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An investigation on the green manufacturing of zinc oxide nanoparticles using leaf extract from Ruta chalepensis and their effectiveness against fungal infections

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Abstract

Recently biosynthesis of nanoparticles received a scientific approach in the research field. ZnO nanoparticles have received remarkable attention due to their unique chemical and physical properties. Nanoparticles are studied extensively for their specific catalytic, antimicrobial, wound healing and anti-inflammatory properties. The main aim was to compare the yield, nature and antimicrobial activity of synthesized nanoparticles by using biological methods. Zinc oxide nanoparticles are known to be one of the most multifunctional inorganic nanoparticles. A recent study highlights the environmentally friendly method of producing zinc acetate dihydrate with ZnO nanoparticles (Nps) by utilising an extract from the leaves of the Ruta chalepensis plant. Also the prepared ZnO nanoparticles have been characterized by UV- vis absorption spectroscopy, and antifungal activity of ZnO nano particles was done by well diffusion method against pathogenic organisms like Fusarium oxysporum, Ustillago maydis, Aspergillus flavus, Aspergillus niger. This study also suggests that green synthesized Zn nanoparticles can be used as an alternative to existing antimicrobial agents.

Key words: *Zinc oxide nanoparticles, UV- vis spectroscopy, Ruta chalepensis, Antifungal activity.*

Introduction:

Nanoscience and nanotechnology have explored several new applications in biological science and biomedical research. Zinc Oxide nanoparticles have been studied for their various applications in many fields including medicine. Nanoparticles are synthesised using chemical and environmentally friendly procedures. Thus, we require an environmentally friendly and cost-effective technique for nanoparticle synthesis (Happy Agarwal et al., 2017; Zargar et al., 2017). Green techniques utilizing plants, fungi, bacteria, and algae have been introduced and modified due to the high doses of chemicals and harsh conditions used in the chemical manufacturing of NPs (Wani and Shah, 2012). In biosynthesis or green synthesis, plant extracts are used to synthesize the metal nanoparticles using metal salts. Each nanoparticle's surface properties are specifically based on the nature of the plant extract. Ag, Cu, Au, Pt, and Pd metal ions are widely reduced by converting various plant extracts to nanoparticles with unique surface properties. These noble metal nanoparticles conversely impose higher toxicity to the environment and have limited applications in biomedical science. Metal oxide nanoparticles contain different surface-based chemical and nontoxic properties. Compared to other metal nanoparticles, ZnO nanoparticles from green synthesis are potentially effective and beneficial for clinical and environmental applications.In recent years, ZnO has been most commonly employed for the breakdown of numerous organic contaminants. In addition, microbial contamination is a major concern in the healthcare and nutrition industries. In recent years, there has been a substantial focus on the development of antimicrobial agents and exterior coatings. Antimicrobial properties of nanoparticles have recently received a lot of attention. Oil glands are present in leaves having strong deterrent odors. Leaves and young stems of plants have high content of alkaloids, furocoumarins, comourins, flavonoids, phenols, amino acids and saponin. Essential oil was extracted by many researchers of Iran (Russian et al., 2002), Greece (zakou et al., 2001), Turkey (Baser et al. 1996) and India (Bagchi et al., 2003) from leaves of these plants. There are several species of Rutaceae family which have been used as medicinal plants such as *Ruta graveolens, Ruta patavina, Ruta corsica, Ruta angustifolia, Ruta montana, Ruta bracteosa*, etc.

Materials And Methodology:

Plant material and preparation of leaf extract:

Ruta chalepensis L. leaves were collected and shed dried and powder was prepared using a mixer grinder. 5 g of powder of *Ruta chalepensis L.* was dissolved in 100 millilitres of distilled water and magnetically spun for an hour at 70 degrees Celsius. After cooling to ambient temperature, the resultant solution was filtered using Whatman filter paper. The filtrate from the aqueous extract was used as a reduction agent.

Zinc oxide nanoparticle synthesis:

In order to create zinc oxide nanoparticles, 4 grams of dihydrate of zinc acetate was mixed in 60 ml of distilled water, and the mixture was stirred for ten minutes to create a 0.3M zinc acetate dihydrate was created. solution. 60 ml of the dihydrate of zinc acetate solution were mixed with 40 ml of the generated plant extract. After that, the mixture was agitated for a period of twenty minutes. To produce zinc oxide nanoparticles, the reaction mixture has been dried at 80°C for an entire night before being calcined for two hours at 100°C. The antibacterial qualities of the brown-colored zinc oxide nanoparticles that were produced following calcination were examined.

UV-Vis spectra analysis:

The sample's maximum absorbance was determined using UV-Vis spectrophotometry. Using visible and ultraviolet absorption spectrum analysis within the 350–450 nm region, the optical characteristics of nanoparticles composed of zinc oxide were examined.

Evaluation of zinc oxide nanoparticles' antifungal properties:

Strains of fungus: A. niger A. flavus, Ustillago maydis, and Fusarium oxysporum were tested for antimicrobial properties.

Assessing Antifungal Efficacy With The Agar Diffusion Assay:

Using the agar well diffusion method, the antifungal activity of the plant extract was evaluated against four types of fungi: A. niger flavus, Ustillago maydis, and Fusarium oxysporum. Potato dextrose agar plate was prepared and swabbed with 100 µl a mature broth culture of a specific fungus strain using a sterile L-shaped glass rod. The wells were prepared with sterile cork borers in each petri plate, 4 mm size of well are formed in plate. 40 µl sample of ZnO nanoparticles added in well. Nystatin served as a positive control. The plates were incubated for 48–72 hours at 28ºC. Following the incubation period, each well's zone of inhibition was evaluated and zinc oxide nanoparticle activity was tracked.

Outcome And Conversation:

The nanoparticles of zinc oxide were created using Ruta chalepensis leaves extract as the natural decreasing agent for the first time in this study utilizing an environmentally friendly, economical, and safe method. When compared to chemical synthesis, green production of ZnO nanoparticles is proven to be significantly safer and more environmentally friendly (Ogunyemi et al., 2019)

Figure.1- photograph of Ruta chalepensis plant

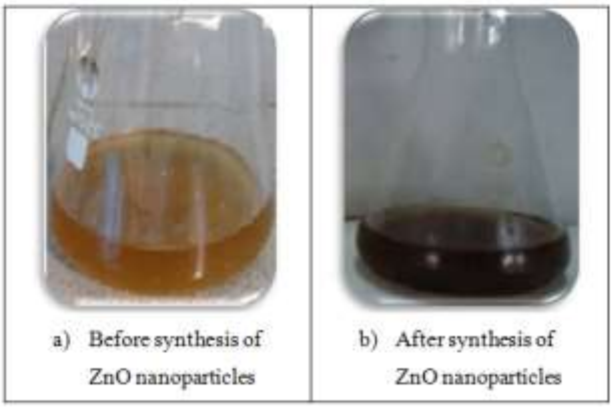


Figure 2: ZnO nanoparticle biosynthesis employing Ruta chalepensis plant leaf extract

Zno Nanoparticle Biosynthesis:
Visual and optical analysis of nanoparticles formation:
Combining Ruta chalepensis leaf extracts with zinc acetate dihydrate to cause physio-chemical reactions in the mixture. The reaction mixture's color somewhat shifted from light brown to dark brown. The formation of a brown precipitate at the conclusion of the procedure verified the production of ZnO nanoparticles. Plant extracts are enriched in flavonoids and phenolic compounds that strongly facilitate precipitation of Zn ions into Zn nanoparticles. The synthesis was further confirmed by spectral analysis. Figure 1 shows the absorption spectra for ultraviolet of an extract of biologically produced nanoparticles made from zinc oxide having a peak of absorption at 370 nm. The precipitate was dried in a hot air oven to generate zinc oxide nanopowder.

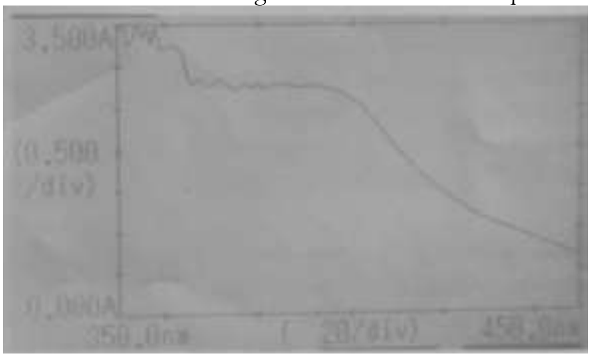


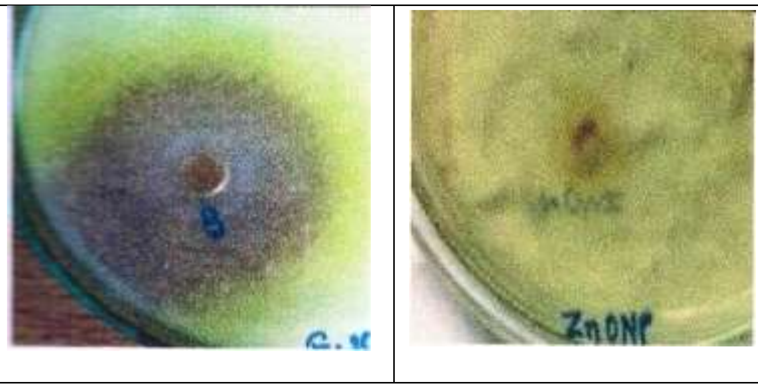
Figure 3: Biosynthesized ZnO nanoparticles UV-vis range of absorption

Antifungal Activity Of Zno Nanoparticles:
Mechanism of action:
Antimicrobial activities of metallic NPs are due to their high aspect ratio (size to surface ratio). The nano particles interfere with cellular processes once entering the microbes. Also, the nano particles' surface adhesion with the microbial cell surface leads to its immobilization.

Antifungal Activity:
The ZnO nanoparticles showed antifungal efficacy against Fusarium oxysporum, Ustillago maydius, Aspergillus flavus, and Aspergillus niger (Figure 4). The greatest zone of inhibition against Fusarium oxysporum measured 32 mm in diameter, followed by 30 mm against Ustillago maydis, 25 mm against Aspergillus flavus, and 13 mm against Aspergillus niger (Table 1).

Table-1. Antifungal Activity Of Zno Nanoparticles On Various Selected Pathogenic Fungal Strains

Sr.No.	Name of the Bacteria	Zone of inhibition (mm)	
		ZnO Nanoparticles	Nystatin (50µg/ml)
1	*Fusarium oxysporum*	32	12
2	*Ustillago maydis*	30	15
3	*Aspergillus flavus*	25	10
4	*Aspergillus niger*	13	10



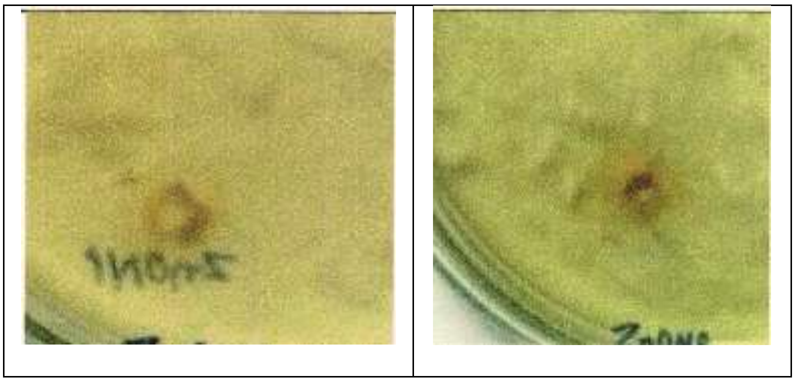


Figure 4: ZnO nanoparticles' antifungal activity against strains of Aspergillus flavus, Aspergillus niger, Ustillago maydis, and Fusarium oxysporum

Conclusion:

The current study describes an eco-friendly and straightforward way to green synthesis of ZnO nanoparticles utilizing Ruta chalepensis leaf extract, which acts as an effective reducing and stabilizing agent. Many biomolecules found in plants, including proteins, enzymes, amino acids, polysaccharides, alkaloids, alcoholic compounds, and vitamins, may play a role in the bioreduction, synthesis, and stability of metal nanoparticles. The prepared ZnO nanoparticles were examined using UV-visible spectroscopy, which revealed an absorption peak at 370 nm. For the first time, a green novel and environmentally friendly pathway using a natural extract of Ruta chalepensis extract as an effective oxidizing/reducing chemical agent was demonstrated. The produced ZnO nanoparticles had good antifungal efficacy against Fusarium oxysporum, Ustillago maydis, Aspergillus flavus, and Aspergillus niger strains.

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Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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