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Synthesis of Zinc oxide nanoparticles (ZnO NPs) using aqueous leaf extract of Zanthoxylum armatum DC for photocatalytic degradation of Organic dye

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Abstract - Plant-mediated production of ZnO NPs is explored in this study, namely the use of Zanthoxylum armatum DC. leaf extract as a reducing and capping agent. UV-Vis, UV-DRS, FTIR, FESEM, EDS, and TEM were all used to analyse the produced ZnO NPs. Degradation of a model organic dye pollutant (MB) was used to examine the photocatalytic activity of ZnO NPs when exposed to UV irradiation from a metal halide lamp (70.2 W• m^{-2} UV and 452.5 W•m^{-2} visible irradiation intensity). ZnO NPs produced in a lab were tested for their antioxidant properties using DPPH radical. In addition, Gram-positive (Bacillus subtilis) and Gram-negative (Escherichia coli) harmful bacteria were tested for susceptibility to green produced ZnO NPs antimicrobial activity.

Keywords - Zanthoxylum armatum DC, Photocatalytic Activity, Gram-positive (Bacillus subtilis) and Gram-negative (Escherichia coli).

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INTRODUCTION

Using the aqueous leaf extract of Zanthoxylum armatum DC. as both a reducing agent and a capping agent, we describe here for the first time a green synthesis of ZnO NPs. The plant is in the family Rutaceae. Studies have shown that the aqueous leaf extract of Zanthoxylum armatum DC. includes phenols, tannins, alkaloids, flavoniod, terpenoids, and steroids, making it a valuable medicinal plant. Zanthoxylum armatum DC. leaf extract mostly contains phenolic acid, terpenoids, and flavoniod, all of which act as reducing agents. The direct reduction of Zn+2 ions into their respective ZnO NPs is facilitated by phenolic acids, which are water-soluble phytochemicals. Stabilization of produced ZnO NPs is aided by the participation of numerous phenolic acid molecules. Antioxidant, antidiabetic, antibacterial, anticancer, and cytotoxic activities have been found in Zanthoxylum armatum DC. leaf extract [3-5]. The primary phenolic ingredient in Zanthxylum armatum DC. leaf extract is gallic acid. In addition to its other uses, gallic acid is useful as a stabilising cap [3].

A typical approach would involve adding 10 mL of a 5 (W/V)% aqueous leaf extract of Zanthoxylum armatum DC. to a 250 mL Erlenmeyer flask along with 0.5 g of Zn (CH3COO)2 and 80 mL of Milli-Q water. The reaction mixture was then heated to 70 degrees Celsius and stirred for 60 minutes. A white precipitate formed after gradually adding 10 mL of 1M NaOH to the reaction fluid; this is evidence of the creation of ZnO NPs.

EXPERIMENTAL

Material

Zinc acetate dihydrate extra pure (Zn $(CH_3COO)_2{\cdot}2H_2O$), sodium hydroxide pellets (NaOH) and MB were purchased from Merck (Mumbai, India) and used without further purification. The leaves of Zanthoxylum armatum DC. were collected from forest of Uttar Pradesh, India, in May 2020. Botanical aspects of collected plant materials were identified and authenticated by plant taxonomist Dr. Pankaj Gupta, Professor, , SunRise University,

Alwar(Rajathan), India. Milli-Q water was used throughout the synthesis process.

Preparation of aqueous leaf extract of Zanthoxylum armatum DC.

Zanthoxylum armatum DC. leaves were washed in water and then in Milli-Q water to remove dust particles, and then they were shade dried for 15 days to remove moisture. After being dried, the leaves were chopped up and blended into a powder. After weighing out 5 grammes of powder, it was added to 100 cc of Milli-Q water in an Erlenmeyer flask and heated for 20 minutes at 60 degrees Celsius. The resulting extract was filtered through Whatman NO.1 qualitative cellulose filter paper (diameter 125 mm; pore size 11 m); the paper and the filtrate were then kept at 4^0C for later use.

Aqueous leaf extract of Zanthoxylum armatum DC. as a reducing and capping agent in the green production of ZnO NPs

Ten millilitres (mL) of a 5 (W/V) % aqueous leaf extract of Zanthoxylum armatum DC is used in a typical operation. To a 250 mL Erlenmeyer flask, we poured 0.5 g of Zn $(CH_3COO)_2$ and 80 mL of Milli-Q water. The reaction mixture was then heated to 70 degrees Celsius and stirred for 60 minutes. A white precipitate formed after gradually adding 10 mL of 1M NaOH to the reaction fluid; this is evidence of the creation of ZnO NPs. The generated ZnO NPs were centrifuged, filtered, and washed with Milli-Q water three times and once with ethanol to eliminate the contaminants and unreacted precursors. After that, the acquired product was dried at room temperature in a vacuum.

Figure 1 (a) *Zanthoxylum armatum* DC. leaf extract (inset plant image) (b) Thesolution mixture formed after addition of $Zn(CH_3COO)_2.2H_2O$ to the extract (c)*Zanthoxylum armatum* DC. leaf mediated ZnO NPs formed after addition of NaOH base to the solution mixture at 70 ^{0}C

Characterization Techniques

Degradation of Methylene Blue by Photocatalysis with ZnO Nanoparticles

Under ultraviolet (UV) irradiation, the produced ZnO NPs' photocatalytic activity was tested on the organic dye pollutant methylene blue (MB) (200-380nm). Several factors, including initial dye concentration, pH of the solution, and photocatalyst dose, were found to optimise the photocatalytic degradation of MB organic dye. Initial solutions of 5, 10, and 15 mg/L of MB dye were made. During this time, the dye solution's pH was carefully controlled between 3 and 11. Different amounts of photocatalyst were tested in their ability to degrade MB (25 mg, 50 mg and 75 mg). An open-air batch photoreactor was used to degrade MB dye via photocatalysis; 100 mL of MB solution (5 mg.L-1) and 75 mg of the ZnO NPs were mixed in a cylindrical Pyrex glass vessel with a volume of 250 mL. For the first 60 minutes, the mixture was agitated in the dark to reach an adsorption-desorption equilibrium. A metal halide lamp (70.2 Wm^{-2} UV and 452.5 Wm^{-2} visible) was then placed 10 cm above the surface of the reaction solution in the photoreactor vessel, and 3 mL of sample solution was taken from the reactor every 30 minutes for 180 minutes. Afterward, the collected samples were centrifuged, and the resulting supernatant was examined. Using a UV-Vis spectrophotometer, the absorbance of each solution was measured between 200 and 800 nm. The concentration of MB in the solution was then calculated from the sample's absorption rate as a function of time. Measuring the absorbance maximum (max = 665 nm) of MB as a function of time allowed us to assess the degree to which photocatalytic degradation had occurred. As shown in (Eq.1), ZnO NPs were found to be effective in degrading MB by a proportion equivalent to their concentration in the sample.

$$\text{Degradation}\ (\%) = [(C_0 - C_t)/C_0] \times 100$$

"C_0" is initial dye concentration (mg L^{-1}) before addition of catalyst and irradiation "C_t" is the concentration MB after addition of catalyst at a time "t" irradiation.

Evaluation of generated ZnO NPs for diffusion utilising an aqueous leaf extract of Zanthoxylum armatum DC in agar wells.

The agar-well diffusion method was used to research the antibacterial properties of ZnO NPs. After preparing Mueller Hinton Agar plates with sterile cotton swabs, the standard cultures of test bacteria were then equally dispersed throughout the surface. A sterile cork borer was used to create four wells in each plate. For the negative control, we have 50 L of 3% DMSO (Dimethyl sulfoxide), for the positive control we have 50 L of 25 g/mL reference antibiotic solution Streptomycin, and for the ZnO NPs we have 50 L of 100 g/mL ZnO NPs. Compounds, antibiotics, and DMSO were left to diffuse for 1 hour at room temperature. Plates were sealed with lids and placed in a 37 0C incubator for 24 hours. After the plates were incubated, we looked for the zone of inhibition, where no bacteria could grow. The compounds'

antibacterial activity was quantified by measuring the average diameter of the inhibition zone, which was then represented in millimetres. ZnO NPs concentrations were each tested in triplicate across two separate trials, and the average values of inhibitory zone diameters were recorded.

RESULTS AND DISCUSSION

UV-Vis, FT-IR, XRD, FE-SEM and TEM characterization of synthesized Ag NPs using an aqueous leaf extract of Zanthoxylum armatum DC.

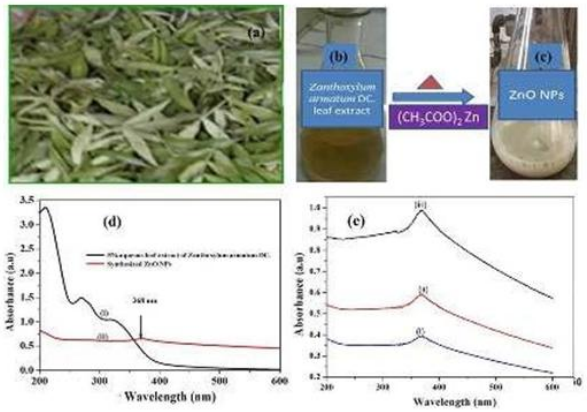


Figure 2 (a) Images of leaves of *zanthoxylum armatum* DC. (b) Visual observation of aqueous leaf extract of *Zanthoxylum armatum* DC.(light yellow) (c) ZnO NPs (white colour precipitate) (d) UV-Vis absorption spectrum of (i) aqueous leaf extract of *Zanthoxylum armatum* DC. (ii) synthesized ZnO NPs (e) UV-Vis absorption spectra of (i, ii ,iii) synthesized ZnO NPs using 1%, 3 %, 5 %aqueous leaf extract of *Zanthoxylum armatum* DC. respectively.

Figure 2 shows the direct proof for ZnO NPs production. Figure 2 displays the UV-Vis absorption spectra of both synthetic ZnO NPs and an aqueous leaf extract of Zanthoxylum armatum DC (b). Alkaloids, phenolic acid, flavonoids, tannins, terpenoids, and carbohydrates in the aqueous extract of Zanthoxylum armatum DC's leaves cause a high absorption band at 260 and 335 nm. ZnO NPs can be formed as the result of electron transitions from the valence band to the conduction band, as evidenced by the presence of a distinctive absorption band at 368 nm [1]. In addition, the spectral lack of the 260 and 335 nm peaks in ZnO NPs shows that the reducing and capping agents for the synthesis of ZnO NPs are the alkaloids, phenolic acid, flavonoids, tannins, terpenoids, and carbohydrates phytochemicals found in Zanthoxylum armatum DC leaf extract.

Green synthesis of ZnO NPs was analysed in relation to the content of plant extract. The concentration of plant extracts is found to play a significant influence in the eco-friendly production of ZnO NPs. The UV-Vis spectra of ZnO NPs produced using 1%, 3%, and 5% aqueous leaf extract of Zanthoxylum armatum DC. are displayed in Figure 2. (e). The absorbency of produced ZnO NPs increases with increasing concentrations of aqueous leaf extract. The peaks that are thought to result from light being absorbed and scattered by NPs. As leaf concentration increases from 1% to 5%, a blue shift occurs in the surface plasmon resonance (SPR) at 372 nm to 368 nm in the UV-Vis absorption spectrum. In order to achieve the most efficient green synthesis of ZnO NPs, it was determined that a 5% concentration of aqueous leaf extract was the optimal concentration of plant extract. It demonstrates that the bioactive chemicals found in a 5% aqueous extract of the leaf are sufficient to totally reduce the Zn+2 ions in the reaction mixture.

ZnO nanoparticles UV-DRS spectra shown after synthesis (Figure 3). This spectra revealed a band gap of 3.36 eV for ZnO NPs that were as-synthesized utilising an aqueous leaf extract of Zanthoxylum armatum DC.

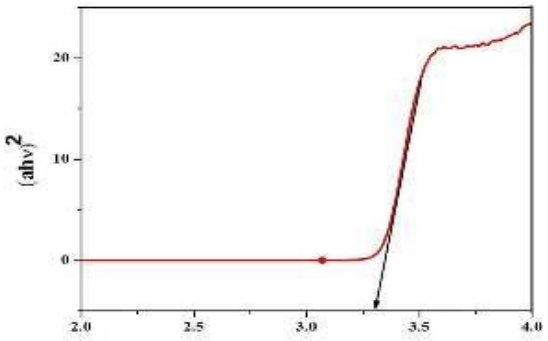


Figure 3: UV- Vis Diffuse Reflectance spectra of synthesized ZnO NPs

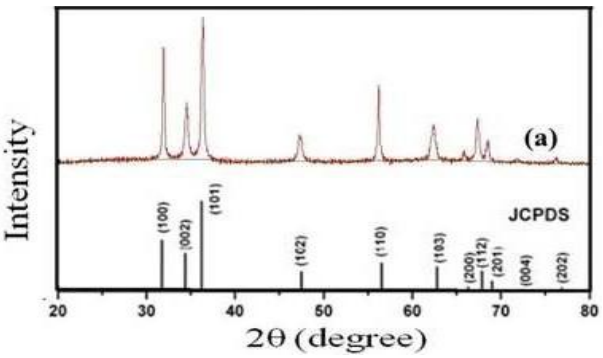


Figure 4: Powder XRD patterns of synthesized ZnO NPs by using aqueous leafextract of *Zanthoxylum armatum* DC

ZnO XRD spectra are shown in Fig.4 (a) and (b). The presence of ZnO NPs was confirmed by the presence of X-ray diffraction peaks at (100), (002), (101), (102), (110), (103), (200), (112), (201), (004), and (202) planes, respectively. ZnO NPs XRD results are consistent with those reported by the Joint Committee on Powder Diffraction (JCPDS file No. 36-1451). Scherrer's equation (Eq. 1) was used

to calculate the average crystallite sizes of the ZnO NPs; the width of the (101) plane was found to be 71.35 nm.

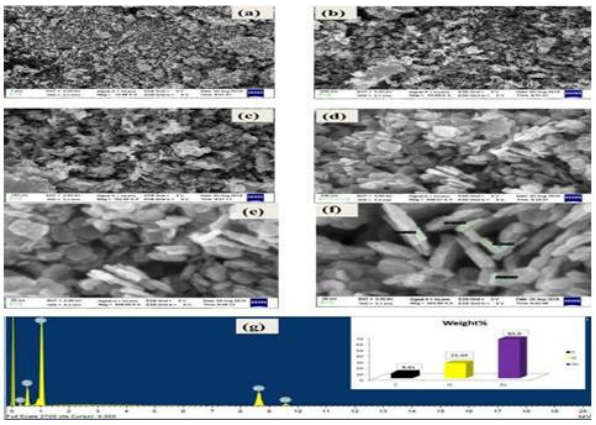
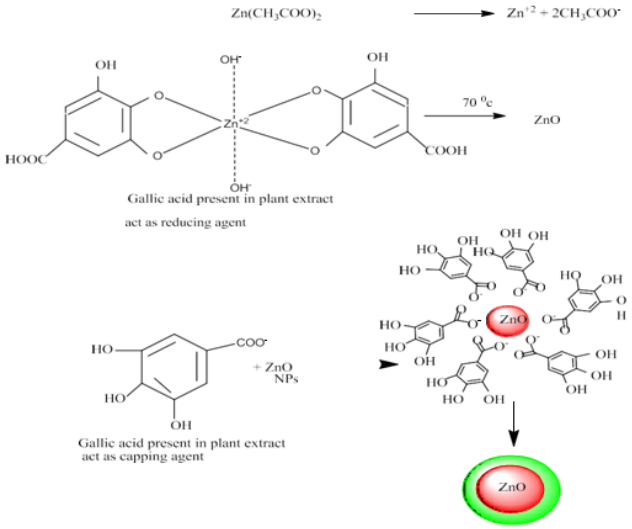


Figure 5: FESEM micrograph of green synthesized ZnO NPs in different magnification ranges (a)1µm (b)200nm (c, d) 100 nm (e, f) 20 nm show hexagonalshape. and (g) EDS spectrum of synthesized ZnO NPs

Plausible mechanism for the synthesis of ZnO NPs



Scheme 1 (a) plausible reduction mechanism for the formation of ZnO NPs using *Zanthoxylum armatum* DC. leaf extract as a reducing agent, (b) plausiblecapping of plant extract through the carboxyl (COO^-) group

Tannins, phenols, alkaloids, terpenoids, flavonoids, and steroids can be found in the aqueous leaf extract of the medicinal plant Zanthoxylum armatum DC., which is a member of the Rutaceae family. Antioxidant, antidiabetic, antibacterial, and cytotoxic activities have been found in Zanthoxylum armatum DC. extract. The main phytochemical component of Zanthoxylum armatum DC. aqueous leaf extract is phenolic acid, which is water soluble and directly responsible for the reduction of zinc ions into their corresponding nanoparticles. Multiple molecules of phenolic acid are involved, which aids in stabilising the produced ZnO NPs. The primary phenolic component of Zanthoxylum armatum DC. aqueous leaf extract is gallic acid. Gallic acid has been shown to have reducing and stabilising effects [3-5]. As can be seen in Scheme, gallic acid contained in aqueous leaf extract of Zanthoxylum armatum DC. served as both a reducing and capping agent during the synthesis of ZnO NPs. The production of ZnO NPs with Zanthoxylum armatum DC. leaf extract as the capping agent is depicted in [Scheme 1(a)]. The extract of the leaves of Zanthoxylum armatum DC. contains gallic acid, which functions as a capping agent. zinc oxide nanoparticles (NPs) with a gallic acid -COO- group attached to the top (Scheme 1. 1(b)).

Photocatalytic studies Photocatalytic degradation of MB

Initially, blank experiments were performed with the synthesized ZnO NPs in the absence of irradiation where no degradation of MB dye was observed. Similarly, experiments were done with MB dye without the catalyst in the presence of UV- visible light irradiation and there was no degradation observed. Effect of pH, the effect of catalyst dosage, the effect of dye concentration on photocatalytic degradationof MB were investigated.

Antioxidant activity

To quickly and simply ascertain antioxidant activity, DPPH is a useful tool. The free radicals produced by DPPH were neutralised by ZnO NPs. In the presence of ZnO NPs, the colour of the DPPH solution shifts from deep violet to pale yellow. Absorbance at 517 nm is found to decrease monotonically when ZnO NPs concentration rises. The absorbance drop is indicative of ZnO NPs' ability to scavenge free radicals. Antioxidant efficiency (percent inhibition) of ZnO NPs is shown to increase with ZnO NPs concentration in Figure 6(a),(b). Good evidence toward antioxidant activity is provided by the observation that a concentration of 450 g/mL of ZnO NPs scavenged free radicals with an efficiency of 18.4% (Figure 5(b)) [Table 2].

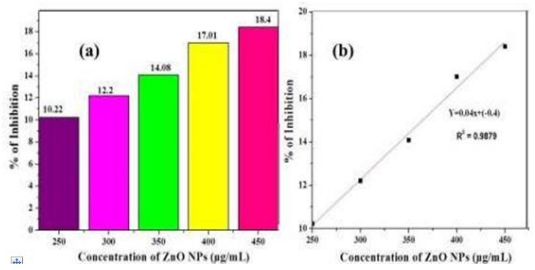


Figure 6: (a),(b)The antioxidant efficacy of ZnO NPs against DPPH·

Table 1: The antioxidant efficacy of ZnO NPs at different concentrations against DPPH·

S.No	Concentration of ZnO NPs (µg/mL)	Percentage of DPPH scavenging effect (mean ± S.D)
1.	250	10.22 ± 0.02
2	300	12.03 ± 0.03
3.	350	14.08 ± 0.01
4.	400	17.01 ± 0.02
5.	450	18.4 ± 0.02

CONCLUSION

This research reports the synthesis of ZnO NPs from an aqueous leaf extract of Zanthoxylum armatum DC., as well as its catalytic, antioxidant, and antibacterial applications. Zanthoxylum armatum DC. aqueous leaf extract includes active functional groups including alkaloids, phenolic acid, flavonoids, tannins, terpenoids, and carbohydrates, which serve as a stabilising and reducing agent for the synthesis of ZnO NPs of uniform size and without aggregation. The absorbance peak of the produced ZnO NPs was found to be 368 nm in the UV-Visible spectrum. Analysis of UV-Vis spectra showed that ZnO NPs had formed. ZnO NPs appeared almost perfectly hexagonal in scanning electron microscopy and transmission electron microscopy images. ZnO NPs are composed of Zn, O, and C, as shown by EDS analysis. Crystallinity was demonstrated by the XRD pattern, which showed distinct peaks indicating that the produced ZnO NPs were highly crystalline. The absorption bands of ZnO NPs were identified in the FTIR spectrum as having maxima at 554 cm-1 and 450 cm-1. Synthesized ZnO NPs show enhanced photocatalytic activity for MB dye degradation. DPPH levels, which indicate the antioxidant capacity approach shown that raising the ZnO NPs concentration led to a higher proportion of inhibition. This substantiates ZnO NPs' antioxidant properties, and the particles also demonstrated strong antibacterial activity against B. subtilis and E. coli.

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