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Characterization and antibacterial activity of zinc oxide nanoparticles (ZnONPs) synthesized from plant leaf *Citrullus colocynthis* (Tamil name: Aatru thummatti)

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Abstract

Nanotechnology has grown in importance in modern material science research in recent decades. Green nanoparticles have sparked widespread interest due to their inherent characteristics of rapidity, environmental friendliness, and cost-effectiveness. In this study, zinc oxide nanoparticles were successfully synthesized for the first time using *Citrullus colocynthis* leaf extract. SEM with EDAX was used to investigate the morphology of zinc oxide nanoparticles. The crystalline nature of the nanoparticles was exposed through X-ray diffraction (XRD) experiments, which was responsible for reduction, stabilization, and capping agents. The disc diffusion method was used to test the antibacterial activity of zinc oxide against *Pseudomonas aeruginosa, E. coli, Klebsiella pneumoniae, and Staphylococcus aureus.* The ZnO nanoparticles had a strong antibacterial activity against the tested bacterial strains.

Keywords: zinc oxide nanoparticles, *Citrullus colocynthis*, characterization, antibacterial activity

Introduction

Biosynthesis of nanoparticles is a bottom-up approach to nanoparticles synthesis that involves the use of biological sources or components. One of the most common biological entities used is plant-mediated synthesis, in which bioactive phytochemicals obtained from plants are used to make nanoparticles. Plant extract-mediated nanoparticles synthesis has recently become one of the biggest common alternatives to traditional methods. Toxic chemicals and harsh conditions for reduction and stabilization are avoided in biosynthesis or green synthesis [1]. Biological sources have been used to successfully synthesize a variety of metal and metal oxide nanoparticles (2-5). ZnO nanoparticles (ZnO NP) have sparked a lot of interest due to their semi-conductive properties, high catalytic and photochemical activity, and unusual antifungal, antifungal, antibacterial, wound healing, and UV filtering properties [6]. Environmentally friendly, non-toxic, safe, and biocompatible ZnO NPs are suitable for biological applications [7-8]. ZnO, along with the other four zinc oxide compounds, has been classified as a commonly accepted safe (GRAS) substance by the US Food and Drug Administration [9]. ZnO nanoparticles have recently been used in food packaging and matrices. ZnO, which is integrated into the packaging matrix, has preservative properties for food [10]. Sunscreens, paints, and coatings, as well as antibacterial creams, ointments, and lotions, self-cleaning glass, and ceramics, all contain ZnO NPs [12]. The current study describes a green method for producing zinc oxide nanoparticles that uses *Citrullus colocynthis* aqueous leaf extract as a reducing/capping agent.

Material and Methods

Citrullus colocynthis leaves were collected in Chidambaram, Tamil Nadu, and Cuddalore District. All glassware was sterile, distilled water washed and dried in a hot air oven before use.

Preparation of Citrullus colocynthis leaf extract

Citrullus colocynthis leaves were collected and washed several times with running water before being cleaned with distilled water. 20g of which was boiled for 30 minutes in 100ml of double distilled water. After cooling, the aqueous extract was filtered with a Whatman No.1 filter and processed at 40°C for later use.

Synthesis of zinc oxide nanoparticles and optimization of synthesis parameters

Zinc nitrate hexahydrate was used to make zinc oxide nanoparticles. Leaf extract was applied in amounts of 5ml, 10ml, and 15ml to a 50ml solution of zinc nitrate hexahydrate at a 1M concentration. The colour shift was compared to aqueous leaf extract solution precipitates that were used as a control. Finally, the paste was collected in a ceramic crucible and annealed for 2 hours at 4000C in the Muffle furnace, producing a light yellow pigment powder that was carefully collected. With the aid of a mortar and pastel, the ingredients were powdered into a fine powder that was easy to identify.

Anti-bacterial assay of ZnO NPs

The antibacterial activity of ZnO NPs synthesized with Citrullus colocynthis extract was tested using the agar disc diffusion method against *Pseudomonas aeruginosa, Staphylococcus aureus, E.coli, Klebsiella pneumoniae, and*

Staphylococcus aureus. Bacterial cultures were subculture on a nutrient agar medium. Using sterile cotton swabs, each strain was evenly distributed onto the individual plates. Autoclaving was used to sterilize filter paper discs (Whatman no.1, 9mm diameter). Every paper disc was filled with ten milliners of the nanoparticles solution and allowed to air dry. The discs were placed on top of previously inoculated agar. After 24-hour incubation at 370°C, the different levels of the zone of inhibition of bacteria were measured.

Results

XRD analysis

X-Ray Diffraction (XRD) measurements were used to investigate the crystalline phase structure and size of metal oxide nanoparticles. The crystal lattice indices and particle size of ZnONPs were measured using X-ray diffraction patterns. XRD patterns of ZnO-NP are shown in figure 1. Diffraction peaks were found at 31.77^0, 34.44^0, 36.26^0, 47.52^0, 56.58^0, and 62.85^0, 67.91^0, respectively, corresponding to lattice planes (100), (002), (101), (102), (110), (103), (112), which is in good agreement with powder ZnO obtained from (JCPDS-89-1397) attributable to a hexagonal phase of ZnO. The particle size was calculated using Debye-Scherrer from the extreme peak corresponding to the (101) plane.

$$D=k\lambda/\beta\cos\theta$$

The particle size was calculated to be 34nm using FWHM (Full with Half Maximum of the peak located at 2=36.260), k=Scherrer's constant=X-ray wavelength (1.5406 A0), and =Bragg's angle of diffraction.

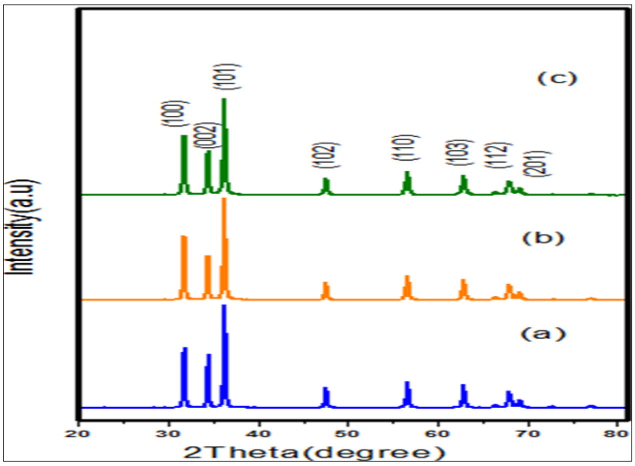


Fig 1: XRD patterns of ZnO NPs using *Citrullus colocynthis* leaf extracts (a) 5 ml (b) 10 ml (c) 15 ml. (show unassigned peaks)

FTIR spectra analysis

The zinc oxide nanoparticles from *Citrullus colocynthis* had an FTIR spectrum in the range of 3424-452 cm^{-1}, which helped distinguish the potential functional groups involved in the nanoparticles. The presence of strong, wide bonds was revealed in the FT-IR spectra due to the O-H stretching of alcohol groups. The strong, deep absorption bands at $3424cm^{-1}$ represent the stretching of alcohol groups O-H. The C=C stretching vibrations of primary amines are responsible for the absorption band at about $1632cm^{-1}$. The elongation The O-H vibrations of aromatic groups are responsible for the vibration bounds observed at $1382cm^{-1}$. The $1110cm^{-1}$ band shows the presence of C-O stretching in alcohol, carboxylic acids, an ester, and other group compounds. The stretching vibration of zinc oxide NPs is verified by the band at $452cm^{-1}$.

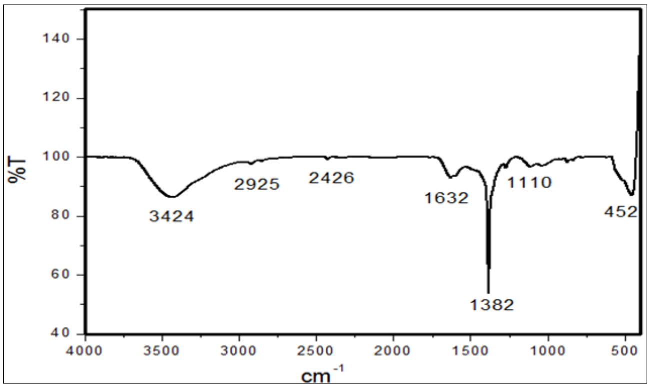


Fig 2: FTIR Spectrum of Zinc oxide nanoparticles (ZnO NPs) synthesized using *Citrullus colocynthis* aqueous leaf extract.

SEM with EDAX

A scanning electron microscope is used to study the surface morphology of the resulting powder. The morphology of zinc oxide nanoparticles is spherical and well distributed with an agglomeration shape, as shown in the figure (fig 3 (a), which is consistent with previous studies. agglomeration shape, on the other hand, has been observed, which is most likely due to the high surface energy of ZnO-NPs that occurs when they are synthesised in an aqueous medium, as well as densification, which results in a narrow space between particles. The EDS spectrum of ZnO-NPs shows only peaks of zinc and oxygen atoms, as seen in the limit, indicating that the ZnO-NPs prepared are basically impurity-free. Zinc has been correctly identified since the identification lines for the major emission energies for zinc and oxygen correspond to peaks in the spectrum [13].

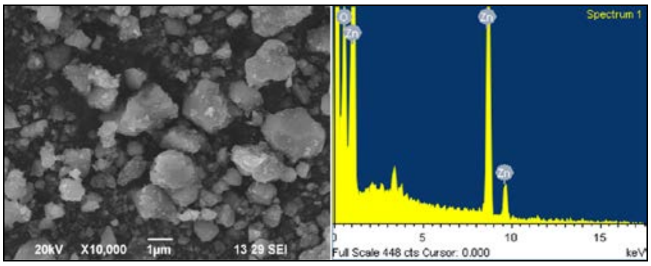


Fig 3: SEM with EDAX imgae of zinc oxide (ZnONPs) synthesized using *Citrullus colocynthis aqueous* leaf extract.

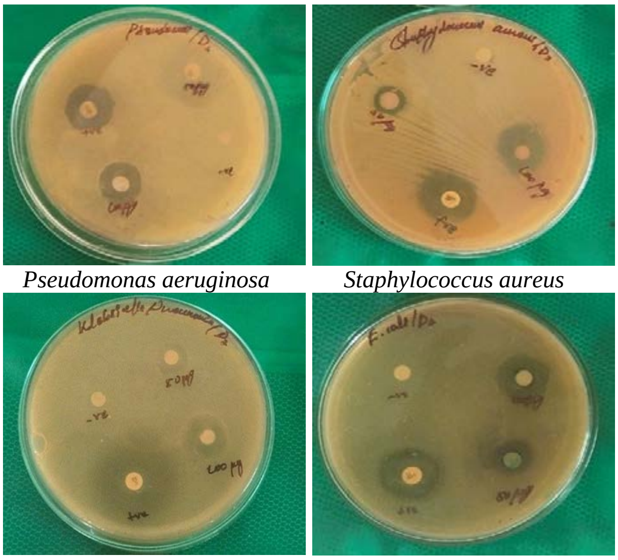
Antibacerial activity

Table 1: Antibacterial activity of ZnO-Sample *Citrullus colocynthis against* bacterial pathogens

S. no	Bacterial pathogens	Zone of Inhibition (mg/µL)			
		50 µg	100 µg	Positive	Negative
1	*Pseudomonas aeruginosa*	-	13.32±0.54	15.28±0.48	-
2	*Staphylococcus aureus*	08.12±0.46	12.04±0.82	15.27±0.64	-
3	*Klebsiella pneumoniae*	09.26±0.72	14.64±1.08	17.38±0.82	-
4	*Escherichia coli*	09.41±0.12	13.48±0.74	15.08±0.62	-

-: No zone of inhibition. Values expressed in mean ± standard deviation.

The ZnO *Citrullus colocynthis was* analyzed for antibacterial activity against selected bacterial pathogens on MHA agar plates. The ZnO *Citrullus colocynthis displayed* significant antibacterial activity against selected pathogens according to the concentration used. The maximum growth inhibitory activity was observed against *Klebsiella pneumonia* followed by *Escherichia coli*, and *Pseudomonas aeruginosa*. However, the ZnO *Citrullus colocynthis exhibited* lesser inhibitory activity against *Staphylococcus aureus* when compared with other tested bacterial pathogens. The standard antibiotic Chloramphenicol recorded zone of inhibition ranged from 15 to 17 mm.



Pseudomonas aeruginosa *Staphylococcus aureus*

Klebsiella pneumoniae *Escherichia coli*

Fig 4: Antibaceterial activity image of zinc oxide (ZnONPs) synthesized using *Citrullus colocynthis aqueous* leaf extract.

Conclusion

With an average size of 34 nm and a hexagonal shape, zinc oxide nanoparticles formed quickly by biosynthesis using Citrullus colocynthis leaf extract are inexpensive, non-toxic, and environmentally friendly, according to the findings. Biosynthesized zinc oxide nanoparticles have higher antibacterial activity than commercial drugs. As a result, the study results are expected to have far-reaching effects in the pharmaceutical and biomedical fields.

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