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The effectiveness of Nanoparticles Dandelion Leaf Extract on some types of (*candida*) isolated from the Respiratory System

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Abstract:

This study aimed to biosynthesize nanoparticles from zinc oxide by a green synthesis process using Taraxacum officinale extracts. A Spectroscopy device is an essential tool for studying the optical properties of nanomaterials, where absorption is measured as a function of wavelength to determine multiple properties such as energy gap, UV-Vis, particle size, FTIR, and the presence of biomolecules and metal oxides. Structural analysis of X-ray diffraction (XRD) reveals the formation of pure hexagonal phase structures of zinc oxide nanoparticles. The surface morphologies of zinc oxide nanoparticles observed under scanning electron microscopy (SEM) indicate most hexagonal zinc oxide crystals. Biosynthesized zinc oxide nanoparticles exhibit strong antimicrobial and antifungal behavior when using the agar drilling method. Synthesized zinc oxide nanoparticles actively exhibit the three strains of *Candida* spy, namely *C. albicans*, C. *glabrata*, C. *tropicalis.*

Keywords: *Candida spy*, Dandelion leaves, green synthesis, Zinc oxide nanoparticles

1-Introduction

Fungal infections, particularly those caused by *Candida* species such as *Candida albicans*, are significant contributors to infectious disease-related mortality worldwide [1]. *Candida albicans* is a common symbiotic fungus that colonizes the mouth, pharynx, digestive system, vagina, and skin of healthy individuals. Clinical diagnoses indicate that nearly 50% of the population suffers from dermatological, mucosal, superficial, and localized disorders, some of which progress to life-threatening invasive diseases [2]. *Candida* exists naturally in the body but becomes opportunistic in cases of immune suppression, such as during chemotherapy, organ transplantation, or diabetes [3].

Nanobiotechnology has emerged as a transformative field, particularly in the synthesis of metal oxide nanoparticles (NPs) [4,5], offering versatile solutions across various sectors including energy [6], water sanitation [7], medicine

[8], agriculture [9], and materials science [10]. Among these, zinc oxide (Zano) nanoparticles have attracted considerable interest due to their chemical stability, non-toxic nature, cost-effectiveness, biocompatibility, and biodegradability [11,12]. Although several physical and chemical methods exist for synthesizing Zano NPs—such as laser ablation, vapor deposition, and hydrothermal techniques—many are costly, energy-intensive, and environmentally hazardous [11,13]. As a result, green synthesis using biological systems like plants has gained traction as a safer and more sustainable alternative. Plants contain phytochemicals such as phenols, flavonoids, saponins, tannins, and alkaloids, which can effectively reduce metal ions and aid in nanoparticle formation [14,15].

This study focuses on the use of the dandelion plant in the green synthesis of Zano nanoparticles, highlighting its extensive medicinal benefits. Dandelion is used as a preventive and therapeutic agent for liver diseases, inflammation, arthritis, burns, asthma, general weakness, and heart rate regulation. It also supports immunity, menstrual health, and is used in the treatment of rheumatism, eczema, digestive and respiratory disorders. Additionally, it exhibits antiseptic, anti-emetic, antioxidant, antibacterial, and antifungal properties, and may be useful in managing cancer, blood clotting, insomnia, and anemia. As a completely non-toxic and edible plant, both the aerial parts and roots of dandelion are utilized in food and medicine [16].

2- Research Gap:

Due to the prevalence of fungal diseases and resistance to antibiotics, the current study aimed to find an alternative to antibiotics using nano zinc.

3-Materials and Methods

3.1Preparation of Chicory Leaf Extract

This study was conducted in the laboratory of the College of Education for Pure Sciences during the period from January 1, 2024, to June 1, 2024

Dandelion leaves were collected from local markets in Thi-Qar Governorate and were cleaned of impurities, then washed with distilled water and dried under a period of 3-7 days. Grinding of dandelion leaves with an electric grinder where weight of 20 g of dry vegetable powder was taken with 200 ml of ethanol alcohol (70%) in a glass flask and then placed on the magnetic Marg device for three hours and then left the solution to settle for one night in the refrigerator at a temperature of 4 °C, then filtered using three layers of medical gauze to get rid of impurities and then placed in test tubes with a volume of 10 ml and discarded centrifuge and speed 4500 cycles/flour and for a period of 10 minutes, and then take the filtrate by syringe and passed through a Millipore filter with diameter 0.22 am. After that, the plant extract is put in Petri dishes and left in the laboratory until the extract dries and becomes a dry powder [17].

3.2 Green Bio fabrication of the Zano-NPs

Prepared by dissolving 2.5 g of zinc oxide in 20 ml of ethanol 70% and the mixture was stirred using the magnetic motor for 20 minutes at room temperature 25 degrees and 25 ml of vegetable extract at a concentration of 1 mg/ ml drop by drop was added to the zinc oxide mixture, stirring for two hours, then separating the precipitate from the solution using a centrifuge. After that, a white precipitate appears; the white precipitate is washed with ethanol and distilled water several times, and then the drying process was carried out at a temperature 100 pm for 3 hours [18].

3.3 Characterization of the Biogenic Zano-NPs

Zinc oxide biosynthetic nanoparticles were characterized using various methods, including visible ultraviolet (UV) spectroscopy (Shimadzu), Japan, to determine the surface plasmon resonance of zinc oxide nanoparticles. Infrared spectroscopy analysis (Shimadzu, Japan) was used to determine the main functional groups of biosynthetic zinc oxide nanoparticles. Biosynthetic zinc oxide nanoparticles were subjected to X-ray powder diffraction (XRD) (Rigaku, Japan) in order to verify their crystalline nature and determine their crystal size. In contrast, the morphology of the prepared zinc oxide particles is determined by scanning electron microscopy (SEM) analysis (Broker Germany).

3.4 Antifungal Effectiveness of Zano-NPs

The sensitivity of three strains of *Candida* was evaluated, namely *C. albicans*, *C. glabrata*, and *C. tropicalis,* to bio-zinc oxide nanoparticles, where the effectiveness of bio-zinc oxide particles against laboratory fungal strains was evaluated. Using the method of drilling with acres, A specific amount of of yeast stuck was taken for all types of *Candida* and spread on SDA by a sterile swab by three repeaters for each type of mushroom *Candida* spp, and then the dishes were left to dry for 20 min. Then, four holes were made on the SDA by a cork piercer with a diameter of 6 mm, where100µL zinc oxide nanoparticles were put in the drilling at the following concentrations 100, 200, 300 mg/ ml and control factor (DMSO), and dishes were incubated at 37 °C for 24-48 hours. After that, the inhibitory diameters around the hole containing zinc oxide nanoparticles was measured. The concentrations of the difference was measured by the graduated ruler, and the rate of the measurement was taken for samples. As well as the minimum inhibitor at concentrations of 5,10,15 mg/ ml [19].

3.5 Statistical analysis

The data were statistically analyzed using SPSS version 25, according to a Completely Randomized Design (CRD) with three replications. Two-way ANOVA was used for analysis, and means were compared using the LSD test at a significance level of 0.05. Additionally, the Chi-square test was applied for heterogeneous samples at a significance level of 0.05.

4-Results and Discussion

Physical and Chemical Characterization of Green-Synthesized Zinc Oxide Nanoparticles

4.1 UV–Vis spectroscopy

The UV–Vis spectroscopy instrument is an important tool for studying the optical properties of nanomaterials, where absorption is measured as a function of wavelength to determine multiple properties such as energy gap and particle size [20]. After optical observation of the synthesis of zinc oxide nanoparticles (Figure 1), the solid powder of these particles was collected and dispersed in deionized water using ultrasound for five minutes to ensure a homogeneous distribution of the particles. Then the synthesis process was verified using UV-visible spectroscopy. The resulting spectrum (190-900 nm) shows the absorption of zinc oxide nanoparticles manufactured by the green method using the plant extract, which is the ciliary leaf extract. The spectra showed a characteristic absorption peak at 326 nm (Figure 2), corresponding to the wurtzite hexagonal phase of zinc oxide in its crystalline state [21].

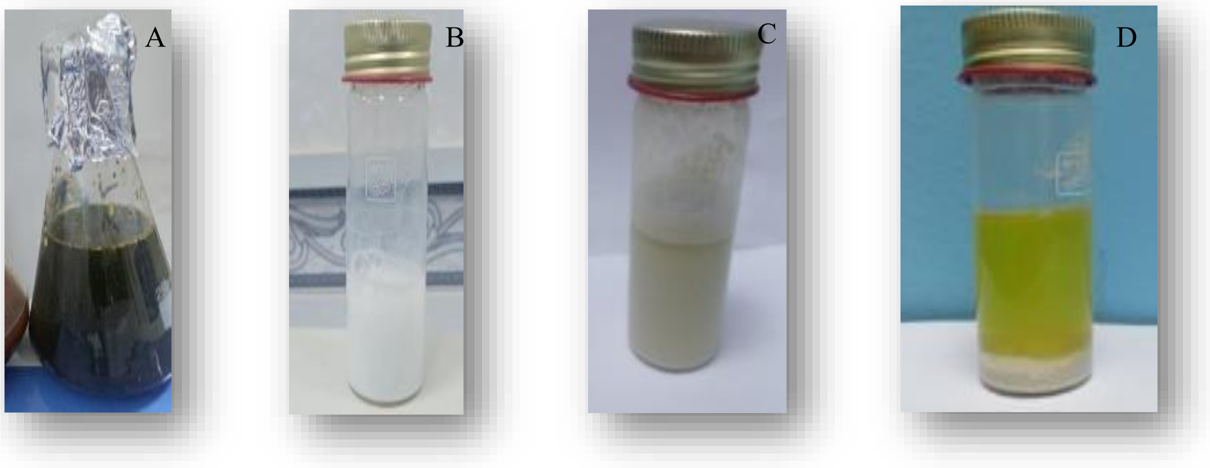


Figure 1: Zinc Oxide Synthesis of Plant Extract A: Dandelion leaf extract, B: Zno, C: Zno + Dandelion leaf extract, D: white precipitate

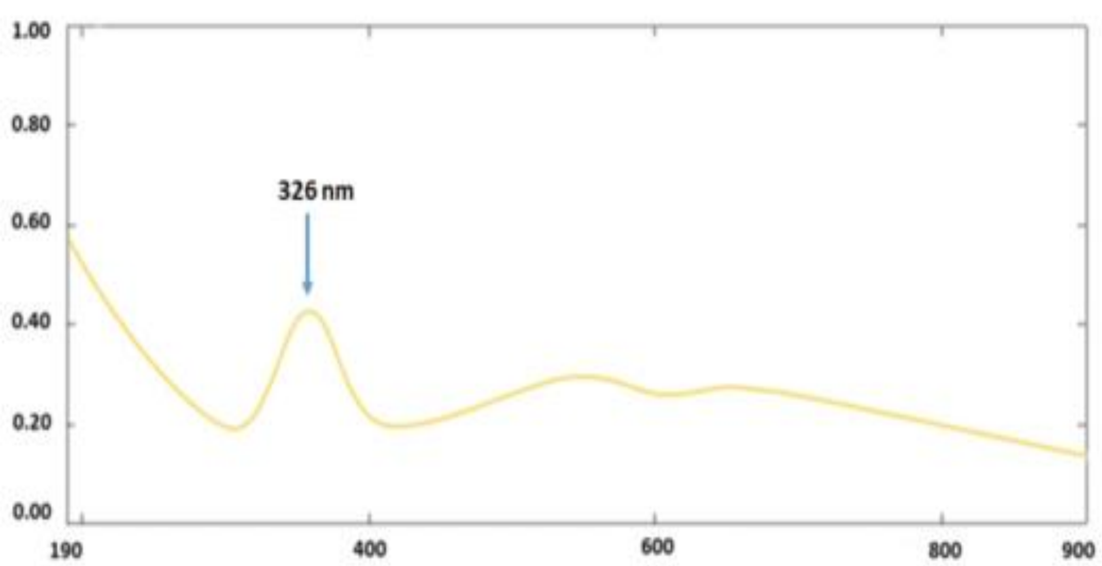


Figure 2: UV spectrum of ZnO –NPs using extract *Chicory Leaf*

4.2 Scanning Electron Microscope - SEM

SEM is an advanced tool for examining and analysing sample surfaces in fine detail. It is based on using a beam of electrons to scan the surface of the sample, where it is scanned point by point, and then the reflected electrons or secondary electrons emitted from the sample are detected to form a three-dimensional image with high clarity [22]. Scanning electron microscopy (SEM) was used to determine the structure of the reaction products formed when the zinc oxide nanoparticles were synthesized using dandelion leaves (Figure 3). SEM was used to study the crystalline nanoparticles of zinc oxide formed after the reaction process. The SEM image showed that the nanoparticles formed were individual zinc particles. The particles were aspherical or oval in shape, which is a pattern common in crystalline particles of zinc oxide. The diameter of these particles ranges from 54-87 nm, which means that within the nanoscale. Nanoscale enhances the active surface area of the particles, making them more effective in many a Other studies [23]. It was observed that zinc nanoparticles are present and appear in a homogeneous distribution in some regions while they collect in others. The observed zinc oxide nanoparticle sizes ranged from 6.5 nm to 7.5 nm. This distribution and agglomeration can be caused by biosynthesis conditions, which affect the shape and final size of the nanoparticles.

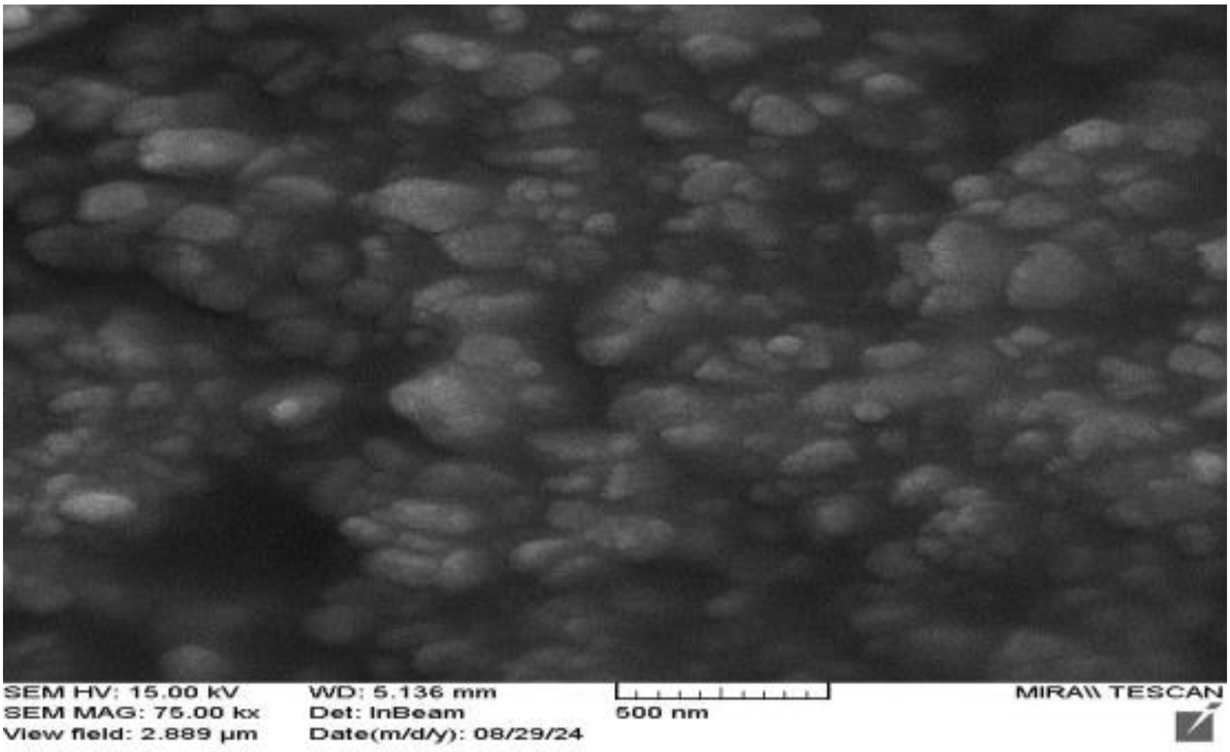
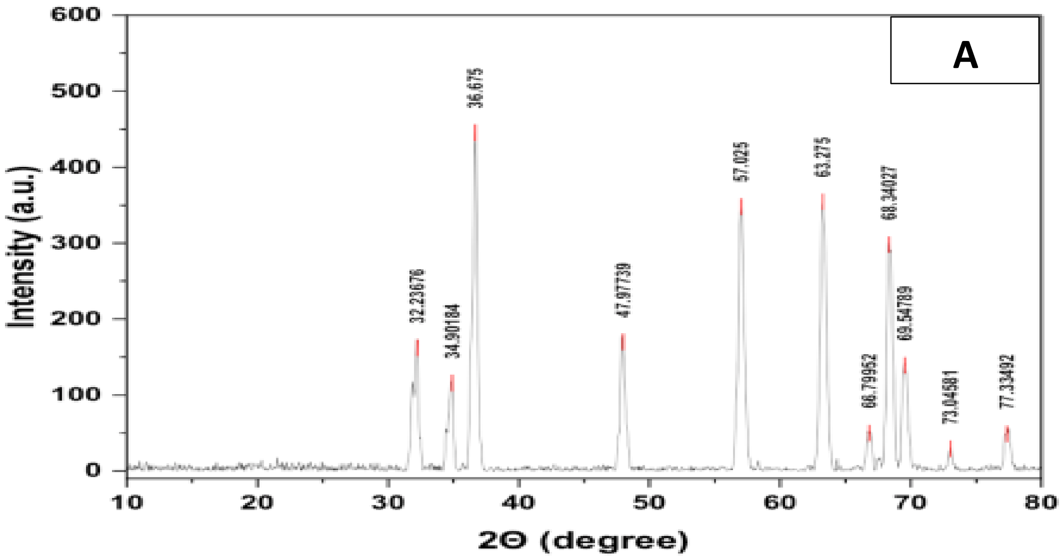


Figure3:SEM images of ZnO –NPs using extract *Chicory Lea*

4.3 X-ray Diffraction(XRD)

X-ray Diffraction is an analytical tool used to study the crystal structure of materials. The device relies on sending a beam of X-rays to the material sample and then measuring the diffraction pattern resulting from the rays' interaction with atoms within the material [24]. (Figure 4 a,b) illustrate the analysis of X-ray diffraction XRD of zinc oxide nanoparticles synthesized using a plant extract of dandelion leaves. All of the resulting peaks showed a match with the zinc oxide nanoparticles, indicating that the base material in the particles is zinc oxide 2Ɵ position of the peaks at angles 32.237°, 34.902°, 36.675°, 47.977°, 57.025°, 63.275°, 66.800°, 68.340°, 69.548°, 73.046°, 77.335°. Diffraction peaks matched the following crystalline levels: (100), (002), (101), (102), (110), (103), (200), (112), (004), (202). The sharp and clear peaks indicate the crystalline nature of the nanoparticles, which have a hexagonal crystal structure [25]. These levels were verified using the Joint Committee on Powder Diffraction Standards (JCPDS) 01-083-6338. This shows an identical crystal structure of zinc oxide in nanoparticles. These levels were verified using the JCPDS No. 01-083-6338. This indicates an identical crystal structure of zinc oxide in nanoparticles. Crystallite Sizes were determined using the Debye-Scherrer equation based on the width of dominant peaks in X-ray diffraction analysis. The result was a crystal size of 19.432 nm, highlighting the effect of the plant extract on reducing crystal size. Zinc oxide particles are formed in conditions that contribute to the formation of high-quality crystals. The absence of large shifts in the XRD tops from plant extracts indicates the similarity of crystal growth mechanisms, where zinc oxide helps stabilize the natural hexagonal crystal structure. The sharp and pronounced peaks in the patterns reflect a high degree of crystallization, indicating the formation of small but well-organized crystals, and using the Shearer equation to calculate the size of crystals highlights that particles are small in size, which enhances their crystalline and optical properties [25]. These findings are consistent with the study of [26], where zinc oxide nanoparticles synthesised using plant extracts derived from *Matricaria chamomilla* L., *Olea europaea* olive leaves and *Lycopersicon esculentum* M. XRD technology was used to confirm the pure crystal shape of the nanoparticles. The results showed that the smallest crystal size of the particles was 48.2 nm, which matches the measurements obtained using TEM microscope technology. These results reflect the great potential of biosynthesis using plant extracts to produce crystalline nanoparticles with characteristic properties that can be controlled based on the type of plant used.



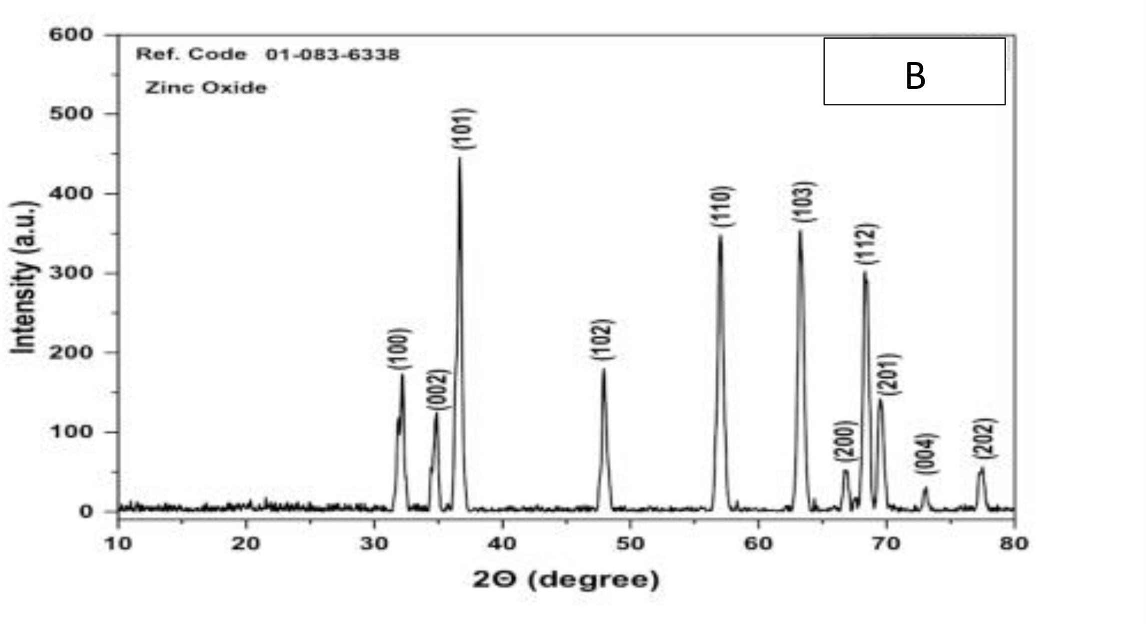


Figure 4. XRD (A: Crystal levels Miller Indices (hkl) of ZnO , B: XRD pattern of ZnO), using *Chicory Leaf*

4.4 Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FTIR) is an important technique to determine functional groups and chemical reactions on the surface of nanoparticles, such as zinc oxide nanoparticles (ZnO). By studying the absorption spectrum in the spectral range 400-4000 cm^{-1}, it is possible to determine the types of bonds and functional groups that exist on the surface of these particles, giving us information about their chemical composition and stability [12].

The formation of nanoparticles using infrared spectroscopy (IR) can clearly reflect the role of different functional groups in this process. In the case of zinc nanoparticles prepared using dandelion, the presence of the top 3411 cm^{-1} indicates the presence of hydroxyl (OH) or amines (N-H) groups in the preparation of nanoparticles (Figuer 5) These groups can act as capping agents for nanoparticles. Hydroxyl helps stabilize the particles, preventing the particles from clumping together. These groups can also help adjust the size and shape of nanoparticles. Amines, especially in primary amines, may also contribute to the reaction with zinc during the formation process and help regulate nanosynthesis. The appearance of a peak of 2924 cm^{-1} indicates the presence of alkanes within the range of C-H vibrations in the nanoprocess, where they may act as a surface cover for particles, limiting their interaction with each other and preventing the aggregation of nanoparticles. The peak of 1569 cm^{-1} indicates the presence of N-H groups or C=C bonds, referring to the presence of amines or alkenes. Amines may play an important role in the formation of nanoparticles as they work to prevent particle aggregation. Alkenes C=C bonds can act as compounds that contribute to the fixation of nanoparticles via double bonds with the surface of the nanoparticles. The peaks of 1412 cm^{-1} and 1056 cm^{-1} indicate the presence of C-O bonds, therefore indicating the presence of alcohols or ethers. These groups may contribute to the interaction of zinc with plants during synthesis[27], as alcohol and ether groups may be involved in binding nanoparticles and preventing them from clumping. A peak of 562 cm^{-1} indicates the presence of bonds between carbon and halogens C-Br or C-I, indicating the presence of halogenated compounds in the formation process. Halogens may be involved in the reaction process, helping to control particle size and formation. Halogens can also act as stabilizers using dandelion extract during the nanoparticle preparation process. The compounds in the extract play a crucial role in stabilizing the nanoparticles and preventing their aggregation, forming stable and homogeneous nanoparticles. Functional groups such as hydroxyl (O-H), alkanes and amines contribute to the stability of particles and adjust their size and shape, making them suitable for various nano applications [27].

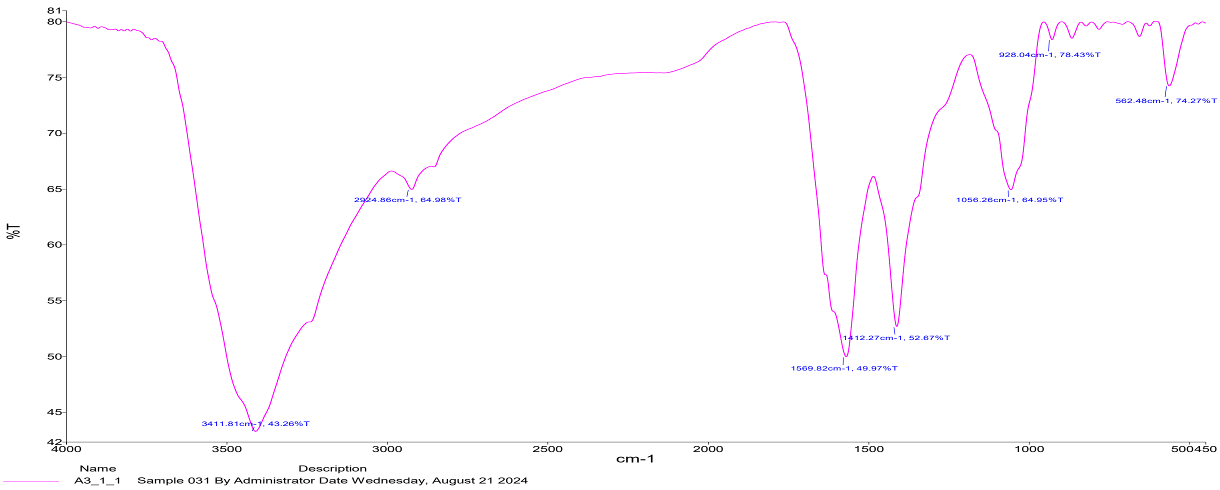
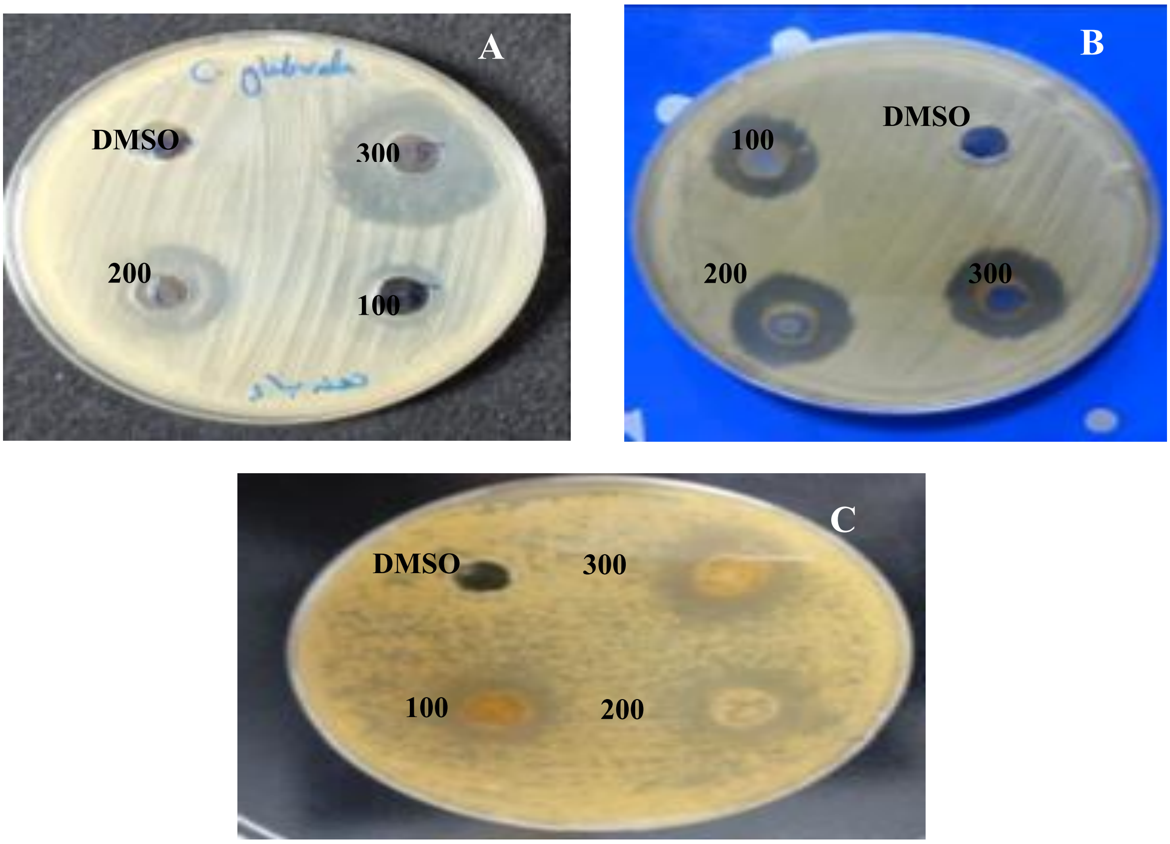


Figure 5: Infrared spectroscopy using Fourier conversion of zinc oxide nanoparticles prepared using dandelion leaves

5- Evaluation of Anticandidal Effectiveness of the Bioinspired ZnO-NPs

The inhibitory activity of zinc oxide nanoparticles extracted of dandelion leaves against strains of Candida species isolated from the respiratory tract was studied. The results showed high inhibition efficacy compared to the control sample, which did not show any inhibitory efficacy. The results in table (1) and figuer(6). show the effect of zinc oxide nanoparticles extracted from dandelion leaves on *Candida* species, and the results were evaluated by agar propagation method by drilling, as *C. albican* showed high sensitivity to ZnO-NPs at a concentration of 300 mg/ ml with an inhibition zone of 28.66±1.52, and the highest inhibitory activity of *C. glabrata* was also at a concentration of 300 mg/ml with inhibition zones of 29.00±1.00, while *C. tropicalis* had a higher inhibitory activity of 29±1.73 at 300 mg/ ml concentration. This result is consistent with the study of [28], which indicates that zinc oxide nanoparticles are effective as antifungals and antibacterials when coated with other substances. Their anti-particle effectiveness depends on the shape of the particles, exposure time, concentration, pH and biocompatibility [29].

Also, the result is consistent with the study of [30], which indicated that zinc oxide particles are biologically protected from highly pomegranate peel aqueous extract against *C. tropicalis*, *C. albican*, *Cglabrata* at concentrations (100 µg/disc) with an inhibition diameter of 24.18±0.32, 20.17±0.56 and 26.35±0.16, the mechanism of antifungal action based on the ability of zinc oxide nanoparticles (NPs) to penetrate the fungal membrane by diffusion and cell phagocytosis. Within the cytoplasm, the presence of zinc oxide nanoparticles (NPs) disrupts the normal functioning of the mitochondria, thereby stimulating the generation of reactive oxygen species (ROS) and the subsequent release of Zn^{2+} ions. Excessive generation of reactive oxygen species (ROS) and accumulation of zinc ions (Zn^{2+}) led to damage to DNA [31,30].



Figuer 8. Inhibitory Activity Zinc Oxide Nanoparticles Dandelion Leaves A: *C. glabrata, B: C. tropicalis, C: 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		
C. albicans	23.33 ± 1.15	26.33 ± .57735	28.66±1.52	26.11	0
C. glabrata	23.66 ±.57735	26.66±1.15470	29.00±1.00	26.44	0
C. tropicalis	24.00 ± 2.00	26.00±1.73205	29.00±1.73	26.33	0

5.1 Minimum Inhibitor Evaluation of Anticandidal Effectiveness of the Bioinspired ZnO-NPs

Table (2) shows the lowest inhibitory concentration of zinc oxide nanoscale for the extract of dandelion leaves, as it was less effective inhibitory *C. abican* at a concentration of 5 mg/ ml with inhibition zones of 1.15470± 1.33, and *C. glabrata* is less effective at 5 mg/ ml with inhibitory zones of 1.00±1.73, while C. *tropicalis* reached the lowest inhibition of 2.66±2.30 at a concentration of 5mg/ml. The statistical analysis results showed no significant differences among the three concentrations of dandelion leaf extract at a significance level of 0.05.

These results are consistent with the findings of [28] that zinc oxide nanoparticles show inhibitory activity towards *C. albicans* at a concentration of 5.8 mg/ ml and contradict with the study of [32] where zinc oxide nanoparticles recorded an inhibition rate of 11 mm, while no inhibition was shown at concentrations of 500, 50, 10, 2, 1 and 0.5 mg/ML.

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		
C. albicans	1.15471.33±	5.66±.57735	8.50±1.32	5.1667	0
C. glabrata	1.00±1.73	6.66±2.30	8.66±.57735	5.44	0
C. tropicalis	2.66±2.30	6.00±1.73205	15.66±.57735	8.11	0

6-Conclusions

The biosynthesis of zinc oxide nanoparticles (ZnO) was compatible with a simple and novel green synthesis procedure involving chicory leaf extract as a reducing and covering agent. Successful zinc oxide nanoparticle biosynthesis was tested through FTIR, XRD, SEM and UV-VIS analyses, and XRD. Results confirm the observed crystal size at 19.432 nm. Biosynthetic zinc oxide nanoparticles showed substantial levels of anti-Candida strain activity. It has been found that biosynthetic zinc oxide nanoparticles may be used as active antimicrobial agents.

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