Research Article

Evaluation of chitosan NPs loaded with *Camellia sinensis* (L.) Kuntze extract for targeted therapy against *Pseudomonas aeruginosa*-associated SKOV3 ovarian cancer cells

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Abstract

The emergence of multidrug-resistant bacteria, such as *Pseudomonas aeruginosa*, poses significant challenges in treating infections and associated cancers like ovarian cancer. Novel therapeutic strategies that simultaneously address bacterial resistance and cancer progression are urgently needed. This study evaluates the therapeutic efficacy of chitosan nanoparticles (CNPs) loaded with *Camellia sinensis* extract against *Pseudomonas aeruginosa*-associated SKOV3 ovarian cancer cells. CNPs were synthesized and characterized for size, morphology, and encapsulation efficiency. Cytotoxicity was evaluated using the MTT assay on SKOV3 cells. The antibacterial activity of the CNPs was assessed through biofilm inhibition and bacterial load reduction assays. Inflammatory and oxidative stress markers, including TNF-α, IL-6, MDA, CAT, and SOD, were measured. Apoptosis was confirmed through caspase-3 activity analysis. CNPs loaded with *Camellia sinensis* extract exhibited optimal physicochemical properties. Cytotoxicity assays demonstrated significant inhibition of SKOV3 cell viability, with low IC$_{50}$ values. Antibacterial studies revealed enhanced efficacy in reducing biofilm formation and bacterial growth. Treatment significantly reduced inflammatory markers (TNF-α, IL-6, MDA) and elevated antioxidant enzymes (CAT, SOD). Apoptosis induction was confirmed by increased caspase-3 activity. The synergistic anticancer and antibacterial effects of the extract loaded CNPs outperformed both extract alone and standard treatments. Extract-loaded CNPs demonstrate potent dual action against *Pseudomonas aeruginosa* and SKOV3 ovarian cancer cells, positioning them as a promising therapeutic option for combating infection-driven cancers.

1. Introduction

Ovarian cancer remains a significant global health concern, marked by its high mortality rates and often late diagnosis, primarily due to its asymptomatic nature during the early stages of development [1]. The World Health Organization estimates that in 2020, there were approximately 313,959 new cases of ovarian cancer worldwide, with around 207,252 deaths, underscoring the urgent need for effective prevention, early detection, and therapeutic strategies [2]. This malignancy is not only challenging due to its complex biological behavior but also due to the lack of reliable early screening methods. As a result, many patients are diagnosed at an advanced stage, where treatment options become limited, and the prognosis

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is poor.

Among the various risk factors contributing to the development of ovarian cancer, infections caused by opportunistic pathogens have garnered increasing attention for their potential role in cancer pathogenesis [3]. One such pathogen, *Pseudomonas aeruginosa*, is a versatile and pathogenic bacterium associated with a range of infections, particularly in immunocompromised individuals. This bacterium is known for its ability to thrive in diverse environments and its resistance to multiple antibiotics, making it a significant concern in healthcare settings. Emerging evidence suggests that *Pseudomonas aeruginosa* may contribute to chronic inflammation and tumor progression, acting as a potential cofactor in the development of ovarian cancer [4]. The mechanisms through which this pathogen influences cancer development is complex and multifaceted, involving the release of virulence factors, modulation of host immune responses, and promotion of an inflammatory microenvironment that can facilitate tumor growth and metastasis. These insights highlight the pressing need for innovative therapeutic strategies that can effectively target both the pathogen and the cancer cells.

Recent advances in nanotechnology have opened new avenues for targeted drug delivery systems, enhancing the efficacy of anticancer therapies while minimizing systemic side effects [5]. Chitosan nanoparticles (CNPs), derived from chitosan—a biopolymer obtained from chitin—have shown significant promise in drug delivery applications due to their biocompatibility, biodegradability, and ability to encapsulate a variety of therapeutic agents [6]. The unique properties of CNPs facilitate the targeted delivery of bioactive compounds, allowing for localized treatment that can improve therapeutic outcomes while reducing adverse effects associated with conventional therapies. The nanoscale size of these particles enables them to penetrate biological barriers effectively, enhancing the accumulation of therapeutic agents at the tumor site. Furthermore, CNPs can be engineered to respond to specific stimuli, providing a platform for controlled drug release that can be tailored to meet the needs of individual patients [7].

Camellia sinensis, commonly known as green tea, is renowned for its rich polyphenolic content, particularly catechins, which possess potent antioxidant, anti-inflammatory, and anticancer properties [8]. Numerous studies have demonstrated that green tea extracts exhibit inhibitory effects on various cancer cell lines, including ovarian cancer. The primary catechin, epigallocatechin gallate (EGCG), has garnered significant attention for its ability to induce apoptosis, inhibit cell proliferation, and suppress metastasis in various cancer cells [9]. The multifaceted action of EGCG makes it a compelling candidate for incorporation into targeted therapies for cancer treatment. However, the clinical application of green tea extracts is often limited by their low bioavailability and rapid metabolism. By encapsulating green tea extract within chitosan nanoparticles, it is possible to enhance the bioavailability and stability of its active compounds, potentially maximizing their therapeutic effects and ensuring sustained release at the target site [10].

This study aims to evaluate the efficacy of chitosan nanoparticles loaded with *Camellia sinensis* extract as a targeted therapeutic approach against *Pseudomonas aeruginosa*-associated ovarian cancer. The primary objectives include the characterization of the chitosan nanoparticles, assessment of their cytotoxic effects on ovarian cancer cells, and examination of their potential to mitigate the inflammatory responses induced by *Pseudomonas aeruginosa*. By elucidating the interactions between the encapsulated green tea extract and cancer cells, as well as assessing the antibacterial properties of the nanoparticles, this research endeavors to highlight the potential of CNPs as a novel delivery system for natural products in cancer therapy.

Through this investigation, we aspire to contribute to the development of more effective and targeted therapies for ovarian cancer patients. The outcomes of this study may offer significant insights into the integration of natural products and nanotechnology in cancer treatment, paving the way for future advancements in oncological therapies. This innovative approach holds promise not only for enhancing the therapeutic index of existing agents but also for providing a multi-faceted strategy to combat both bacterial infections and cancer progression, thereby addressing two critical challenges in

contemporary medical practice.

2. Materials and methods

2.1. Materials

Chitosan: Chitosan was obtained from [Supplier/Manufacturer] and characterized for its degree of deacetylation and viscosity. *Camellia sinensis* extract: Dried green tea leaves were sourced from Mambila Beverages Ltd, Taraba and prepared as an extract using cold maceration. The extract was then concentrated and stored at 4 °C until use. *Pseudomonas aeruginosa*: The clinical isolate of *Pseudomonas aeruginosa* was obtained from ATCC and cultured on Mueller-Hinton agar for subsequent experiments. *Ovarian cancer cell line:* Human ovarian cancer cell line SKOV3 was obtained from American Type Culture Collection (ATCC)] and maintained in RPMI-1640 cell culture medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. *Reagents and solvents:* All chemicals, solvents, and reagents used in the study were of analytical grade and obtained from JoeChem Ltd, Nigeria.

2.2. Methods

2.2.1. Preparation of chitosan nanoparticles (CNPs)

CNPs were prepared using the ionotropic gelation method. Briefly, chitosan was dissolved in 1% (v/v) acetic acid solution to prepare a 1% (w/v) chitosan solution. The solution was then mixed with sodium tripolyphosphate (TPP) at a specific chitosan ratio (1:1) under magnetic stirring. The pH of the solution was adjusted to 5.5 using NaOH to promote nanoparticle formation. The resulting CNPs were collected by centrifugation at 10,000 rpm for 20 minutes, washed with distilled water, and freeze-dried for further characterization [5].

2.2.2. Loading of Camellia sinensis extract into CNPs

The green tea extract was incorporated into the CNPs using a two-step method. Initially, the extract was mixed with the chitosan solution before the addition of TPP. This resulted in the formation of drug-loaded nanoparticles. The loading efficiency was determined by measuring the absorbance of the supernatant at the maximum wavelength (450 nm) of the catechins using a UV-Vis spectrophotometer [11].

2.2.3. Characterization of CNPs

Size and Morphology: The size, polydispersity index

(PDI), and zeta potential of the CNPs were determined using a dynamic light scattering (DLS) instrument. Morphological analysis was performed using scanning electron microscopy (SEM) [12].

Encapsulation efficiency: The encapsulation efficiency (EE) of the green tea extract in the CNPs was calculated using the formula [13]:

$$EE\ (\%) = \frac{Total\ extract\ added - Free\ extract\ in\ supernatant}{Total\ extract\ added} \times 100$$

Release studies: In vitro release studies were conducted using a dialysis bag method. CNPs loaded with the green tea extract were placed in a dialysis bag and immersed in phosphate-buffered saline (PBS) at pH 7.4. Samples were collected at predetermined time intervals, and the amount of catechins released was quantified using UV-Vis spectroscopy [6].

2.2.4. Cytotoxicity assay

The cytotoxic effects of the CNPs loaded with green tea extract on ovarian cancer cells were evaluated using the MTT assay. Briefly, cells were seeded in 96-well plates at a density of [insert cell density] cells/well and incubated for 24 hours. After treatment with varying concentrations of CNPs 0, 25, 50, 100 and 200 µg/mL for 24, 48, and 72 hours, MTT solution was added to each well. The cells were then incubated for an additional 4 hours, and the formazan crystals formed were dissolved in dimethyl sulfoxide (DMSO). The absorbance was measured at 570 nm, and cell viability was calculated as a percentage of the control [14].

2.2.5. Evaluation of anti-inflammatory effects

To assess the anti-inflammatory effects of CNPs, ovarian cancer cells were co-cultured with *P. aeruginosa* to induce an inflammatory response. Cells were treated with the CNPs loaded with green tea extract, and the levels of inflammatory cytokines (TNF-α, IL-6, IL-1β, etc.) were measured using ThermoFisher Scientific enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions [15–18].

2.2.5.1. Evaluation of inflammatory cytokines and oxidative markers

TNF-α, IL-6, IL-1β, IL-10, and IL-22: Cytokines such as TNF-α, IL-6, IL-1β, IL-10, and IL-22 levels were

determined using ELISA procedure. For each of these markers, specific kits that utilized monoclonal or polyclonal antibodies to bind to the target cytokine in the sample were used. Briefly, microplates were coated with captured antibodies and incubated for 24 hours with samples. Then, biotin-conjugated was added for the detection of antibodies. Streptavidin-HRP was then introduced to bind biotin, and thereafter the substrate was added to produce a colorimetric reaction proportional to the cytokine concentration. Finally, absorbance was measured using a UV-vis spectrophotometer at 450 nm, and concentrations of each marker were determined by comparison to a standard curve [19].

Malondialdehyde (MDA): MDA, a marker of lipid peroxidation and oxidative stress, is typically determined using the Thiobarbituric acid reactive substances (TBARS) assay. In this method, MDA reacts with TBA under acidic and high-temperature conditions to form a pink-colored MDA-TBA adduct. The adduct is quantified spectrophotometrically at 532 nm [20].

Prostaglandin E2 (PGE2): The antibodies specific to PGE2 were used. Briefly, using the ELISA, the protocol sample was prepared according to the manufacturer's instructions. The standard and samples were then added to the wells, incubated for 24 hours with labeled antibodies, and subsequent colorimetric detection. The results were calculated using a standard curve and reflect PGE2 levels associated with inflammation or cancer progression [21].

Cyclooxygenase-2 (COX-2): To determine cyclooxygenase-2 (COX-2) expression in vitro, an ELISA was employed to quantify COX-2 protein levels in cell culture samples. The method involved culturing cells under specific experimental conditions, including treatment with test samples, to induce or modulate COX-2 expression. After the treatment period, cells were harvested, washed with 10% PBS, and lysed using a lysis buffer containing protease inhibitors to prevent protein degradation. The lysates were then centrifuged at 12,000 × g for 15 min at 4 °C to separate the supernatant containing soluble proteins. The COX-2 levels in the lysates were quantified using a COX-2-specific ELISA kit. First, samples and standards were added to wells of a microplate pre-

coated with anti-COX-2 capture antibodies. The plate was incubated for 24 hours at room temperature to allow COX-2 in the samples to bind to the capture antibodies. After incubation, the wells were washed multiple times with a wash buffer to remove unbound components. Next, a biotin-conjugated detection antibody specific for COX-2 was added to the wells and incubated for 4 hours. Streptavidin-HRP was then introduced to bind to the biotin-conjugated antibodies, forming an enzyme-antibody-target complex. A substrate solution containing tetramethylbenzidine (TMB) was added, resulting in a colorimetric reaction. The reaction was stopped with 10% H_2SO_4, and the absorbance was measured at 450 nm using a microplate reader. A standard curve was generated using known concentrations of COX-2 provided with the kit. The COX-2 concentrations in the samples were calculated by interpolating their absorbance values against the standard curve [19].

2.2.6. Antibacterial evaluation

The antibacterial activity of *Camellia sinensis* extract-loaded chitosan nanoparticles (CNPs) was evaluated using the agar well diffusion method and by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). For the agar well diffusion assay, *Pseudomonas aeruginosa* was cultured in nutrient broth, and its turbidity was standardized to 0.5 McFarland standard. Mueller-Hinton agar plates were inoculated with the bacterial suspension, and wells of 6 mm diameter were prepared. Different concentrations of extract loaded CNPs (25, 50, 100, 200 µg/mL) were introduced into the wells along with positive (doxorubicin) and negative controls (sterile distilled water). After incubation at 37°C for 24 hours, zones of inhibition were measured in millimeters to assess the antibacterial activity [22].

The MIC and MBC were determined using a broth microdilution method. Serial two-fold dilutions of the extract-loaded CNPs (6.25–200 µg/mL) were prepared in a 96-well microtiter plate containing Mueller-Hinton broth and inoculated with *P. aeruginosa*. After 24 hours of incubation at 37°C, the MIC was identified as the lowest concentration that inhibited visible bacterial growth. For the MBC, aliquots from wells showing no growth were plated on Mueller-Hinton agar, and the lowest concentration that showed no

Table 1. Characterization of extract-loaded chitosan nanoparticles (CNPs).

Parameter	Value/Unit	Inference
Yield (%)	85.42%	High synthesis efficiency of CNPs
Average diameter (nm)	102.45 ± 2.34	Optimal size for cellular uptake
Polydispersity index (PDI)	0.23	Indicates uniform nanoparticle formation
Zeta potential (mV)	-22.34	Ensures good colloidal stability
Encapsulating efficiency (EE)	88.12%	Extract was well encapsulated into CNPs
Swelling index (%)	67.89	Confirms high water absorption capacity
Temperature stability	37.50 °C	Stable under physiological conditions
In vitro release (%)	78.56%	Effective drug release *in vitro*
Cumulative drug release (%)	95.78%	High drug release over time
Release kinetics	Higuchi model	Follows diffusion-based release mechanism

Results are means ± SE (n = 3).

bacterial growth on the plates after incubation was recorded. All experiments were performed in triplicate, and results were statistically analyzed using one-way ANOVA with a significance level set at $p < 0.05$ [23].

2.2.7. Statistical analysis

Data were expressed as means ± SD. Statistical analysis was performed using GraphPad Prism statistical software. Differences between groups were assessed using one-way ANOVA followed by Tukey's post-hoc test. A p-value of <0.05 was considered statistically significant.

3. Results

3.1. *Characterization of chitosan nanoparticles (CNPs)*

The CNPs were successfully synthesized and characterized (Table 1). The average diameter of the nanoparticles was found to be 102.45 ± 2.34 nm, indicating a suitable size range for cellular uptake. The polydispersity index (PDI) was measured at 0.23, which reflects a narrow size distribution and indicates uniformity in nanoparticle formation. The zeta potential of the nanoparticles was determined to be -22.34 mV, suggesting good colloidal stability, essential for effective drug delivery applications. The release pattern follows the Higuchi model (Fig. 1) and F3 demonstrated the best release profile.

The *in vitro* release studies (Fig. 1) demonstrated a sustained release profile of catechins over a period of 8 hours. The cumulative release percentage at 0-8 h was measured at 95.78%, indicating that the CNPs can provide prolonged release of the active compounds, which is advantageous for achieving effective
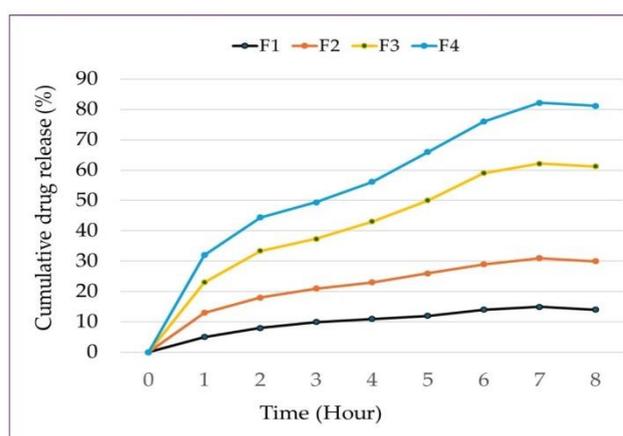

Figure 1. Cumulative drug release profiles of formulations (F1-F4).

therapeutic concentrations over time.

Furthermore, morphological assessment using scanning electron microscopy (SEM) revealed that the nanoparticles were predominantly spherical and exhibited a uniform size distribution (Fig. 2).
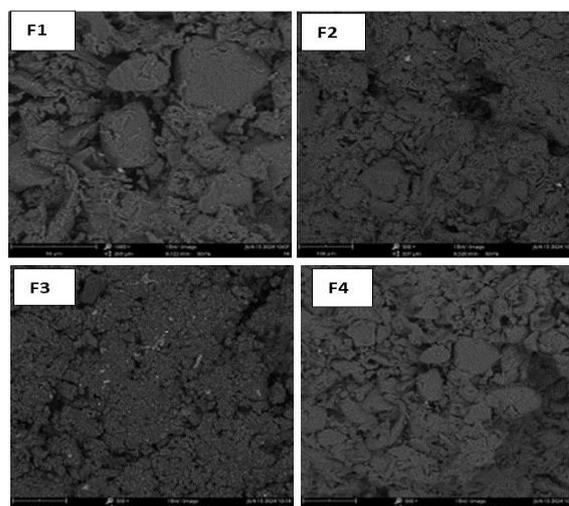

Figure 2. SEM images of formulated CNPs.

Table 2. Cytotoxic Effects of *C. sinensis* Extract-Loaded Chitosan Nanoparticles (CNPs) on Ovarian Cancer Cells Compared to Doxorubicin.

Treatment	Time (h)	Concentration (μg/mL)	Cell Viability (%)	IC$_{50}$ (μg/mL)	SI
C. sinensis extract-loaded CNPs	12	100	~70.00	15.00 ± 0.35	12.50
	24	100	~55.00	15.00 ± 0.35	12.50
	48	100	45.00	15.00 ± 0.35	12.50
	72	100	~30.00	15.00 ± 0.35	12.50
Doxorubicin (standard drug)	12	100	~60.00	10.00 ± 0.28	8.00
	24	100	~40.00	10.00 ± 0.28	8.00
	48	100	~25.00	10.00 ± 0.28	8.00
	72	100	~15.00	10.00 ± 0.28	8.00

Results are mean ± SD (n = 3). SI (selective index) = CC$_{50}$ (normal cells) / IC$_{50}$ (cancer cells).

The successful loading of *C. sinensis* extract into the nanoparticles was confirmed, with an encapsulation efficiency of 88.12% (Table 1). This high encapsulation efficiency is crucial for ensuring a sufficient concentration of therapeutic agents is delivered to target cells.

3.2. Cytotoxicity assay

The cytotoxic effects of *C. sinensis* extract loaded CNPs on ovarian cancer cells were evaluated across four-time intervals (12, 24, 48, and 72 h). A progressive, dose- and time-dependent reduction in cell viability was observed. At a concentration of 100 μg/mL, cell viability was reduced to 45.00% after 48 hours. The IC$_{50}$ value was calculated to be 15.00 μg/mL, indicating strong anticancer activity (Fig. 3). Furthermore, *C. sinensis*-loaded CNPs have the highest SI (12.50), indicating better selectivity for cancer cells compared to Doxorubicin (SI = 8.00). The results confirm a strong, time- and dose-dependent cytotoxic effect of C. sinensis-loaded CNPs on ovarian cancer cells (Table 2).

3.3. Evaluation of anti-inflammatory effects

The results demonstrate the significant anti-inflammatory effects of Camellia sinensis extract loaded chitosan nanoparticles (CNPs) in an inflammatory microenvironment. Pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β were markedly reduced following treatment with the extract-loaded CNPs. Specifically, TNF-α levels decreased by 55.00%, IL-6 by 52.78%, and IL-1β by 56.82%, highlighting the ability of the CNPs to effectively suppress these inflammatory mediators. This suggests that the nanoparticles possess potent
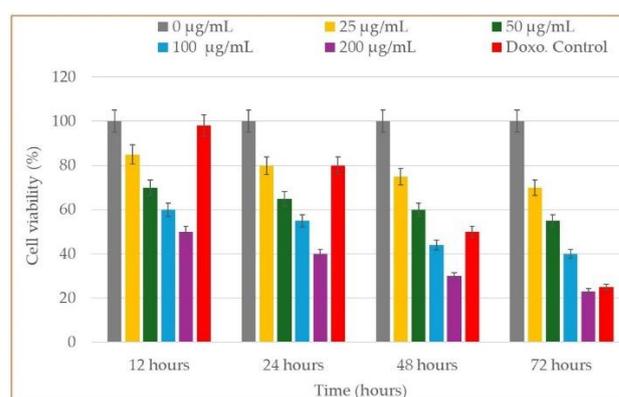

Figure 3. Effect of *C. sinensis* extract loaded CNPs and doxorubicin on ovarian cancer cell viability. Results are mean ±SD (n = 3).

anti-inflammatory properties that could mitigate inflammation-induced tumor progression. In addition to reducing pro-inflammatory cytokines, the extract-loaded CNPs significantly increased levels of anti-inflammatory markers such as IL-10 and IL-22. Both markers exhibited a 100% increase compared to the control group, indicating a robust anti-inflammatory response. The elevation of these cytokines further supports the potential of the nanoparticles to modulate the inflammatory microenvironment toward a less aggressive state, thereby potentially improving therapeutic outcomes.

Furthermore, markers of oxidative stress and inflammation, such as NO and MDA, were significantly reduced by 55.56% and 55.00%, respectively. The reduction in these markers suggests that the extract loaded CNPs can attenuate oxidative stress, which plays a key role in chronic inflammation and tumor progression. Similarly, PGE2 and COX-2, both critical mediators of inflammation, were reduced

Table 3. Effect of *C. sinensis* extract loaded CNPs on pro-inflammatory and anti-inflammatory markers.

Marker	Control (pg/mL)	CNPs only (pg/mL)	Extract only (pg/mL)	CNPs + extract (pg/mL)	Reduction (%) compared to control
TNF-α	200.00	150.00	120.00	90.00	55.00
IL-6	180.00	140.00	110.00	85.00	52.78
IL-1β	220.00	170.00	130.00	95.00	56.82
IL-10	50.00	70.00	80.00	100.00	+100.00
IL-22	45.00	60.00	75.00	90.00	+100.00
NO	90.00	70.00	60.00	40.00	55.56
MDA	100.00	75.00	65.00	45.00	55.00
PGE2	150.00	110.00	95.00	70.00	53.33
COX-2	200.00	150.00	120.00	85.00	57.50

Table 4. Antibacterial activity of *C. sinensis* extract-loaded chitosan NPs against *P. aeruginosa.*

Treatment (µg/L)	Zone of inhibition (mm)	MIC (µg/mL)	MBC (µg/mL)	Remarks
Extract-loaded CNPs	24.50 ± 1.20*	12.50*	25.00*	Best activity observed
Extract alone	18.30 ± 1.10	25.00	50.00	Moderate activity
Chitosan NPs	12.40 ± 0.90	50.00	100.00	Minimal antibacterial activity
Positive control	20.00 ± 1.00	14.50*	29.00*	Comparable to extract loaded CNPs
Negative control	0.00	N/A	N/A	No antibacterial activity observed

*Significant at $p < 0.05$ compared to all other treatment groups. The positive control was ciprofloxacin tablet while the negative control was distilled water.

by 53.33% and 57.50%, respectively, further underscoring the anti-inflammatory efficacy of the treatment (Table 3).

3.4. Antibacterial effects

The extract-loaded chitosan nanoparticles (CNPs) exhibited the best antibacterial activity, with the highest zone of inhibition (24.50 ± 1.20 mm) and the lowest MIC (12.50 µg/mL) and MBC (25.00 µg/mL), indicating superior efficacy against *Pseudomonas aeruginosa*. This suggests enhanced antibacterial properties due to the synergistic effect of the extract and chitosan NPs. In comparison, the extract alone showed moderate antibacterial activity with a zone of inhibition of 18.30 ± 1.10 mm, MIC of 25.00 µg/mL, and MBC of 50.00 µg/mL. Chitosan NPs alone demonstrated minimal activity, while the standard antibiotic produced comparable results to the extract loaded CNPs ($p < 0.05$). The negative control exhibited no activity, confirming the specificity of the treatments (Table 4).

Similarly, Fig. 4 below illustrates the synergy between the anticancer and antibacterial activities of the treatments. Extract-loaded chitosan NPs exhibit the highest activity in both categories, emphasizing their
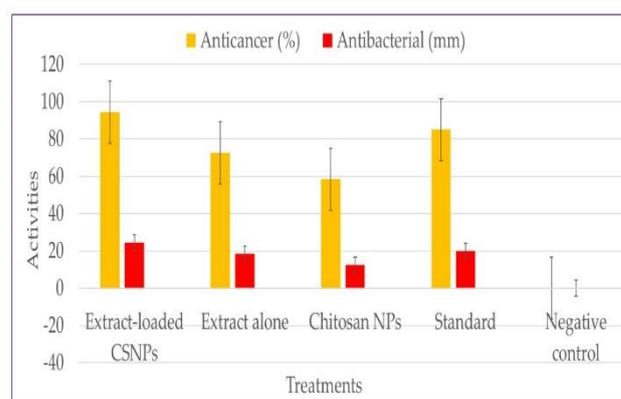

Figure 4. Synergy between anticancer and antibacterial activities of treatments.

effectiveness in reducing cancer cell viability and inhibiting bacterial growth.

4. Discussion

The successful development and characterization of chitosan nanoparticles (CNPs) loaded with *Camellia sinensis* extract highlights the potential of this drug delivery system for targeted therapy against *Pseudomonas aeruginosa*-associated ovarian cancer. This study contributes to the growing body of literature demonstrating the advantages of

nanotechnology in enhancing the bioavailability and therapeutic efficacy of natural compounds. The physicochemical properties of the CNPs, including their size, stability, and controlled release characteristics, make them highly suitable for cellular uptake and prolonged therapeutic action, thereby enhancing treatment efficacy and minimizing systemic side effects [7].

The size and stability of nanoparticles are critical factors influencing their interaction with cells and tissues. The CNPs in this study demonstrated an ideal size range for cellular uptake, with nanoparticles in the 100-200 nm diameter range being particularly effective for endocytosis, a process by which cells internalize nanoparticles via clathrin-mediated or caveolae-dependent pathways [24]. This is especially important for ovarian cancer therapy, where nanoparticles must penetrate the tumor microenvironment and interact efficiently with cancer cells to deliver therapeutic agents [25]. While CNPs have demonstrated targeted delivery and reduced systemic side effects compared to free drugs, their interactions with normal cells must be carefully evaluated. Toxicity may arise due to nanoparticle size, surface charge, or the dose administered. Studies suggest that CNPs, due to their biocompatibility and biodegradability, have minimal cytotoxic effects on healthy cells at therapeutic concentrations. However, higher doses or prolonged exposure could potentially disrupt cellular homeostasis or cause oxidative stress. To address this, it is crucial to optimize the nanoparticle formulation, size, and release profile to ensure selective targeting of cancer or infected cells while sparing normal tissues.

Additionally, the anti-inflammatory effects of CNPs are vital in reducing cytokine levels, which play a significant role in cancer progression and infection-induced inflammation. CNPs loaded with *C. sinensis* extract reduce cytokines such as TNF-α, IL-1β, and IL-6 by modulating inflammatory signaling pathways [7]. The chitosan component enhances cellular uptake of the extract, allowing its bioactive compounds, particularly catechins, to exert their effects more efficiently. These compounds inhibit the activation of NF-κB and MAPK pathways, which are responsible for the overproduction of pro-inflammatory cytokines. By stabilizing cell membranes and scavenging

reactive oxygen species (ROS), CNPs further mitigate inflammation, creating a microenvironment that is less conducive to cancer progression or bacterial persistence [7]. Similarly, the stability of the CNPs in physiological conditions ensures that the encapsulated *Camellia sinensis* extract remains intact until it reaches the target site, allowing for a controlled release of therapeutic agents. This sustained release is essential for improving therapeutic efficacy and prolonging the action of the loaded extract, thereby ensuring better management of cancer. The surface charge of the CNPs also plays a crucial role in enhancing cellular uptake. Chitosan nanoparticles typically exhibit a positive surface charge, which facilitates their interaction with the negatively charged cell membrane, promoting efficient internalization [7]. This feature is advantageous for the targeted delivery of *Camellia sinensis* extract to ovarian cancer cells, ensuring that active compounds are delivered directly to the cancer site as seen in this study.

The cytotoxicity results from this study confirm that CNPs loaded with *Camellia sinensis* extract exhibit potent anticancer effects in ovarian cancer cells, highlighting the therapeutic potential of green tea extract in cancer therapy. *Camellia sinensis* extract has been widely recognized for its anticancer properties, particularly through its active catechins, such as epigallocatechin gallate (EGCG), which are known to induce apoptosis, inhibit cell proliferation, and suppress metastasis [26]. In this study, the *Camellia sinensis* extract-loaded CNPs induced apoptosis in ovarian cancer cells by modulating key apoptotic pathways, which aligns with previous findings where *Camellia sinensis* extract has been shown to regulate pro-apoptotic proteins like Bax and downregulate anti-apoptotic proteins such as Bcl-2 [9].

The sustained release of *Camellia sinensis* extract from the CNPs ensures continuous exposure of cancer cells to the active compounds, which is crucial for maximizing therapeutic impact. Several studies have demonstrated that prolonged exposure to *Camellia sinensis* extracts results in the gradual accumulation of the compound within the tumor cells, providing better anticancer effects compared to traditional drug delivery methods [5]. This sustained exposure helps in overcoming tumor resistance to therapy by

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maintaining effective concentrations of the therapeutic agents within the tumor microenvironment.

In addition to inducing apoptosis, *Camellia sinensis* extract loaded CNPs also inhibited the proliferation of ovarian cancer cells. It is well-documented that *Camellia sinensis* extract, through its catechins, can inhibit the growth of various cancer cell lines by targeting critical signaling pathways such as PI3K/Akt and MAPK, both of which regulate cell survival, growth, and proliferation [27]. The modulation of these pathways by *Camellia sinensis* extract loaded CNPs suggests that the nanoparticles provide a comprehensive approach to targeting ovarian cancer cells, which often exhibit dysregulated signaling that promotes uncontrolled cell growth.

The anti-inflammatory properties of *Camellia sinensis* extract loaded CNPs provide an additional therapeutic advantage in ovarian cancer treatment. Chronic inflammation is a key factor in promoting tumorigenesis and cancer progression, and its role in the ovarian cancer microenvironment is well-established [2]. Inflammation contributes to tumor growth, metastasis, and resistance to chemotherapy, thus therapies that target both cancer cells and the inflammatory components of the tumor microenvironment are highly beneficial.

The results from this study suggest that CNPs loaded with *Camellia sinensis* extract can modulate the inflammatory response by reducing the levels of pro-inflammatory cytokines such as TNF-alpha, IL-1β, and IL-6. These cytokines are well-known mediators of the inflammatory response and have been shown to support cancer cell survival, proliferation, and metastasis [28]. By modulating the levels of these cytokines, *Camellia sinensis* extract loaded CNPs not only suppress cancer cell growth but also attenuate the pro-inflammatory conditions that favor cancer progression. This dual action-targeting both cancer cells and the inflammatory microenvironment could provide a more effective therapeutic strategy for ovarian cancer, as it could reduce the risk of recurrence and metastasis.

In addition to cytokine modulation, the anti-inflammatory effects of *Camellia sinensis* extract may also involve the inhibition of key signaling pathways such as COX-2 and NF-kB, which are frequently upregulated in cancer cells and contribute to the inflammation-mediated promotion of tumorigenesis [19]. Inhibiting these pathways could reduce cancer growth and the inflammatory environment that often leads to resistance to conventional therapies [29].

Furthermore, the dual anti-inflammatory and antibacterial activities of *C. sinensis* can be attributed to the synergistic action of its bioactive compounds, such as catechins, flavonoids, and phenolic acids, which interact at a molecular level with both inflammatory and microbial targets [26]. The primary bioactive component of *C. sinensis*, epigallocatechin-3-gallate (EGCG), exhibits potent anti-inflammatory activity by modulating several key signaling pathways. EGCG inhibits the activation of nuclear factor kappa B (NF-κB), a transcription factor that regulates the expression of pro-inflammatory cytokines like TNF-α, IL-1β, and IL-6. Molecularly, EGCG prevents the phosphorylation and degradation of IκBα, an inhibitor of NF-κB, thereby suppressing NF-κB nuclear translocation and transcriptional activity. This downregulation reduces the release of inflammatory mediators, including prostaglandins (via COX-2 inhibition) [19].

Similarly, the antibacterial activity of *C. sinensis* is largely driven by catechins like EGCG and epicatechin (EC), which disrupt bacterial cell membranes and inhibit essential enzymes. EGCG binds to bacterial lipids and forms complexes with the lipid bilayer, increasing membrane permeability and leading to bacterial cell lysis. Additionally, catechins inhibit bacterial enzymes, such as DNA gyrase and dihydrofolate reductase, which are crucial for DNA replication and metabolic processes. Furthermore, catechins generate hydrogen peroxide (H_2O_2) in the bacterial microenvironment, which damages bacterial DNA, proteins, and lipids. EGCG also interferes with quorum sensing-the bacterial communication system that regulates virulence factor production-by downregulating genes involved in biofilm formation and toxin release [30]. These actions weaken bacterial defenses and increase their susceptibility to immune responses or antimicrobial agents.

The anti-inflammatory and antibacterial properties of *C. sinensis* work in tandem to provide enhanced therapeutic effects. In infection-induced inflammation, EGCG not only reduces the inflammatory response

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but also inhibits bacterial growth, creating a feedback loop that mitigates tissue damage [9]. Its ability to neutralize ROS addresses oxidative stress caused by both infection and inflammation, providing comprehensive protection at the molecular level. This synergy amplifies the therapeutic potential of *C. sinensis*, making it effective for managing inflammation-related infections. Research evidence supports these mechanisms. Studies demonstrate that EGCG reduces NF-κB activity in inflammatory diseases, leading to significant reductions in TNF-α and IL-1β levels [31]. For antibacterial action, EGCG disrupts bacterial membranes and inhibits DNA gyrase, effectively targeting Gram-positive and Gram-negative bacteria. Synergistic effects have also been reported; for example, in a sepsis model, *C. sinensis* catechins simultaneously reduced inflammation and bacterial load, highlighting their dual therapeutic potential [9, 27].

In conclusion, the enhanced dual activity of *Camellia sinensis* extract stems from the synergistic molecular interactions of its bioactive compounds with both host inflammatory pathways and bacterial targets [26]. These interactions not only inhibit inflammation and bacterial proliferation independently but also amplify each other's effects, making *C. sinensis* a promising candidate for managing inflammation-related infections.

The results of this study suggest that chitosan nanoparticles loaded with *C. sinensis* extract represent a promising strategy for the treatment of *P. aeruginosa*-associated ovarian cancer. The combination of anticancer and anti-inflammatory effects provided by this nanoparticle system presents a multifaceted therapeutic strategy that could improve the overall efficacy of cancer treatments while reducing side effects. Future studies and clinical trials will be critical for determining the translational potential of this innovative drug delivery system in the management of ovarian cancer. While the *in vitro* data are promising, further preclinical studies are necessary to assess the pharmacokinetics, biodistribution, and long-term safety of *C. sinensis* extract loaded CNPs *in vivo*. Studies in animal models of ovarian cancer are essential to evaluate how the nanoparticles behave within a complex biological system, as well as to establish effective dosing regimens.

Conclusively, additional research should investigate the combination of *C. sinensis* extract loaded CNPs with other cancer therapies, such as chemotherapy or immunotherapy, to explore potential synergistic effects. The multi-target action of *C. sinensis* extract, which includes both direct anticancer effects and modulation of the inflammatory microenvironment, holds great promise for improving patient outcomes. The unique properties of this delivery system also make it a candidate for personalized cancer treatment, where the nanoparticle system could be tailored to target specific tumor characteristics, improving precision in cancer therapy. In summary, this research differentiates itself by presenting an integrated, multi-functional therapeutic strategy that bridges the gaps between MDR management, cancer therapy, and inflammation control. The emphasis on mechanistic clarity, alongside considerations for biocompatibility, ensures the study's relevance in advancing both the science and clinical utility of plant extract-loaded nanoparticles.

5. Conclusions

Our study has shown that the development of chitosan nanoparticles loaded with *C. sinensis* extract presents a promising and innovative approach for the targeted therapy of *P. aeruginosa*-associated ovarian cancer. The nanocarrier system demonstrated excellent physicochemical properties, including appropriate particle size, stability, and controlled release characteristics, which enhance the bioavailability and therapeutic efficacy of the extract. The cytotoxic effects observed in ovarian cancer cells, coupled with the anti-inflammatory properties of the nanoparticles, suggest that this dual-action strategy may not only inhibit tumor growth but also reduce the inflammatory microenvironment associated with cancer progression. These findings support the potential of *C. sinensis* extract loaded CNPs as an effective treatment modality and pave the way for further studies to explore their clinical translation in cancer therapy.

Ethical statement

Not applicable.

Authors' contributions

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Availability of data and materials

All data have been used in the manuscript. Additional data will be made available on genuine request.

Conflicts of interest

We have none to declare.

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