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Development And Characterization Of Electrospun Nanofiber Scaffolds Containing Phytoconstituents For Tissue Regeneration

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ABSTRACT

The aim of this research work was to prepare a Nanofibrous scaffolds of Nelumbo Nucifera Rhizomes for tissue regeneration. Electrospun Nanofibers of Nelumbo Nucifera Rhizomes was prepared and evaluated for Field emission scanning electron microscopy, Fourier transform-infrared spectroscopy analysis, Zeta potential, X-ray diffraction, Differential Scanning Colorimetry, Swelling index, *In-vitro* drug release study, Antibacterial activity, *In-vitro* anti-inflammatory study and Accelerated stability study. Here excision wound model was used to evaluate the tissue regeneration activity of formulation *in- vivo*. FESEM reveals the formation of smooth nanofibers with a uniform diameter. The degree of swelling of Nelumbo Nucifera Rhizomes Nanofiber in simulated wound fluid (pH 7.4) was found at 390.12% up to 12 hr. Within 24 hr 96% drug was released from Nelumbo Nucifera Rhizomes Nanofiber Scaffolds. The antibacterial action was enhanced by the Nelumbo Nucifera Rhizomes nanofibers by showing larger zone of inhibition. Nelumbo Nucifera Rhizomes nanofibers exhibited around 64.16% of anti-inflammatory activity at 200 µg/ml. Tissue Regeneration Activity of nanofibrous scaffolds of Nelumbo Nucifera in Wistar Rats demonstrates considerable potential for advancing wound healing in rats. It also revealed that prepared scaffolds increase the platelets count that is necessary for acceleration of Tissue Regeneration.

KEYWORDS: Tissue Regeneration, Scaffolds, Nelumbo Nucifera Rhizomes, Nanofibers

INTRODUCTION

In order to cover the injury and resist infection, wound dressing is crucial. The antibacterial qualities of honey pastes, animal fats, and herbal medicines have made them useful materials for wound healing throughout history[1]. In order to create wound dressings that promote the best possible healing environment and accelerate healing while minimizing pain and scarring, more biological approaches are being employed these days due to the development of new biopolymers, antibiotics, and fabrication methods. However, the process of wound healing is still gravely hampered by infection and inflammation brought on by bacteria and cells [2]. Wound healing is an intricate method

of tissue regeneration that promotes the formation of new tissue to provide the body with the required extrinsic barrier. With current technology, the class of diabetic, non-healing, and ulcer wounds demonstrated only a minimal improvement in dressing materials[3- 4]. Wound healing is a complicated process involving several biological, physical, and chemical elements. While being a continuing, overlapping process, wound healing can be divided into the following stages: The four stages are (1) haemostasis; (2) inflammation; (3) proliferation; and (4) remodelling and scar tissue development. After tissue injury, bleeding starts immediately[5]. The proper and simple method of electrospinning has four essential parameters: 1. Micro-pomp (feeding system), 2. Spinneret, 3. Collector, and 4. High voltage-power supply [6]. In the biomedical fields, electrospun nanofiber has found extensive use in wound treatment and tissue engineering [7]. Kyziol et al. created sodium alginate nanofiber which was ciprofloxacin hydrochloride-loaded using the electrospinning technique. According to their study, the scaffolds are promising for a variety of biomedical applications, including drug delivery systems and wound healing, because they contain both biopolymer and cross-linker [8]. An essential therapeutic tool for wound healing is a wound dressing. A dressing placed over the injured area is intended to prevent more harm and microbial invasion while also creating an environment that promotes wound healing and reduces the creation of scars. A range of products were developed, antibacterial gauge dressings [9]. The following characteristics of a perfect candidate for wound dressing:

- Site-specific drug administration;
- High concentration of drug at the wound site;
- Structural and biological similarity to the extracellular matrix protein found naturally;
- Control of cellular activity and provision of support;
- Maintaining the proper level of differentiation within the cellular section;
- Biodegradable and biocompatible;
- having no negative consequences [10]

MATERIAL and Methods

Material

PVA (Molecular Weight= 85, 000 Da) were procured from Sigma Aldrich, Mumbai. Nelumbo Nucifera silver nanoparticles was prepared by chemical reduction method. All the chemicals used during the research have been received and used without any modification or purification.

Fabrication of Nelumbo Nucifera nanoparticles loaded electrospun nanofibers scaffolds

Certain amount of Nelumbo Nucifera silver nanoparticles was dissolved in 2 ml of purified water, and then 10 g PVA (10 wt. %) was added to the solution. This solution was stirred continuously and heat for 20 h at 100^0C. The solution was then filled into a 10 mL syringe with a hypodermic needle and inserted in electrospinning machine. Flow rate was kept 0.5 ml/min. The needle was connected to high voltage power supply and is placed horizontally on a clamp. The 15 cm distance was kept from the tip of the needle to collect the nanofibers. 15 kV voltages were applied to the needle. Due the application of voltage the droplet of polymeric solution was elongates and solvent get evaporated. Then, the nanofibers was attract to the collector plate in mat form. The nanofibers mats then dried for 24 h at 80^0 C under vacuum [11-13].

Characterization of Nelumbo Nucifera Rhizomes Nanofibers

Field Emission Scanning Electron Microscopy (FESEM)

FESEM is a microscope that works with electrons instead of light. These electrons are liberated by a field emission source. Thin films of the sample were prepared on the SEM grid by just dropping a very small amount of the sample on the grid and gold-coated through sputter coater. The extra material was removed using a blotting paper and then the film on the SEM grid was allowed to dry by putting it under a mercury lamp for 5 min [14-15].

Fourier transform-infrared spectroscopy (FT-IR) analysis

Infrared spectroscopy gives information on the vibrational and rotational modes of motion of a molecule and hence is an important technique for the identification and characterization of a substance. Infrared spectra were collected by using Fourier Transform Infrared Spectrometer (IR Prestige Fourier Transform Infrared Spectrophotometer, Shimadzu, Japan). FT-IR measurement was carried out to identify the possible interaction between them, in the diffuse reflectance mode at a resolution of 4 cm^{1} using KBr pellets and the spectrum was recorded in the wavelength interval 4000 to 400 cm^{1} [16-17].

Zeta Potential

The zeta potential measurement of electrospun nanofibers mats is different from powder samples that require specimen holder preparation prior to use a commercial Zetasizer. For this technique, the first two acrylic plates are machined and assembled to form a micro fluidic channel (150 µm high, 2.0 mm wide and 30 mm long). A frame is formed outside the hole where electrospun nanofibers are spun to cover around the frame. Two electrodes for the measurement of streaming currents are housed in the top plate. A programmable micro pump was used to apply fluid pressure with a controlled flow rate (0.1 to 1.6 mL/min^{-1}) [18].

X-ray Diffraction (XRD) Studies

X-ray diffraction (XRD) measurement of the samples were carried out using powder X-ray diffractometer instrument (BRUKER aXS-D8 ADVANCE) in the angle range of 2-80 ºC 2Ø operated at a voltage of 30 kV and a current of 30mA with CuKα radiation. The scanning rate ($2Ø/min^{-1}$) was set at 10 ºC/min [19].

Differential Scanning Colorimetry (DSC)

The isothermal behaviour of nanofibers was investigated using DSC (STA 449 F3) technique over a temperature range of 50-400◦C in ambient air. The sample showed two types of peaks: endothermic peaks and exothermic peaks [20].

Swelling Index

Using simulated wound fluid, the swelling index of the nanofibers was evaluated. The fibre specimens were cut into 2 × 2 cm pieces, weighted (WI) and immersed for 5 minutes in 2 mL of simulated wound fluid. Using tissue paper, any extra fluid was wiped off the specimen when it was taken up, and the fibre that had absorbed the fluid was weighed again (WA) [21]. The swelling index was calculated using the following Equation:

Swelling index (%) = $(WA-WI)/WI \times 100$

WI: Initial weight specimen

WA: Weight of specimen after swelling

In- vitro drug release study

A Shimadzu UV-1800 spectrophotometer was used for recording the drug release profile from nanofibers throughout the period of 24 h by UV-visible spectroscopy experiment. To simulate the fluid in a wound, pseudo-extracellular fluid (PECF) was prepared. The sample was placed in an orbital shaker at 37 ºC and 30 rpm. After that, a piece of nanofiber membrane (1 x 1 cm) was immersed in 3 ml of pH 8±0.5 PECF solution. At different intervals (1, 2, 3, 4, 5, 6, 12 and 24 hours), an aliquot was removed and examined using a UV spectrophotometer at wavelengths between 200 and 400 nm [22-23].

Antibacterial Activity

For *S. aureus* and *E. coli* electrospun nanofiber mats were analysed for its anti-bacterial activity. Mueller Hinton Agar plates having microbial suspensions (1×106 cells m/L) was kept for overnight growth. Before being put on the agar plates, nanofiber samples were prepared and cut into tiny discs with a diameter of 1.5 cm. After that, the agar plates were placed in an incubator set to 37°C for a full day. We used Vernier calliper to measure the inhibition zone diameter [24-25].

In -vitro Anti-inflammatory Assay

The protein denaturation method was used for the *in-vitro* anti-inflammatory assay. In test tubes, about 0.2 ml of egg albumin (derived from fresh hen eggs), 2.8 ml of phosphate buffer saline (PBS, pH 6.4), and 2.0 ml of different concentrations of Nelumbo Nucifera Rhizomes nanofibers were added. In 5.0 mL of reaction mixture, the final concentration of each nanofiber was 100, 200, µg/mL. The control consist of 5.0 mL of distilled water. After 15 minutes at 37 °C in a BOD incubator, test tubes were heated to 70 °C for 5 min. After test tubes were cooled, a UV Spectrophotometer was used to detect the absorbance at 660 nm. To find the absorbance, aspirin (100, 200 µg/mL) was utilized as the standard and handled identically. Using the following formula, the percentage inhibition of protein denaturation was determined:

% inhibition = 100 × [Vt / Vc - 1]

Where, Vt = absorbance of test sample, Vc = absorbance of control [26]

Preparation of Nelumbo Nucifera Rhizomes Nanofibers Scaffolds

Scaffolds are the novel delivery system that facilitates the regrowth of injured or wounded tissues by applying topically as a combination of bioactive compounds, biomaterials, and cells. For the preparation of Nelumbo Nucifera Rhizomes nanofibers scaffolds, Bar shaped adhesive tape and prismatic covrlay is used. Then non-woven cloth layer is placed on bar shaped adhesive tape. At the center of bar shaped adhesive tape the Nano medicine loaded Nano fiber mat was placed and again the mat is covered with non-woven cloth layers to form a Scaffolds.

In-vivo Tissue Regeneration Activity

Experimental animals

Wistar Albino Rats of either sex weighing between 200-270 g was used for the study. The age of the animals was 6-8 w. For each wound model, animals were divided into four groups of six animals each (n = 6 per group).

Housing Conditions Bedding Materials: Autoclaved corn cob; 12: 12 light and dark cycle; Temperature 20-23 ⁰C; Relative Humidity 30-70 %

Diet: Standard laboratory Diet (VRK, Nutritional Solution, Sangli, India)

• Randomization, Grouping and numbering of animals

The animals used were not pregnant and nulliparous. After being divided into groups of 4 at stepwise, they were numbered for identification. The rats were anaesthetised and furs was removed from both the sides of the back and animals were closely observed.

• Mortality

Animals were observed for infection signs and mortality throughout the 14 day's time period.

- **Body weight**

The individual weights of the animals were noted weekly. Animals that survived the test, which lasted 14 days, were weighed and executed humanely [27-28].

Accelerated Stability Study

The Nanofibers were subjected to stability as per ICH guidelines at the following conditions. The sample was kept in a stability chamber. Accelerated stability study was carried out at 40 °C±2°C and 75% RH± 5 % RH. The Nanofibers were subjected to stability for a period of 6 months. The samples were withdrawn every 1 month and subjected to FESEM [29].

RESULTS AND DISCUSSION

Field Emission Scanning Electron Microscopy (FESEM)

Fig. 1 reveals that there is the formation of smooth nanofibers with a uniform diameter as well as there is no bead formation. This is due to the fact that the increased viscosity of solution restricts the formation of beads free nanofibers. Wherein, PVA concentration was 10 wt%, the flow rate was 0.5ml/h and voltage applied was 15 kV.

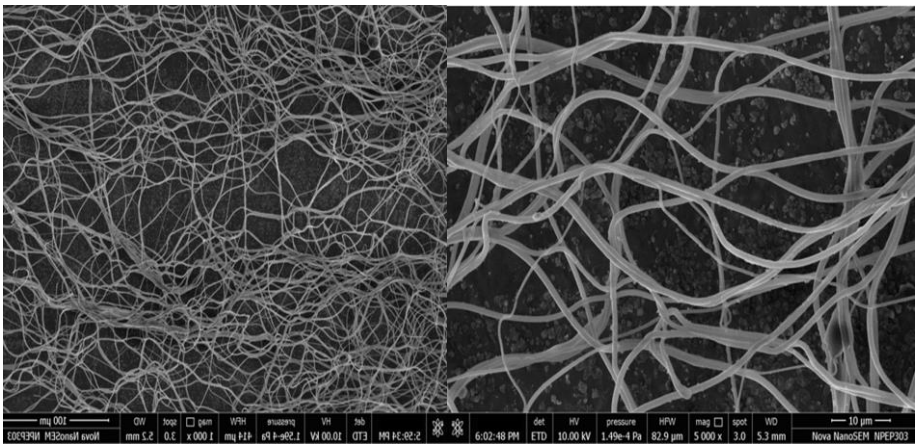


Fig. 1. FESEM OF NELUMBO NUCIFERA RHIZOMES NANOFIBER

As shown in Table 1 the FTIR spectrum of Nelumbo Nucifera Rhizomes Nanofiber exhibits characteristic bands due to the bending vibration of C-H shows peaks at 599.86, 684.73, 707.88, 835.18, and 989.48. The stretching vibrations of C-O shows peak at 1.49.28, C=C at 1382.96, C=O at 1658.78, C-H at 2980.02, 3080.32, 3199.91, and O-H at 3234.62. The frequencies are matching to the frequencies of functional groups present in structure so FTIR study is confirmatory for identification of coumpound.

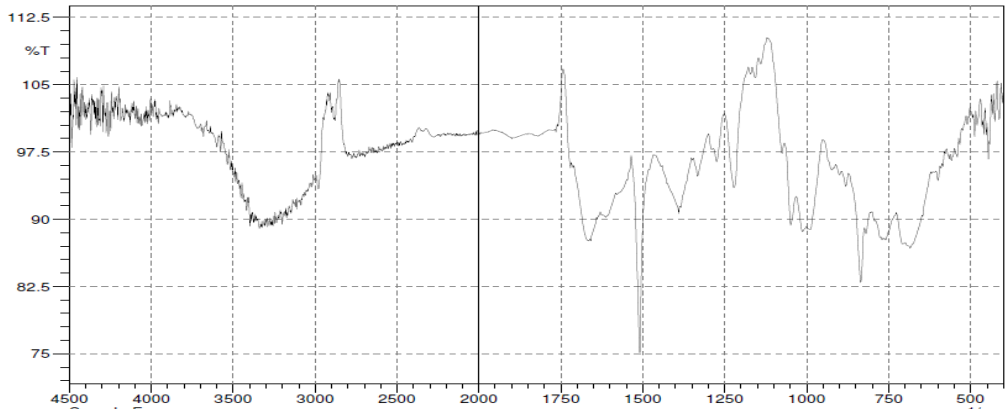


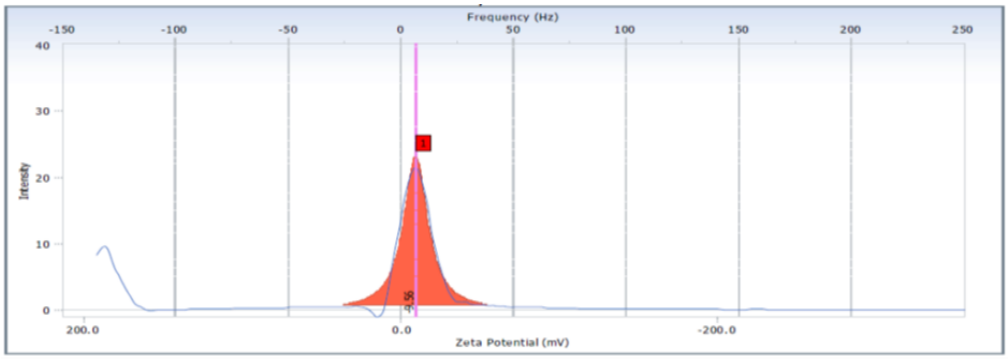
Fig. 2. FTIR OF NELUMBO NUCIFERA RHIZOMES NANOFIBER

TABLE- 1 INTERPRETATION OF FT-IR DATA OF NELUMBO NUCIFERA RHIZOMES NANOFIBER

Peak No.	Position (cm^{-1})	Functional Group	Peak No.	Position (cm^{-1})	Functional Group
1	599.86	C-H (B)	8	1544.98	C=C (S)
2	684.73	C-H (B)	9	1658.78	C=O (S)
3	707.88	C-H (B)	10	2980.02	C-H (S) Alkane
4	835.18	C-H (B)	11	3080.32	C-H (S) Alkane
5	989.48	C-H (B)	12	3199.91	C-H (S) Alkane
6	1049.28	C-O (S)	13	3221.12	-O-H (S)
7	1382.96	C=C (S)	14	3234.62	-O-H (S)

Zeta Potential

Zeta potential of the Nelumbo Nucifera Rhizomes Nanofiber was determined by electrophoretic light scattering using Zeta meter, Delsa Nano.



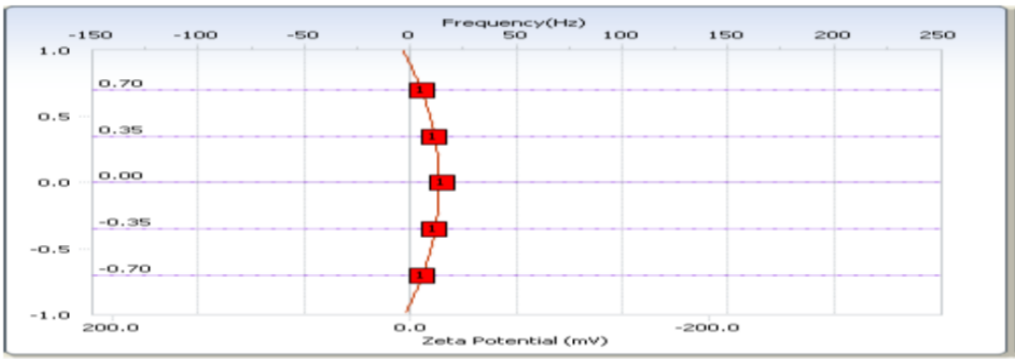


Fig. 3. ZETA POTENTIAL OF NELUMBO NUCIFERA RHIZOMES NANOFIBER

Zeta potential is an important parameter to analyse the long term stability of the nanofiber. Generally, higher potential values, both (+) or (-), indicates long term stability because of electrostatic repulsion between particles with same charges avoid aggregation. Zeta potential for Nelumbo Nucifera Rhizomes Nanofibers was found to -9.56 mV (Fig.3) it means Nelumbo Nucifera Rhizomes Nanofibers are moderately stable as well as posse's greater adsorption efficiency.

X-ray Diffraction (XRD) Studies

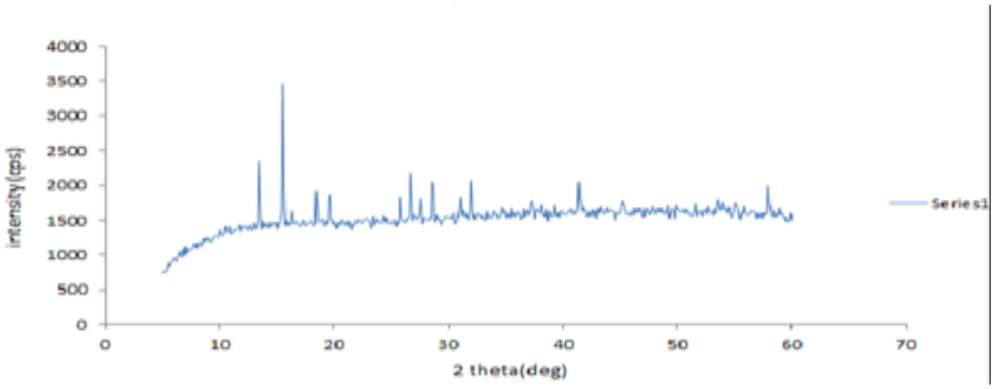


Fig. 4. XRD PATTERN OF NELUMBO NUCIFERA RHIZOMES NANOFIBER

X-ray diffraction (XRD) measurement of the samples was carried out using a powder X-ray diffractometer instrument ((BRUKER aXS-D8 ADVANCE) in the angle range of 2-80 ºC 2Ø. operated at a voltage of 30 kV and a current of 30mA with CuKα radiation. The XRD pattern of Nelumbo Nucifera Rhizomes nanofibers is shown in Fig. 4. The x-ray powder diffractogram of Nelumbo Nucifera Rhizomes nanofibers exhibited series peaks which indicate the crystalline nature of the drug.

Differential Scanning Colorimetry

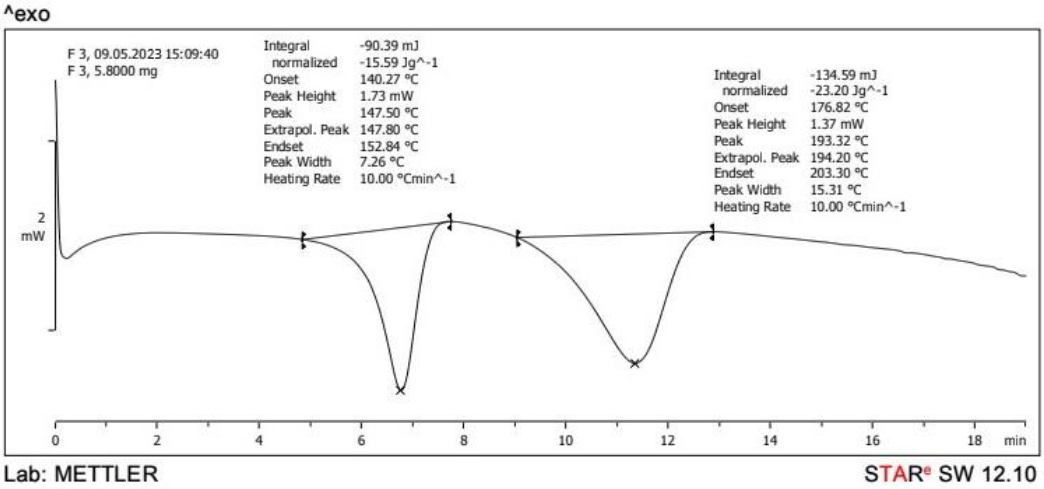


Fig. 5. DSC OF NELUMBO NUCIFERA RHIZOMES NANOFIBERS

The DSC thermogram of Nelumbo Nucifera Rhizomes nanofibers showed two endothermic peaks, first peak at 147.50˚C for drug and second peak at 193.32˚C for polymers (Fig.5).

Swelling Index

For wound healing applications, a scaffold with a noticeable swelling property is preferred because it can hold moisture from exuding wound beds. The degree of swelling of Nelumbo Nucifera Rhizomes Nanofiber in simulated wound fluid (pH 7.4) was 210.10%, 290.23%, 315.03%,333.32% 348.17%, 366.29%, 390.12% for the time intervals of 1, 2, 4, 6, 8, 10, and 12 h, respectively.

Preparation of Nelumbo Nucifera Rhizomes Nanofiber Scaffolds

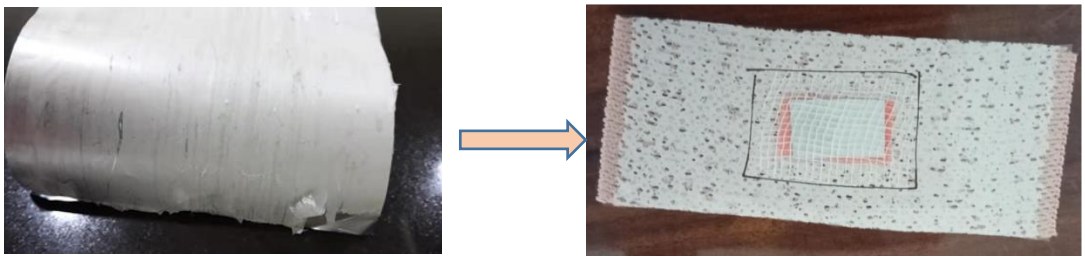


Fig. 6. NELUMBO NUCIFERA RHIZOMES NANOFIBER SCAFFOLDS

As shown in Fig. 6 Nelumbo Nucifera Rhizomes Nanofiber Scaffolds was prepared.

***In- vitro* drug release study**

In- vitro drug release study was carried out. Percentage cumulative drug released at different time intervals (1h, 2h, 3h, 4h, 5h, 6h, 12 h, and 24 h) was found out. Data shows in Table 2 that there is linear release from 1 h to 12 h, whereas burst release was observed at 24 h. Within 24 h 96% was released from Nelumbo Nucifera Rhizomes Nanofiber Scaffolds

TABLE- 2 PERCENTAGE CUMULATIVE DRUG RELEASE

TIME (h)	% CDR
1	9
2	19
3	26
4	30
5	35
6	52
12	63
24	96

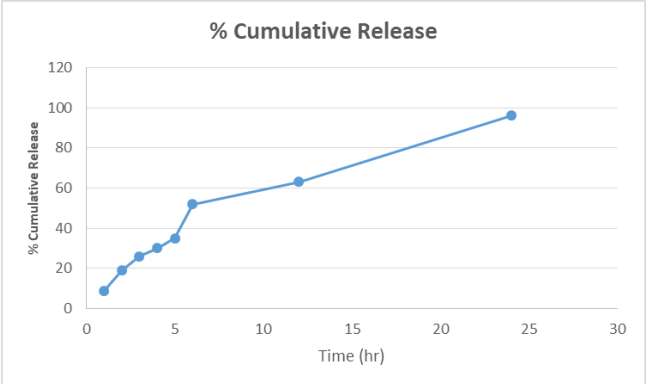


Fig. 7. PERCENTAGE CUMULATIVE DRUG RELEASE OF NELUMBO NUCIFERA RHIZOMES NANOFIBERSSCAFFOLDS

Antimicrobial Activity

Skin is covered in a large amount of *E. coli* bacteria, which can cause serious damage to an injured area of the skin. When comparing Nelumbo Nucifera Rhizomes nanofibers to individual nanofibers, the Table 3 shows the significantly larger zone of inhibition. The increased antibacterial activity of the Nelumbo Nucifera Rhizomes nanofibers appears to be responsible for the observed effects. The antibacterial action was enhanced by the Nelumbo Nucifera Rhizomes nanofibers.

TABLE -3 ANTIMICROBIAL ACTIVITY OF NELUMBO NUCIFERA RHIZOMES NANOFIBER

Microorganisms	Zone of Inhibition of Nelumbo Nucifera Rhizomes Nanofiber (mm)
E. coli	30
S. aureus	26

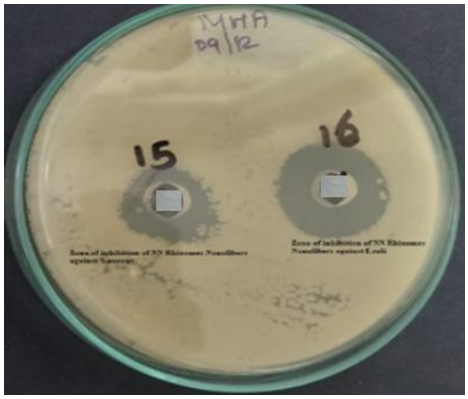


Fig. 8. ZONE OF INHIBITION OF NELUMBO NUCIFERA RHIZOMES NANOFIBERS SCAFFOLDS

In -vitro Anti-inflammatory Assay

As shown in Table 4 the anti-inflammatory action against denaturation of the protein was reported to be maximum at 200 µg/ml. In addition, at high concentrations, the efficacy against albumin denaturation was significantly higher compared to that of reference drugs. Nelumbo Nucifera Rhizomes nanofibers exhibited around 64.16% of anti-inflammatory activity at 200 µg/ml.

TABLE -4 ZONE OF PERCENT INHIBITION OF PROTEIN BY NELUMBO NUCIFERA RHIZOMES NANOFIBERS

Nelumbo Nucifera Rhizomes nanofibers / Standard	Concentration (µg/ml)	Percent Inhibition (%)
Aspirin	100	40.12
	200	53.33
Nelumbo Nucifera Rhizomes nanofibers	100	55.17
	200	64.16

In-vivo Tissue Regeneration activity

For conduction of *In-vivo* Tissue Regeneration activity permission was obtained, from the Institutional Animal ethics Committee. Tissue Regeneration Activity of nanofibers scaffolds Containing Novel Formulation of Nelumbo Nucifera Rhizomes Nanofibers in rat physiology revealed a notable acceleration in the healing process compared to vehicle groups. Rats subjected to the

formulation displayed a significant reduction in wound area on the 4th, 8th, and 14th post-treatment days, (Table 9) indicating heightened tissue repair. The repeated measurements showed that the wound area consistently got smaller over time. This proves that the formulation helps in healing the tissue well. Tissue Regeneration Activity of scaffolds Containing Novel Formulation of Nelumbo Nucifera in Wistar Rats demonstrates considerable potential for advancing wound healing in rats. Table 11 shows the increase in platelets count in group 4 (Nelumbo Nucifera Rhizomes nanofibers Scaffolds) than standard and marketed formulation. Platelets helps blood clot, and they contain special proteins called growth factors. Platelets play a crucial role in the healing process. So increase in platelets counts indicates that the Nelumbo Nucifera Rhizomes nanofibers Scaffolds heals the wound faster.

TABLE -5 BODY WEIGHTS DETERMINATION OF VEHICLE GROUP

Sr. No	Animal Number	Body Weight		
		Day 0	Day 7	Day 14
1	1	244g	257g	269g
2	2	235g	243g	257g
3	3	251g	263g	270g
4	4	225g	239g	251g
5	5	228g	242g	256g
6	6	229g	232g	250g

TABLE – 6 BODY WEIGHTS DETERMINATION OF STANDARD GROUP (BETADINE CREAM)

Sr. No	Animal Number	Body Weight		
		Day 0	Day 7	Day 14
1	1	216g	238g	250g
2	2	232g	247g	262g
3	3	218g	230g	245g
4	4	241g	253g	267g
5	5	228g	241g	255g
6	6	233g	247g	259g

TABLE -7 BODY WEIGHTS DETERMINATION OF NELUMBO NUCIFERA RHIZOMES NANOFIBERS

Sr. No	Animal Number	Body Weight		
		Day 0	Day 7	Day 14
1	1	243g	255g	269g
2	2	212g	225g	240g
3	3	262g	275g	283g
4	4	225g	239g	251g
5	5	269g	281g	289g
6	6	263g	278g	284g

TABLE – 8 BODY WEIGHTS DETERMINATION OF MARKETED FORMULATION

Sr. No	Animal Number	Body Weight		
		Day 0	Day 7	Day 14
1	1	211g	227g	240g
2	2	261g	275g	289g
3	3	242g	256g	270g
4	4	214g	226g	241g
5	5	219g	231g	253g

6	6	212g	218g	255g

TABLE -9 WOUND CLOSURE RATE (SQ.CM) ON VARIOUS DAYS

Group Details	Animal No.	Wound Closure Rate (sq.cm) on various days				
		0 day	Day 5	Day 8	Day 11	Day 14
G1 (Vehicle group)	1.	0%	19%	30%	55%	60%
	2.	0%	20%	29%	54.%	66%
	3.	0%	18%	40%	51%	61%
	4.	0%	22%	42%	58%	62%
	5.	0%	18%	36%	56%	68%
	6.	0%	18%	38%	57%	70%
G2 (Standard group)	1.	0%	41%	59%	79%	96%
	2.	0%	43%	60%	82%	96%
	3.	0%	47%	68%	86%	95%
	4.	0%	45%	65%	78%	96%
	5.	0%	48%	58%	88%	95%
	6.	0%	47%	60%	87%	96%
G3 (Nelumbo Nucifera Rhizomes nanofibers Scaffolds)	1.	0%	33%	50%	69%	92%
	2.	0%	36%	53%	73%	93%
	3.	0%	39%	55%	77%	95%
	4.	0%	35%	50%	70%	93%
	5.	0%	37%	51%	75%	96%
	6.	0%	38%	53%	88%	97%
G4 (Marketed Formulation)	1.	0%	37%	55%	71%	81%
	2.	0%	39%	58%	72%	86%
	3.	0%	42%	59%	77%	91%
	4.	0%	41%	60%	75%	85%
	5.	0%	46%	58%	76%	87%
	6.	0%	43%	60%	77%	88%

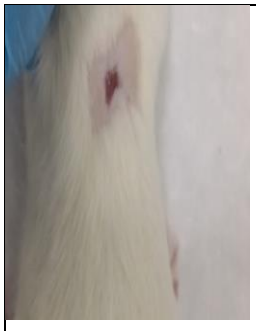
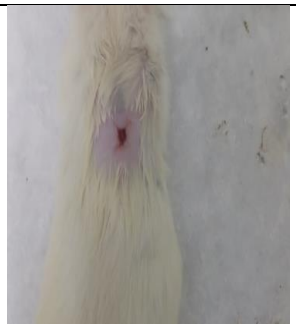
			
G1 Vehicle group	G2 Standard group	G3 Nelumbo Nucifera Rhizomes Nanofibers scaffolds	G4 Marketed Formulation

Fig. 10. HEALING OF WOUND OF ANIMAL IN EACH GROUP

TABLE 10 EFFECT OF NELUMBO NUCIFERA RHIZOMES NANOFIBERS SCAFFOLDS ON WOUND AREA

Groups	Day 0	Day 5	Day 8	Day 11	Day 14
Vehicle group	10 mm	12.3 mm	12.5 mm	12.5 mm	14mm
Standard group	10 mm	10.2 mm	8 mm	7 mm	5 mm
Nelumbo Nucifera Rhizomes Nanofibers Scaffolds	10 mm	8.5 mm	7.1 mm	5.4mm	3.2 mm
Marketed Formulation	10 mm	9.6 mm	8.2 mm	7.5 mm	6.9 mm

TABLE 11 EFFECT OF NELUMBO NUCIFERA RHIZOMES NANOFIBERS SCAFFOLDS ON PLATELET COUNTS

Group Details	Animal No.	Platelets Count (per µL) on various days				
		0 day	Day 5	Day 8	Day 11	Day 14
G1 (Vehicle group)	1.	124	130	141	159	168
	2.	136	132	152	165	176
	3.	148	136	163	171	180
	4.	126	130	152	162	169
	5.	128	131	140	168	183
	6.	132	134	146	164	188
G2 (Standard group)	1.	133	164	175	200	233
	2.	122	160	177	206	240
	3.	138	166	182	201	248
	4.	141	170	188	209	250
	5.	150	189	197	215	248
	6.	124	164	178	211	238
G3 (Nelumbo Nucifera Rhizomes nanofibers Scaffolds)	1.	133	170	200	235	260
	2.	128	167	205	233	254
	3.	140	168	189	219	259
	4.	127	172	198	239	265
	5.	121	178	203	241	300
	6.	148	169	199	239	306
G4 (Marketed Formulation)	1.	128	162	178	204	241
	2.	135	166	177	209	239
	3.	159	164	182	200	250
	4.	134	169	181	201	253
	5.	146	159	187	211	268
	6.	129	163	188	203	240

Accelerated Stability Study

According to ICH guidelines, optimized batch of Nelumbo Nucifera Rhizomes nanofibers Scaffolds were stored at 40±2˚C / 75±5% RH for a period of 6 months and formulation was evaluated by FESEM.

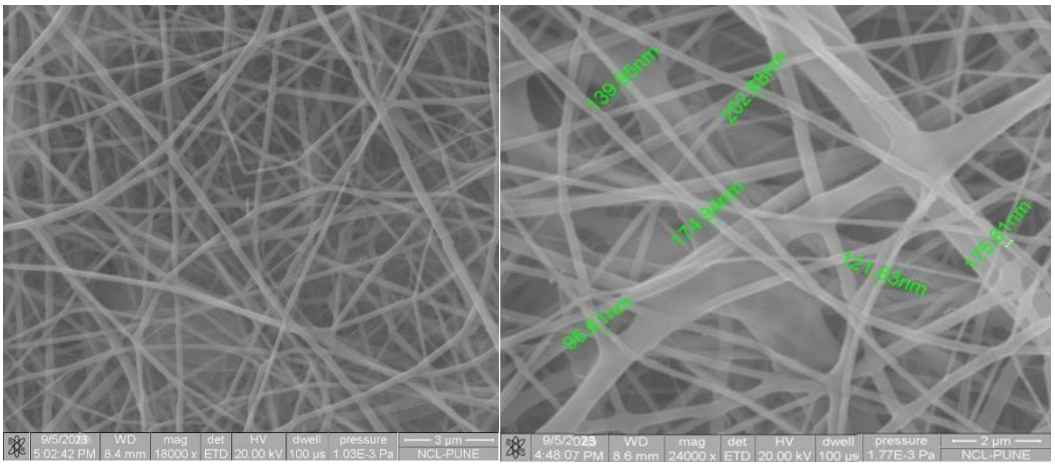


Fig. 11. FESEM OF NELUMBO NUCIFERA RHIZOMES NANOFIBERS LOADED (OPTIMIZED BATCH AFTER 6 MONTHS)

After a period of 6 months, the Nelumbo Nucifera Rhizomes Nanofibers were observed for any change in morphological parameters. There is no significant change taken place in the morphology of formulation before and after stability. Hence, Nelumbo Nucifera Rhizomes nanofibers prepared were found to be stable. From the results, it can be assumed that the nanofibers mats were stable for at least 6 months.

CONCLUSION

Nelumbo Nucifera Rhizomes nanofibers scaffolds accelerate the Tissue Regeneration due to their antibacterial and *In-vitro* anti-inflammatory activity as well as platelet count. Parameters like Field emission scanning electron microscopy (FESEM), Fourier transform-infrared spectroscopy (FT-IR) analysis, zeta potential, X-ray diffraction (XRD), Differential Scanning Colorimetry (DSC), Swelling index and In-vitro drug release study confirms the formation of nanofibers scaffolds**.** An accelerated stability study of the formulation was conducted, and the nanofibers scaffolds was found stable up to the period of six months. This combination may effective in tissue regeneration *in- vivo. In- vivo* study represents the acceleration of tissue regeneration.

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