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Original Article

Eco-Friendly Fabrication of SnO_2-Curcumin Nanoparticles via *Pterocarpus marsupium* Extract: Unveiling Potent Antimicrobial and Anticancer Applications

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ABSTRACT

Background: Curcumin-SnO_2 Nanoparticles (NPs) are advanced materials that combine organic molecules and inorganic substances in a single nanoscale platform to create hybrids with unique and enhanced properties. The synergy between the two components often results in improved functionality, stability and efficacy. **Objectives:** The current work was conducted on Curcumin-SnO_2 NPs synthesized using an eco-friendly method with *Pterocarpus marsupium* extract and evaluated their antimicrobial and cytotoxic effects on MDA-MB-231 cancer cells. **Materials and Methods:** The synthesized Curcumin-SnO_2 NPs were characterized using various methods, including XRD, SEM, PL and UV-visible spectroscopy analyses. The antimicrobial property of Curcumin-SnO_2 NPs was assessed by the disc diffusion method against various pathogens. The cytotoxicity of Curcumin-SnO_2 NPs on MDA-MB-231 cancer cells was assessed by a WST-1 assay. **Results:** Characterization of Curcumin-SnO_2 NPs using X-ray Diffraction (XRD) revealed a tetragonal structure. Photoluminescence (PL) spectra ranging from 350 to 550 nm indicated the presence of tin and oxygen vacancies (surface defects). The Curcumin-SnO_2 NPs exhibited higher antimicrobial activity against *Bacillus megaterium, Klebsiella pneumoniae* and *Candida albicans* than conventional antibiotics. The Curcumin-SnO_2 NPs effectively inhibited the viability of breast cancer cells. **Conclusion:** These findings suggest the potential use of Curcumin-SnO_2 NPs in healthcare institutions to improve patient outcomes.

Keywords: Curcumin, Nanoparticles, *Pterocarpus marsupium*, MDA-MB-231 cancer cells.

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INTRODUCTION

Multifunctional nanomaterials have emerged as powerful biocidal agents that can be activated by ultraviolet and visible light spectra, making them highly versatile for various applications. Specific wavelengths of light trigger antimicrobial actions in these materials, effectively targeting and inactivating pathogens.[1] Moreover, the properties of these nanomaterials are tunable. We can adjust their chemical, physical and biological characteristics to meet specific needs.[2] For instance, we can tailor their size, shape and functional surface groups to optimize their interaction with microbial cell structures, enhancing their effectiveness as biocidal agents.[3] These materials harness the unique properties of organic molecules and inorganic substances to create innovative solutions with improved capabilities.[4]

Curcumin is a bioactive compound primarily extracted from the root of the turmeric plant (*Curcuma longa*), which belongs to the ginger family.[5] Historically, curcumin has been utilized extensively in traditional medicine systems such as Ayurveda in India and traditional Chinese medicine. In these practices, it is revered for its potent anti-inflammatory, antibacterial and healing properties.[6] Practitioners have prescribed turmeric for various health conditions, including pain relief, improving digestion and combatting infections. Tin oxide (SnO_2), a semiconductor, stands out for its exceptional photocatalytic properties.[7] SnO_2 nanoparticles possess a unique ability to generate Reactive Oxygen Species (ROS), highly reactive molecules that can cause oxidative damage to bacterial cells, leading to their inactivation.[8] When exposed to light, especially UV light, SnO_2 nanoparticles absorb photons, exciting electrons from the valence band to the conduction band and creating electron-hole pairs (e/h^+pairs). These pairs then migrate to the surface of the SnO_2 nanoparticles, a crucial separation for the subsequent reactions that generate ROS.[9] The combination of SnO_2 and curcumin creates a potent antimicrobial agent capable of generating high levels of ROS,

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which can effectively inactivate a wide range of pathogenic microorganisms.

Clinical industrial-based microbial infections are a critical, life-threatening issue for the human living system.[10] However, multifunctional organic-inorganic nanoparticles induce oxidative stress, generating excess Reactive Oxygen Species (ROS) that can damage microbial cells.[11] Their biocidal activity is highly acceptable to human systems. SnO_2 (tin oxide) nanoparticles are known for generating ROS, highly reactive molecules that can cause oxidative damage to bacterial cells, leading to their inactivation. Moreover, when incorporated with foreign impurities, Curcumin enhances the biocidal activity of the SnO_2 surface matrix.[12] The incorporation of Curcumin into the SnO_2 surface matrix not only improves its biocidal activity but also modifies its surface properties. Curcumin can alter the surface charge, hydrophilicity and active sites of SnO_2. This modification is crucial in disrupting microbial cell walls, making them more susceptible to the oxidative damage caused by ROS generated by SnO_2.[13] These changes can increase the interaction between the nanoparticles and microbial cells, leading to more efficient antimicrobial action. In this investigation, the Curcumin-SnO_2 NPs were successfully synthesized through a green process using *Pterocarpus Marsupium* extract as a capping agent. *Pterocarpus Marsupium*, commonly known as the Indian Kino Tree, is a significant medicinal plant with a rich history in traditional Ayurvedic medicine.[14] The extract from this tree contains various bioactive compounds that confer numerous health benefits, including anti-diabetic, anti-inflammatory, antioxidant, wound healing, antimicrobial, cardioprotective, gastroprotective, anti-cancer, antipyretic, analgesic and neuroprotective effects. The resulting nanomaterials were characterized for their optical, structural and antimicrobial properties. The resulting NPs were characterized by their optical, structural and antimicrobial properties. Curcumin was primarily used to enhance the antimicrobial and biocidal properties of the SnO_2 surface matrix. Additionally, Curcumin can reduce the cost of the nanoparticles and improve their biosafety.

MATERIALS AND METHODS

Materials

The following high-purity chemicals such as tin chloride and curcumin were used as precursors without further purification.

Plant extract preparation

10 g of fresh *Pterocarpus marsupium* (Vengai leaf) were added to 100 mL of double-distilled water and boiled at 50-60°C for 10 min. The *Pterocarpus marsupium* extract was then filtered using Whatman No. 1 filter paper and stored in a refrigerator at 4°C to preserve its bioactive properties.

Preparation of SnO_2 NPs

0.1 M tin chloride solute was dissolved in 100 mL of *Pterocarpus marsupium* extract and stirred for 6 hr at 80°C and A black precipitate formed. The precipitate was then dried at 120°C and the SnO_2 NPs were annealed at 600°C for 5 hr. Thus, green-synthesized SnO_2 NPs were obtained.

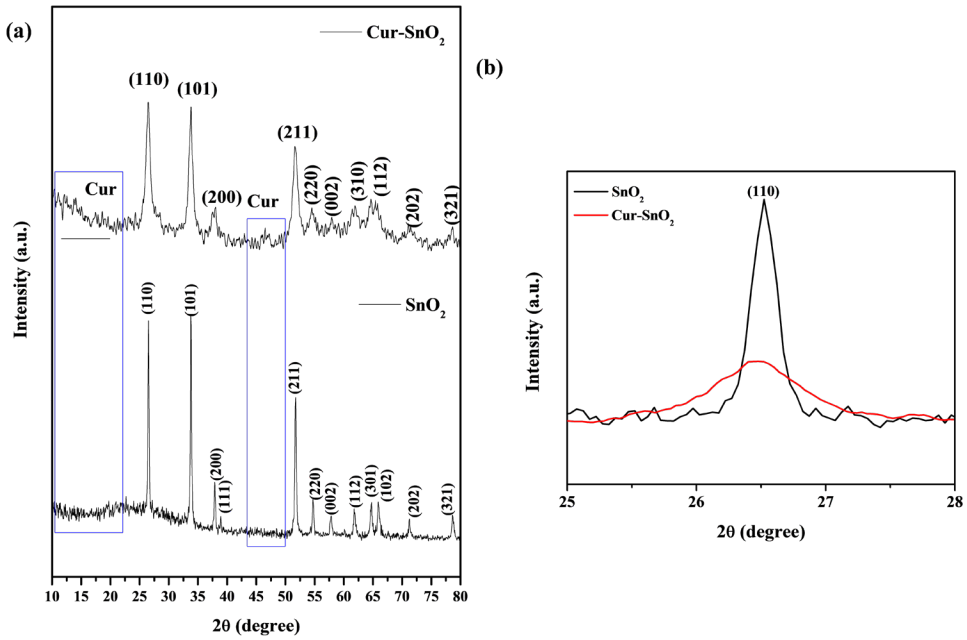

Figure 1: (a)XRD pattern of SnO_2 and Curcumin-SnO_2 NPs and (b) Enlarged patterns of SnO_2 and Curcumin-SnO_2 NPs at 25 to 28°.

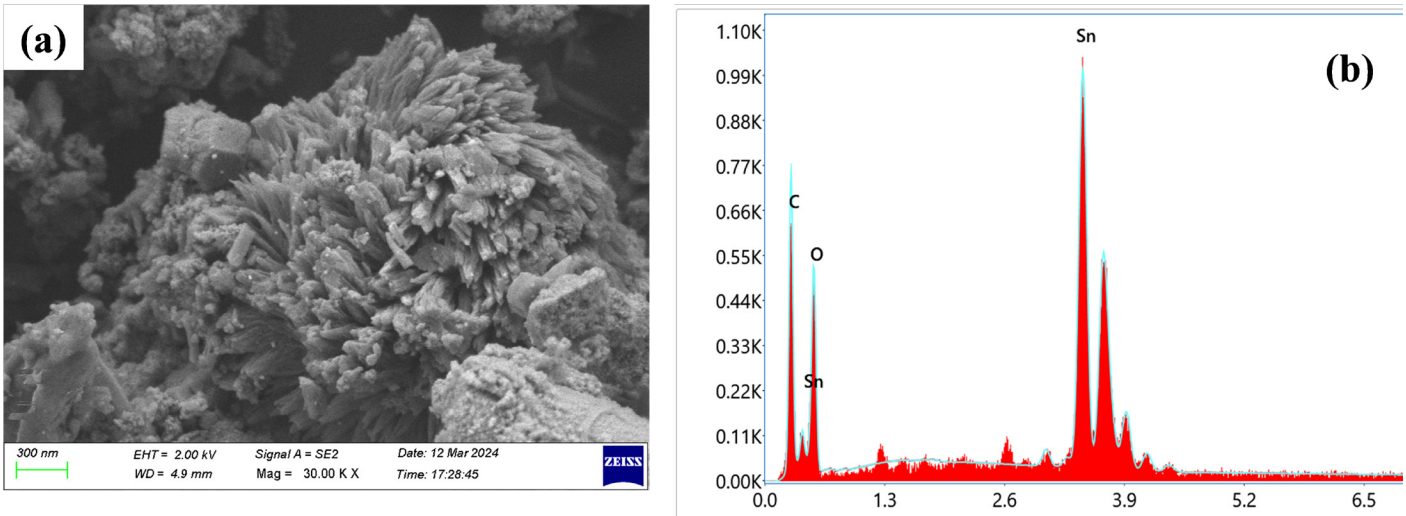

Figure 2: (a) FE-SEM and (b) EDAX spectra of Curcumin-SnO_2 NPs.

Preparation of Curcumin-SnO_2 NPs

500 mg of SnO_2 NPs solution was mixed with 100 mg of curcumin (dissolved in ethanol). The Curcumin-SnO_2 NPs that formed a yellow precipitate were heated for 12 hr at room temperature under a magnetic stirrer. The yellow precipitate was washed with deionized water and ethanol several times. The yellow precipitate solution was centrifuged for 40 min at -3°C at 15,000 rpm. The final curcumin-SnO_2 matrix was dried at 200°C for 2 hr.

Characterization techniques

The curcumin-SnO_2 NPs were characterized by an X-ray diffractometer (model: X'PERT PRO PANalytical). The diffraction patterns were recorded in the range of 25°-80° for the curcumin-SnO_2, where the monochromatic wavelength of 1.54 Å was used. The samples were analyzed using Field Emission Scanning Electron Microscopy (Carl Zeiss Ultra 55 FESEM) with EDAX (model: Inca). The absorbance of curcumin-SnO_2 NPs was studied between 200 and 1100 nm by Lambda 35 spectrometer. Photoluminescence spectra were measured using a Cary Eclipse spectrometer.

Antimicrobial assay

Using the disk diffusion method, the antibacterial activity of curcumin-SnO_2 NPs was tested against *Bacillus megaterium, Klebsiella pneumoniae* bacterial strains and fungi *C. albicans*. Petri plates were prepared with 25 mL of Mueller Hinton Agar (MHA) and potato dextrose media fungi *C. albicans* and the pathogen was swabbed onto the media in the plates. The antimicrobial activity was tested at 1, 1.5 and 2 mg/mL concentrations, with the quantity of curcumin-SnO_2 NPs dispersed in 5% sterilized Dimethyl Sulfoxide (DMSO). The inhibition zones were measured after 24 hr of incubation at 37°C. Streptomycin (10 µg) and Fluconazole (10 µg) served as positive control. Assays were conducted in triplicate.

Anticancer activity

Cells were grown in 96-well plates at 1×10^6 cells/well for the cell viability assay and then incubated for 24 hr. The cells were grown at 37°C for 24 hr and then treated with curcumin-SnO_2 NPs at different dosages (1.56, 3.125, 6.25, 12.5, 25, 50, 100 and 200 µg/mL). After 24, 48 and 72 hr of treatment, fresh medium was used and WST-1® (10 µL) reagent was mixed in each well for 3 hr at 37°C. The inhibition percentages were calculated by measuring absorbance with an ELISA reader at 460 nm to assess cell viability.

Statistical analysis

The GraphPad Prism software was utilized for the statistical analyses and the values are illustrated as the mean±SD of triplicate assays (n=3). The values are analyzed by the one-way ANOVA and Tukey's *post hoc* assay and $p<0.05$ was set as significant.

RESULTS AND DISCUSSION

X-ray Diffraction (XRD)

Figure 1 shows that X-ray diffraction patterns of green synthesis of curcumin-SnO_2 NPs and XRD peaks appear at angles (2θ) of 26.52°, 33.64°, 37.38°, 38.53°, 51.24°, 54.55°, 57.87°, 61.70°, 64.30°, 65.81°, 71.16° and 78.71° and correspond to (110), (101), (200), (111), (211), (220), (102), (301), (202) and (321) hkl planes for SnO_2-Curcumin NPs, those results exactly matched with the tetragonal SnO_2 crystalline structure (JCPDS No. 41-1445).[15] Interestingly, the nanocrystalline curcumin diffraction peak was observed at 17°; these results indicate that the combination of curcumin and SnO_2 phases corresponds to the formation of intermolecular hydrogen bonds between the curcumin-SnO_2 surface matrix. Debye's Scherrer formula calculated the curcumin-SnO_2 NPs at 40 nm.[16]

Morphological and chemical composition analysis

The FE-SEM image of the green synthesized Curcumin-SnO_2 NPs, as shown in Figure 2a, reveals that the Curcumin-SnO_2 NPs

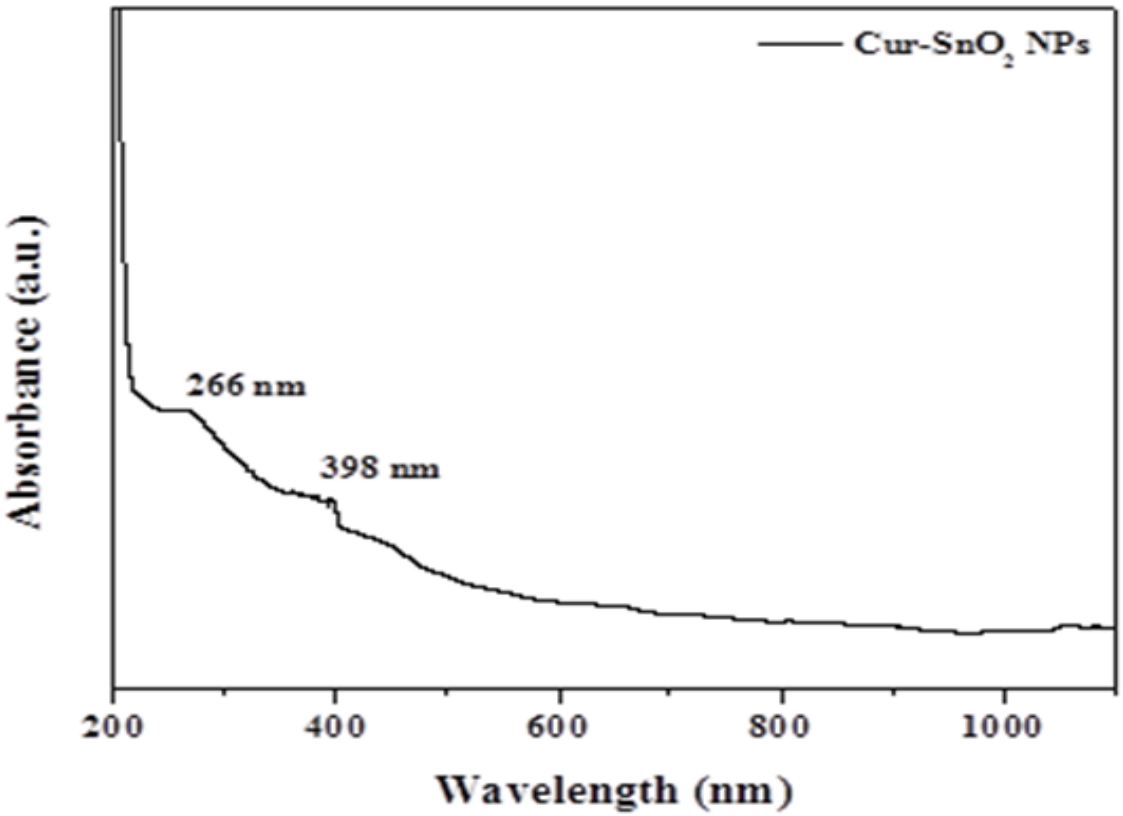

Figure 3: UV-vis absorbance spectrum of Curcumin-SnO_2 NPs.

exhibit nanoflakes with sharp edges. The average thickness of the NPs is 50 nm, as determined by XRD, a result that is in excellent agreement with the FE-SEM image. The chemical composition of the synthesized Curcumin-SnO_2 NPs was analyzed using an EDAX spectrum, as shown in Figure 2b. The atomic percentages of the Curcumin-SnO_2 NPs were found to be 6.64% for Sn (Tin), 28.78% for O (Oxygen) and 64.58% for C (Carbon) (Table 1). The higher carbon atomic percentage observed in the prepared Curcumin-SnO_2 NPs is attributed to the carbon and oxygen molecules derived from the curcumin molecules, confirming that curcumin molecules are coating the SnO_2 surface matrix.

UV-vis spectroscopy

Figure 3 illustrates the UV-vis absorbance spectrum of Curcumin-SnO_2 NPs, with distinct absorbance edge peaks at 266 nm and 398 nm. These peaks correspond to the unique optical properties of the combined Curcumin and SnO_2 surface matrix. The peak at 266 nm is attributed to Curcumin, a naturally occurring polyphenolic compound known for its characteristic absorption in the ultraviolet region.[17] The electronic transitions within Curcumin's molecular structure include π-π^* transitions, commonly observed in conjugated systems. In addition, SnO_2 peaks were observed at 398 nm and its absorbance in the UV region could be associated with its electronic band structure (semiconductor), specifically the band-gap transitions. The organic-inorganic combination suggests a strong interaction between the Curcumin molecules and the SnO_2 surface matrix.

Photoluminescence spectroscopy (PL) analysis

The Photoluminescence (PL) spectrum of green-synthesized Curcumin-SnO_2 NPs is shown in Figure 4, with an excitation wavelength of 325 nm. The PL emission values are observed at 355, 379, 421, 442, 455, 480, 505 and 527 nm for Curcumin-SnO_2 NPs. The UV (near band edge: NBE) emission at 355 and 379 nm is attributed to the bandgap linked to near band edge emission and electron transitions induced by oxygen vacancies.[18] The violet emission observed at 421 nm corresponds to Sn interstitial vacancies. The blue emission monitored at 442, 455 and 480 nm is due to doubly ionized oxygen vacancies. The green emissions at 505 and 527 nm are attributed to single oxygen vacancies.[18]

Antimicrobial activity

Infections caused by Gram-positive (G+) and Gram-negative (G-) bacteria and fungi resistant to multiple drugs have become increasingly common worldwide over the past few decades. This rise in resistant microbes has led to significant clinical challenges, severely impacting patient outcomes and the effectiveness of current treatments.[19] The prevalence of drug-resistant microorganisms is hampering progress in the biomedical sector and posing substantial risks to patient safety, particularly in hospital settings where infections can spread rapidly. Addressing

Table 1: Chemical composition analysis of Curcumin-SnO_2 NPs.

Element	Weight %	Atomic %	Error %	Net Int.	R	A	F
C K	38.32	64.58	11.13	189.84	0.8423	0.1888	1.0000
O K	22.75	28.78	12.40	145.83	0.8564	0.1012	1.0000
Sn L	38.93	6.64	3.96	527.56	0.9136	0.8960	1.0055

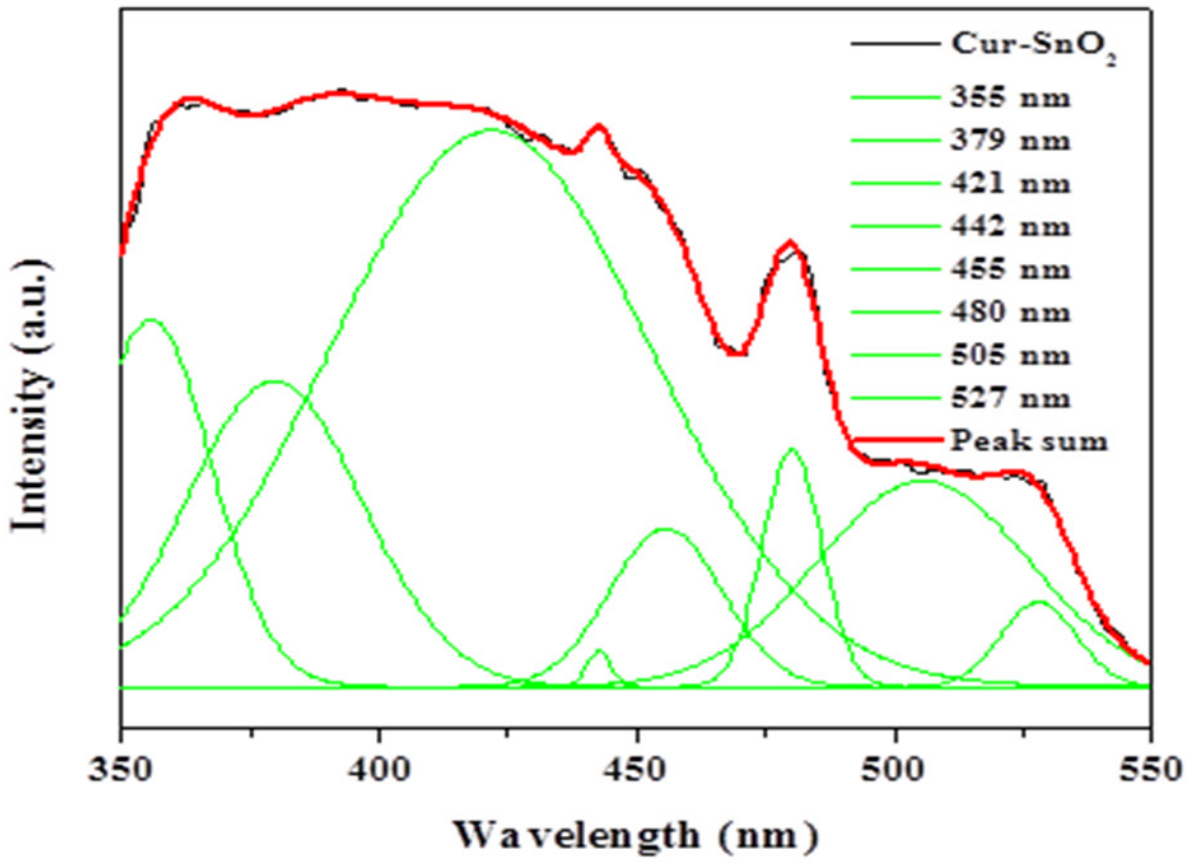

Figure 4: PL spectrum of Curcumin-SnO_2 NPs.

this growing problem requires the development of new antimicrobial strategies and treatments to combat cost-effective and high-killing efficacy pathogens.[20]

The present work shows green synthesized curcumin-SnO_2 NPs tested against *Bacillus megaterium, Klebsiella pneumoniae* and *Candida albicans* strain employing the well diffusion method in Figure 5a, b. The antimicrobial activity was carried out in different concentrations of curcumin-SnO_2 NPs (1, 1.5 and 2 mg/mL) and conventional antibiotics Streptomycin and Fluconazole. Increasing the concentration of NPs also increased antimicrobial activity. The curcumin-SnO_2 NPs containing 2 mg/mL showed a significant zone of inhibition of nearly 10-12 mm for *Bacillus megaterium, Klebsiella pneumoniae* and *Candida albicans* strain, respectively. The antibacterial efficacy of SnO_2-curcumin NPs is frequently influenced by Reactive Oxygen Species (ROS), primarily related to the size, surface area and increase in oxygen vacancies.[21] Antimicrobial activity is higher in the smaller particle size and more oxygen vacancies of curcumin-SnO_2 NPs. In the present work, the FE-SEM results show a nanoflake thickness of 50 nm and with sharp edges.[22] The PL spectra of green emission (oxygen vacancies) were observed at 505 and 527 nm, responsible for more ROS generation.[23] Because of their smaller size and large interfacial area, they can easily penetrate bacterial membranes, increasing their antibacterial efficiency- both synergistic effects of curcumin-SnO_2 NPs and the ability to kill microbes.

The surface morphology is a crucial parameter for antibacterial activity

In the present work, FE-SEM images of Curcumin-SnO_2 revealed a nanoflake-like structure. Detailed examination of the nanoparticle edges showed the presence of irregular ridges on the outer surface. These surface irregularities and rough edges are significant because they facilitate the adhesion of Curcumin-SnO_2 to bacterial cell walls. This adhesion process is essential as it damages the bacterial cell membrane, enhancing

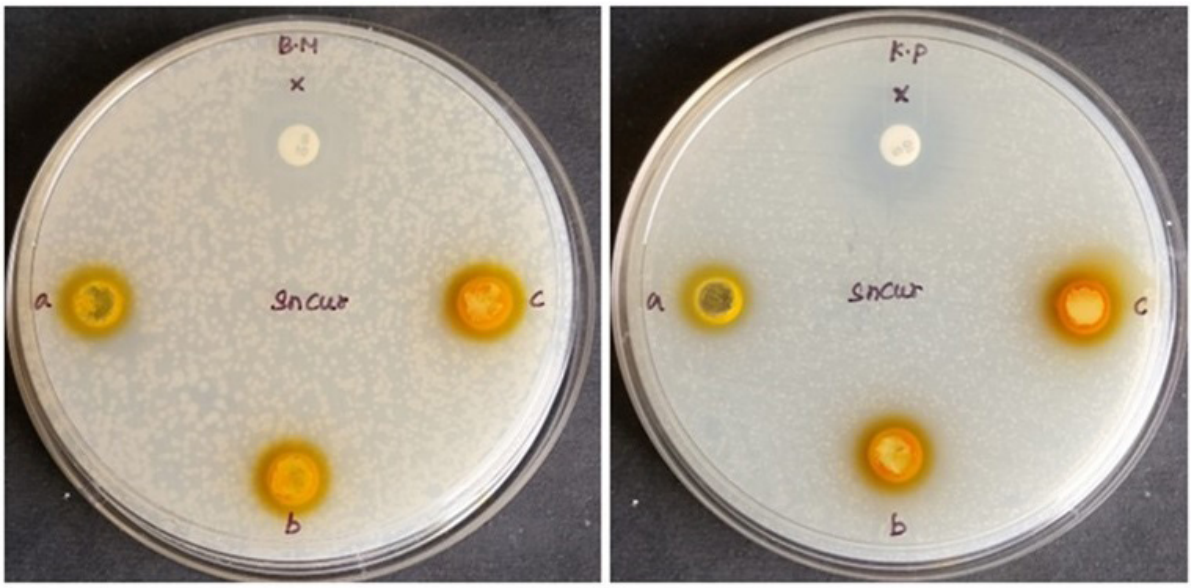

Figure 5a: Antibacterial activity of Curcumin-SnO_2 NPs tested against *Bacillus megaterium* and *Klebsiella pneumoniae* bacterial strain.

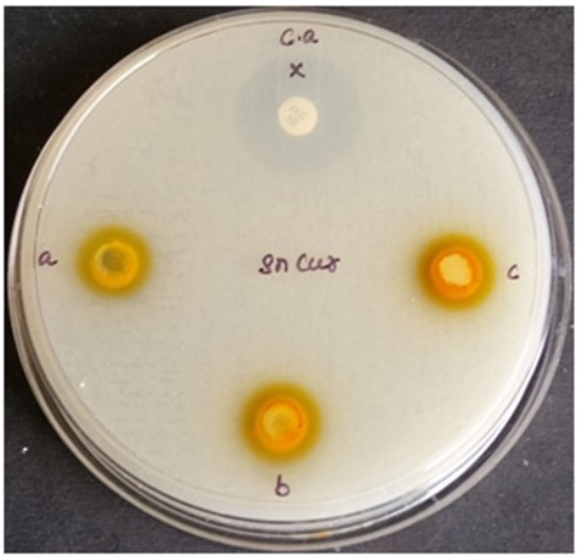

Figure 5b: Antifungal activity of Curcumin-SnO_2 NPs.

the antibacterial efficacy of Curcumin-SnO_2. The rough surface and irregular ridges create more contact points with bacterial cells, disrupting their integrity and making it difficult for bacteria to survive, exhibiting potent antibacterial properties.

Curcumin-SnO_2 NPs inhibit the viability of breast cancer MDA-MB-231 cells

The effect of Curcumin-SnO_2 NPs on the growth of MDA-MB-231 cells was assessed by a WST-1 assay (Figure 6). The cells were treated with several dosages (1.56-200 µg/mL) of Curcumin-SnO_2 NPs for 24, 48 and 72 hr. Curcumin-SnO_2 NPs treatment demonstrated dose-dependent and time-dependent inhibition of MDA-MB-231 cell viability, as indicated by the WST-1 assay results (Figure 6). The IC_{50} concentration of Curcumin-SnO_2 NPs was 52.93, 34.74 and 18.19 µg/mL at 24, 48 and 72 hr, respectively. The results indicated that Curcumin-SnO_2 NPs have a stronger inhibitory activity on the viability of MDA-MB-231 cells.

In many cell culture techniques, measuring cell viability is essential in determining the number of healthy cells. WST-1 can be transformed into a water-soluble formazan by cellular dehydrogenases. Because of its high solubility, the formazan produced by WST-1 has a wider linear range and greater sensitivity.[24] The effect of the sample agents at different times can be seen by adding WST-1 once. Assessing the vitality of cells is an essential way to evaluate how cells react to their surroundings.[25] The WST-1 test exhibits improved accuracy and permits the assessment of several samples without generating radioactive waste, which is currently essential for researching cellular viability.[26] In this work, the influence of Curcumin-SnO_2 NPs on the MDA-MB-231 cell growth was investigated by the WST-1 technique at 24, 48 and 72 hr. The findings showed that

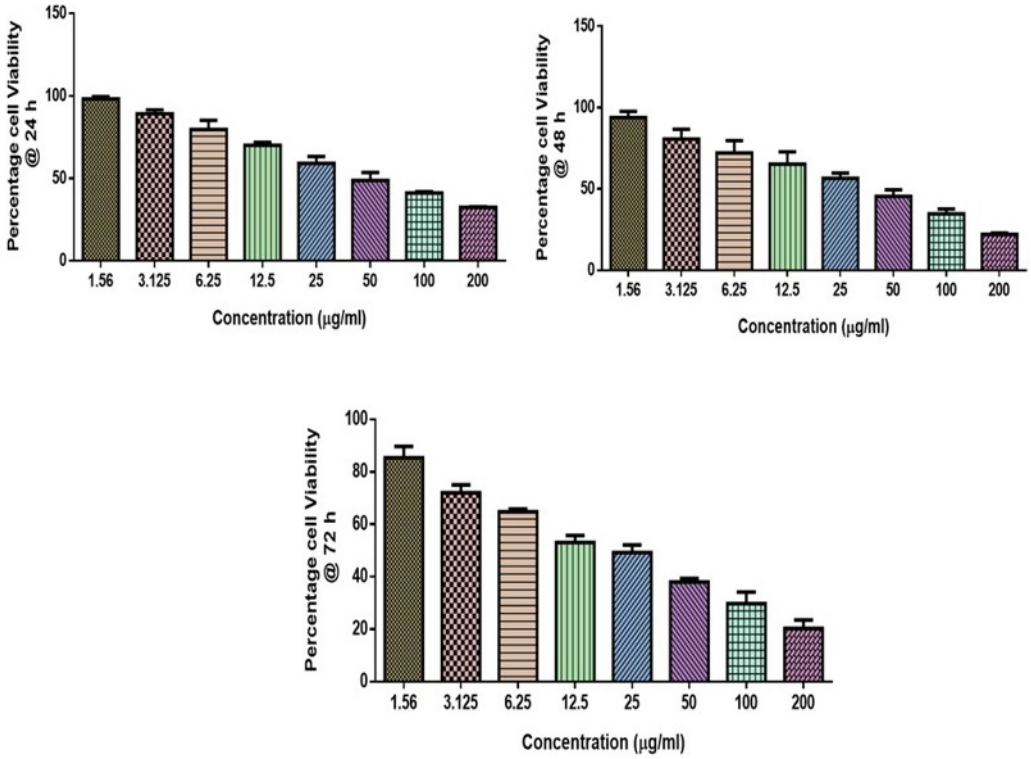

Figure 6: The effect of Curcumin-SnO_2 NPs treatment on MDA-MB-231 cell viability was assessed by the WST-1 cell viability assay. The MDA-MD-231 cells treated with various dosages (1.56-200 µg/mL) of Curcumin-SnO_2 NPs for 24, 48 and 72 hr showed a remarkable decrease in viability.

Curcumin-SnO_2 NPs treatment demonstrated dose-dependent and time-dependent inhibition of MDA-MB-231 cell growth. Apoptosis is a key cell death mechanism in cancer treatment, but abnormalities can lead to abnormal proliferation and division. Apoptotic cell shrinkage, chromatin aggregation and mRNA degradation.[27] Overactive or deficient pathways can cause unrestricted cell growth, therapy resistance and tumor recurrence. Maintaining a balance between cell growth and apoptosis is crucial for cancer prevention.[28]

The surface morphology is an important parameter for anticancer activity

Curcumin-SnO_2 NPs possess a distinctive nanoflake morphology characterized by high surface ridges, which exhibit enhanced affinity for cell surfaces. This unique structure results in increased cellular uptake and improved therapeutic effectiveness. The high surface ridges provide numerous contact points that facilitate the attachment of the Curcumin-SnO_2 nanoparticles to cell membranes, promoting more efficient internalization by the cells. Moreover, another critical factor influencing the effectiveness of these nanoparticles is their tendency to accumulate. This accumulation leads to the formation of particle clusters, which can significantly impact cellular uptake. The formation of clusters not only increases the overall surface area of the nanoparticles available for interaction with cancer cells. The larger surface area ensures more extensive contact with cancer cells, potentially improving the Curcumin-SnO_2 nanoparticles' ability to induce cell death and inhibit tumor growth.

CONCLUSION

The present study has found that synthesized Curcumin-SnO_2 NPs have a significant effect on inhibiting the viability of MDA-MB-231 cells. The Curcumin-SnO_2 NPs have also shown potential antibacterial effects against various pathogens. Hence, the findings suggest that Curcumin-SnO_2 NPs exhibit the potential to be a prospective therapeutic agent in the future. Nevertheless, further extensive analyses are still required to fully comprehend the various therapeutic roles of the Curcumin-SnO_2 NPs against cancer and other diseases.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

NPs: Nanoparticles; **SnO_2:** Tin oxide; **ROS:** Reactive oxygen species; **DMSO:** Dimethyl sulfoxide; **G+:** Gram-positive; **G-:** Gram-negative; **MDA-MB-231:** Breast cancer; **PL:** Photoluminescence; **XRD:** X-ray diffraction; **SEM:** Scanning electron microscopy; **MHA:** Muller-Hinton agar; **DMEM:** Dulbecco's modified Eagle's medium; **FBS:** Fetal bovine serum; ***B. subtilis*:** *Bacillus subtilis*; ***K. pneumonia*:** *Klebsiella pneumonia*.

SUMMARY

The present work was focused on synthesizing and characterizing the SnO_2-Curcumin NPs via *Pterocarpus marsupium* Extract for enhanced antimicrobial and anticancer effects against breast cancer cells. The synthesized SnO_2-Curcumin NPs were characterized using several methods, including UV-vis spectroscopy, XRD, SEM, EDX and Photoluminescence (PL) analyses. Also, the antimicrobial property of the disc diffusion method against various pathogens and WST-1 assay was done to assess the SnO_2-Curcumin NPs against MDA-MB-231 cells. The results of SnO_2-Curcumin NPs have a crystalline nature, clustered morphology and cuboidal structures. The Curcumin-SnO_2 NPs exhibited higher antimicrobial activity and inhibited the viability of breast cancer cells. These findings demonstrate that Curcumin-SnO_2 NPs are a promising therapeutic drug for the treatment of breast cancer cells.

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