Research Article

Formulation, Evaluation and Anti-Microbial Activity of Pomegranate Peel Extract Incorporated Hydrogel Beads

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Received: 10-05-2026; **Revised:** 23-07-2026; **Accepted:** 30-07-2026; **Published online:** 20-08-2026.

ABSTRACT

Innovative hydrogel-based formulations containing natural biologically active compounds offer promising strategies for infection control. This study evaluated the potential of pomegranate peel extract (PPE)-infused hydrogel beads, which were developed and examined their phytochemical analysis and antimicrobial activity. Hydrogel beds were developed using sodium alginate and crosslinked with calcium chloride, incorporating varying concentrations of PPE. The formulated beds were examined for their organoleptic properties, swelling behaviour, pH, solubility, bead size, and uniformity. Antimicrobial screening against Gram-positive *Staphylococcus aureus* (ATCC 29213) confirmed the retention of PPE bioactivity. The findings indicate that PPE-loaded hydrogel beads provide antimicrobial efficacy. These results highlight the potential of PPE-infused hydrogel beads as an innovative, natural, and effective approach for managing infected wounds and promoting tissue repair, supporting their potential advancement toward clinical applications.

Keywords: Pomegranate peel extract, Hydrogel beads, Antimicrobial activity, Staphylococcus aureus, Sodium alginate, Gram-positive bacteria.

INTRODUCTION

The skin forms a natural shield against external exposure and performs multiple vital protective roles. When its structural integrity is disturbed by either acute trauma or persistent irritation, a complex cascade of biological events begins, ultimately aiming to restore the damaged tissue and re-establish the barrier function. Skin wounds involve disruption of normal anatomical organization and function due to significant tissue breakage. Effective management of wounds remains challenging because of the intricate series of overlapping healing events.[1] The repair process results from a coordinated sequence of responses, including blood clotting, inflammation, synthesis of extracellular matrix and ground substance, new blood vessel formation, re-epithelialization, contraction of the wound, and extensive tissue remodelling.[2]

Infections are among the primary factors contributing to delayed wound closure, whether caused by bacteria or fungi. The severity of infection-related complications depends largely on the immune status of the host and the microbial load present at the wound site. A wound with normal immune responses and adequate cleaning may tolerate a limited number of pathogenic organisms without impairing recovery; however, when the microbial count exceeds a critical threshold, infection may develop. Invasive fungal infections continue to be a major global concern, particularly in immune compromised individuals. *Candida albicans* is a major prevalent fungal pathogen found in wounds and represents a widespread cause of opportunistic fungal diseases, characterized by high mortality and increased healthcare burden, and prolonged hospitalization. Its virulence in open wounds is linked to multiple pathogenic mechanisms, including biofilm formation. Therapeutic options are further restricted by rising resistance to antifungal medications.[3]

A hydrogel refers to a three-dimensional network produced through polymer chain polymerisation or cross-link formation. Owing to this distinctive internal network, hydrogels can absorb a limited amount of aqueous and physiological fluids while maintaining their structural stability. Such materials demonstrate several beneficial characteristics, including high biocompatibility, degradability, and suitability as vehicles for therapeutic delivery. As a result, hydrogels have been widely utilized in diverse sectors, including the food industry, environmental remediation, and medical technology. This study is investigates the extraction of pomegranate peel using Soxhlet extraction, phytochemical characterization of the extract, formulation of extract-loaded hydrogel beads, and evaluation of their physicochemical and antimicrobial properties.[4]

Hydrogel beads represent a distinct type of hydrogel structure that can be generated by injecting precursor hydrogel solutions into predefined moulds or by applying syringe-based dripping techniques. The selected fabrication method is essential for determining the beads' mechanical robustness, structural consistency, and overall physicochemical characteristics. For instance, physical crosslinking processes that depend on electrostatic interactions between chitosan and other components can markedly influence both the surface morphology and mechanical stability of chitosan hydrogel beads. In contrast, chemical crosslinking increases the internal crosslink density within the beads, thereby enhancing their functional performance. Hydrogel beads refer to macroscopic,

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spherical hydrogel structures with diameters generally in the millimetre range, distinguishing them from micro scale particles. These spherical hydrogels are typically produced by dripping polymer solutions into crosslinking media using syringes or by filling solutions into rigid moulds to establish shape.

Hydrogel beads have gained importance across food, biomedical, and environmental applications because of their distinctive spherical form and high surface-to-volume ratio.[5] Their bead-like configuration improves practical utility, and their reduced size facilitates incorporation into food matrices without altering sensory characteristics. Owing to their visually appealing spherical appearance, hydrogel beads have attracted significant attention in contemporary food research. Their diameters commonly fall within the millimetre range. Researchers have reported bead sizes within the approximate range of 3–4 mm and 2–6 mm using different preparation techniques.

Plants have developed the ability to synthesize diverse secondary metabolites as protective responses to biotic and abiotic environmental pressures. These metabolites possess chemically diverse structures and include groups such as alkaloids, tannins, vitamins, and phenolic constituents. Since ancient periods, plants have been extensively utilized in traditional medicine for their capacity to enhance immune function and alleviate various diseases. Numerous crude extracts and isolated phytochemicals have been shown to exhibit the wide range of biological properties, including antimicrobial, anticancer, and antioxidant effects.[6] Research investigations have further revealed their potential to promote relaxation of smooth muscles in vertebrate tissues. Natural compound are generally extracted from plants as complex mixtures, which may contribute to synergistic interactions among certain physiological activities. The concentration of bioactive molecules varies across plant tissues but is frequently abundant in peels, which are commonly treated as waste. Consequently, interest is growing in designing new approaches to utilize fruit and plant peels for industrial purposes, including cosmetic formulations, livestock feed, and natural food preservatives and colorants.[7]

Pomegranate (*Punica granatum L*., family *Punicaceae*) is a sweet-tasting fruit recognized for its nutritional qualities and therapeutic potential. It possesses a rich diversity of natural products, such as minerals, alkaloids, and phenolic substances, including flavonoids like anthocyanins and catechins and tannins such as ellagitannins and ellagic acid derivatives such as punicalagin, punicalin, and pedunculagin. These bioactive constituents are found in particularly high concentrations in the peel, which constitutes approximately half of the fruit. Extracts prepared from pomegranate peel have demonstrated anti-inflammatory, anticancer, and antimicrobial activities and have also been linked to potential benefits in managing cardiovascular conditions, hypertension, obesity, and diabetes and improving fertility in animals. Reported antibacterial effects span a range of various Gram-Positive and Gram-Negative bacteria such as *Escherichia coli, Listeria monocytogenes, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella species, Bacillus species, Vibrio parahaemolyticus, Clostridia,* and *Yersinia enterocolitica.*[8] While several extraction approaches have been applied to highlight the potential of pomegranate peel as a reservoir of bioactive agents for industrial exploitation, outcomes reported in different studies remain variable due to the absence of standardized extraction protocols for isolating the most potent components. This lack of methodological uniformity complicates reliable evaluation of the effective and safe use of pomegranate peel in sectors such as food preservation and pharmaceutical manufacturing.[9]

MATERIALS AND METHODS

Collection and Identification of Plant Material

Pomegranate fruits (*Punica granatum L.) were* procured from the local Lucknow, India, market, rinsed thoroughly, and manually peeled. The peels were air-dried at ambient temperature, processed into a fine powder using a blender, and kept under refrigerated conditions for subsequent analysis. The collected plant material was identified and authenticated by a qualified botanist.

Preparation of Pomegranate Peel Extract by Soxhlet Extraction

Fresh pomegranate fruits were cleaned with water and peeled by hand, and the peels were air-dried at room temperature. The dried peels were ground into a fine powder using a laboratory grinder and preserved in a tightly closed and air-tight container until extraction. The 10-gm powdered peel was processed through the Soxhlet method using a hydro alcoholic solvent system consisting of ethanol and water in a 70:30 (v/v) ratio. The extraction process was continued until the solvent in the siphon tube turned clear, signifying complete extraction of soluble constituents. The extract obtained was concentrated under reduced pressure using a rotary evaporator and subsequently dried to yield a concentrated residue. The Concentrated residue was kept in a desiccator for subsequent use.[10-12]

Preliminary Phytochemical Evaluation

Preliminary phytochemical analysis of the extract was performed using standard qualitative tests to detect the presence of alkaloids, flavonoids, phenolic, saponins, glycosides, and terpenoids.[13]

Formulation of Hydrogel Beads

Dried pomegranate peel extract (PPE) powder was used as the active component for bead formulation. For each formulation, a predetermined quantity of PPE was dissolved in warm distilled water (60°C) and stirred magnetically for 30 min to obtain a homogeneous solution. Separately, the gelling polymer solution (sodium alginate) was prepared by dissolving the polymer in distilled water at the same temperature and stirring for 30 min, as shown in Table1. The PPE solution was subsequently added drop wise to the polymer solution with continue stirring and mixed for an

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additional 30 min to ensure uniform dispersion of the extract. A 50 mL syringe fitted with a stainless-steel needle was clamped vertically and preheated to prevent premature gelation. The PPE–polymer mixture was transferred into the syringe and injected drop wise into a chilled calcium chloride crosslinking bath using a controlled dripping technique. Upon contact with the ionic solution, the droplets instantly formed spherical hydrogel beads due to ionic gelation. The beads were allowed to remain in the crosslinking bath for duration of 30 minutes to complete gelation, collected by filtration, and air-dried at room temperature overnight. Subsequently, the semi-dried beads were subjected to high-vacuum drying for 24 hours to eliminate residual moisture. Finished PPE beads (formulations F1, F2, and F3) were stored in airtight containers inside a desiccator until further use and evaluation.[14-19]

Table 1: Composition of PPE-loaded hydrogel bead formulations

Formulation code	Polymer used	Crosslinking Agent	Pomegranate Peel Extract (PPE)
F1	Sodium alginate	Calcium Chloride	5% (w/v)
F2	Sodium alginate	Calcium Chloride	10%(w/v)
F3	Sodium alginate	Calcium Chloride	15% (w/v)

Evaluation Parameters

Optical Microscopy of PPE-Based Hydrogel Beads

Optical microscopy was conducted to determine the surface morphology, size, and shape uniformity of the PPE-based hydrogel beads. Freshly prepared hydrogel beads were carefully blotted with filter paper to remove excess surface moisture. Individual beads were positioned on a glass microscope slide, and a coverslip was carefully lowered to avoid deformation. The samples were imaged under a compound light microscope (bright-field mode) at varying magnifications. Images were captured using an attached digital camera. The diameter of the beads was determined using calibrated image analysis software, and an average size was determined by measuring at least 50 randomly selected beads. The recorded micrographs were analyzed for bead roundness, surface smoothness, and aggregation behaviour. Measurements and imaging were carried out at ambient temperature.

Organoleptic Evaluation

The formulated hydrogel beads were assessed for colour, aroma, taste, and consistency by visual and sensory inspection.[20]

Solubility

Solubility of the hydrogel beads was assessed in distilled water and physiological pH media.[21]

pH Determination

The stability of PPE-based hydrogel beads was monitored by evaluating changes in bead pH and physical integrity over time. The freshly hydrogel beads were stored under ambient conditions in sealed glass vials containing distilled water. The pH of the surrounding storage medium was determined using a properly calibrated benchtop pH meter.[22]

Swelling Properties

Swelling analysis was performed by immersing pre-weighed beads in phosphate buffer (pH). The percentage swelling was calculated at predetermined time intervals.

Antimicrobial Screening

The antimicrobial activity of PPE-loaded hydrogel bead formulations was evaluated under aseptic conditions as per established guidelines. All media and agar were prepared according to the manufacturer's instructions.

The antimicrobial activity of hydrogel bead formulations was determined using the agar diffusion method. Nutrient agar media were formulated and sterilized prior to inoculation with the *Staphylococcus aureus* test strain using a sterile cotton swab to obtain uniform lawn growth. Hydrogel beads of formulations F1, F2, and F3 were placed aseptically onto the agar surface at marked positions, while a disc loaded with a standard antibiotic served as the positive control (S). The plates were kept at 37°C for 24 hours for incubation, after which the zones of inhibition surrounding each sample were observed and measured using a ruler.[23]

Bacterial Strains

The hydrogel beads were tested against a range of Gram-positive bacteria strains, including *Staphylococcus aureus* (ATCC 29213). Bacterial cultures were grown overnight in Mueller-Hinton broth at 30°C and standardized to OD_{600} = 0.1, corresponding to ~1.5×10^8 CFU/mL, before inoculation on agar plates.[24]

Agar Well-Diffusion Assay

Sterile Mueller-Hinton agar plates were prepared, and wells of 15 mm diameter were created using a sterile borer. The bacterial culture was evenly spread onto the agar surface using sterile cotton swabs. Hydrogel beads containing PPE were placed in the wells, while the positive control (standard antibiotic) wells received penicillin. The plates were left for the beads to settle and release the extract into the agar, and then kept for incubation at 37°C overnight. After incubation, the diameters of the zones of growth inhibition were measured in millimetres.[25-28]

Statistical Evaluation

All experiments were conducted in triplicate, and results were expressed as mean ± standard deviation.

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RESULTS AND DISCUSSION

Phytochemical Screening

The phytochemical analysis of PPE revealed the presence of multiple bioactive compounds. Alkaloids were detected using Mayer's reagent, where the formation of a creamy white precipitate indicated a positive result. Carbohydrates were confirmed by Benedict's test, producing a brick-red precipitate after boiling. Steroids and terpenoids were identified using the Salkowski test, where a red colouration indicated their presence. Proteins and amino acids were tested using the Biuret reagent, which showed a negative result as indicated by a brown colour. Tannins and phenolic compounds were confirmed with ferric chloride, producing blue-green to black colouration. Saponins were detected through persistent froth formation when PPE was mixed with distilled water. Flavonoids were identified using an alkaline reagent, giving an intense yellow colour, and glycosides were confirmed by the Keller-Killani test, which produced a reddish-brown colouration (Figure 1). These results indicate that PPE contains a wide variety of bioactive compounds with potential therapeutic applications.[29]

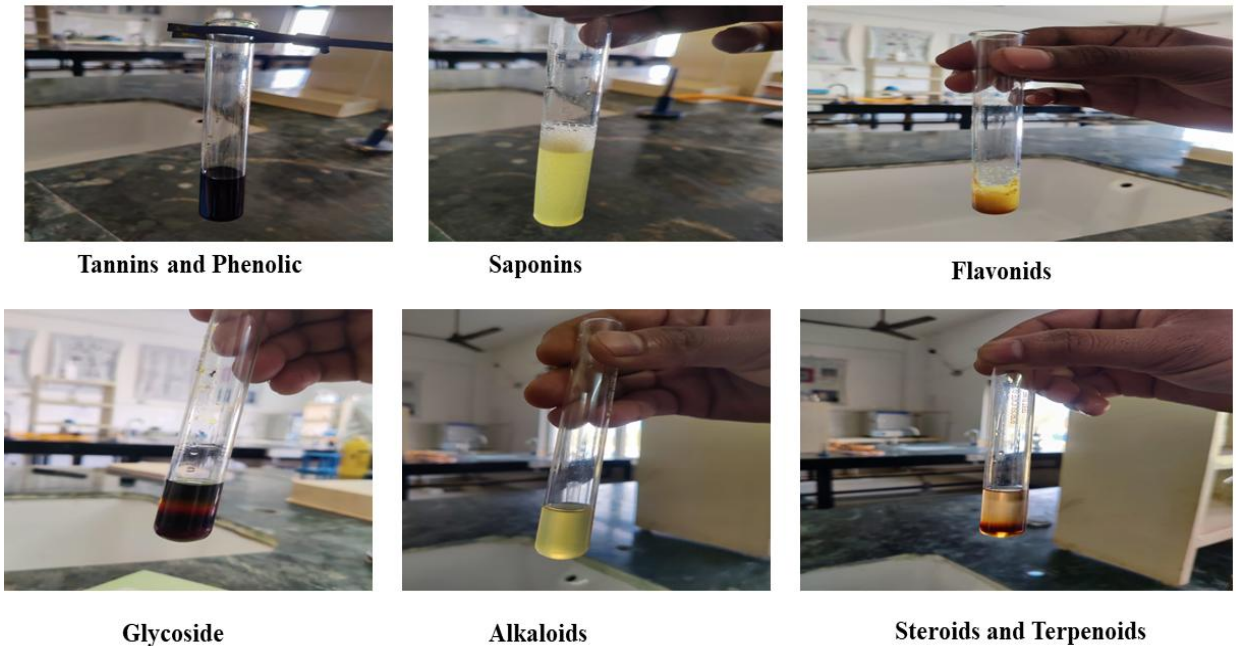

Figure 1: Phytochemical Screening

Development of Formulation

PPE was successfully encapsulated into sodium alginate hydrogel beads by ionic gelation using $CaCl_2$ as the crosslinking agent. Three formulations were developed by varying the PPE concentration (5–15% w/v) while keeping the polymer and cross linker composition constant. Visual inspection revealed that all formulations produced spherical beads with smooth surfaces. However, bead uniformity and structural strength differed with increasing PPE loading. The fabrication of PPE-based hydrogel beads required evaluation and refinement of droplet–gelation processing parameters to achieve reproducible spherical morphology and internal structural integrity. Initial PPE solutions, when injected into aqueous gelation media, dispersed rapidly due to high miscibility, resulting in film-like aggregates instead of discrete particles.

Increasing polymer concentration enhanced viscosity but led to extrusion as continuous threads rather than droplets, indicating that viscosity-driven flow resistance interfered with droplet breakup dynamics. These observations highlight that aqueous media do not provide sufficient immiscibility or interfacial tension control for PPE bead production. Organic gelation media, including hydrophobic and miscible solvents, were investigated to improve droplet retention. While hydrophobic oils reduced direct solvent exchange and slowed droplet spreading, insufficient cooling rates produced uneven bead surfaces and droplet coalescence prior to gelation. Attempts to employ cold oil improved surface hardening, yet the beads remained irregular and poorly reproducible. These findings suggest that controlled droplet stabilization requires rapid heat dissipation to trigger immediate shell formation and prevent interfacial relaxation.[30-32]

Organoleptic Evaluation

The physical characteristics of PPE-based hydrogel beads (F1–F3) were assessed in terms of shape, surface texture, color, and structural integrity (Table 2). F1 beads exhibited a spherical shape with smooth surfaces and pale brown coloration and maintained complete integrity. F2 beads were slightly oval with minor surface roughness, light yellow in color, and remained intact. In contrast, F3 beads showed an irregular shape with a wrinkled surface and dark brown coloration and were partially broken, indicating reduced structural stability at higher PPE loading. These observations suggest that increasing PPE content can adversely affect bead morphology and mechanical strength.

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Table 2: Organoleptic Evaluation of PPE-loaded hydrogel bead formulations

Formulation	Shape	Surface	Color	Integrity
F1	Spherical	Smooth	Pale brown	Intact
F2	Oval	Slightly rough	Light yellow	Intact
F3	Irregular	Wrinkled	Dark brown	Partially broken

Solubility

The solubility behaviour of the hydrogel beads was evaluated in different solvents and media (Table 3). All formulations were insoluble in distilled water, ethanol (70%), and phosphate buffer (pH 7.4). In distilled water, the beads swelled while retaining their original shape, demonstrating their hydrogel nature. No swelling was observed in ethanol, and beads remained intact. In phosphate buffer, the beads exhibited moderate swelling, reflecting their pH-sensitive hydration behaviour and potential for controlled release applications.

Table 3: Solubility of PPE-loaded hydrogel bead formulations

Solvent/Medium	Solubility	Observation
Distilled Water	Insoluble	Swelled, retained shape
Ethanol (70%)	Insoluble	No swelling, beads intact
Phosphate Buffer (7.4)	Insoluble	Swelled moderately

pH Measurement

The pH of the hydrogel beads varied with PPE concentration (Table 4).

Table 4: pH Measurement of PPE-Loaded Hydrogel Bead Formulations

PPE-loaded hydrogel bead formulations	pH
F1	5.49
F2	6.23
F3	6.47

F1 exhibited a slightly acidic pH of 5.49, F2 showed pH 6.23, and F3 had pH 6.47. The enhancement in pH with higher PPE content may be due to the alkaline nature of some polyphenolic components found in the extract. The measured pH values indicate that the beads are within a mild-acidic to near-neutral range, suitable for topical or oral applications.

Swelling Properties

Swelling analysis was conducted to evaluate water uptake and hydration characteristics of the beads overtime (Table 5). The swelling index (SI) was calculated using the formula: Swelling Index (SI)=Ws-Wi/Wi, where Wi is the initial weight and Ws is the swollen weight.

Table 5: Swelling studies of PPE-loaded hydrogel bead formulations

Time (hrs)	Initial Weight (Wi) in gm	Swollen Weight (Ws) in gm.	Swelling Index (SI)
1	0.100	2.50	1.5
2	0.100	0.290	1.9
4	0.100	0.320	2.2

Antimicrobial Activity of PPE-Loaded Hydrogel Beads

The antimicrobial efficacy of PPE-loaded hydrogel bead formulations (F1–F3) was evaluated against Gram-positive bacteria to examine the retention of bioactive compounds following bead fabrication. Beads were evaluated at the equivalent of the highest extract concentration (40 mg/mL) to evaluate the maximal inhibitory potential. All formulations demonstrated significant antibacterial activity, confirming that the encapsulation process preserved the functional components of pomegranate peel extract.

Activity against Gram- Positive Bacteria

Visual inspection of the agar plates inoculated with *Staphylococcus aureus* demonstrated distinct zones of inhibition around all hydrogel bead formulations, indicating antimicrobial activity. Formulation F2 exhibited the largest clear inhibition zone, followed by F1, while F3 showed the smallest zone among the formulations. The standard antibiotic control displayed a well-defined inhibition zone (table 6), confirming the validity of the assay. The relative clarity and diameter of the inhibited areas around each formulation suggest different extents of antibacterial potency mediated through the hydrogel beads (Figure 2).

Table 6: Antimicrobial Activity of PPE-Loaded Hydrogel Bead Formulations

PPE-loaded hydrogel bead formulations	Zone of inhibition (mm)	Remark
F1 (Hydrogel beads)	12	Moderate antimicrobial activity
F2 (hydrogel beads)	15	Strong antimicrobial activity
F3 (Hydrogel beads)	08	Mild antimicrobial activity
Penicillin (Standard antibiotic)	22	Excellent antimicrobial activity (positive control)

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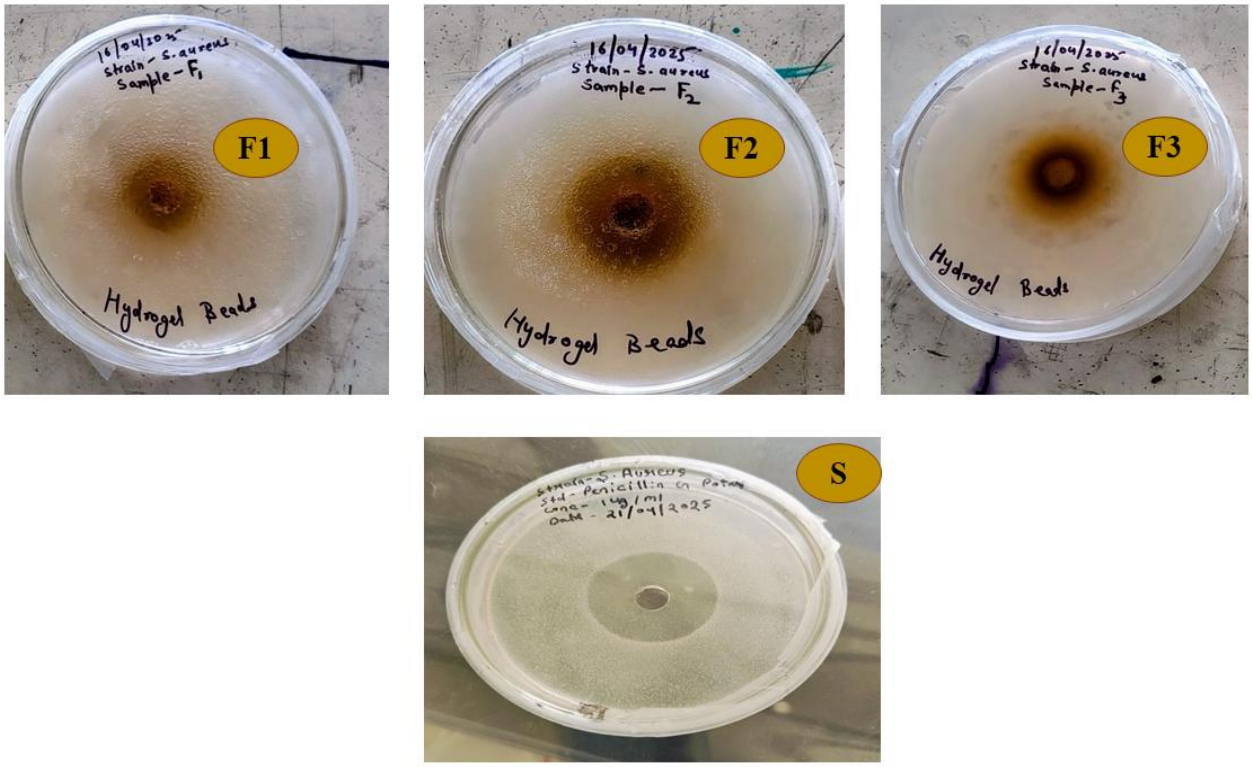

Figure 2: Antimicrobial Activity of PPE-Loaded Hydrogel Beads

CONCLUSION

In this study, PPE-infused hydrogel beads were successfully formulated and evaluated for physicochemical properties, antimicrobial activity, and potential wound-healing applications. The beads exhibited uniform spherical morphology, optimal swelling behaviour, suitable pH, and good structural integrity, indicating their suitability for topical administration. Antimicrobial screening revealed significant inhibitory activity against the Gram-positive bacterial strain *Staphylococcus aureus* (ATCC 29213). This strain is a methicillin-sensitive S. aureus (MSSA) reference organism commonly used for quality control in laboratory antimicrobial susceptibility assays, particularly for MIC determination. It is a wound-isolated clinical strain (Wichita), mecA-negative, weak β-lactamase-producing, and widely employed in drug discovery and antibiotic testing. The observed inhibition confirmed that the encapsulated PPE retained its bioactive potential. The hydrogel matrix provided sustained release of PPE, enhancing its therapeutic efficacy and suggesting improved local infection control. Overall, these findings demonstrate that PPE-loaded hydrogel beads are biocompatible, effectively suppress microbial growth, and exhibit promising wound-healing potential. This study highlights the utility of these natural polymeric beads as an innovative, safe, and cost-effective strategy for managing infected wounds and promoting tissue repair. However, further in vivo and preclinical studies are warranted to establish their clinical applicability in therapeutic and cosmetic wound-care products.

Acknowledgments

The authors gratefully acknowledge R.G.S. College of Pharmacy, Lucknow, for providing the required facilities and support for conducting this research. We also extend our sincere thanks to all the staff and colleagues who contributed their expertise and assistance during the study.

Author Contributions

Conceptualization: PS; Investigation, writing—original draft preparation: SK;

Writing—review and editing: P.S. All authors have reviewed and approved the manuscript.

Data availability

All data obtained during this study included in this manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

The work described has not been submitted elsewhere for publication, in full or in part, and all authors participated in the work and have agreed to the content of the manuscript.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article

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Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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