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Eco-Friendly Synthesis of Zinc Oxide Nanoparticles Using *Parthenium hysterophorus* Flower Extract for Antimicrobial and Antibiofilm Applications

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ABSTRACT

Parthenium hysterophorus, a highly invasive weed from the Asteraceae family, is widely recognized for its detrimental ecological impact. However, recent studies highlight its significant antimicrobial and antibiofilm potential. This plant is rich in bioactive compounds, including alkaloids, flavonoids, phenols, terpenes, and pseudoguaianolides, which contribute to its therapeutic properties. Among its emerging applications, the green synthesis of nanoparticles using *P. hysterophorus* extracts has gained attention due to its eco-friendly and cost-effective nature. In particular, zinc oxide (ZnO) nanoparticles derived from *P. hysterophorus* exhibit remarkable antibacterial and antibiofilm activities. These nanoparticles effectively disrupt bacterial biofilms, a key factor in antimicrobial resistance (AMR), which is responsible for over a million deaths annually. Studies indicate that ZnO nanoparticles synthesized from *P. hysterophorus* extracts inhibit bacterial adhesion, impede biofilm formation, and display potent antibacterial properties against multidrug-resistant pathogens. Furthermore, green-synthesized ZnO nanoparticles leverage the plant's rich phytochemical profile to enhance their efficacy while adhering to sustainable production principles. Although most research focuses on leaf-derived ZnO nanoparticles, the potential of flower-mediated synthesis remains largely unexplored. Expanding studies in this area could unveil novel antimicrobial strategies with enhanced specificity and potency. The integration of plant-based nanotechnology in biomedical applications presents a promising avenue for combating biofilm-associated infections and addressing global health challenges. Despite its medicinal potential, *P. hysterophorus* remains an aggressive invasive species, necessitating further research to harness its benefits while mitigating its ecological threats. Our significance of *P. hysterophorus*-derived nanoparticles in antimicrobial and antibiofilm applications, offering sustainable and effective solutions against drug-resistant infections.

INTRODUCTION

Environmental sciences, medicine, and pharmaceuticals have all seen substantial changes as a result of nanotechnology. The broad-spectrum antibacterial, antibiofilm, antioxidant, and anticancer capabilities of zinc oxide nanoparticles (ZnO NPs) have made them a potential choice among various nanomaterials (*Raghunath & Perumal, 2017*; *Sirelkhatim et al., 2015*). However, the use of toxic chemicals, high energy input, and toxic by products in traditional ZnO NP synthesis processes raises concerns about the environment and human health (*Kumar et al., 2020*; *Rajendran et al., 2018*). Plant-mediated or "green" production of ZnO NPs has drawn more interest as a sustainable substitute since it provides a biocompatible, economical, and environmentally acceptable method (*Sharma et al., 2019*; *Iravani, 2011*).

The Asteraceae family's very invasive plant Parthenium hysterophorus has been the subject of much research due to its phytochemical characteristics and potential uses in medicine. Alkaloids, flavonoids, phenolics, and terpenoids are among the many bioactive substances found in *P. hysterophorus*, despite its reputation as an aggressive weed. These compounds have antibacterial, antioxidant, and anti-inflammatory qualities (*Aditya et al., 2021*; *Sinha et al., 2014*). By serving as stabilising and reducing agents, these phytochemicals make it easier to synthesise biocompatible ZnO NPs with increased biological activity (*Khalil et al., 2017*; *Singh et al., 2018*). However, although research has examined the synthesis of ZnO NPs from a variety of plant sources, little is known about the environmentally friendly synthesis of ZnO NPs utilising *P. hysterophorus* flower extracts, especially when it comes to antibacterial and antibiofilm applications(*Gopalakrishnan et al., 2021*; *Agarwal et al., 2018*).

Because of their strong resistance to traditional antibiotics, biofilms—structured microbial populations encased in an extracellular polymeric substance—present a serious problem in both medical and industrial settings (*Hall-Stoodley et al., 2004*;

Koo et al., 2017). Over a million fatalities are caused by antimicrobial resistance (AMR), which is a serious threat to global health and calls for the creation of new antimicrobial drugs (*Murray et al., 2022*; *WHO, 2021*). By breaking down bacterial adhesion, entering biofilm structures, and producing reactive oxygen species (ROS) that worsen bacterial cell death, ZnO NPs have shown strong antibiofilm action (*Azam et al., 2012*; *Padmavathy & Vijayaraghavan, 2008*).

The objective of this study is to examine the antibacterial and antibiofilm characteristics of ZnO NPs and synthesise them using a green, ecologically acceptable method employing *P. hysterophorus* flower extract. In the fight against drug-resistant illnesses, this work advances sustainable antimicrobial compounds by combining plant-based nanotechnology with biomedical research, potentially providing an alternative to traditional antibiotics.

2. Material and Methods:

2.1 Materials :

Parthenium hysterophorus (flowers), Zinc sulfate (1mM), double distilled water, Whatman Filter paper, Ethanol.

2.2 Methods:

2.2.1 Sample collection**:**

The floral extract of *Parthenium hysterophorus* was obtained from plants collected in Nagthane, Satara District, Maharashtra, India.

2.2.2 Extraction:

For extraction of sample Decoction method was used. To do this, 10 g of washed flowers were soaked in 100 ml of double-distilled water at 60°C for 15 minutes. The mixture was then filtered using Whatman filter paper and stored at 4°C for future use *(Datta et al., 2017; Iqbal et al., 2022)*.

2.3 Synthesis of Nanoparticles**:**

A floral extract was mixed with a 1 mm zinc sulfate solution in a 9:1 ratio and incubated for 24 hours to form nanoparticles. A colour change indicated the presence of zinc oxide nanoparticles *(Leyu et al., 2023, Kumar et al., 2014)*.

2.4 Characterization of Nanoparticles:

For confirmation of ZnO NPs characterization of Nanoparticles was conducted by using Analytical techniques such as UV-Visible Spectroscopy for understanding optical properties showing absorption at specific wavelengths. Fourier Transform Infrared Spectroscopy (FTIR) This technique identifies functional groups in nanoparticles by detecting characteristic peaks. X-ray Diffraction (XRD) measures and identifies the crystalline forms of nanoparticles by analysing diffraction angles. The crystalline size of ZnO NPs was calculated using the Debye-Scherrer equation. Surface morphology of nanoparticles was observed using Scanning Electron Microscopy (SEM). These methods made it possible to examine the morphological, structural, and chemical characteristics of the powdered calcined eggshell, offering important new information about its composition and development of nanoparticles of zinc oxide.

2.5 Antimicrobial assay :

Well diffusion method was used for antibacterial assay and it is carried out by using Sterile MH agar. Different concentrations of *P. hysterophorus* ZnO NPs was prepared by dissolving in water and were poured in wells on sterile MH agar containing 0.1 ml bacterial suspension. These plates were incubated for 24hrs at 37°C. The experiment was replicated in triplicates. Similarly, Antifungal assay targeting *Candida albicans* were performed using the same procedure *(Archana et al., 2021)*. After incubation the zone of inhibition diameter measurements were made around the each well. For comparison with reference standard the same procedure was replicated with standard drug as test compounds that is Streptomycin and Ketoconazole for Antibacterial and Antifungal assay respectively.

2.6 Antibiofilm assay :

The ability of bacteria to form biofilms was tested using a modified method from O'Toole and Kolter (1998). In a sterile 96-well plate, 100 µl of nutrient broth and 50 µl of fresh bacterial suspension were added to each well. For the standard test, 100 µl of streptomycin (1 mg/ml) was used. Test wells received 20 to 100 µg/ml of the compound. After incubating at 37°C for 24 hours, the contents were removed, and the wells were washed with 200 µl of sterile saline to eliminate free-floating bacteria. The remaining biofilms were stained with 0.1% crystal violet for 20 minutes at room temperature. Excess stain was washed off with deionized water, and the plates were fixed with 200 µl of 96% ethanol. The optical density of the stained biofilms was measured at 630 nm using an ELISA microplate reader (*Sahar et.al., 2020*).

3. Result:

3.1 ZnO Nanoparticle Synthesis:

The green synthesis of ZnO nanoparticles using *Parthenium hysterophorus* flower extract involves employing bioactive compounds in the plant as natural reducing and stabilizing agents *(Ansam et al., 2022)*. The process typically begins with preparing the floral extract, which is then mixed with a zinc precursor (e.g., zinc sulphate) under controlled conditions to facilitate nanoparticle formation. Refer to Figure 5.1 which displays Zinc oxide (ZnO) nanoparticles powder after process. ZnO is an inorganic compound which occurs rarely in nature. It is generally found in crystalline form. The synthesis of ZnO NPs was indicated by a colour change in the plant extract. Over time, the reaction mixture changed from yellow to brown.

3.2 Characterization of ZnO nanoparticle:

3.2.1 U.V. Visible Spectroscopy :

The UV absorption spectrum of the synthesized zinc oxide nanoparticles (ZnO NPs) ranged from 250 to 600 nm, with the highest absorption observed at 330 nm (*Sahar et.al., 2020*). Figure 2. Shows the absorption spectrum of ZnO NPs of *Parthenium hysterophorus.*

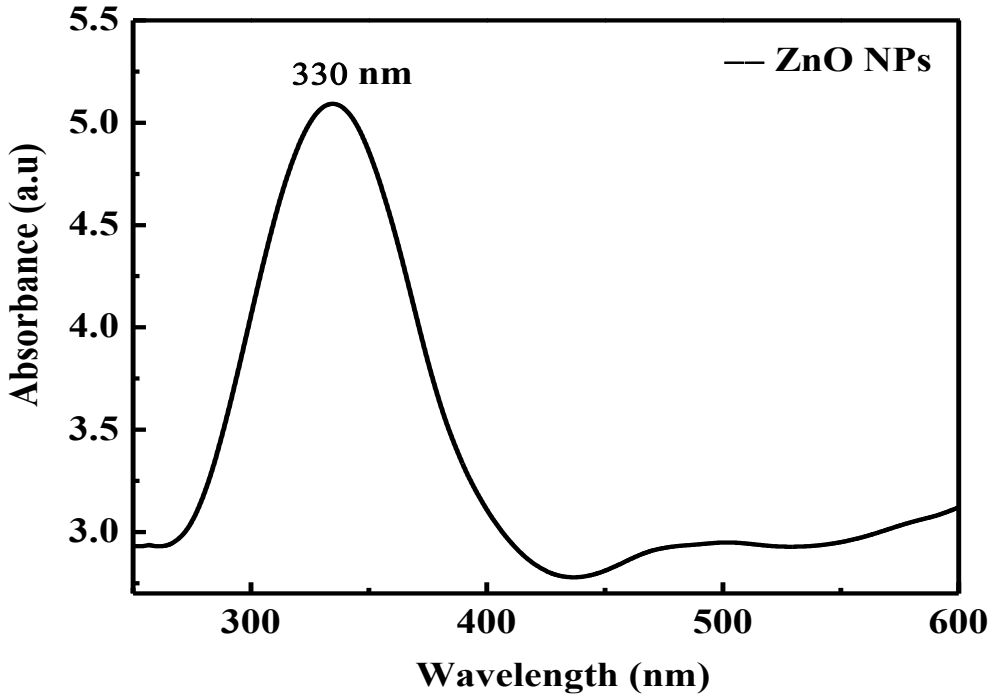


Fig 3.2.1 UV visible spectrum of PH-ZnO NPs.

3.2.2 Fourier Transform Infrared Spectroscopy (FTIR):

The FTIR analysis of *Parthenium hysterophorus* floral extract identified key functional groups, including O-H stretching (alcohols or phenols) at 3299 cm^{-1}, C=C stretching (aromatic compounds) at 1634 cm^{-1}, C-H bending (alkanes or alkyl groups) at 1380 cm^{-1}, and C-O stretching (ethers or esters) at 1112 cm^{-1}. For ZnO nanoparticles synthesized from the floral extract, similar functional groups were observed with slight shifts, such as O-H stretching at 3320 cm^{-1}, C-H stretching at 2929 cm^{-1}, C=C stretching at 1601 cm^{-1}, C-H bending at 1380 cm^{-1}, and C-O stretching at 1103 cm^{-1}. Additionally, a new peak at 543 cm^{-1} was identified, corresponding to Zn-O stretching, confirming the formation of ZnO nanoparticles. The shifts in peaks indicate interactions between the extract's biomolecules and ZnO during nanoparticle synthesis *(Dennis et al., 2024)*.

Figure 3. Shows The IR spectra of *Parthenium hysterophorus* aqueous extract and ZnO nanoparticles were compared using infrared spectroscopy.

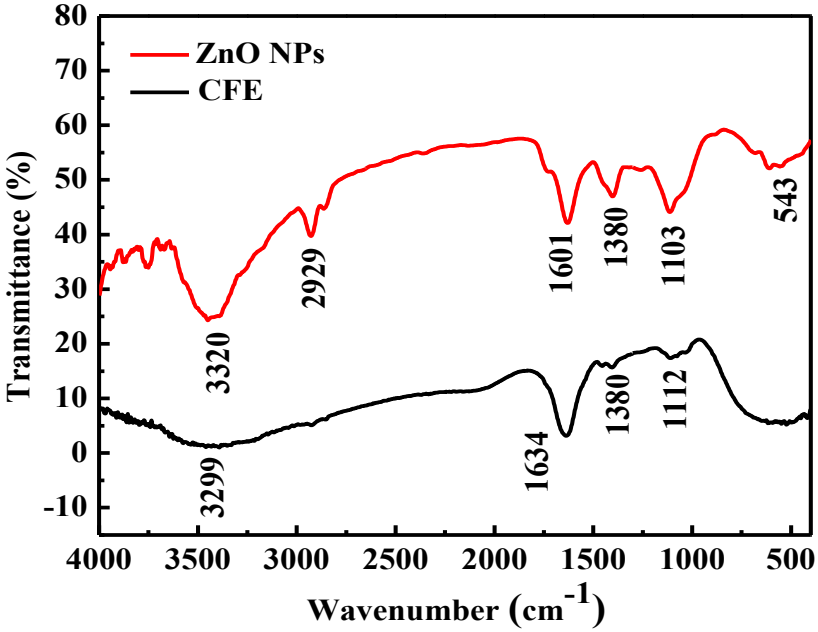


Figure 3.2.2 FTIR spectrum of PH-ZnO NPs

3.2.3 X-ray diffraction (XRD):

The XRD pattern of ZnO nanoparticles (ZnO NPs) matched the Miller indices and reference data for ZnO NPs, indicating a polycrystalline structure. The nanoparticles, synthesized using the aqueous floral extract of *Parthenium hysterophorus*, were consistent with the JCPDS reference card (01-079-0208). The XRD peaks appeared at 30.56, 34.44, and 36.42, corresponding to the Miller indices (100), (002), and (101), respectively. The crystallite size was calculated using the Debye-Scherrer formula:

$$D = \lambda / \beta \cos\theta$$

Here, k=0.9 (constant), λ=1.54 A° (Cu Kα radiation wavelength), β is the peak's FWHM (in radians), θ is the diffraction angle, and D is the crystal size. The crystallite size of ZnO NPs was calculated to be 16.60 nm *(Ahmed et al., 2021)*.

Figure 4. Shows the crystalline structure of synthesized ZnO NPs, illustrated using measured XRD diffraction patterns.

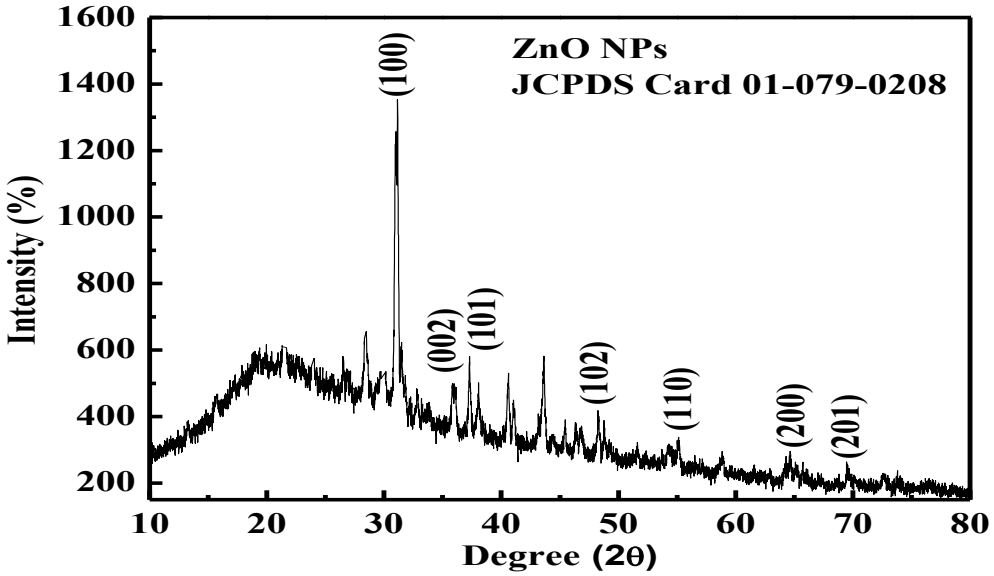


Fig 3.2.3: XRD spectrum of PH- ZnO NPs.

3.2.4 Scanning Electron Microscopy (SEM):

Figure 3.2.4. Shows the morphology of prepared ZnO NPs of *Parthenium hysterophorus* floral extract.

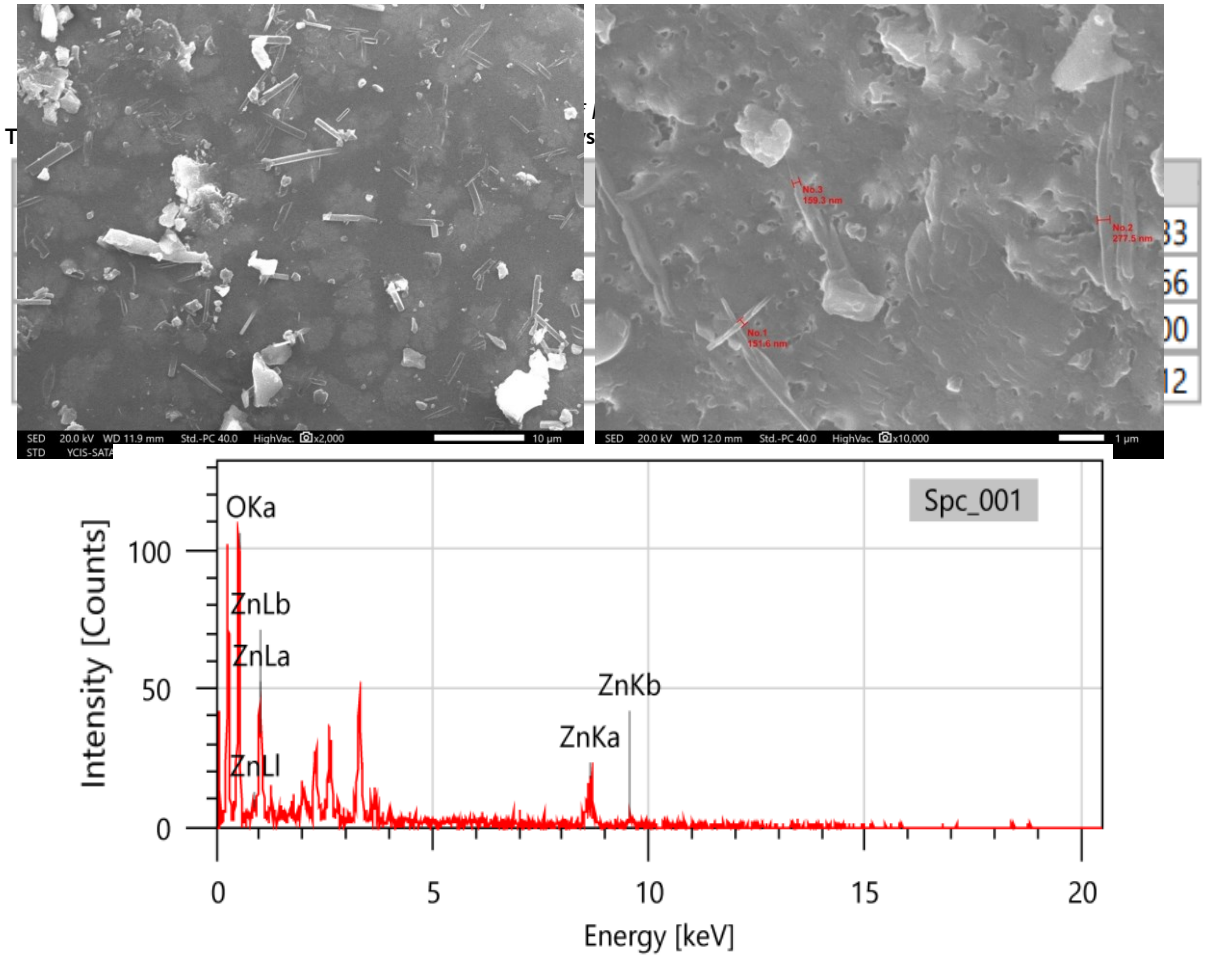


Fig 3.2.5 EDS spectrum of PH- ZnO NPs at 20.0 keV.

As seen in Fig.3.2.4. ZnO NPs exhibit in spherical and rod in shape. Sample was coated with a 4 nm gold layer to avoid charging effects during analysis. The nanoparticles have a relatively smooth surface, with some rough patches or facets visible. They show slight agglomeration, a common occurrence in dry powder form. Their sizes range from 50 nm to 200 nm, and they display diverse shapes, including spherical and rod-like structures. The size distribution is non-uniform, with noticeable variation in particle sizes *(Ansam et al., 2022)*. EDS (Energy dispersive spectroscopy) gives confirmation of the desirable groups are present in Nanoparticles that are zinc (Zn) & oxygen (O).

3.3 Antimicrobial Assay:

3.3.1 Antibacterial and antifungal activity:

Antimicrobial assays, involving antibacterial and antifungal evaluations, were conducted to assess the antimicrobial efficacy of PH- ZnO nanoparticles. The plates were subjected to examination to determine the zone of inhibition after 24 hours of incubation.

Figures 3.3 series and Table 2 illustrate bacterial and fungal candidates alongside the observed inhibition zones resulting from treatment with ZnO nanoparticles. Among these, the PH-ZnO NP displayed the t activity against *and S.aureus*, *B.subtilis*, *P.vulgaris* and *C.albicans* shows the zone of inhibition at 250 mm, 500 mm, 750 mm, 1000 mm concentration.

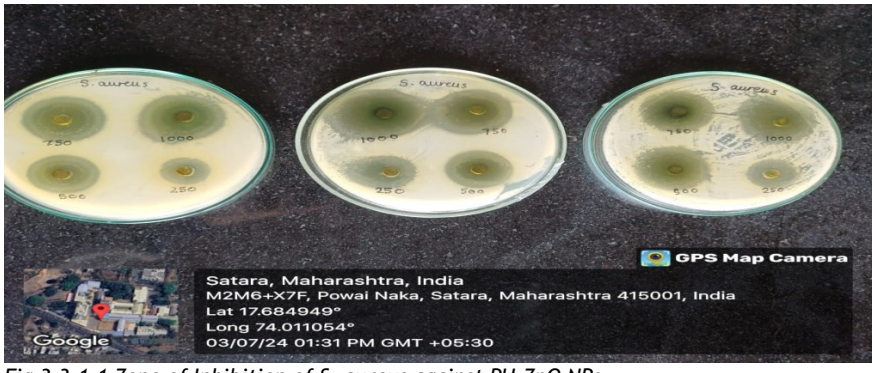


Fig 3.3.1.1 Zone of Inhibition of S. aureus against PH-ZnO NPs.

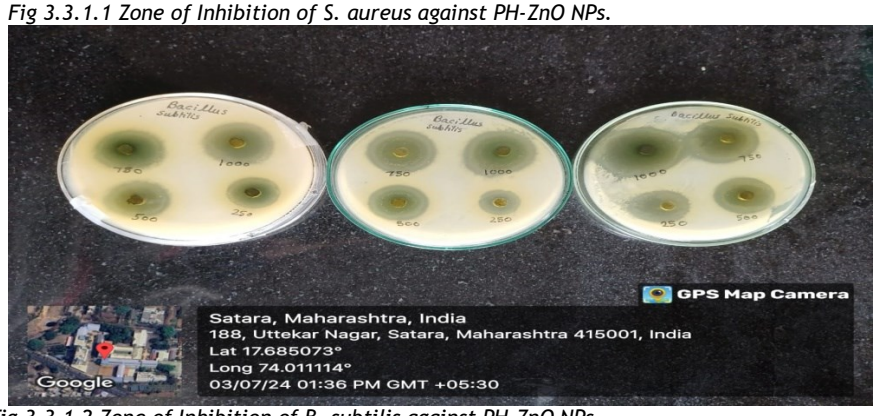


Fig 3.3.1.2 Zone of Inhibition of B. subtilis against PH-ZnO NPs.

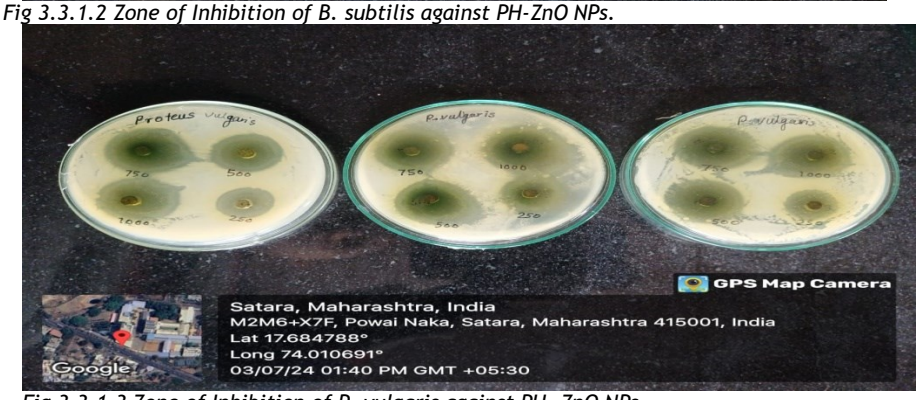


Fig 3.3.1.3 Zone of Inhibition of P .vulgaris against PH- ZnO NPs.

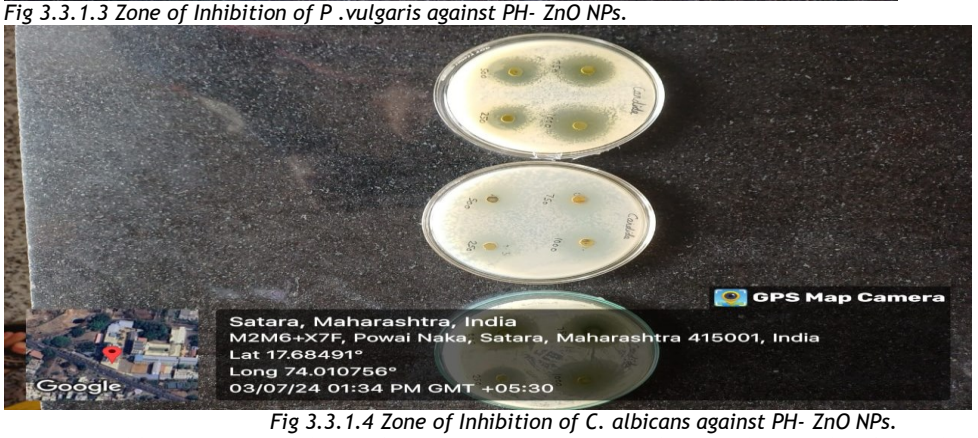


Fig 3.3.1.4 Zone of Inhibition of C. albicans against PH- ZnO NPs.

Table 2: Zone of inhibition due to PH -ZnO NPs against different bacterial and fungal candidates.

Gram Positive *Staphylococcus aureus*

250	6	8	8	7.333333333
500	9	13	10	10.66666667
750	10	12	11	11
1000	15	17	18	16.66666667

Gram Positive *Bacillus subtilis*				
250	7	9	7	7.666666667
500	9	11	9	9.666666667
750	11	13	13	12.33333333
1000	12	12	14	12.66666667

Gram Negative *Proteus vulgaris*				
250	7	9	6	7.333333333
500	11	12	10	11
750	13	14	14	13.66666667
1000	13	15	14	14

Gram Negative *Candida albicans*				
250	7	9	6	7.333333333
500	11	12	10	11
750	13	14	14	13.66666667
1000	13	15	14	14

Note- The standard drugs used for bacterial and fungal candidates were Streptomycin and ketoconazole respectively.

3.4 Antibiofilm assay:

An antibiofilm assay targeting three organisms, namely *S. aureus*, *P. aeruginosa* and *C. albicans*, was conducted. Tables 3, 4 and 5, along with Figures respectively, present the IC-50 values and the inhibitory impact of PH-ZnO nanoparticles against the aforementioned organisms, compared with those of standard drugs for comparison. The PH-ZnO NPs showed good antibiofilm activity against bacterial candidates, with 84.59 and 89.07 as IC50 values for *S.aureus* and *P.aeruginosa* respectively. Consequently, the standard drug Streptomycin displayed an IC50 value of 80.12 for both bacterial subjects. On the contrary, for yeast candidate (*Candida albicans)* IC50 values were 99.25 and 71.12 for standard drug miconazole and ZnO NPs respectively.

3.4.2.1 Antibiofilm assay of ZnO NPs against *S.aureus*.

Fig 3.4.2.1 Antibiofilm assay of ZnO NPs against S.aureus.

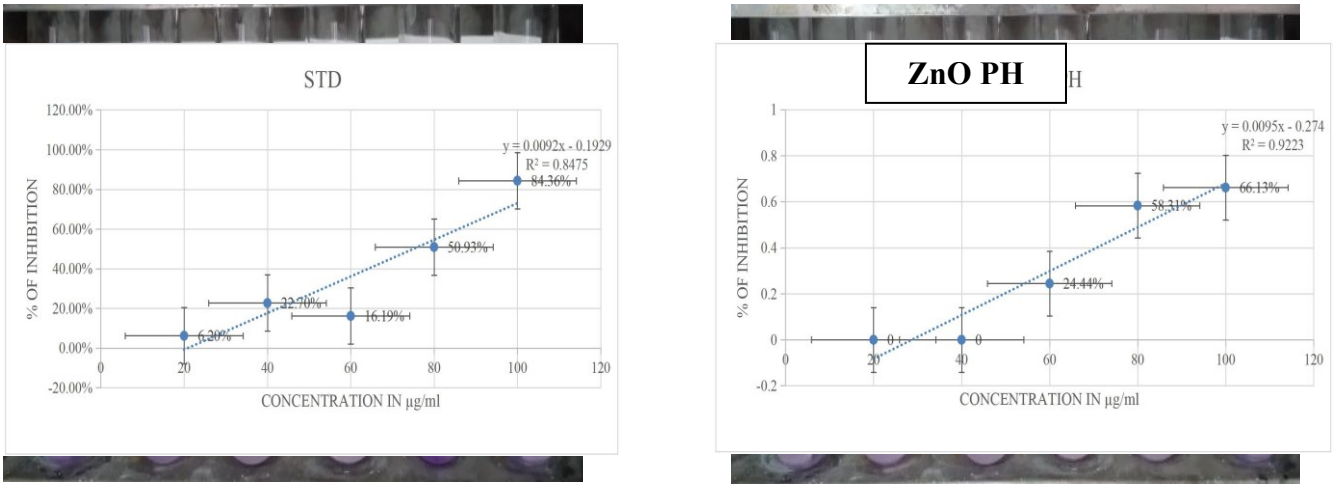


Fig 3.4.2.2 Graphical representation of percent of inhibition (IC50) of S.aureus biofilm formation by standard drug and ZnO NPs.

Table No. 03- Effects of compound against *S. aureus* after staining withCrystal Violet

SR NO	SAMPLE CODE	Concentration(µg/ml)	Absorbance				% Of Inhibition	IC50 (µg/ml)
			Test 1	Test 2	Test 3	Mean		

1	Control		2.303	2.303	2.303	**2.303**	-	-
2	Standard	20	2.16	2.18	2.14	**2.16**	**6.20%**	**80.12**
	(Streptom-ycin)	40	1.78	1.80	1.78	**1.78**	**22.70%**	
		60	1.93	1.95	1.91	**1.93**	**16.19%**	
		80	1.13	1.13	1.13	**1.13**	**50.93%**	
		100	0.37	0.35	0.38	**0.36**	**84.36%**	
3	ZnO-PH	20	-	-	-	**-**	**-**	**84.59**
		40	-	-	-	**-**	**-**	
		60	1.75	1.76	1.73	**1.74**	**24.44%**	
		80	0.96	0.98	0.94	**0.96**	**58.31%**	
		100	0.78	0.80	0.76	**0.78**	**66.13%**	

Note: ABS- Absorbance, Conc - Concentration.

3.4.2.2 Antibiofilm assay of ZnO NPs against *P.aeruginosa*.

Fig 3.4.2.3 Antibiofilm assay of ZnO NPs against P. aeruginosa.

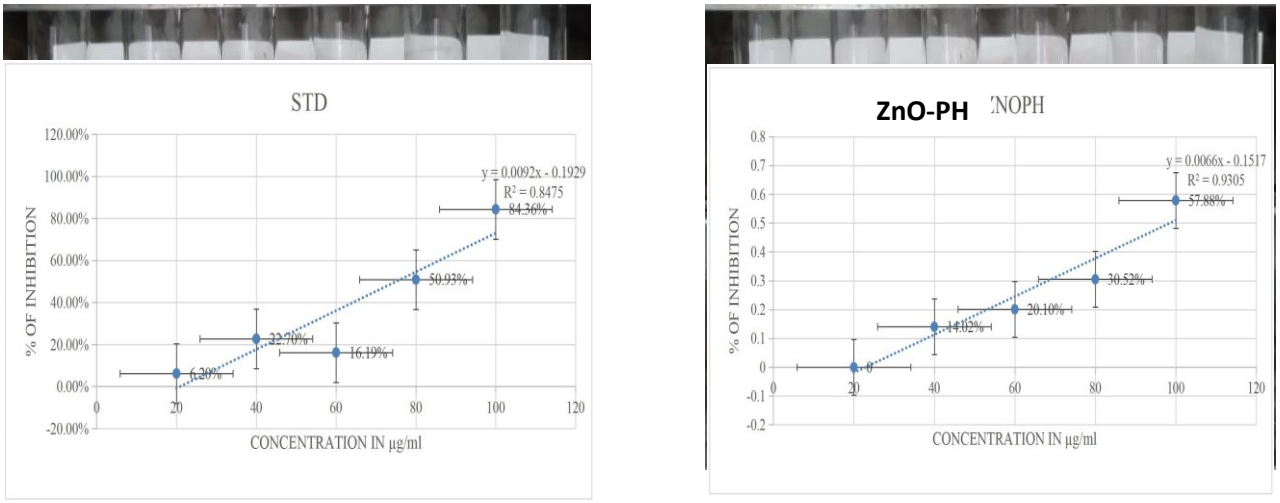


Fig 3.4.2.4 Graphical representation of percent of inhibition (IC50) of P.aeruginosa biofilm formation by standard drug and ZnO NPs.

Table No. 04- Effects of compound against *P.aeruginosa* after staining withCrystal Violet

SR NO	SAMPLECODE	Concentration(µg/ml)	Absorbance				% Of Inhibition	IC50 (µg/ml)
			Test 1	Test 2	Test 3	Mean		
1	Control		2.303	2.303	2.303	**2.303**	-	-
2	Standard	20	2.16	2.18	2.14	**2.16**	**6.20%**	**80.12**
	(Streptomy-cin)	40	1.78	1.80	1.78	**1.78**	**22.70%**	
		60	1.93	1.95	1.91	**1.93**	**16.19%**	
		80	1.13	1.13	1.13	**1.13**	**50.93%**	
		100	0.37	0.35	0.38	**0.36**	**84.36%**	
4	ZnO-PH	20	-	-	-	**-**	**-**	**89.07**
		40	1.98	1.97	1.98	**1.98**	**14.02%**	

		60	1.84	1.85	1.84	**1.84**	**20.10%**	
		80	1.60	1.61	1.60	**1.60**	**30.52%**	
		100	0.97	0.97	0.97	**0.97**	**57.88%**	

3.4.2.3 Antibiofilm assay of ZnO NPs against *C.albicans:*

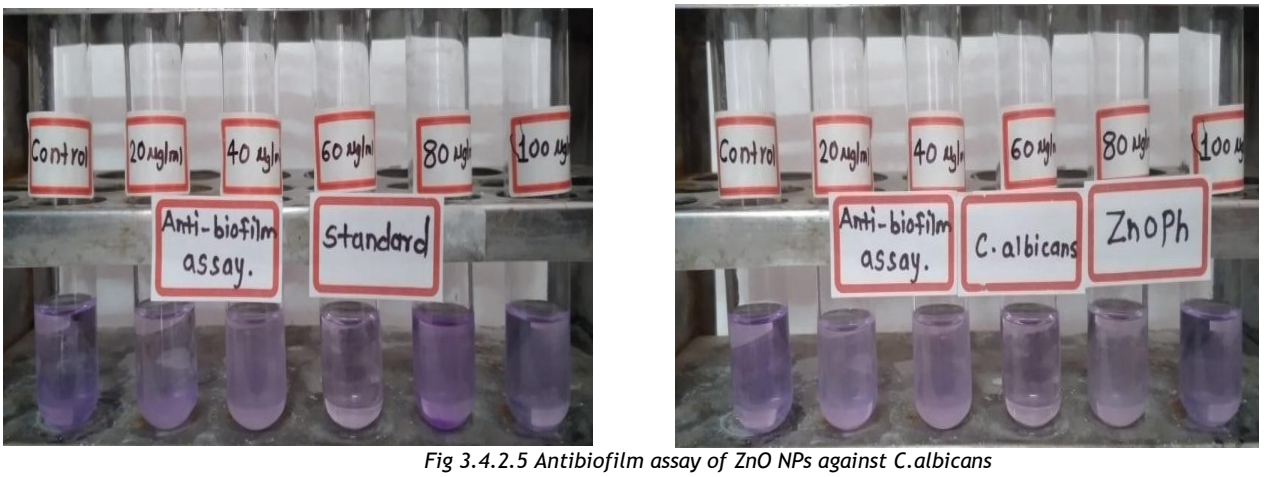
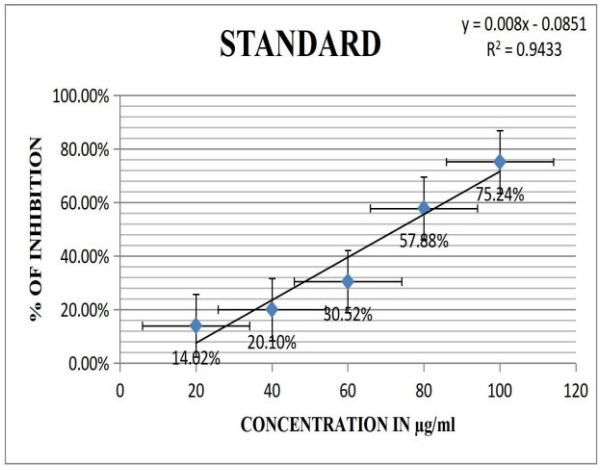


Fig 3.4.2.5 Antibiofilm assay of ZnO NPs against C.albicans



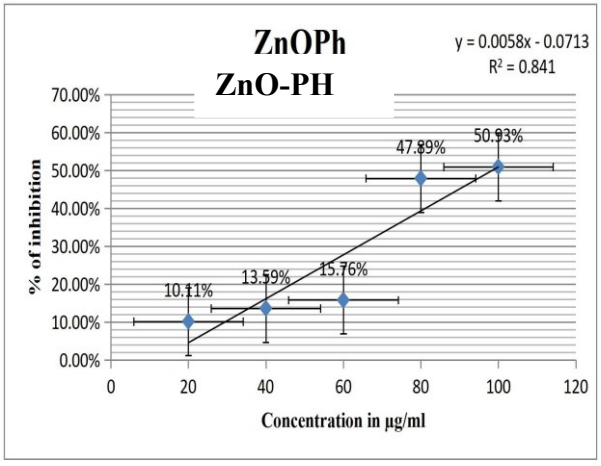


Fig 3.4.2.6 Graphical representation of percent of inhibition (IC50) of C.albicans biofilm formation by standard drug and ZnO NPs.

Table No. 05- Effects of compound against *C. albicans* after staining with Crystal Violet

SR NO	SAMPLE CODE	Concentration(µg/ml)	Absorbance				% Of Inhibition	IC50 (µg/ml)
			Test 1	Test 2	Test 3	Mean		
1	Control		2.303	2.303	2.303	**2.303**	-	-
2	Standard	20	1.98	1.97	1.98	**1.98**	**14.02%**	
	(Miconazol -e)	40	1.84	1.85	1.84	**1.84**	**20.10%**	**71.12**
		60	1.60	1.61	1.60	**1.60**	**30.52%**	
		80	0.97	0.97	0.97	**0.97**	**57.88%**	
		100	0.57	0.56	0.57	**0.57**	**75.24%**	

4	ZnO-PH	20	2.07	2.08	2.07	**2.07**	**10.11%**	**99.25**
		40	1.99	1.99	1.99	**1.99**	**13.59%**	
		60	1.94	1.95	1.94	**1.94**	**15.76%**	
		80	1.20	1.21	1.20	**1.20**	**47.89%**	
		100	1.13	1.13	1.13	**1.13**	**50.93%**	

DISCUSSION

According to (Minha et.al., 2020). Metals like silver (Ag), copper (Cu), and gold (Au) are commonly used for synthesizing nanoparticles with plant extracts, but their high toxicity to humans and animals limits their medical applications. In contrast, zinc oxide (ZnO) nanoparticles are considered safe (GRAS) and have low toxicity. Their size-dependent properties make ZnO NPs more suitable for various applications compared to other metal nanoparticles. The synthesized ZnO nanoparticles (ZnO-NPs) showed UV absorption in the range of 250–600 nm, with maximum absorption at 330 nm. According to (Dennis et al., 2024). FTIR analysis confirmed key functional groups such as O-H, C=C, C-H, and C-O in both *Parthenium hysterophorus* floral extract and the synthesized ZnO-NPs, with a new Zn-O stretching peak at 543 cm^{-1} indicating nanoparticle formation. XRD analysis revealed a polycrystalline spherical rod and irregular structure consistent with the JCPDS reference card (01-079-0208), with peaks at 30.56°, 34.44°, and 36.42° corresponding to Miller indices (100), (002), and (101) which shows similarity to (Iqbal et al., 2022). The crystallite size was calculated as 16.60 nm using the Debye-Scherrer formula. The nonuniform size distribution highlights the structural variety of the nanoparticles. These results, showcasing well-defined shapes and manageable agglomeration, suggest superior quality and adaptability of our ZnO NPs compared to others. These results demonstrate the successful synthesis of high-quality ZnO-NPs, with better crystallinity, smaller size, and efficient biomolecule interactions compared to other nanoparticles, enhancing their potential for biomedical and environmental applications.

This study showed that the tested nanoparticles had strong antibiofilm activity against *S. aureus* and *P. aeruginosa*. Unlike the control group, the nanoparticle-treated group showed degraded cell masses and weakened biofilms. At higher doses, the nanoparticles significantly inhibited biofilm formation, with 89.07% inhibition for *P. aeruginosa* and 84.59% for *S. aureus* these results co-ordinate with (Sahar et.al., 2020). These results suggest that these nanoparticles are highly effective and may outperform other nanoparticles in antibiofilm activity.

CONCLUSION

In conclusion, the synthesis of Zinc oxide nanoparticles (ZnO NPs) derived from *Parthenium hysterophorus* have garnered significant attention for their potent antimicrobial activity. As antibacterial agents, ZnO NPs are effective against a wide range of pathogens, including both Gram-positive and Gram-negative bacteria. Their mechanisms include disrupting bacterial membranes, generating reactive oxygen species (ROS), and interfering with microbial proteins and genetic material. These nanoparticles also demonstrate strong antifungal activity by destabilizing fungal cell walls and inducing oxidative stress, which makes them versatile tools in combating various infections. Enhanced versions, such as ZnO-Ag nanoparticles, have shown even greater efficacy, working effectively at lower concentrations.

The research demonstrated that the extract possesses notable antimicrobial activity against various pathogens, potentially attributed to its rich phytochemical composition, which interferes with microbial growth and biofilm formation. These eco-friendly and cost-effective approaches yield biocompatible nanoparticles suitable for medical use. With their dual functionality and sustainable production methods, ZnO NPs are poised to revolutionize both antimicrobial treatments and therapies in the future.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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- Phytochemical Investigation, Antioxidant Properties and In Vivo Evaluation of the Toxic Effects of Parthenium hysterophorus By Javed Iqbal 1,Ayaz Ali Khan 1,*ORCID,Tariq Aziz 2,*ORCID,Waqar Ali 1ORCID,Saeed Ahmad 3,Shafiq Ur Rahman 4,Zafar Iqbal 5,Anas S. Dablool 6ORCID,Mashael W. Alruways 7ORCID,Abdulraheem Ali Almalki 8,Abdulhakeem S. Alamri 8ORCID andMajid Alhomrani 8
- .Allelochemicals in Parthenium in response to biological activity and the environment D.K. PandeyPhysiology Section, Directorate of Weed Science Research, Maharajpur, Jabalpur (Madhya Pradesh) E-mail : daya-pandey@hotmail.com
- Antimicrobial, Antioxidant and Cytotoxic Activity of Green Synthesized Copper Nanoparticle of Parthenium Hysterophorus L.Archana Rai1, Rohit Lall21,2 Department of Molecular and Cellular Engineering, Jacob School of Biotechnology and Bioengineering,Sam Higginbottom University of Agriculture, Technology & Sciences, Allahabad, India.
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