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**A dissertation report on
“Comparative analysis of antioxidant and antibacterial properties of leaf extract and zinc nanoparticles synthesized from leaf extract of *Ziziphus nummularia*”**

By

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Under the supervision of

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Introduction

➤ Nanotechnology:

- It is the science and technology that deals with particles at the nanoscale (about 1-100 nanometers).

➤ Nanoparticles:

- A nanoparticle or ultrafine particle is usually defined as a particle of matter between 1 and 100 nanometers (nm) in diameter for example Zn Nps, Au Nps, Ag Nps, etc.

➤ Zinc nanoparticles:

- Second most abundant metal oxide after iron.
- Less toxic and biodegradable.
- Used as an active ingredient in antibacterial cream, lotions, and ointments.
- Reduces oxidative stress in cells.

Ziziphus nummularia

Ziziphus nummularia

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Rosales

Family: Rhamnaceae

Genus: *Ziziphus*

Species: *Z. nummularia*



- Commonly occurring much-branched thorny shrub.
- Occupies almost all habitats except saline patches and sand dunes.
- Leaves used in scabies and skin diseases.
- Alkaloid fraction in bark shows analgesic activity.

Objective

The main purpose of this study was to obtain the following objective:

1. To perform green synthesis of zinc nanoparticles using *Ziziphus nummularia*.
2. To perform a comparative study on the antioxidant activity of Zn Nps and *Ziziphus nummularia*.
3. To perform a comparative study on the antioxidant of Zn Nps and *Ziziphus nummularia*.

Methodology

► Preparation of methanolic leaf extract:

Leaves of *Ziziphus nummularia* were collected, washed thoroughly, and then air dried for two days followed by crushing.



Leaf powder(50g) was homogenized in methanol(250ml).



The mixture was kept in an orbital shaker for 24 hrs at 50rpm and 37°C, and then the extract was obtained by the filtration process.

Methodology

► Green Synthesis of Zinc nanoparticles :

Methanolic extract and Zinc acetate solution was taken in ratio of 1:3 (50ml extract, 150 ml zinc acetate)



The solution was then titrated against NaOH so as to maintain the pH of the solution.



The solution was kept in an orbital shaker for 24 hrs at 150rpm and 37°C.



The solution was then centrifuged so as to wash the green synthesized nanoparticles.

► Comparative Analysis of Antioxidant Activity

- 1. DPPH radical scavenging activity:** Ziziphus leaf extract and ZnO NPs have added one mL at various concentrations (20, 40, 60, 80, 100, 120, and 140 μ L/mL) to 4 mL of a 0.004% methanol solution of DPPH. After that, they were maintained in a dark, room-temperature environment for 30 minutes. At 517 nm, the absorbance was measured in comparison to a methanol blank.
- 2. Phosphomolybdate(TAC) assay:** It is a crucial in vitro antioxidant assay to assess the total antioxidant capacity (TAC) of the plant extract. The assay's basic premise is that Mo (VI) is converted to Mo (V) by an extract or a substance with antioxidant capability, resulting in green phosphate Mo (V).

► **Comparative Analysis of Antibacterial Activity:**

The antibacterial activity of ZnNps and the leaf extract was determined by using the MIC and Agar well diffusion method.

1. MIC(Minimum Inhibitory Concentration):

This minimal concentration of an antibiotic needed to halt microbial growth is calculated using a semi-quantitative test approach. An inoculum of bacteria was previously added using this technique to tubes of growth broth that contained a test amount of preservative. The test's result was the lowest antimicrobial concentration that created a clear solution, which meant that there was no growth or very little of it.

2. Agar well diffusion method:

The petri plates were filled with nutrient agar. After that spreading of inoculum was done followed by the making of wells. Each plate was divided into 4 quadrants each having negative control(nutrient agar), positive control(ampicillin), leaf extract and the ZnNps solution.

Results and discussion

► Preparation of methanolic extract

Methanolic extract of *Ziziphus nummularia* was prepared and its yield was 400ml.

► Green synthesis of Zinc nanoparticles

The change in color of the solution of methanolic leaf extract of *Ziziphus nummularia* and 5mM zinc acetate solution, from greenish to brownish indicated the formation of nanoparticles.

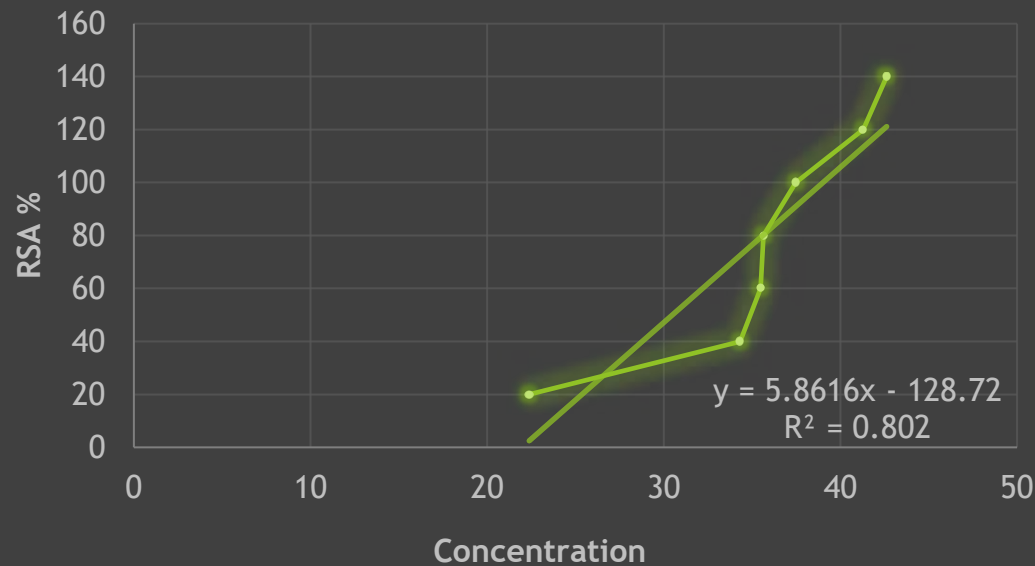
The weight of zinc nanoparticles so formed from 200 ml of leaf extract and zinc acetate in the ratio of 1:3 was found to be 40 mg.



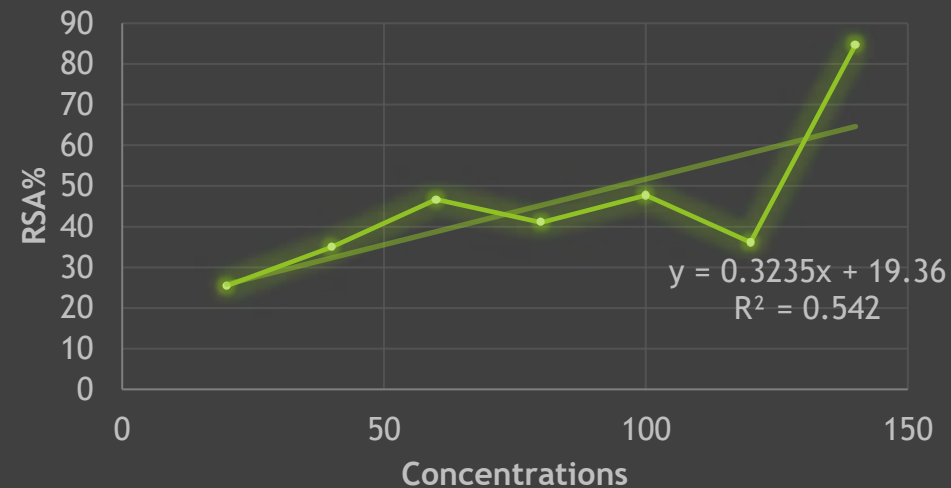
► Comparative analysis of antioxidant activity:

1. DPPH Radical Scavenging activity:

Radical scavenging activity
leaf extract



Radical scavenging activity
shown by
Zinc nanoparticles

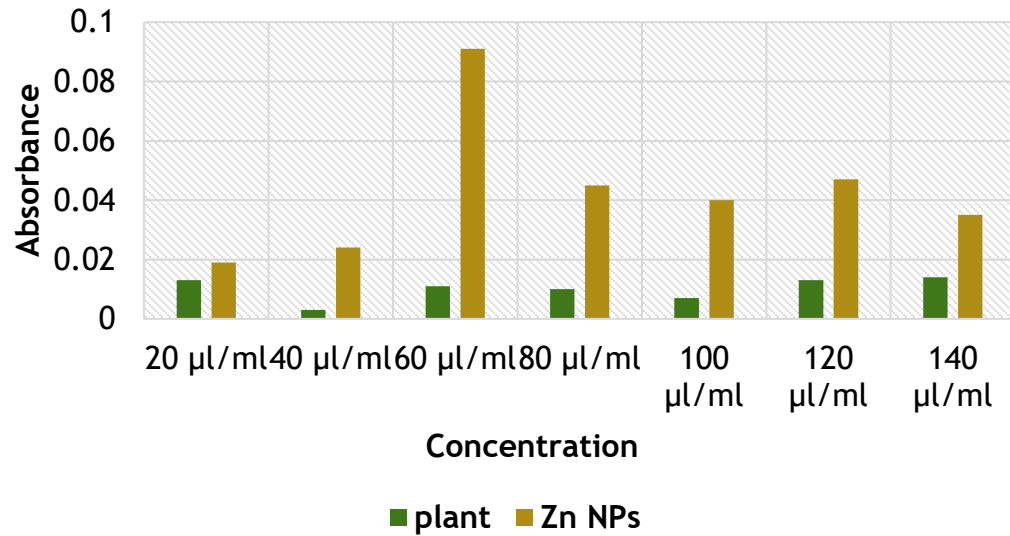


The IC₅₀ value for *Ziziphus nummularia* leaf extract is **30.49 µg/ml** and that for ZnNPs was found to be **94.71 µg/ml**. So we can say that antioxidant activity. So we can say that our leaf extract is showing better antioxidant activity as compared to the nanoparticles.

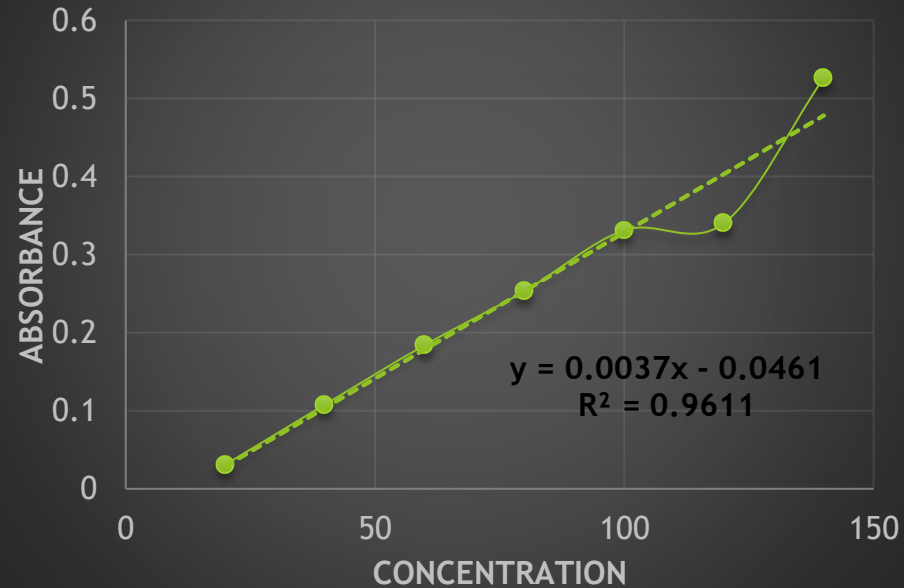
► Comparative analysis of antioxidant activity:

2. Phosphomolybdate TAC (Total Antioxidant Activity) assay:

Absorbance shown by the samples(Leaf extract and ZnNPs)



Absorbance shown by standard(Ascorbic Acid)



The IC₅₀ value for *Ziziphus nummularia* leaf extract is **30.49 µg/ml** and that for ZnNPs was found to be **94.71 µg/ml**.

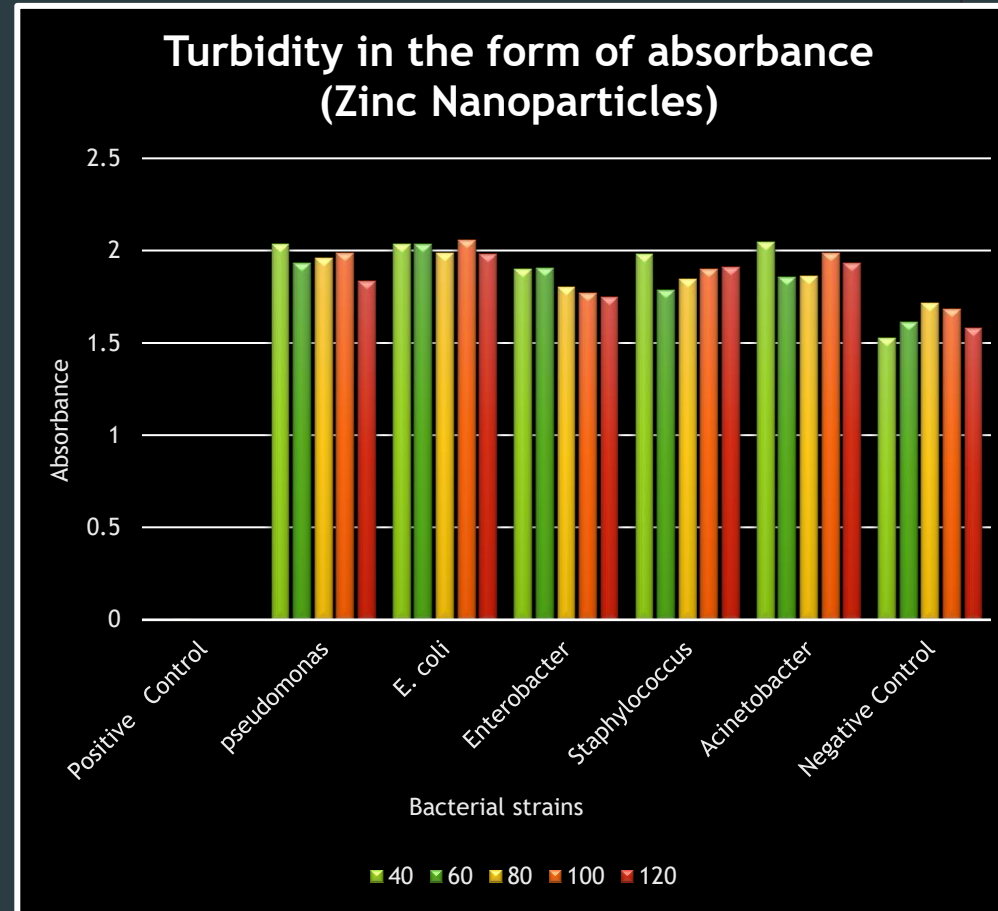
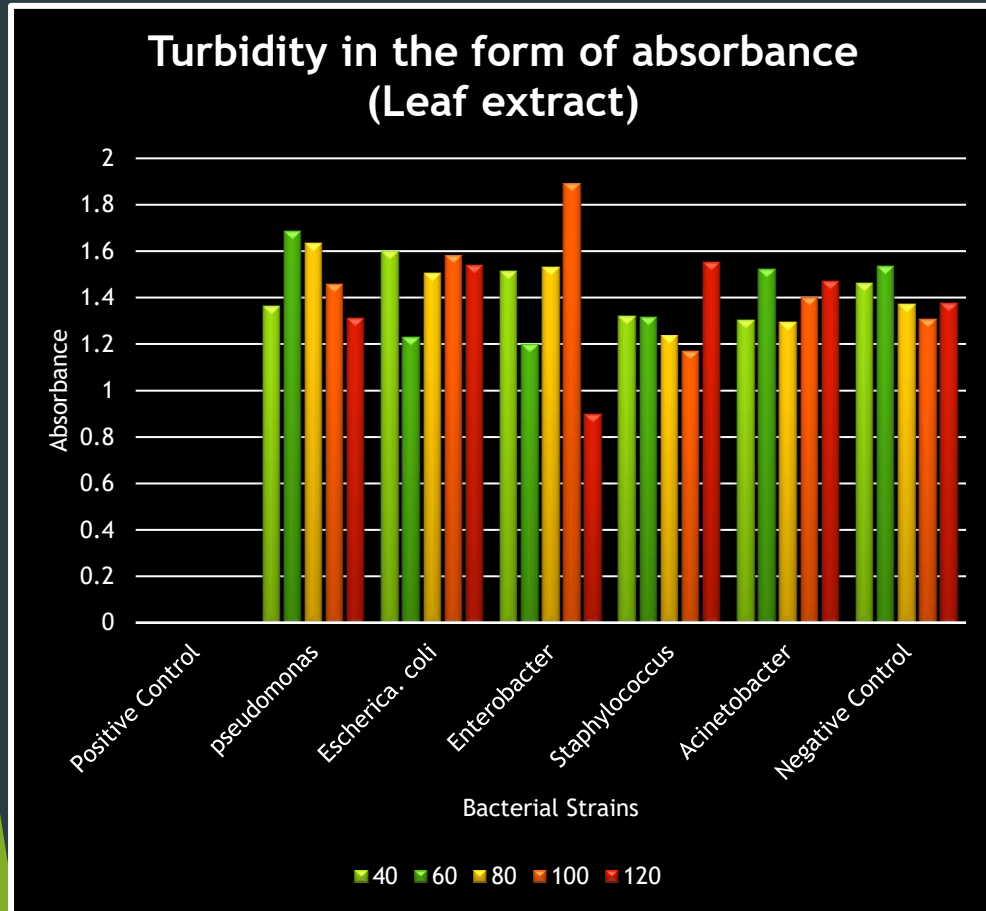
Concentrations of leaf extract and ZnNps in AEAC:

S. no	Concentration($\mu\text{g/ml}$)	Y(absorbance met. LE)	X(concentration in AEAC)
1.	40	0.013	15.973
2.	60	0.003	13.270
3.	80	0.011	15.432
4.	100	0.01	15.162
5.	140	0.007	14.351

S. no	Concentration($\mu\text{g/ml}$)	Y(absorbance ZnNPs)	X(concentration in AEAC)
1.	40	0.019	17.595
2.	60	0.024	18.946
3.	80	0.091	37.054
4.	100	0.045	24.622
5.	140	0.04	23.27

► Comparative analysis of antibacterial activity:

1. MIC (Minimum Inhibitory Concentration):



2. Agar well diffusion method:



Escherichia coli sp.



Staphylococcus sp.



Enterobacter sp.



Acinetobacter sp.



Pseudomonas sp.



Control

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Thank You!