

Preparation and characterization of electrospun PVA nanofibrous mat with Bryophyllum pinnatum for biomedical applications.

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Research Article

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Abstract

The present study represents the development of Nano mat from the mixer of PVA and *Bryophyllum pinnatum* (*B. pinnatum*) leaves extract that provides better performance as a biomedical material, especially in wound healing applications. After electrospinning, the mat underwent a series of analyses to determine its properties and structural composition. Scanning electron microscopy (SEM) was used to examine its morphological structure, revealing the detailed arrangement and surface features of the nanofibers. A moisture management tester (MMT) assessed the mat's ability to handle moisture, offering insights into its practical application potential. Fourier transform infrared (FTIR) spectroscopy was conducted to confirm the presence of chemical components derived from *B. pinnatum* within the nanofibers, ensuring the integration of the plant's properties into the material. These combined techniques provided a comprehensive evaluation of the mat's functional and structural attributes. Furthermore, the antibacterial performance of the nanofiber mat against *Staphylococcus aureus* (*S. aureus*) was studied. Result shows, the developed mat reveals an outstanding antibacterial property by forming a 10 mm inhibition zone against the abovementioned bacterial cells. MMT shows the rating of top wetting time, bottom wetting time and OMMC is good where top absorption rate is very good & bottom absorption rate is fair. The SEM analysis demonstrated the fiber formation having different diameter at a value of 174.45 nm along with a moderate level pore size distribution having 51.45% porosity and FTIR spectra analysis corroborate the availability of *B. pinnatum* components within PVA-*B. pinnatum* extract which is attributed to the better absorbing properties that could make it a promising wound healing material candidate.

Introduction

Nanofiber membranes possess several distinctive characteristics that make them highly versatile. Their specific surface area can range widely, from 1 to 35 m² per gram, depending on the diameter of the fibers. This substantial surface area enhances their effectiveness in various applications. Additionally, these membranes exhibit robust pore interconnectivity, allowing efficient fluid or gas flow. They can also incorporate active chemical components or functional properties at the nanoscale, further broadening their potential uses.(Barhate et al., 2006) There are a number of techniques for creating nanofibers, including forcespinning, bicomponent spinning, melting, drawing, and flash-spinning, electrospinning. (Nayak et al., 2012) Electrospinning is a remarkable and efficient technique for creating ultrafine, continuous fibers at the sub-micron or nanoscale. This method can produce fibers with diameters ranging from just a few tens of nanometers to several microns, offering exceptional precision and versatility in fiber fabrication. Electrospinning is the method that produces nanofibers which is the most versatile.(Gibson et al., 2001; Greiner & Wendorff, 2007; Khajavi & Abbasipour, 2012) Electrospinning is a versatile and increasingly popular method for creating fibers through the application of electrostatic forces. It allows the production of polymer fibers with incredibly small diameters, ranging from as little as 2 nanometers to several micrometers. This technique accommodates solutions made from both natural and synthetic polymers, making it highly flexible for diverse uses. Over the years, interest in

electrospinning has grown substantially in both academic research and commercial industries, reflecting its broad applicability and potential.(Bhardwaj & Kundu, 2010)

Nanostructures, primarily composed of nonwoven fibers, have colloidal length scales, meaning their widths are primarily between tens and hundreds of nm. Also, numerous techniques for generating scaffold have been studied.(Geng et al., 2005; Shahid & Khan, 2022) A variety of materials may be utilized to form nanofibers, and the choice of material is dictated by how the developed substances will be used in the end. The ideal choice for constituent components in the creation of the nanofiber scaffold is to use substances that are naturally occurring. In basic healthcare, the application of medicinal plants to human skin for wound dressing, burn and wound healing, as well as antibacterial, antiviral, and antifungal applications for treating skin infections, has a long history.(Bihani & Mhaske, 2020; Shahid et al., 2022) Because natural antibacterial agents are harmless and environmentally benign, they can be a more effective substitute for synthetic particles like silver nanoparticles, which are employed in wound dressings.(Abid et al., 2019; Shahid & Khan, 2022; Shahid et al., 2022) Most widely utilized pharmaceuticals are made mostly from natural resources, such as plants or herbs. From this perspective, *B. pinnatum* has been traditionally used in various folk medicine practices but commonly known as the air plant, cathedral bells, life plant, or "Pathorkuchi" in some regions, is a succulent plant that belongs to the Crassulaceae family.(Akinpelu, 2000) A class of particularly active compounds called bufadienolides is found in the leaves. Bufadienolides, such as bryotoxin A, B, and C, have antitumorous, antibacterial, cancer-preventive, and insecticidal properties. They also have a structure and activity with digoxin and digitoxin, two additional cardiac glycosides.(Akacha et al., 2016; Mbachu et al., 2019; McKENZIE et al., 1987; Yamagishi et al., 1989) *B. pinnatum* functions as a tonic to enhance respiration, help with toxin removal, and enhance general vigor.(Ofokansi et al., 2006) It might have use, mostly as a wound treatment in the form of extracts from *B. pinnatum* leaves. PVA is utilized as a carrier with the extract of *B. pinnatum* leaves in order to make things more ordered and better than they would be if they were considered independently during manufacturing.

Experimental

Materials

Fresh matured leaves of *B. pinnatum* were collected from the garden of Textile Engineering College, Noakhali, Bangladesh. Then these were cleaned by fresh water and dried in oven dryer at 45⁰c for 48 hours. Dried leaves were converted into powder by using electronic blender. PVA (molecular weight 115000, viscosity 6.8-9.2, Degree of hydrolysis 86-89%) and silver nitrate were collected from Merck, Germany and distilled water was used as solvent to prepare PVA solution. Absolute methanol was used where the purity was 99%. These chemicals were not further purified as it was of analytical grade.

Preparation of PVA solution

A 10% PVA solution was prepared by dissolving 10 grams of PVA powder in 100 milliliters of distilled water. To achieve a homogeneous and crystal-clear solution, the mixture was stirred at 70°C for 4 hours using a magnetic stirrer.

The required amount of PVA for getting weight percentage of the polymer solution was calculated using following formula:

$$WP\% = \frac{Mp}{Mp+Ms}$$

Here, Mp= mass of polymer (g), WP%= Weight percent, Ms= mass of solvent (ml).

Preparation of *B. pinnatum* extract

Prepared *B. pinnatum* powder was dissolved with methanol of 10% solution at room temperature for 24 hours where 10 gm *B. pinnatum* powder along with 100 ml methanol solvent was used. Then filtration process was done for removing leave substrate with the help of nylon mesh and evaporation process was performed at 40°C for 4 hours to remove methanol solvent from the solution. After preparing the leave extract, it was stored at 4°C for further use.

Preparation of PVA- *B. pinnatum* solution

Glassware has been washed and cleaned with distilled water for preparing the PVA-*B. pinnatum* solution. The solution was made from direct leaf extract. 30% PVA with 70% fresh leaf extract was used. A magnetic stirrer was used for preparing homogeneous as well as uniform solution at warming condition for 1 hour unless it aggregates. Then the solution has been filtrated to get the final solution (Fig-1) which was taken at a syringe of 30 ml and set in pump of electrospinning machine.

Parameter optimizations of electrospinning

In order to make a nanofibrous mat, the setup of an electrospinning machine [Model: TL-Pro-BM, Company: Tong Li Tech, Origin: China] has been demonstrated by Fig-2 and the machine can works with the following parameters: High voltage supply (-11 KV and +21 KV), a drum collector (Diameter-158mm, Length-500mm, RPM-500), a syringe (30ml), a syringe pump (TL-F6, Tong Li Tech, China), a heater setup (0.4-0.5 KW), and a needle (24 gm/pc).

A 30 ml syringe was filled up by the solution to be used and set on the pump of placed over electrospinning machine. Then required pressure has been set (2.5ml/hr.). The solution was passed through the pipe and needle from where nanofiber comes out. Negative and positive charges are situated at upper part and lower drum respectively. Nanofibrous mat was formed by the mutual action of both charges. An aluminum foil paper has been wrapped over the drum as the produced fiber from the foil surface can easily be collected. Finally, machine was run until the expected layer of mat has been produced. The sequence of production process for PVA electrospun nanofibrous mat is shown in the Fig-3.

Characterizations

Morphological observation

The SEM (SU 1510, Hitachi, Japan) was used to observe the morphological orientation of the nanofibers at magnifications of 2000 and 5000, with a voltage of 5KV, to obtain images of the proper quality. Additionally, the software ImageJ 1.50i was used to measure the porosity of the created nanomat using SEM pictures.

Antibacterial Assay

Antimicrobial sensitivities of the nanofibrous mat were measured using the Kirby-Bauer disc diffusion method (AATCC 6538) against gram-positive *Staphylococcus aureus*. The zone of inhibition (ZOI) of the sample was also measured. This qualitative test evaluated the antimicrobial characteristics of the diffusible antibacterial agent on the treated textile material.

To conduct the experiment, an agar plate was streaked with cultivated bacteria (1.5×10^5 CFU/ml) of *S. aureus*. A single bacterial colony was inserted into a tube containing 9 ml of TSB using a loop and mixed thoroughly. The broth was then inoculated and shaken for two hours at 45°C to meet the 0.5 MacFarland standard.

A sterile cotton swab, soaked in the broth culture, was repeatedly wiped over the MHA plates. Nanofibrous mats and a 10 mm x 10 mm disk were then placed on the plate surface. The agar plate was incubated for 24 hours at 37°C. Following incubation, the ZOI and bacterial growth were observed, and the ZOI was measured using calipers.

FTIR analysis

To measure the infrared transmittance, an FTIR-4700 made in Japan by JASCO International Co. Ltd. was used. With a resolution of 4 cm^{-1} , the spectra of the investigated band, which were gathered using column chromatography, were recorded in the $500\text{--}4000 \text{ cm}^{-1}$ range. FTIR analysis produces a unique spectral fingerprint for each molecule or chemical structure, making it an excellent method for chemical identification.

Tensile Strength Measurement

The sample was performed, tensile strength test using the ASTM D5034 test procedure. The effective tensile strength and elongation were measured using universal tensile testing equipment (Testometric, M250-3CT, India) with a precise load cell capacity of 300N. The samples were divided into 150mm long by 100mm wide strips, each of which was clamped with a 25mm clamp. Initially, a measurement distance of 75mm was used. A steady tensile speed of 300 mm per minute was maintained. The experiments were conducted in natural settings.

MMP

A moisture management tester (M290, SDL Atlas, UK), the AATCC 195-2009 procedure was followed to measure the Moisture Management Properties (MMP). This test evaluates the three-dimensional dynamic liquid transportation characteristics of nanofibers. For the purpose of classifying the nanofibrous mat, factors such as absorption rate, wetting time, one-way transit capability, spreading/drying rate, and overall moisture management capacity were assessed.

Result and Discussion

Optimization of parameters and morphological observation

Sample development allowed the determination of optimal electrospinning parameters. Due to the surface tension of the solution preventing the formation of a Taylor cone, which is essential for fiber production, no fibers were generated when the applied voltage was lower than +22 kV and greater than -12 kV. For the purpose of preparing the sample, positive voltages greater than 22 kV and negative voltages less than 12 kV were used. Since the viscosity of the solution and the pump flow rate are correlated, higher viscosity results in higher flow rates and vice versa.

This is the reason that the flow rate of nanomat sample kept 2.0 ml/hr. A consistent gap of 14 cm was kept between the collector and the needle tip. The produced sample's SEM picture, displayed in Fig-4, verifies the creation of a nanofiber with an average diameter of 174.45 nm when viewed at a 5K magnification. It is important to note that the proven nanofiber nature is crucial for effectiveness since it permits oxygen from the air to reach the skin through the minuscule pores that are present in it. These little pores not only provide airflow but also hinder bacterial infiltration into the injured region, promoting prompt healing. Fig-4 indicates that the mat has 51.45% porosity, which is ideal for cell adhesion and proliferation. This increased porosity could result from the nanofibers' mesho structure, which produces this effect when they overlap.

Investigations of moisture management properties

The purpose of the moisture management test is to assess how moisture interacts with a material to determine its hydrophilic, hydrophobic, and moisture transfer performance. The sample moisture management property is categorized using a number of indicators, including wetting time, wetted radius, absorption rate, spreading rate, and one-way transport capacity.

The nanofibrous mat should provide excellent comfort at the dermal level of human skin along with its biological function. Thus, the ability to transport liquids, such as water or sweat, from the skin to the surrounding environment is crucial. Fig-5 illustrates the results for the moisture management properties of the PVA and *B. pinnatum* mixture. The wetting time (in seconds) for the top and bottom surfaces of the PVA/*B. pinnatum* nanomat is 3, indicating good wetting properties due to the highly amorphous structure of the PVA polymer. Additionally, the absorption rate for the top and bottom surfaces ranges

from 4 to 3. However, the top and bottom wetted radius and spreading speed were graded as poor. Finally, the one-way transport index and overall moisture management were rated as excellent to good, highlighting the nanomat's effective water penetration characteristics.

Antibacterial activity

A nanofibrous mat's antibacterial efficiency is critical for biological applications. Fig-6 illustrates the inhibition zone of *B. pinnatum* nanofibrous mat to *S. aureus* bacteria. Although *S. aureus* generally has a stronger cell wall that prevents it from being destroyed by biocides (natural or artificial), the produced mats had outstanding antibacterial resistance with an inhibition zone of 10 mm. This could occur as a result of the ethanolic extraction method of *B. pinnatum* leaves, where antibacterial components might be present. Organic acids, flavonoids, bufadienolides, phenols, phenylpropanoids, triterpenoids and some ubiquitous compounds are the main chemical components found in *B. pinnatum*. (Supratman et al., 2001) Because Bufadienolides, Flavonoids, Phenols, Phenylpropanoids, Triterpenoids have antibacterial site in their chemical compounds.

Inhibition of Na^+/K^+ -ATPase activity in bacteria or disruption of cell membrane integrity are the reason of bufadienolides shows antibacterial properties. Flavonoids exhibits disruption of cell membrane function, interfering with DNA replication, and inhibition of bacterial enzyme activity. (Hua et al., 2022) Phenols have the ability to damage bacterial cell membranes and have antioxidant effects. Phenylpropanoids may cause bacterial cell membrane integrity to be disrupted or interfere with the formation of white blood cells. Triterpenoids helps to inhibition of important metabolic pathways or disruption of the function of bacterial cell membrane. (Kumar et al., 2023)

Once more, the amount of *B. pinnatum* leaf extracts in the PVA solution is crucial. Additionally, because the generated nanomat has tiny pores, its mesho structures can effectively block any bacteria from penetrating and causing infections in the surrounding area. (Islam et al., 2021)

FTIR

The sample shown in Fig-7 contained components of *B. pinnatum* and PVA polymer, as confirmed by the FTIR spectra.

The FTIR spectra of *B. pinnatum* showed phenolic groups, C–H stretching, and C–H bending bands, which align with prior literature (Harsha, Aditya et al., 2008). Peaks identified at 3300 cm^{-1} (O–H stretching), 2850 cm^{-1} (C–H stretching), and 1100 cm^{-1} (C–O–R stretching) were also attributed to the PVA polymer, consistent with the characteristic peaks of both *B. pinnatum* and PVA as documented. The hydroxyl groups of the PVA polymer chains interact with free hydroxyl groups (–OH) and electronegative oxygen in deoxykalanchide molecules to form hydrogen bonds.

Tensile properties

As PVA-*B. pinnatum* nanomat will be utilized in biomedical applications, it is necessary for it to have a minimal mechanical property, particularly breaking strength. In this instance, the breaking strength has been a significant impact.

The force versus elongation behavior during tensile testing is displayed in Fig-8. The horizontal axis represents elongation (mm), and the vertical axis represents force (N). It was discovered that the nanomat's maximum strength was 9.10N at 4.97438 mm of elongation which is long enough for any items regarding to biomedical applications.

Conclusion

In this experiment, we explained the development of PVA-*B. pinnatum* nanofibrous mat and its characterization. We focus our discussion on the potentiality of promising applications in the field of biomedical which is ensured by the antibacterial efficiency against *S. aureus* bacteria. The presence of different chemical constituents of the herb was confirmed by FTIR. Test of moisture management properties defined excellent to good one way transport index and overall moisture management resulted in water penetration identity. Tensile strength was good enough for the application of biomedical. So, the developed nanofibrous mat would be a potential antibacterial material. Therefore, this study depicts that PVA-*B. pinnatum* nanofibrous mat can be used as a biodegradable biomedical product.

Declarations

Data Availability

Data will be made available on request.

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Contributions

Md. Anwar Hossen: Conceptualization, Research design, Supervision and final approval.

Md. Shablu Khan: Result analysis and interpretation.

Md. Lokman Ali: Methodology, Background study & writing editing.

Md. Golam Mortuza Limon: Resources and Characterization.

Abdullah Nur Uddin: Draft writing and review.

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Ethics declarations

Competing interests

The authors declare that they have no financial competing interests.

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Figures

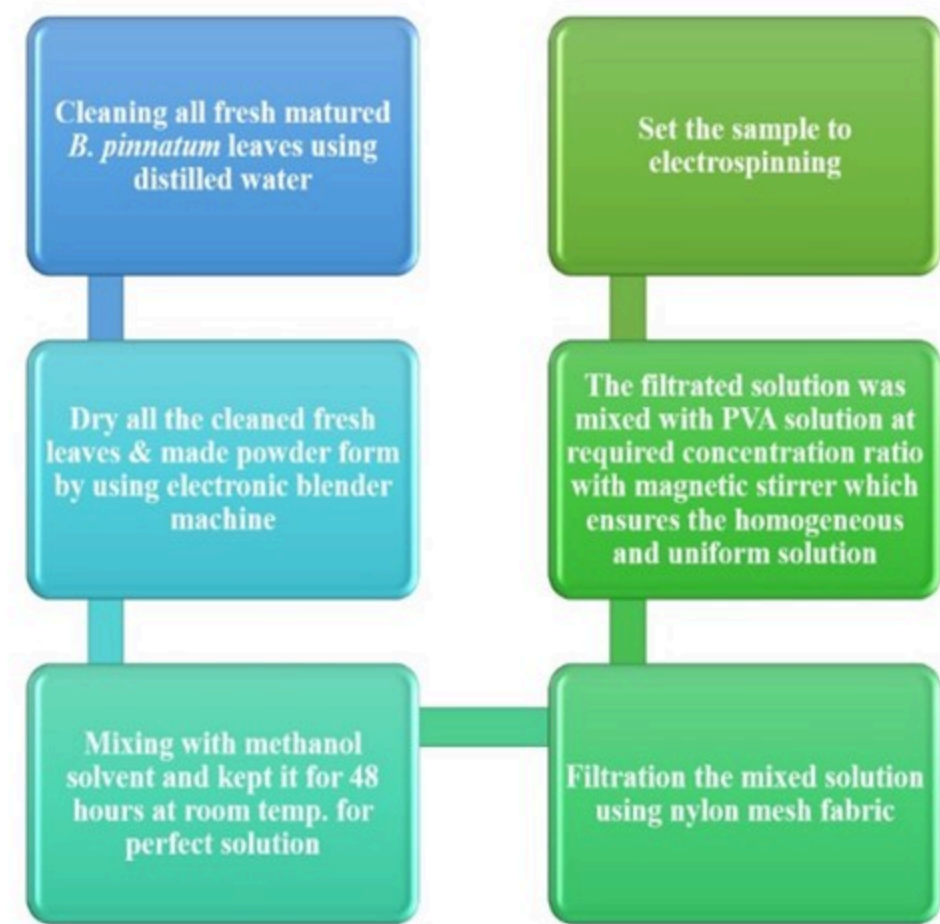


Figure 1

Solution preparation



Figure 2

Electrospinning Machine

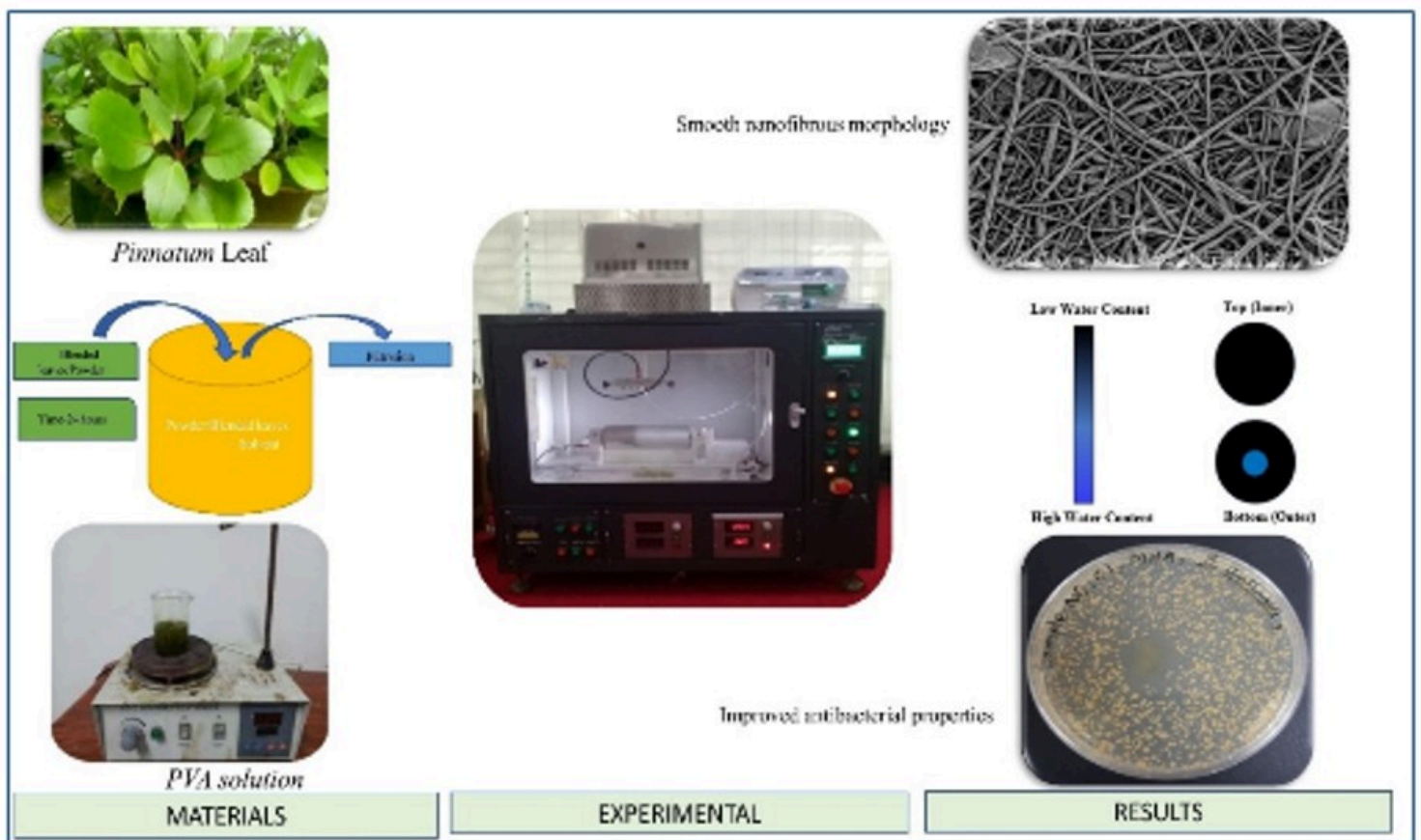


Figure 3

Scheme of electrospinning process of PVA-*B. pinnatum* nanofibrous mat.

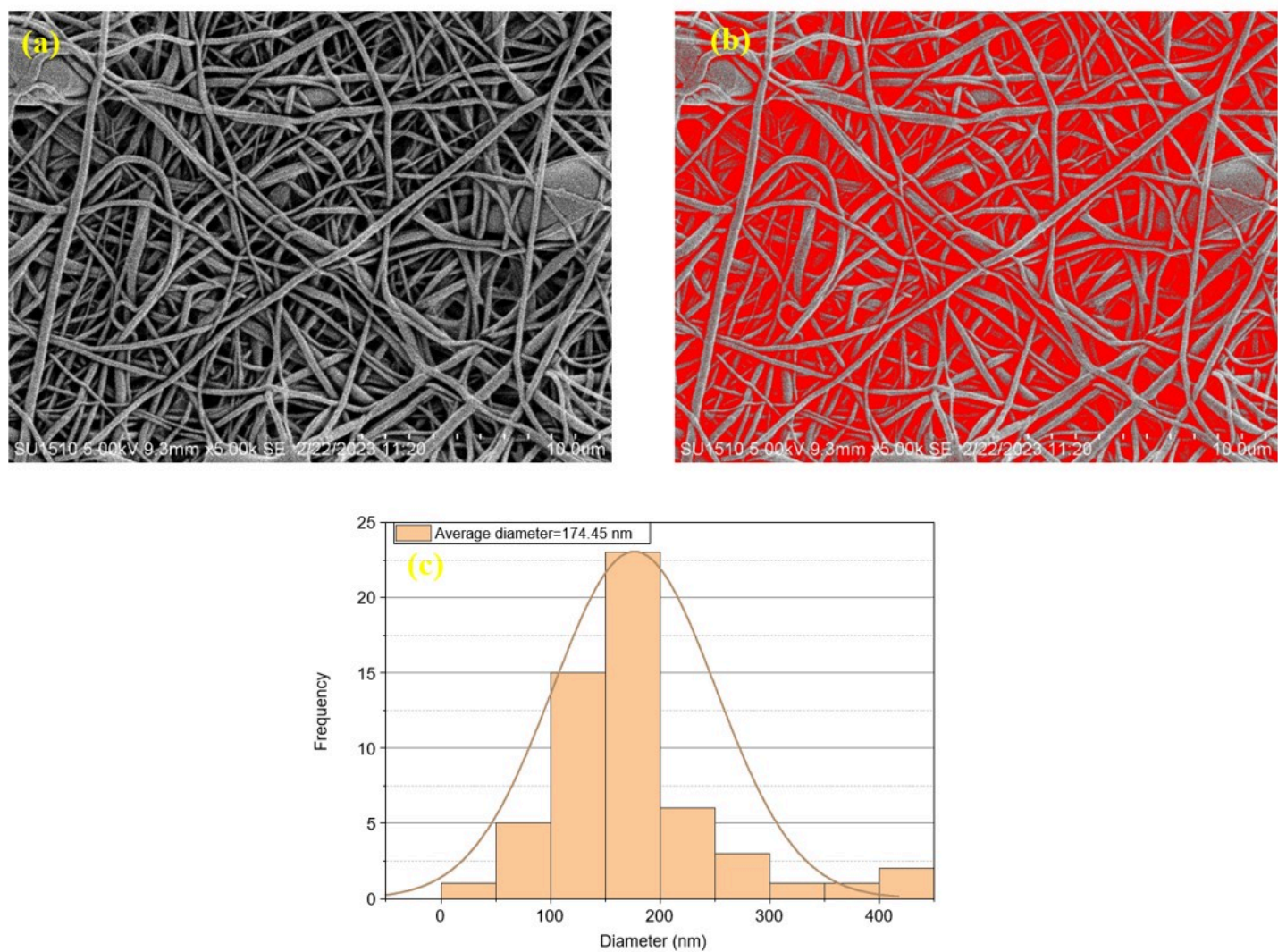


Figure 4

(a) SEM image of the developed nanomat, (b) porosity determination of modified SEM image, (c) fiber diameter distribution histogram

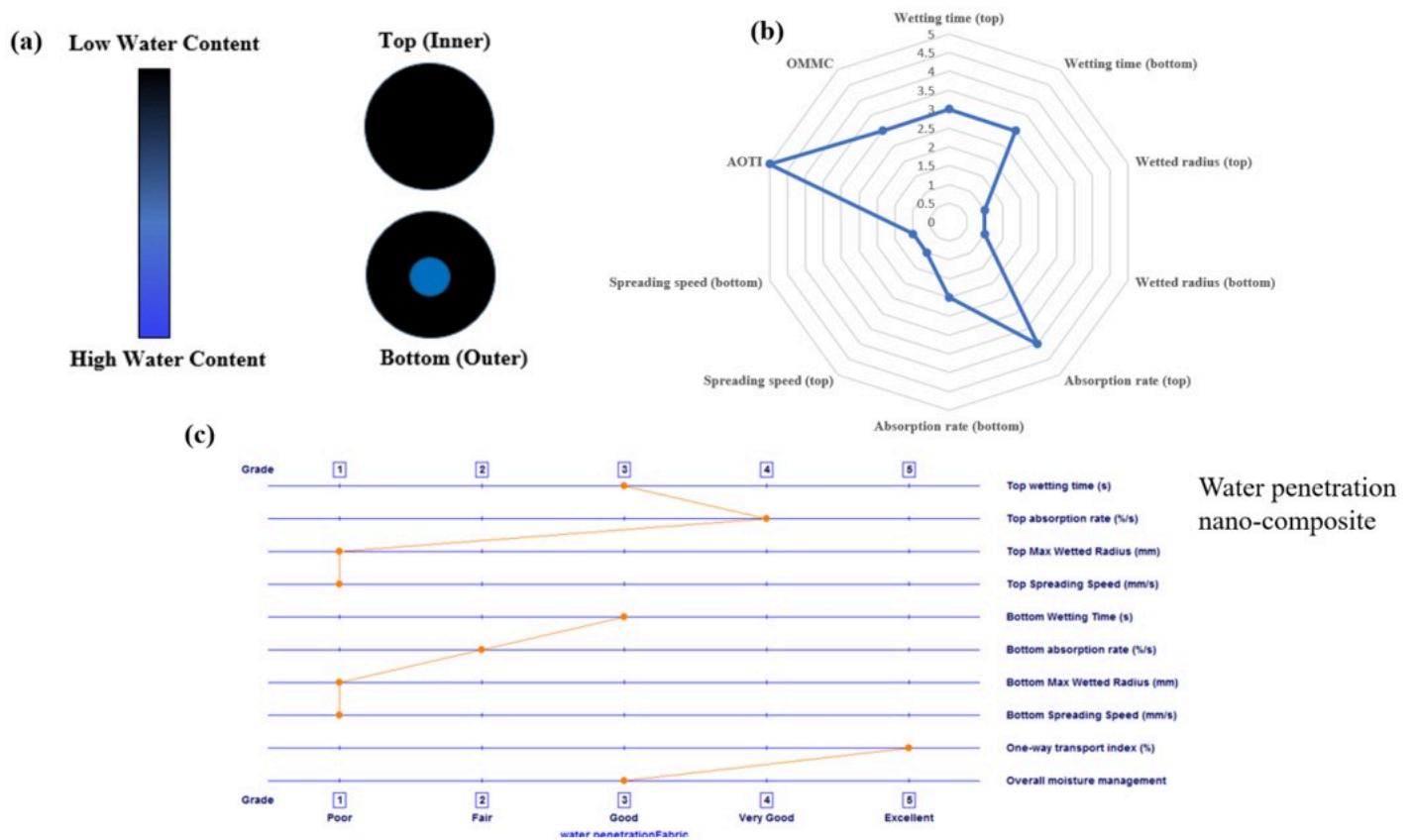


Figure 5

(a) Water location vs time diagram (b) radar diagram, (c) grading from 1 to 5 rating with properties of the developed nanomat

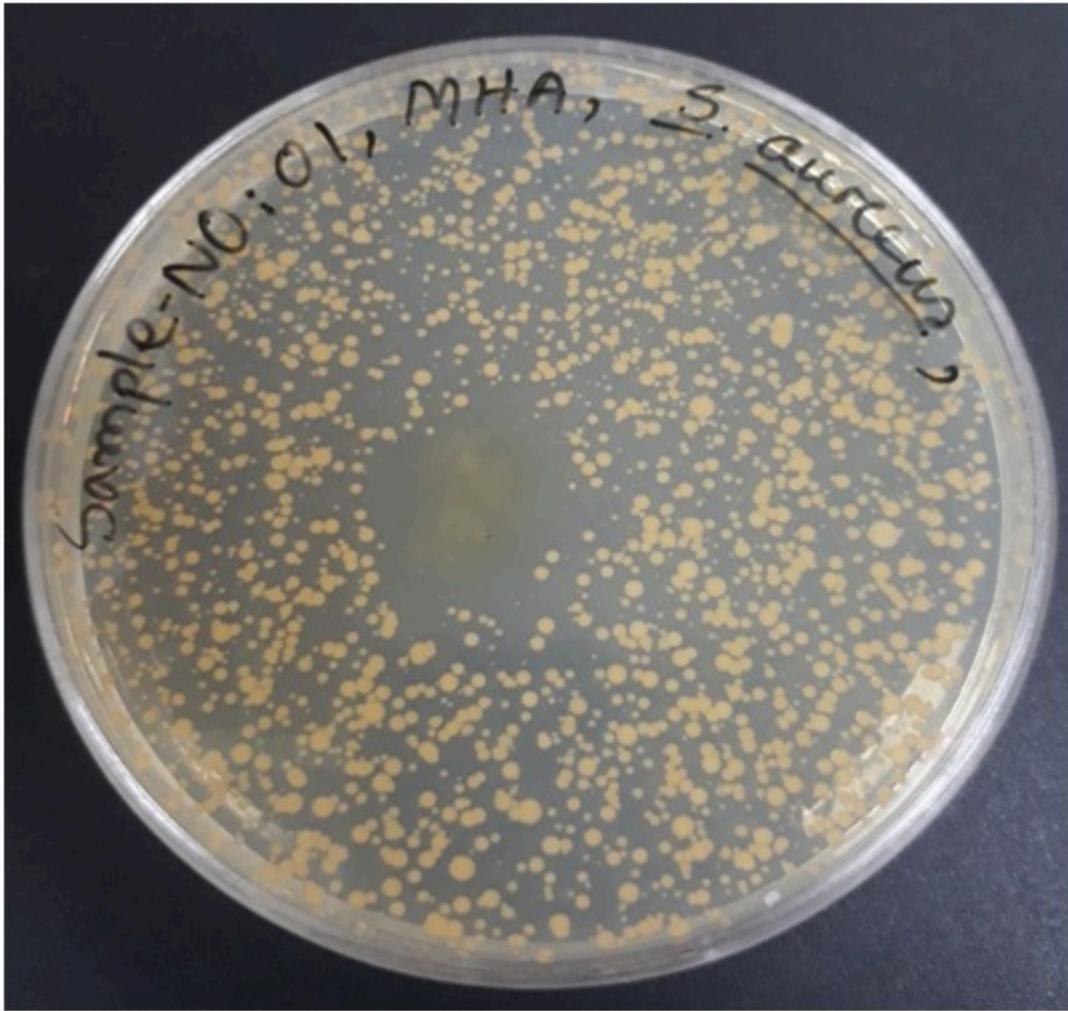


Figure 6

Inhibition zone to *S. aureus* bacteria

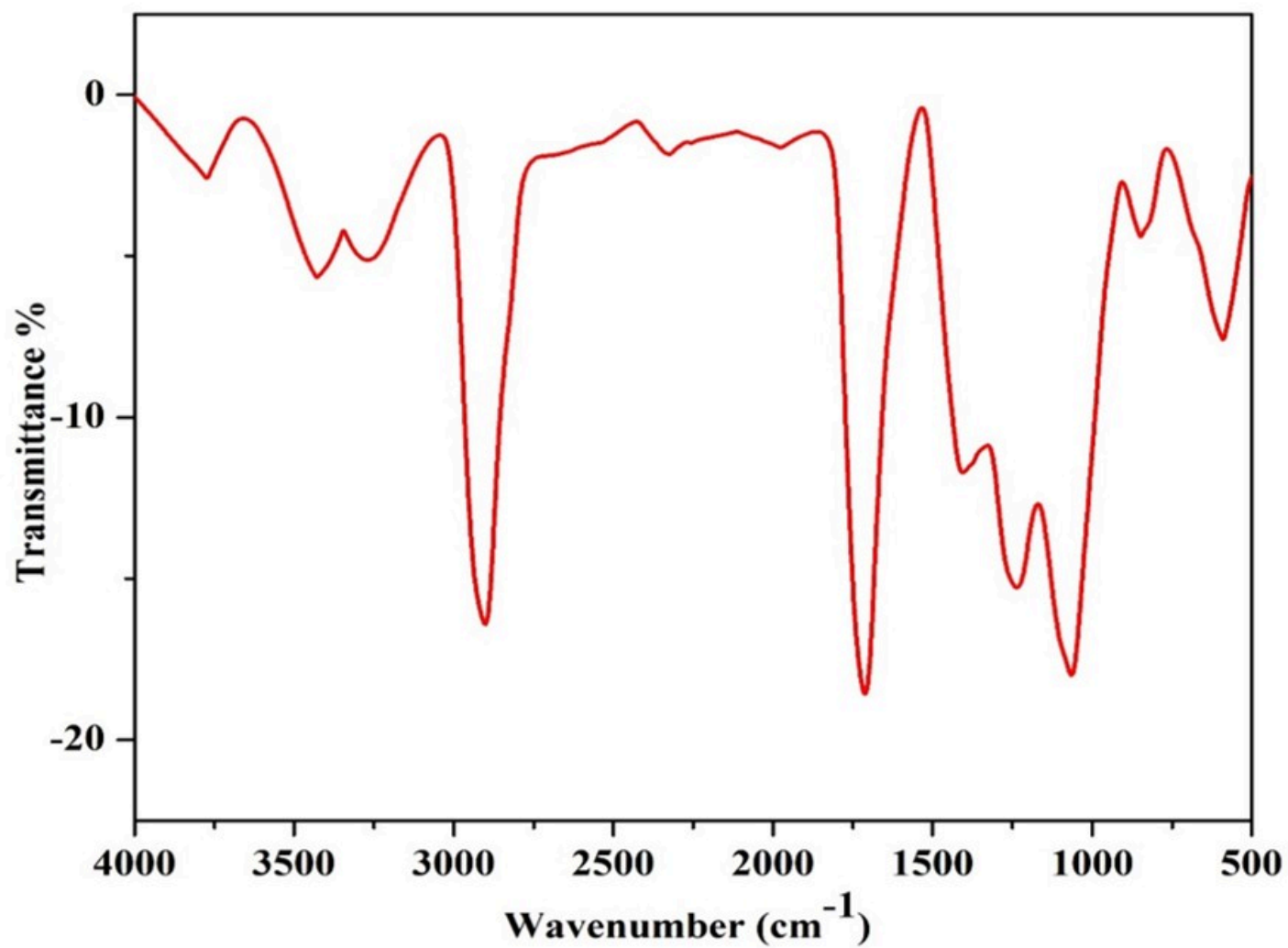


Figure 7

FTIR spectra of developed nanomat showing individual peaks

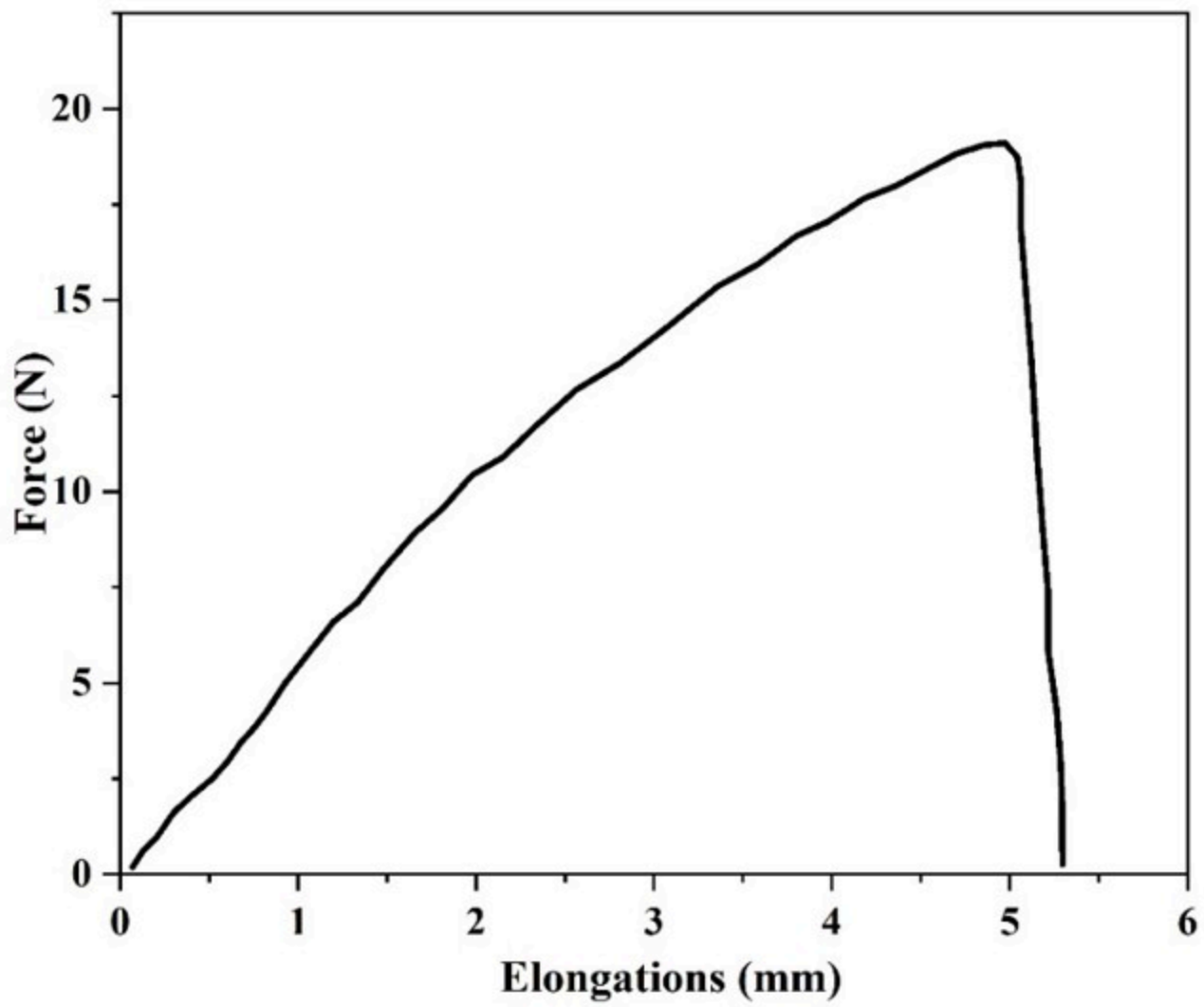


Figure 8

Force vs elongation curve of developed nanomat