RESEARCH ARTICLE

Fabrication, Evaluation and *In-vitro, In-vivo* Efficacy of Electrospun Nanofiber Mats of *Tinospora cordifolia* Extract and Curcumin

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ABSTRACT

The current research work highlights the fabrication and evaluation of nanofiber mats prepared using electrospinning technology containing *Tinospora cordifolia* extract and curcumin incorporated in chitosan polymer. The study of the prepared mats was carried out to optimize the formulations, to check *in-vitro* antibacterial activity, and to establish the effectiveness in diabetic wound healing by conducting an animal study. The nanofiber mats of curcumin and leaf extract of *T. cordifolia* using chitosan as a base polymer were prepared, optimized, and evaluated for antibacterial activity *in-vitro*, while their ability to enhance the wound healing process was checked using an animal model. The research parameters included the rate at which the wound contracted besides the time taken for epithelialization by using the wound healing model- excision technique. The results we found are encouraging for further studies. The nanofibers showed good antibacterial potential and faster healing of wounds. The research finished by developing an improved new formulation that effectively combats bacterial resistance, a common problem in the diabetic wound healing process. The mixture will not only fight resistant bacteria, but it will also help diabetic wounds heal faster because the *T. cordifolia* leaf extract is known to improve blood vessel growth and tissue remodeling.

Keywords: *Tinospora cordifolia*, Chitosan, Nanofiber mats, Antibacterial activity, *In-vivo* wound healing study.

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INTRODUCTION

Medicinal application of plants may be sketched back to the actual beginning of human civilization. Plants having medicinal activities have been used in several ancient medicinal systems to address various diseases. Herbal medicine is the primary form of therapy for around 75 to 80% of people in many undeveloped countries because of its high cultural acceptance, compatibility with the human body, and fewer adverse effects.[1] *Tinospora cordifolia*, belonging to the Menispermaceae family, is a very valuable plant due to its constituent compounds and pharmacological properties. The term often used for it in Sanskrit is guduchi. The entire plant, including the leaves, stems, and roots, have important medicinal effects. Furthermore, *Tinospora* has been recognized for its antipyretic, antispasmodic, anti-inflammatory, immunomodulatory, and anticomplementary characteristics. Moreover, *Tinospora* has been shown to have hepatoprotective, antidiabetic, antioxidant, and anticancer properties. The plant's stem, root, and leaf extracts have been shown to have antibacterial activities against harmful microorganisms.[2, 3]

Curcumin, a bioactive compound found in turmeric, has beneficial characteristics in the treatment of inflammatory diseases, metabolic syndrome, pain, renal dysfunction, and the control of inflammatory and degenerative eye conditions. This chemical exhibits antioxidant action by effectively scavenging free radicals, regulating enzymes involved in the neutralization of free radicals, and inhibiting enzymes that produce these reactive species. Curcumin is a well-known wound healer compound that has the capacity to promote cell death by reducing the levels of cell survival proteins, hence displaying anticarcinogenic effects.[4,5] Although curcumin has several benefits, it has faced substantial criticism since it has low bioavailability caused by chemical instability, insufficient absorptivity, a high metabolic rate, and quick removal from the body. Using nanocarriers to encapsulate curcumin is a promising approach to enhancing its biological effectiveness by boosting its solubility, bioavailability, circulation length, and retention inside the body. This helps overcome the physiological challenges associated with curcumin.

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Nanofibers may be synthesized using various polymers and display a wide range of physical properties. Electrospinning is a direct and effective technique for generating nanofibers of varying dimensions by employing electricity force to remove threads having charge from a solution of polymer, with or without the inclusion of herbal extracts. Moreover, electrospinning can administer drugs with a high degree of control and precision.[6] An important advantage of this technology is its ability to operate with agility and flexibility, allowing for the achievement of desired surface features such as a high ratio of volume to surface area and desirable perviousness. The use of this technology offers benefits for spinning many kinds of materials, such as natural, synthetic, and mixed polymers.[7]

The electrospinning technology allows for the direct encapsulation of both hydrophilic and hydrophobic pharmaceuticals inside the electrospun fibers. The use of electrospun fibers can decrease the minimum dose needed for medication, leading to a decrease in the absorption of the medication into the bloodstream and the appearance of unwanted side effects. Electrospun nanofibers can improve the effectiveness of drug encapsulation and decrease sudden release, giving this method a competitive edge over other drug delivery methods.[7,8] Electrospun membranes may be used as drug-delivery implants for antibacterial, antifungal, and anticancer treatments by including particular biomolecules. Electrospinning is a very beneficial technology widely used in several areas.[9]

Electrospinning is a money-saving method for producing various fibers used in the field of medicine, *viz*. scaffolds, wound coverings & medicinal implants, which are used to create artificial tissues. Cell adhesion may be enhanced by applying a layer of collagen to biodegradable fibers, which aid as an actual extracellular material. Nanofibers are used in biomedicine for tissue engineering, which includes introducing cells into an electrospun scaffold for treatment or entirely replacing the target. Furthermore, nanofibers-based wound dressings are very efficient in avoiding microbial infections by efficiently isolating the wound.[8,9]

MATERIALS AND METHOD

Materials

Curcumin, chitosan, and other chemicals were obtained from Sanjay Chemicals, located in Mumbai. The leaves of *T. cordifolia* were collected from the college's medicinal garden.

Methods

Plant material identification

Curcumin was procured as a gift sample from Sanjay Chemicals in 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 were carefully examined throughout the taxonomy.

Extraction of T. cordifolia

Leaf extraction was conducted systematically. By drying the substance, excess moisture was eliminated, and then grinding it into a fine powder enhanced its surface area. Methanol and acetone were used as solvents for extraction in a soxhlet apparatus. An extract with bioactive properties, which can dissolve in solvents, was developed after being subjected to a temperature of 40°C for 16 hours.

Nanofiber production by electrospinning

Typically, the process of electrospinning fiber creation may be divided into three separate stages: The Taylor cone and the commencement of a jet, the occurrence of whipping instability, and the deposition of the resulting fiber.[10] Figure 1 illustrates the typical configuration for electrospinning.

The adaptable electrospinning method was used to make nanofibers. Solution preparation required trifluoroethanol-dissolved chitosan, curcumin, and *T. cordifolia* leaf 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.[11]

Fabrication of curcumin and TSC loaded nanofiber mats

Chitosan solutions containing curcumin (CS) (0.25–1% w/v) and *T. cordifolia* extract (TSC) (0.5% v/v), total of nine formulations were prepared with changing concentrations of the curcumin as it is one of the main constituents of the formulation, the electrospinning parameters like RPM and needle distance were kept constant and specially viscosity and voltage were altered to check the effect on the formulation were electrospun into nanofiber mats. The batches were manufactured as mentioned in Table 1. Solvent removal by vacuum drying produced curcumin-loaded nanofiber mats for regulated drug delivery and tissue engineering.[12]

Development of nanofiber mats

Table 2 outlines the process of electrospinning to create nanofibrous mats loaded with *T. cordifolia* extract + curcumin and based on chitosan. The composition of the spinning solution is detailed, consisting of 1 g of chitosan and 0.1 g of curcumin dissolved in 10 mL of trifluoroethanol. Key electrospinning parameters such as feed rate (1.5 mL/h), optimized voltage (21 kV), and viscosity (225 mPa s) are highlighted as critical for achieving desired nanofiber production and properties.

Following electrospinning, the nanofibrous mats undergo vacuum drying at room temperature for 24 hours. This step is essential for solvent removal and structural stabilization of the nanofibrous mats. Subsequently, cross-linking with a 25% glutaraldehyde/ethanol solution at 4°C for 24 hours enhances the stability and mechanical strength of the nanofibrous structure. Washing the mats with ultrapure water post-cross-linking eliminates excess cross-linking agents and other contaminants.

The final step involves overnight freeze-drying of the nanofibrous mats to remove moisture and preserve their structure and characteristics. This meticulous approach yields uniform chitosan-based nanofibrous mats with well-dispersed curcumin throughout the matrix, ensuring consistent quality and functionality.

Table 1: Curcumin-loaded nanofiber mats with varying levels of drug content

Formulation	*Spinning solution (% v/v)*	*Curcumin concentration (%)*	*Electrospinning parameters*				
			Flow rate (mL/hr)	*Distance (cm)*	*RPM*	*Voltage (kv)*	*Viscosity (mPa s)*
F1	10	0.25	1.5	10	1000	12	115
F2	10	0.25	1.5	10	1000	15	220
F3	10	0.25	1.5	10	1000	17	110
F4	10	0.50	1.5	10	1000	12	115
F5	10	0.50	1.5	10	1000	15	220
F6	10	0.50	1.5	10	1000	17	120
F7	10	1	1.5	10	1000	12	115
F8	10	1	1.5	10	1000	15	220
F9	10	1	1.5	10	1000	21	225

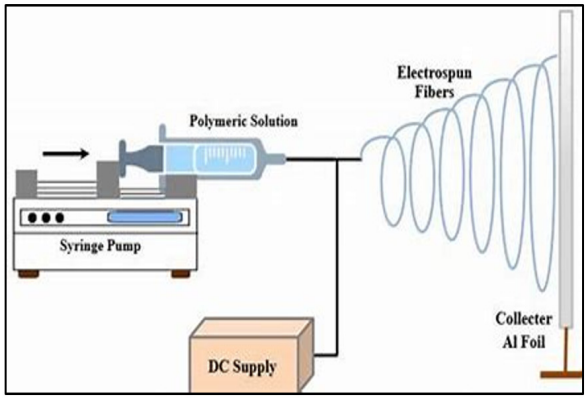

Figure 1: Typical configuration for electrospinning

Table 2: Nanofiber production with electrospinning

Parameter	*Value*
Spinning solution	1 g chitosan + 0.1 g curcumin in 10 mL trifluoroethanol + 5 mL TSC extract
Viscosity	225 mPa s
Electrospinning	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

Evaluation of Nanofiber Mats

Antibacterial activity

Nanofibers' antibacterial susceptibility against *Staphylococcus aureus* and *Escherichia coli* was tested using agar diffusion. Nanofiber discs showed antibacterial sensitivity in clear zones. Statistical analysis confirmed the results, ensuring the experiment was reliable and could be repeated.

Wound healing activity

Because the connective tissue matrix is synthesized, wound healing is a basic reaction to tissue damage that restores tissue integrity. The primary protein that makes up the extracellular matrix is collagen, which in the end, determines how strong a wound is.[13]

Wound healing is such a complicated process; however, when it comes to diabetic wound healing, the process even becomes more complicated due to many factors such as increased blood sugar levels, altered microbial flora, increased chances of bacterial resistance, tissue necrosis due to poor angiogenesis, etc.[14] Traditional herbs like *T. cordifolia* and curcumin have many therapeutic benefits; however, curcumin's limited bioavailability is a problem[15]. Electrospun nanofibers are an answer because they increase the effective surface area greatly and make drug delivery better in almost all aspects.[15] The combination in the form of nanofibers can be used in the healing of diabetic wounds where curcumin, the known antibacterial and anti-inflammatory element, plays an important role while *T. cordifolia* is a known antidiabetic drug also helps wound healing by enhancing tissue remodeling by reducing blood sugar, ensuring faster angiogenesis.[14] It is observed that when combined with the extract of the leaves of *T. cordifolia*, which has known antidiabetic and wound healing actions, blended in the chitosan polymer, which is itself a weak antibacterial compound, it can be one of the solutions to the menace of bacterial resistance, which is very common in diabetic wound healing.[14]

Induction of diabetes and wound healing potential

The animal study using diabetic excision wound healing model is conducted at Biocyte Institute of Research and Development, Sangali, Maharashtra, India (CPCSEA/2114/PO/s/20/CPCSEA). Before administering STZ, all the rats underwent an overnight fast. STZ was administered intraperitoneally (i.p.) at a dosage of 50 mg/kg using a recently made 0.1 M cold citrate buffer with a pH of 4.5. Two days following the STZ injection, blood glucose levels were determined with a glucometer. Diabetic rats were defined as those whose blood glucose levels were more than 250 mg/dl. These rats were then used in further studies. Rats are anesthetized using diethyl ether inhalation. The back of the rat is shaved and sterilized with 70% ethanol. In the impressed area, a wound is created by excising the skin, resulting in a wound area of approximately 500 mm^2. Twice a day, a medication is administered to the wound. Wound

measurement, specifically wound contraction, is taken starting from day 5 (the day of wounding). Measurements are taken on days 1, 5, 8, 11, 14. The wound area is traced onto transparent paper on these days to monitor changes over time. The trial runs until the wound heals fully, which ever happens first, or until the fourteenth postoperative day. The following formula was used to calculate the rate of wound healing.[16]

$$\textbf{Wound percentage} = \frac{\text{Initial wound size-specific day wound size}}{\text{Initial wound size}} \times 100$$

RESULTS AND DISCUSSION

Formation of Nanofibers with Varying Viscosity and Voltage

Our experiments corroborated these findings. Batches with the highest viscosity (225 mPa s) and 1% curcumin concentration (F9) exhibited excellent Taylor cone formation, facilitating proper spraying and nanofiber formation. Conversely, batches with lower viscosity and curcumin concentration encountered spraying difficulties and lacked Taylor cone formation, thus precluding nanofiber formation.

Moreover, when electrospinning, increasing the supplied voltage lengthens a steady jet. A stronger tangential electric field at the needle tip and lower static charge density stabilizing the jet are thought to be the causes of this phenomenon.[14] The magnitude and type of applied voltage significantly influence jet stability and Taylor cone initiation shape. In our experiments, proper spraying and Taylor cone formation were observed at 21 kV voltage (F9), while lower voltages yielded inadequate spraying patterns.

The structural characteristics of electrospun nanofibers are profoundly influenced by various factors, including solvent and polymer properties, alongside ambient parameters during the electrospinning process.[17] Working distance, viscosity, conductivity, composition of the polymer solution, nozzle design, temperature, humidity, and applied voltage are all included in this set of factors. In solution-based electrospinning, it is critical to identify and maximize the dominating variables in order to achieve the desired morphology.

The electrospinning process can be delineated into three main stages: initial deformation of a prolate droplet into a Taylor cone and initiation of a jet, followed by whipping or bending instability, and finally, fiber deposition.[18] The formation of a Taylor cone at the nozzle's tip, facilitated by electrostatic charge, is pivotal for initiating the ejection of a single fluid jet. As polymer concentration increases, viscosity typically rises, initially gradually and then rapidly. This viscosity increase leads to a homogenous fiber shape by promoting uniform jet elongation and generation. Using concentrated polymer solutions or raising the molecular weight of the polymer are two methods for improving viscosity during electrospinning. For example, adding high-molecular-weight polyethylene oxide to polylactide solutions increases their viscosity.[19]

Antibacterial Activity

Table 3 displays the antimicrobial effectiveness of nanofibers, including *T. cordifolia* leaf extract, against *E. coli* and *S. aureus*. "M" "extract" refers to the amount of *T. cordifolia* leaf extract in the form of nanofibers. The experiment consists of three discrete concentrations: 0.1, 0.2, and 0.5 g. Compilation of nanofiber composition, including chitosan (CS) and *T. cordifolia* leaf extract. The compositions examined are represented by the letters A–E. When nanofiber samples were tested against *E. coli*, they exhibited an inhibitory zone that was 1-mm in width. Nanofibers derived from *T. cordifolia* leaf extract restrict bacterial growth inside the inhibitory zone.[15] Greater inhibition zones correlate with more potent antibacterial activity. The zone of inhibition was measured in millimeters around the nanofiber samples when tested against *S. aureus*. Greater inhibition zones indicate more antibacterial efficacy. The nanofiber sample (A) consisting only of chitosan (CS) exhibited no inhibitory zones against *E. coli* or *S. aureus*, suggesting little antibacterial activity. Nanofibers with a higher concentration of *T. cordifolia* stem extract (from (B) to (D)) exhibit wider inhibitory zones against both bacterial strains. This demonstrates that increasing the concentration of leaf extract results in a greater degree of antibacterial activity. The control group, which was treated with streptomycin (E), exhibited the largest inhibitory zones against both bacterial strains, confirming the validity of the experimental setting.

Table 3: Summary of antibacterial activity

Nanofiber composition	*E. coli ZOI (mm)*	*S. aureus ZOI (mm)*
Chitosan 1% w/v (A)	No Inhibition (R)	No Inhibition (R)
Chitosan 1% w/v + 0.1 g *T. cordifolia* extract (B)	5.0 ± 1.4	3.0 ± 0.7
Chitosan 1% w/v +0.2 g *T. cordifolia* extract (C)	9.0 ± 2.1	6.0 ± 0.7
Chitosan 1% w/v + 0.5 g *T. cordifolia* extract (D)	12.0 ± 0.7	7.0 ± 1.4
Streptomycin (E)	20.0 ± 0.0	23.0 ± 0.0

Wound Healing Activity

The results are summarised in Table 4.

The formulation showed greater wound healing potential than the standard. On 14th day, 65.78% wound healing was observed in the case of formulation-treated groups. The comparative graphs of the wound healing potential are presented in Figure 2.

From Figure 3 it can be observed that rapid wound healing was noted in the formulation-treated groups as compared to other treated groups. This investigation indicated that nanofiber formulations in their therapeutic equivalent doses promote healing of the diabetic wound model employed in this study.

Girish *et al*.'s study found that using TC during surgery may speed up the healing of surgical wounds. Because of their combined or individual effects with curcumin, which speeds up the healing process, the phytoconstituents in TC may be responsible for its wound-healing properties.[20, 21]

Moreover, curcumin has been shown to promote collagen synthesis and deposition, essential processes for wound closure and tissue remodeling. Collagen provides structural

Table 4: Wound healing of the three groups: Diabetic control, standard, and formulation

Group	*5th day (%)*	*8th day (%)*	*11th day (%)*	*14th day (%)*
Diabetic control	3.27 ± 1.52	8.47 ± 1	19.67 ± 0.57	26.22 ±1
Standard (mupir ocin 2%)	8.77 ± 1.52	23.07 ± 0.57	43.85 ± 1.52	51.72 ± 1.52
Form ulation	6.89 ± 1.73	20.37 ± 1.52	34.48 ± 2.08	65.78 ± 0.70

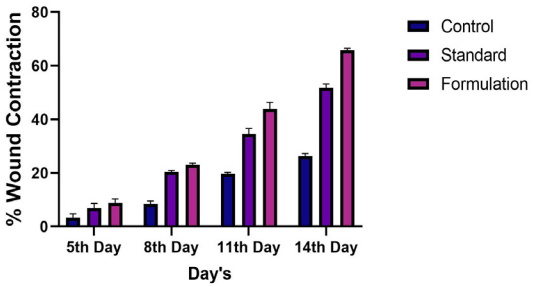

Figure 2: Comparative wound healing potential

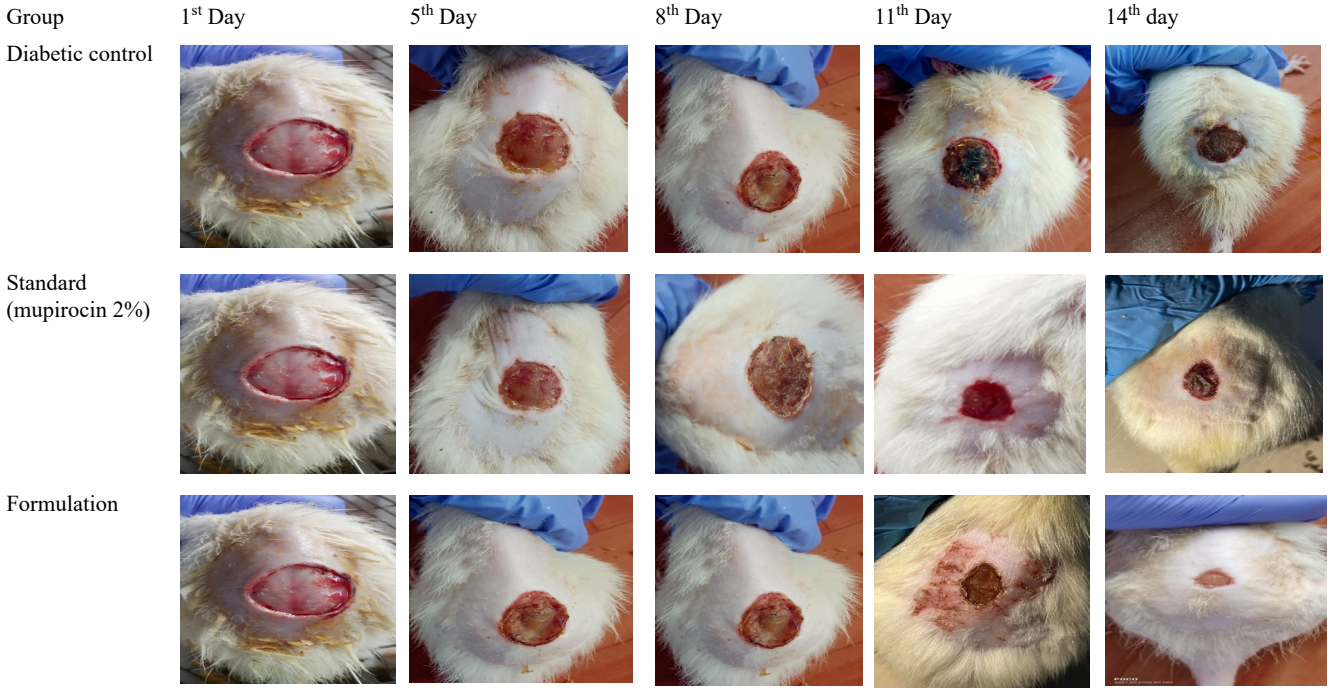

Figure 3: Actual photo images of the wound healing

support to the wound site and helps restore tissue integrity.[22,23] By stimulating collagen production, curcumin accelerates wound closure and promotes the formation of healthy scar tissue. Additionally, curcumin has been reported to enhance angiogenesis, the process of new blood vessel formation. Adequate blood supply is critical for delivering oxygen, nutrients, and immune cells to the wound site, all of which are essential for proper healing. By promoting angiogenesis, curcumin helps improve tissue perfusion and accelerates the healing process.

One of the key benefits of nanofibers is their high surface area-to-volume ratio, which enables efficient absorption and retention of bioactive agents such as growth factors, antimicrobial agents, and other therapeutic compounds. By incorporating these bioactive agents into nanofibers, controlled release kinetics can be achieved, allowing for sustained delivery to the wound site, which is beneficial for promoting tissue regeneration and combating infections.[24-26] The figures clearly indicate that *T. cordifolia* when combined with curcumin, provides an additive effect and provides faster wound healing even in complicated diabetic conditions, as observed in Figure 3

CONCLUSION

The study successfully fabricated and evaluated nanofiber mats utilizing electrospinning technology, incorporating *T. cordifolia* extract and curcumin within a chitosan polymer matrix. The optimized electrospinning conditions, such as those observed in batch F9 with a viscosity of 225 mPas and 21 kV voltage, are crucial for achieving the desired fiber morphology. Through systematic optimization, the formulations exhibited promising outcomes in terms of *in-vitro* antibacterial activity and efficacy in promoting diabetic wound healing, as demonstrated in animal studies and antibacterial studies. Additionally, the inclusion of *T. cordifolia* leaf extract is particularly noteworthy, as it not only contributes to antibacterial activity but also promotes blood vessel growth and tissue remodeling, thereby aiding in the expedited healing of diabetic wounds. This study underscores the promising potential of the developed nanofiber formulation as an effective

strategy for addressing bacterial resistance and enhancing diabetic wound healing. Further research and clinical trials are warranted to validate its efficacy and safety for clinical applications.

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