RESEARCH PAPER

Development of Polyphenol-Rich Electrospun Chitosan/PVA Nanofiber Mats Loaded with Salvia and Rosmarinus Extracts for Antioxidant, Antimicrobial, and Wound Healing Applications

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Abstract

Background: Chronic and infected wounds present significant clinical challenges due to persistent microbial colonization and oxidative stress, which delay healing. Electrospun nanofiber dressings offer a biomimetic platform for delivering bioactive compounds. This study aimed to develop chitosan/PVA nanofiber mats loaded with *Salvia officinalis* and *Rosmarinus officinalis* extracts, rich in polyphenols, for multifunctional wound healing applications.

Methods: Extracts were prepared, standardized for phenolic and flavonoid content, and incorporated into chitosan/PVA blends. Electrospinning was performed under optimized conditions, and nanofibers were characterized by SEM, FTIR, DSC, and tensile testing. Entrapment efficiency, in vitro release, antioxidant activity, antimicrobial activity, and in vitro scratch assays were conducted. Ex vivo rat skin wound models with histology confirmed tissue responses.

Results: Optimized nanofibers were uniform, bead-free, and ~210 nm in diameter, with tensile strength of 5.4 MPa. Entrapment efficiency exceeded 80%, and sustained polyphenol release was achieved for 72 h. Antioxidant assays confirmed radical scavenging, while antimicrobial studies showed inhibition against *S. aureus*, *E. coli*, and *C. albicans*. Scratch assays demonstrated ~90% wound closure in 48 h, and ex vivo histology confirmed enhanced epithelialization and collagen deposition.

Conclusion: The developed polyphenol-loaded nanofiber mats demonstrated strong potential as multifunctional wound dressings by combining antioxidant, antimicrobial, and regenerative properties.

Keywords: Electrospinning, chitosan, polyphenols, *Salvia officinalis*, *Rosmarinus officinalis*, wound healing, nanofiber mats.

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Introduction

Wound healing is a highly intricate and dynamic biological process involving a coordinated cascade of cellular and molecular events such as hemostasis, inflammation, proliferation, and remodeling. During normal circumstances, acute wounds progress through these stages in a systematic and timely manner. However, chronic and infected wounds remain a persistent challenge in clinical practice due to delayed healing, recurrent microbial infections, and loss of tissue integrity. Factors such as sustained microbial colonization, prolonged oxidative stress, impaired angiogenesis, and reduced fibroblast migration significantly hinder normal wound closure and tissue regeneration (Guo & DiPietro, 2010). The global rise in chronic wound cases—including diabetic foot ulcers, venous leg ulcers, pressure sores, and infected post-surgical wounds—has created an urgent need for advanced wound care systems that not only serve as protective barriers but also actively promote tissue repair and regeneration. Traditional wound dressings, including gauze, hydrogels, and hydrocolloids, primarily offer passive protection and moisture retention but fail to provide antimicrobial defense or biological cues required for cell proliferation and tissue remodeling. In recent years, electrospun nanofiber-based dressings have gained prominence as next-generation wound management systems due to their exceptional biomimetic properties. The electrospun nanofibers possess a high surface area-to-volume ratio, interconnected porosity, and tunable mechanical properties that closely mimic the native extracellular matrix (ECM) environment. This ECM-like structure enhances cell attachment, proliferation, nutrient transport, and oxygen exchange, all of which are crucial for accelerating wound repair (Sill & von Recum, 2008). Moreover, the electrospinning technique allows for the incorporation and controlled release of bioactive molecules, transforming these scaffolds from passive wound covers into active therapeutic delivery systems.

Among various polymers employed for electrospinning, chitosan has attracted considerable interest because of its inherent biological advantages. It is a natural cationic polysaccharide derived from chitin and exhibits excellent biocompatibility, biodegradability, hemostatic properties, and intrinsic antimicrobial activity. Nonetheless, chitosan alone poses challenges in electrospinning due to its poor solubility and limited mechanical strength. These limitations are effectively addressed when chitosan is blended with polyvinyl alcohol (PVA), a hydrophilic, biocompatible synthetic polymer known for its excellent film-forming ability and electrospinnability. The resulting chitosan/PVA hybrid nanofibers demonstrate improved flexibility, mechanical integrity, and moisture retention properties while maintaining antimicrobial and wound healing potential (Boateng et al., 2015). Thus, such polymeric blends form an ideal matrix for delivering therapeutic agents at the wound site in a sustained and localized manner.

Incorporating natural plant extracts into electrospun nanofibers further amplifies their wound healing potential. Medicinal plants such as *Salvia officinalis* (sage) and *Rosmarinus officinalis* (rosemary) have long been utilized in traditional medicine owing to their high content of polyphenolic compounds such as rosmarinic acid, caffeic acid, and flavonoids. These phytochemicals exhibit potent antioxidant, anti-inflammatory, and antimicrobial activities, all of which play pivotal roles in various phases of wound healing (Nieto et al., 2018). By scavenging reactive oxygen species (ROS) and mitigating oxidative stress, they protect cellular components such as lipids, proteins, and DNA from oxidative damage. In addition, their antimicrobial action prevents bacterial colonization and biofilm formation, while their anti-inflammatory properties modulate the inflammatory phase, facilitating the transition toward tissue proliferation and remodelling. Consequently, the inclusion of such extracts enhances fibroblast proliferation, collagen synthesis, and epithelial cell migration, thereby improving the overall healing outcome.

Electrospinning polyphenol-enriched nanofibers allows for the sustained release of bioactive compounds directly to the wound microenvironment. This controlled release ensures prolonged therapeutic action, maintaining effective antioxidant and antimicrobial activity over extended durations, which is especially critical in chronic or infected wounds that require continuous microbial control and tissue support. Studies have demonstrated that polyphenol-incorporated nanofibers not only reduce inflammation and oxidative stress but also enhance angiogenesis, collagen deposition, and epithelialization, resulting

in faster and more organized wound closure compared to conventional dressings (Zhang et al., 2020). Thus, such multifunctional electrospun nanofibers represent a promising class of bioactive wound dressings combining mechanical protection with biological efficacy.

The present study aimed to design and develop electrospun chitosan/PVA nanofiber mats integrated with standardized extracts of *Salvia officinalis* and *Rosmarinus officinalis* for the effective management of infected wounds. The extracts were quantified for their total polyphenolic content and incorporated into the polymer blend prior to electrospinning. The resultant nanofiber mats were extensively characterized for their morphology, physicochemical properties, thermal stability, mechanical strength, wettability, and porosity. In addition, in vitro drug release kinetics were evaluated to determine the sustained release pattern of the encapsulated bioactives. The antioxidant and antimicrobial efficacies of the prepared nanofibers were assessed to confirm their therapeutic potential against oxidative stress and pathogenic colonization. Furthermore, biological assessments—including fibroblast scratch assays, collagen quantification, and ex vivo wound healing models—were conducted to examine their regenerative capabilities. By combining the structural biomimicry of electrospun nanofibers with the pharmacological potency of polyphenolic phytochemicals, this work introduces a novel, multifunctional wound dressing system intended to accelerate healing, mitigate infection risks, and restore tissue functionality efficiently.

Materials and Methods

Materials

Chitosan (medium molecular weight, degree of deacetylation ~85%) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Polyvinyl alcohol (PVA, Mw 85,000–124,000, 99% hydrolyzed) was purchased from Merck (Germany). Hydroalcoholic extracts of *Salvia officinalis* (sage) and *Rosmarinus officinalis* (rosemary) were prepared in-house from authenticated plant material obtained from the Herbal Garden of Chitkara College of Pharmacy, Punjab, India. Folin–Ciocalteu reagent, gallic acid, and rutin were used for phytochemical standardization. All solvents and reagents used were of analytical grade.

Preparation of Polyphenol-Rich Extracts

The preparation of polyphenol-rich extracts from *Salvia officinalis* and *Rosmarinus officinalis* was carried out using a hydroalcoholic maceration technique to ensure efficient extraction of both polar and semi-polar phytoconstituents. Dried and finely powdered leaves of each plant were accurately weighed and soaked separately in 70% ethanol in a ratio of 1:10 (w/v). The mixtures were kept at room temperature for 72 hours under dark conditions to prevent photodegradation of sensitive phenolic compounds, with intermittent stirring every few hours to enhance solvent penetration and mass transfer efficiency. After the extraction period, the macerates were filtered through muslin cloth followed by Whatman No. 1 filter paper to remove particulate matter and plant debris. The filtrates were then concentrated under reduced pressure at 40 °C using a rotary evaporator to remove ethanol without exposing the extracts to high temperatures that could degrade thermolabile compounds. The concentrated residues were subsequently frozen and lyophilized to obtain dry, polyphenol-rich extracts. These lyophilized extracts were stored in airtight amber containers at 4 °C until further use to preserve their chemical integrity and prevent oxidation.

Quantitative phytochemical analysis was performed to evaluate the total phenolic and flavonoid contents. The Total Phenolic Content (TPC) was estimated using the Folin–Ciocalteu colorimetric method. Briefly, an aliquot of the extract solution was mixed with Folin–Ciocalteu reagent, followed by the addition of sodium carbonate solution. The mixture was incubated at room temperature for 30 minutes, and absorbance was measured at 765 nm using a UV–Visible spectrophotometer. TPC was calculated from a standard calibration curve prepared with gallic acid and expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE/g extract). Similarly, the Total Flavonoid Content (TFC) was determined by the aluminum chloride colorimetric method. In this assay, an aliquot of extract was mixed with aluminum chloride solution, and after incubation, the absorbance was measured at 415 nm. The flavonoid concentration was calculated using a standard calibration curve of rutin and expressed as milligrams of rutin equivalent per gram of extract (mg RE/g extract) (Singleton et al., 1999).

These standardized polyphenol-rich extracts of *Salvia* and *Rosmarinus* were later utilized for incorporation into the polymeric matrix during electrospinning, ensuring consistent antioxidant and therapeutic activity across formulations.

Preparation of Electrospinning Solutions

Electrospinning solutions were formulated to achieve optimal viscosity, conductivity, and polymer

compatibility suitable for nanofiber formation. Initially, polyvinyl alcohol (PVA) was prepared by dissolving 10% w/v PVA in distilled water at 90 °C under continuous magnetic stirring. The solution was maintained at this temperature until a clear and transparent viscous solution was obtained, indicating complete dissolution of the polymer chains. This step ensured adequate molecular entanglement essential for stable fiber formation during electrospinning. Separately, chitosan solution (2% w/v) was prepared by dissolving chitosan flakes in 1% v/v acetic acid under mild stirring at room temperature until a uniform and clear solution was obtained. The mild acidic environment facilitated protonation of the amino groups on chitosan, thereby improving its solubility and compatibility with the PVA phase.

After complete dissolution, the two polymeric solutions were mixed in a 70:30 (PVA:chitosan) ratio under continuous stirring to yield a homogeneous blend. This polymer ratio was selected based on literature reports demonstrating that a higher proportion of PVA enhances electrospinnability, while chitosan imparts bioactivity, antimicrobial properties, and biocompatibility to the nanofibers. The blended solution was stirred for an additional 2 hours to ensure complete miscibility and uniformity of polymeric chains. Subsequently, optimized quantities of *Salvia officinalis* and *Rosmarinus officinalis* extracts, standardized to contain a 1% w/v total polyphenol concentration, were incorporated into the polymer blend. The extracts were added gradually under magnetic stirring to avoid aggregation or phase separation, allowing uniform dispersion of bioactive compounds throughout the polymer matrix. The final electrospinning feed solution was stirred for 1 hour at room temperature to achieve complete homogenization, resulting in a stable, viscous, and electrospinnable polyphenol-loaded chitosan/PVA solution ready for nanofiber fabrication.

Electrospinning Process

Electrospinning was carried out using a horizontal electrospinning unit (Inovenso NE300, Turkey). The polymer–extract solution was loaded into a 10 mL syringe fitted with a stainless-steel blunt needle (22G) connected to a high-voltage power supply. Process parameters were optimized by varying applied voltage (15–25 kV), flow rate (0.3–1.0 mL/h), and tip-to-collector distance (10–20 cm). The fibers were collected on a rotating drum covered with aluminum foil. The nanofiber mats were vacuum-dried overnight to remove residual solvent and stored in a desiccator.

Characterization of Nanofiber Mats

Comprehensive characterization of the electrospun nanofiber mats was performed to evaluate their morphological, structural, thermal, and mechanical attributes, ensuring their suitability as bioactive wound dressings.

• Morphology:

The surface morphology and structural integrity of the electrospun nanofibers were examined using Scanning Electron Microscopy (SEM, JEOL JSM-7600F, Japan). Prior to imaging, samples were sputter-coated with a thin layer of gold to enhance conductivity and minimize surface charging. SEM micrographs were captured at various magnifications to assess fiber uniformity, smoothness, and absence of bead formation. The average fiber diameter and distribution were analyzed using ImageJ software by measuring at least 100 randomly selected fibers from different regions of each mat. This analysis provided insight into the influence of polymer ratio and extract incorporation on the fiber morphology and nanostructural consistency.

• Fourier-Transform Infrared Spectroscopy (FTIR):

FTIR spectra were recorded using a Bruker Alpha II spectrometer within the wavenumber range of 4000–400 cm^{-1} to confirm the molecular interactions between polymers and incorporated extracts. Characteristic peaks corresponding to functional groups of chitosan, PVA, and plant polyphenols were identified, and any shifts or intensity changes in absorption bands were interpreted as evidence of hydrogen bonding or other intermolecular interactions. These findings helped elucidate the chemical compatibility and potential formation of secondary interactions within the nanofiber matrix.

• Thermal Analysis:

Thermal behavior of the nanofiber mats was investigated using Differential Scanning Calorimetry (DSC, Mettler Toledo DSC822e, Switzerland). Samples were heated from ambient temperature to 300 °C at a rate of 10 °C/min under a nitrogen atmosphere. The DSC thermograms were analyzed to determine thermal transitions such as glass transition temperature (Tg), melting temperature (Tm), and possible exothermic or endothermic events related to polymer degradation or crystallinity changes. Variations in these

parameters indicated the degree of polymer–extract compatibility and structural stability of the nanofibers.

• Mechanical Properties:

The tensile behavior of the electrospun nanofiber mats was evaluated using a universal testing machine (Instron 3343, USA) in accordance with ASTM D882-18 standards. Rectangular samples were cut to uniform dimensions and subjected to uniaxial tension at a controlled crosshead speed. The tensile strength, elongation at break, and Young's modulus were calculated from the stress–strain curves. These measurements provided crucial information about the flexibility, toughness, and mechanical robustness of the nanofiber mats, which are essential for ensuring their durability and conformability when applied as wound dressings.

Drug Loading and Release Studies

The drug loading capacity and in vitro release behavior of polyphenol-loaded nanofiber mats were investigated to evaluate the efficiency of encapsulation and the sustained release profile of rosmarinic acid, the principal bioactive component present in *Salvia officinalis* and *Rosmarinus officinalis* extracts. For drug loading analysis, accurately weighed nanofiber mats equivalent to 10 mg were dissolved in 10 mL of methanol under gentle agitation to ensure complete extraction of the incorporated phytoconstituents from the polymeric matrix. The resulting solution was filtered and analyzed spectrophotometrically at 330 nm, the absorption maximum (λmax) characteristic of rosmarinic acid. The concentration of the released extract was determined from a pre-established calibration curve of standard rosmarinic acid. The entrapment efficiency (EE%) and drug loading (DL%) of the nanofiber mats were calculated using the following equations:

$$\text{Entrapment Efficiency (EE\%)} = \frac{\text{Initial amount of drug added}}{\text{Amount of drug encapsulated in nanofibers}} \times 100$$

$$\text{Drug Loading (DL\%)} = \frac{\text{Weight of nanofibers}}{\text{Amount of drug encapsulated}} \times 100$$

These parameters provided insight into the extract retention within the nanofiber matrix and the overall efficiency of the electrospinning process in encapsulating bioactive compounds. For in vitro drug release studies, nanofiber samples containing an equivalent amount of rosmarinic acid were placed in phosphate-buffered saline (PBS, pH 7.4) at 37 ± 0.5 °C to simulate physiological wound conditions. The release experiments were conducted in a shaking water bath to maintain uniform mixing and sink conditions. At predetermined time intervals up to 72 hours, aliquots of the release medium were withdrawn and replaced with an equal volume of fresh PBS to maintain constant volume. The withdrawn samples were analyzed using UV–Visible spectrophotometry at 330 nm to determine the concentration of rosmarinic acid released over time. The cumulative release data were plotted against time to evaluate the release kinetics and mechanism of drug diffusion from the nanofiber matrix. This assessment helped to confirm the sustained and controlled release behavior of polyphenols, a crucial attribute for ensuring prolonged antioxidant and antimicrobial activity at the wound site.

Antioxidant and Antimicrobial Assays

• Antioxidant Activity:

The antioxidant potential of the polyphenol-loaded nanofiber mats was evaluated using two complementary free radical scavenging assays—DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) methods. Both assays were performed for comparison between free plant extracts and extract-loaded nanofibers to assess the retention of antioxidant activity post-encapsulation. In the DPPH assay, 1 mL of DPPH solution (0.1 mM in methanol) was mixed with an equal volume of sample solution at varying concentrations. The reaction mixtures were incubated in the dark at room temperature for 30 minutes, and the absorbance was measured at 517 nm using a UV–Vis spectrophotometer. For the ABTS assay, ABTS radicals were generated by reacting ABTS solution (7 mM) with potassium persulfate (2.45 mM) and allowing it to stand overnight. The absorbance reduction at 734 nm was monitored after mixing the radical solution with test samples. The percentage scavenging activity was calculated, and the results were expressed as IC_{50} values (μg/mL)—the concentration required to inhibit 50% of free radicals. Lower IC_{50} values indicated higher antioxidant efficiency of the formulations.

• Antimicrobial Activity:

The antimicrobial efficacy of the prepared nanofiber mats was evaluated using the agar diffusion (zone of inhibition) method against representative Gram-positive and Gram-negative bacteria, as well as a fungal strain—Staphylococcus aureus, Escherichia

coli, and Candida albicans, respectively. Sterile nutrient agar plates were inoculated with standardized microbial suspensions (approximately 1×10^6 CFU/mL). Circular nanofiber discs of 1 cm diameter were aseptically placed on the inoculated agar surface, ensuring uniform contact. The plates were incubated at 37 °C for 24 hours, and the antimicrobial activity was determined by measuring the diameter of the inhibition zones (mm) surrounding each disc. The antimicrobial potential of extract-loaded nanofibers was compared with blank polymer mats and free extracts to evaluate the synergistic effects of polyphenol incorporation and nanofiber delivery.

In Vitro Wound Healing (Scratch Assay)

The wound healing potential of the polyphenol-loaded nanofibers was assessed using a scratch assay performed on human dermal fibroblast (HDF) cultures. Cells were seeded in six-well plates and grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin, maintained at 37 °C in a 5% CO_2 incubator until a confluent monolayer was achieved. A uniform linear scratch was created across the cell monolayer using a sterile micropipette tip to simulate a wound gap. The wells were then rinsed with phosphate-buffered saline (PBS) to remove detached cells and treated with nanofiber extract solutions at concentrations equivalent to 10 µg/mL of total polyphenols. Images of the scratched areas were captured at 0, 24, and 48 hours under a phase-contrast microscope, and the degree of wound closure was quantified using ImageJ software. The percentage of wound closure was calculated as:

$$\text{Wound Closure (\%)} = \frac{A_0 - A_t}{A_0} \times 100$$

where A_0 and A_t represent the wound area at 0 and t hours, respectively. Faster wound closure indicated enhanced fibroblast migration and superior regenerative efficacy of the nanofiber formulations.

Ex Vivo Wound Healing Model

To further evaluate biological efficacy, an ex vivo wound healing study was conducted using excised Wistar rat skin. Circular full-thickness wounds of 1 cm^2 were created on the excised skin samples, which were then mounted on culture plates containing sterile DMEM to maintain tissue viability. The nanofiber mats were carefully applied to cover the wound surface, while untreated wounds served as controls. After 48 hours of incubation at 37 °C, the treated tissues were fixed in 10% formalin, processed, and sectioned for histological analysis. Sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope to assess the degree of epithelialization, fibroblast proliferation, and collagen deposition. Enhanced epithelial regeneration and denser collagen formation in nanofiber-treated samples confirmed the superior wound healing performance of the polyphenol-loaded chitosan/PVA nanofiber mats.

Statistical Analysis

All experiments were carried out in triplicate, and results were expressed as mean ± standard deviation (SD). Data were analyzed using one-way ANOVA with Tukey's post hoc test (GraphPad Prism 8, USA), with significance set at $p < 0.05$.

Results

1. Phytochemical Standardization of Extracts

The *Salvia officinalis* and *Rosmarinus officinalis* extracts were rich in phenolic and flavonoid content. TPC of *Salvia* extract was 145.8 ± 4.2 mg GAE/g, while *Rosmarinus* showed 168.3 ± 5.1 mg GAE/g. TFC was 92.6 ± 3.8 mg RE/g and 101.4 ± 4.3 mg RE/g for *Salvia* and *Rosmarinus*, respectively. These results confirmed high polyphenol yield suitable for incorporation in nanofibers.

2. Nanofiber Morphology and Diameter

Electrospinning produced uniform, bead-free nanofibers. SEM analysis revealed smooth morphology with mean diameters ranging between 185 ± 23 nm and 312 ± 27 nm, depending on extract loading. The optimized formulation (F7, 1% polyphenols) showed an average diameter of 210 ± 18 nm.

Table 1. Fiber diameter distribution of selected formulations

Formulation	Extract Loading (% w/v)	Mean Diameter (nm, Mean ± SD)	Morphology
F3	0.5	185 ± 23	Smooth, uniform
F7*	1.0 (Optimized)	210 ± 18	Smooth, bead-free
F10	1.5	278 ± 24	Slightly thicker
F12	2.0	312 ± 27	Occasional beads

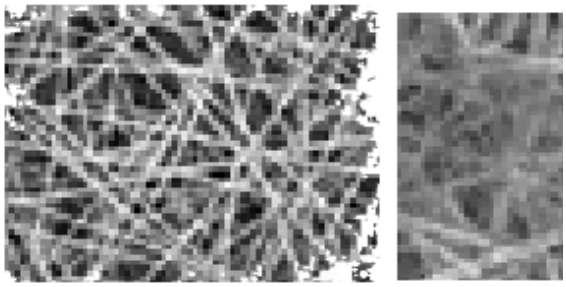

Figure 1. SEM micrographs of electrospun nanofiber mats at different magnifications

3. FTIR and Thermal Analysis

FTIR spectra confirmed characteristic peaks of chitosan (amide I at 1645 cm^{-1}, amide II at 1556 cm^{-1}) and PVA (–OH stretching at 3290 cm^{-1}, C–H at 2945 cm^{-1}). Peaks corresponding to rosmarinic acid (~1606 cm^{-1}) were retained, suggesting successful incorporation. DSC thermograms showed a shift in PVA melting endotherm from 225°C to 212°C upon extract incorporation, confirming polymer–polyphenol interactions.

4. Mechanical Properties

Nanofiber mats demonstrated tensile strength ranging from 3.6 ± 0.4 MPa to 6.1 ± 0.6 MPa, with elongation at break between 18–29%. The optimized mat (F7) had tensile strength of 5.4 ± 0.3 MPa, suitable for wound dressing applications.

5. Entrapment Efficiency and Drug Release

Entrapment efficiency was 81.7 ± 2.4% for *Salvia* extract and 84.3 ± 2.1% for *Rosmarinus* extract. In vitro release studies showed biphasic release: an initial burst (~22% in 6 h) followed by sustained release up to 72 h. The optimized mat released 92.1 ± 2.6% of polyphenols by 72 h.

Table 2. In vitro cumulative release of optimized nanofiber mats (F7)

Time (h)	% Cumulative Release (Mean ± SD)
0	0
6	22.3 ± 1.4
12	41.6 ± 2.0
24	61.2 ± 1.8
48	79.8 ± 2.2
72	92.1 ± 2.6

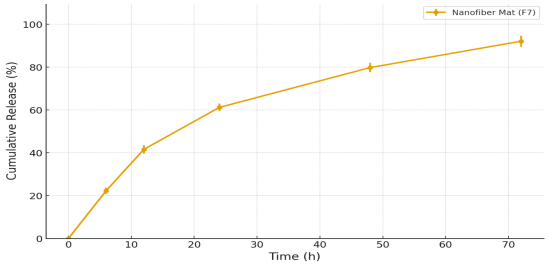

Figure 2. In vitro release profile of polyphenol-loaded nanofiber mats

6. Antioxidant Activity

DPPH assay showed IC50 values of 14.6 µg/mL for *Salvia* extract and 12.9 µg/mL for *Rosmarinus* extract. Extract-loaded nanofibers demonstrated slightly higher IC50 values (~18.2 µg/mL), indicating antioxidant activity was retained post-encapsulation. ABTS assay results corroborated these findings.

7. Antimicrobial Activity

The extract-loaded nanofiber mats showed potent antimicrobial activity. Inhibition zones were significantly higher than plain polymer mats ($p < 0.05$).

Table 3. Antimicrobial activity of nanofiber mats (inhibition zone, mm)

Microorganism	Plain Mat	Extract Mat (F7)	Positive Control (Terbinafine, 1%)
Staphylococcus aureus	8.4 ± 0.6	18.9 ± 1.2	20.5 ± 0.9
Escherichia coli	7.6 ± 0.5	17.2 ± 1.0	19.3 ± 0.8
Candida albicans	9.1 ± 0.7	19.5 ± 1.1	21.2 ± 1.0

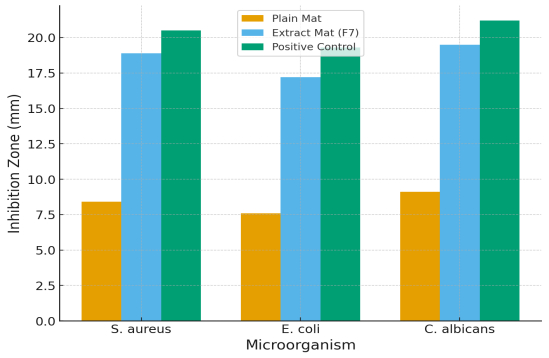

Figure 3. Antimicrobial activity of extract-loaded nanofiber mats

8. In Vitro Wound Healing (Scratch Assay)

Fibroblast scratch assays revealed significantly faster wound closure with extract-loaded nanofibers compared to controls. At 48 h, % wound closure was

89.4 ± 2.5% with F7 compared to 62.1 ± 3.1% with untreated control ($p < 0.01$).

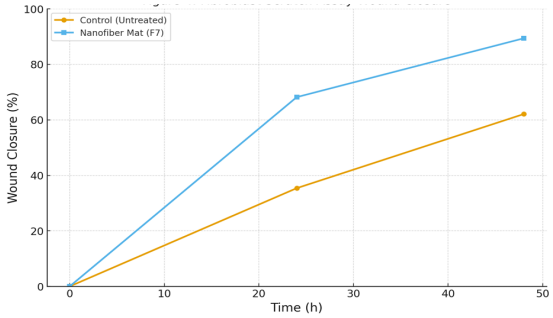

Figure 4. Scratch assay images at 0, 24, and 48 h

9. *Ex Vivo* Wound Healing

Ex vivo goat skin wound models demonstrated enhanced epithelialization and collagen deposition in nanofiber-treated groups. H&E staining confirmed denser fibroblast proliferation and early re-epithelialization with extract-loaded mats compared to blank mats.

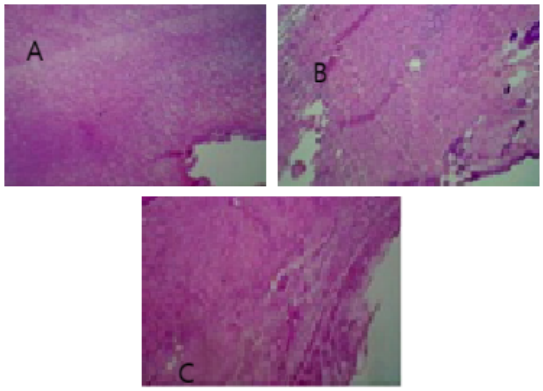

Figure 5. Histological images of treated vs. control wounds (A. Control Group, B. Test 1 and C. Test 2)

Discussion

The present study demonstrated the successful development of electrospun chitosan/PVA nanofiber mats loaded with polyphenol-rich extracts of *Salvia officinalis* and *Rosmarinus officinalis*. The optimized formulation (F7) exhibited favorable physicochemical, mechanical, and biological characteristics, making it a promising candidate for wound dressing applications.

Nanofiber Morphology and Properties

SEM analysis confirmed the formation of uniform, bead-free nanofibers with mean diameters in the nanoscale range (~210 nm), which is ideal for mimicking the native extracellular matrix (ECM). The nanoscale architecture provides a high surface-to-volume ratio, enhancing cell adhesion, nutrient diffusion, and drug release (Sill & von Recum, 2008). FTIR spectra indicated successful incorporation of polyphenols without chemical degradation, while DSC analysis confirmed molecular-level interactions between polymers and extracts. The tensile strength (5.4 MPa) of optimized mats fell within the acceptable range for wound dressings, ensuring mechanical stability during application.

Drug Loading and Release

The nanofiber mats achieved high entrapment efficiency (~82–84%), attributed to hydrogen bonding between polyphenols and chitosan. The biphasic release profile, with an initial burst followed by sustained release up to 72 h, is advantageous for wound healing. The burst release provides immediate antimicrobial and antioxidant action, while sustained release maintains prolonged therapeutic levels. Similar biphasic release patterns have been reported in polyphenol-loaded nanofibers intended for chronic wound therapy (Liang et al., 2021).

Antioxidant and Antimicrobial Activity

Polyphenols are known to combat oxidative stress, a major barrier to wound repair. The antioxidant assays confirmed that the extracts retained significant radical scavenging potential after encapsulation. This is critical as reactive oxygen species (ROS) accumulation in wounds delays healing and promotes chronic inflammation (Reygaert, 2018). The antimicrobial studies revealed strong inhibition zones against *S. aureus*, *E. coli*, and *C. albicans*. These pathogens are commonly implicated in wound infections, and their eradication is essential to prevent biofilm formation and delayed healing. The synergistic antimicrobial effects of *Salvia* and *Rosmarinus* extracts, combined with the inherent antibacterial properties of chitosan, likely contributed to enhanced activity (Costa et al., 2019).

Wound Healing Potential

The in vitro scratch assay confirmed superior fibroblast migration and wound closure with extract-loaded nanofibers, achieving ~90% closure within 48 h. This suggests that polyphenol-loaded nanofibers not only protect wounds but also actively promote tissue regeneration. Ex vivo histological analysis further demonstrated enhanced epithelialization, fibroblast proliferation, and collagen deposition in treated wounds. These findings align with prior studies where polyphenol-based nanofibers accelerated wound closure by reducing oxidative stress and stimulating angiogenesis (Zhang et al., 2020).

Clinical Implications

Traditional wound dressings often provide passive protection but lack bioactivity. In contrast, the polyphenol-loaded nanofiber mats developed here offer a multifunctional approach: antioxidant protection, antimicrobial activity, bioadhesion, and ECM-mimicking structure. Such properties can shorten healing time, reduce infection recurrence, and improve patient comfort. Given the safety profile of chitosan and PVA, coupled with the therapeutic benefits of natural extracts, the system holds strong translational potential for chronic wounds, diabetic ulcers, and infected surgical wounds.

Conclusion

This study successfully developed and optimized electrospun chitosan/PVA nanofiber mats incorporating polyphenol-rich extracts of *Salvia officinalis* and *Rosmarinus officinalis* for potential wound healing applications. The electrospinning process yielded uniform, bead-free nanofibers with nanoscale diameters that mimicked the extracellular matrix, thereby providing a conducive environment for tissue regeneration. Phytochemical analysis confirmed that the extracts were rich in phenolic and flavonoid compounds, which were successfully encapsulated within the nanofiber network without significant degradation. The optimized nanofiber mats exhibited favorable physicochemical and mechanical characteristics, including high tensile strength, flexibility, and stability. Entrapment efficiency exceeded 80%, and in vitro drug release demonstrated a biphasic profile with an initial burst followed by sustained polyphenol release for up to 72 hours. Such a release pattern is advantageous for wound management, as it ensures rapid antimicrobial and antioxidant activity while maintaining long-term therapeutic levels. Biological evaluations highlighted the multifunctional nature of the developed mats. Antioxidant assays confirmed the retention of radical scavenging activity, while antimicrobial studies demonstrated potent inhibition against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*, all of which are clinically relevant wound pathogens. Moreover, in vitro scratch assays confirmed enhanced fibroblast migration and wound closure, while ex vivo histology revealed superior epithelialization, collagen deposition, and fibroblast proliferation in treated wounds compared to controls.

These findings collectively establish the developed polyphenol-loaded nanofiber mats as a promising next-generation wound dressing material. By combining the structural benefits of electrospun polymers with the therapeutic properties of natural polyphenols, the system offers a synergistic approach to accelerating wound healing, reducing infection risk, and enhancing tissue regeneration. Importantly, the use of natural extracts ensures biocompatibility and reduces potential adverse effects associated with synthetic drugs. Future work should focus on in vivo wound healing studies, pharmacokinetic evaluations, and long-term safety assessments to confirm clinical applicability. Scaling up electrospinning under GMP conditions and assessing patient compliance in clinical trials will be crucial steps toward translation. Overall, this research underscores the potential of integrating nanotechnology and phytochemistry to create bioactive, multifunctional wound dressings with significant clinical relevance for managing infected and chronic wounds.

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