



OPEN Green synthesis and functional evaluation of zinc oxide nanoparticles from red dragon fruit peel

Devi-Nair Gunasegavan Rath^{1,4}, Norazalina Saad², Norhaizan Mohd Esa³,
Aswir Abd Rashed⁴, Mohd Fairulnizal Md Noh⁴ & Che Azurhanim Che Abdullah^{1,2,5}✉

Using red dragon fruit peel extract (RDPE) as a biogenic reducing and stabilizing agent, this paper presents an inexpensive, eco-friendly and simple biogenic synthesis of zinc oxide nanoparticles (ZnONPs). These biogenic materials are non-hazardous, environmentally friendly, and incur minimal costs compared to chemical approaches. The successful biosynthesis of ZnONPs was confirmed using several characterization tests, including UV-Vis spectroscopy, particle size analysis, zeta potential analysis, Fourier transform infrared spectroscopy, thermogravimetric analysis, X-ray diffraction spectroscopy, Field-emission scanning electron microscopy and Energy dispersive X-ray spectroscopy. In addition, a Taguchi experimental design was employed to optimize the synthesis conditions, resulting in nine formulations that incorporated the effects of two zinc salt precursors (acetate and nitrate). Among these, acetate-derived ZnONPs produced the desired hydrodynamic size (203.97 ± 1.53 nm) and best colloidal stability (zeta potential: -29.4 ± 0.89 mV) with formulation AR7. The hexagonal wurtzite phase with a crystallite size of 18.00 ± 5.32 nm was verified by X-ray diffraction. FTIR spectra showed the presence of phytochemical functional groups in charge of capping and reduction. FESEM revealed unique flower-like morphology, with an average particle size of 45.85 ± 4.64 nm, proving the efficacy of AR7 as the optimal formulation. The antimicrobial activity tests revealed minimum inhibitory concentration values of 2.50–5.00 µg/mL against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*; while an IC_{50} of 405 µg/mL was obtained with the cytotoxicity test on 3T3-L1 cells after 24 h of incubation. These results imply that the optimal formulation of AR7, mediated from RDPE, presents excellent prospects in reducing environmental wastage for widespread biomedical applications.

Keywords Zinc oxide, Nanoparticles, Dragon fruit peel, Waste, Biogenic synthesis, Biomedical

Zinc oxide (ZnO) has gained economic and industrial attention for its wide range of properties that allow for extensive applications in various sectors. The zinc oxide nanoparticles (ZnONPs) are one of the most essential metal oxide NPs and are explored for their application in diverse fields due to their peculiar physical and chemical characteristics^{1,2}. Initial years have seen their application mainly in the rubber industry, and recently this has emerged for its utilization in biomedical, textile, electrotechnology, biomedical engineering and others^{3–7}. The synthesis of nano-ZnO is deemed advantageous, especially for biomedical aspects, since it assists in the better absorption attributed to the small particle size⁸.

Traditionally, the synthesis of ZnONPs was mostly done using chemical or physical approaches. The commonly used chemical techniques include precipitation, microemulsion, sol-gel, chemical reduction and

¹Nanomaterial Synthesis and Characterization Laboratory, Institute of Nanoscience & Nanotechnology, Universiti Putra Malaysia, Serdang 43400, Selangor, Malaysia. ²Laboratory of Cancer Research UPM-MAKNA (CANRES), Institute of Bioscience, Universiti Putra Malaysia, Serdang 43400, Selangor, Malaysia. ³Department of Nutrition, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang 43400, Selangor, Malaysia. ⁴Nutrition, Metabolism and Cardiovascular Research Centre, Institute for Medical Research, National Institute of Health, Ministry of Health Malaysia, No 1, Jalan Setia Murni U13/52, Seksyen U13 Setia Alam, Shah Alam 40170, Malaysia. ⁵Biophysics Laboratory, Department of Physics, Faculty of Science, Universiti Putra Malaysia, Serdang 43400, Selangor, Malaysia. ✉email: azurhanim@upm.edu.my

hydrothermal method. These approaches often lead to high energy consumption due to the need for high pressure or temperatures^{9–12}. Physical approaches are less commonly used; however, the vapour deposition, plasma and ultrasonic irradiation techniques are among the common techniques that have been reported. These techniques necessitate high energy and robust equipment that incurs a high cost of the product^{10,13}.

Given the drawbacks, recent trends in ZnONPs synthesis have observed the focus towards green approaches or also known as biogenic synthesis. This method promotes the use of non-toxic, renewable materials with efficient and reduced energy consumption, which makes the process more cost-effective and eco-friendlier^{10,12,14–16}. On a general note, the basis for biogenic synthesis of NPs relies on the ability of any organism to reduce and stabilize the metal ions. For these, plants were considered the best candidates for several reasons, including their rich phytoconstituent composition that functions as both reducing and capping agents^{17–19}. Biogenic synthesis generates NPs that are stable, non-toxic, low-cost and environmentally friendly, which is associated with their small size and shapes^{15,20,21}. Based on published findings, it was found that the biological properties, such as antimicrobial activity and biocompatibility, improved with biogenic ZnONPs compared to those of conventional synthesis. The superiority of ZnONPs, being a low-cost and less toxic nanomaterial, has attracted tremendous interest for their applications in various biomedical fields^{22–24}.

Plant extracts are comprised of bioactive compounds (e.g. polyphenols, flavonoids, terpenoids, alkaloids, etc.), known to possess strong reducing properties. These components facilitate the reduction of zinc ions to ZnONPs via electron donation. Upon reduction of the zinc ions, they undergo nucleation, which is a process of synthesizing small clusters of ZnO that continue to grow into ZnONPs. Here, various parameters, especially the plant extract concentration, time, temperature, and others, are acknowledged to impact the size and shape of the synthesized NPs. Apart from this, the bioactive compounds also play their role in stabilizing the ZnONPs via capping that ensures the ZnONPs remain well-dispersed and prevents aggregate formation. In general, the green synthesis includes several steps that initiates with bio-reduction, followed by nucleation, growth and stabilization²⁵.

Among the various plant resources, dragon fruit or pitaya, which belongs to *Cactaceae* and *Hylocereus* families, is considered a promising material. They are categorized into three varieties; which are *Hylocereus undatus* (red skin with white pulp), *Hylocereus polyrhizus* (red skin with red pulp), and *Hylocereus megalanthus* (yellow skin with white pulp)²⁶. Dragon fruit is highly attractive in relevance to their nutritional value, with an extensive amount of essential oils, antioxidants, polyphenols, vitamins and other nutrients²⁷. Nevertheless, the peels, which are considered inedible, account for about 33% of the total fruit weight. However, this portion of fruit has been recognized as a good source of pigments that can potentially be utilized for various applications^{28,29}.

In this context, increasing trends were noticed with the exploration of these once considered waste resources for transformation into value-added products. The red dragon fruit (*H. polyrhizus*), specifically, has been recognized as a substantial reservoir of betacyanins, whereby the peels were demonstrated to harbor a higher content compared to the pulp^{30–33}. In addition, terpenoids (limonene, retinoid, lutein, lycopene), flavonoids (quercetin, kaempferol, isoflavones, naringenin, isorhamnetin) and tannins (hydrolyzed and condensed form) are also found to be present in the peels. Moreover, a recent study has highlighted the presence of higher flavonoid content in their peels compared to their flesh³⁴. Nevertheless, the betacyanins that form the majority composition of the red dragon fruit peels (RDPE) portray low stability in the food matrix. For these reasons, nano-based technologies have been proposed as a significant alternative towards stability enhancement of these labile bioactive compounds²⁸.

Biogenic ZnONPs, are one approach to the nanotechnological application of transforming waste materials such as RDPE for various applications. Their unique characteristics impart remarkable potential in multiple industries, ranging from environmental to healthcare. One interesting aspect of their wide applicability in biomedical field is attributed to their antimicrobial potential, that are found to inhibit a wide range of microorganisms^{35,36}. Moreover, biogenic ZnONPs have also portrayed excellent cytotoxicity in cancer cells, with their ability to induce cell death through multiple pathways, such as apoptosis³⁷. ZnO is a transition metal oxide and semiconductor with known characteristics of owning a wide band gap (around 3.3 eV). Hence, when the radiation has energy larger than the band gap of ZnO, electron-hole pairs are formed, and the electrons are then promoted to the conduction band. Consequently, these holes within the valence band possess strong oxidizing characteristics, which promote the generation of oxidizing sites. These sites are capable of oxidizing the water molecules or hydroxide anions and hence, form strong oxidizing species^{38,39}.

This reaction channels redox chain reaction along with reactive oxygen species (ROS) generation, formed by hydroxyl radicals, hydroperoxide radicals and superoxide radical anions as the pathways of bactericidal actions^{39,40}. Oxidative stress in the bacterial cell can be induced through ROS generation, which leads towards inhibition of protein synthesis and DNA replication⁴¹. Under these circumstances, the conductivity of ZnO elevates near the band gap of the UV spectrum, characterized by the presence of high emission energy. This electronic excitation tends to destabilize the charges present within the cytoplasmic membranes, leading towards their rupture. In addition, ZnO also tends to deteriorate the cytoplasmic membrane by releasing zinc ions from the ZnO dissolution in aqueous solution.

Moreover, a group of researchers studied the underlying antibacterial mechanism of action and presented a potential approach. It was deduced that ZnONPs tend to attach to Gram-positive and Gram-negative bacterial cells via different pathways. The teichoic acid and lipoteichoic acid found within the peptidoglycan layer and membrane, respectively, are identified as negative charge present on the cell surface. Under such scenario, the positively charged ZnONPs are attracted to the cell surface via electrostatic interactions, whereby the arising differences in electrostatic gradient lead to cell surface penetration and damage^{39,42,43}. Despite the multifaceted benefits highlighted, safety and potential toxicity remain the critical aspects in their applications. It is highly vital to understand their interactions with biological systems and optimize their antimicrobial efficacy while keeping potential toxicity minimal.

In the face of multiple available studies on RDPE, we specifically aim to establish an eco-friendly and simple biogenic synthesis that generates ZnONPs with improved characteristics. Besides, a strong justification for this study was correlated to the restricted exploration of the RDPE-mediated biomedical grade ZnONPs. Moreover, although past investigations offered fundamental validation, these findings fall short in delivering comprehensive comparisons when different precursor salts are utilized, along with limited aspects of systematic optimization hence raising uncertainties on the reproducibility.

Therefore, in this study, different approaches of optimization using Taguchi experimental design are applied, and the biogenic synthesis of ZnONPs from RDPE with two types of precursor salts is streamlined. Moreover, a detailed characterization parameter, along with biological and cytotoxicity assessments of the synthesized material, was explored and discussed. Collectively, this research embarks on the venture to elucidate the potential of biogenic ZnONPs towards a diverse range of biomedical prospects. Promising outcomes of this work would channel further investigation into enhancing bioactivity and bioavailability with advanced nano-encapsulation techniques that are deemed advantageous in certain biomedical applications.

Materials & methods

Chemicals and reagents

Zinc nitrate hexahydrate [Zn(NO₃)₂·6H₂O] (AR grade, 99%) and zinc acetate dihydrate (AR grade, 97%) [Zn(CH₃CO₂)₂·2H₂O] were procured from Thermo Fisher Scientific (UK). Sodium hydroxide [NaOH] was sourced from R & M Chemicals (UK). Ultra-pure water was used in all preparations of solutions and obtained from the MiliQ Integral Water Purification System (Merck Milipore, Darmstadt, Germany). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution and dimethyl sulfoxide (DMSO) were obtained from Thermo Fisher Scientific (UK).

Test organisms, cell lines and culture media

Tryptic soy and Mueller-Hinton broths were sourced from Nawah Scientific Inc. (Cairo, Egypt). The bacterial cells (*Escherichia coli* ATCC 8739 and *Staphylococcus aureus* ATCC 29213) and the fungi (*Candida albicans* ATCC 10231) were procured from Nawah Scientific Inc. (Cairo, Egypt). The 3T3-L1 cells of green epithelial monkey cells were purchased from the American Type Culture Collection (ATCC) and cultured in DMEM medium supplemented with 10% fetal bovine serum.

Raw material and sample processing

Red dragon fruits (*Hylocereus polyrhizus*) were purchased from a selected farm in Klang Valley. The collected fruit samples were ensured to be of appropriate maturity based on the Federal Agricultural Marketing Authority (FAMA) index of maturity. The collected fruit samples were weighed and cleaned thoroughly to remove any remaining dirt, debris or particles before processing. The peels were then manually separated, and the weight of each sample was recorded. The samples were then chopped into smaller pieces and freeze-dried. The freeze-dried samples were then ground into a fine powder and sieved using several test sieves of 200 mm x 50 mm with mesh sizes in the range of 50–250 microns (Retsch, Verder Scientific, Germany). These samples were labelled accordingly and stored in an airtight container at 4 °C, until further analysis.

Optimization of extract

The freeze-dried powders were subjected to the hot-water extraction method as described in⁴⁴, with slight modifications. A combination of different parameters was tested as per the Taguchi statistical model, as presented in Table 1. In brief, the samples were added to deionized water at several concentrations (2–8% w/v) and were

Parameters	(A)		
Levels			
	1	2	3
[Extract] w/v %	2.0	4.0	8.0
Time (min)	30	45	60
Agitation (rpm)	100	150	200
Runs	(B)		
	[Extract] w/v%	Time (min)	Agitation (rpm)
1	2	30	100
2	2	45	150
3	2	60	200
4	4	30	150
5	4	45	200
6	4	60	100
7	8	30	200
8	8	45	100
9	8	60	150

Table 1. Process parameters and Taguchi experimental design for optimization of RDPE extract.

subjected to hot-water extraction at 100 °C. Several durations (30–60 min) and agitation levels (100–200 rpm) were evaluated to achieve the desired efficiency. The resulting mixture was centrifuged at 4000 rpm for 20 min and was filtered using hardened ashless filter paper with a diameter of 125 mm (Whatman). The collected supernatant was then transferred into a 50 mL centrifuge tube and kept chilled at 4 °C until use. The efficiency of extraction was evaluated via yield determination and desired colour intensity.

Green synthesis of ZnONPs using RDPE

ZnONPs were synthesized from RDPE using two types of zinc salt precursors: [Zn(CH₃CO₂)₂·2H₂O] and [Zn(NO₃)₂·6H₂O], in reference to previously described protocols with slight modifications^{45,46}. To begin, the respective precursor salt was prepared according to the desired concentration in 200 mL of deionized water, and left to stir and well-homogenize. This was then followed by the addition of RDPE, where several amounts were tested to study the effect of the extract volume ratio on precursor salt concentration. For temperature and pH optimization, reactions were carried out under different conditions, while maintaining the total duration constant at 2 h for each experiment. These parameters were randomized and tested according to the Taguchi statistical model (Table 2). These levels were established based on literature reviews and preliminary experiments^{47,48}.

The color change from red to yellow indicates the initiation of the reducing effects by the extract. Upon the reaction, the mixture was first cooled and centrifuged at 6000 rpm for 10 min. The supernatant was discarded, and the resulting pellet was then thoroughly washed with water three times to remove any leftover impurities. The pellets were then left to dry in an oven at 60 °C for 2 days. The dried pellets were ground into fine powders using a mortar and pestle and stored in an airtight container, which was kept in a desiccator till further use. The synthesized ZnONPs powder was characterized to determine the best parameter combination, and also to compare and determine the differences observed between the two precursor salts. The results were analyzed statistically using Minitab21 software following the Taguchi experimental design.

Characterizations of the synthesized biogenic ZnONPs

The synthesized biogenic ZnONPs were confirmed using UV-Vis spectroscopy (Jenway 7315, Staffordshire, UK). The particle size and polydispersity index (PDI) were performed using a particle sizer (Malvern Instruments, Nano S, Malvern, UK). These aspects were determined as initial characterization, to identify the optimal samples for further analysis. The selected representative samples for each precursor was then analyzed for their stability, identification of functional groups, measure of weight loss against temperature, crystallinity, morphology and elemental composition analysis using the following instrumentation: Zetasizer (Zetasizer Nano ZS90, Malvern, UK), Fourier transformed-infrared (FT-IR) spectroscopy (FTIR-spectrum 400, Perkin Elmer, USA), Thermogravimetric (TGA) (Q500 instrument (TA Instruments), X-ray diffraction (XRD) (SmartLab, Rigaku, Tokyo, Japan) and Field-emission scanning electron microscopy (FE-SEM) equipped with Energy dispersive X-ray (EDX) spectroscopy (JSM 7600 F, JOEL, USA); respectively.

Antimicrobial activity

The antimicrobial activity of selected ZnONPs was studied against two bacteria and one fungus: *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 29213 and *Candida albicans* ATCC 10231; respectively, via the broth microdilution technique⁴⁹. The microorganisms were first cultured in tryptic soy broth medium, followed by Mueller-Hinton broth with turbidity adjustment to a 0.5 and 2.0 McFarland standard, respectively, for the bacterial and fungal cells. In brief, the samples were added to the first well, followed by serial dilutions in the

Parameters	(A)			
Levels				
	1	2	3	
[Precursor] (M)	0.05	0.10	0.20	
Extract volume (mL)	40	100	200	
pH	8	10	12	
Temperature (°C)	25	60	90	
Runs	(B)			
[Precursor] (M)	Extract (mL)	pH	Temperature (°C)	
1	0.05 40	8	25	
2	0.05 100	10	60	
3	0.05 200	12	90	
4	0.10 40	8	90	
5	0.10 100	10	25	
6	0.10 200	12	60	
7	0.20 40	8	60	
8	0.20 100	10	90	
9	0.20 200	12	25	

Table 2. Process parameters and Taguchi experimental design for optimization of biogenic synthesis of ZnONPs.

Run	Extract (w/v %)	Time (min)	Agitation (rpm)	Yield (g)	Colour	Colour intensity (%)
1	2	30	100	78.40	Light red	50.00
2	2	45	150	73.68	Light orange	0.00
3	2	60	200	78.97	Light orange	0.00
4	4	30	150	66.07	Thick red	100.00
5	4	45	200	70.05	Light red	50.00
6	4	60	100	70.67	Red	75.00
7	8	30	200	51.03	Red	75.00
8	8	45	100	27.42	Red	75.00
9	8	60	150	42.21	Red	75.00

Table 3. Optimization of extraction in relation to their yield and colour intensity.

concentration range of 0.02–10 µg/mL. Negative control (broth only) and positive control (inoculated broth) were added to confirm the test validity. Visual examination of the incubated plate was performed via turbidity detection. The minimum inhibitory concentration (MIC) values of the samples were determined upon 24 h incubation at 37 ± 1 °C via absorbance measurement.

Cytotoxicity assay

The selected ZnONPs sample and RDPE were also subjected to cytotoxicity evaluation via MTT assay. 3T3-L1 cells of green epithelial monkey cells with a concentration of 1.0×10^4 cells/mL were prepared and plated onto 96-well plates (100 µL/well). The diluted ranges of sample extracts were added to each well in the range of 10–1500 µg/mL and were incubated for 24 h. At the end of the sample incubation, MTT solution was added and incubated for 3 h. Upon solubilization of the purple formazan crystals using DMSO, the optical density of the samples was measured using an ELISA reader at 570 nm. The cytotoxicity was recorded as the drug concentration contributing to 50% cell growth inhibition (IC_{50}) using the formula below. Based on the determined cell viability %, graphs were plotted against their respective concentration. The acceptance criteria of the assay were based on the absorbance value; whereby lower values indicate decreased cell proliferation and vice versa compared to the control.

$$Cell\ viability\ (\%) = \frac{Absorbance\ sample_{Mean}}{Absorbance\ control_{Mean}} \times 100\%$$

Statistical analysis

Statistical analysis was performed using Minitab statistical software (Version 21.0) for Taguchi experimental design and optimization. The statistical differences between the optimized samples were analyzed using SPSS software (version 28.0). All results were expressed as mean and standard deviation. Normality test was performed and determined by Shapiro-Wilk, where data is normally distributed at a probability of $p > 0.05$. The mean differences of the optimized samples between groups were compared using One-way Anova, and significance was accepted at the probability of $p < 0.05$.

Results and discussion

Extract optimization

Table 3 presents the yield and intensity of the obtained extracts, concerning the combination of different parameters as per the Taguchi statistical model. Based on the results, it was found that R4 gave the intended outcome, with the desired colour intensity. Although higher yields were seen with R1 – R3, the desired colour intensity of red/thick red was not achieved. Similarly, although the desired colour intensity was almost attainable with R7 – R9, the yield was comparatively lower than the other runs. Hence, the best conditions to obtain the desired yield were with 4% (w/v) extract (R4 – R6), and R4 was selected based on the desired colour intensity of thick red.

In addition, to further strengthen this finding, the main effect plot on means and SN ratios (Fig. 1A and B) indicates that the best parameter combination was at 4% (w/v) extract concentration, agitated at 100 rpm for 30 min. Hence, the nearest representative is the R4, with a slight alteration of the agitation level.

Biogenic synthesis of ZnONPs

Taguchi optimization with zinc nitrate hexahydrate salt $[Zn(NO_3)_2 \cdot 6H_2O]$

Figure 2 shows the UV absorption peak analysis of the ZnONPs. The UV absorption region for ZnONPs was detected around 350 nm, which aligns with past literature⁵⁰. Based on the results, only NR2 – NR7 indicated successful formation of ZnONPs among the nine runs.

The yield, particle size and PDI of the synthesized biogenic ZnONPs with zinc nitrate hexahydrate as per the Taguchi array are presented in Table 4. Based on the combination of different parameters, it was observed that the particle size falls within the range of 110–190 nm for five samples (NR2 – NR6), while a larger size was attained with the NR7 sample (570 nm). The lowest PDI was noticed with NR2, NR3 and NR6 (0.10–0.21), while the other three samples showed higher PDI in the range of 0.35–0.53. Based on Table 5, it was concluded that the extract volume (1st rank) imparted the greatest influence, followed by precursor concentration (2nd rank), pH

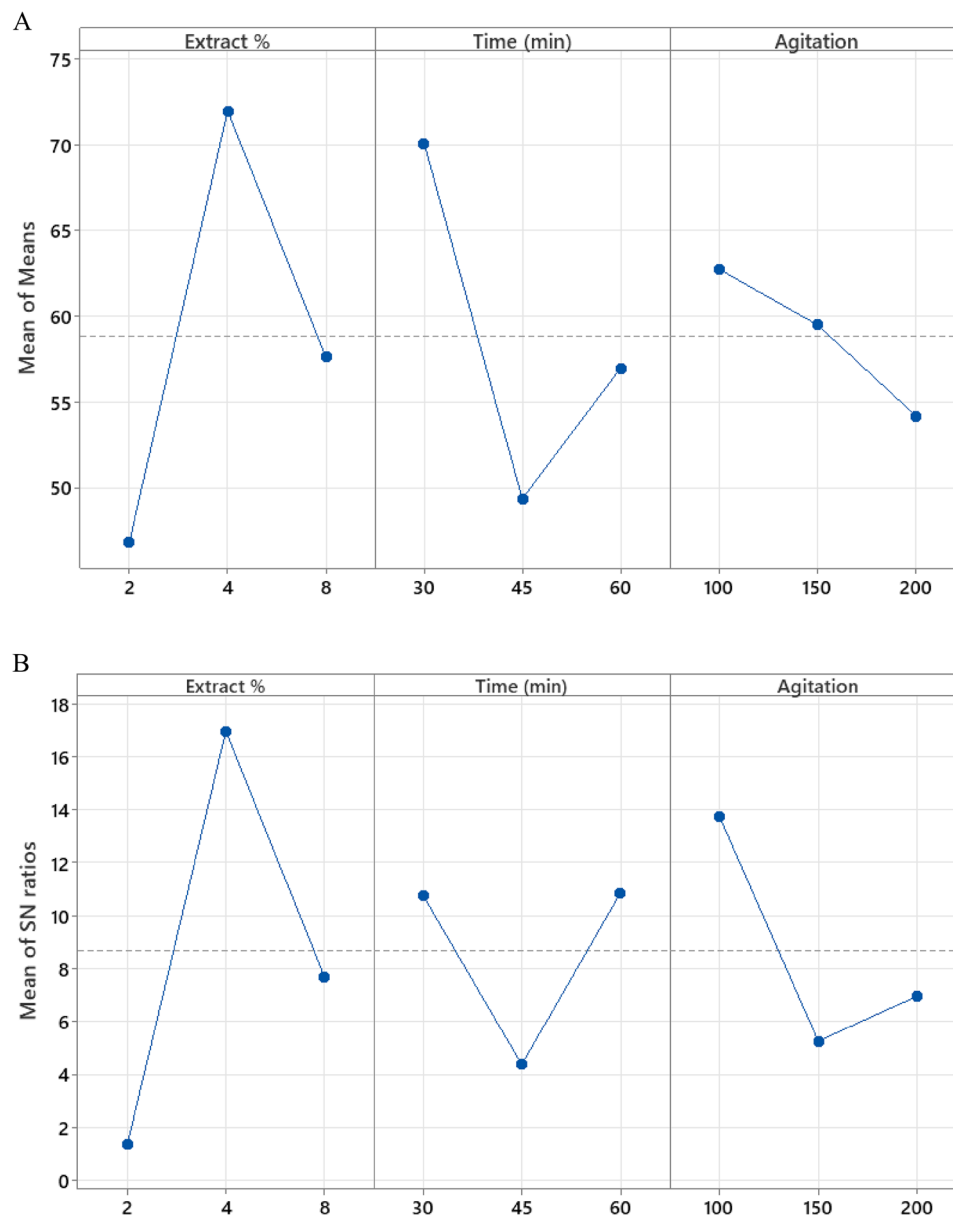


Fig. 1. The main effects plot for (A) means and (B) SN ratios for optimization of extract.

(3rd rank) and temperature as the final factor. This ranking was determined as per the delta values, with stronger impact signified by larger values.

Taguchi statistical analysis presents the best combination of parameters with 0.10 M precursor concentration, 200 mL of extract, pH 10 and temperature of 25–90 °C (Fig. 3). This finding was further verified with the desired white/creamish white colour of the synthesized NPs. Based on the combined outcomes, NR2 and NR3 samples were selected as the best representatives for further characterization and comparison with biogenic ZnONPs synthesized using zinc acetate dihydrate precursor salt.

Taguchi optimization with zinc acetate dihydrate salt $[\text{Zn}(\text{CH}_3\text{CO}_2)_2 \cdot 2\text{H}_2\text{O}]$

Figure 4 shows the UV absorption peak analysis of the ZnONPs. As mentioned above, the UV absorption region for ZnONPs was detected around 350 nm and among the nine runs, only AR3, 4, 7 and 8 indicated successful formation of ZnONPs.

The yield, particle size, and PDI of the synthesised biogenic ZnONPs, as determined by the Taguchi array, are presented in Table 6. Based on the combination of different parameters, it was observed that the particle size of the successfully synthesized ZnONPs falls within the range of 150–290 nm in four samples (AR3, 4, 7, 8). The lowest PDI was noticed with AR7 (0.12), while the other three samples showed higher PDI in the range of 0.30–0.51. As presented in Table 7, the impact of the optimization factors showed a different trend with acetate precursor, whereby the temperature holds the 1st rank, followed by pH (2nd rank), precursor concentration (3rd rank) and extract volume as the least important factor. This was significantly different from nitrate-derived

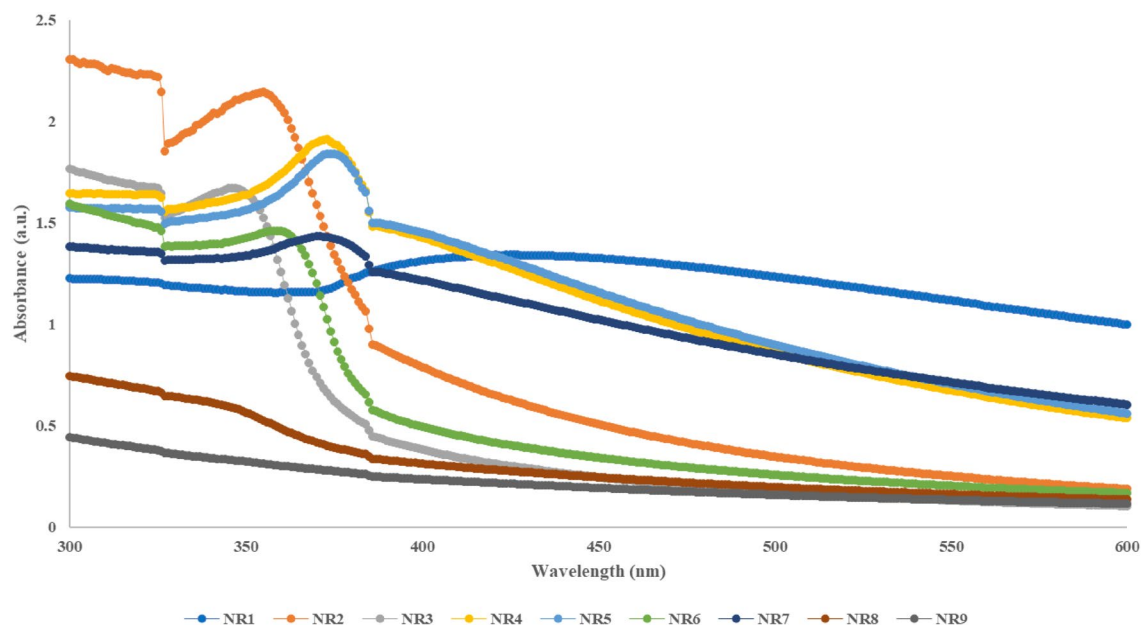


Fig. 2. UV-Vis spectra for biogenic ZnONPs synthesized using zinc nitrate hexahydrate precursor salt.

Sample	[Precursor] (M)	Extract (mL)	pH	Temp (°C)	Yield (g)	Particle size (nm)	PDI	Colour
NR1	0.05	40	8	25	1.04	499.93 ± 24.48	0.44 ± 0.03	Brown
NR2	0.05	100	10	60	2.32	162.17 ± 1.96	0.10 ± 0.03	White
NR3	0.05	200	12	90	6.30	133.77 ± 1.85	0.17 ± 0.02	White
NR4	0.10	40	8	90	1.08	187.77 ± 13.08	0.35 ± 0.03	Light brown
NR5	0.10	100	10	25	1.07	126.40 ± 1.31	0.38 ± 0.07	Light brown
NR6	0.10	200	12	60	4.82	117.90 ± 4.36	0.21 ± 0.03	Light brown
NR7	0.20	40	8	60	0.33	569.10 ± 22.64	0.53 ± 0.02	Light brown
NR8	0.20	100	10	90	0.95	376.50 ± 12.04	0.47 ± 0.12	Brown
NR9	0.20	200	12	25	3.08	128.47 ± 3.50	0.42 ± 0.04	Brown

Table 4. Yield, particle size and PDI of the synthesized biogenic ZnONPs using zinc nitrate hexahydrate precursor salt.

Level	Precursor concentration (M)	Extract (mL)	pH	Temperature (°C)
1	89.58	140.06	111.36	84.58
2	48.89	74.48	53.97	95.28
3	119.98	43.90	93.12	78.59
Delta	71.10	96.16	57.39	16.68
Rank	2	1	3	4

Table 5. Response table for means of the nitrate-derived biogenic ZnONPs.

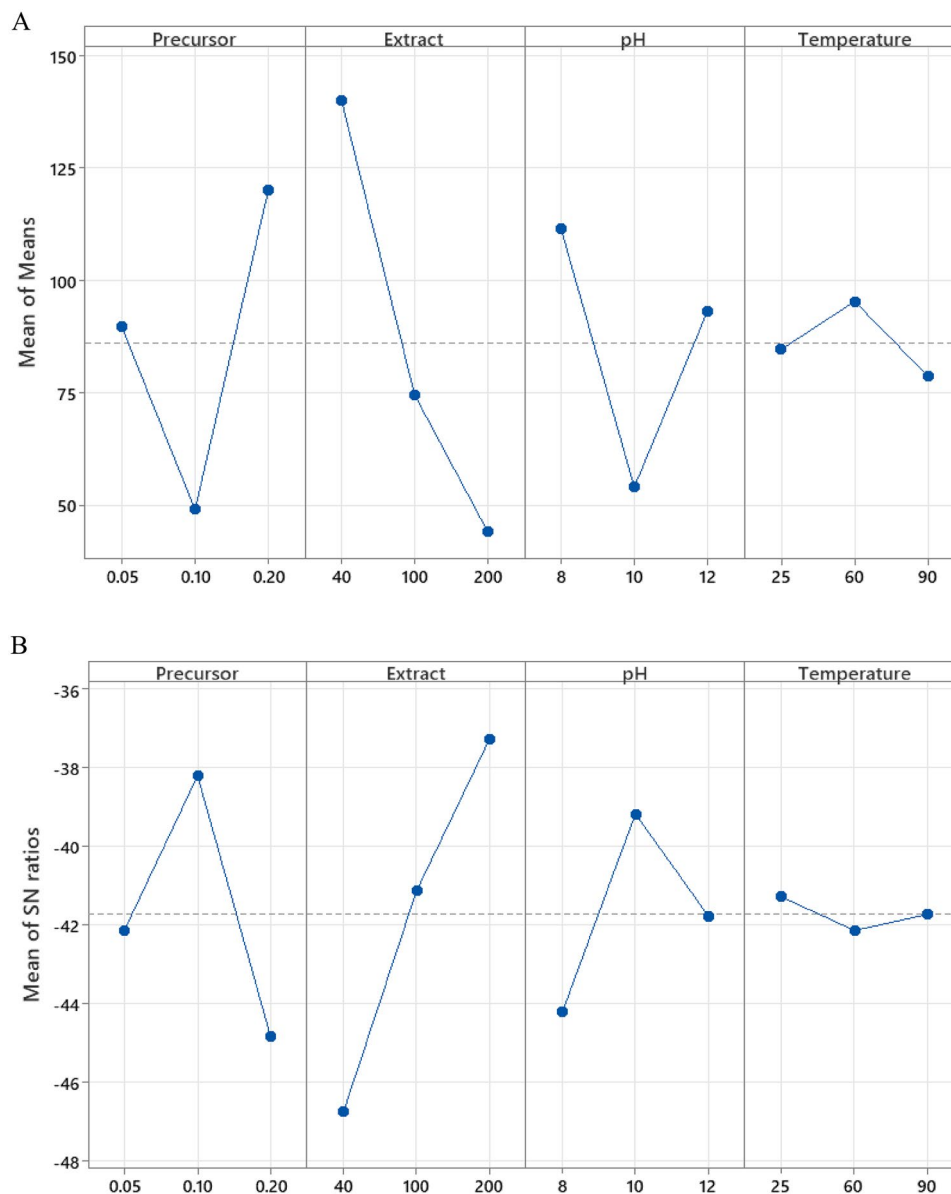


Fig. 3. The main effects plot for (A) means and (B) SN ratios for optimization of nitrate-derived biogenic ZnONPs.

synthesis with respect to temperature and extract volume which presented an opposite effect. Therefore, it could be summarized that among the four parameters investigated, the extract volume and temperature tend to greatly impact the synthesis process, with the inclusion of different precursor salts. The larger delta values signify higher impact and form the basis of this ranking.

Taguchi statistical analysis presents the best combination of parameters with 0.05 M precursor concentration, 40 mL of extract, pH 8 and temperature of 60 °C (Fig. 5). This finding was further verified with the desired white/creamish white colour of the synthesized NPs. Based on the combined results, AR4 and AR7 samples were selected as the best representatives for further characterization and comparison with the nitrate-derived ZnONPs.

Characterization and optimization of the selected ZnONPs

Zeta potential analysis

Zeta potential defines colloidal stability and is a typical measurement of the particle's surface charge. In this study, the zeta potential of the biosynthesized ZnONPs falls within the range of −18 to −20 mV and −23 to −30 mV using nitrate and acetate precursor salt, respectively (Fig. 6). The results obtained revealed that the negatively charged molecules found on the biogenic ZnONPs are involved in capping and stabilizing the NPs^{51,52}. Similar findings were reported in several other studies, and remarkably, it was established that NPs synthesized with ± 25 mV are more likely to possess higher stability. However, the colloidal dispersion stability with absolute zeta potential has been established with ± 30 mV for ZnONPs^{52–54}. Therefore, it could be presumed that lower

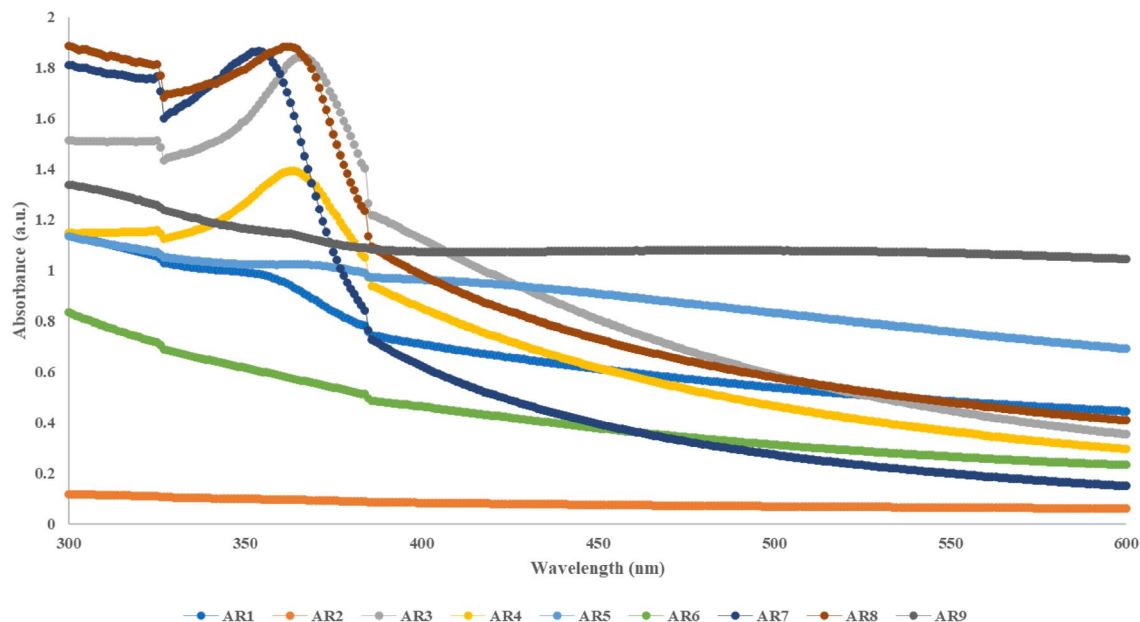


Fig. 4. UV-Vis spectra for biogenic ZnONPs synthesized using zinc acetate dihydrate precursor salt.

Sample	[Precursor] (M)	Extract (mL)	pH	Temp (°C)	Yield (g)	Particle size (nm)	PDI	Colour
AR1	0.05	40	8	25	1.27	137.38 ± 34.76	0.44 ± 0.08	Brown
AR2	0.05	100	10	60	1.05	158.50 ± 42.36	0.27 ± 0.05	Brown
AR3	0.05	200	12	90	0.72	252.30 ± 0.69	0.30 ± 0.02	Light brown
AR4	0.10	40	8	90	2.59	280.90 ± 32.26	0.51 ± 0.13	White
AR5	0.10	100	10	25	3.21	715.53 ± 190.29	0.64 ± 0.175	Brown
AR6	0.10	200	12	60	10.04	147.50 ± 13.16	0.47 ± 0.14	Brown
AR7	0.20	40	8	60	9.77	203.97 ± 1.53	0.12 ± 0.01	White
AR8	0.20	100	10	90	5.09	147.23 ± 1.42	0.38 ± 0.03	Light brown
AR9	0.20	200	12	25	3.24	1116.77 ± 670.24	0.73 ± 0.24	Brown

Table 6. Yield, particle size and PDI of the synthesized biogenic ZnONPs using zinc acetate dihydrate precursor salt.

Level	Precursor concentration (M)	Extract (mL)	pH	Temperature (°C)
1	61.36	70.77	49.98	219.91
2	129.04	114.66	173.84	59.08
3	165.26	170.23	131.84	76.67
Delta	103.90	99.46	123.86	160.83
Rank	3	4	2	1

Table 7. Response table for means of the acetate-derived biogenic ZnONPs.

colloidal stability is associated with possible aggregation of NPs^{55,56}. Based on the data, it was concluded that the synthesized ZnONPs achieved better stability using acetate salt, compared to nitrate salt. In addition, the AR7 formulation presented better colloidal stability, which limits potential aggregation. This result suggests AR7 as the most stable and optimal formulation of the synthesized NPs.

FTIR spectroscopy

The FTIR spectra of RDPE and the biosynthesized ZnONPs are presented in Fig. 7, along with the details of the peaks and their corresponding functional groups as elaborated in Table 8.

RDPE extract showed bands at 3331 cm^{-1} (O-H stretching), 1736 cm^{-1} (C=O stretching), 1609 cm^{-1} (C=C stretching), $1016\text{--}1407\text{ cm}^{-1}$ (C-F stretching) and $758\text{--}894\text{ cm}^{-1}$ (C-H bending). In terms of ZnONPs, similar patterns were visible with both precursor salts; with the bands at $3300\text{--}3389\text{ cm}^{-1}$ (O-H stretching), $1550\text{--}1630\text{ cm}^{-1}$ (C=C stretching), $1016\text{--}1411\text{ cm}^{-1}$ (C-F stretching), $880\text{--}960\text{ cm}^{-1}$ (C=C bending of alkenes) and $600\text{--}700\text{ cm}^{-1}$ (ZnO stretching). The presence of the ZnO stretching vibration confirms the formation of the ZnO structure, as reported in past findings^{57,58}. This formation was correlated and attributed to the presence of phytocompounds in the RDPE, such as pectin, betanin, betacyanins, phyllolactin and others with various functional groups that are believed to play their role as the reducing, capping and stabilizing agents during the synthesis^{57,58}.

TGA and differential TGA (DTG) analysis

The thermal stability and degradation pattern of biogenic ZnONPs were established by TGA and DTG analysis, as depicted in Fig. 8. Nitrate-derived biogenic ZnONPs present stable thermal degradation up to $900\text{--}950\text{ }^{\circ}\text{C}$, with an approximate weight loss of 9.37% and 7.38% for NR2 and NR3 samples, respectively. Specifically, for NR2, the weight loss was observed in ranges between 60 and 560 , $560\text{--}800$, $800\text{--}950\text{ }^{\circ}\text{C}$ with mass losses of 8.25%, 0.89% and 0.23%, respectively. In addition, the intensity DTG peak at 250 , 660 and $865\text{ }^{\circ}\text{C}$ was observed with the derivative rate at 0.99 , 0.11 and 0.02 mg/min . Conversely, the weight loss for NR3 was observed in the ranges between 50 and 185 , $185\text{--}660$, $660\text{--}900\text{ }^{\circ}\text{C}$ with mass losses of 1.92%, 4.88% and 0.58%, respectively. In

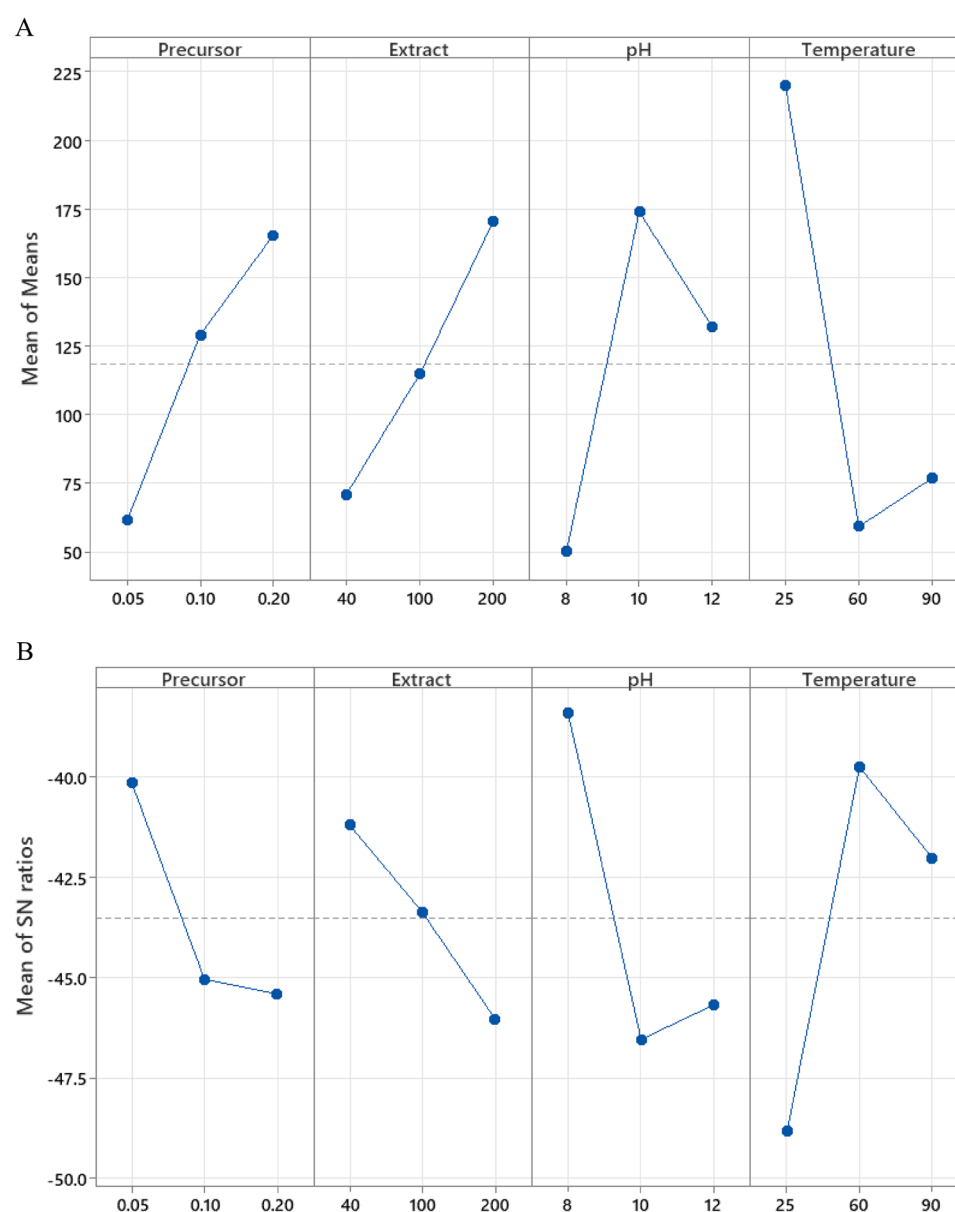


Fig. 5. The main effects plot for (A) means and (B) SN ratios for optimization of acetate-derived biogenic ZnONPs.

addition, the intensity DTG peak at 104, 360, 700 and 840 °C was observed with the derivative rate at 0.04, 0.18, 0.10 and 0.02 mg/min.

On the other hand, acetate-derived biogenic ZnONPs present stable thermal degradation up to 900–920 °C, with an approximate weight loss of 13.80% and 15.93% for AR4 and AR7 samples, respectively. Specifically, for AR4, the weight loss was observed in ranges between 60 and 520, 520–750, 750–920 °C with weight losses of 12.14%, 1.02% and 0.64%, respectively. In addition, the intensity DTG peak at 335, 680 and 860 °C was observed with the derivative rate at 2.11, 0.10 and 0.03 mg/min. Conversely, the weight loss for AR7 was observed in the ranges between 50 and 140, 140–600, 600–900 °C with weight losses of 3.91%, 10.14% and 1.88%, respectively.

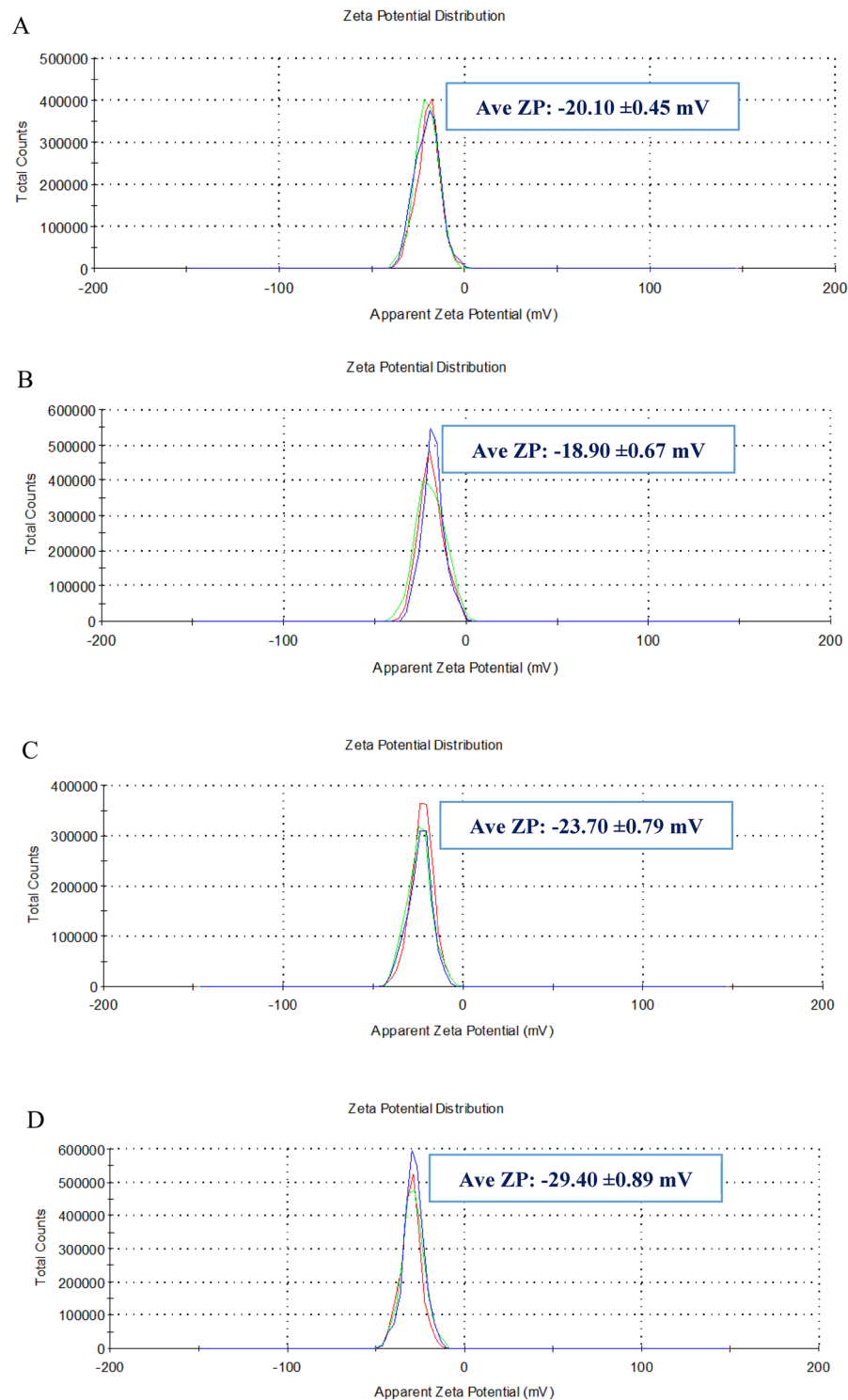


Fig. 6. Zeta potential distribution graph for (A) NR2, (B) NR3, (C) AR4 and (D) AR7.

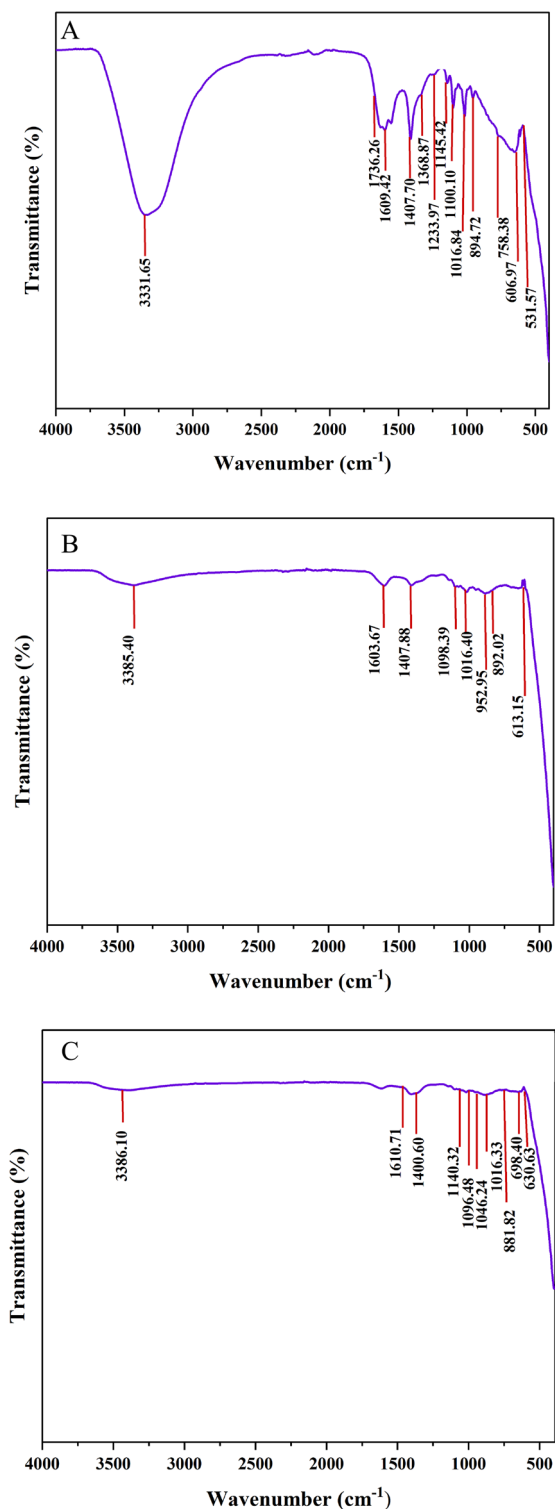


Fig. 7. FTIR spectra of (A) RDPE and biogenic ZnONPs (B) NR2, (C) NR3, (D) AR4, (E) AR7.

In addition, the intensity DTG peak at 206 and 670 °C was observed with the derivative rate at 1.56 and 0.31 mg/min. Based on the described details of each biogenic sample, it could be assumed that they portray high thermal stability, as subsequent incremental temperature rise did not yield significant losses. This phenomenon is strongly attributed towards the presence of phytochemicals in the biogenic ZnONPs, which function as the stabilizing and reducing agents^{59,60}.

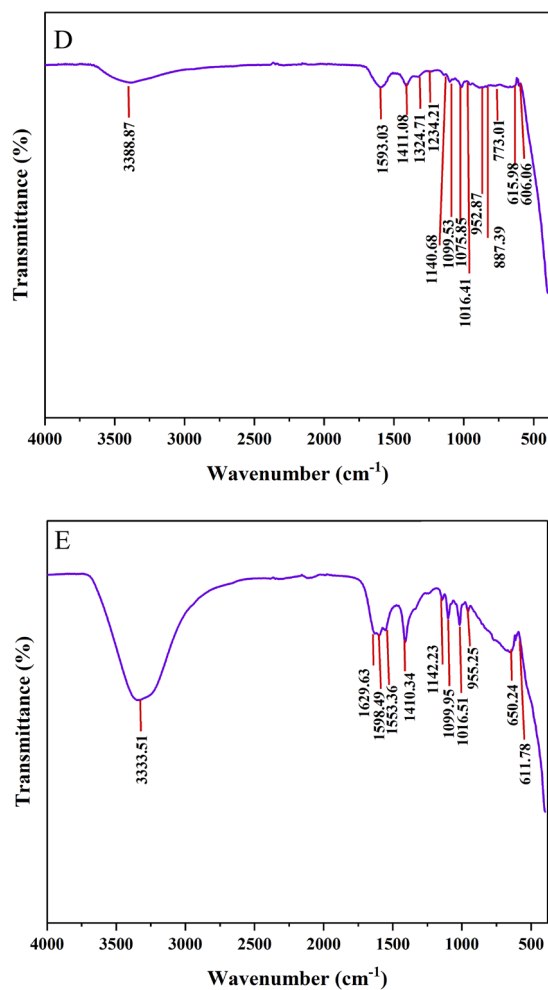


Fig. 7. (continued)

Wavenumber (cm ⁻¹)	Functional group
RDPE	
3331	O-H stretching
1736	C=O stretching
1609	C=C stretching
1016–1407	C-F stretching
758–894	C-H bending
Nitrate-RDPE-derived ZnONPs (NR2, NR3)	
3300–3386	O-H stretching
1600–1610	C=C stretching
1016–1407	C-F stretching
880–960	C=C bending of alkenes
610–700	ZnO stretching
Acetate-RDPE-derived ZnONPs (AR4, AR7)	
3300–3389	O-H stretching
1550–1630	C=C stretching
1016–1411	C-F stretching
880–960	C=C bending of alkenes
600–650	ZnO stretching

Table 8. The functional group of RDPE and biogenic ZnONPs.

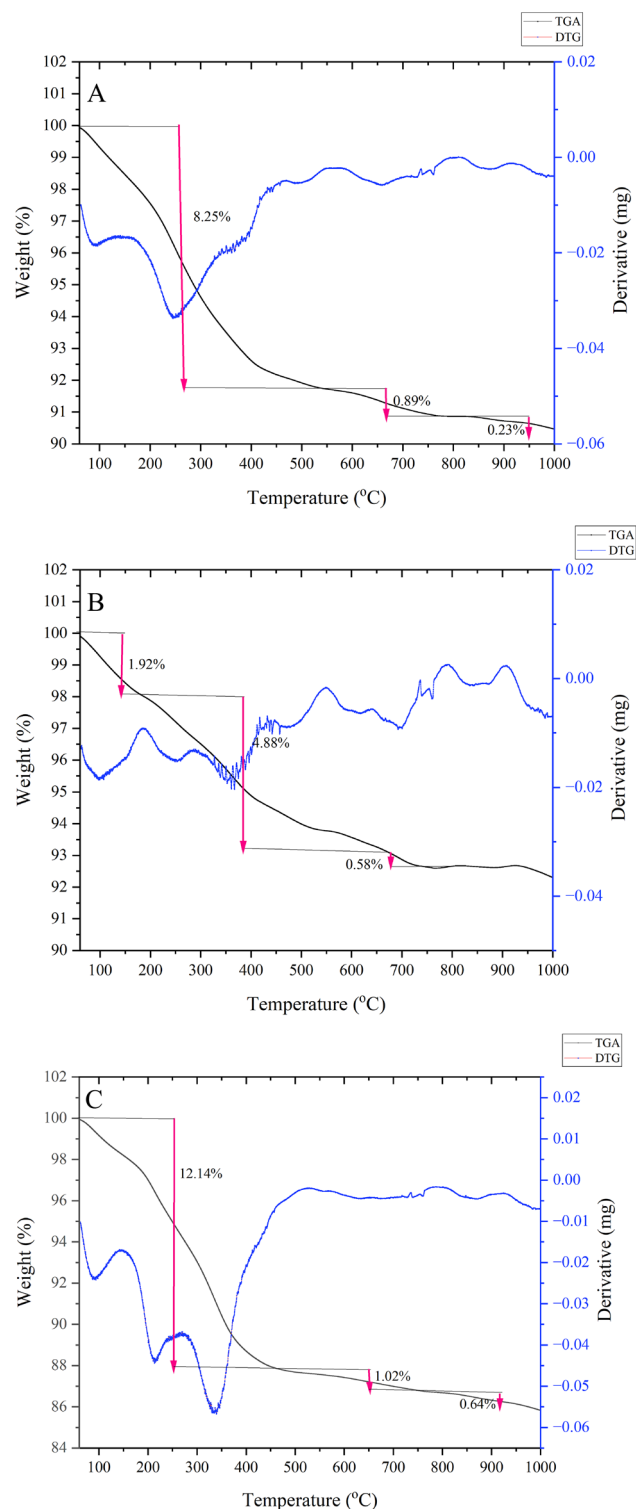


Fig. 8. TGA-DTG curve of the biogenic ZnONPs (A) NR2, (B) NR3, (C) AR4 and (D) AR7.

XRD analysis

The XRD spectrum detecting the crystalline lattice structure of the biosynthesized ZnONPs is depicted in Fig. 9. Similar patterns were evident among all four samples, with lattice orientation planes of (100), (002), (101), (102), (110), (103), (200), (112), (201), (004) and (202) that correspond to their respective 2θ intensity peaks, as presented in Fig. 9. These spectra were correlated to the hexagonal wurtzite structure of ZnONPs, which was proven and established by the Joint Committee on Powder Diffraction Standards (JCPDS card no. 36-1451).

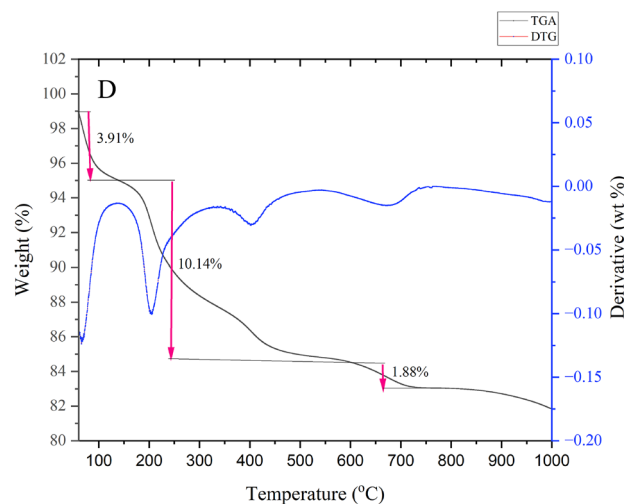


Fig. 8. (continued)

In addition, the crystallite size was also determined for each sample, as calculated using the Scherrer equation. The average crystallite sizes of NR2, NR3, AR4 and AR7 were calculated as 20.29, 15.80, 18.33 and 18.00 nm, respectively (Table 9). The spectra in Fig. 9 indicate the presence of narrow and strong diffraction peaks, suggesting that the synthesized NPs are of optimal crystalline structure and high purity, indicated by the absence of impurities. Our results obtained here were verified as similar to past findings, as reported by multiple researchers^{59,60}.

Morphology and elemental composition

Surface morphology of the synthesized biogenic ZnONPs was studied using FESEM analysis. These results are represented in Fig. 10. The images showed an aggregate of closely packed irregular spherical shapes for NR2 and NR3, whereas irregular hexagonal shapes were observed with AR4. These images represent the common patterns of biogenic ZnONPs, as published in several findings^{60,61}. Interestingly, AR7 stands out with a distinct pattern, indicative of flower-shaped particles that are rarely found.

The size of the synthesized NPs measured from the FESEM micrograph falls within the range of 32–54 nm ($n=20$), with specific sizes of 54.00 ± 7.39 nm, 32.00 ± 5.34 nm, 50.21 ± 7.39 nm and 45.85 ± 4.64 nm for NR2, NR3, AR4 and AR7; respectively (Fig. 10). This finding differed from the particle size measurement via the DLS technique, which indicated larger sizes in the range of 133–280 nm. These differences in particle size analysis via FESEM and DLS are correlated with the bias of the latter technique towards measuring larger particles or even aggregates. In short, DLS measures the hydrodynamic size while FESEM allows the measurement of individual particles^{46,62}. The irregular patterns of the synthesized NPs suggest that the presence of a large surface area could have potentially led to particle aggregation^{52,63}. In summary, it could be deduced that the possibility of sporadic aggregation during synthesis may account for the increased particle size distribution with the DLS technique, even though FESEM reports that most particles were smaller and within the nanoscale range.

EDX analysis, as depicted in Fig. 11 revealed the presence of three major elements: zinc (Zn), oxygen (O) and carbon (C), which also correlates with past findings. The presence of two fundamental elements, Zn and O, was reported to signify the purity of elemental compositions. In addition, the presence of the C element was interrelated to bioactive compound capping of ZnONPs during synthesis^{64,65}. However, it was noted that one of the samples (NR2) indicated a trace amount of the aluminium (Al) element, which is considered an impurity. This finding was possibly attributed to the use of aluminium foil during the synthesis, which highlighted the need for more precautionary measures to prevent such a scenario in the future.

Summary and statistical comparison of optimized samples

Table 10 presents a summary of the characterization of the optimized samples along with their statistical validation. The particle size measured via FESEM and crystallite size obtained via XRD indicate significant differences among all four samples (NR2, NR3, AR4 and AR7). However, in terms of PDI measurement, only AR4 showed a significant difference from the other three samples. The zeta potential of the nitrate-derived samples (NR2, NR3) showed no differences from each other. In contrast, the zeta potential was found to be significantly different among the acetate-derived samples themselves (AR4, AR7), as well as when compared to nitrate-derived samples. Overall, the NR3 and AR7 samples were chosen as representative materials of each precursor salt to be subjected to antimicrobial assessments in relevance to their smaller particle and crystallite sizes.

Antimicrobial activity

Based on the broth microdilution method, the antimicrobial activity of the biogenic ZnONPs was determined in terms of MIC, which is defined as the minimum concentration at which the growth of respective microorganisms

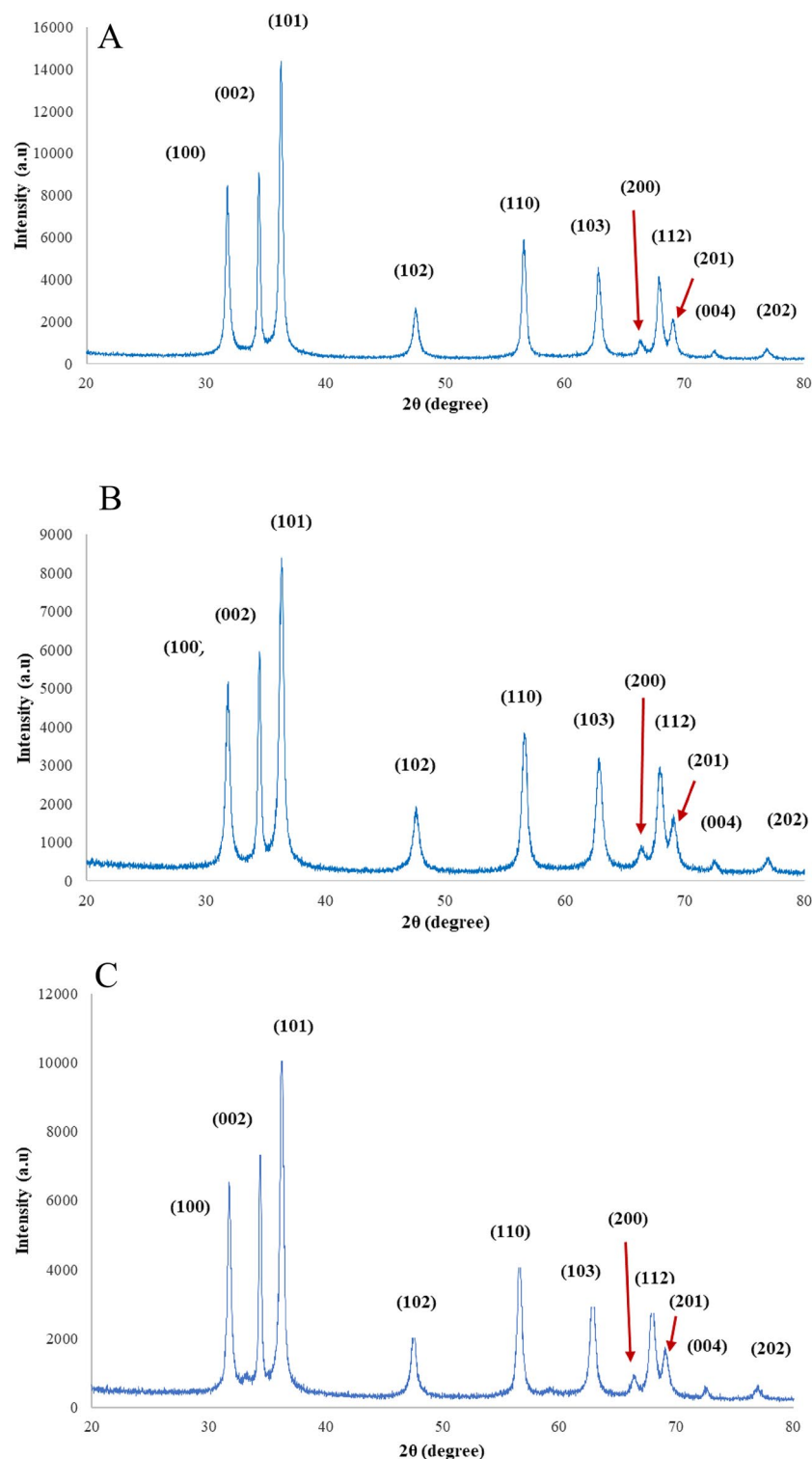


Fig. 9. XRD spectra of the biogenic ZnONPs (A) NR2, (B) NR3, (C) AR4 and (D) AR7.

is not visible. NR3 and AR7 were selected for antimicrobial activity assessments as mentioned above. The results presented effective antibacterial and antifungal activities of both samples against all three microorganisms, with similar MIC values. Specifically, the MIC values of 2.50 µg/mL were recorded against *E. coli* and *C. albicans*, while higher values were determined against *S. aureus* at 5.00 µg/mL (Table 11). This finding was further confirmed with the visual turbidity observation of the samples analyzed against each microorganism (Table 12). As shown, both samples indicated a colour change to turbid at 1.25 µg/mL against *E. coli* ATCC 8739 and *C. albicans* ATCC 10231; while a much rapid turbidity change was observed against *S. aureus* ATCC 29213 (2.50 µg/mL). The

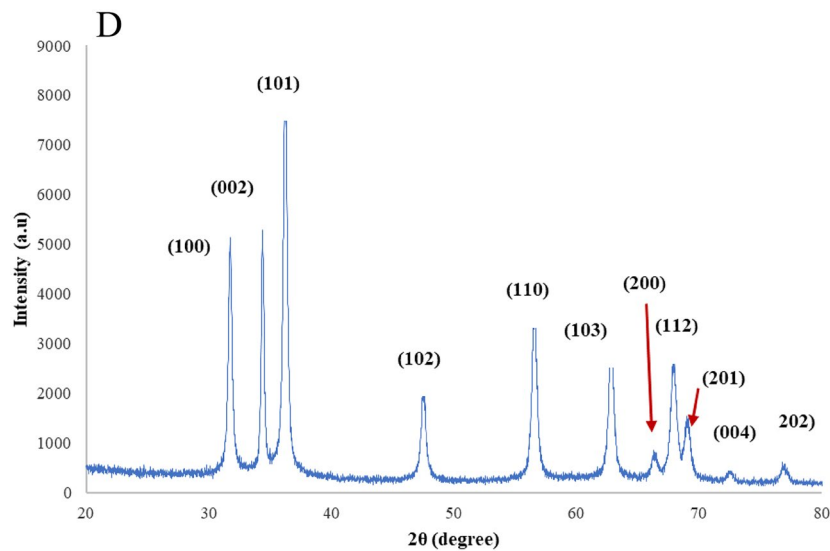


Fig. 9. (continued)

Lattice planes	Peaks			
	NR2	NR3	AR4	AR7
(100)	31.78	31.80	31.77	31.75
(002)	34.42	34.44	34.41	34.41
(101)	36.26	36.29	36.26	36.24
(102)	47.76	47.56	47.54	47.53
(110)	56.60	56.63	56.60	56.58
(103)	62.85	62.87	62.85	62.85
(200)	66.48	66.57	66.50	66.47
(112)	67.94	67.97	67.94	67.94
(201)	69.05	69.08	69.06	69.05
(004)	72.53	72.53	72.54	76.55
(202)	78.27	76.15	79.72	76.55
Average crystallite size (nm)	20.29 ± 6.16	15.80 ± 5.15	18.33 ± 6.21	18.00 ± 5.32

Table 9. Crystallite size of the synthesized biogenic ZnONPs.

absorbance readings corresponding to these results, along with the visual representation of these changes, are presented as supplementary material for further clarification (Table S1, Figure S1 and Figure S2).

Previous findings reported MIC values of biogenic ZnONPs around 30–65 µg/mL, 7.8–62.5 µg/mL and 128 µg/mL for *E. coli*, *S. aureus* and *C. albicans*, respectively. In comparison to past studies, our findings recorded a much lower MIC values, suggesting our RDPE-derived ZnONPs, are a more potent antibacterial and antifungal agent^{61,66,67}. One possible explanation for this scenario was interrelated to the presence of hydrogen peroxide (H₂O₂) molecules on the surface of ZnONPs. These H₂O₂ molecules were believed to exert their antibacterial activities via cell membrane penetration.

Yamamoto and colleagues⁶⁸ have described this phenomenon whereby the increase in NPs concentration and time also leads to a proportional rise in H₂O₂ production, and hence contributes towards significant antimicrobial activities. In another incident, researchers have also highlighted the relevance of pore creation on the cell membranes of microbial cells, as well as the initiation of toxic oxygen radicals within microbial cells, in exerting the antimicrobial effects. The antimicrobial effects of ZnONPs are presumed to be exerted by destroying the balance of the entry and exit of minerals via their entry across the cell membranes, along with leakage of intracellular proteins and enzymes, followed by cell growth inhibition and death^{69,70}.

Past literature has highlighted several potential mechanisms that exert their bactericidal actions through bacterial surface adsorption, generation of various intermediates and electrostatic interactions. The electrochemical gradient is generated by the action of hydrogen ions across the cell membrane, which facilitates the diffusion of metallic ions. This mechanism is strongly associated with the NPs' size; whereby smaller particles will yield better electrostatic interactions. Overall, it was observed and confirmed that the targeted inhibitory action of ZnONPs is interdependent on various factors, including concentration, size, and time of interaction. Likewise, although the action of ZnONPs affects several structures, it was concluded that their main mechanism

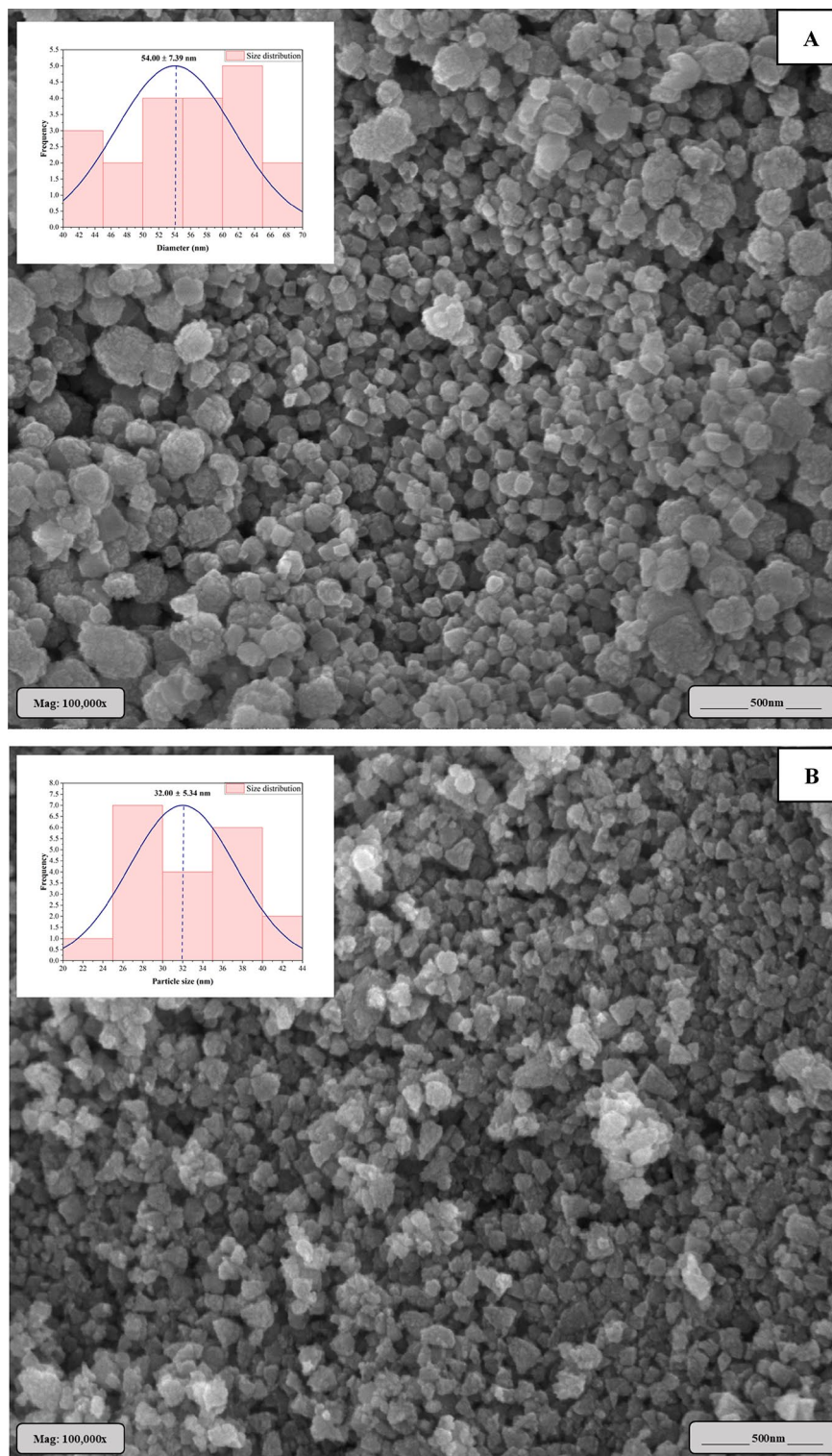


Fig. 10. FESEM micrographs of the biogenic ZnONPs (A) NR2, (B) NR3, (C) AR4 and (D) AR7.

was exerted against the cytoplasmic membrane, which indirectly generates secondary effects upon membrane rupture³⁹.

On a general note, ZnONPs tend to interact with the cell membrane upon contact, which leads to cell membrane damage and structural disruption²⁵. As the cell integrity is affected, it leads to permeability changes and eventual bacterial cell lysis. Moreover, ZnONPs are also found to generate ROS upon exposure to light or moisture⁷¹. On the whole, the antibacterial mechanism of action is closely correlated to their size, shape and concentration. For example, smaller-sized NPs with larger surface area facilitate greater contact with bacterial

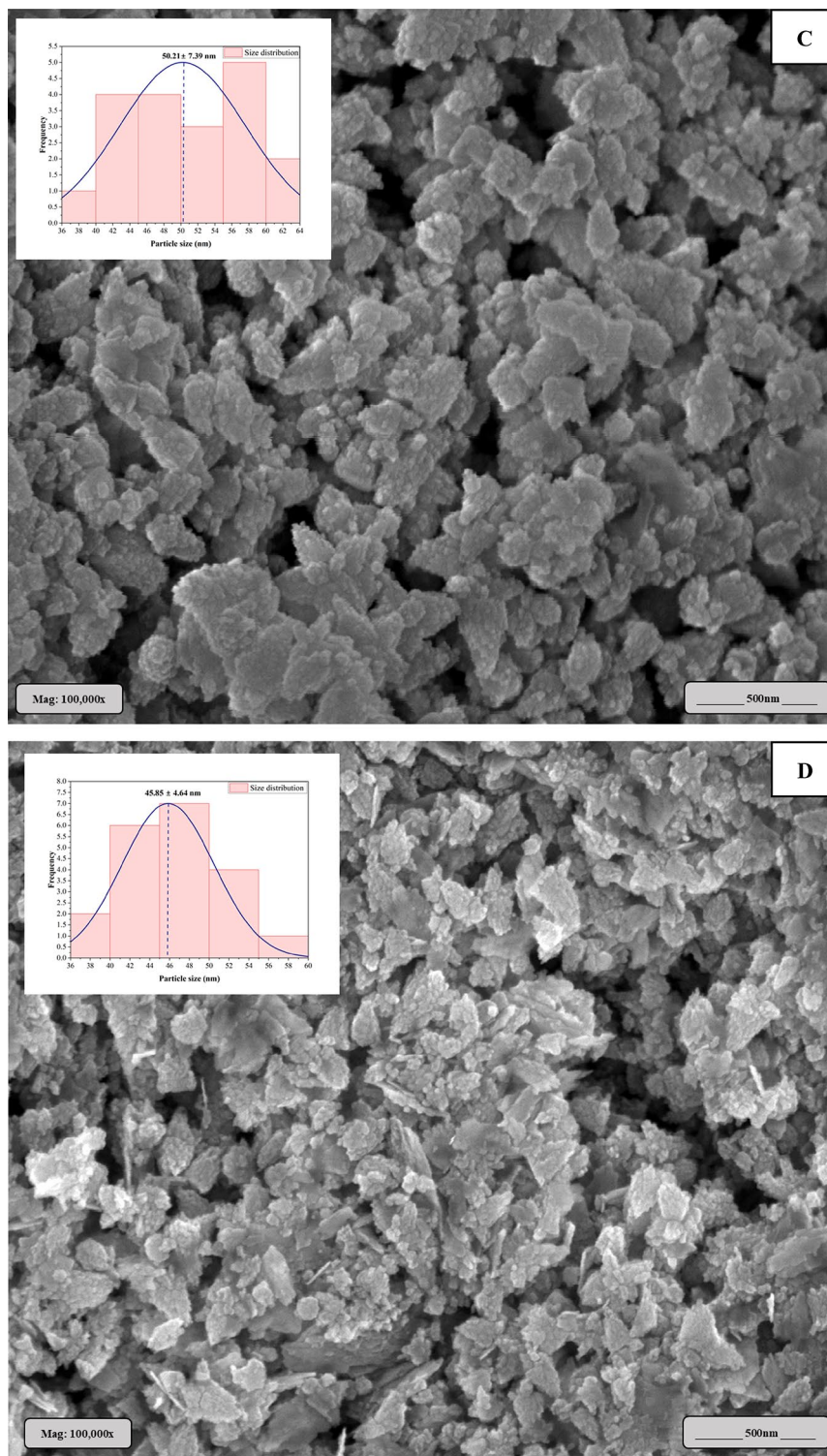


Fig. 10. (continued)

cells, resulting in enhanced activity. Additionally, oxygen annealing on ZnO enhances the number of oxygen atoms that conduct greater adsorption of oxygen atoms and amplifies ROS formation, which further enhances their antimicrobial properties⁷². Moreover, ZnONPs tend to induce cytoplasmic shrinkage and disrupt cell walls, resulting in cytoplasmic spillage⁷³. Past findings have revealed the potential of ZnONPs as potent bactericidal agents that affect both Gram-positive and Gram-negative bacteria via direct interaction with the bacterial cell wall, thereby compromising its integrity. Furthermore, the multifaceted attack on bacterial cells minimizes the likelihood of developing resistance²⁵.

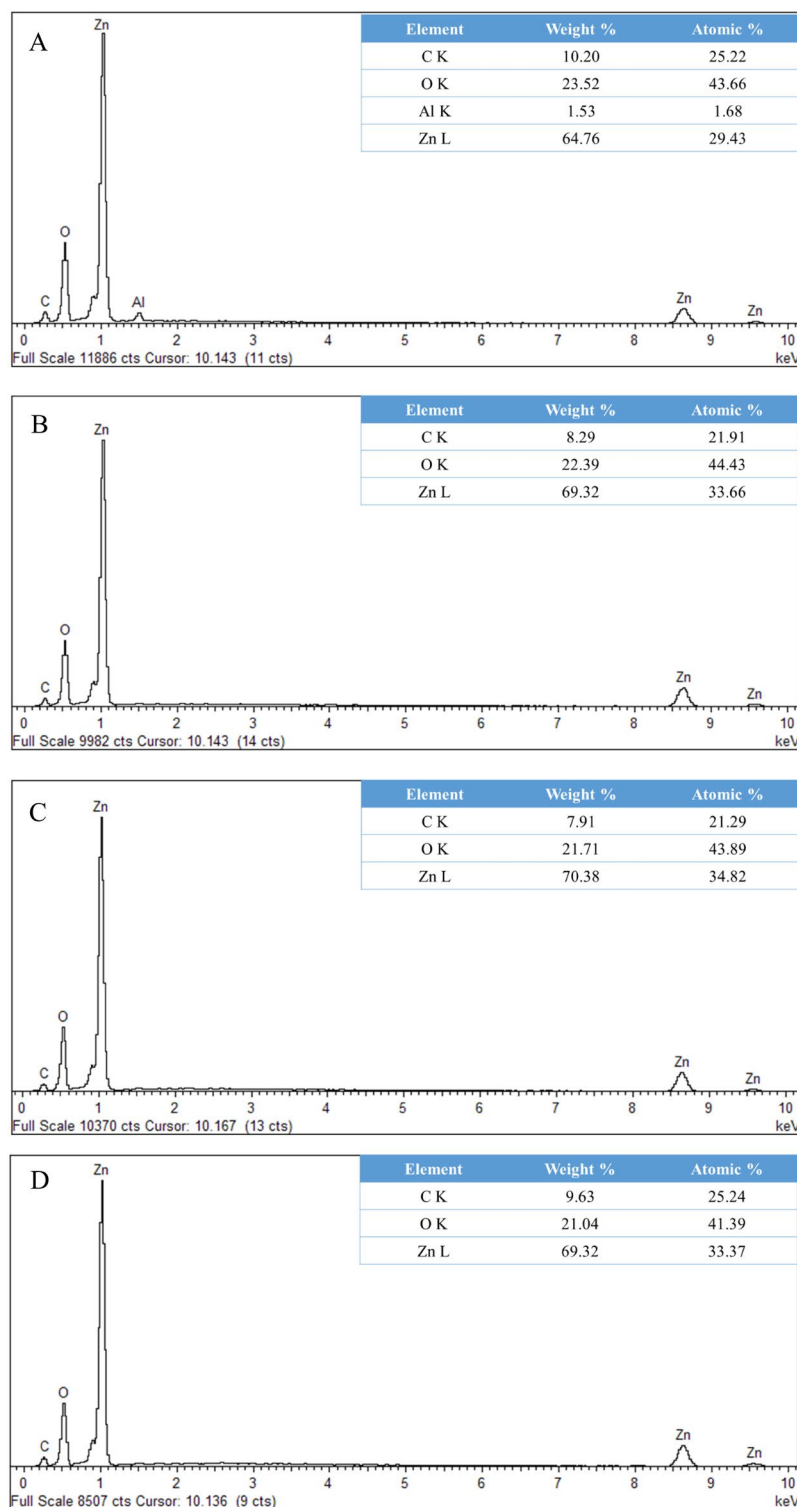


Fig. 11. EDX spectra of the biogenic ZnONPs (**A**) NR2, (**B**) NR3, (**C**) AR4 and (**D**) AR7.

On the other hand, literature has also highlighted diverse efficacy on their antifungal abilities in association with their structural, size and concentration variations²⁵. The antifungal potential of ZnONPs is correlated in several ways. In general, the action of ZnONPs as an antifungal agent is exerted through cell wall penetration that allows infiltration of the small-sized NPs, which affects the structure and increases permeability, compromising the cell's integrity. Moreover, the presence of NPs also stimulates the generation of ROS, which eventually leads to cell death by imposing harmful effects on DNA, proteins and other essential cellular components. Collectively, the infiltration of small-sized NPs that affect cell integrity and also stimulate ROS generation leads to cell

	NR2	NR3	AR4	AR7	<i>p</i> value
Particle size	53.70 ± 9.79 ^a	31.00 ± 6.09 ^b	48.00 ± 7.26 ^c	43.90 ± 5.13 ^d	All < 0.001**
PDI	0.10 ± 0.03 ^a	0.17 ± 0.02 ^a	0.51 ± 0.13 ^b	0.12 ± 0.01 ^a	NR2:NR3–0.109 NR2:AR7–0.855 NR3:AR7–0.318 AR4: All – <0.001**
Zeta potential	–20.10 ± 0.45 ^a	–18.90 ± 0.67 ^a	–23.70 ± 0.79 ^b	–29.40 ± 0.89 ^c	NR2:NR3–0.249 Others – < 0.001**
Crystallite size	20.29 ± 6.16 ^a	15.80 ± 5.15 ^b	18.33 ± 6.21 ^c	18.00 ± 5.32 ^d	AR4:AR7–0.020** Others – < 0.001**

Table 10. The differences in characterization of the optimized samples. *Data shown are means of triplicate. ** $p < 0.05$ are significant differences based on One-Way Anova between different types of precursor salt synthesized ZnONPs materials. Mean data accompanied by different superscript letters indicate significant differences at $p < 0.05$ based on Tukey HSD post-hoc test comparison, respective to each characterization parameter.

Sample	Microorganisms	MIC (µg/mL)
NR3	<i>E. coli</i> ATCC 8739	2.50
	<i>S. aureus</i> ATCC 29213	5.00
	<i>C. albicans</i> ATCC 10231	2.50
AR7	<i>E. coli</i> ATCC 8739	2.50
	<i>S. aureus</i> ATCC 29213	5.00
	<i>C. albicans</i> ATCC 10231	2.50

Table 11. MIC values of the biogenic ZnONPs.

Concentration (µg/mL)/ Microorganisms	<i>E. coli</i> ATCC 8739	<i>S. aureus</i> ATCC 29213	<i>C. albicans</i> ATCC 10231
NR3			
10.00	Clear	Clear	Clear
5.00	Clear	Clear	Clear
2.50	Clear	Turbid	Clear
1.25	Turbid	Turbid	Turbid
0.63	Turbid	Turbid	Turbid
0.31	Turbid	Turbid	Turbid
0.16	Turbid	Turbid	Turbid
0.08	Turbid	Turbid	Turbid
0.04	Turbid	Turbid	Turbid
0.02	Turbid	Turbid	Turbid
AR7			
10.00	Clear	Clear	Clear
5.00	Clear	Clear	Clear
2.50	Clear	Turbid	Clear
1.25	Turbid	Turbid	Turbid
0.63	Turbid	Turbid	Turbid
0.31	Turbid	Turbid	Turbid
0.16	Turbid	Turbid	Turbid
0.08	Turbid	Turbid	Turbid
0.04	Turbid	Turbid	Turbid
0.02	Turbid	Turbid	Turbid

Table 12. Turbidity detection of the biogenic ZnONPs.

death, and these mechanisms hinder fungal growth and proliferation. Previous studies have investigated the effectiveness of ZnONPs against *Candida sp.* isolates, which proved their capability as an antifungal agent⁷⁴. The general concept of ZnONPs to inhibit microbial cells is illustrated in Fig. 12. Overall, the combinatory action of physical disruption, ROS generation, and release of zinc ions creates a synergistic effect, allowing ZnONPs to be highly effective against a broad spectrum of microorganisms.

In summary, AR7 was selected as the most optimal formulation specifically to account for excellent stability and unique flower-like morphologies. Importantly, the particle size, crystallinity and thermal stability were considered adequate as evidenced by past studies.

Cytotoxicity assay

Cytotoxicity is defined as the killing ability of synthesized chemicals, naturally occurring toxins or immune-mediator cells. Based on cell viability, IC_{50} was not determined for RDPE up to the tested concentration, while the AR7, representing the selected ZnONP sample, denotes cytotoxic effects at 405 $\mu\text{g/mL}$. Figure 13 illustrates this outcome, where 3T3-L1 cells subjected to RDPE demonstrated viability, while those treated with AR7 exhibited approximately 50% inhibition following 24 h of incubation.

This indicates that AR7 exhibits cytotoxic effects in a dose-dependent manner. The differential cytotoxic response can be explained by multiple factors, such as (i) the physicochemical characteristics of the AR7 ZnONP, including reduced particle size, increased surface reactivity, and the potential production of ROS; (ii) the existence of surface-bound impurities or synthesis byproducts that may elevate cellular stress; (iii) discrepancies in cellular uptake between RDPE and AR7-treated cells; and (iv) differences in antioxidant defense mechanisms present in the cells when subjected to various materials. The collective influence of these factors may enhance the cytotoxic profile of AR7 relative to the biocompatible RDPE extract.

In a recent study, it was reported that cell viability indicated a sharp decline at concentrations above 125 $\mu\text{g/mL}$, for green ZnONPs sourced from *Helichrysum cymosum*⁵⁶. Comparatively, our biogenic ZnONP indicated approximately two-fold higher concentration for the possible safe limit. It was inferred that ZnONPs penetrate cells through membrane channels, cell membrane, transport proteins or endocytosis, which could potentially endanger the function of some organelles such as mitochondria. Overall, past literature has mostly attributed ZnONPs cytotoxicity effects towards oxidative stress, lipid peroxidation and ROS generation, leading to DNA mutation, breakage and DNA destruction^{56,75,76}.

Study limitation and future directions

Nevertheless, we would also like to address certain limitations of our study. This study focused on a comprehensive outcome focusing on the utilization of the RDPE extract for synthesis optimization and identification of biogenic ZnONPs with the best properties. In this context, the aspects of antimicrobial assessment and cytotoxicity were conducted as a preliminary study to ensure the safety profile of the biogenic ZnONPs. Certain critical aspects of this remain to be explored, which include the microscopic imaging techniques as a validation tool for the mechanistic pathways against the treated cells. Also, future work will investigate the inhibition zones of these ZnONPs, along with those advanced nano-encapsulated formulations, to provide a more conclusive finding on their antimicrobial properties.

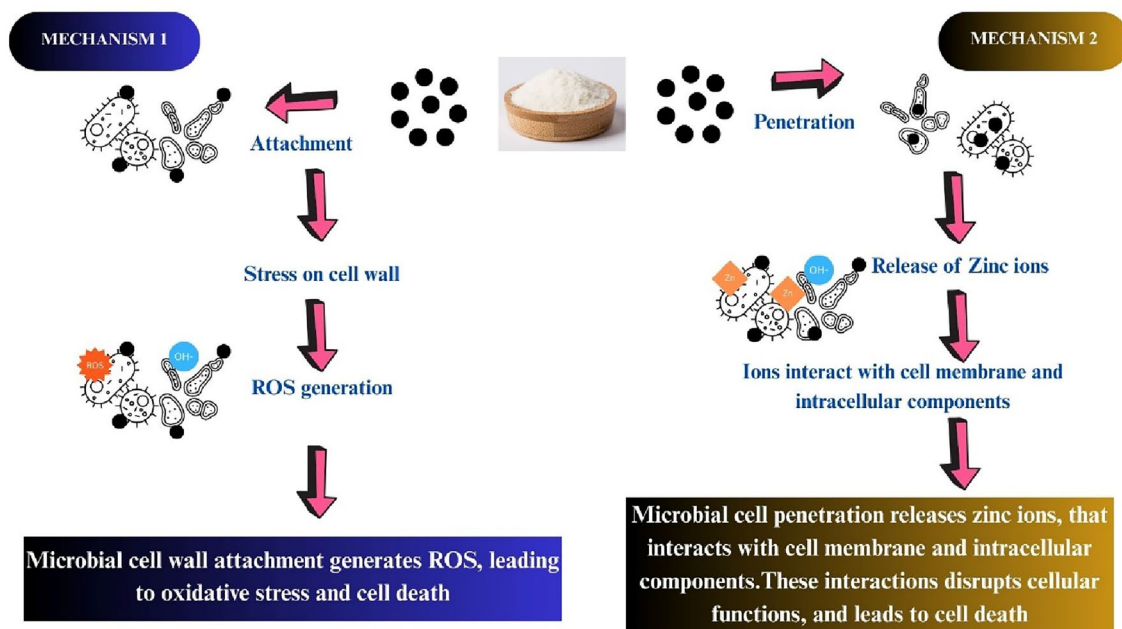


Fig. 12. Mechanism of action concept of biogenic ZnONPs action against microbial cells. (Adapted from^{25,39}).

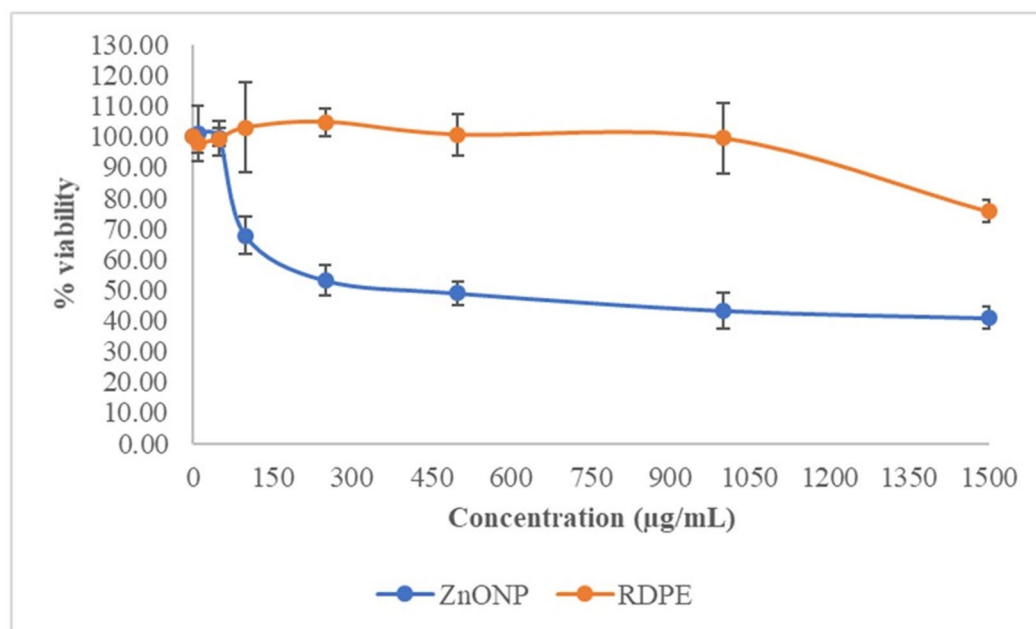


Fig. 13. Effect of ZnONP and RDPE ON 3T3-L1 cells upon 24 h exposure.

Conclusion

This study presented a promising aspect of biogenic synthesis of ZnONPs using RDPE as the reducing and capping agent, in a green, cost-effective and sustainable manner. The applied characterization techniques presented excellent characteristics compared to past findings. Comparative analysis using different precursor salts revealed superior properties of the acetate-derived ZnONPs against the nitrate salts, with AR7 identified as the optimal one. Nevertheless, there were no significant differences in the precursor-representative samples when subjected to antimicrobial assessments. In terms of cytotoxicity, comparative evaluation presented cytotoxic effects of the biogenic ZnONPs, while the RDPE was considered safe, suggesting an outlook for proper usage of the NPs. Herein, we highlight that our research incorporates Taguchi optimization to methodically determine the optimal synthesis parameters, leading to a distinctive and unique flower-like morphology with enhanced colloidal stability. Additionally, an enhanced biosafety profile and improved antimicrobial efficacy were exhibited by the optimized ZnONP, AR7. Overall, this research work emphasized the suitability of valorizing RDPE as a natural source for biogenic synthesis of ZnONPs with the desired characteristics. Future insights will explore the biomedical prospects of the optimized formulation, AR7, along with integration of nano-encapsulation technologies, focusing on antidiabetic applications.

Data availability

The authors declare that the data supporting the findings of this study are available within the paper. The crystallographic data for the structures reported in this study have been deposited at the Cambridge Crystallographic Data Centre (CCDC) under deposition numbers CSD 2472732–2472735. Should any raw data files be required in another format, they are available from the corresponding author upon reasonable request.

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Author contributions

Conceptualization: [Rathi DNG; Abdullah CAC]; Methodology: [Rathi DNG; Abdullah CAC]; Formal analysis and investigation: [Rathi DNG]; Writing - original draft preparation: [Rathi, DNG]; Writing - review and editing: [Rathi DNG; Abdullah CAC; Norhaizan ME; Norazalina S]; Funding acquisition: [Rathi DNG; Abdullah CAC; Norhaizan ME; Aswir AR; Mohd Fairulnizal MN]; Supervision: [Abdullah CAC; Norhaizan ME; Norazalina S; Aswir A; Mohd Fairulnizal MN].

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Declarations

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The authors declare no competing interests.

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Not applicable.

Additional information

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Correspondence and requests for materials should be addressed to C.A.C.A.

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