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Phyla Nodiflora Derived Silver Nanoparticles In Hydrogel Formulations: A New Approach To Wound Management

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Abstract

A vital component of healthcare is wound healing, which includes the healing of both acute and chronic wounds. Prolonged inflammation brought on by immunological dysregulation, increased reactive oxygen species, and matrix metalloproteinase activity make chronic wounds difficult to heal. Recent developments in hydrogel dressings supplemented with antioxidants and immunomodulatory medicines have the potential to improve chronic wound healing. This work investigates the synthesis of green-synthesized silver nanoparticles (AgNPs) from the medicinal plant *Phyla nodiflora* , which has a variety of pharmacological properties, intending to develop a novel phytopharmaceutical hydrogel. Ethanolic extraction was employed to obtain bioactive compounds from *Phyla nodiflora* , followed by comprehensive phytochemical analysis revealing a notable amount of alkaloids, flavonoids, tannins, and phenolic compounds. The green synthesis of AgNPs has been examined by UV spectroscopy, zeta potential measurement, SEM with EDAX, and HRTEM. The hydrogels were evaluated for their pH, viscosity, homogeneity, spreadability, drug content, and release profiles. The optimized formulation demonstrated exceptional spreadability and prolonged medication release, making it appropriate for the treatment of chronic wounds. The hydrogel's biocompatibility and wound-healing capabilities were shown in *In-vitro* experiments, suggesting that it may find use in clinical settings for the treatment of chronic wounds in the future.

Keywords: Wound Healing, Chronic Wounds, Silver Nanoparticles, *Phyla nodiflora*, Green Synthesis, Phytopharmaceutical Hydrogel

Introduction

A vital component of healthcare is wound healing, which includes the restoration of both acute and chronic wounds. Due to immunological dysregulation, pro-inflammatory macrophages, increased reactive oxygen species, and higher matrix metalloproteinase activity, chronic wounds have extended inflammation and poor healing. Chronic wound healing is hampered by biological biofilms. Recent developments in the study of wound healing have focused on immunomodulatory treatment approaches, including altering the phenotypes of macrophages, controlling the expression of miRNA, and employing both pro- and anti-inflammatory medications. Antioxidant-infused hydrogel dressings show promise for hastening wound healing and laying the

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groundwork for upcoming advancements in the treatment of chronic wounds(1).

The skin contains several subsets of stem cells that have multipotent qualities when it is wounded, therefore understanding the roles of different cell types in wound healing is essential. The functional and phenotypic diversity among these cell types has been revealed by advances in single-cell technologies, and this knowledge can inform the development of therapeutics targeted at improving tissue damage and wound healing. The study of the clinical and molecular foundations of wound rehabilitation is crucial because chronic, non-healing wounds, which are frequently linked to aging and illnesses like diabetes, entail substantial financial costs(2).

Native to southern India, *Phyla nodiflora* is a medicinal plant with a wide range of pharmacological activity, such as hypotensive, anti-inflammatory, antioxidant, anti-tumor, antibacterial, anti-diarrheal, anticancer, and diuretic effects(3–5). It is used in traditional medicine to treat women in state, diarrhea, dysuria, and digestive issues. It is eaten in Ceylon, but it is sipped like tea in the Philippines.In Pakistan's tribal people, the herb is also utilized as a traditional cosmetic and cure for many skin ailments(6).

The green synthesis of silver nanoparticles (AgNPs) has garnered attention for their broad-spectrum antibacterial properties, making them suitable for biomedical applications such as wound care. This environmentally friendly synthesis method employs plant extracts, reducing harmful chemical pollutants. The green synthesis process rapidly produces metal nanoparticles under mild conditions, leveraging phytochemicals like terpenoids, aldehydes, alkaloids, amino acids, ketones, flavonoids, and carboxylic acids. This method is cost-effective, environmentally sustainable, and avoids generating hazardous by products(7,8).

Hydrogels, known for promoting epithelial renewal and maintaining a moist wound environment, show significant potential in wound dressing applications(9). This study aims to develop a novel phytopharmaceutical hydrogel for wound healing, utilizing *Phyla nodiflora* -derived silver nanoparticles synthesized through green methods. Using an ethanolic extract of *Phyla nodiflora* silver nanoparticles was synthesized and incorporated into a carbopol hydrogel formulation. Various formulation trials were conducted, with the optimal formulation selected based on comprehensive evaluation parameters.

Materials and Methods

Extraction and evaluation of the plant

Preparation of Ethanolic Extract of *Phyla nodiflora*

The plant was collected from an herbal garden in Chennai, Tamil Nadu, and authenticated by a botanist. The entire *Phyla nodiflora* plant was air-dried and subsequently ground into a coarse powder. Three kilograms of this dried material were subjected to maceration with ethanol for seven days. Following maceration, the extract was filtered and concentrated using a rotary evaporator under reduced pressure and low temperature. The yield of the ethanolic extract was then calculated and documented.

Preliminary Phytochemical Screening

The ethanolic extract of the plant subjected to preliminary phytochemical screening to identify the presence of alkaloids, proteins, saponins, starch, amino acids, steroids, terpenoids, tannins, flavonoids, anthraquinones, and glycosides using conventional qualitative methods(10).

Quantitative Analysis

The quantitative analysis of the ethanolic extract of *Phyla nodiflora* was conducted to determine the precise concentrations of these phytochemicals.

Estimation of Total Phenol Content- Folin Ciocalteu method

Using gallic acid as a standard, the Folin-Ciocalteu technique was used to calculate the total phenol concentration.

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Making a calibration curve from standard gallic acid solutions at 200 mg/mL, 300 mg/mL, 400 mg/mL, and 500 mg/mL was the process. To perform the experiment, combine 1 mL of Folin reagent, 5 mL of distilled water, and 1 mL of the sample. One mL of 10% sodium carbonate was added after five minutes, and the mixture was allowed to stand at room temperature for an hour. At 725 nm, absorbance was measured. Gallic acid equivalent (GAE) in milligrams per gram of dry extract weight was used to express the results.

Estimation of Tannin Content- Folin-Denis method

The total tannin content was measured using the Folin-Denis technique. The extract (1 mg/mL) was produced as a stock solution. 0.5 mL of the Folin-Denis reagent, 0.5 mL of distilled water, and 0.1 mL of the extract were combined for the experiment. After adding 1 mL of 15% sodium carbonate to the mixture, it was allowed to stand at room temperature in the dark for half an hour. At 700 nm, absorbance was measured. The standard used was 1 mg/mL of tannic acid, and the data were displayed on an estimation graph(11).

Estimation of Flavonoids -Aluminium chloride Colorimetric method

Quercetin served as a benchmark for measuring the number of flavonoids. Quercetin dilutions at 100, 200, 400, 500, 600, 800, and 1000 µg/mL were used to create calibration curves. Using the aluminium chloride complex-forming assay, 1 mL of the sample solution was combined with 4 mL of distilled water. Following a 5-minute incubation period, 300 µL of sodium nitrite and 300 µL of aluminium chloride were added, respectively. Finally, sodium hydroxide (two milliliters) was added.

Estimation of Alkaloid Content - Harborne's method

After combining the extract with 10% acetic acid in ethanol, it was left to stand for four hours. The extract was filtered and then concentrated to one-fourth of its original volume on a water bath. Ammonium hydroxide concentrate was added drop by drop till precipitation happened. After allowing the mixture to settle for three hours, the supernatant was disposed. Following a 0.1 M ammonium hydroxide rinse, the precipitates were dried, weighed, and cleaned(12). The following formula was used to determine the percentage of alkaloids:

$$\text{Percentage of alkaloid} = \frac{\text{weight of sample}}{\text{Weight of alkaloids}} X\,100$$

Green Synthesis of *Phyla nodiflora* Silver Nanoparticles (Pn-Ag-Np)

Preparation of *Phyla nodiflora* Silver Nanoparticles

A 10 mL volume of the ethanolic extract (1 mg of extract dissolved in 10 mL of ethanol) was mixed with 90 mL of $AgNO_3$ solution (0.04 g dissolved in 100 mL of distilled water) and heated for 1 hour until a color change was observed. The solution was kept in the dark for 24 hours. The nanoparticles were purified by washing three times with sterile distilled water via centrifugation at 10,000 rpm for 10 minutes.

Characterization of *Phyla nodiflora* Silver Nanoparticles (Pn-Ag-Np)

The synthesised *Phyla nodiflora* Silver Nanoparticles (Pn-Ag-Np) were characterized for further analysis.

UVSpectroscopy

The reduction of Ag^+ to Ag^o was monitored using a Perkin Elmer UV-Vis spectrophotometer (Lambda 365 model). Absorption scans were conducted over a wavelength range of 200 to 800 nm.

Particle Size Determination

The average mean diameter and size distribution of the nanoparticles were determined using Dynamic Light Scattering (Malvern Zetasizer). The dried nanoparticles

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were dispersed in water to obtain the necessary light scattering intensity.

Zeta Potential Measurement

The surface charge of Pn-Ag-Np was measured using a Malvern Zetasizer equipped with zeta cells and polycarbonate cells with gold-plated electrodes, using water as the medium for sample preparation. This measurement is crucial for assessing the stability of the nanoparticles.

Scanning Electron Microscopy (SEM) with EDAX

Scanning Electron Microscopy (SEM) was conducted to evaluate the surface morphology of *Phyla nodiflora* silver nanoparticles (Pn-Ag-Np). The nanoparticles were prepared by first drying the sample thoroughly to eliminate any moisture content. The SEM analysis provided high-resolution images of the surface features, morphology, and particle size distribution of the Pn-Ag-Np.

High-Resolution Transmission Electron Microscopy (HRTEM)

HRTEM analysis of Pn-Ag-Np was performed to evaluate the size, shape, and dispersion of the nanoparticles. The detailed structural properties and lattice fringes were observed, confirming the nanoscale dimensions and crystalline nature of the synthesized nanoparticles(13.)

Antioxidant Activity of Pn-Ag-Np

DPPH Assay for the synthesised Pn-Ag-Np

Various concentrations of standard ascorbic acid and samples (100, 200, 400, 800, and 1000 µg/mL) were prepared in distilled water. Equal volumes of different concentrations of ascorbic acid and DPPH were mixed and incubated at room temperature in the dark for 30 minutes. Absorbance was measured at 517 nm. The scavenging activity was calculated using the formula:

$$Scavenging\ activity\ (\%) = \frac{Ac - As}{AC}\ X\ 100$$

Nitric Oxide Scavenging Assay for the synthesised Pn-Ag-Np

After preparing a 3 mL reaction mixture in distilled water with different concentrations of standard ascorbic acid and samples (100, 200, 400, 800, and 1000 µg/mL) and sodium nitroprusside (10 mM in phosphate-buffered saline), it was incubated at 37°C for 4 hours. Griess reagent (0.5 mL) was added after incubation, and the absorbance was measured at 546 nm(14). The following formula was used to get the % inhibition:

$$Percentage\ inhibition = \frac{Abs\ control - Abs\ sample}{Abs\ control}\ X\ 100$$

Formulation of Pn-Ag-Np loaded Hydrogel

Hydrogels were formulated using carbopol-940, propylene glycol, methyl paraben, propyl paraben, and triethanolamine. Once the hydrogel base components were mixed, Pn-Ag-Np was added to the hydrogels, and the final volume was adjusted to 50 mL using distilled water. The formulations were stirred under magnetic stirring at 700 rpm for 1 hour at room temperature. Triethanolamine was then added dropwise with vigorous stirring until gel consistency and appropriate pH were achieved. Various formulations trials in Table 1 were prepared and evaluated by following parameters.

Evaluation Parameters for Pn-Ag-Np Loaded Hydrogel (Pn-Ag-Hg)

pH

A pH meter was used to measure each Pn-Ag-Hg formulation's pH. The standard deviation (SD) was determined after the experiments were carried out in triplicate.

Viscosity

The viscosity for each Pn-Ag-Hg formulation was determined by means of a Brookfield Viscometer. The trials were run three times, and the standard deviation was computed.

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Table 1: Formulation trials of Pn-Ag-Np hydrogel

Formulation Code	Pn-Ag-Np (g)	Carbopol (g)	Propylene Glycol (mL)	Methyl Paraben (g)	Propyl Paraben (g)	Triethanolamine (mL)	Distilled Water (mL)
F1	0.5	1	25	0.01	0.01	0.5	25
F2	1	1	25	0.01	0.01	0.5	25
F3	1.5	1	25	0.01	0.01	0.5	25
F4	2	1	25	0.01	0.01	0.5	25
F5	0.5	2	25	0.01	0.01	0.5	25
F6	1	2	25	0.01	0.01	0.5	25
F7	1.5	2	25	0.01	0.01	0.5	25
F8	2	2	25	0.01	0.01	0.5	25

Homogeneity

A visual inspection was used to assess the homogeneity of each Pn-Ag-Hg formulation. Aggregate content in the formulations was investigated. The trials were completed in triplicate, and the standard deviation was computed.

Spreadability

Every Pn-Ag-Hg formulation's spreadability was assessed. It was measured in terms of how long it took for two slides, subjected to a given weight, to slide off the gel that was placed between them. A spreadability (S) calculation was made.

$$S = \frac{M\ X\ L}{T}$$

Where, M = weight tied to upper slide, L = length of glass slide, T = time taken to separate the slides

Drug Content analysis by UV spectroscopy

Each Pn-Ag-Hg formulation was dissolved in a predetermined amount in 100 milliliters of pH 6.8 phosphate buffer. After filtering the mixture, phosphate buffer (pH 7.4) was used as the blank in a spectrophotometric analysis of the solution at 410 nm. The formulation trials were run through three times, and the standard deviation was calculated.

***In-vitro* drug permeation study**

The research adopted a diffusion cell apparatus that covered a receptor compartment with a membrane saturated in glycerol and used phosphate buffer as the dissolving media. To ascertain drug permeation over time, samples were examined while the medium was agitated at 50 rpm. On the basis of drug release profiles, the optimal formulation was chosen for additional assessment and research. The best formulation was chosen for more analysis and research.

Release Kinetics study for the for the optimized F4 formulation

The *In-vitro* release kinetics were evaluated for the optimized formulation to investigate the mechanism of drug release(15). The data were analyzed using the following mathematical models, such as zero order kinetics, first order kinetic, Higuchi kinetic, Kors Meyer - Peppa's Model and Hixson-Crowell cube root law

***In- vitro* cell line study using NHDF cell lines for the optimized F4 formulation**

MTT assay for the optimized F4 formulation

After being planted onto a 96-well plate at a density of 1 x 10^4 cells per well in 100 µL of DMEM media, Normal Human

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Dermal Fibroblast (NHDF) cells were allowed to develop for a full day. The medium was changed to several concentrations of the optimized F4 formulation (25, 50, 100, and 500 µg/mL) after the first incubation. After that, the plates were incubated for a full day more. After that, each well received 10 µL of the 5 mg/mL MTT reagent, and the plates were incubated for an additional 4 hours. The resultant purple formazan crystals were made soluble by filling every well—including the control wells—with 100 µL of dimethyl sulfoxide (DMSO) (without treatment). After giving the plates, a gentle shake in order to ensure complete mixing, they were left at room temperature in the dark for around half an hour. The absorbance was then measured with a microplate reader at 570 nm. Using the formula below, the percentage cell viability (CV) was determined:

$$CV\ \% = \frac{Absorbance\ of\ test\ sample}{Absorbance\ of\ control}\ X\ 100$$

In-vitro Wound Scratch Assay for the optimized F4 formulation

The migration rates of NHDF cells were assessed using the scratch assay method. Cells were seeded into each well of a 24-well plate at a density of 2 x 10^5 cells and incubated with complete medium at 37°C and 5% CO_2 until a confluent monolayer was formed. The monolayer cells were then scraped horizontally with a sterile P200 pipette tip to create a scratch. The debris was removed by washing the cells with PBS. Subsequently, the cells were treated with various concentrations of the optimized F4 formulation (10, 25, and 50 µg/mL) diluted in serum-free DMEM. Untreated cells served as the control, and cells treated with allantoin (50 µg/mL) served as the positive control.Images of the scratch, representing the wound, were captured at 0 hours using phase contrast microscopy at 50x magnification. After 24 hours of incubation with the F4 formulation, a second set of images was taken. The migration rate was determined by analyzing the images using "ImageJ" software to measure the percentage of the closed area compared to the initial wound area at 0 hours. An increase in the percentage of the closed area indicated cell migration(16). All experiments were performed in triplicate, and the data were recorded and analyzed statistically using GraphPad Prism version 10.

$$Wound\ Closure\ \% = \frac{Measurement\ at\ 0\ hour - Measure\ at\ 24\ hour}{Measurement\ at\ 0\ hour}\ X\ 100$$

Statical analysis

All experimental data were presented as mean ± standard deviation (SD). The MTT assay, *In-vitro* wound scratch assay, and hydrogel formulation parameters were each conducted in triplicate to ensure reliability and reproducibility. The statistical analysis was performed using GraphPad Prism version 10 software. One-way ANOVA followed by Tukey's post-hoc test was used to assess the statistical significance between different groups. The significance level was set at $p < 0.05$ for all analyses.

Results and discussion:

Extraction

The Percentage yield of the ethanolic extract was found to be 31.6% W/W.

Preliminary phytochemical screening

A thorough analysis of the phytochemical content of *Phyla nodiflora* 's ethanolic extract has revealed a wide range of bioactive chemicals. The rich chemical profile of this plant species is highlighted by the presence of alkaloids, proteins, saponins, starch, amino acids, steroids, terpenoids, tannins, flavonoids, anthraquinones, and glycosidesshown in Table 2. Because of these chemicals' noteworthy biological activity and possible pharmacological effects, *Phyla nodiflora* is an ideal choice for additional research in the fields of medicine and Pharmaceuticals.

Total Phenol content

The phenol content analysis of the ethanolic extract of *Phyla nodiflora* yielded

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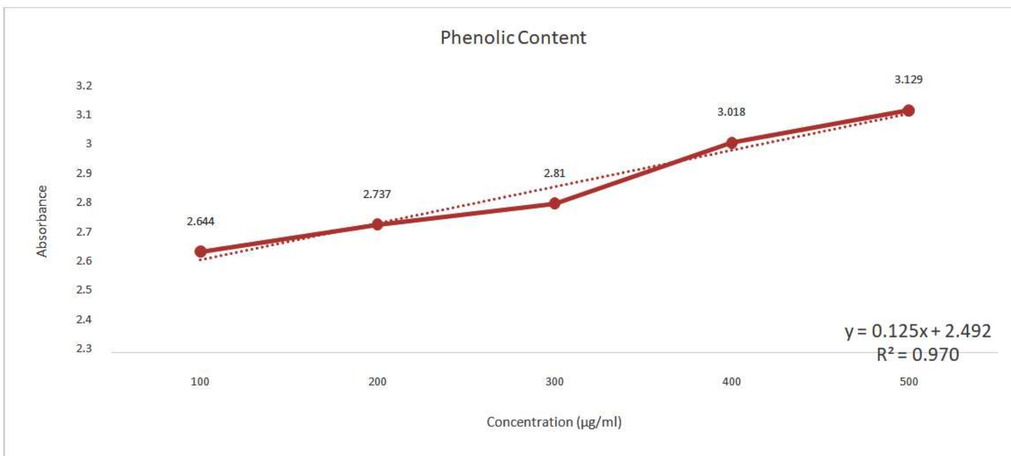
Table 2:Preliminary phytochemical screening of *Phyla nodiflora* ethanolic extract

S. No	Phytochemical analysis	Report
1.	Alkaloids	+
2.	Proteins	+
3.	Saponins	+
4.	Carbohydrates	+
5.	Amino Acids	+
6.	Steroids	+
7.	Terpenoids	+
8.	Tannins	+
9.	Flavonoids	+
10.	Anthraquinones	+
11.	Glycosides	+



Graph 1: The calibration curve of Gallic acid for determination of Phenol content in the *Phyla nodiflora* extract

value of 0.16 mg GAE/g, calculated using the regression equation y = 0.1251x + 2.49. The graph was illustrated in Graph 1. This finding underscores the significant presence of phenolic compounds in the sample, crucial for their antioxidant properties and potential health benefits

Total Tannin content

The tannin content estimation of the ethanolic extract of *Phyla nodiflora* revealed a value of 4.00 mg TE/g, derived from the regression equation y = 0.6495x + 0.7357. The graph was shown in Graph 2. This indicates a substantial presence of tannins in the sample, essential for various biological activities and applications.

Total Flavonoid content

The analysis of flavonoid content of the ethanolic extract of *Phyla nodiflora* resulted in a value of 0.749 mg QE/g, obtained from the regression equation y = 0.0997x + 0.0693. The graph was illustrated in Graph 3. This highlights the significant

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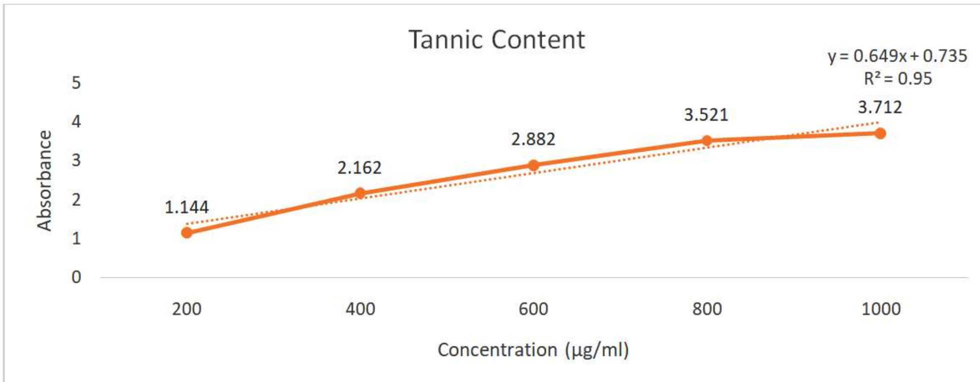
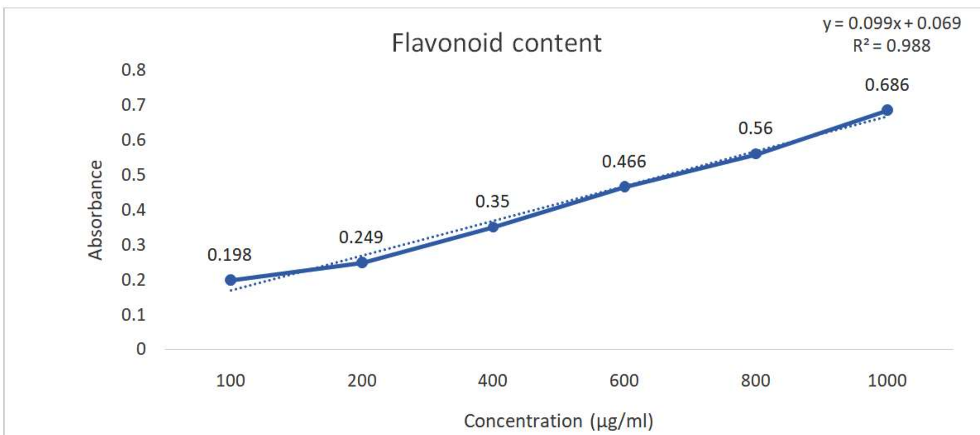


Graph 2: The calibration curve of Tannic acid for determination of Tannin content in the *Phyla nodiflora* extract



Graph 3: The calibration curve of Quercetin for determination of flavonoid content in the *Phyla nodiflora* extract

concentration of flavonoids in the sample, known for their diverse biological activities and health-promoting effects

Total Alkaloid Content

The significant alkaloid content was found to be 23.5% w/w in the ethanolic extract of *Phyla nodiflora* highlights its potential pharmacological importance**.**

Characterization of *Phyla nodiflora* Silver Nanoparticles (Pn-Ag-Np)

UV Spectroscopy

The spectrum's detected UV peak at 410 nm suggests the existence of silver nanoparticles Figure 1. This peak relates to silver nanoparticles' surface plasmon resonance (SPR) absorption feature, in which incoming light causes conduction electrons on the nanoparticle surface to oscillate collectively. Because of their size and shape, silver nanoparticles usually exhibit an SPR peak in the 400–450 nm range, which verifies the presence of silver nanoparticles in the solution.

Zeta size and zeta potential

The zeta size measurement of 76.53 nm suggests the average size of the silver nanoparticles in the solution. This parameter

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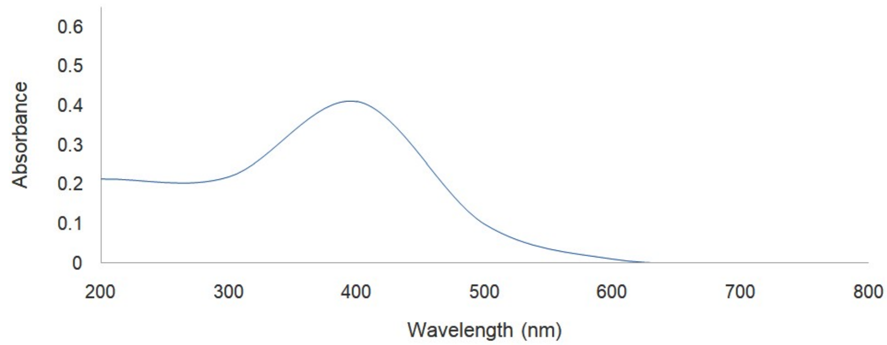

Figure 1: UV Spectroscopy of synthesis silver nanoparticle

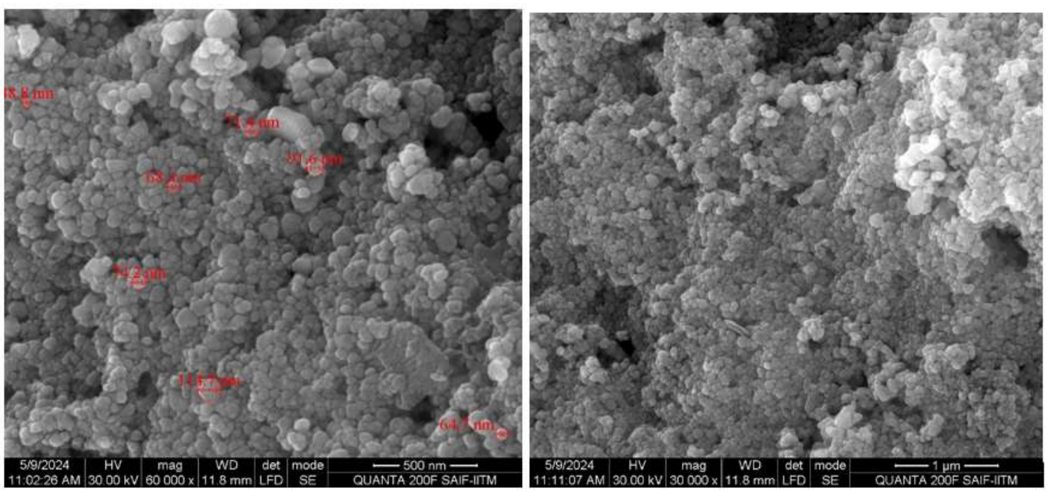

Figure 2: SEM analysis of synthesised Pn-Ag-Np

is crucial for understanding the physical dimensions of the nanoparticles. The negative zeta potential value of -30.72 mV indicates the surface charge of the nanoparticles. The high negative potential suggests good stability, as the nanoparticles repel each other, inhibiting aggregation.

SEM analysis

The SEM image, captured at 60,000x magnification, reveals the nanostructure of the silver nanoparticles synthesized from the ethanolic extract of Phyla nodiflora. The nanoparticles, with diameters ranging from 64.7 nm to 114.7 nm, are uniformly distributed within the matrix shown in figure 2. Analysis conducted using a QUANTA 200F at SAIF-IITM demonstrates the potential of this silver nanoparticles for wound healing applications due to its enhanced antimicrobial properties.

EDAX

The EDAX analysis of the synthesized nanoparticle indicates that the primary element present is silver (Ag), with a weight percentage (wt%) and atomic percentage (at%) both being 100% shown in Figure 3A. This suggests the nanoparticles are predominantly composed of silver, with

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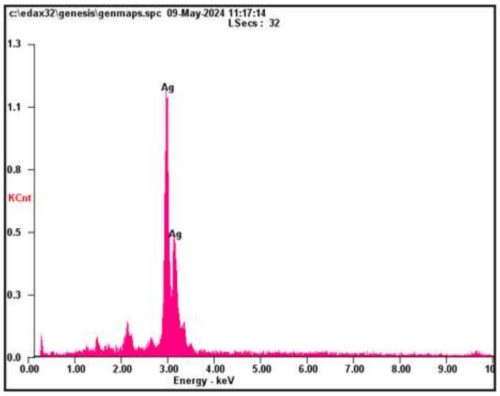

Element	Wt%	At%
AgL	100.00	100.00
Matrix	Correction	ZAF

Figure 3A: EDAX analysis of synthesised Pn-Ag-Np

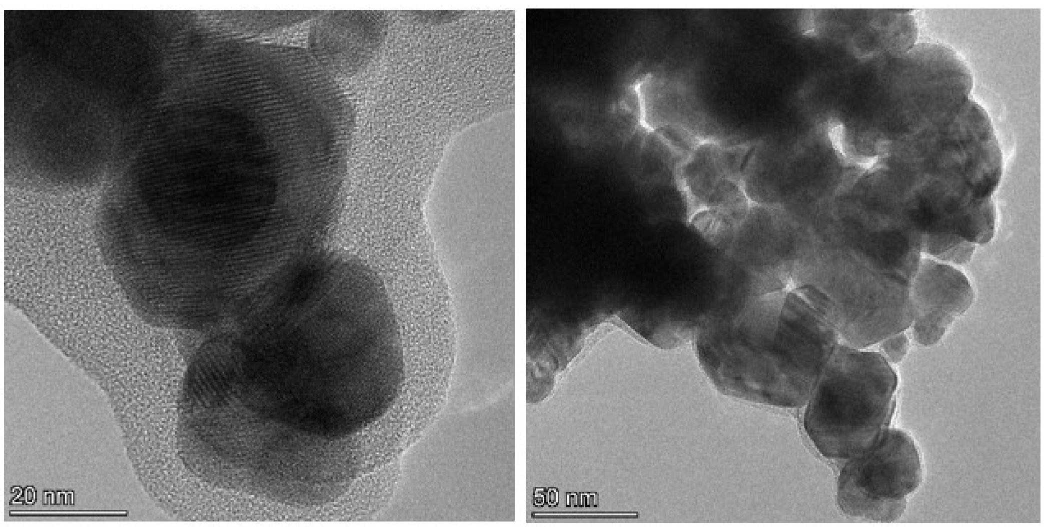

Figure 3B: HRTEM analysis of synthesised Pn-Ag-Np

no significant impurities detected. The matrix weight percentage correction and At% ZAF values further confirm the purity and elemental composition, ensuring the accuracy of the elemental analysis

HRTEM

TEM analysis of the silver nanoparticles synthesized from the ethanolic extract of Phyla nodiflora shows well nanostructures within the matrix. The high-resolution images, taken at scales of 50 nm and 20 nm, confirm the successful incorporation and uniform dispersion of the nanoparticles shown in Figure 3B nanostructures are critical for the wound healing offering improved antimicrobial activity and biocompatibility.

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Antioxidant Activity of Pn-Ag-Np

DPPH Assay for synthesisedPn-Ag-Np

The DPPH assay results indicate that the synthesized nanoparticles exhibit strong antioxidant activity, which increases with concentration. The test sample's scavenging activity, reaching 95.5% at 1000 μg/mL, reflects significant free radical neutralization capability shown in Graph 4. This concentration-dependent antioxidant activity makes these nanoparticles suitable for applications in pharmaceuticals and cosmetics where oxidative damage prevention is essential. The slight variations between the test and standard values are within acceptable ranges, confirming the nanoparticles' effectiveness.

Nitric Oxide scavenging of synthesisedPn-Ag-Np

The synthesized nanoparticles demonstrated notable NO scavenging activity, with 77.89% inhibition at 100 μg/mL and 98.59% at 1000 μg/mL shown in Graph 5. These high levels of activity are beneficial for medical applications aimed at controlling inflammation and oxidative stress. The potent NO scavenging properties suggest potential use in developing anti-inflammatory drugs and treatments for conditions associated with excessive NO production.

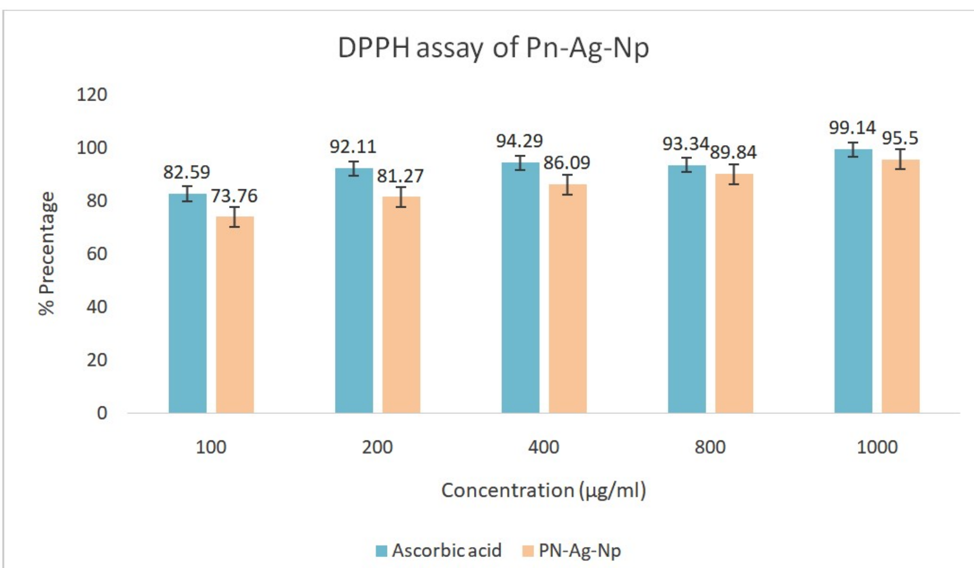
Evaluation parameter of formulated*Phyla nodiflora* silver nano loaded Hydrogel (Pn-Ag-Hg)

pH

The pH of hydrogel formulations is crucial as it affects the stability and compatibility with the skin. Formulation F4 exhibited a pH of 7.0 ± 0.1, which falls within the acceptable range for topical applications. The slightly higher pH can enhance the solubility of the active ingredients and promote better absorption into the skin. The results in Table 3 demonstrate that F4 maintained a consistent pH level, indicating stability and suitability for dermatological use.

Viscosity

Viscosity plays a critical role in the spreadability and application characteristics of hydrogels. Formulation F4 demonstrated a



Graph 4: DPPH assay of Pn-Ag-Np

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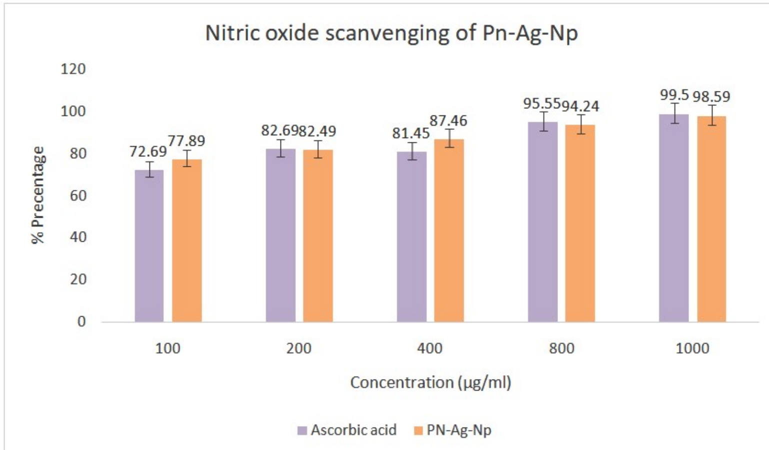

Graph 5: Nitric oxide scavenging of Pn-Ag-Np

Table 3: Physicochemical evaluation of the Pn-Ag-Hg for various trials

Parameter	F1	F2	F3	F4	F5	F6	F7	F8
pH	6.8 ± 0.1	6.9 ± 0.1	6.9 ± 0.1	7.0 ± 0.1	6.7 ± 0.1	6.8 ± 0.1	6.8 ± 0.1	6.9 ± 0.1
Viscosity (cP)	1500 ±50	1520 ±55	1530 ± 60	1550 ±65	1600± 70	1620 ± 75	1640 ± 80	1660 ± 85
Homogeneity	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Spreadability (g cm/s)	20 ± 1	21 ± 1	22 ± 1	23 ± 1	18 ± 1	19 ± 1	20 ± 1	21 ± 1
Drug Content (%)	95 ± 2	96 ± 2	97 ± 2	98 ± 2	94 ± 2	95 ± 2	96 ± 2	97 ± 2

viscosity of 1550 ± 65 cP, which is suitable for easy application and adherence to the skin surface. The viscosity values across formulations F1 to F8 Table 3 show that F4 falls within the desired range, ensuring optimal handling and effectiveness during application.

Homogeneity

Homogeneity is essential to ensure uniform distribution of active ingredients within the hydrogel matrix. All formulations, including F4, exhibited uniform consistency without visible aggregates or phase separation, the results are shown in Table 3. This uniformity indicates that F4 can deliver consistent dosing and efficacy, crucial for therapeutic applications.

Spreadability

Spreadability influences patient compliance and ease of application. Formulation F4 demonstrated a spreadability of 23 ± 1 g cm/s, indicating good glide over the skin surface. The results in Table 3 show that F4 achieved superior spreadability compared to other formulations, suggesting enhanced user experience and better coverage of the affected area.

Drug Content

The drug content in hydrogels determines the amount of active ingredient available for therapeutic action. Formulation F4 exhibited a high drug content of 98 ± 2%, indicating efficient encapsulation and stability

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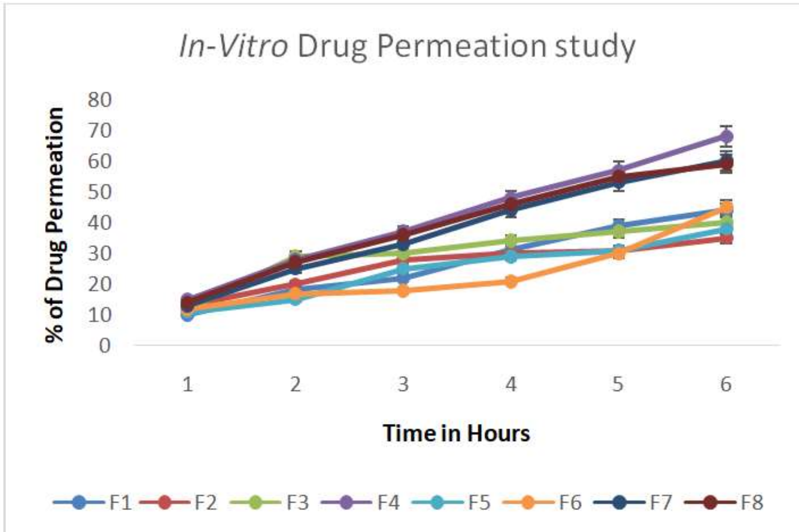

Graph 6: *In-vitro* drug permeation study for various formulation trials in 6 hours

of Pn-Ag-Np within the hydrogel matrix. The data presented in Table 3 highlight that F4 maintained consistent drug content, ensuring reliable and effective delivery of therapeutic agents.

Drug Release

The drug release profile is crucial for achieving therapeutic efficacy over time. Formulation F4 demonstrated a cumulative drug release of 78 ± 5% over the study period, indicating sustained release characteristicsshown in Table 3. The higher drug release observed in F4 suggests that it can provide prolonged therapeutic effects, which is beneficial for chronic wound management and healing acceleration.

***In-vitro* drug permeation study**

The drug release profile is crucial for achieving therapeutic efficacy over 6 hours. Formulation F4 demonstrated a cumulative drug permeation release of 68 ± 5% over the study period, indicating sustained release characteristics in Graph 6. The higher drug release observed in F4 hence it is choosed as the optimized formulation and also suggests that it can provide prolonged therapeutic effects, which is beneficial for chronic wound management and healing acceleration.

Table 4: Release kinetics of Optimized F4 formulation

Release Kinetic Model	r^2
Zero-order Kinetics	0.943
First-order Kinetics	0.975
Higuchi Model	0.986
Korsmeyer-Peppas Model	0.988
Hixson-Crowell Model	0.964

Release Kinetics for Optimized Formulation (F4)

The release kinetics of formulation F4 were analyzed using various mathematical models to understand the mechanism of drug release. The r^2 values for each model are summarized below Table 4:

Based on the r^2 values, the drug release from the optimized formulation F4 follows the Korsmeyer-Peppas model (0.988) and Higuchi model (0.986), indicating that the release mechanism is predominantly diffusion-controlled.

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In- vitro cell line study using NHDF cell lines for the optimized F4 formulation

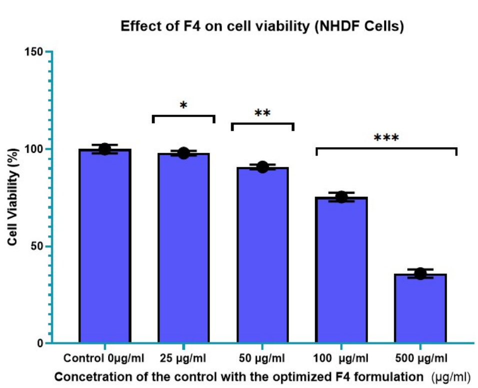
MTT assay for the optimized F4 formulation

The cytotoxicity of the optimized Pn Ag-Hg hydrogel formulation (F4) was assessed using the MTT assay on NHDF cells, as depicted in Graph 7. The control group (0 µg/mL) exhibited a cell viability of 100% ± 5%. At a concentration of 25 µg/mL, cell viability slightly decreased to 95% ± 4%, with a statistically significant reduction compared to the control (p = 0.02). Increasing the concentration to 50 µg/mL further reduced cell viability to 90% ± 6%, with a higher significance level (p = 0.01). A more pronounced cytotoxic effect was observed at 100 µg/mL, where cell viability decreased to 80% ± 7% (p = 0.001). The highest concentration of 500 µg/mL resulted in a substantial drop in cell viability to 40% ± 10%, with a highly significant difference compared to the control (p < 0.001). These findings indicate a clear dose-dependent cytotoxic effect of the F4 formulation, which is critical for determining its safe therapeutic range.

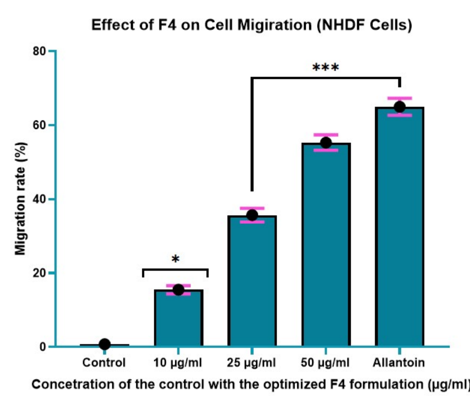
In-vitro Wound Scratch Assay for the optimized F4 formulation

The scratch wound healing assay assessed the wound closure capability of NHDF cells treated with various concentrations of the F4 formulation are shown in Figure 4 and Graph 8. Figure 4 illustrates the wound closure at o hours and 24 hours post-treatment. Control cells showed minimal wound closure after 24 hours. In contrast, treatment with F4 at 10 µg/mL, 25 µg/mL, and 50 µg/mL resulted in dose-dependent increases in wound closure. In graph 8 the control group (0 µg/mL) had a baseline migration rate of 0% ± 1%. Treatment with 10 µg/mL of F4 increased the migration rate to 15% ± 3%, with a statistically significant difference compared to the control (p = 0.05). A further increase in concentration to 25 µg/mL resulted in a migration rate of 30% ± 4%, showing a highly significant difference from the control (p = 0.001). At 50 µg/mL, the migration rate rose significantly to 50% ± 5% (p < 0.001). The positive control, Allantoin, produced a migration rate of 60% ± 3%, which was also highly significant compared to the control (p < 0.001). These results demonstrate that F4 enhances cell migration in a dose-dependent manner, suggesting its potential utility in wound healing and tissue regeneration applications.

The research effectively showcased the formulation of a phytopharmaceutical hydrogel that integrated silver nanoparticles from *Phyla nodiflora* that were green synthesized. The thorough phytochemical



Graph 7: Effect of F4 on cell viability



Graph 8: Effect of F4 on Cell Migration (NHDF Cells)

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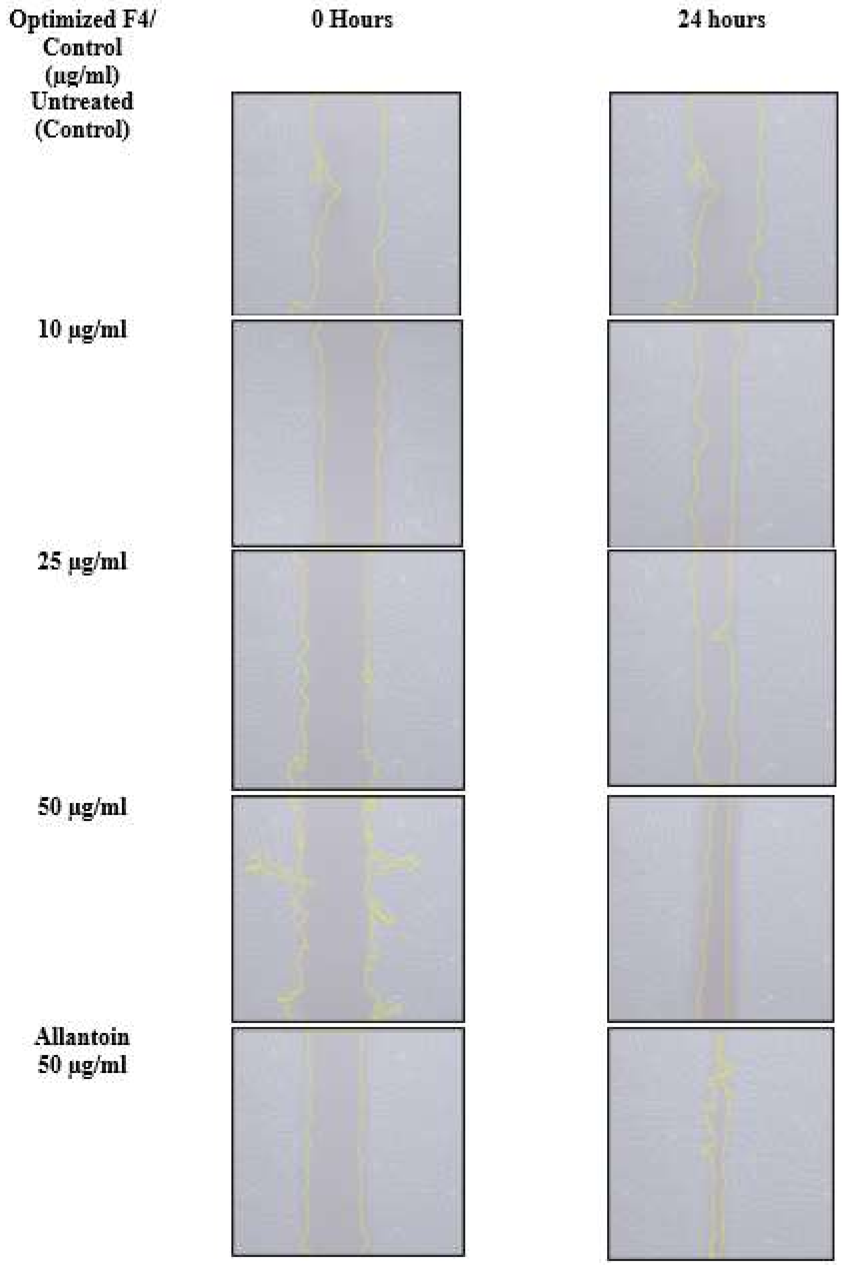

Figure 4: The *In-vitro* wound scratch assay using NHDF Cells

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investigation verified the existence of bioactive substances, which are essential for the hydrogel's medicinal potential. The nanoparticles' proper size, stability, and shape were revealed by characterization, which guaranteed their effectiveness in the hydrogel matrix. Assessing the hydrogel's physicochemical characteristics and conducting *In-vitro* studies validated its appropriateness for wound healing purposes, especially in the treatment of persistent wounds.

Conclusion

Promising outcomes in wound healing have been observed with the hydrogel matrix incorporating *Phyla nodiflora* silver nanoparticles. The anti-inflammatory and antioxidant characteristics of the ethanolic extract promote wound healing. The hydrogel shows promise as a viable alternative for treating chronic wounds, and its environmentally friendly manufacturing further augments its therapeutic potential.

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Conflict of Interest

The corresponding author states that there is no conflict of interest

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