ORIGINAL ARTICLE

Green synthesis of ginger-encapsulated zinc oxide nanoparticles: Unveiling their characterization and selective cytotoxicity on MDA-MB 231 breast cancer cells

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ABSTRACT

Zinc oxide nanoparticles (ZnO-NPs) were synthesized using ginger (*Zingiber officinale*) extracts in a green synthesis approach and evaluated their *in vitro* cytotoxicity effect on the MDA-MB 231 breast cancer cell line. The bottom-up approach was employed to develop the green-synthesized ginger-encapsulated ZnO-NPs (GZnO-NPs) without using hazardous substances. The most substantial Fourier-transform infrared absorption peak of the ginger root extract was seen at 1634.24 cm^{-1}. The peak also confirmed the presence of ginger root extract-encapsulated ZnO-NPs at 1556.79, 1471.54, and 1019.83 cm^{-1}. It indicates that the biomolecules found in plant extracts behave as capping agents, aiding in the formation of nanoparticles. The mean particle sizes (PSs) of optimized GZnO-NPs of the ratios 1:2 were found to be 104.01 ± 7.12 nm with a zeta potential of −11.5 ± 1.31 mV. The X-ray diffraction and scanning electron microscope analysis confirmed that the prepared nanoparticles were spherical and crystalline, with PS ranging from 100 to 150 nm. The GZnO-NPs were subjected to MTT assay and cellular migration potential, and it was found that the inhibitory concentration on the MDA-MB 231 (breast) cancer cell line and scratch area showed a dose-dependent efficacy. The successfully green-synthesized GZnO-NPs effectively induced cell death in the MDA-MB 231 cancer cell line. The scratch assay results confirmed that prepared GZnO-NPs inhibited the proliferation and migration of cancerous cells.

Key words: Cellular migration potential, ginger root extract, green synthesis, MTT assay, zinc oxide nanoparticles

INTRODUCTION

The most common type of cancer overall, and particularly among women, is breast cancer. More than 2.26 million new instances of breast cancer were diagnosed as of 2020. The uncontrolled growth and division of aberrant cells

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lead to the development of cancer. These cancerous cells can infiltrate nearby tissues and spread to other areas of the body through the lymphatic system or bloodstream. Radiation therapy, chemotherapy, hormone therapy, targeted therapy, immunotherapy, and surgery (such as a lumpectomy or mastectomy) are all common treatments.[1]

Nanotechnology is an emerging field that has found widespread applications in various disciplines, including agriculture, food processing, material science, and health care. Particles with sizes ranging from 1 to 100 nm can be created through physical, chemical, and biological processes.[2] Many metal nanoparticles (such as silver, gold, and platinum) have been discussed and researched. They have unique qualities and are many times more effective than their bulk counterparts. Although these revolutionary nanoparticles are extremely effective, their manufacturing requires the use of costly precursors. A metal-oxide semiconductor material, such as zinc oxide (ZnO) nanoparticles, could be used as an alternative.[3] These unique properties are a result of their reduced size and high surface-to-volume ratio. Because of their anticancer, antifungal, and antibacterial capabilities, ZnO nanoparticles (ZnO-NPs) have been intensively explored in the medical field.[4-7] However, conventional manufacturing processes are often costly, time-consuming, and environmentally harmful. As a result, innovative, cost-effective, efficient, and environmentally acceptable techniques of producing nanoparticles are required.[8,9] One such alternative to the current standard synthesis techniques is known as "green synthesis." Various plant components, including roots, fruits, leaves, seeds, and stems, have been demonstrated to be effective in synthesizing ZnO-NPs. Plants contain a high concentration of phytochemicals, which function as stabilizers and reducers during the synthesis process.[10,11]

Ginger (*Zingiber officinale* Roscoe, Zingiberaceae) is a popular dietary condiment worldwide.[12] Ginger extract has been found to have anticancer activities against pancreatic cancer cells through inducing reactive oxygen species-mediated autosis.[13] Ginger's active components regulate several cell signaling pathways associated with cancer, such as signal transducer and activator of transcription 3, nuclear factors, and among others.[14] Ginger contains various flavonoids and polyphenolic compounds that could facilitate the green synthesis of ZnO-NPs. Recent research has demonstrated the use of aqueous ginger extract to produce gold and silver nanoparticles.[15,16]

Numerous carrier systems loaded with ginger have been discovered in recent investigations, although their specific applications remain unclear. Therefore, this study aimed to synthesize ginger-encapsulated ZnO-NPs (GZnO-NPs) and investigate their anticancer efficacy against the MDA-MB 231 cancer cell line.

MATERIALS AND METHODS

Sigma-Aldrich, Germany, provided the zinc nitrate (Zn $[NO_3]_2$) hexahydrate. MDA-MB 231 breast cancer cell line was purchased from NCCS in Pune, India. Emsure, Germany, supplied the dimethyl sulfoxide (DMSO) and sodium hydroxide. Methanol was purchased from JT Baker™, Pulau Penang, Malaysia, and sodium chloride from Systerm® Chemicals Selangor, Malaysia. All other reagents and chemicals were of the highest analytical caliber.

Preparation of the ginger root extract

A weighed quantity of coarse ginger powder was macerated in different quantities of methanol:water mixture (1:2, 1:4, and 1:8). The content was stirred and shaken on a regular basis to ensure thorough extraction. The micelle was removed from the marc by filtration after 2 days. The micelle was then isolated from the menstruum by evaporation in a 40°C oven.[17]

Preparation of ginger-encapsulated zinc oxide nanoparticles

An 11.5 g of Zn $(NO_3)_2$ hexahydrate was dissolved in 40 mL distilled water and transferred onto an analog hot plate stirrer (Model: D0300, TEquipment, Long Branch, NJ) maintained at 60°C and 700 rpm. Using a dropper, 10 mL of a ginger solution made from ginger extracts in ratios of 1:2, 1:4, and 1:8 was added to the mixture. Drop by drop, a 2M sodium hydroxide solution was added to the mixture until the pH of the solution reached 12. The resultant solution was left for 2 h at the same condition at 60°C and 700 rpm. Then, the solution was incubated (Memmert IN30, Germany) overnight at room temperature. The two-layer solution was observed the next day, and the upper layer was removed using a separating funnel. The pellet was placed in distilled water and centrifuged for 15 min at a speed of 6000 rpm. The developed ginger encapsulated zinc oxide nanoparticles were carefully collected for further analysis.[18,19]

Ginger-encapsulated zinc oxide nanoparticle characterization

Particle size (PS), zeta potential (ZP), and poly-dispersible index (PDI) of prepared GZnO-NPs were carried out using a Zetasizer-Malvern (Zetasizer Nano ZS; Malvern Instruments Ltd., Malvern, UK). Drug encapsulation efficiency (EE) and drug loading (DL) of GZnO-NPs were performed to optimize the NP formulation.

Scanning electron microscope (SEM) (JSM-6701F, JEOL Legacy, Japan), Fourier-transform infrared (FTIR) (Nicolet iS5, Thermo Fisher Scientific, US) spectrophotometer, and X-ray diffraction (XRD) (Shimadzu, Tokyo, Japan) were used to investigate the morphological and structural properties of GZnO-NPs.

MTT-based cell viability assay

In vitro safety testing of developed GZnO-NPs and ginger

powder was carried out on the MDA-MB 231 cancer cell line. The cells were selected in 24-well tissue culture plates with the density of 25000 cells per well. Followed by overnight incubation at 37 degree and CO_2 maintained 5%. On the next day, reference and test preparations (1, 2.5, 5, 10, and 25 µg/mL) were administered in accordance with the needs of the experiment. After 24 h of seeding, each well received 50 µL of the prepared MTT solutions (1 mg/mL) and incubated in a CO_2 incubator for 2 h. The medium used in each well was then withdrawn, and the formazan crystals generated by the cells were broken down by adding 500 µL of DMSO to each well, which was followed by pipetting 200 µL of the resulting solution into a 96-well plate.[20] The value of absorbance was determined using a multiwall plate reader at 570 nm and a standard wavelength of 630 nm.[21]

Cellular migration potential

A scratch assay was used to quantify the *in vitro* migration of cells over a gap created using scratch. It is being used in cancer research to assess the proliferation and migration of cancerous cells. MDA-MB 231 cells were seeded into a 24-well plate, with 0.025×10^6 cells per well. These cells were incubated for 24 h in a CO_2 incubator. To represent a wound, the cell monolayer was scraped with a sterile pipette tip. The culture medium containing 2.5 and 5 µg GZnO-NPs after washing one time with phosphate-buffered saline to remove cellular trash was filled in wells. Images were taken with digital microscopes (Olympus CH30, Japan) at defined time points of 0 and 24 h, and the gross number of cells that migrated to the scratch location was taken into account in both the control groups and treatment.[22]

RESULTS AND DISCUSSION

Characterization of ginger-encapsulated zinc oxide nanoparticles

The PS, PDI, ZP, % EE, and % Drug loading (DL) of GZnO-NPs were determined and are summarized in Table 1. The mean PSs of GZnO-NPs of the ratios 1:2, 1:4, and 1:8 were found to be 104.01 ± 7.12, 616 ± 18.78, and 614 ± 17.41 nm, respectively, and the PDI was observed with a range of 0.295 ± 0.05–0.616 ± 0.04. The

Table 1: Particle size, zeta potential, poly-dispersible index, entrapment efficacy (%), and drug loading (%) of prepared ginger-encapsulated zinc oxide nanoparticles

Parameters	GZnO-NPs (1:2)	GZnO-NPs (1:4)	GZnO-NPs (1:8)
Particle size (nm)	104.01±7.12	616±18.78	614±17.41
Zeta potential (mV)	−11.5±1.31	−18.7±1.25	−0.336±0.02
Poly-dispersibility index	0.488±0.02	0.616±0.04	0.295±0.05
Entrapment efficacy (%)	92.62±8.17	74.54±8.19	62.85±5.27
Drug loading (%)	39.11±3.37	19.20±2.41	15.68±2.55

GZnO-NPs: ginger-encapsulated zinc oxide nanoparticles

ZP values of all three prepared formulations indicate a negative charge (−18.7 ± 1.25–−0.336 ± 0.02). The mean PSs of GZnO-NPs of the ratios 1:2 were found to be 104.01 ± 7.12, the lowest among other formulations. The PDI of GZnO-NPs (1:2) was observed with a value of 0.488 ± 0.02, indicating that PS distribution was a little higher. The *ZP* values of GZnO-NPs (1:2) were found to be −11.5 ± 1.31. The ratio of the solvent has an impact on PS. As the concentration of methanol is increased as compared to water, i.e., 1:2 has a higher concentration of methanol in comparison with 1:4 and 1:8 due to the lower dilution ratio of water. The concentration of biomolecules tends to be higher in the organic phase of the 1:2 ratio mixture. The formulated GZnO-NPs in a mixture of 1:2 showed a small particle size due to the fact that a higher concentration of capping agent (Ginger biomolecules in the organic phase) reduced the particle size. The loaded ZnO-NPs with negative potential values showed stability and electrostatic repulsion, which limits the particle's tendency to aggregate while storing the nanoformulation.[23]

The percentage EE and DL of ginger in GZnO-NP formulations [Table 1] were approximately from 62.85% ± 5.27%–92.62% ± 8.17% and 15.68% ± 2.55%–39.11 ± 3.37%, respectively. The percentage EE and DL of ginger in GZnO-NP (1:2) formulation were highest among others and approximately 92.62% ± 8.17% and 39.11 ± 3.37, respectively. Based on the PS, EE, and DL results, the GZnO-NP (1:2) formulation was selected for further characterization. Ahmed *et al*. reported that NPs with drug loading of >10% were considered high. It was proposed that the feeding concentration would determine the loading of ginger into NPs. A high DL was seen in conjunction with the rise in ginger feeding concentration. The loading content dropped at a decreased feeding concentration.[24] It was also proposed that the lengthy overnight loading period might have an impact on the loading procedure.

Morphology and structural properties of ginger-encapsulated zinc oxide nanoparticles (1:2)

In this study, the FTIR absorption spectrum of ginger root extract, GZnO-NPs, and blank ZnO-NPs is presented in Figure 1. Strong absorption of GZnO-NPs was seen at 2160.64, 1556.79 $cm^{-1,}$ and 1396.17 cm^{-1}. The ginger extract spectrum at 1634.24 cm^{-1} was shifted to 1556.79 cm^{-1} in GZnO-NPs. The shifting may be due to the capping or stabilizing characteristics of ginger. The presence of ZnO was also confirmed from GZnO-NP peak at 2160.64 and 1396.17 cm^{-1}. The absorption at the peak of 1556.79 cm^{-1} confirmed the presence of 8.43% of N-H bonds.[25] On the other hand, infrared spectra of ZnO-NPs and G ZnO-NPs showed broad bands at 3285.28 cm^{-1} and 3287.01 cm^{-1} conveying the water molecule's symmetrical stretching vibrations.

The morphology and molecular surface structure of GZnO-NP (1:2) revealed slightly agglomerated NPs with a

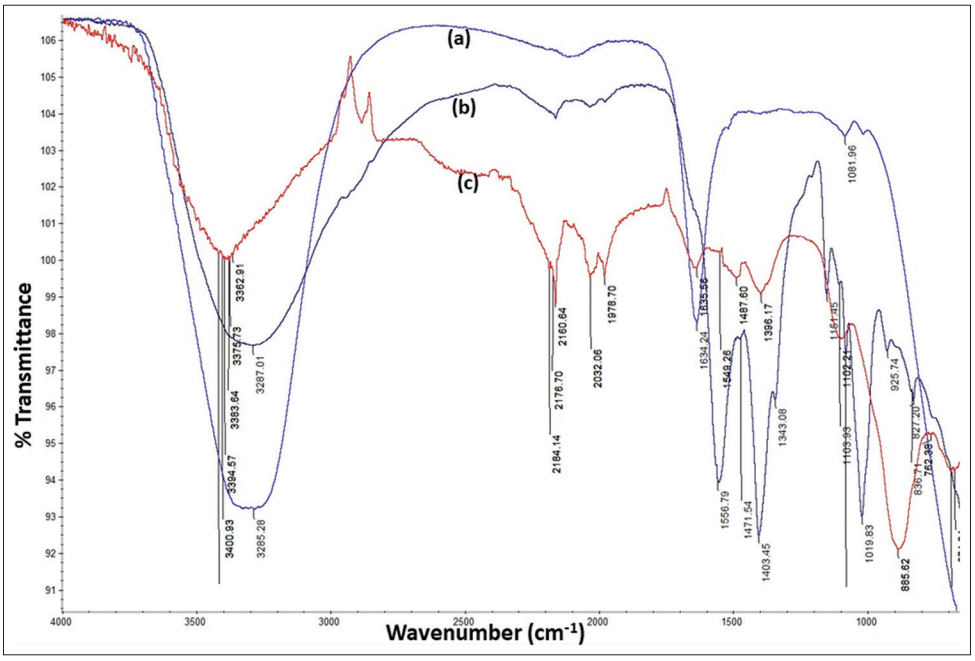

Figure 1: Fourier-transform infrared spectrum of (a) ginger extract (blue color), (b) ginger-encapsulated zinc oxide nanoparticles (1:2) (violet color), and (c) blank zinc oxide nanoparticles (violet color)

spherical shape, a rough surface, and PSs ranging between 100 and 150 nm [Figure 2]. The porous network formed by rough surfaces and homogenous morphologies allowed the molecules of the ginger extract to be adsorbed. The SEM results were similar to those previously reported.[26]

The data obtained from the XRD patterns, as shown in Figure 3, revealed the characteristics of the crystalline phase and the purity of the samples, corresponding to GZnO-NPs and ginger extract. At diffraction angles of 2 θ = 31.2°, 34.8°, 37.13°, 48.7°, 57.2°, 63.5°, and 69.2° in the formulated GZnO-NP crystal peaks were seen to be broad and sharp. Comparing green-synthesized GZnO-NPs to ginger extract, the strong XRD peaks revealed the development of extremely crystalline NPs. The GZnO-NPs' strongest 2 θ peaks are 31.2°, 34.8°, and 37.13°, which fit with the standard database (PCPDFWIN card no: 89–1397). Ginger root extract has distinct crystal peaks at 2 = 14.1, 18.2, and 23.1, which is identical to the results Zhao *et al*. reported.[26] The strong and distinct peaks proved the remarkable purity and crystalline clarity of the generated GZnO NPs.

Cell viability outcome

The most reliable method for determining cell proliferation is the MTT assay, which has a low standard deviation value. Anticancer activity of ginger and GZnO-NPs was screened on breast cancer cell line (MDA-MB 231) at concentrations ranging from 1 to 25 µg/mL. At the highest concentration of 25 µg/ml, ginger powder does not reduce cell growth when tested alone [Figure 4], however, GZnO NPs showed a significant reduction at 25 µg/mL [Figure 5].

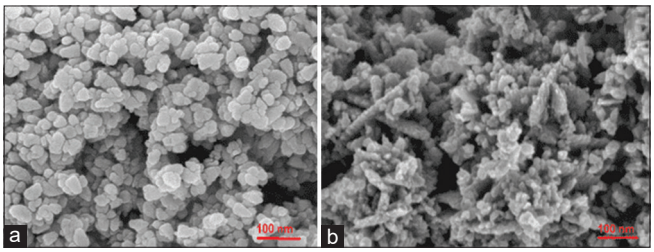

Figure 2: Scanning electron microscope image of (a) ginger-encapsulated zinc oxide nanoparticles (1:2), (b) ginger powder

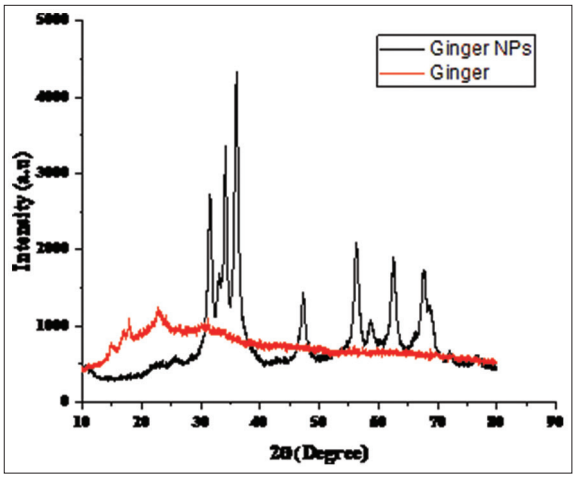

Figure 3: X-ray diffraction pattern of ginger-encapsulated zinc oxide nanoparticles (black color) and ginger extract (red color) showing crystalline phases and purity

It indicates that the ginger powder at concentration 25 µg/ml might not reduce cancer cell proliferation. Nevertheless,

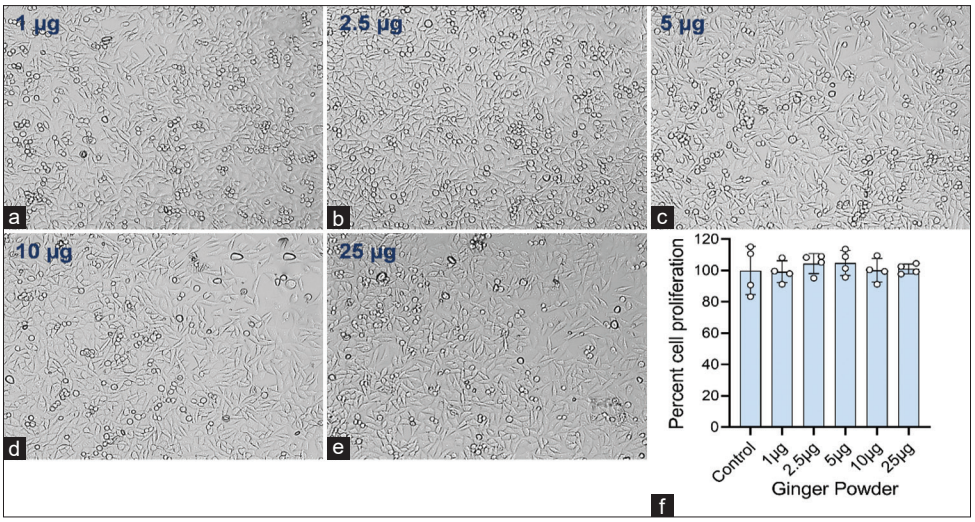

Figure 4: Cell proliferation of MDA-MB 231 cells exposed to various concentrations of ginger powder: (a) 1 µg/mL, (b) 2.5 µg/mL, (c) 5 µg/mL, (d) 10 µg/mL, (e) 25 µg/mL, and (f) bar chart represents the different concentrations and their relative cell proliferation

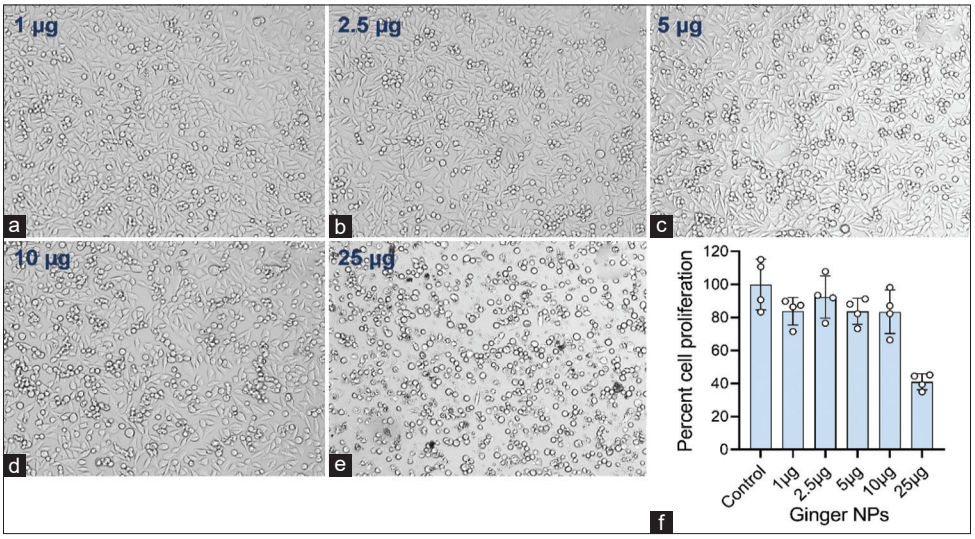

Figure 5: Proliferation of MDA-MB 231 cells subjected to different concentrations of ginger-encapsulated zinc oxide nanoparticles (1:2): (a) 1 µg/mL, (b) 2.5 µg/mL, (c) 5 µg/mL, (d) 10 µg/mL, (e) 25 µg/mL, and (f) bar chart represents the different concentrations and their relative cell proliferation

GZnO-NPs showed effective dose-dependent suppression of MDA-MB 231 cell proliferation. GZnO-NPs showed effective dose-dependent suppression of MDA-MB 231 cell proliferation. An arrest in the cell cycle or cell death could cause a decrease in cell viability.[27]

Cellular migration potential

GZnO-NPs were employed in the cellular migration investigation to assess the efficacy at 2.5 and 5 µg of concentration [Figure 6]. At 24 h, the majority of cells migrated to the wound area in the control group; however, after treatment with GZnO-NPs, the cells migrated to the scratch region in a dose-dependent manner. The migration potential of the cells was also affected with the increase in dose of GZnO-NPs (2.5ug to 5ug). As evident from Figure 6, relatively lesser number of cells migrated towards the wound area in the group treated with 2.5ug and 5ug of GZnO-NPs as compared with significantly higher migration potential of the untreated control cells.The fact that there was a noticeable difference between the two doses indicated that the GZnO-NPs prevented cell migration to the scratch location. The migration of the test formulation showed a concentration-dependent behavior.[21]

CONCLUSIONS

Ginger root extract proved to be a successful reducing and capping agent in the green synthesis of ZnO-NPs. These GZnO-NPs were characterized methodically through PS analysis, ZP measurement, FTIR, SEM, and XRD analysis. The results obtained confirmed the crystalline nature of GZnO-NPs, and morphological studies

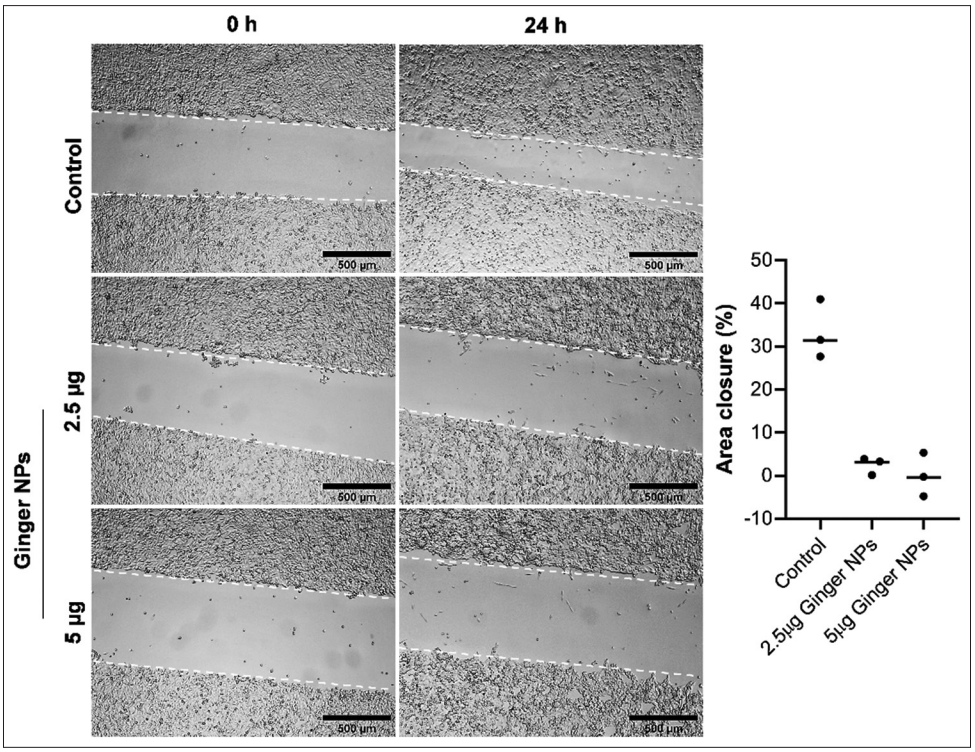

Figure 6: *In vitro* migration potential of ginger-encapsulated zinc oxide nanoparticles treated MDA-MB 231 cells exposed to different concentrations of 2.5 µg, 5 µg, and bar chart quantitatively represents the extent of wound closure area compared with control

revealed well-agglomerated, spherical particles with a rough surface, measuring approximately 100–150 nm. Furthermore, the presence of functional groups in FTIR spectra aided in the bio-reduction and stabilization of Zn ions during the green synthesis of GZnO-NPs. Notably, in MTT assays and cellular migration studies, ginger-loaded ZnO-NPs demonstrated their ability to induce cell death in the MDA-MB 231 cancer cell line. This induction of cell death aligns with a primary goal in cancer therapy research employing ZnO-NPs. The green-synthesized nanomaterials hold significant potential in the pharmaceutical sector, offering opportunities for the synthesis of valuable nanodevices and the development of innovative medications.

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Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Smolarz B, Nowak AZ, Romanowicz H. Breast cancer-epidemiology, classification, pathogenesis and treatment (review of literature). Cancers (Basel) 2022;14:2569.
2. Oyeyemi OT, Babalola BM, Akinmulero TC, Adesida PA, Oyeyemi IT. Molluscicidal activities of green-synthesized alstonia congensis silver nanoparticles. Asian Pac J Trop Biomed 2023;13:176-83.
3. Basnet P, Inakhunbi Chanu T, Samanta D, Chatterjee S. A review on bio-synthesized zinc oxide nanoparticles using plant extracts as reductants and stabilizing agents. J Photochem Photobiol B 2018;183:201-21.
4. Feris K, Otto C, Tinker J, Wingett D, Punnoose A, Thurber A, *et al.* Electrostatic interactions affect nanoparticle-mediated toxicity to gram-negative bacterium *Pseudomonas aeruginosa* PAO1. Langmuir 2010;26:4429-36.
5. Xiao FX, Hung SF, Tao HB, Miao J, Yang HB, Liu B. Spatially branched hierarchical ZnO nanorod-TiO2 nanotube array heterostructures for versatile photocatalytic and photoelectrocatalytic applications: Towards intimate integration of 1D-1D hybrid nanostructures. Nanoscale 2014;6:14950-61.
6. Djearamane S, Xiu LJ, Wong LS, Rajamani R, Bharathi D, Kayarohanam S, *et al.* Antifungal properties of zinc oxide nanoparticles on *Candida albicans*. Coatings 2022;12:1864-79.
7. Liew KB, Janakiraman AK, Sundarapandian R, Khalid SH, Razzaq FA, Ming LC, *et al*. A review and revisit of nanoparticles for antimicrobial drug delivery. J Med Life 2022;15:328-35.
8. Raveendran P, Fu J, Wallen SL. Completely "green" synthesis and stabilization of metal nanoparticles. J Am Chem Soc 2003;125:13940-1.
9. Narayanan S, Sathy BN, Mony U, Koyakutty M, Nair SV, Menon D. Biocompatible magnetite/gold nanohybrid contrast agents via green chemistry for MRI and CT bioimaging. ACS Appl Mater Interfaces 2012;4:251-60.
10. Zong Y, Li Z, Wang X, Ma J, Men Y. Synthesis and high photocatalytic activity of Eu-doped ZnO NPs. Ceram Int 2014;40:10375-82.
11. Subramaniyan V, Kayarohanam S, Ashok Kumar J, Kumarasamy V. Impact of herbal drugs and its clinical application. Int J Res Pharm

Sci 2019;10:1340-5.

12. Raj LF, Jayalakshmy E. Biosynthesis and characterization of zinc oxide NPs using root extract of *Zingiber officinale*. Orient J Chem 2015;31:51-6.
13. Akimoto M, Iizuka M, Kanematsu R, Yoshida M, Takenaga K. Anticancer effect of ginger extract against pancreatic cancer cells mainly through reactive oxygen species-mediated autotic cell death. PLoS One 2015;10:e0126605.
14. Nafees S, Zafaryab M, Mehdi SH, Zia B, Rizvi MA, Khan MA. Anti-cancer effect of gingerol in cancer prevention and treatment. Anticancer Agents Med Chem 2021;21:428-32.
15. Faisal S, Jan H, Shah SA, Shah S, Khan A, Akbar MT, *et al*. Green synthesis of zinc oxide (ZnO) nanoparticles using aqueous fruit extracts of *Myristica fragrans*: Their characterizations and biological and environmental applications. ACS Omega 2021;6:9709-22.
16. Mao QQ, Xu XY, Cao SY, Gan RY, Corke H, Beta T, *et al*. Bioactive compounds and bioactivities of ginger (*Zingiber officinale* roscoe). Foods 2019;8:185.
17. Kayarohanam S, Subramaniyan V, Janakiraman AK, Kumar SJ. Antioxidant, antidiabetic, and antihyperlipidemic activities of dolichandrone atrovirens in albino wistar rats. Res J Pharm Technol 2019;12:3511-6.
18. Suica-Bunghez IR, Ion RM, Teodorescu S, Sorescu AA, Stirbescu RM, Stirbescu NM. Fitochemical and antioxidant characterization of autumn crocus (*Colchicum autumnale*) flowers and roots plant extracts. J Sci Arts 2017;17:539-46.
19. Dasgeb B, Kornreich D, McGuinn K, Okon L, Brownell I, Sackett DL. Colchicine: An ancient drug with novel applications. Br J Dermatol 2018;178:350-6.
20. Raafat M, El-Sayed AS, El-Sayed MT. Biosynthesis and anti-mycotoxigenic activity of *Zingiber officinale* roscoe-derived metal nanoparticles. Molecules 2021;26:2290.
21. Kumar A, Sah DK, Khanna K, Rai Y, Yadav AK, Ansari MS, *et al*. A calcium and zinc composite alginate hydrogel for pre-hospital hemostasis and wound care. Carbohydr Polym 2023;299:120186.
22. Sahar Q, Kushagra K, Khalid R. Dihydroquercetin (DHQ) has the potential to promote apoptosis in ovarian cancer cells: An *in silico* and *in vitro* study. J Mol Struct 2023;1271:1340-93.
23. Javed R, Zia M, Naz S, Aisida SO, Ain NU, Ao Q. Role of capping agents in the application of nanoparticles in biomedicine and environmental remediation: Recent trends and future prospects. J Nanobiotechnology 2020;18:172.
24. Ahmed SA, El-Murr A, Abd Elhakim Y, Metwally MM, Gharib AAEA, Amer SA, *et al*. Comparative study on ginger powder and ginger extract nanoparticles: Effects on growth, immune-antioxidant status, tissue histoarchitecture, and resistance to *Aeromonas hydrophila* and *Pseudomonas putida* infection in *Oreochromis niloticus*. Fishes 2023;8:259.
25. Soner D. Green synthesis of zinc oxide nps using zingiber officinale root extract and their applications in glucose biosensor. El Cezerî J Sci Eng 2020;7:1191-200.
26. Zhao X, Zhu H, Chen J, Ao Q. FTIR, XRD and SEM analysis of ginger powders with different size: Structure characteristic of ginger powders. J Food Process Preserv 2015;39:2017-26.
27. Alyamani AA, Albukhaty S, Aloufi S, AlMalki FA, Al-Karagoly H, Sulaiman GM. Green fabrication of zinc oxide nanoparticles using phlomis leaf extract: Characterization and *in vitro* evaluation of cytotoxicity and antibacterial properties. Molecules 2021;26:6140.