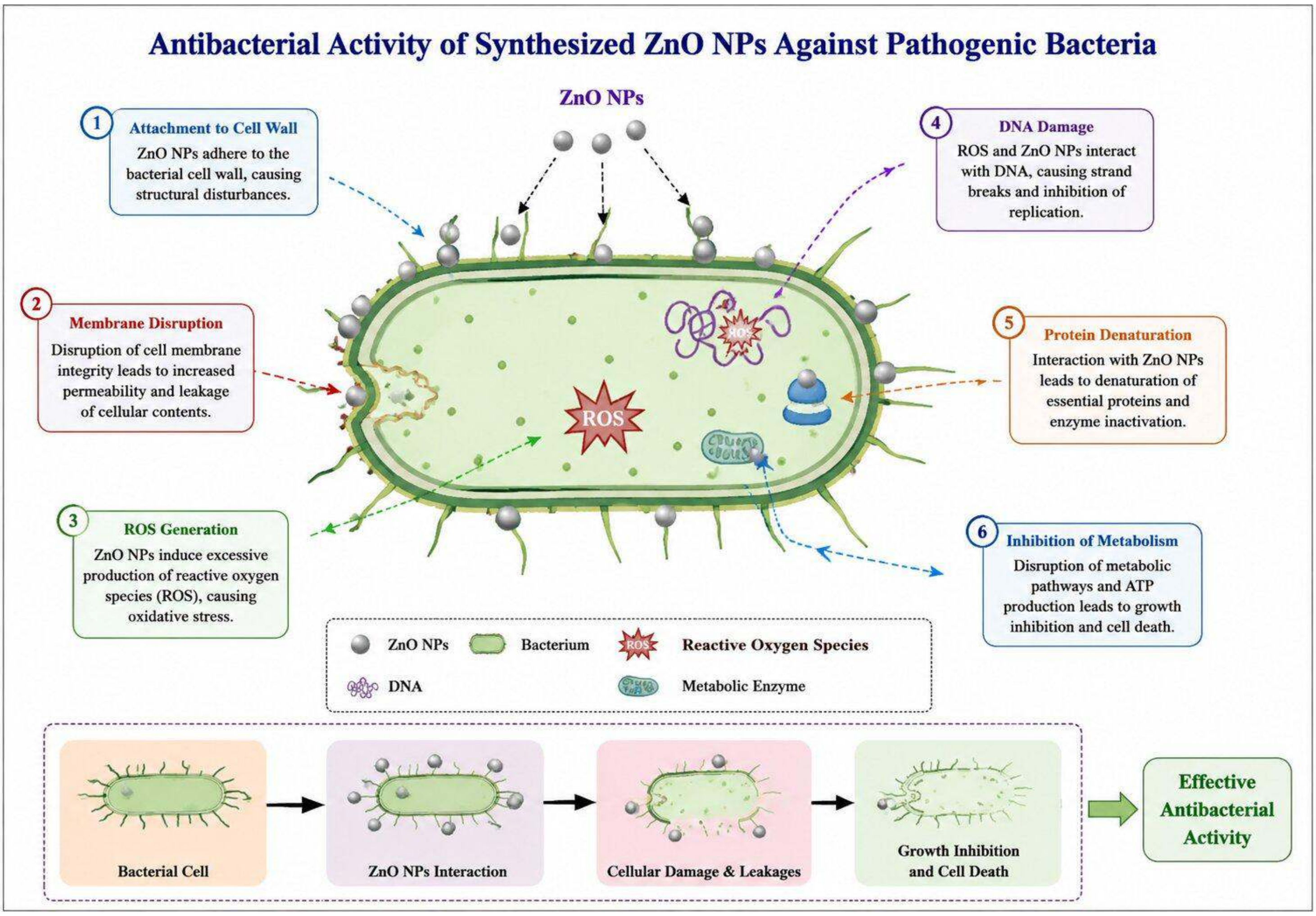

Fig.4: Schematic Representation of Antibacterial activity of Green synthesized ZnO NPS against Pathogenic bacteria

Table 1: Antibacterial Activity of Synthesized ZnO Nps Against Pathogenic Bacteria

S.NO	Microorganism	Zone of Inhibition (mm) Positive Control	**ZnO NPs** (mm) (10µl)	**ZnO NPs** (mm) (20µl)
1.	*Escherichia coli*	25.00 ± 0.58^{a}	16.00 ± 0.33^{b}	17.00 ± 0.58^{b}
2.	*Pseudomonas aeruginosa*	18.00 ± 0.33^{a}	17.00 ± 0.58^{a}	18.00 ± 0.33^{a}
3.	*Salmonella sp*	20.00 ± 0.58^{a}	15.00 ± 0.33^{b}	14.00 ± 0.58^{b}
4.	*Staphylococcus aureus*	22.00 ± 0.33^{a}	13.00 ± 0.58^{b}	13.00 ± 0.33^{b}

Fig. 4: Antibacterial activity of green synthesized ZnO nanoparticles against pathogenic bacteria: (A) Escherichia coli, (B) Pseudomonas aeruginosa, (C) Salmonella sp., and (D) Staphylococcus aureus showing zones of inhibition by agar well diffusion method.

Conclusion

In the present investigation, an eco-friendly and sustainable approach was employed for the biosynthesis of zinc oxide nanoparticles (ZnO NPs) using dry grass extract of *Cyperus pangorei* as a natural reducing and stabilizing agent. The successful formation of ZnO nanoparticles was confirmed through UV–Visible spectroscopy, which exhibited a characteristic absorption peak, while FTIR analysis revealed the participation of bioactive phytochemicals such as phenolics, flavonoids, hydroxyl, and amine groups in nanoparticle synthesis and stabilization. XRD analysis confirmed the crystalline hexagonal wurtzite structure of ZnO nanoparticles, and SEM micrographs demonstrated predominantly spherical and aggregated nanoparticles with particle sizes below 30 nm.

The synthesized ZnO nanoparticles exhibited significant antibacterial activity against both Gram-positive and Gram-negative bacterial pathogens including *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Salmonella* sp. The synthesized ZnO nanoparticles exhibited concentration-dependent antibacterial activity against both Gram-positive and Gram-negative bacteria. Statistical analysis revealed significant differences ($p < 0.05$) among treatments, indicating the effectiveness of ZnO nanoparticles against pathogenic bacterial strains. In addition, the nanoparticles showed promising effects on plant growth and stress tolerance, suggesting their potential utility in sustainable agriculture and nano-enabled crop management strategies. The present findings emphasize the biological significance of *Cyperus pangorei* as a rich source of phytochemicals for green nanomaterial synthesis. Compared with conventional chemical methods, the phytogenic synthesis approach offers advantages such as low toxicity, environmental compatibility, cost-effectiveness, and enhanced biological activity, making biosynthesized ZnO nanoparticles promising candidates for biomedical, antimicrobial, and agricultural applications.

Acknowledgment and Author Contribution:

Plant sample collection and laboratory experiments and discussion write ups were done by Dr. Rohit Gavankar, Dr. Deepa Verma and sample characterization were done by Dr Virendra K Sangode.and Dr.Ankita Tekade. Authors wish to Thanks Anacon Laboratory Pvt ltd for Characterization of nanomaterial and providing essential lab facilities. The findings of this work demonstrate that *Cyperus pangorei* can serve as a valuable biological source for the production of functional zinc oxide nanoparticles with potential applications in antimicrobial therapy, sustainable agriculture, and other nanobiotechnological fields. The study also contributes to the advancement of green nanotechnology by promoting safer and more ecofriendly nanoparticle fabrication methods.

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