



Development and Characterization of Electrospun Piper Betle - *Azadirachta indica* Leaf Extract Incorporated Polyvinyl Alcohol Nanofibrous Composite Mats

Nadim Mahmud^{1*}, Md. Humayon Kabir¹, Md. Arif Rabbani¹, Shamsuzzaman Sheikh² and Ayub Ali¹

¹Department of Textile Engineering, Dhaka University of Engineering and Technology, Bangladesh

²Frontier Fiber Technology and Science, University of Fukui, Japan



Abstract

Innumerable infectious diseases threaten human health safety that result in numerous deaths each year despite significant progress in medical science. Owing to medicinal properties, natural plant extracts had been used to heal wounds for many years. Recent studies found that nanofibrous mats produced by electrospinning have great potential applications in wound dressings. Incorporating plant extracts into electrospun nanofibrous structures will enhance antibacterial properties of nanomats in wound dressing application. This research aims to develop nanofibrous mats by electrospinning of Piper Betle (Paan) and *Azadirachta indica* (Neem) leaves extract composite loaded in Polyvinyl Alcohol polymer foundation to enhance the antibacterial and healing properties of the mats. Solution of Piper Betle-*Azadirachta indica* leaves extract in various ratios were mixed in the PVA solution, to create Piper Betle-*Azadirachta indica*/PVA aqueous solutions then electrospun to develop Piper Betle-*Azadirachta indica*/PVA nanomats. Developed nanomats were characterized to investigate morphological structure, moisture management properties, functional groups and antibacterial activities against *Staphylococcus aureus* bacteria.

Keywords

Electrospinning, Piper betle, *Azadirachta indica*, Antibacterial, *Staphylococcus aureus*

Background

Introduction

Infectious diseases continue to pose catastrophic threats to humankind and civilizations, such as seen in the COVID-19 epidemic. These dangers emanate from phyla of microbes that cause diseases for instance viral infection, bacterial infection, fungal infection etc. killing millions of people worldwide. They are mostly zoonotic which spread between animals and humans and have an animal reservoir [1]. To restrain pandemics, the world has recently realized the significance of antimicrobial products for protection from harmful microorganisms.

Antimicrobial properties are well-known in natural products which were used as medicine from time immemorial and furthermore play an important part in present-day modern medicinal research [2]. Electrospun nanofibrous mats produced with natural antimicrobial agents are the newest and most promising type of biomedical textile product with unique features [3]. Because of the morphological similarities with the body's Extracellular-matrix (ECM), high porosity and high surface area to volume ratio, the continuous and flexible nanostructure electrospun favors quick tissue regeneration,

wound fluid transportation and ensures breathability for cellular growth and proliferation, making it suitable for wound dressing and drug delivery systems [4].

Recently many researchers have reported on the success of electrospun nanofibrous mats production from various natural products like turmeric, aloe-vera, clove, *Azadirachta indica*, chitosan, collagen, gelatin, silk, alginate; incorporated with synthetic polymers such as Polyglycolide (PGA), Polylactic Acid (PLA), Polyvinyl Alcohol (PVA), and Poly(ϵ -caprolactone) (PCL) and their blends [5-19]. These nanofibers mats have demonstrated high performance in various biomedical applications, especially in wound dressings, tissue

***Corresponding author:** Nadim Mahmud, Department of Textile Engineering, Dhaka University of Engineering and Technology, Bangladesh, Tel: +88-01690253158

Accepted: May 22, 2023

Published online: May 24, 2023

Citation: Mahmud N, Kabir H, Rabbani A, et al. (2023) Development and Characterization of Electrospun Piper Betle - *Azadirachta indica* Leaf Extract Incorporated Polyvinyl Alcohol Nanofibrous Composite Mats. Aspects Nanotechnol 5(1):94-102

engineering, biosensors, drug release and so forth because of their excellent biocompatible properties. Following the effectiveness of numerous antimicrobial wound dressing materials incorporating natural biocides, the development of antibacterial nanofibrous mats made from polyvinyl alcohol (PVA) incorporated with a mixture of *Azadirachta indica* (Neem) and Piper Betle (Paan) leaves extract has yet to be explored.

The aim of this study is to develop nanofibrous mats using natural plants Piper Betle (Paan) and *Azadirachta indica* (Neem) leaves extract mixture embedded in Polyvinyl alcohol (PVA) synthetic polymer in different concentrations using the electrospinning technique as well as to characterize nanomats potentiality in the medical textile application. Thus, the objectives of this research study are characterizing and analyzing electrospun nanofibrous mats of PVA embedded with Piper Betle and *Azadirachta indica* leaf extracts nanofiber mats for various medical uses owing to the inherent healing properties of these natural materials. Also, to determine the optimal processing parameters for a smooth electrospinning process PVA embedded with Piper Betle and *Azadirachta indica* leaf extracts, such as solution ratio, applied voltage, collector distance, and flow rate and measure the nanofiber size, arrangement, nanomats moisture behaviour, bonding nature and using different testing equipment such as SEM, MMT, FTIR etc. Finally investigate the antibacterial activity of produced nanomats against gram-positive (*S. aureus*) bacteria. In conclusion, this study aims to develop antibacterial nanomats from Piper Betle and *Azadirachta indica* leaves natural extract.

Literature review

Natural products are very popular in medical use because of their diverse biological qualities and bioactive components, which have been shown to be effective against a wide range of medical conditions. Aloe Vera is a plant that has long been used to heal burn wounds in traditional medicine. Owing to its ability to activate fibroblasts and accelerate the healing process by collagen synthesis and maturation [20]. It also devises a variety of biological qualities including antidiabetic, anti-inflammatory, etc. Electrospinning was used to create core-sheath nanofibrous mats of silk fibroin/poly(vinyl alcohol)/Aloe Vera as a new vitamin E delivery mechanism [21].

Another natural plant, henna contains phenolic compounds that have been widely utilized as a medicinal herb. Lawsone (2-hydroxy-1,4-naphthoquinone), is a red-orange dye existent in the henna plant leaf that has antibacterial, anthelmintic, immunomodulatory, anticancer, antioxidant, UV protecting, wound healing, and antimicrobial properties [22-24]. By coaxial electrospinning, encapsulated lawsone in PCL/gelatin polymers in the core-shell architecture to generate PCL/Gelatin/lawsone nanofibrous mats, PCL in the outer shell polymer, and gelatin-lawsone blend in the inner core. The scaffolds released lawsone for 20 days, and a 1% lawsone-loaded mat improved wound healing by improving re-epithelialization after 14 days and has great properties that can be utilized as a wound dressing or medicine [25].

The nanofibrous mats prepared from nigella/PVA solution incorporated with silver nanoparticles showed antibacterial activity against *Staphylococcus aureus* (*S. aureus*) bacteria while assessed using the agar diffusion method [26]. The nanofibrous mats with an average fiber diameter of 223 ± 20.5 nm, create an inhibition zone of 18.66 ± 2 mm. Furthermore, the nanomats demonstrated improved moisture and thermal properties. Clove, an essential oil produced from cloves, is found naturally in the flower buds of the clove tree. Eugenol possesses antibacterial, antioxidant, analgesic, and anti-inflammatory effects, all of which help the wound heal faster [27]. Eugenol, on the other hand, has a low water solubility and is unstable in the presence of chemical and enzymatic degradation, as well as losses through volatilization and heat decomposition.

Curcumin/thiocarbohydrazide modified gelatin non-toxic nanofibrous mat was developed for wound healing applications, using 50% acetic acid as a solvent to avoid the use of toxic fluorinated solvents, and then cross-linking it with N-(3-dimethylamino propyl)-N'-ethylene carbodiimide hydrochloride. Curcumin was articulated as an amorphous nano solid dispersion in the mat [28]. The higher the crosslinking percentage, the higher the hydrophobicity of the fiber mat and the lower the release rate. In comparison to 2 and 3% curcumin, 1% curcumin in the nanofibrous mat exhibited optimum slow and steady release at neutral pH, which could favor the effective release of curcumin at the wound site, as well as higher mechanical strength and effective no inhibition.

To include the intrinsic therapeutic characteristics of this natural herb for biomedical application, an electrospun nanofibrous mat was created from a mixture of poly (vinyl alcohol) (PVA) and Mikania micrantha extract [29]. Which bacterial resistance was evaluated using the disc diffusion method against Gram-positive (*S. aureus* - ATCC 6538) bacteria for the sample developed at the maximum mixing ratio of Mikania micrantha extract (80%), which shows the formation of an inhibition zone up to 12 mm. Thymol-based wound dressings were developed with two layers of electrospun nanofibers; A top layer of silk fibroin/poly(caprolactone) to act as a physical barrier at the wound site and a bottom layer of silk fibroin/hyaluronic acid/Thymol to aid wound healing and prevent infection [30]. Electrospinning honey and curcumin longa (turmeric) extract loaded in polyvinyl alcohol polymers to produce antibacterial nanofibrous mats with improved moisture management qualities than electrospun polyvinyl alcohol nanofibrous mats alone [31]. By using the electrospinning technique to create polyvinyl alcohol/honey/turmeric extract nanofibers, which improved moisture control and antibacterial activity.

Numerous medical materials incorporating natural biocides were developed for antibacterial sensitivity using the electrospinning technique. Although the healing benefits of Piper Betle (Paan) are widely known in Asian countries, no research has been conducted on its leaf extract in medical textile applications. In addition, the antibacterial sensitivity of leaf-extract nanofibrous mats derived from Piper Betle and *Azadirachta indica* has yet to be explored. The aim of this

research is therefore to develop and characterize nanofibrous mats based on Piper Betle and *Azadirachta indica* mixed bio compounds using polyvinyl alcohol (PVA) as the base polymer.

Electrospinning

Researchers are developing electrospun nanofibers that include antimicrobial compounds such as nanoparticles and antibiotics in wound dressings to prevent infection [32]. The newest and most promising type of biomedical textile product for wound dressing and drug delivery process gaining popularity with unique properties is electrospun nanofibrous mats produced with natural medicinal compounds. The electrospinning technology is popular for manufacturing nanoscale size fiber or nanofiber approximately 1 to 100 nanometres in diameter [33]. This mechanism constitutes three simple parts, a high-voltage power supply, and a metallic needle placed over some distance from an aluminum collector. In the electrospinning technique, a jet of the polymeric solution is injected through the metallic needle into an electrostatic force field formed due to the high-voltage difference between the metallic needle and collector [34] (Figure 1).

Electric forces stretch the polymer jet, resulting in a significant reduction of diameter thus forming nanofibers which are then gathered onto the aluminum collector and fabricating nanofibrous mats [35]. Due to the nanoscale structure, electrospun nanofibrous mats have unique properties that offer diverse applications in the filtration and the biomedical industry. Different structures and properties of electrospun nanofibers can be obtained by using different polymers and by changing machine parameters such as applied voltage and needle to collector distance.

Piper betle

Piper Betle, a member of the Piperaceae family, is a heart-

shaped deep green leaf that grows on a root climbing vine and is a traditional herbal medicinal plant used in Asian countries for a variety of health advantages [36]. The extracts and essential oil of Piper Betle leaves have been found to contain a broad scope of bioactive compounds such as polyphenols, terpenes, and other chemicals [37]. Different contemporary standard procedures were used to determine the structural and functional characteristics of the extract and essential oil bio-actives. The majority of Piper Betle leaves' health advantages also were attributed to its functional phenolic components [38]. In order to extract bioactive chemicals from plant sources, a variety of extraction procedures are used. Methanol, ethanol, acetone, ethyl acetate, and other common solvents are employed in extraction because of their biodegradability and simplicity of handling, as well as low toxicity [39]. To extract functional substances efficiently, it's critical to choose the right extraction procedure (Figure 2).

Due to its potency, researchers have suggested that Piper Betle could be used as a natural antioxidant source in a variety of applications such as medicine, food, and pharmaceuticals. The need for bioactive chemicals derived from natural plants is on the rise. So, the utilization of natural materials and their products is growing every day, and they are employed in a variety of applications. Dried leaves extract of Piper Betle using ethanol by ultrasound-assisted founds Hydroxychavicol (66.55%), eugenol (11.92%), isoeugenol (2.90%) and 4-allyl,12-diacetoxybenzene (3.21%). An appropriate extraction procedure is used to extract the chemicals present in Piper Betle leaves [40].

Azadirachta indica

Azadirachta indica, also recognized as *Azadirachta indica*, has gained a worldwide reputation in the past few decades because of its extensive spectrum of medical benefits.

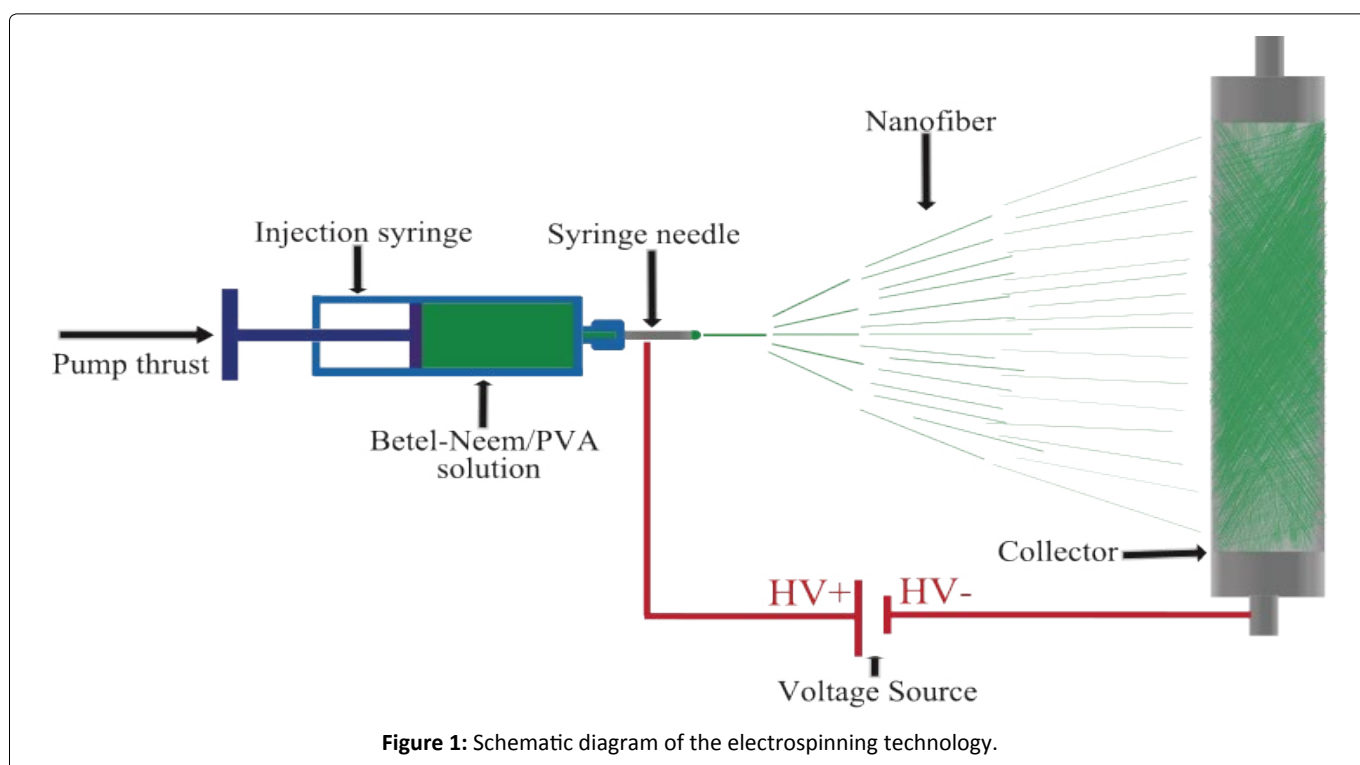
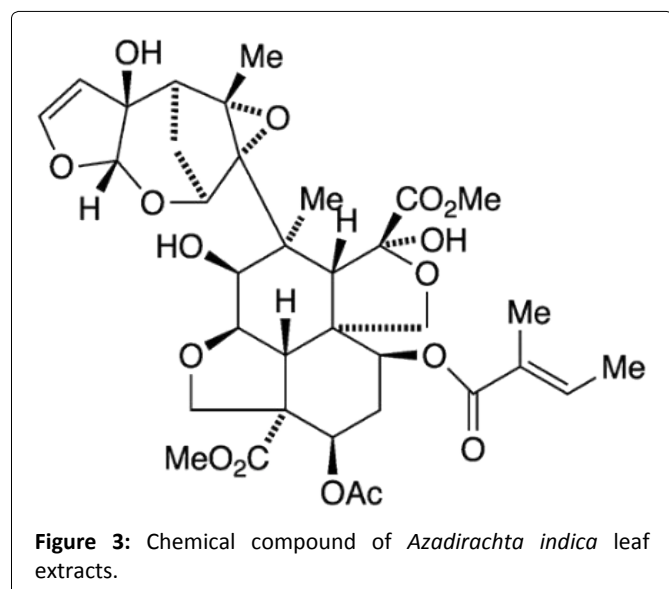
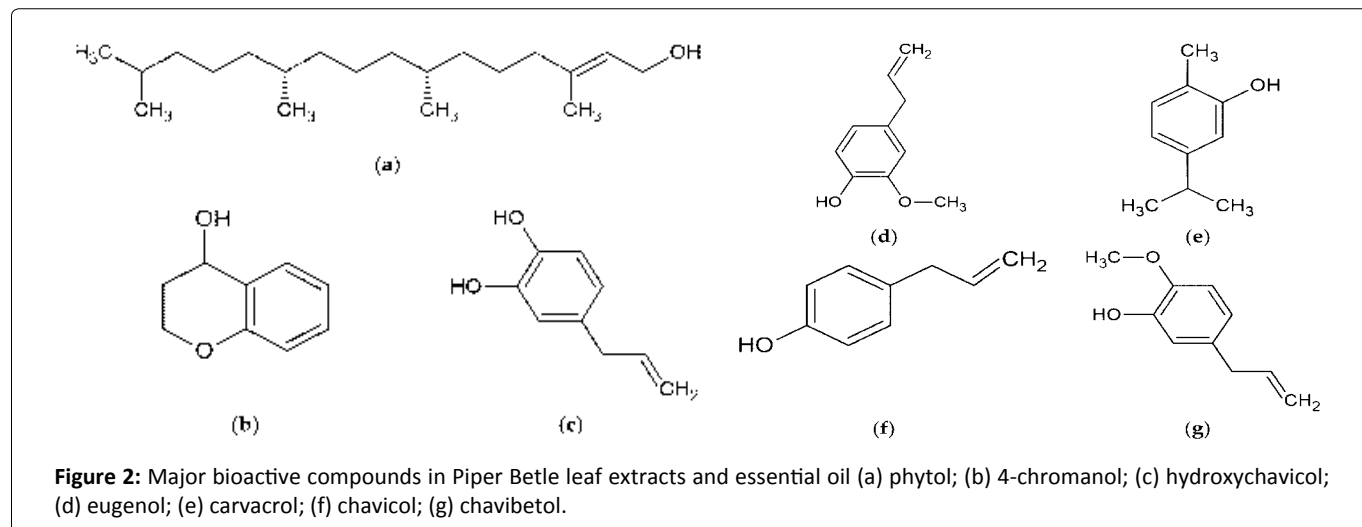


Figure 1: Schematic diagram of the electrospinning technology.



Azadirachta indica encompasses a broad range of bioactive compounds that are both chemically and structurally diverse [41]. The therapeutic constituents of *Azadirachta indica* leaf extract have been found to exhibit antiviral, antifungal, antibacterial, immunomodulatory, anticarcinogenic, anti-inflammatory, antiulcer, antimalarial, antioxidant, antimutagenic and antihyperglycaemic properties [42]. A 10% aqueous extract of leaves has antiviral action against the vaccinia and variola viruses [43]. The methanolic extract of *Azadirachta indica* leaves has been shown to exhibit antiviral properties against group-B Coxsackie viruses. Trichophyton, Microsporium, Epidermophyton, Geotricum, Trichosporon, and Candida are all susceptible to *Azadirachta indica* leaf extracts, which are attributed to *Azadirachta indica*'s volatile sulphides and the limonoid gedumin. Mahmoodin, a limonoid secluded from *Azadirachta indica*, is found to have powerful antibacterial properties as a result *Azadirachta indica* oil has shown to have antibacterial action toward a wide range of Gramnegative and Grampositive bacteria, including Mycobacterium tuberculosis and Streptomycin-resistant strains [44]. *Azadirachta indica* is added to electrospinning polymer solutions to create active nanofibrous wound

dressings. Antibacterial compounds extracted from *Azadirachta indica* leaves with methanol were incorporated into a polyvinyl alcohol/chitosan solution and electrospun into nanofibrous using a bi-layered system (Figure 3).

Because chitosan is brittle and difficult to collect after electrospinning, the PVA solution was electrospun first as a backdrop, followed by the chitosan/*Azadirachta indica*/PVA solution. The mat was smooth, degradable, porous (91%) with homogenous pictures, and had a minimum and maximum diameter of 152 and 298 nm, respectively. *Azadirachta indica* increased the creation of microorganism inhibitory zone, heat stability, absorbability, and synergistic antibacterial efficacy against *Staphylococcus aureus* germs of electrospun mats [45].

Polyvinyl alcohol

Polyvinyl alcohol (PVA) is a synthetic polymer constituting a vinyl group, linked by only carbon-carbon bonds [46]. Different areas of biotechnology and biomedicine field have used PVA extensively for being a water-soluble, biodegradable polymer with excellent biocompatibility, and also has the ability to self-crosslink resulting from large concentrations of hydroxyl groups on its side chains [47]. PVA is a semi-crystalline polymer with excellent chemical and thermal properties. As well as being non-hazardous, PVA causes no harm to the skin when comes into contact. Thus, properties allow PVA can be used as a base polymer in nanomats to impart strength and incorporate functional components [48].

Method

Materials

Polyvinyl alcohol (PVA, Mw = 115000, Degree of polymerisation: 1700-1800, Viscosity: 26-32 cps, 99% hydrolyzed granules) was purchased from Loba Chemie Pvt. Ltd. The PVA solution was made with distilled water as a solvent. Fresh leaves of *Azadirachta indica* and Piper Betle were collected from Gazipur, Bangladesh then washed with distilled water and dried in shade. Methanol with a purity of 99% was obtained from Merck in Germany. Leaves were extracted with ethanol to obtain functional compounds. All of

Table 1: Solution ratio with respective sample identity.

Sample code	<i>Azadirachta indica</i> extract (%)	Piper Betle leaf extract (%)	PVA (%)	Technique
N1 (80:20)	40	40	20	Monolayer
N2 (50:50)	25	25	50	Monolayer
PVA	0	0	100	Monolayer

the compounds were analytical reagent grade and were not purified further.

Preparation of PVA solution

In 100 mL of distilled water, 10 grams of PVA powder was dissolved. The solution was then agitated for 3 hours at 60 °C temperature using a magnetic stirrer to obtain a homogeneous, crystal-clear solution. For the amount of PVA required to generate a weight percentage of polymer solution the following equation was used:

$$WP\% = \frac{M_p}{M_p + M_s}$$

Where, WP% = Weight Percent, Mp = Mass of polymer, Ms = Mass of solvent

Preparation of *Azadirachta indica* and piper betle leaf extract

Methanol was used to prepare the separate extracts of *Azadirachta indica* and Piper Betle leaves by the maceration technique. For that, into two flasks containing 100 mL of 100% methanol (MeOH), a dried leaf of 10 grams each of the *Azadirachta indica* and Piper Betle was dissolved. To complete maceration, the flasks were kept at room temperature for 24 hours. This was followed by filtering the macerate through nylon mesh. As a result, a final clean solution of *Azadirachta indica* and Piper Betle extract of methanol was obtained. The extract was then stored at 4 °C for further use.

Preparation of piper betle-*Azadirachta indica*-PVA electrospinning solution

A homogenous Piper Betle-*Azadirachta indica* solution was produced at a (50:50) ratio from separate leaf extracts of *Azadirachta indica* and Piper Betle methanol solutions. 50 mL *Azadirachta indica* and 50 mL Piper Betle were combined in a washed glass beaker and stirred for 1 hour to make Piper Betle-*Azadirachta indica* solution. Following that, the Piper Betle-*Azadirachta indica* solution was mixed with PVA solution Piper Betle-*Azadirachta indica*/PVA in various proportions, such as 80/20 and 50/50 (v/v percent). For one hour at 40 °C, a magnetic stirrer was employed to make the Piper Betle-*Azadirachta indica*/PVA solution uniform and homogeneous. Because PVA molecules tend to cluster and dissociate from Piper Betle-*Azadirachta indica* extract at low temperatures, Piper Betle-*Azadirachta indica*/PVA was kept warm. After that, the prepared solution is placed in a syringe and injected into the electrospinning machine's pump.

Electrospinning setup and process

This experiment used the electrospinning of Tong Li Tech;

Model: TI- Pro- BM; Origin: China; which constitutes of high voltage supply (-12 KV and +26 KV), a syringe pump (TL- F6, Tong Li Tech), a drum collector (Diameter: 158 mm, Length: 59 mm), a heater (0.5 KV), and needles (20 G). The syringe was filled with the required amount of Piper Betle-*Azadirachta indica*-PVA solution, then placed into the syringe pump and piped to the needles. The syringe pump was used to supply the Piper Betle-*Azadirachta indica*/PVA solution from the syringe to the needles at a constant rate of 1.5 ml/h. Aluminum foil was wrapped around the drum collector's surface. The drum collector was connected to a negative voltage supply and the positive voltage supply was connected to needles. Needles to collector drum distance was set to 20 cm and the drum rotation speed was kept at 250-300 rpm (Table 1).

Sample identification

The overall experimental design with respective sample identity has been as Table 1.

Results and Discussion

SEM Morphological analysis

The morphology and diameter of the developed mat nanofibers were investigated using scanning electron microscopy (SEM), and images of the scaffolds is shown Figure 4. The average diameter of fibres measured with SEM image analysis software has been found to be 170 nanometers for N1 (80:20) nanomat and 110 nanometers for N2 (50:50) nanomat. Nanofibers have a cylindrical shape and slight structural imperfections also very small pores were formed in the nanomats. As a result of such tiny pores, the air is able to circulate in the wound as well as bacteria are restricted to access the wound. By increasing Piper Betle and *Azadirachta indica* leaves extract in the PVA polymers, increased diameter and a more porous structure nanocomposites was observed (Figure 4).

Moisture management properties

It is essential for nanomats to maintain the right conditions of moisture at the dermal level of human skin in order to be used for biomedical applications. The abilities of developed mats to transfer liquids (water or perspiration) to the external environment and their interaction with liquid have been evaluated. Samples developed by electrospinning the mixture of Piper Betle-*Azadirachta indica* and PVA moisture management properties has been presented according to the AATCC-195-2009 standard. The wetting time, absorption rate and spreading radius (mm) of the top surface of N1 (80:20) is found to be 2.808, 11.585 and 25.0 respectively and of N2 (50:50) samples to be 120, 0 and 0 respectively. Given the behaviour of absorption rate, the N1 (80:20) top layer shows a better grade than the N2 (50:50), it may be likely due to

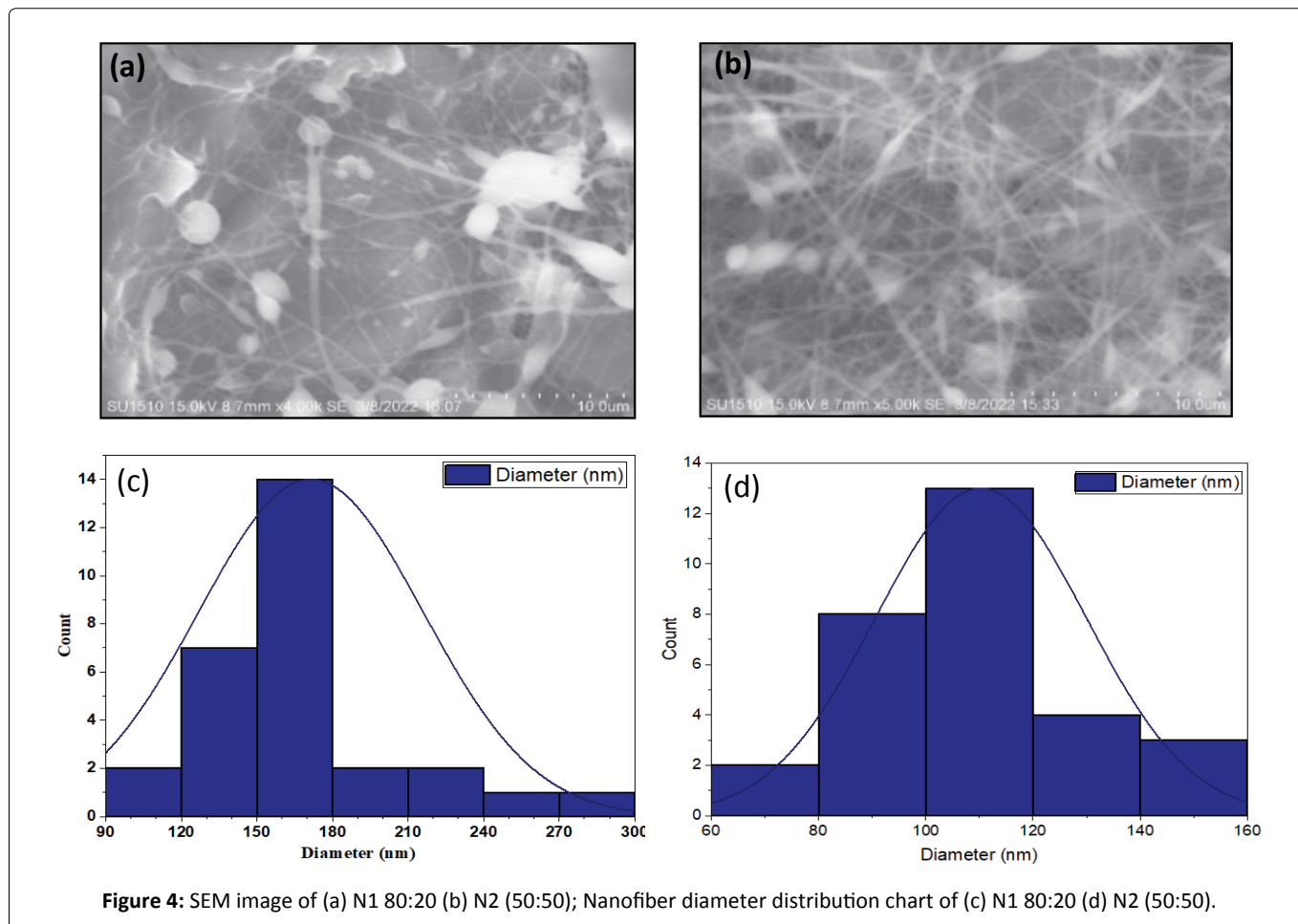


Figure 4: SEM image of (a) N1 80:20 (b) N2 (50:50); Nanofiber diameter distribution chart of (c) N1 80:20 (d) N2 (50:50).

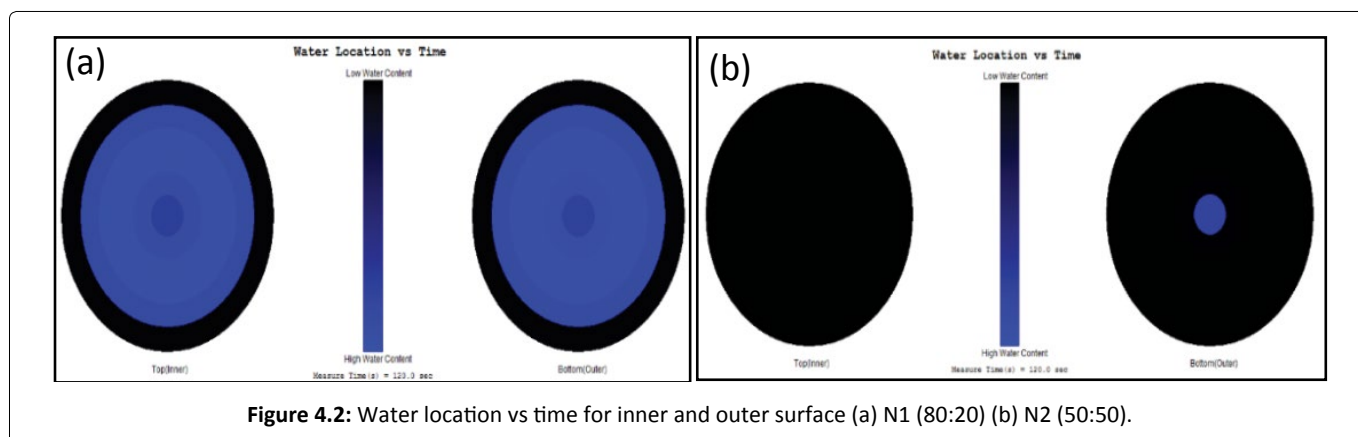


Figure 4.2: Water location vs time for inner and outer surface (a) N1 (80:20) (b) N2 (50:50).

the presence of the natural extract of the N1 (80:20) sample (Table 2 and Figure 4.2).

According to the moisture management test, AATCC standard the N1 (80:20) sample was rated as a slow absorbing and slow drying fabric, while N2 (50:50) was rated as water penetration fabric. Therefore, Piper Betle-*Azadirachta indica*:PVA nanomats are best suited for wound dressings at an 80:20 Piper Betle-*Azadirachta indica* and PVA ratio, whereas a 50:50 Piper Betle-*Azadirachta indica* and PVA ratio is not appropriate.

Analysis of fourier transforms infrared spectroscopy (FTIR)

The presence of functional groups in both samples of N1

(80:20) and N2 (50:50) Piper Betle-*Azadirachta indica*/PVA nanofibrous mats were found using infrared spectroscopy. Phenolic compounds have been confirmed on both of the samples via absorption peak 3740 cm^{-1} wave number (aromatic O-H stretching) by virtue of Piper Betle. *Azadirachta indica* leaf characteristic absorption peaks at wave numbers 2861 cm^{-1} (C-H stretching); 1520 cm^{-1} (Aromatic C=C Bending) and 1720 cm^{-1} (C=O stretching) have been found in the FTIR spectrum of both nanomats. Furthermore, the polymer yields an absorption peak at 3300 cm^{-1} wave number (alcohol O-H stretching of hydroxyl) and 2920 cm^{-1} wave number (alkyl C-H stretching) as well as 1095 cm^{-1} (C-O-C stretching) owing to the PVA (Figure 4.3).

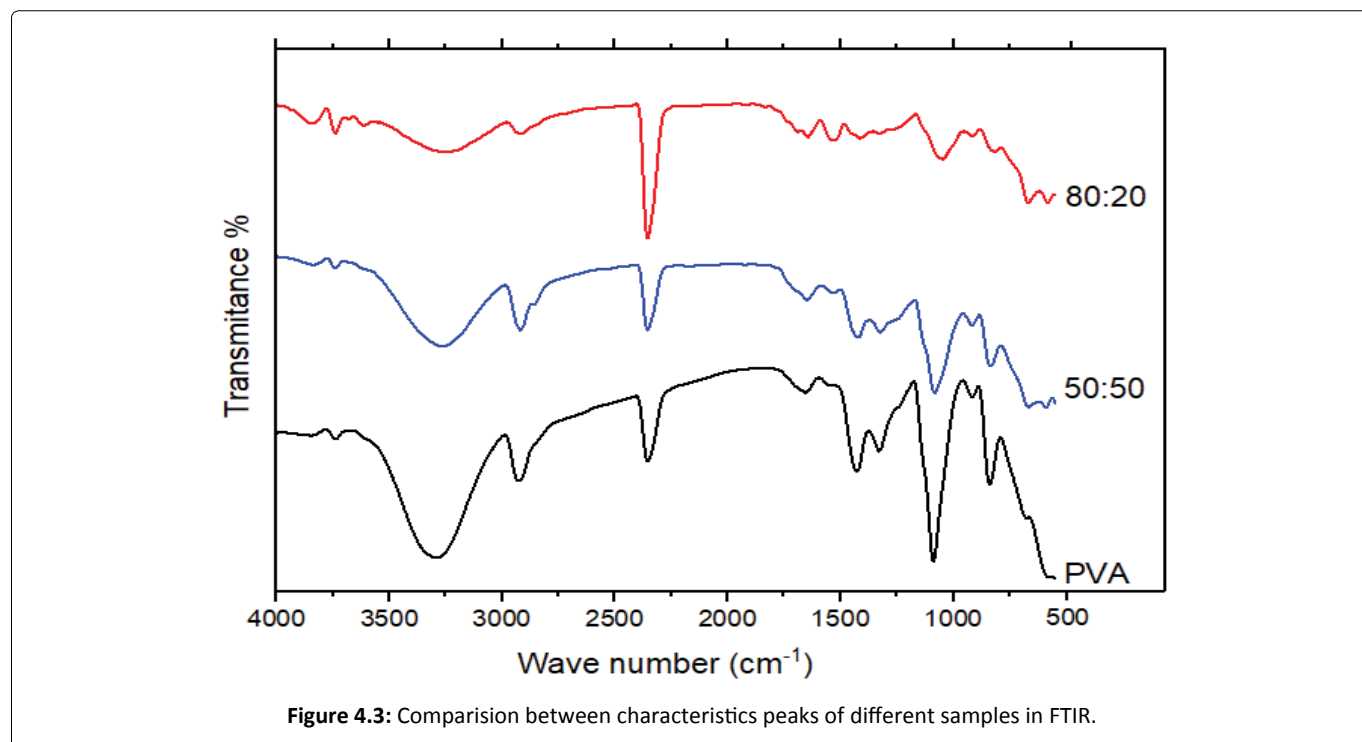


Figure 4.3: Comparison between characteristics peaks of different samples in FTIR.

Table 2: Result of moisture management properties.

Sample:	N1 (80:20)		N2 (50:50)	
Surface:	Top surface	Bottom surface	Top surface	Bottom surface
Wetting Time	2.808	2.808	120	5.804
Absorption	11.586	8.527	0	6.325
Max wetted radius (mm)	25.0	25.0	0	5.0
Spreading speed	5.311	4.928	0	0.8346
One way transport Capacity	-169.1058		112.5502	
Test Description	AATCC-195-2009		AATCC-195-2009	
Rating	Slow absorbing and slow drying fabric		Water penetration fabric	

Table 3: Antimicrobial activity.

Identity	Bacterial concentration	<i>Staphylococcus aureus</i>	Incubation time	Incubation temperature
N1 (80:20)	1.5×10^5 CFU/ml	10.63 ± 2	24 hrs	37 °C
N2 (50:50)	1.5×10^5 CFU/ml	3.89 ± 2	24 hrs	37 °C
PVA	1.5×10^5 CFU/ml	No ZOI	24 hrs	37 °C

Antibacterial assay

In wound dressings applications, the antibacterial activity of the nanofiber mat is very essential to determine the efficacy of empirical antimicrobial agents. The antibacterial properties of the developed nanomats against *S. aureus* are discussed in this section. The susceptibility of *S. aureus* bacteria against developed nanomats was tested using disk diffusion assays. In antibacterial tests against *S. aureus*, no zone of inhibition (ZOI) was observed around the nanofibrous mat produced from only PVA polymer. But, there was inhibition zone of 10.63 ± 2 mm around N1 (80:20) nanomat and 3.89 ± 2 mm around N2 (50:50) nanomat. So, the developed nanomats have antibacterial properties because of Piper Betle and

Azadirachta indica leaves extract loaded in Polyvinyl Alcohol polymer foundation (Table 3).

Conclusion

This research has successfully developed nanofibrous composite mats comprised of Piper Betle (Paan) and *Azadirachta indica* leaves extract incorporated on polyvinyl alcohol (PVA) polymer using the electrospinning method. The analysis of the SEM image shows that the novel developed nanofibrous mat has an average diameter of 170 nanometers in N1 (80:20) mats and 110 nanometers in N2 (50:50) mats. These nanocomposite mats have a slightly larger diameter and more porous structure than the PVA nanomat. Compared

to the N2 (50:50) ratio, the N1 (80:20) ratio of Piper Betle-*Azadirachta indica*:PVA nanomats exhibit better moisture management properties and is compatible with the wound dressing application. The existence of Piper Betle has been proved by the identification of the phenolic functional compound, as well as characteristic absorption peaks of *Azadirachta indica* leaf have been found in the FTIR spectrum of both nanomats. Mats developed to combat *Staphylococcus aureus* (*S. aureus*) bacteria shown a inhibition zone of 10.63 ± 2 mm around N1 (80:20) nanomat and 3.89 ± 2 mm around N2 (50:50) nanomat, proving substantial antibacterial activity. Piper Betle and *Azadirachta indica* leaves extract loaded in Polyvinyl Alcohol polymer electrospun nanomats has great potential in wound dressing application. However, the influence of various processing parameters such as electrospinning parameters, solution concentration, surface tension and humidity, *Azadirachta indica*-Piper Betle extraction method, mixing ratio, and PVA concentration may be investigated in future research.

Author Agreement

All authors have seen and approved the final version of the manuscript being submitted and declared that the article has not been accepted for prior publication and is not under consideration for publication elsewhere.

Declaration of Competing Interest

Authors declare no conflict of interest on this research paper.

Acknowledgements

This research work is a part of the thesis in Department of Textile Engineering, Dhaka University of Engineering and Technology, Bangladesh.

References

1. Das A, Satyaprakash K (2018) Antimicrobial properties of natural products: A review. *Pharma Innovation J* 7: 532-537.
2. Gyawali R, Ibrahim SA (2014) Natural products as antimicrobial agents. *Food Control* 46: 412-429.
3. Adamu BF, Gao J, Jhatial AK, et al. (2021) A review of medicinal plant-based bioactive electrospun nano fibrous wound dressings. *Materials & Design* 209: 109942.
4. Ambekar RS, Kandasubramanian B (2019) Advancements in nano fibers for wound dressing: A review. *European Polymer Journal* 17: 304-336.
5. Durand B, Marchand C (2016) Smart features in fibrous implantable medical devices, in smart textiles and their Applications. *Elsevier* 257-307.
6. Ali A, Shahid M (2019) Polyvinyl alcohol (PVA)-*Azadirachta indica* (Neem) nanofibrous mat for biomedical application: Formation and characterization. *Journal of Polymers and the Environment* 27: 2933-2942.
7. Ali A, Shahid A, Hossain D, et al. (2019) Antibacterial bi-layered polyvinyl alcohol (PVA)-chitosan blend nanofibrous mat loaded with *Azadirachta indica* (neem) extract. *International Journal of Biological Macromolecules* 138: 13-20.
8. Naseri N, Algan C, Jacobs V, et al. (2014) Electrospun chitosan-based nanocomposite mats reinforced with chitin nanocrystals for wound dressing. *Carbohydr Polym* 109: 7-15.
9. Barrientos IJH, Paladino E, Szabo P, et al. (2017) Electrospun collagen-based nanofibres: A sustainable material for improved antibiotic utilisation in tissue engineering applications. *International Journal of Pharmaceutics* 531: 67-79.
10. Chen JP, Chang GY, Chen JK (2008) Electrospun collagen/chitosan nanofibrous membrane as wound dressing. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 313: 183-188.
11. Hajiali H, Shahgasempour S, Reza Naimi-Jamal M, et al. (2011) Electrospun PGA/gelatin nanofibrous scaffolds and their potential application in vascular tissue engineering. *International Journal of Nanomedicine* 6: 2133-2141.
12. Hong S, Kim GH (2010) Electrospun polycaprolactone/silk fibroin/small intestine submucosa composites for biomedical applications. *Macromolecular Materials and Engineering* 295: 529-534.
13. Ranjbar-Mohammadi M, Rabbani S, Hajir Bahrami S, et al. (2016) Antibacterial performance and in vivo diabetic wound healing of curcumin loaded gum tragacanth/poly (ϵ -caprolactone) electrospun nanofibers. *Materials Science and Engineering: C* 69: 1183-1191.
14. Niranjana R, Kaushik M, Thamarai Selvi R, et al. (2019) PVA/SA/TiO₂-CUR patch for enhanced wound healing application: In vitro and in vivo analysis. *International Journal of Biological Macromolecules* 138: 704-717.
15. Abdel-Mohsen A, Abdel-Rahman RM, Kubena I, et al. (2020) Chitosan-glucan complex hollow fibers reinforced collagen wound dressing embedded with aloe vera. Part I: Preparation and characterization. *Carbohydrate Polymers* 230: 115708.
16. Ajmal G, Bonde GV, Mittal P, et al. (2019) Biomimetic PCL-gelatin based nanofibers loaded with ciprofloxacin hydrochloride and quercetin: A potential antibacterial and anti-oxidant dressing material for accelerated healing of a full thickness wound. *International Journal of Pharmaceutics* 567: 118480.
17. Xu F, Weng B, Gilkerson R, et al. (2015) Development of tannic acid/chitosan/pullulan composite nanofibers from aqueous solution for potential applications as wound dressing. *Carbohydrate Polymers* 115: 16-24.
18. Selvaraj S, Duraipandy N, Kiran MS, et al. (2018) Anti-oxidant enriched hybrid nanofibers: Effect on mechanical stability and biocompatibility. *International Journal of Biological Macromolecules* 117: 209-217.
19. Esmaeili E, Arshaghi TE, Simzar H, et al. (2020) The biomedical potential of cellulose acetate/polyurethane nanofibrous mats containing reduced graphene oxide/silver nanocomposites and curcumin: Antimicrobial performance and cutaneous wound healing. *International Journal of Biological Macromolecules* 152: 418-427.
20. Isfahani FR, Tavanai H, Morshed M (2017) Release of aloe vera from electrospun aloe vera-PVA nanofibrous pad. *Fibers and Polymers* 18: 264-271.
21. Kheradvar SA, Jhamak N, Hadi T, et al. (2018) Starch nanoparticle as a vitamin E-TPGS carrier loaded in silk fibroin-poly (vinyl alcohol)-Aloe vera nanofibrous dressing. *Colloids and Surfaces B: Biointerfaces* 166: 9-16.

22. Rahmoun NM, Zahia BA, Benabdallah M, et al. (2013) Antimicrobial activities of the henna extract and some synthetic naphthoquinones derivatives. *American Journal of Medical and Biological Research* 1: 16-22.
23. Nawasrah A, AlNimr A, Ali AA (2016) Antifungal effect of henna against *Candida albicans* adhered to acrylic resin as a possible method for prevention of denture stomatitis. *International Journal of Environmental Research and Public Health* 13: 520.
24. Ahamed MI, Inamuddin, Lutfullah, et al. (2016) Turmeric/polyvinyl alcohol Th (IV) phosphate electrospun fibers: Synthesis, characterization and antimicrobial studies. *Journal of the Taiwan Institute of Chemical Engineers* 68: 407-414.
25. Hadisi Z, Nourmohammadi J, Nassiri SM (2018) The antibacterial and anti-inflammatory investigation of lawsonia inermis-gelatin-starch nano-fibrous dressing in burn wound. *International Journal of Biological Macromolecules* 107: 2008-2019.
26. Ali A, Islama SM, Mohebullaha MD, et al. (2021) Antibacterial electrospun nanomat from nigella/PVA system embedded with silver. *The Journal of the Textile Institute* 112: 561-567.
27. Mouro C, Simões M, Gouveia IC (2019) Emulsion electrospun fiber mats of PCL/PVA/chitosan and Eugenol for wound dressing applications. *Advances in Polymer Technology* 2019.
28. Kulkarni AS, Gurav DD, Khan AA, et al. (2020) Curcumin loaded nanofibrous mats for wound healing application. *Colloids and Surfaces B Biointerfaces* 189: 110885-110885.
29. Islam MA, Beguma HA, Alib A, et al. (2021) Antibacterial electrospun nanofibers from poly (vinyl alcohol) and mikania micrantha with augmented moisture properties: Formation and evaluation. *The Journal of the Textile Institute* 112: 1602-1610.
30. Varshney N, Ajay KS, Poddar S, et al. (2020) Soy protein isolate supplemented silk fibroin nanofibers for skin tissue regeneration: Fabrication and characterization. *International Journal of Biological Macromolecules* 160: 112-127.
31. Shahid MA, Ali A, Jamal MSI, et al. (2020) Antibacterial wound dressing electrospun nanofibrous material from polyvinyl alcohol, honey and curcumin longa extract. *Journal of Industrial Textiles* 51: 455-469.
32. Homaeigohar S, Boccaccini AR (2020) Antibacterial biohybrid nanofibers for wound dressings. *Acta Biomaterial* 107: 25-49.
33. Varghese RJ, El Hadji Mamour Sakho, Parani S, et al. (2019) Introduction to nanomaterials: Synthesis and applications. *Nanomaterials for Solar Cell Applications* 75-95.
34. Davoodi P, Gill EL, Wang W, et al. (2021) Advances and innovations in electrospinning technology. *Biomedical Applications of Electrospinning and Electrospaying* 45-81.
35. Unnithan AR, Arathyram R, Kim CS (2015) Electrospinning of Polymers for Tissue Engineering. *Nanotechnology Applications for Tissue Engineering* 45-55.
36. Adhikari J, Ghosh M, Das P, et al. (2022) Polycaprolactone assisted electrospinning of honey/Betle with chitosan for tissue engineering. *Materials Today Proceedings* 57: 307-315.
37. Chakraborty D, Shah B (2011) Antimicrobial, antioxidative and antihemolytic activity of Piper Betle leaf extracts. *International Journal of Pharmacy and Pharmaceutical Sciences* 3: 192-199.
38. Lei D, Chan C-P, Wang Y-J, et al. (2003) Antioxidative and antiplatelet effects of aqueous inflorescence piper betle extract. *Journal of Agricultural and Food Chemistry* 51: 2083-2088.
39. Majumdar B, Chaudhuri SR, Ray A, et al. (2002) Potent antiulcerogenic activity of ethanol extract of leaf of piper betle Linn by antioxidative mechanism. *Indian Journal of Clinical Biochemistry* 17: 49-57.
40. Ali A, Lim XY, Wahida PF (2018) The fundamental study of antimicrobial activity of piper betle extract in commercial toothpastes. *Journal of Herbal Medicine* 14: 29-34.
41. Mossini AG, De Oliveira SKP, Kimmelmeier C (2004) Inhibition of patulin production by penicillium expansum cultured with neem (*Azadirachta indica*) leaf extracts. *Journal of Basic Microbiology* 44: 106-113.
42. Subapriya R, Nagini S (2005) Medicinal properties of neem leaves: A review. *Current Medicinal Chemistry-Anti-Cancer Agents* 5: 149-156.
43. Singh O, Khanam Z, Ahmad J (2011) Neem (*Azadirachta indica*) in context of intellectual property rights (IPR). *Recent Research in Science and Technology* 3: 80-86.
44. Badam L, Joshi S, Bedekar S (1999) In vitro antiviral activity of neem (*Azadirachta indica*. A. Juss) leaf extract against group B coxsackieviruses. *The Journal of Communicable Diseases* 31: 79-90.
45. Sahne F, Mohammadi M, Najafpour GD, et al. (2016) Extraction of bioactive compound curcumin from turmeric (*Curcuma longa* L.) via different routes: A comparative study. *Pakistan Journal of Biotechnology* 13: 173-180.
46. Sun D et al. (2015) Antimicrobial materials with medical applications. *Materials Technology* 30: B90-B95.
47. Alvarado Y, Muro C, Javier I, et al. (2019) Polymer nanoparticles for the release of complex molecules. *Materials for Biomedical Engineering* 135-163.
48. Singh R, Sanjeev G, Bhasha S, et al. (2021) Biopolymers and their classifications. *Biopolymers and Their Industrial Applications* 21-44.

DOI: 10.36959/758/545