RESEARCH ARTICLE

Formulation and Evaluation of Electrospun Nanofiber Mats of Curcumin and Leaf Extract of *Tinospora cordifolia*: Designed for Accelerating Diabetic Wound Healing

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ABSTRACT

Traditional herbs like *Tinospora cordifolia* and curcumin have many therapeutic benefits. However, curcumin's limited bioavailability is a problem. Electrospun nanofibers are an answer because they increase the effective surface area greatly, and make drug delivery better in almost all aspects. The combination in the form of nanofibers can be greatly useful in the treatment of diabetic wound healing, where curcumin, the known antibacterial and anti-inflammatory element, plays an important role. It is observed that when combined with extract of leaves of *T. cordifolia,* which has known antidiabetic and wound healing actions, blended in the chitosan polymer, which is itself a weak antibacterial compound hence can be one of the solutions to the menace of bacterial resistance which is very common in diabetic wound healing. This research aims to create and test electrospun nanofiber mats of curcumin and *T. cordifolia* leaves extract blended in chitosan as a base polymer in the treatment of diabetic wound healing. Leaves of the plant *T. cordifolia,* which were collected from the institute's botanical garden, were subjected to drying and then prone to solvent extraction using methanol and acetone, resulting in the production of bioactive extracts. The curcumin was obtained as a gift sample from Sanjay Chemicals, Mumbai. Nanofibers were electrospun from chitosan, *T. cordifolia* leaf extract and curcumin. Characterization included scanning electron microscope (SEM), water contact angle measurement, TGA/DSC analysis, drug release studies, fourier-transform infrared (FTIR) analysis, swelling tests and *in-vitro* release profiling. The nanofiber mats of curcumin and leaves extract of *T. cordifolia* using chitosan as a base polymer were prepared and evaluated at various parameters. The results we found are very promising. The nanofibers formed by using electrospinning techniques show good content uniformity as well as good diameter which was checked using SEM. The study confirmed no component interactions using differential scanning calorimetry (DSC). The good hydrophilicity of mats was found to ensure good retention time. The study concluded with the formation of a novel formulation that can fight the menace of bacterial resistance, which is very common in the diabetic wound healing process. The formulation will ensure not only countering bacterial resistance but also the faster healing of diabetic wounds, as *T. cordifolia* leaves extract is known to improve angiogenesis and tissue remodeling.

Keywords: *Tinospora cordifolia*, Electrospinning, Curcumin, Chitosan nanofiber mats.

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INTRODUCTION

The utilization of plants for therapeutic purposes dates back to the inception of human civilization. Various traditional systems of medicine have employed medicinal plants to treat ailments. Herbal medicine is the major treatment for around 75 to 80% of individuals in numerous underdeveloped nations because of its superior cultural acceptability, compatibility with the human body, and fewer negative effects.[1]

Tinospora cordifolia, a member of the Menispermaceae family, is a precious plant in terms of its components and pharmacology. It is generally referred to as guduchi in Sanskrit. It possesses significant therapeutic properties in all components, including leaves, stems, and roots. Additionally, *Tinospora* has been identified as possessing antipyretic, antispasmodic, anti-inflammatory, immunomodulatory, and anticomplementary properties. Furthermore, it has been observed that *Tinospora* possesses hepatoprotective, antidiabetic, antioxidant, and anticancer activities. The stem, root, and leaf extracts of the plant have been observed to possess antimicrobial properties against pathogenic microbes.[2,3]

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Curcumin, an active constituent present in turmeric, exhibits advantageous properties in the treatment of inflammatory illnesses, metabolic syndrome, pain, and kidney dysfunction, as well as the management of inflammatory and degenerative ocular disorders. The antioxidant activity of this substance is demonstrated through its ability to scavenge free radicals, modulate enzymes responsible for neutralizing free radicals, and inhibit enzymes that generate these species. Curcumin has demonstrated its wound-healing properties and its ability to induce cell death by decreasing cell survival proteins, hence exhibiting anticarcinogenic actions.[4,5]

Despite numerous advantages, curcumin has been subject to significant criticism due to its limited bioavailability resulting from chemical instability, inadequate absorptivity, elevated metabolic rate, and rapid elimination from the body. The utilization of nanocarriers to encapsulate curcumin is an attractive option for enhancing its biological efficacy by improving its solubility, bioavailability, circulation duration, as well as retention inside the body, consequently overcoming its physiological obstacles.[6]

Nanofibers can be produced using different polymers and exhibit diverse physical characteristics. Electrospinning is a straightforward and efficient method for producing nanofibers of different sizes by applying electric force to draw charged threads from polymer solutions, with or without the addition of herbal extracts. Furthermore, electrospinning can deliver medications in a controlled and precise manner.[5] One notable benefit of this technology is its operational agility and flexibility, which enables the attainment of acceptable surface qualities such as a high surface-to-volume ratio and desired porosity. Utilizing this technique is advantageous in spinning several types of materials, including natural, synthetic, and blended polymers.[7]

It is possible to encapsulate both hydrophilic and hydrophobic medicines directly into the electrospun fibers using the electrospinning method. The utilization of electrospun fibers has the potential to minimize the minimal dosage requirement of the medicine, resulting in a reduction in systemic absorption and the occurrence of undesirable side effects. Electrospun nanofibers can enhance the efficacy of drug encapsulation and reduce burst release, hence conferring a competitive advantage to this approach in comparison to alternative drug delivery systems.[7, 8] The utilization of electrospun membranes as drug-delivery implants for antibiotic, antifungal, and anticancer purposes can be achieved through the incorporation of specific biomolecules. Electrospinning is a very advantageous technique that finds extensive utility across many domains of biomedical engineering, including medication delivery, tissue engineering, antibacterial research, and wound dressing.[9]

Electrospinning has demonstrated its efficacy as a highly economical technique for the production of diverse medical fibers, including scaffolds, medical implants, and wound dressings utilized in the fabrication of artificial human tissues. The behavior of scaffolds is analogous to that of the extracellular matrix found in actual tissues. Cell attachment can be facilitated by coating biodegradable fibers with collages, which function as an extracellular matrix. The utilization of nanofibers in the field of biomedicine involves the process of tissue engineering, wherein cells are introduced into an electrospun scaffold to either treat or substitute biological targets. In addition, wound dressings composed of nanofibers are highly effective in preventing microbial infections by effectively isolating the wound.[8,9]

MATERIALS AND METHOD

Plant Material Identification

T. cordifolia leaves were collected from the College's medicinal garden, and curcumin was procured as a gift sample from Sanjay Chemicals Mumbai. Plant samples were sent to the Botany Department of Sangola Science College for precise identification. Leaf shape, arrangement, and venation for *T. cordifolia* morphology, it was carefully examined throughout the taxonomy.

Extraction of *T. cordifolia*

Systematic leaf extraction occurred. Drying removed superfluous moisture, then pulverising into a fine powder increased surface area. In a soxhlet device, methanol and acetone were used for solvent extraction. A bioactive, solvent-soluble extract was obtained after 16 hours at 40°C.

Curcumin Procurement

Sanjay Chemicals, Mumbai, India, gifted curcumin, solvents, and chemicals for analysis.

Nanofiber Production by Electrospinning

The adaptable electrospinning method was used to make nanofibers. Solution preparation required trifluoroethanol-dissolved chitosan, curcumin and *T. cordifolia* leaves extract. Electrospinning required putting the spinning solution into a syringe and applying DC voltage at a predetermined feed rate. Vacuum drying and glutaraldehyde cross-linking stabilized nanofibrous structure after electrospinning.[10] Morphological characterization using scanning electron microscope (SEM) revealed nanofiber shape, diameter, and distribution.

Fabrication of Curcumin and TSC Loaded Nanofiber Mats

Chitosan solutions containing various amounts of curcumin and *T. cordifolia* extract were electrospun into nanofiber mats. Variable amounts produced *T. cordifolia* extract and curcumin-loaded nanofiber mats with varying drug levels. Solvent removal by vacuum drying produced curcumin-loaded nanofiber mats for regulated drug delivery and tissue engineering.[11]

Morphological Characterisation

The nanofiber mats' average fiber diameter and distribution were determined using SEM. Characterization of nanofiber morphology helped assess their appropriateness for various applications.

Surface Hydrophilicity Measurement

The surface hydrophilicity of curcumin and TSC-loaded nanofiber mats was tested using a water contact angle machine. A little water droplet on the nanofiber surface monitored the contact angle over time. Water spreads easier on surfaces with lower contact angles, indicating hydrophilicity. Higher contact angles indicate hydrophobic surfaces. Investigating time-dependent wetting behavior with dynamic contact angle measurements helped comprehend nanofiber surface properties.[12]

Thermogravimetric Analysis and Differential Scanning Calorimetry

Thermal degradation characteristics of chitosan-*Tinospora*-curcumin-loaded electrospun mats were studied using thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). The TGA method heated samples in N_2 and O_2 atmospheres, while the DSC method recorded heat flow in N_2 atmospheres. These analyses revealed thermal stability and decomposition behavior, helping explain material features.[13]

Drug Release Determination

It immersed extract-loaded nanofiber mats in PBS solution and collected supernatant at intervals to study curcumin and *T. cordifolia* leaves extract (TSC) release. Using UV spectrophotometry, the amounts of released curcumin and TSC were found. This assessed release kinetics and sustained release, essential for regulated drug delivery.[14]

Fourier Transform Infrared Spectroscopy Analysis

The distinctive chemical peaks of CQ extract and POSS nanoparticles were analyzed utilizing ATR in fourier transform infrared spectroscopy (FT-IR) analysis. This method revealed molecular vibrations and bond forms, indicating chemical structure and material applications.[15]

Swelling Study

The water absorption capacity of nanofiber mats was assessed using a swelling test. The samples were submerged in a PBS solution, and their weights were monitored as time progressed. The swelling rate was computed to evaluate the water absorption capability, facilitating comprehension of the material's response under physiological circumstances.[16]

In-vitro Drug Release Profile

Substance leakage into the PBS solution was evaluated during bilayer composite incubation. UV spectrophotometry determined the release rate *via* absorbance. This illuminated release kinetics and controlled drug delivery uses.

RESULTS

The extraction cycle data for getting an extract from a sample using a soxhlet device can be seen in Table 1. The same sample is extracted in each cycle to maximize extraction efficiency. The obtained extracted substance's weight is recorded in grams during each cycle. This sample underwent four extraction cycles. Each cycle yielded 17.5 grams of extract. Summing the extraction cycle weights yields 70.0 grams of extract after four cycles. This table shows the uniformity of extract yield across cycles, illustrating the extraction process. It helps evaluate the extraction method's efficiency and effectiveness.

Table 1: Weight of extract obtained from *T. cordifolia* leaves using solvent extraction

Extraction cycle	*Weight of extract (grams)*
Cycle 1	17.5
Cycle 2	17.5
Cycle 3	17.5
Cycle 4	17.5
Total	70.0

Table 2 details the electrospinning process for curcumin-loaded chitosan-based nanofibrous mats. It describes the nanofibrous mat production process, properties, and prospective uses. The spinning solution's components and amounts are provided. The spinning solution was 1 g of chitosan and 0.1 g of curcumin in 10 mL of trifluoroethanol. Electrospinning parameters are given. The feed rate was 1.5 mL/h, and the voltage was 21 kV. These factors are crucial for electrospinning nanofiber production and properties. Electrospun nanofibrous mats were vacuum-dried at room temperature for 24 hours. To remove solvents and stabilize the nanofibrous structure, this step is crucial. Next, 25% glutaraldehyde/ethanol solution cross-linked the nanofibrous mats for 24 hours at 4°C. The nanofibrous structure is stabilized and mechanically improved by cross-linking. Nanofibrous mats were washed with ultrapure water after cross-linking. This procedure removes excess cross-linking agents and other mat contaminants. Furthermore, the nanofibrous mats were freeze-dried overnight. Freeze-drying dehydrates nanofibers to preserve their structure and characteristics.[17,18] The approach produces "uniform chitosan-based nanofibrous mats loaded with curcumin." It produced nanofibrous mats with consistent curcumin dispersion in the chitosan matrix. The table finds that nanofibrous mats can be used in tissue engineering, drug delivery, and medicinal research. Nanofibrous mats are versatile and useful in many fields.

Table 3 shows the composition of spinning solutions used to make curcumin-loaded nanofiber mats with different drug concentrations. A table shows the amount of curcumin (w/v) in each spinning solution and different amounts of TSC. The spinning solutions contain chitosan (CS) and TSC.

Table 2: Nanofiber production with electrospinning

Parameter	*Value*
Spinning solution	1 g Chitosan + 0.1 g curcumin + 0.1% TSC in 10 mL trifluoroethanol
Electrospinning parameters	Feed rate: 1.5 mL/h, voltage:21 kV
Vacuum drying	24 hours at room temperature
Cross-linking	24 hours at 4°C in 25% glutaraldehyde/ ethanol (1% v/v)
Rinse	Ultrapure water
Freeze drying	24 hours
Result	Uniform chitosan-based nanofibrous mats loaded with *T. cordifolia* extract and curcumin.
Potential applications	Diabetic wound healing. Tissue engineering, drug delivery, biomedical research,

Table 3: Curcumin-loaded nanofiber mats with varying levels of drug content

Spinning solution	*Curcumin concentration (w/v)*
CS + Chitosan + 0.1% TSC	0.05%
CS + Chitosan + 0.2% TSC	0.1%
CS + Chitosan + 0.5% TSC	0.25%

Each spinning solution contains 0.05 to 0.25% curcumin (w/v). The spinning solution's curcumin content is a weight/volume proportion. This shows the curcumin concentration in the spinning solution relative to its volume. The spinning solutions contain 0.1, 0.2, and 0.5% w/v *T. cordifolia* extract. This permits nanofiber mat drug content to vary, impacting their characteristics and uses.

Figure 1 shows representative SEM images of nanofiber mats after preparation. The photos reveal that all nanofiber mats have a nonwoven structure with randomly aligned fibers. Statistical research shows that C-TCS nanofiber mat fiber diameter uniformity is reduced considerably.[19, 20] Importantly, the mean fiber diameter of all four mats was 100 to 200 nm, similar to native skin ECM collagen fibrils (50–500 nm).

Figure 2 shows that the dressing materials that mimic the native skin ECM are crucial to skin tissue engineering, especially for chronic hard-to-heal wounds like diabetic ulcers. In this study, blend electrospinning was used to make curcumin and TCS nanofiber mats, and Chitosane was encapsulated into nanofibers to make C-TSC 0.1, C-TSC-0.2, and C-TSC-0.5 mats. A polymeric combination of chitosan and TSC had outstanding electrospinnability, and trifluoroethanol did not impair spinning.

Based on the weight losses of the two degrading phases, the electrospun mats' biopolymeric and co-spinning agent components are 1:1. It is crucial to emphasize that the stabilizing cross-linking technique has no impact on the thermal properties of the nanofibrous mats. This suggests no deterioration when exposed to NH^{4+} vapors and UV rays. DSC was conducted to examine further the electrospun membrane's chemical composition and thermal characteristics (Figure 3). The PEO powder exhibited a distinct melting behavior at around 70°C, but no noticeable transition was observed for curcumin.

The surface hydrophilicity of curcumin and TSC-loaded nanofiber mats was determined by dynamically testing and recording the water contact angle. Figure 4 reveals that the nanofiber mats are very wettable and hydrophilic, with an average water contact angle of 0. The water droplet was completely absorbed in 5 seconds on two nanofiber mats.

The *in-vitro* drug release behavior of curcumin and TSC nanofiber mat was evaluated by calculating accumulated release rates over 10 hours in PBS. Figure 5 demonstrates a consistently even release pattern during the 10-hour incubation period, with no noticeable sudden release occurrence. Furthermore, the average cumulative release rate was 84.0% after a duration of 10 hours. The stable and continuous release of curcumin and TCS nanofiber mat was expected to extend administration and increase wound dressing efficiency.

The liquid absorption capability of single and bilayer wound dressings was tested by swelling. Swelling tests were conducted by measuring the weight changes of samples at 4, 24, and 48 hours in a solution of 1X PBS. Figure 6 compares single and bilayer wound dressing swelling percentages. Swelling results revealed that chitosan's water absorption capacity increased after 24 hours but reduced at 48 hours due to its low stability. Statistical analysis reveals a notable disparity in the

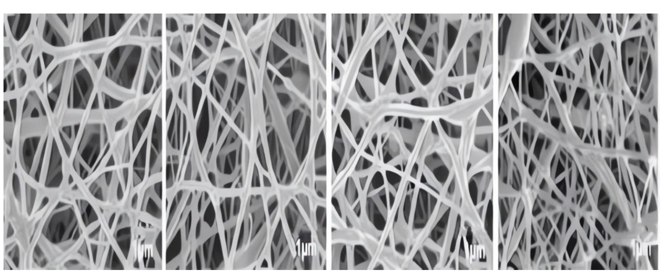

Figure 1: The representative SEM images

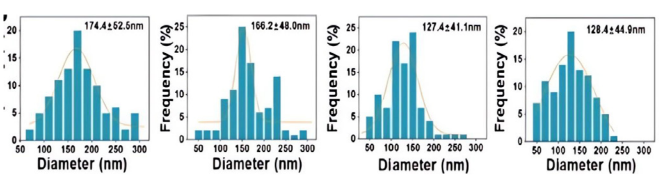

Figure 2: The fiber diameter distribution analysis of nanofiber mats using a blend electrospinning strategy

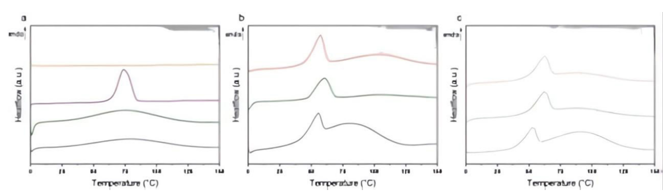

Figure 3: Thermogravimetric analysis and differential scanning calorimetry

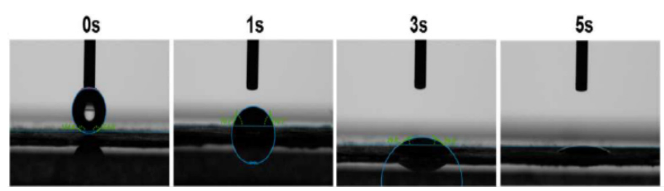

Figure 4: Water angle test photographs

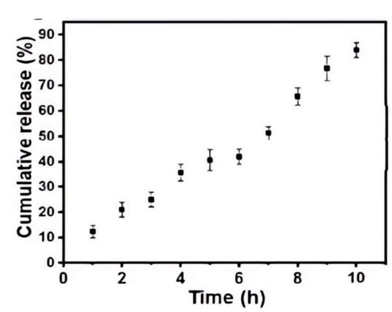

Figure 5: Drug release from nanofiber mats

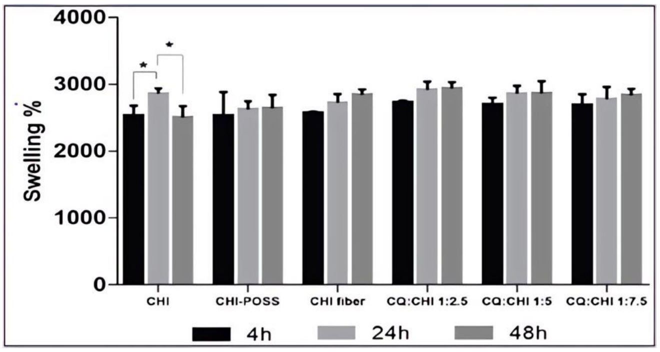

Figure 6: The swelling percentages of wound dressings for 4, 24 and 48 hours

swelling percentage between the chitosan control group (CHI) at 4 and 24 hours and between 24 and 48 hours.

DISCUSSION

We successfully prepared and assessed nanofiber mats incorporating curcumin and *T. cordifolia* leaf extract, with chitosan serving as the base polymer. Our findings are highly promising. Utilizing electrospinning techniques, we achieved excellent content uniformity and observed favorable diameter characteristics, as confirmed by SEM analysis.[21,22] DSC analysis revealed no component interactions, ensuring the integrity of the formulation. Furthermore, the mats exhibited notable hydrophilicity, indicating prolonged retention time. Our study culminated in the development of a novel formulation to treat the challenge in diabetic wound healing.[23] This formulation will not only address bacterial resistance but also accelerate diabetic wound healing, leveraging the angiogenic and tissue-remodeling properties of *T. cordifolia* leaf extract. The study provides a rationale for the purported applications of *T. cordifolia* within the conventional medicinal framework, specifically in the treatment of diverse infectious ailments.[24]

CONCLUSION

The work shows that curcumin-loaded nanofiber mats can be customized for drug content and application by changing the spinning solution composition. The nanofiber mats' homogeneity and desired qualities, confirmed by SEM imaging and hydrophilicity tests, make them suitable for the treatment of diabetic wound healing. *In-vitro* medication release and liquid absorption tests show that nanofiber mats can improve wound dressing efficiency and drug administration. Moreover, this study advances biomaterials and nanotechnology, presenting viable answers for biomedical difficulties and opening the way for the investigation, culminating in the development of an innovative formulation capable of combating the prevalent issue of bacterial resistance in the context of diabetic wound healing. The formulation of *T. cordifolia* leaves extract not only effectively combats bacterial resistance but also promotes expedited healing of diabetic wounds. This is due to the extract's recognized ability to enhance angiogenesis and tissue remodeling. Future antibacterial and animal studies will help to establish the claims.

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