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In Vitro Evaluation of the Synergistic Antibacterial and Antibiofilm Activities of *Parietaria muralis* Extract and Chitosan Nanoparticles

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Abstract

Herbal medicines incorporated with nanomaterials have garnered considerable attention in biomedicine; however, the antibacterial potential of combining chitosan with *Protoparmeliopsis muralis* (*P. muralis*) extract has not yet been investigated. The present study aimed to evaluate the synergistic effect of *P. muralis* and chitosan nanoparticles (CS/NPs) against selected pathogenic bacteria. Antibacterial activity was assessed using the minimum inhibitory concentration (MIC) assay, while antibiofilm effects were determined by the crystal violet assay. A mixture containing 10% *P. muralis* extract and 0.5% CS/NPs was prepared, and the synergistic interaction was further examined by the checkerboard titration method. The results revealed that CS/NPs exhibited stronger antibacterial activity than *P. muralis* extract alone. Furthermore, the checkerboard assay confirmed a synergistic effect between *P. muralis* and CS/NPs. The combined treatment also significantly enhanced antibiofilm activity, highlighting its potential as a promising strategy for controlling bacterial growth and biofilm-associated infections. In conclusion, these findings indicate that the combination of *P. muralis* extract and chitosan nanoparticles may serve as a potential candidate for further studies focused on the identification and development of novel antibacterial and antibiofilm compounds. Future research, including mechanistic studies and in vivo evaluations, is necessary to better understand and validate the observed effects.

Key words: Chitosan, Lichen, Antibacterial, Antibiofilm

Introduction

Over the past decades, the prevention and treatment of infectious diseases have remained major public health challenges. These infections are associated with prolonged hospital stays, increased healthcare costs, and high mortality rates (Sydnor and Perl, 2011). One of the major obstacles in the treatment of bacterial infections is antibiotic resistance (Kolář, 2022). Antibiotic resistance arises through multiple mechanisms and is often associated with a variety of virulence factors, among which biofilm formation plays a key role by contributing to both resistance to antibiotics and evasion of host immune responses (Schroeder et al, 2017). Biofilm formation is an important survival strategy that enables bacteria to persist in

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the presence of antibiotics. Biofilms are estimated to account for approximately 60% to 85% of bacterial infections in humans (Abebe, 2020). These infections are particularly difficult to treat because the biofilm matrix restricts the penetration of antimicrobial agents (Singh et al, 2022). Therefore, the discovery and development of novel antibiofilm and antibacterial agents capable of preventing biofilm formation, disrupting established biofilms, and treating biofilm-associated infections are urgently needed. New strategies for combating multidrug-resistant (MDR) bacterial strains include the use of nanoparticles, phage therapy, and secondary metabolites. Among these, secondary metabolites from medicinal plants have attracted considerable attention due to their antibacterial, antifungal, anticancer, anti-inflammatory, and antioxidant properties, and they may represent a promising and innovative approach against pathogenic bacteria (Bhatti et al, 2022).

Lichens have long been used in traditional medicine and are known for their potential in treating bacterial infections (Shrestha and Clair, 2013). In this context, *Protoparmeliopsis muralis*, a member of the lichen family, has shown promising biomedical applications due to its favorable physicochemical properties, including biocompatibility, biodegradability, and non-toxicity (Rashki et al, 2021). Moreover, this lichen contains a variety of bioactive compounds such as flavonoids, phenolics, phenolic acids, terpenoids, alkaloids, tannins, and quinones, which exhibit a wide range of biological activities, including antioxidant, antibacterial, antitumor, antiviral, and antifungal effects (Shrestha and Clair, 2013; Bhatti et al, 2022).

Chitosan (CS), a biodegradable polysaccharide, is widely used in nanotechnology due to its broad range of applications (Rashki and Valadbeigi, 2017).

CS is a naturally occurring biopolymer with promising biomedical potential, attributed to its physicochemical properties such as biocompatibility, biodegradability, and non-toxicity. Studies have demonstrated its therapeutic activities, including antibacterial, antiviral, and antioxidant effects, as well as its ability to inhibit biofilm formation (Gao and Wu, 2022; Varma and Vasudevan, 2020). Furthermore, several studies suggest that the integration of herbal medicines with nanotechnology provides a promising strategy to enhance their thermal stability, bioavailability, biological activity, and mechanical properties (Rashki et al, 2021).

Based on these encouraging results, the present study focused on the synergistic antimicrobial activity of chitosan–lichen extract against pathogenic bacteria. To the best of our knowledge, this is among the first studies to investigate the potential effects of this combination. Therefore, the aim of this work was to evaluate the antibacterial and antibiofilm activities of chitosan nanoparticles combined with *P. muralis* extract against pathogenic bacteria.

Materials and Methods

Preparation of Chitosan Nanoparticles

Chitosan nanoparticles were synthesized by the ionotropic gelation method (Algharib et al, 2022; Huang et al, 2016). Briefly, 10 mg of chitosan powder (degree of deacetylation 95%, molecular weight 100–300 kDa; Acros Organics, USA) was dissolved in 1% (v/v) glacial acetic acid (Merck, Germany, pH 5) and stirred at 25 °C for 24 h. A Sodium tripolyphosphate (TPP, Merck, Germany) solution (1 mg/mL in deionized water, pH 4) was then added dropwise under continuous stirring at 25 °C for 4 h. The suspension was centrifuged at 14,000 rpm for 40 min, the pellet was washed with deionized water, and the product was freeze-dried for 24 h.

Preparation of Methanolic Extract of *P. muralis*

The lichen *P. muralis* was collected from Ilam Province, Iran. Dried material (50 g) was extracted with 500 mL of methanol

using a Soxhlet apparatus for 48 h. The extract was concentrated by evaporation at 70 °C for 8 h, dried, and subsequently dissolved in 5% dimethyl sulfoxide (Merck, Germany) for experimental use (Aydın et al, 2013).

Characterization of Chitosan Nanoparticles

The structural features of CS, TPP, and the prepared CS/NPs were analyzed using Fourier Transform Infrared (FTIR) spectroscopy. FTIR spectra of the samples were recorded in the 400–4000 cm^{-1} range using a Nicolet™ Magana 550 FT-IR spectrometer. The morphology and dispersion of the nanoparticles (NPs) were examined by scanning electron microscopy (SEM). The particle size distribution and zeta potential of the synthesized chitosan nanoparticles were determined using dynamic light scattering (DLS) and electrophoretic light scattering techniques with a Zeta sizer instrument at 25°C (Hoang et al, 2022).

Bacterial Isolates

To evaluate the antibacterial activity of the aforementioned compounds, four bacterial clinical isolates from urine samples including *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, and *Pseudomonas aeruginosa* were employed.

Biofilm Detection

Biofilm formation was assessed using the microtiter plate assay. Bacterial suspensions (200µL, 0.5 McFarland standard) were inoculated into 96-well plates and incubated at 37°C for 24 h. Wells were washed twice with PBS, fixed with 200µL methanol (Merck, Germany) for 20 min, and air-dried. Cells were stained with crystal violet (Merck, Germany), washed with PBS, and absorbance was measured at 630 nm using an ELISA reader. Isolates were classified according to the criteria described by O'Toole (2011), in which an OD630 value of ≤ 0.38 indicated weak biofilm formation, values between 0.38 and 0.70 indicated moderate biofilm formation, and values ≥ 0.70 indicated strong biofilm formation.

Antimicrobial Activity Assay

Minimum inhibitory concentrations (MICs) of CS/NPs and *P. muralis* extract were determined by the broth microdilution method according to CLSI guidelines (CLSI, 2018). Serial dilutions of the test samples (2500–4.9µg/mL) were prepared in 96-well plates containing Mueller Hinton Broth (Merck, Germany). Each well was inoculated with 10µL of bacterial suspension (1.5×10^5 CFU/mL) and incubated at 37 °C for 18–24 h. MIC was defined as the lowest concentration that inhibited visible bacterial growth (Aydın et al., 2013; Meletiadis et al, 2009).

Inhibitory Effect on Biofilm Formation

The effect of CS/NPs and *P. muralis* extract on biofilm inhibition was evaluated using the microtiter plate technique (Aydın et al, 2013; O'Toole, 2011). Bacterial suspensions were inoculated into 96-well plates, followed by the addition of CS/NPs and *P. muralis* extract at concentrations of 250 and 500µg/mL. Plates were incubated at 37 °C for 24 h, then washed with PBS. Wells were fixed with 200µL methanol for 20 min, air-dried, stained with crystal violet, and washed with PBS. Absorbance was recorded at 630 nm.

Synergistic Effect of CS/NPs and *P. muralis* Extract

The combined antibacterial effects of CS/NPs and *P. muralis* extract were assessed using the checkerboard titration method (Meletiadis et al, 2009). Fractional inhibitory concentration (FIC) indices were calculated, where the FIC of CS-NPs was defined as the MIC of CS-NPs in the presence of *P. muralis* divided by the MIC of CS-NPs alone, and the FIC of *P. muralis* was defined as the MIC of P. muralis in the presence of CS-NPs divided by the MIC of P. muralis alone. The overall FIC index was

obtained by summing these two values, and the interaction was interpreted as synergistic when the FIC index was < 1, additive when it was equal to 1, and antagonistic when it was > 1.

Statistical Analysis

All experiments were performed in triplicate, and the results are expressed as mean ± standard error of mean (SEM). Statistical analyses were carried out using GraphPad InStat 3 (GraphPad Software, San Diego, CA, USA). The normality of data distribution was evaluated using the Shapiro–Wilk test.

Differences among multiple groups, including antimicrobial and antibiofilm activities of chitosan nanoparticles (CS/NPs), *P. muralis* extract, and their combinations against different bacterial isolates, were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test. Comparisons between two groups were analyzed using the independent Student's t-test.

Results

FTIR Analysis of CS/NPs

The FTIR spectrum of the fabricated CS/NPs (Figure 1) exhibited a broad peak at ~3422 cm^{-1}, more pronounced than that of chitosan, corresponding to O–H and N–H stretching vibrations. The bands at ~2922 and 2896 cm^{-1} can be attributed to asymmetric C–H stretching of $–CH_3$ and $–CH_2$ groups (Jayakumar et al., 2011). In CS/NPs, the splitting of the 1630 cm^{-1} peak into two distinct bands suggests NH_2 bending vibrations and deacetylation, consistent with other FTIR analyses. Additionally, bands at ~1092 and 1087 cm^{-1} correspond to C–O stretching, while the appearance of signals near ~1100 cm^{-1} in TPP-containing samples aligns with phosphate group vibrations (Cushnie et al, 2014).

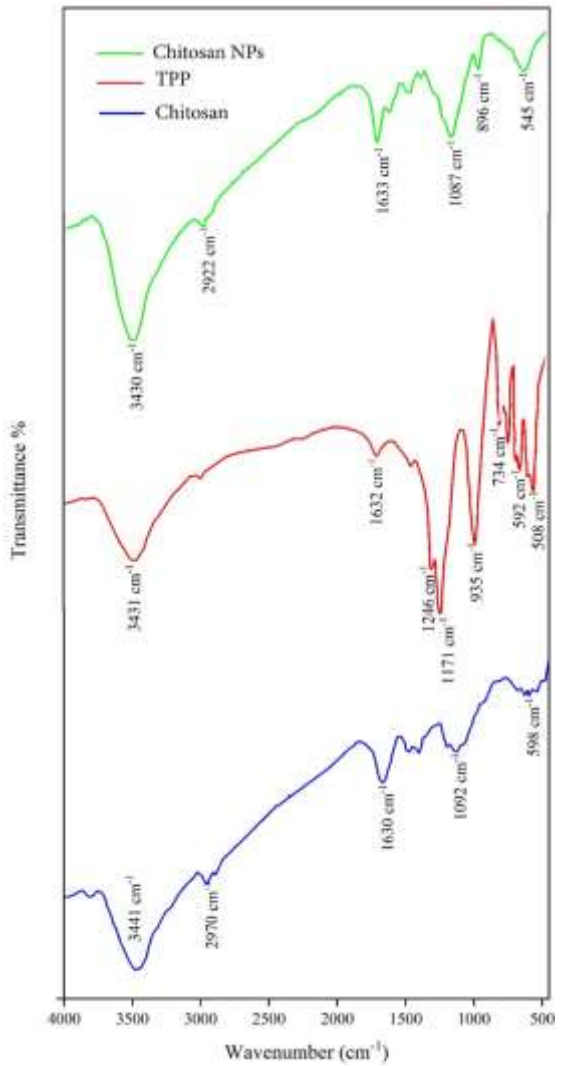


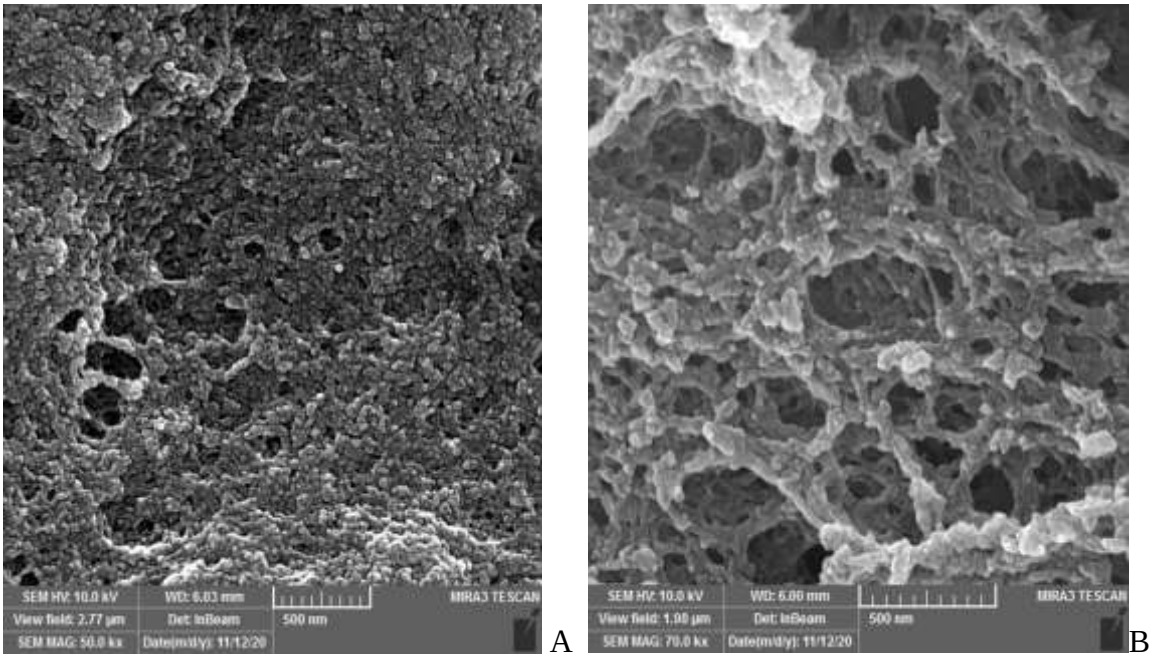
Figure 1: FTIR spectra of chitosan (CS), sodium tripolyphosphate (TPP), and the prepared chitosan nanoparticles (CS-NPs).

Characterization of CS/NPs

SEM imaging demonstrated that the prepared nanoparticles exhibited a uniform spherical morphology with smooth surfaces and narrow size distribution (Figure 2), which is advantageous for biomedical applications (Hoang et al, 2022). The particle size and zeta potential of chitosan nanoparticles in the current study using the ionotropic gelation method was in the range of 100–300 nm and +30 to +50 mV, respectively.

Antibacterial Activity of CS/NPs

CS/NPs exhibited significantly (P≤0.05) higher antibacterial activity than *P. muralis* extract against all tested isolates. Gram-positive bacteria (*S. aureus* and *E. faecalis*) were more susceptible than Gram-negative bacteria. MIC values of CS/NPs were 78.125, 156.25, 625, and 1250 µg/mL for *S. aureus*, *E. faecalis*, *E. coli*, and *P. aeruginosa*, respectively. Corresponding MICs for *P. muralis* extract were 156.25, 312.5, 2500, and 1250 µg/mL, respectively (Table 1).



A B

Figure 2: Representative scanning electron microscopy (SEM) images of the synthesized chitosan nanoparticles (CS-NPs) showing the morphology of the particles at magnifications of (A) 50,000× and (B) 70,000×.

Table 1: Antibacterial activity of chitosan nanoparticles (CS/NPs) and *P. muralis extract* against *S. aureus, E. faecalis, E. coli*, and *P. aeruginosa* local clinical isolates

Bacteria	CS/NPs		*P. muralis*	
	MIC µg/ml	MBC µg/ml	MIC µg/ml	MBC µg/ml
S. aureus	78.125	78.125	156.25	625
E. feacalis	156.25	625	312.5	1250
E. coli	312.5	625	2500	2500
P. aeroginosa	1250	1250	1250	2500

Antibiofilm Activity of CS/NPs

The antibiofilm potential of CS/NPs and *P. muralis* extract is presented in table 2. The results demonstrated that these compounds were able to reduce biofilm formation at various concentrations, although the degree of inhibition varied depending on the bacterial species and the specific compound. Generally, the compounds at lower concentrations (0.5MIC) significantly decreased the initial adhesion of bacterial cells to surfaces, while higher concentrations (1MIC) exhibited a stronger antibiofilm effect. These findings suggest that the studied compounds not only possess antibacterial activity but also can inhibit the development of stable biofilms, which is crucial for preventing drug resistance and enhancing the efficacy of antibacterial treatments.

Table 2: Inhibitory effects of chitosan nanoparticles (CS/NPs) and *P. muralis* extract on *S. aureus*, *E. faecalis*, *E. coli*, and *P. aeruginosa* local clinical isolates biofilm formation. MIC: minimum inhibitory concentration; 0.5 MIC: half of the MIC value. Data are presented as mean ± SEM. Means with different letters within the same row are significantly different (P≤0.05)

Bacteria	CS/NPs		*P. muralis*	
	1 MIC	0.5 MIC	1 MIC	0.5 MIC
S. aureus	$^{A}65 \pm 2.6$	$^{B}55 \pm 2.9$	$^{C}51.3 \pm 1.8$	$^{D}46 \pm 1.5$
E. feacalis	$^{A}60 \pm 2.1$	$^{B}57.7 \pm 1.4$	$^{C}49.3 \pm 0.9$	$^{D}45.3 \pm 1.4$
E. coli	$^{B}48 \pm 1.5$	$^{B}44.3 \pm 1.2$	$^{C}37.3 \pm 1.4$	$^{D}27.3 \pm 1.4$
P. aeroginosa	$^{B}43 \pm 0.9$	B40.3 ± 0.9	$^{C}32.3 \pm 1.4$	$^{D}26.6 \pm 0.9$

CS/NPs markedly suppressed biofilm formation by 65±2.6% in *S. aureus*, 60±2.1% % in *E. faecalis*, 48±1.5% % in *E. coli*, and 43±0.9% in *P. aeruginosa*, whereas *P. muralis* extract showed significantly (P≤0.05) lower inhibition.

Notably, a comparison between Gram-positive and Gram-negative bacteria indicated that Gram-positive strains were relatively more sensitive to the antibiofilm effects of the compounds, which may be related to differences in cell wall structure and adhesion mechanisms.

Synergistic Effect of CS/NPs

FIC index values for the combination of CS/NPs and *P. muralis* extract were 0.39, 0.41, 0.64, and 0.75 for *S. aureus, E. faecalis, E. coli,* and *P. aeruginosa*, respectively, indicating synergism.

Discussion

The physicochemical analysis confirmed the successful fabrication of chitosan nanoparticles (CS/NPs) through ionic gelation with sodium tripolyphosphate (TPP). FTIR spectra revealed a characteristic broad peak at 3422 cm^{-1}, corresponding to stretching vibrations of hydroxyl, water, and amine groups, which is in agreement with earlier findings on chitosan-based nanoparticles (Varma and Vasudevan, 2020). The shift of the amide peak from 1630 cm^{-1} to two distinct bands in the CS/NPs spectrum further indicated strong electrostatic interactions between amino groups of chitosan and phosphate groups of TPP, signifying successful crosslinking and partial deacetylation. The appearance of phosphate-related signals provided additional confirmation of the crosslinking process.

SEM imaging demonstrated that the prepared nanoparticles exhibited a uniform spherical morphology with smooth surfaces and narrow size distribution. Such morphological consistency is considered advantageous for biomedical applications, as uniform particle size can enhance stability, reproducibility, and bioavailability. In particular, nanoscale dimensions increase surface area-to-volume ratio, facilitating stronger interactions with bacterial membranes and improved drug or metabolite loading capacity (Xie et al, 2015).

Methanolic extracts of *P. muralis* demonstrated concentration-dependent antibacterial activity against all tested bacterial isolates. Gram-positive bacteria were more sensitive than Gram-negative isolates, likely due to differences in cell wall structure. Gram-negative bacteria possess a lipopolysaccharide-rich outer membrane that acts as a selective barrier, restricting the entry of many hydrophobic compounds. By contrast, Gram-positive bacteria, which lack this outer membrane, are more susceptible to direct interactions with phytochemicals (Xie et al, 2015; Okoth et al, 2013).

The antibacterial potential of *P. muralis* has been documented in several reports. Valadbeigi et al. demonstrated stronger activity of methanolic extracts compared to aqueous fractions, particularly against *S. aureus* and *S. epidermidis* (Valadbeigi et al, 2020). Almola et al. also reported higher

inhibitory activity against Gram-positive than Gram-negative strains, supporting our observations (Almola et al, 2021). The bioactivity of *P. muralis* is largely attributed to secondary metabolites, including flavonoids, phenolic acids, terpenoids, alkaloids, tannins, and quinones (Bhatti et al, 2022; Shrestha and Clair, 2013; Xie et al, 2015; Okoth et al, 2013). These compounds exert diverse pharmacological effects such as antioxidant, antiviral, antibacterial, antifungal, and antitumor activities.

The antibacterial mechanisms of these metabolites have been widely studied. Alkaloids can intercalate into DNA or disrupt bacterial cell walls, leading to impaired replication and structural instability. Flavonoids interfere with nucleic acid synthesis, inhibit energy metabolism, and alter membrane permeability (Okoth et al, 2013). Phenolic compounds and terpenoids destabilize bacterial membranes by inserting into lipid bilayers and causing leakage of intracellular contents (Valadbeigi et al, 2020). Anthraquinones, on the other hand, can promote the generation of reactive oxygen species, leading to oxidative damage and bacterial cell death (Almola et al, 2021). The synergistic presence of these metabolites in *P. muralis* extract likely accounts for its broad-spectrum but moderate antibacterial effect.

Our findings revealed that CS/NPs inhibited the growth of *S. aureus*, *E. faecalis*, *E. coli*, and *P. aeruginosa* at MIC values of 78.125, 156.25, 625, and 1250 µg/mL, respectively. These results are consistent with the previous reports, although MIC values vary depending on bacterial strains and nanoparticle properties. For example, Valian et al. reported an MIC of 0.625 µg/mL against *S. mutans* (Valian et al, 2020), while Divya et al. demonstrated activity against *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *S. aureus* with MICs ranging from 64–512 µg/mL (Divya et al, 2017). Vimal et al. reported values of 128–512 µg/mL against different Gram-negative and Gram-positive pathogens (Vimal et al, 2013). Collectively, these studies highlight the consistent antibacterial efficacy of CS/NPs, although variability can be expected due to nanoparticle size, degree of deacetylation, and preparation method.

The antibacterial mechanism of CS/NPs is primarily attributed to their cationic amino groups, which enable electrostatic interaction with negatively charged bacterial cell surfaces. This disrupts membrane integrity, increases permeability, and results in leakage of intracellular contents such as proteins, nucleotides, and electrolytes. This disruption is typically more pronounced in Gram-positive bacteria due to their relatively permeable peptidoglycan layer, whereas Gram-negative bacteria are partially protected by their outer membrane barrier (Godoy-Gallardo et al., 2017; Kong et al., 2010). In addition to membrane disruption, CS/NPs have been reported to bind to bacterial DNA, thereby inhibiting replication and transcription, or chelate essential trace metals required for bacterial growth (Varma and Vasudevan, 2020).

Both *P. muralis* extract and CS/NPs demonstrated inhibitory effects on biofilm formation, with CS/NPs showing superior activity. At MIC concentrations, CS/NPs reduced biofilm formation by up to 65% in *S. aureus*. This is consistent with the previous studies showing that chitosan nanoparticles disrupt extracellular polymeric substances (EPS), prevent bacterial adhesion, and impair quorum sensing pathways (Varma and Vasudevan, 2020; Divya et al, 2017). Interestingly, we observed more pronounced inhibition in Gram-negative isolates, which may be due to differences in biofilm matrix density and nanoparticle penetration efficiency. The ability to inhibit biofilm formation is of particular clinical relevance, as biofilm-associated infections are notoriously

resistant to conventional antibiotics and represent a major therapeutic challenge.

The most significant finding of our study was the synergistic antibacterial and antibiofilm effect observed when CS/NPs were combined with *P. muralis* extract. The FIC index values confirmed synergy against all tested isolates. This effect likely arises from complementary mechanisms: CS/NPs enhance adhesion to bacterial surfaces and disrupt cell membranes, facilitating increased uptake of phytochemicals from *P. muralis*. The metabolites, in turn, may interfere with metabolic processes or generate oxidative stress, leading to enhanced bacterial killing.

Synergistic combinations of chitosan with plant extracts have been documented in earlier studies. Etemadi et al. (2019) reported that chitosan combined with turmeric aqueous extract showed enhanced activity against MRSA strains. Aldayel et al. demonstrated synergy between chitosan and *Capsicum annuum* extract against *S. aureus*, *S. typhimurium*, and *P. aeruginosa* (Aldayel et al, 2020). Tantala et al. found that chitosan films containing fingerroot and holy basil essential oils could reduce *Listeria monocytogenes* to undetectable levels within 12 hours (Tantala et al, 2019). Collectively, these findings reinforce the concept that nanotechnology-based systems can augment the antimicrobial efficacy of natural bioactive compounds.

The growing prevalence of antibiotic-resistant pathogens and biofilm-associated infections underscores the urgent need for alternative antimicrobial strategies. The results of the present study demonstrate that CS/NPs and *P. muralis* extract, individually and in combination, offer promising antibacterial and antibiofilm activities against clinically important bacteria. Importantly, the observed synergy suggests that lower doses of each agent may be sufficient when used in combination, thereby potentially reducing toxicity and minimizing the risk of resistance development (Varma and Vasudevan, 2020).

Given the biocompatibility and biodegradability of chitosan, CS/NPs have high translational potential in biomedical applications such as wound dressings, coatings for medical devices, and drug delivery systems. Similarly, the use of *P. muralis* extract, which is derived from a natural and sustainable source, aligns with the global movement toward eco-friendly and plant-based therapeutics. However, further investigations, particularly in vivo studies and molecular assays, are required to fully elucidate the mechanisms of action and to confirm safety and efficacy in clinical settings.

In summary, the current study demonstrated that CS/NPs exhibit potent antibacterial and antibiofilm activity, *P. muralis* extract shows moderate but notable antibacterial effects, and their combination results in synergistic enhancement. The underlying mechanisms involve complementary interactions between nanoparticle-mediated membrane disruption and phytochemical-driven metabolic interference. These findings highlight the potential of integrating nanotechnology with plant-derived compounds to develop innovative antimicrobial strategies against resistant and biofilm-associated infections. Future studies should focus on optimizing nanoparticle formulations, evaluating cytotoxicity, and conducting in vivo efficacy testing to pave the way for clinical translation.

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Conflict of interest

The authors report no competing financial or personal interests that could have biased this work.

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ارزیابی برون‌تنی هم‌افزایی فعالیت‌های ضدباکتریایی و ضدبیوفیلمی عصاره پاریتاریا مورالیس (گلسنگ) و نانوذرات کیتوزان

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چکیده

داروهای گیاهی ترکیب شده با مواد نانو نگاه‌های قابل توجهی را در حوزه پزشکی زیستی به خود جلب کرده‌اند؛ با این حال، توان ضدباکتریایی ترکیب کیتوزان با عصاره گلسنگ *پروتوپارملیوپسیس مورالیس* (*Protoparmeliopsis muralis*) تاکنون مورد بررسی قرار نگرفته است. هدف از پژوهش حاضر، ارزیابی اثر هم‌افزای عصاره *پروتوپارملیوپسیس مورالیس* و نانوذرات کیتوزان (CS/NPs) بر روی باکتری‌های بیماری‌زای منتخب بود. فعالیت ضدباکتریایی با استفاده از آزمون حداقل غلظت مهارکنندگی (MIC) مورد ارزیابی قرار گرفت و اثرات ضدبیوفیلمی آن نیز با آزمون **کریستال** ویوله سنجیده شد. مخلوطی حاوی ۱۰ درصد عصاره *پروتوپارملیوپسیس مورالیس* و ۰/۰۵ درصد نانوذرات کیتوزان تهیه گردید و تعامل هم‌افزا از طریق روش **چکربورد** مورد بررسی بیش‌تر قرار گرفت. CS/NPs فعالیت ضدباکتریایی قوی‌تری نسبت به عصاره *پروتوپارملیوپسیس مورالیس* از خود نشان داد. علاوه بر این، آزمون چکربورد تأیید کرد که میان *پروتوپارملیوپسیس مورالیس* و CS/NPs اثر هم‌افزایی وجود دارد. ترکیب آن‌ها فعالیت ضدبیوفیلمی را نیز به طور قابل توجهی افزایش داد که پتانسیل آن را به عنوان رویکردی نویدبخش برای کنترل رشد باکتری‌ها و عفونت‌های مرتبط با بیوفیلم برجسته می‌سازد. در مجموع، یافته‌های این مطالعه نشان می‌دهد که ترکیب عصاره **گل‌سنگ** و نانوذرات کیتوزان می‌تواند به عنوان یک گزینه امیدوارکننده برای مطالعات آتی با هدف شناسایی و توسعه ترکیبات جدید ضدمیکروبی و ضدبیوفیلم مطرح شود. با این حال، انجام پژوهش‌های بیش‌تر، از جمله بررسی‌های مکانیسم اثر و ارزیابی‌های درون‌تنی، برای درک بهتر و تأیید اثرات مشاهده‌شده ضروری است.

کلمات کلیدی: کیتوزان، گلسنگ، ضد باکتریایی، ضد بیوفیلمی

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