Current Trends in Biotechnology and Pharmacy
Vol. 20 (Supplementry Issue 1A), March 2026, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2026.1s.18

Comprehensive evaluation of a wound healing formulation incorporating *Phyla nodiflora* silver nanoparticles

Reshma Devi Senthilnathan[1], Hemalatha S[1]*, V. Gayathri[2], Komala M[3], and R. Chandru[4]

[1]Department of Pharmacognosy, Sri Ramachandra Faculty of Pharmacy, Sri Ramachandra Institute of Higher Education and Research (DU), Porur Chennai-600 116, Tamil Nadu, India

[2]Head/Test Facility Management, Centre for Toxicology and Developmental Research (CEFTE), Sri Ramachandra Institute of Higher Education and Research (DU), Porur, Chennai-600 116, Tamil Nadu, India

[3]School of Pharmaceutical Sciences, Vels Institute of Science Technology and Advanced Studies (VISTAS), Chennai-600 117, Tamil Nadu, India

[4]Department of General Surgery, Sri Ramachandra Institute of Higher Education and Research (DU), Porur, Chennai-600 116, Tamil Nadu, India

*Corresponding author: hemacognosy@gmail.com

Abstract

The developed hydrogel formulation incorporating *Phyla nodiflora* derived silver nano particle for wound healing application. LCMS analysis of the ethanolic extract identified key bioactive compounds that facilitated the green synthesis of silver nanoparticles (Pn-Ag-Np). These nanoparticles were incorporated into a carbopol-based hydrogel and characterized using FTIR analyses to confirm structural integrity and compatibility. Stability studies for the formulated hydrogel conducted over three months demonstrated excellent viscosity retention, minimal variations in pH, and consistent drug content and *In-vitro* drug release studies at the 1st, 2nd, and 3rd months showed sustained release, with maximum permeation observed at 14 hours. SEM analysis confirmed nanoparticle sizes between 2.4 nm and 31.7 nm, ensuring uniform distribution within the hydrogel matrix. Antioxidant assays such as DPPH and nitric oxide scavenging demonstrated significant free radical neutralization, while anti-inflammatory studies, including proteinase inhibition, lipoxygenase inhibition, and protein denaturation assays, highlighted the formulation's efficacy in modulating inflammatory pathways. Additionally, the hydrogel exhibited antimicrobial activity against Gram-positive *Staphylococcus aureus, Bacillus cereus and Gram-negative Pseudomonas aeruginosa, Salmonella typhi* bacteria, with strong antifungal action against *Aspergillus niger, Candida albicans*. These findings suggest that the *Phyla nodiflora* silver nanoparticle hydrogel formulation is a promising wound healing agent with sustained drug release, potent antioxidant, anti-inflammatory properties, and broad-spectrum antimicrobial activity, making it a potential therapeutic option for enhanced wound care and tissue regeneration.

Keywords: Wound healing, *Phyla nodiflora*, Silver nanoparticles, Hydrogel, Sustained drug release

1. Introduction

Expanding upon our previously published study on the green synthesis of *Phyla nodiflora*-derived silver nanoparticles and their potential role in wound healing, the present work focuses on their incorporation into a hydrogel-based delivery system and a detailed exploration of its physicochemical and biological properties. In our initial investigation, the synthesized silver nanoparticles demonstrated promising antioxidant, antimicrobial, and healing-supportive effects.[1] Encouraged by these outcomes, we extended our research to develop a hydrogel formulation—designated as Pn-Ag-Hg—that harnesses the bioactivity of silver nanoparticles within a stable,

Vol. 20 (Supplementry Issue 1A), March 2026, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2026.1s.18

biocompatible matrix intended for topical wound care.

Nanotechnology-based systems have transformed the field of drug delivery, offering enhanced bioavailability, controlled release, and targeted therapeutic effects. Among various nanomaterials, silver nanoparticles have shown immense potential due to their broad-spectrum antimicrobial action and capacity to modulate oxidative and inflammatory responses, which are critical in the wound healing process. [2] When embedded within hydrophilic polymeric structures like hydrogels, their performance is further improved through sustained release, better skin adherence, and reduced toxicity.

To ensure the quality and reproducibility of the formulation, LC-MS analysis of *Phyla nodiflora* ethanolic extract was conducted to identify key phytoconstituents responsible for nanoparticle formation. The resulting silver nanoparticles were incorporated into a carbopol-based hydrogel, and their compatibility with other excipients was confirmed through Fourier-transform infrared spectroscopy (FTIR). Entrapment efficiency was evaluated, demonstrating effective loading of bioactive agents into the hydrogel matrix without structural disruption.

A comprehensive stability study was carried out over a period of three months on all prepared formulations. Parameters such as pH, viscosity, drug content, and homogeneity were evaluated under both room temperature and accelerated storage conditions. Among the tested variants, the F4 formulation exhibited superior physicochemical stability with minimal changes across all time points. *In-vitro* drug permeation studies conducted on all formulations further reinforced the selection of F4, as it showed a sustained and controlled release profile extending up to 14 hours, making it well-suited for prolonged wound coverage and therapeutic action.

Based on these findings, F4 was chosen for advanced characterization. Scanning Electron Microscopy (SEM) confirmed a uniform distribution of nanoparticles within the hydrogel, with significantly reduced particle size, while Energy Dispersive X-ray (EDAX) analysis verified the elemental composition and successful incorporation of silver nanoparticles. Following this, F4 underwent biological efficacy testing. Anti-inflammatory activity was assessed through proteinase inhibition, lipoxygenase inhibition, and protein denaturation assays, all of which revealed significant inhibition rates, suggesting strong modulation of inflammatory mediators. The formulation was also tested for antimicrobial activity and demonstrated notable efficacy against Gram-positive and Gram-negative bacteria, as well as fungal strains such as *Candida albicans*.

Overall, this study offers a valuable contribution to the development of nanocarrier-based drug delivery systems. It aligns well with the journal's focus on formulation development, controlled drug release, and novel therapeutic strategies. The integration of green-synthesized nanoparticles into a stable hydrogel matrix presents a promising and sustainable approach for managing wound-related infections and inflammation.[3] These findings provide a strong foundation for further translational research and potential clinical application in wound care and regenerative medicine.

2. Materials and Methods

The ethanolic extract of *Phyla nodiflora*, previously characterized in our earlier study, was utilized for the green synthesis of silver nanoparticles. Building on its confirmed phytochemical profile, the extract facilitated effective reduction and capping during nanoparticle formation. LC-MS was used to reaffirm the presence of key bioactives. Subsequent analyses focused on nanoparticle characterization and formulation performance.

Liquid Chromatography–Mass Spectrometry (LC-MS) Analysis

The ethanolic extract of *Phyla nodiflora* was subjected to LC-MS analysis to

Vol. 20 (Supplementry Issue 1A), March 2026, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2026.1s.18

identify bioactive constituents involved in silver nanoparticle synthesis. The presence of flavonoids, phenolics, and alkaloids—which are involved in the reduction and capping processes during the production of nanoparticles—was confirmed by the analysis. After being weighed, the dried extract was diluted in ethanol and filtered. An electrospray ionization (ESI) source running in positive ionization mode was used to inject the material into an LC-MS system. Components were separated using a reverse-phase C18 analytical column according to retention time and polarity. Solvents A and B made up the mobile phase, which was administered using a gradient elution procedure. Both low and relatively high molecular weight molecules were detected by the mass spectrometer, which scanned a broad m/z range.[4]

FTIR Analysis

Fourier Transform Infrared (FTIR) spectroscopy was performed to evaluate potential chemical interactions between the excipients used in the hydrogel formulation and the synthesized silver nanoparticles (Pn-Ag-Np). Two sample sets were prepared for analysis: one consisting of the hydrogel formulation containing Pn-Ag-Np (Pn-Ag-Hg) and the other containing only the synthesized silver nanoparticles. Both samples were dried and finely ground to form uniform powders. The powdered samples were then compressed into thin pellets using potassium bromide (KBr) as a matrix. The FTIR spectra were recorded in the range of 4000–400 cm^{-1} using an FTIR spectrophotometer. The obtained spectra were analyzed to identify the functional groups potentially involved in the interaction between the hydrogel components and the nanoparticles.[5]

Stability Studies (ICH Guidelines) for 3 months

Formulations F4, F7, and F8 were chosen for further analysis because they were the most preferred formulations based on previously published *In-vitro* drug permeation data. To evaluate formulations F4, F7 and F8's long-term physicochemical stability under typical storage, a three-month stability study was carried out. In order to replicate real-world situations and forecast the hydrogel's behaviour over extended periods of time, the study entailed storing them at room temperature (25°C ± 2°C/60% RH ± 5% RH) and under accelerated temperature (40 °C ± 2 °C/75% RH ± 5% RH). Visual appearance, pH, viscosity, spreadability, drug content, and homogeneity were among the important factors evaluated. A digital pH meter was used to detect the pH, a Brookfield viscometer was used to assess viscosity, and a spectrophotometric method at 410 nm was used to quantify the drug content. Visual examination and uniformity testing were used to confirm homogeneity.

***In-vitro* Drug Permeation Study**

A study was conducted to assess the sustained release potential of hydrogel formulations (F4, F7, F8) after three months of stability testing under ICH-recommended storage conditions. Samples stored at room temperature and under accelerated conditions were placed between the donor and receptor compartments using a semi-permeable membrane. The donor compartment was loaded with the hydrogel formulation, while the receptor compartment was filled with phosphate-buffered saline. Aliquots were withdrawn at regular intervals, filtered through 0.45 µm syringe filters, and analyzed spectrophotometrically at 410 nm. The optimized formulation was identified based on its consistent, sustained drug permeation profile without an initial burst effect, even after three months of storage.

SEM and EDAX Analysis of the Optimized Formulation

The optimized hydrogel formulation was dried at room temperature and mounted on a metal stub using double-sided adhesive carbon tape. A thin layer of gold was then applied using a sputter coater to enhance the sample's conductivity. Scanning Electron Microscopy (SEM) was performed under

Vol. 20 (Supplementry Issue 1A), March 2026, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2026.1s.18

appropriate accelerating voltage and magnification to examine the surface morphology and microstructure of the hydrogel. The same gold-coated sample was subjected to Energy Dispersive X-ray Analysis (EDAX) using an integrated detector attached to the SEM system. [6] Elemental mapping was carried out to identify and quantify key elements such as silver, carbon, and oxygen. The resulting spectra were processed using dedicated software to determine the presence and relative abundance of elements within the hydrogel matrix.

In-vitro Anti-inflammatory Assays of Optimized Formulation [7]

The anti-inflammatory potential of the optimized hydrogel formulation was assessed using a series of *In-vitro* assays designed to simulate key inflammatory pathways. These included the inhibition of proteinase activity, lipoxygenase (LOX) activity, and protein denaturation—all of which are considered relevant markers of inflammatory response in wound healing.

Proteinase Inhibition Assay

Proteinase enzymes contribute to tissue degradation and inflammation at wound sites. In this assay, trypsin was used as the standard proteinase enzyme. A reaction mixture containing trypsin and varying concentrations of the optimized formulation was incubated with casein substrate. After the incubation period, the reaction was terminated, and the undigested proteins were quantified by measuring absorbance at 210 nm. The percentage inhibition of proteinase activity was calculated.

Lipoxygenase (LOX) Inhibition Assay

LOX enzymes are responsible for the biosynthesis of leukotrienes, which are potent mediators of inflammation. The assay involved incubating sodium linoleate with soybean lipoxygenase in the presence of different concentrations of the optimized formulation. The formation of conjugated dienes was monitored spectrophotometrically at 234 nm. A decrease in absorbance indicated effective inhibition of LOX activity.

Protein Denaturation Assay

Protein denaturation is a key event in the development of inflammation, particularly in autoimmune and chronic inflammatory conditions. In this assay, egg albumin was incubated with the optimized formulation at various concentrations, followed by heating. The turbidity of the solution was measured at 660 nm. A reduction in absorbance indicates inhibition of protein denaturation.

Each assay was performed in triplicate, and results were expressed as mean ± standard deviation. The optimized formulation showed significant dose-dependent inhibition across all three models, indicating its strong anti-inflammatory potential. These findings support the therapeutic relevance of the formulation in modulating inflammatory responses during wound healing.

Antimicrobial Assay of Optimized Formulation using Agar well method

The antimicrobial efficacy of the optimized F4 hydrogel formulation was assessed using the agar well diffusion method against a panel of clinically relevant wound pathogens. This evaluation included both Gram-positive and Gram-negative bacteria as well as fungal species commonly implicated in infected wounds. The selected test organisms were *Staphylococcus aureus* and *Bacillus cereus* (Gram-positive), *Pseudomonas aeruginosa* and *Salmonella typhi* (Gram-negative), along with *Candida albicans* and *Aspergillus niger* representing fungal strains. Freshly prepared nutrient agar and Sabouraud dextrose agar plates were inoculated with standardized microbial suspensions using the lawn culture technique. [8] Wells were bored into the agar surface, and 100 µL of the optimized hydrogel was carefully introduced into each well. The plates were incubated at 37°C for 24 hours for bacterial strains and at 28°C for 48 hours

Vol. 20 (Supplementry Issue 1A), March 2026, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2026.1s.18

for fungal cultures. After incubation, zones of inhibition were measured around each well to determine antimicrobial activity. The results were compared with standard antibiotics—streptomycin (1mg/ml - 20 µl) for bacterial strains and Ketaconazole (1mg/ml - 20 µl) for fungal species.

3. Results

Liquid Chromatography–Mass Spectrometry (LC-MS) analysis

The ethanolic extract of *Phyla nodiflora*, previously reported for its phytochemical richness, was investigated to identify the constituents responsible for its bioreductive potential in silver nanoparticle synthesis. Liquid Chromatography–Mass Spectrometry (LC-MS) was employed to detect major bioactive compounds such as flavonoids, phenolics, and alkaloids, which are known to facilitate both reduction and stabilization of metal ions (Table 1 and Fig. 1). The most abundant class of compounds was flavonoids, which are potent electron donors in green synthesis protocols. Glycosylated flavonoids, such as kaempferol-3-O-glucoside and naringenin, were also identified, suggesting enhanced solubility and bioavailability in aqueous systems. Phenolic acids, represented by protocatechuic acid and rosmarinic acid, have been extensively documented for their high redox potential and capacity to chelate metal ions. Their presence enhances the plausibility of *Phyla nodiflora* as a phytogenic precursor for silver nanoparticle synthesis. Terpenoids and triterpenoid compounds such as linalool, ursolic acid, oleanolic acid, and betulinic acid are well documented for their anti-inflammatory, antimicrobial, and wound-healing effects. Ursolic and oleanolic acids in particular show potent anti-inflammatory activity via NF-κB and other signaling pathways, as well as broad-spectrum antibacterial properties against Gram-positive bacteria [9]. Betulinic acid has demonstrated anti-inflammatory activity in various *in-vivo* edema models and also exhibits antimicrobial potential. [10] Stigmasterol and campesterol, common steroidal phytosterols, have been associated

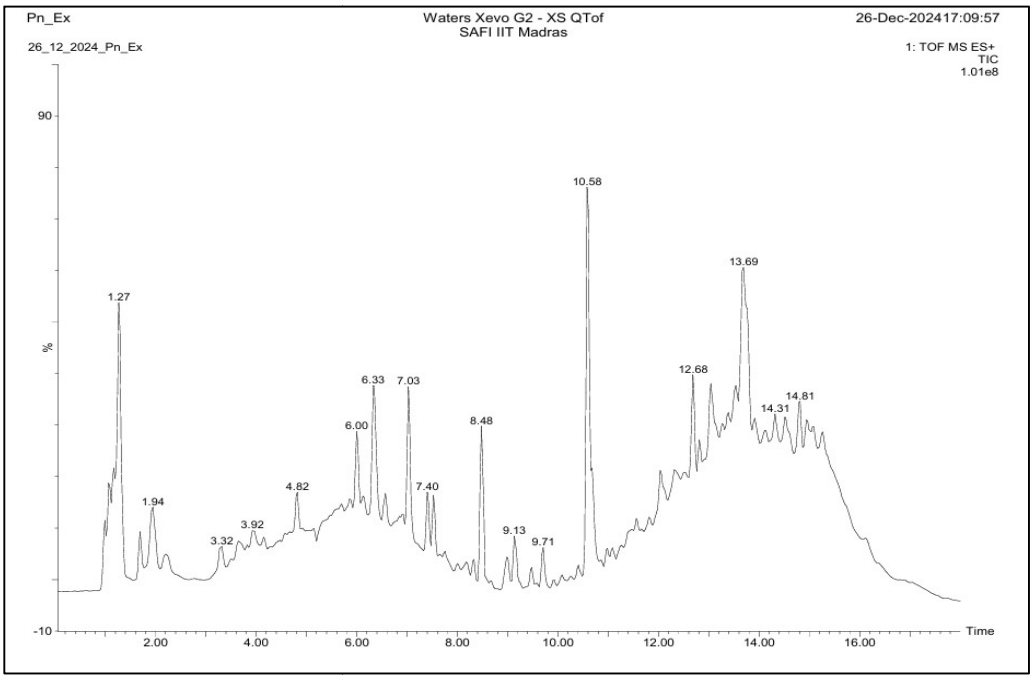


Fig. 1: LC-MS Chromatogram of *Phyla nodiflora* Ethanolic Extract

Phyla nodiflora silver nanoparticles

Vol. 20 (Supplementry Issue 1A), March 2026, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2026.1s.18

Table 1: LC-MS Profile of Bioactive Compounds Identified in *Phyla nodiflora* Ethanolic Extract

S. No	Retention Time (min)	Mass (m/z)	Classification	Possible Compound	Chemical Name
1.	1.27	231.0818	Flavonoid	Quercetin	3,3',4',5,7-Pentahydroxyflavone
2.	1.94	170.0401	Flavonoid	Apigenin	4',5,7-Trihydroxyflavone
3.	3.32	453.8491	Flavonoid	Rutin	Quercetin-3-rutinoside
4.	3.92	452.7036	Flavonoid	Luteolin	3',4',5,7-Tetrahydroxyflavone
5.	4.82	699.3563	Flavonoid	Kaempferol Glycoside	Kaempferol-3-O-glucoside
6.	6	677.3702	Flavonoid	Naringenin	4',5,7-Trihydroxyflavanone
7.	6.33	144.1023	Phenolic Acid	Protocatechuic Acid	3,4-Dihydroxybenzoic acid
8.	7.03	325.2286	Phenolic Acid	Rosmarinic Acid	(R)-α-[[3-(3,4-Dihydroxyphenyl)-1-oxo-2-propenyl]oxy]-3,4-dihydroxybenzenepropanoic acid
9.	7.4	172.1317	Terpenoid	Linalool	3,7-Dimethyl-1,6-octadien-3-ol
10.	8.48	398.1041	Terpenoid	Ursolic Acid	3β-Hydroxy-urs-12-en-28-oic acid
11.	9.13	679.5117	Terpenoid	Oleanolic Acid	3β-Hydroxy-olean-12-en-28-oic acid
12.	9.71	520.403	Terpenoid	Betulinic Acid	3β-Hydroxy-lup-20(29)-en-28-oic acid
13.	10.58	194.5561	Steroid	Stigmasterol	(3β,22E)-Stigmasta-5,22-dien-3-ol
14.	12.68	212.0193	Steroid	Campesterol	(3β)-Ergost-5-en-3-ol
15.	13.69	774.2745	Xanthone	Xanthone	Phenolic aldehyde derivative
16.	14.31	701.4932	Triterpenoid Saponin	Ginsenoside	Triterpenoid saponin derivative
17.	14.81	340.2619	Tannin	Gallic Acid	3,4,5-Trihydroxybenzoic acid

with membrane-stabilizing and anti-inflammatory properties potentially enhancing cellular resilience in wounded tissue[11]. The presence of gallic acid, a xanthone-derived tannin compound, further supports the multifunctional character of the extract, providing antioxidant and astringent activities beneficial for skin repair and regeneration [12]

FTIR Spectroscopic Analysis of Pn-Ag-Np and Hydrogel Formulation Ingredients

The FTIR spectrum of Pn-Ag-Np revealed several distinct absorption bands corresponding to functional groups of plant-derived phytoconstituents that facilitated nanoparticle synthesis and stabilization. A prominent broad peak at 3309 cm^{-1} indicated O–H stretching vibrations, suggesting the

Vol. 20 (Supplementry Issue 1A), March 2026, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2026.1s.18

presence of hydroxyl groups, aliphatic chains, and aromatic rings. Low-frequency bands at 779.22 cm^{-1} and 425.97 cm^{-1} were also recorded, confirming the formation of silver nanoparticles stabilized by bioactive molecules (Fig. 2A).

When the Pn-Ag-Np were incorporated into the hydrogel formulation ingredients, the FTIR spectrum demonstrated additional peaks arising from the presence of formulation excipients, including carbopol. New absorption bands at 2945.5 cm^{-1} (C–H stretching of methylene groups) and 1701.3 cm^{-1} (C=O stretching), possibly due to carboxylic acid or ester functionalities, were observed. The low-frequency Ag–O bands at 615.66 cm^{-1} and 506.5 cm^{-1} were retained in the spectrum of the combined formulation (Fig. 2B), indicating that the nanoparticles remained chemically stable during the formulation process.

Stability study

A three-month stability study was conducted on hydrogel formulations (F4,F7,F8) under room temperature (Table 2) and Accelerated conditions (Table 3) (40 ± 2°C / 75% RH) in accordance with ICH Q1A(R2) guidelines. To evaluate their physicochemical integrity and determine the most stable candidate for further development. The study assessed parameters such as pH, viscosity, physical appearance, homogeneity, spreadability and drug content. In the first month, all formulations retained acceptable pH levels and viscosity, with no visible signs of phase separation or microbial growth. However, as storage time progressed, variations in physical stability began to emerge among formulations.

Drug content varied significantly over time, with F4 consistently maintaining the highest retention 22 ± 1 % at room

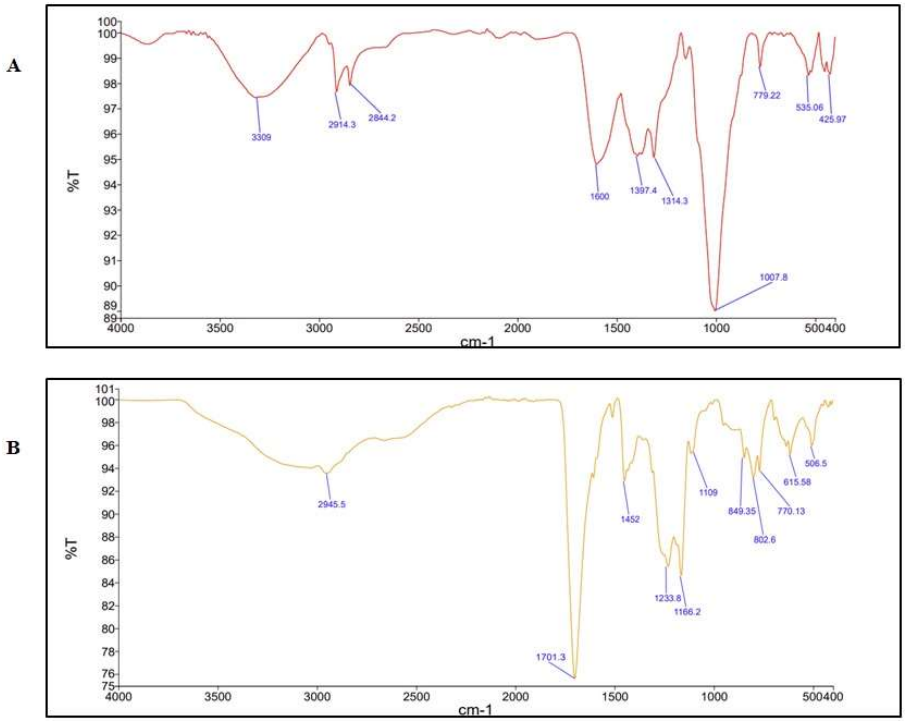


Fig. 2: (A) FTIR Spectra of Synthesised Pn-Ag-Np, (B) FTIR Spectra of Pn-Ag-Np with formulation ingredients

Vol. 20 (Supplementry Issue 1A), March 2026, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2026.1s.18

Table 2: Stability profile of hydrogel formulations (F4,F7,F8) stored at room temperature (25 ± 2°C / 60% RH) for 3 months, evaluating pH, viscosity, drug content, physical appearance, and homogeneity

Room Temperature (25°C ± 2°C/60% RH ± 5% RH)			
At the end of 1st month			
	F4	F7	F8
colour	Pale grey	Pale grey	Pale grey
pH	7.1 ± 0.1	6.9 ± 0.1	7.0 ± 0.1
Viscosity(cp)	1550 ± 65	1640 ± 80	1660 ± 85
Homogenity	Good	Good	Good
Spreadability (g cm/s)	24 ± 1	21 ± 1	22 ± 1
Drug Content (%)	99 ± 2	98 ± 2	99 ± 2
At the end of 2nd month			
colour	Pale grey	Pale grey	Pale grey
pH	7.0 ± 0.1	6.8 ± 0.1	6.9 ± 0.1
Viscosity	1530 ± 65	1610 ± 80	1630 ± 85
Homogenity	Good	Good	Good
Spreadability (g cm/s)	23 ± 1	20 ± 1	21 ± 1
Drug Content (%)	98 ± 2	97 ± 2	98 ± 2
At the end of 3rd month			
colour	Dark Grey	Dark Grey	Dark Grey
pH	6.9 ± 0.1	6.7 ± 0.1	6.8 ± 0.1
Viscosity (cp)	1510 ± 65	1590 ± 80	1610 ± 85
Homogenity	Good	Good	Good
Spreadability (g cm/s)	23 ± 1	19 ± 1	20 ± 1
Drug Content (%)	98 ± 2	96 ± 2	97 ± 2

Table 3: Stability profile of hydrogel formulations (F4,F7,F8) stored under accelerated conditions (40 ± 2°C / 75% RH) for 3 months, assessing changes in physicochemical parameters including pH, viscosity, drug content, and physical consistency

Accelerated condition (40 °C ± 2 °C/75% RH ± 5% RH)			
At the end of 1st month			
	F4	F7	F8
colour	Pale grey	Pale grey	Pale grey
pH	6.9 ± 0.2	6.7 ± 0.2	6.8 ± 0.2
Viscosity (cp)	1550 ± 75	1620 ± 90	1640 ± 95
Homogenity	Good	Good	Good
Spreadability (g·cm/s)	22 ± 1	18 ± 1	19 ± 1
Drug Content (%)	97 ± 2	95 ± 2	96 ± 2
At the end of 2nd month			
colour	Pale grey	Pale grey	Pale grey
pH	6.8 ± 0.2	6.6 ± 0.2	6.7 ± 0.2
Viscosity (cp)	1520 ± 75	1600 ± 90	1620 ± 95
Homogenity	Good	Good	Good
Spreadability (g·cm/s)	21 ± 1	17 ± 1	18 ± 1
Drug Content (%)	96 ± 2	94 ± 2	95 ± 2
At the end of 3rd month			
colour	Dark Grey	Dark Grey	Dark Grey
pH	6.7 ± 0.2	6.5 ± 0.2	6.6 ± 0.2
Viscosity (cp)	1490 ± 75	1570 ± 90	1590 ± 95
Homogenity	Good	Good	Good
Spreadability (g·cm/s)	20 ± 1	16 ± 1	17 ± 1
Drug Content (%)	95 ± 2	93 ± 2	94 ± 2

Vol. 20 (Supplementry Issue 1A), March 2026, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2026.1s.18

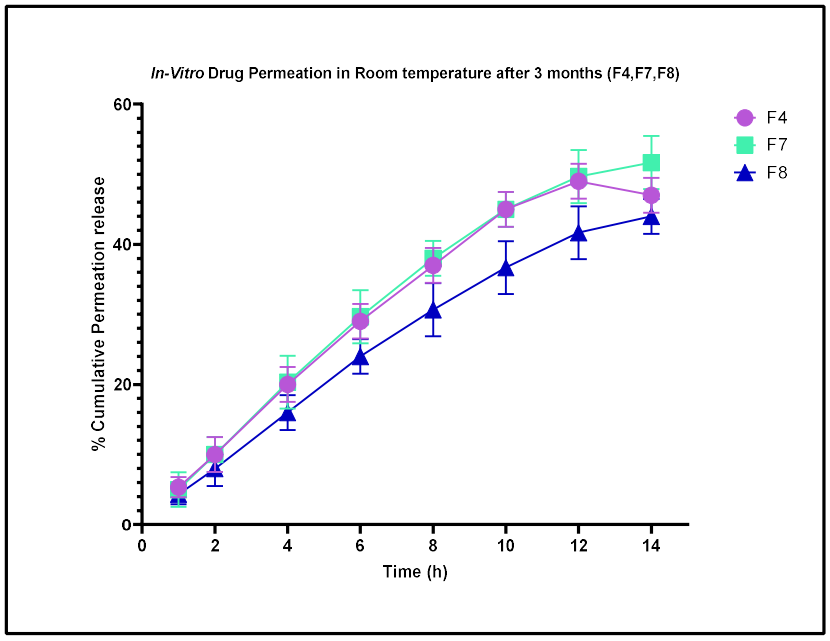


Fig. 3: In-vitro *drug permeation profile of the optimized Pn-Ag-Hg hydrogel formulation at room temperature.* The formulation F4 exhibited a sustained release pattern extending up to 14 hours, indicating its potential for prolonged topical therapeutic action. Results are expressed as mean ± standard deviation (SD, n = 3).

temperature and 20 ± 1 % under accelerated conditions after three months. F4 maintained consistent pH and viscosity, with no visible signs of degradation, and remained smooth, homogeneous, and free from clumping or microbial contamination.

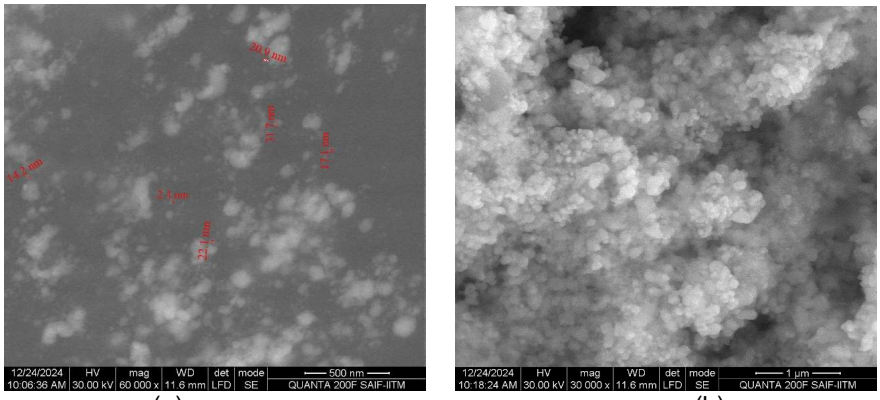
F4 demonstrated excellent physicochemical stability under both storage conditions, with minimal variation across all parameters. This stability profile is in agreement with other herbal silver nanoparticle formulations, where stability is attributed to effective polymer selection and proper nanoparticle integration.

In-vitro drug permeation study after the 3rd Month

The *in-vitro* drug permeation profiles of the hydrogel formulations F4, F7 and F8 were assessed at both room temperature (Fig. 3) and accelerated temperature (Fig. 4). Over the course of the 14-hour period, the cumulative drug release from all three formulations increased gradually and steadily, demonstrating their ability to provide regulated delivery even after extended storage. With a cumulative drug release of almost 50% ± 0.02 at the 12 hour and 47% ± 0.02 at the 14th hour, F4 continuously demonstrated the greatest and most consistent release profile at room temperature. In the accelerated stability condition at 40°C/75% RH,F4 maintained superior release characteristics with 49% ± 0.01 at 12 hours and 48% ± 0.03 at 14 hours, indicating that thermal stress did not significantly compromise its controlled release capacity. F4 maintained superior release characteristics, indicating that thermal stress did not significantly compromise its controlled release capacity. Based on these results, F4 was selected for

Vol. 20 (Supplementry Issue 1A), March 2026, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2026.1s.18

Fig. 4: In-vitro *drug* permeation profile of the optimized Pn-Ag-Hg hydrogel formulation at 40 ± 2°C and 75% RH (accelerated stability conditions). The F4 formulation exhibited enhanced permeation over time, indicating a possible thermally responsive release behavior. Data are presented as mean ± standard deviation (SD, n = 3)



(a) (b)

Fig. 5a & b: SEM analysis of the optimized F4 Pn-Ag-Hg hydrogel formulation.image depicts the surface morphology of silver nanoparticles synthesized using Phyla nodiflora, showing a uniform and well-dispersed nanoparticle distribution within the hydrogel matrix

further detailed analysis and development as the optimized hydrogel candidate for effective sustained topical therapeutic delivery.

SEM and EDAX Analysis of the Optimized Formulation

The SEM micrograph (Fig. 5) revealed a smooth, interconnected hydrogel

Vol. 20 (Supplementry Issue 1A), March 2026, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2026.1s.18

matrix with evenly distributed silver nanoparticles. The nanoparticles ranged in size from 2.4 to 31.7 nm, indicating a nanoscale dispersion ideal for biological applications. This uniform distribution without signs of aggregation highlights the stabilizing effect of the Carbopol polymer matrix, aided by solubilizing agents such as triethanolamine and propylene glycol. These components help regulate particle size and prevent agglomeration. Such morphology is comparable to earlier findings by Sell et al. (2012) [13], who reported similarly stabilized silver nanoparticles (25–35 nm) in chitosan-PVA hydrogels. The reduced particle size enhances the surface area-to-volume ratio, improving interactions with tissues and supporting antibacterial and antioxidant activities.

EDAX analysis (Fig. 6 and Table 4) confirmed the successful incorporation of silver into the hydrogel system. The elemental profile showed a high content of Carbon (70.47%) and Oxygen (28.00%), with minor amounts of Potassium (0.44%) and Silver (1.09%). The low silver content is attributed to its controlled dosage and the dilution effect of the polymeric and phytochemical matrix. These findings are consistent with the results reported by Fahad et al. (2023)[14], affirming the stability and integration of silver nanoparticles in plant-based hydrogel systems.

Overall, the SEM and EDAX data together validate that the F4 hydrogel is a homogenous, nanoscale, and chemically stable formulation—critical attributes for effective wound healing, sustained release, and safe topical application.

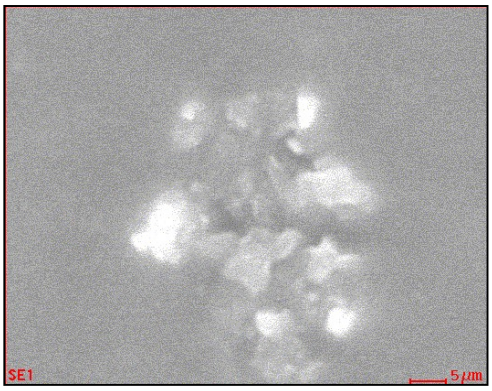
In-vitro Anti-inflammatory Assays of Optimized F4 Formulation

Proteinase Inhibition Assay

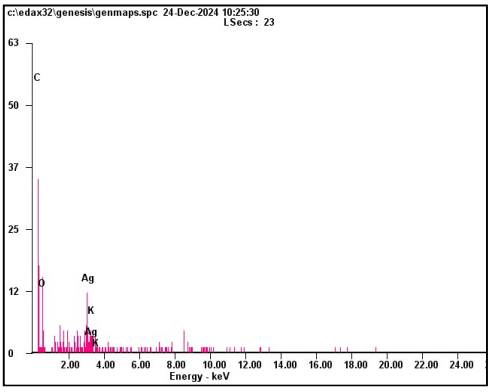
The proteinase inhibitory activity of the optimized F4 Pn-Ag-Hg hydrogel was evaluated to determine its anti-inflammatory potential. As shown in Fig. 7, the formulation demonstrated a clear concentration-dependent inhibition of proteinase enzymes. At the lowest concentration of 100 µg/mL,

Table 4: EDAX analysis of the optimized F4 Pn-Ag-Hg hydrogel formulation

Element	*Wt%*	*At%*
CK	59.23	70.47
OK	31.34	28.00
AgL	08.22	01.09
KK	01.21	00.44
Matrix	Correction	ZAF



(a)



(b)

Fig. 6a & b: EDAX analysis of the optimized F4 Pn-Ag-Hg hydrogel formulation.spectrum confirms the elemental composition, with prominent peaks corresponding to silver (Ag), carbon (C), oxygen (O), and potassium (K), validating the successful incorporation of AgNPs into the hydrogel

Phyla nodiflora silver nanoparticles

Vol. 20 (Supplementry Issue 1A), March 2026, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2026.1s.18

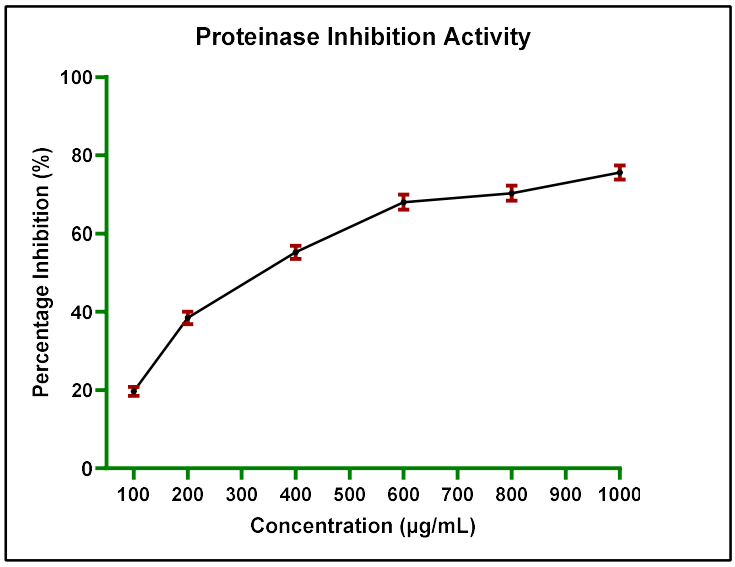


Fig. 7: Proteinase Inhibition Activity of F4 Pn-Ag-Hg hydrogel. The graph shows a concentration-dependent increase in inhibition of proteolytic enzymes. Values are expressed as mean ± SD (n = 3).

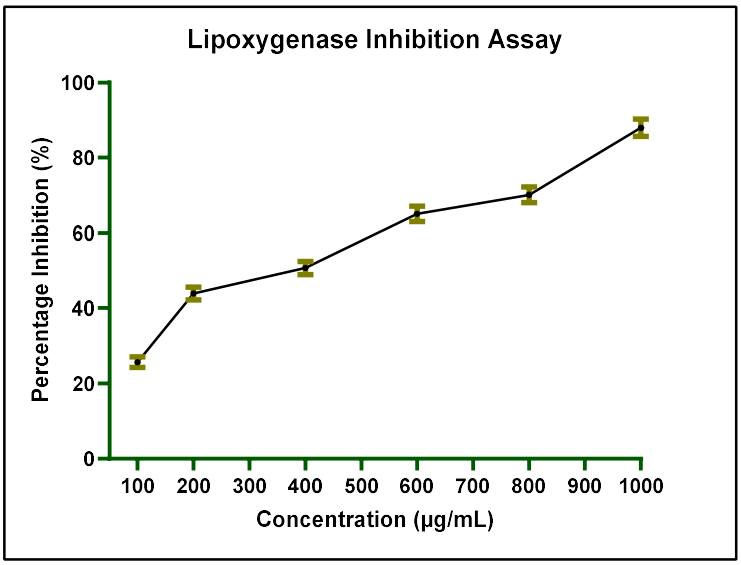


Fig. 8: Lipoxygenase Inhibition Assay of F4 Pn-Ag-Hg hydrogel. The graph displays concentration-dependent inhibition of LOX activity. Results are expressed as mean ± SD (n = 3).

inhibition was recorded at 19.7% ± 1.2, while at 1000 µg/mL, it reached a significant 75.6% ± 1.8. This suggests strong anti-proteolytic potential, which is beneficial in managing chronic inflammatory wounds where protease overactivity leads to extracellular matrix degradation and delayed healing. The mechanism underlying this inhibition appears

Vol. 20 (Supplementry Issue 1A), March 2026, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2026.1s.18

to involve the synergistic activity of bioactive phytochemicals from *Phyla nodiflora* and the incorporated silver nanoparticles. The polyphenolic compounds are likely to chelate with metal ions at the active site of serine proteases, thereby reducing their enzymatic activity. Silver nanoparticles may also contribute via enzyme binding or conformational changes that render the protease inactive.

Lipoxygenase (LOX) Inhibition Assay

The F4 hydrogel formulation, incorporating *Phyla nodiflora*-mediated silver nanoparticles (Pn-Ag-Hg), demonstrated notable lipoxygenase (LOX) inhibitory activity, indicative of its anti-inflammatory potential. As illustrated in Fig. 8, the inhibition increased progressively with concentration, reaching a maximum of 87.9% ± 2.4 at 1000 µg/mL. Lipoxygenase is a key enzyme in the biosynthesis of pro-inflammatory mediators such as leukotrienes, which contribute to vascular permeability, edema, and leukocyte infiltration at sites of tissue injury. The strong LOX inhibition may be attributed to the presence of flavonoids and polyphenols from *Phyla nodiflora*, known to interfere with enzymatic oxidation of fatty acids. In addition, silver nanoparticles may influence enzyme conformation or disrupt signaling pathways involved in inflammatory responses. The hydrogel matrix provides an optimal environment for localized retention, controlled release, and prolonged action of the active constituents. Together, these properties enhance tissue exposure to anti-inflammatory agents, improving therapeutic outcomes in wounds characterized by chronic inflammation. F4's sustained LOX inhibition suggests that the formulation not only alleviates inflammation but also prevents secondary tissue damage, making it a promising candidate for treating inflamed or non-healing wounds.

Protein Denaturation Assay

The F4 Pn-Ag-Hg hydrogel displayed significant inhibition of heat-induced protein denaturation, demonstrating strong anti-inflammatory potential. As shown in Fig. 9, the formulation achieved a maximum

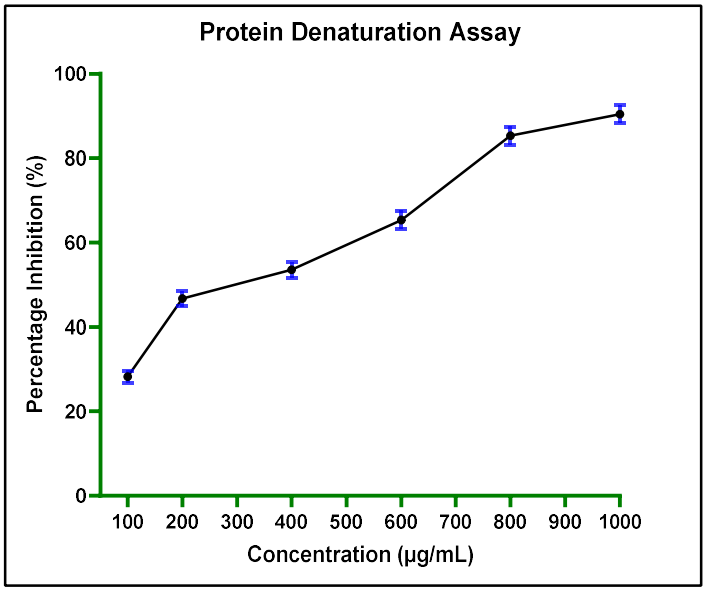


Fig. 9: Protein Denaturation Inhibition by F4 Pn-Ag-Hg hydrogel. The graph shows significant concentration-dependent inhibition of protein denaturation. Data are presented as mean ± SD (n = 3).

Phyla nodiflora silver nanoparticles

Vol. 20 (Supplementry Issue 1A), March 2026, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2026.1s.18

Table 5: Zone of inhibition (in mm) exhibited by the optimized hydrogel formulation against selected bacterial and fungal pathogens at different concentrations (125–1000 µg/ml), compared with standard antibiotics (Streptomycin and Ketoconazole at 1 mg/ml, 20 µl)

Microorganisms	Zone of Inhibition in mm				
	Bacterial organisms				
	1-125 µg/ml	2-250 µg/ml	3-500 µg/ml	4-1000 µg/ml	5-(1 mg/ml - 20 µl) Streptomycin
Staphylococcus aureus	-	8	10	12	33
Bacillus cereus	-	6	9	11	22
Pseudomonas aeruginosa	-	4	5	8	19
Salmonella typhi	-	5	7	8	25
Fungal Organisms					
Microorganisms	1-125µg/ml	2-250 µg/ml	3-500 µg/ml	4-1000 µg/ml	5-Ketaconazole (1mg/ml - 20 µl)
Aspergillus niger	-	4	5	7	12
Candida albicans	-	10	13	16	30

inhibition of 90.4% ± 2.1 at 1000 µg/mL, indicating effective stabilization of protein structure under stress conditions. Protein denaturation is a well-established marker of inflammation, as denatured proteins can act as neo-antigens, triggering immune responses and prolonging inflammatory cascades. The inhibitory effect is likely attributed to the combined action of *Phyla nodiflora* phytoconstituents and silver nanoparticles. Flavonoids and tannins are known to stabilize protein conformation through hydrogen bonding and hydrophobic interactions, while silver nanoparticles may adsorb denatured proteins, preventing their recognition by immune cells.[15] The hydrogel matrix plays a key role by ensuring sustained release and optimal interaction of active compounds with inflammatory proteins.[16] This not only prolongs the anti-inflammatory action but also reduces the likelihood of irritation or systemic exposure.

Antimicrobial Assay of Optimized F4 Formulation

The optimized F4 Pn-Ag-Hg hydrogel exhibited significant antimicrobial properties, particularly against Gram-positive bacteria and fungal strains. As shown in Table 5 and in Fig. 10, the formulation demonstrated an 8 mm zone of inhibition against *Salmonella typhi* and *Pseudomonas aeruginosa* (Gram-negative), while displaying stronger activity against Gram-positive bacteria due to their simpler membrane structure and thicker peptidoglycan layers, which are more susceptible to silver ion penetration. The antimicrobial mechanism is likely mediated by silver nanoparticles inducing membrane disruption via oxidative stress and electrostatic interaction, compounded by the phytochemicals of *Phyla nodiflora*. These compounds may further enhance bacterial membrane permeability and destabilize essential proteins. Notably, the F4 hydrogel showed potent antifungal activity, producing a 16 mm inhibition zone against *Candida albicans* and 7 mm against *Aspergillus niger*. The enhanced antifungal effect supports its potential use in managing wound infections complicated by fungal colonization. Although the antibacterial effect was slightly lower than that of the standard drug streptomycin, the broad-spectrum efficacy and sustained release properties of F4 make it a promising candidate for treating polymicrobial and fungal-infected wounds.

Vol. 20 (Supplementry Issue 1A), March 2026, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2026.1s.18

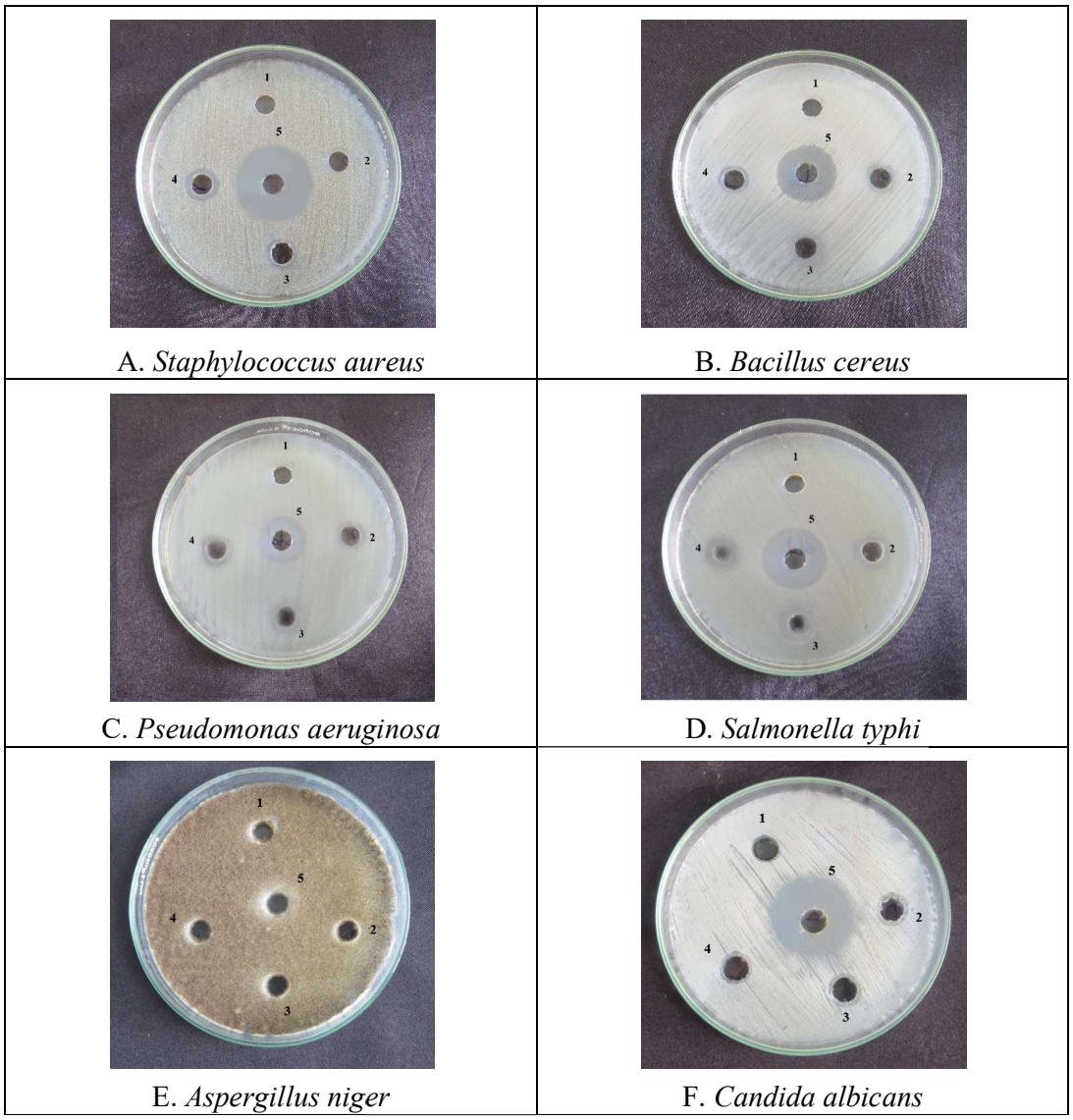


Fig. 10: Antibacterial and Antifungal activity of F4 Pn-Ag-Hg hydrogel. Zones of inhibition against selected bacterial and fungal strains are shown.

4. Discussion

The study presents a silver nanoparticle-based hydrogel formulation using *Phyla nodiflora* extract, focusing on wound healing efficacy, stability, and multifunctional bioactivity. The strategy involves integrating phytochemically rich plant extracts with green-synthesized silver nanoparticles into a Carbopol-based hydrogel to enhance therapeutic performance. LC-MS analysis of the *Phyla nodiflora* extract revealed a diverse profile of bioactive compounds, including flavonoids, phenolic acids, and anti-inflammatory triterpenoids.

Vol. 20 (Supplementry Issue 1A), March 2026, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2026.1s.18

This phytochemical diversity facilitated efficient reduction and capping of silver ions during nanoparticle synthesis, which was further supported by FTIR spectra showing characteristic peaks for O–H, C=O, and Ag–O functional groups—indicating successful nanoparticle formation and compatibility with the hydrogel matrix.

Among the various formulations analyzed, F4 emerged as the most stable and effective candidate. It maintained consistent physicochemical parameters such as pH, viscosity, homogeneity, and drug content over a three-month period. A comprehensive stability study was conducted under ICH Q1A(R2) guidelines, including storage at room temperature (25 ± 2 °C / 60% RH) and accelerated conditions (40 ± 2 °C / 75% RH). F4 demonstrated minimal deviation across all evaluated parameters, confirming its robustness and shelf-life stability. *In-vitro* drug release studies further established F4's ability to provide sustained release for up to 14 hours without an initial burst effect, making it ideal for prolonged wound coverage and therapeutic action.

Biologically, F4 exhibited significant dose-dependent anti-inflammatory activity, effectively inhibiting proteinase, lipoxygenase, and protein denaturation—key mediators in chronic wound inflammation. In addition, it demonstrated superior antifungal efficacy, particularly against *Candida albicans*, reinforcing its role as a potent antimicrobial dressing. This comprehensive evaluation highlights the translational potential of the F4 hydrogel formulation for topical wound healing, bridging traditional herbal wisdom with modern nanopharmaceutical strategies through a stable, bioactive, and biocompatible delivery platform.

5. Conclusion

The developed F4 hydrogel formulation, incorporating *Phyla nodiflora*-derived silver nanoparticles, offers a promising bio-inspired approach to wound healing. Rooted in green nanotechnology and phytopharmaceutical principles, it demonstrated sustained drug release (up to 14 hours), excellent physicochemical stability under ICH guidelines, and significant anti-inflammatory and antimicrobial activity. Key functional attributes include inhibition of proteinase, lipoxygenase, and protein denaturation, along with strong antifungal efficacy against *Candida albicans*. The combination of traditional herbal constituents, silver nanoparticles, and a Carbopol-based hydrogel matrix positions F4 as a multifunctional, stable, and biocompatible formulation for effective topical wound care.

References

1. Raziyeva K, Kim Y, Zharkinbekov Z, Kassymbek K, Jimi S, Saparov A. Immunology of acute and chronic wound healing. Biomolecules 2021;11(5):700.
2. Xu Z, Han S, Gu Z, Wu J. Advances and Impact of Antioxidant Hydrogel in Chronic Wound Healing. Adv Healthc Mater 2020;9(5):e1901502.
3. Venugopal DC, Senthilnathan RD, Maanvizhi S, Madhavan Y, Sankarapandian S, Ramshankar V, et al. Preparation and Characterization of Silymarin Gel: A Novel Topical Mucoadhesive Formulation for Potential Applicability in Oral Pathologies. Gels 2023;9(2):139.
4. Mohan C, Devi S. LC-MS and HPTLC Profile of Crude Ethanol Extract of Plant Michelia champaca Linn. Pharmacognosy Res 2024;16(3):490–4.
5. Mohandoss Kiruba, V Vijayan, Hemalatha S. Carrageenan-Induced Arthritic Joint Inflammation in Rats: Evaluating the Anti-Arthritic Potential of Phytosomal Cissus quadrangularis Ethanolic Extract. African journal of Biological sciences 2024;6(13):1715–35.
6. Senthilnathan RD, Hemalatha S, Gayathri V, Komala M, Chandru R. Phyla Nodiflora Derived Silver Nanoparticles In Hydrogel Formulations: A New Approach To Wound Management. Curr Trends Biotechnol Pharm 2024;18(3s):240–56.
7. Mohandoss Kiruba, V Vijayan, S Hemalatha. Cissus quadrangularis Phytosomes' Bioactivity Assessment: Cytotoxic, Anti-Inflammatory and Antioxidant

Vol. 20 (Supplementry Issue 1A), March 2026, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2026.1s.18

Properties. Asian J Biol Life Sci 2025;13(3):714–22.
8. Baveloni FG, Meneguin AB, Sábio RM, Camargo BAF de, Trevisan DPV, Duarte JL, et al. Antimicrobial effect of silver nanoparticles as a potential healing treatment for wounds contaminated with Staphylococcus aureus in wistar rats. J Drug Deliv Sci Technol 2025;103:106445.
9. Kashyap D, Sharma A, S. Tuli H, Punia S, K. Sharma A. Ursolic Acid and Oleanolic Acid: Pentacyclic Terpenoids with Promising Anti-Inflammatory Activities. Recent Pat Inflamm Allergy Drug Discov 2016;10(1):21–33.
10. Fontanay S, Grare M, Mayer J, Finance C, Duval RE. Ursolic, oleanolic and betulinic acids: Antibacterial spectra and selectivity indexes. J Ethnopharmacol 2008;120(2):272–6.
11. Goswami M, Priya, Jaswal S, Gupta G Das, Verma SK. A comprehensive update on phytochemistry, analytical aspects, medicinal attributes, specifications and stability of stigmasterol. Steroids 2023;196:109244.
12. Bai J, Zhang Y, Tang C, Hou Y, Ai X, Chen X, et al. Gallic acid: Pharmacological activities and molecular mechanisms involved in inflammation-related diseases. Biomedicine & Pharmacotherapy 2021;133:110985.
13. Sell SA, Wolfe PS, Spence AJ, Rodriguez IA, McCool JM, Petrella RL, et al. A Preliminary Study on the Potential of Manuka Honey and Platelet-Rich Plasma in Wound Healing. Int J Biomater 2012;2012:1–14.
14. Aldakheel F, Mohsen D, El Sayed M, Alawam K, Binshaya A, Alduraywish S. Silver Nanoparticles Loaded on Chitosan-g-PVA Hydrogel for the Wound-Healing Applications. Molecules 2023;28(7):3241.
15. Feng Y, Jin C, Lv S, Zhang H, Ren F, Wang J. Molecular Mechanisms and Applications of Polyphenol-Protein Complexes with Antioxidant Properties: A Review. Antioxidants 2023;12(8):1577.
16. Ninan N, Goswami N, Vasilev K. The Impact of Engineered Silver Nanomaterials on the Immune System. Nanomaterials 2020;10(5):967.