Synthesis, characterization, and evaluation of chitosan nanoparticles: Antimicrobial activities and cytotoxicity impact on cosmetics

Mohamed N. Abd El-Ghany[1], Rawan A. El-Sheikh[2]

[1]*Botany and Microbiology Department, Faculty of Science, Cairo University, Egypt*
[2]*Faculty of Biotechnology, MSA University*

Cosmetic products are prone to microbial contamination, with studies showing that 40–70% of used cosmetics contain harmful fungi like *Penicillium* spp., *Aspergillus fumigatus*, and *Candida albicans*, posing infection risks, especially for immunocompromised individuals. This study aims to synthesize and characterize chitosan nanoparticles (CSNPs) to prevent microbial contamination in cosmetics and determine the minimum effective dose required for complete fungal inhibition. CSNPs were synthesized via chitin deacetylation and characterized using Transmission Electron Microscopy (TEM), Dynamic Light Scattering (DLS), Zeta potential analysis, and Fourier-transform infrared (FTIR). TEM confirmed spherical nanoparticles of 100–110 nm, while DLS showed a polydisperse system with a peak near 100 nm. Zeta potential analysis indicated around 25 mV surface charges, suggesting minimal electrostatic repulsion. FTIR had several peaks, the first at 663.23 $cm^{-1,}$ which represents C-H bending, alkynes. Bands appearing at 1643.14, and 3354.21 cm^{-1} represented C=C stretching, alkene, and N-H stretching of aliphatic primary amine. The antifungal efficacy of CSNPs was tested using a spore germination inhibitory assay against *Aspergillus flavus*, the most potent fungus from cosmetic samples. Germination decreased with increasing CS-NP concentration. The Minimum inhibitory concentration was at 25 µg/mL. CSNPs also showed an inhibitory effect against *Staphylococcus aureus*, *Streptococcus mutans*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae, Candida albicans*, and *Aspergillus niger* using the agar well diffusion method, showing inhibition zones comparable to standard antibiotics. TEM analysis of *Aspergillus flavus* spores revealed severe structural malformation, including cell wall damage, cytoplasmic leakage, and membrane collapse. Cytotoxicity assessment via MTT assay on HSF melanocyte cells demonstrated an IC50 of 30 µg/mL, indicating selective antimicrobial activity with minimal toxicity. These findings highlight CSNPs as a promising antimicrobial agent for cosmetics and biomedical applications.

Keywords: Chitosan nanoparticles, Antimicrobial activity, Cosmetic contamination, Minimum Inhibitory concentration, Cytotoxicity

ARTICLE HISTORY
Received: March 21, 2025
Revised: May 16, 2025
Accepted: May 18, 2025

CORRESPONDENCE TO
Mohamed N. Abd El-Ghany,
Botany and Microbiology Department, Faculty of Science, Cairo University
Email: Mabdelghany@sci.cu.edu.eg
DOI: 10.21608/nasj.2025.370274.1006

INTRODUCTION

Cosmetic and personal care product usage has heightened concerns regarding fungal microbial contaminations of these products. Most cosmetics do not come with expiration or production dates which allow users to continue using products past their recommended use period [1]. Cosmetic products support microbial growth because they contain organic compounds, inorganic substances as well as minerals and water content [2]. Giving cosmetics to others through sharing tools like brushes and lipsticks enables bacterial transfer of skin microflora and creates health risks mainly targeting individuals with weakened immune systems. Scientific studies demonstrate that approximately 70% of used cosmetics have been found to contain dangerous fungi including *Penicillium spp., Aspergillus fumigatus,* and *Candida albicans* that contribute to infections, allergic reactions, and respiratory diseases [3].

Microbes have adapted to chemical preservatives in cosmetics which has rendered them less successful as preservatives. Rising customer interest in chemically unadulterated cosmetics has prompted the industry to develop alternative antifungal agents [4]. Bio-nanotechnology provides an effective solution to combat microbial contaminants that affect cosmetics. Nanotechnology in cosmetics has become extensive because it improves the skin penetration of ingredients while extending stability and advancing delivery methods [5].

Chitosan-based nanoparticles stand out as an optimal choice among nanomaterials due to their unique combination of biodegradability, biocompatibility, and powerful antimicrobial properties, making them both safe for human use and environmentally friendly [6]. Chitosan nanoparticles have drawn scientific attention for their potent antimicrobial properties, derived from chitin [7].

Chitosan nanoparticles exhibit substantial antifungal properties that qualify them as superior preservative alternatives to conventional products. Their ability to interact closely with fungal cells enables small CSNPs to penetrate cell membranes which disrupts the growth cycle of fungi. CSNPs enable sustained microbial protection in cosmetics due to their controlled release abilities [8].

The antimicrobial properties of chitosan nanoparticles exceed those presented by other nanoparticles including zinc oxide and titanium dioxide. Chitosan nanoparticles stand apart from metal-based nanoparticles since they decompose within the body while avoiding hazardous cell toxicity [9]. The synthesis of chitosan nanoparticles occurs through different combinations of chemical, physical, and biological methods. Three primary production techniques for chitosan nanoparticles are ionic

gelation, microemulsion, and solvent evaporation. The synthesis techniques determine three critical aspects that affect both the size and electrical properties and long-term stability of nanoparticles [10].

To achieve effective and stable nanoparticles within cosmetic forms, professionals need to conduct suitable oxide characterization through Transmission Electron Microscopy, Dynamic Light Scattering, Zeta potential analysis, and Fourier-transform infrared spectroscopy [11]. The Minimum Inhibitory Concentration tests show that chitosan nanoparticles maintain strong antifungal properties when used at minimal dosages. Chitosan nanoparticles show promise as contamination-preventing agents for cosmetic products since they maintain product safety together with desired performance [12].

The usage of chitosan nanoparticles supports current trends because they present both ecological sustainability and efficient alternative solutions to synthetic preservatives. The challenges for the implementation of large-scale production of chitosan nanoparticles alongside formulation compatibility work alongside regulatory approval requirements. More investigation is necessary to integrate Chitosan nanoparticles into commercial cosmetics efficiently and ensure long-term stability when used in commercial cosmetic products [13].

The antimicrobial effects of CSNPs function through various mechanisms that begin when the compounds attach to microbial membranes by electrostatic forces which increases membrane permeability and emptied intracellular elements until cell death occurs. Oxidative stress occurs when secreted CSNPs create ROS which damages essential cellular components like lipids proteins and DNA. Accumulation of metal ions through their chelation ability acts as a dual mechanism to minimize microbial growth because it interferes with essential nutrient availability for microbes [14].

Studies demonstrate that chitosan nanoparticles show beneficial effects on toxic behavior by harming cancer cells without harming regular human cells. Surface modification of nanoparticles together with the chosen cell type affects their cytotoxic properties during testing. Research evidence supports CS-NP advancement as a biomedical tool for multiple applications along with the requirement to optimize nanoparticles for therapeutic success without compromising security [15].

MATERIALS AND METHODS

Cosmetics Sample Collection and Fungal Isolation

Cosmetics samples, including mascara, lipsticks, pen lipsticks, liquid formulations, and blush brushes, were collected from cosmetic testers in shopping malls. Swabs were stored in sterile containers and transported under control to the laboratory for further analysis. The swabbed samples were used to inoculate agar plates to facilitate the isolation of fungal species present in the cosmetics. The plates were prepared with a nutrient-rich medium suitable for fungal growth. Czapex Dox Agar medium was prepared according to [16] for isolating fungal species. Initially, 20g/L of sucrose, 1 g of K_2HPO_4, 2 g of $NaNO_3$, 0.5 g of $MgSO_4$, and 0.5 g of KCl were measured and added to a sterilized glass flask containing ethanol and H_2SO_4. Additionally, 0.01 g/L of $FeSO_4 \cdot 7H_2O$ and 15 g/L of agar were included, then the flasks were filled up to 30 mL of distilled water. The mixture was autoclaved at 121 °C and 1.5 bars for 15 minutes to ensure sterilization. After cooling, 15 mL of the solidified medium was poured into petri dishes, each labeled with the appropriate dilution factors, and each swab was streaked across the surface under aseptic conditions to prevent cross-contamination. Plates were then incubated at appropriate temperatures for a designated period to encourage the growth of fungal colonies. After incubation, the distinct fungal colonies were observed and isolated for further characterization and testing.

Synthesis of Chitosan Nanoparticles

Acetylated chitosan nanoparticles were synthesized using the ionotropic gelation process. All Glassware was cleaned and sanitized using aqua regia. To prepare 25 mL of chitosan solution, 100 mg of 0.4% ascorbic acid was mixed with 25 mL of deionized water. Additionally, 0.5 mL of 2% acetic acid was added to another 25 mL of deionized water to prepare another 25 mL of chitosan solution. Both solutions are magnetically stirred for one hour. The pH was adjusted to 4.8 using 0.5 M NaOH and agitated for 30 minutes. During this period, 1.5 mL of Tripolyphosphate solution (TPP) was introduced into each solution. The mixture was then centrifuged at 4,000 rpm and 4°C for one hour, then cooled before proceeding to physicochemical characterization according to [17].The concentration of chitosan nanoparticles prepared was 100 ppm.

Characterization of the Chitosan Nanoparticles

The diluted colloidal solution of chitosan nanoparticles was subjected to ultrasonication for five minutes to reduce particle aggregation. A Jeol JEM-1400 Transmission electron microscopy (TEM) was employed to examine the characterization of chitosan, including size, morphology. For analysis, a drop of the synthesized nanoparticle solution was placed on a grid, which was then secured in the specimen holder for TEM examination. Dynamic light scattering (DLS) was utilized to measure the size distribution using Zetasizer Nano- ZS equipped with a 633 nm laser. Fourier-transform infrared (FTIR) spectra of freeze-dried Chitosan nanoparticle were obtained by a Fourier Transform Infrared spectrometer (FTIR 6100). Spectra were collected in the range of 4000 to 400 cm−1 with a resolution of 4 cm^{-1}. The average particle size distribution was then analyzed to determine the charge and characteristics of the particles according to [17] [18].

The minimum inhibitory concentration (MIC)

The minimum inhibitory concentration is the lowest concentration that stops visible growth of a fungus after a set incubation time. The minimum inhibitory concentration was determined using a broth microdilution method to evaluate the antifungal activity of the synthesized chitosan nanoparticles and the spore germination was monitored using light microscope. A fungal spore suspension was prepared by harvesting fungal colonies grown on Czapek Dox Agar and adjusting the spore concentration to 2×10^6 CFU/mL using sterile saline. Chitosan nanoparticle solutions were serially diluted in a 96-well microplate, with final concentrations ranging from 0.0625 mg/mL to 8 mg/mL in 100 µL of nutrient broth. Each well was inoculated with 100 µL of the fungal suspension to achieve a final concentration of 5×10^5 CFU/mL. Positive controls contained fungal suspension without nanoparticles, while negative controls contained only nutrient broth. The plates were incubated at 30 °C for 16 hours, after which the MIC was identified as the lowest concentration of nanoparticles that inhibited visible fungal growth [19].

Leakage of proteins and DNA

The leakage of proteins and DNA from *A. flavus* cells was studied to investigate the probable mechanism of CSNPs affecting cells [20]. Spore suspension solutions of *A. flavus* were prepared in saline solution (NaCl, 0.9% w/v). The fungal spore suspensions were incubated for 24 h at 30 °C with CSNPs at their respective MIC values for spore germination inhibition. Control spore suspension sets (without CSNPs) were used as blanks. Spore suspension in each case was centrifuged for 15 min at 4000 rpm at 4 °C. The absorbance of the supernatant was read at 280 and 260 nm to estimate the proteins and DNA leaked from the cells.

Antimicrobial Activity Testing

The antimicrobial activity of chitosan nanoparticles (CSNPs) was evaluated using the agar well diffusion method [20]. Investigated fungal and bacterial strains, including *Staphylococcus aureus, Streptococcus mutans, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Candida albicans*, and *Aspergillus niger*, were tested using nutrient agar [20] and Czapeks dox agar media as previously mentioned. The prepared media were sterilized and poured into Petri dishes (20–25 mL per dish) and allowed to solidify at room temperature. A microbial suspension was prepared in sterilized saline equivalent to the McFarland 0.5 standard (1.5×10^5 CFU/mL), with turbidity adjusted to OD = 0.13 at 625 nm. Sterile cotton swabs were dipped into the adjusted suspension and spread evenly onto the dried agar surface. Wells of 6 mm diameter were created using a sterile borer, and 100 µL of CS-NP solution was added to each well. The plates were incubated at 37°C for 24 hours for bacterial strains and at suitable temperatures for fungal strains.

Following incubation, the zones of inhibition were measured in millimeters. Ampicillin and Gentamicin were used as standard antibiotics for Gram-positive and Gram-negative bacteria, respectively, while Nystatin was used as a standard antifungal drug. DMSO served as the negative control.

Cytotoxicity assay

The cytotoxicity of the prepared CSNPs was tested against the normal human cell line: human skin fibroblast cells (HSF). Cells were plated in a 6-multiwell plate (104 cells/well) for 24 h before the treatment with CSNPs to allow the attachment of the cells to the wells of the plate. Different concentrations (0, 1, 2, 3, 4, 5 and 6 µg/mL) were added to the cell monolayer, triplicate wells were composed for each dose. Monolayer cells were incubated with CSNPs at 37 °C for 48 h in an atmosphere of 5% CO_2. Cells were fixed, washed, and stained with Sulfo-Rhodamine-B after incubation. Acetic acid was used to wash the excess of stain. The attached stain was recovered using Tris-EDTA buffer. Microplate reader (Meter tech.

Σ 960, USA) was used to measure the color intensity [20]. The relation between cell survival (as a percentage of the control) and CSNPs concentration was plotted to get the survival curve of the normal human melanocytes (HSF cell line) after being subjected to CSNPs. Cell survival (%) was calculated as follows:

Survival (%) = (It/Ic) x 100

Where the color intensity of the treated cells and Ic is the color intensity of the control cells.

Statistical Analysis

Differences between samples were analyzed using one-way ANOVA, followed by Duncan's multiple comparisons test (SPSS version 22). Results were expressed as mean ± S.E., with statistical significance set at $p < 0.001$ for very high significance.

RESULTS

Isolation of fungi from different cosmetics samples

Among the fungi investigated from various cosmetics samples, the most abundant species is *Aspergillus flavus*, with a count of approximately 509. *Aspergillus niger* is the second most abundant, with a count of around 220. The remaining species, including *Candida albicanus, Alternaria alternata, Cladosporium sp, Rhizopus sp, fusarium oxysporum, and Penicillium chrysogenum,* have significantly lower counts, ranging from nearly zero to around 100 (Figure 1).

Characterization of the Chitosan Nanoparticles

TEM: The TEM test shows spherical chitosan nanoparticles with a relatively uniform size distribution. The measured diameters of the nanoparticles range from approximately 99.8 nm to 124 nm (Figure 2).

DLS: The graph illustrates the size distribution of chitosan nanoparticles. The nanoparticles exhibit a prominent peak centered around 100 nm (Figure 3).

Zeta Potential Distribution: Zeta potential measurement helps determine whether nanoparticles have a sufficient surface charge. The zeta potential distribution graph shows a single, sharp peak centered around 25 mV (Figure 4).

FTIR: FTIR spectra of chitosan nanoparticles had several peaks, the first at 663.23 cm−1 which represents C-H bending, alkynes. Bands appearing at, 1643.14, and 3354.21 cm−1 represented C=C stretching, alkene, and N-H stretching of aliphatic primary amine, respectively as shown in Table 1.

The effect of chitosan nanoparticles on fungal spores

In this experiment, the germination percentage was assessed at varying concentrations of chitosan nanoparticles to evaluate their inhibitory effects on fungal growth. The germination rate, plotted on the

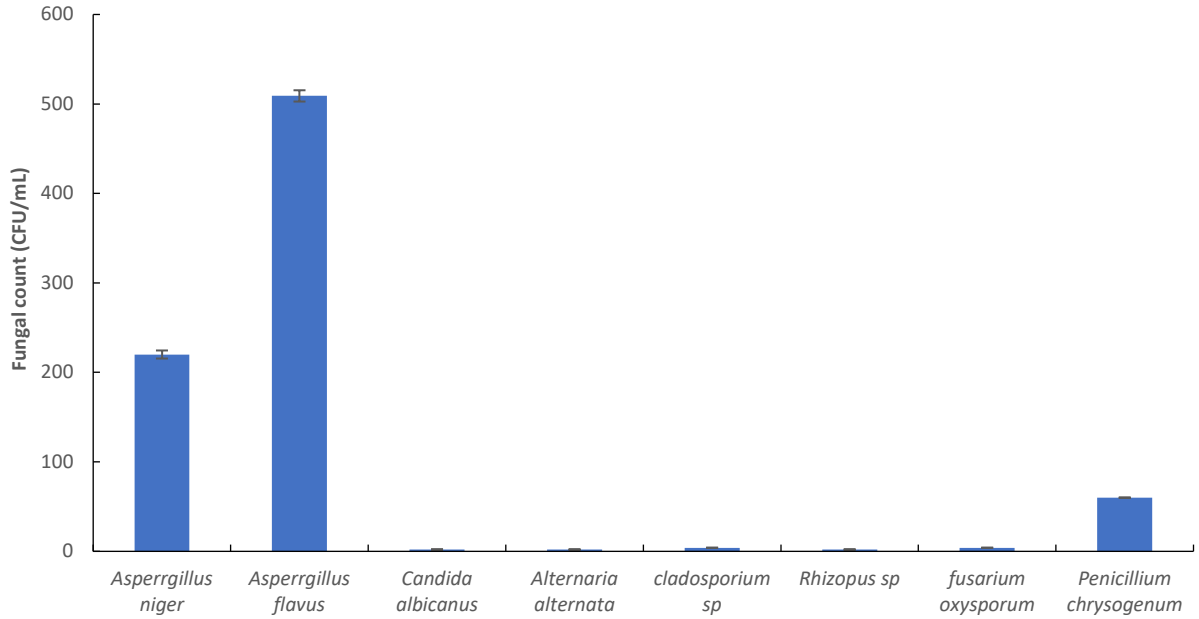

Figure 1. The figure shows the counts of different fungi species isolated from the cosmetics sample. Results are ± standard deviation

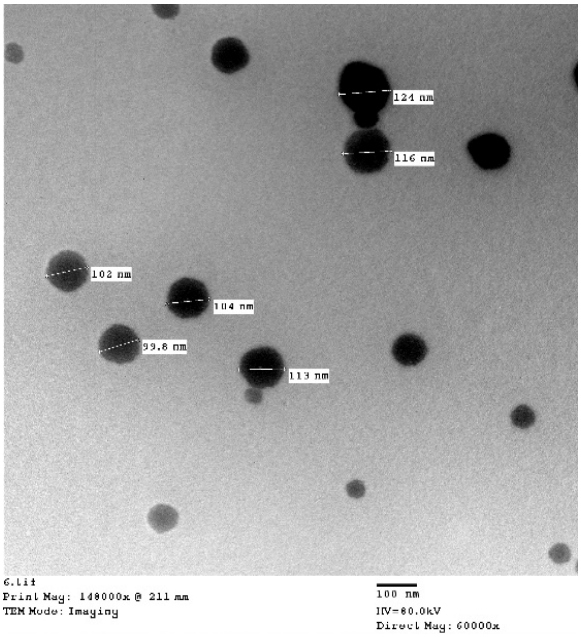

Figure 2. Transmission electron microscope of chitosan nanoparticles

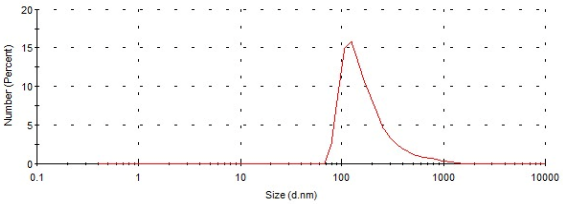

Figure 3. DLS of chitosan nanoparticles

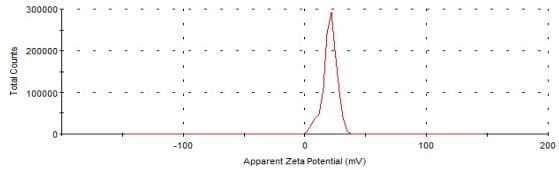

Figure 4. The zeta potential distribution of chitosan nanoparticles.

Table 1. FTIR spectra characteristics and functional groups.

Assignment	wavelength (cm^{-1})
C-H bending	663.23 cm^{-1}
C=C stretching	1643.14 cm^{-1}
N-H stretching	3354.21 m^{-1}

vertical axis, was observed to be 100% in the absence of chitosan nanoparticles. However, as the concentration of nanoparticles increased, the germination percentage declined. At concentrations of 25 µg/mL, the germination rate dropped to nearly 0% (Figure 5).

Leakage of protein and DNA

To demonstrate the effect of chitosan nanoparticles on *A. flavus* spores, the leakage of proteins and DNA was tested at wavelengths 280 and 260 nm, and the absorbance was 3 ± 0.41 and 2.7 ± 0.32 respectively.

Cytotoxicity assay

The cytotoxicity assay shows the effect of the sample on HSF cell line to determine the IC50 as shown in Figure 6. The IC50 was 30 (µg/mL).

Antimicrobial Activity Testing

The safe dose of chitosan nanoparticles was further tested against different bacteria (gram +ve and gram—ve) as well as some unicellular and filamentous fungi to verify its antimicrobial efficacy, as shown in Table 2.

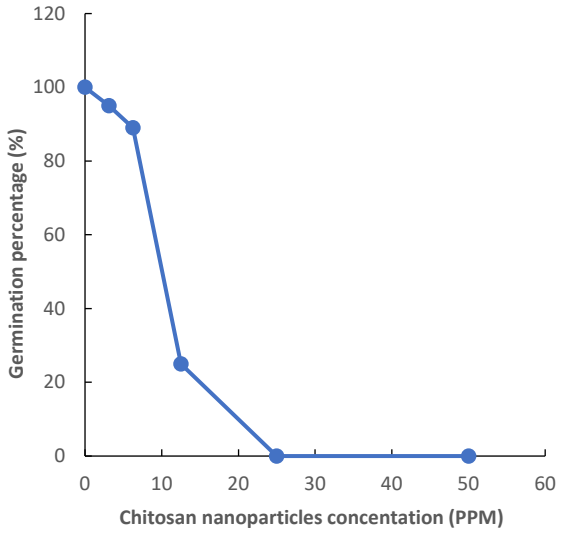

Figure 5. The effect of chitosan nanoparticles with different concentrations on the germination percentage of *A. flavus*

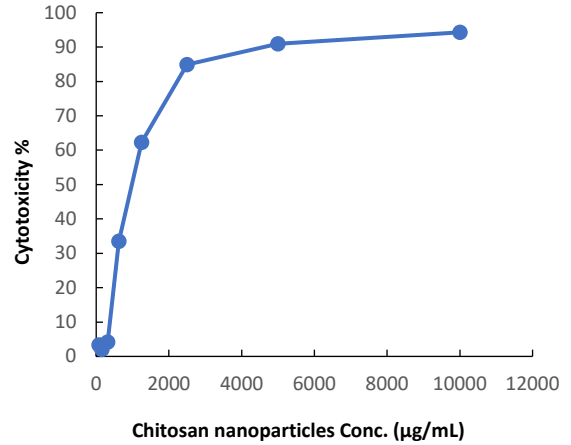

Figure 6. The figure shows cytotoxicity test of synthesized chitosan nanoparticles against HSF cells at different concentrations.

Table 2. Mean ± standard deviation (mm), with a well diameter of 6 mm and a tested volume of 100µl.

Sample Microorganism	Inhibition zone (mm)	Standard antibiotic
Gram negative bacteria		Gentamicin
Escherichia coli (ATCC:10536)	25±1.2	27±1.0
Klebsiella pneumonia (ATCC:10031)	26.4±1.1	25.3 ±0.6
Gram positive bacteria		Ampicillin
Staphylococcus aureus (ATCC:13565)	20.9±0.8	21.3±0.6
Streptococcus mutans (ATCC:25175)	27.5±0.5	28.3±0.6
Fungi		Nystatin
Candida albicans (ATCC:10231)	20.6±0.8	21.7±0.6
Aspergillus niger (ATCC:16404)	18.9±0.4	19.3±0.6

DISCUSSION

The fungal isolation from various cosmetic samples revealed a significant presence of microbial contamination, emphasizing the need for effective antimicrobial agents in cosmetic formulations. Among the fungal species identified, *Aspergillus flavus* was the most abundant, with a count of approximately 509 isolates, followed by *Aspergillus niger* with 220 isolates, and other species present in lower quantities. These findings underscore the high risk of microbial contamination in cosmetic products, which may pose health hazards, particularly to immunocompromised individuals, which is in alignment with other studies as those in 2023 by Alshehrei [1] who proved that microbial contamination was detected in approximately 70% to 90% of the cosmetic products. Fungi, which can act as pathogenic organisms, were particularly prevalent in low-quality cosmetic items, with *Aspergillus*, *Rhizopus*, and *Penicillium* being isolated. Among these, *Aspergillus* emerged as the most identified fungal genus.

Chitosan nanoparticles (CSNPs) were synthesized using the ionotropic gelation method and characterized through various techniques to mitigate this contamination. Transmission Electron Microscopy (TEM) analysis revealed that the CSNPs were spherical, with diameters ranging between 99.8 nm and 124 nm, within the optimal size range for effective antimicrobial activity. Dynamic Light Scattering (DLS) measurements showed a polydisperse system with a major peak centered around 100 nm, confirming the uniformity of the nanoparticle distribution. Zeta potential analysis demonstrated a peak at approximately 25 mV, indicating a moderately stable nanoparticle formulation that prevents aggregation while maintaining bioactivity. The FTIR spectral analysis of chitosan nanoparticles confirmed the presence of key functional groups responsible for their bioactivity. The peak at 663.23 cm^{-1} indicates C–H bending, suggesting hydrocarbon components, while the 1643.14 cm^{-1} band corresponds to C=C stretching, confirming unsaturated bonds likely involved in nanoparticle stability. The broad peak at 3354.21 cm^{-1} is characteristic of N–H stretching, typical of primary amines, which play a critical role in the antimicrobial activity of chitosan by facilitating interactions with microbial cell membranes. These findings validate the successful formation and functional integrity of the chitosan nanoparticles. These characterization results align with previous studies [21][18], which reported that Transmission Electron Microscopy (TEM) revealed spherical particles ranging in size from 0.78–1.15 nm up to 60–120 nm for CSNPs loaded with 9 mg/mL of metabolites. Dynamic Light Scattering (DLS) showed a size increase from 63.1 ± 13.87 nm to 175.1 ± 51.67 nm (9 mg/mL). Zeta potential analysis indicated strong positive surface charges across all samples, increasing from +41.1 ± 3.35 mV to +52.6 ± 3.75 mV with higher metabolite concentrations, suggesting good colloidal stability. FTIR spectra of chitosan nanoparticles had several peaks, the first at 661.46 cm^{-1} which represents C-H bending, alkynes. Bands appearing at, 1645.84, and 3356.60 cm^{-1} represented C=C stretching, alkene, and N-H stretching of aliphatic primary amine, which contribute to the bioactivity and stability of the nanoparticles. These results highlight how increasing metabolite concentration alters CSNP structure and enhances their antifungal effectiveness. Further insights into the antifungal properties of CSNPs were obtained through germination inhibition assays. The results indicated a strong dose-dependent suppression of fungal spore germination, particularly in *Aspergillus flavus*. At concentrations above 25 µg/mL, germination was almost entirely inhibited, suggesting that CSNPs effectively prevent the early stages of fungal development. These results align with previous studies [22], which reported that CSNPs at 31.6 µg/mL concentrations were capable of completely inhibiting fungal growth. The ability of CSNPs to prevent germination is particularly important for cosmetic applications, as it can help reduce the persistence and spread of fungal contamination in stored products.

The structural effects of CSNPs on fungal spores were further examined using Transmission Electron Microscopy (TEM). Leakage assays were conducted to assess membrane integrity following CSNP treatment. The results demonstrated a substantial release of intracellular contents, including DNA and proteins, indicating severe membrane damage. The absorbance readings at 260 nm (2.27±0.32) confirmed DNA leakage, while readings at 280 nm (3.00±0.41) confirmed protein leakage. These findings strongly suggest that CSNPs induce permeability changes in fungal and bacterial cell membranes, ultimately leading to cell lysis. Before treatment, the fungal spores exhibited intact cell walls and well-defined cytoplasmic structures, indicating normal cellular integrity. However, after treatment with CSNPs, the TEM images showed severe structural damage, including cell wall disruption, cytoplasmic leakage, and membrane collapse. These observations strongly suggest that CSNPs interfere with fungal cell integrity, leading to cell death.

The observed damage aligns with the results of the leakage assay, which confirmed the release of intracellular components such as DNA and proteins, indicating increased membrane permeability. This mechanism of action is consistent with previous studies [23], which demonstrated that the positively charged CSNPs interact with negatively charged microbial membranes, causing destabilization and leakage of essential cellular contents. The ability of CSNPs to disrupt fungal structures at the nanoscale level reinforces their potential as an effective antifungal agent.

The structural disintegration of fungal spores, particularly *Aspergillus flavus*, were highly susceptible to CSNPs at concentrations exceeding 25 µg/mL. The combination of germination inhibition, intracellular leakage, and direct structural damage highlights the multifaceted antimicrobial activity of CSNPs. These findings confirm that CSNPs prevent fungal growth and induce irreversible cellular damage, making them a promising alternative to conventional antifungal agents in cosmetic formulations.

The antimicrobial activity of CSNPs was evaluated using the agar well diffusion method, demonstrating significant inhibition against bacterial and fungal pathogens. For Gram-negative bacteria, *Escherichia coli* exhibited an inhibition zone of 25±1.2 mm, comparable to the 27±1.0 mm inhibition observed with Gentamicin. Similarly, *Klebsiella pneumoniae* showed an inhibition zone of 26.4±1.1 mm, slightly exceeding the 25.3±0.6 mm inhibition of the standard antibiotic. In the case of Gram-positive bacteria, *Staphylococcus aureus* and *Streptococcus mutans* displayed inhibition zones of 20.9±0.8 mm and 27.5±0.5 mm, respectively, which were like the effects of Ampicillin (21.3±0.6 mm and 28.3±0.6 mm, respectively). Regarding antifungal activity, *Candida albicans* (20.6±0.8 mm) and *Aspergillus niger* (18.9±0.4 mm) exhibited inhibition zones close to those of Nystatin (21.7±0.6 mm and 19.3±0.6 mm, respectively). These findings highlight the broad-spectrum antimicrobial potential of CSNPs, positioning them as a viable alternative to conventional preservatives in cosmetic formulations.

The cytotoxicity of chitosan nanoparticles (CSNPs) was assessed in HSF cells using the MTT viability assay, yielding an IC50 value of 30 µg/mL. This indicates a moderate, dose-dependent cytotoxic effect, suggesting that CSNPs may be safe for use within a specific concentration range. The observed cytotoxicity, while within an acceptable threshold, highlights the need for further optimization of nanoparticle formulations to ensure an appropriate balance between antimicrobial efficacy and biocompatibility for potential cosmetic applications [24]. Comparative studies have reported varying IC50 values for CSNPs across different cell lines. For instance, a study demonstrated that chitosan nanoparticles exhibited an IC50 value of 5.3 µg/mL against human gastric carcinoma MGC803 cells after 48 hours of treatment, indicating higher sensitivity of cancerous cells to CSNPs [25]. In contrast, a study on fibroblast 3T3 cells showed that even at a high concentration of 4 mg/mL, chitosan and chito-oligosaccharides (COS) resulted in 67.2% and 78.5% cell viability, respectively, with no detectable IC50, indicating low cytotoxicity and good biocompatibility with normal cells [26]. Additionally, studies on normal human gingival fibroblasts reported that unloaded CSNPs did not induce cytotoxic effects at concentrations up to 600 µg/mL and rather promoted cell proliferation. These findings suggest that CSNPs exhibit selective cytotoxicity, being more toxic to cancerous cells while maintaining biocompatibility with normal human cells [27]. Differences between samples were analyzed using one-way ANOVA, followed by Duncan's multiple comparisons test (SPSS version 22). Results were expressed as mean ± S.E., with statistical significance set at $p < 0.001$ for very high significance. These findings underscore the importance of tailoring CSNP formulations to enhance their therapeutic efficacy while ensuring safety for

normal human cells, particularly in cosmetic and pharmaceutical applications.

Chitosan nanoparticles (CSNPs) exhibit notable antimicrobial properties and biocompatibility, positioning them as promising alternatives to conventional synthetic preservatives like parabens and phenoxyethanol. CSNPs have demonstrated efficacy against a broad spectrum of microorganisms, including bacteria and fungi, making them suitable for applications in cosmetics and pharmaceuticals. Their biocompatibility and biodegradability further enhance their appeal as natural preservatives [28]. In contrast, synthetic preservatives such as parabens and phenoxyethanol have raised health concerns due to their potential endocrine-disrupting effects. Studies have indicated that parabens can mimic estrogen, potentially leading to hormonal imbalances and other health issues. Similarly, phenoxyethanol has been associated with skin irritation and nervous system effects in infants. These concerns have led to increased scrutiny and regulatory restrictions on the use of such synthetic preservatives in personal care products [29].

Given these factors, CSNPs offer a safer and more environmentally friendly alternative to traditional synthetic preservatives, aligning with the growing consumer demand for natural and sustainable cosmetic ingredients. The antimicrobial capacity of CSNPs demonstrates promising properties, but analysts need to conduct additional research due to known limitations. These limitations must be addressed before their widespread application in cosmetics. One of the primary concerns is cytotoxicity, as high concentrations of CSNPs have shown dose-dependent toxic effects on human cells, requiring careful optimization to ensure safety, especially for long-term use on skin [30]. Additionally, CSNPs may suffer from stability issues under varying environmental conditions, such as temperature and pH, which could impact their shelf life and effectiveness [31]. Their nanoscale size also raises skin penetration and systemic absorption concerns, warranting further investigation into their biodistribution and potential side effects [32]. Addressing these limitations through targeted research, such as surface modification, controlled-release systems, and comprehensive vivo testing, will be essential to harness the full potential of CSNPs in cosmetic formulations [33]. Chitosan nanoparticles are made from natural polymer which can be easily functionalized to obtain the desired targeted results and is also approved by GRAS (Generally Recognized as Safe by the United States Food and Drug Administration) [34-35]. Further investigation of how CSNPs work with standard antimicrobial treatment agents might produce knowledge about novel joint treatment methods for superior microbial management. The field needs research that tests CSNPs through safety and efficacy evaluation within actual cosmetic formulations to determine their practical utilization in cosmetics. The cosmetic industry can implement CSNPs as efficient, sustainable preservatives by solving remaining implementation issues.

CONCLUSION

In conclusion, the results of this study support the potential of CSNPs as an effective antimicrobial agent for cosmetic applications. The potent inhibitory effects against bacteria and fungi and a moderate cytotoxic profile suggest that CSNPs can be a viable alternative to conventional cosmetic preservatives. The antimicrobial mechanisms, including membrane disruption and intracellular leakage, align with existing literature, further validating the efficacy of CSNPs. However, variations in cytotoxicity across studies emphasize the importance of optimizing nanoparticle properties to enhance safety and efficacy. Future research should focus on improving the stability and biocompatibility of CSNPs while ensuring their effectiveness against microbial contamination. Additionally, regulatory considerations must be addressed to facilitate their commercialization. Given the increasing demand for natural and sustainable preservatives, CSNPs hold significant promise as a next-generation antimicrobial agent for the cosmetic industry.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding this study.

REFERENCES

[1] Alshehrei, F. (2023). Isolation and Identification of Microorganisms associated with high-quality and low-quality cosmetics from different brands in Mecca region -Saudi Arabia. *Saudi Journal of Biological Sciences*, *30*(12), 103852. https://doi.org/10.1016/j.sjbs.2023.103852

[2] Martins, A., & Marto, J. (2023). A sustainable life cycle for cosmetics: From design and development to post-use phase. *Sustainable Chemistry and Pharmacy*, *35*, 101178. https://doi.org/10.1016/j.scp.2023.101178

[3] Osilo, C., Okoli, U., Ekegbo, C., Uche-Anebonam, V., & Nwachinemere, N.(2023). Evaluation of microbial risk associated with the use of shared makeup applicators

and the rate of hygiene practices among beauticians. *World Journal of Biology Pharmacy and Health Sciences*, *16*(2), 134–141. https://doi.org/10.30574/wjbphs.2023.16.2.0474

[4] Głaz, P., Rosińska, A., Woźniak, S., Boguszewska-Czubara, A., Biernasiuk, A., & Matosiuk, D. (2023). Effect of Commonly Used Cosmetic Preservatives on Healthy Human Skin Cells. *Cells*, *12*(7), 1076. https://doi.org/10.3390/cells12071076

[5] Nhani, G., Di Filippo, L., De Paula, G., Mantovanelli, V., Da Fonseca, P., Tashiro, F., Monteiro, D., Duarte, J., & Chorilli, M. (2024). High-Tech Sustainable Beauty: Exploring Nanotechnology for the Development of Cosmetics Using Plant and Animal By-Products. *Cosmetics*, *11*(4), 112. https://doi.org/10.3390/cosmetics11040112

[6] Korniienko, V., Husak, Y., Diedkova, K., Varava, Y., Grebnevs, V., Pogorielova, O., Bērtiņš, M., Korniienko, V., Zandersone, B., Ramanaviciene, A., Ramanavicius, A., & Pogorielov, M. (2023). Antibacterial Potential and Biocompatibility of Chitosan/Polycaprolactone Nanofibrous Membranes Incorporated with Silver Nanoparticles. *Polymers*, *16*(12), 1729. https://doi.org/10.3390/polym16121729

[7] Mawazi, S., Kumar, M., Ahmad, N., & Mahmood, S. (2023). Recent Applications of Chitosan and Its Derivatives in Antibacterial, Anticancer, Wound Healing, and Tissue Engineering Fields. *Polymers*, *16*(10), 1351. https://doi.org/10.3390/polym16101351

[8] N S, S., Pillai, D., & Shanmugam, R. (2024). The Antifungal Activity of Chitosan Nanoparticle-Incorporated Probiotics Against Oral Candidiasis. *Cureus*, *16*(9), e70093. https://doi.org/10.7759/cureus.70093

[9] Wathoni, N., Herdiana, Y., Suhandi, C., Abdelwahab Mohammed, A. F., El-Rayyes, A., & Narsa, A. C. (2024). Chitosan/Alginate-Based Nanoparticles for Antibacterial Agents Delivery. *International Journal of Nanomedicine*, *19*, 5021. https://doi.org/10.2147/IJN.S469572

[10] Akdaşçi, E., Duman, H., Eker, F., Bechelany, M., & Karav, S. (2024). Chitosan and Its Nanoparticles: A Multifaceted Approach to Antibacterial Applications. *Nanomaterials*, *15*(2), 126. https://doi.org/10.3390/nano15020126

[11] Jagadeesh, P., Rangappa, S., & Siengchin, S. (2023). Advanced characterization techniques for nanostructured materials in biomedical applications. *Advanced Industrial and Engineering Polymer Research*, *7*(1), 122-143. https://doi.org/10.1016/j.aiepr.2023.03.002

[12] Iqbal, N., Shoaib, A., Fatima, Q., Farah, M., & Raja, V. (2024). Synthesis and antifungal efficacy of chitosan nanoparticles against notorious mycotoxigenic phytopathogens. *Plant Stress*, *14*, 100614. https://doi.org/10.1016/j.stress.2024.100614

[13] Desai, N., Rana, D., Salave, S., Gupta, R., Patel, P., Karunakaran, B., Sharma, A., Giri, J., Benival, D., & Kommineni, N. (2023). Chitosan: A Potential Biopolymer in Drug Delivery and Biomedical Applications. *Pharmaceutics*, *15*(4), 1313. https://doi.org/10.3390/pharmaceutics15041313

[14] Qadeer, A., Khan, A., Khan, N., Wajid, A., Ullah, K., Skalickova, S., Chilala, P., Slama, P., Horky, P., Alqahtani, M., & Alreshidi, M. (2024). Use of nanotechnology-based nanomaterial as a substitute for antibiotics in monogastric animals. *Heliyon*, *10*(11), e31728. https://doi.org/10.1016/j.heliyon.2024.e31728

[15] Zoe, L., David, S., & Rajabalaya, R. (2023). Chitosan nanoparticle toxicity: A comprehensive literature review of in vivo and in vitro assessments for medical applications. *Toxicology Reports*, *11*, 83-106. https://doi.org/10.1016/j.toxrep.2023.06.012

[16] Watanabe, T. (2002). Pictorial Atlas of Soil and Seed Fungi. In CRC Press eBooks. Informa. https://doi.org/10.1201/9781420040821

[17] Fan, W., Yan, W., & Ni, H. (2012). Formation mechanism of monodisperse, low molecular weight chitosan nanoparticles by ionic gelation technique. *Colloids and surfaces. B, Biointerfaces*, *90*, 21–27. https://doi.org/10.1016/j.colsurfb.2011.09.042

[18] Abdel-Nasser, A., Fathy, H. M., Badr, A. N., Barakat, O. S., & Hathout, A. S. (2025). Chitosan nanoparticles loaded with Lactobacillus rhamnosus bioactive metabolites: Preparation, characterization, and antifungal activity. *Heliyon*, *11*(2).https://doi.org/10.1016/j.heliyon.2025.e41875

[19] Abo El-Ela, F., Hassan, W., Amer, A., & El-Dek, S. (2023). Antifungal Activity of Chitosan Polymeric Nanoparticles and Correlation with Their pH Against Mucor circinelloides Causing Mucormycosis, Along with Penicillium notatum and Aspergillus Species. *Current Microbiology*, *81*(1), 47. https://doi.org/10.1007/s00284-023-03555-

[20] Khalil, N., Abd El-Ghany, M., & Rodríguez-Couto, S. (2019). Antifungal and anti-mycotoxin efficacy of biogenic silver nanoparticles produced by *Fusarium chlamydosporum* and *Penicillium* chrysogenum at non-cytotoxic doses. *Chemosphere*, *218*, 477-486. https://doi.org/10.1016/j.chemosphere.2018.11.129

[21] Wael, K., Abdelgawad, A., Elsherbiny, D., & El-Naggar, M. (2024). Chitosan nanoparticles for antimicrobial applications. Fundamentals *and Biomedical Applications of Chitosan Nanoparticles*, 363-404. https://doi.org/10.1016/B978-0-443-14088-4.00001-0

[22] Abo El-Ela, F., Hassan, W., Amer, A., & El-Dek, S. (2023). Antifungal Activity of Chitosan Polymeric Nanoparticles and Correlation with Their pH Against Mucor circinelloides Causing Mucormycosis, Along with Penicillium notatum and Aspergillus Species.

Current Microbiology, *81*(1), 47. https://doi.org/10.1007/s00284-023-03555-y

[23] Ing, L., Zin, N., Sarwar, A., & Katas, H. (2012). Antifungal activity of chitosan nanoparticles and correlation with their physical properties. *International journal of biomaterials*, *2012*, 632698. https://doi.org/10.1155/2012/632698

[24] Rashidipour, M., Abbaszadeh, S., Birjandi, M., Pajouhi, N., Ahmadi, S., Goudarzi, G., Shahryarhesami, S., Moradi Sarabi, M., & Babaeenezhad, E. (2024). Antimicrobial activity and cytotoxic and epigenetic effects of tannic acid-loaded chitosan nanoparticles. *Scientific Reports*, *14*(1), 1-12. https://doi.org/10.1038/s41598-024-80771-x

[25] Qi, L. F., Xu, Z. R., Li, Y., Jiang, X., & Han, X. Y. (2005). In vitro effects of chitosan nanoparticles on proliferation of human gastric carcinoma cell line MGC803 cells. *World Journal of Gastroenterology: WJG*, *11*(33), 5136. https://doi.org/10.3748/wjg.v11.i33.5136

[26] Thirukanthan, C. S., NurIslamiah, K. M., Sung, Y. Y., Sifzizul, T. S. M., & Effendy, A. W. M. (2021). Characterization and cytotoxicity of low-molecular-weight chitosan and chito-oligosaccharides derived from tilapia fish scales. *Journal of Advanced Pharmaceutical Technology & Research*, *12*(4), 373-377.https://doi.org/10.4103/japtr.japtr_117_21

[27] Frigaard, J., Jensen, J. L., Galtung, H. K., & Hiorth, M. (2022). The Potential of Chitosan in Nanomedicine: An Overview of the Cytotoxicity of Chitosan Based Nanoparticles. *Frontiers in pharmacology*, *13*, 880377. https://doi.org/10.3389/fphar.2022.880377

[28] Bakshi, P. S., Selvakumar, D., Kadirvelu, K., & Kumar, N. S. (2018). Comparative study on antimicrobial activity and biocompatibility of N-selective chitosan derivatives. *Reactive and Functional Polymers*, *124*, 149-155. https://doi.org/10.1016/j.reactfunctpolym.2018.01.016

[29] Chatterjee, S., Adhikary, S., Bhattacharya, S., Chakraborty, A., Dutta, S., Roy, D., ... & Rajak, P. (2024). Parabens as the double-edged sword: Understanding the benefits and potential health risks. *Science of The Total Environment*, 176547. https://doi.org/10.1016/j.scitotenv.2024.176547

[30] Jafernik, K., Ładniak, A., Blicharska, E., Czarnek, K., Ekiert, H., Wiącek, A. E., & Szopa, A. (2022). Chitosan-Based Nanoparticles as Effective Drug Delivery Systems—A review. *Molecules*, *28*(4), 1963. https://doi.org/10.3390/molecules28041963

[31] Rampino, A., Borgogna, M., Blasi, P., Bellich, B., & Cesàro, A. (2013). Chitosan nanoparticles: preparation, size evolution and stability. *International journal of pharmaceutics*, *455*(1-2), 219–228. https://doi.org/10.1016/j.ijpharm.2013.07.034

[32] Rahman, M., & Mondal, M. (2024). Stability, challenges, and prospects of chitosan for the delivery of anticancer drugs and tissue regenerative growth factors. *Heliyon*, *10*(21), e39879. https://doi.org/10.1016/j.heliyon.2024.e39879

[33] Costa, E., Silva, S., & Pintado, M. (2022). Chitosan Nanoparticles Production: Optimization of Physical Parameters, Biochemical Characterization, and Stability upon Storage. *Applied Sciences*, *13*(3), 1900. https://doi.org/10.3390/app13031900

[34] Pangestuti, R., Kim, SK. (2010). Neuroprotective properties of chitosan and its derivatives. Mar Drugs. 8(7):2117–28. doi: 10.3390/md8072117.

[35] El-Basiouny, N.M.,Soliman, S.M.,Khalil, N.M., Abd El-Ghany, M.N. (2025). Chitosan and alginate/*Aspergillus flavus*-mediated nanocomposite films for preservation of postharvest tomatoes Int. J. Biol. Macromol., 297, Article 139559.