

Rhynchosia capitata driven bioproduction of Zinc oxide nanoparticles, characterization and multifaceted therapeutic applications

Zakir Ullah

Quaid-i-Azam University

Javed Iqbal

javed89qau@gmail.com

Bacha Khan University

Banzeer Ahsan Abbasi

Rawalpindi Women University

Farhat Gul

Quaid-i-Azam University

Shumaila Ijaz

Shenzhen University Medical School, Shenzhen University

Sobia Kanwal

Allama Iqbal Open University

Mahboobeh Mahmoodi

University of California

Mohsin Kazi

King Saud University

Tariq Mahmood

Quaid-i-Azam University

Research Article

Keywords: Rhynchosia capitata, ZnONPs, antimicrobial, antioxidant, cytotoxicity, and anti-cancer

Posted Date: May 30th, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-4442285/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

The current study aimed to formulate zinc oxide nanoparticles (ZnONPs) employing an aqueous extract derived from *Rhynchosia capitata* (RC). Utilizing several spectroscopic methods (UV-visible spectroscopy, XRD, FTIR, SEM, EDX, DLS), the synthesis of ZnONPs was evaluated. ZnONPs have a mean crystallite size of about 32.15 nm, as determined by the Scherrer equation. Moreover, *in vitro*, biological assessments showed notable therapeutic potential. Furthermore, the disc diffusion method was precisely employed with antibacterial and antifungal properties across a range of concentrations (100–1500 µg/ml). Among the bacteria, *B. subtilis* displayed a prominent inhibition zone, measuring 24 mm at 1500 µg/ml. Similarly, susceptible fungal strains demonstrated a zone of inhibition measuring 29 mm. Antioxidant potentials were determined using different assays. The radical-scavenging capabilities were quantified using a DPPH assay and revealed a significant result of 73%. Further, reducing power was calculated and found to be $69 \pm 1.77\%$, and the total antioxidant capacity was found to be 88 ± 4.8 . Brine shrimps cytotoxicity assay was performed and displayed an LC_{50} value of 2.20 µg/mL, affirming biocompatibility. The biocompatibility potential of ZnONPs was determined against red blood cells, HEK, and VERO cell lines at different concentrations to confirm their biosafety and biocompatibility. An alpha-amylase (AA) inhibition assay was performed and showed a percent inhibition of 70%. Particularly noteworthy was the ZnONPs efficacy against (Hep-2 liver) cancer cells (IC_{50} :44.90). The ZnONPs exhibited promising non-toxic attributes and displayed substantial potential for diverse biological applications in various domains. The outcomes of this study are anticipated to contribute valuable insights and open new avenues for further research and development of ZnONPs in a multitude of biomedical applications.

Research Highlights

- Green zinc oxide nanoparticles (ZnONPs) were fabricated utilizing the extracts of *Rhynchosia capitata*.
- Various spectroscopic and microscopic instruments were used to confirm the synthesis of ZnONPs.
- Diverse biological assays have been performed to confirm the effectiveness of the formulated nanoparticles.

1. Introduction

Nanotechnology is a promising, emerging, and multidisciplinary area of science and engineering that concentrates on the synthesis, characterization, and applications of nanoparticles [1]. Nanoparticles exhibit distinguished features such as small size (1 to 100 nanometers), different shapes, large surface area to volume ratios, surface energy, etc., which enhance their catalytical properties [1, 2]. Over the past few decades, nanotechnology has gained considerable attention from the nano-taskforce due to its unique and distinct features related to particle size and structure [1, 2, 3]. Nanoparticles have emerged as groundbreaking solutions to address various technological and environmental challenges and have a

wide range of applications in catalysis, tissue engineering, pharmaceuticals, energy, bioremediation, agriculture, and drug delivery [4, 5, 6, 7, 8, 9, 10]. Nanoparticles can be synthesized using different synthesis protocols including physical, chemical, and Biological (green) synthesis approaches [11, 12]. However, the chemicals employed in physical and chemical synthesis protocols have adverse effects on the biotic and abiotic components of the environment [8, 9, 13]. Keeping in view the side effects associated with physical and chemical methods, consequently, the development of green synthesis methods has proven to be simple, cost-effective, energy-efficient, eco-friendly, and sustainable strategies for making nanoparticles owing to the abundant availability and reaction kinetics [10, 14]. The green synthesis of nanoparticles is non-toxic and biocompatible, resulting in the fabrication of nanoparticles with diverse sizes, shapes, compositions, and both physicochemical and biological potentials [15, 16, 17, 18].

The primary mechanism sustaining the phytofabrication of nanoparticles involves the presence of phytochemicals [19, 20, 21, 22]. Key phytochemicals responsible for reducing, stabilizing, and capping nanoparticles encompass carboxylic acids, aldehydes, ketones, amides, quinones, flavonoids, terpenoids, polysaccharides, and proteins [23]. Numerous research studies have revealed the efficiency with which plant extracts and phytochemicals can reduce and stabilize metal ions, surpassing microbial methods and yielding more stable nanoparticles [24, 25, 26, 27]. The scientific community has made joint collaborative and dedicated efforts among chemists, chemical technologists, and researchers towards the development of a wide range of metal and metal oxide nanoparticles [28, 29]. ZnONPs have gained considerable attention from the scientific community due to their remarkable attributes, including high electrical conductivity, stability, and notable catalytic, antibacterial, and antiviral properties [30]. ZnONPs belong to the family of group II-VI semiconductor materials featuring a considerable band gap of approximately 3.3 eV and a significant excitation energy capacity of 60 eV [31]. These nanoparticles find extensive use in various applications such as pottery, transparent materials, flexible polymers, ointments, lubricants, dyes, adhesives, and ceramic compounds [32]. ZnONPs are frequently employed for their outstanding antimicrobial, antioxidant, anti-inflammatory, and anticancer properties in the treatment of various ailments [33]. So far different medicinal plants mediating ZnONPs have been synthesized using green chemistry protocols [34]. The current research aimed to synthesize ZnONPs utilizing *Rhynchosia capitata* leaf extracts. Traditional medicines have a wide range of pharmacognostic properties (cholagogue, demulcent, diuretic, laxative, refrigerant, and vulnerary properties) [35]. Furthermore, it is also used to treat urinary system disorders like cystitis and nephritis, hinting at potential kidney and bladder ailment treatments [36]. The synthesis of as-prepared ZnONPs was confirmed using different characterization techniques. Finally, the prepared ZnONPs were analyzed for diverse bioactivities.

2. Material and methods

Collection of Plant and extract preparation

Following the established protocols and obtaining authorization, the *Rhynchosia capitata* (RC) (Leguminosae) medicinal plant was collected from Rumli Quaid-I-Azam University main campus, in Islamabad, Pakistan. The plant was investigated to meet all applicable national, International, and Institutional laws and regulations. The obtained samples were approved and taxonomically permitted by experts and taxonomists at <https://qau.edu.pk>, under voucher number SAS-571. The plant sample was carefully and thoroughly rinsed with distilled water and allowed to dry in the shade for around seven weeks. Using a previous approach [2], the material was crushed into a fine powder and kept in an airtight container. The extract was prepared by mixing 40 grams of dry *R. capitata* powder and 500 mL of distilled water and heated to 70-75 °C for approximately four hours. After bringing the solution down to room temperature, filter papers were used repeatedly to remove the pure aqueous phase.

2.1. Synthesis of *R. capitata* extract ZnONPs

The reaction mixture (100 milliliters of filtered *R. capitata*-mediated extract for three hours at 75° C with three grams of zinc acetate salt was made by using an already optimized protocol [2,6], and the nanoparticle was fabricated. Upon completion, the reaction mixture was centrifugated for 30 minutes at 3000 rpm in centrifuge. Similarly, the subsequent powder was carefully collected and preserved for later use after being placed at 500 °C for 60 minutes in a furnace. Furthermore, using the appropriate analytical instruments, the synthesized ZnONPs were thoroughly examined and characterized. Afterward, an assessment of the produced nanoparticles was conducted aimed at multiple purposes in biology (Figure. 1).

2.2 RC@ZnONPs characterization approaches

Several microscopic and spectroscopic techniques were used to determine and validate the synthesis of ZnONPs along with other physical and chemical features [2,6,37]. The synthesis of ZnONPs was demonstrated by the color shift in the zinc acetate solution after it was incubated with aqueous plant extract. But to pinpoint the precise absorption peak, ZnONPs were carefully examined with a UV-Vis spectrophotometer (UV-4000, Germany) within the 200–800 nm wavelength range. To validate the presence of distinct functional groups absorbed on the surface of the ZnONPs FT-IR analysis was performed. This analysis was conducted using an IR200 FT-IR spectrometer (Alpha Brucker, Germany) over a wavenumber range ranging from 400-4000 cm⁻¹. Using an energy-dispersive X-ray (EDX) detector equipped with a scanning electron microscope (SEM), the morphological characteristics and elemental makeup of the ZnONPs were thoroughly investigated. For this analysis, a small number of dried nanoparticles was precisely prepared and mounted on a sample stub, ensuring accurate characterization of the nanoparticles surface morphology and elemental composition. By using a high-resolution X-ray diffractometer (Empyrean diffractometer, PANalytical) with a 45 kV voltage and copper K α radiation at a wavelength of $\lambda=1.5406$ Å, the crystalline structure of the ZnONPs was revealed. Through a thorough study, and by utilizing the Scherrer equation the size and the crystallographic characteristics of the nanoparticles were determined. In addition, the polydispersity index (PDI) and size distribution range of the particles in a solution were determined using the Dynamic Light Scattering (DLS) approach. The

electrostatic charge present on a particle's surface was found using zeta potential. Important information about the ZnONPs' size distribution and colloidal stability was gleaned from these experiments.

2.3. Bioactivities of RC@ZnONPs

2.3.1. Assessing Cytotoxicity with *Artemia salina*

Using a previously developed methodology, an *Artemia salina* (A.S) cytotoxicity test was carried out to assess the cytotoxic potential of the as-prepared ZnONPs [2,6,20]. This procedure is a well-known approach for figuring out how harmful naturally occurring substances are. The eggs of *A. salina* (Oceanic Star, USA) were raised on a hatching tray with a saline solution containing 3.8 g/L. To guarantee that mature nauplii hatched, the hatching tray was then put into the incubator and kept under constant illumination for 24 hours at a regulated temperature of 27 °C. Twenty mature nauplii have been placed into individual glass vials to assess the cytotoxicity potential. Employing a Pasteur pipette, a range of ZnONPs concentrations (50–1200 µg/mL) were incorporated into these vials. Mature nauplii, vincristine sulfate, and saline solution in a glass vial were employed as positive controls, while dimethyl sulfoxide (DMSO) was used for negative controls. The vials were then kept in an incubator for twenty-four hours at a regulated temperature of 30° C. After the incubation period, each vial's dead *A. salina* was counted thoroughly. The GraphPad (version 8.0.0) was used to examine the data collected for this evaluation.

2.3.2. Antidiabetic (Alpha-Amylase Inhibition) Assay

The alpha-amylase inhibition studies were utilized to investigate the antidiabetic efficacy of RC@ZnONPs [6,21]. The study aims to assess the antidiabetic potential by controlling the alpha-amylase (which breaks down carbohydrates in animal pancreas and saliva) enzyme [38]. The potential therapeutic application of ZnONPs as medication for diabetes was examined. In this experiment, a reaction mixture was prepared on a microplate by adding 30 µL of ZnONPs, 40 µL of starch solution, 15 µL of phosphate-buffer saline (pH 7), and 25 µL of alpha-amylase successively. The reaction mixture was incubated at 50 °C for 90 minutes. Subsequently, the mixture was added with 90 µL of iodine solution and 20 µL of 1 M HCl. The positive and negative controls used were acarbose and DMSO, respectively. To quantify the assay's results, optical density (O.D) readings were taken at 540 nm using a reader (SN64001849) [2,6]. The % inhibition was determined by employing the formula:

$$\% \text{ Inhibition} = (\text{Sample Abs} - \text{Abs of negative control}) / (\text{Abs of blank} - \text{Abs of negative control}) \times 100.$$

Furthermore, the concentration at which 50% of the inhibition was attained was determined by calculating the median lethal concentration (LC₅₀) value using GraphPad 8.0.0.

2.3.3. Antibacterial activity of RC@ZnONPs

Using the disc diffusion method (DDM) [2,6,22] on several bacterial strains, the antibacterial efficiency of the obtained RC@ZnONPs was evaluated. The strains were subcultured in a nutrient broth medium and

were incubated at 37 °C for 24 hours. Strains were streaked on an agar medium and then allowed to dry. The filter discs were then put on the media after being loaded with (50 µl) of ZnONPs at concentrations (100-1500 µg/mL). Soon after, the plates were incubated for 24 hours at 37 °C in a controlled environment. After incubation, zones of inhibition (ZOI) on the plates were observed. The oxytetracycline and 5% DMSO were the positive and negative controls respectively in the reaction. Origin-2018 was used to examine the data.

2.3.4. Antifungal activity of RC@ZnONPs

An antifungal experiment of RC@ZnONPs was assessed using an autoclaved Sabouraud dextrose liquid medium (SDA) [21]. After a 24-hour incubation period at 37°C in an incubator, several fungal strains were subcultured in SDA (DM 1063, Batch no. 450416). For the liquid fungal cultures, the optical density was set at 0.5. Later, using autoclaved cotton swabs, the fungal strains' broth cultures were evenly spread over an SDA medium. Various concentrations of ZnONPs, ranging from 100-1500 µg/mL, were added to the medium. The fungal strains were cultured in the pre-made Petri plates for 72 hours at 37 °C. After 48 hours of the incubation period, ZOI was determined. Ampicillin/amphotericin-B and DMSO were utilized as a positive and negative control respectively.

2.3.5. Testing blood compatibility using RC@ZnONPs

A hemocompatibility experiment [2,6,21] was conducted to evaluate the possible impact of zinc oxide nanoparticles (ZnONPs) on the safety of human red blood cells (RBCs) and to ascertain if ZnONPs had any negative effects on human RBCs. The process commenced with the addition of 90 microliters of blood to the EDTA (ethylenediaminetetraacetic acid) tube. The RBCs were then separated from the blood and a pellet was produced by centrifuging the mixture for 12 minutes at 3000 rpm. The pellet was then thoroughly washed with phosphate-buffered saline (PBS) to get rid of any residual ingredients. 200 µL of pure erythrocytes were added to 9.8 mL of PBS buffer to produce erythrocyte suspensions. Furthermore, ZnONPs were introduced at different doses ranging from 17 to 200 µg/mL to 100 µL of the erythrocyte solution. The mixture was centrifuged for 15 minutes at 3000 rpm after being incubated for one to two hours at 37°C. The supernatant was then transferred to a 96-well plate, and the absorbance at 540 nm was measured using a microplate reader to ascertain the amount of hemoglobin released. In this experiment, the positive and negative controls were Triton X-100 and DMSO, respectively. The hemolysis percentage was calculated with the formula:

$$\% \text{ Hemolysis} = (\text{Sample absorbance} - \text{Negative Control}) / (\text{Negative control}) \times 100.$$

2.3.6. Evaluation of RC@ZnONPs' Antioxidant Capabilities

Antioxidants have significance for combating free radicals and supporting the restoration of normal cell activity. The antioxidant capacity of produced RC-ZnONPs was assessed in this study using three antioxidant investigations [39]. To produce free radicals, 25 milliliters of methanol were dissolved with 2.4 milligrams of 2,2-diphenyl-1-picrylhydrazylhydrate (DPPH). Following the experimental formula, 180

μL of the reagent solution and 20 μL of ZnONPs were put on a 96-well plate. The plate was then placed in darkness for 60 minutes. At different doses (5-100 μg/mL), ZnONPs' capacity to scavenge free radicals was examined. DMSO was utilized as the negative control and ascorbic acid (AA) as the positive control. With the aid of a microplate reader, observations were made at a wavelength of 517 nm. The proportion of DPPH scavenging was computed using the following formula:

$$\% \text{ scavenging} = [(\text{Abs of control} - \text{Abs. of sample}) / \text{Abs. of control}] \times 100$$

$$\text{DPPH scavenging} = 1 - (\text{Abs of the sample}) / (\text{Abs of control}) \times 100.$$

Moreover, the total reducing power (TRP) of ZnONPs was evaluated in a similar manner using the potassium-ferricyanide method [26]. In this study, aspartic acid (AA) served as the positive control and DMSO as the negative control. At 630 nm, the optical density was determined using a microplate reader. In addition, TAC was calculated using the phosphomolybdenum approach, which measures a test solution's capacity to neutralize free radicals [34]. By adding a 0.2 mL solution of sodium phosphate, sulfuric acid (0.6 M NaHPO₄, H₂SO₄, H₂O 28 mL), and 4 mM ammonium heptamolybdate (NH₄)₆Mo₇O₂₄ · 4H₂O) while maintaining an acidic pH, the TAC reagent was made. The reaction mixture that was obtained was incubated in the water bath at 95 °C for 90 minutes. Ascorbic acid was utilized as the positive control and DMSO as the negative control. Ascorbic acid equivalents (gAAE/mg), or micrograms per milligram of the substance, were used to describe the results. At a wavelength of 695 nm, optical density was measured with a microplate reader.

2.3.7. Cytocompatibility Assessment of RC@ZnONPs

Several cell lines (HEK (human embryonic kidney cells) and VERO (renal epithelial cells derived from an African green monkey, *Chlorocebus* sp.) cells) were used to assess the cytotoxicity [39, 40] of the synthesized ZnONPs. The cell lines were sourced from the National Institute of Health (NIH) in Islamabad (<https://www.nih.org.pk>). In each well of a 96-well plate, approximately 10,000 cells (1×10^4 cells) were seeded. Subsequently, various concentrations of ZnONPs, spanning from 5-100 μg/mL, were introduced into the plates containing these cell lines. Following treatment, the cells were kept at 37 °C in a CO₂ incubator for 24 hours. After the incubation period, each well was given 10 μL of 4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide (MTT; 5 mg/mL). For around three hours, the cells were cultured so that formazan crystals could form. After dissolving the formazan crystals in around 100 μL of DMSO, the mixture was incubated for an additional 50 minutes. Next, at 570 nm, the absorbance value was measured with an Eliza microplate reader. The percentage of cell inhibition was calculated using the following method to evaluate the viability of the cells:

$$\% \text{ Cell Inhibition} = 100 - (\text{Cell Inhibition}).$$

Here, cell inhibition was calculated as:

$$\text{Cell Inhibition} = ((\text{O.D. of treated cells}) / (\text{O.D. of control})) \times 100.$$

2.3.8. Cytotoxicity and Anti-Cancer Assay of RC@ZnONPs

In the territory of cancer therapy, NPs are frequently employed either as nanocarriers, combined with active drugs or as independent agents. Nano-drug systems are favored due to their enhanced bioavailability, extended activity, and improved metabolic stability [27,48]. This work examined the antiproliferative effects of the yielded nanoparticles using the Hep-2 liver cancer cell obtained from the NIH. 10,000 cells (1×10^4) were planted into each well of a 96-well plate for the experiment, and the plates were left in the incubator overnight. ZnONPs were subsequently added to the 96-well plate at varying quantities, ranging from 5-100 $\mu\text{g/mL}$. After that, these treated cells remained for 24 hours at 37 °C in a CO₂ incubator. After the incubation period, each well receives 10 μL of 4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide (MTT; 5 mg/mL). To allow the formazan crystals to form, the cells were cultured for around three hours. After dissolving the formazan crystals with DMSO (100 μL) solvent, the combination was incubated for a further hour. Next, an ELISA was used to measure the absorbance value at 570 nm.

To evaluate the percent cell viability, the formula utilized was:

$$\text{Cell Viability (\%)} = (\text{Abs of test compound}) / (\text{Abs of control (untreated cells)}) \times 100.$$

Furthermore, the calculation of cell inhibition was carried out using the formula:

$$\text{cell inhibition} = (\text{O.D. of treated cells} / \text{O.D. of control}) \times 100.$$

3. Results and discussion

3.1. Synthesis of ZnONPs

In the present research, extracts from RC leaves were used as stabilizing agents and bio-reductants to produce ZnONPs. In the extracts of *R. capitata*, various Biomolecules (**Figure.2**) were screened out (**Table 1**). The observable shift in color from light brown to yellow white serves as a clear indicator of ZnONPs production. This color alteration is a consequence of specific optical properties, primarily arising from surface plasmon vibrations. A broad spectrum of characterization approaches, including UV spectroscopy, FT-IR, XRD, SEM, EDX DLS, and Zeta potential analysis, were used to thoroughly assess the biocompatible nature of the yielded ZnONPs.

Table. 1. The basic phytochemical evaluation of *R. capitata*.

S/No	Secondary Metabolites	Indicators
1	Phenol	+++
2	Flavonoids	+++
3	Terpenoids	+++
4	Saponins	+++
5	Tannins	+++
6	Steroids	+++
7	Glycosides	–
8	Phytosterol	++
9	Coumarins	+
10	Proteins	++
11	Carbohydrates	+++
12	Anthocyanin& BetaCyanin	–
13	Phlobatannins	–
14	Quinone	++
15	Fats and Fixed Oils	+++

3.2. UV-Vis Spectrophotometry of RC@ZnONPs

A significant aspect of our study involved the utilization of UV-Vis spectrophotometry to monitor and characterize the bioreduction process. The effective reduction of the precursor solution to ZnONPs was evident through observable changes in color as the aqueous extracts were introduced. A visible transition from a lighter brown hue to a whiter brown shade indicated a pronounced increase in the efficiency of the bioreduction process. By scanning the reaction mixture over a range of wavelengths (200 to 700 nm), Particularly noteworthy were the absorbance peaks detected within the 377 nm wavelength. These peaks aligned well with the characteristic Surface Plasmon Resonance (SPR) range associated with ZnONPs confirming the successful synthesis of ZnONPs (**Figure. 3 (A)**). Importantly, the absence of any apparent blue or red shifts at the peak point emphasized that the nanoparticles exhibited a consistent and steady SPR behavior. Remarkably, our research outcomes, as derived from the understandings provided by UV-Vis spectroscopy, were found to align agreeably with the conclusions drawn from an earlier study [30,34,35]. This consistency across studies underscores the strength and validity of our findings.

3.3. X-ray diffraction spectroscopy of RC@ZnONPs

XRD was used to assess the crystalline properties of ZnONPs. A thorough X-ray diffraction study was incorporated on annealed biogenic ZnONPs at a temperature of 100 °C. The resulting XRD pattern exhibited distinct Bragg peaks that precisely matched the expected crystalline reflections from the JCPDS pattern 00-036-1451, illustrated in **Figure 3 (B)**. These peaks were observed at specific angles: 22.62 (012°), 018 (60.16°), 024 (55.07°), 030 (65.54°), 104 (36.08°), 110 (40.33°), 113 (50.91°), 116 (57.81°), and 214 (62.63°), as elaborated in **Table 2**. The size of the ZnONPs was determined to be approximately 32.15 nm using Debye Scherrer's equation. These findings align with previous research conducted by [36,37], reinforcing the reliability of the results. It is noteworthy that the presence of Bragg peaks consistent with the pure phase of ZnO signifies the complete crystallinity of the biogenic ZnONPs.

Table: 2. (2 theta) value and Bragg peak of RC@ZnONPs

S. No	2 theta value	Bragg peak
1	22.62	012
2	36.08	104
3	40.33	110
4	50.91	113
5	55.07	024
6	57.81	116
7	60.16	018
8	62.63	214
9	65.54	030

3.4. FTIR of RC@ZnONPs

The molecular vibrations and the existence of functional groups or biomolecules that are essential to the synthesis and stability of ZnONPs were examined using FTIR spectroscopy. Different absorption bands that correspond to different chemical vibrations and functional groups were seen in the FTIR spectra, as shown in **Figure 4**. The stretching vibrations of the OH plane bending were shown by the absorption bands seen at 662.2 cm⁻¹. Similarly, the absorption bands at 839.93 cm⁻¹, which correlate to the bending and rocking vibrations of molecules containing carbon and hydrogen, were used to determine the presence of a C-H bond. The absorption bands at 927.43 cm⁻¹ were used to identify the stretching vibrations of C-O. Moreover, the absorption bands at 1013.93 cm⁻¹, which correlate to the stretching vibrations of carbon-oxygen bonds, clearly showed the presence of a C-O-C bond in the sample. The C-O-

C bond was linked to the absorption band at 1107.77, and the peak indicated the stretching vibrations of the CN amide III bond at 1253.30 cm⁻¹. Additionally, the stretching of C-O inorganic carbonate was found at 1422.90 and the CH aliphatic bending group was observed at 1370.21 cm⁻¹. Furthermore, the Diketones functional groupings have been determined at 1535.24–1640.78. In the same way at 2865 C-H alkane medium to strong, at 3027.24 C=C bond and 3629.2 O-H strong bond of alcohol were observed. This absorption feature provides an understanding of the presence of compounds containing variant functional groups. The information gathered from these FTIR absorption bands was summarized in **Table 3**, allowing for a comprehensive absorption of the molecular components and functional groups present in the sample under investigation. Our findings are also in good agreement with the earlier reported studies [33,34,35,36,37].

Table. 3. Functional group associated with the surface area of the RC@ZnONPs

S. NO	Wavenumber cm ⁻¹	Functional groups capped up on NPs
1	662.2	OH plane bending
2	839.93	C-H bond
3	927.43	C-O stretching
4	1013.93	C-O-C bond
5	1107.77	C-O-C bond
6	1253.30	CN amide III bond
7	1370.21	CH aliphatic bending group
8	1422.90	Stretching C-O inorganic carbonate
9	1535.24-1640.78	Diketones
10	2865	C-H alkane medium to strong
11	3027.24	C=C bond
12	3629.2	O-H strong bond of alcohol

3.5. Zeta Potential of RC@ZnONPs

In the case of ZnONPs, mediated by RC, their size and charge characteristics were evaluated using two techniques: Dynamic Light Scattering (DLS) and ζ potential. In this context, the ZnONPs produced using RC exhibited a negative charge in terms of zeta potential. This negative charge arises due to the high adsorption of phytochemicals on the surface of the nanoparticles. This charge plays a pivotal role in stabilizing the nanoparticles by creating electrostatic repulsions between them. As a result, the nanoparticles are less likely to aggregate or clump, ensuring their dispersion and maintaining their

individuality in the solution. The polydispersity index (PDI) is a parameter that provides perceptions of the distribution of particle sizes within a sample. A lower PDI value, as indicated by the value of 0.428 in **Figure 5(A)**, suggests a more uniform size distribution among the nanoparticles. This uniformity reveals the high-quality nanoparticles with consistent characteristics, making them suitable for various biological activities. Furthermore, the measured zeta potential value of -19.4 mV for the ZnONPs (**Figure 5B**) falls within the range of -30 mV to +30 mV, strongly considered anionic which is generally measured optimal for nanoparticle stability. This means that the ZnONPs negative charge is within the stable range, reinforcing their suitability for various applications. Interestingly, previous research involving [6,35,38,39,40,41], also yielded findings consistent with the results obtained in the present study.

3.6. Scanning Electron Microscopy of RC@ZnONPs

In this study, SEM analysis was conducted to examine the physical appearance of the ZnONPs that were produced with the assistance of RC. As illustrated in **Figure 6 (A)**, the SEM images depict the ZnONPs synthesized through RC mediation. The images demonstrate that these nanoparticles possess a spherical morphology. Moreover, the mean crystal diameter of the nanoparticles is reported to be 32.15 nm, a measurement that was determined through the XRD analysis. This SEM analysis provides valuable insights into the physical characteristics of the nanoparticles. The spherical shape of the particles suggests that the synthesis process yielded uniform structures, which is essential for consistent properties and potential applications [42,43,44].

3.7. Energy-dispersive X-Ray Spectroscopy

In the case of the ZnONPs synthesized with the involvement of RC. Through EDS analysis, it was confirmed that the ZnONPs mainly consisted of zinc (Zn) and oxygen (O). **Figure 6 (B)** visually represents the EDS spectrum, highlighting strong signals corresponding to zinc and oxygen elements. Only zinc and oxygen in the spectrum reinforce the idea of single-phase purity, indicating that the nanoparticles were primarily composed of these two elements. The EDS spectrum also showed minute amounts of additional elements, such as chlorine (Cl), in addition to zinc and oxygen. These trace components may come from the synthesis process, or the sample being prepared for analysis, among other sources. However, the overall purity and quality of the ZnONPs are unaffected by their slight presence [43,44,45,46].

3. 8. Pharmacological assays

3.8.1. Brine-shrimp cytotoxicity assessment

The brine shrimp lethality assay (BSLA) was utilized to evaluate the potential detrimental impact of green ZnONPs on freshly hatched *A. salina* while estimating cytotoxicity [6]. The objective of the study was to examine ZnONP cytotoxicity at different concentrations, particularly between 50 µg/mL and 1200 µg/mL. The estimation's findings demonstrated a dose-dependent relationship between the ZnONPs'

cytotoxic potential. 90% of the newly hatched *A. salina* died by the ZnONPs at the maximum dose tested (1200 µg/mL). On the other hand, 10% died at the lowest dose (50 µg/mL). The ZnONPs' capacity to produce cytotoxic effects was further supported by this concentration-dependent reaction. The calculated LC₅₀ value for the ZnONPs was measured at 2.20 µg/mL, indicating the concentration at which 50% of the brine shrimp population exhibited mortality. As the nanoparticle concentration increased, so did their cytotoxic potential, a trend that was visually depicted in **Figure 7**. These findings are consistent with prior research on Zinc-mediated nanoparticles. Similar studies involving [6,32,45,46,47,48] have yielded results in alignment with our research, highlighting the reproducibility and validity of the observed effects. In the current study vincristine sulfate, used as a positive control, demonstrated an LC₅₀ value of 1.975 µg/mL, indicating its higher cytotoxic potential in comparison to the ZnONPs.

3.8.2. Antidiabetic assessment (Alpha-amylase inhibition (AA))

The research examined how effective ZnONPs are at inhibiting Alpha-amylase activity at various concentrations: The inhibitory potential of ZnONPs was notable across a sort of concentrations, from **50-1100 µg/mL**. As the concentration of ZnONPs decreased, the rate of Alpha-amylase inhibition slowed down (**Figure 8**). The highest inhibition (70%) was achieved at the maximum concentration (1100 µg/mL). Inhibition percentages were 42% at 700 µg/mL and 25% at 500 µg/mL. However, positive control (surfactin: ~83%) showed greater inhibition than tested ZnONPs. The research findings regarding green ZnONPs from RC are confirmed by similar outcomes observed with ZnONPs derived from other plants: [6,21,33,38,39,47,48,49,50].

3.8.3. Antibacterial assay of RC@ZnONPs

The present study employed an antibacterial assay to evaluate the efficacy of biogenic ZnONPs against a range of bacterial strains, including *Staphylococcus aureus* (ATCC 23235), *Lactobacillus acidophilus* (ATCC 4356), *Bacillus subtilis* (ATCC 23857), *R. jostii*, *Pseudomonas aeruginosa* (ATCC 15442), and *Escherichia coli* (ATCC BAA-2471), at varied dosages. These bacterial strains were carefully chosen, with *S. aureus*, *L. acidophilus*, *B. subtilis*, and *R. jostii* representing gram-positive bacteria, while *P. aeruginosa* and *E. coli* are gram-negative bacteria. The results of the current investigation showed that *B. subtilis* was vulnerable to the effects of ZnONPs at 1500 µg/mL, which induced a ZOI that measured 24 mm. As the concentration was lowered to 100 µg/mL, the ZOI dropped to 6 mm. Similarly, strain *L. acidophilus* displayed a ZOI of 23.25 mm at 1500 µg/mL and 6 mm at 100 µg/mL. In the case of *E. coli*, ZOI was 22.70 mm when treated with 1500 µg/mL of ZnONPs, while at 100 µg/mL, the ZOI was 6 mm. Comparable trends were noted for other strains of *P. aeruginosa*, where a ZOI of 22.75 mm was observed at 1500 µg/mL, and this reduced to 6 mm at 100 µg/mL. The susceptibility of *S. aureus* to ZnONPs was evident, with a ZOI of 23 mm at 1500 µg/mL and 6 mm at 100 µg/mL. Similarly, *R. jostii* displayed vulnerability, exhibiting a ZOI of 23.78 mm at 1500 µg/mL and 8 mm at 100 µg/mL.

Incorporated in **Table 4** are the recorded ZOI values, where a positive control involved the administration of 10 mg of pure oxytetracycline medication. Particularly, oxytetracycline displayed higher efficiency compared to any singular concentration of the test sample. In essence, our findings suggest a promising potential for biogenic ZnONPs in terms of antibacterial activity, aligning well with prior research in this area [2,6,7,19,40,49,50,51,52]. The potential efficiency of these ZnONPs might be attributed to the presence of bioactive functional groups associated with the nanoparticles. These functional groups play a pivotal role in capping and stabilizing the ZnONPs, and their presence correlates with a significant level of antibacterial activity, as depicted in **Figure 9**.

Table. 4. List of strains and their ZOI value at different concentrations.

Bacterial strains	ZOI (µg/ml)
B. subtilus	24
L. acidophilus	23.25
S. aureus	23
E. choli	22.7
P. aeruginosa	22.75
R. jostii	23.78
Oxytetracycline	30

3.8.4. Antifungal activity of RC@ZnONPs

The study aimed to evaluate the antifungal competence of ZnONPs synthesized using RC as a mediator. These ZnONPs were tested against multiple fungal strains, including *Aspergillus flavus* (Strain ID: FCBP:0064), *Aspergillus niger* (Strain ID: FCBP:0918), *Candida albicans* (Strain ID: FCBP:478), *Mucor racemosus*, and *Fusarium solani* (Strain ID: FCBP:0291) at different concentrations (1500 to 100 µg/mL). In this study, *A. flavus* emerged as a susceptible strain, revealing a ZOI measuring approximately 27 ± 0.71 mm at 1500 µg/mL. Furthermore, its Minimum Inhibitory Concentration (MIC) value was determined to be around 8.2 ± 1.4 mm at the lower concentration of 100 µg/mL. Similarly, *A. niger* demonstrated a ZOI of about 28.75 ± 0.71 mm at 1500 µg/mL, while *M. racemosus* displayed a ZOI of roughly 29 ± 0.71 mm at the same concentration. *C. albicans* exhibited constrained growth within a ZOI of approximately 28 ± 1.41 mm, and *F. solani* strains displayed a ZOI of 27.50 ± 0.71 mm (as indicated in **Figure 10**). It is important to note, however, that none of the tested samples showed a degree of inhibition greater than that of Amp-B, a known antifungal agent. This data is consistent with previous studies [2,5,7,21,32,39,41,42,50,51,52,53] that indicate ZnONPs interact with fungi and their hyphae to impede fungal growth and produce Reactive Oxygen Species (ROS). This current study's outcomes parallel those of previous investigations conducted which showcased a significant and dose-dependent antifungal activity, a correspondence that further encourages the credibility of the present findings.

Table. 5. ZOI of different fungal strains at various concentrations.

Fungal strains	ZOI (µg/ml)
A. flavus	27
F. solani	27.5
A. niger	28.75
C. albicans	28
M. racemosus	29
Ampicillin	38

3.8.5. Antihemolytic effects of RC@ZnONPs on Human RBC

The criteria for categorizing a biological substance's hemolytic activity were as: A substance is classified as hemolytic when its activity level reaches a minimum of 5%, while it is categorized as slightly hemolytic if the activity falls within the range of 2-5%, and non-hemolytic if the activity is less than 2% [2,6,24]. The current assessment involved subjecting human RBCs to varying concentrations of ZnONPs, ranging from 200-17 µg/mL. The data collected from this experiment revealed several important findings. The synthesized nanoparticles exhibited non-hemolytic behavior at lower concentrations, specifically at 17, 37, and 71 µg/mL. The concentration range 75–125 µg/mL, showed a somewhat hemolytic response. However, at above 125 µg/mL, the nanoparticles exhibited clear hemolytic activity. These outcomes align with earlier studies involving ZnONPs mediated by substances such as [2,5,53,54,55]. The current study's results contribute to the body of evidence indicating that the biosynthesized ZnONPs are biocompatible and non-hemolytic at lower concentrations (**Figure 11 (A)**).

3.8.6. Antioxidant activities of RC@ZnONPs

This study examined RC@ZnONPs antioxidant properties (TAC, TRP, and DPPH free radical scavenging). The plant extract contains phenolic compounds known for their ability to scavenge ROS, complementing the properties of ZnONPs. Various Concentrations (5–100 µg/mL) were used in the current assay. A substantial capacity to scavenge DPPH radicals was evident at a concentration of 100 µg/mL, demonstrating a scavenging rate of 73% (depicted in **Figure (11-B)**). This data suggests that a diverse range of antioxidant substances potentially contributes to stabilizing and reducing ZnONPs via the RC leaf extract involvement. Moreover, the highest recorded total antioxidant activity index was $88 \pm 4.8\%$ at 100 µg/mL of ZnONPs, whereas the lowest TAC activity observed was $3.0 \pm 1.7\%$. **Figure (11-C)** graphically illustrates the TAC results, Showing the capacity of the compounds to scavenge ROS. The TRP was conducted to investigate deeper into the existence of antioxidant entities associated with ZnONPs. This inquiry aimed to elucidate reductones, molecules that contribute hydrogen atoms to enhance antioxidant potential, thereby serving as agents that mitigate potential chain damage induced by free radicals. The utilization of biogenic ZnONPs has provided considerable evidence of their vigorous antioxidant abilities. A proportional decline in reducing power was observed as the concentration of

ZnONPs decreased. When the concentration hit 100 µg/mL, the reduction in the value was at $69 \pm 1.77\%$. In contrast, the lowest result at a concentration in the 5 µg/mL range was measured at $12 \pm 1.36\%$ (as illustrated in **Figure 11-D**). Our findings align with prior research involving biogenic ZnONPs synthesized from sources such as [2,5,6,7,54,55,56,57]. This collective body of evidence underscores the significant potential of biogenic ZnONPs as potent antioxidants.

3.6. Biocompatibility assay of RC@ZnONPs

To measure the biocompatibility of ZnONPs, VERO and HEK293 cell lines were utilized in the assessment. Prior to being allowed to adhere to DMEM medium for 24 hours, cells were first seeded into a 96-well plate. Afterward, different concentrations of ZnONPs, ranging from 5 to 100 µg/mL, were added to the cells. The MTT cell viability assay, following the methodology established by Devanesan et al. in 2021 [58], was employed to measure the biocompatibility features of the ZnONPs. The cellular response to ZnONPs treatment displayed a dose-dependent pattern. The assay outcomes revealed that at a concentration of 100 µg/mL, ZnONPs hindered cell growth by approximately 46%, indicating the biologically safe nature of biogenically synthesized ZnONPs. Notably, both VERO and HEK293 cell lines possess inherent mechanisms to respond to ROS from external sources. Prior studies have indicated that lower concentrations of ROS are non-lethal to these cell lines. However, once ROS concentrations surpass a certain threshold, the viability of both cell types becomes compromised [58]. The IC_{50} values for ZnONPs were determined to be 208.14 µg/mL for VERO cells and 344.90 µg/mL for HEK293 cells, representing the concentrations at which ZnONPs inhibit cell growth by 50 (**Figure.12**). The findings regarding ZnONPs mediated by RC were consistent with previous studies of ZnONPs [58,59,60].

3.8.7. Anticancer activity of the RC@ZnONPs

In this study the impact of the nanoparticles on cancer cells, specifically Hep-2 cancer cell lines, was assessed by introducing varying doses of nanoparticles ranging (5-100 µg/mL), allowing them to incubate for 24 hours. The primary objective was to evaluate the antiproliferative effects of the ZnONPs. The study found that the viability of Hep-2 cells was inhibited in a dose-dependent manner. Results from the MTT test exhibited a gradual decrease in cell viability with rising quantities of ZnONPs, as depicted in **Figure 13**. Notably, the calculated IC_{50} for ZnONPs was determined to be 44.90 µg/mL. In addition to quantitative assessments, an inverted microscope was utilized to scrutinize cellular morphology across different ZnONPs concentrations. As shown in **Figure 13**, the results indicated that biosynthesized ZnONPs had a potent and substantial impact on Hep-2 cell lines, validating their antiproliferative properties. Collectively, these understandings suggest that green-synthesized ZnONPs possess noteworthy power versus Hep-2 cell lines. Our findings align with prior reports on biosynthesized ZnONPs [2,60,61,62,63,64,65,66]. This research has the potential to contribute significantly to the ongoing pursuit of innovative and effective approaches to combating cancer. Overall, the biocompatibility and anticancer analysis are shown in illustration (**A**).

4. Conclusion

This research study inquired into the utilization of RC, known as a medicinal plant due to its established therapeutic properties. Markedly, these findings highlighted the multifaceted biological functionalities of ZnONPs, showcasing their biocompatible properties. The outcomes demonstrated a dose-dependent behavior in the ability of ZnONPs across various biological activities. Future research endeavors should encompass comprehensive in vitro and in vivo studies employing diverse animal models to clarify the pharmacological significance across various bioactive contexts. Comprehensive in vitro and in vivo studies should explore ZnONPs' complete range of biological activities, extending beyond cancer to integrate infectious diseases and neurodegenerative disorders. Investigating potential interactions between ZnONPs and existing cancer therapies or natural compounds can enhance therapeutic outcomes. Integrating ZnONPs into advanced nanomedicine platforms or drug delivery systems can improve targeted delivery to cancer cells. Collaboration among oncology, nanotechnology, pharmacology, and biotechnology experts can accelerate ZnONPs development and application. This research has the potential to advance our understanding of biogenic ZnONPs and potentially transform cancer treatment, particularly in liver cancer. The complete approach should combine scientific consistency, clinical translation, and interdisciplinary cooperation to fully connect the promise of this innovative approach.

Declarations

Author Contributions: ZK, J.I and TM designed and conceived the study idea. ZU completed the experiments. FG, BAA, S.I, S.K, and M.M analysed the data and performed visualizations and statistical data analysis. ZU and JI wrote the original draft. ZU, JI, T.M, BAA reviewed and edited the manuscript. M.K reviewed the manuscript and provided funds. TM provided the resources and supervision. All authors made valuable revisions and edited the manuscript and approved the last version.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All the raw data in this research can be obtained from the corresponding authors upon reasonable request.

Acknowledgements: The authors express their genuine gratitude to the Researchers Supporting Project Number (RSP2024R301) at King Saud University, Riyadh, Saudi Arabia.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Ahire SA, Bachhav AA, Pawar TB, Jagdale BS, Patil AV, Koli PB. (2022). The augmentation of nanotechnology era: A concise review on fundamental concepts of nanotechnology and applications in material science and technology. Results Chem, 100633.

2. Ullah Z, Iqbal J, Gul F, Abbasi BA, Kanwal S, Elsadek MF, Mahmood T. Biogenic synthesis, characterization, and in vitro biological investigation of silver oxide nanoparticles (AgONPs) using *Rhynchosia capitata*. *Sci Rep*. 2024;14(1):10484.
3. An C, Sun C, Li N, Huang B, Jiang J, Shen Y, Wang Y. Nanomaterials and nanotechnology for the delivery of agrochemicals: strategies towards sustainable agriculture. *J Nanobiotechnol*. 2022;20(1):1–19.
4. Jain N, Gupta E, Kanu NJ. Plethora of Carbon nanotubes applications in various fields—A state-of-the-art-review. *Smart Sci*. 2022;10(1):1–24.
5. Ullah R, Ullah Z, Iqbal J, Chalgham W, Ahmad A. Aspartic Acid-Based Nano-Copper Induces Resilience in *Zea mays* to Applied Lead Stress Via Conserving Photosynthetic Pigments and Triggering the Antioxidant Biosystem. *Sustainability*. 2023;15(16):12186.
6. Abbasi BA, Iqbal J, Yaseen T, Zahra SA, Ali S, Uddin S, Chalgham W. Exploring Physical Characterization and Different Bio-Applications of *Elaeagnus angustifolia* orchestrated nickel oxide nanoparticles. *Molecules*. 2023;28(2):654.
7. Ullah Z, Gul F, Iqbal J, Abbasi BA, Kanwal S, Chalgham W, Mahmood T. Biogenic Synthesis of Multifunctional Silver Oxide Nanoparticles (Ag₂ONPs) Using *Parietaria alsinaefolia* Delile Aqueous Extract and Assessment of Their Diverse Biological Applications. *Microorganisms*. 2023;11(4):1069.
8. Mazari SA, Ali E, Abro R, Khan FSA, Ahmed I, Ahmed M, Shah A. Nanomaterials: Applications, waste-handling, environmental toxicities, and future challenges—A review. *J Environ Chem Eng*. 2021;9(2):105028.
9. Barhoum A, García-Betancourt ML, Jeevanandam J, Hussien EA, Mekkawy SA, Mostafa M, Bechelany M. Review on natural, incidental, bioinspired, and engineered nanomaterials: history, definitions, classifications, synthesis, properties, market, toxicities, risks, and regulations. *Nanomaterials*. 2022;12(2):177.
10. Ullah Z, Iqbal J, Abbasi BA, Akhtar W, Kanwal S, Ali I, Mahmood T. Assessment of Gus Expression Induced by Anti-Sense Os PPO Gene Promoter and Antioxidant Enzymatic Assays in Response to Drought and Heavy Metal Stress in Transgenic *Arabidopsis thaliana*. *Sustainability*. 2023;15(17):12783.
11. Ahire SA, Bachhav AA, Pawar TB, Jagdale BS, Patil AV, Koli PB. (2022). The augmentation of nanotechnology era: A concise review on fundamental concepts of nanotechnology and applications in material science and technology. *Results Chem*, 100633.
12. Noviana E, Ozer T, Carrell CS, Link JS, McMahon C, Jang I, Henry CS. Microfluidic paper-based analytical devices: from design to applications. *Chem Rev*. 2021;121(19):11835–85.
13. Abid N, Khan AM, Shujait S, Chaudhary K, Ikram M, Imran M, Maqbool M. Synthesis of nanomaterials using various top-down and bottom-up approaches, influencing factors, advantages, and disadvantages: A review. *Adv Colloid Interface Sci*. 2022;300:102597.
14. Ahmed SF, Mofijur M, Nuzhat S, Chowdhury AT, Rafa N, Uddin MA, Show PL. Recent developments in physical, biological, chemical, and hybrid treatment techniques for removing emerging contaminants

- from wastewater. *J Hazard Mater.* 2021;416:125912.
15. Sharma A, Thatai KS, Kuthiala T, Singh G, Arya SK. Employment of polysaccharides in enzyme immobilization. *Reactive Funct Polym.* 2021;167:105005.
 16. Chouke PB, Shrirame T, Potbhare AK, Mondal A, Chaudhary AR, Mondal S, Chaudhary RG. Bioinspired metal/metal oxide nanoparticles: A road map to potential applications. *Mater Today Adv.* 2022;16:100314.
 17. Pachaiappan R, Rajendran S, Show PL, Manavalan K, Naushad M. Metal/metal oxide nanocomposites for bactericidal effect: A review. *Chemosphere.* 2021;272:128607.
 18. Abbasi BA, Iqbal J, Zahra SA, Shahbaz A, Kanwal S, Rabbani A, Mahmood T. Bioinspired synthesis and activity characterization of iron oxide nanoparticles made using *Rhamnus Triquetra* leaf extract. *Mater Res Express.* 2020;6(12):1250e7.
 19. Islam F, Shohag S, Uddin MJ, Islam MR, Nafady MH, Akter A, Cavalu S. Exploring the journey of zinc oxide nanoparticles (ZnO-NPs) toward biomedical applications. *Materials.* 2022;15(6):2160.
 20. Kaliramna S, Dhayal SS, Kumar N. Structural and optical studies of ZnO doped PMMA thin film and its photocatalytic and antibacterial activities. *Opt Mater.* 2022;133:112891.
 21. Iqbal J, Abbasi BA, Yaseen T, Zahra SA, Shahbaz A, Shah SA, Ahmad P. Green synthesis of zinc oxide nanoparticles using *Elaeagnus angustifolia* L. leaf extracts and their multiple in vitro biological applications. *Sci Rep.* 2021;11(1):20988.
 22. Sharma I, Sharma MV, Haque MA, Simal-Gandara J. (2023). Antifungal action and targeted mechanism of Bio fabricated zinc oxide (ZnO) nanoparticles against *Ascochyta fabae*. *Heliyon*, 9(9).
 23. Wyss KM, Luong DX, Tour JM. Large-scale syntheses of 2D materials: flash joule heating and other methods. *Adv Mater.* 2022;34(8):2106970.
 24. Umapathi A, Kumawat M, Daima HK. Engineered nanomaterials for biomedical applications and their toxicity: a review. *Environ Chem Lett.* 2022;20(1):445–68.
 25. Ijaz S, Iqbal J, Abbasi BA, Ullah Z, Yaseen T, Kanwal S, Cho WC. Rosmarinic acid and its derivatives: Current insights on anticancer potential and other biomedical applications. Volume 162. *Biomedicine & Pharmacotherapy*; 2023. p. 114687.
 26. Murali M, Kalegowda N, Gowtham HG, Ansari MA, Alomary MN, Alghamdi S, Amruthesh KN. Plant-mediated zinc oxide nanoparticles: Advances in the new millennium towards understanding their therapeutic role in biomedical applications. *Pharmaceutics.* 2021;13(10):1662.
 27. Hiba H, Thoppil JE. Medicinal herbs as a panacea for biogenic silver nanoparticles. *Bull Natl Res Centre.* 2022;46(1):9.
 28. Khan A, Ahmad M, Sultan A, Ullah Z, Majeed K, Ullah T, Zafar M. (2023). Contribution to pollen morphology of *Astragalus* L. section *Aegacantha* Bunge (Galegeae, Fabaceae) and its systematic significance. *Palynology*, (just-accepted), 1–14.
 29. Abuzeid HM, Julien CM, Zhu L, Hashem AM. Green Synthesis of Nanoparticles and Their Energy Storage, Environmental, and Biomedical Applications. *Crystals* 2023, 13, 1576.

<https://doi.org/10.3390/cryst13111576>.

30. Shamaila S, Sajjad AKL, Farooqi SA, Jabeen N, Majeed S, Farooq I. Advancements in nanoparticle fabrication by hazard free eco-friendly green routes. *Appl Mater Today*. 2016;5:150–99.
31. *Rhynchosia capitata* (Heyne ex Roth) DC., *Prodr.* 2: 386. 1825; Gamble, Fl. Pres. Madras 374(264). 1918; Sanjappa, *Legumes Ind.* 237. 1992.
32. Praveena R, Sadasivam K, Kumaresan R, Deepha V, Sivakumar R. Experimental and DFT studies on the antioxidant activity of a C-glycoside from *Rhynchosia capitata*. *Spectrochim Acta Part A Mol Biomol Spectrosc.* 2013;103:442–52.
33. Ramachandran S, Vamsikrishna M, Gowthami KV, Heera B, Dhanaraju MD. Assessment of cytotoxic activity of *Agave cantula* using brine shrimp (*Artemia salina*) lethality bioassay. *Asian J Sci Res.* 2011;4(1):90–4.
34. Kaur N, Kumar V, Nayak SK, Wadhwa P, Kaur P, Sahu SK. Alpha-amylase as molecular target for treatment of diabetes mellitus: A comprehensive review. *Chem Biol Drug Des.* 2021;98(4):539–60.
35. Manasa, D. J., Chandrashekar, K. R., Kumar, M. P., Suresh, D., Kumar, D. M., Ravikumar, C. R., ... Murthy, H. A. (2021). Proficient synthesis of zinc oxide nanoparticles from *Tabernaemontana heyneana* Wall. via green combustion method: Antioxidant, anti-inflammatory, antidiabetic, anticancer and photocatalytic activities. *Results in Chemistry*, 3, 100178.
36. Ramalingam V, Sathya PM, Srivalli T, Mohan H. Synthesis of quercetin functionalized wurtzite type zinc oxide nanoparticles and their potential to regulate intrinsic apoptosis signaling pathway in human metastatic ovarian cancer. *Life Sci.* 2022;309:121022.
37. Fakhari S, Jamzad M, Kabiri Fard H. Green synthesis of zinc oxide nanoparticles: a comparison. *Green Chem Lett Rev.* 2019;12(1):19–24.
38. Shamhari NM, Wee BS, Chin SF, Kok KY. (2018). Synthesis and Characterization of Zinc Oxide Nanoparticles with Small Particle Size Distribution. *Acta chimica Slovenica*, 65(3).
39. Shamhari NM, Wee BS, Chin SF, Kok KY. (2018). Synthesis and Characterization of Zinc Oxide Nanoparticles with Small Particle Size Distribution. *Acta chimica Slovenica*, 65(3).
40. Hadi AJ, Nayef UM, Mutlak FAH, Jabir MS. Laser-ablated zinc oxide nanoparticles and evaluation of their antibacterial and anticancer activity against an ovarian cancer cell line: in vitro study. *Plasmonics*; 2023. pp. 1–11.
41. Shahanaz L, Shalini AS. Zinc oxide nanoparticles synthesized using *Oldenlandia Umbellata* leaf extract and their photocatalytic and biological characteristics. *J Indian Chem Soc.* 2023;100(2):100917.
42. El-Shamy OA, Deyab MA. Improvement of the corrosion resistance of epoxy coatings with the use of a novel zinc oxide-alginate nanoparticles compound. *Mater Lett.* 2023;331:133402.
43. Dangana RS, George RC, Agboola FK. The biosynthesis of zinc oxide nanoparticles using aqueous leaf extracts of *Cnidioscolus aconitifolius* and their biological activities. *Green Chem Lett Rev.* 2023;16(1):2169591.

44. Chinnathambi A, Alharbi SA, Lavarti R, Jhanani GK, On-Uma R, Jutamas K, Anupong W. Larvicidal and pupicidal activity of phyto-synthesized zinc oxide nanoparticles against dengue vector aedes aegypti. *Environ Res.* 2023;216:114574.
45. Rajasingam M, Gopinath SC, Anbu P, Vijayakumar S. Biological synthesis of zinc-oxide nanoparticle using wide-spread *Lentinus sajor-caju* extract as a carrier for natural-compounds. *Mater Today Commun.* 2023;35:105973.
46. Sarkheil M, Johari SA, An HJ, Asghari S, Park HS, Sohn EK, Yu IJ. Acute toxicity, uptake, and elimination of zinc oxide nanoparticles (ZnO NPs) using saltwater microcrustacean, *Artemia franciscana*. *Environ Toxicol Pharmacol.* 2018;57:181–8.
47. Bibi S, Ullah R, Burni T, Ullah Z, Kazi M. Impact of Resorcinol and Biochar Application on the Growth Attributes, Metabolite Contents, and Antioxidant Systems of Tomato. *Lycopersicon esculentum* Mill. *ACS Omega*; 2023.
48. Tahir H, Rashid F, Ali S, Summer M, Abaidullah R. (2023). Spectrophotometrically, Spectroscopically, Microscopically and Thermogravimetrically Optimized TiO₂ and ZnO Nanoparticles and their Bactericidal, Antioxidant and Cytotoxic Potential: A Novel Comparative Approach. *J Fluoresc*, 1–15.
49. Dhobale S, Thite T, Laware SL, Rode CV, Koppikar SJ, Ghanekar RK, Kale SN. (2008). Zinc oxide nanoparticles as novel alpha-amylase inhibitors. *J Appl Phys*, 104(9).
50. Nasab SB, Homaei A, Karami L. Kinetic of α -amylase inhibition by *Gracilaria corticata* and *Sargassum angustifolium* extracts and zinc oxide nanoparticles. *Biocatal Agric Biotechnol.* 2020;23:101478.
51. Salem NM, Awwad AM. Green synthesis and characterization of ZnO nanoparticles using *Solanum rantonnetii* leaves aqueous extract and antifungal activity evaluation. *Chem Int.* 2022;8(1):12–7.
52. Venkataraju JL, Sharath R, Chandraprabha MN, Neelufar E, Hazra A, Patra M. Synthesis, characterization and evaluation of antimicrobial activity of zinc oxide nanoparticles. *J Biochem Technol.* 2014;3(5):151–4.
53. Narayanan PM, Wilson WS, Abraham AT, Sevanan M. Synthesis, characterization, and antimicrobial activity of zinc oxide nanoparticles against human pathogens. *BioNanoScience.* 2012;2:329–35.
54. Jayaseelan, C., Siva, D., Kamaraj, C., Thirugnanasambandam, R., Kumar, V. G., Subashni, B., ... Saravanan, D. (2024). Phytosynthesis of zinc oxide nanoparticles for enhanced antioxidant, antibacterial, and photocatalytic properties: A greener approach to environmental sustainability. *Environmental Research*, 251, 118770.
55. Khazaei Z, Behnamian M, Dezhsetan S, Estaji A. Green synthesized ZnONPs Using *Foeniculum vulgare* l. seed extract and investigation of antioxidant activity in *Ganoderma lucidum*. *S Afr J Chem.* 2024;78:147–57.
56. Shnawa BH, Jalil PJ, Al-Ezzi A, Mhamedsharif RM, Mohammed DA, Biro DM, Ahmed MH. Evaluation of antimicrobial and antioxidant activity of zinc oxide nanoparticles biosynthesized with *Ziziphus spina-christi* leaf extracts. *J Environ Sci Health Part C.* 2024;42(2):93–108.

57. Muthuvel A, Jothibas M, Manoharan C. Effect of chemically synthesis compared to biosynthesized ZnO-NPs using *Solanum nigrum* leaf extract and their photocatalytic, antibacterial and in-vitro antioxidant activity. *J Environ Chem Eng.* 2020;8(2):103705.
58. Abbasi, B. H., Shah, M., Hashmi, S. S., Nazir, M., Naz, S., Ahmad, W., ... Hano, C.(2019). Green bio-assisted synthesis, characterization and biological evaluation of biocompatible ZnO NPs synthesized from different tissues of milk thistle (*Silybum marianum*). *Nanomaterials*, 9(8), 1171.
59. PP V. In vitro biocompatibility and antimicrobial activities of zinc oxide nanoparticles (ZnO NPs) prepared by chemical and green synthetic route—a comparative study. *BioNanoScience.* 2020;10(1):112–21.
60. Chandrasekaran S, Anusuya S, Anbazhagan V. Anticancer, anti-diabetic, antimicrobial activity of zinc oxide nanoparticles: A comparative analysis. *J Mol Struct.* 2022;1263:133139.
61. Seshadri VD. Zinc oxide nanoparticles from *Cassia auriculata* flowers showed the potent antimicrobial and in vitro anticancer activity against the osteosarcoma MG-63 cells. *Saudi J Biol Sci.* 2021;28(7):4046–54.
62. Vidhya E, Vijayakumar S, Prathipkumar S, Praseetha PK. Green way biosynthesis: Characterization, antimicrobial and anticancer activity of ZnO nanoparticles. *Gene Rep.* 2020;20:100688.
63. Hassan HFH, Mansour AM, Abo-Youssef AMH, Elsadek BE, Messiha BAS. Zinc oxide nanoparticles as a novel anticancer approach; in vitro and in vivo evidence. *Clin Exp Pharmacol Physiol.* 2017;44(2):235–43.
64. Sivakumar P, Lee M, Kim YS, Shim MS. Photo-triggered antibacterial and anticancer activities of zinc oxide nanoparticles. *J Mater Chem B.* 2018;6(30):4852–71.
65. González SE, Bolaina-Lorenzo E, Pérez-Trujillo JJ, Puente-Urbina BA, Rodríguez-Fernández O, Fonseca-García A, Betancourt-Galindo R. Antibacterial and anticancer activity of ZnO with different morphologies: a comparative study. *3 Biotech.* 2021;11:1–12.
66. Guruviah K, Annamalai SK, Ramaswamy A, Sivasankaran C, Ramasamy S, Barabadi H, Saravanan M. (2020). Comparative antimicrobial and anticancer activity of biologically and chemically synthesized zinc oxide nanoparticles toward breast cancer cells. *Nanomed J*, 7(4).

Figures

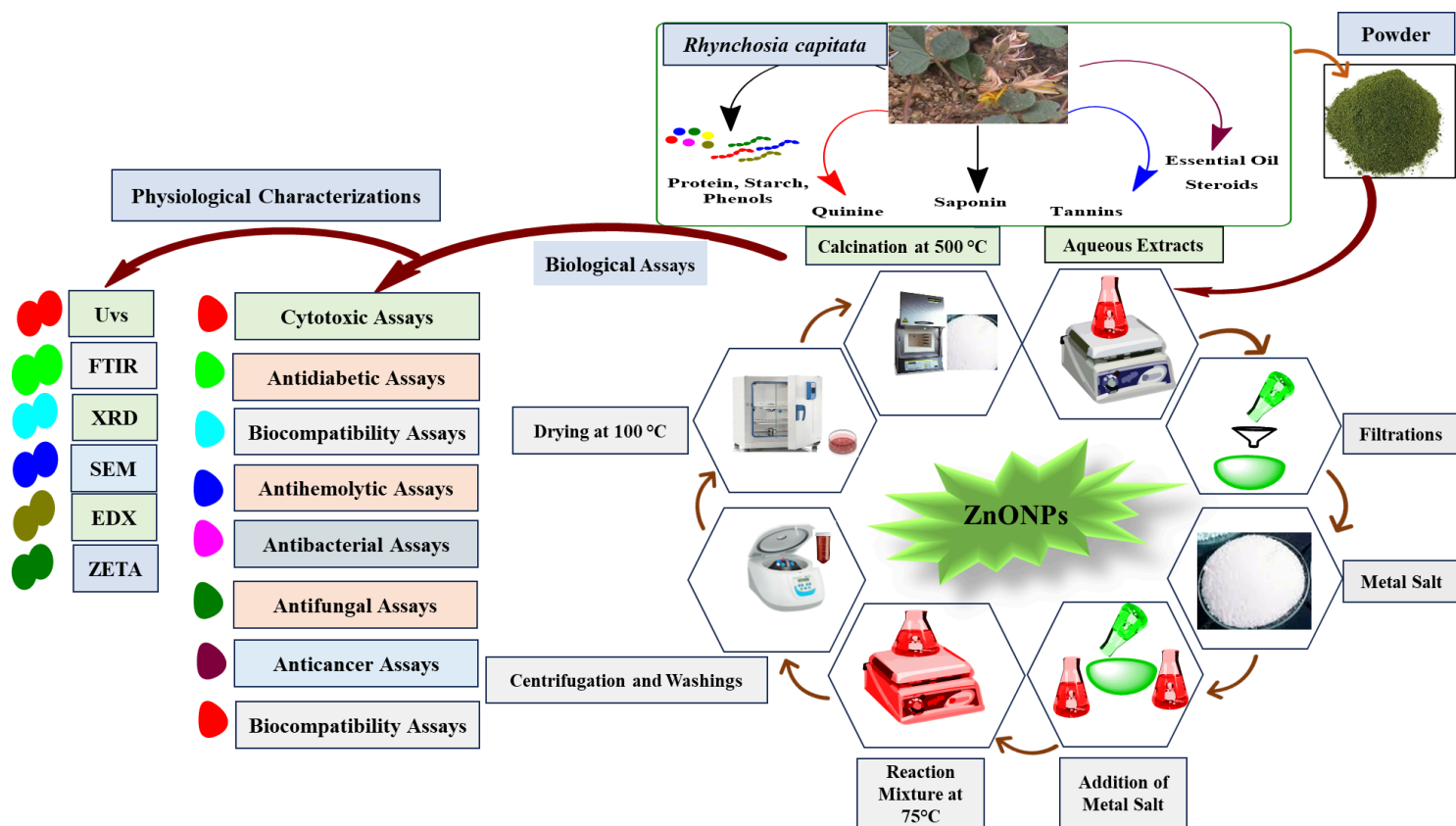


Figure 1

The green synthesis, characterization, and biological activities of ZnONPs mediated by *R. capitata* are illustrated in the graphic diagram.

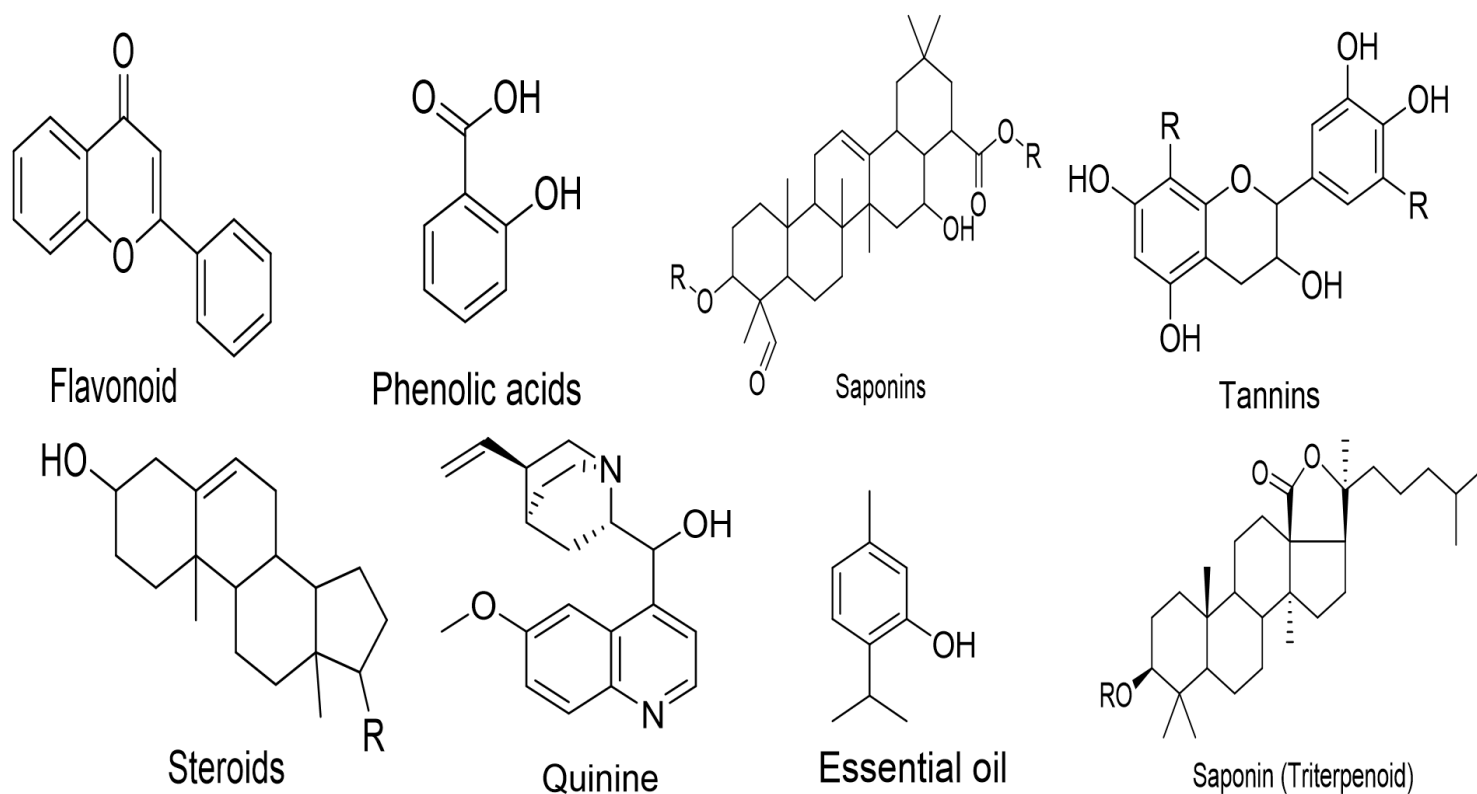


Figure 2

Structures of some Compounds produced via Chemo draw.

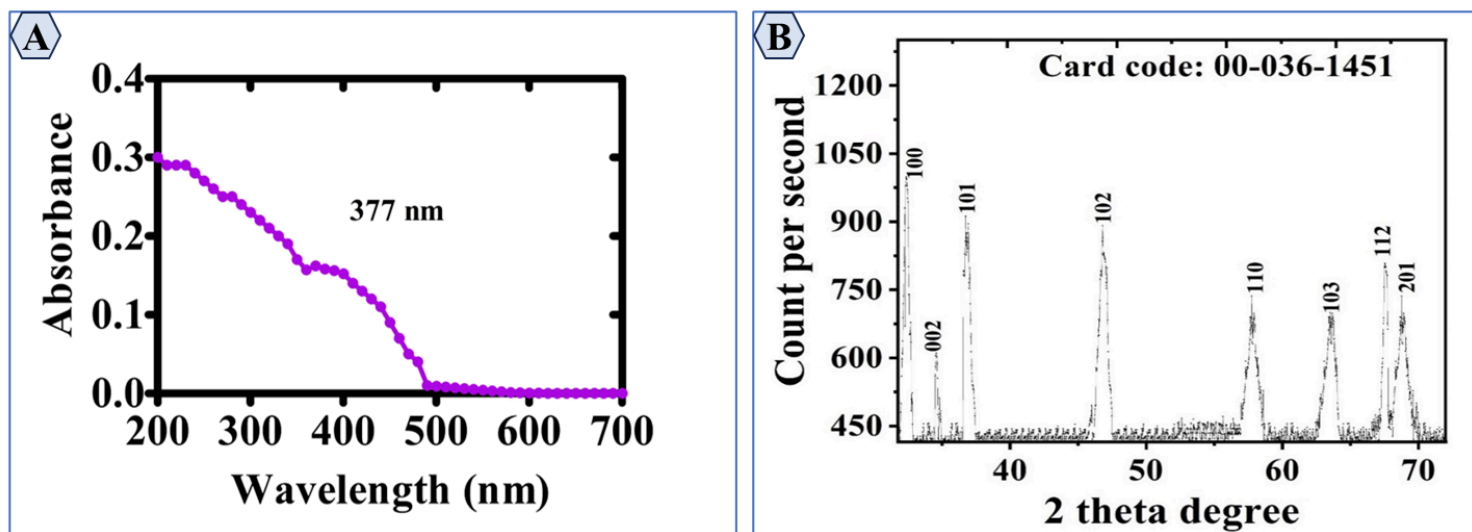


Figure 3

(A) Optical spectrum of Biologically produced calcinated ZnONPs. **(B)** XRD spectra of ZnONPs developed by RC.

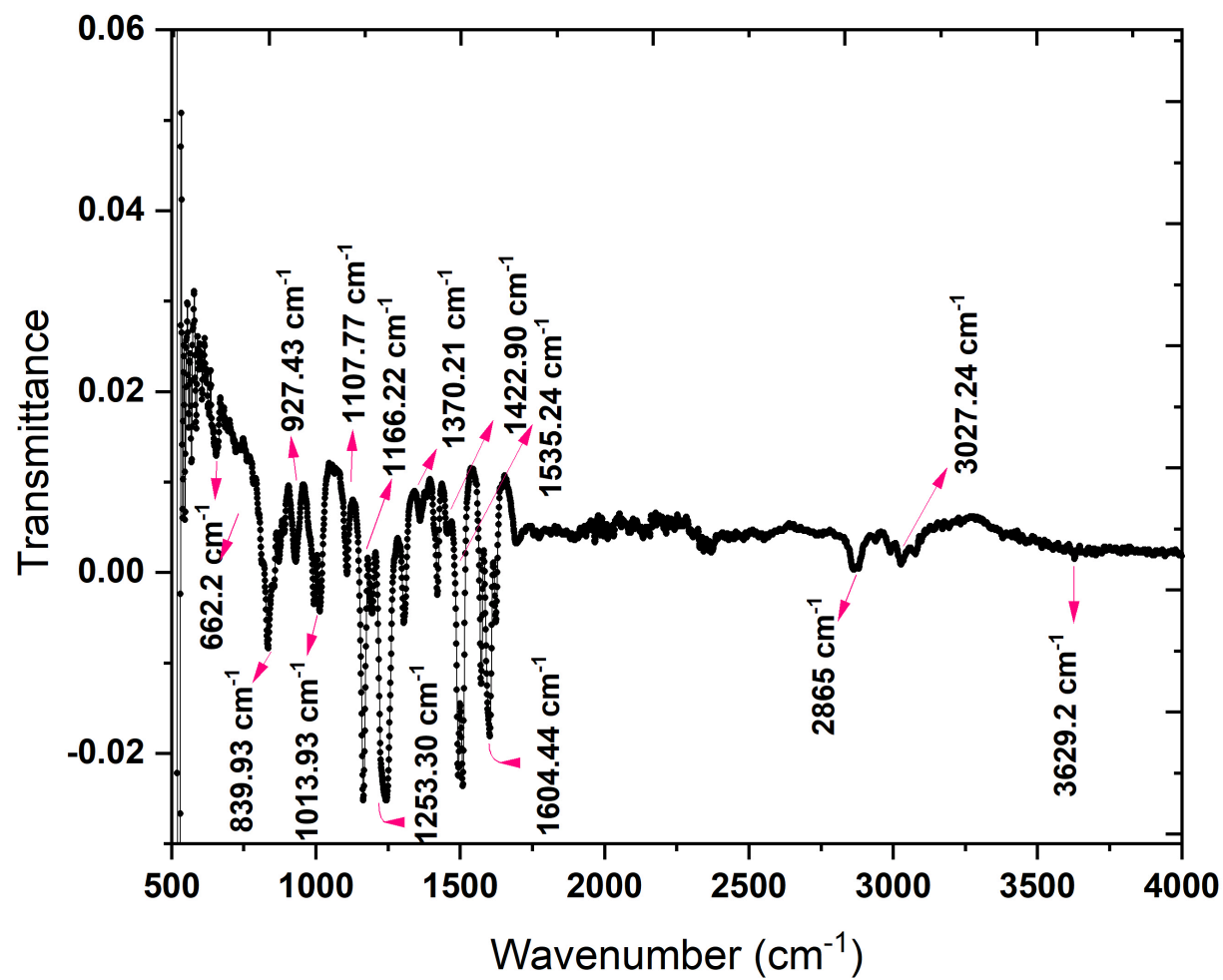


Figure 4

FTIR spectra of biogenic RC@ZnONPs.

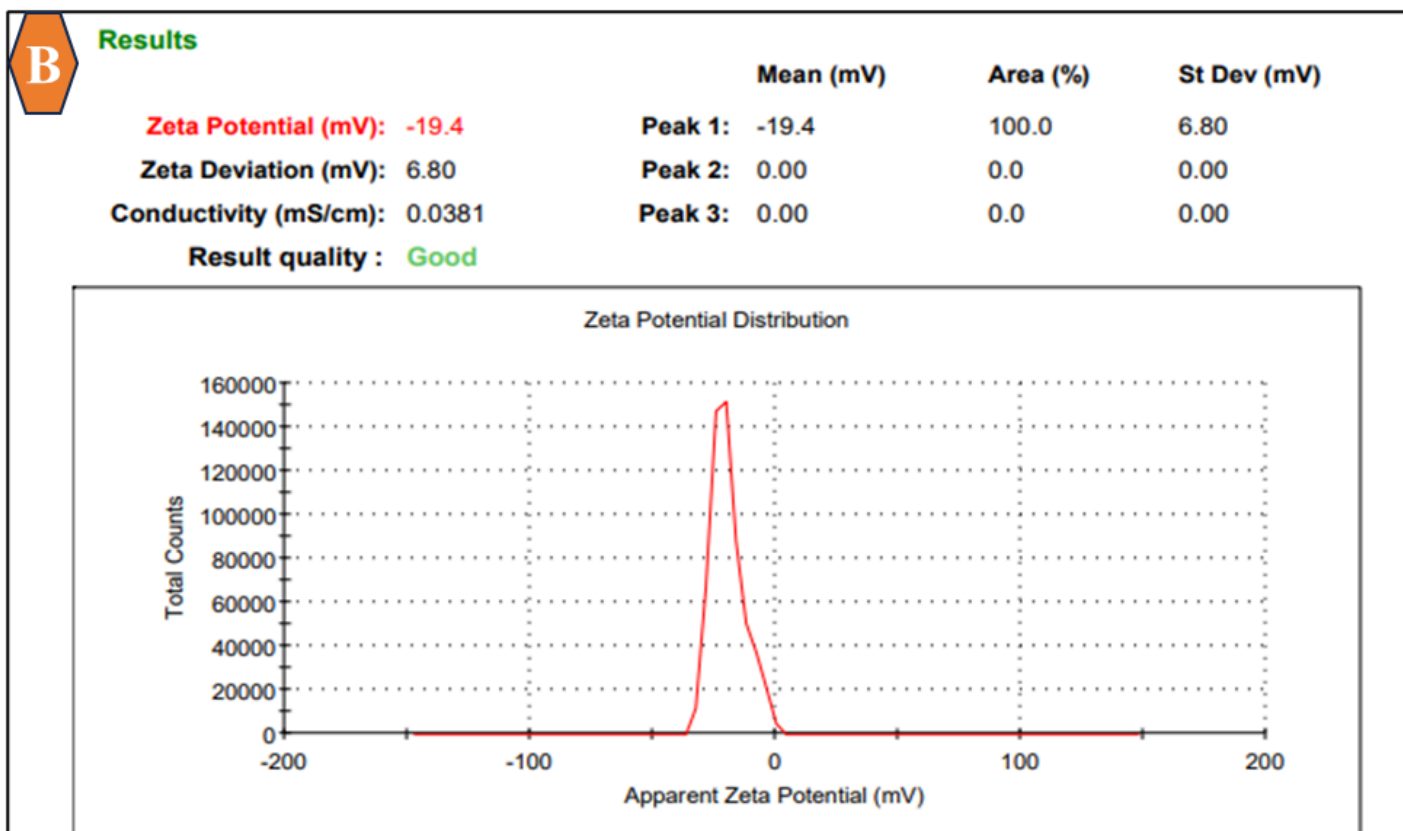
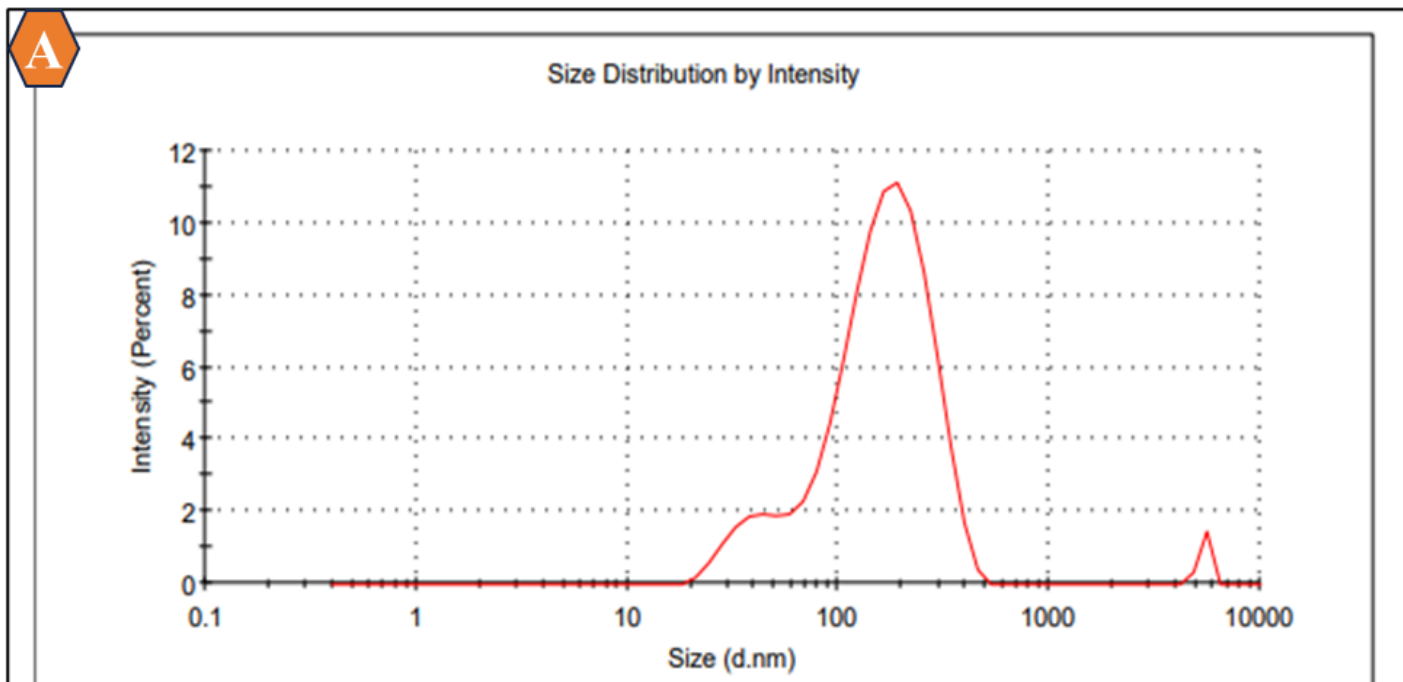


Figure 5

(A) Particle size distribution of ZnONPs (B) Zeta potential measurement of RC@ZnONPs.

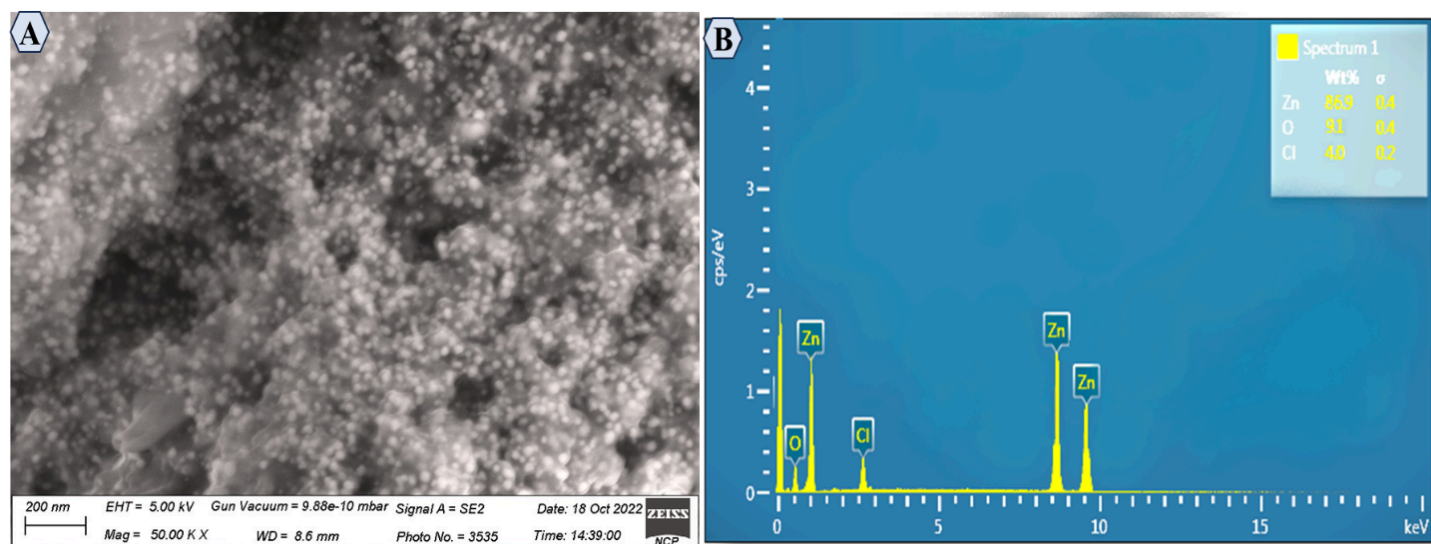


Figure 6

(A) Biogenic RC@ZnONPs SEM examination. **(B)** RC@ZnONPs' elemental composition as determined by EDX.

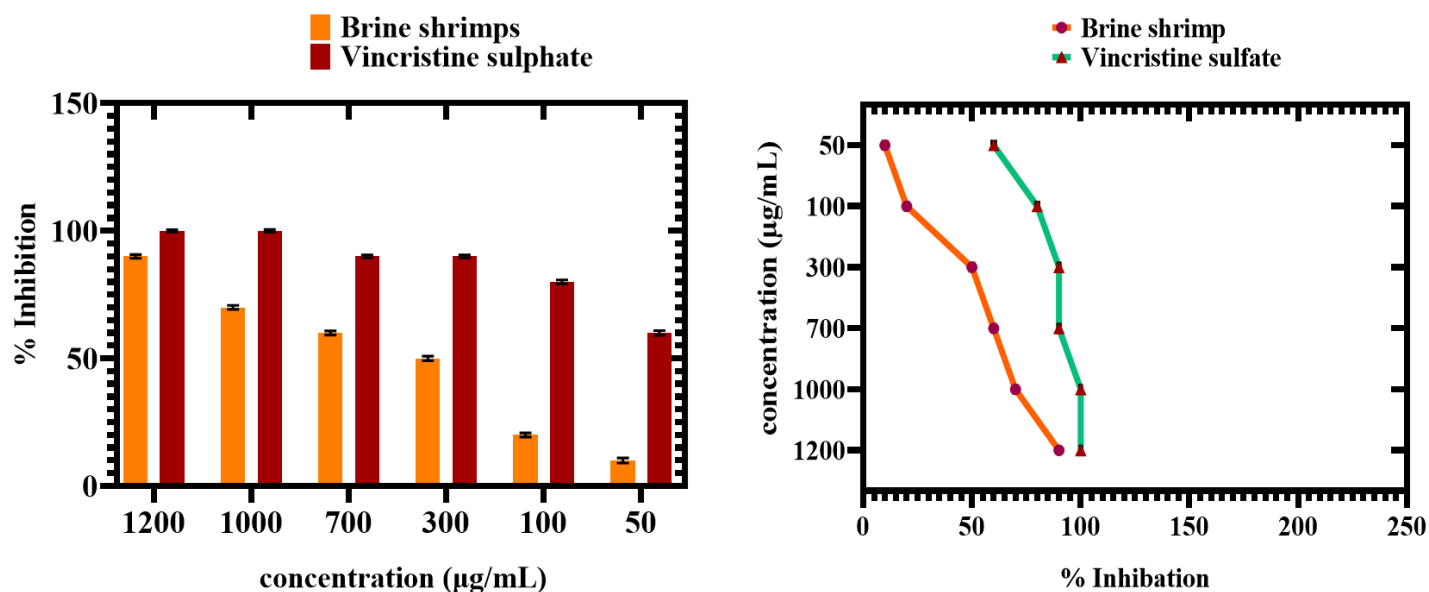


Figure 7

Cytotoxic effects of RC@ZnONPs on brine shrimp.

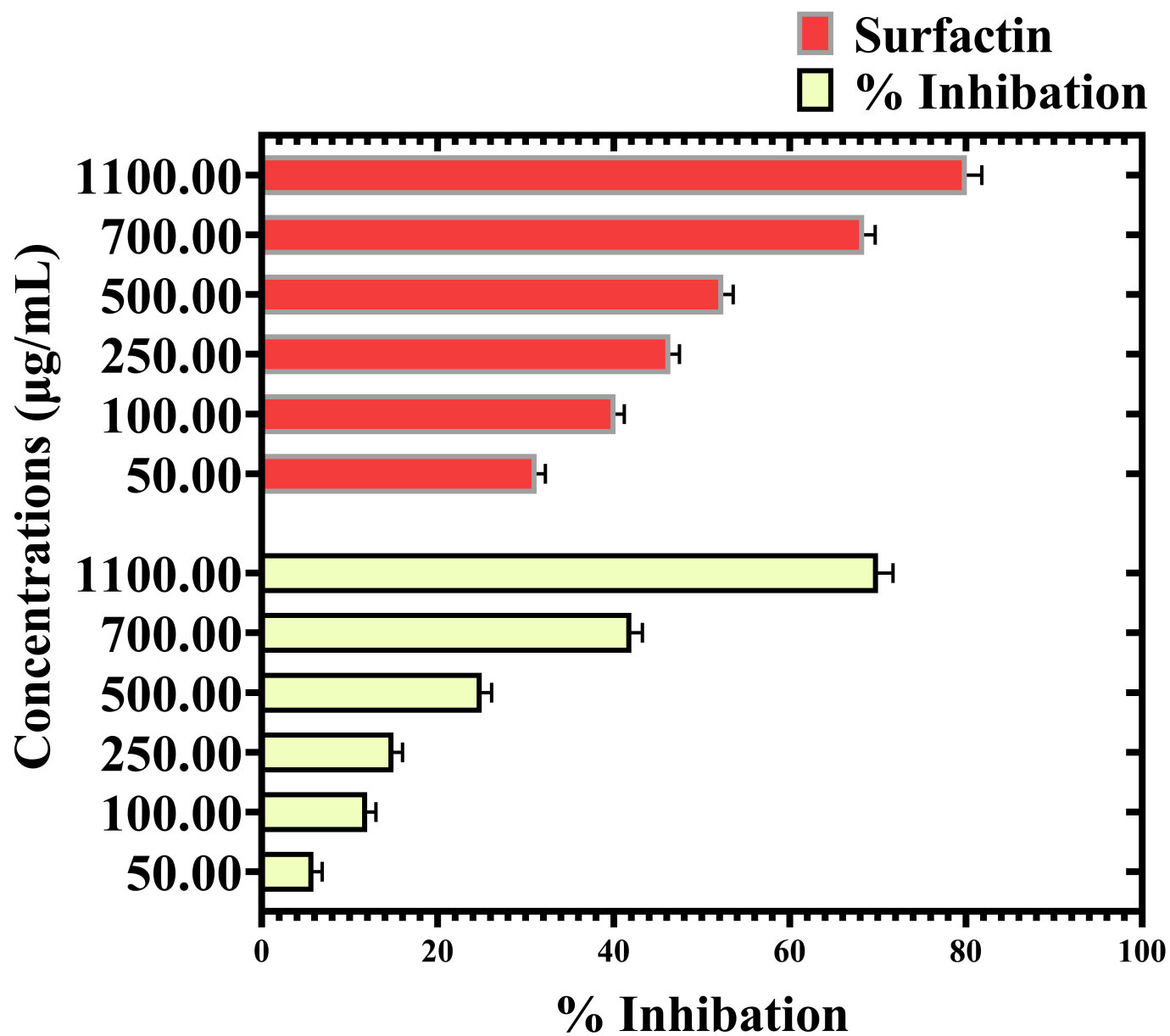


Figure 8

Antidiabetic potential of ZnONPs mediated RC against *alpha-amylase*.

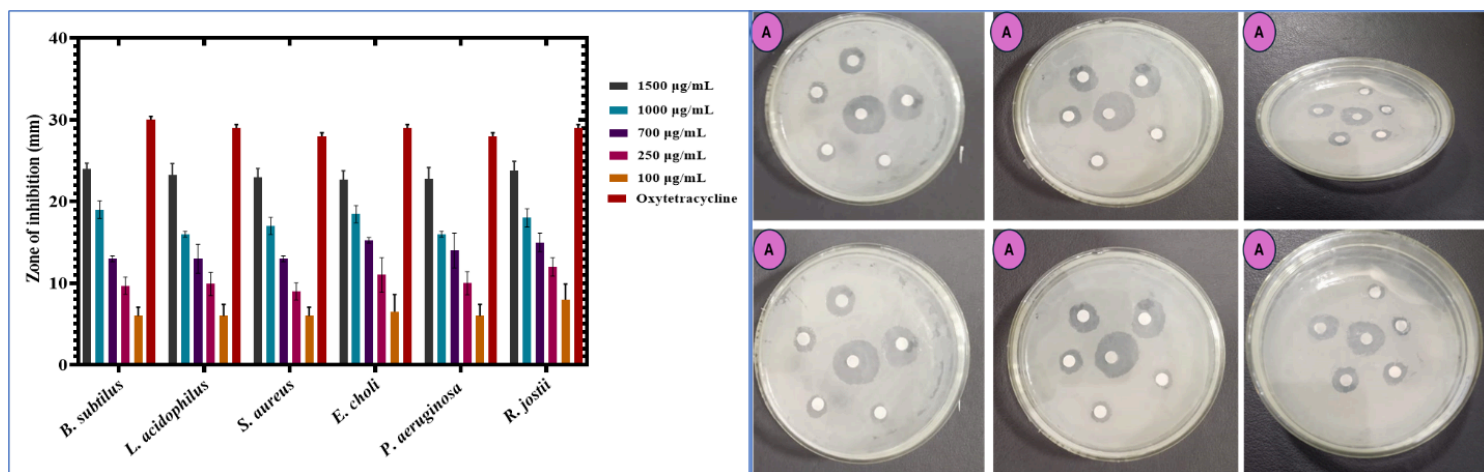


Figure 9

Antibacterial potential of mediated ZnONPs against various concentrations.

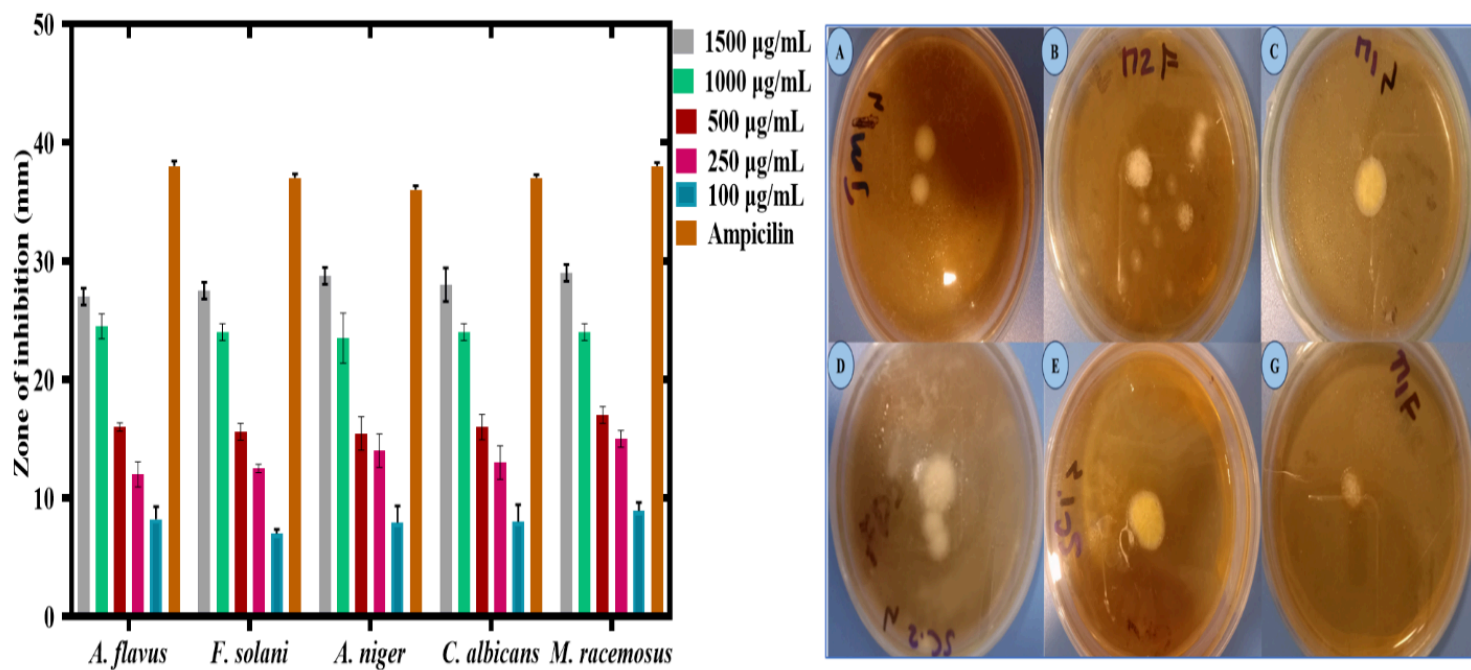


Figure 10

Shows the Antifungal potential of biogenic ZnONPs at various concentrations.

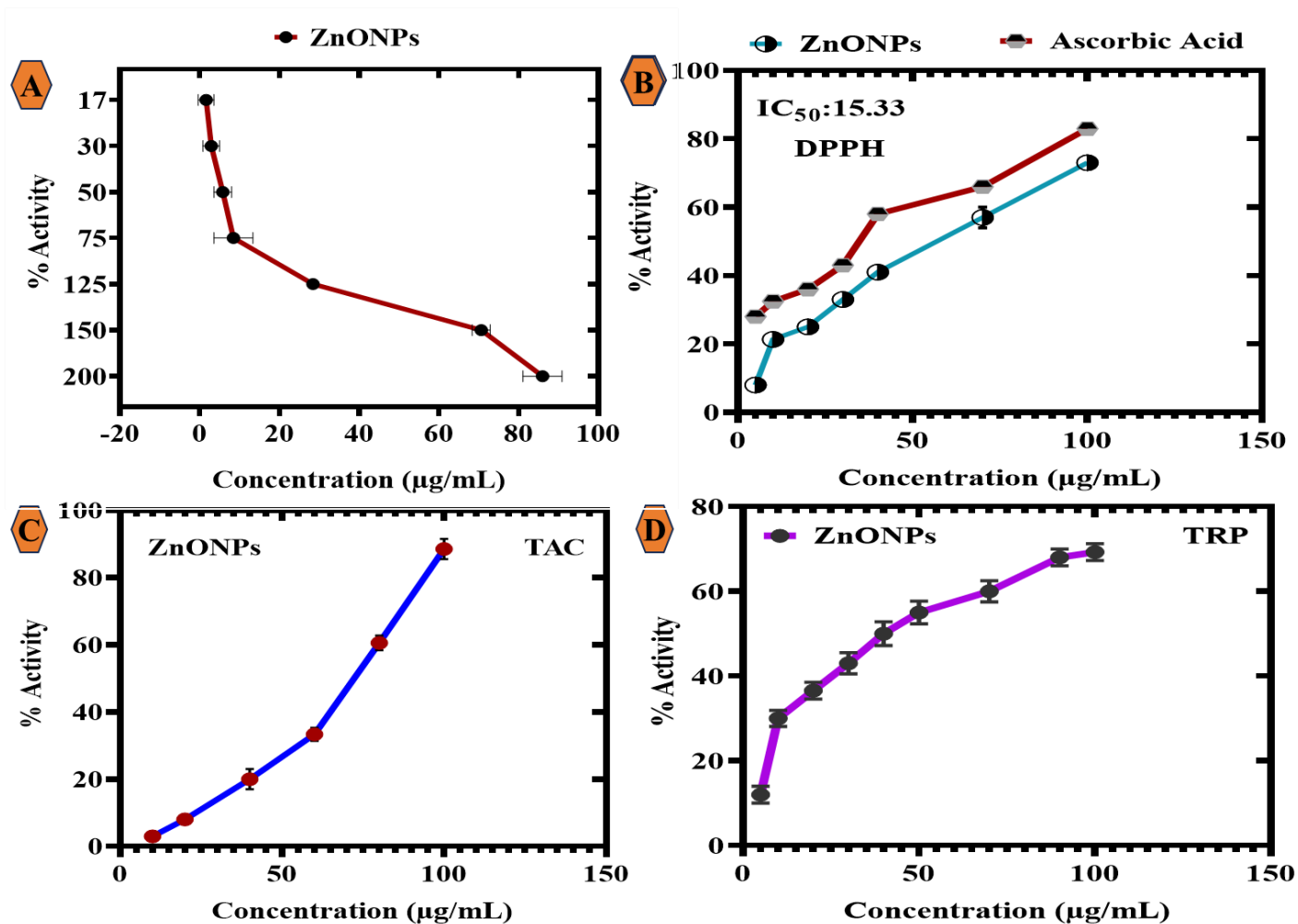


Figure 11

(A) The anti-hemolytic potential of RC-mediated ZnONPs. (B) Antioxidant (DPPH) potential (C) Antioxidant (TAC) potential (D) Antioxidant (TRP) potential of ZnONPs of *R. capitata* mediated ZnONPs.

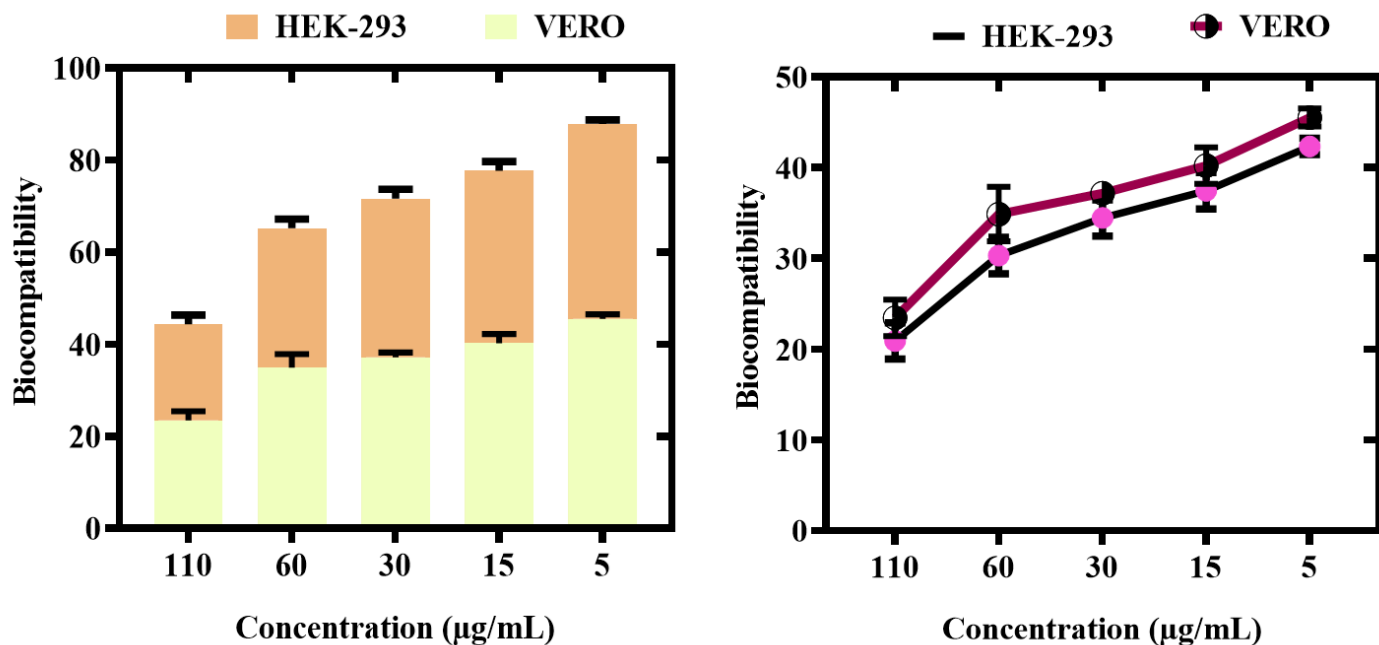


Figure 12

ZnONPs' potential for biocompatibility against HEK-93 and VERO cell lines. The proportion of viable VERO and HEK cell lines in the presence of ZnONPs, doxorubicin, and untreated cells at varying concentrations.

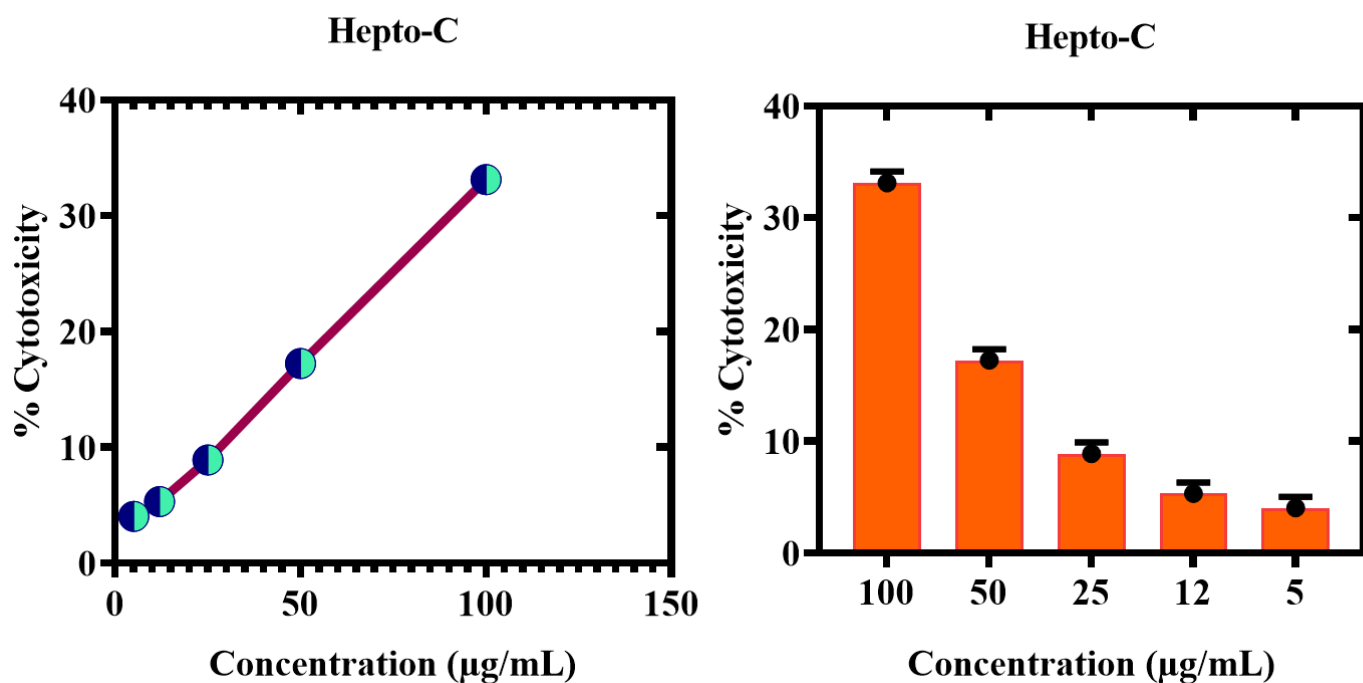


Figure 13

Hep-2 cell line susceptibility to the cytotoxic potential of RC@ZnONPs.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [floatimage1.png](#)
- [floatimage15.png](#)