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ORIGINAL: Garlic Extract–Loaded Caffeic Acid–Grafted Chitosan Nanoparticles: Enhanced Anticancer and Antimicrobial Efficacy

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ABSTRACT

Introduction: COVID-19 has been associated with multiple organ injuries, contributing to increased morbidity and mortality in hospitalized patients. This study aimed to investigate the prevalence of cardiac, renal, and hepatic injuries among deceased and ICU-admitted COVID-19 patients at Imam Sari Hospital.

Methods: Garlic extract was encapsulated into caffeic acid–grafted ChNPs and characterized using dynamic light scattering (DLS), scanning electron microscopy (SEM), and *In vitro* release assays.Cytotoxicity was assessed using MTT assays on HT29 colon cancer cells (3.125–100 µg/mL, 24 h) and blank chitosan nanoparticles (12.5–200 µg/mL) on HEK293 normal cells. Antimicrobial activity was tested against *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* using the microbroth dilution method.

Results: ChNPs had an average size of 230.9 ± 6.1 nm with spherical morphology. Encapsulation significantly slowed garlic compound release (58% over 72 h vs. rapid release from free extract). Garlic-loaded ChNPs reduced HT29 cell viability to ~16% at 100 µg/mL ($p < 0.001$), showing dose-dependent cytotoxicity. Blank ChNPs exhibited low toxicity in normal cells. The nanoformulation also enhanced antimicrobial efficacy, reducing MICs and MBCs up to eight-fold, especially against *K. pneumoniae* (MIC: 15.62 µg/mL; MBC: 31.25 µg/mL).

Conclusion: Garlic-loaded caffeic acid–grafted ChNPs significantly improve anticancer and antimicrobial activity while maintaining biocompatibility, suggesting their potential for use in cancer therapy and infection control.

Introduction

Garlic (*Allium sativum* L.) is an ancient medicinal plant widely acknowledged for its antifungal, antiviral, antibacterial, and anticancer properties. These bioactivities are attributed to sulfur-rich compounds like diallyl trisulfide, allicin, diallyl disulfide, vinyldithiins, and allyl cysteine [1, 2]. Evidence suggests that garlic exerts anticancer effects by modulating reactive oxygen species, scavenging free radicals, preventing DNA damage, and activating key detoxification enzymes, such as glutathione S-transferase [3, 4]. Allicin and related sulfur compounds also exhibit antimicrobial efficacy against pathogenic and drug-resistant bacteria [5]. Despite these promising attributes, the direct clinical use of garlic extract is limited by its volatility, instability, and poor absorption [6].

In recent years, nanotechnology-based drug delivery enhances phytochemical extracts' stability, solubility, and controlled release [6]. Encapsulating garlic extract in suitable nanocarriers protects its labile sulfur-containing compounds from premature degradation, extending circulation time and improving uptake in target cells or tissues.

Chitosan (CS), a partially deacetylated derivative of the natural polymer chitin, has garnered attention for its biocompatibility, biodegradability, and low toxicity, in addition to its hemostatic, fungistatic, and bacteriostatic properties [7, 8]. The cationic nature of chitosan facilitates ionic interactions with negatively charged molecules, thereby improving encapsulation efficiency and release control [9]. We grafted caffeic acid onto chitosan to enhance antioxidant properties and stabilize garlic's sulfur compounds, building on prior work.

Several studies have already demonstrated successful encapsulation of botanical extracts in chitosan nanoparticles (ChNPs)-including Pomegranate [10], limonene [11], Cassia fistula leaf [12], avocado callus [13], Clitoria ternatea [14] improving bioactivity, stability, and targeted delivery. Despite preliminary evidence that garlic-loaded chitosan nanoparticles boost the retention of sulfur compounds and antimicrobial potency [15], there is limited information on about their efficacy against specific cancer cell lines. Moreover, the mechanistic basis of these antimicrobial effects remains to be fully elucidated. This study advances prior work by using caffeic acid-grafted ChNPs to improve garlic extract delivery, targeting HT29 colon cancer cells and key pathogens.

Our specific objectives are to (i) synthesize and evaluate garlic extract–encapsulated caffeic acid–grafted ChNPs for enhanced stability, (ii) investigate their anticancer efficacy against HT29 colon cancer cells, and (iii) assess their antimicrobial activity against a panel of pathogenic bacterial strains.

Material and methods

Garlic extract was prepared by macerating 5 g of garlic powder in 100 mL distilled water with continuous stirring at room temperature for 48 h. The suspension was filtered using Whatman paper, concentrated under reduced pressure at 40–50 °C using a rotary evaporator (R100, Jhal Tajhiz, Iran), and stored at 4 °C at 10 mg/mL [16].

Preparation of garlic extract

viral entry into liver tissue. It is also possible that liver injury in these patients results from.

Synthesis of Caffeic Acid–Grafted Chitosan Nanogels

Caffeic acid–grafted chitosan nanogels were synthesized via self-assembly. In brief, 0.5 g of high-molecular-weight chitosan [Sigma-Aldrich) was dissolved in 100 mL of 0.1% [w/v) acetic acid at 250 rpm for 1 hour. Afterward, 85 mL of methanol was added and stirred for 20 minutes. Separately, 221 mg of caffeic acid were dissolved in 668 µL of ethylene dichloride and then introduced into the chitosan-methanol mixture. The mixture was sonicated for 5 minutes and subsequently shaken at 250 rpm for 5 hours at room temperature. The pH was then raised to 8.5–9.0 via 1 M NaOH. Nanogels were collected by centrifugation (9000 rpm, 15 min), washed with ethanol and water, freeze-dried, and stored at –80 °C [17]. Caffeic acid was chosen to enhance antioxidant properties and stabilize garlic's sulfur compounds.

Encapsulation of Garlic Extract into Chitosan Nanogel

For encapsulation, 170 mg of garlic extract was dispersed in 170 mg ethanol and then combined with 170 mg of freeze-dried caffeic acid–grafted nanogels. Distilled water was added to 35 mL total volume, followed by 5 minutes of sonication at 70 Hz. The suspension was shaken for 1 hour at room temperature to complete encapsulation.

Characterization of chitosan nanogels

Particle Size Distribution

The hydrodynamic diameter was measured using a DLS(Zetasizer-ZS, Malvern). Samples were diluted in distilled water, and the polydispersity index (PDI) was recorded [18].

Morphological Analysis

Nanogel morphology was examined using Scanning Electron Microscopy (SEM). Samples were sputter-coated with a thin gold layer (10 nm) to enhance conductivity. SEM images were captured under high vacuum conditions at an accelerating voltage to assess particle morphology [19].

In vitro Cytotoxicity Assessment

Cytotoxicity on HT-29 Cells

Cell Culture Conditions: HT-29 human colon adenocarcinoma cells (National Cell Bank of Iran, Pasteur Institute, Iran) were cultured in high-glucose Dulbecco's Modified Eagle Medium (DMEM; Gibco, Thermo Fisher Scientific, USA) supplemented with 10% (v/v) fetal bovine serum (FBS; Gibco) and 1% (v/v) penicillin-streptomycin (100 U/mL penicillin, 100 µg/mL streptomycin; Gibco) in a humidified atmosphere containing 5% CO_2 at 37 °C [2]. The culture medium was replenished every 48–72 hours. Cells were passaged upon reaching ~80% confluence, and viable cells were counted using the trypan blue exclusion method before further experiments [20].

Treatment Procedure: Garlic extract–chitosan nanoparticle suspensions were prepared in serum-free DMEM at a 1 mg/mL stock concentration, then diluted to working concentrations (3.125–500 µg/mL) immediately before treatment. HT-29 cells (5,000 cells/well) were seeded into 96-well plates and allowed to adhere 24 hours prior to replacing the medium with nanoparticle suspensions. All treatments were performed in triplicate.

MTT Assay: Cell viability was assessed after 24, 48, and 72 hours of treatment using the MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) assay. After incubation with 10 µL of 5 mg/mL MTT solution for 3–4 hours, the medium was replaced with 100 µL of dimethyl sulfoxide (DMSO; Sigma-Aldrich, USA) to dissolve formazan crystals. Absorbance was measured at 570 nm using a microplate reader (BioTek, USA) [11].

$$\%inhibition = [\frac{Abs.of\ f\ control - Abs.of\ treated}{Abs\ of\ treated}] \times 100$$

Cytotoxicity on Normal Cells (HEK-293)

The cytotoxicity of blank chitosan nanoparticles was evaluated on HEK-293 normal cells using the MTT assay. Cells were treated with concentrations ranging from 12.5 to 200 µg/mL for 24 hours. Cell viability was determined as described above. Results showed minimal cytotoxicity at lower concentrations, with significant effects observed only at 100 and 200 µg/mL ($p < 0.05$).

Antimicrobial Activity

The antimicrobial activity of chitosan nanoparticles containing garlic extract was evaluated against four bacterial strains: *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 15442), and *Klebsiella pneumoniae* (ATCC 43816). These bacterial strains were obtained from the microbiological bank of the Pasteur Institute of Iran. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the nanoparticles were determined using the broth microdilution method. Briefly, bacterial suspensions were prepared to match turbidity equivalent to 0.5 McFarland standard.

Each well was inoculated with 10 µL of the bacterial suspension and incubated for 24 hours at 37 °C. MIC values were determined as the lowest concentration that inhibited visible bacterial growth. To determine the MBC, samples from wells showing no growth were streaked onto agar plates and incubated for an additional 24 hours at 37 °C. The MBC was defined as the lowest concentration that resulted in no bacterial colony growth [21].

Statistical Analysis

All experiments were conducted in triplicate. Data are reported as mean ± standard deviation (SD). Statistical significance was determined by one-way ANOVA followed by Tukey's post hoc test using Prism software version 9. A p-value< 0.05 was considered statistically significant [10].

Results

The particle size of the prepared chitosan nanoparticles containing garlic extract

Dynamic light scattering (DLS) analysis revealed that the garlic extract–chitosan nanoparticles had a mean hydrodynamic diameter of 230.9 ± 6.1 nm (Figure1A), demonstrating a size distribution suitable for biomedical applications.

Morphological Analysis of Chitosan Nanogels

The morphology of the synthesized chitosan nanogels containing garlic extract was analyzed using Scanning Electron Microscopy (SEM). The images showed that the nanoparticles were predominantly spherical with a smooth surface morphology (Figure1B). The surface morphology confirmed the successful synthesis of well-structured chitosan nanogels.

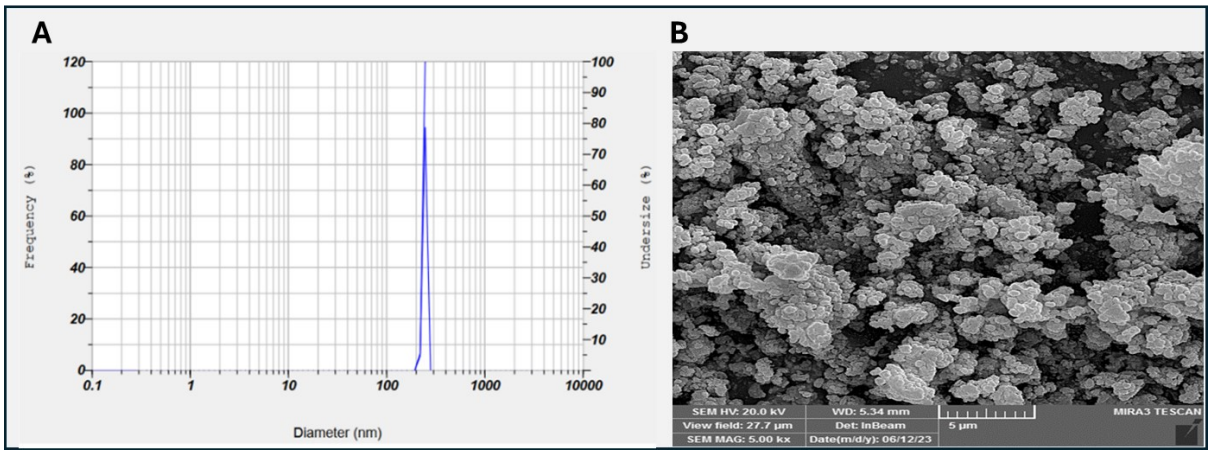

Figure 1. (A) DLS analysis of garlic extract–loaded chitosan nanoparticles (Garlic ChNPs), showing an average particle size of 230.9 ± 6.1 nm. (B) SEM image of Garlic ChNPs, highlighting their spherical morphology with a smooth surface.

In vitro Release Profile of Garlic Extract

Figure 2 illustrates the cumulative release of free and chitosan-encapsulated garlic extract in a PBS-SDS release medium over 72 hours. To better mimic physiological or *in vivo* conditions, SDS was included in the phosphate-buffered saline (PBS) to enhance garlic compounds' solubility and diffusion characteristics. As shown in Figure 2, the free garlic extract achieved near-complete release (100%) within 72 hours. In contrast, the garlic-loaded chitosan nanocarrier released only 58% of the encapsulated extract over the same time frame.

Cytotoxicity Assessment

In vitro Cytotoxicity on HT29 Colon Cancer Cells

MTT assays were performed to assess the cytotoxic effect of free garlic extract and

garlic extract–chitosan nanoparticles at concentrations ranging from 3.125 to 100 µg/mL on HT29 cells at 24 hours post-treatment.

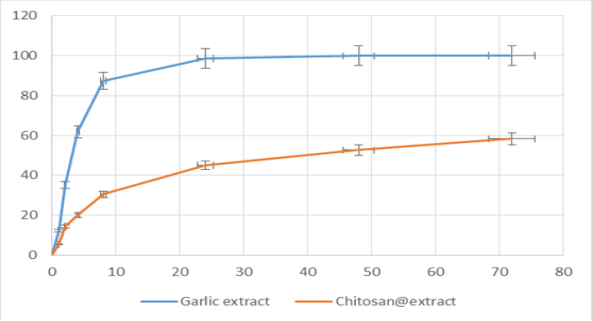

Figure 2. *In vitro* cumulative release profiles of free garlic extract and garlic-extract-loaded chitosan nanoparticles in PBS–SDS medium over 72 hours at 37 °C. The free extract rapidly approached complete release (~87% within 8 hours and 100% by 72 hours

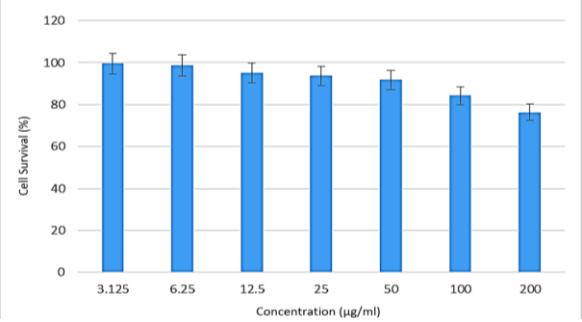

Figure 3. Dose-dependent cytotoxic effects of garlic extract–loaded chitosan nanoparticles on HT29 colon cancer cells after 24 hours of treatment. Concentrations ranging from 3.125 to 100µg/mL significantly reduced cell viability in a graded manner ($p<0.05$ for 3.125µg/mL; $p<0.001$ for ≥25µg/mL), indicating that higher doses led to more pronounced inhibition of HT29 cell proliferation. Error bars represent the standard deviation from triplicate measurements.

As shown in Figure 3, treatment with increasing concentrations of garlic-loaded ChNPs resulted in a significant, dose-dependent reduction in cell viability. Specifically, exposure to 3.125 µg/mL of garlic-loaded ChNPs reduced cell survival to 76.26 ± 0.18% ($p < 0.05$), while 6.25 µg/mL further decreased viability to 63.17 ± 0.26%. Higher concentrations- 12.5, 25, 50, and 100 µg/mL- induced progressively greater cytotoxic effects, yielding cell viabilities of 51.74 ± 0.26%, 44.16 ± 0.28%, 32.19 ± 0.23%, and 16.36 ± 0.59%, respectively ($p < 0.001$ for concentrations ≥25 µg/mL) (Figure 3). These data demonstrate a concentration-dependent inhibitory effect of garlic-loaded ChNPs on HT29 cell proliferation, suggesting that the encapsulation of garlic extract in chitosan nanoparticles enhances its anticancer potency. Lower doses induced moderate cytotoxicity, whereas the higher doses led to a marked decrease in cell viability, underscoring the potential therapeutic implications of this formulation for colorectal cancer treatment.

Cytotoxicity on HEK-293 Cells

Normal HEK293 cells were exposed to varying concentrations of blank chitosan (12.5, 25, 50, 100, and 200 µg/mL) for 24 hours, and cell viability was assessed by the MTT assay. As depicted in Figure 4, there was no significant decrease in cell viability at concentrations up to 50 µg/mL compared to the control group ($p > 0.05$). Although cell mortality did increase at 100 and 200 µg/mL ($p < 0.05$ vs. control), the overall impact remained modest, indicating that the synthesized chitosan exhibits minimal cytotoxicity at lower concentrations (Figure 4). These findings suggest that the blank chitosan formulation is generally biocompatible and safe for use in subsequent applications at or below 50 µg/mL.

Antimicrobial Evaluation of Garlic Extract–Loaded Chitosan Nanoparticles

To determine the antimicrobial efficacy of free garlic extract and garlic-loaded chitosan nanoparticles (ChNPs), a microbroth dilution assay was performed against *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 43816), *Staphylococcus aureus* (ATCC 6538), and *Pseudomonas aeruginosa* (ATCC 15442). As shown in Table 1, garlic-loaded ChNPs exhibited significantly lower minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

values than free garlic extract for all test organisms.

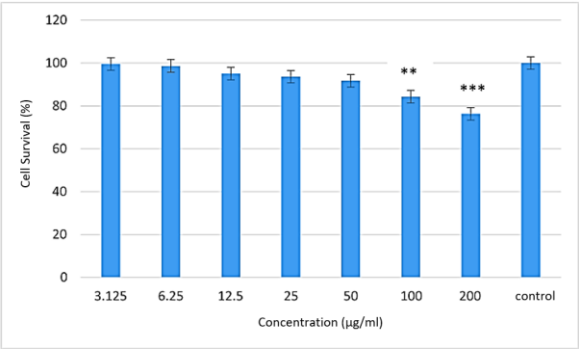

Figure 4. Viability of normal HEK293 cells treated with various concentrations of blank chitosan (12.5–200 µg/mL) for 24 hours, as determined by the MTT assay. No significant cytotoxic effects were observed at concentrations up to 50 µg/mL. However, cell mortality increased at 100 and 200 µg/mL ($p < 0.05$ vs. control), indicating relatively low toxicity of the blank chitosan at lower doses.

Among the Gram-negative strains, *K. pneumoniae* demonstrated the greatest susceptibility to the encapsulated formulation, with MIC and MBC values of 15.62 and 31.25 µg/mL, respectively. While E. coli also showed enhanced sensitivity toward the nanoparticle formulation (MIC: 62.5 µg/mL; MBC: 125 µg/mL), it's MIC and MBC values remained somewhat higher than those observed for *K. pneumoniae*. The encapsulated garlic extract was equally effective against Gram-positive *S. aureus* and Gram-negative *P. aeruginosa*, reducing the MIC to 31.25 µg/mL and the MBC to 62.5 µg/mL in both cases- approximately four- to eight-fold lower than the values for free garlic extract.

Table 1. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Free Garlic Extract and Garlic-Loaded Chitosan Nanoparticles against Selected Bacterial Strains

Bacteria strain	MIC Garlic Extract (µg/mL)	MBC Garlic Extract (µg/mL)	MIC Garlic-Loaded Chitosan (µg/mL)	MBC Garlic-Loaded Chitosan (µg/mL)
Escherichia coli	500	500	62.5	125
Klebsiella pneumoniae	125	250	15.62	31.25
Staphylococcus aureus	250	500	31.25	62.5
Pseudomonas aeruginosa	250	500	31.25	62.5

Discussion

This study successfully synthesized garlic-loaded caffeic acid–grafted chitosan nanoparticles and evaluated their potential for controlled release, antimicrobial activity, and cytotoxicity against colon cancer cells. The results demonstrate that encapsulating garlic extract within chitosan nanoparticles enhances its bioavailability, prolongs release, and significantly improves its antimicrobial and anticancer properties compared to free garlic extract. Below, these findings are discussed in the context of existing research.

The newly synthesized nanoparticles were spherical, with an average diameter of about 230.9 nm, as determined by SEM and DLS analyses. This dimension falls within a range recognized as advantageous for drug delivery, given that chitosan or chitosan-coated nanoparticles smaller than 300 nm have been shown to enhance cellular uptake through clathrin-mediated endocytosis- leading to improved internalization by both phagocytic and non-phagocytic cells [22, 23]. Indeed, prior investigations of chitosan-based nanocarriers confirm that such reduced particle sizes can significantly boost therapeutic efficacy, partly by facilitating more efficient intracellular delivery.

Scanning electron microscopy showed that most of the nanoparticles were round, had a smooth surface, and were uniform at about 100 nanometers. This outcome aligns well with similar findings in other studies [24].

The controlled release profile observed in this study demonstrated that garlic-loaded nanoparticles released 58% of the extract over 72 hours, compared to the rapid and complete

release of free garlic extract within 8 hours. Similar sustained release patterns have been reported for other bioactive compounds encapsulated in chitosan nanoparticles [25, 26], highlighting their ability to improve stability and reduce burst release effects. Implementing nano-formulated drug delivery systems enables a tightly regulated, long-term release of therapeutic agents. This approach reduces side effects and injection frequency and ensures stable drug levels over an extended period, ultimately enhancing treatment efficiency and patient outcomes [27, 28].

Garlic's broad-spectrum antimicrobial properties have been linked primarily to sulfur-based compounds like allicin [29]. Encapsulating garlic extract in chitosan nanoparticles significantly reduced MIC and MBC values for *K. pneumoniae*, *E. coli*, *S. aureus*, and *P. aeruginosa*. These results align with previous studies showing that chitosan nanoparticles enhance the antimicrobial activity of encapsulated compounds by improving their interaction with bacterial cell walls, altering their integrity and leading to inhibition or eradication of the pathogens evaluated [30, 31]. This finding underscores the synergistic effect of chitosan's cationic charge and garlic's sulfur compounds [29, 31, 32].

Garlic-loaded chitosan nanoparticles exhibited dose-dependent cytotoxicity against HT29 colon cancer cells, significantly reducing cell viability at concentrations ≥25 µg/mL. Our results are consistent with previous studies on garlic extract and its derivatives, which have shown selective cytotoxicity against cancer cells [33-35]. Significantly, the encapsulation of garlic extract in chitosan nanoparticles enhanced its anticancer efficacy, likely due to improved cellular uptake and controlled release [22, 36]. Of note, blank chitosan nanoparticles exhibited minimal impact on normal HEK293 cells, supporting the safety profile. These findings highlight the potential of garlic-loaded chitosan nanoparticles as a targeted anticancer therapy with reduced off-target effects. Chitosan nanoparticles generally demonstrate low cytotoxicity and are considered safe for biomedical applications, though their toxicity may vary with changes in size and concentration [37, 38].

These findings address critical challenges in conventional garlic extract usage-instability and rapid degradation- by leveraging a chitosan-based delivery approach. The study demonstrates that garlic-loaded chitosan nanoparticles offer robust antimicrobial and anticancer capabilities with reduced toxicity in normal cells. Future studies should include *in vivo* validation to investigate pharmacokinetics, biodistribution, and therapeutic efficacy in animal models while clarifying the molecular pathways underlying cell death and bacterial inhibition through detailed mechanistic analyses.

Conclusion

In summary, garlic extract–loaded caffeic acid-grafted chitosan nanoparticles exhibited enhanced anticancer effects against HT29 cells and superior antimicrobial activity against key pathogens while remaining low cytotoxicity to normal cells. By stabilizing garlic's sulfur compounds, this formulation overcomes traditional limitations, offering a cost-effective, biocompatible platform for cancer therapy and infectious disease management. The results pave the way for further *in vivo* studies and industrial or clinical translation.

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Authorship

M. Mirhashem: Investigation, Data curation,

Writing- original draft. N. Sartipnia: Methodology, Formal analysis. S. Taheri: Conceptualization, Supervision, Writing-review & editing.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

1. El-Saadony MT, Saad AM, El-Wafai NA, Abdo SM, Algarni EA, Abd El-Hack ME, et al. Garlic bioactive substances and their therapeutic applications for improving human health: a comprehensive review. Front Immunol. 2024;15:1277074.

2. Jikah AN, Olawoye B, Kayode RMO. A review of the therapeutic potential of sulfur compounds in *Allium sativum*. Meas Food. 2024;13:100195.

3.Farhat Z, Hershberger PA, Freudenheim JL, Aga DS, Mu L. Types of garlic and their anticancer and antioxidant activity: A review of the epidemiologic and experimental evidence. Eur J Nutr. 2021;60(3):1-25.

4. Mitra S, Lami MS, Uddin TM, Das R, Islam F, Anjum J, et al. Diallyl disulfide: a bioactive garlic compound with anticancer potential. Front Pharmacol. 2022;13:943967.

5. Oyawoye O, Oladipo EK, Adelusi OB, Faboro O, Ojelade BS, Oyeniran OS, et al. Antioxidant potential and antibacterial activities of *Allium cepa* (onion) and *Allium sativum* (garlic) against multidrug resistance bacteria. Bull Natl Res Cent. 2022;46(1):214.

6. Lu X, Li C, Li M, Qiu J, Zhang J, Ma X, et al. Improving the bioavailability and bioactivity of garlic bioactive compounds via nanotechnology. Crit Rev Food Sci Nutr. 2022;62(30):8467-96.

7.Elkomy MH, Ali AA, Eid HM. Chitosan on the surface of nanoparticles for enhanced drug delivery: A comprehensive review. J Control Release. 2022;351:923-40.

8. Jafernik K, Ładniak A, Blicharska E, Czarnek K, Ekiert H, Wiącek AE, et al. Chitosan-based nanoparticles as effective drug delivery systems—a review. Molecules. 2023;28(4):1963.

9.Raza ZA, Khalil S, Ayub A, Banat IM. Recent developments in chitosan encapsulation of various active ingredients for multifunctional applications. Carbohydr Res. 2020;492:108004.

10. Soltanzadeh M, Peighambardoust SH, Ghanbarzadeh B, Mohammadi M, Lorenzo JM. Chitosan nanoparticles as a promising nanomaterial for encapsulation of pomegranate (Punica granatum L.) peel extract as a natural source of antioxidants. Nanomaterials. 2021;11(6):1439.

11. Alipanah H, Farjam M, Zarenezhad E, Roozitalab G, Osanloo M. Chitosan nanoparticles containing limonene and limonene-rich essential oils: potential phytotherapy agents for the treatment of melanoma and breast cancers. BMC Complement Med Ther. 2021;21(1):186.

12. Ganesan A, Rengarajan J. Green synthesis of chitosan nanoparticles using Cassia fistula leaf extract: evaluation of antimicrobial, antioxidant, antibiofilm, and cytotoxic activities. 3 Biotech. 2024;14(10):223.

13.Abo El-Fadl R, El-Shamy S, El-Rahman SSA. Enhancing the biochemical constituents in avocado callus using encapsulated chitosan nanoparticles. Egypt J Chem. 2022;65(12):769-82.

14. Mobasher M, El-Kordy E, El-Haddad A. Clitoria ternatea extract-loaded chitosan nanoparticles ameliorate diabetes and oxidative stress in diabetic rats. Indian J Biochem Biophys. 2023;60(7):501-15.

15. Elghobashy SA, Mohamed MI, El-Sayed HS, Younis NA, Elnagar K, Elshamy AI, et al. Thyme/garlic essential oils loaded chitosan–alginate nanocomposite: Characterization and antibacterial activities. e-Polymers. 2022;22(1):997-1006.

16. Samira O, Benali A, Selles SMAH, Benhalla S, Bouzid HA, Rezzoug M, et al. Recent advances in the extraction of bioactive compounds from plant matrices and their use as potential antioxidants for vegetable oils enrichment. J Food Compos Anal. 2024;129:105995.

17. Aytekin AO, Morimura S, Kida K. Synthesis of chitosan–caffeic acid derivatives and evaluation of their antioxidant activities. J Biosci Bioeng. 2011;111(2):212-6.

18. Mohammadi M, Mirabzadeh S, Shahvalizadeh R, Hamishehkar H. Development of novel active packaging films based on whey protein isolate incorporated with chitosan nanofiber and nano-formulated cinnamon oil. Int J Biol Macromol. 2020;149:11-20.

19. Hajizadeh H, Peighambardoust SJ, Peighambardoust SH, Peressini D. Physical, mechanical, and antibacterial characteristics of bio-nanocomposite films loaded with Ag-modified SiO2 and TiO_2 nanoparticles. J Food Sci. 2020;85(4):1193-202.

20. Memari F, Kaviani M, Ghasemi F, Tavakoli-

Yaraki M. Tumor-inhibitory effects of zerumbone against HT-29 human colorectal cancer cells. Int J Toxicol. 2022;41(5):402-11.

21. Sarangi A, Bhattacharyya S, Lahiri S, Ghosh S, Pal A, Chakraborty A, et al. Formulation of Garlic Essential Oil-assisted Silver Nanoparticles and Mechanistic Evaluation of their Antimicrobial Activity against a Spectrum of Pathogenic Microorganisms. Curr Top Med Chem. 2024;24(22):2000-12.

22. Aibani N, Rai R, Patel P, Cuddihy G, Wasan EK. Chitosan nanoparticles at the biological interface: implications for drug delivery. Pharmaceutics. 2021;13(10):1686.

23. Tahara K, Sakai T, Yamamoto H, Takeuchi H, Kawashima Y. Improved cellular uptake of chitosan-modified PLGA nanospheres by A549 cells. Int J Pharm. 2009;382(1-2):198-204.

24. Pawaskar NK, Prabhu P, Joseph B, Mallayasamy SR. Synthesis and characterization of chitosan nanoparticles: Insights from *in-vitro* analysis. Res J Pharm Technol. 2021;14(10):5343-8.

25. Ryu JH, Yoon HY, Sun IC, Kwon IC, Kim K. Tumor-targeting glycol chitosan nanoparticles for cancer heterogeneity. Adv Mater. 2020;32(51):2002197.

26.Buyuk N, Celebi N, Aydin T, Asilturk M. Synthesis of chitosan nanoparticles for controlled release of amiodarone. Indian J Pharm Sci. 2020;82(1):108-18.

27. Sartipzadeh O, Naghib SM, Seyfoori A, Rahmanian M. Microfluidic-assisted synthesis and modelling of monodispersed magnetic nanocomposites for biomedical applications. Nanotechnol Rev. 2020;9(1):1397-407.

28. Herdiana Y, Wathoni N, Shamsuddin S, Muchtaridi M. Drug release study of the chitosan-based nanoparticles. Heliyon. 2022;8(1):e08674.

29. Ahmadi H, Morshedloo MR, Mumivand H, Maggi F, Neko HT. A new antibacterial insight of herbal chitosan-based membranes using thyme and garlic medicinal plant extracts. J Clean Prod. 2022;334:130114.

30. Garg U, Chauhan S, Nagaich U, Jain N. Current advances in chitosan nanoparticles based drug delivery and targeting. Adv Pharm Bull. 2019;9(2):195-204.

31. Olivas-Flores J, López-Meneses AK, Plascencia-Jatomea M, Fernández-Benavides AA, Moreno-Ibarra GM, Castillo-Yáñez FJ, et al. Antimicrobial effect of chitosan nanoparticles and Allium species on Mycobacterium tuberculosis and several other microorganisms. Microorganisms. 2024;12(8):1605.

32. Sindhu M, Remya S, Sivaprakasam P, Ayyadurai N, Tamilselvan C, Muthusamy S. Nanoencapsulation of garlic essential oil using chitosan nanopolymer and its antifungal and anti-aflatoxin B1 efficacy *In vitro* and in situ. Int J Biol Macromol. 2023;243:125160.

33. Vijayakumar S, Vaseeharan B, Malaikozhundan B, Gopi N, Ekambaram P, Pachaiappan R, et al. Garlic clove extract assisted silver nanoparticle–Antibacterial, antibiofilm, antihelminthic, anti-inflammatory, anticancer and ecotoxicity assessment. J Photochem Photobiol B. 2019;198:111558.

34. De Greef D, Barton EM, Sandberg EN, Croley CR, Pumarol J, Wong TL, et al. Anticancer potential of garlic and its bioactive constituents: A systematic and comprehensive review. Semin Cancer Biol. 2021;73:1-15.

35. Hashemy SI, Mahmoodzadeh H, Sajadimajd S, Ramazani A, Mohammadi E, Zarenezhad E, et al. PEGylated lecithin–chitosan–folic acid nanoparticles as nanocarriers of allicin for *In vitro* controlled release and anticancer effects. Appl Biochem Biotechnol. 2023;195(7):4036-52.

36. Fathima E, Shanmugan S, Kandasamy R. Enhanced cellular uptake, transport and oral bioavailability of optimized folic acid-loaded chitosan nanoparticles. Int J Biol Macromol. 2022;208:596-610.

37.Thandapani G, Prasad S, Sudha PN, Sukumaran A. Size optimization and *In vitro* biocompatibility studies of chitosan nanoparticles. Int J Biol Macromol. 2017;104:1794-806.

38. Rodrigues S, Dionísio M, López CR, Grenha A. Biocompatibility of chitosan carriers with application in drug delivery. J Funct Biomater. 2012;3(3):615-41.