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Preparation of Zinc Oxide Nanoparticles using *Azima tetracantha* Lam. Leaf Extract and Its Potential for the Removal of Contaminants from Water

E. Sirumbayee[1], S. Anusuya[1,2,*]

[1]*Department of Botany, Bishop Heber College, Tiruchirappalli – 620 017, Tamilnadu, India.*
[2]*Department of Botany, St. Joseph's College, Tiruchirappalli – 620 002, Tamilnadu, India.*

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ABSTRACT

Biologically synthesized metal oxide nanoparticles are gaining considerable interest in the removal of contaminants from surface water. In the present work, zinc oxide nanoparticles (ZnO NPs) were prepared using the biocomponents of *Azima tetracantha* Lam. leaves and evaluated for their photocatalytic and antifungal activity. Physico-chemical characterization of developed nanoparticles was carried out by UV–Vis spec, FTIR, DLS and TEM. The nanoparticles were characterized by a peak at 360 nm in the UV–Vis spectrum. FTIR spectra confirmed the presence of functional groups of leaf extract and nanoparticles. The size of nanoparticles as analyzed by DLS was 167 nm with a zeta potential of -19 mv which highlighted the colloidal stability of nanoparticles. TEM analysis revealed the spherical shape of the nanoparticles with a size range of 18-25 nm. In view of photocatalytic activity, the results showed that nanoparticles potentially degraded the organic dyes (methylene blue, methyl violet, methyl red, safranin and eosin) depending upon the time exposure to solar irradiation. Further, ZnO NPs strongly inhibited the germination of *Aspergillus niger* and *Candida albicans* spores *in vitro*. Our results suggest that biogenic ZnO NPs could be exploited as nanostructured catalysts and opens up the possibility of ZnO NPs application in water treatment plants and textile industries as it is fast, clean and environment friendly.

1. Introduction

Clean potable water is a requirement worldwide for healthy life, but is often limited. The discharge of heavy metals, pesticides and organic dyes in water bodies leads to eutrophication and reduces reoxygenation capacity. It causes severe damage to aquatic organisms by hindering the infiltration of sunlight and pose a serious threat to environment and human health [1]. Existing water treatment technologies demand a high maintenance cost and large area. Thus, there is an urgent need to develop a technology to afford clean water. Environmental nanotechnology has stimulated the development of novel and cost-effective technologies for remediation, pollution detection, catalysis and removal of contaminants from water [2]. Accordingly, nanocatalysts are effective in the removal of contaminants from water due to their remarkable physico-chemical properties. For instance, CNTs [3], and several metal and metal oxide nanoparticles including TiO_2 [4-7], ZnO and zirconium oxide [8], Ag nanocatalyst [9] has been demonstrated for its effective degradation of organic dyes and elimination of biological contaminants from water owing to its minimal environmental impact and no secondary pollution. The bioactive nanoparticles, in particular ZnO NPs have immense potential in treatment of water due to its eco-friendly nature and extensive antimicrobial activity [10, 11].

Plants contain natural compounds and novel secondary metabolites which act as a potential precursor for the synthesis of nanoparticles in a non-hazardous way. They act as reducing and stabilizing agents for the bioreduction reaction to synthesize nanoparticles [12]. Different parts of the plant promote the synthesis of metallic nanoparticles at room temperature with fast reaction kinetics [13]. Leaves of *Centella asiatica, Murraya koenigii, Alternanthera sessilis* and *Piper nigrum* were known to contain bioactive compounds which are involved in the synthesis of nanoparticles by eco-friendly method [14]. The synthesis of ZnO NPs using orange juice was reported by Jha et al. [15]. The advantage of using ZnO nanoparticles is that they strongly inhibit the action of pathogenic microbes when used in small concentration [16]. Gunalan et al. [10] showed that the green synthesized ZnO NPs exhibited a stronger inhibitory effect against fungal strains: *Aspergillus flavus, Aspergillus nidulans, Trichoderma harzianum*, and *Rhizopus stolonifer*. Lonnen et al. [6] reported the use of TiO_2 nanoparticles to inactivate bacteria (*E. coli, Pseudomonas aeruginosa*) and fungi (*Candida albicans, Fusarium sloani*) under solar light irradiation. The fungicidal mechanism of biosynthesized metallic nanoparticles has more inhibitory potential than commercially available antibiotics such as fluconazole.

Azima tetracantha Lam. belonging to salvadoraceae is an ornamental plant. It is well known for its medicinal value due to the presence of alkaloids (azimine, azcarpine and carpine) and terpenoids in their leaves. In our present work, the accurate principles of green chemistry were applied for the synthesis of zinc oxide nanoparticles using the leaf extract of *A. tetracantha* Lam. as a capping agent. It is aimed to explore the photocatalytic degradation of organic dyes such as methyl violet, safranin, eosin methylene blue and methyl orange. Furthermore, inhibitory effect of biogenic ZnO NPs was evaluated against the tested fungal strains in contaminated water.

2. Experimental Methods

2.1 Biological Material

The healthy plant samples of *A. tetracantha* Lam. were collected from Cauvery river bank and authenticated at Rapinat Herbarium, Tiruchirappalli, Tamilnadu. The fungus *Aspergillus niger* and *Candida albicans* was obtained from MTCC (Microbial Type Culture Collection and Gene Bank), Chandigarh, India and was maintained on Potato Dextrose Agar.

2.2 Extraction and Qualitative Phytochemical Analysis

The leaves of *A. tetracantha* Lam. was thoroughly washed with running tap water followed by double distilled water, dried at room temperature for two weeks and made into powder for further analysis. Leaf powder (10 g/100 mL) was extracted with double distilled water in a water bath at 80 °C for 4 hr. The aqueous extract was dried using a rotary evaporator

*Corresponding Author:anusathsar.rajesh@gmail.com(S. Anusuya)

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(Yamato RE 601) and subjected to qualitative tests for the identification of various phytochemical constituents using the standard procedures [17].

2.3 Biological Synthesis of ZnO Nanoparticles

ZnO NPs were synthesized by direct precipitation method using zinc sulphate and sodium hydroxide as precursors. To the aqueous zinc sulphate (0.2 M), sodium hydroxide (0.4 M) was added drop-wise at room temperature under vigorous stirring. The white suspension obtained was centrifuged at 5000 rpm for 20 min and washed three times with sterile double distilled water. The obtained product was calcined at 500 °C in air atmosphere for 3 hr. To 30 mL (0.2%, w/v) aqueous leaf extract, 30 mL (0.5 M) ZnO NPs was added and stirred at 90 °C for 4 hr. The suspension was continuously stirred for 24 hr at 30 °C centrifuged and freeze dried for further studies.

2.4 Characterization of Zinc Oxide NPs

The bioreduction of ZnO metal ions in the mixture solution was monitored by UV-VIS spectrum (Model 2202, Systronics Ltd.) at room temperature ranging from 200 nm to 500 nm for the confirmation of nanoparticle formation. Infrared spectra of biologically synthesized ZnO NPs were recorded on a Nicolet-560 FTIR spectrophotometer using KBr pellet technique in the range of 400–4000 cm^{-1}. The particle size, size distribution [polydispersity index (PDI)] and zeta potential of ZnO NPs were measured by Zetasizer (Malvern Instruments, UK), based on the dynamic light scattering (DLS) technique. All DLS experiments were carried out at a temperature of 25.0 ± 0.2 °C and repeated several times to check the reproducibility. The morphological characteristics of ZnO NPs were investigated by transmission electron microscope (HRTEM (JEOL model 1200 EX). The freeze-dried nanoparticles were placed on a carbon coated copper grid and then examined using a TEM without any further modification or coating.

2.5 Photocatalytic Activity of ZnO Nanoparticles

The photocatalytic (in presence of sun light) activities of ZnO NPs were studied for the degradation of organic dyes such as methyl violet, safranin, eosin, methylene blue and methyl red under solar radiation. The solution (0.01%, w/v) prepared using organic dyes was added to ZnO NPs suspension (0.01%, w/v), mixed thoroughly in a vortex and kept under solar radiation for 3 hr. The degradation of the organic dyes by nano-suspension was visualized by the gradual change in dye colour and detected through UV-Vis spectrophotometer. The dye degradation (%) was calculated using the following equation,

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

where C_0 is the initial concentration of dye solution; C_t is the concentration of the dye solution after "t" hours in the sun light irradiation.

2.6 Antifungal Activity

For the preparation of conidial suspension, *Aspergillus niger* and *Candida albicans* was grown in a conical flask containing Potato Dextrose Agar for 15 days. To this, 10 mL sterile double distilled water was added to the flask, scraped gently with sterile loop and filtered through sterile Buchner funnel. Initial concentration of conidial suspension was adjusted to 1×10^5 spores mL^{-1} using half strength Czapek's Dox Broth.

To study the antifungal activity of ZnO NPs, the growth of *A. niger* and *C. albicans* was assayed as described by Broekaert et al. [18] with some modifications. To 1 mL of the conidial suspension (1×10^5 spores mL^{-1}), 1 mL of ZnO Nps was added and incubated at 28 °C for 60 hr. Conidial suspension without the addition of ZnO Nps was used as control. Optical readings were taken at 600 nm at every 4-hr interval up to 60 hr. Experiments were replicated thrice and the data was subjected to one-way analysis of variance to determine the significance of individual differences at $p < 0.01$ and 0.05 levels using SPSS 16 software support.

3. Result and Discussion

Biosynthesized metal and metal oxide nanoparticles from plant derivatives have application in medical and commercial sectors including waste water treatment, cosmetics and food industry. The aqueous leaf extract of *A. tetracantha* Lam. revealed the presence of alkaloids, saponins, flavanoids, proteins, amino acids, diterpenes and triterpenes (Table 1). These novel metabolites and the available functional groups promote the fabrication of nanoparticle as capping and stabilizing agent at ambient conditions [14, 19]. Several studies showed the synthesis of ZnO nanoparticles using milky latex of *Calotropis procera*, *Aloe vera* extract [20], *Ocimum basilicum* L. var. *Purpurascens*, *Parthenium hysterophorus* L. [21], *Citrus aurantifolia* extract [22], *Plectranthus amboinicus* [23], and *Nephelium lappaceum* L. (rambutan) peel extract [24].

Table 1 Phytochemical screening of *Azima tetracantha* Lam. leaf extracts

S.No.	Phytoconstituents	Reagents	Aqueous
1	Alkaloids	Wagner, s	+
2	Carbohydrates	Benedict' s	_
3	Glycosides	Borntrager' s	_
4	Saponins	Water	+
5	Triterpenes	Salkowski' s	+
6	Phytosterols	Libermann Burchard' s	_
7	Phenol	Ferric Chloride	_
8	Flavonoids	Alkaline Reagent	+
		Lead Acetate	+
9	Proteins	Xanthoproteic	+
10	Amino acids	Ninhydrin	+
11	Diterpenes	Copper acetate	+

3.1 Characterization of ZnO NPs

The optical properties of green synthesized ZnO NPs measured by UV–Vis spectrometry showed typical exciton absorption at 360 nm (Fig. 1). The shape of the band was symmetrical, suggesting uniform scattering of spherical shape nanoparticles. According to Gupta et al. [25] the absorption edge systematically shifts to the lower wavelength or higher energy with the decreasing size of the nanoparticle.

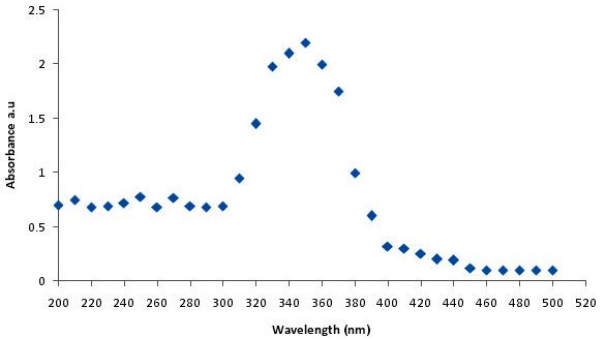

Fig. 1 UV-Vis spectrum of biogenic ZnO NPs

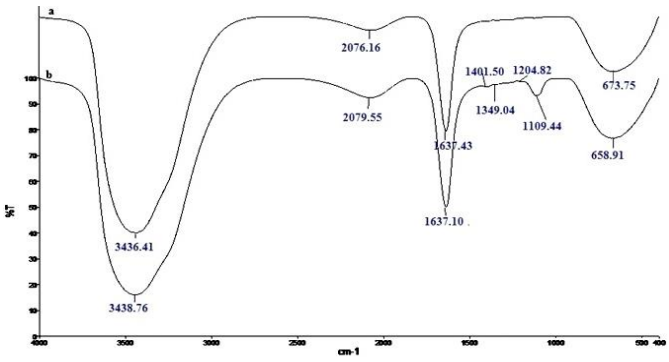

Fig. 2 FTIR spectrum of (a) ZnO and (b) biologically synthesized ZnO NPs

The results were further reinforced by FT-IR analysis, which showed the shifts and difference in areas of the peaks. According to Fig. 2a, it was observed that the bands are at 3436.41 cm^{-1}, 2076.16 cm^{-1}, 1637.43 cm^{-1} and 673.75 cm^{-1}. The peak in the region between 673.75 cm^{-1} is allotted to Zn–O [24]. The bands at 3438.76 cm^{-1} and 1637.10 cm^{-1} are characteristic for hydroxyl group (O–H). The peaks at 1349.04 cm^{-1} and 1109.44 cm^{-1} may be ascribed to –C–O and –C–O–C stretching modes. The band which appeared at 2079.55 cm^{-1} is due to C=C stretching (Fig. 2b). Also, the band located near 515 cm^{-1} is assigned to ZnO stretching vibration. From FTIR analysis, we confirmed that the carbonyl groups have the stronger ability to bind zinc indicating that the metabolites could possibly form the ZnO NPs (i.e, capping of ZnO NPs) to prevent agglomeration and thereby stabilize the medium. This suggests that the biological molecules could play a role in stabilization of ZnO NPs in the aqueous medium.

Size including size distribution is an essential characteristic parameter for nanosuspensions [26]. DLS result showed the monodisperse nature of nanoparticles with particle size of 167 nm (Fig. 3a). The zeta potential was -19 mv (Fig. 3b) which shows its greater affinity towards biological membranes in an aqueous environment [27]. The TEM monograph clearly

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revealed the distribution of spherical shaped ZnO NPs with the size range from 18-25 nm (Fig. 4). Similar size range and shape of ZnO NPs was reported by Yedurkar et al. [28] and Agarwal et al. [29]. The size difference between TEM and DLS analyses might be due to the state of nanoparticles [30].

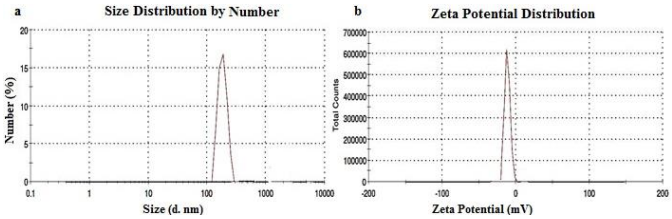

Fig. 3 (a) Dynamic light scattering and (b) Zeta potential of synthesised ZnO NPs

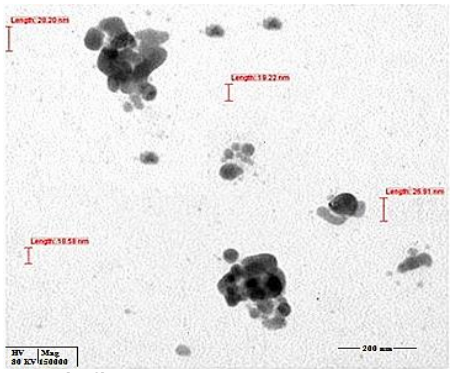

Fig. 4 TEM micrograph of biogenic ZnO NPs

3.2 Photocatalytic Activity

Dye degradation was visually detected by the gradual change in colour. The characteristic absorption peak of different dyes was observed by UV-vis spectrophotometer. The catalytic activity of bio synthesized ZnO nanoparticles was verified by the decrease of peak intensity after 3 hr exposure to solar light as shown in Fig. 5. The electron (e^-) reduction and oxidation ability of methylene blue (MB) was demonstrated at ground state without using catalyzer $NaBH_4$ [31]. The characteristic absorption peak of MB was observed at 657 nm (Fig. 5a). The decrease in absorbance after 3 hr exposure to sunlight indicated the catalytic activity of biosynthesized ZnO NPs. Auld [32] reported the enhanced photocatalytic degradation of MB pollutant dye by ZnO nanoflowers synthesized from proteins of B*. licheniformis*.

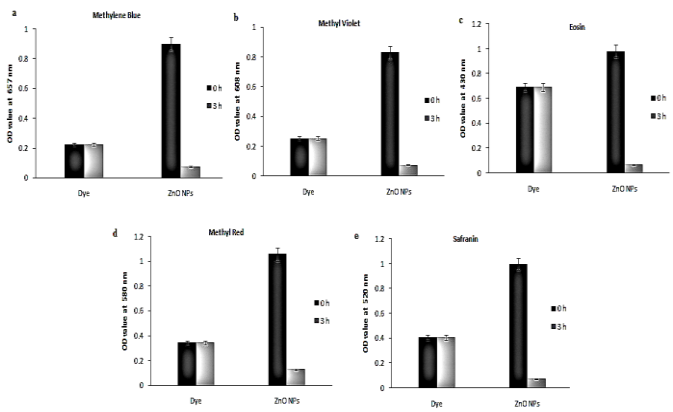

Fig. 5 Photocatalytic activity of biosynthesized ZnO NPs against methylene blue (MB), methyl violet (MV), methyl red (MR), safranin (S) and eosin (E)

Methyl violet (MV) is a mixture of methylene blue and diluted alkalis that are mainly used as dyes for textiles to give deep violet. The relative absorbance of MV band was observed at 608 nm (Fig. 5b). The characteristic absorption peak at 430 nm of eosin (E) was used for monitoring the catalytic degradation activity. The main peak at 430 nm decreased gradually with the exposure time, indicating the photocatalytic degradation of eosin dye (Fig. 5c).

To test the photodegradation of safranin by ZnO NPs, a solution containing dye and ZnO NPs was photo irradiated by sunlight. The absorption spectrum of safranin was recorded at 520 nm. Fig. 5e showed the degradation efficiency of NPs against safranin. It is important to note that degradation of the safranin dye in the absence of NPs resulted in no significant change in the absorption spectra. The characteristic absorption peak of methyl red (MR) was observed at 580 nm (Fig. 5d). Degradation of methyl red (MR) was visualized by decrease in the peak intensity. Visible light was found to be faster in decolorizing dyes in the presence of metal catalyst [33]. The decrease in absorbance indicates the ability of phytoextract capped ZnO NPs to degrade the organic dyes. Photocatalysis, using nanostructures of metal oxide semiconductors like zinc oxide (ZnO), titania (TiO_2), tungsten oxide (WO_3), zinc stannate (Zn_2SnO_4), etc. plays a role in water purification as it is capable of removing chemical as well as biological contaminants [34, 35]. During degradation, the catalysis occurs on the surface region of nanoparticles, therefore increasing the surface area availability which will significantly improve the efficiency of the catalyst [36]. Metal nanoparticles support the electron (e^-) relay from the donor to the acceptor and act as a substrate for the e^- transfer reaction. During e^- transfer reaction, the reactants are adsorbed on the surface of metal and consequently, the reactants gain an e^- and are reduced. Thus, biogenic ZnO NPs act as an efficient catalyst through the electron transfer process in all the above catalytic reactions [37].

In the absence of a photocatalyst, the degradation of dyes under solar light irradiation was very slow, and almost no degradation was observed even after 3 hr. This revealed the strong dye degradation activity (%) of ZnO NPs against the organic dyes tested (Fig. 6).

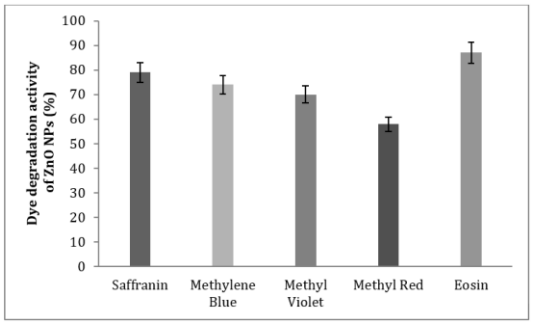

Fig. 6 Dye degradation activity (%) of biogenic ZnO NPs against methylene blue (MB), methyl violet (MV), methyl red (MR), safranin (S) and eosin (E)

3.3 Antifungal Activity

The antifungal activity of ZnO nanoparticles was evaluated by spore suspension method. The inhibitory effect of leaf extract was comparatively lower than nanoparticles. Incubation of *A. niger* with ZnO NPs significantly inhibited the sporulation up to 16 hr. There was no further inhibition after 16 hours of incubation (Fig. 7a). The inhibitory effects of ZnO nanoparticles are correlated with their size and concentration [38, 39].

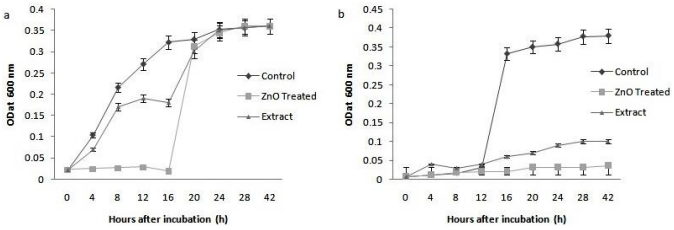

Fig. 7 Inhibitory effect of biogenic ZnO NPs against (a) *A. niger* and (b) *C. albicans*

In case of *C. albicans,* ZnO NPs strongly inhibited spore germination endorsing the interaction with NPs. Even after 48 hr, ZnO NPs continued to inhibit the spore germination (Fig. 7b). The inhibitory effect of ZnO NPs could be attributed to the damage of the cell wall, cell membrane and the extrusion of the cytoplasmic contents resulting in death of the fungi [40-42]. The ZnO NPs might generate reactive oxygen species (ROS) and decrease the production of hydroxyl radicals due to the capping of phytoconstituents. Further, it was observed that excitation of ZnO NPs with light improved the antimycotic effects [43], as it can break up complex long chained organic molecules (normally toxic) into simpler fragments as well as distort cell walls of microbes thereby immobilizing them [34]. Hence the synthesized biogenic ZnO NPs could be used as a potent sanitizing agent for disinfecting the contaminated water.

4. Conclusion

ZnO NPs synthesized by green method is a cost effective and most promising technique for degradation of toxic organic dyes into harmless fragments thereby minimize the impact of industrial wastes in environment. The synthesized ZnO NPs using biological material degraded the organic dyes such as methyl violet, safranin, eosin, methylene blue and

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methyl orange under sunlight. Moreover, the biosynthesised ZnO NPs strongly inhibited the fungal growth which is present in contaminated water.

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