

Green Synthesis of Chitosan-coated Tin Dioxide Nanoparticles Using *Moringa oleifera* Flower Extract Against Breast Cancer via the Caspase-dependent Apoptotic Pathway

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Ahmad Alsulimani¹ , Saleh M. Abdullah¹, Ahmed Ali Jerah¹, Ali M. Hendi² and Abdullah Farasani^{1,3}

Abstract

Background and Purpose: In the present work, chitosan is furnished with tin dioxide (CsSnO₂) nanoparticles (NPs) synthesized using the green route using *Moringa oleifera* (*M. oleifera*) flower extract.

Materials and Methods: These preparation methods are low-cost, easily reproducible, nontoxic, and eco-friendly. X-ray diffraction (XRD), dynamic light scattering (DLS), field emission scanning electron microscope (FESEM), energy dispersive X-ray, Fourier-transform infrared spectroscopy (FT-IR), and photoluminescence (PL) analysis were used to learn more about the CsSnO₂ NPs. The antibacterial property of CsSnO₂ NPs was assessed by the disc diffusion method against various pathogens. The MTT assay was done to assess the cytotoxicity of CsSnO₂ NPs in MCF-7 cells. The expressions of the apoptotic proteins were examined using assay kits.

Results: XRD results demonstrated that the formulated CsSnO₂ NPs exhibit a tetragonal structure. From the FT-IR spectra, the Sn–O stretching peak was observed at 579 cm⁻¹. A FESEM image shows that the CsSnO₂ NPs were formed into a spherical structure. Surface defects, such as tin and oxygen vacancies, are visible in the PL spectra, and the average particle size of CsSnO₂ NPs is 32 nm. Thus, surface defects are essential parameters for biocidal properties. The antimicrobial property of CsSnO₂ NPs and amoxicillin was investigated against bacteria and human fungal pathogens. CsSnO₂ NPs exhibit similar antimicrobial properties as compared to amoxicillin. Also, the dosage of CsSnO₂ NPs elevated their antimicrobial activity. The anticancer property of the green-synthesized CsSnO₂ NPs against MCF-7 cells was further analyzed to study their ability to cytotoxicity and apoptosis induction, like caspase-3, -8, and -9 activation.

Conclusion: The study's outcome revealed that the green synthesized CsSnO₂ NPs are an outstanding candidate for cancer therapy.

Keywords

Chitosan, tin dioxide nanoparticles, *Moringa oleifera*, antimicrobial activity, caspase, anticancer

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Introduction

Cancer is one of the important reasons for mortality globally. Moreover, its burden has increased dramatically for several reasons, such as aging, a growing population, and accelerated socio-economic development (Cao et al., 2021). Breast cancer is a prominent global health issue that mostly affects women and is characterized by a high occurrence rate and substantial death rate. The reported number of recorded cases in 2020 was 2.3 million, resulting in 685,000 deaths worldwide (Wilkinson & Gathani, 2022). Furthermore, breast cancer has ranked second among all cancer types, accounting for approximately 12% of the overall cases. Nonetheless, the

management of breast cancer entails various challenges, such as evaluating risk factors, predicting diagnostics, inhibiting metastasis, selecting proper therapy, and ensuring

¹Department of Medical Laboratory Technology, Faculty of Nursing and Health Sciences, Jazan University, Jazan, Saudi Arabia

²Department of Radiology, College of Medicine, Jazan University, Jazan, Saudi Arabia

³Health Research Centre, Jazan University, Jazan, Saudi Arabia

Corresponding author:

Abdullah Farasani, Department of Medical Laboratory Technology, Faculty of Nursing and Health Sciences, Jazan University, Jazan 45142, Saudi Arabia.
E-mail: aofarasani@jazanu.edu.sa



cost-effective methods (Mazurakova et al., 2022). According to the American Society for Infectious Diseases, multidrug-resistant (MDR) microbes pose a grave threat to the human population (Van Duin & Paterson, 2016). Therefore, developing a therapeutic with anticancer and antibacterial properties is crucial. Consequently, researchers have paid much attention to nanotechnology because it can kill bacteria and fight cancer.

Tin oxide (SnO_2) is an essential n-type metal oxide semiconductor with a broadband gap ($E_g = 3.6$ eV) and has antimicrobial activity against several microbes like viruses, fungi, and bacteria, and antioxidant and photocatalytic properties (Dheyab et al., 2022; Patil et al., 2012). Khanom et al. (2018) examined the antibiotic property of SnO_2 nanoparticles (NPs) in a concentration-dependent manner against numerous pathogens. Synthesized Co- SnO_2 NPs showed that their large surface area and their size are reasons for increased antibacterial activity (Qamar et al., 2017). According to Ahamed et al.'s (2018) research, SnO_2 NPs significantly increase apoptosis in breast cancer cells by inducing oxidative stress. They studied the anticancer properties of SnO_2 NPs against human cancer cells like colorectal (HCT116), lung (A549), and hepatocellular (HepG2) cancer cells. The requirement for environmental sustainability has led to a great deal of focus on developing eco-friendly manufacturing of new, nontoxic, biodegradable materials used to control hospital-mediated cross-contamination by pathogens. As a result, nanocomposites with nanometer size scale, based on biodegradable polymers and organic or inorganic fillers, have developed as a developing area in a variety of medical usages (Roopan et al., 2015; Tammina et al., 2017).

Multimetallic nanocomposites synthesized using different shapes and sizes are more advantageous as they overtake the role of simple metallic NPs concerning function, structural stability, and specificity. The integrated NPs exhibit individual or combined beneficial properties, and incorporating biopolymers such as chitosan adds valuable properties to these synthetic NPs. Various types of NPs synthesized using chitosan have been used for targeting tumor cells in drug delivery. At present, titanium dioxide (TiO_2) is mainly being utilized together with natural polymers like chitosan to synthesize nanocomposites with numerous beneficial properties (Basavegowda et al., 2017; Dobrucka et al., 2021; Wrońska et al., 2021).

Chitosan is commonly utilized in various fields to encapsulate active compounds, enzyme immobilization, and as a medication delivery vehicle. Biodegradability, biocompatibility, non-toxicity, and polycationic nature are noteworthy features of chitosan (Divya & Jisha, 2018). Chitosan can also speed up the process of cell death, revealing the interaction of negatively and positively charged chitosan molecules in the bacterial cell wall that results in the release of microorganisms that die as a result of intracellular components (Karthikeyan et al., 2022). It is also used to

functionalize metal NPs, improving their biocidal efficacy. However, there has never been a report on the biocidal effects of chitosan SnO_2 in green processes. This includes the fact that it can kill bacteria and fungi and, most importantly, fight cancer.

Tin oxide (SnO_2) and tin oxide nanoparticles (SnO_2 NPs) have developed as the most significant metal oxide NPs due to their properties, including their antioxidant and antibacterial properties (Haq et al., 2021; Vidhu & Philip, 2015), biocompatibility, and low operating temperatures. SnO_2 is a transparent conductor due to its stability, high optical transparency, and low electrical resistance, while SnO_2 is an insulator (Kuang et al., 2018; Mientus et al., 2020). NPs of SnO_2 have many applications, including photocatalysis, lithium batteries, solar panels, supercapacitors, catalysis, and gas sensors (Pandit & Ahmad, 2022; Pusawale et al., 2011; Rehman et al., 2021; Zhao et al., 2015).

Moringa oleifera is the most widely used herbal medicine for treating cancer. Its efficacy is attributed to polyphenols such as tannins, flavonoids, and terpenoids present in the plant (Wisitpongpun et al., 2020). The plant is used to treat numerous ailments conventionally and stimulate good well-being. The beneficial parts of the plant include leaves, flowers, seeds, roots, gum, and bark. It is utilized in conventional treatment treating infections and numerous illnesses. It possesses antimicrobial, antidiabetic, anti-inflammatory, anticancer, cardioprotective, neuroprotective, and antioxidant properties (Madi et al., 2016). *M. oleifera* can lower cholesterol, modulate the immune system, is hepatoprotective, increases wound healing, is antihypertensive, and is also a diuretic agent (Ravani et al., 2017). It also improves liver and kidney functionality (Vergara-Jimenez et al., 2017). The NPs and phytochemicals extracted from the plant naturally exhibit potent antibacterial and anticancer properties. Combining NPs with a natural polymer, such as chitosan, might help develop a treatment strategy that could produce better results (Elderderly et al., 2022).

M. oleifera extract has antimicrobial properties, including inhibiting bacterial strains and antifungal activity against fungal pathogens. It may also have antiviral properties against certain viruses (Wisitpongpun et al., 2020). Chitosan-coated tin dioxide NPs show potential antimicrobial properties against bacterial and fungal pathogens. They have potential applications in biomedicine, food packaging, and water treatment. However, safety concerns and environmental impact need further evaluation. Further research is needed to optimize the synthesis and characterize the antimicrobial activity.

In the present study, chitosan-decoded SnO_2 (CsSnO_2) NPs are prepared using the *M. oleifera* flower extract method. The resulting CsSnO_2 NPs were characterized for their optical, structural, and antimicrobial activities. We also evaluated the synergistic anticancer effect of CsSnO_2 NPs synthesized using *M. oleifera* flower extract against breast cancer (MCF-7) cells.

Materials and Methods

Materials

The chitosan (200 kDa, 87% deacetylation degree) and SnCl_4 (98%) were purchased from Sigma Aldrich, USA.

Flowers Extract Preparation

The fresh *M. oleifera* flowers were obtained from commercial sources and 10 g of fresh *M. oleifera* flowers were boiled with 100 mL of double distilled water and agitated with a magnetic stirrer for 1 h at 80°C. The color of the solution turned from watery to a light white-yellowish color. After cooling to 37°C, the solution was filtered using Whatman No. 1 filter paper.

Preparation of Chitosan-encapsulated SnO_2 NPs

At room temperature (RT), 0.1 M tin chloride solute and 1% acetic acid solution of chitosan were mixed with 100 mL of *M. oleifera* flower aqueous extract. The homogenous reaction chitosan-Sn metal ion mixture solution was heated on a hot plate at 80°C for 4 h with magnetic stirring. The final solution was cooled to 37°C and centrifuged at 8,000 rpm for 15 min and then dried at 120°C for 2 h. The resultant CsSnO_2 NPs were annealed in the atmosphere for 5 hours at 800°C before being used for further characterization.

Characterization Studies

The CsSnO_2 NPs were characterized using an X-ray meter (X'PERT PRO Analytical). CsSnO_2 NPs diffraction peaks were noted in a 20°–80° range at a wavelength of 1.54 Å. The CsSnO_2 NPs were examined using a field emission scanning electron microscope (Carl Zeiss Ultra 55 FESEM) with energy dispersive X-ray (EDX) spectrometry (model: Inca). The Nano-Plus instrument was used to measure particle size's dynamic light scattering (DLS). A Perkin-Elmer spectrometer in the 400–4,000 cm^{-1} range was utilized to obtain the Fourier-transform infrared spectroscopy (FT-IR) spectra. The photoluminescence spectrum (PLS) was measured at 37°C using a luminescence spectrophotometer (Perkin-Elmer LS-5513) and a xenon lamp.

Antimicrobial Activity

The antibacterial properties of the CsSnO_2 NPs were assessed using the well diffusion technique. The CsSnO_2 NPs were dissolved using a 1 mL solution of 5% dimethyl sulfoxide (DMSO) as a stock solution. Pathogen species such as strains like *Staphylococcus aureus* (*S. aureus*), *Streptococcus pneumoniae* (*S. pneumoniae*), *Bacillus subtilis* (*B. subtilis*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Shigella dysenteriae* (*S. dysenteriae*), *Escherichia coli* (*E. coli*) and fungal activity *Candida albicans* (*C. albicans*) were cultured on agar plates,

and then the 6 mm wells were developed on the agar plate's surface. These wells were then filled with the synthesized CsSnO_2 NPs. Following the 24-h incubation, the inhibition zones around wells were evaluated, and the data were tabulated (Hameed et al., 2013, 2015).

Cell Culture

The MCF-7 and 3T3 (embryonic fibroblasts from mouse) cells were procured from ATCC, USA. The cells were kept at 37°C and cultivated using a Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% antimycotic mixtures in an incubator with 5% CO_2 .

MTT Analysis

Cytotoxicity of CsSnO_2 NPs was evaluated against MCF-7 and 3T3 cells using MTT analysis. The cells were added on 96-well plates and treated using varying nanocomposite dosages (2, 4, 16, 32, 64, 128, and 256 $\mu\text{g/mL}$) for 24 h. Later, about 20 μL of MTT was mixed and incubated for 4 h. The formazan deposits formed were mixed with DMSO (150 μL). Absorbance was taken in a spectrophotometer at 570 nm. The percentage of cell viability was noted. The results were derived from triplicate analysis. Cellular morphology was studied under the optical microscope at (20 $\times$) magnification.

Measurement of Activities of Caspase 3, 8, and 9

Control cells and IC_{25} and IC_{50} concentrations of CsSnO_2 NPs prepared using *M. oleifera* flower extract-treated cells were kept for 24 h of incubation. The activity of caspases was determined using the manufacturer's instructions for the assay kits. The kit contains specific substrates tagged with chromophore *p*-nitroanilide (*pNA*), which is later released during caspase activity and is studied at 405 nm (Iyappan et al., 2021).

Statistical Analysis

The GraphPad Prism software was utilized for the statistical analyses, and the values are illustrated as the mean $\pm$ SD of triplicate assays ($n = 3$). The values are analyzed by one-way analysis of variance (ANOVA) and Tukey's *post hoc* assay.

Results

X-ray Diffraction (XRD) and DLS Analysis of the CsSnO_2 NPs

The XRD diffraction peaks of the CsSnO_2 NPs are illustrated in Figure 1a. The diffraction peaks (2θ) are positioned at 26.59°, 33.81°, 37.93°, 43.94°, 51.75°, 54.52°, 58.09°, 61.93°, 64.80°, 71.56°, and 78.80° with corresponding (hkl)

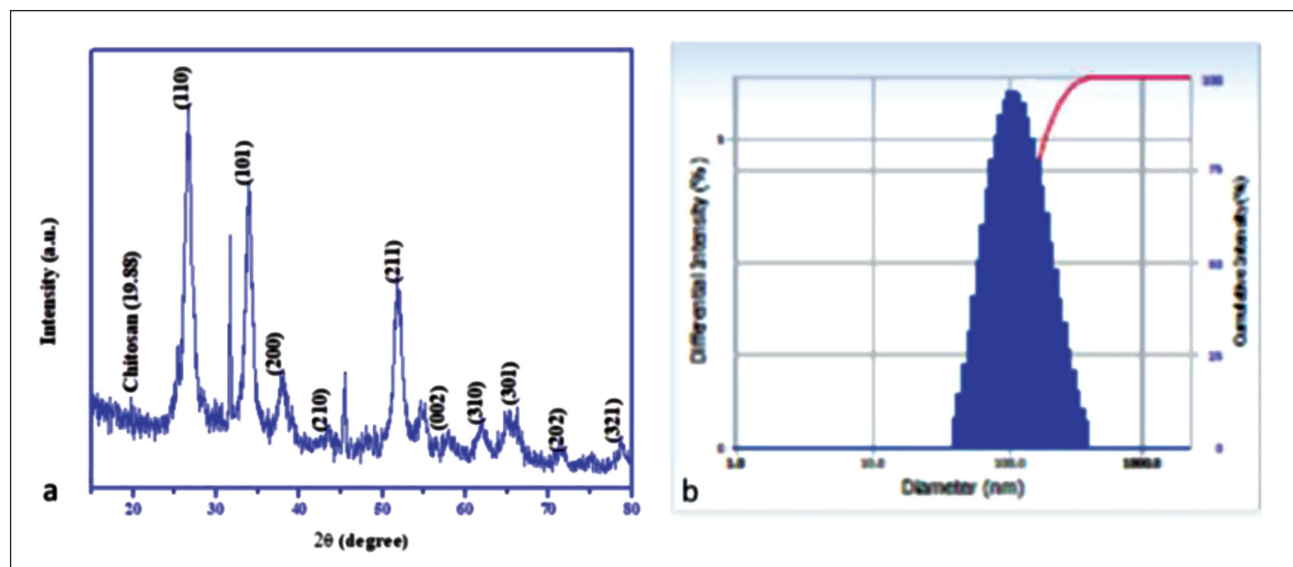


Figure 1. X-ray Diffraction (XRD) Patterns of Chitosan-decoded SnO_2 (Cs SnO_2) Nanoparticles (NPs) (a) and Dynamic Light Scattering (DLS) Spectrum of Cs SnO_2 NPs (b).

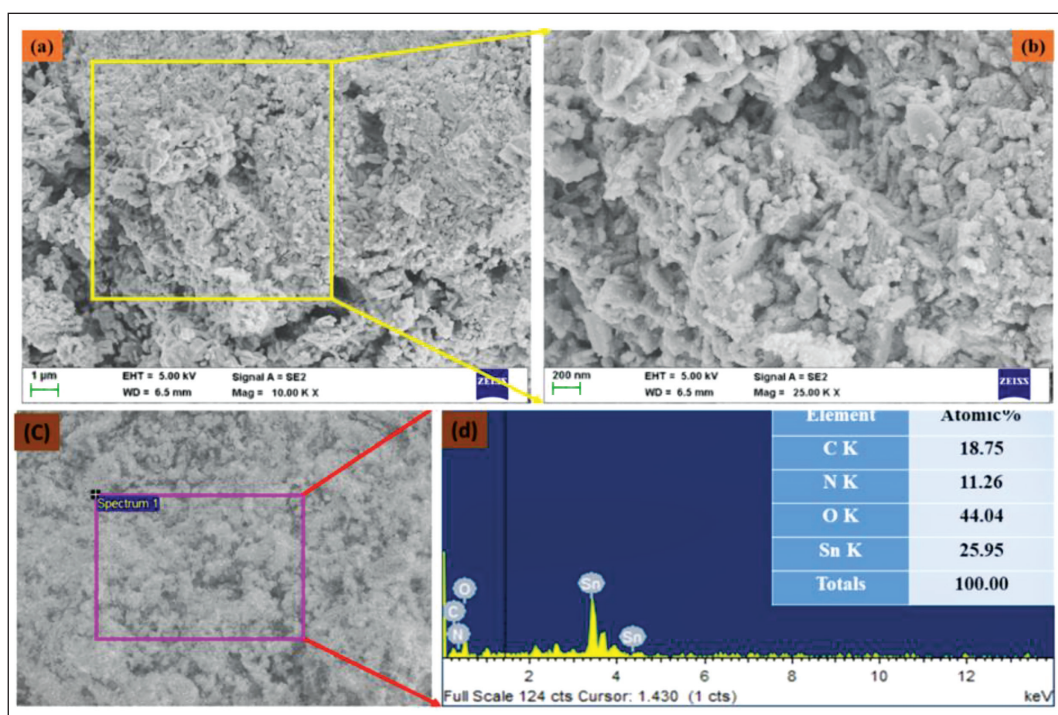


Figure 2. Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray (EDX) Analysis of the Synthesized Chitosan-decoded SnO_2 (Cs SnO_2) Nanoparticles (NPs). Lower (a,c) and Higher (b) Magnification Field Emission Scanning Electron Microscopy (FE-SEM) Microphotographs and EDAX (d) Analysis of Cs SnO_2 NPs.

planes of (110), (101), (200), (210), (211), (220), (002), (310), and (301), respectively. The particle size distribution of Cs SnO_2 NPs in an aqueous solution was investigated using

the DLS method (Figure 1b). The hydrodynamic diameter of the Cs SnO_2 NPs was observed at 114 nm, which may be Cs SnO_2 NPs surrounded by aqueous molecules.

SEM and EDX Analysis of the CsSnO₂ NPs

The lower- and higher-magnification field emission scanning electron microscopy (FE-SEM) images of CsSnO₂ NPs are shown in Figure 2a–c. The CsSnO₂ NPs are formed into a spherical structure with an aggregation formation. In addition, the CsSnO₂ NPs surface resembles a sharper edge with a porous structure, which might originate from the accumulation of chitosan molecules on their surface. The EDX analysis of CsSnO₂ NPs revealed an atomic percentage of 18.75% of carbon (C), 11.26% of nitrogen (N), 44.04% of oxygen (O), and 29.95% of tin (Sn) (Figure 2d).

FT-IR and PL Analysis of the CsSnO₂ NPs

The FT-IR spectrum of the *M. oleifera* flower extract-derived CsSnO₂ NPs is depicted in Figure 3a. The observed broad-OH and -NH stretching peaks at 3,456 cm⁻¹ correspond to biomolecules and chitosan molecules. Small intense peaks at 2,922 and 2,849 cm⁻¹ could be caused by asymmetric and symmetric C–H stretching. Figure 3b shows the PLS of CsSnO₂ NPs at a wavelength of 325 nm. The UV emission was noted at 391 nm, 380 nm, and 367 nm, corresponding to the near band edge (NBE) emission.

Antibacterial Activity of CsSnO₂ NPs

The antimicrobial effects of CsSnO₂ NPs were tested against *S. aureus*, *S. pneumoniae*, *B. subtilis*, *K. pneumoniae*, *S. dysenteriae*, *E. coli* and fungal activity *C. albicans* using the well diffusion technique, as illustrated in

Figure 4a. Antimicrobial activity can be seen in the zone of inhibition of CsSnO₂ NPs and traditional antibiotics like amoxicillin (Figure 4b). To elevate the concentration of CsSnO₂ NPs while augmenting the potential antimicrobial activity.

In vitro Study's

CsSnO₂ NPs Decrease the Viability of the MCF-7 Cell Line

MTT analysis was performed to determine the cytotoxic efficacy of CsSnO₂ NPs against MCF-7 and 3T3 cells (Figure 5a and b). CsSnO₂ NPs-treated breast cancer cells displayed a higher percentage of cytotoxicity, whereas normal mouse fibroblast cells showed (Figure 5b) higher viability rates compared to MCF-7. The higher concentration displayed more cytotoxicity and thus reduced the number of viable cells. The CsSnO₂ NPs induces cytotoxicity IC₅₀ concentration at 29.8 µg/mL against MCF-7 cells

CsSnO₂ NPs Increase Apoptotic Protein Expressions in the MCF-7 Cells

Apoptotic protein expressions were analyzed in both the control and CsSnO₂ NPs-treated MCF-7 cells using corresponding assay kits (Figure 6). In comparison to the untreated control cells, the caspase-3, -8, and -9 expressions were decreased; however, the CsSnO₂ NPs-treated expression was increased in the MCF-7 cells. Hence, it was apparent that CsSnO₂ NPs induce apoptosis in the MCF-7 cells.

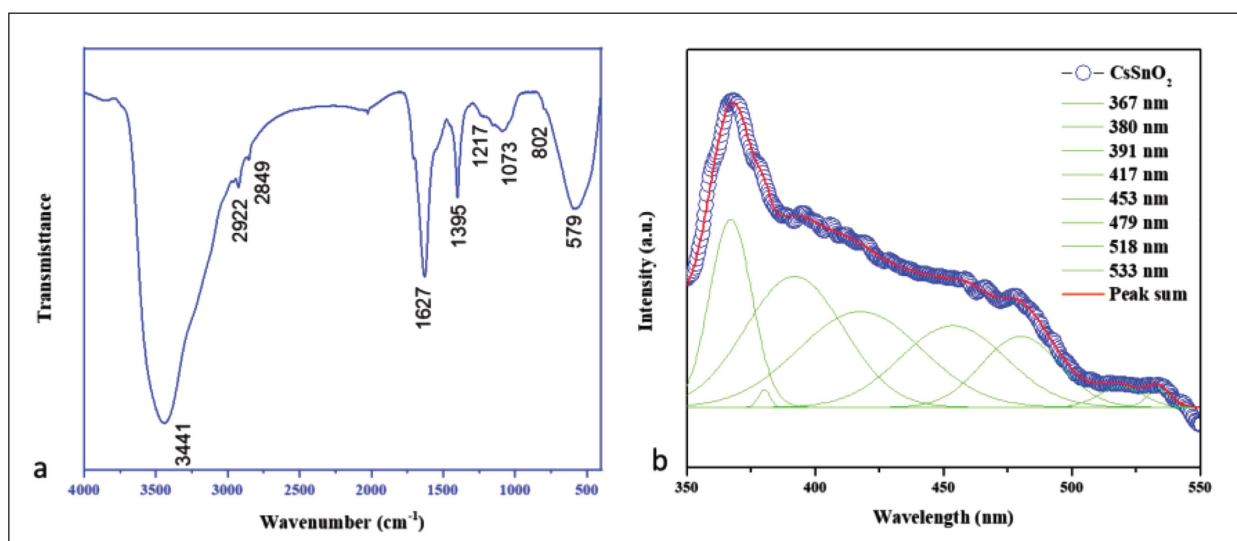


Figure 3. (a) Fourier-transform Infrared Spectroscopy (FT-IR) Analysis of the Synthesized Chitosan-decoded SnO₂ (CsSnO₂) Nanoparticles (NPs). The Fourier-transform Infrared Spectroscopy (FT-IR) Spectrum of the CsSnO₂ NPs Shows Several Peaks, Which Indicate the Presence of the Stretching and Bonding in the NPs and Photoluminescence (PL) Spectrum of CsSnO₂ NPs (b).

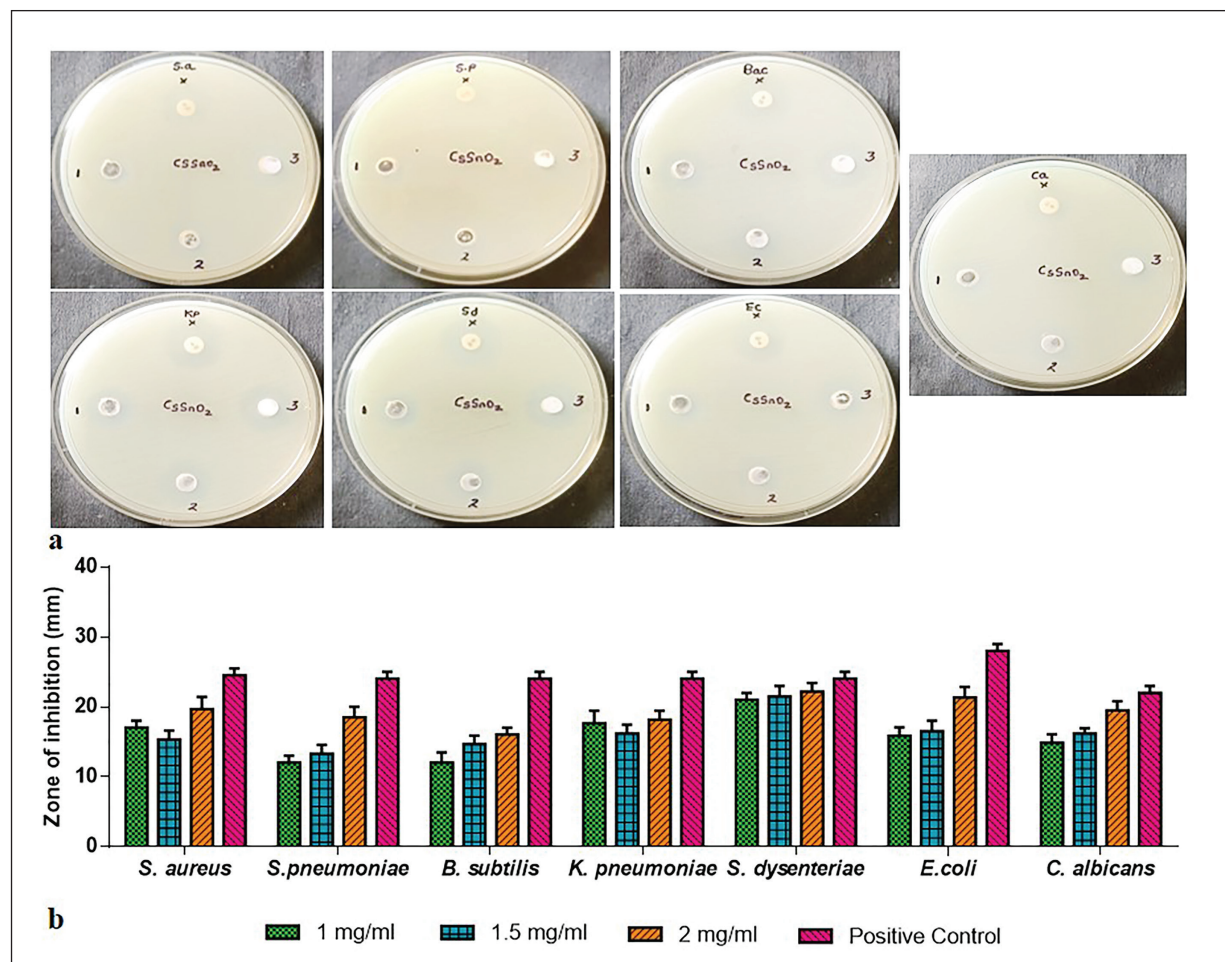


Figure 4. Antibacterial Activity of Chitosan-decoded SnO₂ (CsSnO₂) Nanoparticles (NPs) Tested Against *S. aureus*, *S. pneumoniae*, *B. subtilis*, *K. pneumoniae*, *S. dysenteriae*, *E. coli*, and fungal activity *C. albicans* of the Human Pathogen (a) and Graph Indicates the Zone of Inhibition (ZOI) of CsSnO₂ NPs and Amoxicillin Samples (b).

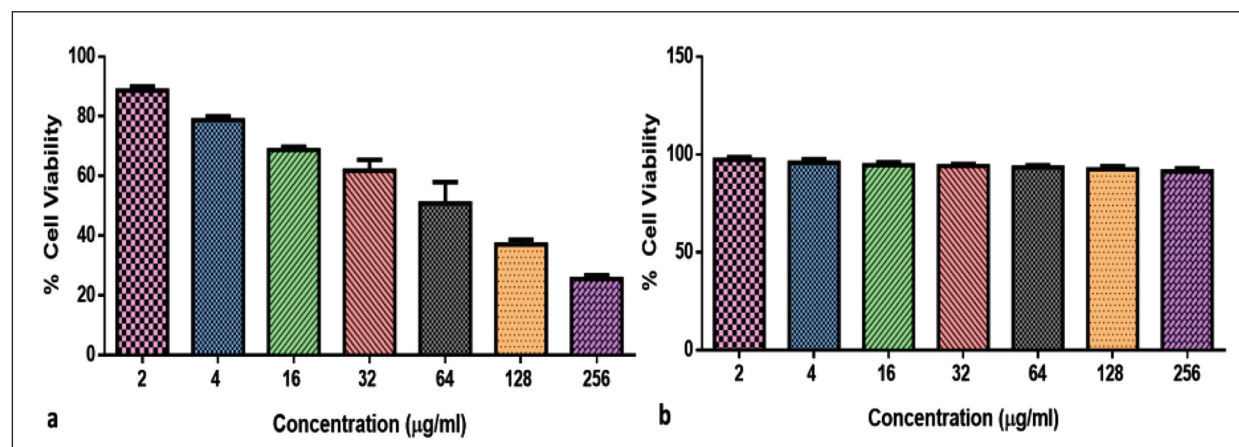


Figure 5. Chitosan-decoded SnO₂ (CsSnO₂) Nanoparticles (NPs) Inhibited the MCF-7 (a) and 3T3 (b) cells. Cells were Supplemented with Varying Concentrations of CsSnO₂ NPs for 24 h. The Cells were Then Investigated Using MTT Analysis. The Data were Given as Mean ± SD of Triplicates.

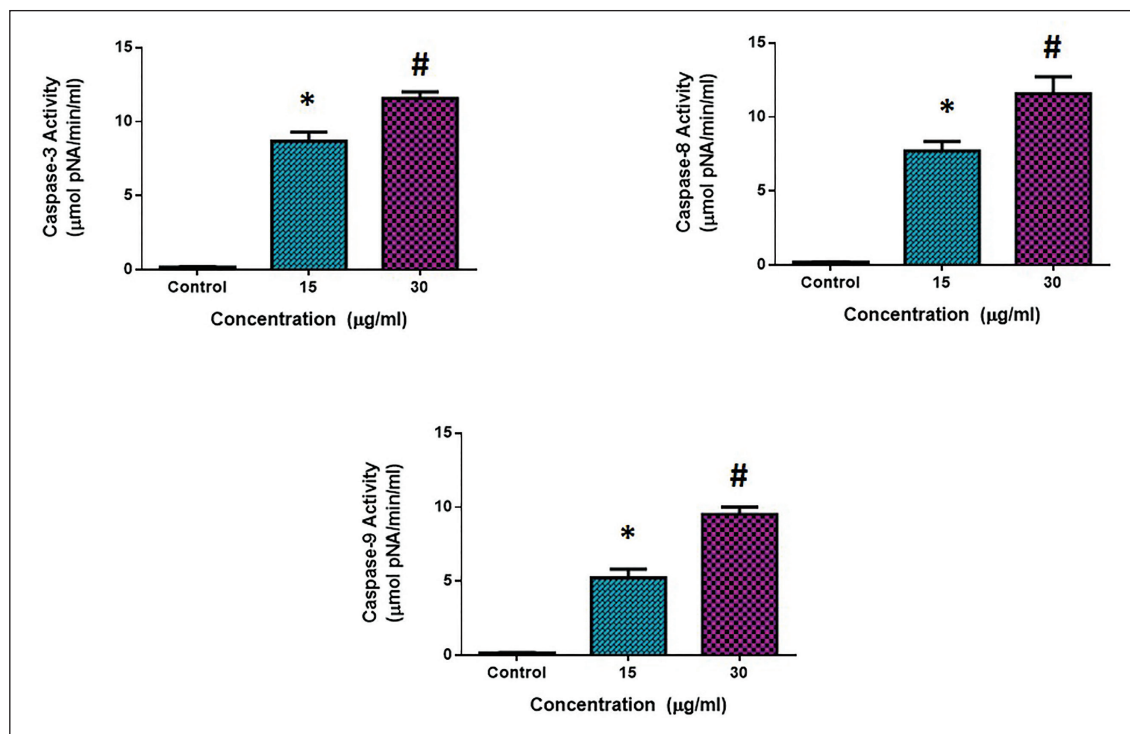


Figure 6. Effect of Chitosan-decoded SnO_2 (CsSnO_2) Nanoparticles (NPs) on the Caspase-3, -8, and -9 Activities. Administration of IC_{25} and IC_{50} Concentrations of CsSnO_2 NPs Remarkably Boosted the Caspase-3, -8, and -9 Activities in the MCF-7 Cells. Data were Represented as Mean $\pm$ SD of Triplicates. The Values are Analyzed by the One-way Analysis of Variance (ANOVA) and Tukey's *Post Hoc* Assay.

Notes: *indicates $p < 0.05$ compared with control; #indicates $p < 0.01$ compared with control.

Discussion

In a green process, *M. oleifera* flower extract was used to synthesize chitosan-decorated SnO_2 NPs. The chitosan molecule was dissolved in acetic acid, and tin chloride solute and *M. oleifera* flower extract were added. Tin cations (Sn^{2+}) were formed from this metal salt. The Sn^{2+} ions are bonded to the chitosan bridge network of hydroxyl (OH) and amine (NH) groups. *M. oleifera* flower extract contains many phytochemicals, such as alkaloids, steroids, flavonoids, tannins, saponins, carbohydrates, and proteins (Saini et al., 2016). Plant extracts were utilized as reducing and stabilizing agents in the green formulation, and chitosan-modified engineered tin dioxide NPs are well dispersed by reduced graphene oxide (Tu et al., 2023). They include many phytochemical constituents, which play a pivotal role as reducing and capping agents of the Sn^{2+} ions needed to form the SnO_2 NPs. CsSnO_2 NPs were constructed and converted due to the Sn-chitosan nucleation solution (Gebreslassie & Gebretnsae, 2021). The XRD diffraction peaks of the CsSnO_2 NPs are illustrated in Figure 1a. The diffraction peaks with corresponding (hkl) planes of (110), (101), (200), (210), (211), (220), (002), (310), and (301), respectively, matched the tetragonal structure of SnO_2 NPs (JCPDS No. 88-0287) (Ragupathy et al., 2022; Wongsaprom et al., 2018). Furthermore, weak chitosan diffraction of 2θ value was

observed at 19.88° . These results confirm that chitosan and SnO_2 hybridization exhibit intermolecular interactions and synergistic effects. Using the Debye-Scherrer equation, the average particle size of CsSnO_2 NPs is 32 nm (Holzwarth & Gibson, 2011). The particle size distribution of CsSnO_2 NPs in the DLS method (Figure 1b) showed that the hydrodynamic diameter of the CsSnO_2 NPs was observed at 114 nm, which may be CsSnO_2 NPs surrounded by aqueous molecules.

The lower- and higher-magnification FE-SEM images of CsSnO_2 NPs are shown in Figure 2a–c. Due to a biopolymer component (chitosan), the characters lacked a well-defined crystalline structure in this instance. Nevertheless, the SnO_2 NPs have been decorated onto the chitosan surface matrix. The EDX analysis of CsSnO_2 NPs revealed an atomic percentage of 18.75% of carbon (C), 11.26% of nitrogen (N), 44.04% of oxygen (O), and 29.95% of tin (Sn) (Figure 2d).

The FT-IR spectrum of the *M. oleifera* flower extract-derived CsSnO_2 NPs are the observed broad-OH and -NH stretching peaks at $3,456\text{ cm}^{-1}$, corresponding to biomolecules and chitosan molecules (Mishra et al., 2015). Small intense peaks at $2,922$ and $2,849\text{ cm}^{-1}$ could be caused by asymmetric and symmetric C–H stretching (Figure 3a). The antioxidants' –C=C and –C–O stretch vibrations in *M. oleifera* flowers are assigned to the bands at $1,397$ and $1,653\text{ cm}^{-1}$. When the reduction occurs on the surface of the particles, these reducing agents can donate electron pairs to the metals (Sn). Because

polyphenols have a negative charge on the surface of the reduced metal, they act as capping agents. The typical chitosan peaks at 1,217, 1,073, and 802 cm^{-1} denote C–O–C stretching in the glucose circle, C–O stretching, and C–H in-plane bending. A metal and oxide (M–O) absorption peak at 579 cm^{-1} wavenumber position was also noted and attributed to Sn–O stretching (Ma et al., 2020; Manohari et al., 2022; Talouki & Ghorbani, 2017).

Figure 3b shows that UV emission was noted at 391, 380, and 367 nm, corresponding to the NBE emission. The violet emission peak occurs at 417 nm and is attributable to the electron transition regulated by defects in the band gap. At 453 and 479 nm, two blue emissions correlate to deep-level emissions, like oxygen vacancies and Sn interstitials. Two green emissions (518 and 533 nm) were also observed, accredited to the singly ionized oxygen vacancy. The oxygen vacancies and Sn interstitial defects on the CsSnO_2 NPs' surface are significant to their ability to kill living things.

To elevate the concentration of CsSnO_2 NPs while augmenting the potential antimicrobial activity (Figure 4a and b), CsSnO_2 NPs biocidal properties can be attributed to one of the following mechanisms: The interaction of chitosan with metal ions (Sn^{4+}) results in increased chitosan adsorption with a positive charge density onto the negatively charged bacterial cell membrane, which leads to more growth inhibition (Amininezhad et al., 2015; Mostafa & Mwafy, 2020). Furthermore, binding the Cs with Sn^{4+} complex formation to the cell DNA blocks protein formation. The high surface area of NPs causes more oxidative stress by reactive oxygen species (ROS) generation (Zhang et al., 2018), which is accountable for their augmented chemical and biological effects. In the present work, from the FESEM results, the CsSnO_2 NPs surface resembles a sharp edge with a porous structure, which might originate from the accumulation of chitosan molecules on their surface. CsSnO_2 NPs with irregular surfaces and rough edges were discovered to adhere to the bacteria and trigger cell membrane damage. As a result, microbes are dead.

MTT results proved that the MCF-7 cell growth was markedly declined by treatment with the green synthesized CsSnO_2 NPs (Figure 5). This might be because of the upstream result of cytotoxicity initiated by increased ROS production (Zhang et al., 2018). Hence, the results substantiate that the CsSnO_2 NPs have a potent cytotoxicity, which correlates with the earlier findings (De Marzi et al., 2013). The tumor cell viability was decreased to 50% when exposed to 37.33 $\mu\text{g/mL}$ concentration of green synthesized CsSnO_2 NPs and the cultures were subjected to further analysis.

Apoptosis can be induced in cancer cells by triggering apoptotic signaling via the death receptor-mediated (extrinsic) and/or the mitochondria-mediated (intrinsic) pathways (Park et al., 2014). The activation of the intrinsic pathway occurs via the involvement of Bcl2 family proteins, including antiapoptotic proteins like Bcl-2 and Bcl-XL, as well as proapoptotic proteins such as Bax and Bak. Caspase is an

enzyme stimulated by caspase 3, which further enhances the caspase-8 and caspase-9 activations (Herr, 2018). Thus, the nanocomposites significantly augmented the activities of numerous proapoptotic proteins and activated caspase 3, 8, and 9. MCF-7 cells administered with the green synthesized CsSnO_2 NPs exhibited more caspase-3, -8, and -9 activities when compared with the control (Figure 6). The study's findings displayed that green synthesized CsSnO_2 NPs prepared using *M. oleifera* flower extract triggered apoptosis in MCF-7 cells by caspase-dependent signaling. Treatment with the green synthesized CsSnO_2 NPs prepared using *M. oleifera* flower extract increased the proapoptotic marker expressions.

The potential cytotoxicity of SnO_2 NPs on breast cancer cells has been demonstrated using many different synthesized SnO_2 NPs from various methods and sources, but the mechanism underlying this cytotoxicity has not been fully explored. It was already highlighted in earlier work that SnO_2 NPs activate the mitochondrial apoptosis signaling by overexpressing Bax/Bcl-2 and downregulating PI3K/AKT/mTOR pathway (Guo et al., 2021). Based on these data, we can speculate as to how SnO_2 NPs might produce their anticancer properties. The antiproliferative properties of SnO_2 NPs prepared by hydrothermal technique against K562 cells were examined (Ahmadabad et al., 2021). Cell growth, migration, and oxidative stress could be blocked, and apoptosis could be induced by SnO_2 NPs in oral tumor cells (Li et al., 2020).

Conclusion

The present study found that the chitosan is decorated with tin dioxide (CsSnO_2) NPs exhibit antimicrobial and anticancer effects on reducing cell viability and inducing apoptosis. The results of the various characterization analyses showed the formation of metallic CsSnO_2 NPs with spherical structure, narrowly distributed NPs with an average size at 114 nm, which may be CsSnO_2 NPs surrounded by aqueous molecules. The antimicrobial properties of the CsSnO_2 NPs and amoxicillin were investigated in human pathogens. CsSnO_2 NPs exhibit similar antimicrobial properties as compared with amoxicillin. The treatment of CsSnO_2 NPs significantly decreased cell viability, increased apoptotic protein expressions, and thereby induced apoptosis in the MCF-7 cells. Hence, the present findings indicate that CsSnO_2 NPs hold the potential to be a highly effective candidate for breast cancer treatment. Furthermore, future research should be conducted to fully comprehend the specific therapeutic mechanisms of CsSnO_2 NPs in breast cancer treatment.

Abbreviations

NPs: Nanoparticles; MDR: Multidrug-resistant; TiO_2 : Titanium dioxide; CsSnO_2 : Chitosan-decoded SnO_2 ; MCF-7: Breast cancer; FESEM: Field emission scanning electron microscope; EDX:

Energy dispersive X-ray; DLS: Dynamic light scattering; PLS: Photoluminescence spectrum; ZOI: Zone of inhibition.

Declaration of Competing Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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ORCID iDs

Ahmad Alsulimani  <https://orcid.org/0000-0002-2131-9163>

Abdullah Farasani  <https://orcid.org/0000-0001-6942-5136>

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