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Biophysical Properties, Antimicrobial Activity, and In Vitro Fibroblast Migration of Propolis-Loaded Electrospun Polycaprolactone Fibers

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ABSTRACT
Propolis, a resinous substance produced by honeybees, possesses potent antioxidant, antimicrobial, and regenerative properties, making it a promising bioactive agent for wound healing. Electrospun Polycaprolactone fibers containing 10% Propolis were prepared using a chloroform–methanol solvent system and compared with Propolis-free control fibers. The samples were characterized for morphology, thermal and mechanical properties, wettability, antimicrobial activity, in vitro wound healing using a fibroblast scratch assay, and molecular docking of pinobanksin with wound-healing-related proteins, followed by statistical analysis using an independent samples t-test. SEM images revealed a uniform fiber network with reduced pore size in Propolis-loaded samples, while WDX confirmed the loading of Propolis extract into the fibers. Propolis slightly increased the melting temperature from 61.6 °C to 63.8 °C. The water contact angle decreased from 113.2° to 64.4°, indicating increased surface wettability and decreased tensile strength from 1.98 MPa to 0.86 MPa. The fibers showed strong antibacterial activity against *E. coli* and *Staphylococcus aureus* with 99.5% reduction, antifungal activity against *Mucor racemosus* with 70% reduction, enhanced fibroblast migration with 84.97% wound closure, and strong pinobanksin binding to wound-related proteins such as 966C (−10.1 kcal/mol) and 4XCT (−9.5 kcal/mol). These results indicate that electrospun fibers containing Propolis can contribute to the development of wound dressings. However, in the scratch assay, electrospun fibers containing Propolis did not perform better than the control sample, a finding that warrants further investigation.
Keywords: Propolis, Electrospinning, Polycaprolactone, Fibroblast Migration, Wound Healing, Antimicrobial Activity

INTRODUCTION

Propolis is a resinous substance produced by honeybees from the buds, leaves, stems, and exudates of various plant species, including poplar, alder, birch, eucalyptus, acacia, and clusia, which is then mixed with beeswax and saliva to form a highly adhesive material that serves as a hive defensive substance [1]. The word Propolis originates from Greek, where “pro” means “in defense” and “polis” means “city,” reflecting its role in protecting the hive [2].

Propolis has a complex chemical composition, which varies depending on its geographical and botanical origin, climate, environmental conditions, and harvesting season, and includes over 300 biologically active compounds such as flavonoids, phenolic acids, terpenes, steroids, amino acids, vitamins (B1, B2, B6, C, E), fatty acids, aromatic acids, alcohols, sugars, esters, and essential oils, with its main constituents being resins and balsams (45–55%), waxes (8–35%), essential oils and aromatics (5–10%), fatty acids (5%), pollen (5%), and other organic and mineral substances [3]. Propolis is classified based on geographical location, flora origin, and color into groups such as Populus, Eucalyptus, Baccharis, Clusia, Macaranga, Betula, Mediterranean, as well as temperate and tropical Propolis, and these classifications affect its chemical composition and biological activities [4].

Propolis exhibits a wide range of biological properties, including immunomodulatory, antioxidant, anti-inflammatory, antibacterial, antiviral, antifungal, hepatoprotective, anticancer, antiallergenic, and photoprotective activities, which make it an attractive ingredient for

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pharmaceuticals, functional foods, cosmeceuticals, and nutraceuticals [3, 4]. The anticancer effects of Propolis are attributed mainly to its phenolic compounds, especially caffeic acid phenethyl ester (CAPE), chrysin, artepillin C, and cardanol, which induce apoptosis, cause cell cycle arrest, inhibit angiogenesis, prevent metastasis, and modulate NF-κB signaling pathways in various cancer cell lines, including human breast (MCF-7, MDA-MB-231), leukemia (HL-60, U937), ovarian (SK-OV-3), lung (NCI-H358), hepatocellular (HepG2), cervical (ME180), pancreatic (PANC-1, BxPC-3), and colon (HCT116) cancers, without significant toxicity to normal cells [2, 5, 6]. CAPE exerts anticancer activity via activation of caspase-3, downregulation of Bcl-2, upregulation of Bax, and stimulation of TRAIL-mediated apoptosis, while chrysin promotes apoptosis through inhibition of PI3K/Akt pathway, downregulation of NF-κB and IAP, and activation of caspase cascades [2]. The immunomodulatory effects of Propolis, including stimulation of nonspecific immunity, activation of humoral immunity, and enhancement of cell-mediated immunity, further contribute to its chemopreventive potential [7, 8].

Propolis is generally regarded as non-toxic, non-genotoxic, and safe when administered at doses of 1.4 mg/kg, equivalent to approximately 70 mg per day. Studies in mice have indicated that even at a dose of 1400 mg/kg/day over 90 days, no histomorphological alterations were observed, establishing this level as the NOEL (No-Observed-Adverse-Effect Level). Additionally, administering ethanolic extracts of Brazilian and Chinese Propolis to 5-week-old mice at doses ranging from 2230 to 4000 mg/kg for 2 weeks did not affect mortality, body weight, or cause histological abnormalities. Topical application of Propolis in acetone at concentrations of 1%, 10%, and 20% on the shaved flank of guinea pigs caused irritation only at concentrations exceeding 20%. Regarding flavonoids, their LD50 has been reported to range from 2000 to 10,000 mg per animal, which corresponds to 8000 to 40,000 mg/kg for a 250 g rat [9].

Polycaprolactone is an FDA-approved, biocompatible and biodegradable polyester with easy electrospinnability, widely used in biomedical applications such as skin regeneration and drug delivery. Propolis is a natural bee product rich in phenolic compounds and flavonoids, exhibiting antioxidant, anti-inflammatory, antimicrobial, and wound-healing properties, making it a promising additive for skin repair and pathogen control [10].

Unlike previous studies, the innovation of this work lies in the combination of a 10% Propolis concentration with molecular docking against wound-healing targets, alongside an integrated analysis of biophysical, physicochemical, and antimicrobial properties and fibroblast migration. The ultimate goal of this study is to design and evaluate electrospun Polycaprolactone fibers containing Propolis extract that exhibit both antimicrobial activity and the ability to promote fibroblast migration, aiming to enhance wound healing and tissue regeneration.

MATERIALS AND METHODS

Materials

Polycaprolactone (average Mn 80,000), Chloroform, and Methanol were purchased from Sigma–Aldrich Chemical Company, USA. With slight changes to the Propolis extraction method, Propolis extract was prepared by first cutting the raw Propolis into thin pieces or flakes to increase the exposed surface area. The prepared pieces were then frozen and subsequently ground into a fine powder. Then, 10 g of the Propolis powder was accurately weighed and transferred to a glass container, followed by the addition of 100 mL of an ethanol–water solution containing 80% ethanol and 20% water, corresponding to a 1:10 (w/v) Propolis-to-solvent ratio, at 25 °C. The container was sealed and shaken thoroughly and then kept in a dark place for 1 week to allow efficient dissolution of bioactive compounds. After the extraction period, the mixture was filtered through a fine cloth to remove insoluble residues. The resulting Propolis extract was collected and stored in a dark glass bottle until further use [11]. The 10% Propolis concentration was selected based on our preliminary optimization experiments, where concentrations below 10% showed insufficient antimicrobial activity.

Fibers Synthesis

First, Polycaprolactone was dissolved in a chloroform–methanol mixture (3:1) to obtain a 20% w/w PCL solution. The solution was stirred continuously using a magnetic stirrer at room temperature until complete dissolution was achieved. Subsequently, Propolis was incorporated at a concentration of 10% relative to the PCL weight and the container was wrapped with aluminum foil to protect the Propolis from light. After complete homogenization, the resulting solution was used for electrospinning. The electrospinning process was performed with a Full Option Lab2 ESI-I device. In this study, two groups were evaluated: a wound dressing containing 10% Propolis and a control sample without Propolis [12]. According to Table 1.

Table 1 Electrospinning method specifications

Solution flow rate	1-2 mL/h
Voltage	17-18 kV
Distance between nozzle and collector	13.5 cm
Ambient temperature	26-29 c
Scan	10 cm
Rotation	120 rpm
Collector type	Aluminum
Syringe volume	5 mL
Needle size	18 Gauge
Needle tip type	Blunt
Connection type	Luer Slip

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Fibers characterization

Fourier-transform infrared spectroscopy (FTIR) analysis

The identification of functional groups in the samples was performed with an FTIR spectrometer (manufactured by PerkinElmer, UK). The samples were analyzed within the wavenumber range of 400 to 4000 cm^{-1}.

Combination of Scanning Electron Microscopy (SEM) and Wavelength-Dispersive X-ray Spectroscopy (WDX)

The Electrospun mats were sputter-coated with gold using a SCD 005 (Bal-Tech, Balzers, Liechtenstein) coater and their morphology was evaluated by scanning electron microscopy (Philips XL30, Netherlands) operated at an accelerating voltage of 25 kV. Propolis also contains nutritional minerals such as calcium, which can be detected by WDX, confirming that the extract is loaded into the fibers [13]. The amount of Calcium absorption on the surface of samples was detected by WDX (WDX MICROSPEC, United States).

Differential Scanning Calorimetry (DSC)

The analysis is performed from room temperature to 100 °C using a SANAF device to investigate the thermal behavior of the sample. In this test, the sample is placed in a designated aluminum pan and positioned inside the instrument chamber alongside an empty reference pan. The device increases the temperature from ambient to 100 °C at a heating rate of 10°C/min and measures the difference between the sample and the reference.

Dry Matter (DM)

The dry matter content was determined using an AMB 50 moisture analyzer. Briefly, a one-gram sample was placed in the device and heated to 160°C to remove moisture. Once the moisture had completely evaporated, the remaining dry mass was recorded, and the dry matter content was displayed by the device. It was done with 3 repetitions.

Wettability

The water contact angles for the polymer films were determined by the sessile drop method, at room temperature and controlled humidity, within 10 s, after placing 5µL drop of water, on the film surface, using a Dino-lite microscope (Taiwan). Contact angle measurements were taken at least three times at different locations on the surface. The average values were used in contact angle analysis and then the angles were determined using ImageJ software.

Tensile Strength Measurements

Tensile strength of fibrous scaffolds was tested using GOTECH testing system (Taiwan). The scaffolds were cut with 2 cm length and 0.5 cm width. The sample strips were held by grips and the tensile force were applied at an extension rate of 1 mm. min^{-1}.

Antibacterial and Antifungal Testing

The antibacterial activity of the samples was evaluated according to the AATCC Test Method 100–1993. Briefly, 1 mL of a bacterial suspension of *Staphylococcus aureus* and *Escherichia coli*, adjusted to a concentration of 1×10^3 $CFU \cdot mL^{-1}$, was inoculated onto electrospun samples with approximate dimensions of 0.5 × 0.5 cm to ensure uniform bacterial coverage. The inoculated samples were incubated at 37 °C for 24 h to allow direct contact between the bacteria and the sample surface. After incubation, the viable bacteria were recovered from the sample surface, and 100 µL of the resulting bacterial suspension was spread onto Mueller–Hinton agar plates. The plates were then incubated under appropriate conditions to allow colony formation. The number of viable bacteria was quantified by counting the colony-forming units (CFU). The antibacterial activity was expressed as the percentage of bacterial reduction, calculated according to the following equation:

(1) $$\text{Bacterial reduction (\%)} = \left(\frac{CFU_{control} - CFU_{sample}}{CFU_{control}}\right) \times 100$$

An attempt was made to follow a similar protocol to evaluate the antifungal effect of the sample against *Mucor racemosus*. For this purpose, 1 ml of a spore suspension of *Mucor racemosus* with an initial concentration of (3.2×10^3) CFU/ml was taken and applied onto electrospun samples with approximate dimensions of 0.5 × 0.5 cm to ensure the surface was uniformly covered with spores. The samples were then incubated under appropriate conditions to allow spores sufficient contact with the sample surface. After a specified contact time, the viable spores on the surface were counted. Colony counting was performed as CFU on plates to directly assess the effect of the sample surface on fungal growth. After a contact time of 24 h at 28 °C, the plain PCL and Propolis-loaded PCL membranes were rinsed with 10 mL of sterile saline on a Petri dish, and the value for colony-forming cells ($CFU \cdot mL^{-1}$) was determined on Potato Dextrose Agar. It was done using the previous formula.

Scratch Wound Assay

Human foreskin fibroblast (HFF) cells were employed to assess the wound healing efficacy of the wound dressing using an in vitro scratch assay. Human foreskin fibroblast (HFF) cell lines were obtained from Yazd Reproductive Sciences Institute. All ethical protocols of the university were followed.

A conditioned-medium approach, rather than direct cell seeding onto the scaffold, was adopted for this assay because the thickness and opacity of the electrospun mats precluded clear visualization of the scratch gap and cell morphology by inverted phase-contrast light microscopy. Such limited optical transparency is a recognized constraint of dense, non-woven polymeric scaffolds, which restricts direct microscopic imaging to superficial regions of the construct [27]. The conditioned-medium format is also an established and validated approach for evaluating the paracrine bioactivity of scaffold-released compounds on fibroblast migration in scratch assays [28].

Initially, wound dressings were incubated in complete culture medium for five days to facilitate the release of bioactive compounds. After incubation, the medium containing the released components was collected and filtered to remove any particulate matter or residues. HFF cells

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(1×10^5 cells/well) were seeded into 6-well plates and allowed to adhere and reach confluence for 24 h at 37 °C in a humidified incubator with 5% CO_2. A uniform linear scratch was then created across the center of each well using a sterile pipette tip to simulate a wound. The wells were gently washed with phosphate-buffered saline (PBS) to remove detached cells and debris. The filtered conditioned medium obtained from the wound dressing incubation was subsequently added to the wells. Cells were incubated again at 37 °C, and wound closure was monitored microscopically. Images of the scratched area were taken at 0 h and after 40 h using an inverted phase-contrast microscope equipped with a digital camera. The migration of fibroblast cells into the wound gap over the 40-hour period was analyzed to determine the percentage of wound closure, reflecting the in vitro wound healing potential of the dressing.

(2) % Wound closure = Average of (([Gap length]$_0$h − [Gap length]$_{40}$h) / [Gap length]$_0$h)

Molecular Docking

Pinobanksin, as one of the most important flavonoids present in Propolis, has been highlighted in various studies for its bioactive properties and potential role in wound healing processes [14, 15]. In this study, the three-dimensional structure of Pinobanksin with chemical identifiers 548-82-3, 3,5,7-Trihydroxyflavanone, (2R,3R)-Pinobanksin, and CID 73202, with a molecular formula of C15H12O5 and a molecular weight of 272.25 g/mol, was retrieved from the PubChem database and prepared for docking. In Figure 1, Pinobanksin Structure is shown.

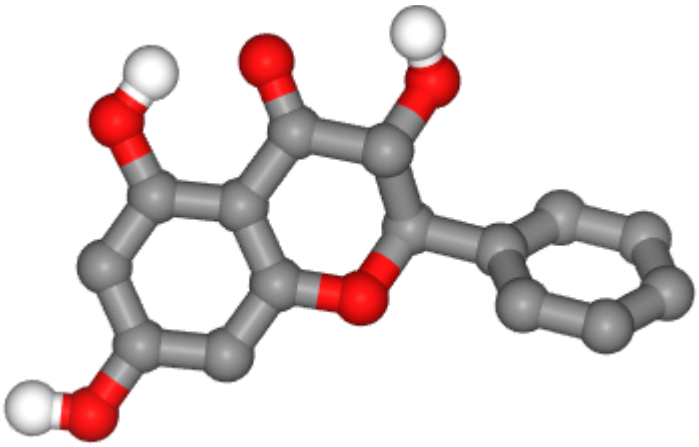

Fig. 1 Pinobanksin Structure

Proteins related to wound healing, including 2AZ5, 3UZP, 5HLP, 6Y8M, 1ALU, 2ZOQ, 3KCX, 4EJN, 4XCT, 1AGW, 3POZ, 5K5N and 966C, were selected as receptors [16-19]. Docking was performed in the online platform SeamDock using the Vina option, applying the software's default settings to determine the optimal binding poses and facilitate the evaluation of Pinobanksin interactions with the active sites of the target proteins.

Statistical Analysis of the Data

The collected data were entered and analyzed using SPSS version 26. To compare the means between two groups, an independent samples t-test was performed. The results indicated that there was a statistically significant difference between the groups in the measured parameter at a significance level of 0.05 ($P < 0.05$).

Ethics approval and consent to participate

No humans or animals were used in the present research.

RESULTS AND DISCUSSION

Propolis FTIR Spectrum

The FTIR spectrum of the prepared Propolis is shown in Figure 2. A broad band at 3418 cm^{-1} is observed, which is attributed to the O–H stretching of phenolic and hydroxyl groups. Absorptions at 2924 and 2850 cm^{-1} correspond to the C–H stretching of methyl and methylene groups. Strong bands at 1728 and 1640 cm^{-1} are related to the C=O stretching of carbonyl groups in esters, ketones, and carboxylic acids. In the range of 1456 to 1275 cm^{-1}, bands are assigned to C–H bending and C–O–H stretching vibrations, while peaks at 1161 and 1028 cm^{-1} correspond to C=C stretching of alkenes and C–O–C stretching of aromatic ether groups, confirming the presence of flavonoids, phenols, and other functional groups in Propolis.

The FTIR results of this study are consistent with the findings of Hegazi and colleagues, as both spectra show the presence of a broad O–H band associated with phenolic and hydroxyl groups, and C=O bands similar to those reported by Hegazi. Both studies also confirm the presence of bands corresponding to C–O–H and bands related to C=C and C–O–C, indicating the presence of flavonoids, phenols, and aromatic ether groups. Minor differences in the exact positions of the peaks may be attributed to the source of the Propolis and extraction conditions, but structurally and chemically, the results are fully comparable [20].

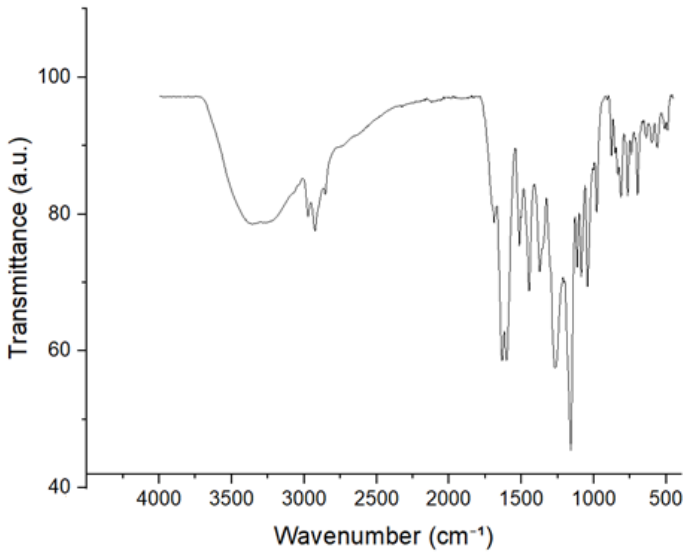

Fig. 2 FTIR spectrum is of Propolis

Results of Scanning Electron Microscopy (SEM) and Wavelength-Dispersive X-Ray Spectroscopy (WDX)

The scanning electron microscopy (SEM) images demonstrated that the incorporation of Propolis extract had a distinctly positive effect on the morphology and structural organization of the electrospun mats. The Propolis-loaded sample (A) exhibited a highly uniform, smooth, and continuous fibrous network with well-defined interconnections and minimal bead formation. This improvement in fiber alignment and homogeneity suggests that the presence of bioactive and viscous components in the Propolis extract contributed to better jet stability during electrospinning, resulting in the formation of well-structured fibers. SEM analysis was performed based on 100 fiber diameter measurements for each sample. The mean fiber diameter was 705.02 ± 197.87 nm for Sample A and 692.43 ± 260.73 nm for Sample B. However, statistically, these results did not show a significant difference $p > 0.05$. In Figure 3, Scanning Electron Microscopy Images of Propolis Electrospun is shown.

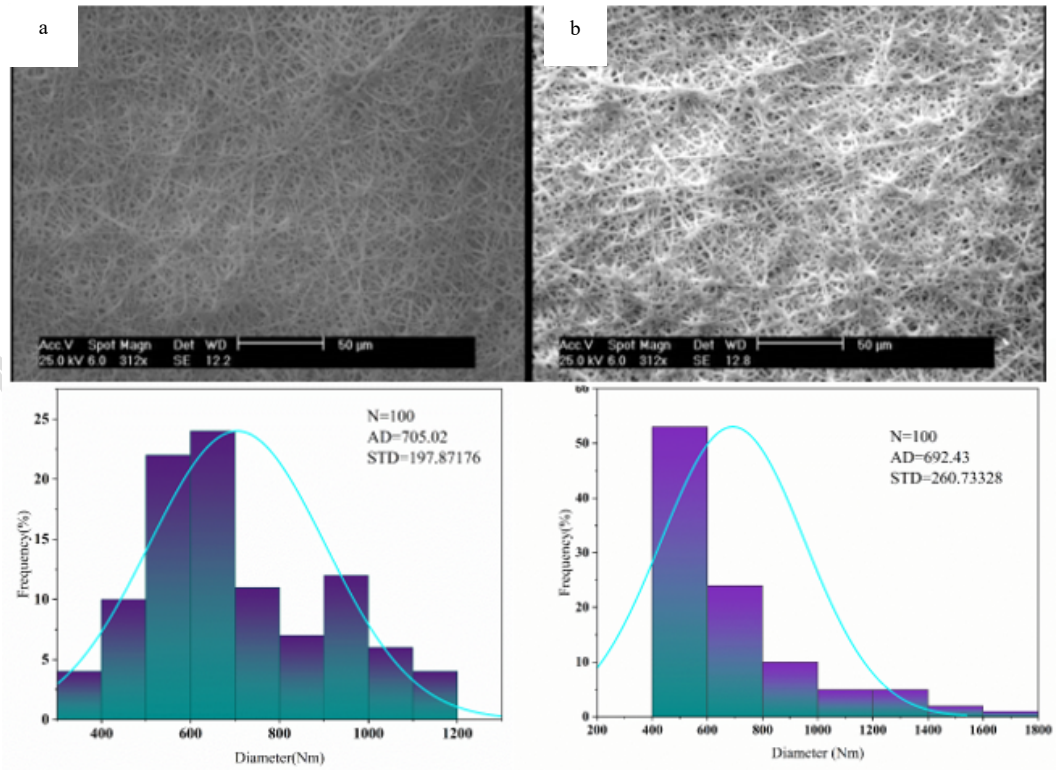

Fig. 3 Scanning Electron Microscopy Images of Electrospun Mats from Propolis (A) and Control (B) Samples

The WDX elemental analysis and calcium mapping provided additional confirmation of the successful incorporation of Propolis into the polymer matrix. Distinct calcium signals were detected throughout the Propolis-loaded fibers, whereas the control sample showed no trace of

calcium. Since calcium is known to be one of the natural inorganic constituents of Propolis and is absent in the neat polymer, its detection verifies that the Propolis extract was effectively loaded within the polymeric fibers. The calcium distribution map showed localized but consistent signals along the fibrous network, particularly at fiber junctions, suggesting that the mineral components of Propolis were evenly dispersed and integrated into the structure. A semi-quantitative comparison of elemental percentages across the samples was not possible because one sample lacked calcium. In Figure 4, WDX Map of Calcium from Propolis fibers is shown.
Salimbeigi et al. also reported that the addition of Propolis to the electrospinning solution resulted in a decrease in the average fiber diameter from 0.722 µm for pure PCL to 0.611 µm for PCL/Propolis, which is consistent with our results in this study [21].

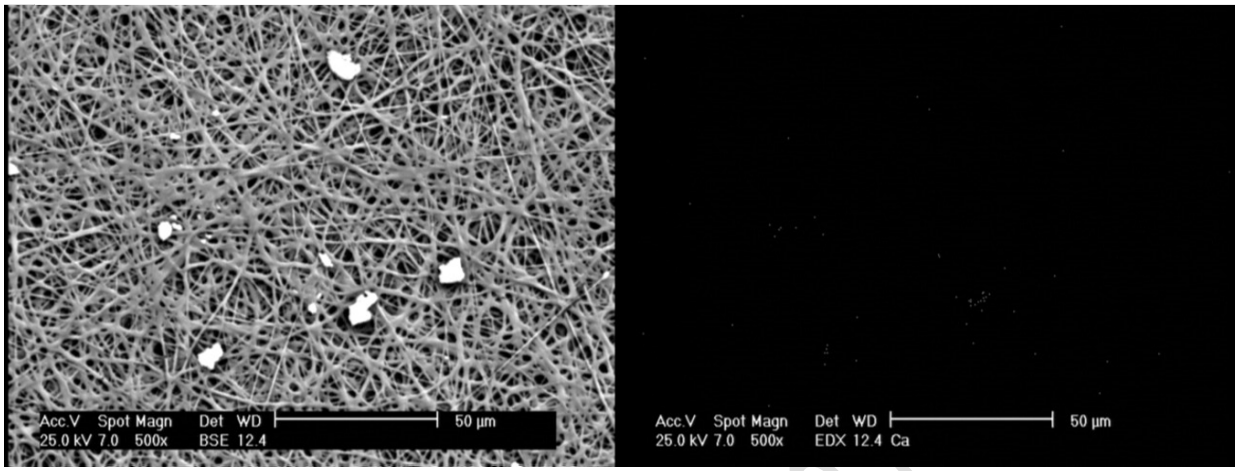

Fig. 4 WDX Map of Calcium from Propolis fibers

Differential Scanning Calorimetry (DSC)

Based on Figure 5, The DSC analysis showed that the control PCL sample exhibited a melting peak at approximately 61.6 °C, whereas incorporation of 10% Propolis shifted the melting peak slightly upward to 63.8 °C. This modest increase in melting temperature suggests that the incorporation of Propolis altered the thermal behavior of the PCL matrix.

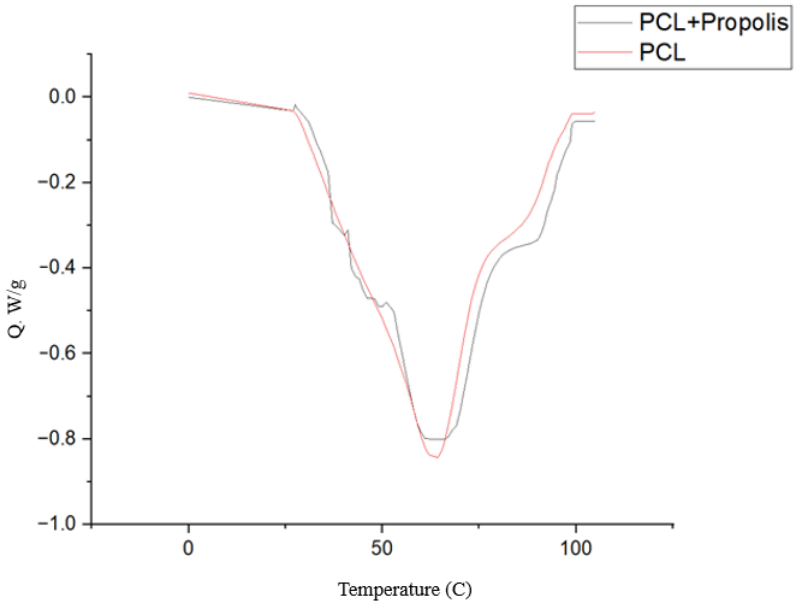

Fig. 5 DSC curves of PCL + 10% Propolis and PCL samples

Dry Matter

The mean dry matter content in PCL + 10% Propolis samples was 95.33±0.22, while in pure PCL it was 97.00±0.18, and the difference between the two groups was statistically significant. The mean difference was -1.67±0.16, indicating that the dry matter content in the Propolis-containing sample was lower than in the pure PCL. The small numerical variation of 1.67 percentage points, indicates that incorporation of Propolis did not meaningfully affect the solid content of the electrospun fibres. The relevant tables 2 and 3 are attached.

Wettability

According to Figure 6, the mean contact angle (as a measure of wettability) of PCL + 10% Propolis samples was 64.40±0.52 degrees, while that of pure PCL was 113.20±0.23 degrees, and the difference between the two groups was statistically significant. The mean difference was -48.80±0.33 degrees, indicating that the contact angle of the Propolis-containing sample was much lower than that of pure PCL, which means the wettability of the Propolis-containing sample was significantly higher than that of pure PCL. The relevant tables 2 and 3 are attached in the appendix, which is due to the presence of phenolic compounds and hydroxyl groups on the surface, leading to reduced contact angle[10]. This behaviour can be primarily attributed to changes in the surface chemical composition of the electrospun fibres. Pure PCL is intrinsically hydrophobic because of its relatively low surface energy and limited availability of polar functional groups at the surface. In contrast, Propolis contains a complex mixture of phenolic acids, flavonoids, and other oxygen-containing compounds bearing polar hydroxyl groups. The incorporation of these constituents into the PCL matrix can increase the density of polar moieties exposed at the fibre–water interface, thereby enhancing hydrogen-bonding interactions with water molecules and reducing the water contact angle [22]. The relevant tables 2 and 3 are attached.

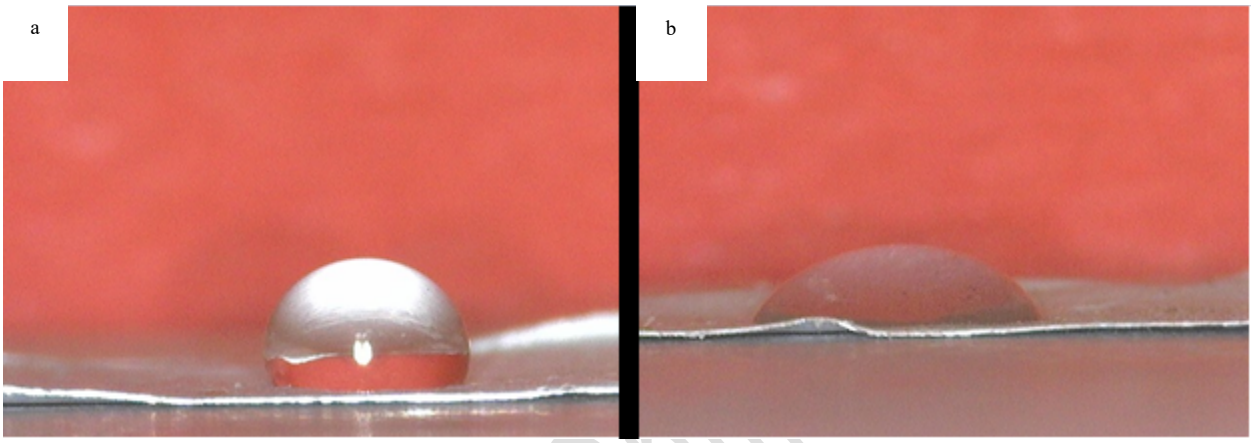

Fig. 6 Water Contact Angle of Electrospun Mats from Control (A) and PCL+Propolis (B) Samples

Tensile Strength Measurements

The average tensile strength of the PCL + 10% Propolis samples were 0.86±0.30, compared to 1.98±0.35 in the pure PCL group. This difference was statistically significant, with a mean difference of -1.12±0.26, showing that incorporating Propolis noticeably reduced the tensile strength of the PCL samples. The relevant tables 2 and 3 are attached in the appendix. The decrease in tensile strength of the Propolis-containing sample compared to the control indicates an increase in surface flexibility and a slight change in the intermolecular bonds of the matrix; This mechanical modification could provide advantages in regenerative applications, as the more flexible matrix can conform to tissue deformation and better withstand mechanical stresses, although for applications requiring high mechanical loading, special attention should be paid to reducing tensile strength. Taken together, these data suggest that the addition of Propolis provides a balance between mechanical and biological properties, such that flexibility and active surface area for cellular interaction are increased without severely compromising the overall structure of the matrix [23]. While reducing tensile strength, Propolis was significantly beneficial for wound dressings in terms of other parameters [24]. The skin is constantly subjected to minor stresses, and its tensile strength has been reported to be approximately 1.8 MPa [25].

The tensile strength of the pure sample was 1.98 MPa and the Propolis-containing sample was 0.86 MPa, both of which are far below the range of native human skin (at least 2.5 MPa). Therefore, the Propolis-containing scaffold is only suitable for non-load-bearing wounds or as a drug delivery layer, and is not appropriate for mobile or high-tension areas. The relevant tables 2 and 3 are attached.

Table 2 Group Statistics of Samples

Variables	Group	N	Mean	Std. Deviation	Std. Error Mean
DM	PCL + 10% Propolis	3	95.33	0.22068	0.12741
	PCL	3	97	0.18028	0.10408
Wettability	PCL + 10% Propolis	3	64.4	0.52029	0.30039
	PCL	3	113.2	0.22913	0.13229
Tensile	PCL + 10% Propolis	3	0.8600	0.29614	0.17098
	PCL	3	1.98	0.34598	0.19975

Table 3 Independent Samples t-Test Results for Group Comparison

	Levene's Test for Equality of Variances		t-test for Equality of Means						
Variables	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference Lower	Upper

DM	Equal variances assumed	0.062	0.815	-10.151	4	0.001	-1.67	0.16452	-2.12678	-1.21322
	Equal variances not assumed			-10.151	3.847	0.001	-1.67	0.16452	-2.13404	-1.20596
Wettability	Equal variances assumed	1.109	0.352	-148.677	4	0.000	-48.8	0.32823	-49.71131	-47.88869
	Equal variances not assumed			-148.677	2.748	0.000	-48.8	0.32823	-49.90099	-47.69901
Tensile	Equal variances assumed	0.059	0.821	-4.260	4	0.013	-1.12	0.26293	-1.85002	-.38998
	Equal variances not assumed			-4.260	3.907	0.014	-1.12	0.26293	-1.85692	-.38308

Antibacterial and Antifungal Results

Table 4 shows the antibacterial activity against *E. coli* and *Staphylococcus aureus*, where the initial count for both bacteria was 1000 CFU. The PCL membranes loaded with 10% Propolis reduced the bacterial count to 5 CFU, corresponding to a 99.5% reduction, while the plain PCL membranes showed no reduction, with counts remaining at 1000 CFU. Antibacterial results against Gram-positive vs Gram-negative show no differential activity pattern. Table 5 presents the antifungal effect against *Mucor racemosus*, with an initial spore count of 3.2×10^3 CFU/ml. The PCL membranes containing 10% Propolis reduced the spore count to 9.6×10^2 CFU/ml, resulting in a 70% reduction, whereas the plain PCL membranes showed no antifungal activity, with the count remaining at 3.2×10^3 CFU/ml. The antibacterial results are consistent with the study by Mohamadinooripoor et al [26]. The greater antibacterial reduction observed in the present study compared with the antifungal reduction may reflect the differential susceptibility of bacteria and fungi to Propolis-derived bioactive compounds, rather than an intrinsically superior antibacterial activity of Propolis [27]. Natural compounds derived from plants—and even animal products—have attracted significant attention as potential antimicrobial agents due to their diverse bioactive constituents and wide-ranging biological activities [28, 29]. The relevant tables 4 and 5 are attached.

Table 4 Antibacterial Effect of 10% Propolis on *E. coli* and *Staphylococcus aureus*

Type of bacteria		Initial Count (CFU/ml)	Count After Contact (CFU/ml)	Reduction (%)
E. coli	PCL + 10% Propolis	1000	5	99.5
	PCL	1000	1000	0
Staphylococcus aureus	PCL + 10% Propolis	1000	5	99.5
	PCL	1000	1000	0

Table 5 Antifungal Effect of 10% Propolis on *Mucor racemosus*

fungus		Initial Count (CFU/ml)	Count After Contact (CFU/ml)	Reduction (%)
Mucor racemosus	PCL + 10% Propolis	3.2×10^3	9.6×10^2	70
	PCL	3.2×10^3	3.2×10^3	0

In vitro Wound-Healing Assay

The results of the cell scratch test showed that the wound closure rate in the control sample was the highest at 98.64±0.68 percent and was statistically placed in a separate group. The fiber treatment without extract showed the lowest percentage of wound closure at 75.92 ± 0.44 percent and was significantly lower than the other groups. Also, the fiber containing extract with a wound closure rate of 84.97 ± 0.45 percent was in the middle between the other two groups. According to Duncan's test, there was a significant difference between all three groups, so that the order of the statistical letters (a, b and c) confirmed a significant difference at the 0.05 level between them. These results indicate that the loading of the extract in the fibers has been able to improve the wound healing process compared to the fibers without extract, although it has not yet reached the level of the control group.

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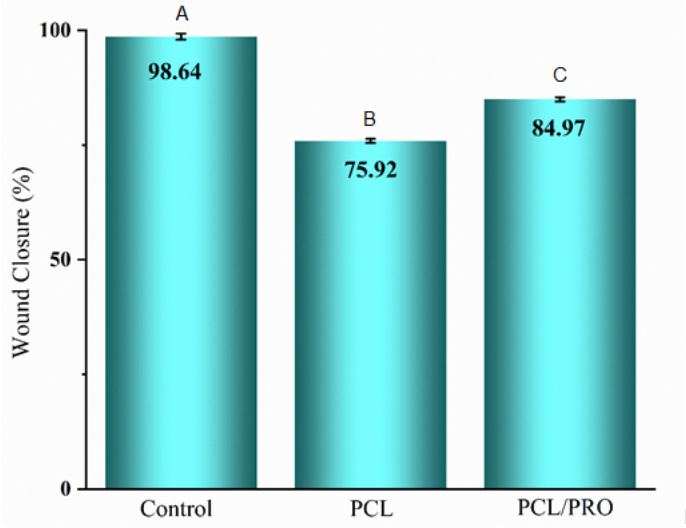

Fig. 7 Quantitative evaluation of Human foreskin fibroblast (HFF) cells migration area after 24 hours of wound healing in the presence of PCL/Propolis mats, with cells cultured without any film serving as the control. Different letters in each column indicate significant differences at the 0.05 level.

Microscopic images show that at time 0 hours, in all three groups including control, A (fibers without extract) and B (fibers containing Propolis extract), cells are present in a scattered manner and no significant adhesion or movement is observed. At 24 hours, in the control group, cells have begun to proliferate and spread, and in sample B, cell density has increased and a suitable adhesion pattern is observed, while in A, growth is slower and the distance between cells is greater. At 32 hours, cells in the control group have reached a high density and have migrated uniformly, while in B, cell density and migration are close to the control, and the cells have an elongated and active appearance; but in A, empty areas are still visible. At 40 hours, the control group covers almost the entire surface and has maximum density, while in B, cell coverage is very high and almost similar to the control, with the difference that in some areas the density is slightly lower. In sample A, there are still areas without cells. But still, the control group was better than the other groups.

It seems likely that in the control group, the absence of oxidative stress maintains optimal ROS balance and allows unhindered cell migration. It seems likely that plain PCL fibers, due to their hydrophobic surface, induce mild ROS production and inflammatory signaling. It also seems likely that Propolis-loaded fibers partially counteract this effect through their phenolic compounds, which scavenge ROS and suppress cytokines, thereby exerting anti-inflammatory actions.

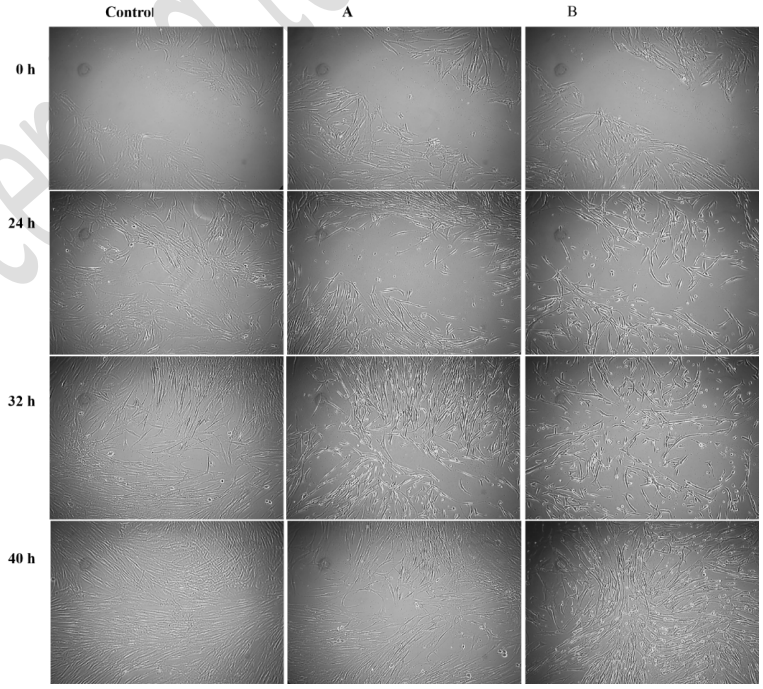

Fig. 8 Assessment of the scratch wound-healing assay on Human foreskin fibroblast (HFF) cells through microscopic observation of stained cells immediately after scratching (0 h) and following 40 hours of wound healing.

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The results of the cell scratch test showed that the highest wound healing rate was observed in the control sample, and the fibers containing Propolis had an intermediate rate, and the fibers without extract showed the lowest performance. This indicates that Propolis loading improved cell migration and adhesion compared to the sample without extract and accelerated the healing process, although it has not yet reached the control level. Therefore, it can be said that Propolis, as an active biological agent, was able to improve the repair function of the matrix. However, our results are consistent with some previous studies on the effect of Propolis extract on cell migration[10], while they are inconsistent with some studies [30, 31].

The results of the scratch assay on HFF cells are likely related to morphological characteristics rather than the presence of the Propolis itself. However, further studies are necessary to evaluate the effect of Propolis concentration on the cellular response [10].

Results of Docking of Wound-associated Proteins with Pinobanksin

Among the proteins studied, 966C exhibited the strongest binding with an affinity of -10.1 kcal/mol, forming hydrophobic contacts with Leu181, Val215, and Tyr240, and hydrogen bonds with Ala182, His218, Glu219, and Leu181. Other proteins also demonstrated significant interactions: 4XCT showed an affinity of -9.5 kcal/mol with hydrophobic contacts involving Leu188, Val223, His226, Leu243, and Tyr248, and hydrogen bonding with Glu227 and Leu188; 4EJN had an affinity of -9.1 kcal/mol with Trp80 mediating hydrophobic interactions and Ser205, Thr211, and Asp292 forming hydrogen bonds. Proteins 3KCX and 3UZP displayed affinities of -8.5 and -8.4 kcal/mol, respectively, with multiple hydrophobic and hydrogen bond contacts including Leu186, Thr196, Asp201, Arg238 for 3KCX, and Ile15, Ile23, Ala36, Lys38, Leu135, Ile148 for 3UZP. Intermediate affinities were observed for 5K5N (-8.3 kcal/mol), 3POZ (-8.3 kcal/mol), 1AGW (-7.5 kcal/mol), 2ZOQ (-7.3 kcal/mol), 2AZ5 (-7.7 kcal/mol), 5HLP (-7.2 kcal/mol), and 6Y8M (-6.4 kcal/mol), each forming distinct hydrophobic and hydrogen bonding interactions with their respective residues. These results indicate that Pinobanksin is capable of engaging in both hydrophobic contacts and hydrogen bonds with multiple key residues across diverse wound-related proteins, suggesting its potential role as a bioactive flavonoid with modulatory effects on proteins involved in wound healing. The relevant table 6 is attached.

Table 6 List of Proteins ID showed their Affinity (kcal/mol) with the Pinobanksin interaction

PDB ID	Affinity (kcal/mol)	Amino acid residues (Hydrophobic contact)	Amino acid residues (Hydrogen bond)
2AZ5	-7.7	Phe124 Leu93	Gln125 Leu93
3UZP	-8.4	Ile15 Ile23 Ala36 Lys38 Leu135 Ile148	Ile15 Met82 Leu85 Gly86
5HLP	-7.2	Ile62 Lys85 Tyr134 Leu188 Asp200	Asp200 Lys85
6Y8M	-6.4	Lys63 Glu64 Pro87	Lys65 Tyr68 Pro87
1ALU	-6.7	Asn63 Glu93 Val96 Pro139 Leu147	Asn63 Glu93 Asn144
2ZOQ	-7.3	Ile190 Asp192 Pro193	Arg104 Phe371 Glu194
3KCX	-8.5	Leu186 Thr196	Asp201 Arg238
4EJN	-9.1	Trp80	Ser205 Thr211 Asp292
4XCT	-9.5	Leu188 Val223 His226 Leu243 Tyr248	Glu227 Leu188
1AGW	-7.5	Leu484 Val492 Val561 Leu630	Glu562 Ala564 Lys514

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3POZ	-8.3	Ala743 Lys745 Leu788 Thr790 Leu844	Met793
966C	-10.1	Leu181 Val215 Tyr240	Ala182 His218 Glu219 Leu181
5K5N	-8.3	Ile62 Phe67 Val70 Ala83 Lys85 Tyr134 Leu188 Asp200	Val135 Cys199

COCLUSION

The present study demonstrated that electrospun fibers containing Propolis possess suitable structural, chemical, and thermal properties for potential wound healing applications. FTIR analysis confirmed the presence of flavonoids, phenols, and aromatic ether groups, consistent with previous studies. SEM and WDX analyses showed that the incorporation of Propolis resulted in thinner and more uniformly distributed fibers, while DSC results indicated only a slight reduction in crystallinity without significant changes in thermal stability. The water contact angle decreased from 113.2° to 64.4°, indicating increased surface wettability. Mechanical testing revealed decreased tensile strength and increased flexibility, which may be advantageous for regenerative applications. Microbial assays demonstrated good antibacterial and antifungal activity, and in vitro wound healing assays indicated improved cell migration and adhesion in fibers containing Propolis compared to fibers without it, although not reaching the level of the control. Molecular docking revealed that pinobanksin, a key bioactive compound in propolis, has the potential to interact with various wound-related proteins through hydrophobic interactions and hydrogen bonding, highlighting its potential role in modulating wound healing. Overall, the incorporation of Propolis into electrospun fibers provides a balanced combination of mechanical, biological, and antimicrobial properties, making these fibers highly promising for wound healing applications. This study has several limitations, including the small sample size without power analysis, limited microbial strain panel, reduced tensile strength below native skin range, lack of in vivo validation, and unvalidated molecular docking results. Future studies should focus on mechanical reinforcement through crosslinking or bilayer structures, direct cell-scaffold contact assays, broader antimicrobial testing, and animal studies to evaluate in vivo wound healing efficacy. Clinical translation will require optimization of compound release kinetics and scaffold design for low-tension wound applications such as pressure ulcers or diabetic wounds.

Consent for Publication

Not applicable.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Conflict of Interests

The authors declare that they have no conflict of interest.

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Authors' Contributions

All authors contributed to the conception and design of the study. All authors read and approved the final version of the manuscript.

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