Journal of King Saud University – Science 34 (2022) 101931

Contents lists available at ScienceDirect

Journal of King Saud University – Science

journal homepage: www.sciencedirect.com

Original article

Fabrication, characterization and *in vitro* evaluation of *Melia dubia* extract infused nanofibers for wound dressing

Sathish Kumar Karuppannan [a,1], Jayandra Bushion [b,1], Raghavendra Ramalingam [a], Subhashini Swaminathan [b], Kantha Deivi Arunachalam [a,*], Avinash Ashok Kadam [c], Rajakrishnan Rajagopal [d], Rengasamy Sathya [e], Sasikala Chinnappan [f,*]

[a] *Center for Environmental Nuclear Research, Directorate of Research, SRM Institute of Science and Technology, SRM Nagar, Kattankulathur, 603203, Kanchipuram, Chennai, Tamil Nadu, India*
[b] *Department of Biotechnology, College of Engineering and Technology, SRM Institute of Science and Technology, SRM Nagar, Kattankulathur, 603203, Kanchipuram, Chennai, Tamil Nadu, India*
[c] *Research Institute of Biotechnology and Medical Converged Science, Dongguk University-Seoul, 32 Dongguk-ro, Ilsandong-gu, Goyang-si 10326, Gyeonggi-do, Republic of Korea*
[d] *Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia*
[e] *Department of Microbiology, Centre for Research and Development, PRIST University, Tamil Nadu 613 403, India*
[f] *Department of Pharmaceutical Biology, Faculty of Pharmaceutical Sciences, UCSI University Kuala Lumpur (South Wing), No.1, Jalan Menara Gading, UCSI Heights 56000 Cheras, Kuala Lumpur, Malaysia*

ARTICLE INFO

Article history:
Received 24 November 2021
Revised 18 February 2022
Accepted 25 February 2022
Available online 1 March 2022

Keywords:
Antibacterial activity
Biocompatible
Biopolymer
Scratch assay
L929 fibroblast cells

ABSTRACT

Open wounds are easily susceptible to infection, so it is necessary to develop a suitable barrier to prevent infection and enhance wound healing. The nanofibers has potential applications in regenerative medicine and tissue engineering. Certain phytochemicals are proangiogenic agents and helps in tissue remodeling. The present study focuses on the fabrication and characterization of *Melia dubia* (MD) leaf ethanolic extract incorporated polycaprolactone (PCL)/gelatin (Gel) electrospun nanofibers (NFs) for efficient wound dressing applications. The existence of bioactive compounds in MD leaf extract was confirmed by gas chromatography-mass spectrometry analysis (GCMS). The fabricated nanofibers are characterized by scanning electron microscope (HRSEM), fourier transform infrared spectroscopy (FTIR), X-ray crystalline diffraction analysis (XRD), contact angle and mechanical strength analysis. Antioxidant and *in-vitro* drug release was evaluated for NFs. Then antibacterial activity was tested against clinical pathogens and the biocompatibility of the NFs was tested on L929 fibroblast cells by MTT and confocal analysis. GCMS analysis of MD leaf extract confirms the existence of numerous bioactive compounds, that support wound healing. HRSEM analysis revealed that MD leaf extract loaded electrospun nanofibers were smooth, ultrafine, and free of beads with 256 ± 89 nm. The incorporation of the MD leaf extract into the NFs reduced their semi-crystalline nature and increased the tensile strength of the NFs. Contact angle analysis confirmed that incorporation of plant extracts enhanced the hydrophilic nature (22.93 ± 4.3°). The continuous sustained release of drug from initial concentration (~74 %). *In vitro* antibacterial analysis revealed that fabricated NFs effectively inhibited the growth of *Escherichia coli* (18.2 ± 1.5 mm), *Staphylococcus aureus* (26.5 ± 2.0 mm), multidrug-resistant *Staphylococcus aureus* (23.0 ± 0.9 mm), and *Pseudomonas aeruginosa* (28.1 ± 1.2 mm). *In vitro* biocompatibility assays revealed that fabricated NFs are not cytotoxic and

Abbreviations: HRSEM, High resolution scanning electron microscopy; FTIR, Fourier transform infrared spectroscopy; XRD, X-ray crystalline diffraction analysis; PCL, polycaprolactone; Gel, Gelatin; NFs, nanofibers; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide; TFE, Trifluoroethanol; DMEM, Dulbecco's Modified Eagle Medium; MD, *Melia dubia*; MHA, Muller Hinton Agar; OD, optical density; PBS, Phosphate-buffered saline; GCMS, Gas chromatography-mass spectrometry analysis; AO, Acridine orange; EtBr, Ethidium bromide; DPPH, Diphenyl-1-picrylhydrazyl.

* Corresponding authors.
E-mail addresses: kanthad.arunachalam@gmail.com (K.D. Arunachalam), sasikala@ucsiuniversity.edu.my (S. Chinnappan).

[1] Sathish Kumar Karuppannan and Jayandra Bushion equally contributed to the work as first authors.

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jksus.2022.101931

enhanced the proliferation of fibroblast. The study indicated the possible ability of *Melia dubia* leaf extract incorporated PCL/Gel NFs in the killing of wound pathogens with biocompatibility towards L929 cells.

1. Introduction

The human skin serves as an anatomical barrier, maintains body hemostasis and protects internal organs from the external damages (Yousef et al., 2021). Replacement of damaged or destroyed tissue in living organisms is referred to as wound healing and it is an intricate and complex process (Krafts, 2010). Normally, wound healing is organized as different phases such as hemostasis, inflammation, proliferation and maturation or remodelling phase. The reepithelization is a process of movement of epithelial cells from surrounding epidermis over denuded surface (Clark et al., 2007). Heavy loads of microbial infection on the injured site slowdowns the wound healing process (Alonso-Montemayor et al., 2021). Wound dressing materials like alginates, hydrogels, sponges, and films are commercially available in the market (Kim et al., 2019). Still wound care management is challenging due to the susceptible bacterial infection (Zhou et al., 2018). Aligned and nonwoven nanofibers with high porosity can be fabricated under the influence of an electric field by a method called electrospinning (Homaeigohar and Boccaccini, 2020). Electrospun nanofibers are used for various applications, such as thermal insulation and filtration, protective covering garments, conductive devices and sensors, wound dressings and tissue engineered scaffolds (Park, 2010). Poly-ε-caprolactone has high mechanical properties compared to other natural polymers, it has excellent processability and good biocompatibility (Unalan et al., 2019). So, the combined PCL and Gel should give improved biocompatibility with enhanced physical and mechanical properties (Sheng et al., 2019). Plant extract mediated wound dressing nowadays is gaining attention due to less toxicity and fewer side effects, inherent medicinal properties, environmentally sustainable, easily available with less cost and good alternatives for wound management (Adamu et al., 2021; Nayeem et al., 2021). Some of the plant extracts incorporated in nanofiber are *Centella asiatica*, *Tecomella undulata*, *Aloe vera*, *Indigofera aspalathoides*, *Calendula officinalis*, etc. (Fatehi and Abbasi, 2020).

The plant *Melia dubia* (MD) of the family Meliaceae is mostly known as Malabar Neem and available in the tropical moist deciduous forests. The *Melia dubia* has extensive medical, pharmacological and ethnomedicinal uses (Goswami et al., 2020). The ethanolic extract of MD increased the synthesis of collagen, deposition of hexosamine, wound contraction rate and tensile strength of wound in female Wistar rats (Thangavel et al., 2019). Objective of the current study is identifying bioactive compounds present in the *Melia dubia* ethanolic extract, fabrication and characterization of MD plant extract incorporated PCL/Gel hybrid NFs, determination of antioxidant property, *in-vitro* drug release of NFs and assessment of antibacterial and wound healing efficiency of NFs using L929 fibroblast.

2. Materials and methods

2.1. Chemicals

Poly-ε-caprolactone (Mw-80,000, Sigma Aldrich, UK), Porcine Gelatin (Gel strength 300-Type A, Sigma Aldrich, USA), Dulbecco's Modified Eagle Medium (DMEM), Phosphate-buffered saline (PBS) (Hi-Media, India), Acetic acid, Ethanol (Analytical reagent), 2,2,2 Trifluoroethanol (TFE), Acridine Orange (AO), Ethidium bromide (EtBr) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), and DPPH (diphenyl-1-picrylhydrazyl) (SRL, India).

2.2. Processing of Melia dubia leaves

MD leaves were cleaned, shade dried for 3 weeks, then made into powder using a mechanical blender. To obtain ethanolic plant extract, 25 g of leaf powder was added in 0.5 L of 70 % ethanol and stirred for 3 h in a shaker. For extraction, the stirred mixture was placed in an ultrasonic water bath at 60 ℃ for 60 min. The extract was then filtered, concentrated and stored at 4 °C. The GCMS analysis of the extract was performed to identify the presence of phytochemical using the mass spectral patterns stored in NIST library (Konappa et al., 2020).

2.3. Electrospinning of nanofiber

Nanofiber was fabricated by ESPIN-Nano, Physics Instrument Co., Chennai, India. PCL (8 %), gelatin (4 %) and plant extract (25 %) (respect to PCL Wt. %) were added to the TFE and kept in stirring condition for 14 h. The polymer mixture was taken in a plastic syringe (5 mL) and 13 kV high voltage was applied. The polymer solution at the needle orifice begun to stretch and the nanofibers were collected on the collector that was kept 14 cm away from the tip of the needle.

2.4. Characterization of synthesized nanofiber

The morphology was identified using HR-SEM (Thermo Scientific Apreo S), Functional groups of the nanofiber were identified using FTIR (Shimadzu, IR Tracer 100), crystalline nature of the compound was determined using XRD (PANalytical, Netherlands), and the wettability of NFs was determined by contact angle analysis (KYOWA DMS-401, Japan), Mechanical property of the NFs was analysed using tensile strength tester (Instron, USA).

2.5. In vitro drug release

50 mg of the plant extract incorporated nanofiber was submerged in 3 mL of PBS (pH 7.4). At different time interval, 3 mL of PBS was taken from the release medium, at the same time replaced with 3 mL of fresh PBS. The different concentration of standard was prepared using plant extract and absorbance was measured at 268 nm.

2.6. Antioxidant assay

The antioxidant activity of NF was examined at a fixed time point. For that 10 mg of PCL/Gel, PCL/Gel/MD NFs are added to 5 mL of 50 μm DPPH (dissolved in methanol) solution and kept in dark for 30 min. The antioxidant activity of the NF was analyzed using UV spectrophotometer at 514 nm. Antioxidant activity was evaluated using equation (1).

$$Antioxidant\ activity(\%) = (A - A1)/A \quad (1)$$

A- denotes absorbance of DPPH solution and A1-denotes absorbance of nanofibers immersed DPPH solution.

2.7. Antibacterial studies

PCL/Gel/MD NFs antibacterial activity was analyzed by disc diffusion method. The clinical pathogens (*Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), Methicillin-Resistant *Staphylococcus aureus* (multidrug-resistant *S. aureus*) and *Pseudomonas aeruginosa* (*P. aeruginosa*)) were obtained from Department of Microbiology, SRM Institute of Medical College Hospital and Research Center, Kattankulathur, Chengalpattu. The bacterial cultures were (1.5×10^8 CFU/mL) spread on Muller Hinton Agar (MHA). Then UV sterilized PCL/Gel and PCL/Gel/MD NFs (30×30 mm) were placed on MHA and incubated at 36 ± 1 °C for 24 h.

2.8. Cell culture

2.8.1. Scratch assay

The cell migration after exposing to nanofiber extracts was measured using scratch assay. L929 (fibroblast cell lines) were cultured in a 6-well culture plate containing complete media and grown up to 90% confluency. Then the cell monolayers were injured and to eliminate cell debris, wounded monolayers were washed thrice using a culture medium. By incubating the sterile mats in DMEM for 24 h, we were able to get PCL/Gel and PCL/Gel/MD NFs scaffold extracts in the medium. The extracts of the scaffold were added to the scratched cell monolayers and incubated for 1 day. Image J is used for wound area calculation. Depending on the wound closure of the control and treatment groups, the wound area percentage was calculated.

2.8.2. MTT and confocal analysis

The fibroblast cells were incubated with the media containing PCL/Gel or PCL/Gel/MD NFs extract for 24 and 48 h. MTT dye was added (5 mg/mL in PBS) to each well and incubated for about 4 h. After the incubation time, the MTT was taken out without disturbing the purple colored formazan crystals. To dissolve the insoluble crystal, solubilizing agent (DMSO) was added. Finally, the optical density (OD) was measured at 570 nm. After 48 h incubation, the live/dead staining done using AO/EtBr (1:1).

3. Results and discussion

3.1. GCMS analysis

GCMS analysis of MD plant extract demonstrated the presence of numerous bioactive compounds that enhances the wound healing process (Fig. 1). The detected compounds are presented in Table 1. The bioactive compounds like squalene, vitamin E, octadecanoic acid, ethyl ester, azafrin, heneicosane, palmitic acid, ethyl ester and 4-epi-cubedol has antioxidant, antibacterial and anti-inflammatory properties (Brigelius-Flohé, 2006; Eswaraiah et al., 2020; Islam et al., 2018; Rosselli et al., 2019; Vanitha et al., 2020). Presence of these bioactive compounds confirms that plant can be suitable for enhancing the wound healing process.

3.2. HR-SEM analysis

The structure and morphology of plant extract-loaded nanofiber were investigated using HRSEM. Fig. 2a & c shows the random orientation of electrospun nanofiber with a bead-free, continuous, smooth, narrow size distribution and also no particulate aggregates were found indicating no phase separation occurred during electrospinning after the incorporation of plant extract. The mean diameter for PCL/Gel and PCL/Gel/MD NFs (Fig. 2b & d) was found to be 319 ± 104 and 256 ± 89 nm, respectively. Previous study reports incorporation of *Gymnema sylvestre* and Henna plant extract into polymer solution decreased the fiber diameter due to higher charge density and conductivity (Ramalingam et al., 2021; Yousefi et al., 2017). PCL/Gel/MD NFs were found to be entangled compared to PCL/Gel NFs. Rosa et al. (2017) reported the entangled NFs helps in improving the nutrition infiltration, new cell integra-

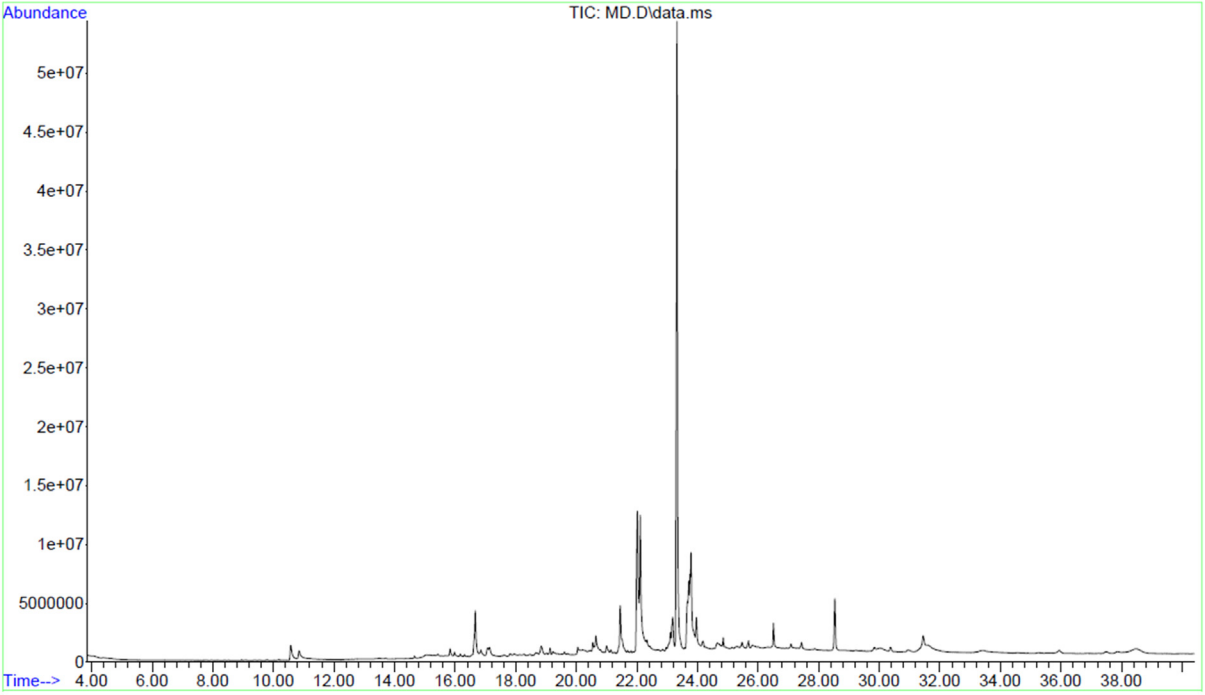

Fig. 1. GC–MS spectrum of ethanolic extract of *Melia dubia* leaf extract.

Table 1
Bioactive compounds present in the ethanolic extract of *Melia dubia* leaf extract.

S. No	Retention Time	Compound	Formula	Molecular weight	Area %
1	10.574	Ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl)propan-2-yl carbonate	$C_{13}H_{22}O_4$	242	1.46%
2	10.847	α-Methyl-α-[4-methyl-3-pentenyl]oxiranemethanol	$C_{10}H_{18}O_2$	170	0.92%
3	14.658	Cyclohexane, 1-ethenyl-1-methyl-2-(1-methylethenyl)-4-(1-methylethylidene)-	$C_{15}H_{24}$	204	0.16%
4	15.053	6-Methyl-cyclodec-5-enol	$C_{11}H_{20}O$	168.1514	1.04%
5	15.428	Cyclohexane, 1-ethenyl-1-methyl-2,4 bis(1-methylethenyl)-, [1S-(1α,2β,4β)]-	$C_{15}H_{24}$	204.18	0.12%
6	15.829	β-ylangene	$C_{15}H_{24}$	204.1878	0.35%
7	16.172	Cedrene	$C_{15}H_{24}$	204.18	0.13%
8	16.306	β-copaene	$C_{15}H_{24}$	204.18	0.11%
9	16.662	1H Cyclopenta[1,3]cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-3bβ,4β,7α,7aS*)]- (β-Cuvebene)methylethyl)	$C_{15}H_{24}$	204.1878	2.91%
10	17.127	4-epi-cubedol	$C_{15}H_{26}O$	222.19	1.19%
11	17.807	Cyclotridecane	$C_{13}H_{26}$	182.2	0.48%
12	18.66	tau.-Cadinol	$C_{15}H_{26}O$	222.19	0.32%
13	18.844	2-Naphthalenemethanol, decahydro-α,α,4a-trimethyl-8-methylene-, [2R-(2α,4aα,8aβ)]-	$C_{15}H_{26}O$	222.19	0.69%
14	19.131	Eicosane, 2-methyl-	$C_{21}H_{44}$	296.34	0.22%
15	19.226	Cholestan-3-ol, 2-methylene-, (3β,5α)-	$C_{28}H_{48}O$	400.37	0.37%
16	19.608	Heneicosane	$C_{21}H_{44}$	296.34	0.13%
17	20.047	10-Heneicosane	$C_{21}H_{42}$	294.32	0.81%
18	20.543	3,7,11,15-tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.3	0.42%
19	20.645	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268.27	0.91%
20	20.995	Phthalic acid, 5-methylhex-2-yl isobutyl ester	$C_{19}H_{28}O_4$	320.19	0.47%
21	21.141	Eicosane	$C_{20}H_{42}$	282.32	0.13%
22	21.447	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.255	3.52%
23	22.013	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.240	9.73%
24	22.108	Palmitic acid, ethyl ester	$C_{18}H_{36}O_2$	284.271	7.90%
25	23.107	8,11-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294.25	0.64%
26	23.177	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292.24	1.95%
27	23.323	Phytol	$C_{20}H_{40}O$	296.302	31.3%
28	23.667	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.24	2.43%
29	23.711	9,12-Octadecadienoic acid, ethyl ester	$C_{20}H_{36}O_2$	308.27	2.87%
30	23.781	Ethyl 9,12,15-octadecatrienoate	$C_{20}H_{34}O_2$	306.25	8.00%
31	23.966	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312.3	1.69%
32	24.182	Isophytol, acetate	$C_{22}H_{42}O_2$	338.31	0.40%
33	24.659	2H-1-benzopyron-2one,7-methoxy-6-(3-methyl-2-butenyl)-	$C_{15}H_{16}O_3$	244.1	1.00%
34	25.289	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366.42	0.27%
35	25.467	4,8,12,16-Tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$	324.3	0.37%
36	25.817	Desoximetasone	$C_{22}H_{29}FO_4$	376	0.30%
37	30.054	17-Pentatriacontene	$C_{35}H_{70}$	490.54	0.77%
38	30.366	Squalene	$C_{30}H_{50}$	410.39	0.33%
39	31.448	Heptacosane, 1-chloro-	$C_{27}H_{55}Cl$	414.39	3.137
40	33.458	Azafrin	$C_{27}H_{38}O_4$	426.27	1.02%
41	35.946	Hentriacontane	$C_{31}H_{64}$	436.5	0.47%
42	37.479	Vitamin E	$C_{29}H_{50}O_2$	430.38	1.47%

tion, oxygen permeation, long-term medication release and wound exudate clearance (Rosa et al., 2017).

3.3. Functional group analysis NFs

The chemical bonding of PCL/Gel NFs and PCL/Gel NFs with MD plant extract was determined by FTIR analysis as given in Fig. 3a. The peaks obtained at 2942 and 1723 cm^{-1} in PCL/Gel nanofiber represents asymmetric $-CH_2$ and C = O stretching vibration that corresponds to PCL. N–H stretching associated with the peaks at 3309 cm^{-1}, C = O amide I stretching at 1648 cm^{-1}, N–H bending associated at 1652 cm^{-1} and stretching at 1040 cm^{-1} associated with C = O corresponds to gelatin. The sole difference between PCL and gelatin is the NH_2 and OH stretchings (Chong et al., 2015). In-plant extract, stretching at 3043 cm^{-1} represents O–H stretching and 1032 cm^{-1} represents the C-O stretching. In PCL/Gel/MD NFs, the plant extract band was overlapped in PCL/Gel NFs at the wavenumber of 3043, 2612, 1211, 1052, 795 and 673 cm^{-1}.

3.4. XRD analysis

Fig. 3b depicts the PCL/Gel & PCL/Gel/MD NFs XRD spectrum. In PCL peaks at 2θ = 21.7° and 24.8° indicate the plane of 110 and 200, respectively indicating the crystalline nature of PCL. The plant and gelatin exhibits the amorphous nature (Fig. 3b). The peak intensity of PCL/Gel/MD NFs is lesser than PCL which is due to the incorporation of plant extract. Gautam et al. (2013) stated that incorporating the amorphous components decreased the intensity and semi-crystalline nature of PCL NFs (Gautam et al., 2013).

3.5. Water contact angle analysis

The hydrophilic property of the nanofibers was analysed using water contact angle analysis. The water contact angle was found to be 51.16 ± 5.26° and 22.93 ± 4.3° for PCL/Gel and PCL/Gel/MD NFs, respectively (Fig. 3c). The wettability of MD plant extract loaded nanofibers was found to be increased when compared to PCL/Gel nanofibers; this can enhance the absorption of wound ooze outs and keeps a moist environment around the wound bed (Yeum et al., 2016). Unalan et al. (2019) reported that the existence of polar phytocomponents and hydrophilic groups in the plant enhances the hydrophilicity of the PCL/Gel NFs (Unalan et al., 2019). The wettability of NFs exhibits a dynamic role in the adhesion and proliferation of the fibroblast cells.

3.6. In-vitro drug release

Fig. 3d demonstrates the drug release profile of PCL/Gel/MD NFs. About 75.9 % of plant extract was entrapped in NFs. In the first

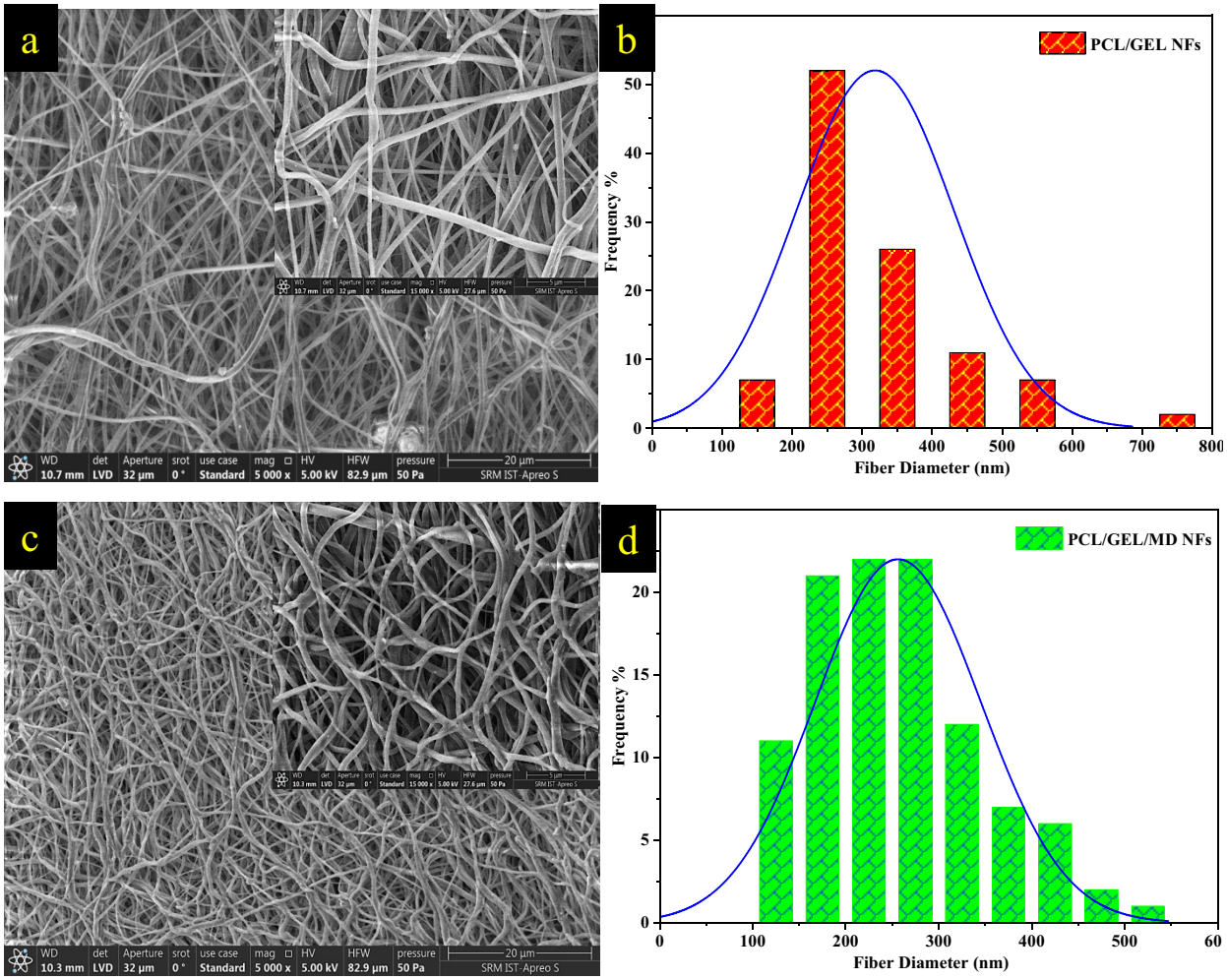

Fig. 2. HRSEM images and histogram plot of (a&b) PCL/Gel and (c&d) PCL/Gel/MD NFs (Magnification-5000X). Insert represents higher magnification image of nanofibers ((Magnification- 15000X).

2 h of incubation, initial burst release was observed which might be due to the dissolution of plant extract present in the surface of the nanofibers. After that, a continuous and sustained drug release was observed up to 72 h, releasing about 74 % of the drug from the initial concentration. The extended drug release of PCL/Gel/MD NFs is due to slow degradation of gelatin entangled with PCL (Xue et al., 2014). The initial burst release of the plant extract helps to reduce the bacterial colonization (Ramalingam et al., 2019b).

3.7. Mechanical strength analysis

Mechanical properties of the NFs plays important role in tissue engineering applications. The incorporation of plant extract into polymer increased their mechanical strength (Table 2). Raghavendra et al. (2019) reported that incorporation of plant extract increased tensile strength, toughness and tensile modulus in nanofibers (Ramalingam et al., 2019a). Selvaraj et al. (2017) fabricated fenugreek incorporated nanofiber with enhanced mechanical property compared to silk fibroin (Selvaraj and Fathima, 2017). Small fiber with densely packed fibrillar structure with more entanglements enhanced the mechanical properties, in case of PCL/Gel NFs alignment of NFs with increased fiber diameter decreased the mechanical property (Lim et al., 2008).

3.8. Antioxidant assay

The excess amount of oxidative stress leads to impaired wound healing. The antioxidants helps to control the oxidative stress in wound site thereby accelerating the wound healing process (Fitzmaurice et al., 2011; Krochmal-Marczak et al., 2021). The antioxidant property of nanofibers was evaluated by antioxidant assay (DPPH) shown in Fig. 4a & b. The DPPH solution exhibits maximum absorbance at 514 nm. PCL/Gel and PCL/Gel/MD NFs exhibited the DPPH scavenging activity of 16.65 ± 0.18 and 49.27 ± 0.53 %, respectively. The antioxidant activity of the PCL/Gel NFs, might be due to the presence of gelatin (radical scavenging peptide) (Mendis et al., 2005). GCMS and FTIR analysis of the MD extract confirms presence of phenolic compounds/groups. Kanthaswamy et al. (2019) fabricated quinone-based chromenopyrazole antioxidant-laden silk fibroin NFs, reduced oxidative stress and enhanced the dense growth of the fibroblasts (Kandhasamy et al., 2019).

3.9. Kirby-Bauer disc diffusion analysis

The antibacterial effect of the nanofibers was assessed using disc diffusion method (Fig. 4c). PCL/Gel NFs has no antibacterial activity against the wound pathogens. Zone of inhibition for MD

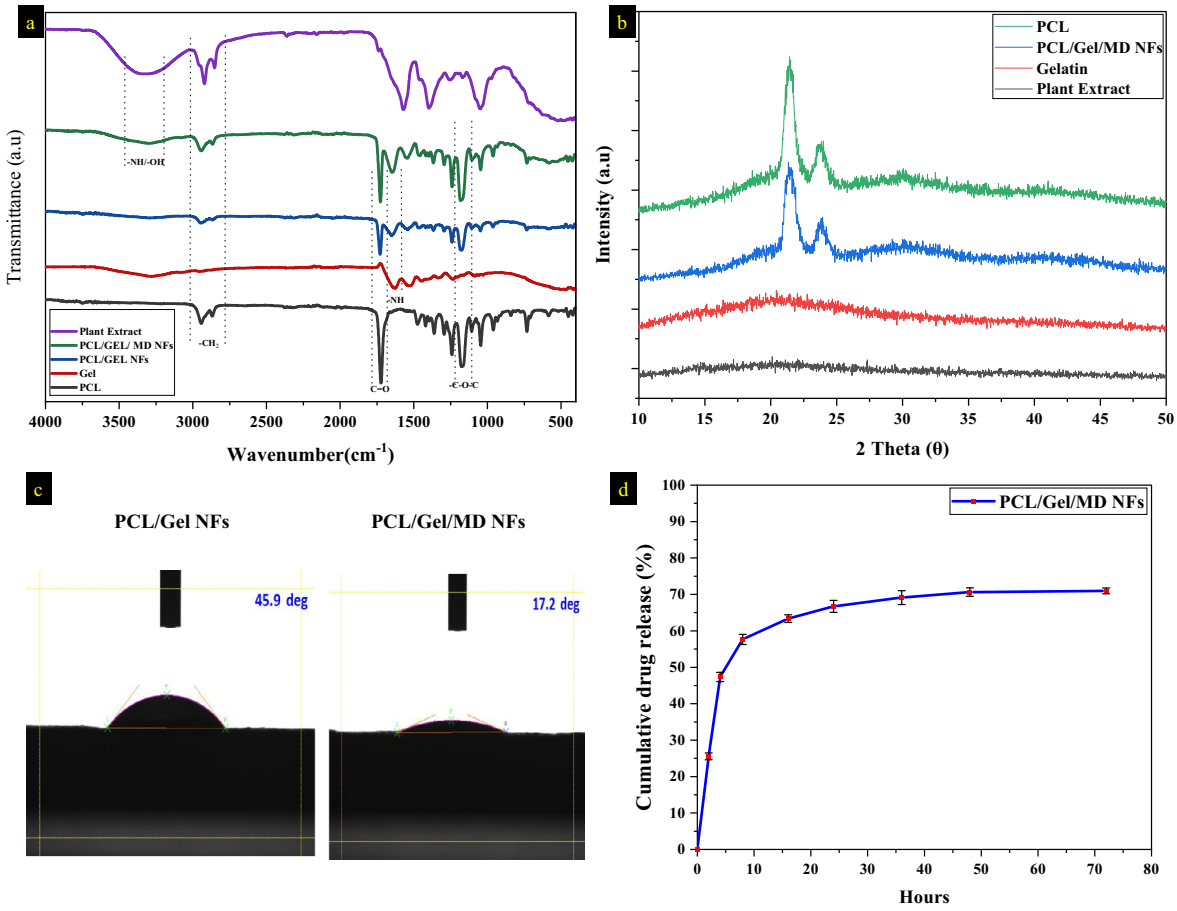

Fig. 3. a) FTIR spectrum of plant extract, PCL/Gel/ MD NFs, PCL/Gel NFs, gelatin, and PCL, b) XRD spectrum of plant extract, gelatin, PCL and PCL/Gel/MD NFs, c) contact angle analysis of PCL/Gel and PCL/Gel/MD NFs, d) cumulative drug release percentage of PCL/Gel/MD NFs.

Table 2
Mechanical properties of PCL/Gel and PCL/Gel/MD NFs.

Sample	Thickness (mm)	Maximum load (N)	Elongation at break (%)	Tensile strength (MPa)
PCL/Gel NFs	0.17 ± 0.01	4.19 ± 0.10	42.05 ± 0.79	2.23 ± 0.08
PCL/Gel/MD NFs	0.19 ± 0.08	7.01 ± 0.12	46.61 ± 2.20	7.84 ± 0.01

plant extract incorporated NFs against *E. coli*, *S. aureus*, multidrug-resistant *S. aureus*, and *P. aeruginosa* were 18.2 ± 1.5, 26.5 ± 2.0, 23.0 ± 0.9 and 28.1 ± 1.2 mm, respectively (excluding fiber diameter). The ethanolic extract of MD leaves have terpenoids, phenolic derivatives, lipophilic compounds and limonoids as bioactive compounds which possess antibacterial activity against clinical pathogens (Goswami et al., 2020; Thangavel et al., 2019). Bioactive compounds inhibit bacterial growth by forming complex with cell wall, extracellular matrix and soluble proteins (El-Zayat et al., 2021). Zheng et al. (2005) reported unsaturated fatty acids exhibited antibacterial activity by inhibiting the bacterial enoyl-acyl carrier protein reductase (Zheng et al., 2005).

3.10. Scratch assay

The cells were scratched artificially in the center, causing the cells to migrate to the damaged location. The pace, pattern and direction of cell migration may all be analyzed using this assay (Kandhasamy et al., 2019). By densitometry analysis, the percentage of wound healing or the cell migration percentage was calculated. These values were comparable to PCL/Gel nanofiber levels. When compared to PCL/Gel NFs, nanofiber containing plant extract enhanced the healing rate, which was observed over a longer period of up to 24 h (Fig. 5a). The mean percentage of cell migration achieved for PCL/Gel was 87.74 % and 97.13 % for the PCL/Gel/MD NFs.

3.11. MTT assay and confocal analysis

Biocompatibility of PCL/Gel and PCL/Gel/MD NFs was assessed by MTT assay and confocal analysis. Compared to control (TCPS), the PCL/Gel and PCL/Gel/MD NFs extract-treated wells showed increased color (purple) intensity. It indicates that PCL/Gel/MD NFs positively enhance the proliferation of L929 cells at 24 and 48 h. Fig. (5b&c) indicates that the plant extract has proliferating ability and no cytotoxicity. MD ethanolic extract enhances collagen

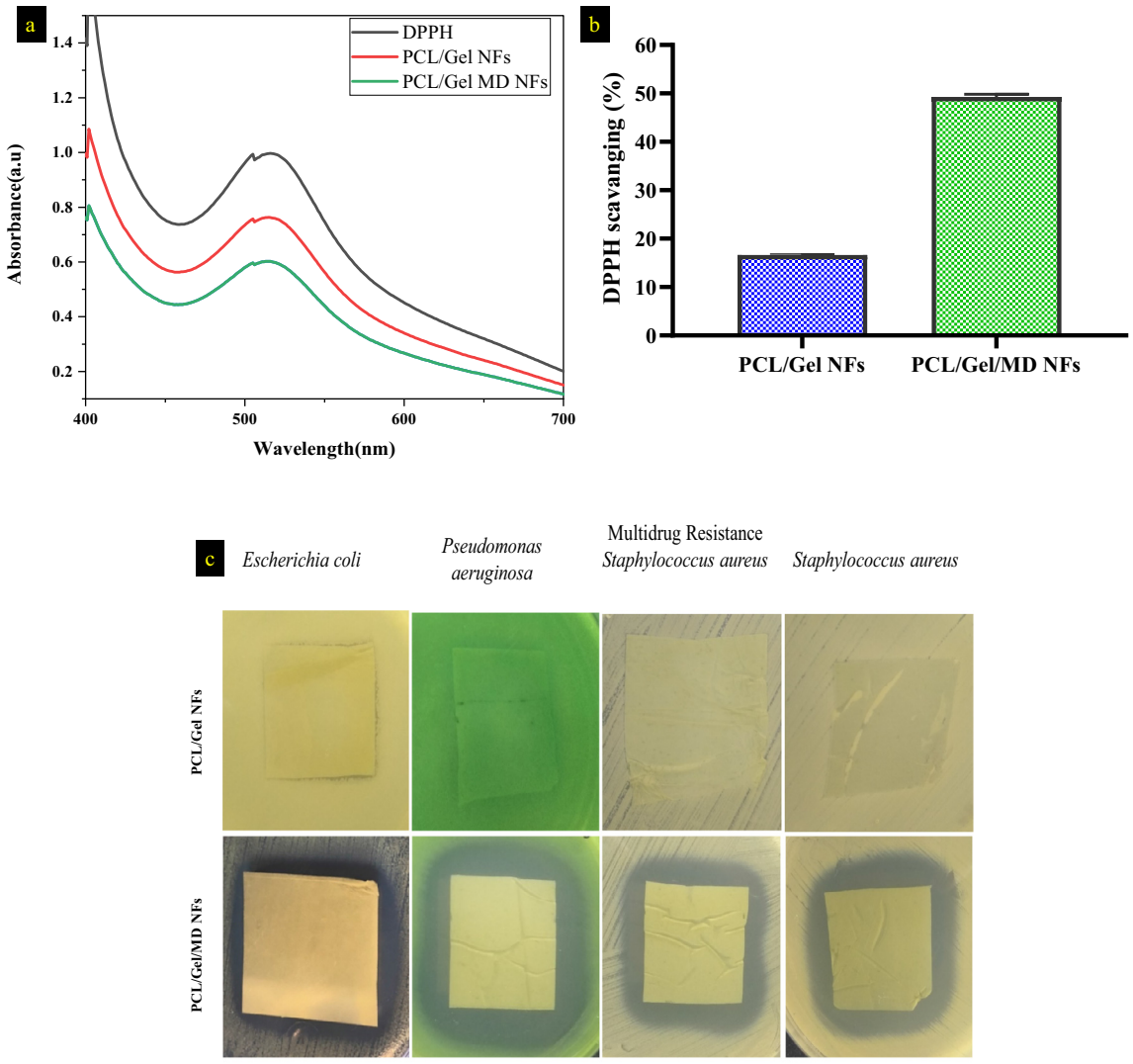

Fig. 4. a) & b) antioxidant activity of PCL/Gel and PCL/Gel/MD NFs after 30 min incubation, c) Antibacterial activity PCL/Gel and PCL/Gel/MD NFs against Gram-positive and negative organisms.

synthesis and hexosamine in wound surfaces (Thangavel et al., 2019). Ajmal et al. (2019) reported that gelatin and flavonoid content in the nanofiber enhanced fibroblast proliferation (Ajmal et al., 2019).

4. Conclusion

In this study, PCL/Gel nanofibers functionalized with *Melia dubia* ethanolic leaf extract has showing great potential as a wound dressing. GCMS analysis of the plant extract confirms the presence of bioactive compounds (which enhance the wound healing process). Morphological analysis showed the synthesized nanofibers are uniform, smooth, and beadless with nanometer dimension. Incorporation of the plant extract into PCL/Gel NFs decreased the crystallinity and mechanical properties. Biofunctionalized nanofiber showed hydrophilic nature and sustained release up to 72 h. Moreover, the *in vitro* analysis of these nanofibers exhibited potential bactericidal action against the wound pathogens; in addition to that, it also enhanced the proliferation and attachment of fibroblasts cells compared to PCL/Gel NFs. In future, the PCL/Gel/MD NFs can be used as effective wound dressing material.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors acknowledge the funding support from Researchers Supporting Project Number (RSP2022R465), King Saud University, Riyadh, Saudi Arabia for this research work. We would like to thank CSIR-CLRI, Chennai for helping in tensile strength analysis. The authors would like to thank Mr. Mohammed Junaid Hussain, Dr. M. R Ganesh and Ms. Shazia Anjum Musthafa, SRM IST, Chennai for helping in confocal microscopic analysis. The authors would like to sincerely thank IIISM, NRC and SCIF, SRM Institute of Science

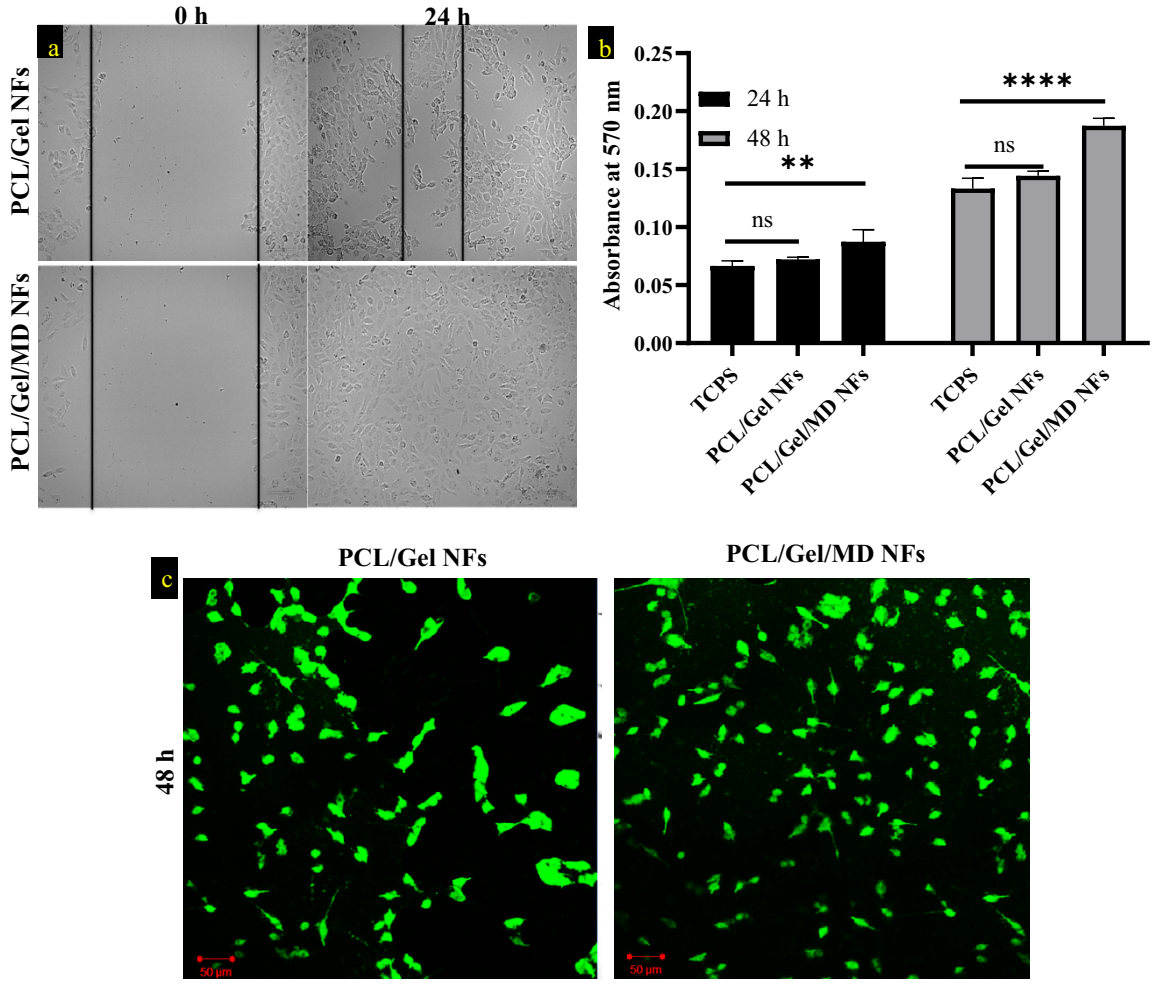

Fig. 5. a) Wound healing assay of PCL/Gel NFs and PCL/Gel/MD NFs, b) MTT analysis of TCPS, PCL/Gel and PCL/Gel/MD NFs at 24 and 48 h. Two way ANOVA was performed. $p > 0.05$ is considered as nonsignificant (ns) c) Confocal image of PCL/Gel and PCL/Gel/MD NFs after 48 h (scale bar- 50 μm).

and Technology, Chennai for providing the necessary facilities required for the successful completion of the work.

References

Adamu, B.F., Gao, J., Jhatial, A.K., Kumelachew, D.M., 2021. A review of medicinal plant-based bioactive electrospun nano fibrous wound dressings. Mater. Des. 209, 109942.

Ajmal, G., Bonde, G.V., Mittal, P., Khan, G., Pandey, V.K., Bakade, B.V., Mishra, B., 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. Int. J. Pharm. 567, 118480.

Alonso-Montemayor, F.J., Reyna-Martínez, R., Neira-Velázquez, M.G., Sáenz-Galindo, A., Aguilar, C.N., Narro-Céspedes, R.I., 2021. A review on antibacterial and therapeutic plasma-enhanced activities of natural extracts. J. King Saud Univ. - Sci. 33 (6), 101513.

Brigelius-Flohé, R., 2006. Bioactivity of vitamin E. Nutr. Res. Rev. 19 (2), 174–186. https://doi.org/10.1017/S0954422407202938.

Chong, L.H., Lim, M.M., Sultana, N., 2015. Fabrication and evaluation of polycaprolactone/gelatin-based electrospun nanofibers with antibacterial properties. J. Nanomater. 2015, 1–8. https://doi.org/10.1155/2015/970542.

Clark, R.A.F., Ghosh, K., Tonnesen, M.G., 2007. Tissue engineering for cutaneous wounds. J. Invest. Dermatol. 127 (5), 1018–1029.

El-Zayat, M.M., Eraqi, M.M., Alfaiz, F.A., Elshaer, M.M., 2021. Antibacterial and antioxidant potential of some Egyptian medicinal plants used in traditional medicine. J. King Saud Univ. – Sci. 33 (5), 101466.

Eswaraiah, G., Peele, K.A., Krupanidhi, S., Kumar, R.B., Venkateswarulu, T.C., 2020. Identification of bioactive compounds in leaf extract of Avicennia alba by GC-MS analysis and evaluation of its in-vitro anticancer potential against MCF7 and HeLa cell lines. J. King Saud Univ. – Sci. 32 (1), 740–744.

Fatehi, P., Abbasi, M., 2020. Medicinal plants used in wound dressings made of electrospun nanofibers. J. Tissue Eng. Regen. Med. 14 (11), 1527–1548.

Fitzmaurice, S.D., Sivamani, R.K., Isseroff, R.R., 2011. Antioxidant therapies for wound healing: a clinical guide to currently commercially available products. Skin Pharmacol. Physiol. 24 (3), 113–126. https://doi.org/10.1159/000322643.

Gautam, S., Dinda, A.K., Mishra, N.C., 2013. Fabrication and characterization of PCL / gelatin composite nano fi brous scaffold for tissue engineering applications by electrospinning method. Mater. Sci. Eng. C 33 (3), 1228–1235. https://doi.org/10.1016/j.msec.2012.12.015.

Goswami, M., Bhagta, S., Sharma, D., 2020. Melia dubia and its importance: a review. Int. J. Econ. Plants 7 (1), 029–033.

Homaeigohar, S., Boccaccini, A.R., 2020. Antibacterial biohybrid nanofibers for wound dressings. Acta Biomater. 107, 25–49. https://doi.org/10.1016/J.ACTBIO.2020.02.022.

Islam, M.T., Ali, E.S., Uddin, S.J., Shaw, S., Islam, M.A., Ahmed, M.I., Chandra Shill, M., Karmakar, U.K., Yarla, N.S., Khan, I.N., Billah, M.M., Pieczynska, M.D., Zengin, G., Malainer, C., Nicoletti, F., Gulei, D., Berindan-Neagoe, I., Apostolov, A., Banach, M., Yeung, A.W.K., El-Demerdash, A., Xiao, J., Dey, P., Yele, S., Jóźwik, A., Strzałkowska, N., Marchewka, J., Rengasamy, K.R.R., Horbańczuk, J., Kamal, M.A., Mubarak, M.S., Mishra, S.K., Shilpi, J.A., Atanasov, A.G., 2018. Phytol: a review of biomedical activities. Food Chem. Toxicol. 121, 82–94. https://doi.org/10.1016/J.FCT.2018.08.032.

Kandhasamy, S., Arthi, N., Arun, R.P., Verma, R.S., 2019. Synthesis and fabrication of novel quinone-based chromenopyrazole antioxidant-laden silk fibroin nanofibers scaffold for tissue engineering applications. Mater. Sci. Eng. C 102, 773–787. https://doi.org/10.1016/J.MSEC.2019.04.076.

Kim, H.S., Sun, X., Lee, J.H., Kim, H.W., Fu, X., Leong, K.W., 2019. Advanced drug delivery systems and artificial skin grafts for skin wound healing. Adv. Drug Deliv. Rev. 146, 209–239. https://doi.org/10.1016/J.ADDR.2018.12.014.

Konappa, N., Udayashankar, A.C., Krishnamurthy, S., Pradeep, C.K., Chowdappa, S., Jogaiah, S., 2020. GC–MS analysis of phytoconstituents from Amomum nilgiricum and molecular docking interactions of bioactive serverogenin acetate with target proteins. Sci. Rep. 10 (1). https://doi.org/10.1038/s41598-020-73442-0.

Krafts, K.P., 2010. Tissue repair. http://dx.doi.org/10.4161/org.6.4.12555 6, 225–233. doi: 10.4161/ORG.6.4.12555.

Krochmal-Marczak, B., Zagórska-Dziok, M., Michalak, M., Kiełtyka-Dadasiewicz, A., 2021. Comparative assessment of phenolic content, cellular antioxidant, antityrosinase and protective activities on skin cells of extracts from three sweet potato (Ipomoea batatas (L.) Lam.) cultivars. J. King Saud Univ. – Sci. 33 (6), 101532.

Lim, C.T., Tan, E.P.S., Ng, S.Y., 2008. Effects of crystalline morphology on the tensile properties of electrospun polymer nanofibers. Appl. Phys. Lett. 92 (14), 141908. https://doi.org/10.1063/1.2857478.

Mendis, E., Rajapakse, N., Kim, S.-K., 2005. Antioxidant properties of a radical-scavenging peptide purified from enzymatically prepared fish skin gelatin hydrolysate. J. Agric. Food Chem. 53 (3), 581–587.

Nayeem, N., Mohammed Basheeruddin Asdaq, S., Alamri, A.S., Alsanie, W.F., Alhomrani, M., Mohzari, Y., Alrashed, A.A., Alotaibi, N., aalhathal, A.S., Alharbi, M.A., Aldhawyan, N.N., Asad, M., Mohammad Ali Abdalla, F., Najmi, S.Y., 2021. Wound healing potential of Dodonaea viscosa extract formulation in experimental animals. J. King Saud Univ. – Sci. 33 (5), 101476.

Park, J.-S., 2010. Electrospinning and its applications. Adv. Nat. Sci. Nanosci. Nanotechnol. 1 (4), 043002. https://doi.org/10.1088/2043-6262/1/4/043002.

Ramalingam, R., Dhand, C., Leung, C.M., Ong, S.T., Annamalai, S.K., Kamruddin, M., Verma, N.K., Ramakrishna, S., Lakshminarayanan, R., Arunachalam, K.D., 2019a. Antimicrobial properties and biocompatibility of electrospun poly-ε-caprolactone fibrous mats containing Gymnema sylvestre leaf extract. Mater. Sci. Eng. C 98, 503–514. https://doi.org/10.1016/J.MSEC.2018.12.135.

Ramalingam, R., Dhand, C., Mayandi, V., Leung, C.M., Ezhilarasu, H., Karuppannan, S. K., Prasannan, P., Ong, S.T., Sunderasan, N., Kaliappan, I., Kamruddin, M., Barathi, V.A., Verma, N.K., Ramakrishna, S., Lakshminarayanan, R., Arunachalam, K.D., 2021. Core-shell structured antimicrobial nanofiber dressings containing herbal extract and antibiotics combination for the prevention of biofilms and promotion of cutaneous wound healing. ACS Appl. Mater. Interfaces 13 (21), 24356–24369.

Ramalingam, R., Dhand, C., Ming Leung, C., Ezhilarasu, H., Prasannan, P., Theng Ong, S., Subramanian, S., Kamruddin, M., Lakshminarayanan, R., Ramakrishna, S., Kumar Verma, N., Deivi Arunachalam, K., 2019b. Poly-ε-caprolactone/gelatin hybrid electrospun composite nanofibrous mats containing ultrasound assisted herbal extract: antimicrobial and cell proliferation study. Nanomaterials 9, 462. https://doi.org/10.3390/nano9030462.

Rosa, R.M., Silva, J.C., Sanches, I.S., Henriques, C., 2017. Simultaneous photo-induced cross-linking and silver nanoparticle formation in a PVP electrospun wound dressing. Mater. Lett. 207, 145–148. https://doi.org/10.1016/J.MATLET.2017.07.046.

Rosselli, S., Maggio, A., Formisano, C., Napolitano, F., Senatore, F., Spadaro, V., Bruno, M., 2019. Chemical Composition and Antibacterial Activity of Extracts of Helleborus bocconei Ten. subsp. Intermedius, 2, 675–679. doi: 10.1177/1934578X0700200611.

Selvaraj, S., Fathima, N.N., 2017. Fenugreek incorporated silk fibroin nanofibers—a potential antioxidant scaffold for enhanced wound healing. ACS Appl. Mater. Interfaces 9 (7), 5916–5926.

Sheng, D., Li, J., Ai, C., Feng, S., Ying, T., Liu, X., Cai, J., Ding, X., Jin, W., Xu, H.e., Chen, J., Chen, S., 2019. Electrospun PCL/Gel-aligned scaffolds enhance the biomechanical strength in tendon repair. J. Mater. Chem. B 7 (31), 4801–4810. https://doi.org/10.1039/C9TB00837C.

Thangavel, P., Pathak, P., Kuttalam, I., Lonchin, S., 2019. Effect of ethanolic extract of Melia dubia leaves on full-thickness cutaneous wounds in Wistar rats. Dermatol. Ther. 32 (6).

Unalan, I., Endlein, S.J., Slavik, B., Buettner, A., Goldmann, W.H., Detsch, R., Boccaccini, A.R., 2019. Evaluation of Electrospun Poly(ε-Caprolactone)/Gelatin Nanofiber Mats Containing Clove Essential Oil for Antibacterial Wound Dressing. Pharm. 2019, Vol. 11, Page 570 11, 570. doi: 10.3390/PHARMACEUTICS11110570.

Vanitha, V., Vijayakumar, S., Nilavukkarasi, M., Punitha, V.N., Vidhya, E., Praseetha, P.K., 2020. Heneicosane—a novel microbicidal bioactive alkane identified from Plumbago zeylanica L. Ind. Crops Prod. 154, 112748.

Xue, J., He, M., Liu, H., Niu, Y., Crawford, A., Coates, P.D., Chen, D., Shi, R., Zhang, L., 2014. Drug loaded homogeneous electrospun PCL/gelatin hybrid nanofiber structures for anti-infective tissue regeneration membranes. Biomaterials 35 (34), 9395–9405.

Yeum, J.H., Park, S.M., Yang, S.B., Sabina, Y., Kim, Y.H., Shin, J.C., 2016. Novel natural polymer/medicinal plant extract electrospun nanofiber for cosmeceutical application. Nanofiber Res. – Reach. New Height. https://doi.org/10.5772/63412.

Yousef, H., Alhajj, M., Sharma, S., 2021. Anatomy, Skin (Integument), Epidermis. StatPearls.

Yousefi, I., Pakravan, M., Rahimi, H., Bahador, A., Farshadzadeh, Z., Haririan, I., 2017. An investigation of electrospun Henna leaves extract-loaded chitosan based nanofibrous mats for skin tissue engineering. Mater. Sci. Eng. C 75, 433–444. https://doi.org/10.1016/J.MSEC.2017.02.076.

Zheng, C.J., Yoo, J.S., Lee, T.G., Cho, H.Y., Kim, Y.H., Kim, W.G., 2005. Fatty acid synthesis is a target for antibacterial activity of unsaturated fatty acids. FEBS Lett. 579, 5157–5162. https://doi.org/10.1016/J.FEBSLET.2005.08.028.

Zhou, J., Yao, D., Qian, Z., Hou, S., Li, L., Jenkins, A.T.A., Fan, Y., 2018. Bacteria-responsive intelligent wound dressing: Simultaneous In situ detection and inhibition of bacterial infection for accelerated wound healing. Biomaterials 161, 11–23. https://doi.org/10.1016/J.BIOMATERIALS.2018.01.024.