

Developing and Screening of a Bacteria-Fighting and Moisture-Controlling *Coccinia grandis* and Poly(vinyl alcohol) Electrospun Nanofibrous Mat

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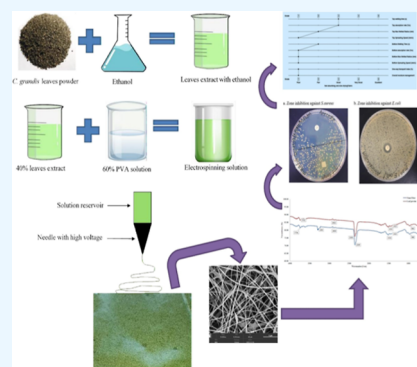
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ABSTRACT: Nanofibers are extensively employed in the antimicrobial industry owing to their remarkable properties and diverse applications. Managing wounds poses a significant and enduring challenge for healthcare systems globally. This study aims to produce and evaluate electrospun nanofiber mats made from poly(vinyl alcohol) (PVA) and *Coccinia grandis* (*C. grandis*) leaf extract, highlighting the medicinal properties of this herbal product for potential biomedical applications (wound dressing). During the evaluations, a 60:40 ratio of PVA to leaf extract was found to be suitable, and the electrospinning process was utilized for production. Scanning electron microscopy was employed for morphological assessment, and an antibacterial assay was conducted against *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) to evaluate cell cytotoxicity. Additionally, Fourier-transform infrared spectroscopy (FTIR) and moisture management behavior (moisture management test) analyses were performed on the fabricated electrospun nanofibrous mat. The formation of small beads was confirmed, with the nanofibers having an average diameter of 295.07 ± 0.0032 nm and a porosity of approximately 76%, which is adequate for oxygen circulation and air ventilation, ensuring skin breathability. Gram-positive bacteria (*S. aureus*) exhibited a zone of inhibition (ZOI) of 14 mm, while Gram-negative bacteria (*E. coli*) showed a ZOI of 10 mm, attributed to the presence of a thick peptidoglycan cell wall in Gram-positive bacteria with no cell toxicity (100% cell viability). FTIR confirms the formation of weak van der Waals bonds and represents H-bonds between PVA polymer and *C. grandis* leaf extract. Furthermore, according to the MMT analysis, the electrospun nanofibrous mat demonstrates rapid absorption and slow drying properties. Therefore, the produced electrospun nanofibrous mat could prove beneficial for wound dressing purposes.



1. INTRODUCTION

The medical industry is shifting toward wound dressings with herbal ingredients rather than metal-based nanoparticles for antifungal and antibacterial benefits. Even today, herbal remedies are still used for basic healthcare, including wound healing and burn injuries, as well as antifungal, antiviral, and antibacterial applications for skin infections.^{1,2} Wound dressing materials must provide a micromost environment, protect against bacterial invasion, limit tissue damage, avoid dehydration, and be biocompatible and biodegradable.³ Natural herbs have medicinal characteristics that can replace metallic particles in present dressing products, making the development of such materials more relevant. Aromatic plants have historically been used in traditional medicine to extend food lifespan because of their ability to inhibit the growth of bacteria, fungi, and yeasts.⁴ Researchers studying infectious diseases have shown a strong interest in natural compounds with biological activity.⁵ Wound care, tissue engineering, and drug research are among the many biomedical applications for electrospun nanofibrous materials.⁶ These notable properties,

such as biocompatibility, high porosity structure, and superior pore interconnectivity and surface area, have the potential to be liable. This technology is very adaptable, low-cost, capable of creating continuous nanofibers, simple to functionalize the surface, industrially relevant, and unique in that it can manufacture fibers ranging in size from several micrometers to nanometers when electrical forces are applied.^{7,8} A flexible method for producing nanofibers with sizes varying from a few nanometers to several micrometers is electrospinning. A polymer solution is subjected to a high voltage, which creates a jet that is drawn in the direction of a collector.⁹ The researchers added a natural antibacterial peptide and an anti-

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Figure 1. Schematic diagram of solution preparation and fabrication [visual representation made by Md. Redwanul Islam].

inflammatory drug to the nanofibers.¹⁰ In vitro and in vivo, the nanofibrous membrane efficiently prevented bacterial growth and decreased the level of inflammation. Furthermore, electrospun nanofibrous mat have been employed in the development of antimicrobial air filtration systems.¹¹ The inclusion of antimicrobial compounds into nanofibers has resulted in the creation of numerous antibacterial materials that might be used in wound dressings, air filtration systems, and other biomedical disciplines.¹⁰

Natural herbs have been investigated as possible biopolymer sources for electrospinning.¹² Aloe Vera gel, for example, has been electrospun with poly(vinyl alcohol) (PVA) to form nanofibers that might be used in wound dressing and tissue engineering.¹³ Basil essential oil has been electrospun into PVA nanofibers with potential antibacterial characteristics for application in food packaging.¹⁴ Ginger,¹⁵ eucalyptus,¹⁶ turmeric,¹⁷ and other natural biopolymers are already employed in the creation of nanofibrous mats for a variety of applications.

Owing to a number of advantageous characteristics such as the soluble nature in water, biological adaptability, drug

delivery, reliability, mechanical attributes, and customizable features, PVA is a polymer matrix that is frequently utilized in electrospinning for biomedical purposes.¹⁸ For single or bilayer approaches, PVA solution with a concentration of 5–10% (w/v) produces good fiber formation.⁹ Furthermore, “green polymer” PVA possesses superior film-forming capabilities and is nontoxic.¹⁹ For tissue engineering applications, qualities such as strong tensile strength and flexibility are preferred. These characteristics provide the electrospun PVA fibers the ability to sustain mechanical loads and give the surrounding tissue structural support.²⁰

Coccinia grandis is a kind of plant native to Asia and Africa that is also known as the ivy gourd or scarlet gourd. It is known as “Telakucha” in Bangladesh. It has been used for a variety of purposes in traditional medicine, including as a therapy for diabetes, fever, and inflammation.²¹ The antioxidant and anti-inflammatory effects of *C. grandis* were examined in the Journal of Ethnopharmacology in 2018. The ethanolic extraction of *C. grandis* was discovered to have substantial antioxidant, anti-inflammatory properties, and antidiabetic efficacy.^{22,23} The antibacterial efficacy of *C. grandis* extracts against many

bacterial species, including *Staphylococcus aureus* and *Pseudomonas aeruginosa*, was investigated in a paper published in the Journal of Applied Biology and Biotechnology in 2022.²⁴ The incorporation of such a *C. grandis* extract into nanostructures is expected to accelerate healing, boost bacterial resistance, minimize air resistance, and function as a viable wound dressing material and filtering media.²⁵

Nonetheless, the production of nanofibrous mats from *C. grandis* extract was paired with a PVA polymer solution, which has yet to be done. As a result, the purpose of this study is to design a nanofibrous mat that uses *C. grandis* as a natural antibacterial agent. The inventiveness of this work can be justified by the fact that it had not been wrapped up previously. The properties of the synthesized nanofibrous mats will be investigated for their possible biological applications. The nanofibrous mat is a promising material for wound treatment in therapeutic applications.

2. MATERIALS AND METHODS

2.1. Materials. The *C. grandis* leaves were purchased from the Jatrabari local market in Dhaka, Bangladesh. Loba Chemical (India) supplied PVA (molecular weight: 115,000 g/mol, 15,000; DP: 1700–1800; viscosity: 26–32 cps, 99% hydrolyzed granules) and Merck, Germany supplied absolute ethanol (99% purity). All of the chemicals were of analytical purity and used without additional purification.

2.2. *C. grandis* Leaf Extraction. The leaves were first thoroughly cleansed with fresh water and sun-dried. The dried leaves were then coarsely pulverized with a blender, and 10 g of this powder was added to a flask containing 100 mL of ethanol. The flask was left at room temperature for 24 h to achieve proper maceration. After being filtered using a nylon mesh, the macerate was employed directly as *C. grandis* extracts.

2.3. PVA Solution Preparation. The PVA solution was made 10% (w/v) by dissolving 10 g of PVA polymer in 100 mL of distilled water. At 70 °C, the solution was agitated in a magnetic stirrer until a crystal clear PVA solution was produced. It was preserved in a glass beaker for future use.

2.4. Electrospinning Solution Preparation. The electrospinning solution was made by combining *C. grandis* leaf extract in a 40:60 ratio with PVA solution. The mixture was stirred in a magnetic stirrer at room temperature for 2 h so that the solution mixed properly. So ultimately, 4 g of *C. grandis* leaf powder was present in 100 mL of the electrospinning solution.

2.5. Fabrication of Nanofibrous Mat. The electrospinning process requires careful consideration when setting up the variables, as they affect the shape and properties of the nanofibrous mat. The fabrication was carried out using a single electrospinning machine (model: TL-Pro-BM; company: Tong Li Tech; origin: China). A total of 40 mL of solution was made, consisting of 14 mL of leaf extract and 26 mL of PVA solution. Figure 1 illustrates the schematic diagram of the solution preparation and the fabrication process of the electrospinning nanofibrous mat. The prepared solution was placed into the machine using a 30 mL syringe, with the settings adjusted to a flow rate of 2.8 mL per hour, a positive voltage of 22–24 kV, a negative voltage of 12–13 kV, a collector distance of 15 cm, and a heater power of 0.35 kW. Nanofibers were consistently fabricated over 8 h using five 20-gauge needles on a rotary collecting drum (500 mm in length, 158 mm in diameter, and rotating at 500 rpm) wrapped in aluminum foil with double-sided tape.

2.6. Characterization. **2.6.1. Scanning Electron Microscopy.** In order to acquire realistic pictures, the orientation and diameter of nanofibrous mat were examined using a scanning electron microscope (ZEISS Sigma 300) at a magnification of 20 KX. Porosity was measured by estimating weight and calculating volume based on material thickness using eqs 1 and 2. Briefly, at 20 °C, the densities of PVA and *C. grandis* extract were 1.19 g/cm³ and 0.57 g/cm³, respectively. To begin, a nanofibrous mat measuring 4 cm × 4 cm was taken, and the weight and thickness were carefully measured using digital balances and micrometers, respectively. A digital thickness meter was used to measure the sample thickness at various points, and an average value of 0.12 mm was taken. Fiber diameter distribution of 50 randomly selected fibers was determined by using ImageJ software.

Density of fabricated nanofibrous mat (g/cm³)

$$\text{density of fabricated nanofibrous mat (g/cm}^3\text{)} = \frac{\text{mass of fabricated mat (g)}}{\text{thickness of the fabricated mat (cm)} \times \text{area of the fabricated mat (cm}^2\text{)}} \quad (1)$$

$$\text{porosity \%} = \left\{ 1 - \frac{\text{density of fabricated mat (g/cm}^3\text{)}}{\text{bulk density of raw polymers (g/cm}^3\text{)}} \right\} \times 100\% \quad (2)$$

2.6.2. Antibacterial Assessment. The antibacterial activity of the developed nanocomposite was tested using the Kirby Bauer agar disc diffusion method with Gram-positive bacteria (*S. aureus*) [American Type Culture Collection (ATCC) 6538]²⁶ and Gram-negative bacteria (*Escherichia coli*) (ATCC 11775).²⁷ Using 1.5 × 10⁴ cfu/mL of subcultured bacteria on tryptic soy agar plates, the qualitative disk diffusion test was carried out to find zone of inhibition (ZOI) and antibacterial activity of the electrospinning nanofibrous mat. Since *S. aureus* and *E. coli* bacteria are primarily responsible for skin wound infections, these two bacteria were selected for the study.

2.6.3. Cell Cytotoxicity. The fabricated nanofibrous mat was further tested using a hemocytometer, a trinocular microscope with camera (Optika, Italy), a biological safety cabinet (model NU400E, NuAire, USA), a CO₂ incubator (NuAire, USA), and a cytotoxicity test. The HeLa cell line from the ATCC²⁸ was used to study cell cytotoxicity. The cells were cultured in 10% fetal bovine serum, 1% penicillin–streptomycin (1:1), and 0.2% gentamicin in Dulbecco's modified Eagle's medium (DMEM, Sigma). 4.0 × 10⁴/200 μL cells were sown in 48-well plates and incubated for 24 h at 37 °C and 5% CO₂. The autoclaved sample was added to each well the next day. After 48 h of incubation, the cytotoxicity was assessed using a reversed light microscope with 10× magnification and triplex wells for the sample. The following formula eq 3 was used to determine the cell viability rate

$$\text{cell viability \%} = \left\{ \frac{\text{(live cells)}}{\text{(total number of cells)}} \right\} \times 100 \quad (3)$$

2.6.4. Fourier-Transform Infrared Radiation Spectroscopy. The elemental composition of PVA nanofibrous mats containing *C. grandis* extract was investigated using an FTIR machine model on an IRPrestige 21, Shimadzu Corporation, Japan. The transmittance spectral profiles were calculated with a 4 cm⁻¹ resolution at the wavenumbers varying from 4000 to 400 cm⁻¹.

2.6.5. Moisture Management Test Behavior. The moisture management test (MMT) qualities were assessed using the AATCC 195-2009²⁹ technique, and the model of the moisture management tester was M290, SDL Atlas, USA. The above-mentioned standard was used to categorize the fabricated electrospun mat based on its liquid interaction by measuring wetting time, absorption rate, maximum wetted radius, and spreading speed of inner and outer surfaces, as well as accumulative one-way transport capacity and overall moisture management capacity (OMMC).

3. RESULTS AND DISCUSSION

3.1. Morphological Analysis of Developed Nanofibrous Mat. The concentration and viscosity of the polymer are the two fundamental factors of an electrospinning solution, whereas the other parameters that determine fiber morphologies include the applied voltage, flow rate, collector distance, solution conductivity, solvent type, and humidity. The major goal of having good qualities is to create bead-free fiber on foil paper.³⁰ But it forms some beads as shown in the SEM image. In this study, the PVA polymer was mixed with different proportions of leaf extract and trials were conducted for electrospinning. The 70:30 and 50:50 electrospun solutions formed droplets during nanofiber formation at the Taylor cone during electrospinning. In contrast, the 60:40 electrospun solution produced small beads in the nanofibrous mat, as shown in Figure 2 along with its SEM image.

The accompanying SEM picture confirmed that the nanofibers were properly formed. Figure 3 presents the histogram of fiber distribution, showing that the average diameter of the fabricated nanofibers was 295.07 ± 0.0032 nm, based on a random sample of 50 fibers. The porosity was 76%, according to eq 2. Tiny pores are beneficial for oxygen circulation and air ventilation in skin breathability. The existence of a nanoporous meshy structure demonstrated the efficacy of the created nanofibrous mat for wound healing. These small holes can help promote early healing by preventing germs from entering the wound (if used as wound dressing materials) while allowing for improved air ventilation.

3.2. Antibacterial Activity. The effectiveness of the electrospinning nanofibrous mat heavily relies on its antibacterial activity, which is crucial for biomedical applications. Figure 4 illustrates the bacterial resistance of the fabricated nanofibrous mat against both Gram-positive and Gram-negative bacteria. The images show the formation of ZOI against these two types of bacteria.

Zone inhibition produced results of 14 mm and 10 mm against *S. aureus* and *E. coli* bacteria, respectively. Gram-positive bacteria are surrounded by a thick peptidoglycan layer, which comes into immediate contact with the extract, resulting in higher bacterial resistance compared to Gram-negative bacteria. The nanomat's ability to exhibit action against Gram-positive and Gram-negative bacteria suggested a broad spectrum of activity, making it feasible to identify antibiotic compounds for biomedical usage.³¹ Bacterial cell membranes include proteins called plasma binding sites that are bound by antibacterial cells. After that, the antibacterial cell starts to function and stops transpeptidase enzymes. Thus, damage to the peptidoglycan layer results from collapse of the amino acid chain. As a result, the bacteria's cell wall is destroyed by this core layer damage. Bacteria then perish. Figure 5 represents the layer of cell wall that is being affected by the leaf extracts. The

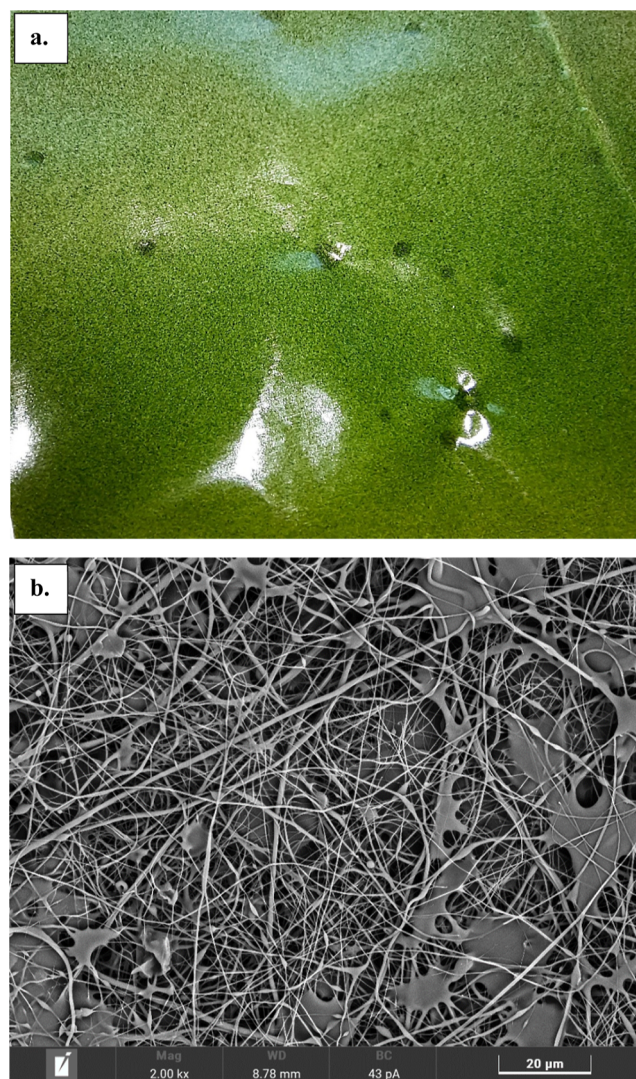


Figure 2. Images of developed nanofibrous mat sample with *C. grandis* leaf extract: (a) original image and (b) SEM image.

ethanol extraction technique aided in increasing the antibacterial activity of the produced sample.

The ethanol extract of *C. grandis* contains phenolic components, flavonoids, tannins, and saponins. Figure 6 depicts five chemicals found in the extracted sample: ferulic acid (1),³² methyl caffeate (2),³³ ligstroside (3),³⁴ trans *p*-coumaric acid (4),³⁵ and kaempferol-3-*O*- β -D-glucoside (5).³⁶ According to studies, *S. aureus* is the principal bacteria that causes the majority of infections in humans, particularly in soft tissues such as the skin and abscesses.³⁷ This experiment demonstrated the efficacy of the *C. grandis* leaf extract against several *S. aureus* strains. *E. coli* is a commensal bacterium found in the human intestine. Nonetheless, certain strains of the bacterium are hazardous and can cause illnesses such as meningitis, septicemia, urinary tract infections, and diarrhea (enteropathogenic *E. coli*).³⁸ The study's findings were similar to previous studies that looked at the antibacterial activity of the *C. grandis* extract against different *E. coli* strains.³¹

3.3. Cell Cytotoxicity. Contrarily, cell culture-based measurement techniques are widely employed in vitro cytotoxicity (cell viability) tests to evaluate possible drug candidates or look into the cytotoxic properties of particular

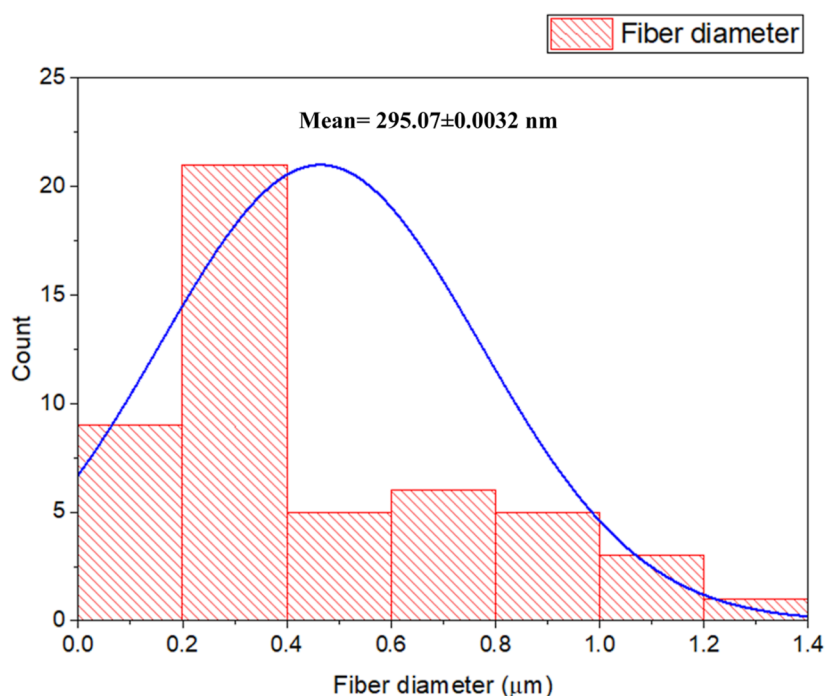
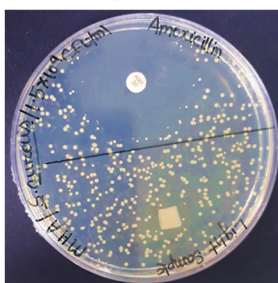


Figure 3. Fiber diameter distribution of fabricated nanofibrous mat.

a. Zone inhibition against *S. aureus*



b. Zone inhibition against *E. coli*

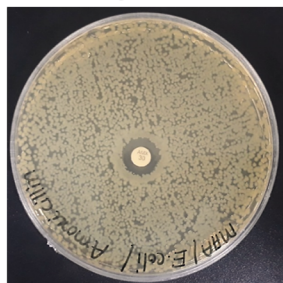


Figure 4. Formation of zone inhibition against 2 types of bacteria [tested and captured from WAFFEN Research Laboratory Limited, Dhaka, Bangladesh].

biomaterials. Huge numbers of nanofibrous mats can be examined quickly, and these methods may provide vital information for upcoming animal studies. Consequently, evaluation of cell survival was necessary to ascertain the biocompatibility of the nanofibrous mat produced in this study.³¹

It was discovered that every cell in the cell line survived (Figure 7) and that no cytotoxicity (100% cell viability) was seen. This nontoxicity is due to the biobased components of the electrospun nanofibrous mat. Consequently, this sample is suitable for animal trials.

3.4. Fourier-Transform Infrared Radiation Analysis.

The presence of PVA polymer in the *C. grandis* nanofibrous mat was confirmed by the FTIR test. The functional groups located from 1600 to 400 cm^{-1} denoted the fingerprint region, and 4000 to 1600 cm^{-1} denoted the functional group region.

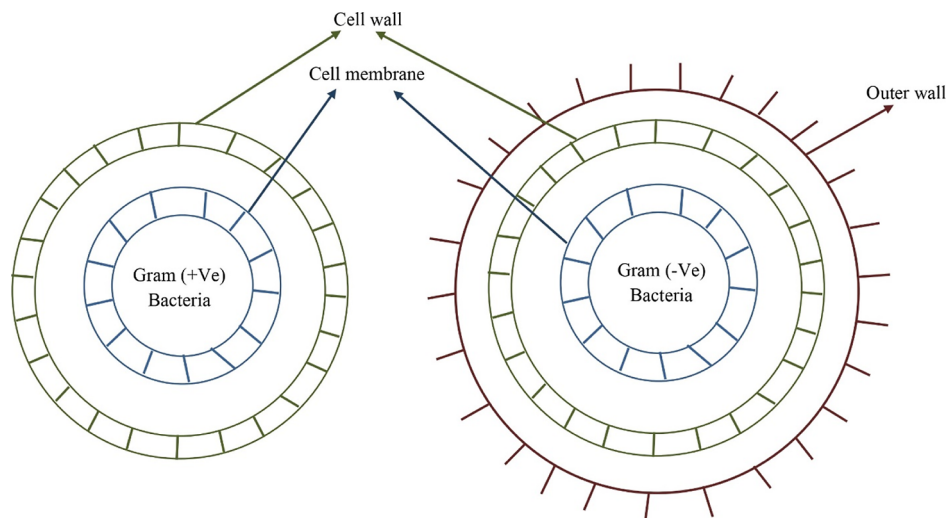


Figure 5. Visual representation of the cell walls of bacteria that are affected with leaf extract.

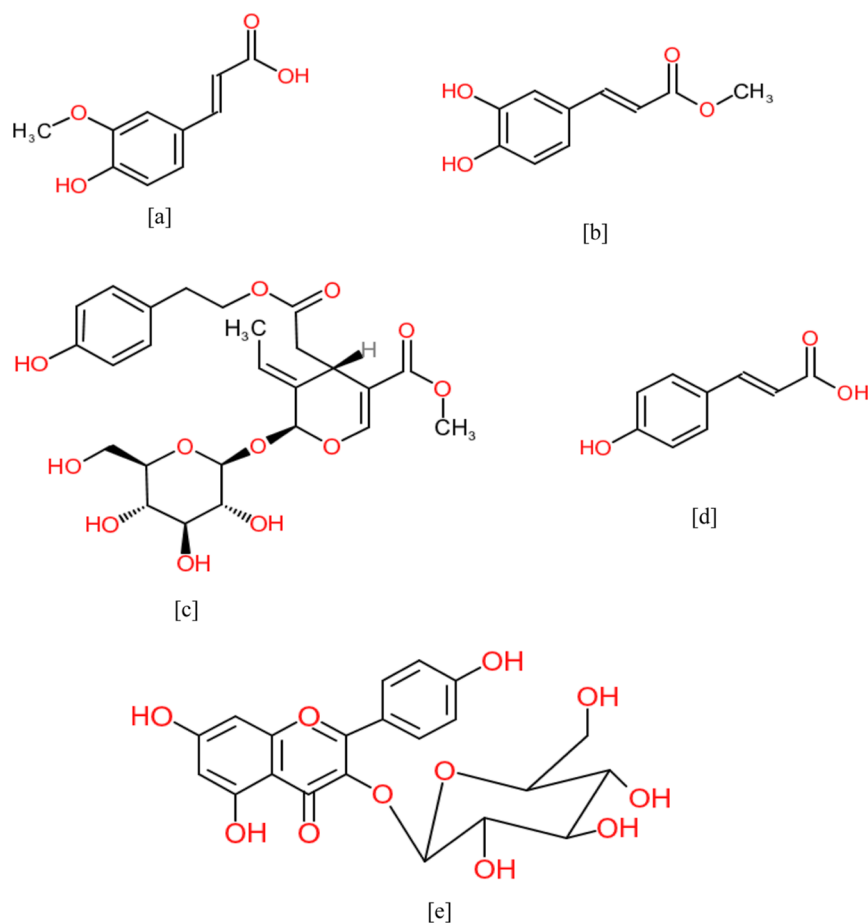


Figure 6. Chemical constituents of *C. grandis* leaf extract: (a) ferulic acid, (b) methyl caffeate, (c) ligstroside, (d) trans *p*-coumaric acid, and (e) kaempferol-3-*O*- β -D glucoside.

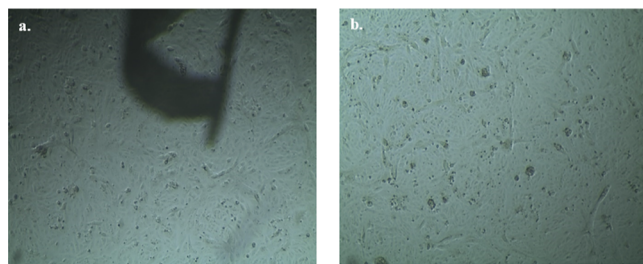


Figure 7. Microscopic image of HeLa cell viability: (a) sample and (b) control.

Figure 8 represents the elementary peaks for *C. grandis* at wavenumbers 1425 cm^{-1} (C=C stretching), 1609 cm^{-1} (C=O stretching), and 2934 cm^{-1} , indicating the presence of alkenes. Moreover, the presence of PVA polymers was found from the characteristic peaks of 3292 cm^{-1} (O–H stretching), 1104 cm^{-1} (C–O–R stretching), and 2845 cm^{-1} (C–H stretching). Kaempferol-3-*O*- β -D-glucoside has a free hydroxyl group and electronegative oxygen present in it.³⁶ The FTIR curve depicted a significant number of individual molecules, and a band broadening after 3000 cm^{-1} points to the development of hydrogen bond.⁹ It is thought that the development of weak van der Waals bonds and representing H-bonds has collaborative potentials within the hydroxyl group of the PVA polymer and *C. grandis* leaf extract, as shown in **Figure 9**.

3.5. Assessment of Moisture Management Test. The appropriate exploitation of a moist environment across the dermal level of human skin is essential for the successful deployment of a nanofibrous mat in biomedical applications, particularly in injury dressing. As a result, investigations focused on how produced mats and liquid interact to determine the amount to which they may transport liquid from the skin to the surrounding environment (e.g., perspiration or water). **Figure 10** depicts the consequence of the produced nanofibrous mat's moisture management behavior. According to the AATCC standard, the inner surface wetting time (9.45 s) and absorption rate (41.76%) were satisfactory. The outer layer took longer, and the absorption rate was likewise low (22.18 s and 4.22%, respectively).

A comparison in **Table 1** displayed the values of MMT properties of several PVA mixed nanofibrous mats with the current work. The maximum wetted radius of the inner and outer surfaces was 10 and 5 mm, respectively. This demonstrated the decreased absorption of the bottom surface due to the sticky characteristic of the PVA polymer, which prevented moisture from sticking to it.

Figure 11 shows that the outside surface absorbed less than the inner surface, allowing for one-way liquid transport from the wound site and perhaps speeding up healing. Nonetheless, using all indicators, the values of the OMMC and one-way transfer capacity (OMTC) revealed that the mat was rapid-absorbing and slow-drying.

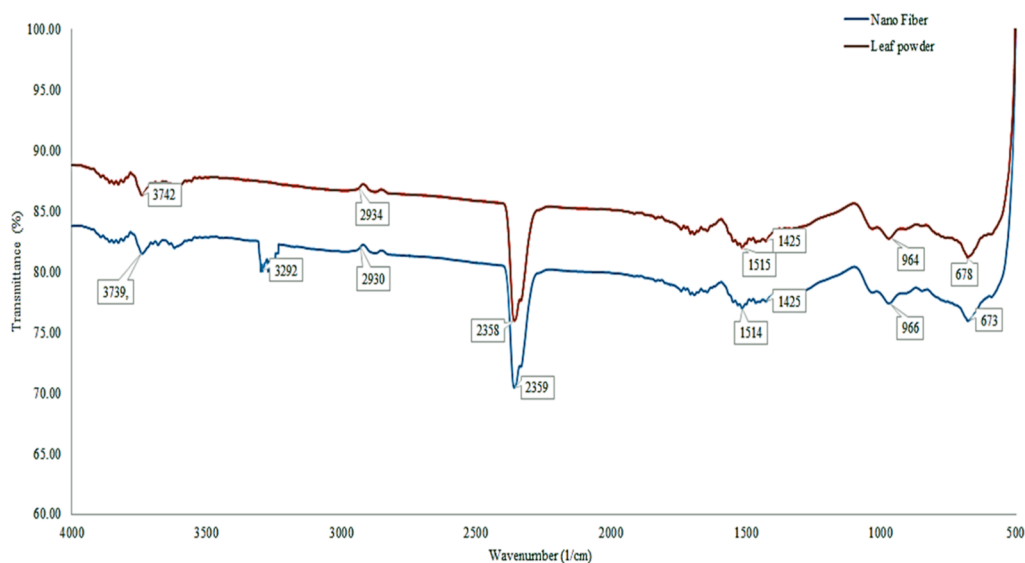


Figure 8. Elementary peaks of *C. grandis* leaf powder and nanofibrous mat.

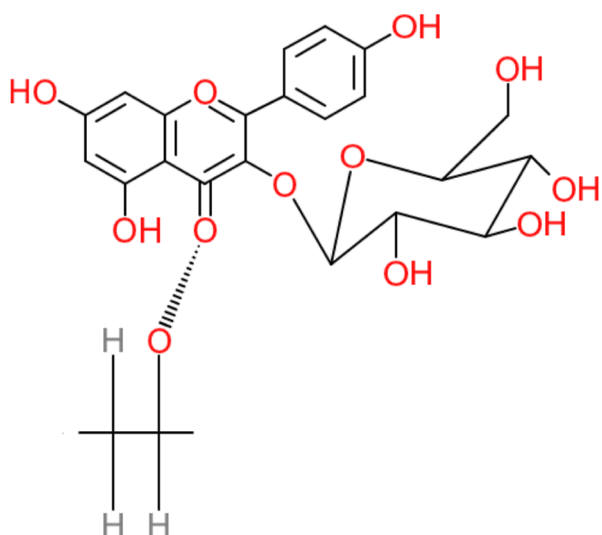


Figure 9. Proposed H-bonding between PVA and *C. grandis*.

4. CONCLUSIONS

This study meticulously fabricated a PVA/*C. grandis* leaf extract electrospun nanofibrous mat by carefully controlling each critical parameter during the production process. Various analyses were performed, including morphological studies, antibacterial assays, cell cytotoxicity, FTIR, and moisture absorption tests. Nanofibers were successfully prepared with an average diameter of 295.07 ± 0.0032 nm, forming small beads and exhibiting a porosity level of 76%. The tiny holes in the fabricated mat enhance oxygen circulation and air ventilation, improving the skin breathability. The produced nanofibrous mat demonstrated excellent antibacterial efficacy, providing outstanding protection against *S. aureus* and *E. coli*, with ZOI measuring 14 and 10 mm, respectively. Additionally, no cell toxicity was observed as all cells in the cell line survived. Proposed hydrogen bonds were identified between the hydroxyl groups of the PVA polymer and the *C. grandis* leaf extract. The fabricated mat was quick-absorbing and slow-drying, which helped maintain a moist environment for wounds. Overall, this biobased electrospinning nanofibrous

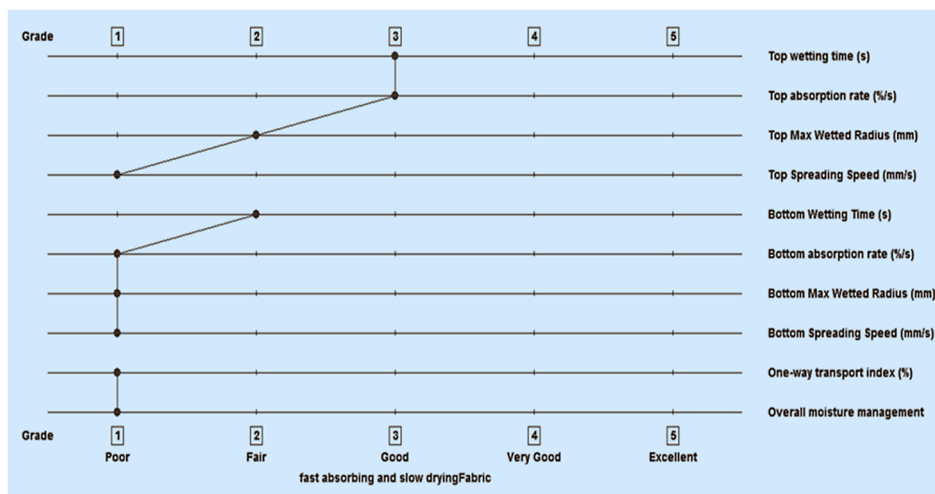


Figure 10. Grading for MMT properties.

Table 1. MMT Values of Different PVA Polymers Incorporated with Various Extracted Materials

| nanofibrous mat | | | wetting time (s) | | absorption rate (% s) | | wetted radius (mm) | | spreading Speed (mm/s) | | ATOI (%) | OMMC | reference |
|-----------------|------------------------|-------|------------------|---------------|-----------------------|---------------|---------------------|---------------------|------------------------|---------------|----------|--------|--------------|
| carrier | extraction mixed | ratio | inner surface | outer surface | inner surface | outer surface | inner surface (max) | outer surface (Max) | inner surface | outer surface | | | |
| PVA | C. grandis leaf | 60:40 | 9.54 | 22.18 | 41.76 | 4.22 | 10 | 5 | 0.567 | 0.2235 | −226.274 | 0 | present work |
| PVA | Mikania micrantha leaf | 40:60 | 6.08 | 6.27 | 79.67 | 6.39 | 10 | 10 | 0.88 | 0.85 | −928.11 | 0 | 39 |
| PVA | Betel leaf | 80:20 | 3.27 | 4.57 | 40.25 | 25.3 | 5 | 5 | 1.44 | 0.58 | −9.57 | 0.0449 | 3 |
| PVA | Nigella sativa | 60:40 | 11 | 8.5 | 6.2 | 85.3 | 5 | 5 | 0.5 | 0.5 | 1034.5 | 0.7 | 40 |

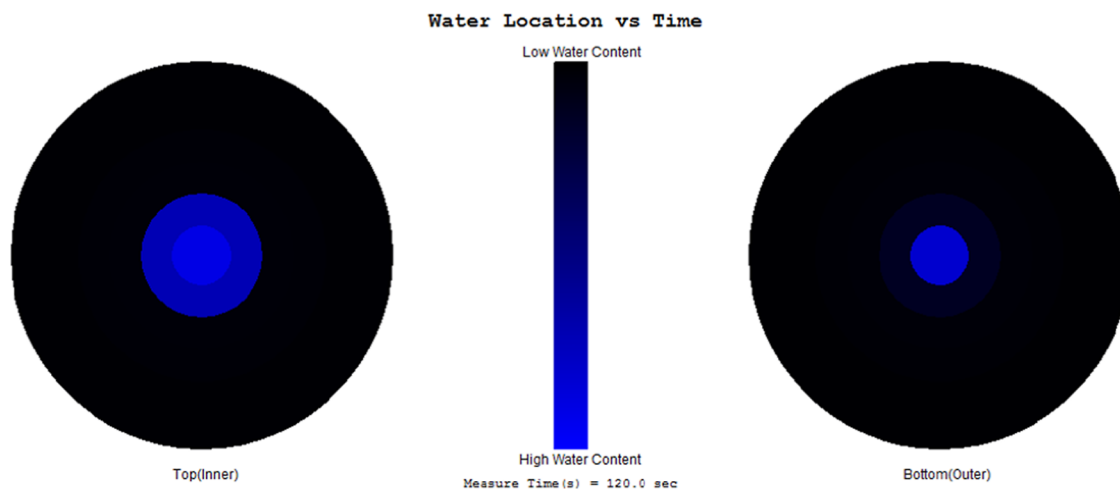


Figure 11. Water absorption vs time diagram [time 2 min].

mat has adequate fiber distribution, antibacterial properties, cell viability, and moisture management, making it a promising candidate for wound dressing materials.

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Author Contributions

FE. Karim designed the methodology and experiment and synthesized the leaf extract; S. Raoha and T. Jahan formulated the electrospun solution and fabricated the nanofibrous mat; R. Islam prepared the original draft with R. Alam; and S. Haque conceptualized and supervised the study and revised the manuscript.

Notes

The authors declare no competing financial interest.

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