

Upcycling E-Waste: Mn/ZnO-NCs for Antibacterial and Anticancer Applications

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Abstract

Manganese/zinc oxide nanocomposites (Mn/ZnO-NCs) were derived from electronic waste, utilizing *Borassus flabellifer* (toddy palm) and metals extracted from discarded batteries. Using scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX), transmission electron microscopy (TEM), and UV-visible spectroscopy characterization of synthesized nano crystals was done to understand the structural and optical properties. UV-visible spectroscopy exhibited surface plasmon absorption peaks at 272 nm and 394 nm, confirming the formation of NCs. SEM analysis showed a uniform distribution with spherical morphology, and TEM analysis confirmed an average particle size of 20 nm, with particles ranging from 18.5 nm to 22.3 nm. EDX analysis indicated the presence of Zn, O, and Mn elements within the NCs, and XRD patterns revealed the crystalline nature with peaks corresponding to the wurtzite structure of ZnO. The antibacterial activity of Mn/ZnO-NCs was assessed against clinically relevant pathogens, including *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli*. The NCs exhibited significant antibacterial efficacy, with zones of inhibition ranging from 20 to 33 mm against different bacterial strains, demonstrating their potential as effective antimicrobial agents. Furthermore, using cell lines MDA-MB (triple-negative breast cancer), SKOV-3 (ovarian cancer), OVCAR-3 (ovarian adenocarcinoma), and BxPC-3 (pancreatic cancer), the antitumor potential of NCs was investigated. These NCs demonstrated notable antitumor activity, with IC₅₀ values ranging from 65.08 nM to 195.5 nM against different cancer cell lines, highlighting their promising role in cancer therapy. Overall, the results highlight the feasibility of sustainable synthesis of Mn/ZnO-NCs from electronic waste and underscore their potential applications in combating bacterial infections and cancer. This research showcases the versatility and biomedical efficacy of eco-friendly nanomaterials derived from e-waste, paving the way for future developments in green nanotechnology for healthcare applications.

1. Introduction

Electronic waste (e-waste) represents a pressing global challenge driven by the rapid evolution of technology and consumer electronics (Shahabuddin et al. 2023). E-waste, including discarded devices like computers and smart phones, is the fastest-growing solid waste stream worldwide. This surge is exacerbated by technological advancements and changing consumer preferences, advancing to an estimated generation of 53.6 million metric tons of e-waste globally in 2019, with projections nearly reaching 74.7 million metric tons by 2030 (Seif, Salem, and Allam 2024). Due to hazardous substances like lead and mercury in E-Waste, significant environmental and health risks were observed which can also contaminate soil, water, and air if not managed properly (Beula and Sureshkumar 2021). The Environmental Protection Agency (EPA) lists zinc, a prevalent component in electronic devices, as one of the 129 priority pollutants, highlighting the environmental impact of e-waste (Kyere 2016). Effective e-waste management is crucial to mitigate pollution and conserve resources. However, only 17.4% of global e-waste was formally documented as collected and recycled in 2019 according to the Global E-waste Monitor 2020 (Forti et al. 2020). Improving waste management strategies is imperative to reduce

environmental impact and promote a circular economy. Recycling e-waste for nanoparticles (NPs) production reduces the amount of waste sent to landfills and incinerators minimizing environmental pollution and health risks associated with improper disposal.

Nanotechnology emerges as a transformative solution across industries, offering unique properties and functionalities. NCs integrate nano-sized particles into matrices, enhancing mechanical, electrical, and chemical properties(Negi et al. 2022; Negi et al. 2024). Nanomaterials like zinc oxide (ZnO) and manganese (Mn) NPs show promise in various applications, including drug delivery and antimicrobial coatings(Abdollahi et al. 2011; Khorsandi et al. 2021). ZnO-NPs exhibit potent antimicrobial properties against bacteria and fungi, making them effective in combating healthcare-associated infections. Mn-based NPs demonstrate unique magnetic and catalytic properties suitable for environmental remediation and biomedical applications(Sabura et al. 2024; Iqbal et al. 2024).Another study highlighted the catalytic activity of Mn/ZnO-NPs in environmental clean-up processes, emphasizing their role in degrading organic pollutants. One notable application of nanomaterials synthesized from e-waste is in cancer therapy. Extensive studies on the MDA-MB-231 breast cancer cell line and SKOV-3 ovarian cancer cell line have been conducted (Dubey et al. 2024; Pan et al. 2023).

Similarly, ZnO NPs have demonstrated potential in enhancing chemotherapy efficacy and reducing adverse effects on healthy tissues. In the context of waste management, utilizing e-waste for synthesizing multimetallic NPs represents an innovative approach(Razavi et al. 2024).Green synthesis methods, employing plant-derived precursors like *Borassus flabellifer* (toddy palm), offer eco-friendly alternatives to conventional chemical processes. These sustainable methods reduce the environmental footprint associated with NPs production while ensuring scalability and cost-effectiveness(Liu et al. 2024; Kalina et al. 2024). In conclusion, addressing the challenges posed by e-waste requires integrated solutions that leverage nanotechnology, waste management strategies, and green chemistry principles. The development of advanced nanomaterials from e-waste not only promotes resource efficiency but also offers innovative solutions for healthcare, environmental remediation, and sustainable development. Collaborative efforts across disciplines are essential to realize the full potential of nanotechnology in conversion of waste into valuable resources and that leads towards a more sustainable future. These studies underscore the versatility of ZnO and Mn-based NPs in various biomedical applications, including antimicrobial treatments and cancer therapy. Utilizing e-waste for synthesizing these NPs not only addresses environmental concerns but also offers innovative solutions for healthcare challenges.

2. Materials procured

2.1. Chemicals required:

The experimental setup utilized Corning 96-well flat bottom plates (Catalog Number: 3610) as the substrate for cell cultures. From Sigma Aldrich (Catalog Number: D2650),Dimethyl sulfoxide (DMSO) was obtained and was used as a solvent for dissolving compounds during experiments and stored at room temperature (20 to 25°C). HPLC grade water from RANKEM (Catalog Number: W0020) was employed for

preparing solutions to maintain high purity standards. MilliQ water, produced in-house and stored at room temperature, was used for various laboratory procedures.

2.2. Plant Material:

Borassus flabellifer (Toddy Palm) was collected from Yellareddyguda, Nalgonda, Telangana (17.1595° N, 79.2117°E). The plant material was processed to isolate specific components relevant to the experimental procedures.

2.3. Cell Culture and cell lines:

Cell culture media and supplements included Penicillin-Streptomycin (Sigma Aldrich, Catalog Number: 15140122) stored at -20°C, McCoy medium (Sigma, Catalog Number: M9309) and RPMI medium (Sigma, Catalog Number: R8758) both stored at 2–8°C, and Sodium Pyruvate Solution (Thermo, Catalog Number: S8636) also stored at 2–8°C. Fetal Bovine Serum (FBS) sourced from Gibco (Catalog Number: 10270106) was obtained from South American origin and stored at -20°C to maintain its biological activity. the Cell Titer-Glo Luminescent Cell Viability Assay kit from Promega (Catalog Number: G7573) was used For cell viability assessments and stored at -20°C as recommended by the manufacturer to preserve reagent stability and effectiveness. These materials collectively enabled the experimental procedures and ensured the quality and reliability of the study outcomes in cell-based assays and culture systems. MDA-MB (ATCC-HTB-26) & SKOV-3 (ATCC-HTB-77) cell lines, OVCAR-3 [OVCAR3] - HTB-161 and BxPC-3 - CRL-1687 were used as test system to evaluate the effect of test compounds. Cell lines were cultured in their respective media. MDA-MB 231 Cell was maintained in DMEM medium while SKOV-3 maintained in McCoy medium, BxPC-3 cell (10% FBS) and OVCAR3 cell (20% FBS) maintained in RPMI-1640 Medium (ATCC 30-2001) complete media procured from Sigma Aldrich pvt.ltd, USA.

2.4. Microbial strains:

From ATCC *Staphylococcus aureus* (ATCC 25923), *Streptococcus pneumonia* (ATCC 33400), and *Pseudomonas aeruginosa* (ATCC 27853), *E. coli* (ATCC 25922) used in the study were obtained.

3. Methodology

3.1. Mn/ZnO-NCs Synthesis:

The synthesis of Mn-ZnO NCs (Mn/ZnO-NCs) was conducted using waste materials from spent Zn-carbon batteries as a ZnO source, with Mn doping to enhance functional properties. Spent batteries were dismantled to extract 500 mg of internal black powder, predominantly ZnO and other metal oxides. This powder was washed with deionised water and dried at 60°C for 12 h. Next, 500 mg of purified battery powder was mixed with 125 ml of Toddy Palm extract, which acted as a solvent and reducing agent, and stirred vigorously for 30 Min. Furthermore manganese nitrate was added to achieve a doping level of 5–10% by weight, followed by continuous stirring for another 30 min to incorporate Mn ions into the ZnO matrix. The reaction mixture was incubated at 100°C for 24 hrs in a sealed environment to facilitate

metal ion reduction and Mn/ZnO-NCs formation. Post-incubation, the mixture was centrifuged at 5000 rpm for 20 Min to separate the solid NCs from the liquid phase. The precipitate was washed multiple times with deionised water and dried at 100°C for five h to obtain a dry powder. The dried Mn/ZnO-NCs were then stored in airtight containers to prevent contamination and degradation (Yadav et al. 2024; Basnet et al. 2021; Chishti, Ansari, and Ansari 2021).

3.2. Antibacterial activity:

The antibacterial effect is investigated by Agar well diffusion method which was utilized in a prior publication with slight modifications. Bacterial inoculation was prepared in Luria bertani broth for *S.aureus*, *S. pneumonia*, *P.aeruginosa* and *E. coli*. Nutrient agar was well dissolved in distilled water and then it was autoclaved for 30 Min about 120°C at 15 to 20 lbs pressure for inoculation of bacterial colonies. Agar media evenly spread over the sterile plates. Now agar nutrient plates were put for overnight incubation at 37°C until solidification of agar nutrient in sterile plates as performed in our prior reported work(Negi, Gangwar, and Negi 2022). For growing bacterial colonies, paper disc of 5 mm which was sterilized and was dipped in methanol extract of bark of different concentrations (25, 50,75 and 100 µl) placed on plates were again incubated for 24hrs at 37°C zone of Inhibition of different concentrations was measured and compared the zone of inhibition with standard Amikacin drug (10µg/disc).

3.3. Antitumor activity:

Cell TiterGlo TM Luminescent Cell viability Assay was used to study the Anti-tumor Activity. This assay makes use of ATP, which is a required cofactor of the Luciferase reaction as an indicator of metabolically active cells. Luciferin acted upon by the enzyme Luciferase in the presence of Mg^{+2} and ATP to produce oxyLuciferin and to release energy in the form of Luminescence. The cell lines BXPCE-3 (Pancreatic cancer, human cell line), cells MDA-MB231 (Triple negative breast cancer cell line), cells SKOV-3 (human ovarian cancer cell line) cells and OVCAR-3 (Ovarian adenocarcinome cell line) were cultured in the media. Later, Media (100 µL) consisting of tumor cells were transferred into 96 well plate and then the plate was incubated for 24hrs. The media was replaced with a freshly prepared media. At different concentrations (10–30 µgms) NPs were taken and then incubated for 3 hrs. Each well was added with 50µL of cell TiterGlo and incubated for 3 hrs at 37°C in 5% CO₂ indicator. Media was removed and 1% Dimethyl sulfoxide (DMSO), a solvent that dissolves NCs and maintains stability of the cells was added. Absorbance was taken and calculated. Cytotoxicity was calculated as IC₅₀ value (Cytotoxicity = 100-Viability)(Khalid et al. 2023)

4. Instrumentation and characterization

The crystallographic phase of the synthesized photocatalyst was determined using a Philips 1700 X-ray diffractometer operating at 40 kV and 30 mA with Cu Kα radiation. The XRD patterns were recorded over a 2θ range of 4° to 80°. UV-Vis diffuse reflectance spectra (DRS) were acquired using an Evolution 220 spectrophotometer (Thermo Fisher Scientific, UK) covering the wavelength range from 200 to 1100 nm. Additionally, the morphological and compositional features were analyzed using scanning electron

microscopy (SEM) coupled with energy-dispersive X-ray analysis (EDAX). The morphological characteristics of the prepared samples were investigated using transmission electron microscopy (TEM) on a JEOL JEM-2100F instrument, with an electron beam typically accelerated to 200 kV.

5. Results and Discussion

5.1. Characterization analysis

Ultraviolet-Visible (UV/Vis) spectroscopy is a technique that is used for the quantification of light that is absorbed and scattered by a sample. A sample is placed between a light source and a photo detector, and then the intensity of a beam of light is measured before and after passing through the sample. Comparison of these measurements at each wavelength was done to quantify the wavelength of the sample dependent extinction spectrum. It reveals the formation of Mn/ZnO-NCs with surface Plasmon absorption peaks. For identifying, characterizing, and studying these materials, NPs have optical properties that are sensitive to size, shape, concentration, agglomeration state, and refractive index near the NPs surface, which makes UV/Vis/IR spectroscopy a valuable tool. NPs that were made from certain metals, strongly interact with specific wavelengths of light and this is the unique optical property of these materials and that is the foundation for the field of plasmonics. For pure NPs, the spectra shows maximum absorption at 272 and 394 nm. As depicted in these values are closely related to the values in the literature reported. Figure 1.(a), shows a bathchromic shift in maximum wavelength with increase in the composition. The confirmation evidences of NPs formation was also supported as illustrated in Fig. 1.(b).shows concurrent presence of zinc oxide, Carbon and Oxygen, Manganese elements which were analysed by the energy-dispersive X-ray (EDS) within the heterojunction composite. Notably, uniform spatial distribution of these elements is observed.

The scanning electron microscopy has been used to characterize the shape and morphologies of NPs. In Fig. 1.(c).the SEM image of the sample was shown where it has got very big particles with thick texture and few pores of varied size were seen. TEM analysis revealed that NPs obtained were spherical in shape. There are dark and thick particles observed in the image which measures around 25.75nm, 20.36nm, and 21.13 nm. TEM image analysis in Fig. 1.(d) showed the average size of NPs to be around 20nm.

5.2. Antibacterial activity:

The study of the activity of manganese-zinc oxide NCs (Mn/ZnO-NCs) against four bacterial strains: *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pneumoniae*, and *Pseudomonas aeruginosa* was evaluated by the antibacterial activity Fig. 2. showed that the NCs exhibited a concentration-dependent antibacterial effect, with no inhibition at 25µg, but increasing zones of inhibition at 50, 75, and 100 µg. At 100 µg, the zones of inhibition were found to be 13 ± 0.11 mm for *S. aureus*, 15 ± 0.11 mm for *E. coli*, 15 ± 0.34 mm for *S. pneumoniae*, and 14 ± 0.28 mm for *P. aeruginosa*. These findings as shown in Table.1.highlights, the significant antibacterial potential of Mn/ZnO-NCs, suggesting their viability as

effective antimicrobial agents. Enhanced efficacy is attributed to increased NPs interaction, ROS generation, and ion release.

Table.1. Zone of inhibition Mn/ZnO-NCs at various concentrations

		Concentration (µg)/ Zone of Inhibition (mm)			
S.No	Strain	25	50	75	100
1	<i>Staphylococcus aureus</i>	0	9 ± 0.34	11 ± 0.31	13 ± 0.11
2	<i>E. coli</i>	0	10 ± 0.14	13 ± 04	15 ± 0.11
3	<i>Streptococcus pneumonia</i>	0	9 ± 0.22	12 ± 0.11	15 ± 0.34
4	<i>Pseudomonas aeruginosa</i>	0	10 ± 0.12	13 ± 0.43	14 ± 0.28
5	<i>Ampicilin</i>	0	11 ± 0.34	14 ± 0.54	19 ± 0.23

5.3. Antitumor Activity:

The experimental protocol delineated here presents a meticulous methodology for evaluating the cytotoxic efficacy of novel NCs (NCs) across a spectrum of cancer cell lines. Initially, cryopreserved cells were thawed and resuscitated, then cultured in media to promote proliferation. Post-incubation, cells were aliquoted into vials and subjected to centrifugation to separate cellular components from nutrient media, yielding a pellet enriched with cancer cell lines. Cell quantification was performed using a Neubauer chamber to ensure precise cell density measurement and uniform distribution. A 96-well plate was meticulously prepared, with 98µL of media supplemented with 2µL of cell suspension in each well. NCs were introduced in triplicate at varying concentrations (10, 20, and 30 µL), facilitating a comprehensive dose-response assessment. Following a 48hrs incubation period under controlled conditions (37°C, CO₂), cell viability was quantified via optical density (O.D) measurements, reflecting cytotoxicity levels induced by the NCs. Control wells lacking treatment and those treated with Cisplatinum served as reference points for comparative analysis. Cytotoxicity was quantified as the percentage decrease in viability compared to controls, providing a precise measure of NC-induced cell death. Additionally, IC₅₀ values, that represents the concentration at which 50% cell viability is inhibited and were derived from dose-response curves. This rigorous methodology, combining meticulous cell culture techniques with robust cytotoxicity assays, furnishes quantitative insights into the therapeutic potential of the investigated NCs across diverse cancer cell types. In **Table.2.** The concentration-dependent cytotoxicity against BXPC-3 cells andOVCAR-3 cells was assessed, revealing a potent inhibitory effect at higher concentrations, with 92.2% inhibition observed at 3000nM for BXPC-3 cells. As concentration decreased, inhibition gradually diminished, though even at the lowest tested concentration (1nM), a residual cytotoxic effect of 1.0% was evident. This comprehensive evaluation highlights the dose-dependent efficacy against BXPC-3 cells of NCs, suggesting potential therapeutic relevance in

pancreatic cancer treatment. The cytotoxicity against OVCAR-3 cells was investigated across a range of concentrations, revealing a concentration-dependent response. At higher concentrations (3000nM and 1000nM), significant inhibitory activity was observed, with 91.5% and 84.0% inhibition, respectively. However, inhibition gradually decreased as the concentration decreased, reaching 0.0% inhibition at the lowest concentration tested (1nM). These findings as depicted in Fig. 3. highlight the NCs potential as a cytotoxic agent against OVCAR-3 cells, emphasizing the importance of dosage in eliciting therapeutic effects. The NCs impact on MDA-MB231cells was evaluated across varying concentrations, revealing a concentration-dependent response as shown in Table.2. Significant inhibition was observed at higher concentrations (3000nM and 1000N), with 92.2% and 88.5% inhibition, respectively. As concentrations decreased, inhibitory effects gradually diminished, reaching 0.0% inhibitions at 1nM. Standard deviation values remained consistent, indicating reliable data. These findings highlight NCs potential cytotoxicity against MDA-MB231cells, emphasizing the importance of dosage in eliciting therapeutic effects. Cytotoxicity of NCs against SKOV-3 cells displayed a concentration-dependent trend. It induced significant inhibitory effects at higher concentrations (3000nM and 1000nM), yielding 94.3% and 93.7% inhibition, respectively. As concentration decreased, inhibition gradually diminished. At 1nM, no notable inhibition was observed as shown in **Table.3**.The data exhibited consistent reliability, with low standard deviation values across concentrations. These findings underscore the compound's potential as a cytotoxic agent against SKOV-3 cells, emphasizing the importance of dosage for therapeutic efficacy.

Table.2.Cytotoxicity of Mn/ZnO-NCs against BXPC-3 cells and OVCAR-3 cells

Concentration	BXPC-3 Cells % inhibition	Std	OVCAR-3 Cells % inhibition	Std
3000nM	92.2	0.2	91.5	0.4
1000nM	81.2	1.4	84.0	0.0
300nM	63.7	1.9	66.2	1.6
100nM	28.9	5.2	36.4	0.3
30nM	15.7	3.6	17.2	2.6
10nM	4.5	3.1	17.7	1.4
3nM	6.6	6.7	15.5	8.8
1nM	1.0	1.2	0.0	0.0

Table.3.Cytotoxicity of Mn/ZnO-NCs against MDA-MB231cells and SKOV-3 Cells

Concentration	MDA-MB231 Cells % inhibition	Std	SKOV-3 Cells % inhibition	Std
3000nM	92.2	0.0	94.3	0.3
1000nM	88.5	0.6	93.7	1.0
300nM	73.0	1.8	84.1	0.1
100nM	45.7	3.0	60.3	1.1
30nM	24.2	9.0	28.5	1.6
10nM	22.3	3.5	13.7	1.8
3nM	18.4	1.7	6.8	1.9
1nM	0.0	0.0	0.0	0.0

The study showed that the synthesised NPs significant antitumor activity against all the cell lines studied. The concentration of compound where percent inhibition is equal to 50 is called the IC_{50} value. The NPs showed IC_{50} values of 195.5nM against BXP3 cells, IC_{50} values of 178.10 nM against OVCAR3 cell lines, IC_{50} values of 128.1nM against MDA-MB231 cells, IC_{50} values of 65.08 nM against SKOV3 cells. The synthesised NPs showed better inhibition of SKOV3 cell lines in comparison to other cell lines studied.

6. Conclusion

This study successfully synthesized Mn/ZnO-NCs from electronic waste, specifically utilizing *Borassus flabellifer* (toddy palm) and extracted metals from discarded batteries. The NCs were thoroughly characterized using the techniques scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX), transmission electron microscopy (TEM), and UV-visible spectroscopy, that confirms their structural and optical properties. The Mn/ZnO-NCs exhibited significant antibacterial activity against clinically relevant pathogens ranging from 20 to 33 mm, demonstrated the NCs potent antibacterial efficacy, indicating their potential application as effective antimicrobial agents. In addition, the antitumor potential of these NCs was evaluated using various cancer cell lines, including MDA-MB (breast cancer triple-negative), SKOV-3 (cancer of ovary), OVCAR-3 (ovarian adenocarcinoma), and BxPC-3 (pancreatic cancer). The Mn/ZnO-NCs showed significant cytotoxicity, with IC_{50} values ranging from 65.08 nM to 195.5 nM across different cell lines, underscoring their promise as effective anticancer agents. The results highlight the feasibility of upcycling electronic waste into valuable nanomaterials with dual functionalities in antibacterial and anticancer applications. This approach not only provides an innovative solution for e-waste management but also contributes to the establishment of sustainable

and green nanotechnology for biomedical applications, paving the way for future advancements in sustainable healthcare solutions

Declarations

I declare that the authors have no competing interests as defined by Springer, or other interests that might be perceived to influence the results and/or discussion reported in this paper.

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NA

Author Contribution

V.P: Methodology, Original draft writing.A.N: Review and editingR.P.V: Formal analysis. R.M: Data validation, Supervision.

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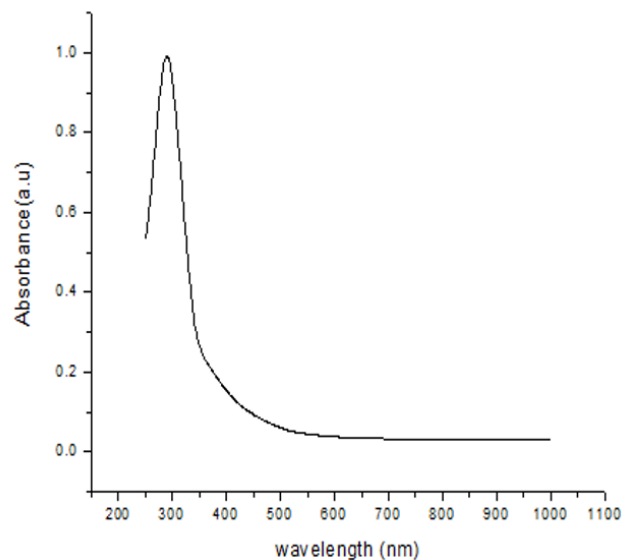
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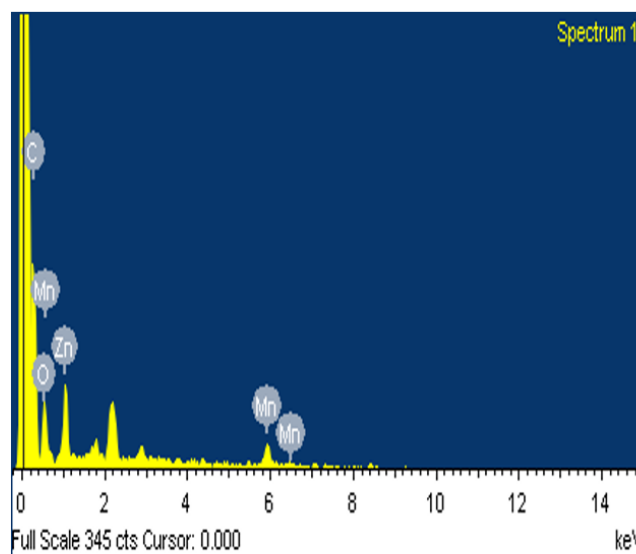
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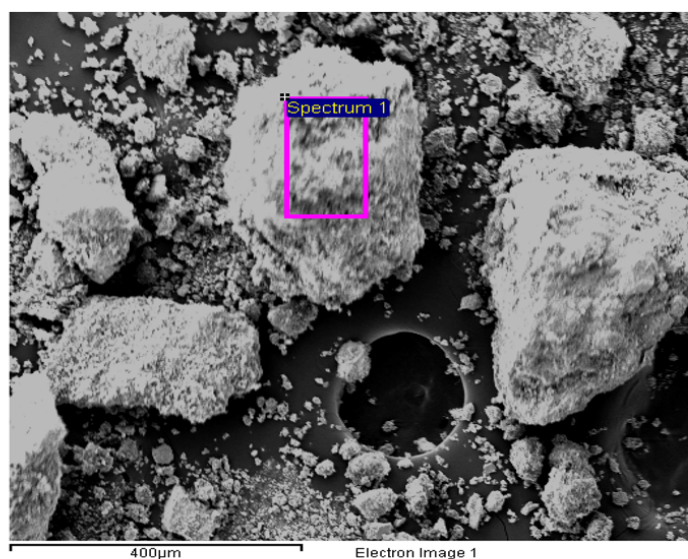
Figures



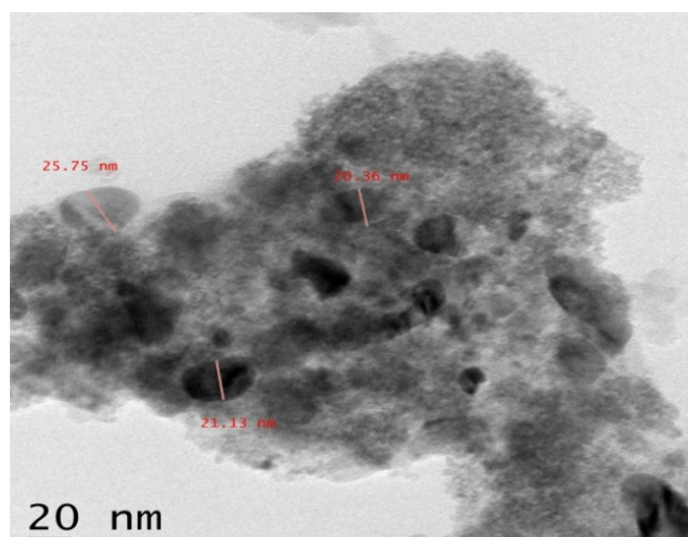
A



B



C



D

Figure 1

(a).UV Visible Spectrum analysis of Mn/ZnO-NCs **(b):** EDX image **(c).**SEM micrograph **(d).**TEM image at 20nm scale

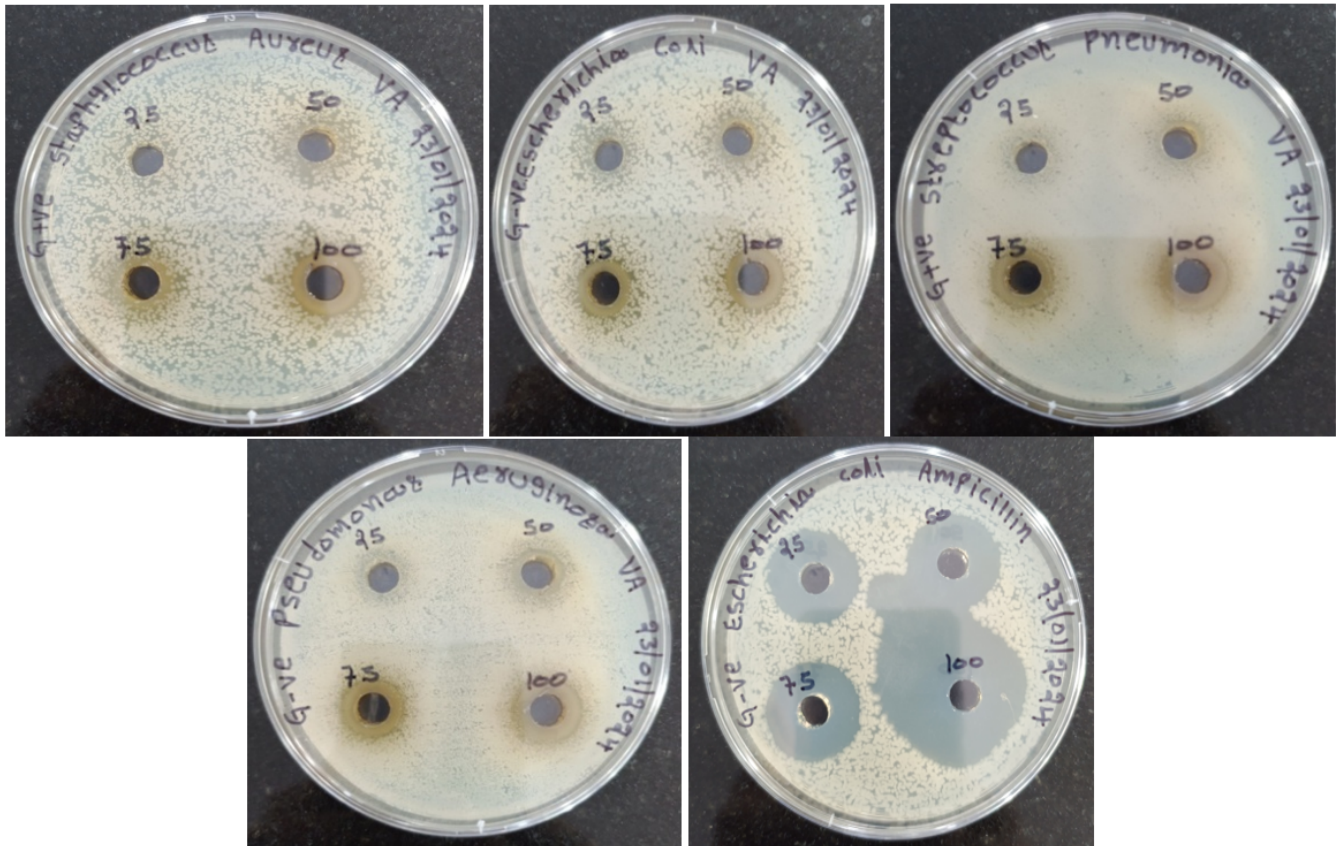


Figure 2

Antibacterial Activity of Mn/ZnO-NCs against each mentioned pathogens.

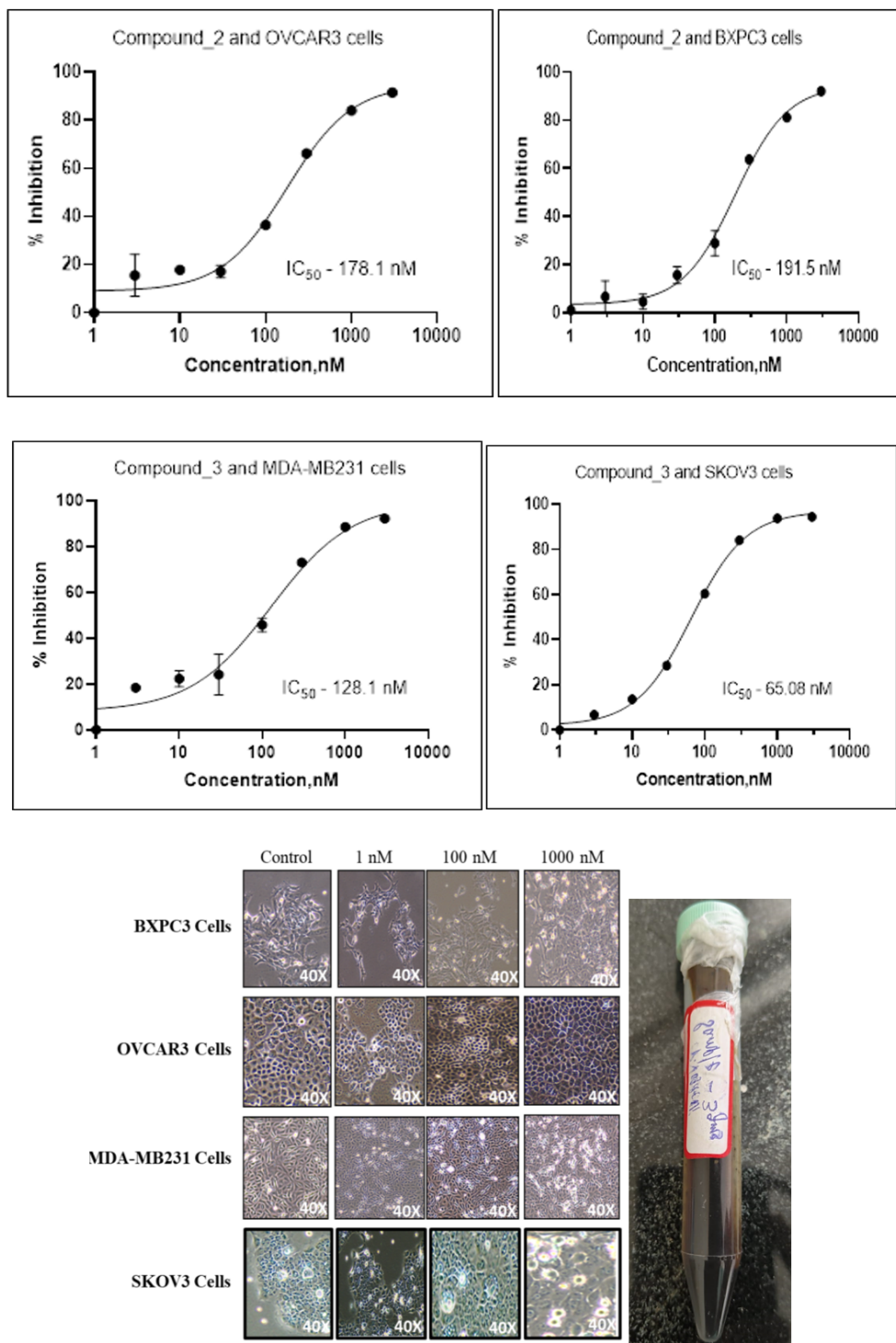


Figure 3

Anticancer activity in BXPCE3 (human pancreatic cancer cell line) Cells, MDA-MB231 (breast cancer cell line, triple-negative) Cells, SKOV3 (cancer cell line of human ovary) Cells and OVCAR3 (cell line of ovarian adenocarcinoma) cell lines treated using NCs.