JPharmSci
Journal of Pharmaceutical Sciences
Open access peer-reviewed Journal
www.jpharmsci.com [ISSN: 1520-6017]
Scopus ELSEVIER

Bioactive Extract of Aegle marmelos Blended with Mupirocin Electrospun Nanofiber for Accelerated Wound Healing

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ABSTRACT

The current study evaluated the topical potential of Nanofibrous, which is a combination of gelatin, chitosan, polyvinyl alcohol (PVA), mupirocin, and leaf extract from Aegle marmelos that has been fabricated for increased wound healing applications. Numerous methods were used to characterize the formulation: FTIR, SEM, XRD, contact angle measurement, phytochemical screening of the herbal extract, calibration curve analysis for API, antimicrobial testing, & cell line studies. The nanofibers' consistent shape and sizes in the nanometer range(2µm, 1µm) were shown by SEM. The 66.7% amorphous nature of the nanofibers was revealed by XRD analysis, and the effective integration of the herbal extract and API was verified by FTIR. Measurements of contact angles for nanofibers which has 34.25o Left angle and 36.50o Right angle revealed good hydrophilicity, which is important for applications involving wound dressings. Invitro drug release profile of formulated nanofiber has the 97% of release over the period of 8 hr. Antimicrobial assays demonstrated effective inhibition against tested pathogens, while cell line studies exhibited biocompatibility and potential for promoting cell proliferation. These findings highlight the promising potential of the developed nanofibers as advanced wound healing scaffolds combining antimicrobial properties with bioactive herbal components.

Keywords: Electrospinning, Wound healing, Nanofibers, Biomaterial, Biocompatible

INTRODUCTION

Wound healing is an intricate process involving hemostasis, where bleeding stops via vasoconstriction and clot formation with platelet activation and growth factors. Inflammation follows, with neutrophils and macrophages clearing debris and releasing cytokines to aid tissue repair. Proliferation then occurs, marked by angiogenesis, collagen production by fibroblasts, and epithelial cell migration for wound closure(1). Remodelling reorganizes collagen and strengthens tissue, though scars remain less strong than original tissue, completing the dynamic, multi-step healing cycle(2). Wound healing is still a major difficulty in the medical sciences, but researchers are making tremendous strides in this area every day. Overcoming several challenges, including as inflammation, infection, and skin regeneration, is part of the process. The utilization of nanomaterials is steadily expanding across several medical research

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

domains, encompassing medication and gene delivery, cell therapy, biosensors, and regenerative medicine, among others. Wound dressing is one of the most significant uses of nanomaterials. Wound dressings have been made with a variety of nanofibers, however scientists are still far from having nanofibers with improved properties(3). Various methods, including as phase separation, sol-gel, self-assembly, electrospraying, and 3D printing have been used to achieve nano-dimensional fibers from polymers to be used in wound dressing(4). Nanofibers are extremely fine fibers with diameters typically between 1 and 100 nanometers, possessing unique properties that set them apart from bulk materials. Their extensive surface area-to-volume ratio, impressive mechanical strength, and distinctive physical and chemical attributes make them highly useful in various applications, such as filtration, medicine, and materials science (5). Electrospinning is the predominant method for producing nanofibers, involving the creation of a charged jet from a polymer solution or melt that solidifies into nanofibers as it travels towards a collector. Additionally, it is a strong and adaptable manufacturing method for creating wound dressings. To achieve optimal fibers for wound dressing, it is essential to adjust key electrospinning process parameters, including the polymer type, concentration, solvent, applied voltage, and the distance between the tip and collector. The fibers' ability to interact with skin cells for adhesion, proliferation, and even differentiation depends critically on their nano-dimension and the size of their pores, which are created by electrospinning. Wound dressings have been made using a variety of natural and synthetic polymers, like gelatin, chitosan, polylactic acid, poly(lactic-co-glycolic) acid , cellulose, alginate, collagen, etc.. either alone or in combination with other polymers(6). Furthermore, the function and applications of the generated nanofibers have been improved by the addition of certain substances, drugs, nanoparticles, and extracts from medicinal plants to the electrospinning mixture. Examples of these applications include accelerating wound healing, cell and stem cell interaction and differentiation, and having antibacterial and antifungal effects. In medical applications, nanofibers have revolutionized drug delivery systems, due to extensive surface area enables the controlled and sustained release of therapeutic agents (7). Advanced wound dressings leverage nanofibers to accelerate healing and reduce infection risks by fostering an optimal environment for cell growth and acting as a barrier against pathogens. In tissue engineering, nanofibers serve as scaffolds mimicking the cellular matrix, supporting cell attachment, proliferation, and differentiation to regenerate tissues. The filtration sector benefits greatly from nanofibers' small pore sizes and large surface area, enabling superior particle capture efficiency compared to conventional filters. They are integral to air and water filtration systems, crucial for maintaining clean environments in healthcare and industrial settings by effectively trapping contaminants like bacteria and viruses(8).

Several techniques, including solution blow spinning, self-assembly, and phase separation, are used to produce nanofibers, each suited to specific applications. Electrospinning is particularly effective for wound healing due to its precise control over fiber diameter, aiding in mimicking the extracellular matrix and promoting cellular interactions. The high surface area of electrospun nanofibers supports cell adhesion, migration, and proliferation. Additionally, its flexibility accommodates various materials to customize nanofiber properties like strength and biocompatibility. (9).

This study explores the development and characterization of electrospun nanofibers composed of gelatin, chitosan, polyvinyl alcohol (PVA), and incorporating mupirocin and herbal leaf extract from Aegel marmelos. Gelatin and chitosan contribute biocompatibility and structural integrity, while PVA enhances mechanical strength and stability. Mupirocin, a potent antibiotic, and Aegel marmelos extract, known for its anti-inflammatory and wound healing properties, are integrated to impart therapeutic functionalities to the nanofibers. And to investigate the synergistic effects of these materials in promoting wound healing by accelerating tissue regeneration, preventing infections, and reducing inflammation. Through comprehensive characterization and evaluations, this study seeks to establish the efficacy and potential clinical applications of these novel electrospun nanofibers in advancing wound care treatments

MATERIALS

Mupirocin, Gelatin were purchased from Himedia. PolyVinyl alcohol were purchased from lobachemicals.com, Chitosan was purchased from Srichem.com and the leaves of Aegle Marmelos were collected near the region of Yelagiri hills, Thirupattur (dt), Tamil Nadu, India

METHODS

PREPARATION OF HERBAL EXTRACT

The harvested leaves are thoroughly washed and shade-dried at room temperature for 15 days. Once dried, the leaves are ground into a coarse powder, and 500g of the powdered leaves are placed in a glass container and then the ethanol is poured until the powder gets sunken in ethanol After the 7 days interval at the occasional mixing of the container and the supernatant solution was collected and refiled the ethanol Repeat the above step for 3 times and the collected solution were placed on the rotary freez evaporator for the separation of solvent After solvent separation the remaining content is kept under the water bath for the further solvent evaporation After the solvent evaporated the creamy extract were obtained The obtained extract was stored in refrigerator for future use .

PREPARATION OF STANDARD CALIBRATION CURVE

A pure medication sample of Mupirocin drug around 10 mg was precisely weighed, diluted in a little amount of water, and then sonicated for ten minutes. More water was added to get the volume up to 10 ml. This was accepted as the standard answer. Appropriate dilutions were prepared from the stock solution to obtain concentrations between 2 and 10 µg/ml, which were then scanned in a UV Spectrophotometer at an max of 220 nm. After that, a calibration curve was drawn with the absorbance (nm) on the Y-axis and the concentration (Hg/ml) on the X-axis.

PHYTOCHEMICAL SCREENING OF HERBAL EXTRACT:

The herbal extract was screened for phytoconstituents, including alkaloids, carbohydrates, glycosides, saponins, phytosteroids, polyphenols, tannins, flavonoids, proteins, and amino acids, in the ethanolic extract following standard procedures.

TEST FOR ALKALOIDS:

a) **Mayer's Test:** Mayer's reagent (Potassium Mercuric Iodide) was added to the filtrates. The formation of a yellow precipitate indicates the presence of alkaloids.
b) **Wagner's Test:** Wagner's reagent (Iodine in Potassium Iodide) was added to the filtrates. The presence of alkaloids is confirmed by the appearance of a brown or reddish precipitate.

AMINO ACIDS:

a) **Ninhydrin test:** After adding 2 ml of ninhydrin reagent and boil it for a few minutes, the 2 ml extract became blue, indicating the presence of amino acids.

ANTHOCYANIN:

Anthocyanin is present when 2 ml of aqueous extract was introduced to 2 ml of 2N HCl and NH3. The mixture appears pink red at first but becomes blue violet after that.

ANTHRAQUINONES

a) **Modified borntrager's test:** Boiling in 2 milliliters of 5% ferric chloride and 2 milliliters of diluted sulfuric acid

CARBOHYDRATES:

a) **Molisch's Test:** In a test tube, two drops of an alcoholic alpha-naphthol solution were added to the filtrates. Carbs are present when the violet ring starts to form at the junction.
b) **Benedict's Test:** Benedict's reagent was applied to the filtrates, and they were then gently heated. The orange-red precipitate indicates the presence of reducing carbohydrates.
c) **Fehling's Test:** Filtrates were heated using Fehling's A&B solutions, neutralized with alkali, and hydrolyzed with dilute HCl. Red precipitate formation suggests the presence of reducing carbohydrates.

FLAVONOIDS:

a) **Alkaline Reagent Test:** To the extracts, a little quantity of sodium hydroxide solution was added. When diluted acid is added, the vivid yellow hue that first formed to show the presence of flavonoids turns colorless.
b) **Lead Acetate Test:** To the extracts, a few drops of lead acetate solution were added. Yellow precipitate development indicates the presence of flavonoids.

GLYCOSIDES:

a) **Modified Borntrager's Test:** The extracts were treated with ferric chloride solution and then placed in boiling water for approximately five minutes. Once cooled, the mixture was extracted using an equal volume of benzene. The benzene layer was separated and treated with an ammonia solution. The appearance of a rose-pink color in the ammoniacal layer indicates the presence of anthranol glycosides.

PROTEINS:

a) **Xanthoproteic test:** The addition of a few drops of concentrated HNO3 to the extract resulted in the formation of a yellow color, indicating the presence of proteins.

PHENOLS:

a) **Ferric Chloride Test:** Ferric Chloride Test: Three to four drops of ferric chloride solution were applied to the extracts. Phenols are present when blue black color begins to form.

SAPONIN:

Saponin was identified by the formation of foam after 5 milliliters of the extract were mixed with 20 milliliters of distilled water and shaken in a graduated cylinder for 15 minutes..

STEROID:

Once one milliliter of extract had been dissolved in ten milliliters of chloroform and an equivalent volume of conc, sulfuric acid was added from the side of the test tube. As the top layer became red, the sulfuric acid layer fluoresced green and yellow. This implies the presence of steroids

TANNIN:

Condensed tannin was visible because to the green hue that results from treating 4 milliliters of the extract with 4 milliliters of FeCl3.

TERPENOIDS:

To detect the possible presence of terpenoids, 2 ml of the extract was mixed with 2 ml of chloroform and 3 ml of concentrated sulfuric acid. Sulfuric acid was then slowly added to form a layer, resulting in a reddish-brown color at the interface, indicating the presence of terpenoids.

Preparation of Nanofibers Electrospinning Method

For the electrospinning solution first the polymers (Chitosan and Gelatin) were dissolved in the suitable solvent Acetic acid and DMSO (93:7) were kept in the mixer for 24 h to obtain polymeric solution and the PVA was dissolved in the water , the active compound Mupirocin and the herbal extract were subjected in to the polymeric solution to obtain the homogeneous mixture. The obtained mixture was stirred well before the electrospinning. The parameters for electrospinning, Flowe rate – 8.33 ml/hr, Voltage-20kv, Distance between drum and syringe = 23 cm After completing the electro spined aluminium foil (collector) was dried for 2 days in open air drying The dried foil was cut into the 1x1 cm and the soaked in the EDC solution for 12hr and the soaked in the PBS solution for 5 hrs in the mechanical shaker The nanofiber was get separated in the PBS solution.

Table 1: formulation table

Ingredients	Amount	Solvent
Gelatin	19%	Acetic acid :DMSO (93:7)
Chitosan	2.5%	
Mupirocin	1%	
Herbal extract	2.5%	
PVA	2.5%	Distilled water

FT-IR Spectrophotometric Study of Nanofibers Formulation

FTIR analyses were carried out to determine if the produced nanofiber compositions included any functional groups. There was no evidence of any

drug-excipient interaction. Using an FTIR Spectrophotometer, the FTIR spectra of each produced formulation were obtained. The scanning range used on them was 500–4000 cm-1.

Scanning Electron Microscopy (SEM)

The fiber formation of nanofiber was viewed and photographed using scanning electron microscope The nanofiber was first mounted on a stub for analysing , before that it was ensured that the stub wss clean and devoid of contaminants. In order to clean the stubs ethanol or IPA was used . Use a conductive material to sputter coat the sample (Gold Sputter coating) Once these procedure was over, load the sample in the HRSEM Instrument chamber The image was captured at desired magnification

XRD STUDIES:

At 25ºC, the XPERT-PRO diffractometer was utilized to describe the herbal extract, nanofiber, and pure medicine. Cu-Kf_0 (λ=1.54060 Å) was used together with 45 kV and 40 mA. Data was collected using a specimen with a length of 10 mm, covering an angular range of 10° to 90° at 2θ. The continuous scan mode was employed with a step size of 0.017° 2θ and a scan speed of 1.0 min^{-1}.

CONTACT ANGLE:

When considering the hydrophilicity of electrospun nanofibers for use in medical applications, particularly as wound dressings that come into touch with blood and injured skin tissue, it was important to consider other factors in addition to chemical and morphological criteria. An ossila goniometer was used to measure the contact angle on a prepared 4 x 4 x 1 mm dentine slab in order to assess the wettability. After applying an irrigant droplet to the sample's surface, the contact angle was determined by analyzing images of the droplets taken with a digital camera that was fastened to a goniometer. (Maker: Ossila; Model: Contact Angle Goniometer)

IN-VITRO DRUG RELEASE:

To evaluate the drug release profile of the nanofiber formulation, an in-vitro drug release study was conducted using a Franz diffusion cell equipped with a cellophane or dialysis membrane. The membrane was pre-soaked in PBS for several hours before the experiment. The nanofiber formulation was placed in the donor compartment, while the receptor chamber was filled with phosphate buffer solution (pH 7.4). The setup was maintained at 37 ± 0.5°C and stirred at 50 rpm using a magnetic stirrer. At predetermined time points (0.5, 1, 2, 4, 6, 7, and 8 hours), 1 mL of the release medium was withdrawn from the receptor compartment using a syringe. To ensure sink conditions, the withdrawn volume was replaced with an equal amount of fresh buffer. The collected samples were analyzed at a wavelength of 220 nm using a UV-Vis spectrophotometer.

ANTIMICROBIAL ACTIVITY:

The process of eliminating or impeding the microorganisms that cause disease is known as antimicrobial activity. Antimicrobials can have antiviral, antifungal, or antibacterial properties. The many mechanisms of action of antibacterial activity work to inhibit infection. The agar well diffusion technique is commonly used to evaluate the antimicrobial activity of plant or microbial extracts. The method typically involves the use of Muller-Hinton agar, which is considered optimal for diffusion studies. The Muller-Hinton agar medium was prepared and sterilized using an autoclaving process at 121°C for 15 minutes. After cooling to 50°C, the medium was transferred onto sterile Petri dishes and given time to solidify. A volume of microbial inoculum that has been evenly distributed throughout the agar plate's whole surface

Various microbiological cultures were inoculated, including Bacillus subtilis, Pseudomonas, E. coli, Streptococci, Staphylococci, and Enterococci. The zones of inhibition were measured in millimeters after the plates were incubated for 24 hours at 37°C. A clear zone of inhibition was produced across all of the isolates in various diameter measures (in millimeters) as a consequence of the antimicrobial activity analysis conducted on the isolated and purified bactericidal activity

CELL LINE STUDIES

IN VITRO MATERIALS AND METHOD

HUMAN DPSCS ISOLATION AND CULTIVATION:

NIH 3T3 cell lines were obtained from the National Center for Cell Science (NCCS), Pune, India. The cells were cultured in high-glucose DMEM medium (Sigma-Aldrich, USA) supplemented with 2% antibiotics (Gibco, Thermo Scientific, USA) and 10% fetal bovine serum. They were maintained in the exponential growth phase at 37°C in a CO_2 incubator (Thermo Scientific, USA) with a controlled environment containing 5% CO_2

CYTOTOXICITY EVALUATION CELL VIABILITY/ MTT ASSAY:

$$\text{Cell viability (\%)} = \frac{OD(\text{test sample}) - OD(\text{blank})}{OD(PC) - OD(\text{blank})} \times 100$$

As previously reported (Koka P et al., 2018), the MTT assay was used to assess the biocompatibility of the membrane over 24, 48, and 72 hours on the NIH-3T3 cell line, alongside a control group. Briefly, the membrane elution medium was used as the experimental group and was incubated with 3T3 cells seeded on 96-well culture plates for 24, 48, and 72 hours. To determine cell viability, 10 µL of stock MTT dye (10 mg/mL) was added to each well, followed by incubation at 37°C for 4 hours. After incubation, 100 µL of DMSO was added to each well to dissolve the formazan crystals. Absorbance at 570 nm was measured using a Synergy Hybrid Multi-Mode Reader (BioTek, Winooski, VT, USA). The percentage of viable cells was calculated using the following formula

RESULTS AND DISCUSSIONS

PHYTOCHEMICAL SCREENING:

The Qualitative examination on plant leaf extract were performed. The results are tabulated in Table.8. In particular, the extract was found to be rich in phytoconstituents like phenols and flavonoids confirming the presence of wound healing activity(10).

Table 2: Preliminary phytochemical screening of Herbal extract

PHYTOCONSTITUENTS	PRESENCE/ ABSENCE
Alkaloids	+
Amino acids	+
Anthocyanin	+
Carbohydrates	+
Flavonoids	+
Coumarins	+
Diterpenes	+
Emodins	+
Fatty acids	+
Glycosides	+
Proteins	+
Phenols	+
Saponin	+
Steroids	-
Tannin	+
Terpenoids	+
Anthraquinones	-

(+) indicates presence (-) indicates absence

According to the table 2 the phytoconstituents present in the sample include alkaloids, amino acids, anthocyanins, carbohydrates, flavonoids, coumarins, diterpenes, emodins, fatty acids, glycosides, proteins, phenols, saponins, tannins, and terpenoids. On the other hand, steroids and anthraquinones are absent from the sample(11)

CALIBRATION CURVE:

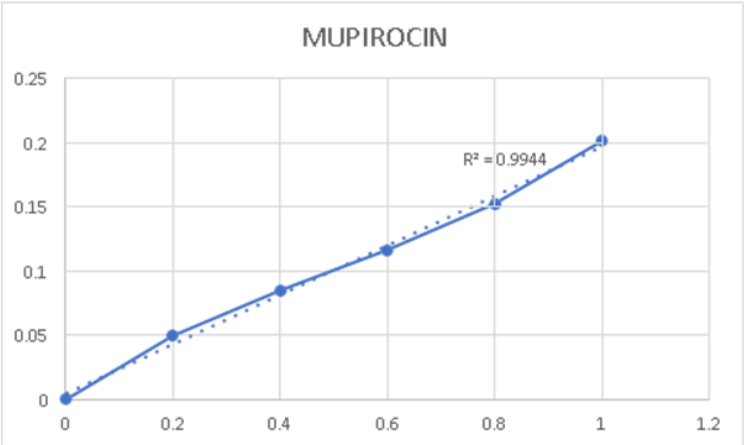


Fig 1: Construction of Mupirocin calibration curve

Table 3: Corresponding to calibration data

Concentration (µg/ml)	Absorbance
2	0.0498
4	0.0851
6	0.1166
8	0.1526
10	0.2014

Drug Mupirocin complied with Beer's Lambert law in the concentration range of 2–10 µg/ml, as demonstrated by the straight line. standard calibration curve that was obtained. The calibration curve was determined to be linear as there was an increase in absorbance with regard to an increase in concentration and the R2 value was 0.9944 (fig1 and table 1) (12

DRUG-EXCIPIENT COMPATIBILITY STUDIES

FOURIER TRANSFORM-INFRA RED SPECTROSCOPY:

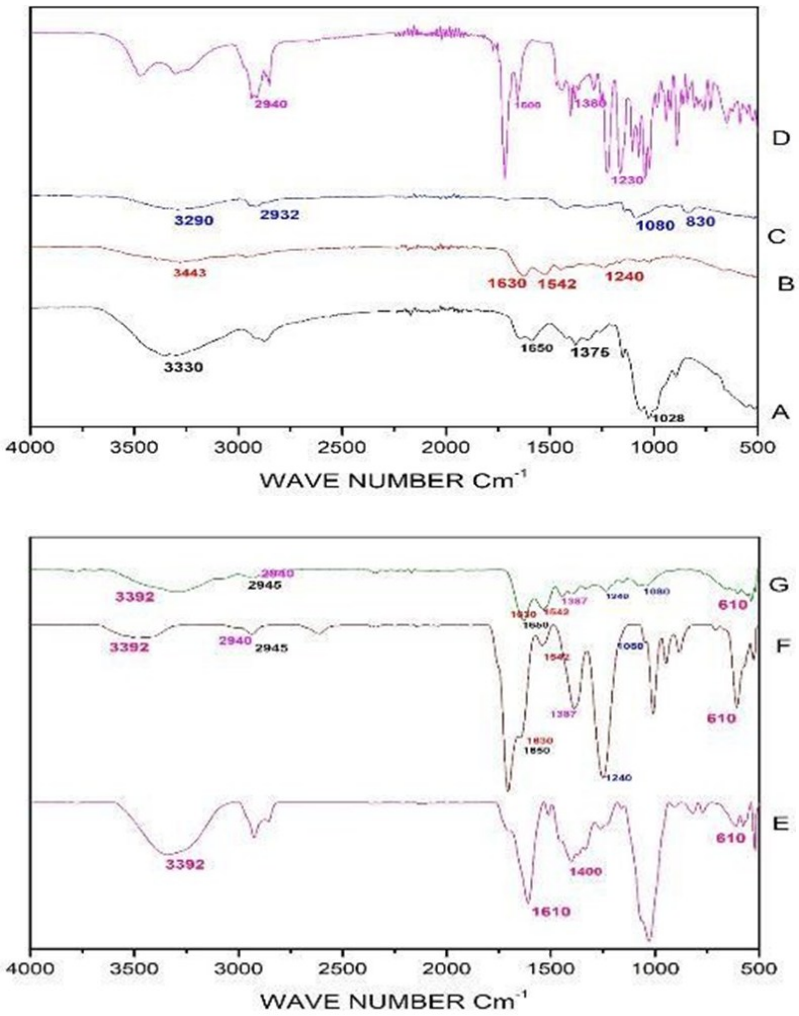


Fig 2: Fourier-transform infrared (FTIR) spectra: A. Chitosan; B. Gelatin; C. PVA; D. Mupirocin; E. Herbal extract; F. Electrospinning Solution; G.Nanofibe

The Fourier-transform infrared (FTIR) spectra for Chitosan, Gelatin, PVA, Mupirocin, Herbal extract, Electrospinning Solution, and Nanofiber are illustrated in (Fig2). There was no overlap of peaks between Mupirocin and excipients like Chitosan, Gelatin, PVA. The peaks wave number corresponding to the functional group are indicated below table 4-7 for the drug A: Chitosan; B: Gelatin; C: PVA; D: Mupirocin E: Herbal extract(13,14,15)

Table4 :A. Chitosan

WAVENUMBER	FUNCTIONAL GROUP
3330	**O-H stretching**
1650	C=O stretching (amide I band)
1375	C-N stretching (amine groups)
1028	C-O stretching (secondary alcohol)

Table4: B. Gelatin

WAVENUMBER	FUNCTIONAL GROUP
3443	C-H stretching
1630	C=O stretching (amide I band)
1542	N-H bending (amide II band)
1240	C-N stretching (amide III band)

Table5: C. PVA

WAVENUMBER	FUNCTIONAL GROUP
3290	O-H stretching)
2932	**C-H stretching**
1080	C-O stretching (secondary alcohol)
830	C-C stretching

Table6:D. Mupirocin

WAVENUMBER	FUNCTIONAL GROUP
2940	C-H Stretching (Alkene)
1600	C=C stretching (alkene groups)
1380	C-H bending
1230	**C-O stretching (ester and alcohol groups):**

Table7: E. Herbal extract

WAVENUMBER	FUNCTIONAL GROUP
3392	O-H stretching
1610	C=C stretching (aromatic rings)
1400	C-H bending (methyl and methylene groups)
610	Bromides (C,H)3=-C-Br (strong)

XRD STUDIES:

XRD patterns ascertains the nature of the compound whether it is crystalline or amorphous and determines various structural characteristics of the crystalline state a-c) shows the x-ray diffraction (XRD) pattern of (a) Herbal extract , (b) Mupirocin and (c) Nanofiber.

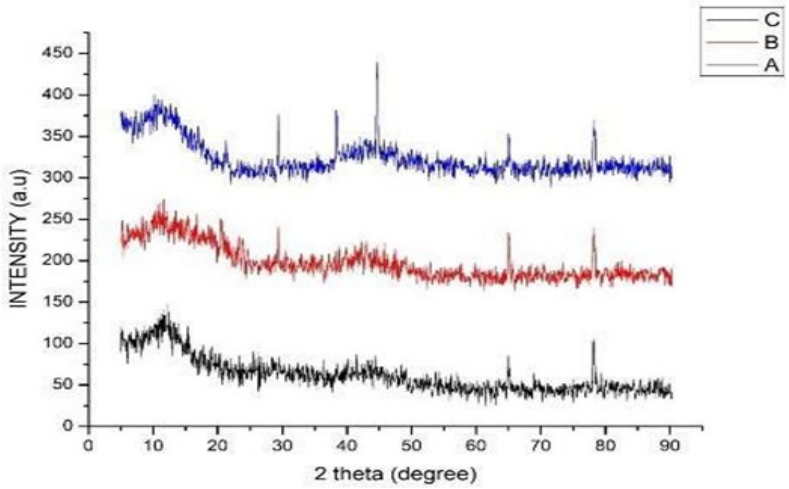


Fig 3:X-ray diffraction (XRD) pattern of (a) Herbal extract; (b) Mupirocin and (c) Nanofiber

For Herbal extract , the peaks were observed at 2 Theta (all degree like 12.302o, 25.541o, 53.704o, 65.006o, 68.960o,79.402o, 78.145o, It shows 51.2% crystalline and 48.8% Amorphous(16).

For Mupirocin , the peaks were observed at 2 Theta (all degree like 20.610o, 23.256o,23.858o, 36.086o, 37.212o,42.928o, 52.886o,52.889o,65.017o, 65.565o, 78.150o). It shows 34% crystalline and 66% Amorphous(17)

For Nanofiber , the peaks were observed at 2 Theta (all degree like 17.061o, 21.286o, 26.716o, 29.378o, 38.372o, 44.665o, 65.026o 78.363o). It shows 33.3 % crystalline 66.7% Amorphous But the spectra of electrospun nanofiber with drug loading and the absorption peaks of Mupirocin and herbal extract did not exhibit any identical characteristic peaks, suggesting that Mupirocin and herbal extract had been bound together. The outcomes which represented in

fig 3 reveals that the materials became more amorphous during the electrospinning process

SCANNING ELECTRON MICROSCOPY (SEM):

The surface morphology of the optimized formulation was investigated using Scanning Electron Microscopy FE-SEM IT800, [JEOL]. The SEM images of formulated Nanofiber was shown in the Fig. . which is prepared by using Electrospinning(14)

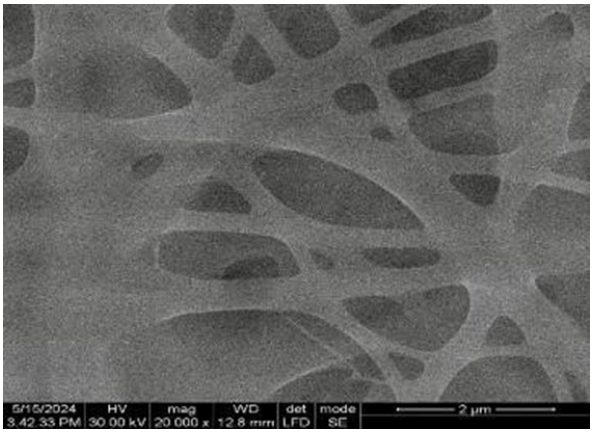


Fig4: SEM Image of Nanofiber - 2µm

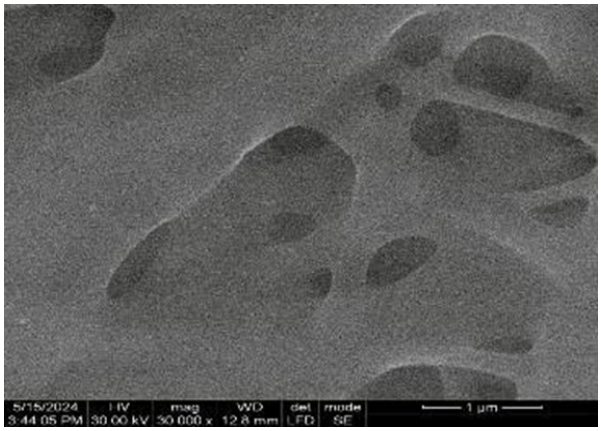


Fig5: SEM Image of Nanofiber - 1µm

Pictures of the prepared nanofibers were taken at different magnifications of 2µm, and 1µm Different diameters of nanofibers were founded has the diameters ranging from 51.8 nm to 350.8 nm shown in figure 4 and 5.

CONTACT ANGLE:



Fig 6: water contact angle for formulated nanofiber

An optimal range less then 90o, indicating a highly hydrophilic surface. This range ensures that the nanofiber material can effectively interact with biological tissues and fluids, promoting better healing and integration. The formulated nanofiber has the 34.25o Left angle and 36.50o Right angle which showed the hydrophilic nature would be appropriate for achieving good performance in biomedical applications. Where the Hydrophilic materials can absorb exudates from wounds, maintaining a moist environment that promotes faster healing. Moist wound environments help reduce the risk of infection and enhance the activity of enzymes involved in tissue repair(18).

IN-VITRO DRUG RELEASE:

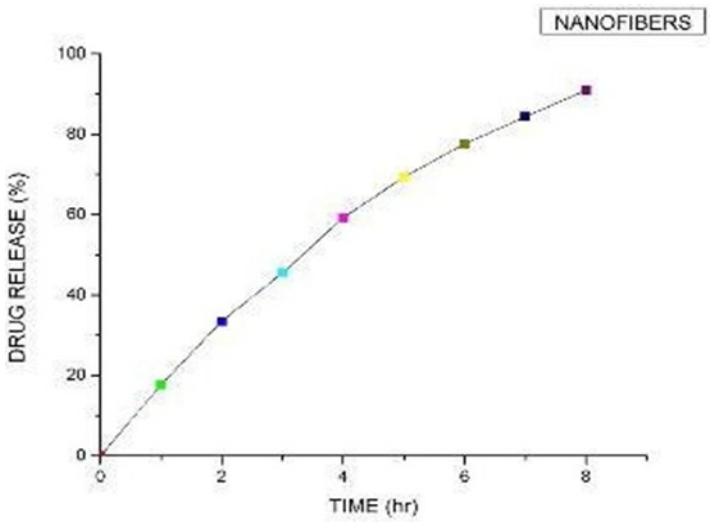


Fig 7: Invitro drug release profile of formulated nanofiber

Table 8 : In vitro Release data of formulated nanofiber

Time (Hours)	% Drug release
0	0
1	17.72
2	33.56
3	45.54
4	59.28
5	69.42
6	77.67
7	84.39
8	97.02

The drug release from nanofiber may be significantly, carried out under sink conditions by Franz diffusion cell apparatus. Nanofiber formulation showed drug release of about 97.02% at the end of 8 hours of study.. In the in-vitro drug release kinetics of Nanofiber, various mathematical models including Zero Order, First Order, Higuchi, Koremeyer-peppas, and Hixson-Crowell were employed. Based on the R2 values, the formulation exhibits significant adherence to both First Order (0.9747) and Hixson crowell (0.9977) kinetics for drug release(17)

ANTIMICROBIAL ACTIVITY:

The table9 shows the zone of inhibition (in mm) of different antibacterial substances (Kanamycin, Tetracycline, and Nanofiber) against various bacterial organisms. The larger the zone of inhibition, the more effective the antibacterial agent is in preventing the growth of the organism. Hence the formulated nanofiber showed greater zone of inhibition compare with the standard which proved that the formulated nanofiber had the potential antimicrobial activity(17,19).

Table9: Zone of inhibition for nanofiber

ORGANISM	Antimicrobial substance	Zone of inhibition (mm)
Bacillus	Kanamycin Tetracycline Nanofiber	24 22 26
E. coli	Kanamycin Tetracycline Nanofiber	19 15 20
Staphylococcus aureus	Kanamycin Tetracycline Nanofiber	20 18 21
Streptococcus	Kanamycin Tetracycline Nanofiber	18 15 20
Pseudomonas	Kanamycin Tetracycline Nanofiber	21 19 22



Fig 8: Invitro Antimicrobial activity for nanofiber (N-Nanofiber, K-Kanamycin T- Tetracycline)

The fig 8 showed that the growth of microbial content compare with the other standards had the greater zone of inhibition which provers that the formulated nanofibers has the potential antimicrobial substance.

CELL LINE STUDIES:

BIOCOMPATIBILITY OF MEMBRANE BY MTT ANALYSIS

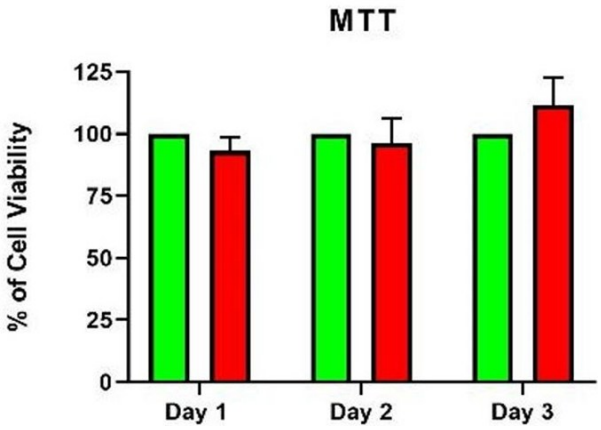


Fig 9: Biocompatibility and cell proliferation levels

The Biocompatibility and cell proliferation levels with control and experimental group (Nanofibers) for different time point of incubation (Day 1, Day 2 & Day 3), graph represented the rate for cell proliferation (Percentile) exponentially increased upon the membrane elute medium when compared to control cells (fig 9 green- standard, red -nanofiber)

CELL MORPHOLOGY STUDY USING PHASE CONTRAST MICROSCOPY:

Morphological evaluation of the Biocompatibility and cell proliferation levels with control and experimental group (Membrane) for different time point of incubation (Day 1, Day 2 & Day 3), using phase contrast microscopy (20x objective)

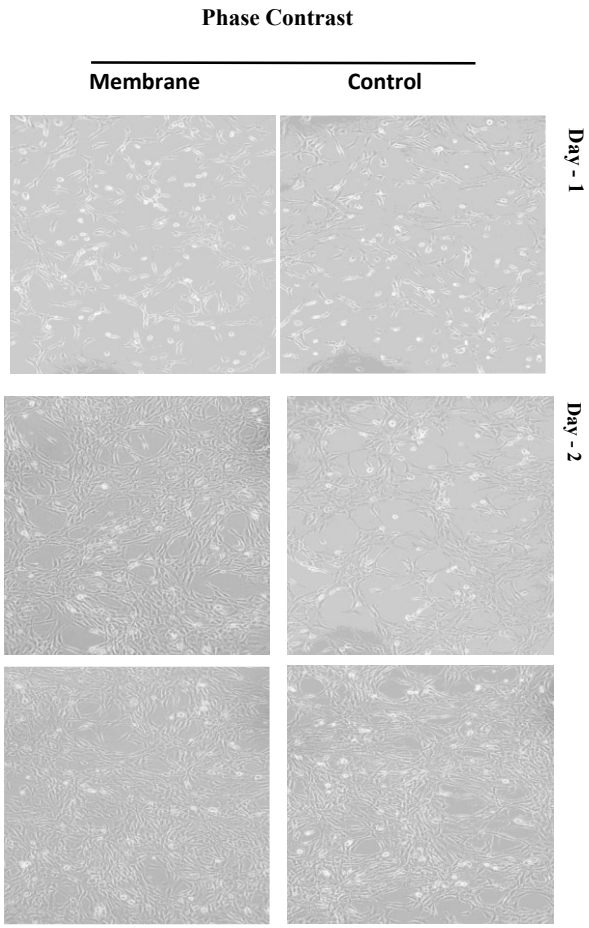


Fig10: Cell morphology using Phase contrast microscopy

The graph illustrated about the cell viability in control and experimental groups (nanofiber) over three days, measured by the MTT assay. Both groups showed similar levels of cell viability on Day 1 and Day 2. By Day 3, the experimental group (Nanofiber) demonstrates a higher cell viability compared to the control group. This suggested that the nanofiber elute medium enhanced cell proliferation over time, with a noticeable increase in cell viability from Day 1 to Day 3 in the experimental group. The data indicated that the nanofiber was a potential biomaterial for improving biocompatibility and cell proliferation(20)

CONCLUSION:

The electrospun nanofibers composed of chitosan, gelatin, PVA, mupirocin, and Aegle marmelos leaf extract exhibited favorable physicochemical properties, sustained drug release, and significant antimicrobial activity, alongside good biocompatibility with cell lines. These characteristics suggest that the formulated nanofibers could serve as effective materials for wound healing applications and other biomedical uses, offering potential advantages in infection management and tissue regeneration Additional in vivo studies are recommended to assess the performance and safety of these nanofibers in clinical settings

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HOW TO CITE: Logeswaran K, Jerad Suresh A, Alan Mathew Punnoose, Vijila Helen Mary, Nagalakshmi S, Bioactive Extract of Aegle marmelos Blended with Mupirocin Electrospun Nanofiber for Accelerated Wound Healing, J. Pharm. Sci., 2025, 1 (11), 576-588. https://doi.org/10.5281/zenodo.17604985