

Phytochemical Analysis, Green Synthesis of Zinc Oxide Nanoparticles Using *Hygrophila Auriculata* leaf extract and Evaluation of Antioxidant, Anti-inflammatory activities

Yash N. Dange¹, Sumangal S. Kale¹, Vijay L. Gurav², Manoj N. Lad², Yogesh A. Pawar¹, Udaysinha C. Patil¹, Dadaso B. Shinde¹, Pravin N. Chavan³, Paresh M. Dhuri^{1*}

Department of Chemistry, Shri Pancham Khemraj Mahavidyalaya, Sawantwadi, 416510, Sindhudurg, Maharashtra, India^{1*}.

Department of Chemistry, Dapoli Urban Bank Senior Science College, Dapoli, 415712, Ratnagiri, Maharashtra, India².

Department of Chemistry, Doshi Vakil Arts College and G.C.U.B. Science and Commerce, Goregaon, 402103, Raigad, Maharashtra, India³.

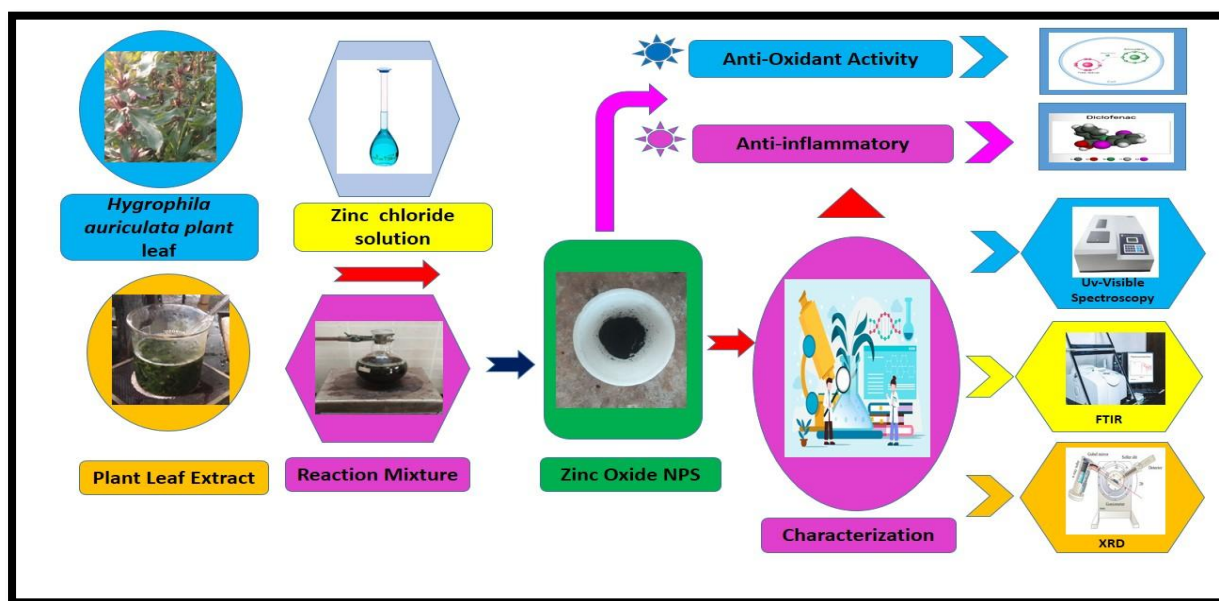
Corresponding author: -

Paresh M. Dhuri^{1*}: - pareshdhuri6642@gmail.com

Abstract

A viable substitute for the conventional chemical synthesis approach is the green synthesis of metal oxide nanoparticles using plant extract. In this work, we describe the biological production of nanostructured zinc oxide particles. Zinc chloride and *Hygrophila auriculata* leaf extract are used to create macro-sized, highly stable zinc oxide nanoparticles. X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and UV-Vis absorption spectroscopy has all confirmed the formation of zinc oxide nanoparticles. The XRD shows size of 16nm which are similar to hexagonal wurtzite structure. FTIR revealed many phytochemicals present in the leaf extract of the *Hygrophila auriculata* plant. 297nm absorption peak has been shows by UV-Vi's. Anti-inflammatory activity was checked and it shows IC₅₀ 88.65 µg/ml. Also, when checked for antioxidant property it shows as comparable moderate inhibition as 94.56 µg/ml. This shows that the resulting zinc oxide nanoparticles were one of the best candidates for this action and possessed antioxidant activity.

Keywords: Green Synthesis, ZnONPs, *Hygrophila Auriculata*, Phytochemical, Anti-oxidant, Anti-Inflammatory.



Graphical abstract

1 Introduction

Nanoparticles, which are atomic or molecular aggregates with a size of less than 100 nm, are the subject of nanotechnology. These are essentially modified forms of basic elements that have had their atomic and molecular properties changed[1]. Because of their distinct chemical and physical characteristics, as well as their high surface area to volume ratio, Nano size inorganic compounds have demonstrated extraordinary antibacterial activity at very low concentrations. Furthermore, at high temperatures and pressures, these particles are more stable. Some of them are known to be harmless and even include mineral elements that are essential to human health[2, 3]. Numerous technologies and industries, including optoelectronics, piezoelectric and magnetic sensors, bio diagnosis, biological labeling, ceramic and rubber processing, environmental protection, biology and the medical field, have employed ZnONPs, which can display a wide range of nanostructures and are thought to be bio safe, nontoxic, and biocompatible[4]. Nanostructured ZnO particles have several advantages over other metal nanoparticles, including reduced cost, UV blocking capabilities, high catalytic activity, huge surface area, white appearance, and impressive uses in agriculture and medicine[5]. Because of its exceptional UV absorption capabilities, zinc oxide (ZnO) is frequently used in personal care products as sunscreens and cosmetics, among other things. ZnO nanoparticles offer remarkable antimicrobial, antibacterial and UV-blocking qualities. Consequently, completed textiles that use ZnONPs in the textile sector also in deodorant[6].

The biological components of plants are typically used to create biosynthesized nanoparticles from plant extracts. Metal-based nanoparticles are frequently synthesized from plant parts, such as leaves, roots, stems and seeds. Additionally, bioactive metabolites were found in plant extracts which are important in lowering and for stabilizing metallic ions[7]. *Hygrophila articulate* also called *Asteracantha longifolia* and *Hygrophila spinosa* and is an upright annual plant that grows all over India, particularly in swampy plains regions. The plant was described as *ikshura*, *ikshagandha* and *kokilasha* indicating that it resembles an Indian cuckoo's eyes[8]. For instance, many other plants have all been employed by researchers[9]. This plant, which belongs to the Ayurvedic class of Vrishya and is used to improve sexual performance. It can also be used to treat blood-related diseases, liver problems and swelling.[10]

2. Material and Methods

2.1 Material

Fresh *Hygrophila auriculate* were collected from the local area of (insuli) Sindhudurg region (western ghat), Maharashtra[11]. Purity 98.5 % Zinc Chloride were purchased from Loba chemie PVT-LTD. Jahangir Villa 107, Wodehouse Rd., Colaba Mumbai (India).Methanol, 2-2- diiphenyl -1 picrylhydrazyl (DPPH) and All reagent and chemical were used as received with no further purification.[12]

2.2 Preparation of *Hygrophila auriculate* Plant Extract

To get rid of dirt particles, the new *Hygrophila auriculate plant* leaves was repeatedly cleaned with deionized water[13]. 20 grams o *Hygrophila auriculate plant* leaves Stir the plant thoroughly in 400 milliliters of deionized water, boil it for 60 minutes and then let it cool to room temperature. Store the filtrate in the refrigerator for a night after filtering using regular filter paper[14].

2.3 Phytochemical Studies

The extract was used to identify the different phytochemicals that were present, as determined by the test mentioned below, which plays a major part in numerous therapeutic applications[10],[8, 15].

1. Alkaloids: - Extract plus one or two drops of Wagner's reagent (along the test tube's side). If a reddish-brown precipitate appears, then it is present.
2. Flavonoids: Extract with two to three drops of NaOH solution, followed by a few drops of dil. HCl. If an intense yellow color develops then is present.
3. Phenolic: 1% FeCl₃ solution plus extract. If a reddish colour appears, it is present.
4. Tannins: 2 ml of 5% FeCl₃ solution plus extract. It might be there if it has a dark green colour.
5. Terpenoids: - Extract + 1 ml chloroform + 1-2 ml concentrated H₂SO₄ on the side. If the layer contact is golden yellow or reddish-brown. It might be there.
6. Saponins: Shake the extract with 2ml of distilled water. If it appears to be 1 cm Foam layer, then it might exist.
7. Steroid: - Extract + 1 ml chloroform + 1-2 ml concentrated H₂SO₄ along the test tube's side. It could appear if it has a reddish-brown colour.
8. Cardiac glycosides: - Extract + 2 ml glacial acetic acid + 1-2 drops FeCl₃ + 1 ml concentrated H₂SO₄ along the test tube's side. It might be there if the interface displays a brown ring.
9. Coumarin - 3 ml of 10% NaOH plus extract. The presence of the test is shown if a yellow color is formed.
10. Proteins: - Add a few drops of NaOH + 1 ml of concentrated HNO₃, heat gradually, and cool. If the yellow color turns orange, it might be there.

2.4 Green Synthesis of Zinc Oxide Nanoparticle (HA-ZnONPs)

A 500 ml round-bottom flask was filled with 100 ml of an aqueous extract of *Hygrophila auriculata* Leaf Extract in order to synthesize ZnONPs. After that, 100 milliliters of a 0.1 M Zinc Chloride solution were added drop wise and well mixed for ten minutes using a magnetic stirrer[16]. The production of ZnONPs causes the reaction mixture solution's deep green color to shift to a deep brown. The mixture deep dark color dropped to laboratory temperature for one night under constant magnetic stirring for five hours. The resulting semi-solid paste was then placed into watch glass after centrifuging at 10,000 rpm for ten minutes [7]. After Five hours of drying in an oven at 100°C, a black powder was produced. This obtained powder material was placed into silica crucible for heating in muffle furnace at 450°C for five hour consequently black color develop means nano powder material achieved. It was used for further analysis[17].

3. Characterization

3.1 X-ray Diffraction (XRD) Analysis

An X-ray diffractometer (XRD-Ultra IV diffractometer, Japan) using Cu K α radiation ($\lambda = 1.5406 \text{ \AA}$) at 40 kV was used to determine the crystalline structure and phase purity of the produced HA-ZnONPs. The 2θ range of 05° to 90° was used to record the diffraction patterns. [18] The Debye-Scherrer equation, $D = (0.9\lambda) / (\beta \cos\theta)$, was used to get the average crystallite size. Where θ is the Bragg diffraction angle, β is the full width at half maximum (FWHM) of the diffraction peak in radians, λ is the X-ray wavelength ($1.5406 \text{ \AA}$), and D is the average crystallite size[12].

3.2 FTIR

The functional groups found in the leaf extract of *Hygrophila Auriculata* and those responsible for the stabilization and reduction of HA-ZnONPs were identified using FTIR analysis[7]. A Bruker Alpha FTIR spectrometer (Germany) was used to record the FTIR spectra. has a resolution of 4 cm⁻¹ in the 4000-400 cm⁻¹ range. The KBr pellet technique was used to prepare the samples[19].

3.3 UV-Visible studies

A UV-Visible spectrophotometer (Shimadzu UV-1800, Japan) was used to examine the formation and optical characteristics of the produced HA-ZnONPs[20]. Using pure water as a blank, the absorption spectra were captured in the 200–800 nm wavelength range. To guarantee that results fell within the spectrophotometers linear range, samples were suitably diluted[21].

4 Bio-Medical activity

4.1 Anti-Oxidant Assay

DPPH free radicals were used to measure the antioxidant activity of test compounds for their ability to scavenge free radicals. Test tubes were filled with 1 ml of various concentrations (20, 40, 60, 80 and 100 µg/ml). After adding 1.5 ml of 0.1% methanolic DPPH to the samples, they were incubated in dark conditions for 30 minutes. After that, the samples were examined for discoloration, ranging from purple to yellow, and the absorbance at 510 nm was measured using a colorimeter[17].

The following formula was used to determine radical scavenging activity:

$$(\%) = \frac{(\text{Absorbance of control} - \text{Absorbance of test sample})}{(\text{Absorbance of control})} \times 100$$

4.2 Anti-inflammatory Assay

Protein denaturation technique for in vitro anti-inflammatory action

0.4 mL of egg albumin (from a fresh hen's egg), 5.6 mL of phosphate buffered saline (PBS, pH 6.4), and 100 µL of a sample with varying concentrations made up the reaction mixture (10 mL). Double-distilled water in a comparable volume was used as a control. The mixes were then heated to 70 degrees Celsius for five minutes after being incubated at 370 degrees Celsius for fifteen minutes[22]. Following cooling, the vehicle was used as a blank to measure their absorbance at 660 nm. In order to determine absorbance, diclofenac sodium at the concentration was utilized as a reference medication and handled in a similar manner. The following formula was used to determine the % inhibition of protein denaturation[23].

$$\% \text{ Inhibition} = C - T / C$$

Where,

T = absorbance of test sample

C = absorbance of control

5. Result and Discussion

5.1 Phytochemical Studies

Numerous phytochemicals that are important in a variety of applications were discovered in the plant extract; Table No. 1 shows that '+' indicates present and '-' indicates absent[16, 24]

Sr. No	Test	Observation
01	Alkaloid	+
02	Flavonoids	+
03	Phenolic	+
04	Tannins	+

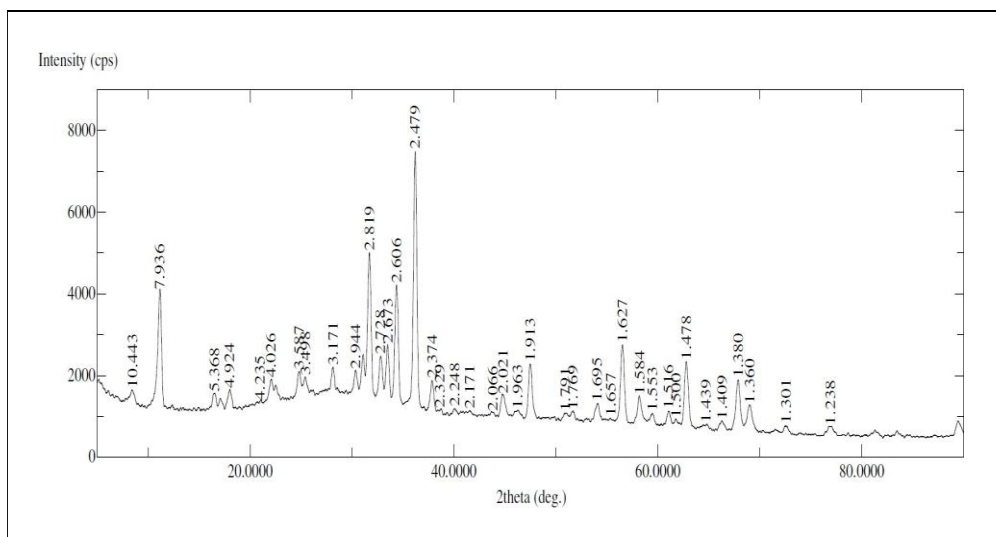
05	Terpenoids	+
06	Saponin	+
07	Steroid	+
08	Cardiac Glycosides	+
09	Coumarin	+
10	Proteins	+

Table 01 Phytochemical Analysis

5.2 Characterization of Nanoparticles

5.2.1 XRD

To assess the phase purity and crystallinity the XRD analysis were performed for ZnONPs and it shows 16nm average size. In Fig.01 the XRD spectrum showed prominent diffraction peaks at 2θ values 31.8° , 34.4° , 36.3° , 47.5° , 56.6° , 62.9° , 66.4° , 67.9° , 69.1° , 72.6° and 77.0° , which Correspond to hkl planes (100), (002), (101), (102), (110), (103), (200) and (112) [21]. These hkl Values are well matched to ZnONPs hexagonal wurtzite structure (JCPDS card no. 36-1451)[11]. The predominant diffraction peaks at 2θ value 31.8° (100), 34.4° (002), and 36.3° (101) are Indicative of polycrystalline nanoparticles as well as confirming phase purity with no extraneous peaks[25].



esters; if silicate elements are present, it may also show Si-O-Si or S-O links. The C-H out-of-plane bending characteristic of alkenes or aromatic compounds is represented by lower wavenumber bands at 905.86 cm^{-1} and 713.34 cm^{-1} [26]. Significantly, the presence of metal-oxygen (Zn-O) stretching vibrations associated with the creation of zinc nanoparticles is confirmed by a series of strong absorptions in the low-frequency band ($674.15\text{--}406.86\text{ cm}^{-1}$). These bands are typical of metal oxides, where stretching modes in the $400\text{--}700\text{ cm}^{-1}$ range are seen in Zn-O. As a result, the spectrum unambiguously shows that metal-oxygen connections and organic functional groups (O-H, C-H and C-O) coexist, demonstrating the creation or presence of metal oxide species in the sample[27].

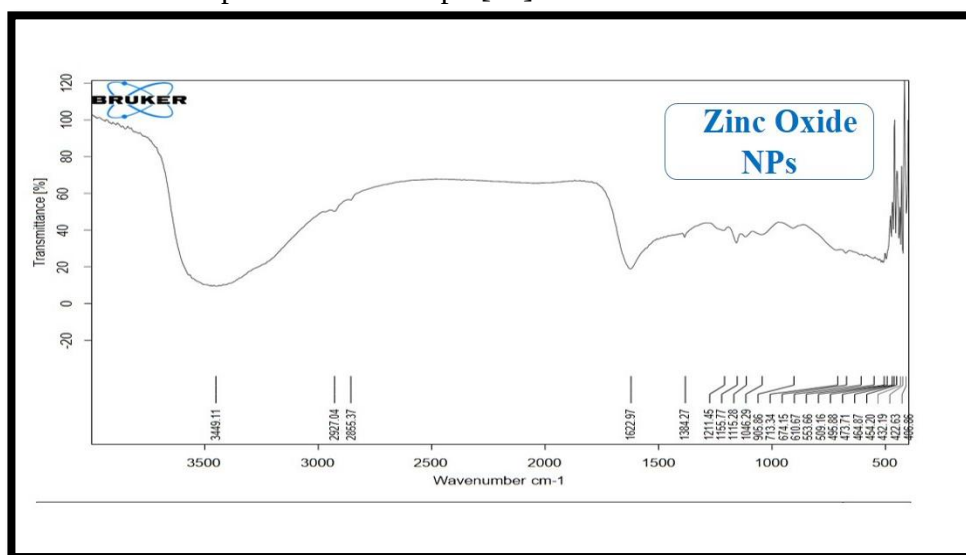


Figure 02 FTIR Peak of Zinc Oxide nanoparticle of *Hygrophila auriculata* leaf extract.

5.2.3 UV-visible studies

UV-Vis spectroscopy in the 200-800 nm region was first used to validate the synthesis of ZnONPs. Green produced ZnONPs absorption spectra revealed a distinctive peak at 297 nm (Fig. 3) [21]. Extracellular zinc bio reduction holds great potential for the green manufacturing of metallic nanoparticles using plant leaf extract materials[27].

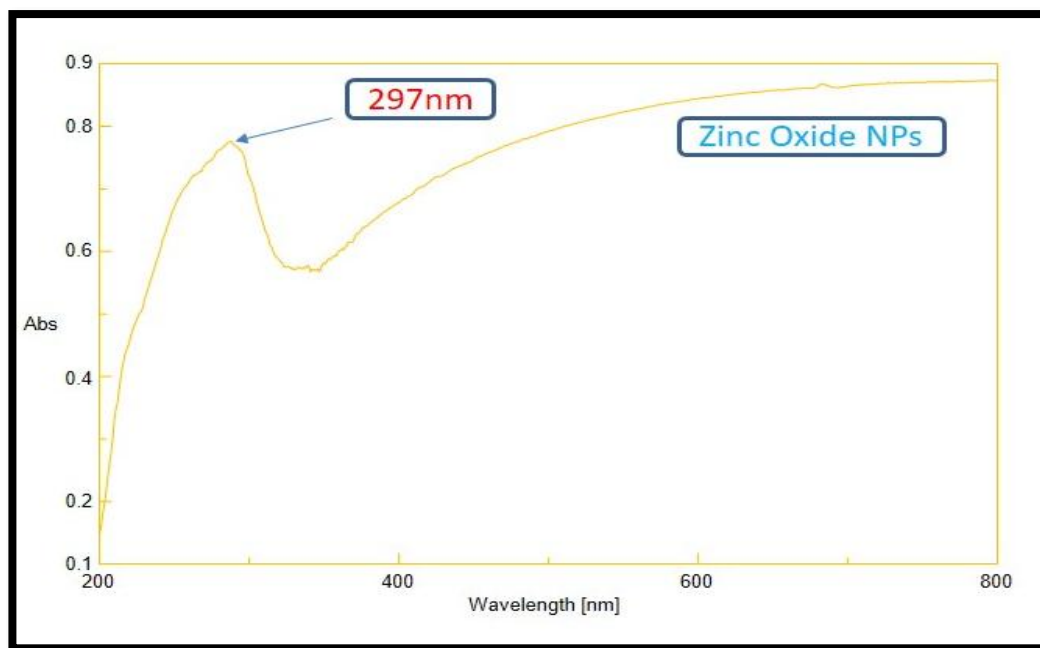


Figure 03 UV spectrum of Zinc Oxide nanoparticle of *Hygrophila auriculata* leaf extract.

5.3 Biomedical Application

5.3.1 Anti-Oxidant Assay

The nanoparticles show good antioxidant activity by using DPPH method. In this IC₅₀ of standard showing 72.49 $\mu\text{g/ml}$ hence HA-ZnONPs sample shows IC₅₀ of 94.56 $\mu\text{g/ml}$. at various concentration as comparable to standard our results are moderate as shown in table 01. % inhibition shown is moderate then standard. Also graph 01 shows comparable data [28].

SR.N O	Sample Code	Concentration ($\mu\text{g/ml}$)	Absorbance at 510nm				% Inhibition	IC ₅₀ ($\mu\text{g/ml}$)
			Test 1	Test 2	Test 3	Mean		
1	Control	-	1.93	1.93	1.93	1.93	-	
2	Standard	20	1.45	1.42	1.40	1.42	26.42%	72.49
	(Ascorbic Acid)	40	1.31	1.29	1.33	1.3	32.12%	
		60	0.95	0.97	0.93	0.95	50.77%	
		80	0.82	0.85	0.79	0.8	57.51%	
		100	0.35	0.32	0.32	0.3	82.90%	
3	(YD8308) HA-ZnONPs	20	1.58	1.59	1.55	1.57	18.65%	
		40	1.40	1.44	1.48	1.44	25.38%	

		60	1.24	1.22	1.29	1.25	35.23%	94.56
		80	1.02	1.06	1.03	1.03	46.63%	
		100	0.84	0.87	0.89	0.86	55.44%	

Table 01 Anti-Oxidant inhibition of Zinc Oxide nanoparticle of *Hygrophila auriculata* leaf extract

Graph 01 Anti-Oxidant inhibition of Zinc Oxide nanoparticle of *Hygrophila auriculata* leaf extract Verses Standard by DPPH Method

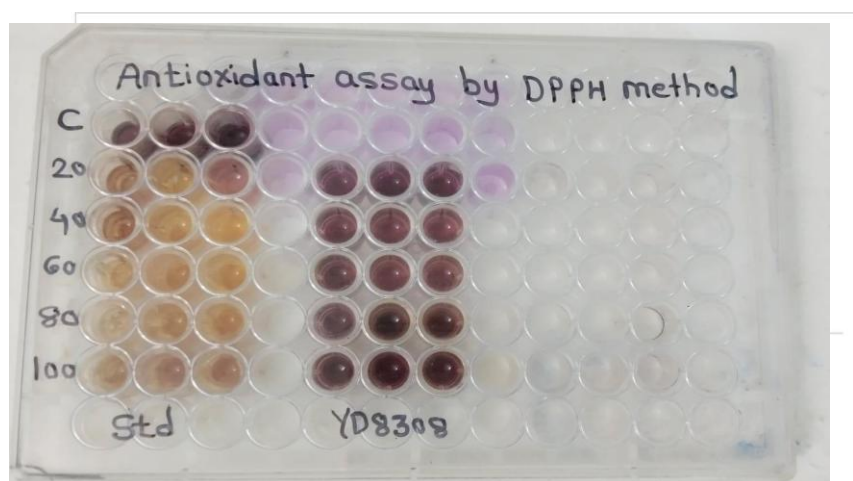


Image 01 of Anti-Oxidant Assay of HA-ZnONPs by DPPH Method

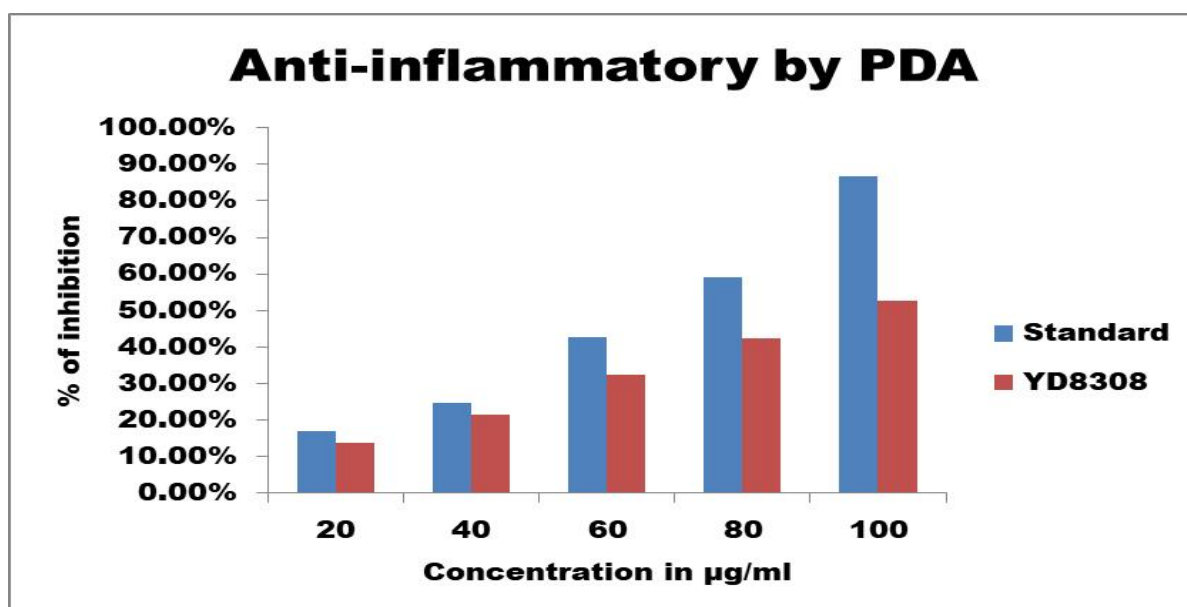
5.3.2 Anti-inflammatory Assay

The generated HA-ZnONPs exhibit strong anti-inflammatory properties. Additionally, HA-ZnONPs have demonstrated a moderate inhibitory capacity against the medication diclofenac sodium. The results reveal that inhibition of Zinc Oxide NPs is 13.63%, 21.42%, 32.46%, 42.20% and 52.59%, while diclofenac sodium medication at concentrations of 20, 40, 60, 80, and 100 mg/ml is 16.98%, 24.52%, 42.76%, 59.11% and 86.79%[29]. Compared to normal diclofenac, which has an IC₅₀ value of 70.91mg/ml, the green production of nanoparticles HA-ZnONPs exhibits IC₅₀ values of 88.60mg/ml. Our findings indicate that due to their strong anti-inflammatory qualities, the aforementioned ZnONPs may be used in drugs and medicine[30].

Sr. No	Sample code	Concentration (µg/ml)	Protein denaturation assay					
			Absorbance at 660nm				%	IC ₅₀
			Test 1	Test 2	Test	Mea		

					3	n	Inhibition n	(µg/ml)
1	Control		1.54	1.54	1.54	1.54	-	70.91
2	Standard (Diclofenac Sodium)	20	1.40	1.37	1.39	1.32	16.98%	
		40	1.20	1.20	1.22	1.20	24.52%	
		60	0.91	0.89	0.93	0.91	42.76%	
		80	0.67	0.65	0.63	0.65	59.11%	
		100	0.21	0.18	0.23	0.21	86.79%	
3	(YD8308) HA-ZnONPs	20	1.32	1.35	1.34	1.33	13.63%	88.65
		40	1.20	1.20	1.25	1.21	21.42%	
		60	1.04	1.03	1.07	1.04	32.46%	
		80	0.89	0.86	0.92	0.89	42.20%	
		100	0.74	0.71	0.76	0.73	52.59%	

Table 02 Anti-inflammatory Activity of Zinc Oxide nanoparticle of *Hygrophila auriculata* leaf extract



Graph 02 Anti-Anti-inflammatory Activity of Zinc Oxide nanoparticle of *Hygrophila auriculata* leaf extract Verses Standard by PDA Method



Image 02 of Anti-Inflammatory Assay of HA-ZnONPs by PDA Method

6. Conclusion

A straightforward, environmentally friendly, and economical process for producing copper oxide nanoparticles from *Hygrophila auriculata* leaf extract is shown in this paper. Without the use of dangerous chemicals, the phytoconstituents in the extract effectively decreased, stabilized, and capped ZnONPs. Crystalline, HA-ZnONPs hexagonal wurtzite structure nanoparticles with nano scale size and stable surface functionalization were found by structural and spectroscopic analyses. Successful nanoparticle synthesis was verified by the measured Zn-O vibrational modes and surface plasmon resonance band. The antioxidant is proof that ZnONPs from *Hygrophila auriculata* are more effective at containing the antioxidant qualities due to their maximum absorption. According to our findings, the aforementioned ZnO NPs may be used in pharmaceuticals and treatments due to their strong anti-inflammatory properties and ability to treat a variety of illnesses, including inflammatory diseases. The ability to produce and consume zinc oxide nanoparticles is expanding, and they may be used to assist humanity in both biomedical and environmental fields. To explore ZnO NPs biological potential *in vitro* and *in vivo* more research is required.

Author contribution

Yash N. Dange made contributions to the research, experimental work and conception. Sumangal S. Kale helps in writing original draft and analysis. Vijay L. Gurav and Manoj N. Lad helped in data curation. Yogesh A. Pawar, Udaysinha C. Patil and Dadaso B. Shinde help in writing review. Pravin N. Chavan helped with data analysis. Paresh M. Dhuri provided conceptualization, methodology, initial draft writing, supervision and guidance.

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Conflict of Interest

The writers affirm that they have no conflicting interests.

Data availability

The article contains the data that supports the study conclusions.

Ethics approval and consent to participate

Not relevant

Consent to publish

Not relevant

Use of AI tools

There was no use of AI tools.

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