

The Effectiveness of Nano Indian Costus Root Extract on Some Types of (Candida) Isolated from the Respiratory System

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Abstract

Introduction. Many methods have historically been used to synthesize nanoparticles (NPs). However, many of these methods have proven to be expensive, producing toxic by-products and necessitating measures to mitigate chemical and physical pollution.

Purpose. Adoption of green nanoparticle synthesis using plant extracts has emerged as a sustainable alternative in the field of nanotechnology. This study explores the use of Indian premium root (Costus) extract for the synthesis of ZnO nanoparticles and their potential antifungal efficacy.

Methods. ZnO nanoparticles were synthesized using Indian costus root extract as a green, plant-based reducing and stabilizing agent. The synthesized nanoparticles were characterized using: UV-Vis visible spectroscopy, scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FT-IR) and X-ray diffraction (XRD).

Results. Characterization confirmed the successful synthesis of ZnO nanoparticles using costus root extract. The nanoparticles showed effective antifungal activity against various *Candida* species, including: *C. albicans*, *C. tropicalis*, *C. glabrata*.

Conclusion. The study demonstrates that Indian costus root extract can be used as an eco-friendly and effective agent for synthesizing ZnO nanoparticles. This green synthesis approach not only minimizes environmental impact but also shows promising antifungal properties, highlighting potential applications across scientific and industrial fields.

Key words: Indian costus, Green synthesis, Zinc oxide nanoparticles, *Candida* spp

INTRODUCTION

Respiratory diseases are common and significant cause of illness and death worldwide. In 2012, respiratory diseases most common causes of hospitalization in kids. In Pakistan, acute respiratory infections make up 30-60% of hospitalized outdoors, including 80% upper respiratory tract infections and 20% lower respiratory tract infections. The most common respiratory problems are: Asthma, Bronchitis, Colds, Cough, Whooping cough [1]. Asthma affects about 300 million people worldwide, and estimated that another 100 million will be affected in 2025 [2-3]. Field of Nano- biotechnology has transformative impact on production of metal oxide nanoparticles, providing remarkable benefits compared to conventional physical and chemical methods. [4,5,6] Plant techniques in green synthesis have received great attention due to their wide range for medical and biotechnological applications [7,8]. Zinc oxide nanoparticles (ZnO NPs) received great interest in many sectors as optics, electricity, packaged food and medicine, due to positive properties like biocompatibility. Low cytotoxicity, cost-effectiveness. Crucial element of zinc oxide nanoparticles (NPs) relates to their ability to cause cell death by facilitating production of reactive oxygen species (ROS) and release zinc ions (Zn²⁺), which exhibit cytotoxic properties [9,10]. In addition, zinc is necessary trace element found in human physiological system. It has biological compatibility due to its low toxicity and high biodegradability, that is attributed to solubility of zinc ions +2 [11]. However, therapeutic use in chemically synthesized zinc oxide nanoparticles is restricted as result of hazardous traits for chemicals used in production [11,12] and therefore, there is increasing tendency towards investigation of environmentally friendly production techniques in zinc oxide nanoparticles (ZnO NPs). Current developments have confirmed use of different plant ingredients as reducing agents in manufacture in zinc oxide nanoparticles [13]. Use of plant-based green synthesis techniques has many benefits that to traditional methods [14-15]. First, virtual methods are arguably properties by simplicity, enhanced safety measures, more sustainability, and more environmentally conscious approach that to traditional physical and chemical alternatives. Also, zinc oxide nanoparticles created via biological methods which have important biological traits, that made

suitable for wide range of biomedical uses. [16,17,18] Using nanoparticles extracted from diverse sectors[19].

MATERIALS AND METHODS

Preparation of Costus Indian Extract

Dandelion leaves were collected from local markets in Thi- Qar Governorate and were cleaned of impurities and washed with distilled water and then dried under period of 3-7 days Indian grinding dandelion leaves with electric grinder where weight of 20 g of dry vegetable powder was taken with 200 ml of ethanol alcohol (70%) in glass flask and then placed on magnetic marg device for three hours and then left solution to settle for one night in refrigerator at temperature of 4 ° C, then filtered using three Layers of medical gauze get rid of impurities and then placed in test tubes with volume 10 ml and discarded centrifuge and speed 4500 cycles / flour and for period 10 minutes, then take filtrate and filter fine filters with diameter Millipore filter syringe μm (0.22 μm) then put plant extract in Petri dishes and left these dishes in laboratory until extract dries and get dry powder[20]

Green Biofabrication of the ZnO-NPs

Prepared by dissolving 2.5 g of zinc oxide in 20 ml of ethanol 70% and mixture was stirred using the magnetic motor for 20 minutes at room temperature 25 degrees and 25 ml of vegetable extract at concentration of 1 mg / ml drop by drop was added to zinc oxide mixture, stirring for two hours, then separating precipitate from solution using centrifuge after that, white precipitate appears, white precipitate is washed with ethanol and distilled water several times, then drying process was carried out at temperature 100 pm duration 3 hours. [21]

Characterization of the Biogenic ZnO-NPs

Zinc oxide biosynthetic nanoparticles were characterized by using various ways, containing visible ultraviolet (UV) spectroscopy (Shimadzu), Japan to determine surface plasmon resonance of zinc oxide nanoparticles. Shimadzu, Japan infrared spectroscopy analysis used to determine basic functional groups for biosynthetic zinc oxide nanoparticles. Biosynthetic zinc oxide nanoparticles were subjected to X-ray powder diffraction (XRD) (Rigaku, Japan) to verify its crystalline nature and determine its crystal size, while morphology of prepared zinc oxide particles is determined by scanning electron microscopy (SEM) analysis (Broker, Germany)

Antifungal Effectiveness of ZnO-NPs

Sensitivity of three strains of *Candida* was evaluated, namely *C. albicans*, *C. glabrata*, and *C. tropicalis* to bio-zinc oxide nanoparticles, where effectiveness of bio-zinc oxide particles against laboratory fungal strains was evaluated. Using the method of drilling with acres, 0.2 of yeast stuck was taken for all types of *Candida* and then published on (SDA) by sterile swab by three repeaters for each type of mushroom *Candida* spp, and then left dishes to dry for 20 and then work of four holes by cork piercer with diameter of 6 mm and then put zinc oxide nanooxide in drilling at the following concentrations 100,200,300 mg / ml and control factor) (DMSO) and incubated dishes 37 m for 24-48 hours and then measured inhibitory diameters around hole containing zinc oxide nanoparticles, and concentrations of difference and measured by the ruler are listed and take rate for them. As well as minimum inhibitor at concentrations 5,10,15 mg/ml[22]

RESULTS

Physical and Chemical Characterization of Green-Synthesized Zinc Oxide Nanoparticles

UV-Vis spectroscopy

UV-Vis spectroscopy device is important tool for studying optical properties of nano materials, where absorption is measured as function of wavelength to determine multiple properties such as energy gap and particle size.[23] After optical observation of synthesis of zinc oxide nanoparticles in shape (1), solid powder of these particles was collected and dispersed in deionized water using ultrasound for five minutes to ensure homogeneous distribution of particles, and then synthesis process was verified using radioscropy. Ultraviolet-visible. The resulting spectrum (900-190) shows absorption of zinc oxide nanoparticles manufactured by green method using different plant extracts, namely Indian costus root extract.

The spectra showed characteristic absorption peaks at 321 Indian costus root extracts, corresponding to wurtzite hexagonal phase of zinc oxide in its crystalline state[24]. [25] studied synthesis of zinc oxide nanoparticles using green synthesis method based on *Calotropis* leaf extract. The nanoparticles produced were characterized using various techniques, including UV-visible spectroscopy, and results of spectroscopy showed characteristic absorption peak at 350 nm



Figure 1: Formation of Zinc Oxide Nanoparticles from Indian Costus Root Extract

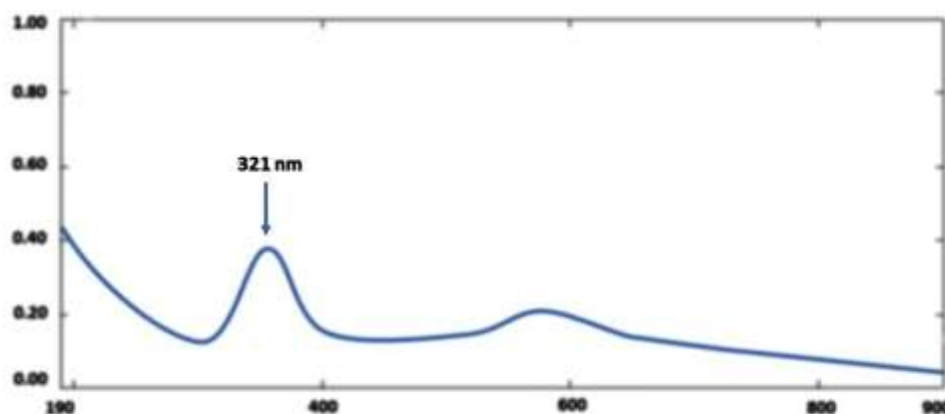


Figure 2 Ultraviolet-visible spectrum (UV-vis) of zinc oxide nanoparticles manufactured using Indian costus root extract

Scanning Electron Microscope - SEM

It is an advanced tool used to examine and analyze sample surfaces in fine detail. Scanning electron microscopy is based on the use of a beam of electrons to scan the surface of a sample, where it is scanned point by point, and then reflected electrons or secondary electrons emitted from the sample are detected to form a three-dimensional image with high resolution [26].

In the study [27], the shape and size of manufactured zinc nanoparticles, specifically how they appear and assemble based on the surface structure of particles, were analyzed, indicating that zinc nanoparticles were prepared and their shape and size were accurately determined by examining the surface. By analyzing the surface using scanning electron microscopy (SEM), it was found that particles clump together, meaning they form into lumpy or clustered structures. This aggregation can affect the properties of the substance, such as its catalytic activity or its chemical and physical properties. In other words, the shape and composition of the surface can play an important role in improving the functional properties of zinc oxide, such as catalytic activity or interaction with other substances.

Figure 6 describes the shape, size, and distribution of zinc oxide nanoparticles using Indian costus. Morphological analysis of zinc oxide nanoparticles using scanning electron microscopy (SEM) focusing on studying the shape and structure of these particles at the microscopic level. According to the description, the results from the SEM image indicate that synthesized zinc nanoparticles have a spherical shape. This means that particles take on a circular or semicircular shape, which is a regular shape and is common in some nanomaterials, especially when synthesized in certain ways such as green synthesis. Spherical and aspherical shapes have many benefits in industrial and environmental applications. For example, spherical particles possess a uniform and defined surface area, making them useful in applications that require equal interactions on all sides of a particle. They are often more stable in physical and chemical compositions.

than irregular shapes. The SEM image showed that nanoparticles that formed had clusters or clusters of them. The diameter of these particles ranges from 70 to 91 nm.

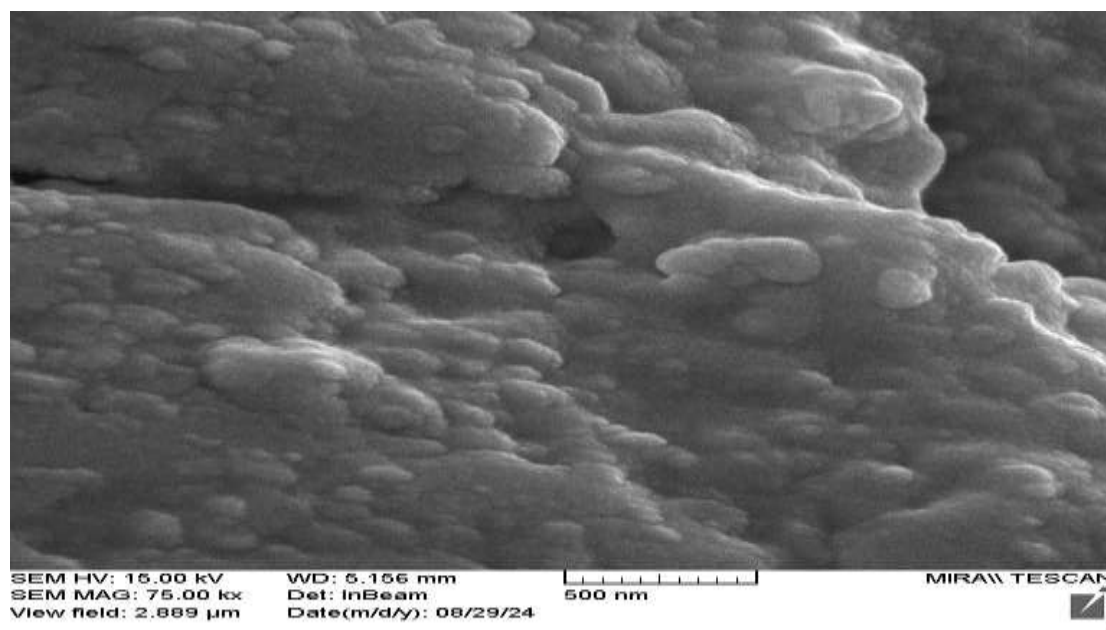


Figure 3 Scanning electron microscopy (SEM) images of zinc oxide nanoparticles synthesized using Indian costus extract

X-ray Diffraction(XRD)

X-ray Diffraction is analytical tool used to study crystal structure of materials. The device relies on sending an X-ray beam to the sample of material, and then measuring diffraction pattern resulting from interaction of rays with atoms within Article [28] Figure (4) illustrates XRD diffraction analysis of zinc oxide nanoparticles synthesized using plant extracts from cress plant, dandelion and Indian costus. All of resulting peaks showed match with zinc oxide nanoparticles, showing that primary material in particles is zinc oxide. Corner location 2θ for strong peaks: Indian premium peaks at angles 32.025° , 34.675° , 36.525° , 47.775° , 56.825° , 63.075° , 66.625° , 68.175° , 69.325° , 72.725° , 77.175° . The diffraction peaks matched the following crystal levels: 100, (002), (101), (102), (110), (103), (200), (112), (004), (202). The sharp and clear peaks indicate to the crystalline nature of nanoparticles, which are characterized by hexagonal crystal structure[29]. These levels were verified using Joint Committee on Powder Diffraction Standards (JCPDS) No. 01-083-6338. This shows identical crystal structure of zinc oxide in nanoparticles. Crystallite Sizes were determined using Debye-Scherrer equation based on width of dominant peaks in X-ray diffraction analysis. The result was Indian costus with size of 24.701 nm. Zinc oxide particles are formed in conditions that contribute to the formation of high-quality crystals, the absence of large shifts in XRD tops resulting from plant extracts indicates similarity of crystal growth mechanisms, where zinc oxide helps to stabilize natural hexagonal crystal structure. The sharp and clear peaks in patterns reflect high degree of crystallization, indicating formation of small but well-organized crystals, and use of Scherrer equation to calculate size of crystals highlights that particles are small in size, which enhances their crystalline and optical properties[29]. These results reflect great potential of synthesizing

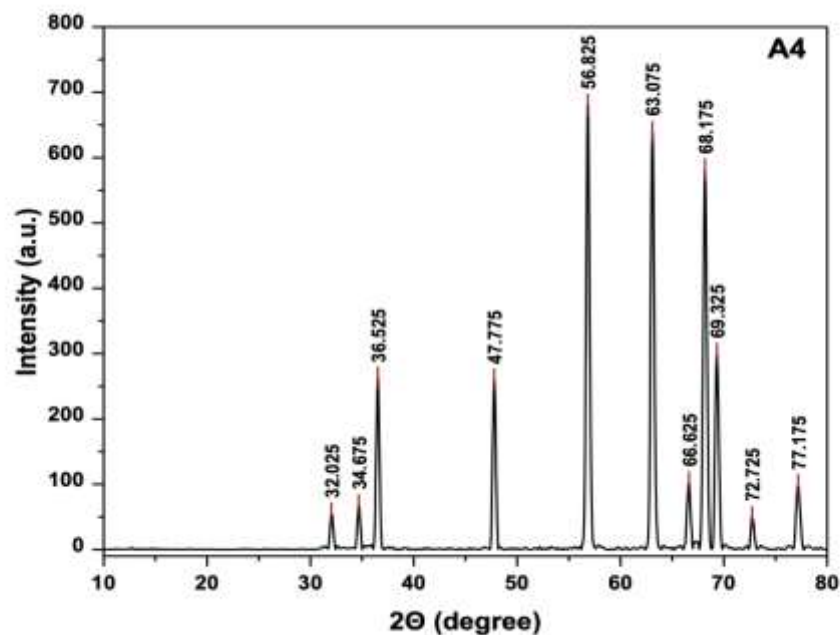


Figure 4 XRD X-ray diffraction spectra for zinc oxide nanoparticles manufactured using Indian costus plant extract

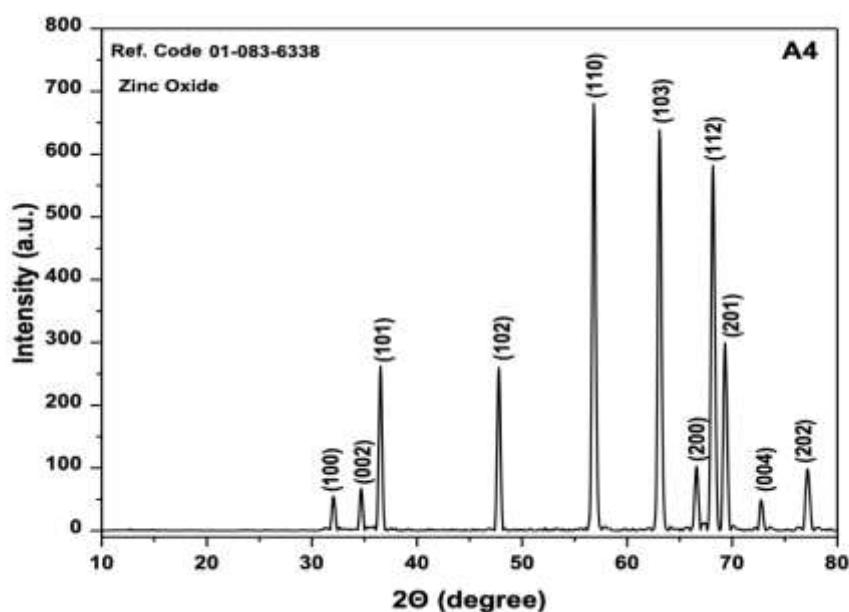


Figure 5: Miller Indices (hkl) crystal levels of X-ray diffraction XRD for zinc oxide nanoparticles manufactured using Indian costus plant extract

Fourier transform infrared spectroscopy

FTIR is important technique used to determine functional groups and chemical reactions on surface of nanoparticles, such as zinc oxide nanoparticles (ZnO). By studying absorption spectrum in spectral range $400\text{--}4000\text{ cm}^{-1}$, it is possible to determine the types of bonds and functional groups that exist on surface of these particles, giving us information about their chemical composition and stability [30], [31]. Figure (6) Infrared spectroscopy using Fourier transform of zinc nanoparticles prepared using Indian costus radicals. When using the Indian costus plant to synthesize ZnO nanoparticles, we can by means of infrared spectroscopy (FTIR) determine functional groups present in nanoparticles and clarify chemical bonds that contribute to their formation. A peak that appears at 3408 cm^{-1} indicates presence of O-H extensions, which are usually result of presence of alcohols in material. These hydroxylated groups can play role in synthesis of nanoparticles by participating in chemical reactions that contribute to stability of particles. Alternatively, this vertex may be caused by N-H extensions of elementary amines, compounds

that can be involved in process of particle synthesis and stabilization. Peaks at 2926 cm^{-1} and 1411 cm^{-1} indicate O-H extensions in carboxylic acids. Carboxylic acids may contribute to influencing properties of zinc oxide nanoparticles through formation of hydrogen bonds, which helps in improving stability and promoting

The peak at 1581 cm^{-1} represents N-H vibrations in amines. Amines may be nanoparticles stabilizers or act as stabilizers that prevent particle aggregation and ensure their survival in nanoscale. The peak at 1059 cm^{-1} refers to C-O extensions in alcohols, which can have role in influencing synthesis of nanoparticles. The peak at 818 cm^{-1} represents the C=C curvature vibrations in alkene compounds. The presence of alkenes may indicate unsaturated organic compounds, which may have role in formation of nanoparticles or in surface reactions that affect the properties of particles. Finally, peak at 780 cm^{-1} may indicate presence of C-Cl bonds in halogen compounds. These bonds can contribute to particle synthesis

The peak at 553 cm^{-1} is most important peak in confirming the formation of ZnO, as it reflects the vibrations of Zn-O. This summit confirms that zinc oxide may have formed successfully and that there are strong bonds between zinc and oxygen ions that form crystal structure of [30],[31] nanoparticles. All these peaks together play key role in understanding the composition of nanoparticles from ZnO, as they help identify compounds on surface of particles. In study[32], infrared spectroscopy of zinc nanoparticles showed peaks attributable to interactions with extract groups. A peak at 468 cm^{-1} representing metallic zinc oxide in nanoparticles appeared. These results suggest that compounds such as phenol, alcohol, amine, and carboxylic acid present in extract may contribute to the conversion of zinc ions into ZnO nanoparticles through chemical reaction between these functional groups and zinc ions, leading to the successful synthesis of nanoparticles. In another study[8], the plant extract of genus *Saussurea* played distinctive role in preventing agglomeration of nanoparticles and formation of secondary structures, where multiple functional groups present in it, such as the hydrate.

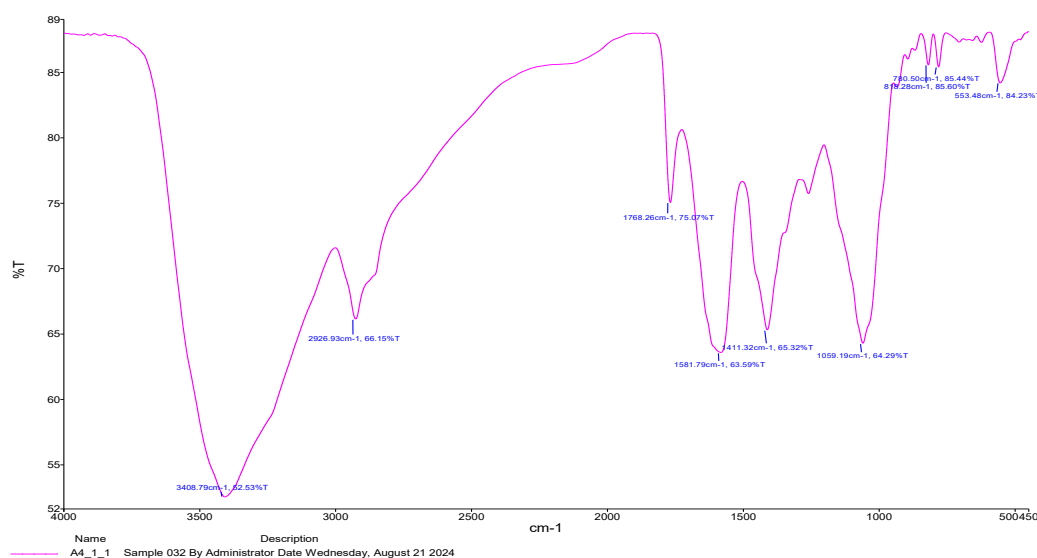


Figure6: Infrared spectroscopy using Fourier transform of zinc nanoparticles prepared using Indian costus plant

Evaluation of Anticandidal Effectiveness of Bioinspired ZnO-NPs

The efficacy biosynthetic zinc nanoparticles against candida pathogens investigated using palacar diffusion method by drilling, where zinc oxide nanoparticles showed highest efficacy against fungi at concentration of 300 mg/ml 31.33 ± 5.7735 , 30.00 ± 1.00 and $29.33 \pm 5.7735\text{ mm}$ in inhibitory areas *C.albican*, *C.glabrata*, *C.tropicalis* against respectively as shown in table. organelles and inactivation of large biological molecular activity representative of proteins or enzymes, cessation DNA replication and disruption antioxidant system through production reactive oxygen species (ROS) [33] It is consistent with study of [34], which indicated that zinc oxide particles are biologically protected from highly pomegranate peel aqueous extract against *C.tropicalis*, *C.albican*, *C.glabrata* at concentrations ($100\mu\text{g/disc}$) with an inhibition diameter of 24.18 ± 0.32 , 20.17 ± 0.56 and 26.35 ± 0.16 .

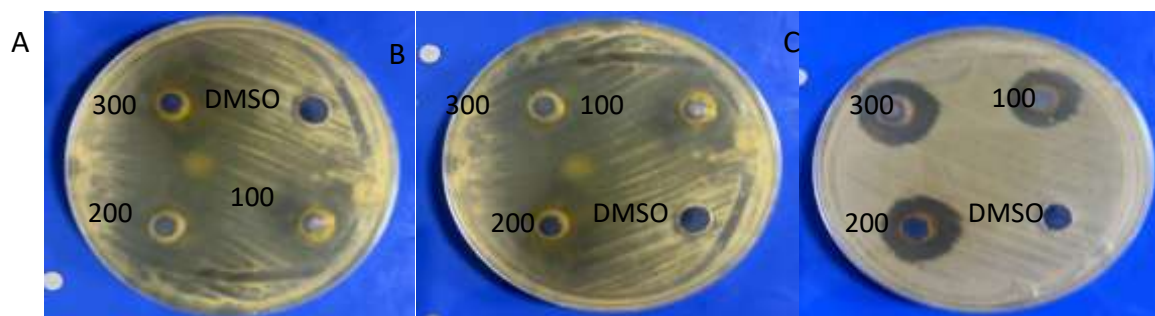


Figure 8: Inhibitory Activity of Zinc Oxide Nanoparticles against *Candida* strains. C: *C. glabrata*, B: *C. tropicalis*, A: *C. albicans*

Table (1) Evaluation of Anticandidal Effectiveness of the Bioinspired ZnO-NPs

Candidal Strains	Inhibition Zone Diameters (mm)			Average	Negative Control
	100mg/ml	200mg/ml	300mg/ml		
<i>C. albicans</i>	25.66±0.57	27.66±0.57	29.33±0.57	27.55	0
<i>C. glabrata</i>	27.00±1.00	29.00±1.00	31.33±.57735	29.11	0
<i>C. tropicalis</i>	26.33±.57735	28.66±.57735	30.00±1.00	28.33	0

Minimum inhibitor Evaluation of Anticandidal Effectiveness of Bioinspired ZnO-NP Table (2) shows that lowest inhibitory activity is at concentration of 5mg/ml for *C. albicans*, *C. glabrata*, *C. tropicalis* with an inhibition diameter of 2.33±.57735, 1.33±1.15 and 4.3333±.57735 mm, respectively, where [35] stated that the properties of nanoparticles such as size, shape, composition, surface charge and thermodynamic directly affect the interaction of nanoparticles with molecules or cells, and these results were consistent with what was recorded in [36] as zinc oxide nanoparticles showed inhibitory activity towards *C. albicans* at a concentration of 5.8 mg/. As previous study showed efficacy of zinc oxide nanosynthetic biosynthesis of aqueous extract *Girardinia diversifolia* leaves against *C. albicans* at concentration of 1mg/ml with inhibition diameter of 20.23±0.65mm

Table (2) Minimum inhibitor Evaluation of Anticandidal Effectiveness of the Bioinspired ZnO-NPs

Candidal Strains	Inhibition Zone Diameters (mm)			Average	Negative Control
	5mg/ml	10mg/ml	15mg/ml		
<i>C. albicans</i>	2.33±.57735	6.33±.57735	6.33±.57735	9.66	0
<i>C. glabrata</i>	1.33±1.15	7.33±1.15	10.66±.57735	6.44	0
<i>C. tropicalis</i>	4.3333±.57735	8.66±1.154	15.33±1.154	9.44	0

DISCUSSION

The current study demonstrates the successful green synthesis of zinc oxide nanoparticles (ZnO-NPs) using Indian costus root extract, highlighting an environmentally friendly and efficient method for nanoparticle fabrication. This green route not only minimizes the use of hazardous chemicals but also exploits the phytochemicals present in the plant extract as reducing and capping agents, contributing to the formation and stability of ZnO nanoparticles.

The characterization of the synthesized ZnO-NPs confirmed their nanoscale structure and crystalline nature. UV-Vis spectroscopy showed a sharp absorption peak at 321 nm, indicating the formation of ZnO nanoparticles with a hexagonal wurtzite structure. This is consistent with findings from other studies that reported similar absorption peaks in green-synthesized ZnO-NPs using various plant extracts.

Scanning Electron Microscopy (SEM) analysis revealed that the nanoparticles were predominantly spherical and exhibited some degree of agglomeration. The particle sizes ranged from 70 to 91 nm, suggesting successful nanoscale synthesis. Aggregation is a common occurrence in green synthesis due to the presence of bioorganic compounds from the plant extract, which may contribute to the formation of clusters. These morphological features are crucial as particle size and shape significantly influence the biological activity of nanoparticles.

X-ray Diffraction (XRD) analysis confirmed the crystalline nature of the synthesized nanoparticles, showing sharp and well-defined peaks corresponding to standard ZnO crystal planes. The Debye-Scherrer equation estimated the average crystallite size to be approximately 24.7 nm. The absence of impurity peaks

further validated the purity of the synthesized ZnO-NPs and indicated that the synthesis method produced high-quality crystals with good structural integrity.

Fourier Transform Infrared (FTIR) spectroscopy identified various functional groups such as O-H, N-H, C-O, and C=C, which were likely involved in the reduction and stabilization of the nanoparticles. The presence of a strong peak at 553 cm^{-1} confirmed the Zn-O bond, indicating the successful synthesis of ZnO. These functional groups, derived from phytochemicals in the Indian costus extract, play a critical role in preventing nanoparticle aggregation and enhancing stability.

The antifungal activity of the biosynthesized ZnO-NPs was evaluated against *Candida albicans*, *C. glabrata*, and *C. tropicalis*. The nanoparticles exhibited significant antifungal effects, with the highest inhibitory activity observed at a concentration of 300 mg/ml. *C. glabrata* showed the highest sensitivity with an inhibition zone of 31.33 mm, followed by *C. tropicalis* (30.00 mm) and *C. albicans* (29.33 mm). The strong antifungal effect is likely due to the generation of reactive oxygen species (ROS) by ZnO-NPs, leading to oxidative stress, damage to cell membranes, and disruption of vital cellular processes in the fungal pathogens these results are consistent by [37,38] .

At lower concentrations (5–15 mg/ml), the ZnO-NPs still exhibited measurable antifungal activity, although to a lesser extent. These findings are in agreement with previous studies that have reported the size, shape, and surface chemistry of nanoparticles as critical determinants of their biological activity. The ability of ZnO-NPs to inhibit *Candida* spp. even at low concentrations underscores their potential as an alternative or complementary antifungal agent This finding contradicts the results reported by [39] .

CONCLUSIONS

Compatible biosynthesis of zinc oxide nanoparticles (ZnO) was achieved by applying simple and novel green synthesis procedure that involves the use of chicory leaf extract as reducing and covering agent. Successful zinc oxide nanoparticle biosynthesis of zinc oxide nanoparticles was performed through FTIR, XRD, SEM and UV-VIS analyses and XRD results confirm observed crystal size at 19.432 nm. Biosynthetic zinc oxide nanoparticles showed strong levels of anti-*Candida* strain activity. It has been found that biosynthetic zinc oxide nanoparticles protect that they may be used as active antimicrobial agents.

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