

Optimizing the Reaction Parameters for Biosynthesized Zinc Oxide Nanoparticles and Evaluation of Their Anti-bacterial, Anti-inflammatory, and Catalytic Degradative Properties

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Abstract: Nanobiotechnology focuses on studying the synthesis and properties of small biological structures. Zinc oxide (ZnO) is a valuable inorganic compound known for its robust properties in the field of medicine. ZnO nano-particles (ZnO-NPs) are metallic nano-particles known for their excellent biological, catalytic, and UV-blocking properties. The purpose of the current research is optimizing the reaction parameters for biosynthesized ZnO-NPs (synthesized by seed-extract of *Lallemantia royleana*) and to investigate their anti-bacterial, catalytic degradative and anti-inflammatory activities. These biosynthesized ZnO-NPs exhibit strong anti-bacterial and catalytic degradative potential. Our research findings indicated that ZnO-NPs effectively destroy the organic dyes and possess strong anti-bacterial properties.

Key words: Nanoparticles, Zinc Oxide, Anti-bacterial, Anti-inflammatory, catalytic activity.

1. INTRODUCTION

Nano-biotechnology is a rapidly developing field that focuses on nano-scale production of materials by biological entities.[1] In recent years, this field garnered significant attention in diagnostics due to the extraordinary capabilities of nano-particles to produce and characterize devices and systems at nano-scale.[2] Noble metal nano-particles [gold, silver, platinum, zinc oxide (ZnO)] are extensively studied as they have spectacular pharmacological and medicinal applications.[3] Plant based production of nano-particles is considered more stable, eco-friendly, economical, and have vast range in form and size.[4,5] Over the past decade In last decade, zinc-oxide nano-particles (ZnO-NPs) have attracted considerable attention due to their enormous applications biological, chemical, physical, medicinal sciences and management of waste-water. [6,7] Over the years many different techniques for synthesizing ZnO-NPs are used which are categorized as liquid and solid phase methods. [8] However, these conventional chemical and physical techniques are often highly expensive and energy intensive or involve variety of toxic chemicals which pose significant health risks and environmental dangers. [9] As a result, the researchers are increasing interested in using green sources to synthesis ZnO-NPs.

Production of ZnO-NPs via bio-genic method is contamination free and eco-friendly procedure. In recent years ZnO-NPs synthesis via plant extract is gaining importance because of the presences of variety of natural components i.e., phytochemicals such as steroids, flavonoids, carotenoids, proteins etc. [10] These natural components of the plant extracts acted as capping and reducing agent for the synthesizing process of ZnO-NPs. [6] Plant extracts used for synthesis of ZnO-NPs is proven to be steadfast, clean and non-toxic in comparison to the conventional physical and chemical procedures. [7,11] ZnO-NPs have robust UV blocking and catalytic properties containing larger surface area [12]. Their anti-bacterial properties were used in textile industry for the inhibition of bacterial growth. [13] Some of the topical plants such as *Hibiscus subdariffa*, *Vitex trifolia*, *Pongamia pinnata*, *Trifolium pretens* and *Punica granatum* were found to be successful for efficient and fast synthesis of ZnO-NPs.[14] *Lallemantia royleana* is an important herb which is related to the Lamiaceae family and is generally been utilized as medicinal herb in Pakistan. It is vastly used and a commonly cultivated herb in Pakistan, India Kazakhstan, Iraq, Russia, China Uzbekistan and some European countries. [15] *L. royleana* has found to have a vast variety of pharmaceutical properties that includes antimicrobial, cephalic astringent, anti-oxidant, anti-inflammatory, anxiolytic, anti-depressant, anti-emetic, hypolipidemic, cardio-vascular and some other protective therapeutic effects.[16]

In present research work we studied the optimization of reaction parameters for biosynthesized ZnO-NPs [8] (synthesized by seed extract of *L. royleana*.) and also studied a few of its biological and catalytic degradative properties.

2. Materials and Method:

2.1. Extraction of mucilage from *Lallemantia royleana* seeds

The seeds of *L. royleana* (5.0 g) were soaked in distilled water (500 mL) for 3 h. The soaked seeds were homogenized via kitchen blender without damaging the seeds. Resultant mucilage was centrifuged @ 6000rpm for 10 mins, for separating seeds. Yellow color clear *L. royleana*-mucilage was collected, and cleansed via vacuum filtration by muslin cloth. Clear LR-mucilage was stowed in the refrigerator at 4°C. The pH of the isolated mucilage was measured.

2.2. Synthesis of Zinc Oxide Nanoparticles

Zinc acetate dihydrate (0.5M; 10mL) was collected in flask having a rounded bottom and was brewed with different volumes of *L. royleana* mucilage (2, 4, 6, 8, 10mL) isolated earlier and total volume was made 20 mL by addition of distilled water. The pH of the reaction mixture was adjusted by using NaOH (1.0 M). The reaction was kept under vigorous stirring for 1 h. The reaction was repeated by varying pH of the reaction mixture.

To confirm the formation of ZnO-NPs the UV-Vis spectra (U-2800 Hitachi, Japan) of solution was recorded in 200-800 nm range.

2.3. Optimizing the reaction parameters

Different reaction parameters such as pH (7-12), volume of *L. royleana* mucilage (2-10 mL) and precursor salt (2-10mL) was varied to control the size, concentration and yield of ZnO-NPs, by varying single parameter at a time while keeping the rest constant. The variables tested were amount of *L. royleana*-mucilage (2-10mL), amount of precursor salt (2-10 mL) and pH (7-12). Synthesized ZnO-NPs were characterized by analytical techniques UV-Vis and FT-IR [8].

2.4. Antibacterial assay

Antibacterial activity was performed as earlier reported well- diffusion method [17, 18]. The solution of nutrient broth prepared in sec. 3.2 was incubated for 24 hours at 37° C with the bacterial strains of *Klebsiella pneumonia*, *Proteus mirabilis* and *Staphylococcus aureus*. These prepared strains were added (1.0 mL each) in sperate flasks containing nutrient agar. This bacterial strain containing agar was poured into petri plates when it cools down to 50°C. The medium was allowed to solidify then by using sterile borer of 8 mm wells were made in the medium. Different concentrations of ZnO-NPs (20, 40, 60, 80, 100 µL/ mL) and the drug Ciprofloxacin (1000 ppm) solution was poured in the wells. Water and mucilage were used as positive control. All these prepared plates were incubated for 24 hours at 37°C. After completion of 24 hours incubation the zones around each well having no bacterial growth were measured.

2.5. Anti-inflammatory activity

Anti-inflammatory activities of biosynthesized ZnO-NPs were carried out via following “the denaturation of proteins” [19]. BSA (0.2 %; v/v) solution prepared in sec. 3.2 was mixed with ZnO-NPs (50 µL) in different concentrations (100%, 50%, 25%). BSA (0.2 %) solution was mixed with water (50 µL) and Diclofenac (100 µg/mL). Water and Diclofenac were used as negative and positive control respectively. All the samples were heated at 72°C for 5 min. and absorbance was measured at 276 nm [20].

Anti-inflammatory activity = [Absorbance of control – Absorbance of test sample / Absorbance of control] x 100

2.6. Catalytic activities of ZnO-NPs

The prepared solutions of Congo-Red dye were mixed (1.0 mL) with distilled water (11.0 mL) in each bottle to make the volume up to 12 mL. ZnO-NPs of different concentrations (1, 2 & 3mL) were added under constant shaking and UV-Vis spectra was taken (at 0min, 15mins & 30mins) in between the range of 200-800nm.

3. Results and discussions

3.1. Extraction of mucilage and Biosynthesis of ZnO-NPs from seeds

A yellow color mucilage was extracted from *L. royleana* seeds for biosynthesis of ZnO-NPs. ZnO-NPs were successfully synthesized by using this seed extract with exceptional stability and their characterization was studied [8].

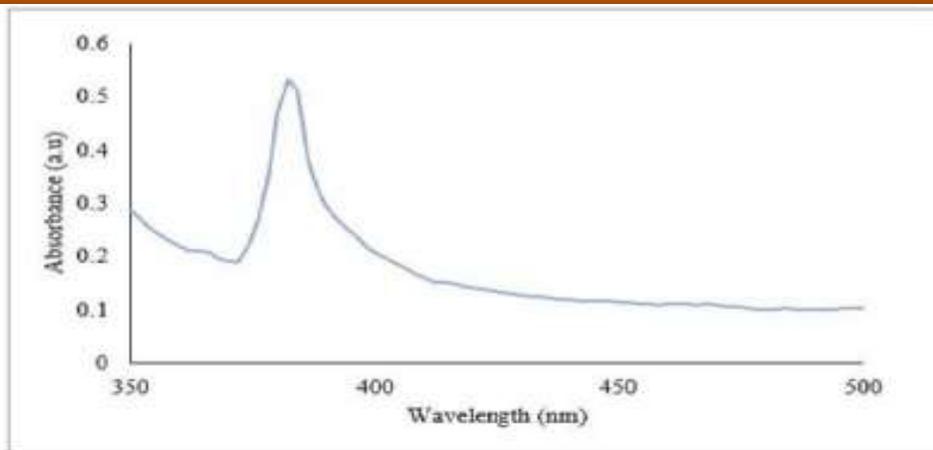


Fig. 1. UV-Vis Spectra of ZnO-NPs

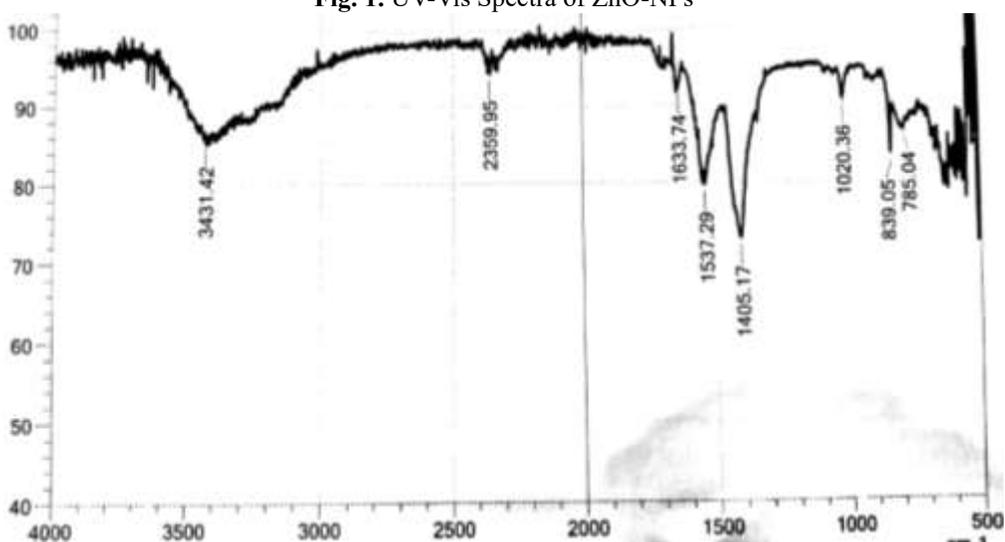


Fig. 2. FT-IR spectra of ZnO-NPs

3.2. Optimizing the reaction parameters

In biosynthesis of ZnO-NPs, the reaction parameters i.e., amount of zinc-acetate-dihydrate, pH, and amount of *LR*-mucilage played a crucial role. For optimizing all the reaction parameters UV-Vis spectroscopy was utilized for monitoring NPs synthesis. The UV-Vis spectra of the NPs are extremely sensitive to the particle's shape concentration and size. Metallic NPs while interacting with light showed collective oscillations similarly ZnO-NPs showed efficient absorbance and effective light scattering potential within the range 250-400nm. During process of synthesizing ZnO-NPs the reaction parameters could be controlled efficaciously by the proper usage of UV-Vis spectroscopy. For optimizing reaction parameters of current research study UV-Vis spectra was measured by varying one parameter at one time while the rest of the reaction condition were kept the same.

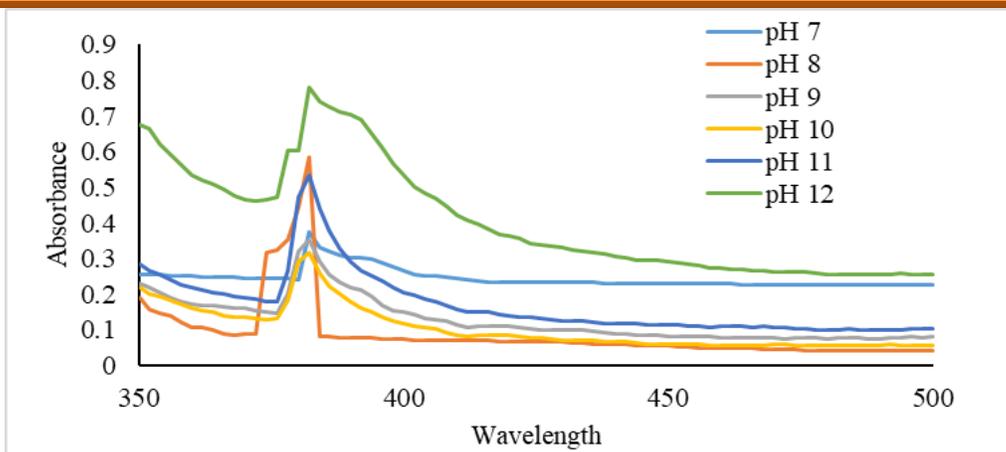


Fig. 3. Variation in Spectra by varying pH

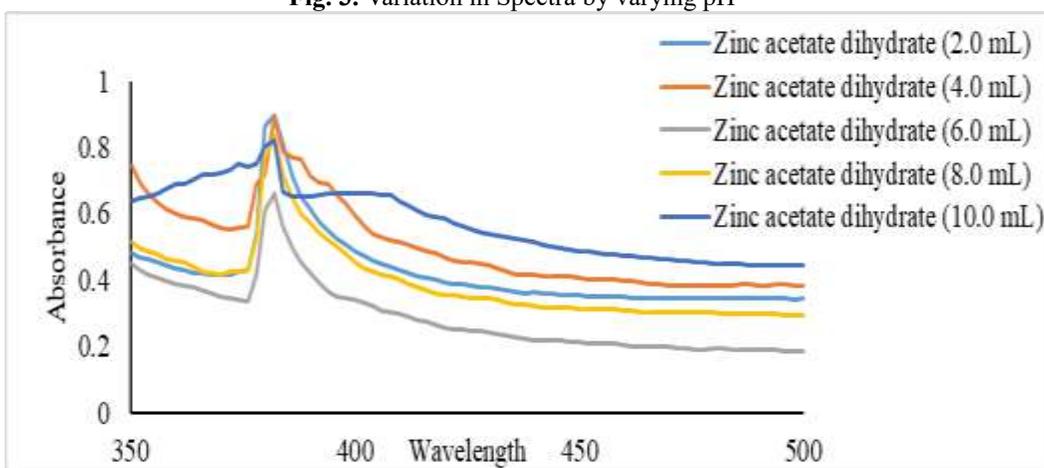


Fig. 4. Variation in Spectra by different volume of Zinc acetate dihydrate

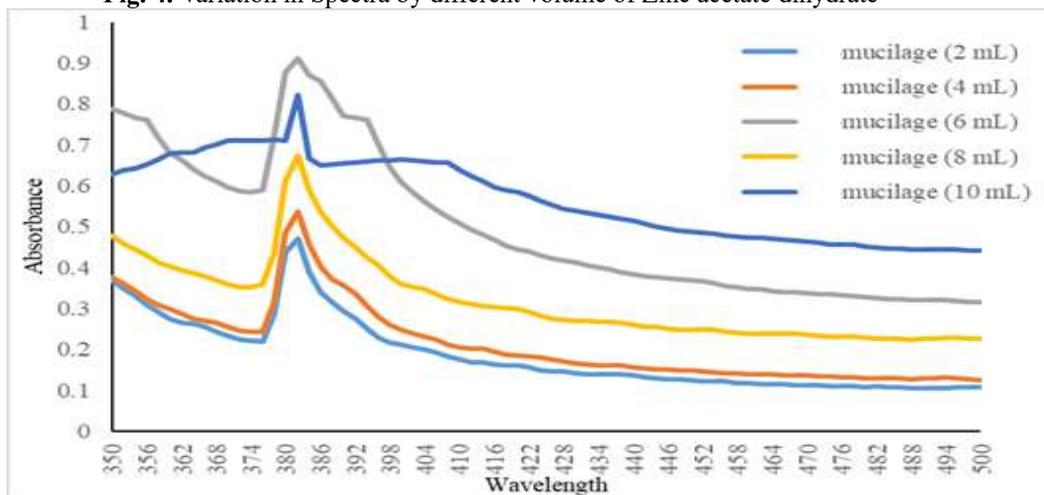


Fig. 5. Variation in Spectra by different volume of *L. royleana*-mucilage

3.3. Antimicrobial assay

The anti-microbial activities of synthesized ZnO-NPs and *L. royleana*-mucilage was examined against four pathogenic bacteria including one Gram positive (*Staphylococcus aureus*) and three Gram negative (*Proteus mirabilis*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). The result showed that ZnO-NPs have greater potential against *Klebsiella pneumoniae* and *Proteus mirabilis* as compared to other tested bacteria i.e., *Pseudomonas aeruginosa* and *Staphylococcus aureus* with the zones of

inhibition ranges from 11-16 including the well. Current results are comparable to the standard antibiotic Ciproflaxin. The *L. royleana*-mucilage showed very negligible activity.

Table 1: Anti-microbial assay of ZnO-NPs

Bacteria	<i>L. royleana</i> -mucilage (mm)	ZnO-NPs zones (mm)				
		20 μ L	40 μ L	60 μ L	80 μ L	100 μ L
<i>Staphylococcus aureus</i>	10	11	13	11	10	13
<i>Proteus mirabilis</i>	10	14	13	11	12	13
<i>Klebsiella pneumoniae</i>	10	16	13	12	13	14
<i>Pseudomonas aeruginosa</i>	10	11	13	10	11	13

3.3.1. Mechanism of action

Anti-microbial activities of NPs depend on i) Bacteria type ii) Physio-chemical properties of NPs

Our results showed great potential of ZnO-NPs to inhibit the growth of all the tested pathogenic bacteria (Fig. 6) which is similar to the findings of Dobrucka *et al* [21].

It is reported by studies that the anti-bacterial activities by these synthesized NPs might be because of the formation of reactive-oxygen-species (ROS). ROS species includes hydroxyl radical, singlet oxygen, hydrogen peroxide and superoxide radicals. These radicals can damage bacterial DNA and its proteins [21].

In the current work ZnO-NPs could be possible source for the formation of ROS which led towards inhibiting the pathogenic bacterial growth.

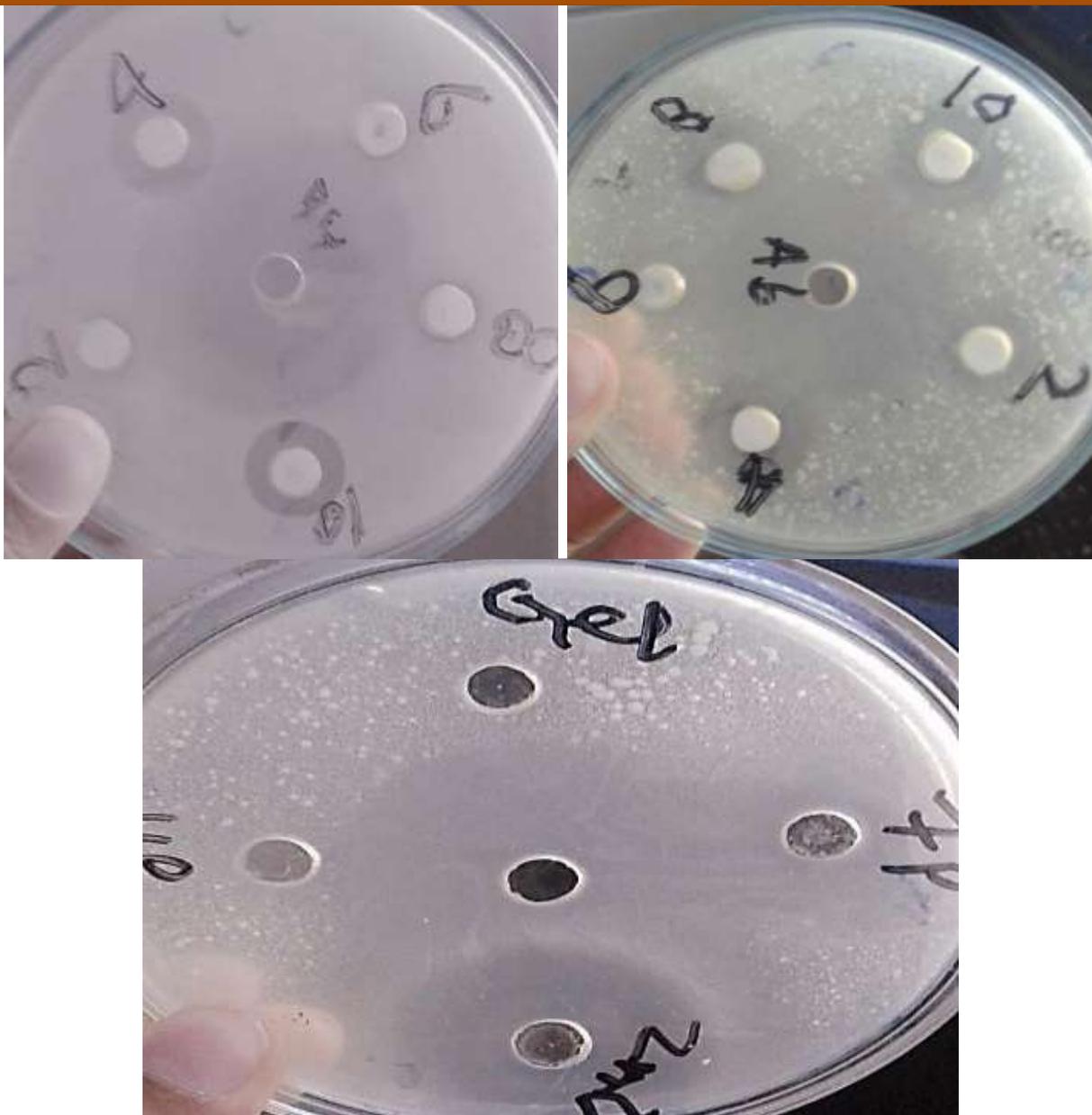


Fig. 6. Anti-microbial activities of ZnO-NPs

3.4. Anti-inflammatory activity

Anti-inflammatory activities of bio-synthesized ZnO-NPs were monitored to know the potential of NPs to be used in medicinal field, which showed that by increasing ZnO-NPs the inhibition activity also increases with increasing the dose as shown in Figure. Rajakumar *et al.* utilized ZnO-NPs synthesized by using leaf extract of *Andrographis paniculata* and reported the anti-inflammatory activity which support the results of our research work [22].

Few other studies also reported the same inhibition effect shown by ZnO-NPs which proved the potential of these NPs to be used in bio-medicinal fields [14, 21].

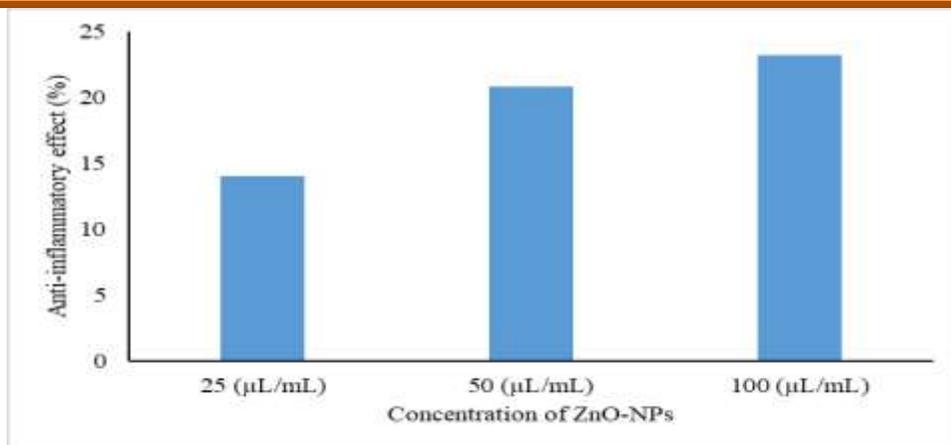


Fig. 7a. Anti-inflammatory effect shown by ZnO-NPs (2.0 mL)

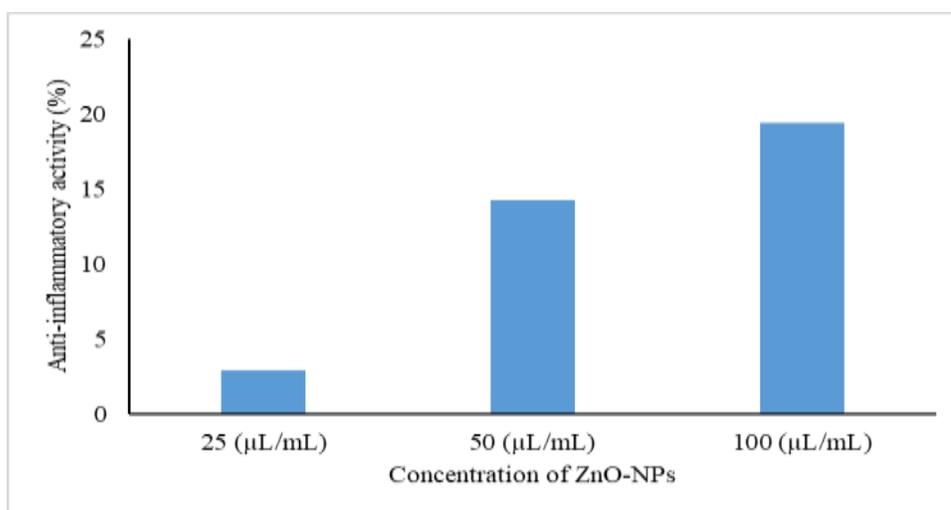


Fig. 7b. Anti-inflammatory effect shown by ZnO-NPs (10.0 mL)

3.5. Catalytic degradation of ZnO-NPs with Congo-Red dye solution

Congo-Red dye was selected as model organic pollutant as its excessively used material in the dye industry. CR ($C_{32}H_{22}N_6Na_2O_6S_2$) is a secondary dye of the salt of sodium 3,3'-([1,1'-biphenyl]-4, 4'-diyl) bis(4 - aminonaphthalene-1-sulfonic acid). It shows highest peak of absorption at 500nm [23].

The CR dye photo-degradative spectra are shown in the figures which revealed the gradual decrease of CR in presence of ZnO-NPs. While the reduction rate of CR is directly proportional to volume of the NPs e.g., increasing volume of ZnO-NPs (3mL > 2mL > 1mL) the rate of degradation increase. Our study results are in harmony with the outcome of Gaur *et al* that proved ZnO-NPs are effective options for removal of industrial dyes [23].

Kumar *et al.*, elaborated the degradation process in terms of production of reactive oxygen species including hydroxyl radical, superoxide radical and hydrogen peroxide which initiates the process of photo-degradation [24]. The production of these radicals on ZnO-NPs surface is because of the redox reaction of water on NPs surface. The studies conducted by Keyikoglu et al showed production of free oxygen radicals via electron-para-magnetic and scavenger techniques that support our research results [25].

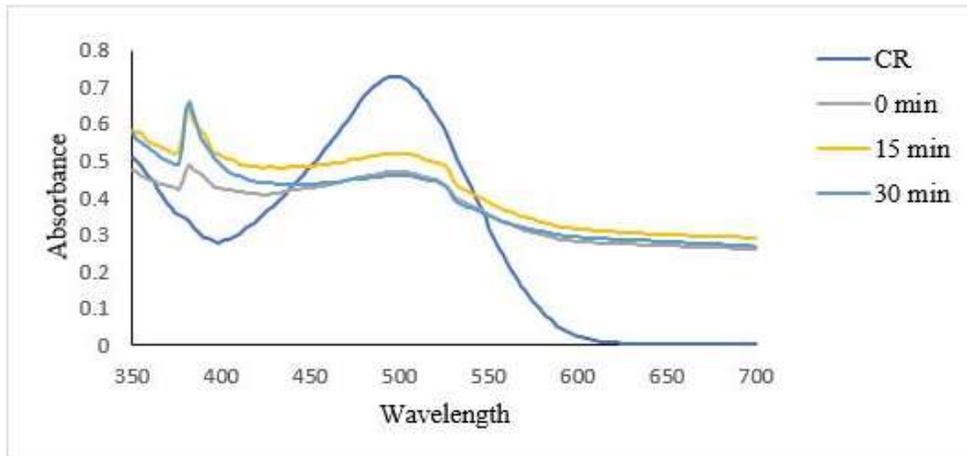


Fig. 8a. Catalytic-degradation of CR with ZnO-NPs (1mL)

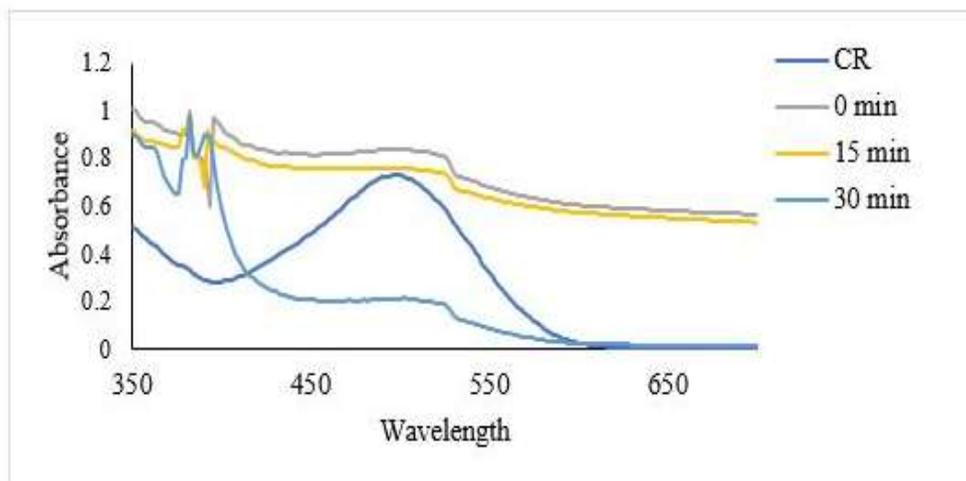


Fig. 8b. Catalytic-degradation of CR with ZnO-NPs (2mL)

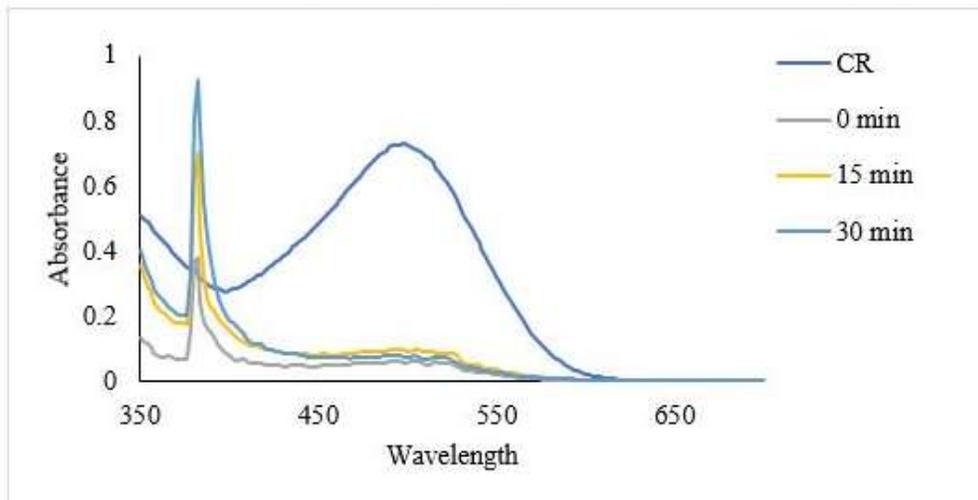


Fig. 8c. Catalytic-degradation of CR with ZnO-NPs (3mL)

4. CONCLUSION

Present research work presents an environment-friendly, cost-effective, efficient and simple method that is based on the approach of green-chemistry for synthesizing of ZnO-NPs by reducing zinc-acetate-dihydrate salt using plant extract as stabilizing and reducing agent. Current findings indicate impressive biological and catalytic properties of the biologically synthesized ZnO-NPs.

The biosynthesized ZnO-NPs are highly bio-compatible and non-toxic in nature. These NPs inhibit the protein denaturation and exhibit highly anti-inflammatory nature. Their non-toxic, anti-bacterial and anti-inflammatory behaviour enables these NPs to be extensively used in bio-medicines.

The current research exhibited that biosynthesized nano-particles by this cost-effective method is ready to be utilized in nanomedicines in response to pathogenic bacteria. Additional detailed studies are needed to understand cellular and molecular mechanism of zinc oxide nanoparticles and their effects on microbes. These ZnO-NPs can be utilized in various applications i.e., biomedicines, catalysis, and magnetic equipment due to their strong magnetic and catalytic potentials.

The synthesized ZnO-NPs has been highly effective against degrading the catalytic dye Congo-Red which proved that these NPs to be efficient in removing contaminations from wastewater. Applications of ZnO-NPs are efficient for removing metal contamination from the wastewater because of their properties i.e., larger surface area, and small size.

SUGGESTIONS

However, further detailed *in-vivo* and *in-vitro* studies are required to know more benefits of these bio-synthesized nano-particles by involving animal models which can assist in synthesis of highly bio-compatible zinc oxide nano-materials.

5. ACKNOWLEDGEMENT

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CONFLICT OF INTEREST: Nil

6. References:

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