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Green Synthesis and Characterization of ZnO Nanoparticles Using *Calotropis Gigantea* Latex and Evaluation of Their Antibacterial Activity

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Abstract: *The concept of green chemistry has provided a new platform for the environmentally propitious synthesis, non-hazardous to the environment and human health. Now a-days synthesis of nanomaterials with plant extracts have been a source of brainwave in designing commercial products for promising applications like biosensors, photo catalysis, antimicrobial and antioxidant technologies, etc. In the present study, zinc oxide (ZnO) nanoparticles (NPs) synthesized by using the latex extract of Calotropis gigantea plant in aqueous medium via green synthesis. The resultant products were thoroughly analyzed using different analytical techniques such as UV-Vis Fourier Transform Infrared spectrophotometer and XRD technique. The XRD patterns reveal that the synthesized NPs crystallized with (101) growth direction. The synthesized NPs are evaluated using its antimicrobial action against Staphylococcus aureus and Klebsiella pneumonia. It is shown that the C. gigantea latex extract capped of ZnO based NPs exhibit better antibacterial activity against selected strains. The as prepared ZnO nanoparticles were used as a photocatalyst to degrade Rhodamine B dye with a catalyst load of 0.2 g and efficiency of 94.14 % degradation of within 220 min.*

Keywords: *Calotropis gigantea*, Nanoparticles, XRD, Zinc Oxide, Green Synthesis

I. INTRODUCTION

Recently, the development of biosynthetic and environmental friendly technology for synthesis of nanomaterials, gaining attention among the research community. The chemical synthesis methods lead to adsorbance of certain toxic chemical species on the surface which causes adverse effects in health care applications. Hence, biological method using plant extracts is one of the valuable alternatives to chemical synthesis [1]. The plants are having the capability to adsorb, hyper-accumulate and degrade certain inorganic metallic ions from their surroundings [2-3]. It is noteworthy to mention here that the phytochemicals of plant tissues capable of significantly reducing environmental contamination [4].

The Calotropis Gigantea commonly called crown flower or giant milk weed used in traditional folk medicine for the treatment of bronchitis, dyspepsia, paralysis, swellings and intermittent fever [5]. Moreover, it exhibits antifungal, antiviral, antibacterial, anticarcinogenic and anticandidal activities [6-14]. That's why, the study of C. gigantean plant has been the subject of interest among various research teams to use for nanoparticles synthesis.

At present, metal oxide nanoparticles (NPs), owing to their optical, electrical, and magnetic properties, are sought after materials for applications in the fields of energy storage, sensors, data storage, optics, transmission, cosmetics, biotechnology, and medicine. To meet the growing demand for NPs, they are manufactured in large scale for industrial and household uses. The increasing use of NPs leads to their release into the environment and serious risk of ecotoxicity [15–17]. Hence, metal oxide NPs emerge as a novel class of environmental xenobiotics, the toxicity/hazard of which

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needs to be assessed on a priority basis. Furthermore, plants, as lifeforms of the first trophic level, are at the forefront of encountering environmental NPs. Therefore, there is a pressing need to obtain vital knowledge on the adverse effects of NPs on plants towards obtaining the crucial environmental impact assessment of NPs.

Among metal oxide NPs, zinc oxide nanoparticles (ZnO NPs) with unique optical and electrical properties are suitable for many applications such as coatings for the solar cells, electronics, and chemical sensors. The ZnONP blocks the UV-radiation and therefore is used in transparent UV-protection films and as a UV-filter in sunscreens [18]. Due to its antimicrobial properties, ZnO NP is included in the linings for canning meat, fish, corn, and peas [19]. Recent studies demonstrate that ZnO NPs hold considerable promise for biomedical applications and therapeutic intervention [20]. The ZnO NP-amended agricultural soils depending on the soil-properties cause phytotoxicity in wheat seedlings [21]. Therefore, the ecotoxicity of the ZnO NPs in diverse organisms is of serious concern [22]. Zinc oxide (ZnO) is considered to be a technologically prodigious material having wide range of applications in the field of sensors, antimicrobial agents, photocatalyst, etc., [23-27] due to its wide band gap (3.37 eV), large binding energy (60 meV), high refractive index, high thermal conductivity, etc., [25-27]. In recent decades, ZnO nanoparticles (NPs) have fascinated more research interest and a lot of work has been done on its synthesis using various plant extracts [28].

Many alternative physico-chemical [29-33] and biological or plant-mediated [34-35] synthesis routes have been used to prepare ZnO NPs. The latter methods using microbes and plant extracts are preferred for the synthesis of NPs, which are considered eco-friendly. The relatively simple bottom-up approach using the plant extracts, also known as green synthesis, minimizes or eliminates hazardous chemicals normally used in physico-chemical synthesis of NPs. Furthermore, green synthesis is preferred for the synthesis of metallic NPs due to reasonable (low) cost, less or no toxicity, and other environmental advantages [36]. Green synthesis of the ZnO NP utilizes the extracts of different plants, including Acalypha indica, Aloe vera, Calotropis gigantea, C. procera, Catharanthus roseus, Coriandrum sativum, and Ixora coccinia [37–41], as well as extracts of the algal seaweeds [42]. Among currently available methods of green synthesis, the present study utilized the alkaline precipitation method to synthesize the ZnO NP by reduction of the precursor, zinc acetate ($Zn(CH_3COO)_2$), using sodium hydroxide (NaOH) along with the milky latex of *C. gigantea*.

There are several routes through which NPs can be synthesized, like Sonochemical, microwave irradiations, alkoxide based route, sol–gel technique, one step solid-state reaction method at room temperature, electrochemical methods, precipitation–pyrolysis, thermal decomposition of precursor or by combination of electro deposition and self-catalytic mechanism etc. In chemical methods, toxic chemicals are highly reactive and pose major environmental and biological problems. Therefore, it was a challenge to find a convenient, mild, nontoxic, natural product to produce metal/metal oxides in an aqueous environment. Amongst various natural materials used for nanoparticle construction, plants seem to be the best candidates because the NPs produced by plants are more stable and the rate of production is fast. Currently, green synthesis of NPs is gaining importance due to its simplicity, inexpensive and eco-friendly. In the present study, latex of *Calotropis gigantea (C. gigantea)* plant was used for the synthesis of ZnO based nanoparticles.

II. EXPERIMENTAL SECTION

2.1. Materials

The zinc acetate dehydrate, sodium hydroxide, was purchased from Sigma aldrich, India. The other all the reagents are of analytical purity grade and have been received from commercial sources.

2.2. Collection of Latex

Fresh milky white latex of was collected by cutting the stem twigs at early morning in Radhanagari area and brought immediately to the laboratory for further studies.

2.3. Synthesis of ZnO Nanoparticles:

ZnO nanostructures were prepared by co-precipitation method. 0.02 M aqueous solution of zinc acetate dihydrate was put into 50 ml of distilled water under vigorous stirring. After 10 min stirring, Latex of Maddar 10.0 ml was added into the above solution. After addition of milky latex, 2.0 M NaOH aqueous solution was introduced into the above aqueous solution, resulting in a white aqueous solution at pH 12, which were then placed on magnetic stirrer for stirring for 2 hr. The precipitate was then taken out and washed repeatedly with distilled water followed by ethanol to remove the impurities for the final products. Then a white powder was obtained after drying at 60 °C in vacuum oven overnight.

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2.4. Characterization of ZnO Nanoparticles :

The UV–Vis absorbance spectra was measured using A Shimadzu double beam UV-visible spectrophotometer in 250–800 nm**.** The FTIR performed recorded using Lambada 7600 PC with wavelength range between 4000 cm^{-1} and 400 cm^{-1}. X-ray diffraction (XRD) measurement was performed using **Bruker Ltd Germany, D2 Phaser** X-ray diffractometer instruments.

2.5. Photocatalytic Dye Degradation studies

ZnO nanoparticles were used as a photo-catalyst in photocatalytic degradation experiments to degrade Rhodamine B under UV light exposure. For the experiment, a specifically made UV chamber was utilized. Distilled water was used to create 0.01g/l Rhodamine B dye solutions. A homogeneous mixture for each dye was obtained through stirring. To improve adsorption and desorption equilibrium processes, 0.2 g of ZnO nanoparticles were added to 100 ml of each dye to create a dye catalyst mixture. The mixture was then vigorously stirred for one hour in a dark room. A benchtop ultraviolet light source was used to illuminate the corresponding dye-catalyst combinations. The longest lighting time was 220 minutes. Every five minutes while the samples were under light, they were spun. Every 20 minutes, around 3 milliliters of each sample were drawn using a syringe, centrifuged, and the supernatant was examined using UV-vis spectrometry.

2.6. Biological activity

The well-diffusion method was utilized to confirm the bioactivity analysis of NPS against two *Staphylococcus aureus* and *Klebsiella pneumonia* strain. Microorganisms were cultivated overnight on nutrient agar plates. A sterile glass spreader was used to dispense 100 µL of microbial suspension. Subsequently, ZnO NPs 50mg/ml was prepared and used further for antibacterial studies. The plates were incubated for one day at 30 ℃ for the bacterial strains. DMSO is used as negative control. The inhibitory zones in millimeters on the agar surface surrounding the well was used to calculate the antimicrobial response. The inhibition zone by ZnO NPs was compared with conventional antibiotics kanamycin.

III. RESULT AND DISCUSSION

All flowering plant parts such as roots, stems, leaves, and fruits contain latex, which is secreted by highly specialized cells called laticifers. Proteins, alkaloids, sterols, fatty acids, starch, glycosides, tannins, saponins, phytosterols, acetogenins, saponins, avanoids, tannins, resins, and enzymatic proteins like proteases, lipases, peptidases, chitnases, esterase papain hevin exhibit a variety of biological activities, including proteolytic, anthelmintic, insecticidal, anti-inflammatory, antioxidant, and anticancer properties. Investigating the therapeutic value of plants as a possible and profitable substitute for synthetic nanomaterials gives some better final products for a range of biological and medical applications. Using the aforementioned justification, we synthesized ZnO NPs for their antibacterial uses using the latex of *C. gigantea*.

3.1. Characterization of ZnO Nanoparticles

3.1.1. UV-Visible Spectroscopy

A UV-Vis spectrophotometer was used to characterize the ZnO nanoparticles. Since the absorption peak was detected at 348 nm, zinc oxide production was verified. This outcome is consistent with previously published findings that showed an absorption peak at 346 nm.

3.1.2. FTIR analysis

The interaction of biomolecules or secondary metabolites from latex, which were responsible for reducing zinc ions to ZnO NPs, produced the functional groups of the ZnO NPs derived from C. gigantea latex. The FTIR spectra showed peaks at 495, 554, 600, 695, and 877 cm^{-1}, which indicate the presence of metal oxide groups and clearly show ZnO NP production. The vibrations of hydroxyl groups from surface-adsorbed water produced a peak at 1636 cm^{-1}. The existence of C–N stretching bonds is shown by the band at 1330 cm^{-1}. The presence of a peak at 1128 cm^{-1} indicated C–O stretching. Additionally, bands corresponding to C-H bonding were found at 1420 cm^{-1} and 1574 cm^{-1}.

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3.1.3 X-ray Diffraction (XRD)

The biological approach for the formation of ZnO nanoparticles using Maddar (*Calotropis Giagantea*) milky latex at room temperature was reported here. The X-Ray diffraction (XRD) pattern reveals the formation of ZnO nanoparticles, which shows crystallinity. **Fig. 1** shows the XRD pattern of the ZnO NPs powder embedded in calotropis matrix synthesized by co- precipitation method. When compare the both the samples, XRD Spectra showed strong diffraction peaks at 31°, 34°, 36°, 47°, 56°, 62°, 66°, 67°, 68°, 72° and 78° degrees of 2-theta which corresponds to (100), (002), (101), (102), (110), (103), (200), (112), (201), (004), (202) crystal planes, which were in significant agreement with the JCPDS file 36145. It can be seen that ZnO NPs embedded in calotropis matrix, XRD peaks were sharp as shown in **Fig. 1**.

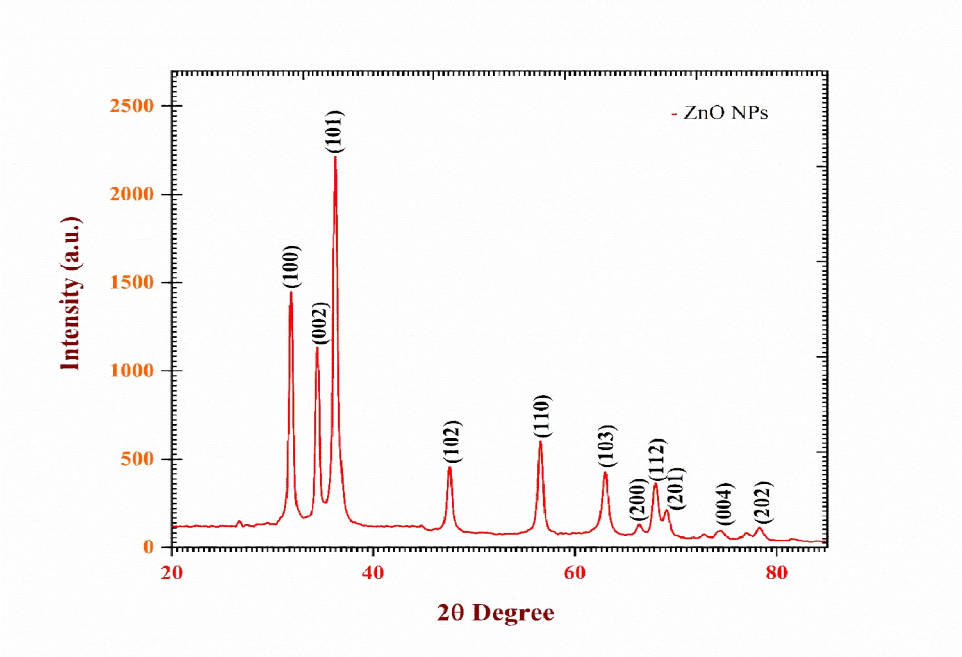

Fig. 1 - XRD graph of biosynthesized Zinc Oxide Nanoparticle

3.2. Photocatalytic Dye Degradation studies

Photocatalytic degradation of the Rhodamine B (RhB) dye was performed with the use of ZnO nanoparticles in the presence of ultraviolet light from a UV transluminator. Data from the UV–vis spectrometry analysis during photocatalytic degradation experiment are shown in **Fig. 2**. ZnO nanoparticles were used in the photocatalytic degradation of Rhodamine B in order to measure the rate of degradation. The amount of dye that deteriorated in 220 minutes was used to assess the rate of degradation. The dyes degraded at the fastest rate in nanoparticles with the smallest crystallite size. This is because there are more reactive sites on a wide surface area, which leads to more photocatalytic reactions. The percentage of dye degradation is provided as

$$d(\%) = \frac{Co - Ct}{Co} X\ 100$$

Where C_O is the initial concentration of the dye and Ct is the concentration of the dye at a particular irradiation time. To ascertain its impact on the dye degradation, 0.2 g of catalyst was utilized in the photo degradation method. A graph of the amount of dye degraded against time of irradiation is shown in **Fig. 2** for the ZnO NPs. It was observed that, photo degradation increased as time increased.

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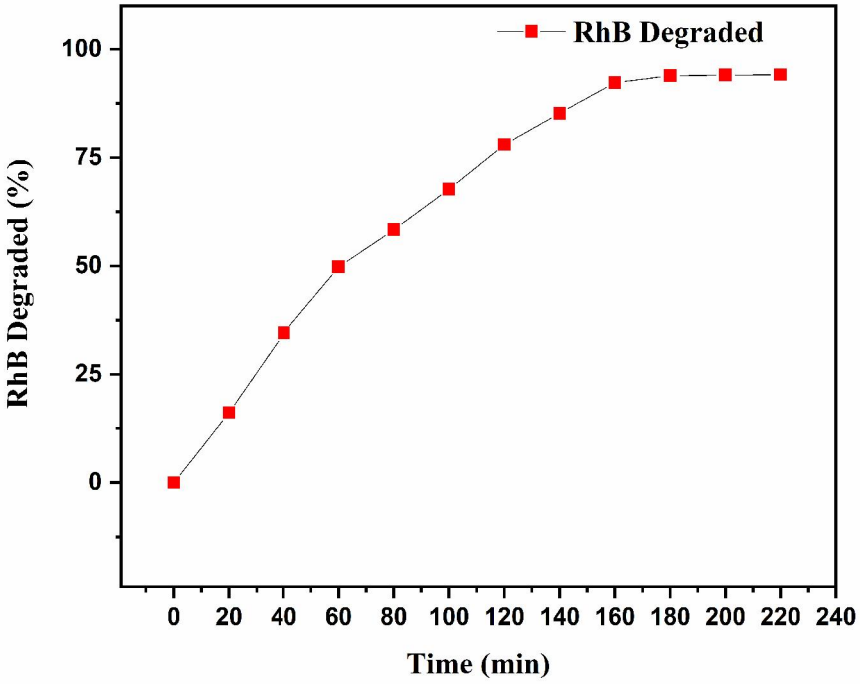

Fig. 2 – Amount of RhB degraded against time.

3.3. Biological Activity

The ZnO NPs made by using latsex of *C. gigantea* show significant antibacterial activity against *Staphylococcus aureus* and *Klebsiella pneumonia*. The maximum zone of inhibition was observed against *S. aureus* by using *C. gigantea* latex based ZnO NPs. The antibacterial properties of latex based ZnO NPs kill and reduce the growth and activity of *S. aureus*, prominently. The biological activity data ZnO NPs given in **Fig. 3**.

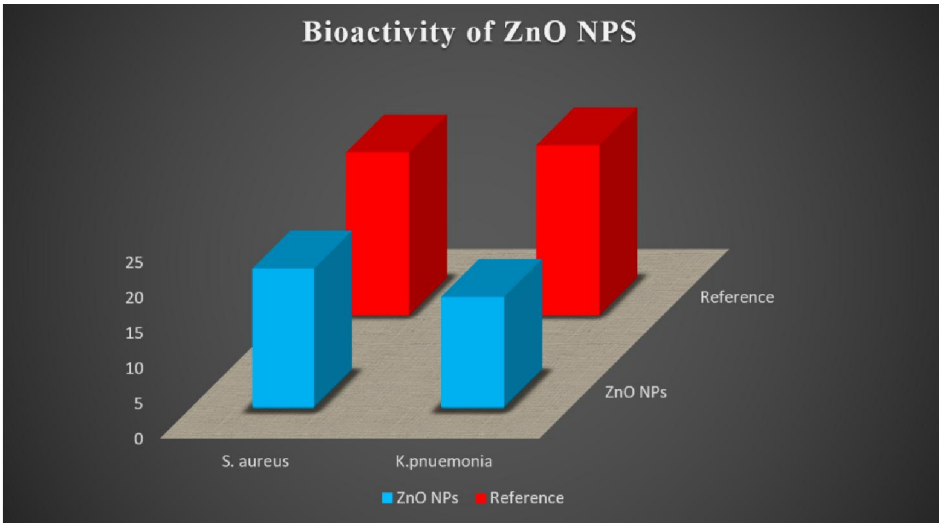

Fig. 3 : **Biological Activity of ZnO Nps prepared using latex of *C. gigantea*.**

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IV. CONCLUSION

The rapid biological synthesis of zinc and copper nanoparticles using latex of *Calotropis Gigantea* provides an environmental friendly, simple and efficient route for synthesis of nanoparticles. The use of plant extracts avoids the usage of harmful and toxic reducing and stabilizing agents. *C. gigantea* latex-mediated ZnO NPs were synthesized by rapid, simple, and eco-friendly methods. The ZnO NPs were created using an aqueous-based latex extract. Using a variety of spectroscopic techniques, the synthesis of ZnO NPs based on *C. gigantea* latex was validated and their physicochemical characteristics were described. The antibacterial activity of *C. gigantea* latex mediated ZnO NPs was assessed using well diffusion method. The maximum zone of inhibition was observed against S. aureus. The results conclude that the ZnO NPs have excellent biocompatibility and broad-spectrum antibacterial activity against selected bacteria. With a 0.2 g catalyst and an efficiency of 94.14% degradation in 220 minutes, the produced ZnO nanoparticles were utilized as a photo catalyst to break down Rhodamine B dye. The synthesis of ZnO nanoparticle is still in its infancy and more research needs to be focused on the mechanism of nanoparticle formation which may lead to fine tuning of the process ultimately leading to the synthesis of nanoparticles with a strict control over the size and shape parameters.

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