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Green Synthesized and Evaluation of ZnO Nanoparticles using *Rhazya stricta* Leaf Extract for Antibacterial and Anticancer Applications

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Abstract

This work aims to synthesize ZnO NPs utilizing *Rhazya stricta* (Harmal Alrazi) aqueous leaf extract in a quick, easy, and environmentally friendly manner. The obtained ZnONPs were characterized using various analytical techniques, which included XRD, FESEM, EDX, and UV-Vis spectroscopy. The XRD analysis confirmed the hexagonal crystal nature with an average crystallite size of 43.4nm. FESEM images indicated a semi-spherical shape with particle sizes ranging from 26.71 nm to 38.40 nm. A characteristic absorption peak was observed at 379 nm for UV-Vis spectra. The biosynthesized ZnO NPs were also investigated as antibacterial agents against *Klebsiella Pneumoniae* (*k.pneumoniae*) and *Staphylococcus aureus* (*S. aureus*). The measured diameters of the inhibition zones were (12mm) and (14 mm), respectively. The cytotoxicity effect of ZnONPs against A549 cell lines showed a higher cytotoxicity of 89.47 ± 1.550 at the high concentration of 100 µg/ml. This is the first attempt using harmal to facilitate the synthesis of ZnONPs as antibacterial and anticancer agents, which can subsequently be exploited as a potential candidate for antibacterial and anticancer agents and might be utilized after further in vivo studies.

Keywords: ZnO; Nanoparticles; *Rhazya stricta*; Antibacterial activity; Anticancer activity.

التصنيع الاخضر و تقييم جسيمات اكسيد الزنك النانوية باستخدام مستخلص اوراق حرمل الرازي للتطبيقات المضادة للبكتيريا والسرطان

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الخلاصة

في هذا العمل، نهدف إلى تخليق جسيمات نانوية من أكسيد الزنك (ZnO NPs) باستخدام مستخلص أوراق نبات الحرمل (*Rhazya stricta*) المائي بطريقة سريعة وسهلة وصديقة للبيئة. تم تمييز الجسيمات

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النانوية المحضرة باستخدام تقنيات تحليلية مختلفة، والتي شملت تحليل حيود الأشعة السينية (XRD)، وتصوير المجهر الإلكتروني الماسح ذو الانبعاث الميداني (FESEM)، والتحليل الطيفي للأشعة السينية المشتتة للطاقة (EDX)، والتحليل الطيفي للأشعة فوق البنفسجية-المرئية (UV-Vis). أكد تحليل XRD الطبيعة البلورية السداسية مع متوسط حجم البلورات 43.4 نانومتر. أظهرت صور FESEM شكل شبه كروي بأحجام جسيمات تتراوح بين 26.71-38.40 نانومتر. لوحظت قمة الامتصاص المميزة عند 379 نانومتر في التحليل الطيفي للأشعة فوق البنفسجية-المرئية. تم أيضًا دراسة الجسيمات النانوية المحضرة كمضادات بكتيرية ضد *Klebsiella pneumoniae* (*K. pneumoniae*) و *Staphylococcus aureus* (*S. aureus*). كانت مناطق التثبيط المقاسة (12 ملم) و (14 ملم) على التوالي. أظهر تأثير السمية الخلوية لجسيمات ZnO النانوية ضد خطوط خلايا A549 سمية خلوية أعلى بنسبة 89.47 ± 1.550 عند تركيز أعلى من 100 ميكروجرام/مل. هذه هي المحاولة الأولى لاستخدام الحرمل لتسهيل تخليق جسيمات ZnO النانوية كمضادات بكتيرية ومضادات للسرطان، والتي يمكن استغلالها لاحقاً كمرشح محتمل للعوامل المضادة للبكتيريا والسرطان، وقد يتم استخدامها بعد مزيد من الدراسات الحية.

1. Introduction

The increasing demand for natural and low-toxicity therapeutics has made medicinal plants a preferred choice for combating infections. Medicinal plants have played a vital role in treating various diseases since ancient times. Medicinal plants contain bioactive compounds in one or more of their parts, such as seeds, roots, leaves, and fruits. These compounds are considered precursors for the synthesis of useful drugs and also act as reducing, capping, or stabilizing agents [1]. It has been proven that plants can be used to synthesis nanoparticles by eco-friendly, facile, simple and safe methods. Plant extract utilization is a straightforward and efficient method for synthesizing metal or metal oxide nanoparticles in large quantities. In contrast to synthesis mediated by bacteria or fungi, plant extracts provide many therapeutic chemicals that can readily convert complex metallic ions into simple ones. Observing the accumulation of metallic ions in plant cells and tissues inspired the idea of applying metal reduction to nanoscale materials [2].

Because of their low environmental impact, ease of manufacturing, resilience against potential microbial harm, and low cost of production and handling, plants are often selected as the principal biological source for ZnO NPs. Furthermore, distilled water is frequently used to make plant extracts, which poses less of a health concern than the production of ZnO NPs with the assistance of microorganisms [3]. Numerous illnesses and ailments, including cancer, diabetes, rheumatoid arthritis, cardiovascular disease, retinal degeneration, Parkinson's disease, hypertension, liver damage, nephritis, asthma, psoriasis, and others, are linked to oxidative stress [4]. Free radicals, which are essentially unpaired oxygen and nitrogen radicals that are free, are typically blamed for these disorders. Free radicals can be generated both endogenously during metabolic processes and exogenously through sources, such as smoke, ozone exposure, ionizing radiation, and certain drugs. When the production of free radicals exceeds the body's antioxidant defence mechanism's capacity to control them, it leads to the onset of various diseases. Although synthetic antioxidant molecules, such as butylated hydroxyanisole, butylated hydroxytoluene, and propyl gallate, are available, their use is limited due to their numerous side effects, carcinogenicity, and toxicity [5].

Consequently, there is a growing inclination towards natural antioxidants and, more recently, towards nanoparticles (NPs). Cancer represents a formidable global health challenge, accounting for a significant portion of worldwide deaths, second only to cardiovascular disease. Conventional cancer treatments like surgery, radiation therapy, and chemotherapy, despite their effectiveness, come with substantial side effects and toxicities [6]. Hence, there

is a pressing need to discover new anti-cancer compounds that are cost-effective with fewer side effects, and plants emerge as promising alternatives [7]. Multidrug resistance, cancer, and diseases related to oxidative stress are global threats requiring urgent attention. The focus has shifted towards natural plants and, more recently, metal nanoparticles (NPs).

Nano science or nanotechnology has rapidly advanced, finding applications in various fields, including industry, computing, medicine, and healthcare [8]. Nanoparticles are particles smaller than 100 nm in size. They possess unique physical and chemical properties, including a large surface area, good thermal conductivity, and distinct characteristics such as size, charge, shape, crystal structure, and surface morphology. These applications span various industries, including agriculture, pharmaceuticals, textiles, nanofertilizers, catalysts in oxidation reactions, water detoxification, dye degradation, and the biomedical and biotechnological sectors [9].

The synthesis of zinc oxide nanoparticles (ZnO NPs) from various plant parts, such as leaves [10], fruits [11], seeds [12], flowers [13], roots, peels [14], rhizomes, and petals [15], is well-established. These plant components possess varying levels and compositions of phytochemicals that function as reducing agents. ZnO NPs synthesized from different plant parts and phytocompounds demonstrate various biological activities, including anti-inflammatory, anti-diabetic [16], cytotoxic [17], antioxidant [18], photocatalytic [19], and antimicrobial properties [20]. Chitosan/zinc oxide nanocomposites synthesized using *Sida acuta* wireweeds have shown enhanced antibacterial and photocatalytic properties [21]. The leaves of *Rhazya stricta* (Harmal Alrazi), a species belonging to the *Apocynaceae* family, have shown many promising biological activities, e.g., antibacterial activity [22], antioxidant and anticancer activity and showed novel findings [23]. However, unlike previous work that only reported the synthesized ZnO NPs from *R. stricta* [24], our study is the first to evaluate their antibacterial and anticancer activity.

The current study utilized an aqueous extract of *Rhazya stricta* leaves to synthesize zinc oxide nanoparticles (ZnO NPs). Characterization was conducted utilizing various methodologies. Lastly, the antimicrobial and cytotoxic effects were also assessed. The antibacterial activity was assessed against two bacterial strains, namely *Staphylococcus aureus* and *Klebsiella pneumoniae*. The cytotoxicity was assessed using the MTT test against the A549 cell lines.

2. Materials and methodology

2.1. Materials

Zinc nitrate ($\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$) was purchased from Thomas Baker, India, sodium hydroxide pellets AR (NaOH) at a purity 98% from Labachemie, India, the Muller–Hinton agar medium from HiMedia, India, and 99% purity ethanol from Duksan, Korea.

2.2. Plant collection and preparation of extract

The plant *Rhazya stricta* (Harmal Alrazi) was collected from Ras al-Khaimah, United Arab Emirates, in January. The leaves were cleaned three times under tap water and then again with deionized water (D.W.) to remove any dust. The leaves were then pulverized into a fine powder using an electric mixer, after which they were left in the shade at room temperature for a month to dry. Next, 500 ml of deionized water (D.W.) was mixed with 10 g of the plant powder and heated to 80 °C for 2 hours. After creating a colored solution, the mixture was filtered through filter paper and centrifuged to remove any remaining impurities. The solution was kept refrigerated.

2.3. Preparation of ZnONPs

5g of Zinc nitrate ($\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$) was dissolved in 150 ml of deionized water and mixed for half an hour on a magnetic stirrer, and 100 ml of Harmal Alrazi extract was added under magnetic mixing for 1h at 80°C. Then, the pH was adjusted to 12 by adding 5M of sodium hydroxide (NaOH) in 20 ml of deionized water dropwise to the solution and the extract. The color changed from colorless to yellow, which is the first sign of ZnO NPs formation. Then, the precipitation was separated by centrifugation and washed with deionized water and ethanol. Finally, the clean precipitation was dried at 75 °C for 3 h to remove any impurities. The calcination process was carried out at 600°C for 2 h. The final product was white.

2.4. Characterization

ZnO NPs were examined by X-Ray diffraction analysis using XRD panalytical Xpert, UK, with Cu K α radiation monochromatic filter in the range of 20°-80°. The morphology, particle size, and size distribution of the synthesized ZnO NPs were studied using field emission scan electron microscope (FESEM- Imaging-EDS-Mapping-line-EBSD/Germany). The Optical properties of ZnO NPs were analyzed using a UV-Visible spectrophotometer (UV-2550 Shimadzu, Japan) with a 100–1100 nm wavelength range.

Antibacterial activity of ZnO NPs

ZnO NPs prepared from Harmal Alrazi leaves were tested against Gram-positive and Gram-negative bacteria using an agar-well diffusion assay. A sterile Petri plate was filled with approximately 20 ml of Muller-Hinton agar (MHA) using the aseptic technique. Using a sterile wire loop, the bacterial species were extracted from their stock cultures. After the organisms had grown, 6 mm diameter wells were made on the agar plates using sterile needles. Various concentrations of ZnO NPs (100, 200, 400, and 800) µg/ml were utilized in the bored wells. The test organisms were incubated overnight at 37°C before measuring and recording the average diameter of the inhibition zone.

2.5. Maintenance of cell cultures

A549, a well-known human lung adenocarcinoma cell line widely used in research to study lung cancer and respiratory diseases, cells were cultured in RPMI-1640 supplemented with 100 units/mL penicillin, 100 µg/mL streptomycin, and 10% fetal bovine serum. The cells were passaged using trypsin-EDTA, reseeded at 80% confluence twice a week, and cultivated at 37 °C.

2.6. MTT Assays

To determine the toxicity of ZnO NPs to the A549 cell line, the MTT test was performed using 96-well plates. The cell line was cultured at a seeding density of 1×10^4 cells per well. Following a 24-hour period, upon reaching a confluent monolayer, cells were subjected to treatment with varying doses of ZnO NPs. After 48 hours, the cells' viability was checked by adding a 28-µL solution of MTT (2 mg/mL) to the medium and incubating them at 37 °C for 2.5 hours. The crystals that remained in the wells were dissolved by adding 130 µL of DMSO (Dimethyl Sulphoxide) and incubating at 37 °C for 15 minutes with shaking after the MTT solution was removed. The absorbency was measured using a microplate reader at a wavelength of 492 nm. The test was conducted three times to ensure accuracy and reliability. The cell growth inhibition rate, which represents the proportion of cytotoxicity, was computed using the following equation:

$$\text{Inhibition rate (\%)} = \frac{A-B}{A} \times 100$$

Where A represents the control's optical density and B the samples' optical density. An inverted microscope with a 40× magnification was used to study the cells, and a digital camera attached to the microscope was used to capture the images.

2.6. Statistical analysis

The collected data were subjected to statistical analysis using an unpaired t-test conducted with GraphPad Prism 6. The values were reported as the average $\pm$ standard deviation of three repeated measurements.

3. Result and Discussion

The XRD pattern of green synthesized ZnO NPs is shown in Fig. 1. The angular range (2θ) of the measured sample was from 20° to 80° . The pattern shows a sharp diffraction peaks at $2\theta = 31.75^\circ, 34.41^\circ, 36.24^\circ, 47.53^\circ, 56.59^\circ, 62.85^\circ, 66.37^\circ, 67.94^\circ$ and 69.08° due to scattering from (100), (002), (101), (102), (110), (103), (200), (112) and (201) crystal planes, respectively. The polycrystalline structure seen in these data suggests a well-crystalline structure. According to the global crystallographic data table card number 01-079-0207 for JCPDS, all the diffraction peaks are well-fitted with the hexagonal structure of ZnO. The ZnO NPs were found to have a corresponding space group of P63mc. The broadness of all the Bragg peaks confirms the nanoscale characteristics of the bioinspired sample. The lattice parameters a and c for the hexagonal ZnO NPs were determined using the interplanar distance relation [25]:

$$\frac{1}{d^2} = \left[\frac{4(h^2 + hk + k^2)}{3a^2} + \frac{l^2}{c^2} \right] \quad (1)$$

Where the reflection planes with Miller indices h, k , and l are represented by the interplanar spacing, d_{hkl} , which is calculated using Bragg's Law. The lattice parameters were obtained with an average value of $a_{\text{exp}} = 3.25 \text{ \AA}$, $c_{\text{exp}} = 5.2 \text{ \AA}$, and $c_{\text{exp}}/a_{\text{exp}} = 1.6$. These values align with the Wurtzite ZnO structure's values of $a = 3.256 \text{ \AA}$ and $c = 5.212 \text{ \AA}$.

The following formula was used to estimate the volume of the hexagonal ZnONPs unit cell [25]:

$$V = 0.866 \cdot a^2 \cdot c \quad (2)$$

For $a = 3.256 \text{ \AA}$ and $c = 5.212 \text{ \AA}$, the volume of the hexagonal ZnO NPs unit cell was calculated according to Equation (2) to be equal to $47.85 \text{ \AA}^3$.

The average crystallite size can be calculated using the Scherrer formula [25].

$$D = \frac{k\lambda}{\beta \cos \theta} \quad (3)$$

Where k is the shape factor, λ has a value of $1.54060 \text{ \AA}$, β is the Full Width at Half Maximum (FWHM), and θ is the diffracting angle. The average crystallite size was found to be 43.4 nm . No peaks related to impurities were seen, affirming the high purity of the synthesized ZnO NPs [26]. At (101) the most intense peak appears, which illustrates the preferred growth plane. This result confirms the successful synthesis of ZnO NPs using the Harmal Alrazi leaf extract.

Other than crystallite size, lattice strain (ϵ) is the source of the XRD peak broadening. Lattice strain in nanoparticle materials is mainly caused by the stress field created by the extra volume of the grain boundaries [27]. The widening of the diffraction line resulting from crystal size (D) changes in proportion to $1/\cos\theta$, whereas the widening resulting from lattice strain (ϵ) changes in proportion to $1/\tan\theta$. The induced lattice strain (ϵ) was calculated using the following equation [28]:

$$\epsilon = \frac{\beta}{4 \tan \theta} \quad (4)$$

The D and ϵ values, along with other structural parameters, for the ZnO NPs that were synthesized are provided in Table 1.

The dislocation density (δ) is a crucial component that influences the characteristics of a material. A high dislocation density signifies a high level of hardness of the material. The dislocation density (δ) was determined by employing the following equation [25]:

$$\delta = \frac{1}{D^2} \quad (5)$$

The drop in microstrain and dislocation density values was evident, but there was an increase in crystallite size. This can be attributed to the improvement taking place in the lattice configuration of the ZnO NPs.

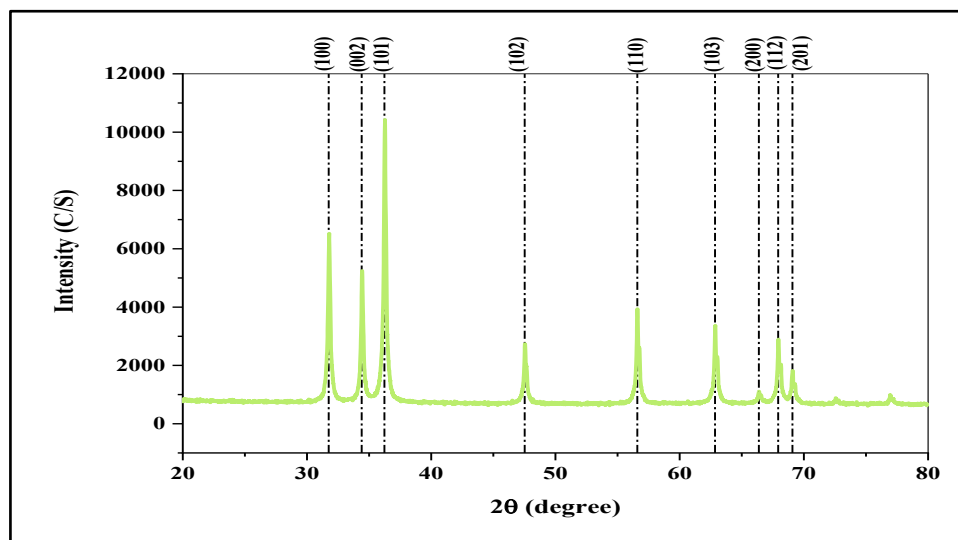


Figure 1: XRD of ZnO NPs from *Rhazya stricta* (Harmal Alrazi) leaf extract.

Table 1: Structural parameters of ZnO NPs from Harmal Alrazi leaves.

2θ (degree)	d_{hkl}	hkl	FWHM	D nm	$\delta \times 10^{15} (m^{-2})$	$\epsilon \times 10^{-3}$
31.7598	2.81519	100	0.1819	37.6	0.70733	2.7899
34.4168	2.6037	002	0.1776	39.2	0.65077	2.5020
36.2443	2.4765	101	0.1861	36.4	0.75473	2.4811
47.537	1.91121	102	0.1661	43.4	0.53090	1.6456
56.5905	1.62505	110	0.1654	44.0	0.51652	1.3405
62.8559	1.47729	103	0.1745	41.1	0.59199	1.2460
66.37	1.40722	200	0.2304	26.7	1.40274	1.5371
67.9489	1.37843	112	0.1405	63.3	0.24957	0.9097
69.0818	1.35856	201	0.1698	42.7	0.54845	1.0763

FESEM images, shown in Fig. 2, depict ZnO NPs characterized by random agglomerations, indicating that the nanoparticles tend to cluster together in a non-uniform manner. ZnO NPs' high surface energy and densification, which results from the small space between nanoparticles, may be the cause of agglomeration [29]. The individual nanoparticles within these agglomerations have a semi-spherical shape, and their particle sizes fall within the specified range of 26.71 to 38.40 nm. Compared to Najoom et al. study [24], the ZnO NPs synthesized in this work demonstrated slightly smaller particles size. However, this information provides insights into the morphology and size distribution of the synthesized ZnO NPs.

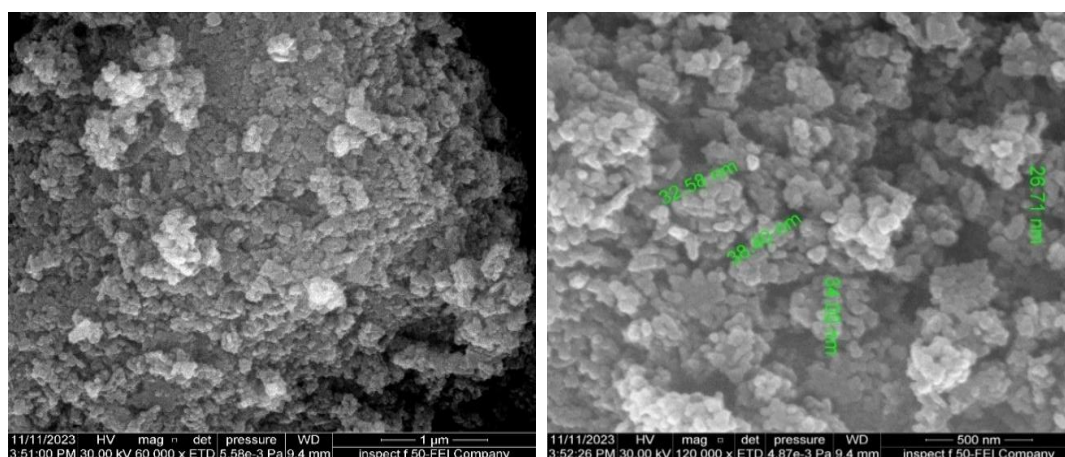


Figure 2: FESEM images of ZnO NPs from *Rhazya stricta* (Harmal Alrazi) leaf extract.

Figure 3 shows the EDX pattern of the synthesized ZnO NPs from Harmal Alrazi leaves extract. The resulting composition from EDX analysis reveals three peaks for zinc with a weight percentage 77.8%, and one peak for oxygen with a weight percentage 22.2%. No additional peaks were observed for other elements, confirming the high purity of the ZnO NPs and verifying their formation. These results are consistent with the XRD analysis. This result is in full agreement with those of El-Khawaga et al. [30].

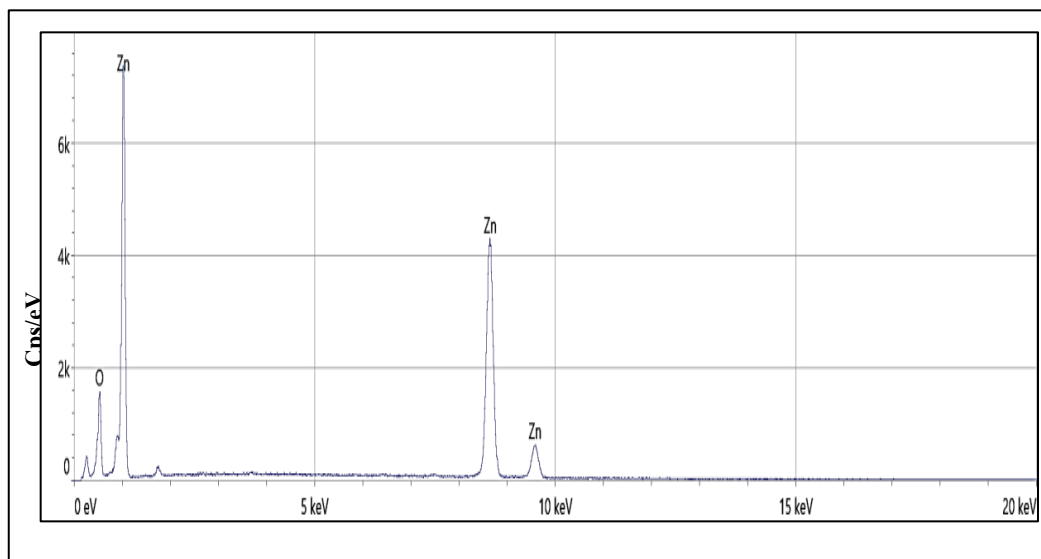


Figure 3: EDX spectrum of ZnO NPs synthesized from *Rhazya stricta* (Harmal Alrazi) leaf extract.

Figure 4 shows the UV-Vis spectrum of ZnONPs synthesized from Harmal Alrazi leaf extract. A high-intensity peak appeared at 379 nm. The result confirmed that phytochemicals present in the leaves extract act as a bio-reductant, which plays a significant role in the ZnO NPs formation. This result supports the report, which showed the absorption spectrum at 360-370 nm undoubtedly represents the occurrence of ZnO NPs [31].

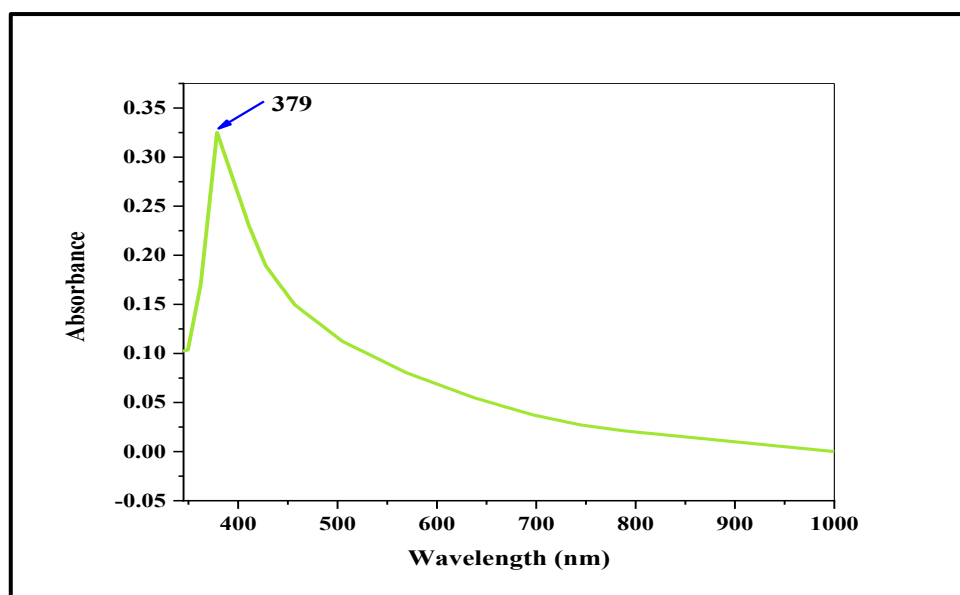


Figure 4: UV-Vis spectrum of ZnO NPs from *Rhazya stricta* (Harmal Alrazi) leaf extract.

3.1. Antibacterial activity

To assess the antibacterial characteristics of ZnO nanoparticles manufactured using green methods, various concentrations of the ZnO NPs sample (100, 200, 400, and 800 $\mu\text{g/mL}$) were examined against two types of bacteria, namely *Staphylococcus aureus* and *Klebsiella pneumoniae*. Deionized water was used as the control (A), and no comparison was made with chemically synthesized or commercial ZnONPs. The bacterium *S. aureus* was selected as the subject of the study to investigate the antibacterial properties of ZnO nanoparticles. This bacterium is a human pathogen that can cause various infections in the skin, respiratory system, and cardiovascular system. Additionally, *K. pneumoniae* was chosen due to its severity in causing infections, producing high mortality rate even when treated properly [32]. Figures 5a and 5b depict the inhibitory zones of ZnO NPs against both gram-positive and gram-negative bacterial strains. The figures also provide the diameter (in millimeters) of the inhibitory zone for each bacterial strain. The results demonstrate that the ZnO nanoparticles exhibit significant antibacterial activity. The concentration of the produced ZnO NPs directly influenced their antibacterial activity. The data clearly demonstrate that the diameters of the inhibitory zones increased as the concentration of the nanoparticles increased for both tested strains. For *S. aureus*, the largest area of inhibition was 14 mm, and for *K. pneumoniae*, it was 12 mm at ZnO NPs concentration of 800 $\mu\text{g/mL}$, as presented in Table 2. Additionally, the ZnO NPs demonstrated superior antibacterial efficacy against gram-positive bacteria compared to gram-negative bacteria. This disparity can be attributed to the small size of the nanoparticles, their high surface-to-volume ratio, and the antibacterial mechanism of ZnO NPs, which is believed to involve interactions with specific cellular compounds. These compounds may be more prevalent or enhanced in gram-positive bacteria as opposed to gram-negative bacteria [33]. These results agree with those of Muslim and Naji [34].

There are two mechanisms of antibacterial activity in ZnO NPs: (1) when nanoparticles are deposited on the surface of bacteria, they rupture their cell wall, releasing intracellular components; and (2) Zn ions are generated and interact electrostatically with the bacterial cell wall, rupturing it, entering the cell and damaging the bacterial cell's constituent parts. Additionally, it leads to the production of reactive oxygen species (ROS), which have the capacity to damage DNA and proteins, ultimately leading to the demise of the bacterial cell. The results demonstrate the toxic properties of green-produced ZnO NPs against the

examined bacterial strains, indicating their potential as a highly efficient antibacterial agent in many industrial and pharmaceutical applications, including food preservation packaging [35].

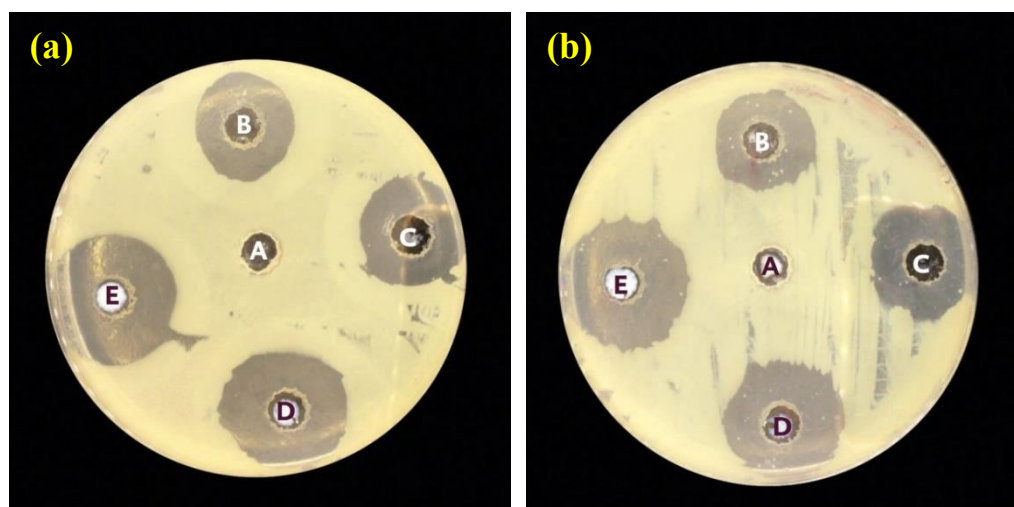


Figure 5: Antibacterial activity of ZnO NPs against (a) *K. pneumoniae* and (b) *S. aureus*. A) the control, and with ZnO NPs of concentrations, B) 100 µg/ml, C) 200 µg/ml, D) 400 µg/ml. and E) 800 µg/ml.

Table 2: Inhibition zone diameters (mm) for ZnONPs.

Bacterial strain	Inhibition zone (mm)				
	Control	100	200	400	800
<i>K.pnumonea</i>	0	9	10	11	12
<i>S.arueus</i>	0	10	10.5	13	14

3.2. Anticancer activity

Discovering pharmaceuticals to combat various forms of cancer is challenging due to their profound impact on public health. The morphology, composition, and dimensions of ZnO nanoparticles significantly impact their efficacy as anticancer agents. A549 cell line was studied against the synthesized ZnO NPs. Figure 6 (a and b) illustrates the morphological alterations of the cells in comparison to the untreated cells (control). It was shown that exposing A549 cells to different concentrations of ZnO NPs for 48 hours greatly reduced their ability to survive, as shown in Figure 7, with a significant decrease in cell viability with the increase in ZnO NPs concentration, especially at the highest concentration of 100 µg/mL. When ZnO NPs were added to A549 cells at concentrations ranging from 6.52 to 100 µg/mL, its cytotoxicity increased from 16.03 ± 1.468 to 89.47 ± 1.550 , which is a huge increase. ZnO NPs may induce cytotoxicity by activating a signaling pathway when a ligand and its corresponding receptor come into contact. The figure shows that the viability of the treated cell line decreased with increasing the ZnO NPs concentration, suggesting that the higher concentrations of ZnO NPs were more harmful to the cancer cell line. However, one limitation of the present study is the absence of experiments on normal (non-cancerous) cell lines. Evaluating the cytotoxicity of ZnO NPs on healthy cells is essential to determine their biosafety and selectivity. This important aspect will be addressed in future work to support the potential clinical applicability of the nanoparticles. Based on our findings, previous research has confirmed that ZnO NPs have a beneficial effect on cancer cells. Previous research has demonstrated that ZnO NPs has the ability to induce cytotoxic effects on many types of cancer cells. Furthermore, it has been proposed that the raised permeability, retention activity, and electrostatic interaction capabilities of ZnO NPs, along with the heightened

reactive oxygen species, are noteworthy. When reactive oxygen species (ROS) are present in cancer cells, they may make zinc oxide nanoparticles (ZnO NPs) more harmful [37]. ROS are molecules that exhibit chemical reactivity and can exist in either non-radical or radical forms. They consist of hydroxyl radical ($\text{OH}^{\cdot}$) and peroxide (O_2^{-2}), oxide (O^{-2}), and superoxide ($\text{O}_2^{\cdot-}$). They are created when neutral molecular oxygen (O_2) combines with other molecules. Nanoparticles (NPs) have unique material properties that make them potentially useful in the fight against cancer. A variety of nanoparticles (NPs), including metal oxide NPs and silver NPs, have been used in the diagnosis and treatment of malignant tumors [37]. Zinc oxide nanoparticles have shown a great deal of promise for use in the identification and management of cancer cells exhibiting aggressive tumors. Zinc oxide nanoparticles have demonstrated unique physical and chemical properties, as well as remarkable biocompatibility and natural degradation capabilities.

Studies indicate that ZnO nanoparticles induce cancer cell death through reactive oxygen species (ROS) generation and mitochondrial damage. ZnO NPs may be able to assist in the long-term, targeted delivery of anticancer medications to cancer cells [38]. The results of this study suggest that zinc oxide nanoparticles may be developed as potential anticancer agents for various types of cancer. Zinc oxide nanoparticles show increased cytotoxicity because zinc induces oxidative stress and upsets the equilibrium of protein activity [39]. There is evidence that ZnO nanoparticles have the amazing ability to induce oxidative stress in cancer cells. This process is a contributing factor to the cytotoxicity of ZnO nanoparticles in relation to cancer cells. ZnO is a material with semiconducting properties, which accounts for its characteristics. ZnO NPs induce the generation of reactive oxygen species (ROS), leading to oxidative stress. If the cell's antioxidant defenses are unable to cope with this stress, it can ultimately lead to cell death [40].

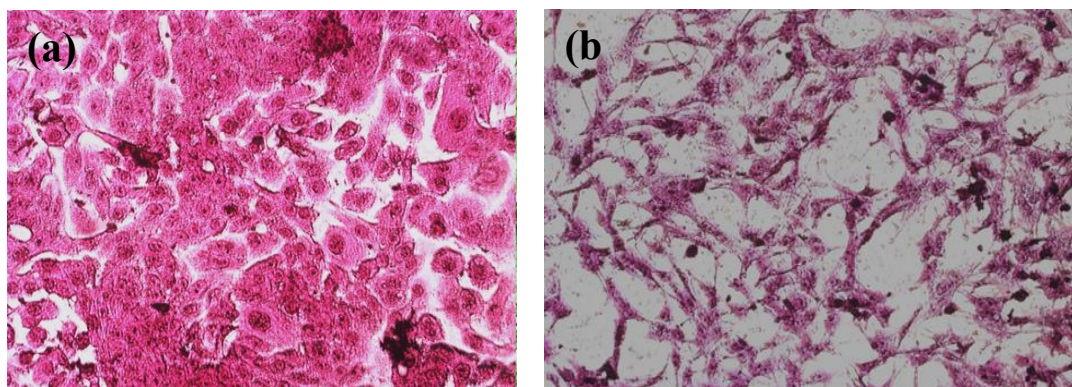


Figure 6: Morphological changes in A549 cancer cells of (a) untreated and (b) treated with synthesized ZnO NPs from *Rhazya stricta* (Harmal Alrazi) leaf extract of 100µg/ml concentration.

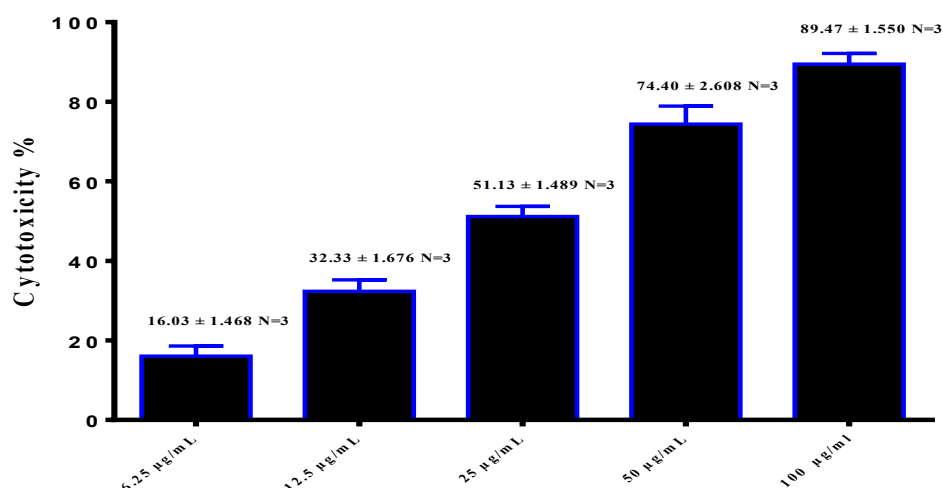


Figure 7: Cytotoxicity of different concentrations of ZnO NPs from *Rhazya stricta* (Harmal Alrazi) leaf extract against A549 cell line.

4. Conclusion

Zinc oxide nanoparticles (ZnO NPs) were successfully produced through the utilization of an aqueous leaf extract of Harmal Alrazi. This method, characterized by its simplicity, speed, ease, and environmentally friendly approach, involved bioactive molecules within the leaves acting as both reducing and stabilizing agents for NPs formation. X-ray diffraction (XRD) analysis revealed a hexagonal structure with an average crystallite size of 43.4 nm. The characteristic absorption maxima appeared at 379 nm. Field emission scanning electron microscopy (FESEM) depicted a semi-spherical shape with a particle size range of 26 to 38 nm. ZnONPs exhibited notable biological activities, including antibacterial and cytotoxic effects. The largest inhibition zone, measuring 14 mm, was observed against the gram-positive bacterium *S. aureus*, while it measured 12 mm against gram-negative bacteria *K. pneumoniae*. A dose-dependent cytotoxic effect was evident, with higher concentrations of ZnO NPs corresponding to increase cytotoxicity, specifically measuring 89.47 ± 1.550 . These findings highlight the potential of *Rhazya stricta*-synthesized ZnO NPs as natural antibacterial and anticancer agents.

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