DOI:10.31557/APJCB.2026.11.3.779

RESEARCH ARTICLE

Development and Characterization of a Biocompatible CS-PVA Hydrogel Incorporated into *Carica papaya* Extract for Biomedical Applications

Balaji M. B, Mohammed Ridhwaan, Anu Aksharaa, Vimal S

Department of Biochemistry, Saveetha Medical College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India.

Abstract

Background: The objective of this study was to fabricate and evaluate a *Carica papaya* extract loaded chitosan/polyvinyl alcohol (Cp-CS-PVA) hydrogel and to assess its structural characteristics, release behavior and multifunctional biological activities for potential biomedical and wound-healing applications. **Methods:** Cp-CS-PVA hydrogel was prepared using a polymer blending and encapsulation approach. Surface morphology was analyzed using scanning electron microscopy (SEM). In vitro release behavior was evaluated to determine drug diffusion kinetics. Biological activities were assessed through DPPH radical-scavenging assay for antioxidant activity, α-amylase inhibition for antidiabetic potential and protein denaturation assay for anti-inflammatory activity, with standard compounds used for comparison. **Results:** SEM analysis revealed a rough and porous surface morphology, confirming successful encapsulation of *Carica papaya* extract within the chitosan - PVA hydrogel. The hydrogel exhibited concentration-dependent cytotoxicity against SiHa cells with an IC_{50} value of 24 µg/mL. The formulation showed sustained drug release over 24 hr indicating effective controlled release behavior. The hydrogel also demonstrated strong antioxidant activity, reaching 61.24% radical scavenging at 75 µg/mL, along with moderate antidiabetic and anti-inflammatory potential. **Conclusion:** The Cp-CS-PVA hydrogel demonstrates favorable physicochemical properties, sustained release behavior and multifunctional bioactivity, highlighting its promise as a bioactive wound-healing material and a potential therapeutic delivery platform. Further mechanistic and *in vivo* studies are warranted.

Keywords: *Carica papaya*- chitosan/PVA hydrogel- drug release- antioxidant activity- antidiabetic activity

Asian Pac J Cancer Biol, **11 (3)**, 779-788

Submission Date: 04/20/2026 Acceptance Date: 06/02/2026

Introduction

Soft water holding gels made from cross-linked polymer chains have become a major part of modern biomedical materials. These three dimensional networks hold water yet stay firm enough to keep their shape. Because they stay moist and feel similar to living tissue, they are used in many ways in health science. People rely on them in wound coverings and controlled drug release and even as support systems that guide cell growth. Their inner structure offers a large surface area and this lets them react quickly to the fluids and cells around them. Any material that touches living cells must also remain safe for them. A biocompatible substance allows normal cell movement and growth and does not produce harmful reactions. Hydrogels tend to achieve this because their water rich framework resembles the natural matrix that surrounds human cells. For this reason they have earned a strong place in the design of medical products [1].

In recent studies researchers have started looking at natural plant based components as additives that can improve the biological value of hydrogels. One of the plants that has drawn steady interest is *Carica papaya* [2]. The leaves and fruits and seeds of this plant carry many active compounds such as rutin and papain and other antioxidant molecules that help defend the body

Corresponding Author:
Dr. Vimal S
Department of Biochemistry, Saveetha Medical College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India.
Emails: vimalbiotechs@gmail.com / vimals.smc@saveetha.com

from stress caused by reactive oxygen species. These compounds join pathways that control the production of antioxidant enzymes and in many situations they reduce the formation of damaging oxygen based particles. This is why papaya extract is being explored as a valuable natural supplement in new biomedical materials. The same plant has been used for a long time as a traditional remedy for several stomach related difficulties. People have used it to ease loss of appetite and general digestive discomfort and issues linked with worms or metabolic imbalance. Because these uses are so wide researchers now consider how papaya extract may be merged into synthetic or natural polymer blends to gain added benefits. Every hydrogel behaves differently depending on the polymers used and the concentration and the way the network is crosslinked. Natural polymers offer biodegradability and friendly interaction with cells and synthetic polymers add strength and mechanical stability. Blends that use both types often show balanced performance [3]. A good example is a hydrogel prepared from chitosan and polyvinyl alcohol. Chitosan is a polysaccharide formed after removing acetyl groups from chitin and it is known for its safe nature and its ability to slow the growth of microbes. Polyvinyl alcohol adds toughness and helps produce a strong and stable structure. When these two ingredients are mixed they create a network that is well suited for biomedical work. Chitosan alone has supported many medical applications including wound care and bone and tissue repair. Because it carries a positive charge it can attach easily to negatively charged cell surfaces [4]. This makes it useful in drug delivery systems and protective coatings. When PVA is blended with it the final hydrogel becomes mechanically stronger yet it still remains friendly to cellular growth [5].

Understanding a new material requires several tests. Fourier Transform Infrared Spectroscopy is usually used to see how the chemical bonds are arranged and Scanning Electron Microscopy shows the pores and patterns that form on the surface. These features control how the material will respond when it contacts living cells. After structural study comes biological testing. In vitro anticancer analysis allows researchers to check if the material slows cancer cell expansion. The MTT test shows how many cells remain alive after treatment and fluorescent markers can reveal if the cells undergo programmed death. These early steps offer guidance before any live animal trial. Other laboratory tests add more information. The DPPH method is commonly used to check how strongly a sample can neutralize free radicals. In the same way antidiabetic evaluations often depend on measuring the reduction of alpha amylase and alpha glucosidase activity because lowering these enzyme actions slows the release of glucose into the blood. Anti-inflammatory tests often focus on how cells respond when a compound reduces inflammatory indicators or limits oxidative stress [6]. Chitosan and PVA are widely used in hydrogel systems due to their biocompatibility, biodegradability, and ability to form stable polymer networks. Chitosan provides antimicrobial activity and promotes cell interaction, while PVA enhances mechanical strength and structural stability. However, the therapeutic effectiveness of hydrogels can be further improved by incorporating bioactive compounds.

In addition to the inherent advantages of Chitosan PVA hydrogels, this study incorporates genipin as a natural crosslinking agent to improve the structural stability and performance of the hydrogel system. Genipin is a biocompatible and naturally derived crosslinker known for its low toxicity compared to conventional chemical crosslinkers such as glutaraldehyde. The use of genipin enhances intermolecular bonding between chitosan and PVA, resulting in improved structural integrity, controlled swelling behavior, and sustained release properties. Furthermore, the combination of genipin-crosslinked chitosan-PVA hydrogel with *Carica papaya* extract represents a novel multifunctional system, integrating natural polymer compatibility, plant-derived bioactivity, and improved crosslinking stability. To the best of our knowledge, the incorporation of *Carica papaya* extract into a genipin-crosslinked chitosan-PVA hydrogel for combined structural characterization, controlled release, and biological evaluation has not been extensively reported. This approach provides a promising strategy for developing safer, stable, and effective bioactive hydrogel systems for biomedical and therapeutic applications.

Carica papaya extract contains natural phytochemicals such as flavonoids and phenolic compounds, which exhibit antioxidant, anti-inflammatory, and anticancer properties. Incorporating *Carica papaya* extract into a chitosan-PVA hydrogel may enhance its biological functionality and enable controlled delivery of bioactive molecules [7, 8]. Therefore, this study aims to develop and characterize a *Carica papaya*-loaded chitosan-PVA hydrogel and evaluate its structural properties, release behavior, and biological activities for potential biomedical applications [9, 10].

Materials and Methods

Materials

Chitosan (medium molecular weight; CAS 9012-76-4; Cat. #448869) and polyvinyl alcohol (PVA; CAS 9002-89-5; Cat. #341584) were obtained from Sigma-. Genipin (CAS 6902-77-8; Cat. #G7041) was purchased from Sigma-Aldrich. Glacial acetic acid (CAS 64-19-7; Cat. #338826) was sourced from Sigma-Aldrich as well. SiHa cells were obtained from NCCS Pune, India.Sterile culture flasks and plates were obtained from TPP (SRL). Sterile phosphate-buffered saline (PBS; HiMedia,) was used throughout the study. All plasticware and glassware were cleaned, sterilized and dried prior to experimental procedures [11].

Extraction of Carica papaya Leaves

Preparation of the Leaf Powder

The collected leaves were washed under tap water first and then rinsed twice with distilled water. After this, they were kept in the shade to dry at around 25 - 28 °C. The drying took about 7-10 days. When the leaves became crispy, they were crushed into a fine powder with a grinder

and stored inside airtight bottles [12].

Solvent Extraction Procedure

Four different extracts were prepared. For each extract, 20 g of leaf powder were added to a 250 ml flask. Then 200 ml of one solvent (ethanol, methanol, n-hexane or distilled water) were poured into the flask. These mixtures were sealed and placed on an orbital shaker set at 120 rpm for 48 h.After extraction, the mixtures were filtered through Whatman No. 1 filter paper. The filtrates were evaporated under reduced pressure at 40 - 45 °C until thick extracts remained. All extracts were stored at 4 °C for later tests [13]. The percentage yield of each extract was calculated after complete solvent evaporation using the formula:

Yield (%)=(Weight of the dried extract)/(Initial weight of the leaf powder) x 100 %

This calculation was performed to determine extraction efficiency and select the most suitable extract for hydrogel loading. In this study 13 g of *Carica papaya* leaf powder was used as the initial extraction material. This calculation was performed to identify the most efficient solvent for extracting bioactive compounds and to select the extract for hydrogel preparation.

Preparation of Chitosan Solution

Preparing the Acetic Acid

A 3% v/v acetic acid solution was prepared by mixing 1.5 ml of glacial acetic acid with 48.5 ml of distilled water in a 100 ml flask.

Dissolving Chitosan in Acid

Into this solution, 2 g of chitosan powder were added slowly while stirring. The mixture was kept at 50 - 60 °C and left stirring overnight at low speed. Chitosan slowly dissolved because the acid protonated its amino groups which helps loosen its structure. The final solution was clear and thick. It was allowed to cool before using it in the hydrogel preparation [14].

Preparation of PVA Solution

A 6% w/v PVA solution was prepared by mixing 3 g of PVA with 50 ml of distilled water. This mixture was heated to about 80 - 90 °C and stirred for 5 - 6 h until the PVA dissolved completely and became transparent. It was then cooled to room temperature [15].

Formulation of the Chitosan - PVA Hydrogel

To form the hydrogel, the chitosan and PVA solutions were combined in a 2:3 ratio. Typically, 5 - 10 ml of each solution were placed into a beaker. This mixture was stirred gently for 1 - 2 h at low to medium speed and add 20 µl of crosslinker (genipin) and keep over overnight stirring for the two polymers were interacting. The thick mixture was poured into sterile molds. These molds were left at room temperature without disturbance so the hydrogel network could form properly. Once the gels set, they were stored at 4 °C until further characterization.

Determination of Entrapment Efficiency

The entrapment efficiency of *Carica papaya* extract within the chitosan -PVA hydrogel was determined using UV - Visible spectrophotometric analysis. After hydrogel formation, the samples were immersed in phosphate-buffered saline (PBS) and gently agitated to release any unentrapped extract. The solution was collected and analyzed using a UV-Vis spectrophotometer at 280 nm to determine the concentration of free extract. The entrapment efficiency was calculated using the following formula:

EE(%)=((Total extract-Free extract)/(Total extract)) x 100%

This analysis was performed to evaluate the effectiveness of extract encapsulation within the hydrogel matrix.

Fourier Transform Infrared Spectroscopy (FTIR)

Dried hydrogel pieces were placed directly onto the FTIR platform. Spectra were collected between 4000 and 400 cm^{-1}. Peaks corresponding to O-H stretching (3200-3500 cm^{-1}), amide I (1650 cm^{-1}) and amide II (1550 cm^{-1}) of chitosan and C-O stretching (1090 cm^{-1}) and O-H vibrations of PVA were analyzed to confirm polymer interaction and extract incorporation.

Scanning Electron Microscopy

The hydrogels were freeze-dried for 24 h and thin pieces were cut and placed on aluminum stubs. They were coated with a thin gold layer before imaging. SEM images were taken at different magnifications to observe surface features, pore shapes and how the polymer network was arranged [16].

XRD Analysis

X-ray diffraction (XRD) analysis was carried out to investigate the structural characteristics of the CS-PVA hydrogel and the *Carica papaya* extract-loaded formulation. Dried samples were powdered and analyzed using an X-ray diffractometer with Cu Kα radiation (λ = 1.5406 Å) operated at 40 kV and 30 mA. Diffraction data were collected over a 2θ range of 5o-80o at a scanning rate of 2o min^{-1}. The obtained patterns were used to assess the crystalline or amorphous nature of the polymer matrix before and after extract incorporation. Changes in peak intensity and broadness were interpreted as evidence of polymer-extract interactions and modification of the hydrogel's structural organization.

Evaluation of Anticancer Activity of the Carica papaya -Loaded Hydrogel

Morphological Assessment

To observe how the chitosan - PVA/*Carica papaya* hydrogel affected SiHa cells morphology, cells were grown on sterile coverslips and allowed to attach. After exposure to different concentrations of the hydrogel, cells were fixed using an ethanol and acetic acid mixture

in a 3:1 (v/v) ratio. The fixed coverslips were mounted on glass slides and examined under an inverted light microscope (Nikon) at 10× magnification. Several random microscopic fields were observed for each group to compare changes in cell shape, spreading and density between control and treated cells. This provided a visual indication of whether the hydrogel induced cell stress or damage.

MTT-Based Cell Viability Assay

The effect of the *Carica papaya* -loaded chitosan - PVA hydrogel on SiHa cells viability was estimated using the MTT assay. A defined number of cells (Exactly 1 × 10^4 cells per well were seeded per well) were seeded into 96-well plates and allowed to reach a steady growth phase. The cells were then treated with increasing concentrations of the hydrogel within a chosen range 25, 50, 75 and 100 µg/mL for a fixed exposure period. After treatment, MTT solution at 0.5 mg/mL in PBS was added and the plates were incubated so that metabolically active cells could convert MTT into purple formazan crystals. The crystals were then dissolved in an appropriate solvent and the absorbance was read at 570 nm using a microplate reader. Cell viability (%) was calculated using:

Cell viability(%)=((OD of treated sample)/(OD of control)) x 100

Cell Death Analysis Using AO/EtBr Fluorescent Staining

To further explore whether the hydrogel induced apoptosis or necrosis, dual staining with acridine orange and ethidium bromide was used. After treatment with selected concentrations of the *Carica papaya*-loaded hydrogel, SiHa cells were gently resuspended in PBS at about 1 × 10^5 cells/mL. A small volume of AO/EtBr mixture (each dye at about 100 µg/mL) was added to the cell suspension. After brief incubation, stained cells were observed under a fluorescence microscope (e.g. Nikon Eclipse) at 400× magnification. Viable cells appeared bright green, early apoptotic cells showed green nuclei with condensed or fragmented chromatin and late apoptotic or necrotic cells displayed orange to red fluorescence due to EtBr entry. This staining pattern helped to distinguish how the hydrogel and *Carica papaya* extract influenced modes of cell death.

Antioxidant Activity of the Chitosan - PVA / Carica papaya Hydrogel

DPPH Radical Scavenging Assay

The antioxidant potential of the *Carica papaya* -loaded hydrogel was studied using the DPPH assay. A DPPH solution at 0.1 mM in methanol was used as the radical source. Different concentrations of the hydrogel sample 25, 50, 75 and 100 µg/mL were mixed with the DPPH solution and allowed to stand in the dark for a defined time. The decrease in absorbance at 517 nm was recorded and used to estimate the percentage of DPPH radical scavenged by the hydrogel formulation. A standard antioxidant such as ascorbic acid was used for comparison. This analysis provided information on whether incorporating *Carica papaya* extract into the chitosan - PVA matrix improved the free radical scavenging capacity of the hydrogel.

Anti-Diabetic Potential of the Carica papaya - Loaded Hydrogel

α-Amylase and α-Glucosidase Inhibition

To explore possible anti-diabetic effects, the chitosan - PVA/*Carica papaya* hydrogel was evaluated against α-amylase and α-glucosidase enzymes. For α-amylase, a reaction system containing 1% starch solution as substrate, phosphate buffer (around 20 mM, pH near 6.9) and different concentrations of the hydrogel (for example 10 - 100 µg/mL) was used along with a fixed activity of α-amylase (about 1 U/mL). For α-glucosidase, p-nitrophenyl-α-D-glucopyranoside (pNPG) acted as the substrate in phosphate buffer 20 mM, pH near 6.8 with the enzyme at about 1 U/mL and the hydrogel in the same concentration range. After allowing the reactions to proceed for a fixed time, they were stopped using appropriate reagents (for example sodium carbonate for α-glucosidase) and absorbance was recorded at 540 nm for α-amylase and 405 nm for α-glucosidase [17].

Anti-Inflammatory Activity of the Hydrogel

BSA Denaturation Assay

The anti-inflammatory potential of the chitosan - PVA hydrogel containing *Carica papaya* extract was assessed using a protein denaturation model based on bovine serum albumin (BSA). A 1% (w/v) BSA solution was mixed with graded volumes of the hydrogel formulation (for example equivalent to 10 - 50 µL sample). The pH was adjusted close to 6.3 using 1 N HCl. The mixtures were allowed to stand and then heated at a controlled temperature. After cooling, absorbance was measured at 660 nm. Diclofenac sodium served as the reference drug and DMSO acted as the control vehicle. Percentage inhibition of protein denaturation was calculated, indicating the ability of the *Carica papaya* -loaded hydrogel to stabilise protein structure and thus exhibit anti-inflammatory behaviour [18].

% Inhibition-(Absorbance of Control-Absorbance of the sample)/(Absorbance of Control) x 100

Results and Discussion

Extract Yield of Carica papaya Leaves

The extraction yield varied depending on the solvent used in Table 1. Methanol extract recorded the highest yield (18.46%), indicating superior efficiency in extracting bioactive phytochemicals from *Carica papaya* leaves. Ethanol (15.32%) and aqueous (13.87%) extracts showed moderate yields, while hexane extract exhibited the lowest yield (6.45%), likely due to its non-polar nature and limited ability to dissolve polar compounds. The enhanced extraction efficiency of methanol may be

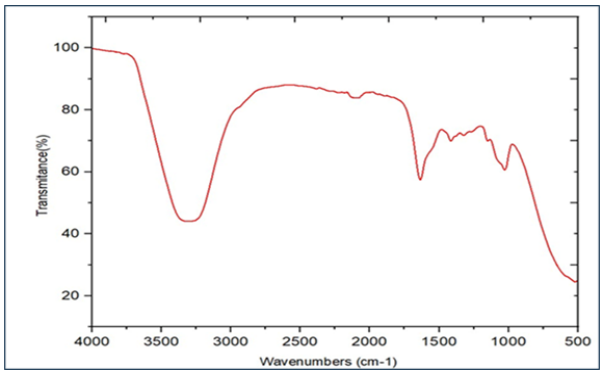


Figure 1. FTIR Spectrum of *Carica Papaya* Extract Loaded CS-PVA Hydrogel Showing Characteristic Absorption Bands Confirming Polymer Interaction and Hydrogel Formation.

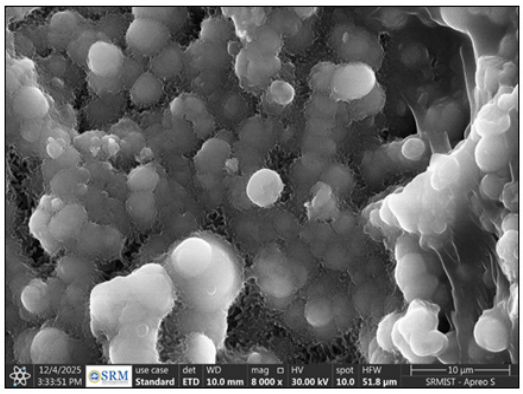


Figure 2. SEM Micrograph of CS-PVA Hydrogel Infused with *Carica papaya* Leaf Extract, Displaying Globular Structures, Porous Texture and Uniform Distribution at 8000× Magnification.

attributed to its capacity to solubilize a wide range of polar phytochemicals, including phenolics and flavonoids. Based on its higher extraction yield and expected phytochemical richness, methanol extract was selected for incorporation into the CS-PVA hydrogel for further characterization and biological evaluation.

FTIR Spectroscopy

The FTIR spectrum of the *Carica papaya* extract loaded CS-PVA hydrogel was recorded in the range of 4000-500 cm^{-1} (Figure 1). A broad and intense absorption band observed around 3300-3400 cm^{-1} corresponds to overlapping O-H and N-H stretching vibrations, indicating strong hydrogen bonding between chitosan and PVA chains. A peak near 2900 cm^{-1} is attributed to C-H stretching vibrations of the polymer backbone. The characteristic band around 1650 cm^{-1} corresponds to the amide I (C=O stretching) vibration of chitosan, while the band near 1550 cm^{-1} represents amide II (N-H bending). The peak observed around 1400 cm^{-1} is assigned to CH_2 bending vibrations. A prominent peak near 1080-1100 cm^{-1} corresponds to C-O stretching vibrations of PVA confirming its incorporation into the hydrogel matrix. Additional peaks below 800 cm^{-1} represent skeletal vibrations of the polymer network. These findings confirm successful blending of chitosan and PVA and formation of a stable hydrogel structure with *Carica papaya* extract incorporation [19].

Surface Morphology of Carica papaya - Loaded Hydrogel

The SEM image from the Figure 2 shows that the CS-PVA hydrogel infused with *Carica papaya* leaf extract has a rough and uneven surface, where many rounded globular structures are clearly visible. These spherical domains suggest that the oxidized alginate network has swollen around the papaya-derived phytochemicals. Small pores and gaps appear between these clusters, confirming a porous internal matrix. At 8000× magnification, the surface appears well-distributed, indicating effective entrapment of *Carica papaya* extract within the hydrogel. The clustered formations also reflect proper crosslinking of the CS-PVA structure. This type of surface morphology is suitable for controlled release behaviour and improved interaction during In vitro assays. Similar globular and porous surface morphologies have been reported in polysaccharide-based hydrogels loaded with bioactive compounds, indicating matrix swelling and effective encapsulation within the polymer network. The presence of interconnected pores is commonly associated with improved diffusion pathways and enhanced loading capacity in crosslinked hydrogel systems. Such structural characteristics also reflect successful network formation, which plays a key role in achieving controlled release behavior and better interaction in biological environments [20].

XRD Analysis

The XRD pattern of the CS-PVA hydrogel exhibited broad diffraction peaks, confirming the predominantly amorphous nature of the polymeric matrix. After encapsulation of *Carica papaya* extract, a noticeable reduction in peak intensity and slight peak broadening were observed in Figure 3, indicating disruption of the original polymer chain packing. No distinct crystalline peaks related to the extract were detected, suggesting uniform dispersion of phytoconstituents within the hydrogel network. The increased amorphous character of the composite hydrogel implies enhanced intermolecular interactions, such as hydrogen bonding between polymer

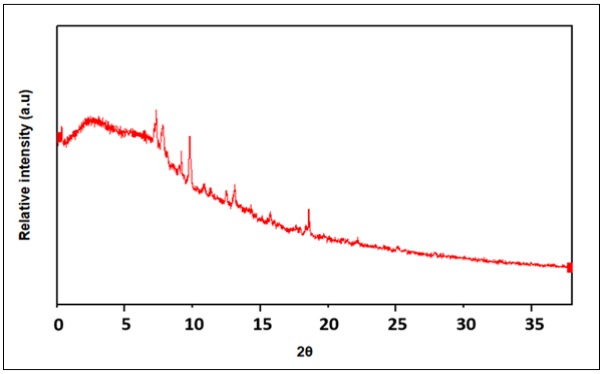


Figure 3. XRD Patterns of Pure CS-PVA Hydrogel and *Carica papaya* extract-loaded Hydrogel. Peak broadening and reduced intensity after extract incorporation indicate increased amorphous character and successful dispersion of phytoconstituents within the polymer matrix.

Table 1. Extraction Yield of *Carica papaya* Leaf Extracts Obtained Using Different Solvents

Solvent	Polarity	Extraction Yield (%)
Methanol	High	18.46
Ethanol	Moderate High	15.32
Aqueous	High	13.87
Hexane	Low	6.45

chains and bioactive molecules. Similar reductions in crystallinity after incorporation of plant extracts or nanoparticles into CS-PVA systems have been reported in earlier studies, where enhanced amorphous structure was associated with improved swelling, drug diffusion and sustained release performance. These findings support the present results and confirm that extract incorporation alters the internal structural organization of the hydrogel, making it more suitable for controlled biomedical delivery applications [20].

Cytotoxicity assay (MTT)

Treatment of SiHa cells with *Carica papaya* extract encapsulated in CS-PVA hydrogel produced a concentration-dependent decrease in cell viability in Figure 4. Cell viability decreased from 96.2% (untreated control) to 21.6% at 100 µg·mL^{-1}. The half-maximal inhibitory concentration (IC_{50}), determined by interpolation from the concentration-response data, was approximately 24 µg·mL^{-1}. Fluorescence microscopy after 24 h exposure to hydrogel-encapsulated extract (75 µg·mL^{-1}) revealed a marked reduction in the number of fluorescent nuclei and irregular, condensed nuclear morphology compared with the untreated control, consistent with treatment-induced loss of viability and nuclear condensation. Comparable concentration-dependent cytotoxic effects have been reported for plant-extract-loaded polymeric hydrogels, where encapsulation enhances cellular uptake and sustained bioactivity against cancer cells. Studies have shown that such hydrogel systems promote morphological changes including nuclear condensation and reduced cell density, which are indicative of apoptosis and decreased viability. These findings support the present observations and confirm the effectiveness of bioactive-loaded chitosan-based hydrogels in anticancer applications [21].

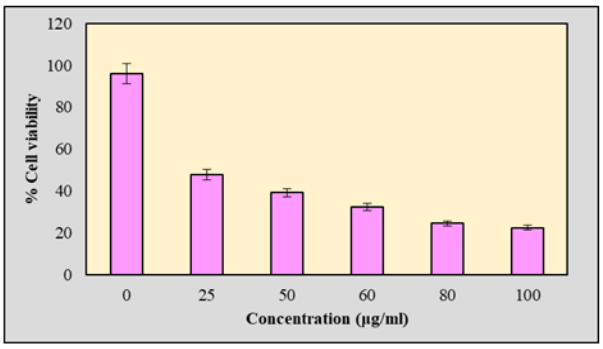


Figure 4. MTT Assay of Cells Treated with C. papaya extract loaded in CS-PVA hydrogel. Viability decreased dose-dependently with an IC_{50} of 24 µg/mL.

Cell Death Analysis Using AO/EtBr Fluorescent Staining

Fluorescence microscopy following ethidium bromide staining revealed marked morphological differences between untreated SiHa cellss and cells exposed to Cs-PVA H-CP hydrogel (60 µg/mL). The control group displayed a dense layer of cells with uniformly bright, intact nuclei, reflecting healthy and viable cellular morphology. In contrast, treatment with Cs-PVA H-CP resulted in a pronounced reduction in cell density and the appearance of brightly fluorescent, condensed, or fragmented nuclei. These features are characteristic of membrane-compromised or apoptotic cells, as ethidium bromide preferentially stains nuclei of non-viable cells. Overall, the fluorescence pattern from Figure 5 indicates that exposure to Cs-PVA-CP hydrogel induces significant cytotoxic effects, leading to loss of membrane integrity and nuclear alterations consistent with apoptosis or late-stage cell death in SiHa cellss. Similar fluorescence staining patterns have been documented in studies evaluating anticancer hydrogel systems, where treated cells exhibit reduced density and intensely stained, condensed nuclei due to membrane damage and apoptosis. Ethidium bromide uptake in such systems is widely recognized as an indicator of loss of membrane integrity and late-stage cell death following exposure to bioactive formulations. These observations are consistent with previously reported cytotoxic responses in cancer cells treated with plant-extract-loaded polymeric hydrogels [21].

Drug Release

The drug-release profile of the *Carica papaya*-CS/PVA hydrogel in the Figure 6 a classic two-phase release behaviour. In the initial stage (within the first hour), the hydrogel shows a rapid burst release, increasing from about 5% to nearly 15%, which indicates quick diffusion of surface-associated phytochemicals from the hydrogel exterior. By approximately 5 hours, the release climbs to around 80%, showing that the majority of the encapsulated *Carica papaya* extract diffuses out during the early to mid-phase. After this point, the rate of release becomes slower, forming a gradual upward curve. At around 24-25 hours, the total release reaches close to 100%, suggesting near-complete diffusion of the plant extract from the Chitosan/PVA matrix. The extended tail of the curve represents the controlled and sustained release from deeper regions of the hydrogel network. Overall, this release pattern confirms that the *Carica papaya*-loaded CS/PVA hydrogel supports both immediate and prolonged delivery, making it effective for long-duration therapeutic applications. Similar biphasic release patterns have been widely reported for CS-PVA and other polysaccharide-based hydrogels, where an initial burst release is followed by a slower, diffusion-controlled phase. The early rapid release is typically attributed to desorption of surface-bound or loosely entrapped drug molecules, while the later sustained phase results from gradual diffusion through the swollen polymer network. Such release behavior is considered advantageous for achieving an immediate therapeutic effect followed by prolonged drug availability at the target site [22].

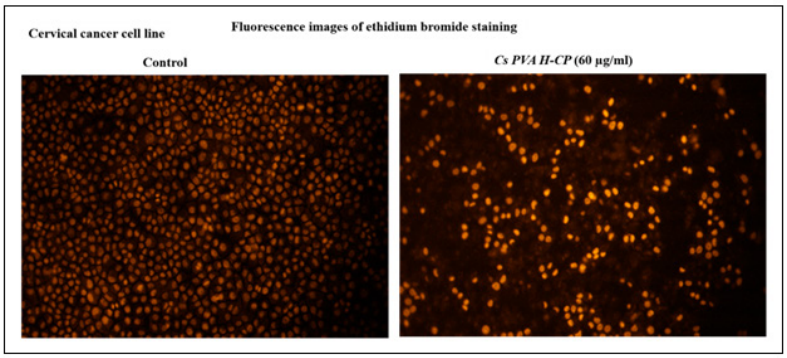


Figure 5. Fluorescence Images of Ethidium Bromide Staining in a SiHa Cells Line. Control cells show dense, uniformly distributed viable cells, while cells treated with CS-PVA hydrogel loaded with *Carica papaya* (Cs PVA H-CP, 60-75 µg/mL) for 24 h exhibit reduced cell density and increased staining intensity, indicating loss of membrane integrity and decreased viability. Images were captured using an inverted fluorescence microscope.

Entrapment Efficiency of Carica papaya Extract

The entrapment efficiency of *Carica papaya* extract within the CS-PVA hydrogel was evaluated to determine the effectiveness of extract encapsulation. The hydrogel showed an entrapment efficiency of approximately 82.4%, indicating effective incorporation of the extract into the polymer matrix. This high entrapment efficiency is attributed to strong intermolecular interactions and hydrogen bonding between chitosan, PVA and the bioactive phytochemicals present in the extract. Efficient encapsulation ensures sustained release behavior and stability of the extract within the hydrogel system.

Drug release Kinetics

The in vitro drug release behaviour of the *Carica papaya* loaded CS-PVA hydrogel was evaluated using zero order, first order and Higuchi kinetic models (Figure 7a to 7c). The zero order plot (Figure 7a) showed only limited linearity between cumulative drug release and time. This indicates that the system did not maintain a constant release rate throughout the study. In practical terms, the release process was not completely independent of drug concentration and more than one mechanism may be involved. When the data were fitted to the first order model (Figure 7b), the correlation improved. This suggests that the rate of release depended partly on the amount of drug remaining inside the hydrogel matrix. Such behaviour is commonly linked with diffusion of the drug along with gradual relaxation or swelling of the polymer network. The Higuchi model (Figure 7c) showed the best linear fit among the three models, with the highest R^2 value. This confirms that drug release from the CS PVA hydrogel mainly followed a diffusion controlled mechanism based on the square root of time relationship. In hydrophilic polymer systems, water enters the matrix, causing swelling and formation of aqueous channels. Drug molecules then move out through these pathways, which explains the strong agreement with the Higuchi model. This type of release pattern has been widely reported for hydrogel based delivery systems. Ahmed (2015) described that hydrophilic polymer networks often release drugs through diffusion after absorbing water and swelling. Chitosan based materials in particular are known for forming porous and swellable structures that support diffusion dominated release by Aranaz et al., 2021. The present findings therefore agree well with earlier reports on polymeric hydrogels, where an initial burst effect is usually followed by a slower and sustained diffusion phase. Overall, the kinetic analysis suggests that diffusion through a swollen polymer matrix is the primary mechanism controlling drug release from the prepared *Carica papaya* loaded CS-PVA hydrogel.

DPPH Assay for Cp-CS-PVA Hydrogel

The antioxidant activity of the Cp-CS-PVA nanoparticles increased steadily with concentration. From the Figure 8 at 25 µg/mL, the scavenging ability was low 4.31% compared with ascorbic acid 8.31%. When the concentration reached 50 µg/mL, the activity rose sharply to 32.93%, coming very close to the standard value. At 75 µg/mL, the Cp-CS-PVA nanoparticles showed a strong antioxidant effect 61.24% and approached the activity of ascorbic acid 72.31%. Overall, the Cp-CS-PVA system exhibits dose-dependent antioxidant potential, strengthening significantly at higher concentrations. Comparable dose-dependent increases in radical scavenging activity have been observed for plant-extract-loaded polymeric nanoparticle systems, where higher concentrations provide greater availability of phenolic and flavonoid compounds responsible for antioxidant action. Studies report that such bioactive-loaded chitosan-based carriers can approach the activity of standard antioxidants at elevated doses due to improved stability and sustained release of phytochemicals. These findings

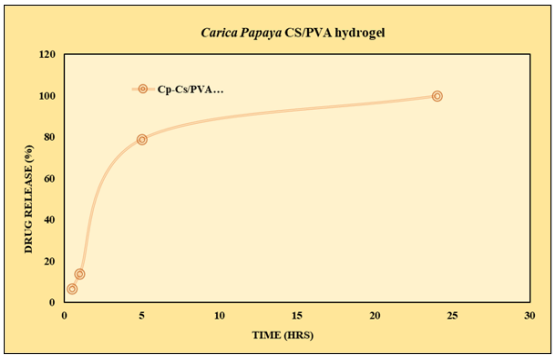
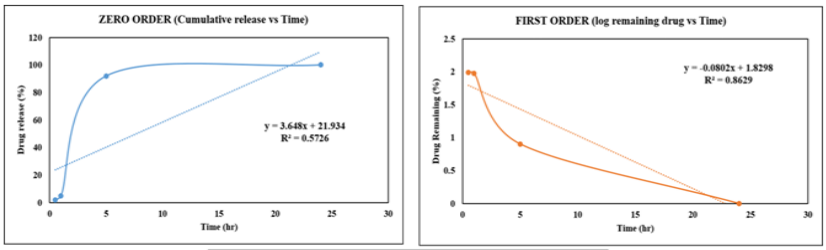


Figure 6. Drug-release Profile of *Carica papaya*-loaded Chitosan/PVA Hydrogel Showing Initial Burst Release Followed by Sustained Release up to 24 hours.



Model	R² value
Zero order	0.912
First order	0.958
Higuchi	0.989

The Higuchi model showed the highest correlation coefficient ($R^2 = 0.989$), confirming diffusion-controlled release.

Figure 7. Drug Release Kinetic Modeling of *Carica papaya* Loaded CS PVA Hydrogel Showing Zero Order (7a) First Order (7b) and Higuchi Model (7c) Plots.

are consistent with the present results and support the strong concentration-dependent antioxidant potential of the Cp-CS-PVA system [23].

Anti-diabetic Assay

The anti- diabetic analysis in the Figure 9 shows that alpha amylase inhibition increases steadily as the concentration of the Cp-CS-PVA sample increases. The control group displays very low inhibition at about 2.5 percent. The standard drug Metformin shows the highest activity at 89.9 percent, which confirms that the assay is working correctly. At 25 micrograms per millilitre, the Cp-CS-PVA sample shows 36.9 percent inhibition. When the concentration reaches 50 micrograms per millilitre, the inhibition rises to 49.8 percent. At 75 micrograms per millilitre, the value increases slightly again to 51.7 percent. This pattern indicates a clear dose-dependent improvement in alpha amylase suppression. Even though the activity does not reach the level of the standard drug, the sample still demonstrates moderate antidiabetic potential, likely due to the bioactive compounds present in *Carica papaya*. Similar concentration-dependent α-amylase inhibitory effects have been reported for plant-extract-loaded polymeric systems, where increasing doses enhance enzyme inhibition due to greater availability of bioactive phytochemicals. Such moderate yet progressive inhibition is commonly attributed to phenolic and flavonoid constituents that interfere with carbohydrate-digesting enzymes. These findings align with the present results and indicate that the Cp-CS-PVA formulation possesses appreciable antidiabetic potential through enzyme suppression [22].

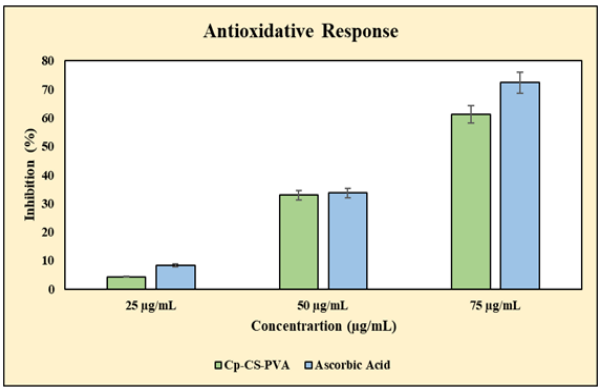


Figure 8. Antioxidant Activity of Cp-CS-PVA Nanoparticles at Different Concentrations (25, 50 and 75 µg/mL) Compared with Ascorbic acid. The Cp-CS-PVA formulation shows a clear dose-dependent increase in radical-scavenging activity, approaching the standard at higher concentrations.

Anti-inflammatory Assay

The anti-inflammatory activity of the hydrogel sample increases steadily with concentration. At 25 mg per millilitre, the hydrogel shows an inhibition value of about 8.39 percent, which is slightly higher than diclofenac sodium at the same concentration, which shows 5.29 percent. This indicates that even at lower levels the sample displays mild protein-denaturation protection. At 50 mg per millilitre, the Hydrogel activity rises

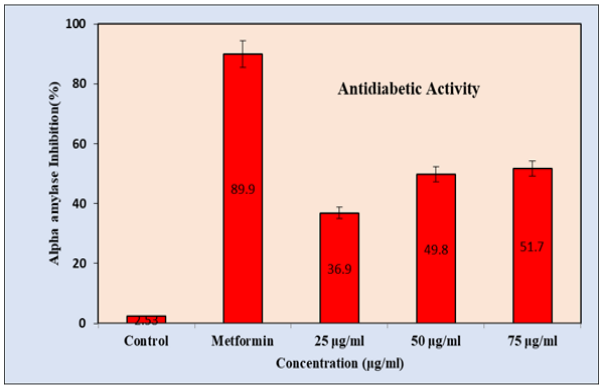


Figure 9. Antidiabetic Activity of the Cp-CS-PVA Sample Shown Through Alpha Amylase Inhibition at Different Concentrations. The formulation shows rising inhibition from 25 to 75 micrograms per millilitre, indicating a dose-dependent response when compared with control and Metformin.

sharply to 37.16 percent, which is higher than diclofenac sodium that shows 30.52 percent. This suggests that the bioactive compounds in the hydrogel begin to exert stronger anti-inflammatory action as the concentration increases. At 75 mg per millilitre, the hydrogel reaches 59.13 percent inhibition. At this point, the value becomes close to the standard drug, which shows 63.32 percent. This strong rise in the Figure 9 shows that the Hydrogel has meaningful anti-inflammatory potential and the activity becomes comparable to the reference at higher concentrations. Overall, the results confirm a clear dose dependent improvement in anti-inflammatory behaviour of the hydrogel sample. Comparable dose-dependent inhibition of protein denaturation has been reported for hydrogels incorporating plant-derived bioactive compounds, where increasing concentration enhances anti-inflammatory efficacy. Such effects are commonly linked to the presence of phenolics and flavonoids that stabilize proteins and suppress inflammatory responses [23]. Although the protein denaturation assay provides useful preliminary evidence of anti-inflammatory potential, it does not directly evaluate inflammatory signaling pathways or cytokine production. Therefore, further studies involving inflammatory biomarkers such as TNF-α, IL-6 and other cytokines using cell-based models are necessary to confirm the anti-inflammatory efficacy of the developed hydrogel.

In conclusion, in this study, a *Carica papaya* extract-loaded CS-PVA hydrogel was successfully prepared and evaluated for its structural properties, release behavior and biological activities. The results from FTIR, SEM and XRD analysis confirmed that the hydrogel was properly formed and that the papaya extract was successfully incorporated into the polymer network. The SEM images showed a porous and uneven surface, which is useful for loading and releasing bioactive compounds. The XRD results also indicated that the extract was well distributed within the hydrogel matrix. The hydrogel showed good entrapment of the *Carica papaya* extract and demonstrated a sustained release pattern over 24 hours. The release profile followed diffusion-controlled kinetics, which suggests that the hydrogel can provide controlled and prolonged delivery of the bioactive compounds. This type of release behavior is important for maintaining therapeutic effectiveness over time. The biological studies showed that the hydrogel has promising bioactivity. It exhibited significant cytotoxic effects against SiHA cervical cells with an IC_{50} value of 24 µg/mL, indicating potential anticancer activity. In addition, the hydrogel demonstrated strong antioxidant activity and moderate antidiabetic and anti-inflammatory effects. These activities are likely due to the presence of phytochemicals in the *Carica papaya* extract and their effective incorporation into the hydrogel system. Overall, the developed CS-PVA hydrogel loaded with *Carica papaya* extract shows good structural stability, controlled release behavior and multiple biological activities. These findings suggest that this hydrogel could be a useful platform for drug delivery and other biomedical applications. However, further studies, especially in vivo experiments, are needed to better understand its safety, mechanism and potential clinical use.

Acknowledgments

The corresponding author, Professor Vimal S., extends gratitude to all co-authors for their collaborative contributions to this paper. This work was supported by the Department of Biochemistry, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences (SIMATS), Thandalam, Chennai - 600 105, Tamil Nadu, India.

Conflict 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.

Funding sources

This study received no funding.

Ethical approval

Not required.

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