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Article

Green Hydrogels Prepared from Pectin Extracted from Orange Peels as a Potential Carrier for Dermal Delivery Systems

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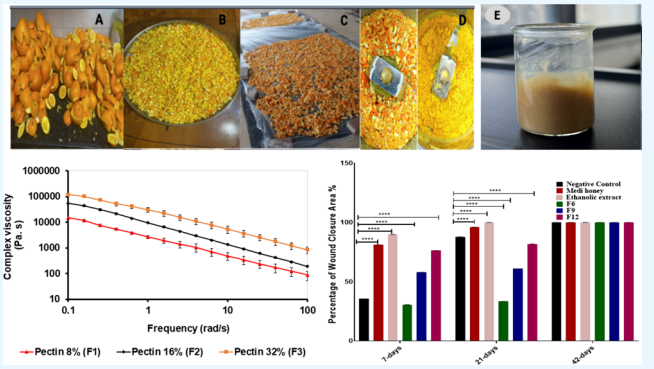
ABSTRACT: Recycling fruit waste, like orange peels, into a green, eco-friendly, low-cost, and sustainable product is an effective way to reduce fruit waste. Pectin is a natural polymer extracted from citrus peels. It is widely utilized as a gelling agent, stabilizer, thickener, emulsifier, and rheology modifier. This study aimed to develop green gels from pectin extracted from orange peels as a carrier for topical preparations. The extracted pectin was characterized by a yield% of 14%, a degree of esterification of 20%, an equivalent weight of 2500 g/mol, a methoxyl content of 14.88%, and a galacturonic acid content of 91.52%. Additionally, the ethanolic orange peel extract was also prepared and compared with pectin hydrogels. Hydrogels were prepared using 8, 16, and 32% pectin concentrations, cross-linked with 0, 20, 40, 60, and 80% $CaCl_2$ concentrations, while the pH of the hydrogel was adjusted to 3.5 and 8.5. Pectin hydrogels exhibited pseudoplastic behavior, viscoelastic properties with elastic modulus G' dominating the viscous modulus G'', and bioadhesive properties. Moreover, the aqueous and ethanolic extractions of pectin from orange peels demonstrated a concentration-dependent antioxidant activity. Pectin hydrogels did not demonstrate wound healing or antimicrobial activities. In contrast, the ethanolic orange peel extract demonstrated improved wound healing, antimicrobial activity against the Gram-positive bacteria *Staphylococcus aureus*, and antioxidant activity. Therefore, the pectin hydrogel could serve as a potential carrier for dermal delivery systems. Moreover, the ethanolic orange peel extract has demonstrated potential as a dermal drug delivery carrier in wound healing and antimicrobial and antioxidant agents.



1. INTRODUCTION

Pectin is an anionic, natural, biocompatible, biodegradable, and renewable polysaccharide that can be safely consumed in any amount.[1] It is a complex natural polysaccharide whose precise structure has not been fully characterized.[1] The challenge in determining the exact chemical structure of pectin stems from its source, location, extraction conditions, and other environmental factors.[2] Notably, 85.5% of commercial pectin is derived from citrus peels (lemon, lime, and orange) and 70% consists of D-galacturonic acid monomers linked by α-(1→4) glycosidic bonds, forming homogalacturonan.[3] The carboxyl groups at C-6 or hydroxyl groups at O-2 or O-3 positions can be partially methyl-esterified or acylated, respectively.[1,2] Figure 1 depicts the chemical structure of pectin, featuring covalently α-1,4-linked D-galacturonic acid units.

Pectin is utilized not only for its gelling property but also as a stabilizer, thickener, emulsifier, and rheology modifier in many pharmaceutical products.[4] Based on its degree of methylation (DM), pectin is divided into high-methoxy pectin (HMP) with DM > 50% and low-methoxy pectin (LMP) with DM < 50%.[5] The degree of methylation has a significant impact on the gelling properties, emulsion stability, and surface tension of pectin.[6]

Pectin exhibits viscosity-enhancing properties, reducing the need for synthetic viscosity enhancers that are characterized by their poor biodegradability. This promotes its use as a rheology modifier from natural sources such as fruit wastes.[7] Since pectin is a versatile gelling agent, it is used to formulate hydrogels from both natural and synthetic HMP and LMP in different patterns. The mechanism of HMP gel formation is mediated by hydrogen bond formation between pectin chains, which relies on an acidic medium at a pH range of 2−3.5. Additionally, a high sucrose concentration of more than 55% is required to produce HMP hydrogel. In contrast, LMP hydrogels are ionically mediated, where their gelling

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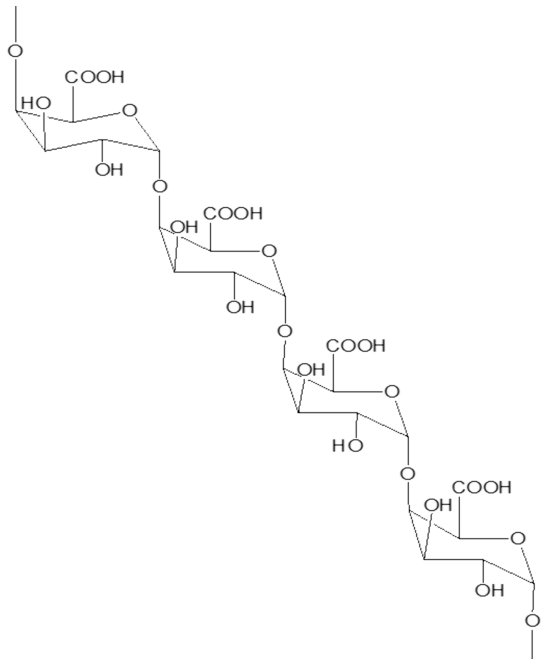

Figure 1. Chemical structure of pectin, which consists of covalently α-1,4-linked D-galacturonic acid units.

mechanism relies on divalent ions such as Ca^{2+}, Fe^{2+}, and Zn^{2+} across a wide pH range of 3−7.[8] Furthermore, pectin demonstrates a broad-spectrum bactericidal effect on both Gram-positive and Gram-negative pathogens.[9] Its mechanism of action involves the binding capacity of the carboxylic acid groups present in the primary backbone of pectin and the acidic pH of pectin, which are considered crucial factors for optimal antibacterial activity.[10] Moreover, pectin displays antioxidant properties due to its chemical structure composition, where the hydroxyl groups of pectin may act as a reducing agent. Hence, it can be utilized in pharmaceuticals and cosmetics.[11,12] Furthermore, pectin promotes wound healing by solubilizing into an acidic environment for wounded skin, acting as a barrier against bacteria or viruses.[13] The presence of hydrophilic groups in the pectin structure is also important for the effective removal of excessive exudate. Finally, the tendency of pectin to bind with active substances such as drugs or growth factors may enhance the wound healing process.[14]

Pectin hydrogels are utilized in various applications due to their soft and flexible nature, high water absorption capacity, biocompatibility, unique structure, and similarity to natural substances.[15] Due to pectin's characteristics, pectin hydrogels serve as carrier systems for analgesics, antibiotics, and tissue repair, facilitating drug release at the absorption site.[16] Additionally, pectin's capacity to form hydrogels under physiological conditions has been evaluated as a novel method to prolong contact time and improve drug penetration for ocular preparations.[17]

All these promising uses motivate the recycling of orange peel waste into pectin. The large quantities of orange peel waste encourage researchers to utilize them as a source of pectin. Orange trees are widely cultivated in the Middle East; hence, orange peels are available in significant amounts. Additionally, orange peel extract contains valuable phytochemicals that exhibit antimicrobial and antioxidant activities.[18] Therefore, pectin hydrogels can be regarded as useful products of low-cost, simple, nontoxic, sustainable, and eco-friendly green gels, suitable for topical applications to deliver antimicrobial, antioxidant, and wound healing agents.

Several methods have been developed to extract pectin from various plant sources.[19] Pectin extraction techniques are divided into conventional techniques, such as acid and aqueous extraction, and nonconventional or innovative techniques, such as ultrasound, microwave, and enzyme-assisted extraction.[20] Conventional methods are influenced by several key factors, including temperature, pH, solvent characteristics, particle size, solid-to-liquid ratio, diffusion rate, and extraction duration.[19] Hosseini et al.[21] investigated the aqueous extraction technique, which relies on distilled water as the sole solvent for pectin extraction without the use of mineral acid. The extraction yield, degree of esterification, and galacturonic acid content are related to three main factors: temperature, extraction duration, and liquid−solid ratio (LSR).[21]

This study aimed to develop an eco-friendly green pectin hydrogel as a carrier system for topical preparations using pectin extracted from orange peels. The extracted pectin, referred to as pectin, was characterized by percent yield (yield %), degree of esterification (DE%), equivalent weight, methoxyl content (MeO%), galacturonic acid content (GaIA %), and Fourier transform infrared spectroscopy (FTIR). An ethanolic extract from orange peels was used as a reference. Hydrogels with various pectin concentrations (8, 16, and 32%) were prepared and cross-linked with different concentrations of $CaCl_2$ (20, 40, 60, and 80%). The pH of hydrogels was adjusted to 3.5 and 8.5. The effects of different rheological factors, including pectin concentration, $CaCl_2$ concentration, and pH, were evaluated to determine the optimal rheological properties of pectin hydrogels. This study aimed to prepare hydrogels using pectin extracted from orange peels with rheological properties comparable to those prepared from commercially available pectin, which was used without modification. The physicochemical, rheological, and bioadhesive properties of the pectin hydrogels were investigated. Additionally, the *in vivo* wound healing, antimicrobial, and antioxidant activities of the pectin hydrogels and ethanolic orange peel extract were evaluated.

2. MATERIALS AND METHODS

2.1. Materials. Orange peels were collected from a local juice market (Nazareth, Palestine). Pectin from citrus peels (product number P9135 and CAS number 9000-69-5) and 1,1-diphenyl-2-picrylhydrazine (DPPH) were purchased from Sigma-Aldrich (Munich, Germany). Ascorbic acid was purchased from Fisher Scientific (PA, USA), water (HPLC-grade) from Tedia Company (Ohio, USA), ethanol (HPLC-grade) from Chem-lab NV (Belgium), absolute ethanol (99.9%) from Echa European agency (Helsinki, Finland), potassium bromide (KBr, spectroscopy grade) from Fisher Scientific (UK), and diethyl ether from Alpha Chemika (Mumbai, India). Dulbecco's phosphate-buffered saline (DPBS) was purchased from EuroClone (Pero, Italy), nutrient agar from NA, Biolab (Budapest, Hungary), Mueller−Hinton agar medium from MHA, Oxoid (Wade Road, UK), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS diammonium salt chromogenic peroxidase substrate) from Abcam (UK), and potassium permanganate ($KMnO_4$) from Riedel-de Haën (Germany). Dimethyl sulfoxide (DMSO) was purchased from Thermo Fisher Scientific (UK). MEDIHONEY Gel (Derma Science) was purchased from a local pharmacy. Ultrapure water was obtained using an EMD Millipore Direct-

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Figure 2. Process of aqueous extraction of pectin from orange peels, including washing fresh orange peels with tap water (A), cutting fresh orange peels into small pieces (B), sun-drying orange peels (C), grinding the dried orange peels (D), aqueous extraction from orange peel powder (E), filtration (F), precipitation of pectin (G), centrifugation, (H) washing pectin with HPLC-grade ethanol (I), obtaining wet pectin (J), drying the pectin (K), and pectin fine powder (L).

Q 5UV system (USA). All other reagents were used as supplied.

2.2. Methods. *2.2.1. Orange Peels.* Orange peels were collected from the local market in Nazareth, Palestine. They were washed using tap water, cut into smaller pieces, placed onto a clean cloth, and left to dry in the sun for two months. Once the peels were well-dried, they were ground into a fine powder. The dry orange powder was stored in a dry place in closed containers until needed.

2.2.2. Aqueous Extraction of Orange Peels. Pectin was extracted from orange peels as described by Hosseini et al.[21] with slight modification. Briefly, 100 g of orange peel powder was added to 2 L of distilled water in a 2 L beaker, achieving a liquid-to-solid ratio (LSR) of 20:1 (v/w). The mixture was heated to 95 °C on a hot plate with continuous stirring for 90 min. After extraction, the mixture was filtered through gauze, and the filtrate was kept cool at room temperature. Subsequently, the pectin was precipitated by adding ethanol (absolute pure, 99.9%) at a 1.5:1 (v/v) ratio and refrigerated at 4 °C for 20 h. To separate the suspended clots of pectin from the ethanol, the mixture was centrifuged at 6000 rpm for 15 min, filtered using filter paper, and washed twice with HPLC-grade ethanol. Finally, the filtrate was dried in a conventional oven at 50 °C for 16 h and then ground into fine powder using a mortar and pestle. The pectin powder was stored in a silica gel desiccator until further use. Figure 2 illustrates the process of the aqueous extraction of pectin from orange peels.

2.2.3. Characterization of Pectin. 2.2.3.1. Yield of Pectin Extraction. The percent yield (yield%) of pectin was calculated using eq 1:[21]

$$\text{yield}\ (\%) = \frac{m}{m_0} \times 100 \tag{1}$$

where m_0 (g) is the weight of dried orange peels and m (g) is the weight of the dried pure pectin.

2.2.3.2. Determination of the Degree of Esterification (DE %). A titration method was employed to calculate the degree of esterification (DE%) of pectin samples, as described by Hosseini et al.[21] A weight of 0.1 g of dried pectin was wetted with HPLC-grade ethanol, dispersed in 20 mL of distilled water free of carbon dioxide, and placed in a sonicator. Then, three drops of a phenolphthalein indicator were added to the mixture and titrated with 0.1 M NaOH. The initial titration volume (V_1) was recorded when a faint pink color appeared. Following this, 10.0 mL of 0.1 M NaOH was added to the mixture and stirred using a magnetic stirrer for 30 min to achieve hydrolysis. Subsequently, 10.0 mL of 0.1 M HCl was added to neutralize the NaOH, and the sample was shaken until the pink color disappeared. A back-titration was then conducted with 0.1 M NaOH. Once a faint pink color reappeared, this volume was recorded (V_2), and the DE% was calculated using eq 2:

$$\text{DE}\ (\%) = \frac{V_2}{V_1 + V_2} \times 100 \tag{2}$$

where V_1 and V_2 are the first and second titration volumes, respectively.

2.2.3.3. Determination of the Equivalent Weight of Pectin. The equivalent weight of pectin was determined as described by Ismail et al.[22] with a slight modification. Initially, a weight of 1.00 g of pectin was placed in a 250 mL conical flask, wetted with 10 mL of HPLC-grade ethanol, dispersed in 200 mL of deionized water free of carbon dioxide, and placed in the sonicator. Then, 2 g of sodium chloride (NaCl) was added to sharpen the end point. The mixture was stirred on a magnetic stirrer at room temperature. After complete dissolution, six drops of the phenolphthalein indicator were

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added and titrated using 0.1 M NaOH until the color turned pink. The equivalent weight of pectin was determined using eq 3:

$$\text{equivalent weight} = \frac{\text{weight of the sample (g)} \times 1000}{\text{volume of NaOH (mL)} \times \text{molarity of NaOH}} \quad (3)$$

2.2.3.4. Determination of the Methoxyl Content (MeO%). The methoxyl content (MeO%) of pectin was determined as described by Wathoni et al.[12] Briefly, 50.0 mL of 0.25 M NaOH was added to the mixture used in the equivalent weight test, stirred vigorously on a magnetic stirrer, and allowed to stand for 30 min in a closed flask at room temperature. Then, 50.0 mL of 0.25 M HCl was added to the mixture and titrated with 0.1 M NaOH until the pink color appeared. The MeO% was calculated using eq 4:

$$\text{MeO (\%)} = \frac{\text{meq of NaOH} \times 31 \times \text{molarity of NaOH} \times 100}{\text{weight of the sample (mg)}} \quad (4)$$

where 31 represents the molecular weight (MW) of the methoxyl group.

2.2.3.5. Determination of the Galacturonic Acid Content (GalA%). The equivalent weight and methoxyl content were used to estimate the GaIA content using eq 5:[12]

$$\text{GaIA (\%)} = [(\text{meq of NaOH for free acid} + \text{meq of NaOH for methoxyl}) \times 176 \times 100] / [\text{weight of the sample (mg)}] \quad (5)$$

where 176 represents the MW of anhydrous GaIA.

2.2.4. Ethanol Extraction of Orange Peels. The ethanol extraction of orange peels was carried out as described by Hanafy et al.[23] Briefly, 500 g of dry powdered orange peels was placed in gauze, closed tightly, and stored in a dark container containing 1200 mL of HPLC-grade ethanol for 2 weeks at room temperature. The container was shaken three times daily to extract the bioactive compounds from the orange peels. Subsequently, the extract was filtered using filter paper, and the solvent was evaporated using a rotary evaporator. The filtrate was stored at room temperature for later use within a week.

2.2.4.1. Analysis of the Ethanolic Orange Peel Extract. A liquid chromatography−mass spectrometry (LC-MS) analysis of the ethanolic orange peel extract was conducted using a Bruker Daltonik UPLC connected with a Bruker Daltonik (Bremen, Germany) Impact II ESI-Q-TOF system for screening the compounds of interest via direct injection. Standards were used to identify m/z with a high-resolution Bruker TOF-MS and determine the exact retention time of each analyte. The liquid chromatographic system comprised a UPLC linked to a Bruker Impact II Q-TOF-MS. Separation was performed using a Bruker Solo 2.0, C18 UPLC column (100 mm × 2.1 mm) with a 2.0 μm pore size; the column temperature was maintained at 40 °C, and the flow rate was set at 0.51 mL/min. The instrument was operated with an Ion Source Apollo II ion funnel electrospray source. The capillary voltage was 2500 V, the nebulizer gas pressure was 2.0 bar, the dry gas (nitrogen) flow was 8 L/min, and the dry temperature was 200 °C. The mass accuracy was <1 ppm; the mass resolution was 50,000 full sensitivity resolution (FSR), and the TOF repetition rate reached up to 20 kHz. The mobile phases were prepared using HPLC-grade deionized water with 0.05% formic acid (solvent A) and acetonitrile (solvent B). The elution program ran a linear gradient over 0−27 min from 5−80% B; 27−29 min, 95% B; 29.1 min, 5% B. The total analysis time was 35 min in both positive and negative mode, with a sample injection volume of 3 μL. All instrumental settings were implemented as described in ref 24. The injected solutions were prepared by dissolving an appropriate amount of the extract in 2.0 mL analytical-grade dimethyl sulfoxide (DMSO), followed by dilution with HPLC-grade acetonitrile. The diluted solutions underwent centrifugation for 2.0 min at 4000 rpm, and 1.0 mL of the supernatant solution was transferred to an HPLC vial. A volume of 3 μL was injected into the UPLC system.

2.2.5. Preparation of Pectin Hydrogels. 2.2.5.1. Effect of Pectin Concentration. Pectin hydrogels (5 g) were prepared by dispersing 8% (F1), 16% (F2), and 32% (F3) of pectin in HPLC-grade water using a magnetic stirrer at room temperature. Once pectin was completely dispersed, the pH was adjusted to 8.5 with 1 M NaOH. Then, 0.5 mL of 40% $CaCl_2$ was added dropwise to pectin hydrogels while stirring continuously on the magnetic stirrer for 5 min. The mixture was left for 24 h to allow cross-linking. The hydrogels were refrigerated at 4 °C overnight for use within 1 day.

2.2.5.2. Effect of $CaCl_2$ Concentration. Pectin hydrogels (5 g) were prepared by dispersing 16% of pectin in HPLC-grade water using a magnetic stirrer at room temperature. Once pectin was completely dispersed, the pH was adjusted to 8.5 with 1 M NaOH. The pectin hydrogel (F4), prepared without adding $CaCl_2$ (0% $CaCl_2$), was used as a control. Then, 0.5 mL of 20, 60, and 80% $CaCl_2$ was added dropwise to prepare F5, F6, and F7 pectin hydrogels, respectively, while stirring continually for 5 min and allowed to sit for 24 h to promote cross-linking. The hydrogels were refrigerated at 4 °C overnight for use within 1 day.

2.2.5.3. Effect of Low and High Pectin Concentration Cross-Linked with 60% $CaCl_2$. Pectin hydrogels (5 g) were prepared by dispersing 8% (F8) and 32% (F9) of pectin in HPLC-grade water using a magnetic stirrer at room temperature. Once the pectin was completely dispersed, the pH was adjusted to 8.5 with 1 M NaOH. Subsequently, 0.5 mL of 60% $CaCl_2$ was added to the pectin hydrogels while continuously stirring for 5 min, after which they were left to cross-link for 24 h. The hydrogels were refrigerated at 4 °C overnight for use within 1 day.

2.2.5.4. Effect of pH. Pectin hydrogel (5 g, F10) was prepared by dispersing 16% of pectin in HPLC-grade water using a magnetic stirrer at room temperature. Once the pectin was fully dispersed, the pH was adjusted to 3.5 with 0.1 M HCl. Then, 0.5 mL of 40% $CaCl_2$ was added to the pectin hydrogel while stirring constantly for 5 min and left to cross-link for 24 h. The hydrogel was refrigerated at 4 °C overnight for use within 1 day.

2.2.5.5. Effect of the Pectin Type. Pectin hydrogel (5 g) was prepared by dispersing 2% of the commercial pectin (Sigma-Aldrich) in HPLC-grade water using a magnetic stirrer at room temperature. Once the pectin was fully dispersed, the pH was adjusted to 8.5 with 1 M NaOH. Then, 0.5 mL of 40 and 60% $CaCl_2$ was added dropwise to prepare F11 and F12 pectin hydrogels, respectively, while stirring continuously for 5 min and letting them sit for 24 h to facilitate cross-linking. The hydrogels were refrigerated at 4 °C overnight for use within 1

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Table 1. Composition and Force of Detachment of Pectin Hydrogels Prepared with Various Pectin Types and Concentrations (8, 16, and 32%) and Cross-Linked with Different $CaCl_2$ Concentrations (20, 40, 60, and 80%)

pectin hydrogels	pectin extracted from orange peels (%)	commercial pectin (%)	$CaCl_2$ concentration (%)	pH	force of detachment (N) (n = 7)
F1	8		40	8.5	304.1 ± 10.2
F2	16		40	8.5	294.0 ± 7.4
F3	32		40	8.5	344.0 ± 2.5
F4	16		0	8.5	165.5 ± 11.4
F5	16		20	8.5	ND[a]
F6	16		60	8.5	352.0 ± 11.7
F7	16		80	8.5	341.9 ± 7.2
F8	8		60	8.5	ND
F9	32		60	8.5	ND
F10	16		40	3.5	ND
F11		2	40	8.5	ND
F12		2	60	8.5	433.5 ± 13.9

[a]ND: not determined.

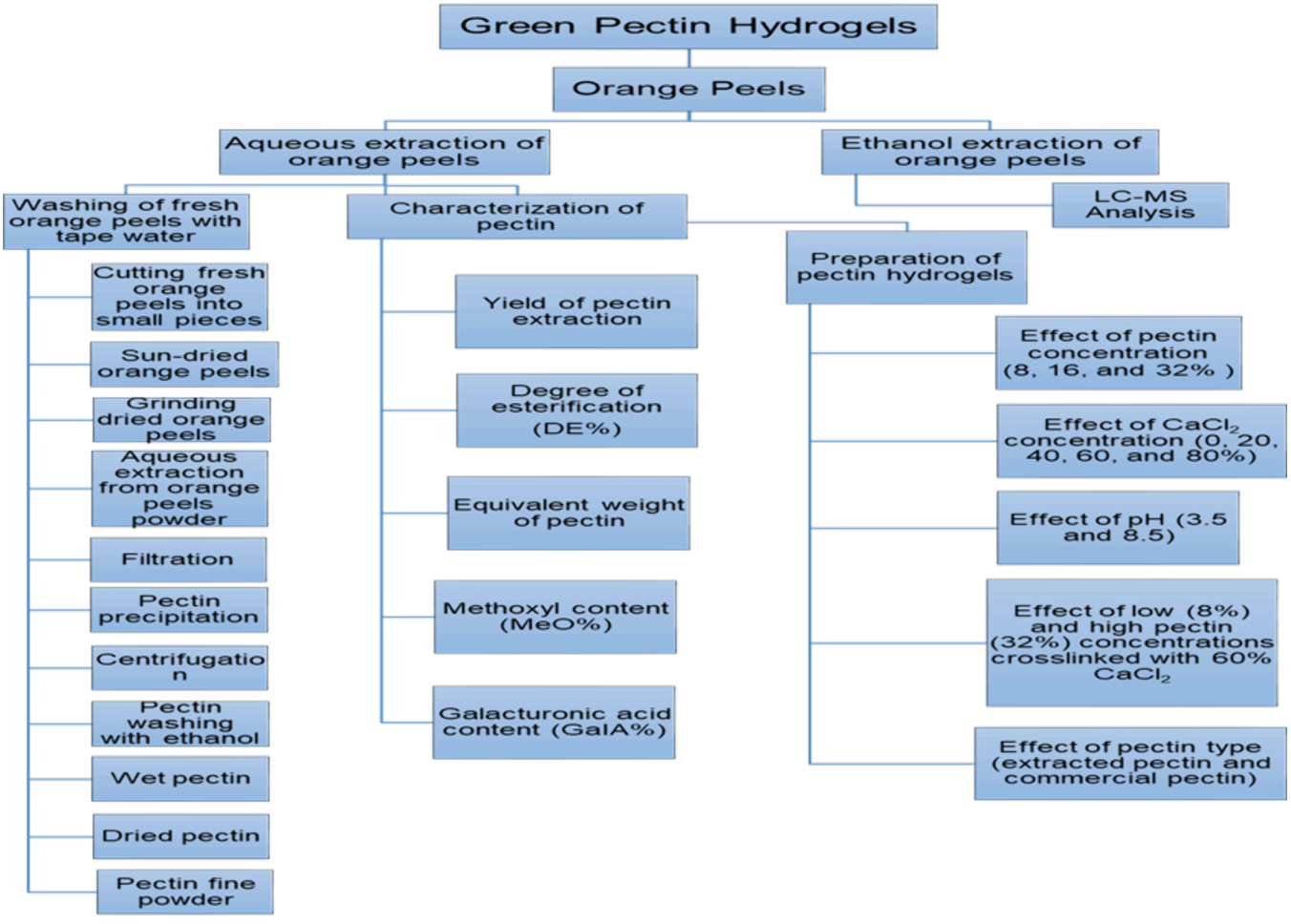

Figure 3. Flow diagram illustrating the method for extracting pectin from orange peels, characterization of the extracted pectin, and various parameters, such as pectin concentration, $CaCl_2$ concentration, pH, and pectin type, that were analyzed to optimize the preparation of pectin hydrogels.

day. Table 1 summarizes the composition of pectin hydrogels (F1−F12).

Figure 3 depicts a flow diagram that illustrates the extraction method of pectin from orange peels, the characterization of the extracted pectin, and various parameters such as pectin concentration, $CaCl_2$ concentration, pH, and pectin type, that were analyzed to optimize the preparation of pectin hydrogels.

2.2.6. Characterization of Pectin Hydrogels. 2.2.6.1. Physical Appearance. Pectin hydrogels were visually evaluated for color change, homogeneity, cloudiness, and microbial growth.

2.2.6.2. pH. The pH of pectin hydrogels was initially determined and measured again after 24 h using a digital pH meter (Jenway 3510, Stone Staffs, UK). The pH measurement of each hydrogel was repeated at least three times, and the mean and standard deviation (SD) were reported.

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2.2.6.3. Rheological Studies. For complex viscosity (η^*), to investigate the flow properties of pectin hydrogels, the complex viscosity curves (η^* (Pa s) vs frequency (rad/s)) were derived from the corresponding frequency sweep tests, performed using a controlled-stress rheometer (CSR) (Anton Paar, MCR 302; Graz, Austria) with a cone and plate geometry of 25 mm diameter and 1° cone angle, and maintained at 37 °C using a temperature control system.

For strain sweep, the deformation of pectin hydrogels over a strain range of 0.01−100% was conducted using a CSR with a cone and plate geometry of 25 mm diameter and 1° cone angle, as described in ref 25. A 0.5 g sample of pectin hydrogel was placed onto the lower plate, allowed to relax, and equilibrated at 37 °C for 1 min. During the experiments, the top cone was oscillated at a frequency of 10 rad/s after being lowered until a 0.1 mm gap separated the cone and plate. The linear viscoelastic region (LVR) was determined for each hydrogel during the strain sweep experiments.

For frequency sweep, the frequency sweep studies for pectin hydrogels were conducted using a CSR with a cone−plate geometry of 25 mm diameter and 1° cone angle over a frequency range of 0.1−100 rad/s and at a selected strain within the LVR, as described in ref 25. This test aimed to investigate the viscoelastic properties of pectin hydrogels characterized by the elastic modulus G' and viscous modulus G''. The frequency dependence of G' and G'' for each hydrogel was determined at 37 °C for at least three replicates.

2.2.6.4. Bioadhesion Studies. The adhesion between rat skin and pectin hydrogels was determined using a TA-XTplus texture analyzer (Stable Micro Systems, Godalming, UK) as described by Carvalho et al.[26] with slight modifications. Male Wistar rats of 180−200 g weight were maintained in the animal care center of the Faculty of Pharmacy, Al-Zaytoonah University of Jordan, Jordan, and were used in the experiment. The experiment was conducted with the approval of the Research Ethics Committee at Al-Zaytoonah University of Jordan (IRB no. 22/04/2021−2022). Fresh skin was obtained from healthy Wistar rats under anesthesia during the procedure. Initially, the hair was shaved, and the skin was cut into pieces with scissors. The skin was cleaned with normal saline, and the adipose tissue was removed from the stratum corneum and epidermis. The prepared skin was stored at −80 °C for later use. On the day of the experiment, the skin was immersed in Dulbecco's phosphate-buffered saline (DPBS) at room temperature for 30 min before the experiment. The rat skin was then fixed using double-sided adhesive tape on a plastic cylindrical support positioned at the plate (base of the measurement apparatus) and held in place using SuperGlue. The hydrogel sample was attached to the upper probe of the instrument and aligned with the plastic cylindrical support to ensure that the sample was in direct contact with the rat skin surface when the upper probe was lowered. Figure S1 illustrates the experimental setup for the bioadhesion study of the pectin hydrogels using the texture analyzer.

The test was run in the tensile mode, with the probe lowered at a speed of 1 mm/s to contact the skin at a force of 0.002 N. It was then withdrawn at a rate of 0.5 mm/s to a distance of 45 mm. Seven replicates were analyzed for each hydrogel. A force−time curve was captured during the experiment, from which the force of detachment was calculated to evaluate the adhesion strength of pectin hydrogels using Texture Exponent 32 software (Hamilton, MA, USA). The adhesion properties of pectin hydrogels were evaluated by altering pectin concentration for F1, F2, and F3 hydrogels, $CaCl_2$ concentration for F2, F4, F6, and F7 hydrogels, and pectin type for F6 and F12 hydrogels.

2.2.6.5. Fourier Transform Infrared (FTIR) Analysis. The FTIR spectra were obtained for pectin extracted from orange peels, commercial pectin, and F6 pectin hydrogel (composed of 16% and cross-linked with 60% $CaCl_2$ for 24 h, at pH 8.5). Powders were ground and thoroughly mixed with KBr. The measurements were carried out using FTIR (Vertex 70 spectrometer, Bruker, Germany) with 10 scans, a resolution of 4 cm^{-1}, and over a frequency range of 3500−500 cm^{-1}.

2.2.7. Biological Activities of Pectin Hydrogels and Ethanolic Orange Peel Extract. 2.2.7.1. Antimicrobial Activity. The antimicrobial activity of pectin hydrogels and ethanolic orange peel extract was assessed by measuring their inhibitory effects against Gram-positive and Gram-negative bacteria using the agar diffusion technique described by Farag et al.[27] Bacterial colonies were prepared in 5 mL phosphate-buffered saline (PBS, 0.5 McFarland standards). Different species of Gram-positive bacteria (*Staphylococcus aureus*, *S. aureus* ATCC6538) and Gram-negative bacteria including *Escherichia coli* (*E. coli*) ATCC8739, *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC9027, and *Proteus mirabilis* (*P. mirabilis*) ATCC12453 were tested. The discs were filled with F4, F6, F8, and F9 pectin hydrogels, F12 commercial pectin hydrogel, and ethanolic orange peel extract. Additionally, 0.03 g of MEDIHONEY Gel, gentamycin (2000 μg/mL), amoxicillin disc (30 μg), and fluconazole (2000 μg/mL) were used as positive controls. The culture plates were incubated at 37 °C for 24 h, and results were observed the following day. A centimeter ruler was utilized to measure the zone of inhibition created around the discs. The test was repeated twice for each hydrogel.

2.2.7.2. Antioxidant Activity. The antioxidant activity of pectin and ethanolic orange peel extract was evaluated using the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay described by Al-Shalabi et al.[28] The ABTS radical was generated by reacting the ABTS salt (3.6 mM in ultrapure water) with $KMnO_4$ solution (2.4 mM in ultrapure water) in dark conditions for 16 h under magnetic stirring at room temperature. To achieve an absorbance of 0.7 at 734 nm, the blue-green solution was diluted 10 times with ultrapure water. A 25 mg/mL stock solution for each sample was prepared and serially diluted to a concentration range of 3.125−25 mg/mL. A weight of 25 mg of pectin was dissolved in 1 mL of ultrapure water, and 25 mg of ethanolic orange peel extract was dissolved in 1 mL of HPLC-grade ethanol mixed with 1 mL of DMSO. Additionally, a stock solution of 25 mg of ascorbic acid dissolved in 1 mL of ultrapure water was used as a control. A volume of 1 mL of the diluted ABTS solution was added to 200 μL of each sample. After 30 min of incubation at room temperature in the dark, the absorbance at 734 nm was measured using a microplate reader. In each trial, solvent blanks (1 mL of ABTS + 200 μL of ultrapure water, 1 mL of ABTS + 200 μL of HPLC-grade ethanol, and 1 mL of ABTS + 200 μL of DMSO) were also run. The experiment was conducted in triplicate. The scavenging activity (%) was determined using eq 6:

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$$\text{scavenging activity (\%)} = \frac{\text{absorbance of the blank} - \text{absorbance of the sample}}{\text{absorbance of the blank}} \times 100 \quad (6)$$

2.2.7.3. In Vivo Wound Healing Study. The wound healing procedure was evaluated for pectin hydrogels and ethanolic orange peel extract, as described by Mahmoud et al.,[29] with slight modifications. Six male Wistar rats were housed individually in plastic cages in a controlled environment (23 ± 2 °C) with unrestricted access to food and water for 3 weeks prior to the experiment, with daily cleaning for the cages. The Research Ethics Committee at Al-Zaytoonah University of Jordan approved the study, which adhered to the university's animal ethics policies (IRB no. 22/04/2021−2022). Rats were anesthetized using diethyl ether while the dorsal hair was shaved. An alcohol pad was then used to sanitize the shaved area. A Flowmaster pen was used to outline a 1 cm^2 area on the dorsal skin, which was carefully cut using sterile scissors. Immediately after the cut, the area was sterilized using an alcohol pad, and all rats received their treatment, as summarized in Table 2.

Table 2. Application of Treatments on Rats' Skin for Wound Healing Activity

rat no.	treatments
1	untreated wound (negative control)
2	MEDIHONEY gel, Derma Science, Inc. (positive control)
3	ethanolic orange peel extract
4	pectin hydrogel (16%) cross-linked with 60% $CaCl_2$ for 24 h (F6)
5	pectin hydrogel (32%) cross-linked with 60% $CaCl_2$ for 24 h (F9)
6	commercial pectin hydrogel (2%) cross-linked with 60% $CaCl_2$ for 24 h (F12)

In this study, the negative control received no treatment (untreated wounds). The positive control received treatment with MEDIHONEY Gel, a commercially available gel known for its wound healing activity, which was used to evaluate the efficacy of pectin hydrogels. The other groups received treatments with ethanolic orange peel extract (Figure S2), pectin hydrogels (F6 and F9) (Figure S3A), and commercial pectin hydrogel (F12) (Figure S3B). The treatments were sticky and applied directly to the open wounds without a secondary dressing. The formulations were applied topically three times daily, and the treatments continued for 42 days. Digital pictures of the wounds were taken on days 0, 3, 7, 14, 21, 28, 35, and 42 from the same position and under consistent conditions for all experimental rats. ImageJ software (Bethesda, MD, USA) was used to measure the wound closure area over time. The percentage of wound closure was calculated using eq 7:

$$\text{percentage of the wound closure area} = \frac{\text{wound area at day zero} - \text{wound area over time}}{\text{wound area at day zero}} \times 100 \quad (7)$$

Three representative time points (7, 21, and 42 days) were selected to improve the clarity and readability of the wound healing analysis.

2.2.8. Statistical Analysis. The data were expressed as means ± standard deviation (SD) obtained from at least three independent measurements ($n \geq 3$). A one-way ANOVA, followed by Tukey's test, was conducted to assess the statistical significance of the force of detachment among various hydrogels. Additionally, a two-way ANOVA with repeated measures followed by Tukey's multiple comparisons was employed to evaluate the percentage of the wound closure area among different groups. All statistical analyses were conducted using Prism-GraphPad 8 (GraphPad Software, Inc., CA, USA). A significance level of $p < 0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Aqueous Extraction of Pectin from Orange Peels.

3.1.1. Extraction Yield. The percent yield (yield%) of pectin extraction, calculated using eq 1, was 14.00%, with the weight of orange peel powder being 50.00 g and that of pectin being 7.00 g (Table 3). In comparison, the yield% of pectin reported

Table 3. Pectin Yield, Degree of Esterification (DE), Equivalent Weight of Pectin, Methoxyl Content (MeO), and Galacturonic Acid Content (GaIA) of Pectin Extracted from Orange Peels

pectin yield (%)	DE (%)	equivalent weight of pectin (g/mol)	MeO (%)	GaIA (%)
14.00	20.00	2500	14.88	91.52

by Hosseini et al.[21] was higher than that obtained in this study (17.95 vs 14.00%). The variation in the yield% might be attributed to various factors such as the extraction technique, fruit maturity, harvesting time, peel thickness, age of the orange tree, irrigation methods, and soil type.[30−32] It was found that the yield% of pectin from orange peels using two conventional extraction techniques, hot distilled water extraction and hot HCl (0.5%) extraction, were 1.75 and 2.95%, respectively.[21] Furthermore, lower yield% values were found in the non-commercial sources of pectin. For instance, the yield% from sunflower heads ranged from 7.4 to 11.6%,[21] carrot peels yielded 8.9−9.1%,[33] and cocoa husks yielded 3.3−7.6%.[34] In contrast, pectin obtained through ultrasound- and microwave-assisted techniques demonstrated higher yield% values of 28.07%[35] and 29.1%,[36] respectively.

The extraction yield of pectin is influenced by three main factors: temperature, extraction duration, and liquid−solid ratio (LSR).[21] Temperature is a significant factor that enhances the pectin extraction yield. Higher temperatures and extended extraction times break down the plant cell wall, facilitating solvent diffusion and pectin extraction.[30] Additionally, higher temperatures enhance the solubility of polysaccharides in the extractant.[21,30] In this study, the extraction was conducted at 95 °C. Although this temperature resulted in a high extraction yield, it may raise costs due to increased energy consumption. Moreover, the pectin extraction yield% increases with increasing LSR.[37] Therefore, excessive amounts of solid material (orange peel powder) can create a dynamic equilibrium with the solvent, resulting in a lower mass transfer rate and reduced pectin yield.[30] The optimum LSR (v/w) was reported to be 20:1,[21] and this ratio was used in our study. The extraction time is another critical variable that directly affects the yield%, with longer extraction durations improving pectin yield. Consequently, our extraction lasted for 90 min. The

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Table 4. Most Abundant Compounds Identified in Ethanolic Extract of Orange Peels by LC-MS and Their Corresponding Retention Times, m/z Measurements, Molecular Weights, and Chemical Classes

RT (min)	m/z meas.	compound name	molecular formula	mass (%)	chemical class
4.86	195.07	3,5-dimethoxy-4-hydroxyacetophenone	$C_{10}H_{12}O_4$	10.90	phenolic compound
10.07	301.07	homoeriodictyol	$C_{16}H_{14}O_6$	9.58	flavonoid
12.35	343.08	eupatilin	$C_{18}H_{16}O_7$	8.09	flavonoid
3.58	167.04	vanillic acid	$C_8H_8O_4$	8.02	phenolic compound
5.09	193.05	ferulic acid	$C_{10}H_{10}O_4$	6.91	phenolic compound
4.9	447.09	*iso*-orientin	$C_{21}H_{20}O_{11}$	5.33	flavonoid
11.93	313.07	galangustin	$C_{17}H_{14}O_6$	4.78	flavonoid
9.41	271.06	naringenin	$C_{15}H_{12}O_5$	4.52	flavonoid

impact of fruit maturity on the pectin yield% was investigated by Azad et al.[38] Their study demonstrated that the pectin yield extracted from lemon pomace varied significantly across the stages of maturity (premature, mature, and over-ripe), where pectin yield decreased as the fruit ripened. This reduction is attributed to the conversion of pectin into protopectin, sugars, and other constituents during the ripening process.[38]

3.1.2. Degree of Esterification (DE%). The degree of esterification (DE%) of pectin, determined using eq 2, was 20.00% (Table 3). The pectin solution turned slightly pink, and the volume of 0.1 M NaOH (V_1) was 2.5 mL. Following neutralization with 0.1 M HCl, back-titration was conducted, and the volume of 0.1 M HCl (V_2) was 10.0 mL, indicated by the appearance of a pink color. The extracted pectin was classified as low-methoxy pectin (LMP) since the DE was below 50%.[39] Carboxylic acid groups of GaIA might exist as free acid, salt (carboxylate), or methyl ester. Hence, DE% of pectin is defined as the ratio of esterified carboxylic acid groups to total carboxylic acid groups. Determining the DE% is crucial as it significantly influences the gel-forming ability, mechanism of pectin gelation, and gel characteristics.[40,41]

The DE% obtained in this study was slightly lower than that reported by Hosseini et al.[21] (20.00% vs 23.00%), extracted from orange peels under comparable conditions (hot-water extraction technique). The DE% of pectin could be impacted by the extraction temperature and duration.[42] Typically, pectin extracted at higher temperatures and with longer extraction times showed lower DE% due to the extreme conditions employed in the extraction process.[21] Additionally, DE% correlates with the fruit's maturity, where increased fruit maturity corresponds to a decrease in DE%.[38] This decrease is attributed to the conversion of pectins into protopectin, which increases the sugar content and makes the fruit softer.[38] Moreover, as the fruit ripens, the activity of pectin methylesterase enzymes increases, hydrolyzing the methyl groups from the pectin chains.[32]

3.1.3. Equivalent Weight of Pectin. The equivalent weight of pectin is defined as the total amount of free GaIA present in the chemical structure of pectin.[43] The equivalent weight of pectin, determined using eq 3, was found to be 2500 g/mol, where the weight of pectin was 1.00 g, the volume of NaOH was 4.0 mL, and the molarity of NaOH was 0.1 M (Table 3). A previous study[44] reported that the equivalent weight of pectin obtained through the conventional extraction technique was 704.46 g/mol. It has been shown that as the equivalent weight of pectin increases, the gel-forming properties also increase.[45]

3.1.4. Methoxyl Content (MeO%). The MeO% of pectin, calculated using eq 4, was 14.88%, where 1000 mg was the weight of pectin, 48.0 mL was the volume of 0.1 M NaOH, and 31 g/mol was the MW of methoxyl group (Table 3). Based on the MeO% of 14.88%, pectin was classified as LMP, where the degree of methylation (DM) was lower than 50%,[46] in agreement with the results of %DE. DM is defined as the ratio of methyl-esterified GaIA units to the total GaIA units, encompassing free and substituted forms of GaIA. Thus, this significant parameter influences the gelling mechanism and gel properties.[47] It has been shown that pectin obtained through the basic extraction technique is classified as LMP, whereas that obtained through the acidic extraction technique is classified as HMP due to the harsh pH conditions.[48]

Previous studies have demonstrated that the temperature and duration of extraction affect DM.[19,28,47,48] In this study, pectin was extracted under harsh conditions of high temperature (95 °C) and extended extraction time (90 min) to obtain pectin with low DM and specific gelling properties.[21] Higher temperatures and longer extraction times promote the demethylation of GaIA units through the thermal hydrolysis of pectin side chains, decreasing DM.[21,49,50]

The MeO% significantly decreased with increasing fruit maturity.[32,38] As the fruit ripens, the activity of pectin methylesterase increases, hydrolyzing the methoxyl groups of pectin, removing methoxyl groups, and consequently reducing the methoxyl content.[32,38] Moreover, the ripening process can increase the susceptibility of pectin to pectin lyase enzymes, leading to the depolymerization of pectin chains.[32]

3.1.5. Galacturonic Acid Content (GalA%). GalA is the monosaccharide that constitutes most of the pectin, followed by L-arabinose, D-galactose, and L-rhamnose. The carboxylic groups or hydroxyls within GalA subunits may be methyl- or O-acetyl-esterified.[1] The O-acetyl-esterification occurs at the O-3 position and can also occur at the O-2 position.[51] The GaIA%, calculated using eq 5, was 91.52%, where 1000 mg was the weight of the pectin sample, 176 g/mol was the molecular weight of anhydrous GaIA, 0.4 was the meq of NaOH for free acid, and 4.8 was the meq of NaOH for methoxyl (Table 3). GaIA% serves as an indicator of pectin purity. According to the International Pectin Procedure Association, the purest citrus pectin contains ~85−90% of anhydrous GaIA, with the minimum acceptable limit set above 65% for it to be suitable for use.[52] Applying higher temperatures for extended periods during pectin extraction results in an increased GaIA content, which could be attributed to the enhanced hydrolysis of pectin side chains.[21] The GaIA% reported by Hosseini et al.[21] was 84.5%, lower than that obtained in our study (91.52%). This discrepancy might be attributed to the maturity stage of the orange; nevertheless, the extraction conditions were similar.

The GalA content varies based on the maturity of the fruit.[38,53] Azad et al.[38] showed that premature lemon pomace exhibited a higher GaIA content than mature lemon pomace.

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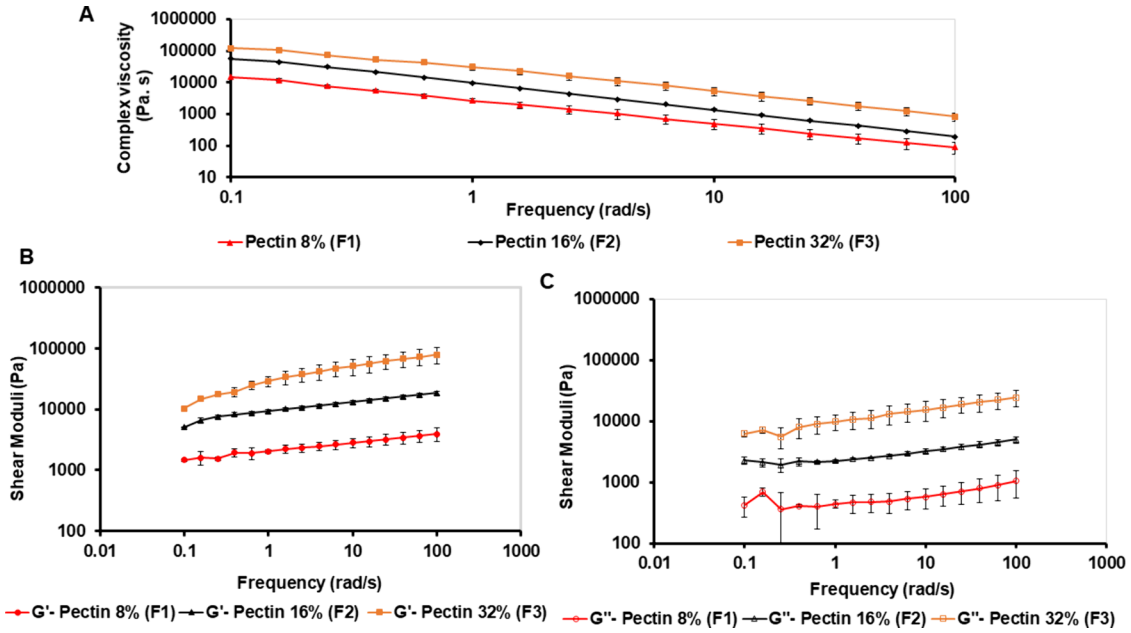

Figure 4. Effect of pectin concentrations (8, 16, and 32%) on the complex viscosity (η^*) (A), Frequency dependence of the storage modulus G' (B), and frequency dependence of the loss modulus G'' (C) of pectin hydrogels F1 (8% pectin), F2 (16% pectin), and F3 (32% pectin) cross-linked with 40% $CaCl_2$ for 24 h (pH 8.5). Data are presented as means ± SD (n = 3).

In contrast, Nidhina et al.[53] demonstrated that the GaIA content in pectin increases with the maturity of jackfruit rags.

3.2. Ethanolic Extraction of Orange Peels. The ethanolic extract of orange peels was sticky and bright orange (Figure S2). According to the phytochemical analysis conducted by Hanafy et al.,[23] the ethanolic orange peel extract contains phenolic compounds, flavonoids, and tannins that are valuable for their antioxidant, antimicrobial, and wound healing properties.

3.2.1. LC-MS Analysis. LC-MS analysis of the ethanolic extract of orange peels identified 40 compounds from various organic families. Table S1 displays the 40 compounds along with their corresponding retention times (RT), relative percentages, and chemical classes. The ethanolic extract included flavonoids (51.6%), phenolics (41.4%), carboxylic acids (5.5%), and coumarins (1.5%). The most abundant compounds found in the ethanolic orange peel extract include 3,5-dimethoxy-4-hydroxyacetophenone, homoeriodictyol, eupatilin, vanillic acid, ferulic acid, *iso*-orientin, galangustin, and naringenin. These compounds, along with their corresponding retention times, m/z measurements, molecular weights, and chemical classes are listed in Table 4. The mass spectra of these compounds are shown in Figures S4−S11.

3.3. Characterization of Pectin Hydrogels. *3.3.1. Physical Appearance.* Hydrogels (F1−F10), prepared from pectin, were homogeneous and yellow. The color intensity was directly related to the concentration of pectin. No precipitation occurred in the pectin hydrogels. F11 and F12 hydrogels, prepared using commercial pectin, were homogeneous and opaque, showing no precipitation. Figure S3 visually illustrates the differences in color, homogeneity, and opaqueness between the extracted pectin hydrogel (F6) and the commercial pectin hydrogel (F12).

3.3.2. pH. The pH of pectin hydrogels F1−F9 and F11−F12 was adjusted to 8.5, whereas the pH of F10 was adjusted to 3.5. Since the pectin extracted from orange peels was classified as LMP, the alkaline condition (pH 8.5) increased the dissociation of the carboxylic groups, thereby promoting gel formation when using ionic cross-linking. Conversely, at pH 3.5, the dissociation of carboxylic acid decreases, reducing calcium bridges and resulting in the low gelling properties of LMP.[8]

The pH values of 3.5 and 8.5 for the pectin hydrogels were selected based on previous studies,[8,54] which examined a wide pH range (3.5−9.5) regarding the gelation of LMP gels. The results showed that the gel strength of LMP was increased concomitant to an increase in pH, with the greatest gel strength and structure found at pH 8.5.[8] This was attributed to the increased dissociation of carboxylic groups of GaIA residues and the de-esterification of pectin at pH 8.5, where the "egg-box" model suggests interactions between LMP and Ca^{2+}.[8,54,55] Therefore, the pectin hydrogels were adjusted at two pH values: pH 3.5, representing the lowest pH range, and pH 8.5, representing the optimal pH.[8]

3.3.3. Rheological Properties. Rheological studies were conducted to evaluate the effect of pectin concentration, $CaCl_2$ cross-linker concentration, pH, and pectin type on the complex viscosity (η^*), LVR, and viscoelastic properties (G' and G'') of pectin hydrogels.

3.3.3.1. Effect of Pectin Concentration. F1, F2, and F3 hydrogels were prepared using 8, 16, and 32% pectin, respectively, and were cross-linked with 40% $CaCl_2$. The complex viscosity (η^*) curves of pectin hydrogels demonstrated non-Newtonian pseudoplastic behavior, where η^* declined linearly with frequency (Figure 4A), in agreement with Kocaaga et al.[56] This pseudoplastic (shear-thinning) behavior is considered a critical property for improving the residence time, spreadability, and adhesion of the polymeric systems for topical applications.[57] It was found that η^* was highly influenced by pectin concentration, where higher concentrations of pectin provide additional binding sites between adjacent free dissociated carboxylic groups mediated by Ca^{2+} ionic bridging, resulting in enhanced elastic characteristics. Additionally, pectin, with its low DE, exhibited a

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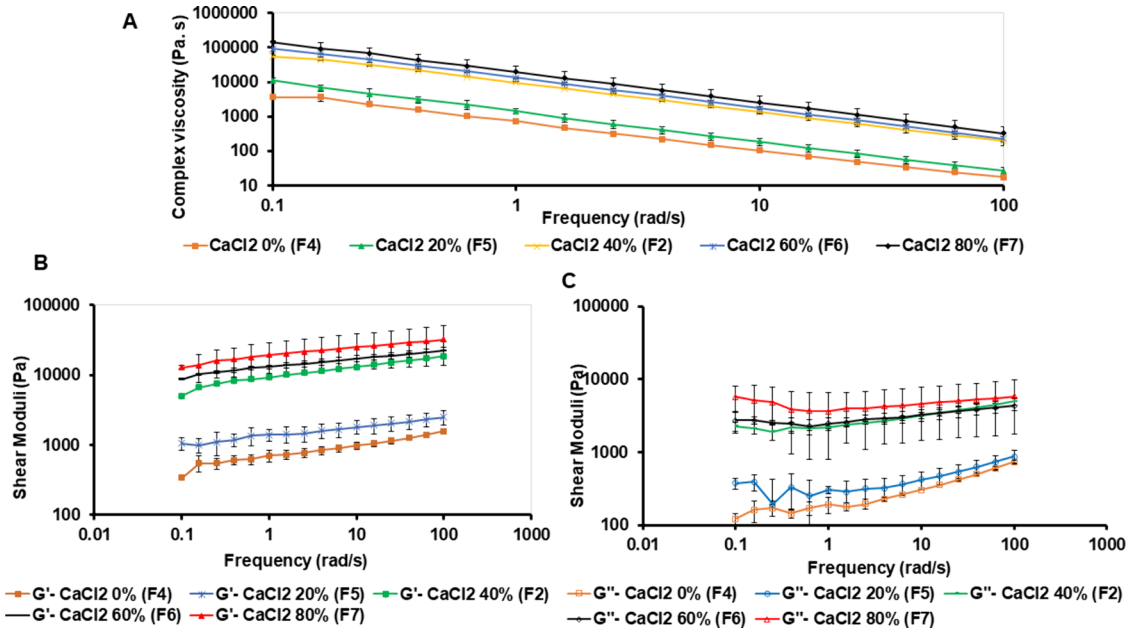

Figure 5. Effect of $CaCl_2$ concentrations (0, 20, 40, 60, and 80%) on the complex viscosity (η^*) (A), frequency dependence of the storage modulus G' (B), and frequency dependence of the loss modulus G'' (C) of pectin hydrogels prepared using 16% pectin and cross-linked with various $CaCl_2$ concentrations (F4 (0% $CaCl_2$), F5 (20% $CaCl_2$), F2 (40% $CaCl_2$), F6 (60% $CaCl_2$), and F7 (80% $CaCl_2$)) for 24 h (pH 8.5). Data are presented as means ± SD (n = 3).

considerably greater number of dissociated carboxylic groups, facilitating the formation of additional calcium bridges.[8,46] Thus, η^* was in the order of F3 > F2 > F1, based on pectin concentration.

The LVR for hydrogels is obtained during strain sweep studies, where G' and G'' remain constant and independent of strain, revealing that the hydrogel structure is preserved and unaffected by strain.[25] The LVRs for F1, F2, and F3 extended over an equal strain range of 0.010−0.398% (Figure S12). Additionally, the critical strain (γ_C) for the three hydrogels was 0.398%, representing the maximum strain value of LVR and the point of departure from the LVR, disrupting the hydrogel structure.[58]

Consequently, the G' and G'' of F1, F2, and F3 hydrogels, obtained during frequency sweep studies, varied based on the concentration of pectin. The viscoelastic properties (G' and G'') followed the order of F3 > F2 > F1 (Figure 4B,C), aligning with the complex viscosity results. The low DE of pectin indicates a remarkably higher presence of dissociated carboxyl groups (nonmethyl-esterified carboxylic acid) compared to the undissociated form (methyl-esterified carboxylic acid). This could promote gel formation via Ca^{2+} bridges between adjacent dissociated (negatively charged) carboxylic groups characterized by the "egg-box" structure.[40] Thus, the variation in the viscoelastic properties among F1, F2, and F3 might be attributed to pectin concentration, where a high pectin concentration ensures a higher prevalence of dissociated carboxylic acids, improving the viscoelastic properties of the hydrogel. This agrees with Han et al.,[59] who demonstrated that as pectin concentration increases, the cross-linking increases, resulting in enhanced mechanical strength and elasticity of the hydrogels. Thus, the viscoelastic properties of hydrogels were highly related to the concentration of pectin and the electrostatic forces formed between the cross-linker and carboxylic groups of LMP.

LMP gels are ionically mediated, where divalent ions such as Ca^{2+} form ionic bridging between two adjacent carboxylic acid groups from different pectin chains. This structure, known as the "egg-box" model, is formed by creating junction zones through organized, side-by-side interactions of GaIA. The "egg-box" model involves particular sequences of GaIA monomers in parallel or neighboring chains that are connected intermolecularly via electrostatic and ionic bonding of carboxyl groups. To stabilize the "egg-box" structure, each involved chain must contain seven continuous carboxyl groups.[60] Additionally, hydrogen bonding and hydrophobic interactions contribute to the formation and stabilization of LMP gels.[61] Reduced DM is favored for LMP gels due to the higher amounts of nonmethylated GalA residues, which increase the potential for forming various "egg-boxes". This results in an LMP-Ca^{2+} mixture that converts from a liquid-like to solid-like gel, exhibiting enhanced rheological properties.[1,62]

3.3.3.2. Effect of $CaCl_2$ Concentration. To study the effect of $CaCl_2$ concentration on the complex viscosity (η^*) and viscoelastic properties (G' and G'') of pectin hydrogels, a series of hydrogels were prepared with a constant pectin concentration of 16% and cross-linked with various $CaCl_2$ concentrations of 0% (F4), 20% (F5), 40% (F2), 60% (F6), and 80% (F7). The η^* curves showed that pectin hydrogels exhibited a pseudoplastic behavior, with η^* decreasing linearly with frequency (Figure 5A). It was found that the η^* of pectin hydrogels was increased concomitant with an increase in $CaCl_2$ concentration. This could be explained by additional Ca^{2+} bridges between the carboxyl groups of adjacent pectin chains, reducing the electrostatic repulsion between similarly charged adjacent pectin chains and enabling the chains to pack more tightly. Such interactions enhance the formation of an "egg-box" structure, resulting in a more rigid structure.[46,63] When comparing η^* of pectin hydrogels, the non-cross-linked F4 hydrogel (0% $CaCl_2$) demonstrated the lowest shear-thinning behavior. In contrast, η^* increased in accordance with $CaCl_2$

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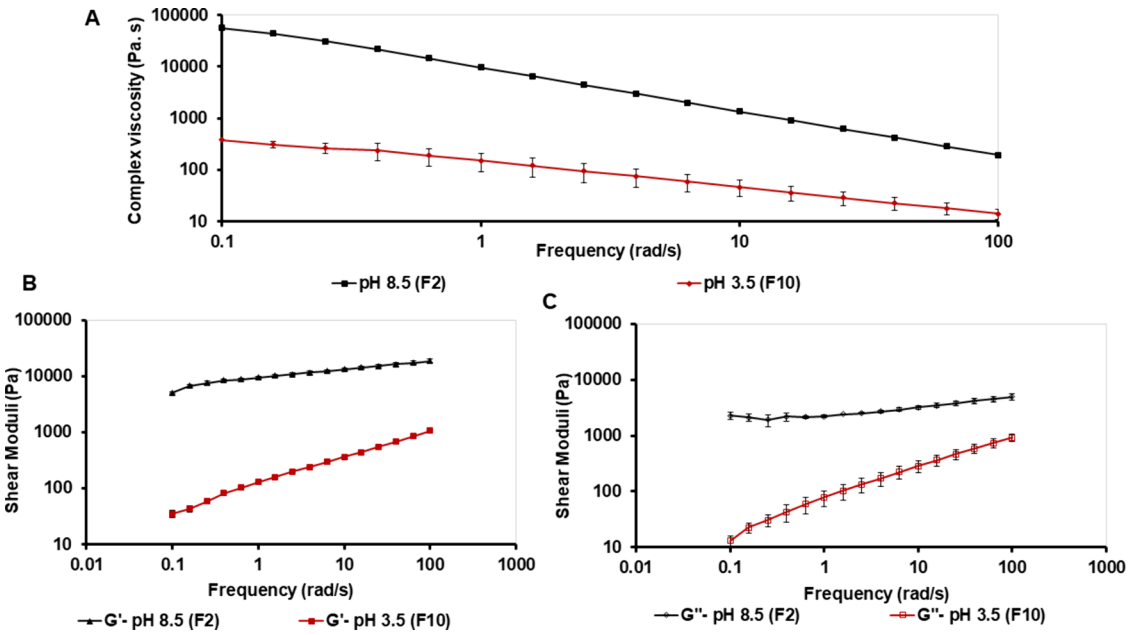

Figure 6. Effect of pH (3.5 and 8.5) on the complex viscosity (η^*) (A), frequency dependence of the storage modulus G' (B), and frequency dependence of the loss modulus G'' (C) of pectin hydrogels F2 (pH 8.5) and F10 (pH 3.5) prepared using 16% pectin and cross-linked with 40% $CaCl_2$ for 24 h. Data are presented as means ± SD (n = 3).

concentration, following the order F7 (80% $CaCl_2$) > F6 (60% $CaCl_2$) > F2 (40% $CaCl_2$) > F5 (20% $CaCl_2$) > F4 (0% $CaCl_2$). This agrees with Kocaaga et al.,[56] who showed that pectin hydrogels exhibit a shear-thinning behavior. Additionally, Cao et al.[46] showed that the viscosity of pectin hydrogel was enhanced at higher $CaCl_2$ concentrations. This is attributed to the rapid gelation induced by the formation of Ca^{2+} ionic bridging with dissociated carboxyl groups of LMP.

The LVR of F4, F5, F6, and F7 extended over strain ranges of 0.010−0.631, 0.010−0.251, 0.010−0.251, and 0.01−0.398%, respectively (Figure S13). The critical strains (γ_C) of F4, F5, F6, and F7 were 0.631, 0.251, 0.251, and 0.398%, respectively. The viscoelastic properties (G' and G'') of F2, F4, F5, F6, and F7 increased with increasing the concentration of $CaCl_2$, where F7 hydrogel cross-linked with 80% $CaCl_2$ exhibited the highest G' and G''. Both G' and G'' were in the order of F7 (80% $CaCl_2$) > F6 (60% $CaCl_2$) > F2 (40% $CaCl_2$) > F5 (20% $CaCl_2$) > F4 (0% $CaCl_2$), consistent with the η^* results (Figure 5B,C). Tran Vo et al.[64] utilized the hot-water acidic extraction technique to extract pectin from citrus pomelo albedo peel (DM 74%). Pectin was further treated with 1 M NaOH to obtain a higher concentration of dissociated carboxylic acid, which is responsible for the pectin gelation mechanism mediated by $CaCl_2$ cross-linking. Pectin hydrogels (3%) were prepared and cross-linked with 7.15, 19.07, and 28.61% $CaCl_2$ at pH 7. It was found that G' and G'' increased with increasing $CaCl_2$ concentration, suggesting an increase in the ionic bridges between dissociated carboxyl groups.

3.3.3.3. Effect of pH. To study the effect of pH on η^* and viscoelastic properties (G' and G'') of pectin hydrogels, F10 (comprising 16% pectin and cross-linked with 40% $CaCl_2$ at pH 3.5) was compared with F2 (comprising 16% pectin and cross-linked with 40% $CaCl_2$ at pH 8.5). Both F2 and F10 hydrogels exhibited pseudoplastic behavior, where η^* decreased with increasing frequency (Figure 6A). Additionally, the η^* of F10 was lower than that of F2. This could be attributed to the low concentration of carboxylate anions, which participate in the formation of Ca^{2+} bridges between adjacent pectin chains at pH 3.5. Tang et al.[65] reported that since the pK_a of pectin is approximately 3.5, at pH > 4.5, more than 99% of the carboxylic acid groups become dissociated and available for electrostatic interactions. Furthermore, Chan et al.[1] demonstrated that at low pH (nearly 3.5), very few carboxylic groups within pectin chains are dissociated. Therefore, hydrogen bonding interactions formed between the undissociated carboxyl groups rather than the conventional egg-box junction zones, resulting in a weak and unstable gel. Moreover, Moreira et al.[66] showed that hydrogels prepared with 2% commercial citrus fruit LMP and cross-linked via Ca^{2+} ions (obtained from 0.184% $CaCO_3$) showed shear-thinning behavior influenced by the pH of hydrogels.

The LVR of F10 extended over a strain range of 0.01−0.251%, with a γ_C of 0.251% (Figure S14). In the frequency sweep tests, F10 (pH 3.5) exhibited lower G' and G'' compared to F2 (pH 8.5) due to the lower pH of the F10 hydrogel (Figure 6B,C). Since the concentrations of pectin and $CaCl_2$ were constant in both F2 and F10 hydrogels, the pH of the hydrogels was the only varied factor. Therefore, G' and G'' were pH-dependent, aligning with the η^* data of F2 and F10. This observation agrees with Yang et al.,[8] who studied the effect of the pH range (3.5−9.5) on the viscoelastic properties of 1% commercial apple LMP hydrogels cross-linked with 6.66% $CaCl_2$. The results showed that as the pH of hydrogels increased, the viscoelastic properties of pectin hydrogels were enhanced due to the higher quantity of charged dissociated carboxylic groups. Additionally, it was found that pectin hydrogel, prepared at pH 8.5, exhibited the highest G' and G'' as well as gel strength.

pH is an additional factor that promotes the gelation mechanism of LMP since sufficient amounts of the dissociated form of carboxylic acids are generated at higher pH values. This promotes the formation of electrostatic forces through Ca^{2+} bridging.[59] As the concentration of Ca^{2+} ions increases, there are more opportunities for cross-linking between

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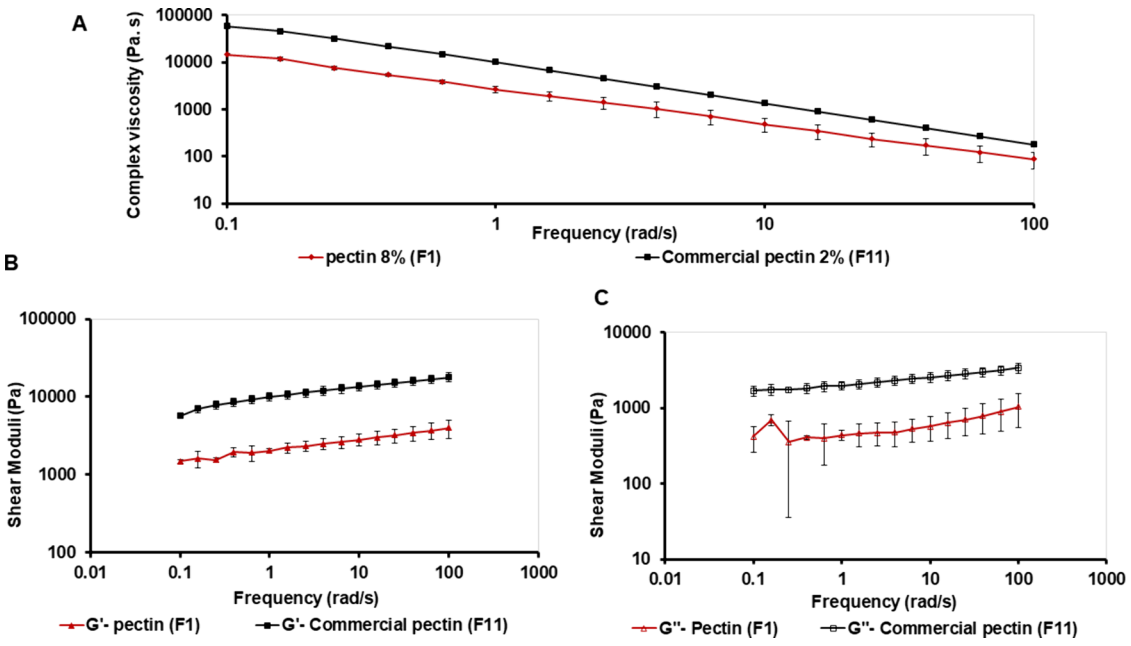

Figure 7. Effect of the pectin type (pectin extracted from orange peels versus commercial pectin) on the complex viscosity (η^*) (A), frequency dependence of the storage modulus G' (B), and frequency dependence of the loss modulus G'' (C) of pectin hydrogels F1 (composed of 8% pectin extracted from orange peels) and F11 (composed of 2% commercial pectin) and cross-linked with 40% $CaCl_2$ for 24 h (pH 8.5). Data are presented as means ± SD (n = 3).

adjacent carboxylic groups, thus enhancing the rheological properties of LMP gels, where gels exhibit a more elastic network.[59] Consequently, high-MW pectin has longer polychains that offer additional binding sites for Ca^{2+} bridges, further enhancing the rheological behavior of LMP gels.[46]

3.3.3.4. Effect of the Pectin Type. To study the effect of the pectin type on η^* and viscoelastic properties (G' and G'') of hydrogels, F11 was prepared with 2% commercial pectin from citrus peels (Sigma-Aldrich) and cross-linked with 40% $CaCl_2$ for 24 h (pH 8.5). The commercial pectin comprised a GaIA content of ≥74.0% (on a dried basis) and methoxy groups of ≥6.7% (on a dried basis), as specified by the manufacturer.

The η^*, G', and G'' of F11 were compared with those of F1, which was prepared with 8% pectin and cross-linked with 40% $CaCl_2$ for 24 h (pH 8.5). The F11 hydrogel exhibited pseudoplastic behavior, with a higher η^* than F1 (Figure 7A). The higher η^* of F11 could be attributed to the fact that the commercial pectin is purer than the pectin extracted from orange peels, as evidenced by its higher GaIA content determined in our lab (121.44 vs 91.52%). Studies have shown that the GaIA content is an indicator of pectin purity, offering additional sites for forming ionic bridges between the dissociated carboxyl groups and $CaCl_2$.[46,67]

The LVR of F11 extended over a range of 0.01−6.31%, with a γ_C of 6.31% (Figure S15). It was found that the LVR of F11 hydrogel extended over a longer region than those of F1−F10 hydrogels prepared from pectin. This implies that F11 is more viscoelastic, capable of withstanding higher strain, and can stretch further without breaking while returning to its original state.[68] The viscoelastic properties (G' and G'') of F11 were higher than those of F1 (Figure 7B,C), consistent with η^* data of F1 and F11 hydrogels. Table 5 summarizes the LVR, the applied strain within the LVR, and the critical strain (γ_C) of F1−F7, F10, and F11 hydrogels.

Table 5. Rheological Parameters of the Strain Sweep Tests (LVR, Applied Strain within the LVR, and Critical Strain (γ_C)) of Pectin Hydrogels

pectin hydrogels	LVR (%)	applied strain within the LVR (%)	critical strain (γ_C, %)
F1	0.010−0.398	0.10	0.398
F2	0.010−0.398	0.10	0.398
F3	0.010−0.398	0.10	0.398
F4	0.010−0.631	0.10	0.631
F5	0.010−0.251	0.10	0.251
F6	0.010−0.251	0.10	0.251
F7	0.010−0.398	0.10	0.398
F10	0.010−0.251	0.10	0.251
F11	0.010−6.310	0.10	6.310

3.4. Bioadhesion of Pectin Hydrogels. *3.4.1. Effect of Pectin Concentration.* The effect of pectin concentration on the bioadhesion of pectin hydrogels (F1, F2, and F3) was examined. F3, which contains the highest pectin concentration (32%), showed significantly better bioadhesion to rat skin, as indicated by a higher detachment force of F3 (344.0 ± 2.5 N) compared to those of F1 (8% pectin) and F2 (16% pectin), which had detachment forces of 304.1 ± 10.2 N ($p < 0.05$) and 294.0 ± 7.4 N ($p < 0.01$), respectively. Additionally, F1 and F2 hydrogels exhibited comparable bioadhesion, where no significant difference was found in their corresponding detachment forces (304.1 ± 10.2 vs 294.0 ± 7.4 N, $p > 0.05$).

The bioadhesive strength between the hydrogels and the skin surface depends on the concentration of the adhesive polymer.[26] It has been shown that as the concentration of pectin increased, the bioadhesive strength also increased.[16] Hydrogels were prepared from pectin with a low DE (20%). This suggests that 20% of the carboxylic groups in pectin chains are methyl-esterified carboxyl groups. This form of carboxylic acid did not form ionic bridges through LMP hydrogel formation. However, it can form bioadhesive

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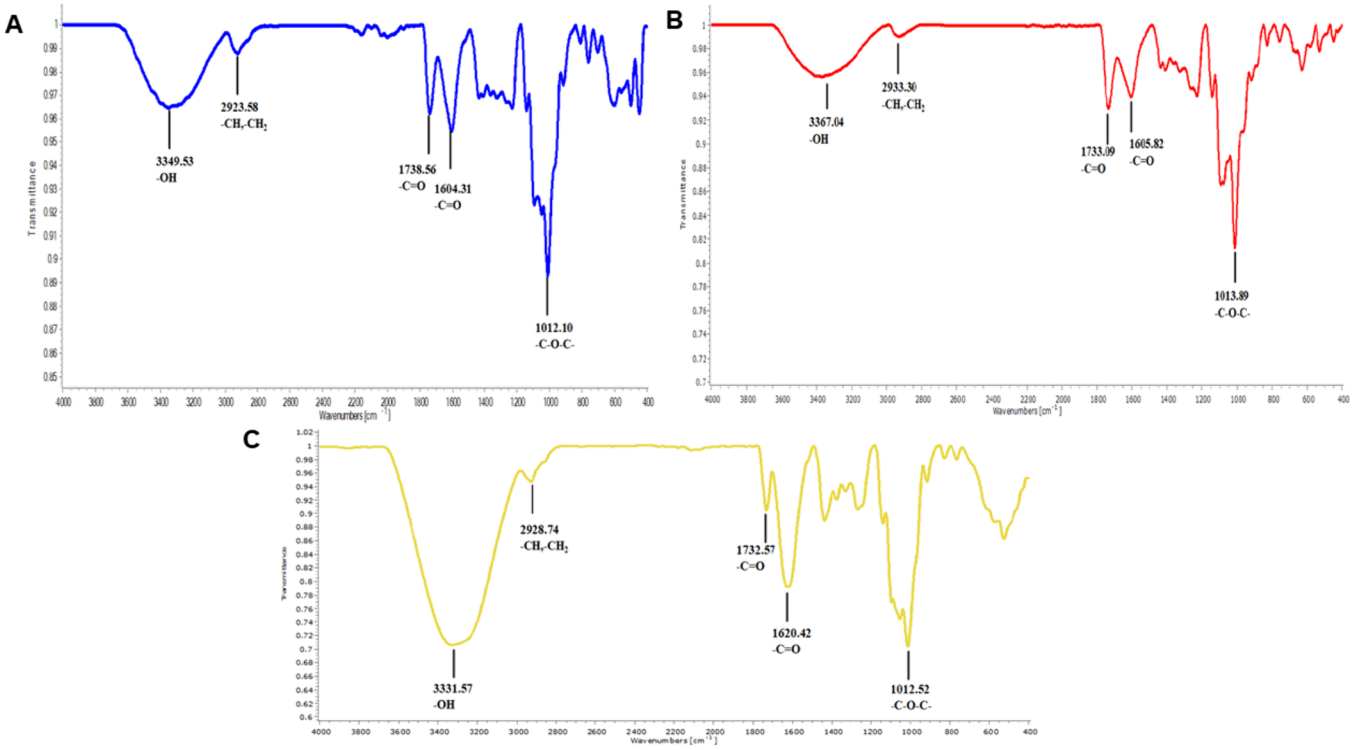

Figure 8. FTIR spectra of pectin extracted from orange peel (A), commercial pectin (B), and F6 hydrogel (16% pectin extracted from orange peel cross-linked with 60% $CaCl_2$ for 24 h, pH 8.5) (C).

interactions with the negatively charged groups on the skin surface.

Therefore, the bioadhesive properties of the hydrogels are attributed to the adhesion mechanism of the adsorption theory, which suggests hydrogen bond formation between the methyl-esterified carboxyl groups of pectin chains and the negatively charged carboxyl and hydroxyl groups present at the surface of the skin.[69,70] This finding aligns with Markov et al.,[16] who studied the effect of various concentrations of the commercial apple low-methoxy pectin (1, 2, and 4%), cross-linked with 7.95% $CaCl_2$, on bioadhesion. The results showed that hydrogels with higher pectin concentrations exhibited greater bioadhesion, where the low methyl-esterified carboxyl groups in pectin chains (accounting for 43% of the total carboxylic groups) participated in adhesion to biological tissues through hydrogen bonding.[16]

3.4.2. Effect of $CaCl_2$ Concentrations. Hydrogels F6 and F7, cross-linked with 60 and 80% $CaCl_2$, respectively, displayed comparable bioadhesive properties, where no significant difference was found between the detachment forces of both hydrogels (352.0 ± 11.7 vs 341.9 ± 7.2 N, $p > 0.05$). Additionally, the bioadhesion of F6 and F7 was significantly higher than that of F4 and F2, cross-linked with 0 and 40% $CaCl_2$, respectively (165.5 ± 11.4 and 294.0 ± 7.4 N, respectively, $p < 0.05$). The results showed that as the concentration of $CaCl_2$ increased, the bioadhesive strength of the hydrogels increased up to 60% $CaCl_2$. At 80% $CaCl_2$ cross-linking, the further increase in the viscoelastic properties of F7 hydrogel did not enhance its bioadhesive properties. This might be attributed to the high rigidity of the F7 hydrogel, which was cross-linked with a high concentration of Ca^{2+} ions.[64] It has been shown that a highly rigid hydrogel network could restrict the adhesion capacity of the functional groups located on the skin surface.[26] This is because highly cross-linked polymers are nonflexible and lack the mobility of individual polymer chains, leading to diminished bioadhesion.[71] Additionally, excess Ca^{2+} might lead to electrostatic repulsion between pectin chains by interacting with other anionic sites in pectin, which can further diminish bioadhesion.[46]

3.4.3. Effect of the Pectin Type. F12 hydrogel prepared with 2% commercial pectin demonstrated higher bioadhesion strength than F6 hydrogel prepared with 16% pectin (433.5 ± 13.9 vs 352.0 ± 11.74 N, $p < 0.01$). The higher bioadhesion strength of F12 can be attributed to the chemical structure of the commercial pectin and its DM or acylation, which varies based on the source of pectin. This is due to the fact that the adhesive properties of polymers can be modified based on the functional groups present on the biological surface, leading to improved bioadhesion.[72] The interactions formed by the natural polymers tend to be weaker.[73] Additionally, the bioadhesive strength is related to the purity of the polymer.[74] Unlike natural polymers, commercial polymers are characterized by their high purity.[67] Therefore, when a high bioadhesive property is required to promote wound healing, extracted pectin can be combined with other polymers, known for their inherent adhesive properties, such as alginate and chitosan, to enhance the bioadhesive properties of pectin hydrogels.[75,76]

Very few studies have explored the bioadhesion of pectin or pectin-containing gels.[16] Most bioadhesive systems have been administered through mucosal membranes.[26] For instance, Markov et al.[16] evaluated the bioadhesion of the low-methoxyl commercial apple pectin (DM = 43%) hydrogels on the serous membranes of the abdominal wall, colon, and liver of laboratory mice. The results demonstrated that 2 and 4% pectin hydrogels exhibited higher bioadhesive properties than 1% pectin hydrogel, indicating that as the concentration of

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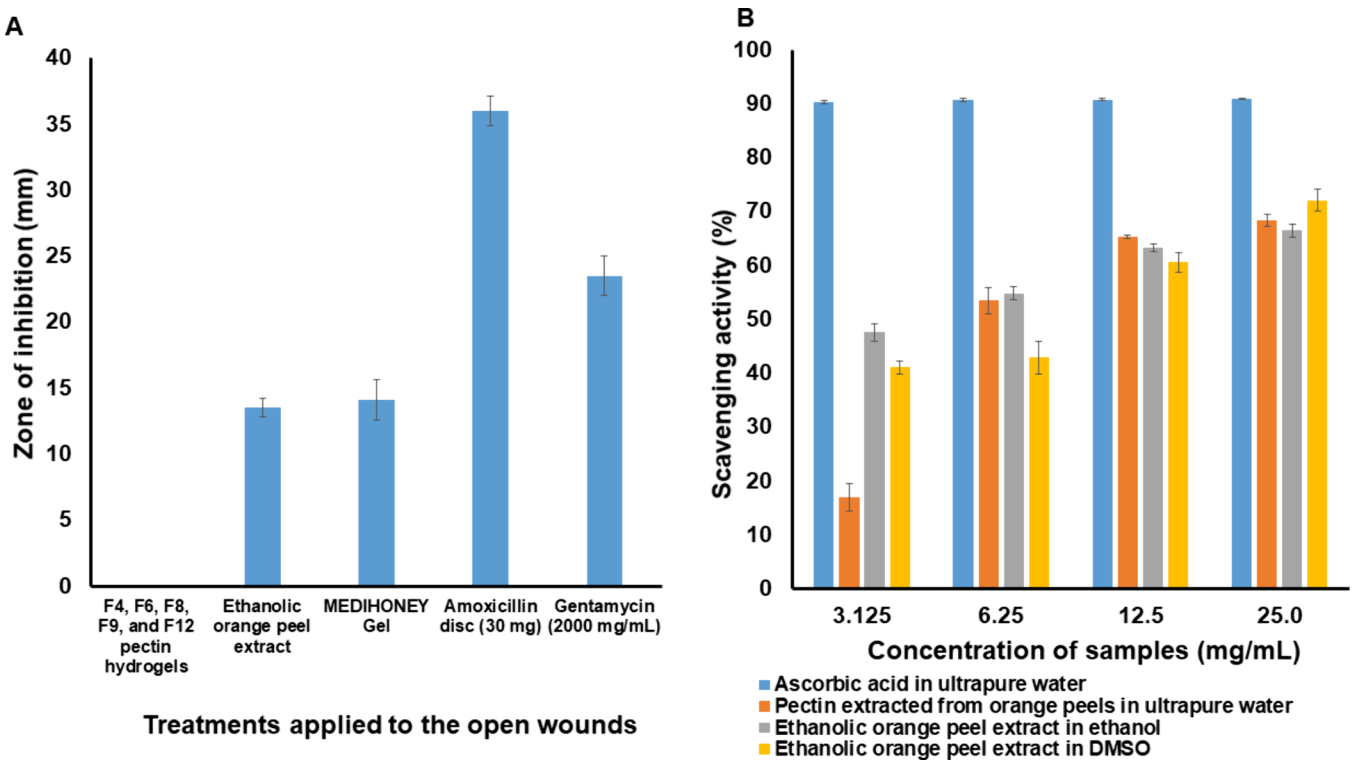

Figure 9. Zones of inhibition of pectin hydrogels, ethanolic orange peel extract, MEDIHONEY Gel, amoxicillin disc, and gentamycin against Gram-positive bacteria (*S. aureus*) by the agar diffusion method. Data are presented as means ± SD (n = 2) (A), and the antioxidant activity of pectin extracted from orange peels, ethanolic orange peel extract, and ascorbic acid was measured using the ABTS assay. Data are presented as the average % scavenging activity ± SD (n = 3) (B).

pectin increases, the bioadhesion of hydrogels also increases. Table 1 summarizes the detachment forces of pectin hydrogels prepared with various pectin types and concentrations (8, 16, and 32%) cross-linked with different concentrations of $CaCl_2$ (20, 40, 60, and 80%).

3.5. FTIR Analysis. The FTIR analysis was performed to identify and compare the distinct peaks that emerged from the functional groups of pectin extracted from orange peels and commercial pectin while also assessing peak intensity, shifting, appearance, or absence as a sign of chemical interactions between the constituents of pectin hydrogels. Figure 8 illustrates the FTIR spectra of pectin extracted from orange peels, commercial pectin, and F6 hydrogel (comprised 16% pectin cross-linked with 60% $CaCl_2$ for 24 h, pH 8.5).

The FTIR spectrum of pectin extracted from orange peels (Figure 8A) displayed several characteristic bands corresponding to OH (broadband 3000−3500 cm^{-1}), CH and CH_2 (stretching bands at 2923 cm^{-1}), C═O (band at 1738 cm^{-1}), carboxylate group (two bands referred to an asymmetric peak at 1604 cm^{-1} and a weak symmetric peak at 1436 cm^{-1}), and the C−O bond (strong band at 1012 cm^{-1}), in agreement with Hosseini et al.[21] The FTIR peaks of the commercial pectin were similar to those observed in the FTIR spectrum of pectin extracted from orange peels (Figure 8A vs Figure 8B).

The FTIR spectrum of the F6 hydrogel (Figure 8C) displayed a 16 cm^{-1} shift of the carboxyl band pectin, which was observed at 1620 cm^{-1} instead of 1604 cm^{-1}, suggesting the formation of an ionic bond between the carboxylate group and Ca^{2+}. The intensity of the carbonyl band at 1738 cm^{-1} was reduced in the hydrogel spectrum due to the basic medium (pH 8.5), suggesting the dissociation of the ester group in the basic medium. The intensity of the OH stretching vibration at 3331.57 cm^{-1} in F6 increased, suggesting the presence of hydrogen bonds in the hydrogel.[64,77]

3.6. Biological Activities of Pectin Hydrogels and Ethanolic Orange Peel Extract. *3.6.1. Antimicrobial Activity.* The antimicrobial activity of pectin hydrogels (F4, F6, F8, F9, and F12) and ethanolic orange peel extract was evaluated against Gram-positive (*S. aureus*, ATCC6538) and Gram-negative (*E. coli*, ATCC8739; *P. aeruginosa*, ATCC9027; and *P. mirabilis*, ATCC12453) bacteria by measuring the zone of inhibition. The diameters of the inhibition zones are shown in Figure 9A. F4, F6, F8, F9, and F12 hydrogels exhibited no zones of inhibition, suggesting a lack of antimicrobial activity. According to Minzanova et al.,[9] pectin nanocomposites and nanoemulsions demonstrated antibacterial activity against *S. aureus* and *E. coli*. Additionally, the pectin solution showed a broad-spectrum bactericidal activity, as reported by Ciriminna et al.[10] The antibacterial activity might be attributed to the binding capacity of the carboxylic acid groups present in the primary backbone of pectin, allowing it to attach to and degrade the outer membrane of microorganisms.[78] Moreover, the acidic pH of pectin is considered a crucial factor for optimal antibacterial activity.[79] Therefore, the absence of antimicrobial activity in pectin hydrogels warranted further investigation.

The zones of inhibition for the ethanolic orange peel extract against *S. aureus* were visible, suggesting effective antimicrobial activity. This could be attributed to the phytochemicals present in the ethanolic extract, including phenolic compounds, flavonoids, and tannins.[80] The antimicrobial activity of the ethanolic extract was comparable to that of the positive control (MEDIHONEY Gel, a commercial Manuka honey). According to Hanafy et al.,[23] ethanolic orange peel extract, when

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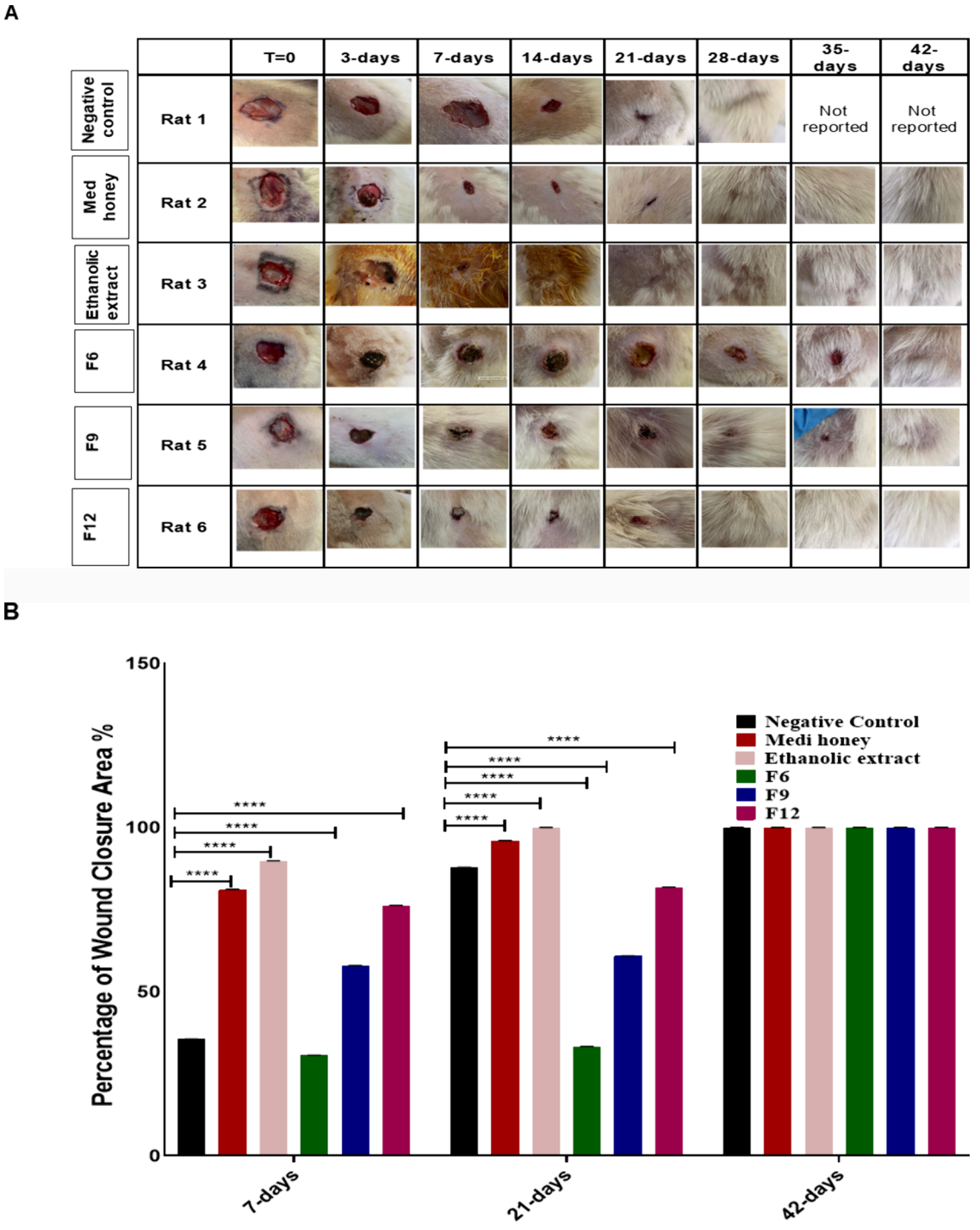

Figure 10. Images depicting the *in vivo* wound healing activity of pectin hydrogels and ethanolic orange peel extract (A) and the percentages of wound closure areas after 7, 21, and 42 days analyzed using ImageJ software. A two-way ANOVA repeated measure followed by Tukey's method for multiple comparisons was applied (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$; $n = 3$ for each group) (B). Data presented as means ± SD.

dissolved in DMSO, exhibited a broad-spectrum antimicrobial against Gram-positive and Gram-negative bacteria. Figure S16 shows images of the zones of inhibition for the pectin hydrogels, ethanolic orange peel extract, MEDIHONEY Gel, amoxicillin disc, and gentamycin against Gram-positive and Gram-negative bacteria, using the agar diffusion method.

3.6.2. Antioxidant Activity. In this experiment, various concentrations were tested to determine the effect of concentration on antioxidant activity. Unlike ascorbic acid (a pure antioxidant compound), the extract contains a significant amount of nonreducing compounds; therefore, concentrations as high as 25 mg/mL were necessary to observe notable antioxidant activity. Serial dilutions were then performed to determine the minimum concentration required for antioxidant activity, which was found to be 3.125 mg/L.

The antioxidant activity of pectin from citrus peels might be attributed to the hydroxyl groups that act as reducing agents.[12] The presence of rhamnose, the RG-I region, and the high

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content of galacturonic acid and phenolic compounds in the pectin structure enhances its antioxidant activity.[11,81] Additionally, the antioxidant activity of the ethanolic orange peel extract might be attributed to the presence of phytochemicals such as phenolic compounds, tannins, and glycosylated flavonoids, including hesperidin, naringin, and gallocatechin.[23]

Figure 9B illustrates the antioxidant activities of pectin extracted from orange peels, the ethanolic orange peel extract, and ascorbic acid, measured by the ABTS assay. The antioxidant activity of pectin extracted from orange peels was 68.34 ± 1.13% for the highest pectin concentration (25 mg/mL), compared to the control sample (ascorbic acid), which showed an antioxidant activity of 90.93 ± 0.12%. The results indicated that the increase in the scavenging activity was concentration-dependent. Thus, pectin could be considered a promising natural antioxidant in pharmaceutical and cosmetic fields. This finding is consistent with Hosseini et al.,[35] who employed the DPPH technique to evaluate the antioxidant activity of pectin and found that the antioxidant activity increased as the concentration of pectin solution increased up to 25 mg/mL. Furthermore, Khodaiyan and Parastouei[82] demonstrated that the antioxidant activity of pectin extracted from black mulberry pomace was ∼75%, compared to those of butylated hydroxyanisole and ascorbic acid of 85 and 90%, respectively. The maximum antioxidant activities of the ethanolic orange peel extract, dissolved in ethanol and DMSO at 25 mg/mL, were 66.5 ± 1.20 and 72.12 ± 2.04%, respectively, indicating its effectiveness as a natural antioxidant. This observation aligns with Shehata et al.,[83] who found that the antioxidant activity of the ethanolic orange peel extract assessed by the ABTS assay was 68.36 ± 0.72%.

3.6.3. In Vivo Wound Healing. The effect of pectin hydrogels (F6, F9, and F12) and ethanolic orange peel extract on the wound healing activity of rat skin was investigated. F6 and F9 (comprised 16 and 32% pectin, respectively) and F12 (comprised 2% commercial pectin) hydrogels were cross-linked with 60% $CaCl_2$ for 24 h. F6 and F12 showed high bioadhesive properties (Table 1), which prolonged the residence time of pectin hydrogel on rat skin and contributed to its maximum wound healing effect. Additionally, F9 (comprised 32% pectin) was studied to evaluate the impact of pectin concentration on wound healing activity. The higher concentration of pectin indicated an increased GaIA content, providing more Ca^{2+} binding sites that enhance water-holding capacity. The hydrophilic characteristics of the hydrogel serve as a barrier against microbial growth, improving the binding affinity for growth factors and accelerating the wound healing process.[46,56]

Pectin hydrogels were applied directly to the open wounds on the dorsal area of the rat without using a secondary dressing such as Tegaderm. This is because the viscoelastic properties, with more elastic (solid-like) behavior, of the pectin hydrogels coupled with high bioadhesive properties ensure the adherence of hydrogels to the wound sites and minimize dislodgement.

F6, F9, and F12 hydrogels showed no significant wound healing activity compared to the positive control (MEDIHONEY Gel). The positive control demonstrated considerable healing on day 21 and achieved complete healing by day 28. Both F6 and F9 showed no wound healing activity on day 28. Conversely, F12 showed a wound healing activity on day 28, similar to that observed with the negative control (no treatment, untreated wound).

The ethanolic orange peel extract showed a remarkable wound healing effect compared to both the negative (untreated wound) and positive controls, with significant healing observed on day 14 and complete healing by day 21. This enhanced wound healing activity might be linked to the beneficial phytochemicals present in the ethanolic orange peel extract, including phenolic compounds, flavonoids, and tannins known for their wound healing activity.[84] Figure 10A illustrates the progression of wound healing over 0, 3, 7, 14, 21, 28, 35, and 42 days. These findings contrast with those of Yi et al.,[71] who found that commercial citrus pectin hydrogel accelerated wound healing more effectively than bulky honey (liquid Manuka honey). They also demonstrated that the unique hydrophilic nature of pectin acts as a barrier against bacteria while binding growth factors, promoting wound healing activity. Moreover, Kocaaga et al.[56] demonstrated that LMP gels have effectively accelerated wound healing.

The wound closure over time was measured using ImageJ software (Bethesda, MD, USA) and quantified using eq 7 (Figure 10B). The difference in the percentage of wound closure areas after 7, 21, and 42 days was analyzed using a two-way ANOVA repeated measure approach. The results indicated significant differences in treatments [$F(5,36) = 64.28$, $p < 0.0001$] and in time [$F(2,36) = 26.79$, $p < 0.0001$], as well as in treatment × time [$F(10,36) = 25.02$, $p < 0.0001$]. Tukey's multiple comparisons revealed a significant difference in the percentage of wound healing in the negative control (untreated wound) compared to other treatment groups at 7 and 21 days. However, by 42 days, no significant differences were detected among the various groups.

3.7. Conclusions. Low-methoxy pectin (LMP) was extracted from orange peels and characterized for yield%, DE %, MeO%, GaIA%, and FTIR analysis. Pectin hydrogels were prepared using various pectin concentrations and cross-linked with various $CaCl_2$ concentrations at pH values of 3.5 and 8.5. It was found that the pectin hydrogels exhibited pseudoplastic (shear-thinning) behavior, where the complex viscosity declined linearly with frequency. Additionally, pectin hydrogels of pH 8.5 exhibited viscoelastic properties where the elastic modulus (G') dominated the viscous modulus (G''), indicating a gel-like structure. Meanwhile, G' and G'' of the pectin hydrogel of pH 3.5 were closely matched. The bioadhesive studies revealed that pectin hydrogels adhered to the skin surface with varying detachment forces based on pectin and $CaCl_2$ concentrations. Moreover, the bioadhesive properties of commercial pectin hydrogels were higher than those of the pectin hydrogels prepared from orange peels. This suggests that commercial pectin is pure and can be modified to suit the functional groups of the skin surface to enhance contact. Pectin hydrogels did not show wound healing or antimicrobial activities, whereas the ethanolic orange peel extract demonstrated wound healing and antimicrobial activities. Additionally, pectin solutions and the ethanolic orange peel extract exhibited more antioxidant activity than ascorbic acid.

In conclusion, LMP hydrogels exhibited viscoelastic and bioadhesive properties, making them ideal carriers for topical drug delivery systems. The wound healing activity of pectin hydrogels and ethanolic orange peel extract should be assessed on a larger group of rats. The ethanolic orange peel extract demonstrated wound healing, antimicrobial, and antioxidant activities. Hence, future work will focus on incorporating the ethanolic orange peel extract into dermal drug delivery systems, such as patches prepared from pectin hydrogels for

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wound healing and treating skin infections. Future studies will also explore the *in vitro* biocompatibility of the pectin hydrogels and their stability over time.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c08449.

Experimental setup for the bioadhesion study of pectin hydrogels; photos of the ethanolic orange peel extract, extracted pectin hydrogel, and commercial pectin hydrogel; compounds identified in the ethanolic orange peel extract by LC-MS; mass spectra of the most abundant compounds in the ethanolic orange peel extract; strain sweep experiments of pectin hydrogels; antimicrobial activity of pectin hydrogels showing the zones of inhibition (PDF)

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Notes

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