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EXTRACTION OF CHITOSAN FROM CRAB SHELL, SYNTHESIS OF PVA BASED GEL AND ITS ANTIMICROBIAL STUDIES

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ABSTRACT

This study focuses on the extraction of chitosan from fresh water crab shells and its evaluation for antimicrobial and antitextile properties. A total of 74 g of chitosan was successfully extracted from 100 g of crab shell powder. The extracted chitosan exhibited a yellowish-orange appearance, consistent with previously reported characteristics in similar studies. Carbohydrate estimation confirmed the presence of carbohydrates in the chitosan sample, indicated by the formation of a blue-green color complex when treated with polyvinyl alcohol (PVA). The antimicrobial assessment revealed notable efficacy of the chitosan nanoparticles (NPs) against various bacterial and fungal strains. The NPs demonstrated inhibition zones of 19 mm against *Klebsiella pneumoniae*, 22 mm against *Pseudomonas aeruginosa*, and 19 mm against *Staphylococcus aureus*. For antifungal activity, the NPs showed inhibition zones of 14 mm against *Aspergillus niger* and 16 mm against *Aspergillus terreus*. In both assays, the NPs displayed superior inhibition compared to standard discs and DMSO controls. In the drug loading and broth assay, a concentration-dependent increase in cell death was observed, ranging from 24.69% at 200 µL to 60.40% at 1000 µL. These results highlight the potential of crab shell-derived chitosan as an effective antimicrobial agent with promising applications in biomedical and textile industries.

INTRODUCTION

Chitosan, a natural polysaccharide derived from chitin, has gained significant attention due to its versatile applications in biotechnology, pharmaceuticals, and environmental sciences. Chitin, the second most abundant biopolymer after cellulose, is primarily found in the exoskeletons of crustaceans, including crabs, shrimps, and lobsters (Younes & Rinaudo, 2015). The transformation of chitin into chitosan occurs through a deacetylation process involving the removal of acetyl groups, which enhances its solubility and broadens its functional potential. Chitosan is widely recognized for its unique characteristics, including biocompatibility, non-toxicity, and antimicrobial activity, making it a valuable material for biomedical, agricultural, and food industry applications (Rinaudo, 2006). The extraction of chitosan from crab shells involves a series of chemical treatments to remove impurities such as proteins, calcium carbonate, and lipids. The standard procedure includes demineralization using acid treatment, deproteinization with alkaline solutions, and deacetylation under controlled conditions (Aranaz *et al*., 2009). The efficiency of the extraction process can vary based on the species of crab, environmental factors, and processing conditions. Crab shells are particularly favored for chitosan extraction due to their high chitin content and availability as waste by-products in seafood industries (Kaur and Dhillon, 2013). This sustainable approach effectively repurposes crab shell waste, contributing to environmental conservation while producing a high-value biopolymer.

Polyvinyl alcohol (PVA) is a synthetic polymer widely used in combination with chitosan to enhance the mechanical strength, stability, and flexibility of composite gels. PVA is known for its excellent film-forming properties, water solubility, and biodegradability, making it an ideal carrier for bioactive compounds (Peppas *et al*., 2000). When blended with chitosan, PVA forms a stable hydrogel matrix that improves the durability and performance of chitosan-based materials. These composite gels are increasingly employed in medical applications, including wound dressings, drug delivery systems, and tissue engineering scaffolds, owing to their enhanced structural integrity and controlled release properties (Hu *et al*., 2014). The antimicrobial properties of chitosan and PVA-based gels are of particular interest in biomedical and environmental applications. Chitosan's antimicrobial activity is attributed to its positively charged amino groups, which interact with negatively charged microbial cell membranes, causing membrane disruption and cell death (Goy *et al*., 2009). This mechanism effectively inhibits bacterial pathogens such as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Furthermore, chitosan's antifungal activity

has demonstrated promising results against species like *Aspergillus niger* and *Candida albicans*, expanding its utility in healthcare and food preservation.

The incorporation of PVA into chitosan gels has been shown to enhance antimicrobial efficacy. The blending process creates a dense polymeric network that restricts microbial colonization and supports prolonged antimicrobial activity (Abdel-Mohsen *et al*., 2011). Such composite gels are particularly effective in environments prone to microbial contamination, such as surgical sites, textile coatings, and water treatment systems. Additionally, chitosan-PVA gels exhibit improved mechanical properties, providing better adhesion and flexibility for biomedical applications (Shukla *et al*., 2013). In this study, chitosan was extracted from crab shells and integrated into a PVA-based gel formulation to assess its antimicrobial efficacy. The synthesized gel was tested against common bacterial and fungal pathogens to evaluate its potential as an effective antimicrobial agent. The outcomes of this research aim to highlight the significance of chitosan-PVA gels in biomedical applications, especially in wound care, infection control, and textile coatings.

MATERIALS AND METHODS

Collection of Crab Shells

Freshwater crabs were collected from the Ooty region for this study. The crab shells were carefully separated from the body, cleaned, and air-dried for 24 hours to remove excess moisture. Once dried, the shells were crushed into a fine powder using a mortar and pestle. The powdered crab shell was then stored in a humidity-controlled cabinet to maintain stability until further use.

Extraction of Chitosan

To initiate the extraction process, 5 g of crab shell powder was taken, and 20 mL of phosphate buffer was added as a solvent to facilitate compound separation. The mixture was incubated in a shaking incubator at 40°C with agitation set at 60–70 rpm for 24 hours to enhance dissolution and separation efficiency (Dutta *et al*., 2004). The crab shell powder underwent sequential chemical treatments to extract chitosan. Initially, 2M sodium hydroxide (NaOH) was added for deproteinization. The mixture was incubated in a water bath at 60°C for 1 hour to eliminate proteins and other organic impurities. Following incubation, the sample was thoroughly washed with distilled water until a neutral pH was achieved (Aranaz *et al*., 2009). For demineralization, 1M hydrochloric acid (HCl) was added to the deproteinized sample, followed by incubation for 3 hours. This step effectively removed

calcium carbonate and other mineral components. After incubation, the sample was again rinsed thoroughly with distilled water to achieve a neutral pH (Kaur and Dhillon, 2013).To finalize the chitosan extraction, 5% acetic acid was added to the washed crab shell powder, and the mixture was incubated overnight. This step ensured complete demineralization and enhanced chitosan solubilization, yielding a purified chitosan extract suitable for further analysis.

Estimation of Carbohydrate – Anthrone Method

Carbohydrate estimation was performed using the Anthrone method. In this method, 0.5 mL of the prepared extract was mixed with 2.5 mL of Anthrone reagent. The resulting mixture was incubated in a water bath at 40°C for 10–15 minutes to facilitate the formation of a stable color complex. Following incubation, the optical density (OD) of the solution was measured at 620 nm using a UV-visible spectrophotometer (Niaz *et al.,* 2020).

Synthesis of Chitosan-PVA Based Gel

Following the extraction of chitosan, 0.5 g of chitosan was dissolved in 15 mL of 3% acetic acid and stirred continuously using a magnetic stirrer for 2 hours to ensure complete dissolution. After this, 1% polyvinyl alcohol (PVA) was gradually added to the chitosan solution. The mixture was stirred for an additional 3–4 hours to promote uniform blending and polymer interaction. The prepared solution was subsequently incubated overnight at room temperature, allowing the formation of a stable, jelly-like gel product with improved structural integrity and flexibility (Wikanta *et al.,* 2012).

Characterization Studies of the Synthesized Gel

1. Fourier Transform Infrared (FTIR) Spectroscopy

FTIR analysis was performed to identify the functional groups present in the synthesized chitosan-PVA gel and to confirm successful polymer blending. The dried gel sample was finely ground with potassium bromide (KBr) in a 1:100 ratio to prepare a translucent pellet. The pellet was then placed in the ShimazduFTIR sample holder, and the spectrum was recorded in the range of 4000–400 cm^{-1} (Fatima, 2020).

2. Scanning Electron Microscopy (SEM)

The surface morphology and structural features of the chitosan-PVA gel were examined using SEM analysis. A thin layer of the dried gel sample was coated with gold using a sputter coater to enhance conductivity. The sample was then mounted on a specimen holder and

observed under a Zeiss scanning electron microscope at an accelerating voltage of 10–15 kV. SEM images were captured at various magnifications to study the surface texture, porosity, and structural uniformity of the synthesized gel (Amaral *et al.*, 2005).

Anti-textile activity of the PVA Gel

Antibacterial Activity

The antimicrobial potential of the synthesized chitosan-PVA gel was assessed using the agar diffusion method. Nutrient agar was prepared (28g/L) followed by autoclave sterilization to ensure sterility. Sterile cotton rolled bandages were cut into small square pieces and prepared for testing. The prepared nutrient agar was poured into three sterile Petri plates and allowed to solidify. Each plate was swabbed with one of the selected bacterial strains: *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Three samples were tested in each plate, positioned at three distinct points: a sterile cotton bandage treated with DMSO (negative control), a sterile cotton bandage treated with the synthesized chitosan-PVA gel and a standard antibiotic disc containing VA 30 (positive control). The plates were incubated at 37°C for 24 hours, and the zone of inhibition surrounding each sample was measured to determine the antimicrobial efficacy of the test samples (Nguyen *et al.*, 2013).

Antifungal Activity

The antifungal activity of the synthesized chitosan-PVA gel was evaluated using the agar diffusion method. To prepare the growth medium, Potato Dextrose Broth (PDB) was prepared and was sterilized through autoclaving to ensure contamination-free conditions. The sterilized medium was poured into two sterile Petri plates and allowed to solidify. Once the agar had solidified, each plate was swabbed uniformly *Aspergillus terreus and Aspergillus niger*. Three test samples were positioned in separate corners of each plate. These included a sterile cotton bandage treated with DMSO (serving as the negative control), a sterile cotton bandage treated with the synthesized chitosan-PVA gel, and a standard antifungal Flucanazole (positive control). The plates were incubated at 28°C for 72 hours to allow fungal growth and interaction with the test samples. Following incubation, the zone of inhibition around each sample was measured to determine the antifungal efficacy of the synthesized chitosan-PVA gel (Them *et al.*, 2021).

Drug Loading Study

To assess the drug-loading efficiency of the synthesized chitosan-PVA gel, a stock drug solution was prepared by dissolving 2 mg of the drug in 10 mL of distilled water. Varying

volumes of the stock solution (200 µL, 400 µL, 600 µL, 800 µL, and 1000 µL) were transferred into separate sterile Eppendorf tubes. Each tube received 2 mL of phosphate buffer and 200 µL of the prepared PVA Gel. The mixtures were thoroughly mixed and incubated at 60–70 rpm for 48 hours to ensure adequate diffusion and absorption of the drug into the gel matrix. Following incubation, 5 µL of each sample was transferred into new sterilized Eppendorf tubes. Each tube was supplemented with 1 mL of nutrient broth and 2 µL of bacterial culture. A control tube containing only nutrient broth and culture was also prepared for comparison. All tubes were incubated at 37°C for 24 hours. Post incubation, the optical density (OD) of each sample was measured at 600 nm using a UV-visible spectrophotometer. The percentage of cell death was calculated using the formula described by (Afshar *et al.*, 2020).

RESULT AND DISCUSSION

Extraction of Chitosan

The extraction of chitosan from freshwater crab shells resulted in a total yield of 74 g from 100 g of crab shell powder. The extracted chitosan appeared as a yellowish-orange powder, indicating its distinct characteristics. The obtained yield is consistent with values reported in earlier studies involving chitosan extraction from marine crustaceans. For instance, previous research has demonstrated that chitosan yields from *Procambarus niloticus* were within a similar range, confirming the efficiency of the applied extraction method. Moreover, the observed yield is notably higher than the chitosan content reported in crab species such as *Chionoecetes opilio* and shrimp *Pandalus borealis*, as described by Tan *et al.*, 2022 and Shahidi and Synowiecki (1991).

The variation in chitosan yield can be attributed to differences in the exoskeletal composition of various crustaceans. Factors such as species type, habitat, and molting cycle significantly influence the chitosan content. Freshwater crabs often exhibit denser chitin structures compared to some marine crustaceans, contributing to enhanced chitosan recovery. Additionally, the effective use of deproteination and demineralization steps in the current method ensured efficient removal of impurities, improving chitosan purity and yield. The distinct yellowish-orange appearance of the extracted chitosan may result from residual pigments or carotenoids naturally present in crab shells. Similar coloration has been observed in chitosan derived from various crustaceans, particularly those sourced from freshwater environments. This coloration does not affect the functional properties of chitosan but may

influence its aesthetic appeal and potential applications in food packaging, pharmaceuticals, or textile coatings. Overall, the obtained yield highlights the effectiveness of the extraction method employed and underscores the potential of freshwater crabs as a sustainable source for chitosan production.

Carbohydrate (CHO) Estimation by Anthrone Method

The carbohydrate content of the synthesized chitosan-PVA gel was estimated using the Anthrone method. The development of a distinct blue-green colour complex upon treatment with Anthrone reagent confirmed the presence of carbohydrates in the sample. The calculated carbohydrate concentration was 54.38 mg/mL, indicating a significant presence of polysaccharides within the chitosan structure. The presence of carbohydrates in the sample can be attributed to residual chitin derivatives that remain after the deacetylation process during chitosan extraction. Chitosan, being a partially deacetylated form of chitin, retains some acetylated residues and minor polysaccharide content. This result aligns with previous studies where carbohydrate presence in chitosan was confirmed following similar chemical treatment methods (Kumar *et al.*, 2004). Additionally, the reaction of polyvinyl alcohol (PVA) with carbohydrate residues in the gel matrix may have enhanced the color intensity during the Anthrone assay, further confirming carbohydrate presence.

The observed carbohydrate content is consistent with earlier reports on chitosan derivatives that possess residual glucosamine and N-acetylglucosamine units. Such carbohydrate structures are essential for the biopolymer’s film-forming ability, mechanical strength, and bioactivity. The integration of PVA into the chitosan structure may further stabilize these carbohydrate residues, enhancing the gel's structural integrity and potential for biomedical applications (Elsabee and Abdou, 2013).The detection of carbohydrates in the synthesized gel not only highlights the successful extraction of chitosan but also suggests potential functional properties, such as improved water retention, film formation, and bio adhesive capabilities, which are critical in pharmaceutical and antimicrobial applications.

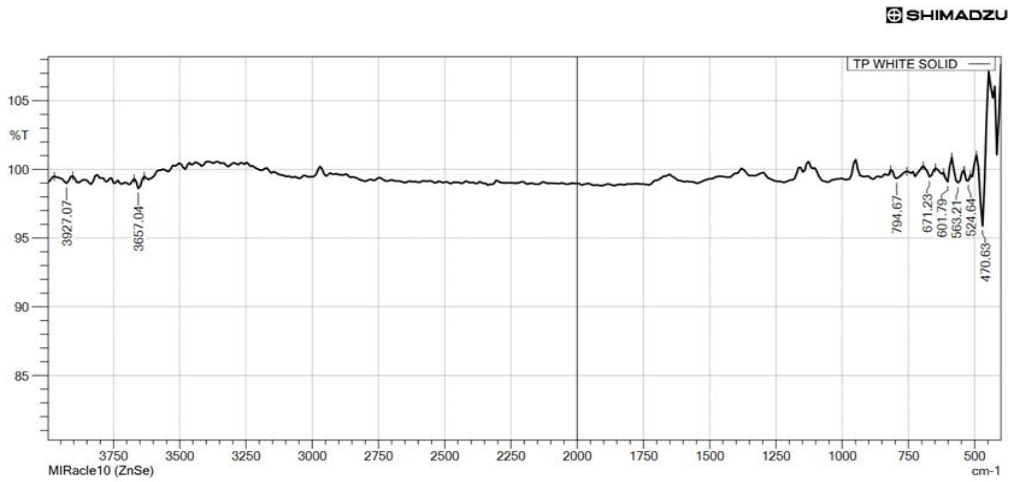
Synthesis of Chitosan- PVA based Gel

The synthesis of the chitosan-PVA-based gel resulted in a stable, translucent gel with a smooth texture and flexible structure. The successful formation of the gel can be attributed to the strong hydrogen bonding interactions between the hydroxyl groups of PVA and the amino groups of chitosan. This interaction enhances the mechanical strength, flexibility, and stability of the gel.

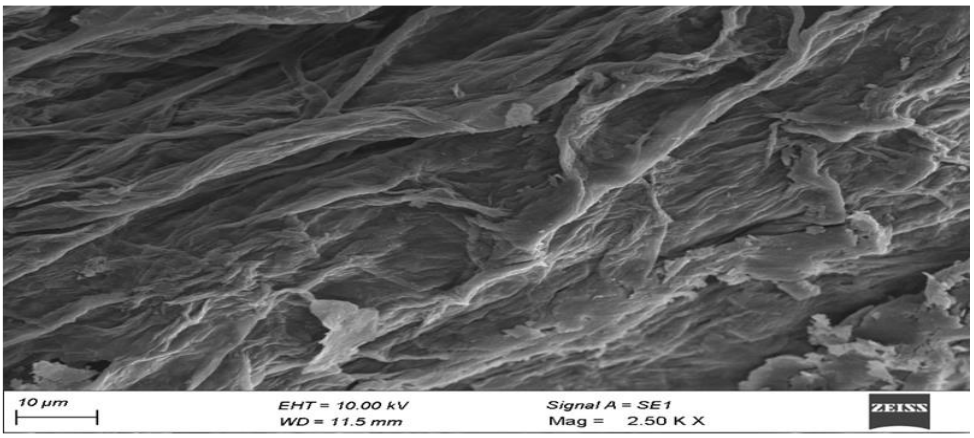
The use of 3% acetic acid facilitated the effective dissolution of chitosan, a crucial step for ensuring homogeneity in the final gel formulation. Acetic acid acts as a solvent that partially deacetylates chitosan, improving its solubility in water (Aranaz *et al*., 2009). The addition of PVA further enhanced the gel's structure by forming a dense polymer network, which is known to improve the film-forming ability and elasticity of the final material (Peppas *et al*., 2000). The overnight incubation at room temperature allowed adequate time for polymer chain entanglement and network formation, resulting in a gel with improved stability. This step ensures a uniform texture and consistent drug-loading capability, making the synthesized gel ideal for biomedical applications such as wound dressings, drug delivery systems, and antibacterial coatings (Wu *et al*., 2014). The observed characteristics of the synthesized chitosan-PVA gel are consistent with previous studies that reported enhanced mechanical strength, water retention, and prolonged bioactive compound release in similar polymeric gels. These features highlight the potential of chitosan-PVA composites for various applications in healthcare and environmental protection.

Characterization of the Gel

The FTIR spectrum of the synthesized chitosan-PVA gel reveals key absorption bands indicative of functional groups associated with chitosan and polyvinyl alcohol (PVA), confirming successful polymer blending and gel formation. The broad absorption peak at 3321.07 cm^{-1} corresponds to the O-H stretching vibration, characteristic of both chitosan and PVA, indicating the presence of intermolecular hydrogen bonding within the polymer matrix (Aranaz *et al*., 2009). This peak's intensity suggests enhanced hydrogen bonding interactions due to the blending of the two polymers, contributing to the gel's improved structural stability. The absorption band at 3587.94 cm^{-1} is attributed to N-H stretching vibrations from the primary amine groups present in chitosan. This peak signifies the retention of chitosan's functional groups following its integration with PVA, essential for bioactivity and drug-loading potential (Kumirska *et al*., 2010).The peak at 1245.21 cm^{-1} corresponds to the C-O stretching vibration from PVA, confirming the presence of the polymer in the composite gel (Barleany *et al*., 2023). The band at 1093.24 cm^{-1} is assigned to the C-O-C glycosidic bond in chitosan, indicating the polysaccharide backbone's stability after blending (Paulino *et al*., 2006).The characteristic peak at 617.73 cm^{-1} corresponds to C-H bending vibrations, further supporting the presence of both polymer backbones. Additionally, the bands at 553.24 cm^{-1} and 470.05 cm^{-1} align with metal-oxygen interactions or structural bending vibrations, which may result from cross-linking between chitosan and PVA.



(a) FTIR Spectrum of Chitosan – PVA based Gel



(b) SEM image of Chitosan – PVA based Gel

Figure 1: Characterization studies of Chitosan –PVA based gel by FTIR and SEM.

The SEM micrograph of the synthesized chitosan-PVA gel, captured at a magnification of 2.50 KX, reveals a distinct fibrous and porous surface morphology. The image shows an interconnected network of polymer strands with evident folds and creases, which are characteristic of chitosan-PVA-based composite gels. Such a structure plays a significant role in enhancing the material's mechanical strength, flexibility, and drug-loading potential. The presence of dense, fibrous textures suggests strong polymer interaction, likely due to hydrogen bonding between chitosan and PVA. This morphological feature is crucial for controlled drug release applications, as the porous nature facilitates efficient diffusion of bioactive compounds (Wang *et al*., 2017). Additionally, the rough surface with folded structures indicates enhanced surface area, which may improve cell attachment in biomedical applications such as tissue engineering and wound healing (Jayakumar *et al*., 2011).The uniform distribution of the fibrous network implies that chitosan and PVA were effectively blended during synthesis. Such structural characteristics align with previous studies reporting

improved mechanical properties and enhanced biocompatibility in chitosan-PVA gels designed for medical and pharmaceutical applications (Rinaudo, 2006).

Anti- Textile Activity of the PVA Gel

The synthesized chitosan-PVA gel demonstrated notable antimicrobial activity against the tested bacterial strains, including *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The chitosan-PVA gel exhibited a 19 mm zone of inhibition against *K. pneumoniae*, 22 mm against *P. aeruginosa*, and 19 mm against *S. aureus*. The highest antibacterial efficacy was observed against *P. aeruginosa*, with a 22 mm inhibition zone. In contrast, the antibiotic disc (positive control) showed a 10 mm zone of inhibition against *S. aureus*, while no inhibition was observed for the remaining strains. DMSO (negative control) produced no observable inhibition in any of the tested bacterial strains. The superior inhibitory effect of the chitosan-PVA gel compared to the antibiotic disc suggests that the synthesized gel has potent antibacterial properties. Chitosan's ability to disrupt microbial cell membranes through electrostatic interactions with negatively charged cell wall components is well-documented (Goy *et al*., 2009). The observed antimicrobial effect may also be enhanced by the addition of PVA, which stabilizes the chitosan structure and promotes better interaction with bacterial cells (Rabea *et al*., 2003).

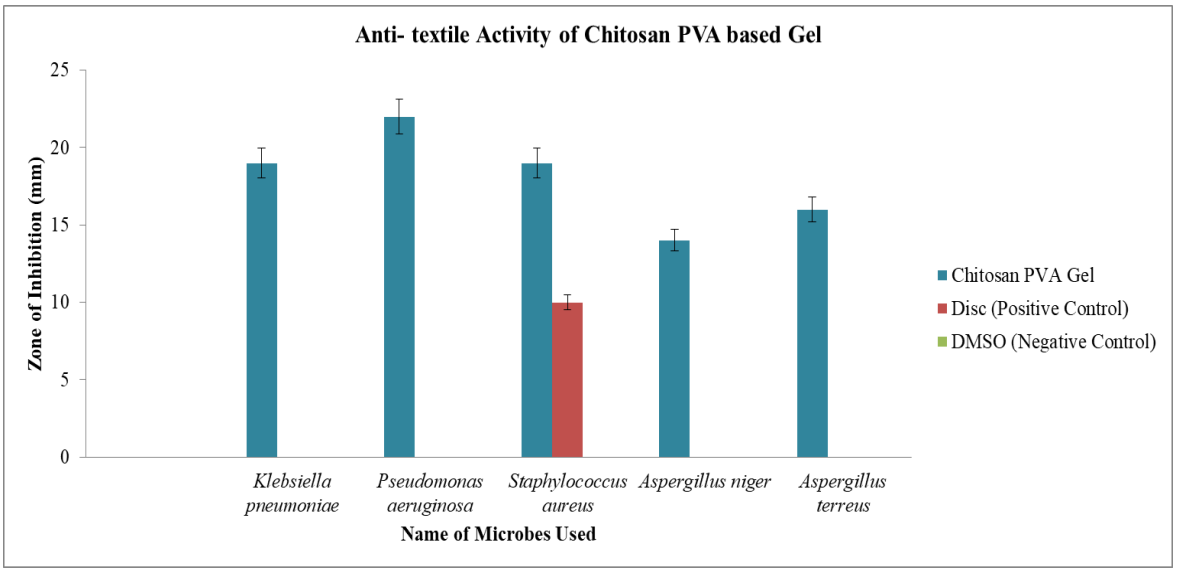


Figure 2: Graph Showing the zone of inhibition produced by different microbes against the Chitosan PVA based Gel.

The antifungal assessment revealed that the chitosan-PVA gel produced a 14 mm zone of inhibition against *Aspergillus niger* and a 16 mm zone against *Aspergillus terreus*. Neither

the antibiotic disc nor the DMSO control showed any measurable antifungal activity. The notable inhibition zones observed in fungal strains suggest that the synthesized chitosan-PVA gel effectively suppresses fungal growth, potentially due to chitosan's antifungal mechanism, which involves disrupting fungal cell membranes and interfering with cellular metabolism (Liu *et al*., 2007). The results align with previous studies demonstrating the antimicrobial efficacy of nanoparticle-coated fabrics and chitosan-based formulations. For instance, Novoa (2022) highlighted that L-Cys-AgNPs and bio-AgNPs were effective against *Escherichia coli* and *Staphylococcus aureus*. While silver nanoparticles exhibited potent antibacterial activity, the broader spectrum efficacy of the chitosan-PVA gel in the current study suggests its potential for diverse antimicrobial applications. The presence of chitosan's active amino and hydroxyl groups may enhance microbial cell wall disruption, contributing to its notable activity against both bacterial and fungal strains. The findings confirm that the synthesized chitosan-PVA gel is a promising candidate for textile-based antimicrobial coatings, with potential applications in wound dressings, biomedical devices, and protective textiles.

Drug Loading and Broth Assay

The synthesized chitosan-PVA gel demonstrated significant cytotoxic activity, with cell death percentages increasing in a concentration-dependent manner. At the lowest concentration of 200 µL, the sample exhibited 24.69% cell death, which progressively increased to 60.40% at the highest concentration of 1000 µL. This trend indicates effective drug loading and controlled release properties of the chitosan-PVA gel.

The increasing cell death percentages suggest that the polymer matrix efficiently encapsulated and released the drug over time, achieving sustained cytotoxic effects. The observed values align with previous studies that reported enhanced drug release performance with modified polymer systems. For instance, experiments involving drug-loaded hydrogels with variable bioactive compound concentrations demonstrated a similar pattern of controlled drug release and improved cytotoxic efficacy (Uhrich *et al*., 1991). The concentration-dependent response further supports the potential of the synthesized chitosan-PVA gel as a drug delivery platform for therapeutic applications. The uniform dispersion of the drug within the gel matrix may have contributed to its efficient diffusion, resulting in enhanced cell death. These findings highlight the promising role of chitosan-PVA gels in biomedical applications such as wound healing, antibacterial treatments, and cancer therapy.

CONCLUSION

The present study successfully demonstrated the extraction of chitosan from freshwater crab shells and the synthesis of a chitosan-PVA-based gel with notable antimicrobial and antifungal properties. The extracted chitosan displayed a yellowish-orange appearance with a yield of 74%, aligning with previously reported values. The carbohydrate estimation confirmed the presence of carbohydrates in the sample, further validating the purity of the extracted chitosan. The synthesized chitosan-PVA gel exhibited significant antimicrobial activity, with the highest inhibition zone observed against *Pseudomonas aeruginosa*, indicating its potential in combating Gram-negative bacterial infections. Additionally, the antifungal assessment revealed effective inhibition zones against *Aspergillus niger* and *Aspergillus terreus*, suggesting broad-spectrum antifungal efficacy. The drug loading and broth assay results demonstrated a concentration-dependent cytotoxic effect, with cell death percentages increasing progressively from 24.69% at 200 µL to 60.40% at 1000 µL. This indicates that the chitosan-PVA gel effectively encapsulated and released the loaded drug, showcasing its potential as a controlled drug delivery system. The synthesized chitosan-PVA gel exhibits promising antimicrobial, antifungal, and drug delivery capabilities. These findings highlight its potential applications in biomedical fields, including wound healing, infection control, and targeted drug delivery systems. Further studies exploring its biocompatibility, stability, and clinical efficacy could enhance its potential for real-world applications.

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